GENETIC DIVERSITY ANALYSIS AND TRAIT PHENOTYPING FOR DROUGHT TOLERANCE IN AMARANTH (*AMARANTHUS SPP.)* GERMPLASM

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ABSTRACT

Drought is a major abiotic stress which causes severe crop loses worldwide. One way of enhancing food security in areas of limited or unpredictable rainfall is to exploit the wide genetic diversity of underutilised crop species with increased drought tolerance characteristics. This study aimed to develop a precise trait phenotyping strategy for drought tolerance in *Amaranthus* spp. (*Amaranthus tricolor*). This research provides a framework to identify the genetic basis of drought tolerance in amaranth germplasm through a panel of 188 amaranth mini core collections.

A 188 amaranth mini core collection, derived from an original collection of 783 accessions, was made up of 131 World Vegetable Center Genebank (AVRDC) accessions, 52 United State Department of Agriculture (USDA) accessions, three commercial African varieties from East-West Seed (E-W) and two commercial local Malaysian varieties. It comprises of 18 species from diverse geographical origins and 120 out of 188 accessions belonging to *A. tricolor*. The choice of sampling strategy through stratification based on morphological database allows the core-set to retain more than 70% of the germplasm entire collection. The multivariate analysis using Jaccard's similarity matrix based on 10 qualitative traits, including leaf, petiole and stem colours, growth habit, branching index, leaf shape and margin, and terminal inflorescence colour, shape and attitude revealed that morphological traits were less capable in demarcating plant-type, namely: grain, vegetable and weed in the 188 amaranth mini core collection.

Structure-like population genetic analysis of a high density DArTseq SNPs was performed in two steps; all 188 amaranth accessions and only 120 *A. tricolor* accessions (3,898 SNP for 183 amaranth accessions and 4, 631 SNP for 118 *A. tricolor* accessions after SNP filering, respectively). Both structures produced three major sub-populations (K=3) and this DArTseq SNPs data generates consistent taxonomic classification of amaranth sub-genera (*Amaranthus Amaranthus*, *Amaranthus Acnida* and *Amaranthus albersia*), although the accessions were less likely demarcated by geographical origin and morphological traits. The genome-wide association study (GWAS) of 10 qualitative traits revealed that there was an association between specific phenotypes and genetic variants within a genome as 25 marker trait associations (MTAs) ($P<0.01$) associated with branching index,

petiole pigmentation, inflorescence colour, and terminal inflorescence shape and attitude were found.

To develop a precise trait phenotyping strategy for drought tolerance in *A. tricolor*, two pilot experiments were evaluated separately to evaluate the effect of drought on shoot and root traits; (1) Transpiration efficiency (TE) and (2) Root morphology, leaf gas exchange, cellular hydration and proline accumulation. In TE experiment, plants were subjected to either a gradual dry down or well-watered conditions. Results showed that TE was significantly higher ($P<0.01$) in water-deficient (WD) plants compared to water-sufficient (WS) plants. There was no significant difference in the fraction of transpirable soil water (FTSW) threshold decline between the amaranth genotypes. In second experiment, two contrasting amaranth varieties (red betalain and green acyanic) were subjected to gradual drought stresses. Genotypes that share similar morphological characteristics, specifically leaf colour may not necessarily have the same drought adaptive features. Green leaf amaranths rapidly reduced relative water content (RWC) as early as 10 days of water treatment (DAT, range: 70%-76%), while red leaf amaranth retained comparatively high RWC at 10 DAT and only began to decline at 15 DAT (range: 59%-61%). Green leaf amaranths showed no changes in proline content, while red leaf amaranths displayed variations in the adjustment of proline accumulation at each time point.

Further, two drought tolerance screening trials were carried out on a sub-set of 44 *A. tricolor* accession to identify germplasms with potential drought-tolerant genotypes. Stress intensity was higher in Trial I (0.73) compared with Trial II (0.31) and low broad sense heritability was found for most of the growth traits (ranged: 0.12 to 0.31). Three drought tolerance indices, namely geometric mean productivity (GMP, $P<0.01$), mean productivity (MP, $P<0.001$) and stress tolerance index (STI, $P<0.05$) were consistent and stable predictors of highly drought tolerant genotypes regardless of different weather conditions. Ten tolerant genotypes and three susceptible genotypes were identified and had consistent drought tolerance performance across the two screening trials. This finding revealed that a change in stem biomass was probably the main mechanisms of drought tolerance in amaranth. Stem biomass was negatively correlated with PSII photochemistry (light-adapted quantum yield, *Fv'/Fm'* and dark-adapted quantum yield, *Fv/Fm*) but positively correlated with RWC under drought stress, i.e. stem biomass

improved yield performance by regulating osmotic adjustment and prevent photoinhibition to the plants. A total of 19 significant ($P<0.01$) MTAs were observed in a combined analysis of 11 drought traits, including yield, stem fresh weight, total leaf area, specific leaf area, days to flowering, days to re-cover, and intracellular CO_2, stomatal limitation, photosynthesis and intrinsic water use efficiency at 50% WHC in 44 *A.tricolor* genotypes across the two trials. Subject to further validation, these markers will be useful for marker-assisted selection for respective traits under target growing conditions.

In conclusion, this research has presented a valuable *A. tricolor* diversity panel with its utility for phenotyping drought tolerance traits. By characterizing the diversity panel using a combination of physiological, morphological and molecular data, accessions with superior drought tolerance traits can be elucidated.

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LIST OF ABREVIATION

Abbreviation	Name
AVRDC	Asian Vegetable Research and Development Center, Thailand
Ci	Intracellular CO_2
DAT	Days after drought stress treatment
DS	Drought scoring
DTR	Days to recover
DTW	Days to wilting
E	Transpiration rate
FTSW	Fraction of transpirable soil water status
Fv/Fm	Dark-adapted quantum yield
Fv'/Fm'	Light-adapted quantum yield
GBS	Genotyping-by-sequencing
GMP	Geometric mean productivity
Gs	Stomatal conductance
GWAS	Genome-wide association mapping
LDW	Leaf dry weight
LFW	Leaf fresh weight
Ls	Stomatal limitation
LWS	Leaf wilting syndrome
MAF	Minimum alle frequency
MP	Mean productivity
MTA	Marker trait association
NTR	Normalised transpiration rate
PC	Principle component
PCA	Principle component analysis
PCoA	Principle coordinate analysis
Pn	Photosynthesis
R/S	Root/shoot ratio
RAD	Root average diameter
RL	Root length
RLPV	Root length per volume

Abbreviation	Name
RSA	Root surface area
RV	Root volume
RWC	Relative water content
SDW	Stem dry weight
SFW	Stem fresh weight
SLA	Specific leaf area
SSI	Stress susceptibility index
STI	Stress tolerance index
TCC	Total chlorophyll content
TE	Transpiration efficiency
TLA	Total leaf area
TOL	Tolerance index
USDA	United State Department of Agriculture, USA
WD	Water-sufficient
WHC	Water holding capacity
WS	Water deficient
WUE	Instantaneous water use efficiency
WUEi	Intrinsic water use efficiency
YP	Yield under normal stress
YS	Yield under drought stress
YSI	Yield stability index

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CHAPTER 1

INTRODUCTION

1.1 Research background

Drought is a major abiotic stress which causes severe crop loses worldwide reducing crop yield significantly (Kogan *et al*., 2019; Fahad *et al*. 2017). The accessibility of water for drinking and agriculture affects the food security of around 1.2 billion people (IPCC, 2012). Given climate change and population growth predictions, current agricultural practices will not be able to support the nutritional requirements of a projected nine billion people by 2050 (UN DESA, 2011). An increasing population growth in the world's poorest regions of South East Asia and Sub-Saharan Africa are not able to rely on the limited availability of natural resources (Vermeulen *et al*., 2012a).

There is a global trend for the increasing frequency and severity of droughts (Dai, 2013) which are expected to increase in severity in the next 30-90 years (Wilhite, 2005). The International Disaster Database recorded 642 drought events from 1900 to 2013 in Africa, The Americas, Asia, Europe and Ocenia, affecting over 2 billion people (Masih *et al*., 2014). The fate of these water-limited ecosystems is critically linked with the consequences of climatic change which includes high temperatures, low rainfall and long dry seasons. Rain fed land is particularly vulnerable to unpredictable rainfall patterns (Magombeyi and Taigbenu, 2008; Kurukulasuriya and Ajwad, 2007). This affects more than 1.1 billion people in South Asia and Sub-Sharan Africa who are largely dependent on the agricultural sector for their livelihood and additionally, 75% of these people live in poverty (Ali *et al*., 2017; Vermeulen *et al*., 2012b).

Whilst changing global climates are bringing an increased risk of food insecurity worldwide, one way of enhancing food security is to improve yields in the agricultural sector. However, this sector is primarily dominated by three major crops; maize (*Zea mays*), rice (*Oryza* spp) and wheat (*Triticum* spp), contributing to an increasingly uniform global diet (Massawe *et al*., 2016; Khoury *et al*., 2014; George *et al*., 2012). Despite being a vital source of approximately 60% of the world's calorific intake, the production of these staple crops is already affected by drought, and the production is unstable due to unpredictable weather (Daryanto *et*

al. 2016; Elliot *et al*., 2014; Kadam *et al*., 2014;). In the quest for yield improvement, tolerance to abiotic and biotic stresses may have been lost in these major crops, making them less resilient to extreme weather and less adapted to low input environments (Hlaváčová *et al*., 2017; Massawe *et al*., 2016; Shiferaw *et al*., 2011).

Therefore, there is an urgent need to explore alternative crops that have the potential to fulfil future food requirements to complement these major crops. One way of enhancing food security in areas of limited or unpredictable rainfall is to exploit the wide genetic diversity of underutilised crop species with increased drought tolerance characteristics (Siwar *et al*., 2013). These crops often contain desirable traits for disease resistance and abiotic stress tolerance (Mabhaudhi *et al*., 2016). Underutilised crops such as amaranth (*Amaranthus* spp.), jute mallow (*Corchorus olitorius*), winged bean (*Psophocarpus tetragonolobus*), Chinese kale (*Brassica oleracea* var. alboglabra) and other crops have potential to achieve high values in markets globally (Ebert, 2014). These crops species maybe widely distributed globally but restricted to a local production and consumption system. With often good adaptation to marginal lands, they constitute an important part of the local diet providing valuable nutritional components, which are often lacking in staple crops (Jain and Gupta, 2013).

Amaranth (*Amaranthus* spp.) belongs to Amaranthaceae family and consists of about 60-70 species which include three cultivated grain (*A. caudatus, A. cruentus* and *A. hypochondriacus*), vegetable crops (*A. blitum and A. tricolor*) and weeds (*A. spinosus, A. virdis, A. retroflexus, A. graecizans, A. dubius and A. hybridus*) (Das, 2012). However, the exact species numbers are still uncertain due to hybridization (Judd *et al*., 2008). Amaranth species are indigenous vegetables commonly consumed in South East Asian, African and South and Central American households and are widely consumed in Malaysia. In tropical markets, amaranth is among the cheapest dark-green leafy vegetables (Varalakshmi, 2004). In Africa, amaranth is an important leafy vegetable because it is highly nutritious and the ease with which it can be grown and cooked (Achigan-Dako *et al*., 2014; Maundu *et al*., 2009).

Amaranth has huge potential as a crop, as it possesses important traits such as highly nutritional quality, low production cost and a rapid growth cycle (Katiyar *et al*., 2000). It contains high level of vitamins and protein, and well-balanced

amino acid profile compared with staple crops (Jain and Gupta, 2013; Rastogi and Shukla, 2013). Being a cheap source of vitamins, amaranth could be among the crops needed to achieve the objective of United Nations Sustainable Development Goal 2, which is to eradicate extreme hunger and poverty, particularly in South East Asia and Sub Saharan Africa. While levels of undernourishment are increasing globally, over reliance on nutrient poor but carbohydrate rich staple crops means malnutrition and micro-nutrient deficiency remain. The availability of cheap and nutritious leafy green vegetables is therefore one way to achieve a healthy lifestyle. Therefore, amaranth could provide easy and cost-effective way to combat malnutrition and to achieve food security (Emokaro *et al*., 2007).

Amaranth is extremely adaptable to adverse environments with no major disease problems, resistance to drought (Barrio and Anon, 2010; Robert *et al*., 2008), high phenotypic plasticity (Khanam and Oba, 2014) and great amount of genetic diversity (Stetter *et al*., 2017; Brenner *et al*., 2000). Amaranth expresses the C_4 carbon cycle, which is more common in grasses but rare in dicots (Stetter *et al*., 2016). Partly as the consequence of its C_4 photosynthesis, amaranth has high water use efficiency and able to maintain CO_2 fixation during drought stress conditions (Omami and Hammes, 2006). Amaranth has the capacity to change its phenotype in response to environmental changes, such as exhibiting an indeterminate flowering habit, growing long tap roots and extensive lateral root systems in response to drought stress (Kadereit *et al*., 2003). The presence of high genetic and phenotypic diversity in amaranth indicates an excellent potential for breeding and varietal development with increased drought tolerance characteristics (Sarker and Oba, 2018; Alemahayu *et al*., 2014).

Therefore, amaranth should be investigated further as a potential crop to extend the use of marginal agricultural lands of arid and semi-arid regions. This will allow an improvement in agricultural system with the purpose of combating hunger and malnutrition in developing countries and to enhance global food security.

1.2 Justification of study

Amaranthus tricolor is an erect and branching annual herbaceous plant (Grubben, 2004). It is thought to have originated in Tropical Asia and is widely cultivated as a commercial vegetable similar to spinach in South and Southeast Asia, and in East

and Southern Africa, however it has limited economic significance (Grubben, 2004). It is now gaining recognition as a healthy food and climate smart crop (Sogbohossou et al., 2018) with leaf protein contents ranging from 12% to 38% (Andini *et al*.,2013). It has high levels of minerals such as potassium (6.4-6.7g/kg), calcium (2.80-3.00g/kg) and magnesium (2.80-3.00g/kg) which may support the intake of recommended daily dietary levels (Shukla *et al*., 2006).

A. tricolor has high rates of photosynthesis and respiration when grown at high temperatures (35°C) and irradiance (Lin and Ehleringer, 1983). *A. tricolor* along with grain amaranth species, *A. cruentus* and *A. hypochondriacus* have been shown to be well adapted in drought stress conditions by reducing leaf area and stabilizing cellular structures through the accumulation of proline (Slabbert and Kruger, 2014). Liu and Stützel (2004) found that *A. tricolor* was capable of maintaining plant growth under drought stress by maintaining water balance (water loss and water uptake) between plant organs. The drought stressed amaranths are also capable of recovering in a short period of time after restoring leaf hydration (Huerto-Ocampo *et al*., 2009; Slabbert *et al*., 2004). However, to date, few studies have recognised leafy vegetable amaranth for its capacity to grow under extreme drought stress conditions, and a precise trait phenotyping strategy for drought tolerance in *Amaranthus* spp. has not yet been elucidated.

Knowledge of genetic diversity and trait variations in crop germplasm is important for plant breeding and for developing plant genetic resources with improved traits (Akin-Idowo *et al*., 2016). Amaranth has a high degree of plasticity and therefore can be difficult to classify based on morpho-physiological traits alone. Correct genotypic identification and preservation is important to maintain ecotypes that have desired traits for breeding programmes (Perez-Gonzalez, 2001). In leafy vegetable breeding, leaf yield is the primary target trait hence it requires proper characterization and strong correlations between other phenotypic traits and leaf yield (Sogbohossou *et al*., 2018). High heritability and genetic advances have been estimated in *A. tricolor* for leaf yield, and a strong correlation with plant height, number of leaves and stem diameter has been observed (Sarker *et al*., 2014; Shukla *et al*., 2006). Therefore, leaf yield in *A. tricolor* could be significantly improved through direct selection of these traits.

Several genomic resources in grain amaranth have been developed through various types of molecular markers, such as random amplified polymorphic DNAs

(RAPDs) (Transue *et al*., 1994), isozymes (Chan and Sun, 1997), amplified fragment length polymorphisms (AFLPs) (Xu and Sun, 2001), and restriction fragment length polymorphisms (RFLPs) (Park *et al*., 2014), simple sequence repeats (SSRs) (Mallory *et al*., 2008), single nucleotide polymorphisms (SNPs) (Maughan *et al*., 2011), genotyping-by-sequencing (GBS) (Stetter & Schmid, 2017; Stetter *et al*., 2017; Wu & Blair, 2017). A whole genome sequence for grain amaranth has recently been made available (Lightfoot *et al*., 2017; Clouse *et al*., 2016). While most of these amaranth marker studies have been useful for evolutionary and phylogenetic analysis between weedy and grain amaranth, further germplasm characterization and marker validation for leafy vegetable amaranth is needed.

From a breeding perspective, the genetic potential of a crop is determined by the combination of genes it contains, their mutual interactions and the interaction with the environment to produce the specific traits phenotype. A genomic study through molecular tools such as Next Generation Sequencing (NGS) or DNA markers could generate significant data on the genetic control of traits and their interaction with the environment. This approach would give species- and trait-specific results, with a deep understanding of the phylogenetic relationships of those crops and it should be possible to identify what the genetic issues and potential of the species are (Mayes *et al*., 2011). Therefore, by characterizing the population structure of *A. tricolor* using a combination of physiological, morphological and molecular data, accessions with superior drought tolerance traits can be identified.

1.3 Aims and objectives

This research seeks to identify vegetable *A. tricolor* accessions with superior drought tolerance traits with the ultimate aim of broadening the number of crop species used in agriculture to mitigate climate change and contribute to healthy diets. This research will provide a framework to identify the genetic basis of drought tolerance in *Amaranthus* spp.

The specific objectives were to:

i. Develop an amaranth mini core collection of 18 *Amaranthus* species, based on qualitative morphological traits, comprised of grain, leafy vegetables

and weedy species differing in geographical origin and morphological traits.

ii. Construct a high-density DArTseq SNP-based population structure in the amaranth core-set.
iii. Develop a rapid and effective phenotypic screening method for drought tolerance traits in vegetable amaranth.
iv. Identify drought tolerance indices and stable traits for vegetable amaranth that can be use in future breeding programmes.
v. Investigate the genetic basis of drought tolerance in vegetable amaranth.

1.4 Thesis layout

Chapter 1: Describes the research background with an urge to develop new crop varieties with increased drought tolerance traits. This section justifies the need to provide a framework in identifying *A. tricolor* accessions with superior drought tolerance traits.

Chapter 2: Provides an extensive review of the existing literature focusing on the need for crop diversification to mitigate climate change and amaranth as one of the potential climate smart crops.

Chapter 3: Presents the morphological characterisation of the entire amaranth germplasm conserved in The World Vegetable Center Genebank, Taiwan (formerly AVRDC) and *A. tricolor* accessions from the United State Department of Agriculture (USDA) through free online access morphological database, leading to the development of amaranth mini core collection (188 accessions comprised of 18 species, including 5 varieties as checks).

Chapter 4: Reports on a construction of a high-density DArTseq SNP-based genetic map of the selected 188 amaranth mini core collection and only 120 *A. tricolor* accessions. GWAS was conducted on 10 morphological traits to demonstrate the effectiveness of the amaranth diversity panel for trait dissections.

Chapter 5: Reports on a pilot study to identify surrogate traits associated with drought tolerance in amaranth. Two experiments were conducted separately to achieve different objectives; Experiment I: Transpiration efficiency of vegetable amaranth in response to terminal drought stress and (ii) Experiment II: Genotypic variation in growth, root morphology and plant physiology of *A. tricolor* in response to gradual drought stress. From this, genotypic variations in growth and physiological responses of amaranth to drought stress were identified.

Chapter 6: Covers drought tolerance screening of 44 *A.tricolor* reference collections from 188 amaranth mini core collections (single seed descent) in two crop growing cycles. The response of *A. tricolor* sub-set under drought stress and its genetic bases are discussed in this section. From this, a dissection of stable traits for drought tolerance in amaranth and the best drought tolerance indices are presented.

Chapter 7: Provides a full in depth critical discussion of all the results in relation to published literature and attempts to draw conclusions for the present research work. Limitations of the research undertaken and future recommendations are also discussed.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction: Crop diversification through a wider use of underutilised crops

With the predicted effects of climate change and a rapidly growing world population, there is a need for adjustment in the agriculture sector such as improved irrigation, better post-harvest storage facilities and higher food production capacity. Consideration has to be given to a wide range of crop species (crop diversification) to determine which crops species match the prevailing climates (Mustafa *et al*. 2019; Chivenge *et al*., 2015).

Underutilised, minor, orphan or neglected crops are indigenous crop species, which are well adapted to marginal lands and typically have low input needs (Mabhaudhi *et al*., 2016). The crops were once cultivated widely by farmers in low input systems, but have become neglected due to large genetic variability, low agronomic value, and lack of socio-economic awareness (Padulosi *et al*., 2002). Underutilised crops have the potential to be better adapted to the adverse effects of climate change due to their wide genetic diversity and adaptive capacity harboured within landraces (Brenner *et al*., 2010; Massawe *et al*., 2005). The identification and development of underutilised crops can increase crop productivity and improve food security in areas of limited or unpredictable rainfall (Dawson *et al*., 2019; Massawe *et al*., 2015).

In comparison to major crops, underutilized crops have large genetic variability due to lower levels of historical selection pressure, with most genetic diversity retained within and among different landraces (Andini *et al*., 2013; Chan & Sun, 1997). Informal traditional farmer seed systems have preserved their genetic diversity through their own local strategies by exchanging germplasm (Massawe *et al*., 2005). Meanwhile, major crops are genetically homogeneous, as a result of formal seed systems and intensively breeding modern hybrids, and the use of certified varieties in major crops has limited their diversification (Mabhaudhi *et al*., 2016).

Crop diversification may supress pest outbreak and reduces pathogen transmission (Lin, 2011), lessens the risk and uncertainties of monoculture and

improves soil conditions which may worsen under future climate conditions (Mustafa *et al*., 2019; Njeru, 2013; Saraswati *et al*., 2011). The potential of underutilized crops has been addressed in 17th Sustainable Development and Goals 2015, which aims to intensify food production and subsequently improve household incomes and guarantee food and nutritional security. Underutilized crops have the potential to help subsistence farmers where at present there is a risk of over-reliance on a limited number of major crops. However, there are challenges in improving and protecting the diversity of underutilized crops to complement with major crops. For instance, there are no thorough efforts in preserving agro-biodiversity within germplasms, lack identification of valuable characteristics and common agronomic traits still remain unexplored (Mayes *et al*., 2011).

Therefore, it is important to identify significant traits in underutilised crops that currently exceed the equivalent trait in major crops, such as drought tolerance, and the need to have a good prospective in markets which will be worth investment from the very limited resources available (Mayes *et al*., 2011). Among underutilised crops, amaranth is considered a promising crop for cultivation in marginal, arid and semi-arid regions because of its nutritional benefits and its ability to withstand drought (Dawson *et al*., 2019; Sarker and Obe, 2018; Allemann *et al*., 1996). This review is an effort to gather information on the potential of amaranth as an alternative crop to support food security with emphasized on understanding the fundamentals of drought tolerance traits in vegetable amaranth.

2.2 Tapping into indigineous knowledge: *Amaranthus spp.*

2.2.1 History

Amaranth is amongst the oldest crops found in the Americas, with archaeological evidence suggesting that grain amaranths were cultivated in Mexico as early as 5000 B.C.E (Sauer, 1950). Grain amaranth *(A. hypochondriacus and A. cruentus*) was an important sacred staple crop and had equal status with other major crops including maize and bean during the reign of Aztec emperor Montezuma II (Sauer, 1950). The grain is native to Mexico and Guatemala and is consumed as a sweet snack named ‘alegria’, where the grains are toasted and mixed with honey and chocolate or milled into flour (Sauer, 1967). It was also popularly cultivated for its rich colour, which was used as dyes in religious rites and cultural roles in pre-Columbian civilizations (Sauer, 1967, 1950). However, the cultivation of grain

amaranth in the Americas was actively suppressed at the end of 5000 B.C.E. and continued to decline after the Spanish conquest, because of its deeply rooted use in indigenous religious ceremonies (Iturbide and Gispert, 1994; Sauer, 1993, 1976).

Later, the Europeans introduced the crop into Europe and by 18th century amaranths were widely distributed to Africa and various parts of Asia as grain and vegetable crops (Sauer, 1993). The production of amaranth in the US began to rise in the late 1970s and by the 1990s there was an improvement in the understanding of grain amaranth in terms of its nutritional value (Brenner *et al*., 2000). Since then, the popularity of grain amaranth has spread to several countries including Mexico, Thailand and Kenya (Lehmann, 1996). Currently, it has worldwide cultivation, mostly in warm temperate and tropical climate regions, as grain or leafy vegetable (Parra-Cota *et al*., 2014).

2.2.2 Origin and evolutionary history

There are two hypotheses that have been proposed for the evolutionary origins of the grain amaranth species. The first hypothesis is based on geographical separation and suggests that the grain amaranth species evolved independently; (i) *A. caudatus* (in Andean region) evolved from *A. quitensis* (subtropical South America), (ii) *A.cruentus* (South Mexico and Central America) evolved from *A. hybridus* (East North America and Central America Highlands), and (iii) *A. hypochondriacus* (North West and Central Mexico) evolved from *A. powellii* (Mexico) (Sauer, 1976, 1967, 1950). The second hypothesis is based on plant morphology by which grain amaranth may have evolved from a single progenitor species *(A. hybridus).* It suggests that either (i) *A. cruentus* arose *from A. hybridus* which in turn hybridised with *A. powellii* and gave rise to *A. hypochondriacus* or (ii) *A. cruentus* arose from *A. hybridus* which eventually hybridized with unknown amaranth and gave rise to *A. caudatus* or (iii) *A. hybridus* may also have hybridized with an unknown amaranth to give rise to *A.quitensis* (Sauer, 1976, 1967, 1950).

Recent studies using molecular markers show that *A. hybridus* is polyphyletic while the grain species are monophyletic, suggesting that all three grain amaranth species arose directly from *A. hybridus* in multiple independent domestication events (Mallory *et al*., 2008), giving convincing evidence to the second hypothesis. Studies based on phylogenies using various types of molecular markers also support the hypothesis that *A. hybridus* could be the progenitor

species of the grain amaranth (Clouse *et al*., 2016; Kietlinski *et al*., 2014; Xu and Sun, 2001; Transue *et al*., 1994).

The ornamental and vegetable type of amaranth, specifically *A. tricolor* was most likely originated in India. It was later introduced to South America, and other tropical and temperate regions (Martin and Telek, 1979). Several domestic varieties of ornamental and vegetable types have been further developed and are extensively cultivated in southern China (Rastogi and Shukla, 2013). Other wild species of amaranth, for example *A. graecizans* and *A. thunbergii* are specifically found in Africa (Alemayehu *et al*., 2014).

2.2.3 Taxonomy and botany description

Amaranth (*Amaranthus* L.) is a C_4 dicotyledonous plant (Kauffman and Weber, 1990). It belongs to the Amaranthaceae family within the order Caryophyllales, which contains nearly 180 genera and 2,500 species (Sauer, 1993). *Amaranthus* along with *Chenopodium* (quinoa and canahua), *Beta* (beet and sugar beet), and *Spinacia* (spinach) are the cultivated genera in the family. *Amaranthus* genus consists of approximately 60-70 species grouped into three sub-genera (Mosyakin and Robertson, 2003); *Amaranthus Acnida*, *Amaranthus Albersia*, *Amaranthus Amaranthus*. Sub-genera *A. Amaranthus* comprises of three cultivated grain species (*A. caudatus, A. cruentus* and *A. hypochondrius*), while sub-genera *A. Albersia* consists of 17 vegetable species (including *A. tricolor, A. blitoides, A. blitum, A. viridis and A. graecizan*) and sub-genera *A. Acnida* consists of weeds (*A. spinosus and A. palmeri*) (Achigan-Dako *et al*., 2014; Das, 2012).

The exact species numbers are uncertain due to hybridization and species concepts (Judd *et al*., 2008). As amaranth had different centres of domestication and origin (Costea *et al*., 2004), cross hybridizations have produced many interspecific hybrids made it difficult to establish the phylogeny and taxonomy of the whole genus (Wassom and Tranel, 2005). Besides, a small number of suitable traits and high phenotypic plasticity intensify the taxonomic complexity (Stetter and Schmid, 2017b). It is possible that only a fraction of available accessions are accounted for as species number despite a large amount of amaranth hybrids (Jacobsen and Mujica, 2003).

The taxonomy and phylogeny of the genus has been investigated using phenotypic traits and genetic markers (Stetter and Schmid, 2017; Jimenez *et al*.,

2013). However, none of the published studies can identify a consistent taxonomic classification (Lanoue *et al*., 1996; Chan and Sun, 1997; Wassom and Tranel, 2005; Das, 2012). Nevertheless, the floral parts and seed morphology have been used for taxonomy identification (Trucco and Tranel, 2011) (Figure 2). The dicotyledonous nature of amaranths precluded them from being classified as a cereal, as true cereals are monocotyledonous grasses. Therefore, grain amaranth is referred to as a pseudo-cereal.

Grain amaranth is characterized by a large to moderately large complex apical inflorescence comprising aggregates of cymes, five tepal lobes and five stamens, variable seed coat colour and well defined flange, utricle circumscissile (Das, 2012). Vegetable amaranth can be distinguished by its inflorescence and indeterminate growth habit, possession of axillary glomerules or short spikes, flower buds from the leaf axil, three tepal lobes and stamens, and has brownish black seed with undifferentiated folded flange. Despite of having well-defined characters to distinguish grain, vegetable and weed amaranth, species differentiation based on morphology features have always been challenging in amaranth. This is because huge dissimilarities are found between and within species (Mandal and Dhangrah 2009), along with large intermediate forms and broad geographical distribution (Mujica and Jacobsen, 2003). Besides that, morphological descriptors are notoriously plastic due to environmental influence (Espitia, 1992; Sauer, 1967).

2.2.4 Breeding system

The Amaranthaceae family is an ancient paleopolyploid, and the species made up of three ploidy levels. Amaranth has experienced whole genome duplication events in its evolutionary history with most species having a haploid chromosome number of n=16 (*A. hypochondriacus*, *A. caudatus*, *A. quitensis*, *A. edulis*, *A. powellii*, and *A. retrolexus* L.) or n=17 (*A. cruentus*, *A. tricolor* L. and A. *spinosus* L.) with exceptions of A. dubius with n=32 (Lighfoot *et al*., 2017; Grant, 1959). Two independent whole genome duplications were occurred in amaranth to give rise to the extant tetraploids (n=16), due to chromosome loss of one homoelog of Chr5 and chromosome fusion of the two homoeologs of Chr1 that explained the reduction from ancestral haploid chromosome number (n=18) in Amaranthaceae family, while n=17 species was presumed to share only one of these chromosomal

Figure 2.1: (A) *A. hypochondriacus* with red inflorescence and partially red-coloured leaves; (B) *A. cruentus* with red inflorescences and partially red-colored leaves; (C) *A. spinosus* with clearly visible spines on the main stem; (D) Green colour *A. tricolor*; (E) Red colour *A. tricolor* and (F) Axillary inflorescence of *A. tricolor* on the main stem (Achigan-Dako *et al*., 2014; Ebert *et al*., (2011).

reduction events (Lighfoot et al., 2017). With the exception of *A. dubius*, amaranth genotypes have been characterised with a high degree of meiotic abnormalities such as multivalent and stickiness of chromosomes, leads to clumping, overlapping of chromosomes and unequal segregation of chromosomes at anaphyse I (Oyelana and Ugborogho, 1992*)*. The grain amaranths are paleo-allo tetraploids (Greizerstein and Poggio 1995).

The breeding system in amaranth species is complex because of the influence of genetics and environmental variations (Hauptli and Jain 1985; Jain *et al*., 1982). The cultivated varieties of amaranth are monoecious (Mosyakin and Robertson, 2003) and primarily self-pollinated (Das, 2016), with female and male flowers arranged in close proximity (Murray, 1940). While some weedy amaranth including *A. tuberculatus* and *A. palmeri* are dioecious (Trucco and Tranel, 2011). Amaranth may combine their natural ability of self and cross pollination through

wind, with average outcrossing of 4%-34% (Brenner and Widrlechner, 1998; Kulakow and Hauptli, 1994; Pal and Khoshoo, 1973).

Whilst some of amaranth species are dioecious, where outcrossing is a must, the variation in outcrossing is dependent on the ratio of staminate to pistillate flowers, and pollinators such as insects could also account for some variability in outcrossing rates (Hauptli and Jain, 1985). It is possible but challenging to produce hybrid amaranth. However, reproductive barriers such as pollen grain sterility and low pollen fertility make F1 seed production difficult (Gudu and Gupta, 1988).

Gene exchange can be difficult due to the differing amaranth species chromosome numbers (Andini *et al*., 2002). In crosses between monoecious and dioecious amaranth species the gender is determined by the pollen parent (Murray, 1940) with a monoecious pollen parent producing female progeny and a dioecious pollen parent producing mixed gender progeny. Observation of natural hybrids show that grain amaranths are cross compatible with several other amaranth species such as *A. arenicola* and *A. australis* (Sauer 1972, 1967, 1957). Amaranth species can be cross incompatible, for example failed outcrosses between grain *A. cruentus* with *A. hypochondriacus* and *A. caudatus*, due to pollen sterility (Greizerstein and Poggio, 1994). However, recently, hand emasculation has been used to successfully produce inter- and intra-specific grain amaranth F1 offspring (Stetter *et al*., 2016).

2.2.5 Cultivation and crop physiology

Amaranth requires less water for cultivation compared to maize, wheat and cotton (*Gossypium hirsutum*) (Kauffman and Weber 1990), although too little water can cause early flowering (Schippers, 2004). It can grow in saline (Sarker *et al*., 2018; Saucedo *et al*., 2017; Huerta-Ocampo *et al*., 2014) or poor fertility soils (Nasir *et al*., 2016) with a low nitrogen requirement (Ejieji and Adeniran, 2010). Soil rich in nitrogen is beneficial to vegetable amaranth as high levels of nitrogen will delay the onset of flowering, thus providing higher leaf yield (Schippers, 2004). Amaranth grows well in high temperature 30/25 °C day/night (Khandaker et al. 2009) with peak photosynthetic rates have been observed at 35ºC (Ehleringer, 1983) and intense solar radiation (Jin *et al*., 2016), while low temperature reduces the vegetative growth (Whitehead *et al*., 2002).

2.2.6 Nutritional characteristics

The nutritional components of amaranth seeds are extensively reviewed in Venskutonis and Kraujalis (2013). Amaranth has a high value of proteins, amino acids, linoleic acid and minerals such as iron, magnesium and calcium in both the grain and leaves (Alvares-Jubete *et al*., 2009; Schnetzler *et al*., 1994). Starch is the main component of the grain (Wu and Corke, 1999) with high fibre content compared to most cereals (Pedersen *et al*., 1987) (Table 2.1). The grain is gluten-free (Alemayehu *et al*., 2014) and the protein consists of high levels of the amino acid lysine which are lacking in maize, wheat and rice (De Ron *et al*., 2017). The sulphur-containing amino acids, normally limited to beans and other legumes, are also high in grain amaranth and ranked second for protein quality after soybean, and approximately 50% higher compared to wheat, rice and maize, with range of 12.0-22.5% (Schoenlechner *et al*., 2008; Gupta and Gudu, 1991). The seed oil is highly unsaturated, containing mostly non-polar liquid compounds, especially triglycerides (Gamel *et al*., 2007). Aside from the highly nutritious component, amaranth seeds also contain other biological substances that are beneficial to the human diet such as protease inhibitors, antimicrobial peptides, lectins and antioxidant compounds (Valdes-Rodrfguez *et al*., 1993).

Vegetable amaranth is an excellent source of vitamin A, caretonoids, ascorbic acid, phenolics and riboflavin, with a cup serving contributing up to 34% of the daily value of magnesium and up to 60% of the daily value of vitamin C (Jiménez-Aguilar & Grusak, 2017). High levels of quercetin glycoside and hydroxycinnamic acid derivatives isomers in amaranth leaves further emphasise the health benefits of this crop (Neugart *et al*., 2017). There is wide genetic variation and large genotype to genotype differences for these nutritional traits which could provide considerable material for future breeding programme to improve human diet (Shukla *et al*., 2018, 2010; Neugart *et al*., 2017; Sarker *et al*., 2014) (Table 2.2).

Table 2.1: Essential amino acid available in amaranth grain.

Essential amino acids(g/100g)	Amaranth	Wheat	Soya	FAO/WHO standard
Lysine	5.95	0.23	2.3	5.4
Leucine	4.2	0.71	2.8	7
Isoleucine	2.71	0.36	1.67	4
Phenylalanine	4.7	0.52	1.8	6
Methionine	0.64	0.18	0.45	3.5
Threonine	3.25	0.28	1.5	4
Tryptophan	1.82	0.13	0.5	1
Valine	3.85	0.42	1.7	5

Source: Teutonico and Knorr (1985).

Table 2.2: Nutritional content in leafy vegetable amaranth.

Species	**Proteins** (fresh mass, mg/g)	**Carbohydrate** (fresh mass, mg/g)	**Minerals** (dry mass, mg/100g)			
			Na	K	Ca	Fe
A. spinosus	9.00±0.19	21.29±1.63	30.00±1.52	2500±0.50	4500±0.93	13.28±0.81
A. viridis	7.85±0.33	10.29±1.17	54.00±7.70	2230±1.20	1995±0.48	15.00±0.62
A. tricolor	6.10±0.26	9.75±1.24	34.00±1.23	3900±1.01	2000±0.56	10.00±0.78
A. blitum	6.15±0.46	11.22±0.95	39.38±1.60	negligible	120.0±1.24	9.00±1.01

Source: Srivastava (2011)

2.3 Genetic improvement

In the past decade, several biochemical and molecular markers have been developed for genome evolutionary and phylogenetic relationship between grain amaranth and its putative weedy progenitor, including allozyme markers (Hauptli and Jain, 1984), isozymes (Chan and Sun, 1997), random amplified polymorphic DNA (RAPD) (Popa *et al*., 2010; Mandal and Das, 2002; Transue *et al*., 1994), amplified fragment length polymorphism (AFLP) (Oduwaye *et al*., 2014; Štefúnovà *et al*, 2014; Costea *et al*., 2006; Wassom and Tranel., 2005; Xu and Sun, 2001), inter simple sequence repeat (ISSR) (Raut *et al*., 2014); simple sequence repeat (SSR) (Kietlinski *et al*., 2014; Suresh *et al*., 2014; Khaing *et al*., 2013, Oo and Park., 2013; Wang and Park, 2013; Lee *et al*., 2008; Mallory *et al*., 2008) and single nucleotide polymorphisms (SNPs) (Wu and Blair, 2017; Stetter *et al*., 2016; Jimenez *et al*., 2013; Maughan *et al*., 2009), bacterial artificial chromosome library (Maughan *et al*., 2008), genetic maps (Maughan *et al*., 2011), transcriptome (Liu *et al*., 2014; Sunil *et al*., 2014; Delano-Frier *et al*., 2011; Riggins *et al*., 2010), chloroplast genomes (Chaney *et al*., 2016), low-copy nuclear loci and chloroplasts regions (Waselkov *et al*., 2018) and draft genome assembly (Lightfoot *et al*., 2017;

Clouse *et al*., 2016; Sunil *et al*., 2014). However, to date there are few corresponding markers available for vegetable amaranth species.

The markers also allow genotyping for germplasm evaluation, core-collection characterisation and recognise redundancy in amaranth (Wu and Blair, 2017). The majority of markers listed above detected high levels of genetic variation within and among amaranth species and admixed accessions, with no specific geographical origin or morphological stratification (Jimenez *et al*., 2013). An admixed population structure or hybrid genotype indicated that frequent hybridization or introgression events had happened and thus produced new gene combinations (Lee *et al*., 2008). This may have occurred due to the cosmopolitan nature of the *Amaranthus* genus, breeding and resources exchange (Khaing *et al*., 2013). While the amaranth marker studies have been useful for evolutionary and phylogenetic studies, further germplasm characterization and marker validation is needed.

2.3.1 Genotype-by-sequencing (GBS)

Nevertheless, with the recent use of SNPs discovery through GBS has proved to be an efficient method in determine the genetic diversity of grain and wild amaranth accessions with consistent geographical origin and morphological classification (Stetter and Schmid, 2017; Wu and Blair, 2017). From this, several subsets of SNPs that captured most of genetic variation in amaranth have been identified and this will aid breeders to efficiently tap the available sequence diversity of the collection to create improved cultivars.

GBS offers a number of advantages, as it is more practical, inexpensive and has driven genotyping to be applied to non-model organisms; i.e does not require reference genome (Andrews *et al*., 2016; Elshire *et al*., 2011). The GBS approach uses next generation sequencing (NGS) technologies for multiplex sequencing of restriction site-associated DNA up to 384 samples on a single sequencing lane with sufficient coverage to call thousands of SNPs (Andrews *et al.*, 2016), making a cost efficient method to genotype large number of samples. GBS uses restriction enzyme digestion to reduce the complexity of genomes, produce consecutive short read sequencing of the sequence fragments around restriction sites (Elshire *et al*., 2011), which makes it possible to analyse plant species with large and complex genomes such as wheat (Poland *et al*., 2012). Further, with the recent use of SNPs

discovery through GBS in amaranth has proved to be the most efficient method to evaluate genetic diversity of amaranth accessions with consistent geographical origin and morphological classification, as well as to validate phylogeny of the *Amaranthus* genus (Stetter *et al*., 2017; Wu and Blair, 2017). GBS was only applied to amaranth when the reference whole genome sequence of the species became available (Lightfoot *et al*., 2017; Clouse *et al*., 2016).

2.3.2 Reference genome

The draft genome of *A. hypochondriacus* produced by Sunil *et al*., (2014) was highly fragmented, containing 367,441 scaffolds, with a scaffold N50 = 35 kb, and was 40% larger than the predicted genome size of 431.8Mb (Bennet & Smith, 1991) or approximately 500 Mb (Lightfoot *et al*., 2017). The second amaranth genome assembly (*A. hypochondriacus*) by Clouse *et al*., (2016) produced substantially more contiguous, 3518 scaffolds with an N50 of 371kb which was still highly fragmented and contained only 377Mb, smaller than the predicted genome size. The genome assembly showed 48% of the genome is comprised of repetitive elements with an additional 1.8% identified as simple sequence repeats (Mallory *et al*., 2008). The sequence consists of over 3,000 scaffolds that have not yet been assembled into the 16 chromosomes of the species.

Recently, Lightfoot *et al*., (2017) have produced very high quality reference genome, highly contiguous, produced 16 chromosome-scale assemblies of amaranth (*A. hypochondriacus*), with contig and scaffold N50 of 1.25Mb and 24.4Mb, respectively. The 16 chromosomes ranged in size from 17.0 to 38.1 Mb. The total sequence length of the assembly spanned 403.9 Mb, representing 93.5% of the predicted genome size. This sequence was based on PacBio single-molecule sequencing, Illumina high throughput reads and Hi-C-based proximity-guided assembly of the n=16 haploid chromosomal complement of amaranth genomes which provided a valuable anchor to all the SNP loci and allele sequences discovered here. The results from this genome assembly indicated that *Amaranthus* underwent whole genome duplication before speciation, which was then followed by further duplication, chromosome loss and fusion events (Lightfoot *et al*., 2017; Stetter and Schimd, 2017; Behera and Patnaik, 1982).

2.4 Understanding the fundamentals of drought tolerance traits in amaranth

2.4.1 An overview on C4 mechanisms

The key feature of C4 photosynthesis is the operation of a CO_2 concentrating mechanisms in mesophyll cells, as the results of evolution and adaptation from high photorespiratory pressures such as low CO_2 atmospheric pressure, high temperature, aridity or salinity (Tipple and Pagani, 2007; Sage, 2004, 2001; Ehleringer *et al*., 1997, 1991) through a series of biochemical and structural modifications around the ancestral C3 photosynthetic pathway (Hatch, 1987).

C4 plants are historically grouped into three distinct biochemical pathways known as enzyme of malate metabolism following the major C4 acid decarboxylation enzyme in the bundle sheath: NAD-dependent malic enzyme (NAD-ME), NADP-dependent malic enzyme (NADP-ME) and PEP carboxykinase (PEPCK) pathway (Hattersly, 1992; Hatch, 1987). However, there was no pure PEPCK-type has been discovered in any C4 species (Sage, 2004) and PEPCK pathway is exist in multiple lineages across different genus and therefore, only NAD-ME or NADP-ME subtype are currently known as distinct C4 biochemical pathway with or without the additional service of PEPCK pathway (Wang *et al*. 2014).

2.4.2 Amaranth responses to drought stress

Amaranth belongs to the NAD-ME subtype of C4 plants (Babayev *et al*., 2014; Ueno, 2001), together with switchgrass (*Panicum virgatum* L.) and pearl millet [*Pennisetum glaucum* (L.) R. Br], which use NAD^+ as cofactor during decarboxylation. Many other cereals belong to the NADP-ME subtype, which use NADP+ as a cofactor, including maize, sugarcane (*Saccharum* spp.), and sorghum (*Sorghum bicolor*) (Edwards and Walker, 1983). The NAD-ME subtype occurs more frequently in dry areas (Taub and Lerdau, 2000) and it exhibits superior water use efficiency under drought conditions, due to its leaf structure and faster leaf curling rates (Ghannoum, 2009), compared to the NADP-ME subtype which exhibits better nitrogen efficiency (Liu and Osborne, 2015). Wild amaranth species, including *A. hybridus*, *A. powelli* and *A. retroflexus* have been shown to have high rates of photosynthesis and rapid growth rates in drier conditions, and have tendency to become invasive in a globally warming climate, competing for resources with cultivated crops (El-Sharkawy, 2016). Grain and vegetable

amaranth have been shown to develop tolerance mechanisms such as osmotic adjustment to maintain leaf turgor, increased root systems and low loss of photosystem II (PSII) (Slabbert and Krüger, 2011, 2004; Liu & Stützel 2002a, b).

2.4.3 Factors contributing to high water use efficiency in amaranth

Partly as a consequence of C4 photosynthesis, amaranth species have a high water use efficiency allowing them to withstand periods of water deficit (Omami and Hammes 2006; Liu and Stützel, 2002a, Lal and Edwards, 1996). Amaranth display a high transpiration rate compare with C3 plants (Hura *et al*., 2007a) and are able to maintain transpiration at early drought stress and hence, keep assimilating CO_2 until the drought becomes severe (Slabbert and Krüger, 2011). One possible reason that amaranth is able to maintain photosynthesis under mild drought stress is that C4 subtype species tend to have instantaneous responses to environmental changes by adjusting their physiological traits such as leaf structure and faster leaf curling rate (Ghannoum, 2009). Liu and Osborne (2015) reported that the NAD-ME Chloridoideae plants that occur in drier habitats have smaller and denser stomata, longer and narrow leaves and high leaf cutilar. The highly elastic leaf characteristics provide the plant with a large capacity to deviate from an ideal osmotic system, which may buffer transient changes in transpiration and contribute to water storage for survival after stomata close (Bartlett *et al*., 2012; Sack *et al*., 2013).

In amaranth, the association of leaves structural traits with photosynthetic rate have been studied by Tsutsumi *et al*., (2017) on 12 different amaranth species under normal conditions. The structural traits of the leaves such as, stomatal density, guard cell length and leaf thickness, interveinal distance and sizes of mesophyll and bundle sheath cells were not significantly correlated with the rate of photosynthesis in amaranth. Nevertheless, these traits could be a possible adjustment for the plants to survive under drought stress, such as denser stomata had rapid controlled during short-term water stress (Franks and Farquhar, 2007), longer and narrower leaves stimulate faster leaf curling rates to save water and high lower leaf cuticular conductance to provide higher internal resistance of leaves (Sack *et al*., 2013).

2.4.4 Limiting factor for photosynthesis in amaranth under drought stress

Chloroplast and mitochondria play a central role in amaranth adaptation to abiotic stress (Huerto-Ocampo *et al*., 2009). The cell-specific expression of the NAD-ME enzymes in C4 leaves are complex involving leaf cell types other than mesophyll and bundle sheath cells (Babayev *et al*., 2014). The NAD-ME enzyme has also been found in vascular parenchyma cells in small amount (Ueno, 2001). In normal conditions, the NAD-ME enzyme had little control over photosynthesis in amaranth and no correlation between NAD-ME with phosphoenolpyruvate carboxylase (PEPC) and PEPC with Rubisco, but positive correlation was found between photosynthesis and Rubisco (Tsutsumi *et al*., 2017). In drought conditions, a new isoform of NAD-ME enzyme was found in the mitochondrial fraction of bundle sheath cells of *A. cruentus* during drought stress and then disappear upon re-watering (Babayev *et al*., 2014). The isoform contributes to the accumulation of CO_2 supplies during drought stress, indicating its potential role in drought adaptation (Babayev *et al*., 2014). An accumulation of drought stress responsive proteins was observed including chloroplast chaperonins that involves in refolding and protein complexes protection (Huerta-Ocampo *et al*., 2009). Drought stress also caused downregulation of proteins such as the Rubisco large subunit, cytochrome b6f, oxygen evolving complexes, and the ascorbate peroxidase mitochondrial thus, reducing the carbon metabolism (Huerta-Ocampo *et al*., 2009). It is not known which enzymes are rate limiting in NADME-type C4 photosynthesis, but in amaranth it may be Rubisco (Tsutsumi *et al*., 2017; von Caemmerer and Furbank, 2016).

2.4.5 Stomatal and non-stomatal limitations in amaranth

Normally, under drought stress, leaf water potential is reduced and initially induces stomatal closure, imposing a decreased supply of CO_2 to the mesophyll cells, and consequently reducing the rate of leaf photosynthesis (Lawlor and Cornic 2002; Williams *et al*., 1999). These adjustments have not been seen in amaranth. Only small changes in leaf water potential have been observed, which in turn only induce small changes in stomatal conductance, imposing in accumulation of intracellular CO_2 which results in photodamage of PSII reaction centres, or development of slowly relaxing excitation energy quenching (Slabbert and Krüger, 2011; Baker and Rosenqivist, 2004). The reductions of photosynthesis in amaranth

have been shown to be independent of stomatal conductance, but because of non-stomatal photosynthesis limitation, which is photoinhibitory injury of the photosynthetic apparatus, and disturbance in enzymatic process of the photosynthesis. The stomatal limitations of photosynthesis are often accompanied by a decrease in the utilization rate of ATP and NADPH for CO_2 assimilation, which can result in decreases in the rate of electron transport and consequently, reduces the maximum quantum efficiency of PSII photochemistry. Unlike C3 plants, the limitation of CO_2 assimilation is due to photorespiration which may maintain the rates of electron transport system, similar to the non-stressed leaves (Ort and Baker 2002). This suggests that closer investigation of PSII functioning could help to identify specific differences in tolerance to water deficit in amaranth.

2.4.6 Osmoprotective regulation on amaranth upon drought stress

In general, a plants resistance to stress depends on its cellular protecting mechanisms and restoration of damage capabilities. Drought stress induces the activation of reactive oxygen species (ROS) which include superoxide (O^{-2}), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\bullet}$) (Mittler, 2002; Neill *et al*., 2002). The ROS are highly reactive, they can disrupt normal metabolism through oxidative damage to organelles particularly photosynthetic apparatus, lipids, protein and nucleic acids (Rout and Shaw, 2001), and can generate photooxidation stress (Wang *et al*., 2013). In amaranth, antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) are increased under drought stress (Slabbert and Krüger, 2014), similar to maize (Köşkeroğlu and Tuna, 2010). SOD is capable of converting superoxide radicals (O^{-2}) into hydrogen peroxide (H_2O_2), and APX uses ascorbate as an electron donor to reduce H_2O_2 (Kuk *et al*., 2003), and GR generates reduced glutathione to remove dioxygen under stress conditions (Hakam and Simeon, 1996). The combined effects of these antioxidant enzymes may maintain the redox balance during oxidative stress. The increased activities of SOD, APX and CAT have been correlated with proline accumulation in amaranth during drought stress (Slabbert and Krüger, 2014). This suggests that the antioxidant defence mechanism is activated by the increase of proline accumulation (Köşkeroğlu and Tuna, 2010; Ahmed *et al*., 2009; Yan *et al*., 2000,). Proline is α-amino acid associated with

osmoprotection roles during drought stress including osmotic adjustment (Zadehbagheri *et al*., 2014; Marek *et al*., 2009), membrane stabilization (Hayat et al., 2012), and gene signalling to activate antioxidizing enzymes that scavenge ROS (de Carvalho *et al*., 2013). Proline accumulation normally occurs in the cytosol where it contributes substantially to cytoplasmic osmotic adjustment (Ashraf and Foolad, 2007), during water stress and decreases rapidly upon re-watering (Hare *et al*., 1998).

Amaranthine is the main betacyanin pigment in amaranth that contributes to the red or purple colour of the plants and has potential as an antioxidant due to its abundance of hydroxyl and imino groups (Strack *et al*., 2003; Cai *et al*., 1998). It had been reported that amaranthine possesses high ROS scavenging activity during drought stress (Neill and Gould, 2003). Red (betacyanic) and green (acyanic) leaves of amaranth have similar chlorophyll content and chlorophyll a/b ratio, hence both have similar light-harvesting capacity (Nakashima *et al*., 2011). However, while it has been observed that RWC, photosynthesis and chlorophyll content are equally reduced under drought stress, photoinhibition is severe in green leaves compares to red. Red leaves display high maximum quantum efficiency of PSII photochemistry (Fv/Fm) and photochemical quenching coefficient during water stress with and increased relative abundance of betacyanin to chlorophyll content (Shao *et al*., 2013). This increased betacyanin contributes to the increased total photoprotective capacity by lowering excitation pressure on PSII via attenuation of potentially harmful excess incident light under water stress (Nakashima *et al*., 2011). The increased of pigment accumulation was not coincide with betacyanin precursor activity, dihydroxyphenylalanine oxidation tyrosinase (DOT) hydroxylation of tyrosine hence, DOT activity may not be necessarily required under certain circumstances such as water stress (Casique-Arroyo *et al*., 2014).

2.5 Conclusion

Amaranthus spp. has been a source of nutritious food for many centuries in Africa, Asia, Central and South America. It is now being consumed and cultivated worldwide and is a promising health food and climate smart crop. It has the potential to alleviate poverty, malnutrition and reliance on staple crops in the face of increasing global droughts. The capacity of amaranth to have wide genetic

variability and better tolerance to drought stress provide new prospect in the development of new crop variety. However, few cultivars are available, and the genetic material is poorly characterised. Therefore, the construction of population structure in amaranth through a combination of physiological, morphological and molecular data, and their association with drought tolerance traits is needed in order to develop a framework for future breeding programmes.

CHAPTER 3

CHARACTERIZATION OF AMARANTH GERMPLASM AND DEVELOPMENT OF A CORE SET USING QUALITATIVE DATA DERIVED FROM GENEBANK MORPHOLOGICAL DATABASE

3.1 Introduction

The principal approach for identifying *A. tricolor* accessions with superior drought tolerance traits is to exploit the diverse genetic resources available within the amaranth germplasm. Diverse crop genetic material provides the opportunity to select better performing genotypes for any trait of interest (Savita, 2006). Sustainable agronomic gains in vegetable amaranth can be achieved by incorporating adaptation and varietal development into breeding programmes. To achieve this, germplasm must be properly assessed and evaluated to improve the genetic resources of commercially important lines.

The assessment of genetic diversity is routinely performed using various markers, including morphological, biochemical and molecular marker (Govindaraj *et al*., 2015). Traditionally, the primary sources of genetic diversity are identified through variation in morphological traits. It provides useful information on the diversity patterns within and among populations (Veasey *et al*., 2008), and agronomic traits of interest can be identified through naked eye observation and certainly expressed under different climatic conditions (Ahmad *et al*., 2018). The characterisation of morphological traits such as the shape, size and colour of the leaf, stem, inflorescence and seed are fast and easy to assess for direct use by farmers or in breeding programmes (Krichen *et al*., 2012). Besides, it provides genetic parameters of specific traits which facilitate plant breeders when selecting potential parental lines (Sarker *et al*., 2014). However, an evaluation of genetic diversity based on morphological traits may be influenced by environmental effects and the complex genetic structure of different morphological traits (Tabatabaei *et al*., 2011; Banerjee and Kole, 2009). Therefore, combined analysis using morphological and molecular markers is routinely performed to produce more accurate data on genetic distances, and genotype and environment interactions (Malviya *et al*., 2012). Nevertheless, morphological data is still worthwhile and necessary to facilitate in the

development of a core set of large germplasm accessions (Archak *et al*., 2016; Upadhyaya et al., 2003), and has been used to successfully characterise genetic variation in a number of amaranth species (Gerrano *et al*., 2017; Akhter *et al*., 2013; Selvan *et al*., 2013; Shukla *et al*., 2010; Pandey *et al*., 2009; Oboh, 2007; Wu *et al*., 2000).

Developing a core set is an efficient approach for characterising and capturing the genetic diversity of large accessions within a germplasm collection (Liu *et al*., 2015). Frankel and Brown (1984) proposed the concept of a core set to resolve redundancy problems, in which the design of a core set should include the maximum possible genetic diversity contained in the entire collection with minimum repetition. General procedures for the development of core collections are the objectives, the size, the sampling strategy, the grouping within the collection, and the number of accessions to be included in the core from each group that will determine the structure of the core set. Multivariate analysis is one way to achieve an effective core set as it able to measure the degree of divergence and ascertains the relative contribution of different characters to the total divergence (Singh *et al*., 2002; Zeven *et al*., 1999). It permits selection of clusters with genetically divergent parents to obtain the desirable recombinants in the selection of segregating generations (Siddique, *et al*., 2016; Akhter *et al*., 2013; Jagadev *et al*., 1991). It has been used successfully to classify genetic diversity based on morphology and phenotypic characteristics within and between species in many crops such as maize (Ali *et al*., 2015), safflower (*Carthamus tinctorius* L.) (Shinwari *et al*., 2014), soybean (*Glycinemax* L. Merr.) (Malek *et al*., 2014).

To date, characterising phenotypic diversity of vegetable amaranth (*A. tricolor*) is very limited and has never been studied properly, and the present study describes the morphological characterisation of the amaranth collection conserved in The World Vegetable Center Genebank, Taiwan (AVRDC) and *A. tricolor* accessions from the United State Department of Agriculture (USDA) using Genebank morphological data, leading to the development of amaranth mini core collection. Qualitative traits such as leaf, stem and petiole colour and shape are chosen for the development of amaranth mini core collection. These qualitative traits are consumer's preferences (Akaneme and Ani, 2013), capable of classifying the genus *Amaranthus* into amaranth species (Gerrano *et al*., 2014) and have been used as quality traits for drought tolerance characteristics

(Nakashima *et al*., 2011). Highly economically and agronomically important species in the genus, grain-type and weed-type amaranth were also incorporated in the genetic diversity analysis, as outliers, in order to assess the level of similarity between different use-type of amaranth species based on morphological features. The diversity of the core set derived from AVRDC and USDA Genebank is compared with their respective whole collections to test the effectiveness of the core set. This study also aimed to identify the level of distinctiveness in qualitative traits within vegetable amaranth with regard to the geographical distribution. This work was also done to provide morphological information on the phenotype-genotype specific data for genome wide association (GWAS) studies in Chapter 4 and drought tolerance screening (Chapter 6).

3.2 Materials and methods

3.2.1 Germplasms evaluation

3.2.1.1 Quality of germplasm passport data

A total of 783 accessions were used for the selection of amaranth core set. Of these, a whole collection of 578 amaranth accessions that have been conserved in AVRDC was obtained as the main germplasm resource (Appendix 3.1a), 179 amaranth accessions were obtained from USDA to incorporate seeds from diverse geographical origins (Appendix 3.1b) and 16 commercialised amaranth varieties, including 12 local Malaysian varieties, as checks (Appendix 3.1c).

The AVRDC Genebank material consists of 18 species from 44 countries, comprising of *A. tricolor* (166 accessions), *A. viridis* (57 accessions), *A. dubius* (36 accessions), *A. hypochondriacus* (34 accessions), *A. spinosus* (28 accessions), *A. sp* (25 accessions), *A. cruentus* (19 accessions), *A. blitum* (17 accession), *A. gracilis* and *A. retroflexus* (four accessions, respectively), *A. graecizans*, *A. hybridus* and *A. thunbergii* (three accessions, respectively), and *A. atropurpureus*, *A. blitoides*, *A. leucorcapus*, *A. mantegazzianus* and *A. palmeri* (one accession, respectively). Thirty-two morphological descriptors (22 qualitative and 10 quantitative traits) were assessed through publicly available AVGRIS database (https://avrdc.org/seed/) (Appendix 3.2). Hundred and seventy four out of 578 accessions (30%) were excluded from further the analysis due to missing data (Table 3.1; Appendix 3.1a). The remaining of 404 accessions (70%),

comprising of 18 species from 39 countries, were subjected to stratification to form a diversity group and identification of a core set.

A large *A. tricolor* (177 accessions) originated worldwide (19 countries) from USDA Genebank, with addition of one accession of *A. hybridus* and *A. retroflexus*, respectively was also obtained to increase the number of materials. Due to large missing data in the *A. tricolor* germplasm, the quality of passport data was examined based on origin country, and 49% of *A. tricolor* accessions having incomplete characterization data (Table 3.2). Five morphological descriptors (qualitative traits) were assessed through publicly available GRIN database (https://npgsweb.ars-grin.gov/gringlobal/search.aspx) (Appendix 3.3).

For 16 commercial varieties, four were African varieties acquired from East-West Seed, Thailand (E-W) and 12 were local Malaysian varieties. The characterizations of these varieties were not readily available through passport data, therefore, an evaluation on morphological characterization (Appendix 3.1c) were carried out by growing the individual varieties in the shade-house conditions (see subheading 3.2.3).

Table 3.1: Quality of passport data of an entire collection of 578 amaranth accessions conserved in the AVRDC Genebank extracted from AVGRIS database ((https://avrdc.org/seed/) and core set selection.

Species	**WHOLE SET (n=578)**			
	Complete	Not complete	Not available	Total
A. atropurpureus	-	1	-	1
A. blitum	1	-	-	1
A. leucorcarpus	1	-	-	1
A. gracilis	4	-	4	4
A.graecizan	3	-	-	3
A.hybridus	3	-	4	3
A.mantegazzianus	1	-	-	1
A.palmeri	1	-	-	1
A.retroflexus	3	1	-	4
A.thunbergii	2	1	2	3
A.blitum	16	1	12	17
A.cruentus	16	3	26	19
A.dubius	31	5	18	36
A.hypochondriacus	30	4	28	34
A.sp	24	1	-	25
A.spinosus	25	3	7	28
A.viridis	53	4	8	57
A.tricolor	144	22	65	166
Total	358	46	174	404
%	**62%**	**8%**	**30%**	**70%**

Table 3.2: Quality of passport data of all 177 *A. tricolor* accessions obtained from USDA Genebank extracted from GRIN database (https://npgsweb.ars-grin.gov/gringlobal/search.aspx) and core set selection.

Country of origin	WHOLE SET		
	Complete	Not complete	Total
Bangladesh	1	-	1
Brazil	-	2	2
China	3	26	29
Hong Kong	12	3	15
India	48	33	81
Indonesia	1	-	1
Madagascar	1	-	1
Malaysia	1	1	2
Papua New Guinea	1	1	2
Puerto Rico	2	-	2
Taiwan	4	3	7
Thailand	1	1	2
Unknown	9	7	16
USA	5	9	14
West Africa	1		1
Zaire	1	-	1
Total	91	86	177
%	**51%**	**49%**	**100%**

3.2.1.2 Identification of a core set from amaranth germplasm using Genebank morphological database

A thorough classification of amaranth resources within each Genebank was obtained. The accessions of individual Genebanks were initially evaluated separately based on their morphological databases. They could not be classified together as the two Genebanks provided different characterisations of morphological passport data. Following van Hintum (1994) and Dwivedi *et al*., (2005), a stratification method was used to identify plant material in a whole collection to be represented in the core set. The whole set of amaranth accession was first divided into non-overlapping groups, and then each group was subjected to hierarchical clustering based on geographical origin and morphological traits, and finally a simple random sample was drawn from within each group. The hierarchical clustering for each group was analysed based on qualitative traits derived from respective Genebanks in order to standardize the selection of amaranth accessions in both AVRDC and USDA germplasm collections.

The accessions were first manually stratified into several groups to provide a better proportion of accessions in each species as well as to obtain an extensive selection of *A. tricolor* accessions in the development of a core-set.

Group 1 consisted of amaranth species with less than 5 accessions (*A. atropurpureus, A. blitoides A. leucocarpus, A. gracilis, A. graecizens, A. hybridus, A. mantegazzianus, A. palmeri, A. retroflexus,* and *A. thunbergii*), while other species that had more than 10 accessions were analysed separately; Group 2 (*A.blitum),* Group 3 (*A. cruentus*), Group 4 (*A. dubius*), Group 5 (*A. hypochondriacus*), Group 6 (*A. sp*), Group 7 (*A. spinosus*), Group 8 (*A. viridis*) and Group 9 (AVRDC-*A. tricolor*) and Group 10 (USDA-*A. tricolor*). All groups (except group 1) were analysed separately in hierarchical clustering, providing more detail selections of accessions with diverse geographical origin and morphological traits within a species. Group 1 was directly selected for the core set as the accessions belonging to this group represented respective species. 14 accessions of unknown country of origin with complete passport information were also assigned for clustering together with other accessions.

3.2.2 Morphological assessment and leaf samples

The distinctness, uniformity and stability of morphological characteristics of the amaranth core set were assessed for each accession in amaranth mini core collection, for comparison with the published Genebank morphological data. Representative plants from each accession were grown in pots under shade-house conditions at the University of Nottingham Malaysia (UNM, latitude 2.940°N, longitude 101.8740°E), with an average daytime temperature of 36°C and night time temperature of 28°C, and average relative humidity of 66%. The seeds were germinated in plastic pots (16 x 12.5 x 14.5 cm) containing 2 kg of compost (Holland peat, Netherlands) in three replications, with one plant per pot. Plants were irrigated daily to field capacity and at 3 weeks old, 3 g of 15N: 15P: 15K fertilizer was applied once to individual pots. 10 qualitative characters based on AVRDC Genebank descriptors were recorded for each plant. Leaf, petiole and stem colours, growth habit, branching index and, leaf shape and margin were recorded at the vegetative phase (7 weeks old) while terminal inflorescence colour, shape and attitude were recorded when the plants had set full inflorescence (approximately 11 weeks after germination). Young leaf material of a single plant in each accession was collected and snap frozen in liquid N_2 and kept in -80ºC freezer for DNA analysis (see chapter 4).

3.2.3 Pure lines development

To increase seeds for further trials, and genotyping purposes, the single plant selections were subjected to controlled pollination. Amaranth species are predominantly self-pollinating species but may also readily out-cross with other varieties (especially wild amaranth species), therefore the panicle flowers were bagged to prevent cross-pollination and to maintain seed purity (Figure 3.1). The flower panicle was bagged at five days after the emergence of inflorescence and in order to minimize seeds dispersal, flower harvest was conducted as soon as over 50% of the parental lines obtained full developed seeds. The flower was dried for two weeks to ease seeds threshing. The 'blow and fly' method was used to collect the seeds to be utilized for drought tolerance assessment. The germination performance of the pure line seeds was tested. 10 seeds per line were sown in trays (5 x 5 cell trays, each cell measures 5 cm in diameter by 10 cm deep) filled with compost (Holland peat, Netherlands) and fully irrigated under shade-house conditions at UNM. The germination performance was scored on a 1 to 4 scale, where 1=excellent and 4=very poor. Approximately, 48% of the total lines (scores of 3 and 4) were excluded from drought tolerance screening.

Figure 3.1: Bagging of panicle flowers to prevent cross-pollination and ready to be harvested at 50% seeds maturity.

3.2.4 Data analysis

3.2.4.1 Dendrogram

The amaranth representative for each group was selected through qualitative traits based-dendrogram. The qualitative data obtained from each Genebank morphological database were subjected to the hierarchical cluster algorithm Jaccard similarity coefficient (Sneath and Sokal, 1973) using the average linkage method (UPGMA-Unweighted pair group method with arithmetic mean) through Genstat 18th software (VSN International, 2015). Average linkage method analysed by Genstat 18th software defines the similarity between a cluster and two merging clusters as the average of the similarities with each of the original clusters. It therefore replaces two merging clusters by their mean, unweighted by cluster size. Jaccard's coefficient similarity measure is used to assess similarities between two objects in which high value indicates that the objects are identical and a low value indicates that the objects are completely distinct. Similar analysis was done to assess morphological diversity in amaranth mini core collection.

The qualitative data were transformed into binary data considering the presence or absence (1/0) of each character and the equation for Jaccard's Index as below:

$Sj = a / (a + b + c)$

where Sj is Jaccard's similarity coefficient and a, b, c defined presence-absence matrix.

3.2.4.2 Representativeness of AVRDC-core set and USDA-core set from whole collection

To characterize variation in the amaranth germplasm derived from each Genebank morphological databases, percentage of frequency distribution and basic descriptive statistics were calculated for the qualitative and quantitative data, respectively. Multivariate analysis of variance (MANOVA) followed by univariate analysis of variance (ANOVA) was performed to test the significance of mean among species for all the quantitative variables.

The degree of representativeness of the AVRDC- and USDA-core set against the whole collection was evaluated using chi-square test which is used to assess the similarity of the distribution frequencies in the whole collections and core collections. The Shannon Weaver diversity index (H') was used as a

comparative measure of phenotypic diversity for each qualitative trait, as described by Perry and Mclntosh (1991):

$$H' = 1 - \sum_{i=0}^{n} Pi\ ln\ Pi$$

where *Pi* is the proportion of accessions in the i^{th} class of an n-class character and n is the number of phenotypic classes of traits. The evenness of H' was calculated by dividing its maximum (H_{max}) value ($\log_c n$). By pooling these traits across the species, the additive properties of H' was used to evaluate the genetic diversity of the qualitative traits between the amaranth species.

In order to further verified the quality of core set developed from AVRDC Genebank, analyses based on quantitative traits were also conducted which include; Levene's test which was used to assess the homogeneity of variance between the whole collection and core set, and Pearson's correlation was used to test whether the core set sampled trait associations are under genetic control.

3.2.4.3 Variability of amaranth mini core collection

Principle component of analysis (PCA) was used to evaluate the contributions of different traits to multivariate polymorphism and conservation of such contributions in the amaranth mini core collection. For this test, qualitative traits were coded as non-binary data as PCA analysis was hampered by existence of more than two possibilities for some of the traits (Zimisuhara *et al*., 2015) and as such, the characteristics were given parametric codes. All statistics were performed using Genstat 18^{th} Edition (VSN International, 2015).

3.3 Results

3.3.1 Identification of AVRDC- and USDA-core set representatives for amaranth mini core collection

The selection of representative from AVRDC and USDA Genebank obtained via hierarchical clustering based on the morphological database in each individual group is presented in Figures 3.2 and 3.3. The dendrogram revealed that, in AVRDC Genebank, *A. cruentus* accessions (Group 3) had the least morphological variability with similarity coefficient of 0.8, while accessions of other species had high variability with similarities coefficient of 0.5. Meanwhile,

in USDA Genebank, *A. tricolor* accessions had low variability with similarity coefficient of 0.8.

The amaranth accessions that grouped in the same cluster were randomly selected provided that the selection had diverse country of origin and non-overlapped qualitative traits. A total of 131 accessions from AVRDC Genebank and 52 accessions from USDA Genebank were selected as representative for amaranth mini core collection. The composition of the AVRDC-core set captures 23% accessions of the entire germplasm. This procedure was able to extract an even proportion of Asian germplasm with 3% from Thailand, 3.2% from Malaysia and 5.4% from Bangladesh (Table 3.3). Of the whole amaranth collection, accessions from Zambia, Cameroon, Peru and Dominican Republic that represented less than 1% in the present analysis were not included in the core set due to the lack of traits variability compared with other accessions. In comparison, USDA-core set captured approximately 28% from the whole set of USDA *A. tricolor* germplasm with an even representation from each country (Table 3.4).

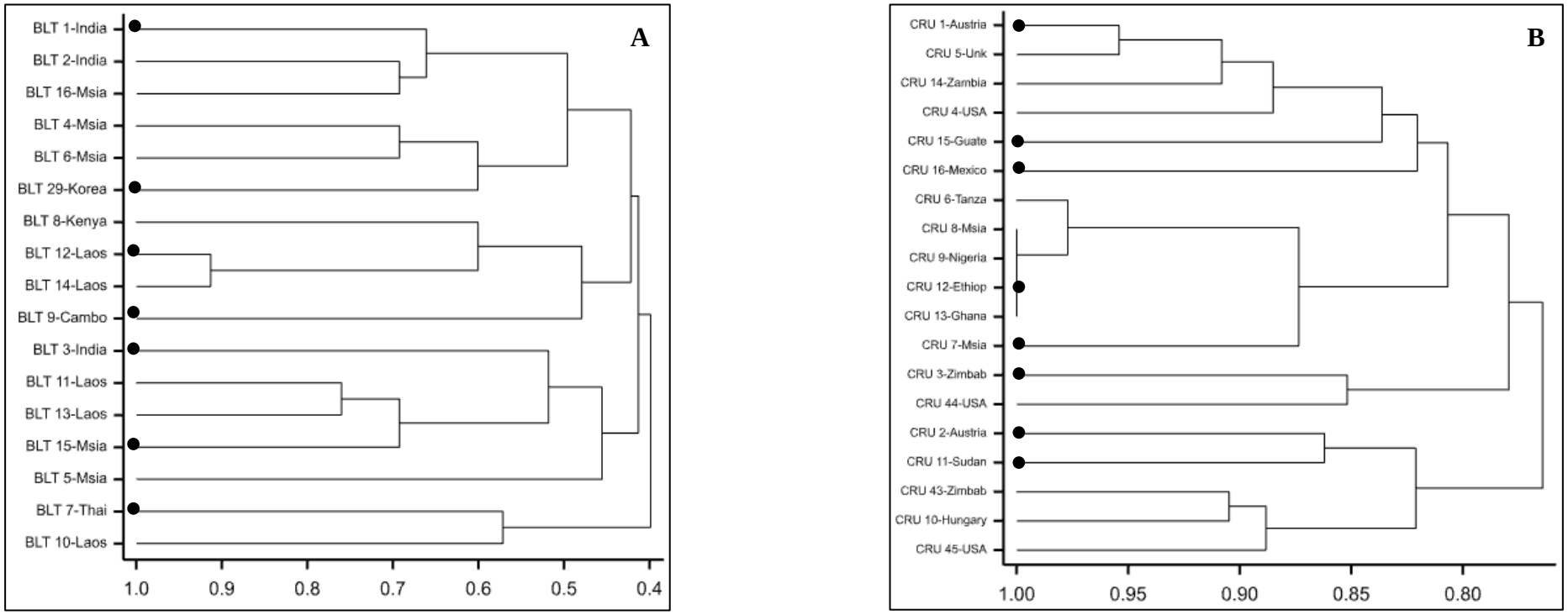
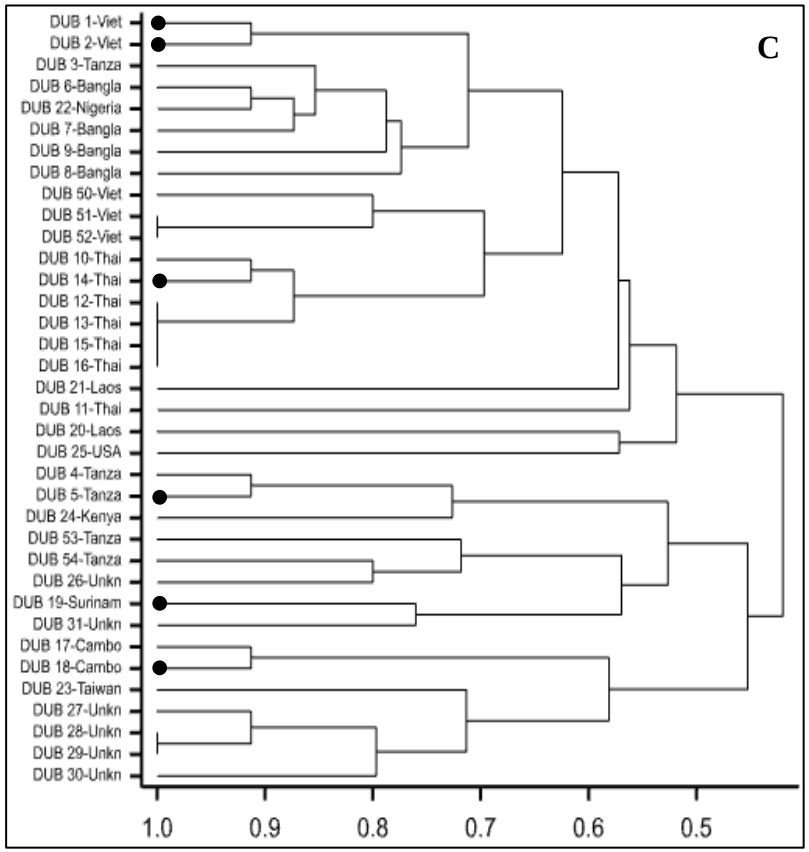


Figure 3.2: Qualitative traits-based dendrogram of amaranth accession from AVRDC Genebank and the bullets in each group represent selection of accessions to be included in the core set. The entry description of each group is presented in Appendix 3.1a. Selection of Group 9 is presented in Appendix 3.4. (A) Group 2: *A. blitum*, (B) Group 3: *A.cruentus*, (C) Group 4: *A. dubius*, (D) Group 5: *A. hypochondriacus*, (D) Group 6: *A. sp*, (E) Group 7: *A. spinosus*, (F) Group 8: *A. viridis* and (G) Group 9: *A. tricolor*.

Figure 3.2: (Continued)



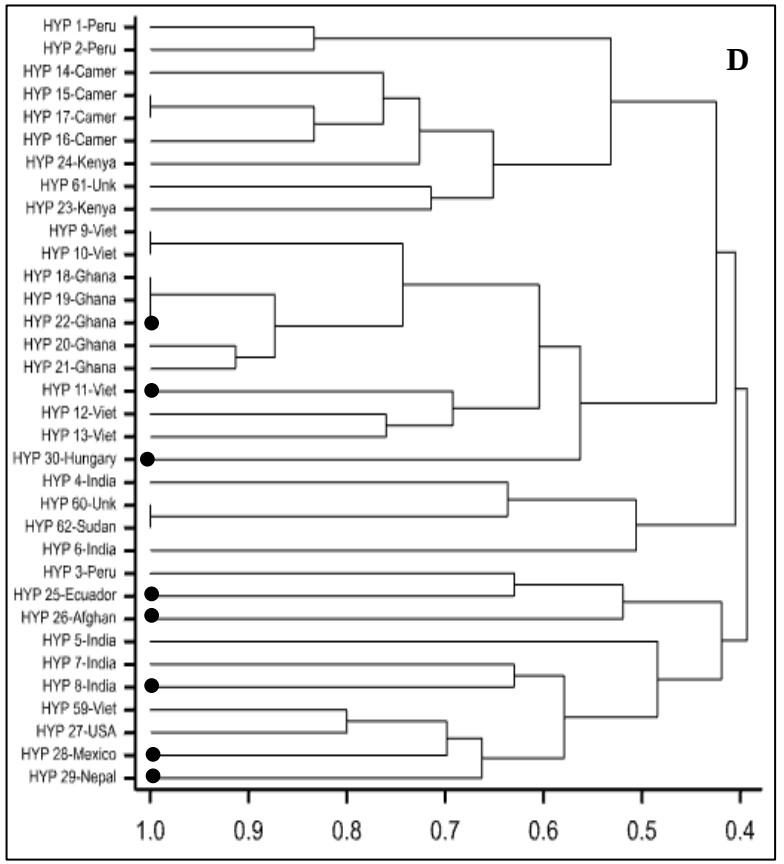
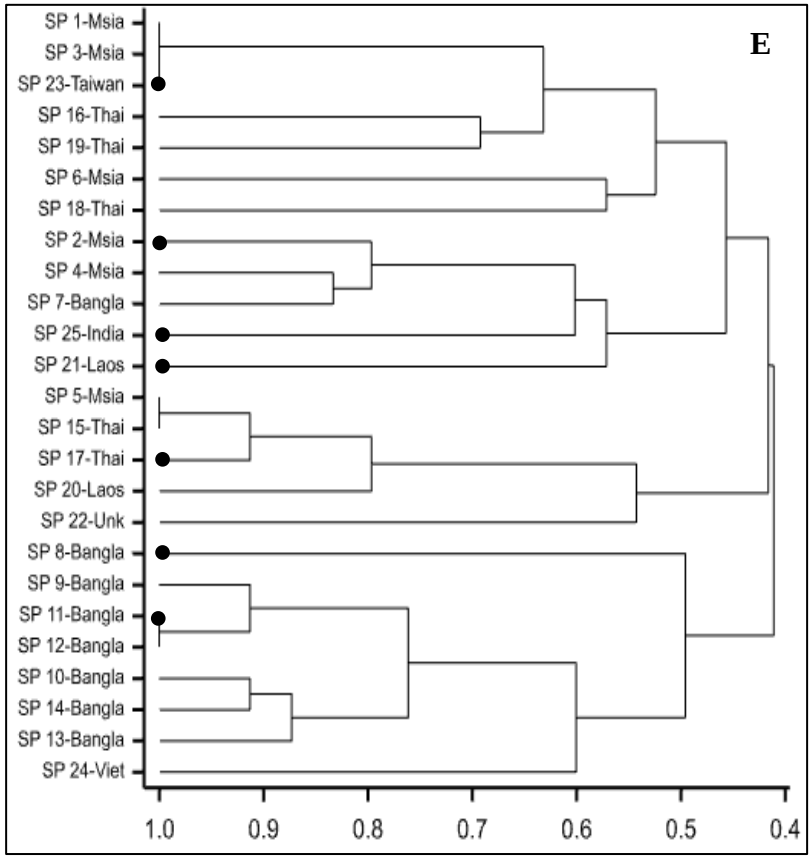


Figure 3.2: (Continued)



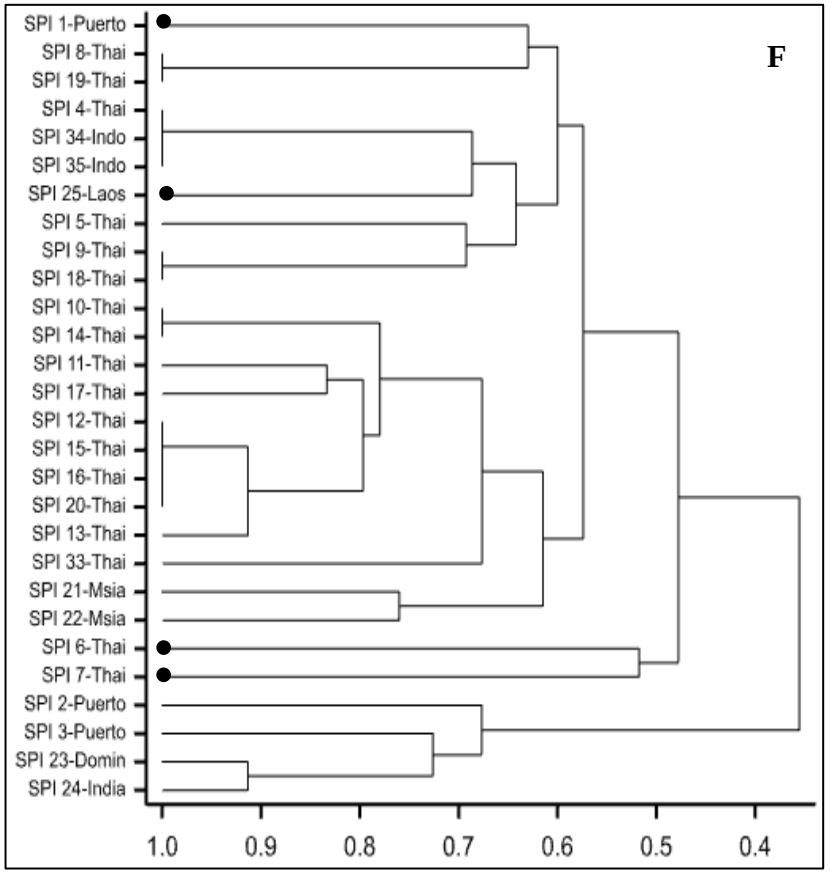
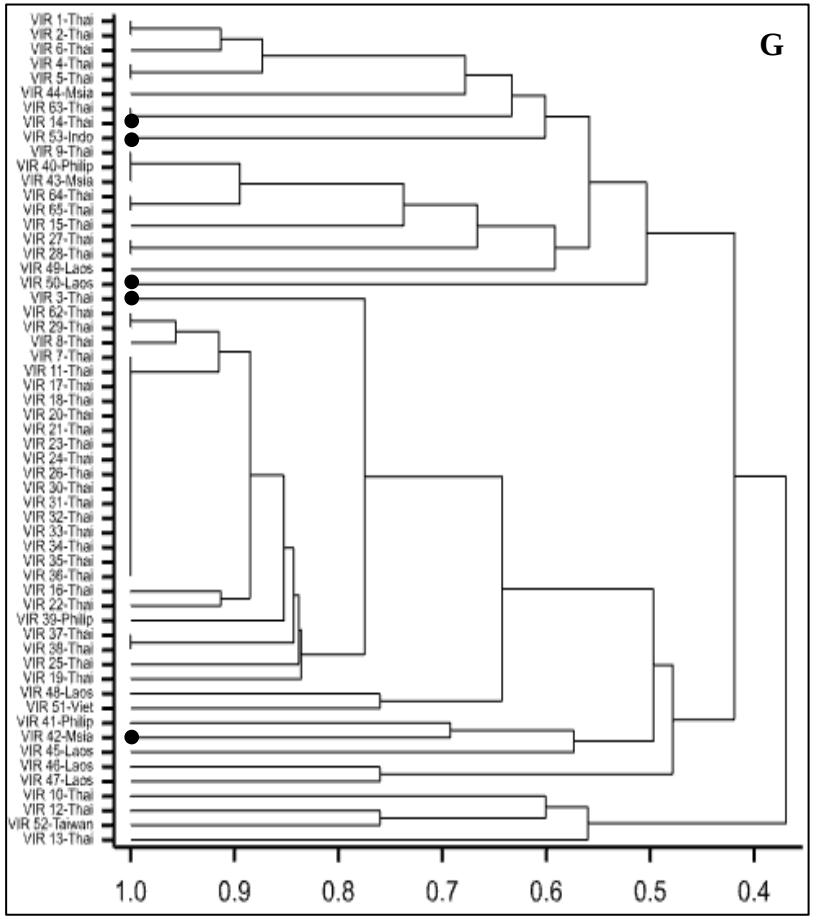
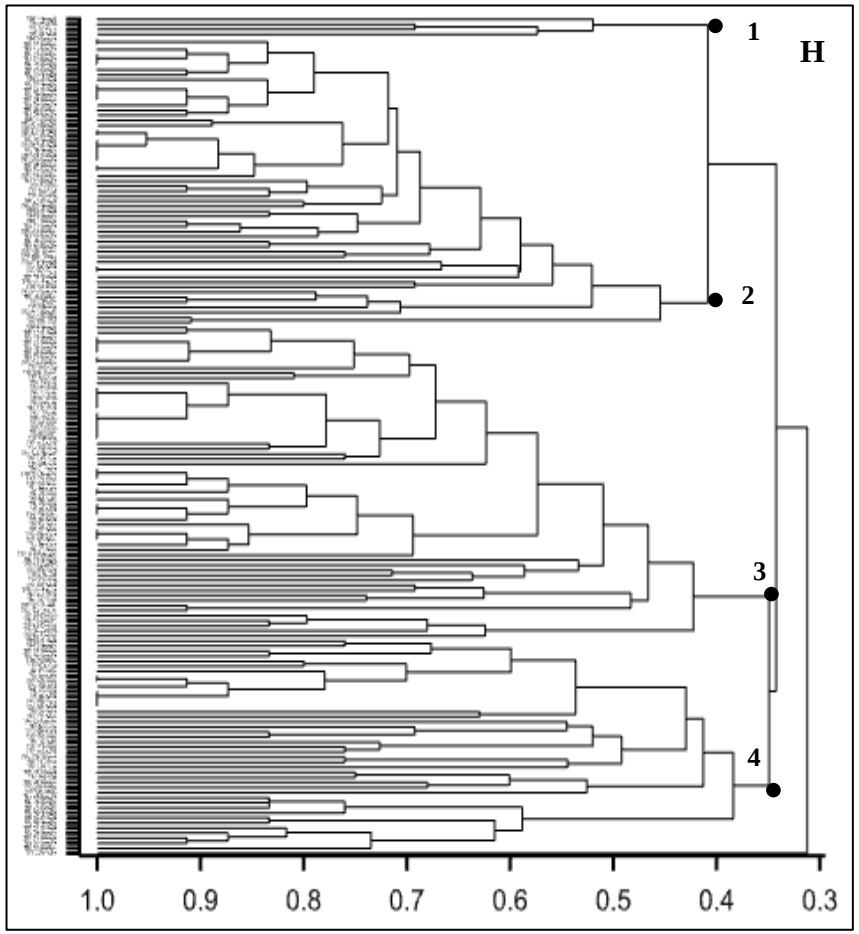


Figure 3.2: (Continued)





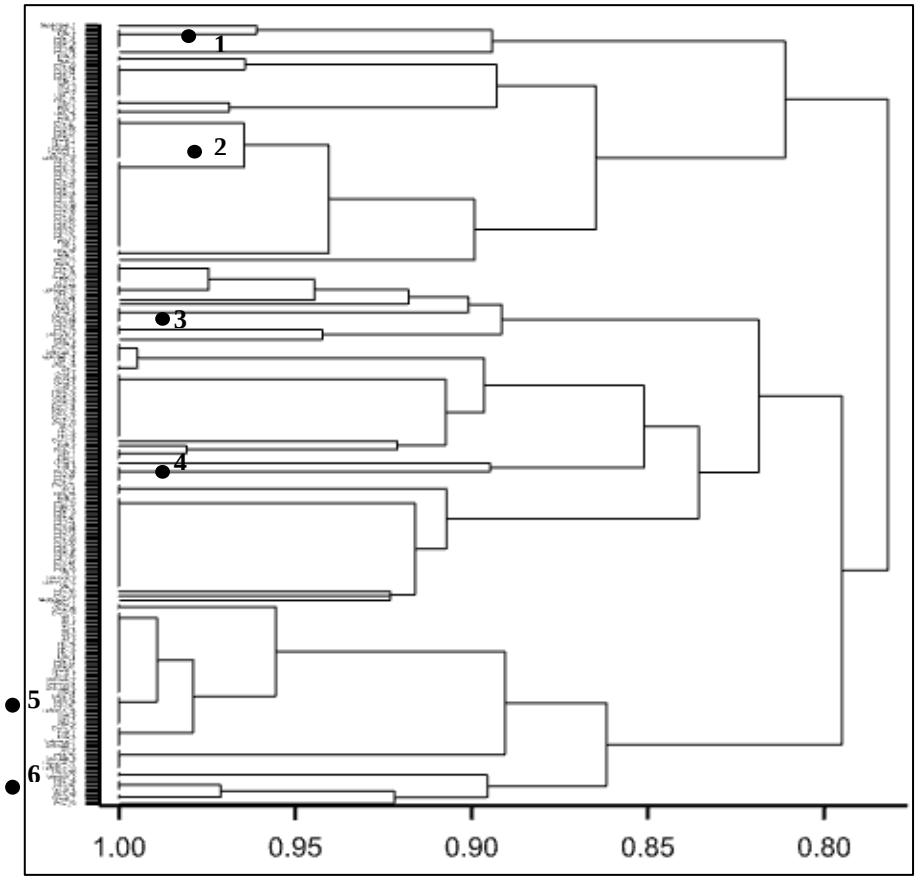


Figure 3.3: Qualitative traits-based dendrogram of *A. tricolor* accession from USDA Genebank (Group 10). The entry description of each group is presented in Appendix 3.1a. The selection of accessions to be included in the core set is presented in Appendix 3.5.

Table 3.3: Geographical region and country of origin of accessions in whole collection and core set of the AVRDC Genebank. The figures in parenthesis were percentage of germplasms representing country of origin from whole collection.

No.	Geographical region	Country of origin	Whole set	Core set
ASIA				
1	East Asia	China	1 (0.2)	1 (0.2)
2	East Asia	Japan	1 (0.2)	1 (0.2)
3	East Asia	Korea	1 (0.2)	1 (0.2)
4	East Asia	Taiwan	8 (2)	5 (1.2)
5	South and South East Asia	Bangladesh	83 (20.5)	22 (5.4)
6	South and South East Asia	Cambodia	14 (3.5)	5 (1.2)
7	South and South East Asia	India	21 (5.2)	12 (3)
8	South and South East Asia	Indonesia	17 (4.2)	5 (1.2)
9	South and South East Asia	Laos	17 (4.2)	5 (1.2)
10	South and South East Asia	Malaysia	47 (11.6)	13 (3.2)
11	South and South East Asia	Philippines	5 (1.2)	2 (0.5)
12	South and South East Asia	Thailand	80 (19.8)	12 (3)
13	South and South East Asia	Vietnam	28 (6.9)	13 (3.2)
14	South and South East Asia	Nepal	1 (0.2)	1 (0.2)
15	West and Central Asia	Afghanistan	1 (0.2)	1 (0.2)
16	West and Central Asia	Pakistan	1 (0.2)	1 (0.2)
17	West and Central Asia	Turkey	1 (0.2)	1 (0.2)
AFRICA				
18	Eastern Africa	Tanzania	6 (1.5)	1 (0.2)
19	Eastern Africa	Zambia	1 (0.2)	0
20	Eastern Africa	Zimbabwe	2 (0.5)	1 (0.2)
21	Sub-Saharan Africa	Cameroon	4 (1)	0
22	Sub-Saharan Africa	Ethiopia	1 (0.2)	1 (0.2)
23	Sub-Saharan Africa	Ghana	6 (1.5)	1 (0.2)
24	Sub-Saharan Africa	Kenya	5 (1.2)	1 (0.2)
25	Sub-Saharan Africa	Nigeria	3 (0.7)	1 (0.2)
26	Sub-Saharan Africa	Senegal	1 (0.2)	1 (0.2)
27	Sub-Saharan Africa	Sudan	2 (0.5)	1 (0.2)
THE AMERICAS				
28	Mesoamerica	Guatemala	1 (0.2)	1 (0.2)
29	Mesoamerica	Mexico	2 (0.5)	2 (0.5)
30	North America	USA	11 (2.7)	5 (1.2)
31	South America	Ecuador	1 (0.2)	1 (0.2)
32	South America	Peru	3 (0.7)	0
33	South America	Suriname	1 (0.2)	1 (0.2)
34	South America	Venezuela	1 (0.2)	1 (0.2)
35	The Caribbean	Dominican Republic	1 (0.2)	0
36	The Caribbean	Puerto Rico	3 (0.7)	1 (0.2)
37	Ocenia	Papua New Guinea	2 (0.5)	2 (0.5)
EUROPE				
38	Europe	Austria	2 (0.5)	2 (0.5)
39	Europe	Hungary	4 (1)	3 (0.7)
Unknown			14 (3.5)	3 (0.7)

List of geographical regions was obtained from International Organization of Standardization by UNSD (United Nation Standard Division): http://www.iucnredlist.org/technical-documents/data-organization/countries-by-regions

Table 3.4: Geographical region and country of origin of *A. tricolor* accessions in whole collection and core set of USDA germplasm. The figures in parenthesis were percentage of germplasms representing country of origin from whole collection.

Geographical origin	Country of origin	Whole set	Core set
ASIA			
East Asia	China	29 (16)	5 (3)
East Asia	Hong Kong	15 (8)	5 (3)
East Asia	Taiwan	7 (4)	3 (2)
South and Southeast Asia	Bangladesh	1 (1)	1 (1)
South and Southeast Asia	India	81 (46)	16 (9)
South and Southeast Asia	Indonesia	1 (1)	1 (1)
South and Southeast Asia	Malaysia	2 (1)	2 (1)
South and Southeast Asia	Thailand	2 (1)	2 (1)
AFRICA			
Central Africa	Zaire	1 (1)	1 (1)
Sub-Saharan Africa	Madagascar	1 (1)	1 (1)
West Africa	West Africa	1 (1)	1 (1)
THE AMERICAS			
North America	USA	14 (8)	5 (3)
Ocenia	Papua New Guinea	2 (1)	2 (1)
South America	Brazil	2 (1)	2 (1)
The Caribbean	Puerto Rico	2 (1)	2 (1)
Unknown		16 (9)	1 (1)

3.3.2 Comparisons of AVRDC- and USDA- core set against their whole collections

3.3.2.1 AVRDC-core set

3.3.2.1.1 Qualitative traits

The comparison of frequency distributions in the whole collection and the core set for each characteristics of 22 qualitative traits revealed that the core set represents high variability of characters among amaranth accessions (Appendix 3.6). For architecture traits, majority of amaranth accessions were monoecious (96%), and the remaining accessions belong to *A. blitum*, *A. cruentus*, *A. dubius*, *A. sp*, *A. spinosus*, *A. viridis* and *A. tricolor* were polygamous. The growth habit for the most of amaranth accessions were erect (88%), while most of *A. viridis* accessions exhibited prostrate growth habit. For branching index, most of *A. cruentus*, *A. dubius*, *A. hypochondriacus* and *A. spinosus* accessions had branches all along the stem, while *A. retroflexus*, *A. blitum*, *A. sp*, *A. viridis* and *A. tricolor* accessions had uniform distribution, classified into three different type of branching index.

For leaf characteristics, the dominant leaf colour was normal green (55%) for all species, except *A. thunbergii* which displayed either purple/pink or margin/vein pigment whilst *A. tricolor* displayed the largest leaf pigmentation

variability among the amaranth species. The dominant petiole pigmentation in amaranth species was green (56%) followed by purple (34%), except *A. graecizans*, which had an even distribution of petiole pigmentation of purple, mixture and white. In addition to green and purple petioles, large colour variation were also displayed in vegetable amaranth, *A. blitum*, *A. sp* and *A. tricolor* accessions including dark green, dark purple, mixture and white. There were various leaf shapes displayed by the accessions across the species with uniform distributions into elliptical (21%), lanceolate (22%), ovatainate (26%) and rhombic (21%). However, there was no distinct leaf shapes for *A. graecizans*, *A. retroflexus*, *A. thunbergii*, *A. graecizans* and *A. retroflexus*. Most accessions displayed either entire (57%) or undulate (41%) leaf margins, with the exception of a few accessions of *A. hypochondriacus*, *A. viridis* and *A. tricolor* which showed crenate leaf margins. Most accessions had rugose prominence leaf veins (99%), and the remaining accessions belonging to *A. viridis* had smooth prominent leaf veins. 93% accessions had no spines in leaf axils, with the exception of a small number of *A. sp* and the majority of *A. spinosus* accessions, which had spines in the leaf axils. 83% of amaranth accessions did not have leaf pubescence and 10% had low leaf pubescence, while the remaining accessions had conspicuous leaf pubescence which was found in few accessions of *A. graecizans*, *A. hypochondriacus* and *A. tricolor*.

For stem characteristics, the majority of amaranth accessions exhibited either green (60%) or purple/pink (39%) stem pigmentation, while some accessions of *A. blitum* and *A. tricolor* had a mixture or white colour stems. The accessions that had low or absent leaf pubescence did not necessarily have low stem pubescence. For example, *A. cruentus* had either low or no leaf pubescence but 33% of the accessions displayed conspicuous stem pubescence. The same condition showed in *A. hypochondriacus* and *A. tricolor* with more accessions having conspicuous stem pubescence while majority of these accessions had no leaf pubescence.

For inflorescence characteristics, the majority of amaranth accessions were green (66%), while the rest were pink (17%) red (10%), yellow (3%), mixture (1%) and others (3%). There was also large genetic variability of inflorescence density, in which 48% of amaranth accessions exhibits dense, 25% were intermediate and 27% were lax. *A. blitum*, *A. cruentus* and *A. spinosus* accessions can have either

presence or absence axillary inflorescence while more than half of *A. dubius*, *A. hypochondriacus*, *A. sp*, *A. tricolor* and *A. viridis* accessions had no axillary inflorescence. 82% of amaranth accessions showed erect terminal inflorescence attitude while 18% were drooping in which the highest contribution to this character was *A. spinosus*. Terminal inflorescence shapes were varied among amaranth species, for example, majority of *A. spinosus* and *A. viridis* had panicle with short branches while majority of *A. blitum* and *A. tricolor* had spike terminal inflorescence shape.

For seed characteristics, the majority of amaranth accessions had an ellipsoid or ovoid seed shape (92%). The main seed coat type was translucent (69%) with the remaining seeds (31%) having an opaque type which belong to *A. cruentus* and *A. viridis*. Most amaranth species produced black seeds (74%), with the exception of *A. cruentus* and *A. hybridus* which had considerable seed colour variation including brown, mixture of pale yellow and black, mixture of pale yellow and pink, and pale yellow. High seeds shattering were found in most *A. cruentus*, *A. hypochondriacus* and *A. spinosus* accessions while the majority accessions of other species had intermediate seed shattering.

Chi-square test proved that there was a homogeneity of traits distribution among the whole collection and core set accessions except for growth habit ($P<0.01$) and terminal inflorescence shape ($P=0.03$) (Table 3.5). In the whole collection, mean Shannon-Weaver diversity index (H') of all traits was 0.57 but the diversity of individual traits varied from 0.06-0.89 (Table 3.6). High diversity was found in most inflorescence traits which include inflorescence colour and density, presence of axillary inflorescence and terminal inflorescence shape. Other than that, branching index, leaf pigmentation, leaf shape, stem pubescence, and seed shattering were also found to have high diversity index. In comparison, the evenness exhibited an increased H' value in 18 qualitative traits in the core set, except a decreased H' value for growth habit, spines in leaf axils and terminal shape inflorescence.

Table 3.5: Chi-square test (x^2) and probability for comparisons of frequency distribution of 22 qualitative traits between whole set and core set of AVRDC amaranth germplasm.

No.	Traits	No. of classes	χ^2	Probability
1	Branching index	4	2.89	0.42
2	Growth habit	2	9.17	0.00
3	Sex type	2	1.39	0.32
4	Leaf margin	4	1.57	0.65
5	Leaf pigmentation	11	11.40	0.33
6	Leaf pubescence	3	0.75	0.64
7	Leaf shape	9	6.96	0.56
8	Petiole pigmentation	6	4.27	0.51
9	Prominence of leaf veins	2	0.98	0.57
10	Spines in leaf axials	2	2.66	0.14
11	Stem pigmentation	4	2.38	0.47
12	Stem pubescence	3	2.26	0.33
13	Inflorescence colour	6	5.30	0.38
14	Inflorescence density	4	2.13	0.52
15	Presence of axillary inflorescence	2	0.21	0.66
16	Terminal inflorescence attitude	2	0.02	1.00
17	Terminal inflorescence shape	5	10.93	0.03
18	Seed coat type	3	1.82	0.43
19	Seed colour	6	3.29	0.69
20	Seed shape	2	0.33	0.59
21	Seed shattering	3	0.01	1.00
22	Germination rate	3	0.42	0.84

Table 3.6: Shannon-Weaver diversity index (H') and its maximum (H_{max}) for the 22 qualitative traits in whole set and core set of AVRDC amaranth germplasm.

		Whole set		Core set	
No.	Traits	H_{max}	H'	H_{max}	H'
1	Branching index	1.39	0.73	1.39	0.78
2	Growth habit	0.69	0.53	0.69	0.20
3	Sex type	0.69	0.23	0.69	0.33
4	Leaf margin	1.39	0.57	1.39	0.59
5	Leaf pigmentation	2.30	0.69	2.40	0.72
6	Leaf pubescence	1.10	0.45	1.10	0.50
7	Leaf shape	2.20	0.79	2.20	0.86
8	Petiole pigmentation	1.79	0.58	1.79	0.66
9	Prominence of leaf veins	0.69	0.06	0.00	0.00
10	Spines in leaf axials	0.69	0.37	0.69	0.20
11	Stem pigmentation	1.39	0.53	1.39	0.59
12	Stem pubescence	1.10	0.72	1.10	0.79
13	Inflorescence colour	1.79	0.68	1.79	0.68
14	Inflorescence density	1.39	0.79	1.39	0.80
15	Presence of axillary inflorescence	0.69	0.88	0.69	0.90
16	Terminal inflorescence attitude	0.69	0.33	0.69	0.67
17	Terminal inflorescence shape	1.61	0.67	1.61	0.64
18	Seed coat type	1.10	0.58	0.69	0.81
19	Seed colour	1.61	0.46	1.39	0.59
20	Seed shape	0.69	0.40	0.69	0.45
21	Seed shattering	1.10	0.89	1.10	0.89
22	Germination rate	1.10	0.53	1.10	0.25

3.3.2.1.2 Quantitative traits

In addition, the amaranth core set were also assessed for quantitative traits, detailed in Appendix 3.7, in order to further verify the quality of core set that was primarily developed based on qualitative traits. The core set captured minimum of 80% range variation for the 10 quantitative traits from the whole collections, deploying a greater part of the genebank collections.

The MANOVA analysis performed on *A. blitum*, *A. cruentus*, *A. dubius*, *A. hypochondriacus*, *A. sp*, *A. spinosus*, *A. viridis* and *A. tricolor* showed that there were significant differences between the eight amaranth species for all 10 quantitative traits ($P<0.01$) (Table 3.7). *A. dubius*, *A. hypochondriacus*, *A. tricolor* had short basal lateral branches in a range of <20cm, while *A. cruentus* and *A. spinosus* had medium length (range >20cm), and *A. blitum*, *A. sp* and *A. viridis* had long basal lateral branch (range >30cm). Overall, grain amaranth species, *A. hypochondriacus* and *A. cruentus* had long top lateral branch (range >10 cm) and recorded the highest plant height (101.7 cm and 141.0 cm, respectively). In comparison, the vegetable-type amaranth species, *A. tricolor* was the smallest plants with shortest basal lateral branch (15.9 cm), the shortest top lateral branch (4.9 cm) and the shortest plant height (49.2 cm). Overall, grain amaranth, *A. cruentus* and *A. hypochondriacus* had long leaves (18.3 cm and 17.4 cm, respectively) but the leaves of *A. cruentus* were thinner (8.7 mm) than *A. hypochondriacus* (11.8 mm). Among the weedy amaranth, *A. dubius* had longer (17.4cm) and thicker (11.8 mm) leaves compared to *A. spinosus*. While in vegetable amaranth, *A. tricolor* showed the highest leaf length (12.6 cm) and leaf width (8.5 mm) compared to *A. blitum* and *A. sp* species. *A. cruentus* and *A. hypochondriacus* had high terminal inflorescence stalk length (32.3 cm and 17.0 cm, respectively) and high terminal inflorescence lateral length (16.1 cm and 16.0 cm, respectively). The highest length of axillary inflorescence belonged to *A. hypochondriacus* (14.1 cm). Other than grain amaranth, weed amaranth *A. dubius* showed similar characteristics as *A. hypochondriacus* with high stalk and lateral inflorescence length.The germination rate of weed amaranth accessions, *A. dubius* and *A. viridis* were rapid (94% and 91%, respectively) while most of other species were slow (75%). *A. blitum* showed the quickest days to flowering within 33 days while other amaranth species need approximately 41-55 days to flowering. In this

germplasm, *A. cruentus* and *A. hypchondriacus* had low 1000-seed weight (0.2 g and 0.4 g, respectively) compared to *A. tricolor* (0.7 g).

The difference among variance of the whole collection and core subset were not significantly different and showed that the variance between these two collections were homogenous, except for mean length of lateral branch length ($P<0.01$) (Table 3.8). This demonstrates that the selection of accessions in the core set optimally represented the range of morphological variation within traits of the two collections. The analysis of trait association was also evaluated in order to identify weather those traits were conserved in the selected amaranth core set (Table 3.9). In a whole collection, days to flowering positively correlated with seed weight ($r=0.23$, $P<0.01$), leaf length ($r=0.45$, $P<0.01$), leaf width ($r=0.37$, $P<0.01$), plant height ($r=0.12$*) but negatively correlated with terminal inflorescence stalk length ($r=-0.25$, $P<0.01$). There is positive correlation between plant heights with terminal inflorescence lateral length ($r=0.35$, $P<0.01$), terminal inflorescence stalk length ($r=0.56$, $P<0.01$), length of axillary inflorescence ($r=0.20$, $P<0.05$), leaf length ($r=0.53$, $P<0.01$), leaf width ($r=0.34$, $P<0.01$) and negatively correlated with seed weight ($r=-0.36$, $P<0.01$). Similar to plant height, leaf characteristics (leaf length and leaf width) are positively correlated to most of the traits studied with the strongest correlation found between the two traits ($r=0.82$, $P<0.01$). In a core set, negative correlations remained on terminal inflorescence stalk length ($r = -0.20$, $P<0.05$), but there was no significant correlation on plant height ($r=0.06$) and occurred to be positively correlated with terminal inflorescence lateral length ($r=0.21$, $P<0.05$). There was also more non-significant correlation between plant heights with other traits in the core set compared to whole set association, which include terminal inflorescence leaf length ($r=0.15$) and leaf of axillary inflorescence ($r=0.14$). Similar to plant height, leaf traits which include leaf length and leaf width displayed reduction of positive correlation between traits association in the core set compared to whole set.

Table 3.7: MANOVA analysis of *A. blitum, A. cruentus, A. dubius, A. hypochondriacus, A. sp, A. spinosus, A. viridis and A. tricolor* on 10 quantitative traits derived from AVRDC morphological database.

No.	Qualitative traits	SOV	d.f.	m.s.	P	No.	Qualitative traits	SOV	d.f.	m.s.	P
1	Mean length of basal lateral branch (cm)	Species	7	1714.2	0.003	6	Terminal inflorescence stalk length (cm)	Species	7	2892.49	<.001
		Error	84	513				Error	84	72.07	
2	Mean length of top lateral branch (cm)	Species	7	1592.76	<.001	7	Terminal inflorescence laterals length (cm)	Species	7	1013.96	<.001
		Error	84	72.95				Error	84	72.67	
3	Leaf length (cm)	Species	7	469.804	<.001	8	Plant height (cm)	Species	7	30241.3	<.001
		Error	84	8.756				Error	84	917	
4	Leaf width (mm)	Species	7	215.005	<.001	9	Days to flowering	Species	7	1591.9	<.001
		Error	84	3.263				Error	84	289.7	
5	Length of axillary inflorescence (cm)	Species	7	182.53	<.001	10	% 1000 seed weight (g)	Species	7	1.16685	<.001
		Error	84	18.16				Error	84	0.08231	

SOV: source of variation, d.f.: degree of freedom, m.s.: mean square, P: probability P-value significant at P<0.05

Table 3.8: Comparison of variance for 10 quantitative traits recorded in the whole set and core set of AVRDC amaranth germplasm.

		Variance		
No.	Descriptor	Whole set	Core set	Probability
1	MLOBLB	125.40	124.00	0.72
2	MLOTLB	14.05	2.16	<0.01
3	LL	19.04	13.78	0.38
4	LW	7.80	8.77	0.83
5	LOAI	5.61	4 04	0.80
6	TISL	94.20	108.10	0.82
7	TILL	27.09	19.74	0.45
8	PH	959.00	1396.00	0.72
9	DTF	106.70	105.50	0.97
10	SW	0.04	0.05	0.90

MLOBLB: Mean length of basal lateral branch, MLOTLB: Mean length of top lateral branch, LL: Leaf length, LW: Leaf width, LOAI: Leaf of axillary inflorescence, TISL: Terminal inflorescence stalk length, TILL: Terminal inflorescence lateral length, PH: Plant height, DTF: Days to flowering and SW: 1000-Seed weight.

Table 3.9: Pearson correlation coefficient among 10 quantitative traits in whole set (below diagonal) and core set (above diagonal) of the World Vegetable Center germplasm.

Traits	SW	TILL	TISL	LOAI	LL	LW	PH	DTF	MLOBLB	MLOTLB
SW		-0.11	-0.24*	0.1	0.06	0.04	-0.33**	0.26**	0.02	0.05
TILL	-0.11*		0.08	0.28	0.19*	0.14	0.15	0.21*	0.07	0.37**
TISL	-0.25**	0.20**		0.29	0.31**	0.29**	0.58**	-0.20*	0.21*	0.30**
LOAI	0.13	0.50**	0.33**		0.16	0.11	0.14	-0.06	0.22	0.52**
LL	0.01	0.32**	0.25**	0.35**		0.82**	0.42**	0.40**	-0.11	0.08
LW	0.05	0.26**	0.24**	0.26**	0.82**		0.29**	0.33**	-0.13	0
PH	-0.36**	0.35**	0.56**	0.20*	0.53**	0.34**		0.06	0.36**	0.34**
DTF	0.23**	0.1	-0.25**	0	0.45**	0.37**	0.12*		-0.12	-0.15
MLOBLB	-0.08	0.07	0.27**	0.14	-0.24**	-0.23**	0.26**	-0.23**		0.62**
MLOTLB	-0.02	0.41**	0.38**	0.45**	0.05	0.01	0.35**	-0.18**	0.60**	

MLOBLB: Mean length of basal lateral branch, MLOTLB: Mean length of top lateral branch, LL: Leaf length, LW: Leaf width, LOAI: Leaf of axillary inflorescence, TISL: Terminal inflorescence stalk length, TILL: Terminal inflorescence lateral length, PH: Plant height, DTF: Days to flowering and SW: 1000-Seed weight.

3.3.2.2 USDA-core set

The comparison of frequency distributions of five qualitative traits between whole collection and core set revealed that the core set represent variability of characters among amaranth accessions (Appendix 3.8). Large variability was observed in

stem and leaf pigmentation among *A. tricolor* accessions originated from diverse geographical origin. Most of accessions from USA displayed either green or amaranthine striped stems with either green or mix colour leaf pigmentation. In contrast, accessions from Hong Kong had green stems with central spot leaf, the accessions collected in China had dominant amaranthine stripes stem with central spot leaf and Indian accessions exhibited mix stem colour with either green or mix colour leaf. The majority of amaranth accessions displayed inflorescence in leaf axils and terminal (95%), had black seed coat (82%) and exhibited ellipsoid or ovoid with rounded bulging perisperm seeds.

A chi-square test showed that there was a homogeneity of distributions among traits between whole set and core set accessions (Table 3.10). The Shannon-Weaver diversity index (H') for individual traits varied from 0.19-0.80 with an overall mean diversity 0.45. Inflorescence shape and, seed colour and shape had low diversity index, while stem and leaf pigmentation had high diversity index. The diversity index (H') of traits in the core set remained the same as whole set except for the increased of H' evenness values in seed colour (Table 3.11). This indicates that the selection of accessions in the core set fairly represented *A. tricolor* accessions from USDA germplasm.

Table 3.10: Chi-square (x^2) test and probability for comparisons of frequency distribution of five qualitative traits between whole set and core set of USDA amaranth germplasm.

No.	Traits	No. of classes	χ^2	Probability
1	Stem pigmentation	6	2.36	0.81
2	Leaf pigmentation	9	7.24	0.52
3	Inflorescene shape	3	2.04	0.38
4	Seed colour	5	3.40	0.63
5	Seed shape	3	0.70	1.00

Table 3.11: Shannon-Weaver diversity index (H') and its maximum (H_{max}) for the five qualitative traits in whole set and core set of USDA amaranth germplasm.

		Whole set		Core set	
No.	Qualitative traits	H_{max}	H'	H_{max}	H'
1	Stem pigmentation	1.79	0.80	1.61	0.95
2	Leaf pigmentation	2.20	0.68	1.95	0.84
3	Inflorescence shape	1.10	0.19	0.69	0.22
4	Seed color	1.61	0.35	0.69	0.63
5	Seed shape	0.69	0.21	0.69	0.24

3.3.3 Composition of amaranth mini core collection

A total of 188 amaranth accessions (131 AVRDC germplasm, 52 USDA germplasm, three E-W-Seed commercial African and two commercial Malaysian varieties) were selected for amaranth mini core collection (Table 3.15). The two commercial Malaysian varieties were selected for inclusion in the core collection due to their ability to tolerate drought stress, as both were initially utilised for drought tolerance screening (Chapter 5), while the three commercial African varieties from E-W Seed were used as checks. The core set comprised of 18 species from 44 countries with Asia contributing the most accessions in the mini core collections (137 accessions), followed by The Americas (24 accessions), Africa (15 accessions), Europe (6 accessions) and unknown (8 accessions) (Appendix 3.9). Of these, 120 accessions belonged to *A. tricolor*, and the number of accessions for each country in individual species is presented in Table 3.12.

Table 3.12: Composition of amaranth core set, 188 amaranth accessions comprised of 18 species originated from 41 countries, 131 accessions from AVRDC Genebank, 52 accessions from USDA Genebank, three African commercialised varieties from E-W Seed, and two local Malaysian commercialised varieties.

Entry	Genotype	Species	Origin country	Germplasm	ID	Entry	Genotype	Species	Origin country	Germplasm	ID
1	AV-ATR	Atropurperus	Indonesia	AVRDC	VI044435	**26**	AV-VIR 4	Viridis	Thailand	AVRDC	VI049001
2	AV-GRA	Graecizans	Hungary	AVRDC	VI036225	**27**	AV-VIR 6	Viridis	Thailand	AVRDC	VI048697
3	AV-GRA SIL	Graecizans ssp	India	AVRDC	VI044403	**28**	AV-VIR 9	Viridis	Malaysia	AVRDC	VI055027
4	AV-GRA ASC	Graecizans ssp	India	AVRDC	VI044388	**29**	AV-VIR 12	Viridis	Laos	AVRDC	VI046127
5	AV-MAN	Mantegazzianus	USA	AVRDC	VI044427	**30**	AV-VIR 14	Viridis	Indonesia	AVRDC	VI044432
6	AV-BLITO	Blitoides	Hungary	AVRDC	VI036227	**31**	AV-CRU 1	Cruentus	Austria	AVRDC	VI036230
7	AV-LEU	Leucocarpus	India	AVRDC	VI044445	**32**	AV-CRU 2	Cruentus	Ethiopia	AVRDC	VI044366
8	AV-PAL	Palmeri	Senegal	AVRDC	VI044473	**33**	AV-CRU 3	Cruentus	Austria	AVRDC	VI036231
9	AV-RET 1	Retroflexus	Viet Nam	AVRDC	VI048310	**34**	AV-CRU 5	Cruentus	Mexico	AVRDC	VI044453
10	AV-RET 2	Retroflexus	Viet Nam	AVRDC	VI048311	**35**	AV-CRU 6	Cruentus	Sudan	AVRDC	VI050473
11	AV-RET 3	Retroflexus	Venezuela	AVRDC	VI033461	**36**	AV-CRU 12	Cruentus	Zimbabwe	AVRDC	VI044457
12	AV-RET 4	Retroflexus	Viet Nam	AVRDC	VI048391	**37**	AV-CRU 14	Cruentus	Malaysia	AVRDC	VI033487
13	US-RET 1	Retroflexus	China	USDA	Ames 26236	**38**	AV-CRU 15	Cruentus	Guatemala	AVRDC	VI044449
14	AV-SPI 1	Spinosus	Puerto Rico	AVRDC	VI044410	**39**	AV-HYB 1	Hybridus	USA	AVRDC	VI044419
15	AV-SPI 4	Spinosus	Thailand	AVRDC	VI040944	**40**	AV-HYB 2	Hybridus	USA	AVRDC	VI044421
16	AV-SPI 5	Spinosus	Thailand	AVRDC	VI048723	**41**	AV-HYB 3	Hybridus	Kenya	AVRDC	VI051004
17	AV-SPI 6	Spinosus	Laos	AVRDC	VI046123	**42**	US-HYB 2	Hybridus	Nigeria	USDA	PI 641052
18	AV-SP 1	Sp	Taiwan	AVRDC	VI050253	**43**	AV-GRA 1	Gracilis	Cambodia	AVRDC	VI056002
19	AV-SP 2	Sp	Thailand	AVRDC	VI049530	**44**	EW-CRU #20866	Cruentus	Tanzania	E-WEST	#20866
20	AV-SP 3	Sp	Laos	AVRDC	VI054799	**45**	AV-HYP 2	Hypochondriacus	Mexico	AVRDC	VI044454
21	AV-SP 4	Sp	Malaysia	AVRDC	VI033471	**46**	AV-HYP 3	Hypochondriacus	India	AVRDC	VI044414
22	AV-SP 5	Sp	India	AVRDC	VI044448	**47**	AV-HYP 5	Hypochondriacus	Hungary	AVRDC	VI036229
23	AV-SP 6	Sp	Bangladesh	AVRDC	VI056563	**48**	AV-HYP 6	Hypochondriacus	Nepal	AVRDC	VI044479
24	AV-SP 7	Sp	Bangladesh	AVRDC	VI056560	**49**	AV-HYP 10	Hypochondriacus	Ghana	AVRDC	VI044365-A
25	AV-VIR 1	Viridis	Thailand	AVRDC	VI049893	**50**	AV-HYP 13	Hypochondriacus	Viet Nam	AVRDC	VI047551

Table 3.12: (Continued)

Entry	Genotype	Species	Origin country	Germplasm	ID	Entry	Genotype	Species	Origin country	Germplasm	ID
51	AV-HYP 14	Hypochondriacus	Ecuador	AVRDC	VI033462-A	**76**	AV-TRI 8	Tricolor	Bangladesh	AVRDC	VI048146
52	AV-HYP 16	Hypochondriacus	Afghanistan	AVRDC	VI044395	**77**	AV-TRI 9	Tricolor	Bangladesh	AVRDC	VI048089
53	AV-BLI 1	Blitum cvg alecereus	India	AVRDC	VI044404	**78**	AV-TRI 10	Tricolor	Bangladesh	AVRDC	VI047848
54	AV-BLI 3	Blitum cvg alecereus	Laos	AVRDC	VI055755	**79**	AV-TRI 11	Tricolor	Bangladesh	AVRDC	VI047795
55	AV-BLI 4	Blitum cvg alecereus	Malaysia	AVRDC	VI055121	**80**	AV-TRI 12	Tricolor	China	AVRDC	VI044420
56	AV-BLI 7	Blitum	Thailand	AVRDC	VI049036	**81**	EW-TRI Thida	Tricolor	Malaysia	E-WEST	Thida
57	AV-BLI 10	Blitum	Korea	AVRDC	VI044447	**82**	EW-TRI Zeya	Tricolor	Malaysia	E-WEST	Zeya
58	AV-BLI 12	Blitum	India	AVRDC	VI044423	**83**	AV-TRI 15	Tricolor	Indonesia	AVRDC	VI042983
59	AV-BLI 13	Blitum	Cambodia	AVRDC	VI056127	**84**	AV-TRI 16	Tricolor	India	AVRDC	VI047439
60	AV-THU 1	Thunbergii	Unknown	AVRDC	VI050456	**85**	AV-TRI 17	Tricolor	Japan	AVRDC	VI048528
61	AV-THU 2	Thunbergii	Unknown	AVRDC	VI050467	**86**	AV-TRI 18	Tricolor	India	AVRDC	VI044446
62	AV-THU 3	Thunbergii	Unknown	AVRDC	VI050468	**87**	AV-TRI 19	Tricolor	India	AVRDC	VI044443
63	AV-DUB 1	Dubius	Viet Nam	AVRDC	VI047576	**88**	AV-TRI 20	Tricolor	Malaysia	AVRDC	VI043725
64	AV-DUB 2	Dubius	Viet Nam	AVRDC	VI047537	**89**	AV-TRI 21	Tricolor	Malaysia	AVRDC	VI043724
65	AV-DUB 6	Dubius	Thailand	AVRDC	VI048985	**90**	AV-TRI 22	Tricolor	Nigeria	AVRDC	VI044438-A
66	AV-DUB 7	Dubius	Tanzania	AVRDC	VI050464	**91**	AV-TRI 23	Tricolor	Laos	AVRDC	VI055809
67	AV-DUB 13	Dubius	Surinam	AVRDC	VI044377	**92**	AV-TRI 24	Tricolor	Pakistan	AVRDC	VI044396-A
68	AV-DUB 15	Dubius	Cambodia	AVRDC	VI057160	**93**	AV-TRI 25	Tricolor	Thailand	AVRDC	VI049129
69	AV-TRI 1	Tricolor	Bangladesh	AVRDC	VI038237	**94**	AV-TRI 26	Tricolor	Thailand	AVRDC	VI049006
70	AV-TRI 2	Tricolor	Bangladesh	AVRDC	VI055356	**95**	AV-TRI 27	Tricolor	Thailand	AVRDC	VI049004
71	AV-TRI 3	Tricolor	Bangladesh	AVRDC	VI055353	**96**	AV-TRI 28	Tricolor	Turkey	AVRDC	VI044389
72	AV-TRI 4	Tricolor	Bangladesh	AVRDC	VI055350	**97**	AV-TRI 29	Tricolor	USA	AVRDC	VI044470
73	AV-TRI 5	Tricolor	Bangladesh	AVRDC	VI048269	**98**	AV-TRI 30	Tricolor	Viet Nam	AVRDC	VI047747
74	AV-TRI 6	Tricolor	Bangladesh	AVRDC	VI048233-A	**99**	AV-TRI 31	Tricolor	Viet Nam	AVRDC	VI050615-A
75	AV-TRI 7	Tricolor	Bangladesh	AVRDC	VI048200	**100**	AV-TRI 32	Tricolor	Viet Nam	AVRDC	VI050613

Table 3.12: (Continued)

Entry	Genotype	Species	Origin country	Germplasm	ID	Entry	Genotype	Species	Origin country	Germplasm	ID
101	AV-TRI 33	Tricolor	Viet Nam	AVRDC	VI050610-A	**125**	AV-TRI 57	Tricolor	Malaysia	AVRDC	VI044426
102	AV-TRI 34	Tricolor	Viet Nam	AVRDC	VI050609-A	**126**	AV-TRI 58	Tricolor	Malaysia	AVRDC	VI055139
103	AV-TRI 35	Tricolor	Viet Nam	AVRDC	VI047603	**127**	AV-TRI 59	Tricolor	Malaysia	AVRDC	VI055062
104	Local PR	Tricolor	Unknown	LOCAL	var. BBS014	**128**	AV-TRI 60	Tricolor	Malaysia	AVRDC	VI033490
105	AV-TRI 37	Tricolor	Taiwan	AVRDC	VI054536	**129**	AV-TRI 61	Tricolor	Malaysia	AVRDC	VI033480
106	AV-TRI 38	Tricolor	Taiwan	AVRDC	VI050214	**130**	AV-TRI 62	Tricolor	Malaysia	AVRDC	VI033474
107	AV-TRI 39	Tricolor	Philippines	AVRDC	VI054572	**131**	AV-TRI 63	Tricolor	Malaysia	AVRDC	VI033473
108	AV-TRI 40	Tricolor	Philippines	AVRDC	VI054571	**132**	AV-TRI 64	Tricolor	Thailand	AVRDC	VI049005
109	AV-TRI 41	Tricolor	Papua New Guinea	AVRDC	VI044450	**133**	AV-TRI 65	Tricolor	USA	AVRDC	VI044379-A
110	AV-TRI 42	Tricolor	Papua New Guinea	AVRDC	VI044407	**134**	AV-TRI 66	Tricolor	Viet Nam	AVRDC	VI047526-A
111	AV-TRI 43	Tricolor	Bangladesh	AVRDC	VI048301	**135**	AV-TRI 67	Tricolor	Viet Nam	AVRDC	VI047387
112	AV-TRI 44	Tricolor	Bangladesh	AVRDC	VI048286	**136**	AV-TRI 68	Tricolor	Taiwan	AVRDC	VI050111
113	AV-TRI 45	Tricolor	Bangladesh	AVRDC	VI048021	**137**	AV-TRI 69	Tricolor	Taiwan	AVRDC	VI049431
114	AV-TRI 46	Tricolor	Bangladesh	AVRDC	VI047929	**138**	US-TRI 1	Tricolor	Bangladesh	USDA	Ames 5368
115	AV-TRI 47	Tricolor	Bangladesh	AVRDC	VI047682	**139**	US-TRI 2	Tricolor	Brazil	USDA	Ames 29504
116	AV-TRI 48	Tricolor	Bangladesh	AVRDC	VI047681	**140**	US-TRI 3	Tricolor	Brazil	USDA	Ames 29505
117	AV-TRI 49	Tricolor	Bangladesh	AVRDC	VI047504	**141**	US-TRI 4	Tricolor	China	USDA	Ames 2017
118	AV-TRI 50	Tricolor	Bangladesh	AVRDC	VI047501	**142**	US-TRI 5	Tricolor	China	USDA	PI 419121
119	AV-TRI 51	Tricolor	Cambodia	AVRDC	VI057270	**143**	US-TRI 6	Tricolor	China	USDA	PI 478310
120	AV-TRI 52	Tricolor	Cambodia	AVRDC	VI056168	**144**	US-TRI 7	Tricolor	Hong Kong	USDA	Ames 2204
121	AV-TRI 53	Tricolor	Indonesia	AVRDC	VI042979	**145**	US-TRI 8	Tricolor	Hong Kong	USDA	Ames 2205
122	AV-TRI 54	Tricolor	Indonesia	AVRDC	VI042978	**146**	US-TRI 9	Tricolor	India	USDA	Ames 2040
123	AV-TRI 55	Tricolor	India	AVRDC	VI059413	**147**	US-TRI 10	Tricolor	India	USDA	Ames 2145
124	AV-TRI 56	Tricolor	India	AVRDC	VI058498	**148**	US-TRI 11	Tricolor	India	USDA	PI 669847

Table 3.12: (Continued)

Entry	Genotype	Species	Origin country	Germplasm	ID	Entry	Genotype	Species	Origin country	Germplasm	ID
149	US-TRI 12	Tricolor	India	USDA	PI 674261	**169**	US-TRI 32	Tricolor	Hong Kong	USDA	PI 674260
150	US-TRI 13	Tricolor	Indonesia	USDA	Ames 2039	**170**	US-TRI 33	Tricolor	India	USDA	Ames 2100
151	US-TRI 14	Tricolor	Madagascar	USDA	Ames 5354	**171**	US-TRI 34	Tricolor	India	USDA	Ames 2101
152	US-TRI 15	Tricolor	Malaysia	USDA	Ames 2029	**172**	US-TRI 35	Tricolor	India	USDA	Ames 2102
153	US-TRI 16	Tricolor	Malaysia	USDA	Ames 29034	**173**	US-TRI 36	Tricolor	India	USDA	Ames 2119
154	US-TRI 17	Tricolor	Papua New Guinea	USDA	Ames 5111	**174**	US-TRI 37	Tricolor	India	USDA	Ames 2120
155	US-TRI 18	Tricolor	Papua New Guinea	USDA	PI 349553	**175**	US-TRI 38	Tricolor	India	USDA	Ames 2121
156	US-TRI 19	Tricolor	Taiwan	USDA	Ames 2199	**176**	US-TRI 39	Tricolor	India	USDA	Ames 2132
157	US-TRI 20	Tricolor	Thailand	USDA	Ames 2024	**177**	US-TRI 40	Tricolor	India	USDA	Ames 2134
158	US-TRI 21	Tricolor	Thailand	USDA	PI 607446	**178**	US-TRI 41	Tricolor	India	USDA	Ames 2135
159	US-TRI 22	Tricolor	USA	USDA	PI 603897	**179**	US-TRI 42	Tricolor	India	USDA	Ames 2138
160	US-TRI 23	Tricolor	USA	USDA	PI 603898	**180**	US-TRI 43	Tricolor	India	USDA	Ames 2223
161	US-TRI 24	Tricolor	USA	USDA	PI 632237	**181**	US-TRI 44	Tricolor	India	USDA	Ames 2224
162	US-TRI 25	Tricolor	West Africa	USDA	Ames 5110	**182**	US-TRI 45	Tricolor	Puerto Rico	USDA	Ames 5117
163	US-TRI 26	Tricolor	Zaire	USDA	Ames 1980	**183**	US-TRI 46	Tricolor	Puerto Rico	USDA	Ames 5118
164	US-TRI 27	Tricolor	China	USDA	Ames 26209	**184**	US-TRI 47	Tricolor	Taiwan	USDA	Ames 1993
165	Local Red	Tricolor	Malaysia	LOCAL	var. BBS027	**185**	US-TRI 48	Tricolor	Taiwan	USDA	Ames 1998
166	US-TRI 29	Tricolor	China	USDA	Ames 26216	**186**	US-TRI 49	Tricolor	USA	USDA	Ames 5134
167	US-TRI 30	Tricolor	Hong Kong	USDA	Ames 5102	**187**	US-TRI 50	Tricolor	USA	USDA	Ames 25153
168	US-TRI 31	Tricolor	Hong Kong	USDA	Ames 5317	**188**	US-TRI 51	Tricolor	Unknown	USDA	PI 633591

3.3.3.1 Uniformity of traits between the Genebank morphological database and the observed traits in shade-house grown plants

Overall, the comparisons between observed traits and the Genebank morphological database were not significantly different (P>0.05), with only 16% accessions from AVRDC Genebank and 12% from USDA Genebank not matching the database. Hence, the Genebank morphological database can be directly used for germplasm selection. The observed traits which include leaf, petiole and stem colours, growth habit, branching index, leaf shape and margin and terminal inflorescence colour, shape and attitude are presented in Appendix 3.10.

3.3.3.2 Variability of traits in amaranth mini core collection

The amaranth core set exhibits large genetic variability especially in leaf, petiole, stem and inflorescence colour (Figure 3.4). In order to assess the patterns of variation, PCA was analysed simultaneously with all the 10 qualitative traits included (Table 3.13). The first four principle components (PCs) contributed 59.08% of the total variation, with PC1 accounting for 20.59% variation and the genetic divergence in the major axis of differentiation were inflorescence, and leaf, petiole and stem pigmentation. PC2 accounting for an additional 14.95% of the total variation, depicted variation in branching index and leaf shape. PC3 and PC4 contributed another 12.51% and 11.03% of the total variation. The dendrogram showed that the amaranth core set had high variability with similarity coefficient of 0.2, but there was no clear demarcation between grain, weed and vegetable amaranth species based on morphological features (Figure 3.5). There was also low level of dictinctness in morphological traits within *A. tricolor* accessions with regard to the geographical distribution. The core set was divided into eight distinct morphological traits;

Cluster 1: accessions with entire leaf margin with drooping terminal inflorescence attitude

Cluster 2: accessions with distinct red inflorescence, ovatainate leaf shape with entire lamina purple/pink colour, and purple petiole and stem

Cluster 3: accession with purple petiole and stem, but no distinct leaf shape

Cluster 4: accessions with green inflorescence, undulate leaf margin with normal green colour, and green petiole and stem

Cluster 5: similar to Cluster 4, except the accession having entire leaf margin

Cluster 6: similar to Cluster 4, but the accessions have no distinct inflorescence characteristics

Cluster 7: accessions with green inflorescence and petiole and purple/pink stem

Cluster 8: accessions with green crunate leaf and erect terminal inflorescence shape

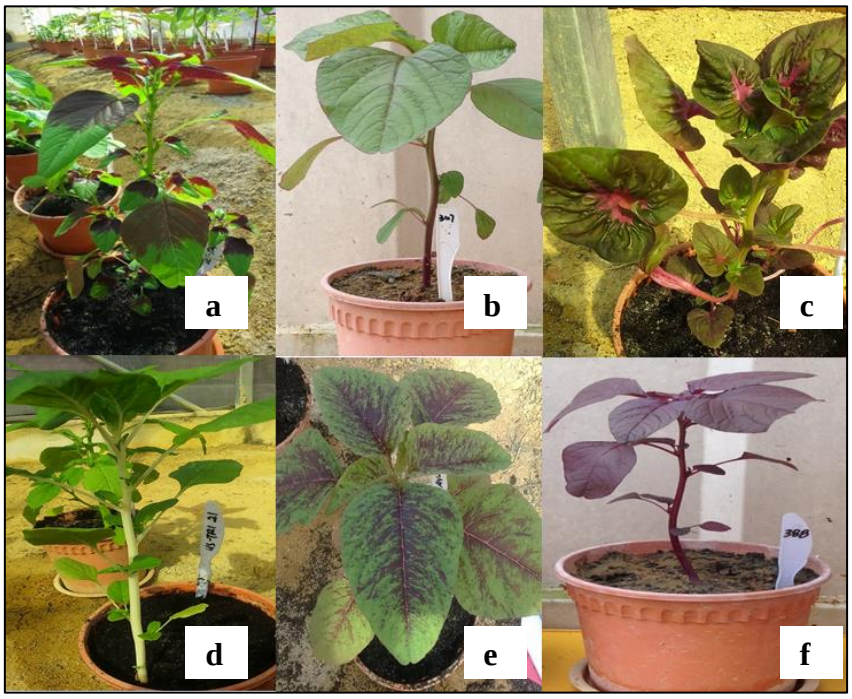


Figure 3.4: The variability of characters among selected *A. tricolor* accessions: (a) basal area pigmented leaf, (b) vein pigmented leaf with red stem, (c) pink spotted leaf with pink petiole and stem, (d) green leaf with white petiole and stem, (e) green leaf with spotted purple, and (f) perfect red amaranth.

Table 3.13: The latent roots (Eigen values) of the first four principle component (PC) analysis for the 10 qualitative traits.

	PC 1	**PC 2**	**PC 3**	**PC 4**
Latent roots	2.06	1.50	1.25	1.10
Percentage variation	20.59	14.95	12.51	11.03
Cumulative percentage variance	20.59	35.54	48.05	59.08
Traits	**Latent vectors**			
Branching index	0.03	0.51	0.46	0.10
Growth habit	0.00	0.10	0.61	-0.44
Inflorescence color	0.51	-0.05	0.14	0.19
Leaf margin	-0.09	0.39	-0.07	0.48
Leaf pigmentation	0.41	0.07	0.27	0.23
Leaf shape	0.08	0.48	-0.29	0.01
Petiole pigmentation	0.47	-0.24	-0.05	0.28
Stem pigmentation	0.55	0.11	0.26	-0.03
Terminal inflorescence attitude	-0.13	-0.49	0.28	0.06
Terminal inflorescence shape	0.11	0.17	-0.29	-0.63

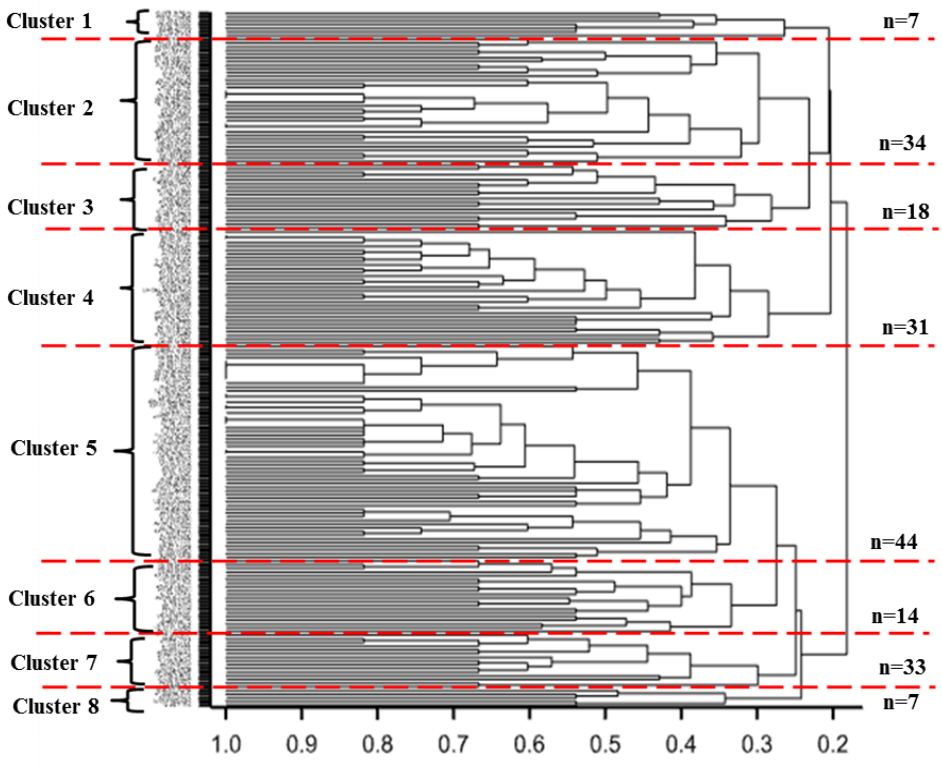


Figure 3.5: Qualitative trait-based dendrogram of the 188 amaranth accession core set. A clear version of accessions in each cluster group is presented in Appendix 3.11. The dendrogram produced eight clusters with n is the number of accessions in each cluster.

3.4 Discussion

The improvement of crop plants depends on the availability of germplasm with beneficial traits of interest (Ojuederie *et al*., 2014). The analysis of morphological diversity has direct benefits in research related to population structure, evolution and plant breeding (Valdiani *et al*., 2014). Traditionally, the curators of Genebanks characterize their materials based on highly heritable selected morphological and agromonical traits (Acquaah, 2007). Although plant description based on morphological traits are highly influenced by environment factors (Sammour *et al*., 2012), some of the traits can determine the potential of agronomic values. For example, quantitative traits such as plant height, number of branches and total leaf area are the most suitable for vegetable production (Sogbohossou and Achigan-Dako, 2014). Qualitative traits such as leaf, stem and petiole colour and shape are mainly influenced by consumer's preferences (Akaneme and Ani, 2013) and capable in classifying genus into amaranth species (Gerrano *et al*., 2014), and different stem and leaves colour of amaranth demonstrate variations in drought

tolerance characteristics (Nakashima *et al*., 2011). The need to conserve these traits with their associations between whole collections and core set is therefore important to maintain co-adapted genetic complexes (Ortiz *et al*., 1998).

Several species in the amaranth mini core collection developed in this study are of high economic importance, including grain (*A. cruentus* and *A. hypochondriacus*) and vegetable crops (*A. tricolor*) as well as invasive weeds (*A. plameri* and *A. retroflexus*) (Costea and DeMason, 2001). Knowledge of the evolutionary relationship between the grain, vegetable and weeds allows for the exploration of the association among agronomically important traits such as drought tolerance. This study is the first of its kind whereby the whole collection of AVRDC germplasm, which is comprised of 18 amaranth species and all *A. tricolor* collection of USDA germplasm, of a diverse geographical origin were investigated thoroughly for morphological diversity. Although similar work has been done by Thapa and Blair (2018), Sogbohossou *et al*., (2014) and Andini *et al*., (2013), these studies only focused on a small number of amaranth species, mostly on grain species and its putative wild progenitors.

Several studies have successfully developed a core set from the large collection of germplasm using qualitative and quantitative traits, with an excellent representation of phenotypic diversity, for example chickpea (Archak *et al*., 2016) and sesame (*Sesamum indicum*) (Xiurong *et al*., 2000). In the present study, amaranth accessions from AVRDC and USDA Genebanks were analysed based on qualitative traits in order to standardize the selection of traits using multivariate analysis. . The standard stratification procedure employed in this study, which include stratifying the entire germplasm collection into taxonomic groups, country of origin and accessions with similar qualtative traits, have been shown to be one of an effective tool for developing mini core collection (Upadhyaya *et al*., 2009; Balakrishnan *et al*., 2000; Xiurong *et al*., 2000).

The degree of phenotypic representativeness between AVRDC- and USDA-core set and the whole collection in the Genebank were accessed through several analyses. The Shannon-Weaver diversity index (H') analysis was used as a measure of richness and evenness in the distribution of accessions in each category (Shannon & Weaver, 1949). The standardized index was classified as high (0.67-1.00) which revealed an even distribution of accessions within the phenotypic classes and polymorphic, intermediate (0.34-0.66), low (0-0.33) which indicate an

extreme unbalanced distribution for individual phenotypic class, a lack of diversity and monomorphic (Moreno *et al*., 2013). In this study, an increased H' evenness values across the traits in the core are an indicative of effective representation of the phenotypic diversity of the entire collection conserved in the AVRDC Genebank. This demonstrated that the selection of qualitative traits of amaranth accessions in the core set had high stability and consistency that may be less influenced by environment. However, *A. tricolor* accessions originated from diverse geographical origins conserved in USDA Genebank showed a low diversity index in inflorescence shape, seed colour and seed shape. In contrast, stem and leaf pigmentation had high diversity index, which showed that these traits were polymorphic with an even distribution among amaranth accessions. The estimates of representativeness acquired in this study showed that the qualitative traits in the core set had reduced the number of positive correlation compared to the whole set, and the plausible reduction in association could be because of sampling effects (Gangopadhyay *et al*., 2010).

The representative of AVRDC- and USDA core set, along with commercialised amaranth varieties were then used as an amaranth mini core collection for phenotypic and molecular diversity studies. In this study, leaf, petiole and stem colour were shown to have high variability and heritability within the amaranth germplasm compared to other morphological traits, similar to results obtained by Thapa and Blair *et al*., (2018), Gerrano *et al*., (2017), Sogbohossou *et al*., (2014), Ahammed *et al*., (2013) and Gerrano *et al*., (2006). Several studies on the genetic variability of amaranth genotypes based on agro-morphological traits have revealed wide genetic diversity within and between amaranth species (Akin-Idowu *et al*., 2016; Sarker *et al*., 2015; Oboh, 2007). The high variation in morphological appearance may be due to (i) a lack of selection pressure because of the artificial or domestication process (Chan and Sun, 1997); (ii) or because of the mixed-mating system of amaranths that may facilitate the natural introgression process (Kulakow and Hauptli, 1994); (iii) or due to polyploidy which leads to gene combination (Andini *et al*., 2013).

Nevertheless, the cluster analysis based on the ten qualitative traits in the present study failed to discriminate species and geographical origin between amaranth accessions. This also happened in Tapha and Blair (2018), in which the cluster developed based on nine qualitative traits on 293 amaranth genotypes,

failed to distinguish grain from weed amaranth species. This is because the same species was found to have variable morphological traits and high plasticity in trait expressions. Furthermore, an evaluation on quantitative traits alone such as plant height and stem diameter was also unable to distinguish species or plant type, but was helpful in identifying accessions with high protein content and interesting vegetable production traits Sarker *et al*., 2017; Andini et al., 2013; Shukla *et al*., 2010).

However, Sogbohossou *et al*., (2014) found out that the mix analysis of 15 qualitative (which include the 10 qualitative traits observed in this study) and 15 quantitative traits on 100 amaranth genotypes was able to show a clear demarcation between grain amaranth and other species. They found that *A. caudatus* and *A. tricolor* to be the most diverse (phenotypically) although there was no clear demarcation in geographical origin between and within amaranth species. The separation of geographical origin within *A. tricolor* accessions was successfully done using mix analysis of phenotypic and nutritional traits (Shukla *et al*., 2010), but contradicted results obtained by Sarker *et al*., (2017), in which the selected analyses used in the study showed no relationship in geographical divergence among the amaranth genotypes. These may be due to the consequences of multiple sites of origin, and because of phenotypic plasticity nature of amaranth (Pandey and Singh, 2011; Brenner *et al*., 2010).

3.5 Conclusion

The multivariate analysis using Jaccard's similarity matrix based on qualitative traits used in this study successfully identified the accessions with diverse morphological traits in the germplasm to be included in the core set. The choice of sampling strategy through stratification allows for the core-set to retain the largest part of the diversity. The analyses failed to discriminate the *Amaranthus* genus into grain, vegetable and weed along with geographical region. This could be due to the large degree of diversity within the genus (intraspecific diversity) in the collections and biased number of accessions used per species. Phenotypic diversity study in amaranth can be improved with either integration of qualitative and quantitative traits, or an inclusion of a larger data of qualitative/quantitaive characters. Nonetheless, this wide variability of genetic resources from diverse geographical origins could be utilized for further improvement in enhancing the genetic potential

of the crops, particularly in the identification of *A. tricolor* accessions with superior drought tolerance traits.

CHAPTER 4

CONSTRUCTION OF A HIGH-DENSITY DArTseq-SNPs BASED POPULATION STRUCTURE IN THE AMARANTH MINI CORE COLLECTION AND GENOME-WIDE ASSOCIATION STUDIES

4.1 Introduction

Correct genotypic identification and preservation of genetic variation is important to maintain ecotypes that have desired traits for breeding programmes (Perez-Gonzalez, 2001). Amaranth has high phenotypic plasticity and a large amount of genetic diversity (Rastogi and Shukla, 2013) and therefore, it is important to characterize the amaranth germplasm, recognize its redundancy and identify intramorphyte variation among the amaranth genotypes (Jimenez *et al*., 2013). Genotyping using molecular markers has been successfully applied to many crops for the development of population structure (Laidò *et al*., 2013), to ascertain genetic diversity within germplasm collections (Cavanagh *et al*., 2013), validate phylogeny of the genus (Stetter *et al*., 2017b), identify QTLs and candidate genes conferring valuable traits (Barilli *et al*., 2018) and generate data for gene expression profiling (Kouzai *et al*., 2016).

Grain amaranth (*A. caudatus, A. cruentus and A. hypochondriacus*) along with its wild putative progenitor (*A. hybridus*) have had various molecular markers applied to them, including random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), single nucleotide polymorphisms (SNPs) among others (refer to subheading 2.2.7), which aimed to identify evolutionary and phylogenetic relationships between the grain amaranth and its possible ancestors. Although useful, these studies used a small number of markers which did not cover the whole genome (Kietlinski *et al*., 2014; Maughan *et al*., 2009 & 2011; Mallory *et al*., 2008). The recent use of SNPs discovery through genotyping-by-sequencing (GBS) has proved to be the most efficient method to evaluate genetic diversity of amaranth accessions with consistent geographical origin and morphological classification, as well as to validate phylogeny of the genus *Amaranthus* (Stetter and Schmid, 2017a & b; Wu and Blair, 2017). While the amaranth marker studies have improved genomic resources in grain amaranth, further germplasm characterization and marker validation is still needed (Wu and Blair 2017) and to

date, very little information is available on the genetic diversity of leafy vegetable *A. tricolor*, with only five or fewer accessions included among other amaranth species in any molecular approaches, including SSR (Khaing *et al*., 2013) and GBS (Stetter and Schimd, 2017).

In this regard, GBS offers a number of advantages; it is more practical, inexpensive and has driven genotyping to be applied for non-model organisms, i.e. does not require a reference genome (Andrews *et al*., 2016; Elshire *et al*., 2011). The DArTseq method based on GBS technology has been successfully applied in several crop species for genetic diversity studies (Brinez *et al*., 2012), linkage mapping (Ho *et al*., 2017; Baloch *et al*., 2016), QTL identification (Barilli *et al*., 2018) and genome wide association studies (GWAS) (Mogga *et al*., 2018). DArTseq is a platform developed by Diversity Arrays Technology Pty Ltd. (Canberra, Australia) for high-throughput genotyping based on sequencing results generated by Next-Generation Sequencing (NSG) technologies. As the choice and number of restriction enzyme to cut down genome complexity will determine the effectiveness of the genomic total coverage, DArTseq provides an intelligent selection of genome fraction by targeting to active genes and low copy DNA areas, and the variant is optimized to a numerous plant species and offered as commercial service by DArT P/L (Li *et al*., 2015). DArTseq generates two types of data i) silicoDArT and ii) SNP markers. SilicoDArT markers are microarray markers that are dominant and scored for the presence or absence of a single allele while SNPs are fragments present in the representation and are co-dominant markers. Further, SNPs genome-wide association mapping (SNPs-GWAS) has rapidly become a powerful tool to identify the relationships between molecular marker, candidate genes or QTL associated with traits in a given population based on linkage equilibrium, known as marker-trait association (MTA). It facilitates understanding of the genetic bases and dissection of complex genes controlling economic traits such as drought tolerance (Li *et al*., 2018). This provides useful information on the degree of genetic variation, and its correlations with agronomic traits.

This present study is the first to utilize the DArTseq platforms in amaranth to investigate the genetic diversity and population structure of amaranth mini core collections (Chapter 3; Subheading 3.3.3). This study aimed to investigate the relationship between *A. tricolor* and other agronomical important species including cultivated, grain and wild amaranth germplasm, of large Asian collections. This

study also aimed to investigate the genetic relationship among a numerically larger group of *A. tricolor* accessions for drought tolerance traits, which were of primary interest. The release of SNP markers from this panel provided inestimable genomic information to conduct a Genome Wide Association Study (GWAS) on morphological traits between 18 species, and drought tolerance traits within vegetable *A. tricolor* accessions.

4.2 Materials and methods

4.2.1 DNA sample preparation

4.2.1.1 DNA extraction

Total genomic DNA of 188 amaranth accessions (subheading 3.3.3) was extracted from young leaves using Qiagen DNEASY plant DNA extraction kit (Qiagen, USA). DNA extraction was repeated for some samples using DNeasy PowerPlant Pro Kit (Qiagen, USA) and modified CTAB protocol (Doyle and Doyle, 1990) until high quality DNA was obtained. Approximately, 50 mg, 100 mg or 200 mg of leaf tissue (using PowerPlant Pro Kit, Qiagen DNEASY Kit or CTAB method, respectively) was ground with stick homogenizer grinder in a 2 µl microcentrifuge tube under liquid nitrogen until leaf sample were ground to a fine powder.

For the CTAB protocol, 200 µg fine powder was mixed with 600 µl of pre-heated extraction buffer (65°C) and 5 µl of 20 mg/ml Proteinase K, shaken vigorously before being incubated at 65°C for 30 minutes under constant agitation (750 rpm). The extraction buffer consists of 3% CTAB, 100 mM TrisHCl (pH 8), 25 mM EDTA (pH 8), 2 M Nacl, 2% SDS, 5% PVP and 4% B-mercaptoethanol. Then, 5 µl of 100 mg/ml RNase A was added and incubated at 37°C for 15 minutes under constant agitation (750 rpm). 610 µl chlorofoam:isoamyl alcohol (CIA) mix 24:1 was added and mixed by shaking 10-20 times, incubated at 50°C for 2 minutes to remove plant polyphenols before centrifuged at 13,500 rpm for 10 minutes at 4°C. 500 µl of aqueous phase was transferred into an equal volume of CIA (500 µl), the solution was then mixed, incubated and centrifuged as the steps above. 400 µl of aqueous phase was transferred into 40 µl of 3 M sodium acetate (pH 5.2) and 1 ml of ice-cold 100% ethanol was added into the mixture, incubated at -20° for 1 hour. To pellet the DNA, the mixture was then centrifuge at 13,500 rpm for 15 minutes at 4°C. Washing DNA pellet was done twice with 500 µl of 70% ethanol and centrifuged at 13,500 rpm for 15 minutes at 4°C, pouring off supernatant each

time. The DNA pellet was allowed to dry before re-suspend in 30 µl sterile distilled water.

4.2.1.2 DNA quantification

The quantification of extracted genomic DNA was confirmed by running the DNA sample on 1% agarose gel electrophoresis and comparing the fluorescence with the standard lambda DNA concentration. To make a 1% gel, agarose (1st BASE, Singapore) was dissolved in 0.5X TBE buffer (R&M Marketing, UK) by slow heating in microwave with occasional swirling. A clear dissolved gel was stained with appropriate amount of 10X SYBR® Safe (Invitrogen, USA) depending on the volume of gel cast tray, e.g. 0.5X in 50 ml minigel. When the gel had set, 5 µl genomic DNA sample that mixed with 1 µl 6X loading dye (NEB, USA) was loaded into the gel together with 1kb ladder (ready-to-use, GeneDireX, USA) and 50 ng, 100 ng and 150 ng lambda DNA (NEB, USA), and the gel was run at 80V for 30 minutes (50 ml minigel). DNA quantification was achieved by comparing the fluorescence intensity of the sample DNA with respective size of lambda DNA under Biorad Gel Doc 2000 USA (Appendix 4.1a). The DNA was further quantified using a Nanodrop spectrophotometer (Thermo Scientific, USA), with 1 µl DNA sample loaded onto the pedestal to know the approximate purity concentration of 1.7-2.0 at A260/A280 and >1.5 at A260/A230. A high quality and integrity of DNA sample was further diluted to a final concentration of 50 ng-100 ng.

4.2.1.3 Restriction enzyme (RE) digestion

The quality of DNA sample was further confirmed through digestion with *HindIII* restriction enzyme (NEB, USA) to check the suitability of DNA sample for DArTseq assay. 1 µl of diluted DNA sample (50 ng-100 ng) was added into a mixture of 1 µl of 10X restriction buffer, 0.2 µl *HindIII* and 7.8 µl sterile distilled water. The solution was vortexed and incubated at 37°C for 1 hour. The digested DNA sample was run on 1% agarose gel, 0.5% TBE buffer at 80V for 30 minutes (Appendix 4.1b). A good digestion of DNA sample was then ready to be sent for DArTseq P/L.

4.2.2 DArTseq assays

Genotyping by sequencing analysis for the 188 mini amaranth collections was performed by using a whole genome profiling services, DArTseq P/L Canberra, Australia. 20 µl of 50 ng μl^{-1} to 100 ng μl^{-1} of high and good quality DNA sample was sent to DArTseq P/L for SNPs and silico DArT marker analysis following the protocol as described by Kilian *et al*., (2012). In brief, this technology is optimized for each organism and application in order to select the most appropriate complexity reduction method. In this study, a combination of a rare cutting RE *PstI* with a set of secondary frequently cutting restriction endonucleases *MseI* was selected as it provided the most appropriate locus coverage, reproducibility and polymorphisms (data not presented). The *PstI*-compatible adapter consists of Illumina flow cell attachment sequence, sequencing primer and a 'staggered' of varying length barcode region. The *MseI*-compatible adapter consists of Illumina flow cell attachment region and *MseI* overhang sequence. The ligated fragments with both a *PstI* and *MseI* adapter were amplified via polymerase chain reaction (PCR) with programmed set to initial denaturation step of 94°C for 1 min, followed by 30 cycles of denaturation at 94°C for 20 s, annealing at 58°C for 30 s and extension at 72°C for 45 s, and final extension at 72°C for 7 min. Equimolar amounts of PCR products from each sample were combined following with a single end sequencing of 77 cycles on an Illumina Hiseq2500.

The resulting sequences from each lane were processed through the application of proprietary DArT analytical pipeline (fastq files). The poor quality sequences were filtered away so that a more stringent selection of barcode regions per sample was accessed for marker calling. The identical sequences were collapsed into ''fastqcall files". These files were used in the secondary pipeline for DArT PL's proprietary SNPs and SilicoDArT (presence, absence or missing of restriction fragments in the representation) marker calling algorithms using DArTsoft14. These representations are informative DNA sequence (approximately 70bp) and each individual's state compared with all others, namely (i) homozygosity with reference allele, (ii) homozygosity with alternate allele, or (iii) heterozygosity, comprising both a reference and an alternate SNP allele. DNA samples of 24 amaranth genotypes were genotyped in two technical replications in order to obtain the reproducibility of the marker data.

4.2.3 Data analysis

4.2.3.1 SNP filtering

The quality and informativeness of the selected SNP datasets were assessed by means of reproducibility (%) >0.97, call rate (%) >0.75 and polymorphism information content (PIC) >0.1. Reproducibility is the proportion of technical replicate assay pairs for which the marker score is consistent. The call rate determined the success of reading marker sequence across the samples, i.e. the proportion of samples for which the genotype call is either "1" or "0", rather than "-". The PIC is an index that evaluates the informative extent of an SNP marker and demonstrates the degree of diversity of the marker in the population, where 0 indicates no allelic variation and a maximum of 1.0 for absolute allele variation. A Venn diagram was used to visualize the SNP loci shared among the *Amaranthus* species. A diagram of overlapping SNP loci was generated using the online program Van de Peer Lab (http://bioinformatics.psb.ugent.be/).

4.2.3.2 Population structure and genetic diversity

Population structure was carried out in two steps. First, to obtain the overall genetic relationships between the 188 amaranth accessions, individual accessions with >50% missing data and SNP loci with >5% missing data were manually removed. The SNP loci were then imputed using Euclidean distance and set with minor allele frequency >5% using TASSEL software (version 5.0). Second, 120 *A. tricolor* accessions were analysed separately as a subset. Two individual accessions with >70% missing values were removed. The SNP loci were filtered for no missing data with MAF >5%.

The population structure was analysed using the Structure-like Population Genetic Analyses R package, LEA (Falush *et al*., 2007; François, 2016). The number of sub-populations was determined using cross-entropy criterion, based on the predictions of a fraction of masked genotypes (matrix completion) and on the cross-validation approach, with runs of eight values of K (K=1:8). A distance matrix was generated using TASSEL software which was used to conduct principal coordinate analysis (PCoA) and a phylogenetic tree based on UPGMA distance. The genetic distance within *A. tricolor* populations was calculated using F-statistic test and the overall population statistic was calculated using the Monte-Carlo test using Adegenet 1.4-1 in R 3.0.3 (Jombart and Ahmed, 2011).

4.2.3.3 Genome-Wide Association Study (GWAS) of morphological traits

DArTseq SNP markers were physically mapped using *Amaranthus hypochondriacus* genome v2.1 (Lightfoot *et al*., 2017), the closest relative of *Amaranthus tricolor*, available in Phytozome (phytozome.jgi.doe.gov) using CLC Genomic Workbench v8 (Qiagen), based on perfect match of aligned sequence tags against the reference genome, with 80% length and similarity fraction (Ho *et al*., 2017). The association study was conducted for 118 *A. tricolor* accessions on 10 morphological traits via mixed linear model (MLM) controlling for Q and kinship (K) as fixed and random effects respectively in TASSEL 5.0. MAF > 0.05 was used to filter SNPs prior to analysis. Q was extracted from results of previous population structure analysis (K=3, R package LEA) and K was calculated using Scaled IBS method implemented in TASSEL 5.0. The qualitative traits including leaf, petiole and stem colours, growth habit, branching index, leaf shape and margin and terminal inflorescence colour, shape and attitude was obtained from previous observations (subheading 3.3.3.1; Appendix 3.10). Marker trait association (MTA) was determined at $P<0.01$. The Manhattan plots of –log(p-values) and the Q-Q (quantile-quantile) plots of expected vs observed p-values for SNP-based genotype-phenotype associations were generated using TASSEL 5.0. Highly significant MTA was then compared with *Arabidopsis thaliana* genes using reference sequence track JBrowse Phytozome at respective position and locus.

4.3 Results

4.3.1 SNP marker discovery

DArTseq analysis generated 74,306 allele sequences of SNP reads from the 188 amaranth accessions. Of these reads, 63,821 SNPs were physically mapped with amaranth reference genome *Amaranthus hyponchondriacus* (Lightfoot et al., 2017), based on aligned sequence tags against the reference genome. The SNP markers were identified with call rate average of 93%, PIC average of 0.15, and reproducibility of 100% reproducibility. Most of the SNPs identified in this study were transition-type mutation which includes A/G (16%) or G/A (15%), and C/T (16%) or T/C (15%) substitution while transversion-type mutation mostly occurred in A/T or T/A (7%). Of these filtered SNPs, 99% were located on the major sequence contigs of the genome while only 1% was located on minor contigs that not have been annotated. To investigate species-specific SNPs among 12 amaranth

species (not included species with one representative), the accessions were manually examined with the unique SNPs presence in one species but was not presence in other species. Six amaranth species that show species-specific SNP loci were assigned into six groups in a Venn diagram (Figure 4.1). *A. thunbergii* showed the highest unique SNPs (26,629), followed by *A. spinosus* (1,008), *A. graecizans* (1,067), *A. tricolor* (820), *A. hypochondriacus* (437) and *A. hybridus* (296). There was only 1,394 SNP shared by all six species group.

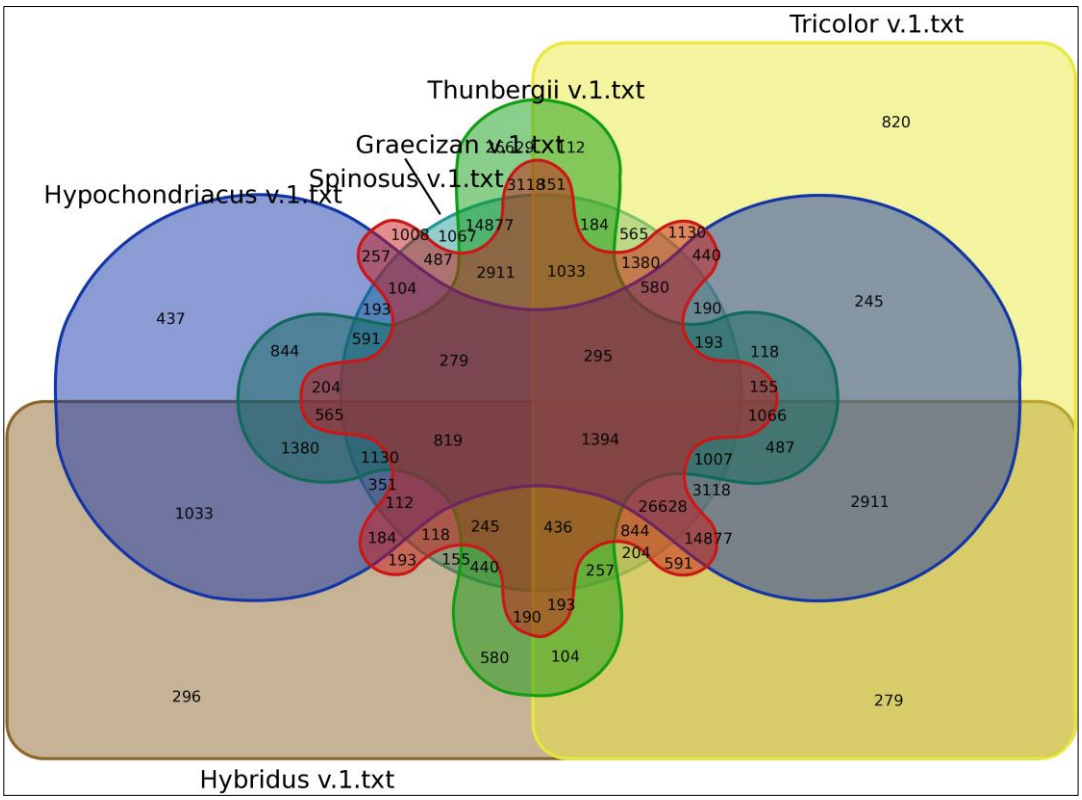


Figure 4.1: Venn diagram to show the presence, average and overlap of SNPs in six different amaranth species.

4.3.2 Population structure and genetic diversity of amaranth collection

Population structure of amaranth was carried out in two steps. For 16 amaranth species, after filtering to remove low quality SNPs, five individual accessions with >50% missing data (AV-ATR, AV-BLITO, AV-SPI 1, AV-SPI 5 and AV-SPI 6) were removed from further analysis. A total of 3,898 SNPs remained for 183 accessions, with 0.1% averaged missing value in SNP loci (median= 0.13%, min=0%, max=0.26%) and 2% averaged missing values in individual-levels (median=0.3%, min=0%, max=23.4%). To obtain a high resolution genetic

estimate within the 120 *A. tricolor* accession subset, stringent filtration was implemented by removing data with missing values. Two individual accessions (AV-TRI 20 and AV-TRI 28) which contribute to 70% of the missing values were removed and a total of 4,637 SNPs remained for the 118 *A. tricolor* accessions. The dataset used to construct genetic distance for all 183 accessions shared 347 SNPs markers with the SNP data set used to construct genetic distance within the *A. tricolor* accessions.

In LEA, choosing the number of clusters is based on the cross-entropy criterion. This criterionis also used by the program admixture (Alexander *et al*. 2011). Population structure analysis demonstrated that the K-values of 16 amaranth accessions and 118 *A. tricolor* subset was K=3 based on minimal cross-entropy (Figures 4.2a & b). The Q-matrix of K=3 is displayed in a bar plot (Figures 4.3 a & b). Each vertical bar represents a single accession and the length of each bar represents the proportion contributed by each sub-population (admixture). The groupings of the sub-populations are similar to the UPGMA phylogenetic tree.

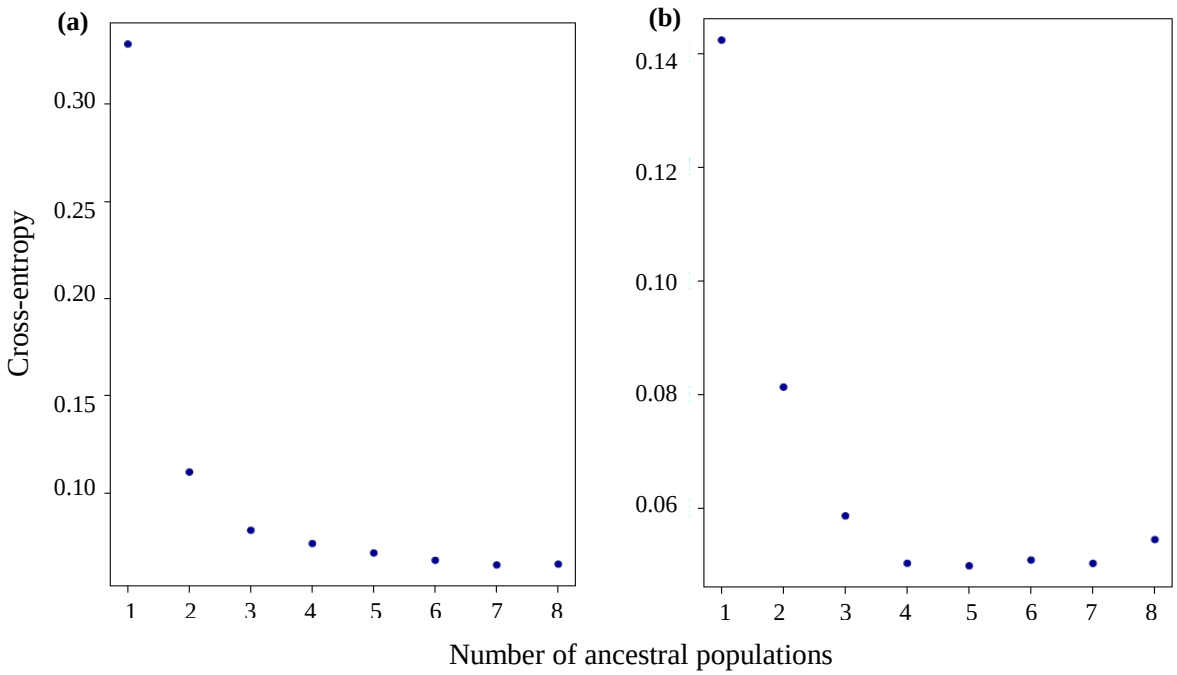


Figure 4.2: Cross-entropy plot for (a) all 183 amaranth accessions comprised of 16 amaranth species and (b) 118 *A. tricolor* accessions. A range of K=1:8 were tested and K=3 was chosen as the cross-entropy curve exhibits a plateau.

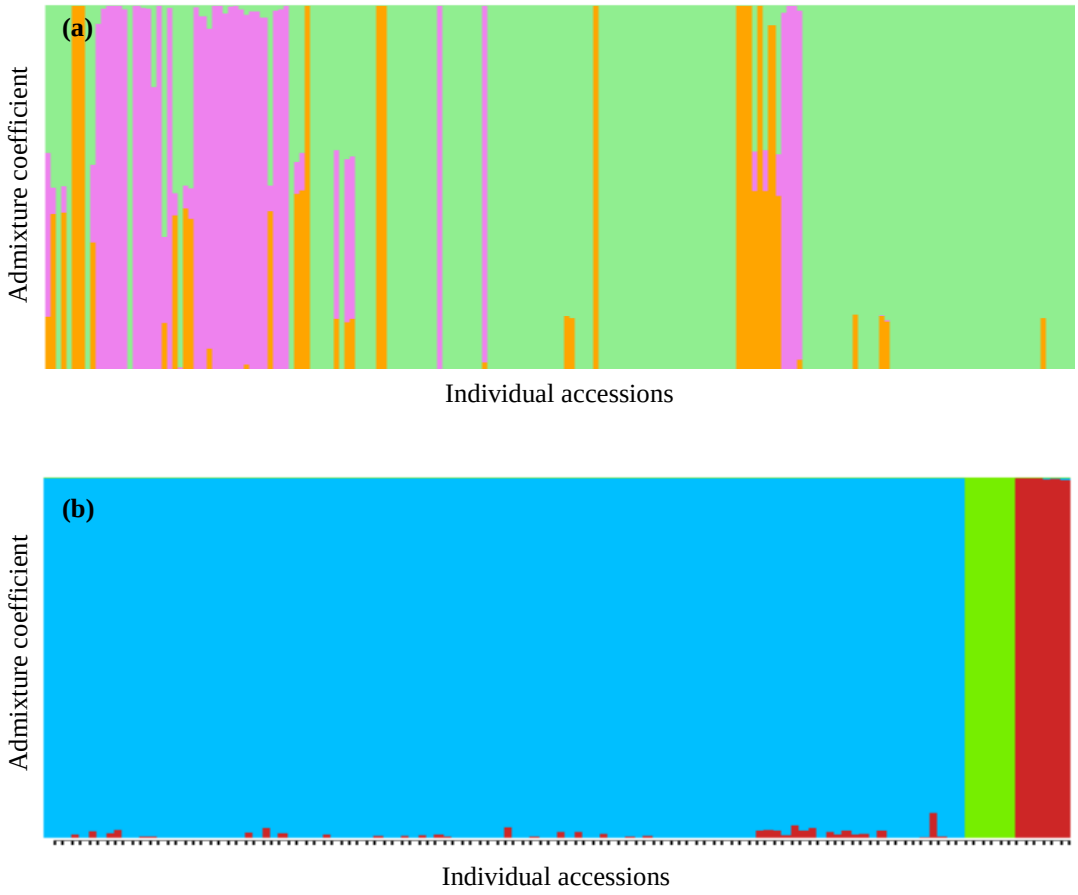


Figure 4.3: (a) Population structure of 16 amaranth species at K=3. Each vertical bar represents a single accession and the length of each bar represents the proportion contributed by each sub-population; green (sub-pop 1), pink (sub-pop 2) and orange (sub-pop 3). (b) Population structure of 118 *A. tricolor* accessions at K=3; blue (sub-pop 1), red (sub-pop 2) and green (sub-pop 3).

The genetic diversity between 16 amaranth species is shown in Figure 4.4a. The amaranth accessions were grouped into two main sub-population (sub-pop A and sub-pop B), and divided into two groups, respectively. The two grain-type amaranth species (*A. hypochondriacus* and *A. cruentus*) both group into sub-pop B together with their putative progenitor (*A. hybridus*), with the exception of one *A. cruentus* accession (AV-CRU 5) which groups into sub-pop A. Other cultivated vegetable-type species such as *A. blitum*, *A. graecizan*, *A. sp* and *A. thunbergii* were closely related with *A. tricolor* (sub-pop A) although several accessions belonged to sub-pop B. The weed-type species such as *A. retroflexus* and *A. viridis* were diverse between the two main clusters.

The genetic diversity of 118 *A. tricolor* accessions is shown in Figure 4.4b. The sub-pop 1 is made up of 105 accessions from 12 countries of origin; sub-pop 2

comprises seven accessions, of which three accessions are from Papua New Guinea and four accessions from USA; and sub-pop 3 consists of six Bangladeshi accessions with distinct morphological traits (branches along the stem, purple-pink stem, purple leaf and petiole, red-green inflorescence and erect terminal inflorescence attitude). In a different dataset of a larger amaranth species, the sub-populations grouping of 118 *A. tricolor* accessions were remained in the same group in sub-pop A1 (Figure 4.4a), except that accession US-TRI-50 diverted away from the rest of accessions. Meanwhile, the two out-grouped *A. tricolor* accessions (AV-TRI 20 and AV-TRI 28) were separated into sub-pop B2 (Figure 4.4a).

The PCoA illustrated the genetic divergence of 16 amaranth species (Figure 4.5a) and among 118 *A. tricolor* accessions (Figure 4.5b). The accession distributions determined by both marker datasets was consistent with the output of the population structure and dendrogram. In 16 amaranth species, accessions in sub-pop A1 and sub-pop B2 showed some dispersal and diversity within each sub-population. Sub-pop A2 clustered tightly together, depicting that little diversity exists within the sub-populations. Sub-pop A2 was also closer with sub-population A1 which may explain the inter-specific admixtures as observed in the dendogram tree. All clusters distributed throughout the 3D-plot although some accessions in sub-pop A1 concentrated towards PCoA1 and one accession belonged to sub-pop B1 located in PCoA3.

In 118 *A. tricolor* accessions, the PCoA displayed a clear division between the sub-populations and showed high dispersal and high genetic diversity within each sub-population. The genetic distance within *A. tricolor* populations calculated using F-statistic test revealed that sub-pop 1 was closer to sub-pop 3 with a value of 0.36 and the highest genetic distance was detected between sub-population 2 and sub-population 3 with value of 0.99 (Figure 4.5c). The overall population statistic calculated using the Monte-Carlo test revealed that there is an overall significant difference between the sub-populations (P=0.002).

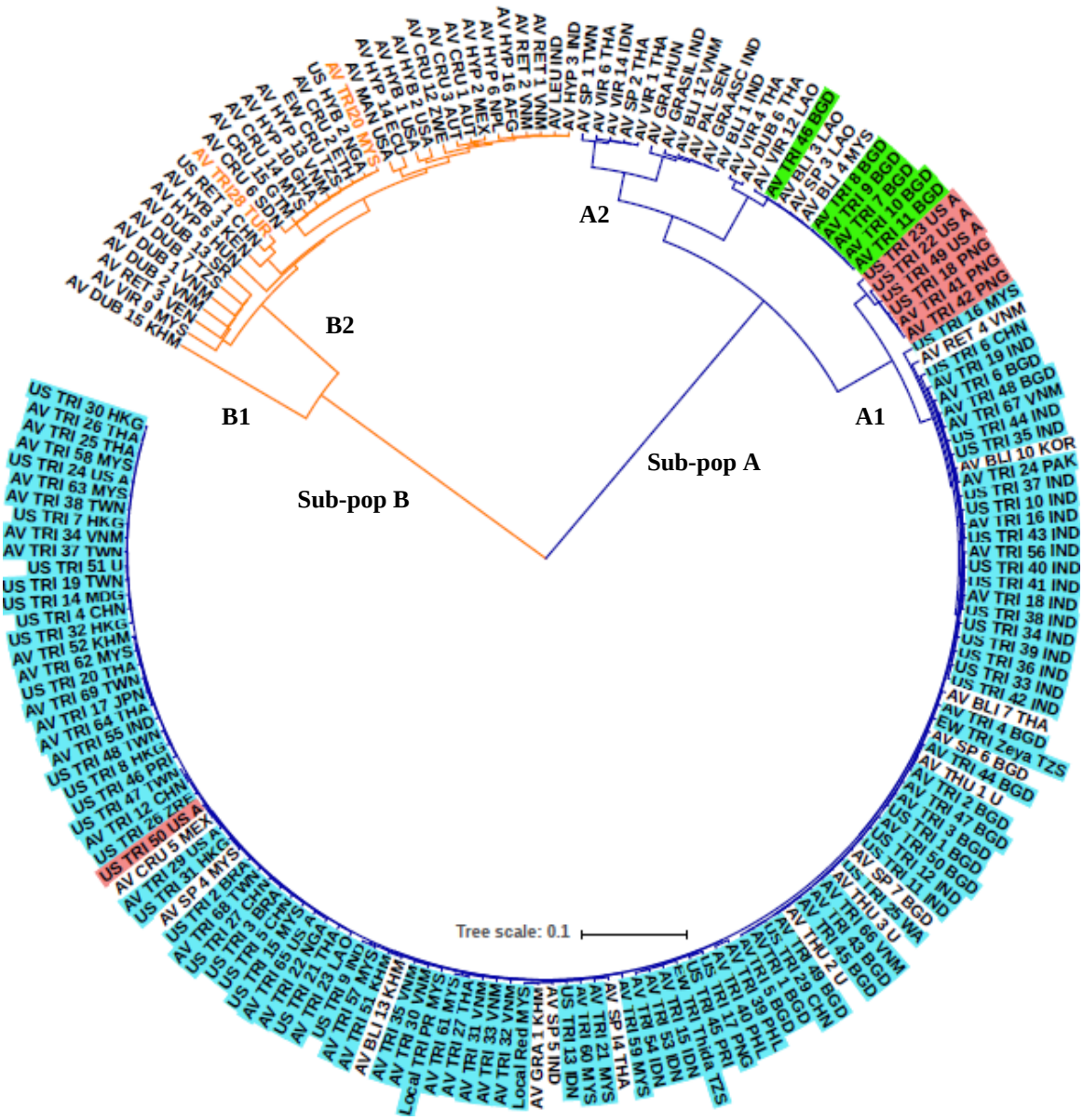


Figure 4.4 (a): UPGMA phylogenetic tree of 16 amaranth species. The accessions divided into two main clusters; sub-pop A (orange clade) and sub-pop B (blue clade), which divided into two sub-clusters A1:A2 and B1:B2, respectively. The highlight color label showed the position of *A.tricolor* accessions according to their second population structure (Figure 4.3b). The two *A. tricolor* accessions written in orange code (AV-TRI 20 and AV-TRI 28) are the out-grouped of *A. tricolor* accessions.

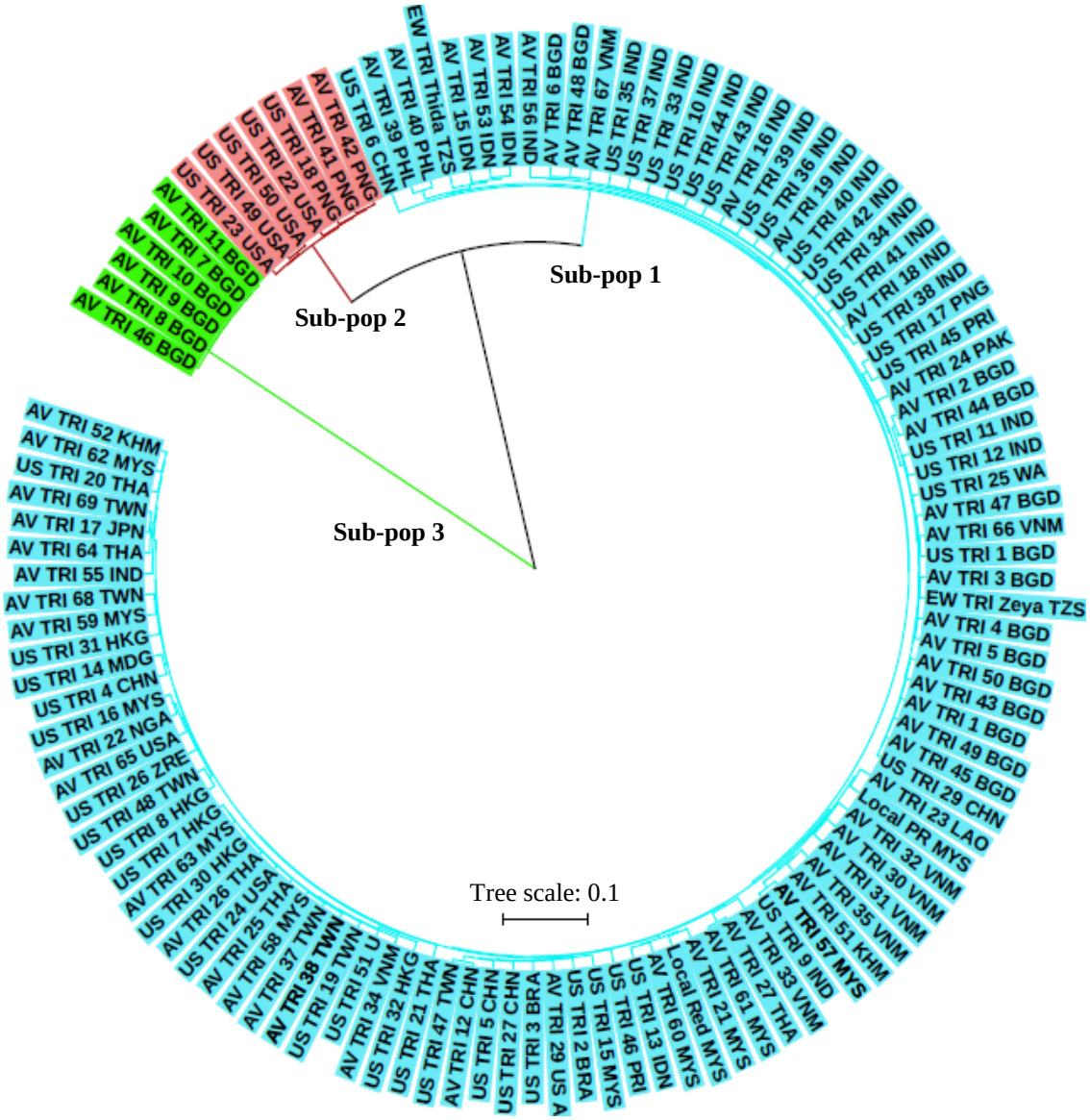


Figure 4.4 (b): UPGMA phylogenetic tree of 118 *A.tricolor* accessions. The accessions divided into three sub-populations; sub-pop 1 (blue), sub-pop 2 (red) and sub-pop 3 (green).

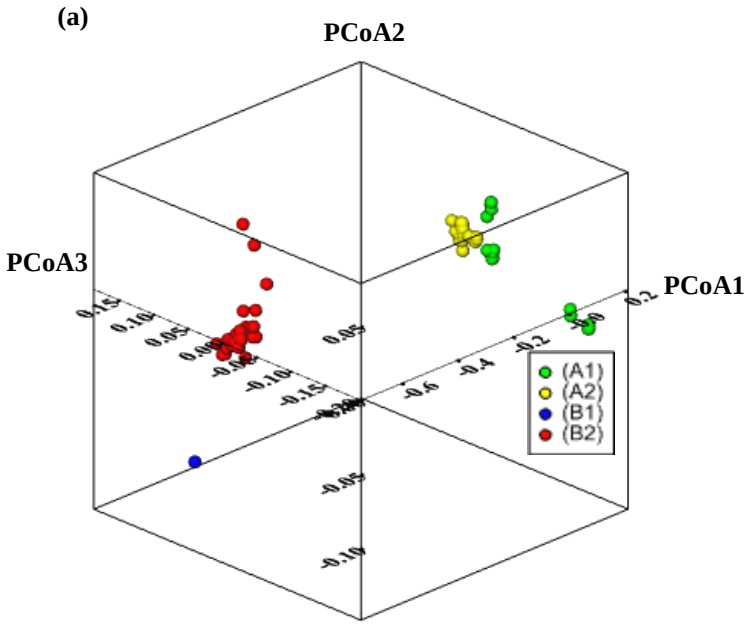


Figure 4.5 (a): 3D-plot principles coordinate analysis (PCoA) of 16 amaranth species. The colour-coded symbols represent sub-populations; sub-pop A1 (green), sub-pop A2 (yellow), sub-pop B1 (blue) and sub-pop B2 (red).

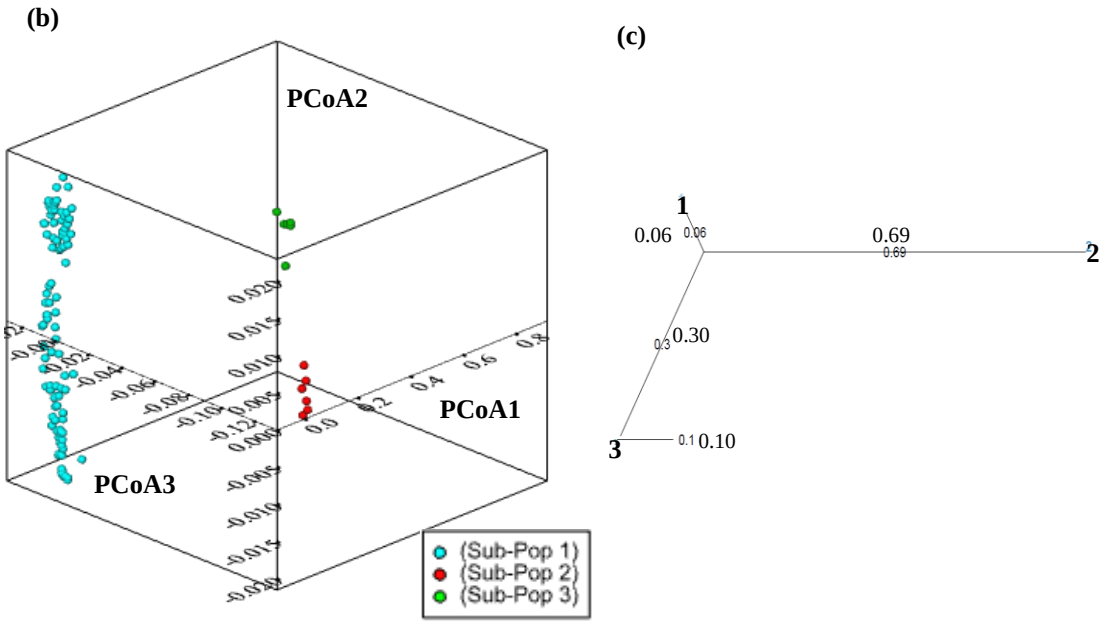


Figure 4.5(b): 3D-plot principles coordinate analysis (PCoA) of 118 *A. tricolor* accessions. The colour-coded symbols represent sub-populations; sub-pop 1 (blue), sub-pop 2 (red) and sub-pop 3 (green). **Figure 4.4(c):** F-statistics of genetic distance for 118 *A. tricolor* accessions at overall population statistic of P=0.002 using Monte Carlo test.

4.3.3 Genome wide association study (GWAS) of 10 morphological traits

GWAS was conducted on 10 morphological traits to demonstrate the effectiveness of the amaranth diversity panel for trait dissection. SNP marker-trait associations (MTA) that had a $P<0.01$ were considered as significant in this study. The analyses revealed 25 significant SNP markers which could lead to the discovery of genes controlling the traits in the amaranth genetic diversity panel (Table 4.1). One SNP marker associated with branching index, eight SNP markers associated with inflorescence color and terminal inflorescene shape, respectively, four SNP markers associated with petiole pigmentation and terminal infloresce attitude, respectively. The markers had low phenotypic variation (<20%), probably due to complex genetic architectures which were controlled by many genes with minor effect. Five significant SNP MTA's were annotated as being homologos to different functional genes in *Arabidopsis thaliana* (Table 4.1). The chromosome and location of SNP markers with similar annotation functions with *Arabidopsis thaliana* are shown in the Manhattan plots of –log(p-values) and the Q-Q (quantile-quantile) plots of expected vs observed p-values (Figure 4.6).

Table 4.1: List of 25 significant marker trait association (MTA) of branching index (BI), inflorescence color (IC), petiole pigmentation (PP), terminal inflorescence attitude (TIA) and terminal inflorescence shape (TIS). MTA's homologous with functional gene in *A. thaliana* is also presented.

Trait	MTA	Chr	Position	R^2	Gene annotation
BI	33406498	Scaffold 1	987614	0.16	Similar to dcaf8: DDB1- and CUL4-associated factor (Xenopus leaves)
IC	33430213	Scaffold 1	24895670	0.14	
	33420510	Scaffold 10	33341934	0.14	
	33416574	Scaffold 11		0.12	
	33421031	Scaffold 15	4478322	0.15	Similar to MIEL:E3 ubiquitin-protein ligase MIEL (*Arabidopsis thaliana*)
	33435567	Scaffold 16	76940235	0.10	
	33439433	Scaffold 3	2496544	0.12	
	33439433	Scaffold 3	6945876	0.12	
	33402825	Scaffold 8	1349607	0.14	
PP	33450147	Scaffold 11	10418610	0.13	Similar to LPEAT1: Lysophospholipid acyltransferase LPEAT1 (*A. thaliana*)
	33439355	Scaffold 3	6759561	0.10	
	33442322	Scaffold 7	14984567	0.12	
	33432644	Scaffold 7	16849506	0.13	
TIA	33444110	Scaffold 13	16813071	0.14	Similar to PCMP-H85: Putative pentatricopeptice repeat-containing At3g13770, mitochondrial (*A. thaliana*)
	33435675	Scaffold 14	34759603	0.10	
	33422961	Scaffold 4	85069467	0.10	
	33431148	Scaffold 9	15273845	0.10	
TIS	33404084	Scaffold 8	7884498	0.10	Similar to Atg06900: Nardilynsin-like *(A. thaliana)*
	33413870	Scaffold 13	18378072	0.14	
	33414222	Scaffold 15	59030699	0.10	
	33416866	Scaffold 5	15234856	0.14	
	33445943	Scaffold 5	9845643	0.10	
	33414588	Scaffold 5	12469356	0.10	
	33431343	Scaffold 8	3993102	0.18	
	33423313	Scaffold 8	3991701	0.18	

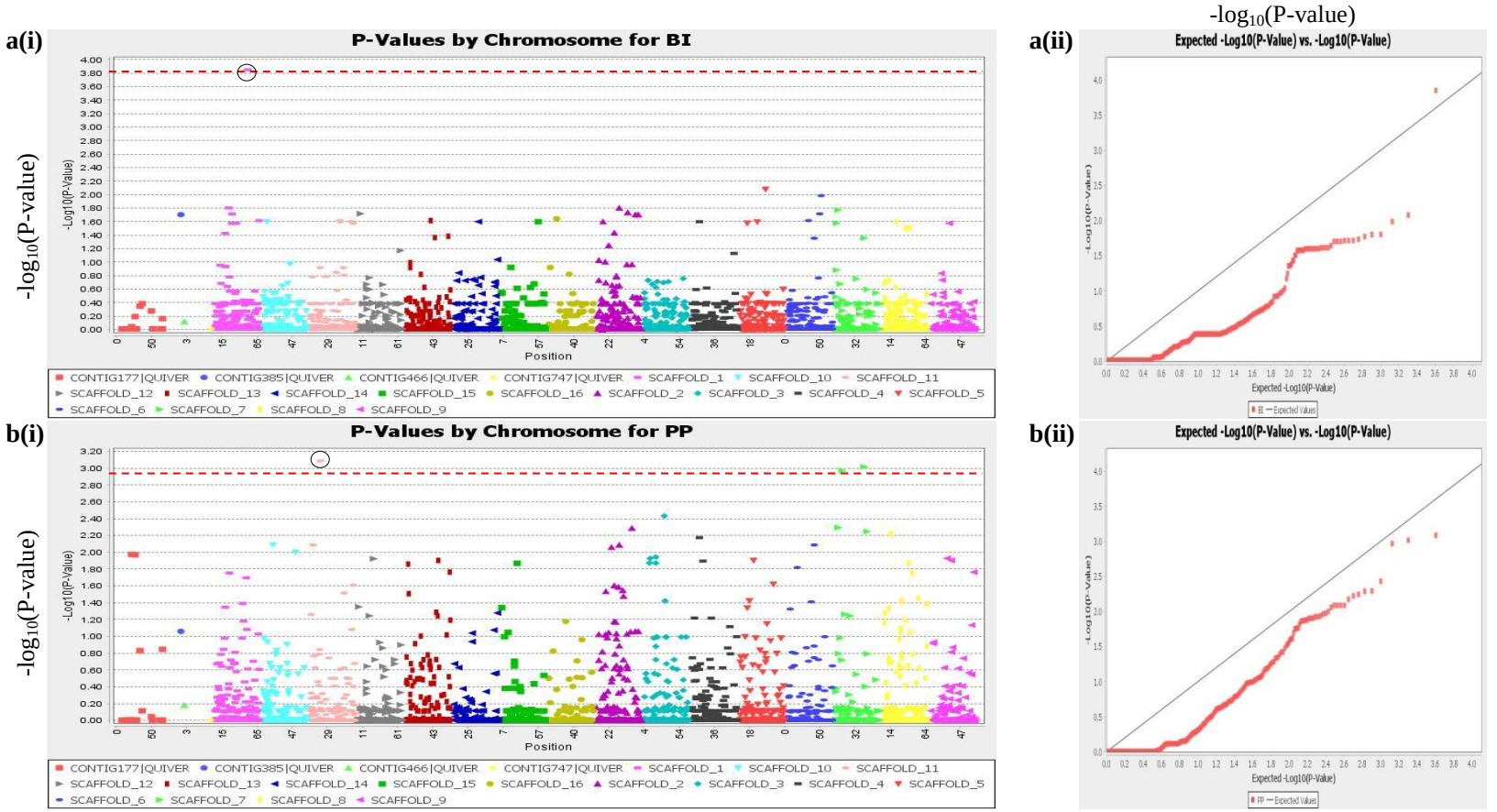


Figure 4.6: Manhattan plots and respective quantile–quantile (Q-Q) plots of the morphological traits evaluated to have significant associations: (a) branching index (BI); (b) petiole pigmentation (PP); (c) inflorescence colour (IC); (d) terminal inflorescence attitude (TIA); and (e) terminal inflorescence shape (TIS). Circle is SNP-based genotype-phenotype associations.

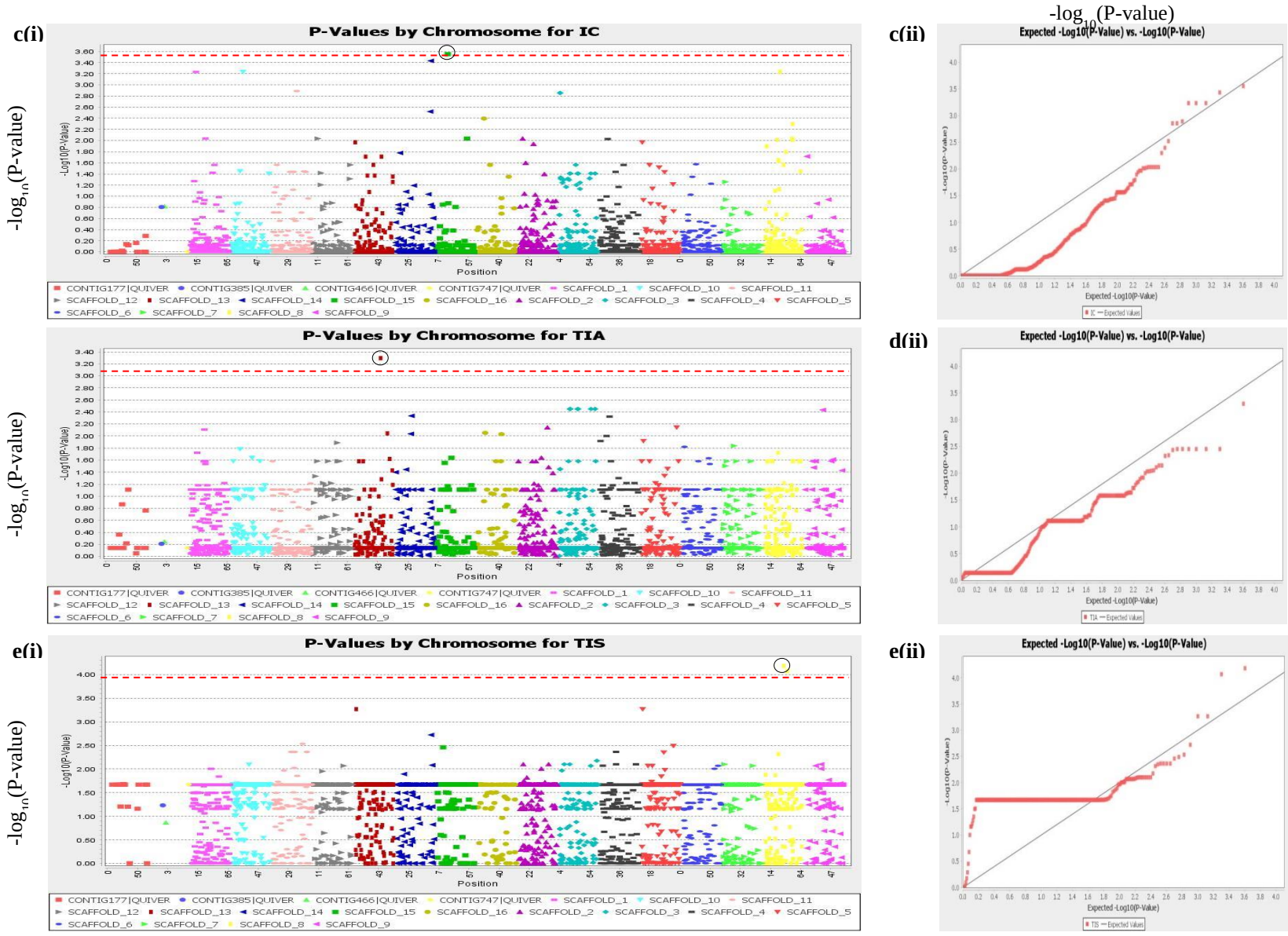


Figure 4.5: (Continued)

4.4 Discussion

4.4.1 Genotyping by sequencing and SNP markers discovery

Genotyping by molecular markers is very valuable because it can provide insights into genetic identification and diversity, which can lead to the discovery of novel alleles, useful in breeding programmes (Nadeem *et al*., 2018). In this study, the evaluation of molecular markers and morphological traits was carried out on single-plants to retain homogeneity of germplasms seed collections, as variations were observed among amaranth plants within one collection. The evaluation of single-plants is necessary as amaranth has high phenotypic plasticity which appears to be heterogamous in field plantings and thus adapts easily to the environmental changes, even though selection within cultivar/landrace has the possibility to be non-reproductive (Guillen *et al*., 1999).

The use of GBS platforms has been shown to be the most efficient method for high-throughput genotyping in amaranth (Stetter and Schmidt, 2017; Stetter *et al*., 2017; Wu and Blair, 2017). DArTseq provides an order of magnitude more markers through an intelligent selection of genome fraction by targeting active genes and low copy DNA areas (Li *et al*., 2015). In this study, a large number of SNP markers (74,306 SNP) were generated through a non-reference based approach (*de-novo*) using *PstI* and *MseI* enzyme cutting in the library preparation step. After aligning the sequence tags against very high quality and full length macromolecules of the *A. hypochondriacus* reference genome for SNP locations (Lightfoot *et al*., 2017), the DArTseq was able to generate relatively high SNP markers (63,821 SNP). The number of SNP loci discovered in this study compared favourably with previous GBS studies that used *ApeKI* single enzyme cutting combined with deep reference-based assembly methods (Wu and Blair, 2017) as well as studies that used two library preparation via reference based and non-reference based assembly methods (Stetter and Schmid, 2017; Stetter *et al*., 2017).

Various densities of SNP distributions in certain parts of the physical map may reflect the occurrence of active and repeat sequence in a chromosome. For example, the high density of SNPs in Scaffold 1 may reflect the euchromatic region where unique and active sequence were highly frequent, and exhibit lower degrees of cytosine methylation (Zhang *et al*. 2010). Methylation-sensitive restriction enzymes used in this study (rare cutter, *PstI*) were effective in enriching genomic DNA for gene-containing regions and reducing genomic clones with repeat

elements (Pootakham *et al*., 2016; Fellers, 2008). Weedy amaranth including *A. thunbergii* and *A. graecizans* possessed more unique SNPs per accession than grain amaranth *A. hypochondriacus* and *A. cruentus*. This finding is reasonable because both species have had far less selection pressure than the cultivated species, which is useful from a breeding perspective, to identify species in amaranth germplasm.

The stringent SNP filtration steps were important in order to get high quality and polymorphism of SNP data to be conducted on the primary interest of the study, the diversity of large number of *A. tricolor* accessions and its phylogeny relationship with other species. GBS data have a high proportion of missing values (Stetter *et al*., 2017) and the number of SNPs retained for the analysis depends on the quality control method (Marees *et al*., 2018). In this study, for a large 16 species amaranth collections, the number of SNPs retained after removal of missing data >50% (AV-ATR, AV-BLITO, AV-SPI 1, AV-SPI 5 and AV-SPI 6), filtering through imputations with MAF>5% was still high with a total of 3,898 SNPs remained for 183 accessions of 16 species. Meanwhile, for 118 *A. tricolor* accessions, the number of SNPs remained after removal of two accessions with missing data >70% (AV-TRI 20 and AV-TRI 28) and MAF>5% was also high with 4,637 SNPs. The range of SNP markers used to evaluate population structure of amaranth collection in this study showed the confidence of DArTseq as a technique for full genome coverage by the new markers. In a study of DArTSeq-based population structure conducted in 67 wild Galapagos tomato accessions (*Solanum cheesmaniae* and *S. galapagense*), 3,974 SNPs used were successfully differentiate the tomato species based on geographical origin (Pailles *et al*., 2017). Besides, 3,974 DArTseq SNPs use to conduct genetic diversity in 80 macadamia accessions (*Macadamia integrifolia*, *M. tetraphylla* and hybrids) were successfully evidenced the historical background and pedigree relationships of the cultivars (Alam *et al*., 2018).

4.4.2 Population structure of 16 amaranth species and 118 *A. tricolor* subset

Population structure analysis on 16 amaranth accessions generates consistent taxonomic classification of amaranth sub-genera which was previously defined using seeds, inflorescence and floral characteristics (Achigan-Dako *et al*., 2014; Das, 2012). Three amaranth sub-genera *Amaranthus Amaranthus*, *Amaranthus Acnida* and *Amaranthus albersia* were well defined in this study, consistent with

other GBS findings by Stetter and Schmid (2017). Subgenus *Amaranthus*, comprised of grain amaranth (*A. hypochondriacus* and *A. cruentus*) and its weed progenitor (*A. hybridus*) were distinguished in sub-pop B. Subgenus *Albersia*, which comprised of vegetable amaranth including *A. tricolor* were distinguished in sub-pop A, together with six out of seven *A. blitum* accessions, all three *A. graecizans* accessions and four of six *A. viridis* accessions. Meanwhile, species belonging to subgenus *Acnida*, which comprised of weedy amaranth, *A. spinosus* and *A. palmeri* were diverse between the two main sub-pops A and B.

Weedy amaranth, *A. spinosus* is a cross-pollinated and subsequent gene flow between populations may occur more rapidly than the primarily self-pollinated amaranth species (Stetter *et al*., 2016). Lee *et al*., (2008) also have stated that varying amounts of outcrossing and frequent interspecific and inter-varietal hybridization have occurred in amaranth accessions even though it is self-pollinated. Therefore, this could explain the admixture between amaranth species. Another important finding was *A. hybridus* that belonged to sub-genenus *Amaranthus* was split into sub-genus *Albersia*. *A. hybridus* is the direct ancestor of cultivated grain amaranth species (Stetter *et al*., 2016; Kietlinski *et al*., 2014; Park *et al*., 2014,), and the split of accessions identity could be due to inter-varietal hybridization.

In this study, genetic differentiation through DArTseq showed a clear demarcation between grain and vegetable amaranth, which has also been observed in many molecular markers studies, including AFLP (Costea *et al*., 2006), SSR (Oo and Park, 2013; Khaing *et al*., 2014; Suresh *et al*., 2014) and GBS (Stetter and Schimdt, 2017), although those studies incorporated far fewer *A. tricolor* accessions. The population structure of both analyses showed similar patterns in differentiating *A. tricolor* accessions, except that the two out-group accessions (AV-TRI 20 and AV-TRI 28) were closely related with other species (subgenus *Amaranthus*). The occurrence of admixed/hybrid genotypes indicated frequent hybridization or introgression events. An experiment based on SSR markers by Khaing *et al*., (2014) revealed that *A. tricolor* scattered to different groups which may imply that *A. tricolor* had large genetic variations. There was also uncertainty in positioning phylogeny of *A. tricolor* accessions among amaranth species, although *A. tricolor* accessions were grouped together in a clade (Stetter and Schmid, 2017). *A. tricolor* had significantly larger estimated genome size among

35 amaranth species, and this suggests that polyploidization likely influenced the genome size of this species (782.7Mbp) (Stetter and Schmid, 2017).

In this study, the species grouping were independent of the accessions geographical origin, contradicting previous GBS findings (Stetter and Schmidt, 2017; Stetter *et al*., 2017; Wu and Blair, 2017). In previous studies, geographical patterns demonstrate that comprehensive origin sampling can assist in understanding the evolution of the species as shown by a strong split of geographic pattern in *A. hybridus* between accessions from Central and South America, which later supports the hypothesis that two different lineage were the ancestors of the grain amaranth (Stetter and Schimdt, 2017). In this study, the genetic differentiation between species and geographical origin was weak, although a strong split of geographical pattern was observed in *A. hybridus* where accessions from America and Africa were divided into two clusters, which may explain the genetic differentiation of hybridus complex (Khaing *et al*., 2013). This is probably due to the cosmopolitan nature of the genus, or the results of human activities such as breeding and resources exchange (Lee *et al*., 2008). Besides, the construction of SNPs library in this study was different from other studies using the GBS method, i.e. complexity reduction method with non-reference based assembly. The combination of methylation-sensitive (rare cutter, *PstI*) and methylation-insensitive enzyme (common cutter, *MseI*) used in this study targeted genome fractions with coding regions, separate low copy sequences from the repetitive regions of the genome (Cruz *et al*., 2013; Tinker *et al*., 2009). This result differ from Wu and Blair (2017) which uses only *APeKI* methylation-sensitive restriction enzyme with reference based assembly method, and able to separate grain amaranths based on geographical origin. However, for a large set of 118 *A. tricolor* accessions, genetic differentiation of Bangladeshi accessions can be distinguished clearly as it clustered together and had distinct morphological characters.

4.4.3 Genome-wide association study

The closely related *A. hypochondriacus* genome was used as the genome reference for association mapping as no *A. tricolor* genome is available to date. The assembled genome is not completely sequenced in which the final assembly span 403.9 Mb (estimated genome size 466Mb), but highly contiguous with contig and scaffold N50 of 1.25 and 24.4 Mb, respectively. Notably, 98% of the assembly

length was scaffolded into 16 chromosomes, representing the haploid chromosome number of the species. The remaining 892 scaffolds are small, representing approximately 2% of the total sequence length (Lightfoot *et al*., 2017). The utilities of the reference quality genome were demonstrated in two ways. First, the study of chromosomal evolution by comparing the amaranth genome to the beet genome enables researchers to better understand amaranth in the context of how plants evolved. Second, the mapping of genetic locus responsible for stem color was able to clarify the scientific understanding of a useful agricultural trait (Lightfoot *et al*., 2017).

The highly significant MTA's found in five morphological traits in this study showed an example on how the features of this DArTseq data can provide a high resolution of mapping opportunities. However, the most significant associations detected in the MLM model had a lower threshold (−log(p−value)<4, except for terminal inflorescence shape, and although the mixed model was superior, but it still leads to at least one false negative and false positive. This could be due to the used of different amaranth species (*A. hypochondriacus*) as reference genome instead of *A. tricolor* genome. This is because, the difficulty of working with plant genomes is that they are highly repetitive and feature excessive structural variation between members of the same species, mostly attributed to their active transposons (Bennetzen, 2000). For example, in the well-studied species *Arabidopsis thaliana*, natural accessions are missing 15% of the reference genome, indicating a similar fraction would be absent from the reference, but present in other accessions (1001 Genomes Consortium, 2016). Moreover, although *A. thaliana* has a small (140 Mb) and not very repetitive genome compared to many other plants, SNPs may be assigned to incorrect positions due to sequence similarity shared between unlinked loci (Long *et al*., 2013). Therefore, more excessive structural variation are expected in a larger *A. tricolor* genome (782.7Mbp) with highly repetitive, and have undergone ancient and recent rounds of polyploidization (Stetter and Schmid, 2017).

4.5 Conclusion

The findings in this study showed that the DArTseq-SNP data generated from 183 amaranth mini core collections comprised of 16 species was capable of differentiating vegetable amaranth, *A. tricolor* from grain amaranth. The species

grouping were independent of accessions' geographical origin. This could be due to the germplasm being registered as where the seeds were donated from which may not be the actual origin of the accession. For a larger *A. tricolor* data set, there was likelihood that a good speciation of *A. tricolor* could be achieved based on combined analysis of molecular marker, geographical origin and morphological traits. GWAS used to conduct a pilot genome association for 10 morphological traits demonstrates the effectiveness of the amaranth diversity panel for trait dissection.

CHAPTER 5

DEVELOPMENT OF SURROGATE SCREENING TECHNIQUES FOR DROUGHT TOLERANCE TRAITS IN VEGETABLE AMARANTH

5.1 Introduction

Plants have various mechanisms to withstand drought stress, and their different morphological and physiological strategies for avoiding drought stress are extensively reviewed in Lamaoui *et al*., (2018), Fang and Xiong (2015), Chatterjee and Solankey (2015), and Kumar *et al*., (2012). In the past two decades, remarkable progress in trait phenotyping for drought tolerance, and the integration of genomic platforms has accelerated the selection of drought resistance cultivars in major crops (Tuberosa *et al*., 2014) such as maize (Cooper *et al*., 2014) and rice (Kumar *et al*., 2014).

For a successful integrated breeding in drought resistant crops, large set of informative drought phenotypic data is needed (Cooper *et al*., 2014). However, for orphan/underutilised leafy vegetables, basic knowledge related to morphological and physiological traits for drought tolerance is still lacking (Sogbohossou *et al*., 2018). Understanding the genetic phenotypic differences in vegetable amaranth in response to water deficit is crucial if new breeds and cultivars are to be developed. It is worth exploring multiple factors that are involved in drought stress before establishing a reliable screening method for the large-scale selection, or for breeding stock. This is due to the requirement for large amounts of space, time-consuming, and expensive and inadequate seed availability of certain genotypes in early generations (Hura *et al*., 2007a).

The ability to develop effective and reliable screening methods for drought tolerance in vegetable amaranth is an important step towards harnessing the potential of amaranth as a future crop for food supply. The key criteria for the development of rapid screening methods is that the technique used must be capable of evaluating plant performance at critical stages of development, use a small amount of plant material and be able to screen large number of plant varieties as short time as possible (Johnson and Asay, 1993). The screening methods should fulfil important requirements for drought tolerance in individual crop plants which

can then be incorporated in breeding programmes to facilitate significant genetic improvement.

Nevertheless, to date, there is no proper rapid screening for drought tolerance, specifically for leafy vegetable amaranth (*A. tricolor*) and very few studies have been carried out on grain amaranth and only fewer *A. tricolor* genotypes to manipulate the growth of the plants upon drought stress (Sarker & Oba, 2018; Tsutsumi *et al*., 2017; Jomo *et al*., 2016; Babayev et al., 2014; Luoh *et al*., 2014; Slabbert and Kruger, 2011, 2014; Hura *et al*., 2007b; Liu and Stützel , 2002a & b, 2004).

Therefore, the present study provides a framework for the development of rapid and effective screening methods for drought tolerance traits in vegetable amaranth. This study also characterized variation in growth and physiological response of amaranth accessions to drought stress. This serves as a pilot study to identify surrogate traits associated with drought tolerance in amaranth, exploiting a small number of plant materials which could then be scaled up to a larger trial.

Two experiments were conducted separately, to evaluate the effect of drought on shoot and root traits;

(i) Experiment I: Transpiration efficiency of vegetable amaranth (*Amaranthus sp.)* in response to terminal drought stress

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*******The version included in the thesis has been slightly modified to ensure consistency of style and usage with other chapters)*

(ii) Experiment II: Variation in growth, root morphology and plant physiology of vegetable amaranth (*Amaranthus tricolor*) in response to gradual drought stress.

5.2 General materials and methods

This section describes the general materials and methods used for the drought screening experiments, including studies in this chapter (Experiment I and Experiment II) and Chapter 6 (Trial I and Trial II).

5.2.1 Experimental site, soil preparation and seed germination

Plants were grown under shade-house conditions at The University of Nottingham Malaysia Malaysia (latitude 2.940°N, longitude 101.8740°E) with an average daytime temperature of 36°C and average night-time temperature of 28°C, and average daily relative humidity of 66% (HOBO ® U30 Weather Station, MA, USA). As not all soil types perform satisfactorily and consistently in pot experiments, the soil used was allowed to fully dry for 10 days to remove excessive soil moisture before potting. The soil was then sieved (0.5 cm x 1 cm) to eliminate large aggregates (Liu and Stützel, 2002a) in order to obtain a uniform soil bulk density. Seedlings were sown in 14 x 10 cell trays (54 cm x 36 cm) and several seeds were planted in each cell. The seedlings were thinned to one plant per cell after the appearance of the first true leaf. 14 days after sowing, seedlings at the 3rd to 4th leaf stage were transplanted into plastic pots with one plant per pot and 5g of fertilizer (15N:15P:15K) was applied once during establishment period, about five days after transplanting. A sample of the soil used was sent to Applied Agricultural Resources Sdn. Bhd. (AAR, Malaysia) for analysis. The minimum and maximum temperature, humidity and photosynthetic active radiation (PAR) during experimental period were recorded from 7 a.m. to 7 p.m. using data logger (HOBO ® U30 Weather Station, MA, USA).

5.2.2 Determination of leaf chlorophyll content

5.2.2.1 Chlorophyll extraction

Chlorophyll was determined non-destructively using an SPAD-502 meter (Konica-Minolta, Japan). To validate the SPAD meter, chlorophyll concentration was determined destructively for 17 amaranth accessions, comprising of two amaranth species; *A. tricolor* and *A. cruentus* (Appendix 3.1c), using methods previously described by Bruinsma (1963). Chlorophyll content was determined destructively on a 2 cm^2 leaf section, enabling chlorophyll content to be expressed in relation to leaf area.

The leaf samples were ground to a fine powder with liquid nitrogen using a micro-centrifuge tube grinder. 2 ml of 80% acetone solution was added to the leaf samples and mixed thoroughly before incubation for 1 hour at 4°C. The leaf samples were then centrifuged at 13,000 rpm for 5 minutes and 1 ml of the extract supernatant was evaluated at an absorbance of 663.6 nm (A663.6) and 646.6 nm

(A646.6) using a UV-VIS Spectrophotometer (Perkin-Elmer, Lambda 5, Massachusetts, USA). Prior to carrying out solvent extraction the SPAD chlorophyll content was recoded on the exact same section of leaf material. Three leaf samples were taken per accession using different parts of leaves consisted of young, matured and old leaf.

Leaf chlorophyll content (µg cm^{-2}) was expressed on a leaf area basis and from this chlorophyll a and b was also derived, as according to the equations made of Porra *et al*., (1989):

Total chlorophyll (µg ml^{-1}) = [Chlorophyll content of the extract (µg ml^{-1}) x Volume of acetone used for extraction (ml)] / Leaf area from which chlorophyll was extracted (cm^2)

Chlorophyll a (µg ml^{-1}) = 12.21 (A663) - 2.81 (A646)

Chlorophyll b (µg ml^{-1}) = 20.13 (A646) - 5.03 (A663)

Total chlorophyll (a+b, µg ml^{-1}) = 17.32 (A646) + 7.18 (A663)

5.2.2.2 Linear function between chlorophyll extracts with SPAD values

Linear functions between chlorophyll content and SPAD values were computed as presented in (Appendix 5.1) and used as a reference to calculate leaf chlorophyll content for subsequent growth measurements. The linear functions calculated were as follows:

Chlorophyll a (µg ml^{-1}): $y = 1.117x + 7.3784$

Chlorophyll b (µg ml^{-1}): $y = 1.1124x + 15.879$

Total chlorophyll (a + b, µg ml^{-1}): $y = 0.6503x + 8.6045$

5.2.3 Growth measurements

Plants were destructively harvested and separated into leaves, stem and roots, and fresh weight (FW) was recorded. Total leaf area (TLA) was measured using a LI-3100 Area Meter (LICOR, Lincoln, Nebraska, USA) at the time of final harvest. Dry weights (DW) of each biomass partitioning were determined after drying at 80°C in an oven for 72 hours.

Yield was calculated as follows:

Yield (g) = Leaf fresh weight (g) + stem fresh weight (g)

Root to shoot ratio (R/S) was calculated as follows:
R/S = Root dry weight (g) / (Leaf + stem dry weight (g)

Specific leaf area (SLA) was then calculated using the following formula:
SLA (cm^2g^{-1}) = Leaf area (cm^2) / Leaf dry weight (g)

5.2.4 Physiological responses

All the measurements were taken at the 3rd fully expanded leaves. Due to natural leaf senescence, it was not possible to take measurement on the same leaf in every occasion, particularly in the final reading of drought stress treatment. For each physiological assessment, the number of reading for an individual plant (technical replicates) was first determined on several leaves at different leaf positions. The final replicates were obtained as the interactions between readings, leaf number and plants were not significant.

5.2.4.1 Total chlorophyll content (TCC)

TCC was measured using a portable Minolta Chlorophyll Meter SPAD-502 (Konica Minolta, Langenhagen, Germany). Readings were taken on the 3rd most fully expanded leaflet, avoiding the midrib section. Three readings were taken per leaf and averaged to give a final reading.

5.2.4.2 Relative water content (RWC)

The 3rd fully expanded leaf was cut into 2 cm x 2 cm sections for each plant and FW was recorded. The leaf sample was then immersed in distilled water for 24 hours in the dark at room temperature to reach saturation. After 24 hours, leaf sample was immediately dry blotted with tissue paper and weighed to obtain the turgid weight (TW). The leaf sample was then oven dried for 24 hours at 80°C and weighed to determine the dry weight (DW).
Relative water content was calculated using the following equation:
RWC (%) = [(FW-DW) / (TW-DW)] x 100
where FW is fresh weight, DW is dry weight and TW is turgidity weight.

5.2.4.3 Photosynthetic gas exchange measurement

The photosynthetic gas exchange of mature leaves was measured with a portable photosynthetic system (LI-6400, LI-COR, Inc., Logan, NE, USA) coupled with a standard red/blue LED broadleaf cuvette (6400-02B, LI-COR, Inc., Logan, NE, USA) and a CO_2 mixer (6400-01, LI-COR, Inc., Logan, NE, USA). The leaf chamber was set to 400 µmol mol^{-1} CO2 concentration, 1500 µmol photon $m^{-2}s^{-1}$ photosynthetically active radiation (PAR), 35°C leaf block temperature, 400 µmol mol^{-1} flow rate and 50-70% relative humidity in the sample to keep the vapour pressure deficit (VPD) in the leaf chamber at approximately 1-1.5 kPa. The gas exchange measurements were taken when a steady state (around 2 to 4 minutes) was obtained on a 2 x 3 cm leaf area and maximum net photosynthetic rate (Pn, µmol CO_2 $m^{-2}s^{-1}$).

Stomatal conductance (Gs, mol H_2O $m^{-2}s^{-1}$), intercellular CO_2 concentration (Ci, µmol CO_2 mol^{-1}), and transpiration rate (E, mmol H_2O $m^{-2}s^{-1}$) were recorded. The measurements obtained were used to calculate instantaneous water use efficiency (WUE) (Wang *et al*., 2016), intrinsic water use efficiency (WUEi) and stomatal limitation value (Ls) (Yin *et al*., 2006):

The equations are as follows:

WUE (µmol mol^{-1}) = Pn/E

WUEi (µmol $mmol^{-1}$) = Pn/Gs.

Stomatal limitation = 1 - Ci/Ca

5.3 Experiment I: Transpiration efficiency of vegetable amaranth in response to terminal drought stress.

5.3.1 Introduction

Genetic phenotypic variation for drought tolerance has been exploited successfully in major crop species such as rice (Zhang *et al*., 2006), maize (Bänziger *et al*., 2004), peanut (Reddy) and wheat (Valkoun, 2001) and to produce cultivars with improved yield under drought stress. Amaranth shows considerable genetic variability and plasticity for drought tolerance (Slabbert and den Heever, 2007) with a high level of intra species variation compared to staple crops (Erum *et al*., 2012; Shukla *et al*., 2010). The crop displays drought-tolerance mechanisms, such as osmotic adjustment (Liu and Stützel, 2002a) and high root to shoot ratio (Liu

and Stützel, 2004) which could be exploited given the high level of genetic variability that exists between and within the species in the genus *Amaranthus*. Understanding the genetic phenotypic differences in amaranth responses to water deficit is crucial for developing new water-use efficient cultivars. A deeper understanding of the different mechanisms of drought tolerance is also required.

Transpiration efficiency has been shown to constitute a large source of yield variation in crops subjected to water deficit (Ratnakumar *et al*., 2009) and has been recognized as a key component of yield variation under drought stress in many crops including, banana (*Musa* spp.) (Kissel *et al*., 2015), grain sorghum (*Sorghum bicolor* (L.) Moench) (Thevar *et al*., 2010), peanut (*Arachis hypogaea* L.) (Krishnamurthy *et al*., 2007) and bean (Ehleringer *et al*., 1991). Yield and biomass have been shown to be positively correlated with high water-use efficiency (WUE) in wheat (Ehdaie *et al*., 1991) and breeding for improved WUE has produced improved drought-tolerant genotypes (Condon *et al*., 2002).

Relatively little data is available on the WUE and mechanisms of drought tolerance in amaranth. However, Liu and Stützel (2004) showed that, in a temperature-controlled greenhouse experiment, WUE in vegetable amaranth was unaffected by drought stress. There is little information available on suitable surrogate traits for drought-tolerance selection in amaranth and therefore a detailed investigation into the water relations of vegetable amaranth in response to water deficit is needed to understand mechanisms of drought tolerance in this C4 plant. In this study, we sought to fill this gap and determine if genetic variation for transpiration efficiency under conditions of drought stress existed in nine vegetable amaranthaccessions.

5.3.2 Materials and methods

5.3.2.1 Plant materials

The plant material consisted of nine accessions of vegetable amaranth; which included three Tanzanian landraces of *Amaranthus cruentus* (B1: Black-seeded amaranth, B2: White-seeded amaranth and B3: Mixed-seeded amaranth). Six accessions belonged to *Amaranthus tricolor*, of which three were local Malaysian red-leafy vegetable varieties (C1: Amaranth perfect red (var. BBS014), C2: Red amaranth and C3: Red amaranth (var. BBS027)), and three were local Malaysian green-leafy vegetable varieties (D1: Dark green pointed leaf (var. Bamboo Dance

008), D2: Green Special Round Leaf (var. 388) and D3: Green amaranth) (Figure 5.1). The morphological characteristics of these accessions are described in Appendix 3.1c.

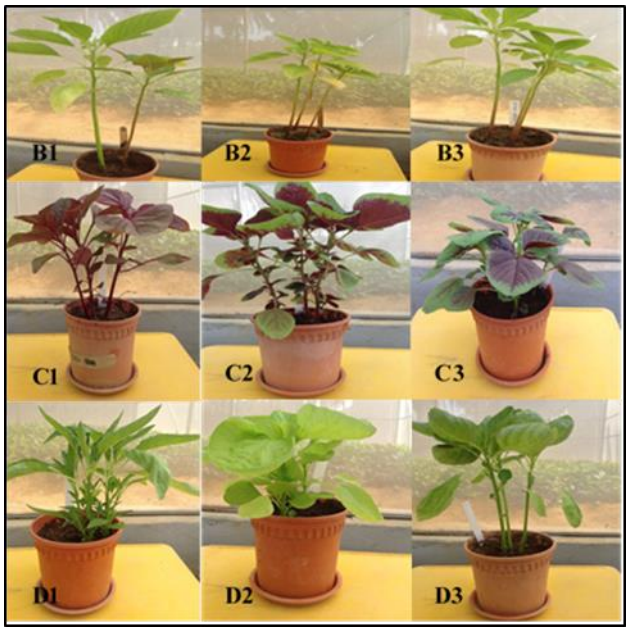


Figure 5.1: Nine amaranth accessions used in this experiment; A. cruentus (BI, B2 and B3) and A. tricolor (CI, C2, C3, D1, D2 and D3).

5.3.2.2 Experimental design

Two treatments were imposed at the vegetative growth stage (26 days after emergence): drought stress (water-deficient, WD) and well-watered control (water-sufficient, WS). The pot size used in this experiment was 16 cm x 12.5 cm x 14.5 cm in size with weight of 2 kg black peat moss mix (Holland Brand, Malaysia). The experimental design was split plot in a randomized complete block design with one initial set (T0) and two water treatments (WS and WD) as main plot, and nine amaranth accessions as sub-plot with four replications. Prior to the onset of the drought treatment, plants were irrigated daily to field capacity. On the first day of transpiration efficiency assessment, the T0 plants were destructively harvested to estimate above-ground dry weight; this date was designated as time 0.

The remaining plants were watered to maximum soil water holding capacity (WHC) and allowed to drain freely for 24 hours. After 24 hours, the pots were sealed with a plastic bag and covered to prevent water loss, except by transpiration (Ray and Sinclair, 1998) (Figure 5.2). Pots were then weighed and initial weight was recorded. Subsequently, pots were weighed every 72 hours. After each

weighing, water was added back to the WS plants to return them to their maximum WHC. For the WD treatment, no further water was added to induce drought. At the time of final harvest, plants were destructively harvested and root, shoot and leaf dry weights were obtained. The watering plan for this experiment is presented in Table 5.1.

Figure 5.2: The saturation pots were sealed with plastic bag after allowed to drain freely for 24 hours to prevent soil evaporation.

Table 5.1: Watering plan of water-sufficient (WS) and water-deficient (WD) plants for transpiration efficiency assessment.

	Seedling Period	**Transplanting**	**Establishment Period**	**Water Treatment Period**	**Harvest**
Days after sowing	Day 0 – Day 13	Day 14	Day 15 - Day 25	Day 26- Day 41	Day 41
WS	Water everyday			Water every 72 hours	
WD	Water everyday			Stop water until the end	
Initial set (T0)	Water everyday				Day 32

5.3.2.3 Growth measurement

5.3.2.3.1 Fraction of trasnspirable soil water (FTSW)

Soil water status in the individual pots was expressed as FTSW status. The daily value of FTSW was estimated as the ratio between the amount of transpirable soil water remaining in the pot and total transpirable soil water.

Daily FTSW was calculated based on Ray and Sinclair (1998) as follows:

FTSW = (Daily pot weight - Final pot weight) / (Initial pot weight - Final pot weight)

Two normalizations were carried out to minimize daily variations in transpiration, according to Devi *et al*., (2009). Briefly, the daily transpiration of the WD plants was divided by the average daily transpiration of the WS plants. All values were standardized against the mean values of the first three days when the plants were still under controlled conditions to produce a normalized transpiration rate (NTR). The experiment continued until the normalized transpiration rate fell below 0.1. The FTSW threshold at which NTR began to decline was calculated using a plateau regression procedure according to the methods of Ray and Sinclair (1998).

5.3.2.3.2 Transpiration efficiency (TE)

TE was calculated for each plant using the following equation:

TE (gk^{-1}) = [Mean shoot biomass at time 0 - Mean shoot biomass at time of harvest] / [(Initial pot weight - Weight of the pot at harvest) + Water added back to the pot].

5.3.2.3.3 Days to wilting (DTW)

Days to wilting (DTW) were recorded as days after initiation of the drought-stress treatment and wilting was recorded pre-dawn.

5.3.2.3.4 TCC

TCC was measured at 2, 8 and 14 days after the imposition of drought treatment (DAT) (*see subheading 5.2.4.1*). Leaf chlorophyll content was calculated using linear equation produced in *subheading 5.2.2.2.*

5.3.2.3.5 Yield, TLA, SLA and R/S ratio

Once the normalized transpiration rate fell below 0.1, plants were destructively harvested and separated into leaves, stem and roots, and FW, DW, TLA were measured (*see subheading 5.2.3*). The SLA and R/S ratio were obtained using formula stated in *subheading 5.2.3.*

5.3.2.3.6 Stress susceptibility index (SSI)

To evaluate drought tolerance of the amaranth accessions, stress susceptibility index (SSI) of shoot traits was determined as the difference between the results obtained under WD and WS conditions.

The SSI was calculated according to Fischer and Maurer (1978) using the following equation:

SSI = [1- (Ypi / Ysi)] / Stress intensity

Stress intensity = 1- (Ys/Yp)

where Ypi is the mean value for the investigated trait under WS conditions, Ysi is the mean trait value under WD conditions, Ys is the mean trait value of all accessions under WD conditions, and Yp is the mean trait value of all accessions under WS conditions.

5.3.2.4 Data analysis

The effect of water treatments and accessions was analysed using Genstat for Windows 16th edition (VSN International 2011). The data were subjected to analysis of variance (ANOVA) with a split plot design. Mean separation among accessions was carried out using Tukey's pairwise comparison and significant differences were identified with letters. Prior to the analysis, the assumption of normally distributed residuals for ANOVA was assessed using Shapiro-Wilk test, whereas the assumption of homogeneity of variance was assessed using Levene's test. The FTSW threshold at which NTR began to decline was calculated using a plateau regression procedure according to the methods of Ray and Sinclair (1998).

5.3.3 Results

5.3.3.1 Influence of drought stress on growth and physiology

There were significant differences between the amaranth accessions for leaf and stem fresh and dry weights under both WS and WD treatments ($P<0.05$) (Table 5.2). The individual ANOVA analysis with a split plot design for all parameters is presented in Appendix 5.2. B2 and B3 had the highest leaf fresh weight in both WS (25.01, 26.80 g respectively) and WD treatments (3.69, 4.12 g respectively). These two accessions also recorded the highest percentage loss in fresh weight under WD treatments for all accessions. In comparison, C3 had the lowest reduction in leaf fresh weight under WD treatment (2.62 g) compared with the WS treatment (8.6 g)

(Table 5.2). There was a significant difference in fresh weight of leaf, stem and root partitioning of individual accessions in WS and WD treatments. For example, the fresh weight of C3 was primarily partitioned into stem (20.28 g), followed by root (15.22 g) and leaf (8.61 g), under WS treatment, and primarily partitioned into roots (3.30 g), followed by leaf (2.62 g) and stem (2.23 g) under the WD treatment.

The root to shoot (R/S) ratio did not change significantly with the WD treatment compared with the WS treatment (P=0.256) (Table 5.2). Accession under the WS treatment did not differ significantly with respect to R/S ratio, whereas there was significant difference recorded among accessions under the WD treatment (P<0.05), with D2 recording the highest R/S ratio (0.80) and B1 the lowest (0.36).

Total leaf area of WD plants was reduced by two-thirds compared with the WS plants (Table 5.3) (P<0.001) (Table 5.3). The highest reduction in TLA was in D2 with an 85% reduction (611.35 cm^2 in WS to 76.90 cm^2 in the WD treatment), whilst the lowest was C3 with a 58% reduction (403.53 cm^2 in WS to 168.43 cm^2 in the WD treatment). The reduction in SLA in WD plants was approximately 50% of the SLA of WS plants (P=0.003), with the exception of D3, which was not significantly reduced under the WD treatment relative to the WS treatment.

Accessions did not differ significantly with respect to days to pre-dawn wilting (ranging from 6 to10 days) (Figure 5.3). The WD plants started to wilt at 6 DAT when the portion of remaining volumetric soil water available for transpiration dropped to 40% compared with WS plants as shown in FTSW (Figure 5.4). The FTSW reached zero transpiration at 14 days after imposition of drought treatment for all accessions.

Total chlorophyll content did not differ significantly between amaranth accessions under either WS or WD treatments at 2, 8 and 14 DAT (Table 5.4). However, the total chlorophyll content was reduced significantly (P<0.001) between 2 DAT and 14 DAT for both treatments. Chlorophyll-a content was higher than chlorophyll-b content in both WS and WD treatments at 2, 8 and 14 DAT. Under severe water deficit conditions (14 DAT), significant differences existed among accessions for chlorophyll-b content, with B3 having the highest (13.85 μgcm^{-2}) and C1 the lowest (4.14 μgcm^{-2}).

The SSI varied significantly among accessions, with the most drought-tolerant accession, C3, recording the lowest SSI (0.83) (P<0.001), and the most

drought susceptible accession, D2, recording the highest SSI value (1.10) ($P<0.001$) (Figure 5.5).

5.3.3.2 Variation in TE in response to soil-water deficit

The total water transpired was significantly reduced under WD conditions compared with WS conditions in all nine accessions ($P<0.001$) (Table 5.5). However, there were no differences among accessions for total water transpired under either treatment. The TE increased significantly for all accessions in the WD treatment relative to the WS treatment ($P<0.001$) with the exception of D3 where the TE was similar under both water treatments. There were no significant differences among accessions with respect to final weight of soil water available for transpiration in pots at the end of WD treatment as FTSW reached zero with a range of 0.48-0.53 kg. The relationship between NTR and FTSW for each amaranth accession is shown in Figure 5.6. The accessions showed the same overall pattern for soil drying and there was no significant difference in the FTSW threshold of the NTR decline (Table 5.5).

5.3.3.3 Correlations

Correlation coefficients for all traits measured for WS and WD treatments are shown in Tables 5.6. Under WS treatments, TE was positively correlated with leaf fresh weight ($r=0.801$, $P<0.05$), root dry weight ($r=0.709$, $P<0.001$) and total yield ($r=0.89$, $P<0.001$), and negatively correlated with R/S ($r=-0.488$, $P<0.001$). Under WD treatments, TE was positively correlated with leaf fresh weight ($r=0.536$, $P<0.001$), leaf dry weight ($r=0.841$, $P<0.001$), stem fresh weight ($r=0.549$, $P<0.001$), stem dry weight ($r=0.790$, $P<0.05$) and root dry weight ($r=0.661$, $P<0.001$), and negatively correlated with R/S ($r=-0.46$, $P<0.05$), SLA ($r=-0.668$, $P<0.001$) and days to wilting ($r=-0.525$, $P<0.001$).

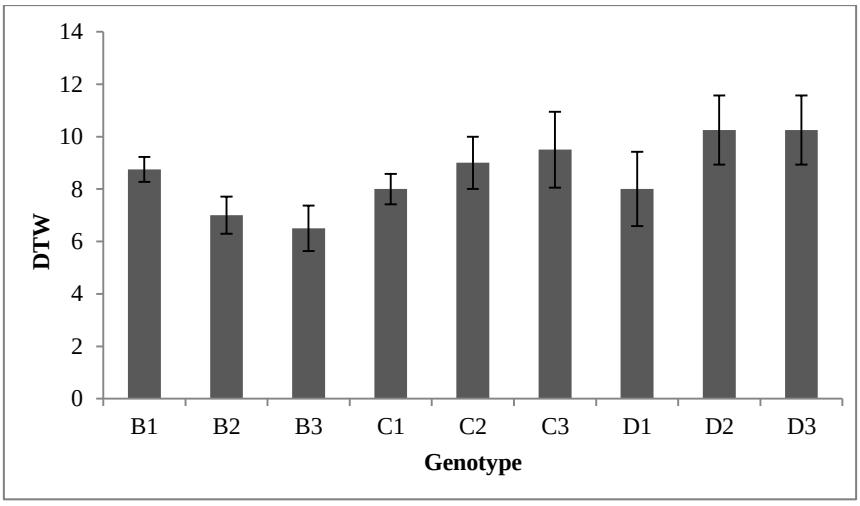


Figure 5.3: Days to pre-dawn wilting (DTW) for nine amaranth accessions in water-deficient conditions (WD). The error bars indicate ± standard error of mean (SE) with n=6.

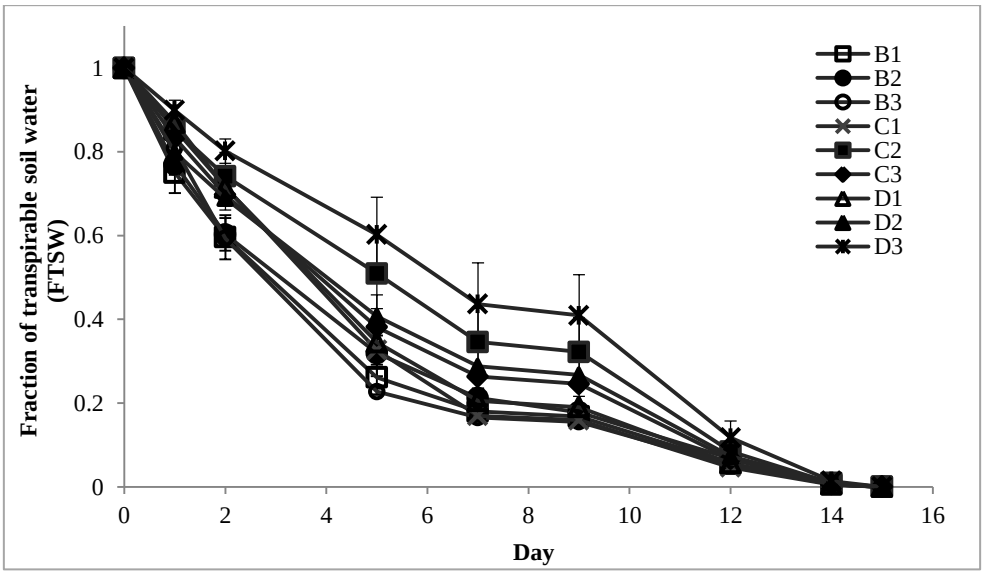


Figure 5.4: Fraction of transpirable soil water (FTSW) reached zero in water-deficient plants (WD) indicating no soil water was available for transpiration after 14 days imposition of drought stress. The error bars indicate ± standard error of the mean with n=6 (SE).

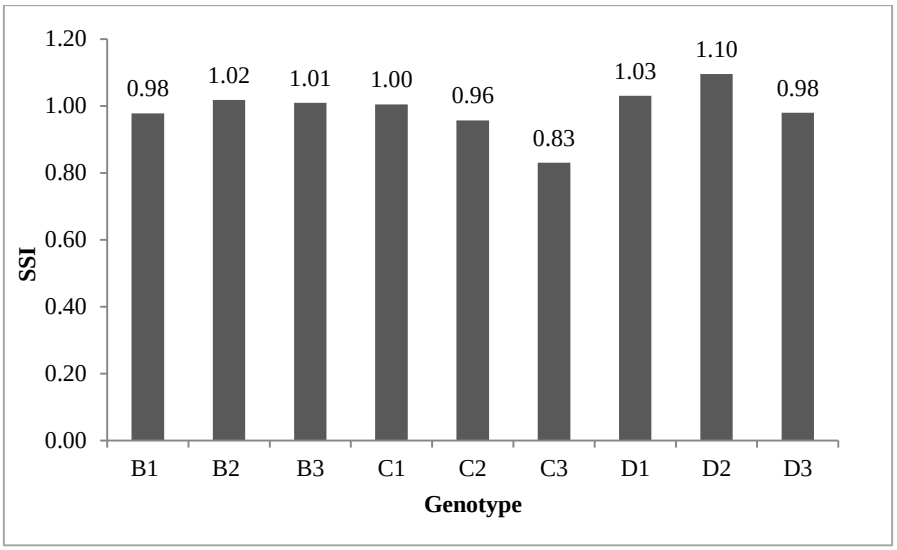


Figure 5.5: Stress susceptibility index (SSI) for yield under drought for nine amaranth accessions. An SSI>1 above-average susceptibility to drought stress.

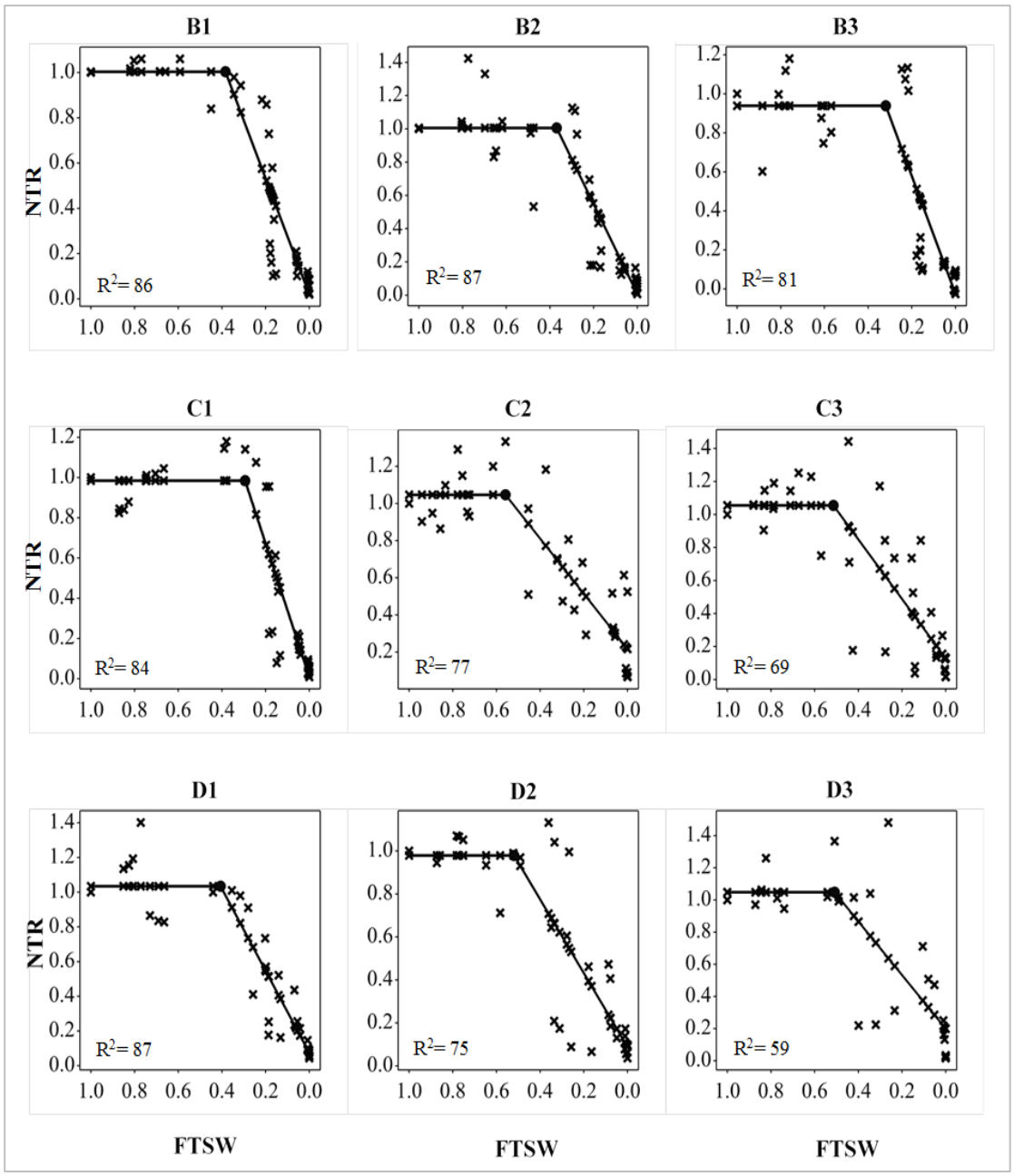


Figure 5.6: A plateau regression to show the relationship between the normalized transpiration rate (NTR) and the fraction of transpirable soil water (FTSW) of nine amaranth accessions. The FTSW threshold is indicated by the breakpoint of the plateau where transpiration starts to decline. R^2 indicates the coefficient of determination between NTR and FTSW.

Table 5.2: Mean of fresh weight (FW) (g) and dry weight (DW) (g) of leaf, root and stem, and root to shoot (R/S) ratio of nine accessions of amaranth under water-sufficient (WS) and water-deficient (WD) conditions, respectively with ± standard error of means (SE).

Accession	LFW (g)		LDW (g)		RFW (g)		RDW (g)		SFW (g)		SDW (g)		R/S (g)	
	WS	WD	WS	WD	WS	WD	WS	WD	WS	WD	WS	WD	WS	WD
B1	18.09±1.04ab	3.26±0.27ab	3.87±0.15abc	2.79±0.29a	18.14±2.75a	4.45±1.57a	3.67±0.09ab	2.35±0.27ab	31.15±5.99ab	6.41±0.85a	6.72±1.52a	3.95±0.50a	0.37±0.06a	0.36±0.05c
B2	25.10±1.84a	3.69±0.31ab	4.78±0.45ab	2.91±0.22a	19.59±3.91a	4.51±0.35a	4.03±0.57a	2.37±0.29ab	32.79±2.44a	5.36±0.64ab	6.73±0.44a	2.98±0.51ab	0.35±0.03a	0.40±0.04bc
B3	26.80±1.16a	4.12±0.26a	5.47±0.38a	3.35±0.27a	20.31±2.42a	4.00±0.40a	4.20±0.16a	2.86±0.33a	27.71±3.61ab	4.30±0.48abc	6.26±0.19ab	3.00±0.16ab	0.36±0.02a	0.46±0.08bc
C1	19.39±1.76ab	3.06±0.38ab	3.95±0.38abc	2.57±0.33ab	17.93±2.11a	3.56±0.44a	2.86±0.32ab	1.54±0.15b	23.14±1.79ab	3.54±0.49bc	3.19±0.55bc	1.55±0.30bc	0.40±0.01a	0.38±0.03bc
C2	18.24±3.23ab	3.61±0.35ab	3.74±0.70abc	2.52±0.47abc	19.14±4.40a	3.41±1.12a	2.72±0.47ab	1.56±0.43b	18.27±3.61ab	2.69±0.76c	2.35±0.68c	1.33±0.36c	0.49±0.09a	0.39±0.04bc
C3	8.61±2.04b	2.62±0.28ab	1.94±0.18c	2.17±0.32abc	15.22±2.45a	3.30±0.86a	1.96±0.23b	1.63±0.20ab	20.28±2.27ab	2.23±0.32c	2.98±0.40c	1.38±0.17c	0.39±0.03a	0.46±0.03bc
D1	18.43±2.52ab	2.52±0.53ab	3.62±0.45abc	1.79±0.47abc	18.89±1.91a	5.52±0.80a	3.05±0.32ab	2.16±0.26ab	25.31±3.11ab	3.20±0.53bc	4.06±0.46bc	1.93±0.24bc	0.40±0.02a	0.59±0.03abc
D2	17.43±4.72ab	1.43±0.44b	2.76±0.54bc	0.97±0.21c	19.06±3.42a	5.11±1.11a	3.24±0.62ab	2.15±0.08ab	20.36±2.13ab	3.57±0.21bc	2.73±0.29bc	1.72±0.10bc	0.60±0.12a	0.80±0.05a
D3	16.56±4.37ab	2.97±1.25ab	3.17±0.50bc	1.19±0.30bc	18.88±2.79a	3.61±0.56a	3.14±0.58ab	1.42±0.20b	16.76±2.99b	3.63±0.24bc	3.02±0.35c	1.18±0.26c	0.51±0.09a	0.62±0.10ab
SED	2.854		0.556		3.242		0.512		3.349		0.733		0.077	
LSD	5.737		1.116		6.548		1.047		6.723		1.471		0.158	
P	<0.001		0.002		0.002		0.019		<0.001		0.002		0.256	

LFW is leaf fresh weight, LDW is leaf dry weight, RFW is root fresh weight, RDW is root dry weight, Stem FW is stem fresh weight, R/S is root to shoot ratio, SED is standard errors of difference between two means of water treatments, LSD is least significant differences of means of water treatments and P is probability (P-value) of the water treatments significantly different at P<0.05. Values in columns identified with the same letter are not statistically different among accession based on Tukey's Pairwise method (P<0.05)

Table 5. 3: Mean of total leaf area (TLA) (cm^2) and specific leaf area (SLA) (cm^2g^{-1}) and total yield (g) ± standard error of means (SE) of nine amaranth accessions under water-sufficient (WS) and water-deficient (WD) conditions.

	TLA (cm^2)		SLA (cm^2g^{-1})		Yield (g)	
Accessions	WS	WD	WS	WD	WS	WD
B1	470.84±119.91a	129.87±29.57a	49.24 ± 6.31ab	9.67±0.79a	121.31±29.96a	45.96±8.12a
B2	784.27±54.25a	164.69±12.43a	57.89±4.08a	9.04± 0.75ab	165.96±10.68a	57.55±5.79a
B3	800.66±30.82a	170.47±22.89a	54.51±4.27a	8.42± 0.05abc	147.48±6.12a	53.40±10.59a
C1	739.23±82.72a	210.79±12.75a	42.53±3.30ab	6.61±0.83abc	186.79±8.16a	85.77±12.06a
C2	714.72±115.82a	215.66±21.46a	36.51± 6.36ab	6.30±0.96abc	243.04±95.63a	91.51±11.31a
C3	403.53±73.82a	168.43±25.46a	28.88± 1.19 b	4.84±0.49c	202.29±26.41a	79.91±12.56a
D1	677.12±90.75a	140.71±42.30a	43.74± 5.46 ab	5.72±0.79bc	189.94±20.67a	97.71±42.79a
D2	611.35±156.89a	75.90±26.00a	37.80±5.90ab	5.00± 0.47c	218.84±45.69a	73.27±8.29a
D3	540.72±157.66a	158.91±54.38a	33.32±7.31ab	6.60± 1.39abc	168.71±40.62a	128.55±25.67a
SED	112		44.94		5.266	
LSD	225.2		90.21		10.573	
P	<0.001		0.003		<0.001	

TLA is total leaf area, SLA is specific leaf area, SED is standard errors of difference between two means of water treatments, LSD is least significant differences of means of water treatments and P is probability (P-value) of the water treatments significantly different at $P<0.05$. Values in columns identified with same letter are not statistically different among accessions based on Tukey's Pairwise method ($P<0.05$)

Table 5.4: Mean of total chlorophyll content, chlorophyll-a and chlorophyll-b (µg cm^{-2}) ± standard error of means (SE) of nine accessions of amaranth under water-sufficient (WS) and water-deficient (WD) conditions at 2 days after treatments (DAT), 8 DAT and 14 DAT.

	2 DAT		8 DAT		14 DAT	
	Total chlorophyll content (µg cm^{-2})					
Accessions	WS	WD	WS	WD	WS	WD
B1	38.81±2.36a	33.25±2.43a	29.17±1.28a	18.36±2.37a	29.63±1.55ab	17.98±11.02a
B2	37.01±1.95a	42.17±2.19a	31.70±2.59a	23.88±5.25a	20.10±4.36ab	22.48±5.56a
B3	37.67±2.00a	39.67±1.06a	37.51±2.50a	42.47±2.67a	34.86±2.64a	39.50±9.77a
C1	37.55±4.02a	42.28±2.33a	28.21±3.87a	31.52±10.59a	18.27±3.27ab	26.25±12.65a
C2	45.95±1.96a	41.42±1.55a	38.09±3.76a	37.62±5.83a	24.37±2.95ab	43.41±5.99a
C3	41.63±2.87a	38.74±1.86a	34.14±2.18a	34.35±7.12a	24.85±3.18ab	34.73±11.21a
D1	37.26±1.98a	43.58±3.03a	37.74±3.97a	38.42±5.11a	31.12±2.12b	36.89±6.93a
D2	33.14±2.47a	37.04±6.09a	32.86±2.29a	38.63±11.09a	30.63±3.47ab	32.61±17.16a
D3	35.08±5.67a	40.47±3.17a	40.99±7.35a	35.44±11.61a	31.62±3.75ab	36.16±8.45a
	Chlorophyll a (µg cm^{-2})					
B1	23.69±1.37a	20.45±1.42a	18.08±0.74a	11.78±1.38a	18.35±0.90ab	11.57±6.42a
B2	22.65±1.13a	25.65±1.28a	19.55±1.51a	15.00±3.05a	12.80±2.54ab	14.19±3.23a
B3	23.03±1.16a	24.19±0.62a	22.94±1.46a	25.83±1.55a	21.39±1.54a	24.09±5.69a
C1	22.96±2.34a	25.71±1.36a	17.52±2.25a	19.45±6.17a	11.73±1.90ab	16.38±7.36a
C2	27.85±1.14a	25.21±0.90a	23.27±2.19a	23.00±3.39a	15.28±1.72ab	26.37±3.49a
C3	25.33±1.67a	23.65±1.08a	20.98±1.27a	21.10±4.14a	15.57±1.85ab	21.32±6.53a
D1	22.79±1.15a	26.47±1.77a	23.07±2.31a	23.47±2.98a	19.22±1.24b	22.57±4.03a
D2	20.39±1.44a	22.66±3.55a	20.23±1.33a	23.59±6.46a	18.93±2.02ab	20.08±9.99a
D3	21.52±3.30a	24.66±1.84a	24.96±4.28a	21.73±6.76a	19.51±2.18ab	22.15±4.92a
	Chlorophyll b (µg cm^{-2})					
B1	16.15±1.38a	12.90±1.42a	10.52±0.75a	4.19±1.39a	10.79±0.90ab	10.42a
B2	15.10±1.14a	18.12±1.28a	11.99±1.52a	7.42±3.07a	7.16±2.32ab	9.62±1.71a
B3	15.49±1.17a	16.66±0.62a	15.40±1.46a	18.30±1.56a	13.85±1.54a	16.56±5.71a
C1	15.42±2.35a	18.18±1.36a	9.96±2.26a	11.89±6.20a	4.14±1.91b	15.37±5.94a
C2	20.33±1.15a	17.68±0.91a	15.73±2.20a	15.46±3.41a	7.71±1.72ab	18.84±3.50a
C3	17.80±1.68a	16.12±1.09a	13.43±1.27a	13.55±4.16a	7.99±1.86ab	13.77±6.56a
D1	15.25±1.16a	18.94±1.77a	15.53±2.32a	15.93±2.99a	11.66±1.24ab	15.03±4.05a
D2	12.84±1.44a	15.12±3.56a	12.68±1.34a	16.05±6.49a	11.37±2.03ab	29.49±5.31a
D3	13.97±3.31a	17.13±1.85a	17.43±4.30a	19.15±6.54a	11.95±2.19ab	14.60±4.94a
SED	4.386		8.42		10.47	
LSD	8.879		16.91		21.21	
P	0.467		0.707		0.636	

SED is standard errors of difference between two means of water treatments, LSD is least significant differences of means of water treatments and P is probability (P-value) of the water treatments significantly different at $P<0.05$

Values in columns identified with same letter are not statistically different among accession based on Tukey's Pairwise method ($P<0.05$)

Table 5.5: Mean of total water transpired (kg) and transpiration efficiency (TE) of nine accessions of amaranth under water-sufficient (WS) and water-deficient (WD) conditions with ± standard error of means (SE). Mean of amount of soil water content in a pot at FTSW=0 with ± SE and FTSW threshold values for nine amaranth accessions were calculated using the linear plateau regression model with ± SE and 95% confidence limit of the threshold.

	Total water transpired (kg)		**TE (gk^{-1})**					
Accession	WS	WD	WS	WD	**Accession**	**Soil water (kg) when FTSW=0 of WD**	**FTSW threshold decline of WD**	**95% CI for FTSW decline of WD**
B1	2.28±0.05	0.94±0.026a	4.62±0.61ab	7.13±0.69a	B1	0.52±0.02a	0.38±0.04a	0.31-0.51
B2	2.41±0.10a	0.89±0.079a	4.79±0.34a	6.74±0.73a	B2	0.52±0.01a	0.37±0.05a	0.29-0.59
B3	2.42±0.09a	0.92±0.041a	4.84±0.19a	6.91±0.44a	B3	0.49±0.01a	0.32±0.05a	0.24-0.47
C1	2.46±0.12a	1.08±0.055a	2.92±0.40bc	3.78±0.36b	C1	0.48±0.02a	0.29±0.01a	0.25-0.37
C2	2.19±0.08a	0.95±0.031a	2.73±0.47c	4.01±0.77b	C2	0.53±0.02a	0.56±0.01a	0.37-0.67
C3	2.25±0.09a	0.98±0.003a	2.19±0.07c	3.60±0.28b	C3	0.46±0.01a	0.51±0.09a	0.29-0.78
D1	2.23±0.05a	0.92±0.048a	3.42±0.33abc	4.09±0.80b	D1	0.49a	0.41±0.04a	0.33-0.52
D2	2.36±0.18a	0.92±0.057a	2.34±0.25c	2.96±0.28b	D2	0.49±0.01a	0.52±0.07a	0.39-0.79
D3	2.43±0.08a	0.97±0.099a	2.41±0.46c	2.40±0.22b	D3	0.51±0.04a	0.51±0.01a	0.06-0.79
SED	0.137		0.667		G	0.0017ns	0.024ns	-
LSD	0.274		1.360		Error	0.001	0.022	-
P	<0.001		<0.001					

Table 5.6: Correlation coefficients (r) for traits associated with water-sufficient (WS) in the bottom diagonal and water-deficient (WD) in the top diagonal for the nine amaranth accessions.

	LFW	LDW	SFW	SDW	RFW	RDW	R/S	TLA	SLA	TCC2	TCC8	TCC14	TWT	TE	Yield	DTW
LFW	1	0.678**	0.318	0.304	-0.057	0.144	-0.572**	0.577**	-0.105	0.049	-0.094	-0.013	-0.053	0.536**	0.755**	-0.173
LDW	0.88**	1	0.398	0.484**	0.163	0.389*	-0.649**	0.392*	-0.546**	-0.001	-0.158	-0.089	0.124	0.841**	0.625**	-0.424**
SFW	0.559**	0.533**	1	0.688**	0.383*	0.510**	-0.126	-0.059	-0.461**	-0.25	-0.282	-0.277	-0.123	0.549**	0.815**	-0.184
SDW	0.562**	0.571**	0.857**	1	0.21	0.808**	-0.142	-0.149	-0.655**	-0.16	-0.287	-0.273	0.022	0.79**	0.600*	-0.413**
RFW	0.435*	0.551*	0.49*	0.482*	1	0.32	0.138	-0.089	-0.236	-0.131	0.252	0.268	-0.038	0.197	0.219	-0.138
RDW	0.594**	0.642**	0.629**	0.724**	0.651**	1	0.262	-0.189	-0.542**	-0.078	-0.274	-0.161	-0.024	0.661**	0.404*	-0.55**
R/S	-0.269	-0.325	-0.364	-0.435	0.029	0.105	1	-0.351	0.286	-0.007	0.017	0.12	-0.191	-0.46**	-0.408*	0.003
TLA	0.701**	0.587**	0.368	0.353	0.152	0.376	-0.001	1	0.474**	0.069	-0.009	0.126	0.198	0.136	0.335*	-0.238
SLA	-0.204	-0.424	-0.407	-0.474	-0.744**	-0.6*	0.106	0.201	1	0.117	0.22	0.288	-0.038	-0.668**	-0.328	0.238
TCC2	-0.325	-0.146	-0.195	-0.264	-0.085	-0.199	0.017	-0.305	-0.09	1	-0.014	0.087	0.17	-0.072	-0.145	-0.115
TCC8	-0.11	0.002	-0.157	-0.124	-0.28	-0.116	-0.022	0.188	0.092	0.312	1	0.668**	-0.106	-0.248	-0.262	-0.039
TCC14	0.093	-0.021	0.038	0.099	0.029	0.042	0.232	0.251	-0.074	-0.208	0.087	1	-0.246	-0.11	-0.22	-0.168
TWT	0.111	0.247	0.51*	0.368*	0.332	0.374	-0.128	-0.034	-0.435*	-0.023	-0.063	-0.009	1	-0.091	-0.08	-0.042
TE	0.801*	0.837	0.739	0.867	0.515	0.709**	-0.488**	0.491*	-0.463	-0.223	-0.086	0.021	0.157	1	0.662**	-0.525**
Yield	0.874**	0.816**	0.857**	0.788	0.502**	0.687	-0.382*	0.595**	-0.321	-0.278	-0.176	0.026	0.312	0.89**	1	-0.232

LFW is leaf fresh weight, LDW is leaf dry weight, SFW is stem fresh weight, SDW is stem dry weight, RFW is root fresh weight, RDW is root dry weight, R/S is root to shoot ratio, TLA is total leaf area, SLA is specific leaf area, TLC2 is total chlorophyll content at 2 days after water treatment (DAT),TLC 8 is total chlorophyll content at 8 DAT, TLC 14 is total chlorophyll content at 14 DAT, TWT is total water transpired and TE is transpiration efficiency**.** P is probability (P-value) significantly different at *P<0.05 and *P<0.001

5.3.4 Discussion

This study was designed to determine the influence of water relations on adaptive strategies to drought in different amaranth accessions. There is a need to resolve whether the variation in TE is an inherent consequence of basic physiological changes regardless of soil drying and subsequently identify suitable surrogate traits for TE as a drought-tolerance selection criterion in amaranth species. Liu and Stützel (2002a) reported that in vegetable amaranth, transpiration during water deficit was regulated through the reduction of leaf expansion and stomatal conductance, and thus prevented leaf dehydration. Leaf area expansion in vegetable amaranths was identified as more sensitive to soil drying when compared with transpiration and stomatal conductance (Liu and Stützel 2002b).

In this experiment, total water transpired by the plants directly affected the TE value as higher total water transpired reduced the TE. There was a similar pattern of total water transpired in both WS and WD treatments among the nine amaranth accessions, with WD plants showing a lower value for total water transpired. This was reflected in higher TE values in WD plants compared with WS plants with the exception of D3 which had similar TE under both water treatments. The similar amount of total water transpired among all accessions under both water treatments suggested that there were other physiological traits that influenced the variation in TE. Sinclair *et al*., (1984) stated that two critical variables accounted for variation in TE in WS plants, which were a difference in the composition of plant products and/or the CO_2 concentration maintained in the leaves.

The response of transpiration to soil water deficit has previously been described using a linear plateau model (Devi *et al*., 2009), which identified the critical soil water content at which transpiration rate started to decline. The FTSW represents the portion of remaining volumetric soil water available for transpiration, and at which threshold, the plants' physiological processes start to decline (Liu and Stützel, 2002a). In the present study, there was a wide range of FTSW threshold values at which the transpiration rate began to decline among the amaranth accessions indicating differences in relation to soil drying. The range of FTSW threshold decline in *A. cruentus* in this experiment (0.32-0.38) was very similar to the range (0.22-0.48) reported by Liu and Stützel (2002b). In contrast, a large difference was found in red *A. tricolor* (0.29-0.56) and green *A. tricolor* (0.41-0.52) in this experiment compared with the range of 0.29-0.44 recorded for

A.tricolor by Liu and Stützel (2002b), possibly as a consequence of the different accessions used in these two studies.

This linear plateau model has also been used as an indicator of stress (Ritchie, 1981). In a study of genotypic responses to transpiration in chickpea, despite no genotypic difference in total water extracted, differences in the pattern of water extraction from the soil profile were observed which consequently affected the pod yield (Ratnakumar *et al*., 2009). The present study showed that there was a difference in the pattern of water extraction, which influenced the TE value. For example, C2 had a high FTSW threshold (0.56) with restricted transpiration during early soil drying which allowed the plants to conserve more water under water deficit conditions and produce a low TE value (4.01 g kg^{-1}). In comparison, B1 had a low FTSW threshold (0.38) and transpiration continued with further soil drying, producing a high TE value (7.13 g kg^{-1}). C1 had the lowest FTSW threshold (0.29) among all accessions, indicating that transpiration declined upon progressive soil drying under relatively drier conditions. However, it is important to note that D3 had high FTSW threshold decline (0.51), but also had similar TE and SLA values for both WS and WD treatments, and a high R/S ratio under both WS and WD conditions. A possible explanation is the greater root density of D3 compared to the other accessions, allowing it to sustain high water uptake at low soil-water content. D3 was able to extract higher amounts of water while sustaining an increased transpiration rate at low soil-water content under the WD treatment and resulted in a similar TE value under the WS treatment.

Plants that perform better under water-deficit conditions are likely to have a high TE value and could be associated with a high threshold for decreased NTR (Devi *et al*., 2009). A higher FTSW threshold could allow the plants to conserve more soil water, better positioning them to endure drought stress (Johnson *et al*., 2009). In the present study, acessions with a high FTSW threshold might have had an opportunity to fully utilize the soil-water content and maximize growth before the experiment was terminated. Accessions such as these are positioned to conserve water during soil drying to the point where transpiration rate is restricted (Gholipoor and Sinclair, 2012). In this study, it is difficult to conclude whether a high FTSW threshold gave a high TE value, as the value of FTSW did not correlate with the TE values. Hence, there is a need to understand the role of TE as a component of the genetic phenotypic differences in the FTSW threshold.

In the present study, a high FTSW threshold was associated with increased drought tolerance, as D3 showed a similar TE value under both WS and WD treatments. D3 had a high FTSW threshold decline, with a lower TE value for WS and WD plants compared to the other accessions. This implied that D3 maximized water-use efficiency instead of utilizing the water for maximizing growth. D3 appeared to have a different mechanism for growth, as soil drying did not significantly alter the TE compared with the WS plants. One explanation could be that lower transpiration under WS conditions led to lower daily transpiration, which would logically drive the transpiration rate of drought stressed plants upward, and consequently the NTR (Kholova *et al*., 2010; Bhatnagar-Mathur *et al*., 2007). Therefore, the maintenance of NTR under drought conditions at similar levels to WS plants results in a lower value for the FTSW threshold at which transpiration begins to decline. Alternatively, this may simply be a consequence of the lower rate of water loss per unit leaf area in the WS plants.

The drought tolerance of the amaranth accessions was expressed as SSI (Fisher and Maurer, 1978). The tolerance of a genotype to drought stress is predicted to be higher if the SSI value is low (Zdravkovic *et al*., 2013). Despite D3 displaying drought tolerance characteristics, it was considered susceptible to drought stress as it had a high SSI value and low yield. In comparison, C3 which also had high FTSW threshold (0.51) and TE value under WD conditions was considered tolerant to drought, as it had a low value of SSI for yield, which can be explained by the low reduction in TLA. The most susceptible accession was D2, which had the highest reduction in TLA. Thus, a low or high FTSW threshold may not necessarily produce a desired amount of crop yield. Kholova *et al*., (2010) reported that two different hybrid lines of pearl millet had low FTSW thresholds. However, one hybrid line also had low yield similar to drought-sensitive lines, compared with high yield achieved by the drought-tolerant lines.

Jomo *et al*., (2016) reported that the total chlorophyll content of amaranth was significantly reduced in response to soil water deficit with *A. tricolor* recording the lowest reduction in chlorophyll compared to other amaranth species. However, the present study showed no significant difference in total chlorophyll between WS and WD plants after 14 days of drought-stress treatment. Drought stress has been shown to alter the ratio of chlorophyll-a and chlorophyll-b content (Anajum *et al*., 2011). In the current study, chlorophyll-a content was higher than chlorophyll-b

content under both water treatments, which was comparable to the results of Jomo *et al*., (2016). Schlemmer *et al*., (2005), reported no effect of drought stress on chlorophyll content in maize, however in contrast, O'Neil *et al*., (2006) reported that chlorophyll was the only measurement affected by drought in maize. Therefore, it could be a trend for amaranth species to react differently to water deficit conditions and might be an adaptation strategy of C4 photosynthesis. Liu and Stützel (2004) reported a negative correlation between WUE and SLA in amaranth. In the present study, the reduction of SLA in WD plants was similar for all accessions, except for D3, demonstrating that SLA was not conclusively responsible for the differences in TE among the amaranth accessions.

5.3.5 Conclusion

The FTSW threshold at which transpiration declined upon progressive soil drying influenced water relations in differing ways for the nine accessions suggesting different adaptive strategies to drought. Amaranth species evaluated in the present study showed similar growth performance relative to transpiration efficiency under controlled and drought stress conditions and high TE may not necessarily be the best indicator for drought tolerance selection traits in amaranth. The mechanisms of TE under drought stress in this present study was not clear, but a consistent high negative correlation between TE and R/S in both controlled and drought conditions could be the possible reason that allows the plants to sustain high water uptake at low soil-water content.

5.4 Experiment II: Variation in growth, root morphology and plant physiology of vegetable amaranth (*Amaranthus tricolor*) in response to gradual drought stress

5.4.1 Introduction

Most plants use more than one strategy at a time to resist drought (Mitra, 2001) and different traits are required to mitigate different types, severity and duration of water shortage (Kamoshita *et al*., 2008). It is necessary to understand the mechanisms that the plants use to confer drought stress (Tuberosa, 2012). Quantifying various drought tolerance traits, at different stress levels can reveal which traits are responsible relative to specific genotypic variations (Mwadzingeni

et al., 2016). Consequently, an interactive response between morphological, physiological and biochemical traits should be included in any study of plants under drought stress.

Different physiological parameters have been shown to be an effective tool for indirect selection for yield under drought stress in various major crops. For instance, screening of deep and vigorous root system for higher yield under drought stress has been recognized in many crops such as wheat (Wasson *et al*., 2012), soybean (Sadok and Sinclair, 2011) and rain-fed rice (Henry *et al*., 2011). Although larger root systems promote greater water uptake which leads to high productivity under water-limited conditions for specific crops variety, restrictions in regulations of water uptake maybe more strategic for a plant to manage limited water availability (Vadez, 2014), as occurred in upland rice (Singh *et al*., 2017). Other than modifying root systems, maintaining high photosynthetic rates (Wang *et al*., 2016), or accelerating chlorophyll decompositions (Chen *et al*., 2016) under drought stress, can be a good predictor for indirect selection of drought tolerant genotypes. Besides, biochemical analysis such as proline contents has been used as a complementary strategy for a selection of high yielding genotypes under drought stress (Mwadzingeni *et al*., 2016; Bowne *et al*., 2012). Accumulation of proline content under drought stress has been associated with osmoprotection roles such as osmotic adjustment, membrane stabilization and activates antioxidant defence mechanisms in amaranth (Slabbert and Krüger, 2014).

Morphological variation in amaranth such as colour pigmentation has been shown to be an indicator for drought tolerance traits in *A. tricolor* and *A.cruentus*, for example, green leaf amaranth (acyanic leaf) and red leaf amaranth (betacyanic leaf) have shown genotypic variation in biomass partitioning (Liu and Stützel, 2004) and photoprotection (Nakashima *et al*., 2011) in response to drought stress. However, information on the correlation between plant physiology at critical growth period and yield in vegetable amaranth is still limited, and relatively little data are available on the growth and mechanisms of drought tolerance in vegetable amaranth (*A. tricolor*) differing in morphological traits.

Therefore, this study was conducted to determine the variation in growth and development and the underlying physiological parameters between two contrasting amaranth varieties subjected to gradual drought stress. The two amaranth varieties with highly contrasting morphological traits, including leaf

pigmentation, plant height, leaf shape and stem diameter were selected to demonstrate the different potential growth responses to drought stress at critical growth period in vegetable amaranth. This study was also used to ascertain the complementary strategy for selection of drought tolerant accessions in response to various drought stresses for future breeding purposes.

5.4.2 Materials and methods

5.4.2.1 Plant materials and growing conditions

The plant material consisted of four *A. tricolor* accessions; two accessions were green leafy vegetables from the USDA Genebank; Green Ames 5134 (GA5134) and Green Ames 15328 (GA15328), and two accessions were local red leafy vegetables; Perfect red amaranth (PR) and Red amaranth (Red) (Figure 5.7). The two local Malaysian amaranths were taller, with smaller stem diameter and broad leaves, and were also been used in previous screening (Experiment I, subheading 5.3) as accessions C1 (PR) and C3 (Red). These two red accessions were included in the mini core collection and consequently GBS data is also available. In contrast, the two green leaf amaranths were shorter, with larger stem diameter, and narrow leaves.

Plants were grown in 31.75 cm x 21.59 cm x 19.05 cm pots, filled with a mixture of 8 kg soil, 1 kg sand to improve aeration for root development and 1 kg black peat moss (Holland peat, Netherlands) to increase WHC of the soil mixture. The soil nutrient analysis is presented in Table 5.7. The mean of 24-hour daily weather data during the experimental period was recorded using data logger (HOBO ® U30 Weather Station, MA, USA) (Figure 5.8).

Figure 5.7: Four *A. tricolor* used in this study; Accession (a) GA5134; (b) GA15328; (c) Red; and (d) PR.

Table 5.7: Soil nutrient analysis for Experiment II.

Soil analysis													
pH in Water (2:5)	C (%)	N (%)	C/N	P (ppm)		Exchangeable Cations (m.e. %)			C.E.C NH_4OAC method (m.e%)	Mechanical Analysis (%)			
				Total	Acid fluoride soluble	K	Ca	Mg		Clay	F Silt	F Sand	C Sand
5.36	1.57	0.14	11.2	295	23.1	1.5	6.44	1.15	7.9	40	12	25	23

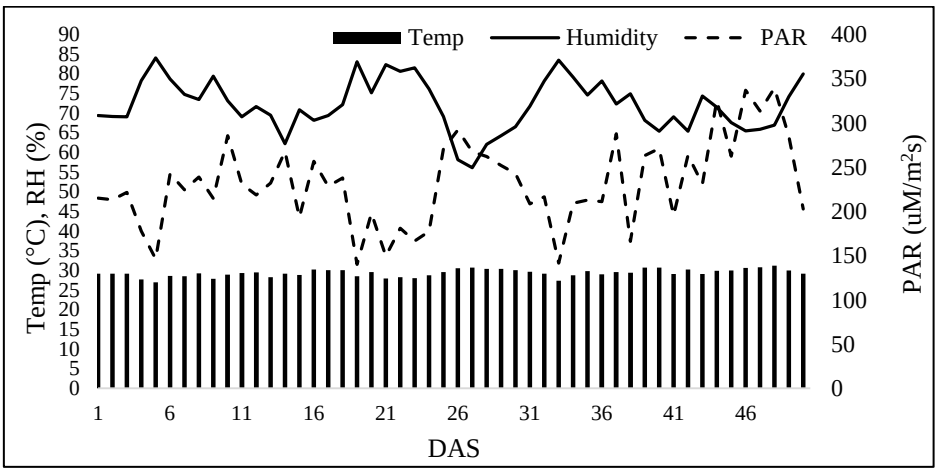


Figure 5.8: Daily weather data collected during the experimental period. Values represent the mean of 24 hours from 16th July 2015 until 5th September 2015.

5.4.2.2 Experimental design

The experimental design was split plot in a randomized complete block design with two water treatments: drought stress (water sufficient, WS) and well-watered control (water deficient, WD) as the main plot, and four amaranth accessions as subplot with four replications and six biological repeats. The watering plan for this experiment is presented in Figure 5.9. Prior to the onset of drought treatment, plants were irrigated daily to field capacity. At 30 days after seeds emergence, four phases of gradual drought stress were imposed to WD plants consecutively, while WS plants were irrigated daily throughout the experimental period to maintain maximum water holding capacity (WHC). The soil water content of WD plants was allowed to fall progressively for five days until reached 50% WHC. At the fifth day of drought treatment (5 DAT), the first six replicates of both WS and WD treatments (Set 1) were harvested for destructive growth measurements. Then, 150 ml water was added daily to the remaining WD plants for another five days to maintain 40% WHC, and at 10 DAT, Set 2 was harvested. Subsequently, 100 ml water was added daily to the rest of WD plants for the next five days to achieve 30% WHC, and Set 3 was harvested at 15 DAT. Lastly, 50 ml water was added daily to the last set of WD plants (Set 4) for five days to obtain 20% WHC, and harvested at 20 DAT.

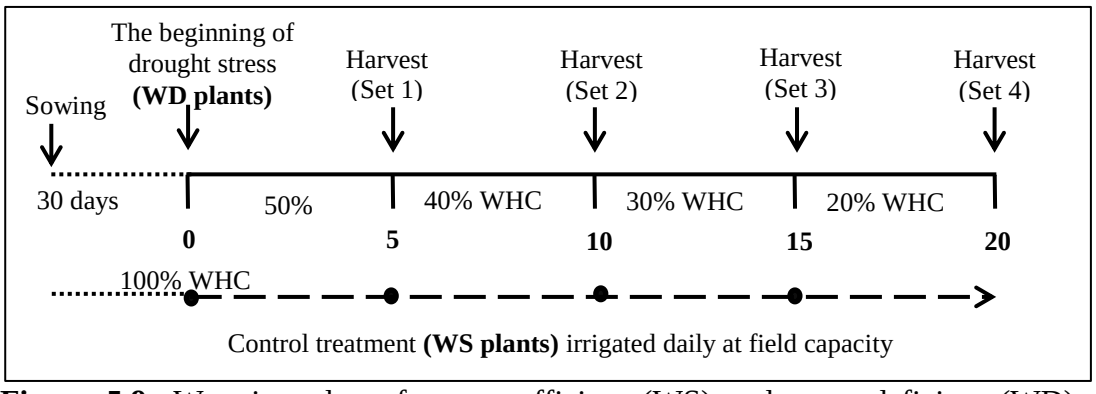


Figure 5.9: Watering plan of water-sufficient (WS) and water-deficient (WD) plants for gradual drought stress. WHC is water holding capacity and DAT is days of drought stress.

The volumetric water content of the soil was measured at the beginning of the drought treatment followed by four measurements at every 5 days for 20 days using portable soil moisture sensor (ML3-ThetaProbe, Delta-T Device, Cambridge, England) (Figure 5.10). The soil had a water content of 35% vol - 40% vol at

maximum WHC. The gradual declined of volumetric water content in WD plants was used to determine the WHC of each time point of drought stress.

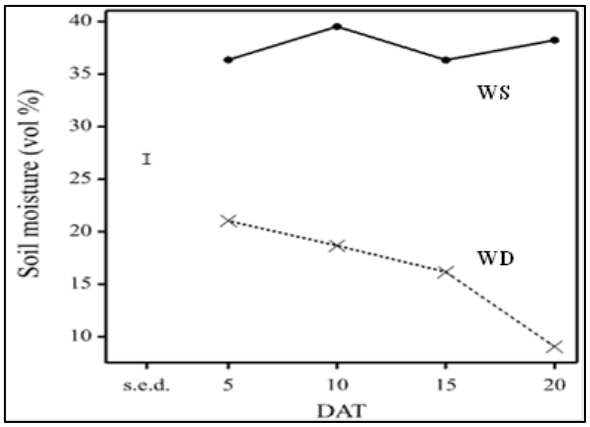


Figure 5.10: Soil volumetric water content (% vol) of well-watered treatment (water-sufficient, WS) and drought-stressed treatment (water-deficient, WD) in 20 days of water treatment (DAT). Values represent mean of six individual of four accessions and error bar represent standard errors of difference (SED).

5.4.2.3 Growth measurements

5.4.2.3.1 Total yield, TLA, SLA and R/S ratio

Plants were destructively harvested at 5, 10, 15 and 20 DAT. The leaves, stem and roots were separated, and FW, DW, TLA, SLA and R/S ratio were determined at each time point (see subheading 5.2.3). Roots were washed thoroughly and kept at -20°C prior analysis and fresh weight was recorded. The dry weight of the roots was measured after analysis has been completed.

5.4.2.3.2 Root morphology analysis

The washed root system of each plant was placed on a transparent tray and evenly spread apart in a thin water layer and images were captured at a resolution of 800 dpi (dots per inch) with a grayscale output using an Epson Expression 836 x L scanning system. Root images were analysed for total root length (RL, cm), root surface area (RSA, cm^2), average root diameter (RD, mm), root volume (RV, cm^3) and root length/root volume (RLPV, $cm.cm^{-3}$) using WinRHIZO 2013 software (V5.0 Regent Instruments, Quebec, Canada) with soil volume set to 6.24 cm^3 based on the weight of soil and the volume of the pots.

5.4.2.4 Physiological responses

In order to obtain consistency in physiological responses at each time point (5, 10, 15 and 20 DAT), measurements were recorded on the same plants of Set 4. Readings were taken on the 3rd most fully expanded leaflet at the top, avoiding the midrib section from 8am to 11am. Two readings were taken per leaf and averaged to give a final reading, with six replications (except for photosynthetic gas exchange and proline analysis where measurements were recorded in four replications). Due to a technical problem, photosynthetic gas exchange measurements could not be measured at 10 DAT. The protocol for TCC, RWC and photosynthetic gas exchange measurement were stated in subheading 5.2.4.

5.4.2.4.1 Light response curve

Before the onset of drought treatment (time 0), a rapid light response curves of photosynthetic assimilation (Pn/I) of each accession was evaluated to identify the maximum photosynthetic CO_2 assimilation (Pn) at different photosynthetic photon flux density (I). The auto program function was performed with photoactive radiation (PAR) set to be 2000, 1750, 1500, 1250, 1000, 750, 500, 250, 100, 50, 25 and 0 µmol photons m^2 s^{-1},with a minimum and maximum waiting time of 120 s and 200 s, respectively, and matching the infrared gas analysers for 50 µmol (CO_2). mol $(air)^{-1}$ difference in the CO_2 concentration between the sample and the reference, which allowed them to be matched before every change in I.

5.4.2.4.2 Determination of proline content

Leaf samples were snap frozen in liquid N_2 and stored at -80°C prior to extraction. Free proline content was estimated by following the method of Bates *et al*., (1973). Briefly, leaf tissue was ground to a fine powder and 200 µg samples were homogenized with 1ml of 3% aqueous sulfosalicylic acid at 14,000 rpm for 5 minutes. 100 µl of the supernatant plant extract was mixed with an acidic reaction mixture (100 µl of 3% sulfosalicylic acid, 200 µl glacial acetic acid and 200 µl acidic ninhydrin). The sample was then incubated in 96°C water bath for 1 hour and the reaction terminated in an ice bath. The reaction mixture was extracted with 3 ml toluene and was allowed to incubate at room temperature for 5 minutes, for organic separation. The chromophore containing toluene was aspirated from the aqueous phase. The absorbance was measured at 520 nm using a UV-VIS

spectrophotometer (Perkin-Elmer, Lambda 5, Massachusetts, USA) using toluene as reference blank.

The proline concentration was determined using a standard curve and calculated on the fresh weight basis using formula given by Bates *et al*., (1973):

Proline concentration (µmoles per g of leaf tissue) = [(µg proline/ml) x (ml toluene) x (ml sulfosalicylic acid)] / [(115.5 µg/µmole) x (g sample)]

where 115.5 is the molecular weight of proline.

The standard curve was produced in 10-fold dilutions (1 ml to 10 ml) of 1 mg/ml proline. The proline dilution factors were added into the acidic reaction mixture and incubated in 96°C water bath for 1 hour. The absorbance was measured at 520 nm using distilled water as the reference blank. A standard curve calibration between absorbance and proline concentration was established and used to calculate free proline content in the leaf sample (Appendix 5.3). The equation of the standard curve is $y = 0.02x + 0.023$.

5.4.2.5 Data analysis

All statistics were performed using Genstat Software for Windows 18th edition (VSN International, 2015). The effect of water treatments, successive time point and accessions was subjected to analysis of variance (ANOVA) with a split plot design. Mean separation among accessions was carried out using Tukey's pairwise comparison and significant differences were identified with letters. Prior to the analysis, the assumption of normally distributed residuals for ANOVA was assessed using Shapiro-Wilk test, whereas the assumption of homogeneity of variance was assessed using Levene's test. Pearson linear correlations were performed to analyse the significant correlations between parameters in both water treatment at each time point.

The net photosynthetic light-response curve (Pn/I) of amaranth accession was constructed using linear-by linear polynomial regression that produce rectangular hyperbolic model as described by Michaelis-Menten (1913) (Ye and Zhaou, 2010):

$Y = (aI\ A_{max}) / (aI + A_{max}) - R_d$

where Y is net photosynthesis, a is the initial quantum efficiency (A_{max}), I is the irradiance, A_{max} is the light saturated photosynthetic rate and R_d is the dark respiration rate.

The linear by linear polynomial equation command from Genstat 18th:

$Y = A + B (1 + D*X)$

where Y is net photosynthesis A, B and D are the parameters estimated by the non-linear regression and X is irradiance.

From the equation above, the light saturated rate of photosynthesis (Pn_{sat}, mmol $m^{-2}s^{-1}$) is determined by the asymptote of photosynthesis at high light, quantum yield of photosynthesis (Pn_q, mol CO_2/mol quantum) corresponds to the initial slope of the curve at low light levels and photosynthetic light compensation point (LCP, μmol $m^{-2}s^{-1}$) is the x-intercept, when $Pn_{sat} = 0$ and photosynthetic and light saturation point (LSP, μmol $m^{-2}s^{-1}$) was determined by the PPFD at which *A* was 99% of the light-saturated net photosynthesis (Li et al., 2014; Peek et al., 2001).

5.4.3 Results

5.4.3.1 The effect of increased severity and duration of drought stress on yield and biomass allocation

Mild drought stress (5 DAT) did not significantly reduce total yield production in any of the amaranth accessions studied, with a yield range of 73.54 g-114.82 g for WS and 74.42 g-90.24 g for WD treatment (Figure 5.12, Appendix 5.4). At 10 DAT, drought stress significantly reduced total yield production by 50% in all amaranth accessions, with a yield range of 141.63 g-200.50 g for WS treatment and 79.00 g-127.42 g for WD treatment ($P<0.001$), except for PR, which able to maintain growth performance at 10 DAT and drought stress only started to affect the PR at 15 DAT. The yield production was severely reduced at further soil drying, with a yield range of 145.11 g-195.42 g for WS and 55.20 g-100.30 g for WD treatment at 15 DAT ($P<0.001$), and a yield range of 153.33 g-211.64 g for WS and 48.23 g-62.73 g for WD treatment at 20 DAT ($P<0.001$). There was no difference observed among amaranth accessions in either WS or WD treatment at any time point.

For fresh weight (FW) biomass, significant interactions between water treatment x time point x accession (WT*TP*A) were observed in fresh weight of leaves (LFW, $P<0.05$) and stem (SFW, $P<0.001$), while significant interactions between any of the two main effects were found in root fresh weight (RFW, $P<0.05$) (Figure 5.13). The FW of green leaf amaranth was equally partitioned into

leaves and stems, then into roots, and only RFW was differ between the two water treatments at 5 DAT. The green leaf amaranth had altered its FW partitioning largely into stem, followed by leaves then into roots by 10 and 15 DAT, and these accessions significantly reduced LFW and SFW under WD treatment by 50%, while maintaining its root growth. In comparison, the red leaf amaranth showed equal FW partitioning into leaves and stems, then into roots at 5, 10 and 15 DAT, and FW reduction occurred mostly in leaves under WD treatment. At 20 DAT, FW partitioning was shifted into leaves, followed by stem, then into roots in all amaranth accessions, and differences were observed among amaranth on the reductions of FW partitioning under WD treatment, i.e. GA15328 showed large reduction in roots, GA5134 in leaves, PR in stems, and Red had equally reductions into leaves, stems and roots.

For dry weight (DW), significant interactions between WT*TP*A were observed in dry weight of leaves (LDW, $P<0.05$) and roots (RDW, $P<0.05$), while significant interactions between WT*G were observed in stem dry weight (SDW, $P<0.01$) (Figure 5.14). As predicted, the pattern of DW partitioning into leaves, stems and roots was the same as FW partitioning, except for 5 DAT, in which the LDW was higher than in SDW although the LFW and SFW were similar. Under WS treatment, at 10 DAT, the green leaf amaranth was recording significantly higher SDW ($P<0.05$) (range: 16.16 g-20.68 g) compared to red leaf amaranth (range: 10.83 g- 12.18 g), while LDW and RDW remained unaltered for the two contrasting amaranth. Nonetheless, by 15 and 20 DAT, the SDW was maintained while LDW and RDW were significantly reduced ($P<0.05$), with red leaf amaranth recording higher LDW and RDW (range: 11.81 g-14.66 g, 3.45 g-6.04 g respectively) compared to green leaf amaranth (range: 6.31 g-10.14 g, 2.26 g-2.60 g respectively). Meanwhile, under WD treatment, green leaf amaranth had significantly higher LDW and RDW ($P<0.05$) (range: 12.58 g-13.11 g, 1.29 g-1.35 g respectively) compared to red leaf amaranth (range: 5.91 g-9.87 g, 0.61 g-2.58 g) at 5 DAT. However, by 20 DAT, the red leaf amaranth showed significantly higher RDW ($P<0.05$) (range: 3.07 g-3.45 g) compared to green leaf amaranth (range: 3.00 g-3.45 g).

The R/S ratio of WS and WD treatments demonstrate an upward trend throughout the experimental period, although significant interaction was only observed between the two main effects (Figure 5.15). Drought stress significantly

increased the R/S ratio of GA15328 and Red at 5 DAT ($P<0.05$) and 20 DAT ($P<0.01$). The variation between the two contrasting amaranths was observed at each time point ($P<0.05$), with red leaf amaranth displaying higher R/S (range: 0.07-0.15 in WS and 0.09-0.24 in WD treatment) compared to leaf green amaranth (range: 0.07-0.08 in WS, 0.08-0.15 in WD treatment).

In TLA, there was significant interaction between WT*TP*A ($P<0.05$) (Figure 5.15). In green leaf amaranth, the TLA of WS plants first increased (range: 1900 cm^2-2725 cm^2 at 5 DAT to 2680 cm^2-3060 cm^2 at 10 DAT), but then decreased at 15 DAT (range: 872 cm^2-2173 cm^2) and 20 DAT (range: 1387 cm^2-2529 cm^2), while TLA of WD plants decreased steadily from 10 until 20 DAT. The decrease in TLA in WS plants at 15 and 20 DAT was due to a reallocation of fresh weight biomass into stems development. In comparison, in red leaf amaranth, the TLA of WS plants increased steadily, and the WD plants were able to sustain its leaf expansion until 10 DAT and only significantly ($P<0.001$) reduced from 15 DAT onwards.

There was a significant interaction between WT*TP*A in SLA ($P<0.05$) (Figure 5.15) but not between the WS or WD treatments. Drought stress did not affect SLA at the first 10 DAT, but variation was observed among the amaranth accessions ($P<0.05$). Further, SLA significantly increased at 15 DAT, with GA5134 having the highest increase, by 54%, from 121 cm^2g^{-1} in WS to 401 cm^2 g^{-1} in WD treatment. However, the SLA was then significantly reduced at 20 DAT, with G15328 having the highest reduction, by 50%, from to 248 cm^2 g^{-1} in WS to 102 cm^2 g^{-1} in WD treatment.

5.4.3.2 The effect of increased severity and duration of drought stress on root morphology

The amaranth accessions showed a typical dicot root structure with one primary root (axial roots) and several orders of lateral roots. Newly grown root structure, i.e. shoot-born roots or adventitious roots were observed under moderate drought stress in less than ten root samples (Figure 5.16a), and deterioration of root systems was observed under severe drought stress (Figure 5.16b). Root morphology which includes RAD, RSA, RL, RV and RLPV was significantly affected at different time points of drought stress, and there was no obvious increasing or decreasing trend throughout the experimental period, in both WS and WD treatments (Figure 5.16a

& b). To summarize, RAD was only affected at early drought stress, in which it significantly increased at 5 DAT ($P<0.05$), then decreased at 10 DAT ($P<0.05$), and remained unaffected at 15 and 20 DAT. Drought stress significantly reduced RSA and RL from 10 DAT until the end of water treatment ($P<0.05$).

The root traits were similar among amaranth accessions under WS treatment, except RAD at 10 DAT, in which red leaf amaranth exhibited a higher RAD compared to green leaf amaranth ($P<0.01$) (range: 0.43 mm-0.55 mm, 0.40 mm-0.42 mm respectively). Under WD treatment, differences among amaranth accessions were only observed at 5 and 10 DAT. At 5 DAT, GA5134 displayed the highest RAD (0.43 m^2) and PR the lowest (0.35 m^2), while GA15328 was recording the highest RV (19.13 m^3) and PR the lowest (7.64 m^3), and by the 10 DAT, PR was exhibiting the highest RL (103.79 m) and GA5134 the lowest (74.34 m).

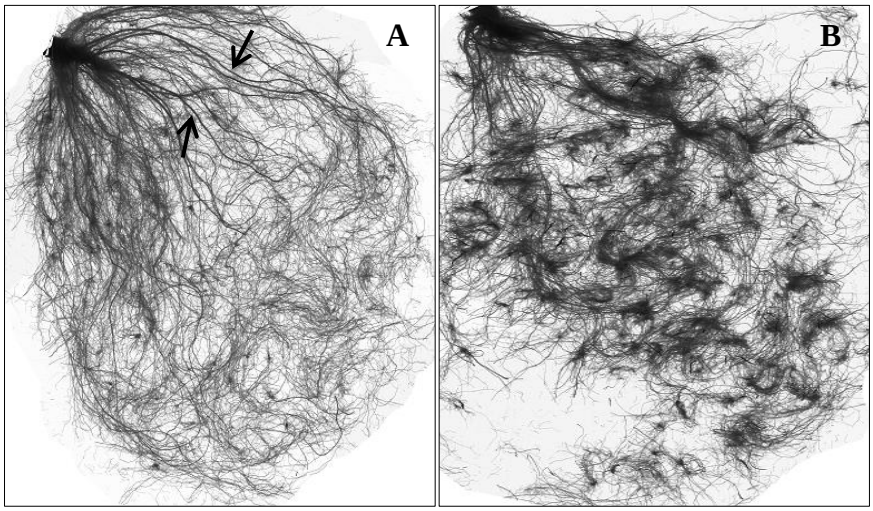


Figure 5.11: Example of sample roots; (a) the arrows are a new grown root structure, i.e. shoot-born roots or adventitious roots observed under moderate drought stress in PR and (b) a deterioration of root systems observed under severe drought stress in GA5134.

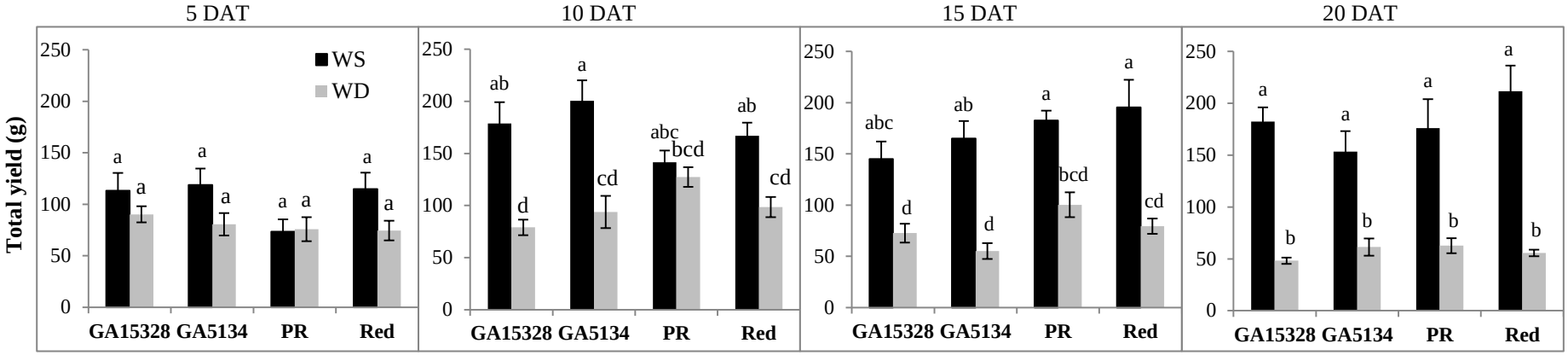


Figure 5.12: The effect of total yield at 5, 10, 15 and 20 days of drought stress (DAT) in four amaranth accessions.

The error bars indicate standard errors of means (SEM), WS is water-sufficient and WD is water-deficient and bars with the same letter are not statistically different between water treatment (WS and WD) and accessions based on Tukey's Pairwise method ($P<0.05$).

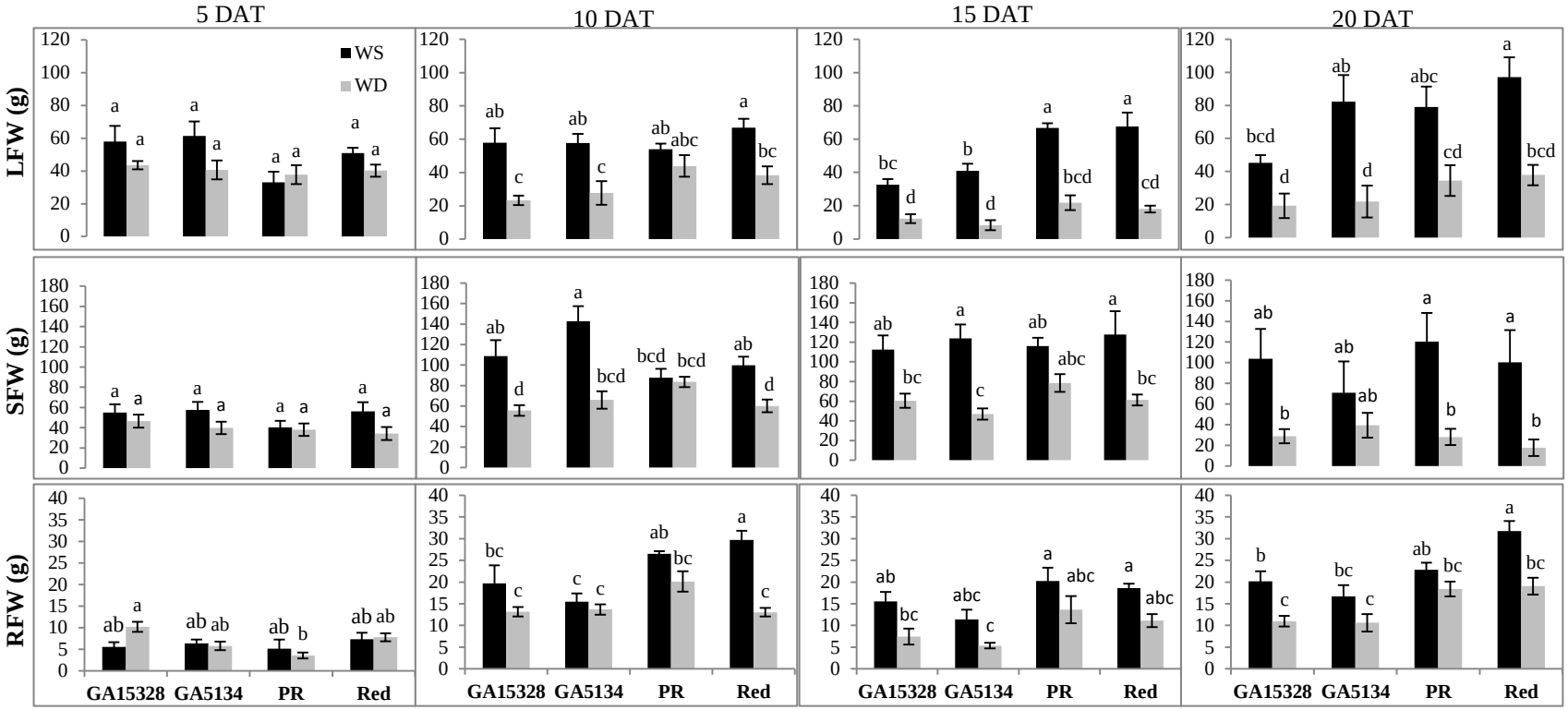


Figure 5.13: The effect of fresh weight of leaves (LFW), stem (SFW) and root (RFW) at 5, 10, 15 and 20 days of drought stress (DAT) in four amaranth accessions.

The error bars indicate standard errors of means (SEM), WS is water-sufficient and WD is water-deficient and bars with the same letter are not statistically different between water treatment (WS and WD) and accession based on Tukey's Pairwise method ($P<0.05$).

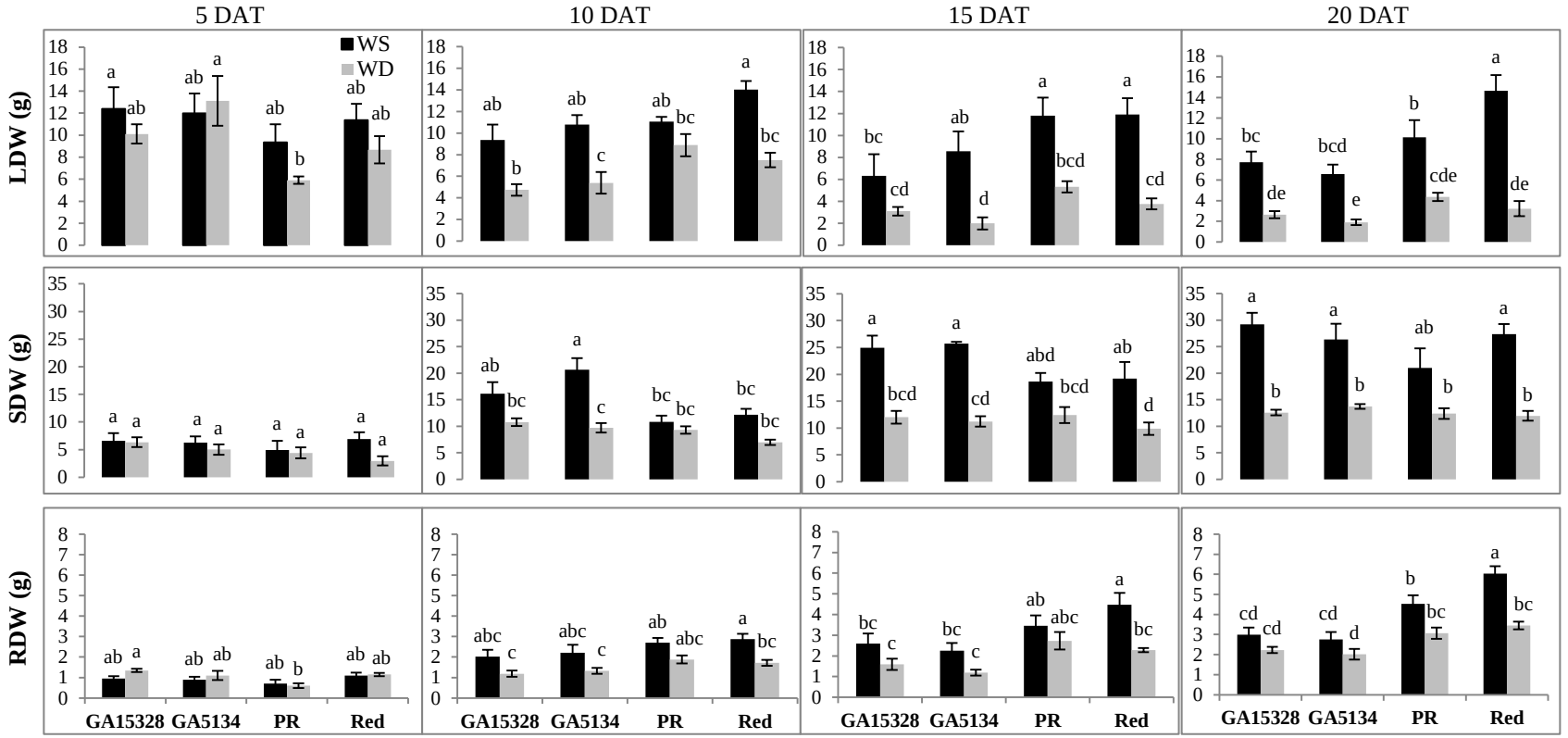


Figure 5.14: The effect of dry weight of leaves (DFW), stem (DFW) and root (DFW) at 5, 10, 15 and 20 days of drought stress (DAT) in four amaranth accessions.

The error bars indicate standard errors of means (SEM), WS is water-sufficient and WD is water-deficient and bars with the same letter are not statistically different between water treatment (WS and WD) and accession based on Tukey's Pairwise method ($P<0.05$).

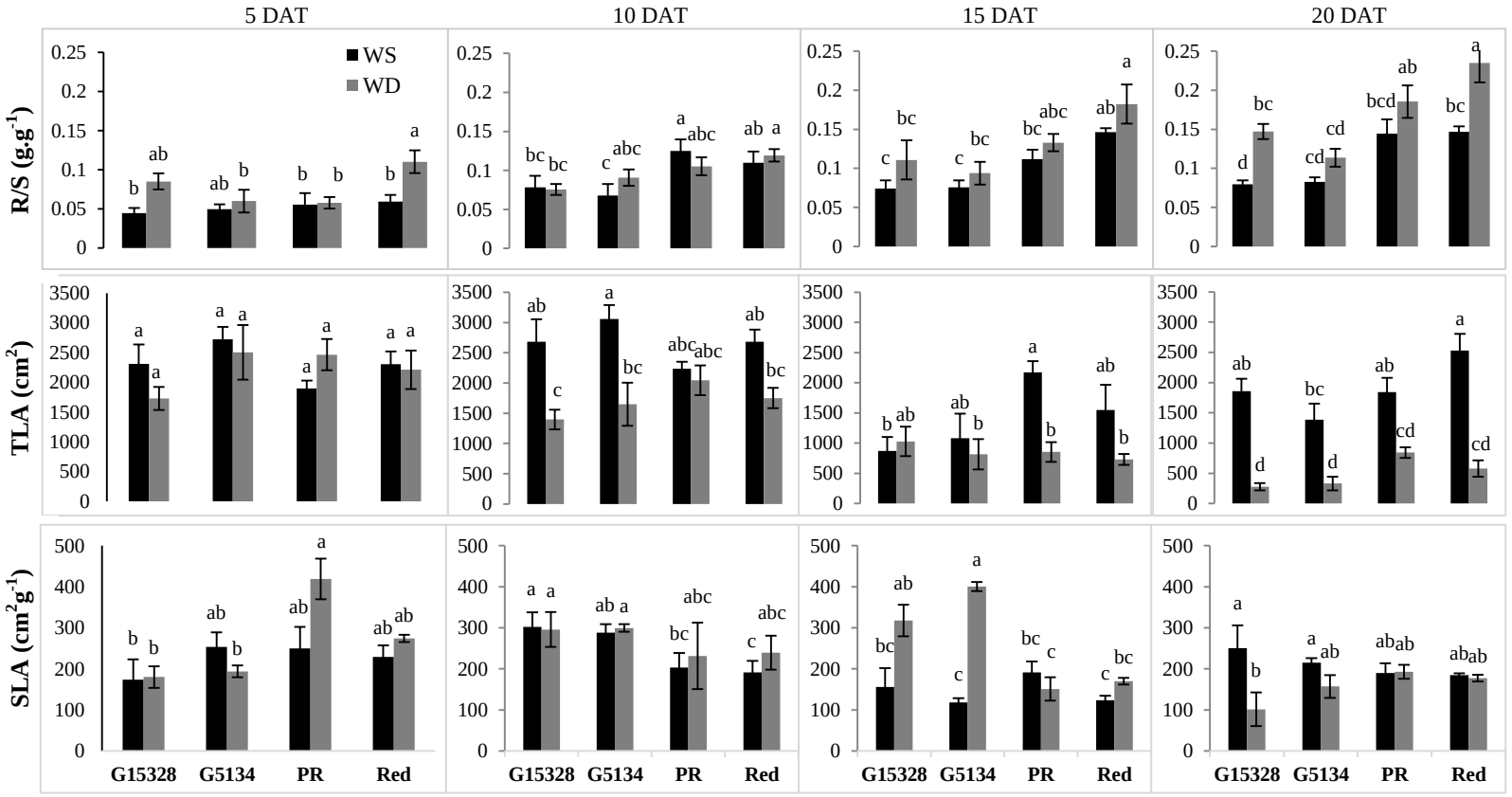


Figure 5.15: The effect of root to shoot ratio (R/S), total leaf area (TLA) and specific leaf area (SLA) at 5, 10, 15 and 20 days of drought stress (DAT) in four amaranth accessions.

The error bars indicate standard errors of means (SEM), WS is water-sufficient and WD is water-deficient and bars with the same letter are not statistically different between water treatment (WS and WD) and accession based on Tukey's Pairwise method ($P<0.05$).

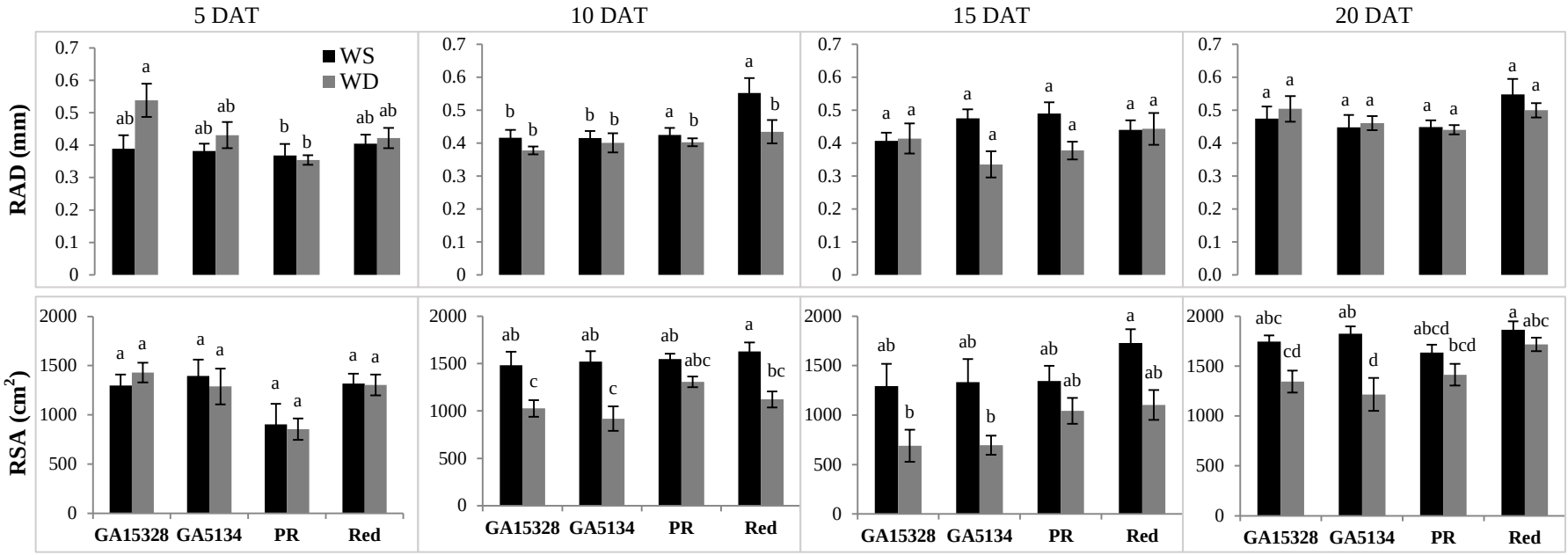


Figure 5.16 (a): The effect of root average diameter (RAD) and root surface area (RSA) at 5, 10, 15 and 20 days of drought stress (DAT) in four amaranth accessions.

The error bars indicate standard errors of means (SEM), WS is water-sufficient and WD is water-deficient and bars with the same letter are not statistically different between water treatment (WS and WD) and accession based on Tukey's Pairwise method ($P<0.05$).

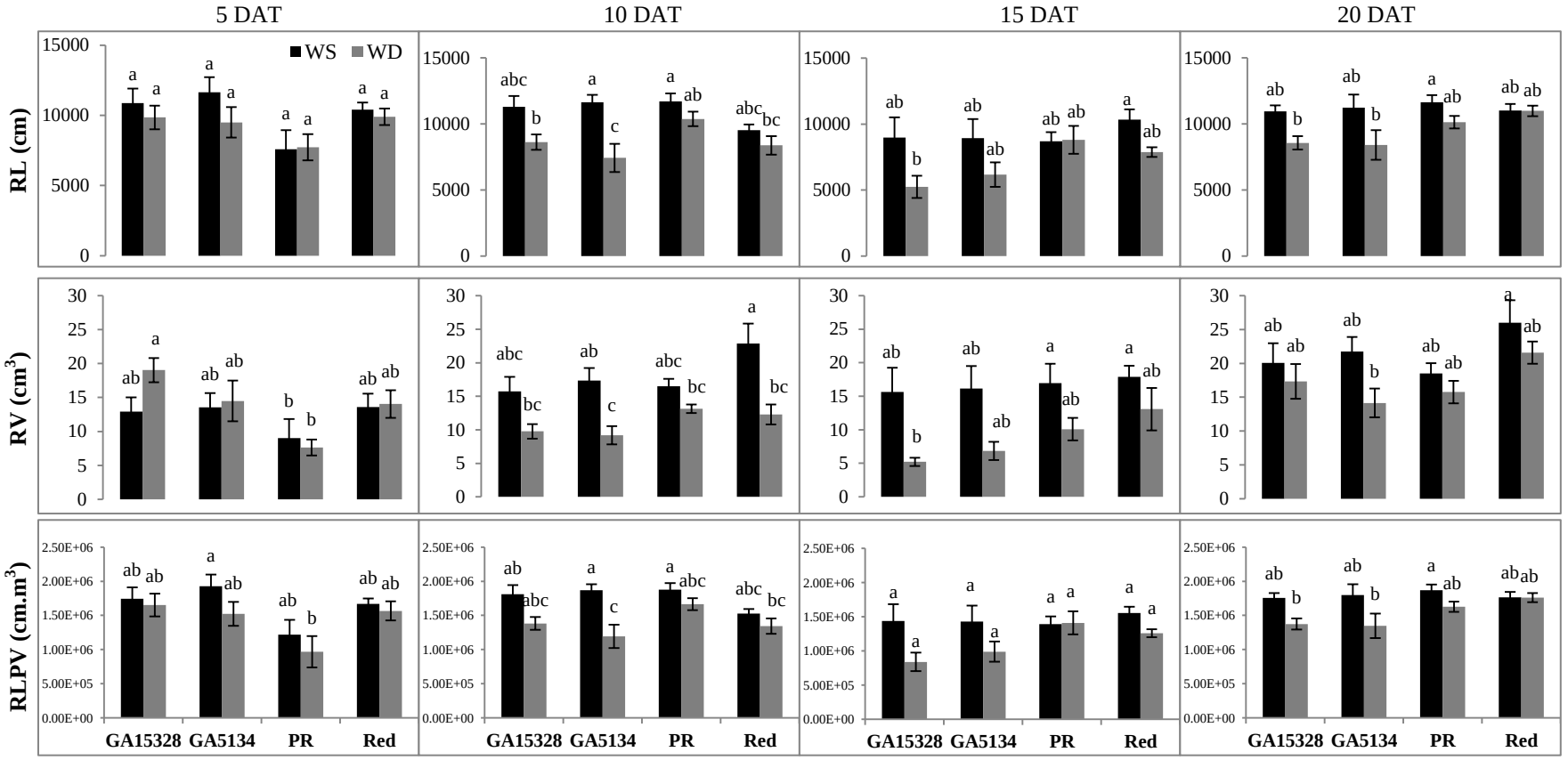


Figure 5.16(b): The effect of root length (RL), root volume (RV) and root length per volume (RLPV) at 5, 10, 15 and 20 days of drought stress (DAT) in four amaranth accessions.

The error bars indicate standard errors of means (SEM), WS is water-sufficient and WD is water-deficient and bars with the same letter are not statistically different between water treatment (WS and WD) and a based on Tukey's Pairwise method ($P<0.05$).

5.4.3.3 The effect of increased severity and duration of drought stress on plant physiology

TCC was only affected in GA15328 under drought stress throughout the experimental period (Table 5.8). Nonetheless, there was a clear decreasing trend in TCC of both green leaf amaranth under WS and WD treatment ($P<0.05$), while the TCC of both red leaf amaranth was maintained throughout the experiment. Despite of differences in pigmentation, the TCC of the two contrasting amaranth were very similar, ranging from 30 µg ml^{-1} to 50 µg ml^{-1} in both WS and WD treatments, with chlorophyll-a content higher than chlorophyll-b content in any drought stress condition.

The light response curve of each accession obtained at time 0 may predict and demonstrate the pattern of photosynthetic gas exchange measurements at corresponding PPFD (Table 5.10 a & b). The light response curve revealed that the Pnmax of the plants under high irradiance was greater for GA15328 (40.23 µmol $m^{-2}s^{-1}$), PR (48.88 µmol $m^{-2}s^{-1}$) and Red (46.86 µmol $m^{-2}s^{-1}$), compared to GA5134 (24.60 µmol $m^{-2}s^{-1}$) (Figure 5.17). As predicted, plants that respond well under high irradiance will have high light saturation points (LSP) and low quantum yield (mol CO_2. mol^{-1} quantum) (Table 5.9). At time 0, GA5134 also had the lowest Gs (range: 0.18-0.24 µmol H_20. $m^{-2}s^{-1}$) and E (range: 4.24-5.42 mmol H_20. $m^{-2}s^{-1}$), while Red had the highest (range: 0.25-0.32 µmol H_20. $m^{-2}s^{-1}$, 6.08-7.49 mmol H_20. $m^{-2}s^{-1}$ respectively). The Ci value of GA5134 was comparatively high together with Red (range: 88.2-142.3 µmol CO_2. mol^{-1}) compared to the other two accessions (range: 45.1-79.4 µmol CO_2. mol^{-1}).

There was a significant interaction between WT*TP*A for Pn ($P<0.01$), Gs ($P<0.01$), Ci ($P<0.05$) and E ($P<0.01$). Pn, Gs and E were significantly reduced over time as plant increased in size under WS and WD treatment in all amaranth accessions ($P<0.05$), and drought stress significantly influenced Pn at 15 DAT and 20 DAT ($P<0.05$) while Gs, E and Ci were unaffected at that time point. In general, photosynthetic measurements of amaranth accessions were maintained at 5 DAT under WS and WD treatment, but interestingly, WD plants of PR had significantly higher Pn, Gs and E compared to WS plants at 5 DAT and this may reflect the capability of PR to sustain its yield performance until 10 DAT under WD treatment (although no photosynthetic measurements were taken at 10 DAT). Further, the Gs of GA15328 was significantly reduced at 5 DAT before remained unaltered at further soil drying.

Significant interaction between WT*TP*A was observed in RWC ($P<0.05$), and RWC only affected at 10 DAT (Table 5.11). The green leaf amaranths rapidly reduced RWC as early as 10 DAT ($P<0.01$) (range: 70%-76% respectively), while red leaf amaranths retained comparatively high RWC at 10 DAT and only began to decline at 15 DAT (range 59%-61%). However, there was no difference among amaranth accessions in either WS or WD treatment at any time point. It is interesting to note that, the RWC of WD treatment can reach approximately 60% under severe drought stress, demonstrating that the amaranth accessions are capable of maintaining a high water status under severe drought stress.

Significant interaction between WT*TP*A was observed in free proline content ($P<0.01$) (Table 5.12). Green leaf amaranths showed no changes in proline content, while red leaf amaranths displayed variations in the adjustment of proline accumulation at each time point. For example, PR showed fluctuating proline accumulations, in which, the proline content of the WD plants (4.94 µmoles g^{-1}) increased approximately three-fold compared to the WS plants (1.36 µmoles g^{-1}) at 5 DAT, and remained unaffected at 10 DAT and 15 DAT before increasing again at 20 DAT by approximately 10-fold higher (WS: 0.63 µmoles g^{-1}, WD: 9.14 µmoles g^{-1}). Meanwhile, the proline content of Red was only affected at 15 DAT, with WD treatment having a three-fold increase (11.54 µmoles g^{-1}) compared to the WS treatment (4.41 µmoles g^{-1}).

5.4.3.4 Correlations

Correlation coefficients for all traits measured for WS and WD treatments are shown in Table 5.6. As yield was the sum of total of leaf and stem fresh weight, yield showed a positive correlation with LFW, LDW and SFW in both WS and WD treatments ($r >0.05$, $P<0.01$) and SDW was also positively correlated with yield under WS treatment ($r=0.79$, $P<0.01$), but not in WD treatment. The associations between physiological traits with yield were distinct in the two water treatments, except RFW ($r >0.4$, $P<0.01$). Under WS treatment, yield was positively correlated with RDW ($r=0.76$, $P<0.01$), R/S ($r=0.46$, $P<0.05$), RAD ($r=0.53$, $P<0.01$), RSA ($r=0.54$, $P<0.01$) and RV ($r=0.58$, $P<0.01$). In comparison, under WD treatment, yield was positively correlated with Pn ($r=0.41$, $P<0.05$), RL ($r=0.38$, $P<0.05$) and TLA ($r=0.53$, $P<0.01$). Lastly, there was no negative correlation between any physiological traits with yield in both WS and WD treatments.

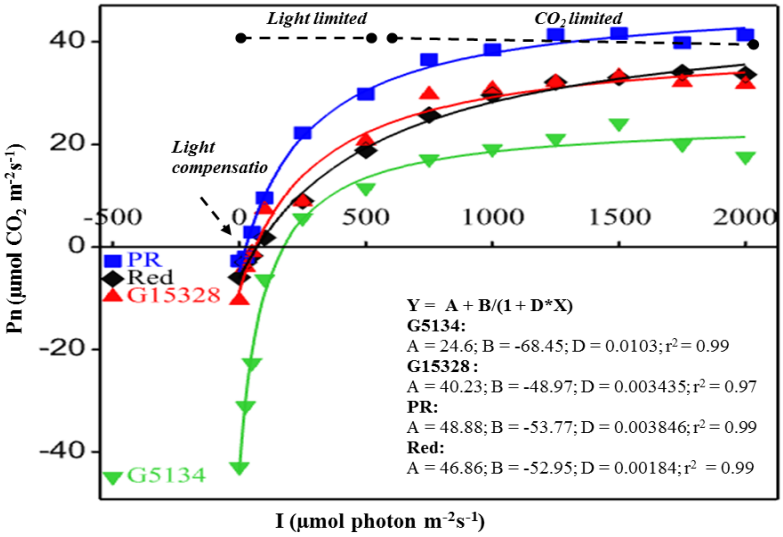


Figure 5.17: Light response curves of net assimilation rate (Pn/I) of four amaranth accessions at time 0. The non-linear regression curve was obtained through rectangular hyperbolic model based on Michaelis-Menten (1913).

Table 5.8: Maximum light saturated rate of photosynthesis (Pn_{max}), quantum yield of photosynthesis (Pn_q), photosynthetic light compensation point (LCP) and light saturation point (LSP) of four amaranth accessions.

Accessions	**Pn_{max}** (μmol $m^{-2}s^{-1}$)	**Pn_q** (mol CO_2/mol quantum)	**LCP** (μmol $m^{-2}s^{-1}$)	**LSP** (μmol $m^{-2}s^{-1}$)
GA5134	24.6	0.0103	173	97
GA15328	40.2	0.0034	63.2	291
PR	48.8	0.0038	26.0	260
Red	46.8	0.0018	70.6	543

Table 5.9: The effect of prolonged soil drying with increased drought severity on total chlorophyll content (TCC) in four amaranth accessions at 5, 10, 15 and 20 days of drought stress treatment (DAT).

TCC (µg ml^{-1})								
Time point (DAT)	GA15328		GA5134		PR		Red	
	WS	WD	WS	WD	WS	WD	WS	WD
0	54.69 ± 0.96 a;z	53.75 ± 1.76 a;z	52.45 ± 1.74 ab;z	55 ± 1.45 a;z	47.26 ± 3.07a;z	48.09 ± 4.72 a;z	43.95 ± 2.99 a;z	45.49 ± 2.25 a;z
5	51.2 ± 1.22 ab;z	43.16 ± 4.75 abc;z	39.87 ± 2.52 a-d;z	46.36 ± 2.89 abc;z	41.87 ± 6.17 a;z	43.88 ± 1.67 a;z	40.34 ± 3.62 a;z	39.26 ± 3.49 a;z
5	38.65 ± 2.60 bc;z	33.81 ± 5.14 c;z	31.61 ± 5.09 cd;z	39.13 ± 4.97 a-d;z	32.34 ± 3.67 a;z	38.51 ± 4.30 a;z	38.11 ± 3.62 a;z	37.38 ± 5.32 a;z
20	34.65 ± 1.63 c;yz	41.06 ± 3.86 bc;z	23.97 ± 3.92 d;y	37.69 ± 3.25 bcd;yz	31.4 ± 3.60 a;yz	43.87 ± 3.58 a;z	39.33 ± 5.72 a;yz	44.88 ± 3.36 a;z
Chla								
0	32.94 ± 0.56	32.39 ± 1.03	31.63 ± 1.47	33.12 ± 0.85	28.61 ± 1.79	29.09 ± 2.75	26.68 ± 1.74	27.58 ± 1.31
5	30.91 ± 0.71	26.23 ± 2.77	24.31 ± 2.97	28.09 ± 1.68	25.47 ± 3.59	26.64 ± 0.97	24.58 ± 2.11	23.96 ± 2.03
15	23.60 ± 1.51	20.78 ± 2.99	19.50 ± 2.28	23.88 ± 2.89	19.93 ± 2.14	23.52 ± 2.51	23.29 ± 2.11	22.86 ± 3.10
20	21.27 ± 0.95	20.25 ± 2.25	15.05 ± 1.52	23.04 ± 1.89	19.38 ± 2.10	26.64 ± 2.08	23.99 ± 3.33	27.22 ± 1.95
Chlb								
0	25.44 ± 0.56	24.89 ± 1.03	24.13 ± 1.47	25.62 ± 0.85	21.1 ± 1.80	21.58 ± 2.76	19.16 ± 1.75	20.06 ± 1.32
5	23.40 ± 0.71	18.70 ± 2.78	16.77 ± 2.98	20.57 ± 1.69	17.94 ± 3.61	19.12 ± 0.98	17.05 ± 2.12	16.42 ± 2.04
15	16.06 ± 1.52	13.23 ± 3.01	11.94 ± 2.29	16.34 ± 2.91	12.37 ± 2.15	15.98 ± 2.52	15.75 ± 2.12	15.32 ± 3.11
20	13.72 ± 0.95	17.47 ± 2.26	7.48 ± 0.52	15.5 ± 1.90	11.82 ± 2.11	19.11 ± 2.09	16.46 ± 3.35	19.7 ± 1.96
SED	2.79		5.69		4.89		5.21	
LSD	3.94		12.33		10.60		10.73	
P (DAT*WT)	<0.01		0.30		0.11		0.75	

Data represent the mean ± SEM (standard errors of means), WS is water-sufficient and WD is water-deficient, SED is standard error of difference between two means, LSD is least significant differences of means and P is probability (P-value) between DAT and water treatments (WT: WS and WD) significantly different at P<0.05. Values in columns identified with the same letter (a, b, c) are not statically different between DAT and WT within accession and values in rows identified with the same letter (x, y, z) are not statistically different between accession and WT at each time point based on Tukey's Pairwise method (P<0.05) respectively.

Table 5.10 (a): The effect of prolonged soil drying with increased drought severity on net photosynthesis (Pn) and stomatal conductance (Gs) intracellular CO_2 (Ci) in four amaranth accessions at 5, 10, 15 and 20 days of drought stress treatment (DAT).

Pn (µmol $m^{-2}s^{-1}$)								
Time point (DAT)	GA15328		GA5134		PR		Red	
	WS	WD	WS	WD	WS	WD	WS	WD
5	42.17 ± 1.37 a;yz	41.4 ± 0.65 ab;yz	31.95 ± 1.64 a;x	35.51 ± 2.04 a;xy	41.53 ± 1.76 a;yz	40.76 ± 1.20 a;yz	39.82 ± 1.03 ab;yz	43.69 ± 1.53 a;z
10	36.37 ± 3.57 abc;z	33.05 ± 2.64 bcd;z	25.07 ± 4.76 abc;z	29.95 ± 2.79 a;z	22.32 ± 3.58 cd;z	34.98 ± 2.17 ab;z	31.91 ± 2.33 cd;z	32.59 ± 1.85 bc;z
15	26.26 ± 0.96 d;z	15.87 ± 1.71 e;y	26.41 ± 0.98 ab;z	14.22 ± 1.00 bc;y	26.84 ± 0.82 bc;z	13.78 ± 1.99 d;y	24.64 ± 1.54 d;z	15.12 ± 1.18 e;y
20	27.76 ± 0.91 cd;z	12.03 ± 0.66 e;y	25.22 ± 1.93 abc ;z	13.21 ± 4.37 c;y	25.33 ± 2.08 bc;z	14.93 ± 2.13 d;y	25.21 ± 1.24 cd;z	12.46 ± 1.66 e;y
SED	2.51		4.12		4.12		2.15	
LSD	5.22		8.82		8.59		4.53	
P (DAT*WT)	<0.05		<0.05		<0.05		<0.01	
Gs (mol H_2O $m^{-2}s^{-1}$)								
Time point (DAT)	GA15328		GA5134		PR		Red	
	WS	WD	WS	WD	WS	WD	WS	WD
5	0.21 ± 0.01 ab;xy	0.24 ± 0.01 ab;xy	0.18 ± 0.01 a;x	0.24 ± 0.01 a;xy	0.22 ± 0.01 a;xy	0.22 ± 0.01 a;xy	0.25 ± 0.02 b;y	0.32 ± 0.01 a;z
10	0.25 ± 0.04 a;z	0.16 ± 0.02 b;yz	0.19 ± 0.05 a;yz	0.18 ± 0.01 a;yz	0.12 ± 0.01 b;y	0.25 ± 0.01 a;z	0.19 ± 0.02 b;yz	0.23 ± 0.02 b;yz
15	0.04 ± 0.01 c;z	0.05 ± 0.02 c;z	0.04 ± 0.00 b;z	0.03 ± 0.00 b;z	0.05 ± 0.01 c;z	0.03 ± 0.01 c;z	0.03 ± 0.01 c;z	0.03 ± 0.00 c;z
20	0.06 ± 0.01 c;z	0.01 ± 0.00 c;z	0.06 ± 0.03 b;z	0.03 ± 0.01 b;z	0.05 ± 0.02c;z	0.04 ± 0.02 c;z	0.03 ± 0.01 c;z	0.02 ± 0.01 c;z
SED	0.02		0.01		0.03		0.02	
LSD	0.03		0.03		0.06		0.04	
P (DAT*WT)	0.53		0.60		0.38		0.60	

Data represent the mean ± SEM (standard errors of means), WS is water-sufficient and WD is water-deficient, SED is standard error of difference between two means, LSD is least significant differences of means and P is probability (P-value) between DAT and water treatments (WT: WS and WD) significantly different at P<0.05. Values in columns identified with the same letter (a, b, c) are not statically different between DAT and WT within accession and values in rows identified with the same letter (x, y, z) are not statistically different between accession and WT at each time point based on Tukey's Pairwise method (P<0.05) respectively.

Table 5.10 (b): The effect of prolonged soil drying with increased drought severity on intracellular CO_2 (Ci) and transpiration (E) in four amaranth accessions at 5, 10, 15 and 20 days of drought stress treatment (DAT).

Ci (µmol CO_2 mol^{-1})								
Time point (DAT)	GA15328		GA5134		PR		Red	
	WS	WD	WS	WD	WS	WD	WS	WD
5	45.10 ± 5.18 a;v	79.4 ± 9.85 a;vwx	88.2 ± 4.84 a;wxy	125.3 ± 7.12a;yz	60.3 ± 6.78 ab;vw	68.10 ± 12.21 ab;vwx	104.7 ± 12.53 a;xyz	142.3 ± 6.48 a;z
10	119.5 ± 15.96 a;yz	34.80 ± 10.04 a;y	142.8 ± 20.58 a;z	111.3 ± 11.96 a;yz	82.1 ± 22.37 ab;z	137.6 ± 9.25 ab;z	212.6 ± 18.17 a;z	135.4 ± 29.84 a;z
15	145 ± 10.87 a;z	159.3 ± 59.96 a;z	108.1 ± 27.88 a;z	184.2 ± 41.52 a;z	168.9 ± 17.02 a;z	188.1 ± 52.29 ab;z	137.60 ± 32.66 ab;z	135.40 ± 44.67 a;z
20	88.4 ± 12.83 a;z	181.5 59.72 a;z	186.5 ± 29.53 a;z	*	212.6 ± 81.79 a;z	25.9 ± 8.63 b;z	92.2 ± 22.99 b;z	192 ± 53.18 ab;z
SED	44.44		42.60		42.10		52.70	
LSD	95.74		89.60		89.00		109.70	
P (DAT*WT)	0.44		0.11		0.44		0.02	
E (mmol H_2O $m^{-2}s^{-1}$)								
Time point (DAT)	GA15328		GA5134		PR		Red	
	WS	WD	WS	WD	WS	WD	WS	WD
5	6.03 ± 0.20 a;yz	6.43 ± 0.22 a;yz	4.24 ± 0.31 a;x	5.42 ± 0.28 a;xy	5.89 ± 0.35 a;y	5.95 ± 0.25 a;y	6.08 ± 0.60 ab;yz	7.49 ± 0.20 a;z
10	5.63 ± 0.83 ab;z	3.92 ± 0.26 b;z	4.29 ± 0.88 a;z	4.13 ± 0.38 a;z	3.42 ± 0.10 b;z	5.69 ± 0.28 a;z	5.14 ± 0.40 b;z	5.57 ± 0.35 b;z
15	1.12 ± 0.23 c;z	1.41 ± 0.53 c;z	0.83 ± 0.09 b;z	0.74 ± 0.08 b;z	1.39 ± 0.23 c;z	0.67 ± 0.21 c;z	0.85 ± 0.31 c;z	0.81 ± 0.14 c;z
20	1.47 ± 0.17 c;z	0.28 ± 0.09 c;y	1.30 ± 0.14 b;z	0.67 ± 0.25 b;yz	1.29 ± 0.34 c;yz	0.49 ± 0.16 c;yz	0.74 ± 0.15 c;yz	0.58 ± 0.32 c;yz
SED	0.40		0.43		0.62		0.39	
LSD	0.83		0.89		1.29		0.81	
P (DAT*WT)	0.10		0.03		0.32		0.01	

Data represent the mean ± SEM (standard errors of means), WS is water-sufficient and WD is water-deficient, SED is standard error of difference between two means, LSD is least significant differences of means and P is probability (P-value) between DAT and water treatments (WT: WS and WD) significantly different at $P<0.05$. Values in columns identified with the same letter (a, b, c) are not statically different between DAT and WT within accession and values in rows identified with the same letter (x, y, z) are not statistically different between accesion and WT at each time point based on Tukey's Pairwise method ($P<0.05$) respectively.

Table 5.11: The effect of prolonged soil drying with increased drought severity on relative water content (RWC) and free proline content in four amaranth accessions at 5, 10, 15 and 20 days of drought stress treatment (DAT).

RWC								
Time point (DAT)	GA15328		GA5134		PR		Red	
	WS	WD	WS	WD	WS	WD	WS	WD
5	87.68 ± 3.50 ab;z	78.5 ± 5.07 bc;z	90.07 ± 1.89 a;z	83.37 ± 2.59 a;z	80.76 ± 3.73 ab;z	73.49 ± 6.99 ab;z	88.23 ± 6.63 ab;z	70.76 ± 4.54 ab;z
10	90.46 ± 1.61 a;yz	70.31 ± 6.01 bc;y	91.81 ± 2.78 a;z	76.78 ± 7.00 b;yz	84.49 ± 3.89 a;yz	84.53 ± 3.63 a;yz	82.33 ± 5.26 a;yz	82.1 ± 5.24 a;yz
15	86.48 ± 2.80 a;z	68.01 ± 4.66 c;yz	83.72 ± 3.54 bc;z	55.83 ± 2.33 c;yz	80.52 ± 5.13 a;z	61.06 ± 1.58 b;yz	84.78 ± 2.61 ab;z	58.79 ± 1.31 c;yz
20	81.76 ± 1.63 ab;yz	66.27 ± 5.65 c;xy	87.05 ± 1.58 a;z	60.29 ± 6.76 bc;x	86.27 ± 4.76 a;z	71.77 ± 6.29 b;xyz	82.8 ± 1.36 a;yz	60.01 ± 3.93 c;x
SED	0.06		0.06		0.06		0.06	
LSD	0.12		0.13		0.13		0.13	
P (DS*WT)	<0.001		0.04		0.02		0.02	
Proline (µmoles g^{-1})								
Time point (DAT)	GA15328		GA5134		PR		Red	
	WS	WD	WS	WD	WS	WD	WS	WD
5	1.46 ± 0.73 a;z	3.92 ± 1.03 a;z	2.51 ± 1.00 ab;z	7.78 ± 2.51 a;z	1.36 ± 0.49 b;z	4.94 ± 1.33 a;z	1.74 ± 0.61 b;z	3.55 ± 0.60 b;z
10	2.27 ± 1.38 a;z	2.13 ± 1.00 a;z	4.35 ± 1.00 ab;z	1.81 ± 0.97 ab;z	5.71 ± 2.20 ab;z	2.13 ± 1.27 ab;z	2.86 ± 1.12 b;z	4.43 ± 2.10 b;z
15	1.62 ± 0.41 a;y	3.17 ± 0.61 a;y	2.03 ± 0.77 ab;y	4.80 ± 1.19 ab;y	4.01 ± 0.32 ab;y	2.61 ± 1.06 ab;y	4.41 ± 1.04 b;y	11.54 ± 2.46 a;z
20	1.67 ± 0.46 a;y	3.84 ± 0.81 a;z	0.55 ± 0.37 b;y	6.15 ± 2.87 ab;yz	0.63 ± 0.14 b;y	9.14 ± 3.73 a;z	1.00 ± 0.53 b;y	6.25 ± 1.64 ab;yz
SED	1.33		1.94		2.10		1.94	
LSD	2.97		4.06		4.40		4.04	
P (DAT*WT)	0.35		0.06		0.01		0.24	

Data represent the mean ± SEM (standard errors of means), WS is water-sufficient and WD is water-deficient, SED is standard error of difference between two means, LSD is least significant differences of means and P is probability (P-value) between DAT and water treatments (WT: WS and WD) significantly different at P<0.05. Values in columns identified with the same letter (a, b, c) are not statically different between DAT and WT within accessions and values in rows identified with the same letter (x, y, z) are not statistically different between accession and WT at each time point based on Tukey's Pairwise method (P<0.05) respectively.

Table 5.12: Correlation coefficients (r) for traits associated with water-sufficient (WS) in the bottom diagonal and water-deficient (WD) in the top diagonal for the four amaranth accessions.

Traits	Ci	E	Gs	LDW	LFW	Pn	Proline	R/S	RAD	RDW	RFW	RL	RLPV	RSA	RV	RWC	SDW	SFW	SLA	TCC	TLA	Yield
Ci	1	-0.12	-0.09	-0.12	0.22	-0.09	0.01	0.22	0.23	0.32*	0.29	0.08	0.04	0.21	0.25	0.24	0.23	-0.13	0.04	0.06	-0.1	0.01
E	*-0.39	1	0.99**	-0.07	-0.21	-0.01	0.07	-0.26	-0.24	*-0.41	**-0.52	*-0.4	*-0.44	**-0.44	*-0.36	0.01	-0.16	0.06	0.31	0.07	0.07	-0.06
Gs	*-0.43	0.98**	1	-0.08	-0.21	-0.03	0.08	-0.28	-0.28	*-0.41	**-0.52	*-0.39	**-0.47	**-0.45	**-0.38	0.04	-0.13	0.08	0.3	0.08	0.05	-0.05
LDW	0.02	-0.22	-0.18	1	0.6**	0.67**	-0.09	*-0.4	0.02	-0.22	0.24	0.37*	0.33*	0.25	0.12	0.01	*-0.36	0.41*	0.05	-0.02	0.74**	0.68**
LFW	0.12	-0.34	-0.25	0.36*	1	0.57**	-0.11	-0.06	0.23	0.03	0.16	0.4**	0.29	0.43**	0.37*	0.05	-0.23	-0.03	0.11	-0.07	0.56**	0.52**
Pn	0.09	-0.01	-0.01	0.39*	0.06	1	-0.25	**-0.5	0.02	**-0.54	-0.05	0.14	0.02	0.07	0.02	0.01	**-0.64	0.12	**0.51	0.07	0.84**	0.41*
Proline	-0.16	0.51**	0.52**	0.06	-0.24	0.07	1	0.12	-0.15	0.12	-0.21	-0.06	0.03	-0.12	-0.14	-0.20	0.01	-0.1	-0.1	0.06	-0.1	-0.15
R/S	-0.33	0	0.11	0.2	0.49**	-0.1	-0.01	1	0.49**	0.87**	0.39*	0.25	0.31	0.49**	0.55**	-0.18	0.2	-0.29	**-0.55	-0.1	**-0.58	-0.28
RAD	-0.19	0.29	0.32	0.18	0.35	-0.11	0.16	0.52**	1	0.46**	0.22	0.15	0.22	0.67**	0.89**	0.16	0.03	*-0.35	-0.36	-0.14	-0.17	-0.17
RDW	-0.25	-0.03	0.05	0.36*	0.66**	0.01	-0.04	0.85**	0.6**	1	0.64**	0.46**	0.52**	0.63**	0.61**	-0.07	0.53**	-0.07	**-0.66	-0.14	**-0.55	-0.04
RFW	-0.34	0.05	0.12	0.3	0.58**	0	0.08	0.82**	0.7**	0.93**	1	0.59**	0.65**	0.56**	0.42*	-0.02	0.41*	0.41*	*-0.43	-0.04	-0.12	0.44**
RL	0.13	*-0.45	*-0.44	0.07	0.35	0.28	-0.36	0.24	-0.12	0.24	0.17	1	0.8**	0.82**	0.56**	0.16	0.15	0.18	*-0.34	-0.05	0.1	0.38*
RLPV	0.14	*-0.45	*-0.45	0.06	0.37*	0.3	-0.31	0.19	-0.16	0.2	0.14	0.99**	1	0.73**	0.54**	0.06	0.26	0.19	*-0.41	-0.12	0.02	0.32*
RSA	-0.07	-0.17	-0.15	0.1	0.51**	0.14	-0.2	0.51**	0.55**	0.6**	0.61**	0.7**	0.66**	1	0.93**	0.22	0.16	-0.1	**-0.45	-0.11	-0.04	0.16
RV	-0.11	-0.02	0.01	0.22	0.52*	0.01	-0.02	0.59	0.84**	0.68**	0.73**	0.4*	0.36*	0.88**	1	0.22	0.13	-0.26	**-0.43	-0.14	-0.12	-0.02
RWC	-0.09	-0.17	-0.17	0.05	0.12	-0.16	-0.04	0.12	-0.06	0.05	0.02	0.23	0.23	0.06	0.06	1	0.11	-0.07	-0.04	0.02	-0.06	-0.03
SDW	-0.17	0.16	0.17	-0.02	0.48*	-0.09	-0.13	0.41*	0.51**	0.75**	0.71**	0.15	0.12	0.53**	0.55**	-0.07	1	0.22	**-0.41	-0.02	**-0.58	0.06
SFW	-0.36	0.31	0.29	0.1	-0.12	0.14	0.21	0.21	0.38*	0.44*	0.53**	-0.03	-0.04	0.29	0.33	-0.29	0.59	1	0.02	0.14	0.25	0.83**
SLA	0.15	-0.34	-0.35	-0.36	-0.28	-0.03	-0.03	-0.23	*-0.47	*-0.43	*-0.41	0.12	0.16	-0.24	*-0.39	0.21	*-0.46	-0.2	1	0.12	0.66**	0.08
TCC	0.01	0.25	0.25	-0.13	-0.36	-0.03	0.33	-0.03	-0.24	-0.18	-0.18	-0.33	-0.32	*-0.38	*-0.37	0.14	-0.22	-0.07	0.08	1	0.03	0.08
TLA	0.15	*-0.43	*-0.42	0.36	0.02	0.33	0.07	-0.13	-0.27	*-0.16	*-0.16	0.23	0.27	-0.08	-0.14	0.25	*-0.42	-0.07	0.7**	-0.08	1	0.53**
Yield	-0.26	0.09	0.13	0.29	0.44*	0.16	0.06	0.46*	0.53**	0.76**	0.8**	0.17	0.17	0.54**	0.58**	-0.2	0.79**	0.84**	-0.34	-0.25	-0.05	1

Ci: intracellular CO_2, E: transpiration, Gs: stomatal conductance, LDW: leaf dry weight, LFW: leaf fresh weight, Pn: photosynthesis, R/S: root to shoot ratio, RAD: root average diameter, RDW: root dry weight, RFW: root fresh weight, RL: root length, RLPV: root length per volume, RSA: root surface area, RV: root volume, RWC: relative water content, SDW: stem dry weight, SFW: stem fresh weight, SLA: specific leaf area, TCC: total chlorophyll content and TLA: total leaf area

5.4.4 Discussion

5.4.4.1 Growth performance is related to differences in biomass allocation patterns

Understanding plant performance and the underlying genetic architecture in various water regimes is important. Trait phenotyping in amaranth is therefore necessary to ascertain the association between amaranth variability and potential yield under water deficit that override the effectiveness of drought tolerance characteristics. In this experiment, the plant physiological parameters were altered in varying degrees throughout the experimental period under control and drought stress conditions. The photosynthesis and yield performance was not affected by mild drought stress (50% WHC), similar to the previous studies carried out by Slabbert and Kruger (2011, 2014) and Liu and Stützel (2004). Although the accessions did not differ significantly with respect to total yield under control and drought stress conditions at any time point, there was a difference observed on the timing of stress responses among the amaranth accessions at which the yield performance started to decline during water-deficit. This was observed in PR, in which the yield was only reduced at severe drought stress (15 DAT, 30% WHC), while Red and the two green leaf amaranth had reductions in yield earlier, at moderate drought stress (10 DAT, 40% WHC). Based on previous study (subheading 5.3: Jamalluddin *et al*., 2018), the differences on the timing of stress responses on yield performance between the two red leaf amaranth could be due to the differences in FTSW threshold, in which PR was able to maintain transpiration in drier conditions, compared to Red.

Drought stress constraints leaf growth to much greater extent than the roots in this experiment. The reduction of yield under drought stress was mainly due to a reduction in TLA. Luoh *et al*., (2014) reported that drought stress inhibits cell expansion and promotes leaf senescence in *A. hypochondriacus* and *A. cruentus*, which later results in decreased transpiration rate, moderating water-use efficiently and thus, reduce cells injury under water stress (Blum, 2004; Mitchell *et al*., 1998). The increased of R/S ratio is also considered to be a drought-tolerance strategy as it enables greater water and nutrient up-take by maximizing absorptive root surfaces (Narayanan *et al*., 2014) and improve water use efficiency (Morison *et al*., 2008). However, a large root system would consume more photosynthetic end products for their own growth and negate shoot growth (Bramley *et al*., 2009). In this study, R/S ratio was only positively correlated with yield under control conditions and an

increased in R/S under drought stress reduces the photosynthetic rate and yield. This reflects on the growth of the two accessions, GA15328 and Red, in which R/S was significantly increased during drought stress, and this may be one of the possible reasons for the reduced yield at early drought stress for these two accessions.

The proportion of above-ground biomass allocations into leaves and stems may also influence the growth performance of amaranth during drought stress. This is because, photosynthetic rate was positively correlated with leaf dry mass but negatively correlated with stem dry mass under drought stress condition in this study. Under drought stress, red leaf amaranths had an equal biomass allocation into leaves and stems, and relatively high in roots. This suggests that the red leaf amaranth had a capacity to distribute nitrogen absorbed by the roots to be equally transported to the leaves, possibly for leaf maintenance or growth during drought stress (Irving, 2015). In contrast, the green leaf amaranth had a biomass allocation primarily into stems and is relatively low level in leaves and roots. This may indicate that these amaranth accessions aimed to store solutes such as amino acids and carbohydrates in stems to be used when water is available, as reported in Vargas-Ortiz *et al*., (2013). Although GA5134 and PR were able to maintain R/S under drought stress conditions at similar levels to well-watered conditions, this led to yield loss in GA5134 faster than PR, a similar result was found in red leaf *A. tricolor* variety in a study conducted by Liu and Stützel (2004).

5.4.4.2 Does root morphology regulate growth performance?

In this study, a destructive approach, i.e WinRHIZO system was used to investigate the effect of gradual drought stress on root growth. For washed root samples, manual settings including washing, cleaning and untangling may have resulted into a large loss of root materials (up to 40%) and compromises certain analyses such as root positioning (Oliveira *et al*., 2000). Nevertheless, this technique is able to show the whole root system (Benjamin and Nielsen, 2006) with parameters such as root length and root diameter which are crucial in water stress adaptation (Osmont *et al*., 2007), and had better root overlap correction efficacy (Meng-Ben and Qiang, 2009).

The changes of root components depend on plant species, cultivar and drought conditions, and not all changes contributed to improved water use

efficiency (Li *et al*., 2011). The pattern of changes among amaranth accessions on specific root components were obvious at 10 DAT (40% WHC), which might be related to regulating photosynthetic demands in leaves and to minimize the energy requires for root construction and maintenance (Lambers *et al*., 2002). In this study, RAD and RSA was positively correlated with other root traits and R/S in both WS and WD treatment. However, high RAD and RSA were associated with better yield under control conditions, but not in drought stress conditions. RSA depends on root hair development, root diameter (Koevoets *et al*., 2016) and root length (Meng-Ben and Qiang 2009). Thus, it seems reasonable that under control conditions, the roots of amaranth accessions were shorter and thicker, similar to spinach (Ors and Suarez, 2017).

Deep rooting was found to be a critical factor for drought resistance as it may influence the ability of the plant to absorb water from deeper layers of the soils (Ors and Suarez, 2017; Franco *et al*., 2008, 2006). In this study, high RL is associated with low stomatal conductance and leaf transpiration rate, and better yield productions under drought stress. RL were varied among amaranth accessions, especially at 10 DAT, in which PR exhibited the highest RL and GA5134 the lowest. A greater RL at this specific time point could be important for PR to stimulate resistance mechanisms by stomatal closing, reducing transpiration and increasing water absorption to regulate photosynthetic assimilation, hence, improved yield production (Ors and Suarez, 2017).

5.4.4.3 Chlorophyll content is less likely to influence variation in leaf gas exchange

The influence of drought stress on C4 photosynthesis was extensively discussed in Liu and Osborne (2015) and Ghannoum (2009) with an emphasis on stomatal and non-stomatal limitation in regulating photosynthesis. It is clear that, in this study, photosynthesis is not dependent on stomatal conductance under drought stress, hence non-stomatal factors such as reduced cellular water status and chlorophyll content amongst others, might be involved (Ghannoum et al., 2009).

Chlorophyll pigmentation is one of the major chloroplast components for photosynthesis, and its alteration upon water-deficit was highly associated with photosynthetic rate (Singh *et al*., 2017; Guo and Li, 1996). Many studies indicated that a stay-green trait has improved yield and transpiration efficiency under water-

limited conditions, including in wheat (Hausemann *et al*., 2002) and sorghum (Verma *et al*., 2004). However, in this study, chlorophyll content was not affected by drought stress, similar results obtained in previous study (subheading 5.3: Jamalluddin *et al*., 2018), but contradicted with findings reported by Jomo *et al*., (2016) and Nakashima *et al*., (2011), in which chlorophyll content reduced under drought stress in different amaranth species. Similar results were observed in some crops such as spinach (Ors and Suarez, 2017) and ornamental shrubs (Toscano *et al*., 2016), whereby chlorophyll content was not closely associated with photosynthesis and yield under drought stress. In contrast, a reduction of chlorophyll content under drought stress was observed in sugar cane (Jangpromma *et al*., 2010) and rice (Singh *et al*., 2017), and has been used as a reliable indicator for screening barley germplasm (Rong-hua *et al*., 2006). This could be due to a degradation of chloroplast membrane, as a result of reactive oxygen species (ROS) activity that causes lipid peroxidation (Jaleel *et al*., 2009).

Nevertheless, it was suggested that the changes of chlorophyll content depended on the degree of drought, plant species, type of cultivar, the timing of drought imposed to the crops (Jangpromma *et al*., 2010) and duration (Toscano *et al*., 2014; Pérez-Pérez *et al*., 2007). Although Shukla *et al*., (2006) and Tsutsumi *et al*., (2017) found out that chlorophyll content and stomatal conductance was positively correlated with photosynthesis in amaranth species under control conditions, the involvement of these two traits may not necessarily be crucial for regulating photosynthetic rate under drought stress. These results reinforce that chlorophyll content cannot be used for drought tolerance parameter in *A. tricolor*, as different cultivars and drought conditions revealed a consistent finding that chlorophyll content was not affected by drought (Jamalluddin *et al*., 2018).

The comparable chlorophyll content in the green and red leaf amaranth suggested that the potential of light harvesting capacities in both leaves were similar, hence the potential risk of photoinhibition was roughly equal (Nakashima *et al*., 2011). Nevertheless, Shao *et al*., (2013) reported that red leaf of *A. tricolor* had a higher tolerance to oxidative damage and photoinhibition was less severe compared to green leaf. Photoinhibition occurs due to the damage of PSII reaction centres, or development of slowly relaxing excitation energy quenching, which resulted from excess intracellular CO_2 (Baker and Rosenqivist, 2004). Abiotic stresses had increased betacyanin content in red leaf amaranth (Khanam and Oba *et*

al., 2014; Shao *et al*., 2013; Ali *et al*., 2009), and this may contributes to the increased of total photoprotective capacity by lowering excitation pressure on PSII via attenuation of potentially harmful excess incident light when stressed (Nakashima *et al*., 2011).

Furthermore, the behaviour and adaptability of photosynthetic rate in amaranth is also dependent on the nitrogen content in leaves. Low photosynthetic rate at lower saturating PPFD was observed in old leaves and shaded-grown young leaves of *A. caudatus*, presumably due to lower nitrogen content in the leaves (El-Sharkawy *et al*., 1968). In this study, GA5134 exhibited the lowest photosynthetic rate at low PPFD among amaranth accessions, similar responses were observed in shady plant, *A. longicaulis* when exposed to high irradiance (Li *et al*., 2014). These findings might suggest that lower nitrogen content reduces the Rubisco carboxylation activity and hence, triggers a leakage of CO_2 photorespiratory and dark respiratory (El-Sharkawy *et al*., 2016). This reveals that further light irradiance upon drier soil water condition may elevate photoinhibition in the plants.

5.4.4.4 RWC as an indicator of plant water status and proline accumulation

The RWC determines leaf water balance in plants during water deficit periods (Uzildaya *et al*., 2012), and estimates the percentage of water in the leaf as a fraction of the total volumetric water that the leaf can hold at full turgor (Blum, 1998). In this experiment, green leaf amaranth immediately reduced RWC (approximately to 70%) at 40% WHC while red leaf amaranth reduced RWC at 30%WHC. *A. tricolor* has been shown to have a high RWC (77%) under severe drought compared to other amaranth species, *A. hybridus* (48%) and *A. hypochondriacus* (33%) (Slabbert and Krüger, 2014). It is interesting to note that, the drop of RWC for WD treatment only reached approximately 60% while other crops normally have a reduced RWC of 30%–20% during severe drought stress (Gindaba *et al*., 2004; Ogbonnaya *et al*., 1998). Some studies have suggested that high RWC is closely related to drought resistance, as observed in a cowpea landrace (Zegaoui *et al*., 2018), arabidopsis (Bac-Molenaar *et al*., 2016) and beans (Rosales *et al*., 2011). Besides, RWC has been successfully used as a screening tool for selecting valuable genotypes in potato (Soltys-Kalina *et al*., 2016). However, RWC was not closely related to drought tolerance in this study, similar studies

observed in maize hybrid, in which RWC may serve as indicator for plant water status but not as a drought resistance parameter (Chen *et al*., 2016).

Nevertheless, RWC is vital to ensuring an accurate assessment of the relative capacity for osmotic adjustment (OA) (Blum, 2016; Sanders and Arndt, 2012). OA is an indicator of plant survival through cell turgor or stomatal conductance, and has also been recognized as a prime adaptive trait for higher yield under drought stress across diverse crops (Blum, 2016). High RWC was associated with high OA in some crops such as castor bean (Babita *et al*., 2010) and sunflower (Rauf and Sadaqat, 2008). OA is regulated through accumulation of organic solutes (such as proline, glycine betaine and total soluble sugar) or inorganic ions (such as K^+ and Ca^{2+}). These solutes may be obtained from inorganic salts in soil and from product of photosynthesis which helps to protect cellular proteins, enzymes and cellular membrane against cell dehydration (Zivcak *et al*., 2016). Proline accumulation is considered as a general marker of drought tolerance (Liu *et al*., 2011) as it permits OA, and negative correlation has been found between OA and RWC under drought stress in faba beans (El-Harty *et al*., 2016) and ornamental shrubs (Toscano *et al*., 2016). This demonstrates that the synthesis of proline became higher as soon as RWC declined. However, in this study, proline content only showed a weak negative and non-significant correlation with RWC under drought stress, and this may suggest that proline content may not be a good reflection of RWC levels or OA in this instance.

Besides, proline content has been associated with higher yield under drought stress in some crops, including cotton (Zhang *et al*., 2014) and chickpea (Ghiabi *et al*., 2013), but in some cases, higher accumulation of proline content was associated with lower yield under drought stress in sunflower (Umar and Siddiqui, 2018) and faba beans (El-Harty, 2016). In this study, proline had a weak negative and non-significant correlation with yield, and higher accumulation of proline was only observed in red leaf amaranth in response to drought stress, while green leaf amaranth had no changes in proline content. As shown by Umar and Siddiqui (2018), high number of leaves, leaf area and RWC, and better plant height under drought stress could be due to the higher accumulation of proline content. Hence, this may prevent leaf senescence and maintain leaf longevity in the red leaf amaranth. Therefore, proline accumulation under drought stress may demonstrate drought tolerance mechanism in amaranth, in which red leaf amaranths may take

the advantage of the capacity to accumulate more proline under stress, but could not be used as a drought tolerance parameter.

Although a high accumulation of proline in this study may not have contributed to osmotic adjustment, it may influence the activation of anti-oxidizing enzymes that scavenge ROS during drought stress, as observed in a study conducted by Slabert and Krüger (2011) in *A. tricolor* genotypes. It was also reported that, proline may sometimes have a slight quantitative contribution to OA but its major effect is in minimizing the cellular damage by stabilizing cellular structures (Shabala and Shabala 2011; Sánchez *et al*., 1998) or modification of cell wall proteome (Maatallah *et al*., 2010). This suggests that the increase of proline production during drought stress may activate anti-oxidative defence mechanism (Ahmed *et al*., 2009; Yan *et al*., 2000) and thus, reduce the oxidative damage to plants' organelles (Rout and Shaw 2001).

5.4.5 Conclusion

The results obtained in this investigation underline the important role of several mechanisms in protecting plants at specific water-deficit conditions. The growth of amaranth accessions evaluated in this study only started to decline at moderate drought stress i.e. 40% WHC. The physiological changes among the two contrasting amaranths (red and green leaves) under drought stress were only affected when leaf water status (RWC) reduced to 70%, in which green leaf amaranth reduced RWC earlier than the red leaf amaranth. A significant adaptation to drought stress in red leaf amaranths was associated with increased proline synthesis. Proline accumulates under stress, but proline, when measured at a single time point, may not serve as a good predictor for indirect selection for drought stressed yield as the value are fluctuated throughout the experiment. Other than that, equal adjustment of biomass (nutrient) allocation into leaves, stems and roots under drought stress are necessary to maintain photosynthetic activity. Equal nutrient allocation between the plant organs is the best indicator for drought tolerance selection traits in amaranth. Nevertheless, the small number of accessions used in this study may limit the information on the role of drought adaptive strategies of amaranth species. Therefore, further studies are required to identify other limitations of photosynthesis under drought stress, for example chlorophyll

fluorescence and betacyanin content which can be used as a surrogate trait for drought stress tolerance.

CHAPTER 6

ASSESSMENT OF DROUGHT TOLERANCE IN VEGETABLE AMARANTH (*AMARANTHUS TRICOLOR*) GERMPLASM: POTENTIAL FOR THE DEVELOPMENT OF IMPROVED DROUGHT TOLERANCE CULTIVARS

6.1 Introduction

Germplasm screening or phenotyping for drought tolerance is an effective way of selecting materials for advanced breeding programmes (Mwadzingeni *et al*., 2016; Passioura, 2012). The preliminary drought tolerance screening conducted in Chapter 5 summarized that genotypic variation of drought mechanisms such as relative water content, proline and photosynthetic capacity, amongst others, were present among amaranth accessions. However, those approaches still require validation for their usefulness in screening germplasm. It is well known that development of drought tolerant cultivars is difficult, primarily due to environmental variations which make drought stress highly variable to observe (Rao, 2002). Several studies have suggested a complementary strategy for selection of drought tolerant genotypes through correlations between yield and yield-related traits, as a surrogate measures (Hoyos-Villegas *et al*., 2017).

Typically, selection should target genotypes with relatively high yields under both stress and non-stress conditions, hence the need to determine stress tolerance indices. The comparative yield performances between stress and non-stress conditions are most often used to quantify the level of drought tolerance of a genotype rather than a direct selection criterion (Farshadfar and Sutka, 2002). Drought tolerance indices are either based on drought resistance or susceptibility of genotypes (Fernandez, 1992). It provides a measure of drought based on loss of yield under drought conditions in comparison to normal conditions (Mitra, 2001).

Appropriate selection of drought tolerance indices will be able to differentiate genotypes into four groups criterion; (i) Group A: genotypes with high yield under both non-stress and stress conditions, (ii) Group B: genotypes with high yield in non-stress condition but low yield in stress condition, (iii) Group C: genotypes with high yield in stress condition but low yield in non-stress condition and Group D: genotypes with low yield in both stress and non-stress conditions

(Fernandez, 1992). The indices have been used in many crops, for example maize (Mhike *et al*., 2012), durum wheat (Talebi *et al*., 2009) and sugar beet (Sadeghian *et al*., 2000).

The aim of this study was to identify potential drought tolerant accessions and to discern their physiological attributes in a reference collection of the mini amaranth core collection. The reference collection is a representative subset of 40 *A. tricolor* accessions assembled based on the germplsms morphological and molecular diversity (Chapters 3 and 4), along with four commercial varieties as checks. As a prerequisite for cultivar development, pure lines were developed (by single seed descent). Their progenies were tested separately in two screening trials to characterize drought tolerance accessions and to discuss plausible mechanism. From this, suitable drought tolerance indices and stable traits can be elucidated. Lastly, the genetic bases (or marker trait association, MTA) of drought tolerance in vegetable amaranth (*A. tricolor*) was determined through GWAS.

6.2 Materials and method

6.2.1 Plant materials

Sixty-two *A. tricolor* accessions were chosen from the amaranth mini core collection and were primarily subjected for drought screening in the first crops growing cycle (Trial I). However, some of the accessions showed very poor growth competency and fitness at the early vegetative phase under optimal conditions and so were excluded from further study. A total of 46 *A. tricolor* sub-set including four check varieties were used as a reference collection of amaranth germplasm (Table 6.1). Two accessions (AV-TRI 20 and AV-TRI 21) were later removed from analysis as most of the replicates died at later stage of drought stress, made it 44 accessions were evaluated for drought tolerance screening.

Table 6.1: List of accessions used for drought tolerance screening in two different trial test environments.

Entry	Accessions	Germplasm	ID	Origin country	Entry	Accessions	Germplasm	ID	Origin country
1	AV-TRI 2	AVRDC	VI055356	Bangladesh	**24**	US-TRI 3	USDA	Ames 29505	Brazil
2	AV-TRI 18	AVRDC	VI044446	India	**25**	US-TRI 6	USDA	PI 478310	China
3	AV-TRI 26	AVRDC	VI049006	Thailand	**26**	US-TRI 13	USDA	Ames 2039	Indonesia
4	AV-TRI 33	AVRDC	VI050610-A	Viet Nam	**27**	US-TRI 14	USDA	Ames 5354	Madagascar
5	AV-TRI 34	AVRDC	VI050609-A	Viet Nam	**28**	US-TRI 15	USDA	Ames 2029	Malaysia
6	AV-TRI 39	AVRDC	VI054572	Philippines	**29**	US-TRI 16	USDA	Ames 29034	Malaysia
7	AV-TRI 40	AVRDC	VI054571	Philippines	**30**	US-TRI 19	USDA	Ames 2199	Taiwan
8	AV-TRI 44	AVRDC	VI048286	Bangladesh	**31**	US-TRI 21	USDA	PI 607446	Thailand
9	AV-TRI 49	AVRDC	VI047504	Bangladesh	**32**	US-TRI 24	USDA	PI 632237	USA
10	AV-TRI 51	AVRDC	VI057270	Cambodia	**33**	US-TRI 25	USDA	Ames 5110	West Africa
11	AV-TRI 53	AVRDC	VI042979	Indonesia	**34**	US-TRI 29	USDA	Ames 26216	China
12	AV-TRI 54	AVRDC	VI042978	Indonesia	**35**	US-TRI 39	USDA	Ames 2132	India
13	AV-TRI 56	AVRDC	VI058498	India	**36**	US-TRI 46	USDA	Ames 5118	Puerto Rico
14	AV-TRI 57	AVRDC	VI044426	Malaysia	**37**	US-TRI 47	USDA	Ames 1993	Taiwan
15	AV-TRI 58	AVRDC	VI055139	Malaysia	**38**	US-TRI 20	USDA	Ames 2024	Thailand
16	AV-TRI 68	AVRDC	VI050111	Taiwan	**39**	US-TRI 30	USDA	Ames 5102	Hong Kong
17	AV-TRI 69	AVRDC	VI049431	Taiwan	**40**	US-TRI 48	USDA	Ames 1998	Taiwan
18	AV-TRI 3	AVRDC	VI055353	Bangladesh	**41**	US-TRI 49	USDA	Ames 5134	USA
19	AV-TRI 11	AVRDC	VI047795	Bangladesh	**42**	US-TRI 51	USDA	PI 633591	Unknown
20	AV-TRI 24	AVRDC	VI044396-A	Pakistan	**43**	Local Red	LOCAL (Check)	var. BBS027	Malaysia
21	AV-TRI 31	AVRDC	VI050615-A	Viet Nam	**44**	Local PR	LOCAL (Check)	var. BBS014	Malaysia
***22**	AV-TRI 20	AVRDC	VI043725	Malaysia	**45**	Thida	E-WEST (Check)	Thida	Tanzania
***23**	AV-TRI 21	AVRDC	VI043724	Malaysia	**46**	Zeya	E-WEST (Check)	Zeya	Tanzania

*Accessions removed from analysis as plant replicates were not survived at later stage of drought stress.

6.2.2 Growth conditions

Two pot experiments (16 cm x 12.5 cm x 14.5 cm) were conducted between 21st May - 6th August 2017 and 2nd November 2017 - 5th February 2018 under shade-house conditions at UNM. The soil used was a mixture of approximately 40% clay, 50% sand and 10% silt and acidic (pH 4.2) with low nutrient organic content (Table 6.2). The high compositions of clay provide greater water and nutrient holding capacity and at the same time the sand provides immediate absorption of water by the plants when watered. High acidic fluoride solute was observed in the soil analysis during Trial I (27.9 ppm) compared to Trial II (1.8 ppm). High fluoride composition in the soil may negatively affect various plants metabolisms by acting on the membranes and the stromal enzymes associated with carbon dioxide fixation and resulting in lower chlorophyll concentrations (Garrec *et al*., 1981). The acceptable acid fluoride soluble in the soils for most of the crops is 2-20ppm, including rice (Weinstein, 1977), but some crops germination is inhibited at 2.5 ppm as reported by Purohit and Sharma (1985) on field cabbage (*Brassica campestris*). However, the high acidic fluoride soluble composition in the soil used in the previous studies (subheading 5.4; Experiment II) had no detrimental effect on the growth of vegetable amaranth.

The averaged photosynthetic active radiation (PAR) and temperature were higher during the first trial of drought treatment imposed on 26th July 2017 until 6th August 2017 (PAR: 309.8-616.9 uM/m^2s; temperature: 30.3-33.1°C, and relative humidity, RH: 61.0-74.8%) compared with the second trials imposed on 11th January 2018 until 5th February 2018 (PAR: 147.8-531.09 uM/m^2s; temperature: 26.6-32.7°C; and RH: 62.2-88.5%) (Figure 6.1). A clear change in weather conditions in the experimental site for both drought screening trials possibly due to high inter-annual climatic variations, implies that the drought screening were conducted in two different environmental conditions, i.e., Trial I was conducted during warm dry condition while Trial II was carried out in warm humid conditions. The onset of drought and the rate of soil depletion were strongly related to the weather condition, which caused extreme and rapid terminal soil moisture depletion (20% water holding capacity) in Trial I (6 days) but was slower in Trial II (10 days).

Table 6.2: Analyses of soil used in drought screening Trial I and II.

Trial	pH in Water (2:5)	C (%)	N (%)	C/N	P (ppm)		Exchangeable Cations (m. e. %)			C.E.C NH_4OAC method (m.e %)	Mechanical Analysis (%)			
					Total	Acid fluoride soluble	K	Ca	Mg		Clay	F Silt	F Sand	C Sand
I	4.23	0.45	0.07	6.4	206	27.9	0.07	0.3	0.08	3.4	44	4	12	40
II	4.21	0.08	0.04	2	124	1.8	0.02	0.12	0.03	2.3	40	6	43	11

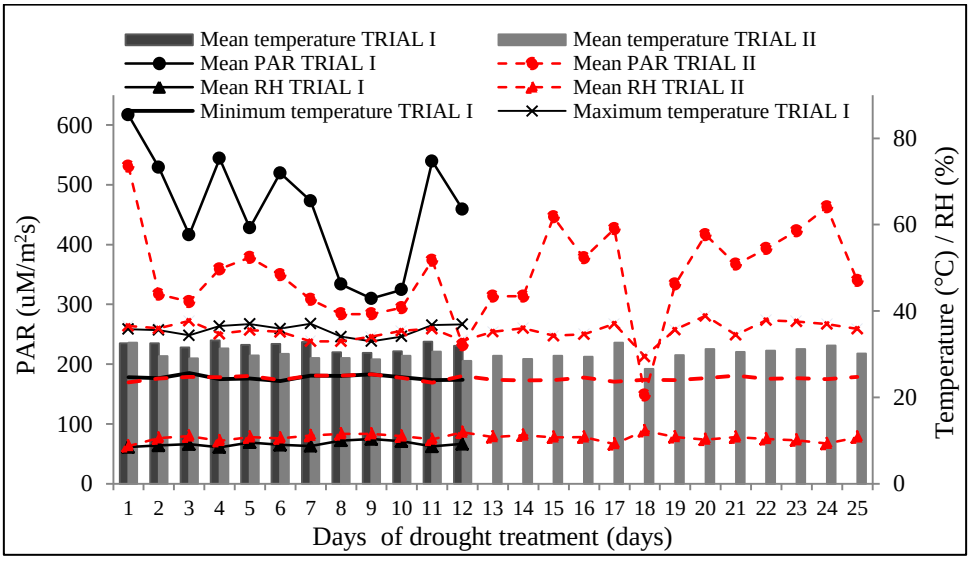


Figure 6.1: Daily weather data collected during the experimental period for drought screening trial I and II. Values represent the mean of 12 hours (7.30am-7.30pm).

6.2.3 Experimental design

Plants were subjected to drought tolerance screening in a split-plot randomized block design with three replications in Trial I and four replications in Trial II (Figure 6.2). In each trial, each block (replicates) consisted of two whole-plots (water treatment: water-sufficient, WS and water-deficient, WD) and within whole-plots split into amaranth accessions.

The water treatment was imposed at the beginning of the vegetative growth stage, when the plants reached the 5-7 mature leaf stage (25 days after emergence in Trial I and 30 days after emergence in Trial II). The WS plants were watered daily throughout experimental period to maintain maximum water holding capacity (WHC) while WD plants were subjected to progressive soil drying with additional of 100ml watered at one day interval to keep all pots consistency. Soil moisture content was measured at the beginning of the drought experiment (0 DAT, 100% WHC) followed by two measurements every 3 days in Trial I and every 5 days in

Trial II, until the soil water was reduced to 20% WHC. The volumetric soil water content was determined using portable soil moisture sensor (ML3-ThetaProbe, Delta-T Device, Cambridge, England).

As screening practice for drought tolerance in shade-house/field require considerable space, time and work which include planting and ascertained measurements at specific time in each time point, one set of block/replication (n=88) was performed at a time and the next block/replications were performed at every 5 day interval. An additional of three replicates of each accession was subjected to re-water assessment during the Trial II experimental period, arranged in a completely randomized design. In the re-watering assessment, the plants were subjected to progressive soil drying without irrigation until they reached terminal wilting (10% WHC) before re-watered to full capacity for 5 days.

Figure 6.2: The 44 *A. tricolor* accessions arranged in completely random within a whole plot of two water treatments.

6.2.4 Growth measurement and physiological response

In this experiment, growth parameters were only obtained for the above-ground biomass allocations which include fresh and dry weight of leaf and stem, TLA and SLA. Plants were destructively harvested and separated into leaves and stem, fresh weight (FW) were recorded. The total yield, fresh and dry weight of leaf and stem, TLA and SLA were determined at 6 DAT and 10 DAT for Trial I and Trial II respectively (see subheading 5.2.3 for the growth measurement protocols). For physiological responses, two different sets of measurements were employed for

both Trials. In Trial I, only a few parameters were recorded to test the suitability of measurements since this was a first attempt to screen a large scale of plants. More detailed physiological responses were determined during Trial II, and the comparisons of accessions performance in the two trials under drought stress treatment were still achievable.

6.2.4.1 RWC

In Trial I, RWC was obtained at 0 and 6 DAT (100% WHC, 20% WHC respectively) and in Trial II, RWC was determined at 0, 6 and 10 DAT (100%, 50% and 20% WHC respectively) (see subheading 5.2.4.2 for RWC protocol).

6.2.4.2 Chlorophyll fluorescence

Chlorophyll fluorescence measurements were taken using a hand held fluorometer FluorPen FP100 (Photon Systems Instruments, Czech Republic). The fluorometer provides a rapid pulse of high intensity light to be absorbed by the leaf inducing fluorescence which is then measured by the sensor. The chlorophyll fluorescence parameters were assessed for quantum yield (QY) of photosystem II (PSII) photochemistry under light adaptation (*Fv'/Fm'*) in both trials (Trial I: 0 and 6 DAT, Trial II: 0, 6, 10 DAT) and under dark adaptation (*Fv/Fm*) which only measured at 0, 6 and 10 DAT in Trial II. For *Fv/Fm* measurement, the leaf was dark-adapted for 20 minutes at pre-dawn (7.00am) using blackout paper (Figure 6.3). The instrument and leaf were covered with blackout cloth during measurement to ensure no other sources of light except from the device. One reading was obtained for each leaf, with two leaves per plant, and averaged to give a final reading.

According to Maxwell and Johnson (2000), *Fv'/Fm'* denotes a maximum efficiency of PSII photochemistry in the light, if all centres were open i.e., the number of fluorescent events for each photon absorbed. *Fv/Fm* is expressed as maximum quantum efficiency of PSII photochemistry i.e., the maximum efficiency at which light absorbed by PSII is used for reduction of Plastoquinone-A (QA).

The equation for QY is as follows:

$Fv'/Fm' = (Fm' - Fo') / Fm'$

$Fv/Fm = (Fm - Fo) / Fm$

where *Fv'* is variable fluorescence, *Fm'* is maximal fluorescence and *Fo'* is minimal fluorescence under light adaptation. *Fv* is variable fluorescence, *Fm* is maximal fluorescence and *Fo* is minimal fluorescence under dark adaptation.

Figure 6.3: Dark-adapted leaf with blackout paper for 20 minutes at pre-dawn

6.2.4.3 Intracellular CO_2 (Ci) response curve

The photosynthetic gas exchange of mature leaves was measured with a portable photosynthetic system (LI-6400, LI-COR, Inc., Logan, NE, USA) at 0 and 6 DAT in Trial II (see subheading 5.2.4.3 for gas exchange measurements protocol). Before the onset of drought treatment during Trial II (time 0), photosynthesis/intracellular CO_2 response curve (Pn/Ci) were evaluated on eight accessions of well-watered plant with one replication per plant to identify the effect of environmental changes and genotypic differences among amaranth accessions. The CO_2 was injected into the open circulating gas-stream of the photosynthesis system using its proper auto-controlled CO_2 injector and the cuvette conditions were maintained at 1500 µmol photon $m^{-2}s^{-1}$ PAR, 35°C leaf block temperature and 50-70% RH in the sample to keep the vapour pressure deficit (VPD) in the leaf chamber at approximately 1-1.5 kPa. The Pn/Ci auto programme function was performed with short-term measurements 6-8 minutes for each data point as the response of photosynthesis. Measurements started with CO_2 (Ca) set to be 400 $\mu mol.mol^{-1}$ and once reached steady state, the CO_2 concentration was gradually lowered to 0 $\mu mol.mol^{-1}$ and then increased stepwise up to 800 $\mu mol.mol^{-1}$ (400, 300, 200, 100, 0, 300, 400, 400, 600, 800 $\mu mol.mol^{-1}$). 10 sequential measurements of net photosynthesis were taken for each Pn/Ci curve.

6.2.4.4 Re-watering assessment

Days to flowering (DTF) and days to wilting (DTW) were recorded as days after the initiation of the drought stress and wilting were recorded pre-dawn. All the plants were rated for leaf wilting scoring (LWS) (Figure 6.4) and drought tolerance symptom (DS) (Figure 6.5) at the same time of DTW were recorded. The LWS and DS scoring on a scale of 0-5 and 0-6, respectively with 0=healthy and higher score was severe. Days to recovery (DTR) were attained after the plants were re-watered at 10% WHC for 5 days. DS were recorded twice, first during the day of DTR were observed and second on the 5th day of recovery.

Figure 6.4: Scale for leaf wilting scoring:
0: leaves healthy, 1: flat, leaves start to fold, 2: V-shaped, 3: U-shaped, 4: O-shaped and 5: leaves tightly fold (Fen et al., 2015; O'Toole and Cruz, 1979).

Score	Scoring
0	No symptoms
1	Slight tip drying
2	Tip drying extended to 1/4 length in most leaves
3	1/4 to 1/2 of the leaves fully dried
4	More than 2/3 of full leaves fully dried
5	Drooping stem but leaves still pigmented
6	All plants apparently dead
7	< 5 leaves senescence
8	< 10 leaves senescence
9	> 10 leaves senescence
10	Not recover

Figure 5. 18: Scale for drought tolerance symptom: 0-6 scales for drought scoring (DS) before re-water and 7-10 scales for DS at recovery. Modified scale according to Fen *et al*., (2015) and De Datta *et al*., (1988).

6.2.4.5 Drought tolerance indices

To evaluate drought tolerance of the vegetable amaranth accessions, seven drought tolerance indices were evaluated for their usefulness in identifying drought tolerant accessions, based on total yield as the relative difference between the results obtained under WS and WD conditions. The drought tolerance indices were calculated using the following relationships:

(1) Stress susceptibility index, SSI = [1-(Ysi/Ypi)]/SI (Fischer and Maurer, 1978)

(2) Stress tolerance index, STI = (Ypi*Ysi)/Yp^2 (Fernandez, 1992)

(3) Drought resistance index, DI = [Ysi*(Ysi/Ypi)]/Ys (Lan, 1988)

(4) Tolerance index, TOL = Ypi-Ysi (Hossain *et al*., 1990)

(5) Geometric mean productivity, GMP = $\sqrt{(Ypi * Ysi)}$ (Fernandez, 1992)

(6) Mean productivity, MP = (Ypi+Ysi)/2 (Hossain *et al*., 1990)

(7) Yield stability index, YSI = Ysi/Ypi (Bouslama & Schapaugh, 1984)

where Ysi is yield of accession in WD condition, Ypi is yield of accession in WS condition, SI is stress intensity = 1-(Ys/Yp), Ys is total yield mean in WD condition and Yp is total yield mean in WS condition

6.2.5 Data analysis

The combined data of growth measurements and physiological responses across the two screening trials (total yield, LFW, LDW, SFW, SDW, TLA and SLA, RWC at 100% and 20% WHC, and *Fv'/Fm'* at 100% and 20% WHC) of the 44 vegetable amaranth were analysed using REML (Restricted Maximum Likelihood) to examine the interaction of accessions and water treatments over two trial test environments. Significance of means was estimated using Wald-statistics by keeping water treatments x accessions (WT* A) as fixed effects and Trials*WT*A as random effects. To compare the performance of accessions in two different trial test environments, the data were subjected to ANOVA with a split plot design, and one-way ANOVA was used to examine the variations among amaranth accessions at each time point.

Genotypic (σg^2), and phenotypic (σp^2) variances as well as broad sense heritability (H_B) were all calculated on an entry mean basis. Heritability on an experimental unit basis was calculated according to Pace *et al*., (2014) as follows:

$H_B = \sigma g^2/\sigma p^2$

$\sigma g^2 = [(MSg - MSe)/r]$

$\sigma p^2 = \sigma g^2 + MSe$

where MS is mean square of accession (g) and error (e) and r is number of replication (n=3, n=4 for Trial I, Trial II respectively).

Yield obtained under control conditions was used as a standardized indicator to evaluate the ability of amaranth to maintain yield performance under

drought stress and to assess their changes in physiological responses under WS and WD conditions at each time point. Manhattan distances computed from the values of total yield on WS treatment were subjected to hierarchical clustering, to group together the accessions with similar shoot growth under control conditions. Cluster analysis was performed using the average linkage method. The grouping for re-water assessment was using cluster analysis evaluated on Trial II as this experiment was conducted simultanuosly with drought screening in Trial II. Pearson linear correlations, principle component analysis (PCA) and biplot were performed to analyse the significant correlations between parameters. All data were processed using Genstat Software for Windows 18th edition (VSN International, 2015).

The P/Ci curve was evaluated using Excel fitting tool (EFT) non-linear curve fitting routine which derives a suite of C4 photosynthetic parameters (Bellasio *et al*., 2015; 2016). The curve is evaluated for non-stomatal limitation: (a) the capacity for carboxylation by PEPC (Vpmax), which determines the initial slope of the curve and (b) carboxylation by Rubisco and/or generation of PEP by PPDK (Vmax), which limit the asymptote of the curve (von Caemmerer, 2000).

Genome wide association study (GWAS) was conducted via mixed linear model (MLM) controlling for Q and kinship (K) as fixed and random effects respectively in TASSEL 5.0. SNPs data (see subheading 4.3.1) filtered at minimum allele frequency >0.05 for the 44 *A.tricolor*. Data that obtained across two screening trials such as total yield, LFW, LDW, SFW, SDW, TLA, SLA, RWC at 20% WHC, and *Fv'/Fm'* 20% WHC were combined through REML analysis (estimated means) and used for association study.

6.3 Results

6.3.1 Drought and weather conditions

The clay composition in the soil used during Trial I was higher than in Trial II, this may reflect the significantly higher soil water content (% vol) acquired during maximum water holding capacity (Figure 6.6). The onset of drought and the rate of soil depletion were strongly related to the weather conditions, which causes extreme and rapid terminal soil moisture depletion (20% WHC) in Trial I (6 days) but was slower in Trial II (10 days).

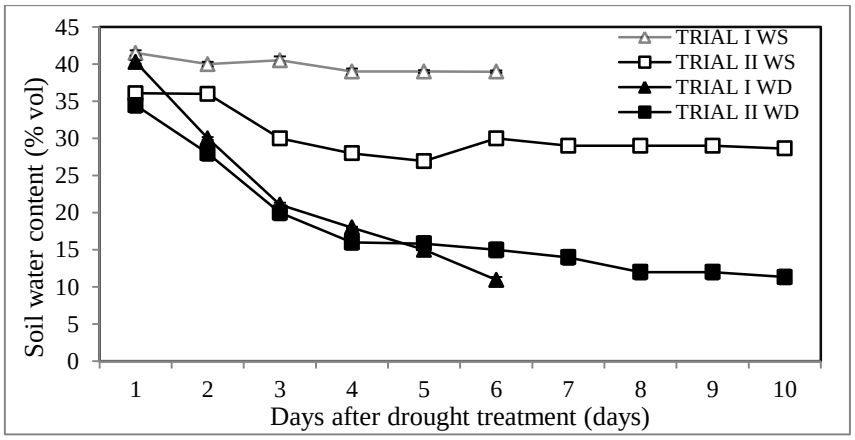


Figure 6.5: Soil volumetric content (% vol) of water-sufficient (WS) and water-deficient (WD) treatments in Trial I and Trial II for 6 and 10 days of drought treatment, respectively. Values represented mean of three and four individual of 44 accessions in Trial I and Trial II respectively. Error bar represents SEM.

6.3.2 Combined analysis of water treatment, accessions and trial environments on growth and plant physiology

Table 6.3 summarizes the estimates of treatment effects from restricted (or residual) REML combined analysis for growth and physiology traits of 44 vegetable amaranth accessions evaluated across the two trial environments. Significant differences were observed on the interactions between water treatment and accessions across the two trials for yield ($P<0.001$), TLA ($P<0.001$), LFW ($P<0.001$), SFW ($P<0.001$), RWC at 20% WHC ($P<0.05$) and *Fv'/Fm'* at 100% WHC ($P<0.01$). Meanwhile, the main effects of water treatment and accessions were significant across the two trials for SDW and *Fv'/Fm'* at 20% WHC even though there were no significant interactions examined between the two main effects. The individual ANOVA analysis with a split plot design for all parameters in each Trial is presented in Appendix 6.1, the ANOVA analysis for grouping is presented in Appendix 6.2, and the mean for each accession in each water treatment are presented in Appendix 6.3.

The mean yield of WS treatment was significantly lower ($P<0.001$) in Trial I (15.78 g) compared to Trial II (48.11 g) ($P<0.001$) and the percentage of yield reductions was found to be higher in Trial I (73%) compared to Trial II (30%). Subsequently, the biomass partitioning parameters including leaf and stem fresh weight and dry weight, TLA and SLA were higher in Trial II compared with Trial I under both WS and WD treatments. The relative reductions of RWC at 20% WHC

in response to drought stress was significantly higher in Trial I (33%) compared with Trial II (12%). The *Fv'/Fm'* at 100% WHC was higher in Trial I (0.65) compared to Trial II (0.54), and significant higher relative reduction was also occurred in *Fv'/Fm'* at 20% WHC for Trial I (13%) compared to Trial II (2%).

Table 6.3: Restricted maximum likelihood (REML) combined analysis for growth and physiology traits of 44 vegetable amaranth accessions (A) evaluated across the two screening trials (Trial I and Trial II) and two water treatment (WT: water sufficient, WS and water-deficient, WD).

		Yield	TLA	SLA	LFW	LDW	SFW	SDW	RWC 100%WHC	RWC 20%WHC	Fv'/Fm' 100%WHC	Fv'/Fm' 20%WHC
Estimated variance												
Random effect												
Trial		468.60	64634	4491	93.83	0.88	142.76	0.83	1.01	0.00	0.0049	0.0000
Trial*WT		2.40	2645	1213	1.02	0.06	0.23	0.00	2.08	7.10	0.0001	0.0003
Trial*A		23.50	12758	2052	9.06	0.04	10.32	0.11	0.58	0.00	0.0001	0.0000
Trial*WT*A		-4.30	-193	1121	-1.46	0.02	-0.72	-0.01	-0.05	0.00	-0.0007	0.0000
Error trial I		19.82	4554	2042	6.316	0.14	4.75	0.10	32.01	227.10	0.0030	0.0075
Error trial II		123.30	39889	33123	35.52	0.54	41.82	0.41	39.91	153.50	0.0066	0.0077
Wald tests for fixed effects												
Fixed effect	**d.f.**											
WT	1	63.39***	12.64***	5.62*	51.98***	1.51ns	90.02***	22.46***	0.77ns	76.65***	0.01ns	5.44*
A	43	59.06*	62.99*	30.44ns	65.23*	102.68***	68.06**	92.78***	67.57*	51.01ns	71.5**	78.19***
WT*A	43	132.96***	91.3***	26.51ns	204.03***	48.86ns	116.47***	55.82ns	37.86ns	61.2*	67.73**	42.92ns

TLA: total leaf area, SLA: specific leaf area, LFW: leaf fresh weight, LDW: leaf dry weight, SFW: stem fresh weight, SDW: stem dry weight, and RWC: relative water content and Fv'/Fm': light-adapted chlorophyll fluorescence quantum yield at 100% and 20% water holding capacity (WHC). Chi-square (χ^2) probability significance difference at P<0.05, <0.01, <0.001 (*, **, *** respectively) and non-significant (ns).

6.3.3 Genotypic variations in growth in response to drought stress

Cluster analysis constructed based on yield performance under WS conditions classified the 44 amaranth accessions into three groups in Trial I and five groups in Trial II (Figure 6.7). The clusters significantly categorized the groups into high, medium and low yield performance; Trial I: Group 1 (highest, 20.04 g), Group 2 (lowest, 8.08 g) and Group 3 (medium, 14.22 g), and Trial II: Group 1 (second highest, 57.84 g), Group 2 (highest, 70.04 g), Group 3 (medium, 45.81 g), Group 4 (medium, 37.93g) and Group 5 (lowest, 23.98 g). Accessions AV-TRI 18, AV-TRI 26, AV-TRI 44, AV-TRI 11, US-TRI 21, US-TRI 46 and US-TRI 51 displayed the highest yield performance in the two different trial environments while accessions US-TRI 6 and US-TRI 47 had the lowest yield. It is interesting to note that groups that belonged to higher and medium category had larger relative reductions in yield performance in response to drought stress compared with the group in the lowest category in both Trials (Table 6.4).

In Trial I, significant interaction of WT*Group was observed in all growth traits ($P<0.05$), except SLA, although a significant reduction of SLA was found in WD treatment ($P<0.001$) (Table 6.4). Under WS conditions, highest (Group 1) and medium (Group 3) category had biomass partitioning primarily in leaf while the lowest category (Group 2) in stems (Figure 6.8), but drought stress shifted the biomass partitioning primarily into stems for all groups (Figure 6.9). In comparison, in Trial II, significant interaction of WT*Group was only found in yield, LFW and SFW ($P<0.001$ respectively), while TLA, LDW, SDW and SLA demonstrates a significant reductions under WD treatment ($P<0.05$) and differences existed among groups (Table 6.4). The highest (Group 1) and lowest (Group 4 and 5) category had biomass partitioning mainly in stem while other groups had equal biomass allocation in leaf and stems (Figure 6.10). However, similar to Trial I, drought stress causes the biomass partitioning to be altered primarily into stem rather than leaf for all groups (Figure 6.11).

Low broad sense heritability was found for most of the growth traits in Trial I (ranged from 0.12 to 0.31), and exceptionally low for yield under both water treatments (0.08, 0.06 respectively). Meanwhile, moderate broad sense heritability was examined for most of the growth traits in Trial II (ranged from 0.27-0.53), and remarkably low SLA under WD treatment (0.09) (Table 6.4).

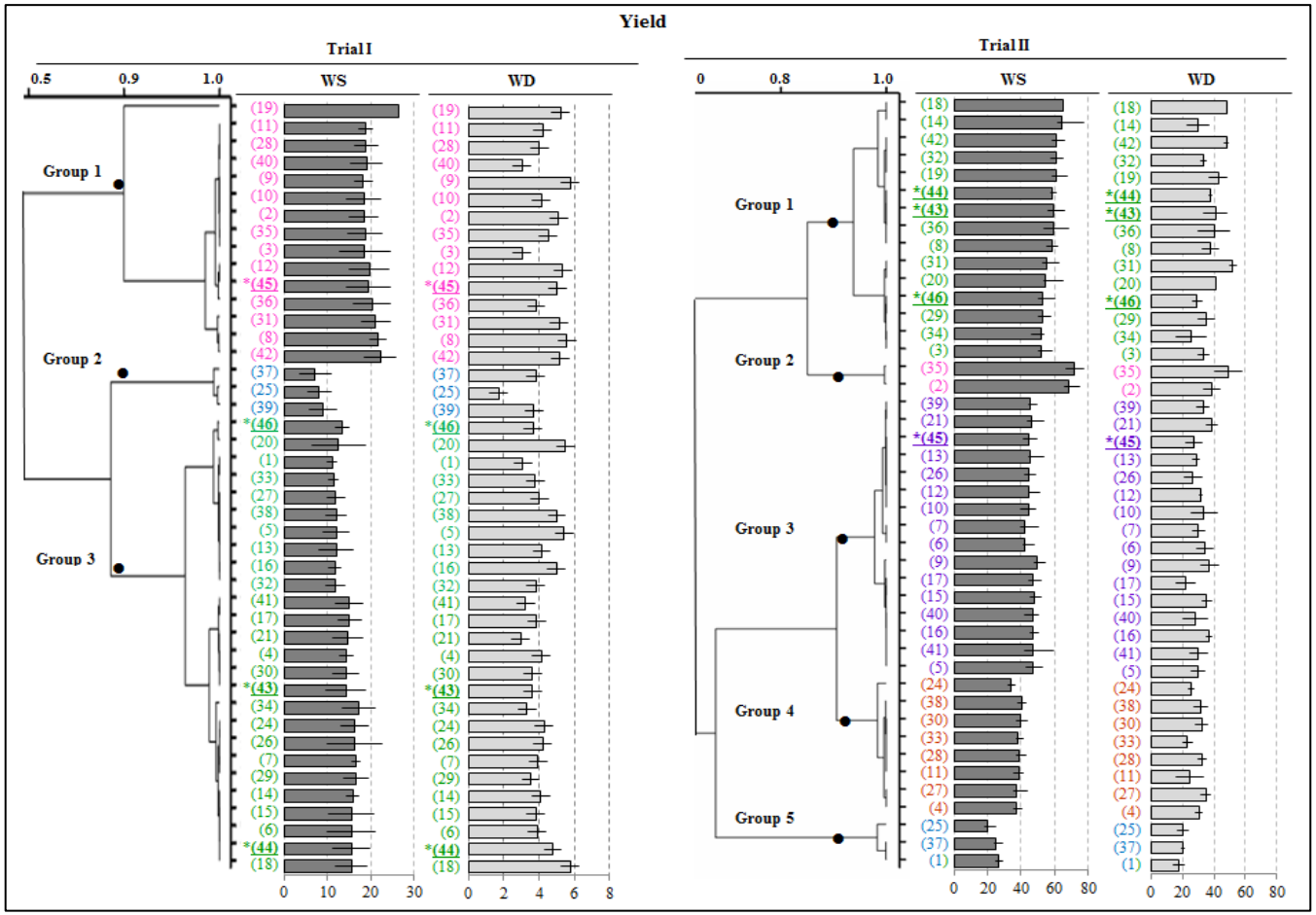


Figure 6.6: Cluster analysis constructed based on yield performance under water-sufficient (WS) conditions classified the 44 amaranth accessions into three groups in Trial I and five groups in Trial II, and their yield performance under water-deficient (WD) conditions. Data represents mean and error bar indicates standard error of mean (SE). The underline accession is checks variety.

Table 6.4: Effects of water treatment (WT: water-sufficient, WS and water-deficient, WD) on growth traits including yield, fresh weight of leaf (LFW) and stem (SFW), dry weight of leaf (LDW) and stem (SDW), total leaf area (TLA) and specific leaf area (SLA) in 44 vegetable amaranth accessions evaluated in groups based on yield performance under control conditions in the two screening trials (Trial I and Trial II).

Yield and biomass partitioning, Trial I

	Yield (g)		LFW (g)		SFW (g)		TLA (cm^2)		LDW (g)		SDW (g)		SLA (cm^2g^{-1})	
	WS	**WD**	**WS**	**WD**	**WS**	**WD**	**WS**	**WD**	**WS**	**WD**	**WS**	**WD**	**WS**	**WD**
Mean	15.78	4.21	8.42	1.81	7.37	2.4	265.49	129.29	1.15	1.09	0.94	0.78	239.62	118.78
σ^2_G (F pr.)	3.14ns	0.12ns	2.56*	0.15*	3.37*	0.12ns	2.74*	2.67*	0.06*	0.05*	0.05*	0.02*	1.77*	1.39ns
H_B	0.08	0.06	0.17	0.29	0.28	0.12	0.05	0.14	0.26	0.31	0.27	0.27	0.07	0.14
Group														
1	20.04a	4.63a	10.35a	1.90a	9.69a	2.72a	312.3a	138.1a	1.39a	1.18a	1.23a	0.83a	231.2a	115.6a
2	8.08c	3.09b	3.13c	1.18b	4.95b	1.91b	97.7c	78.50b	0.39c	0.67b	0.50c	0.58a	237.2a	113.9a
3	14.22b	4.10a	7.91b	1.83b	6.31b	2.28b	257.8b	130.0a	1.09b	1.09a	0.83b	0.77a	244.7a	121.2a
WT	***		***		***		***		ns		***		***	
Group	***		***		***		***		***		***		ns	
WT*G	***		***		***		***		*		***		ns	

Yield and biomass partitioning, Trial II

	Yield (g)		LFW (g)		SFW (g)		TLA (cm^2)		LDW (g)		SDW (g)		SLA (cm^2g^{-1})	
	WS	**WD**	**WS**	**WD**	**WS**	**WD**	**WS**	**WD**	**WS**	**WD**	**WS**	**WD**	**WS**	**WD**
Mean	48.11	33.24	23.23	14.51	24.88	18.73	682.32	440.83	2.48	2.00	2.27	2.02	305.06	257.44
σ^2_G (F pr.)	91.33*	35.73*	37.69*	13.98*	42.57*	19.10*	5.95*	3.16*	0.56*	0.43*	0.38*	0.36*	4.02*	1.38ns
H_B	0.39	0.27	0.50	0.30	0.46	0.36	0.02	0.01	0.49	0.45	0.43	0.53	0.06	0.05
Group														
1	57.84b	38.43a	28.28a	16.94a	29.56b	21.48b	834.4a	527.8a	3.05a	2.37a	2.52b	2.24b	281.2b	229.7a
2	70.40a	44.00a	26.78ab	15.43ab	43.62a	28.57a	685.0b	407.3ab	3.14a	2.15ab	3.89a	3.14a	215.2b	184.3a
3	45.81c	31.59b	23.48b	14.78ab	22.32c	16.81cd	668.8bc	446.9ab	2.39b	2.01b	2.07a	1.90c	302.6b	243.1a
4	37.93d	29.34b	17.81c	12.15b	20.12cd	17.19c	558.2c	364.3b	2.13b	1.73b	2.29bc	2.03bc	279.8b	320.2a
5	23.98e	19.35c	8.67d	6.57c	15.31d	12.78d	247.7d	200.2c	0.53c	0.62c	0.91d	0.83d	565.0a	353.7a
WT	***		***		***		***		***		**		*	
Group	***		***		***		***		***		***		***	
WT*G	***		***		***		ns		ns		ns		ns	

Data represents grand mean, genotypic variance with significance level among accessions (σ^2_G (F pr.)), broad-sense heritability (H_B), mean of grouping and significance level of ANOVA analysis. Probability significantly different at P<0.05 (*), P<0.01 (**), P<0.001 (***) or non-significant (ns). Values in columns identified with the same letter are not statistically different among accession based on Tukey's Pairwise method (P<0.05).

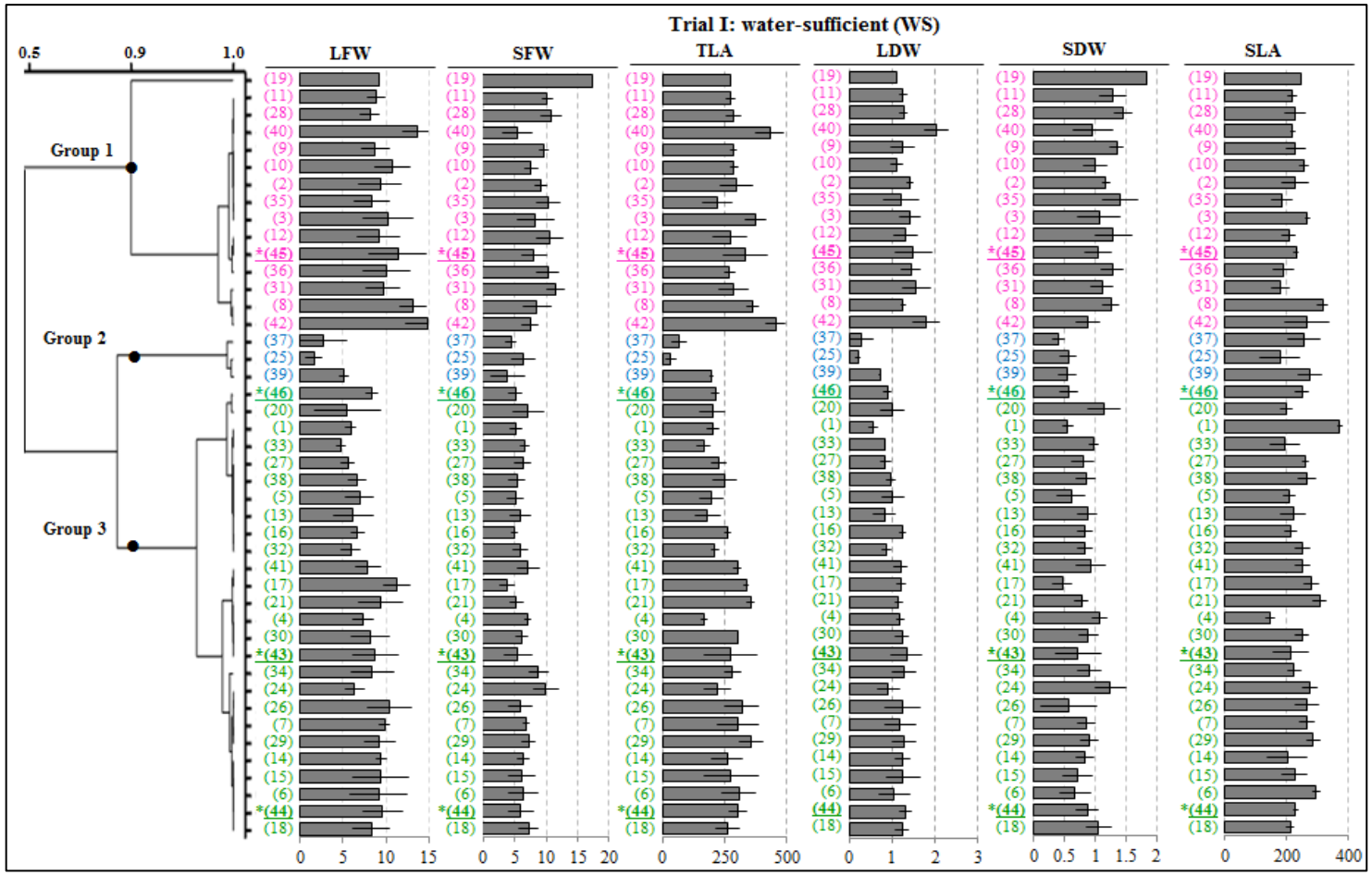


Figure 6.7: Biomass partitioning of 44 amaranth accessions under water-sufficient (WS) conditions in their respective groups in Trial I. Data represents mean and error bar indicates standard error of mean (SE) for leaf fresh weight (LFW), stem fresh weight (SFW), total leaf area (TLA), leaf dry weight (LDW), stem dry weight (SDW) and specific leaf area (SLA). The underline accession is checks.

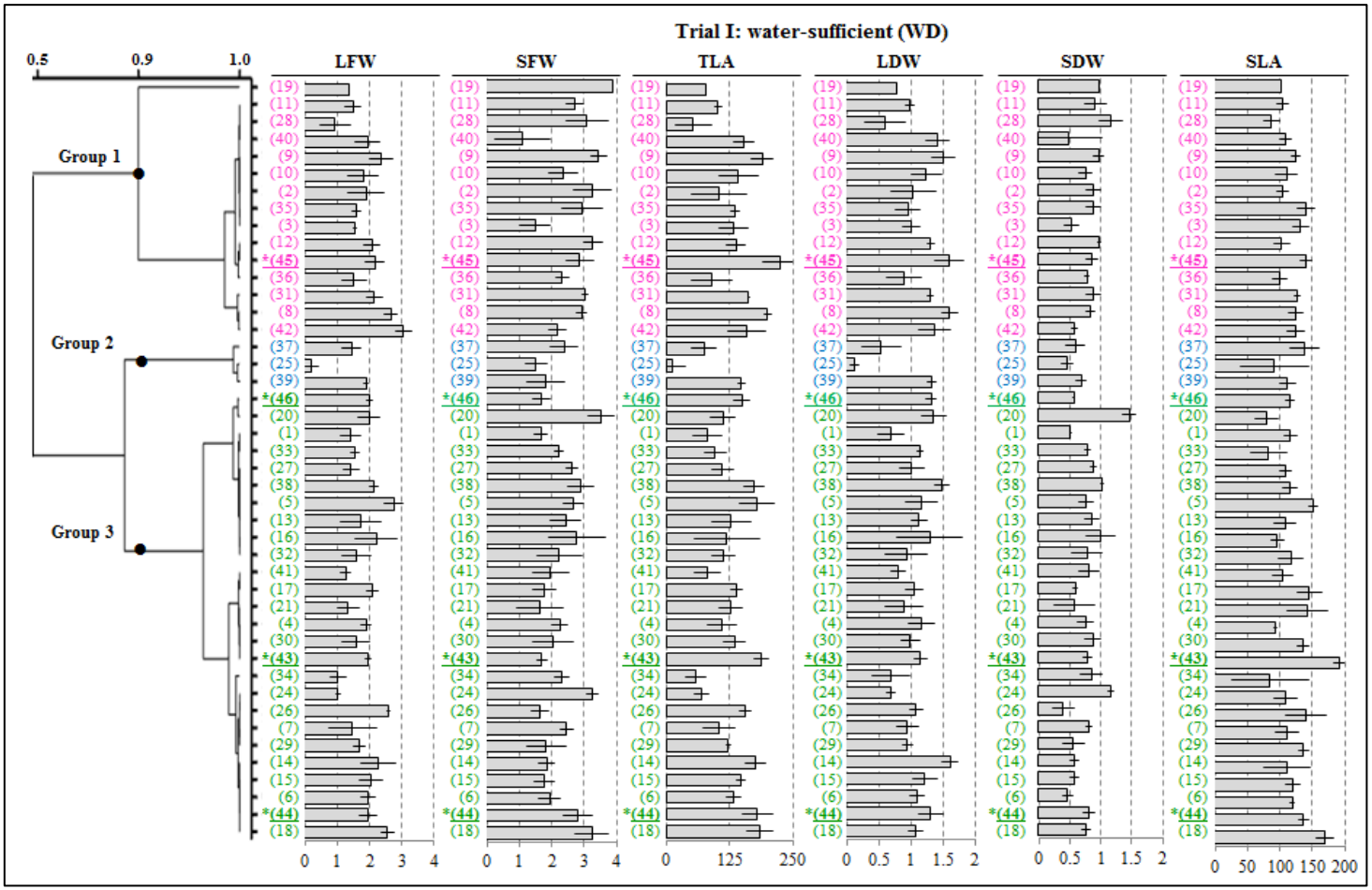


Figure 6.8: Biomass partitioning of 44 amaranth accessions under water-deficient (WD) conditions in their respective groups in Trial I. Data represents mean and error bar indicates standard error of mean (SE) for leaf fresh weight (LFW), stem fresh weight (SFW), total leaf area (TLA), leaf dry weight (LDW), stem dry weight (SDW) and specific leaf area (SLA). The underline accession is checks.

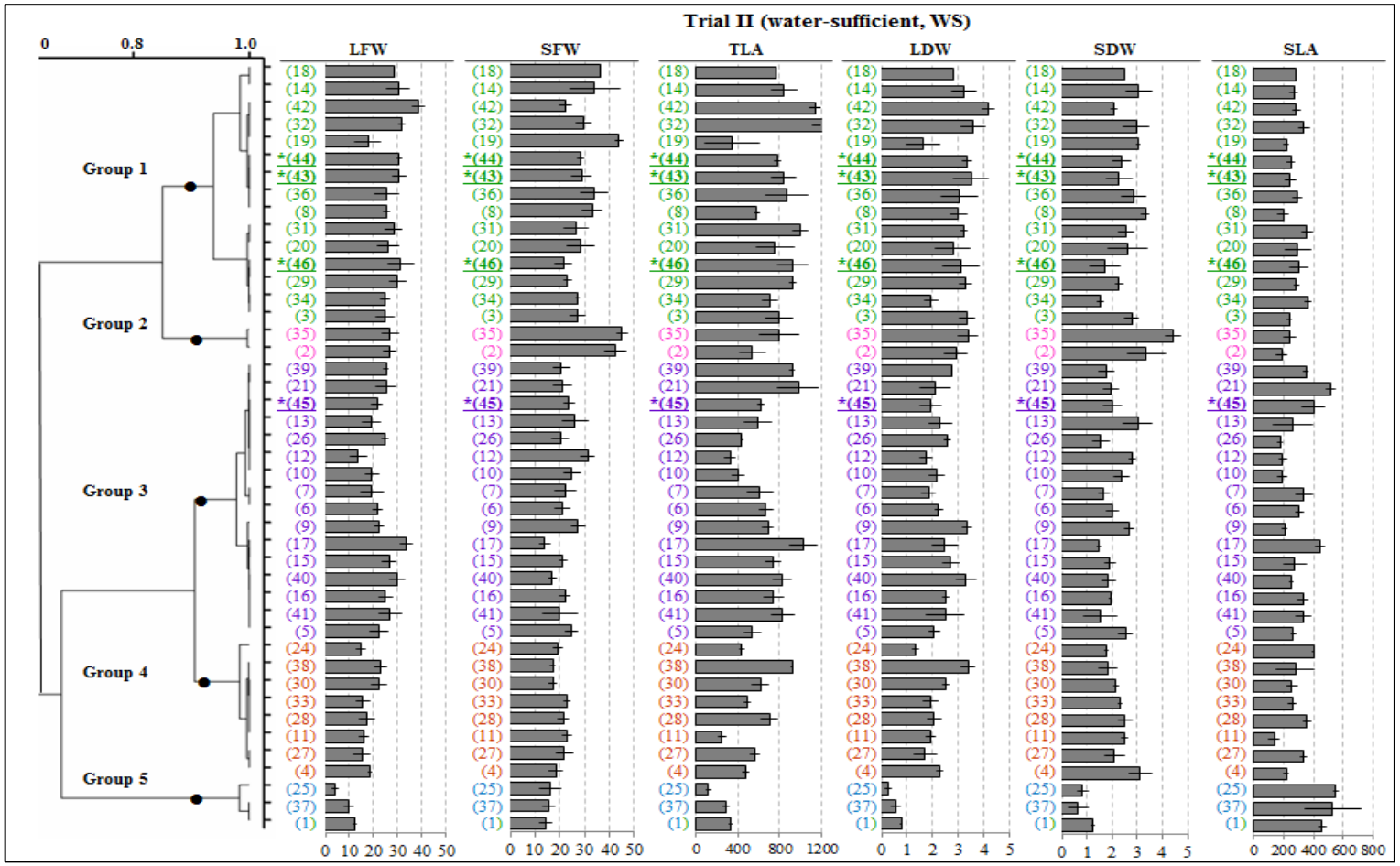


Figure 6.9: Biomass partitioning of 44 amaranth accession under water-sufficient (WS) conditions in their respective groups in Trial II. Data represents mean and error bar indicates standard error of mean (SE) for leaf fresh weight (LFW), stem fresh weight (SFW), total leaf area (TLA), leaf dry weight (LDW), stem dry weight (SDW) and specific leaf area (SLA). The underline accession is checks.

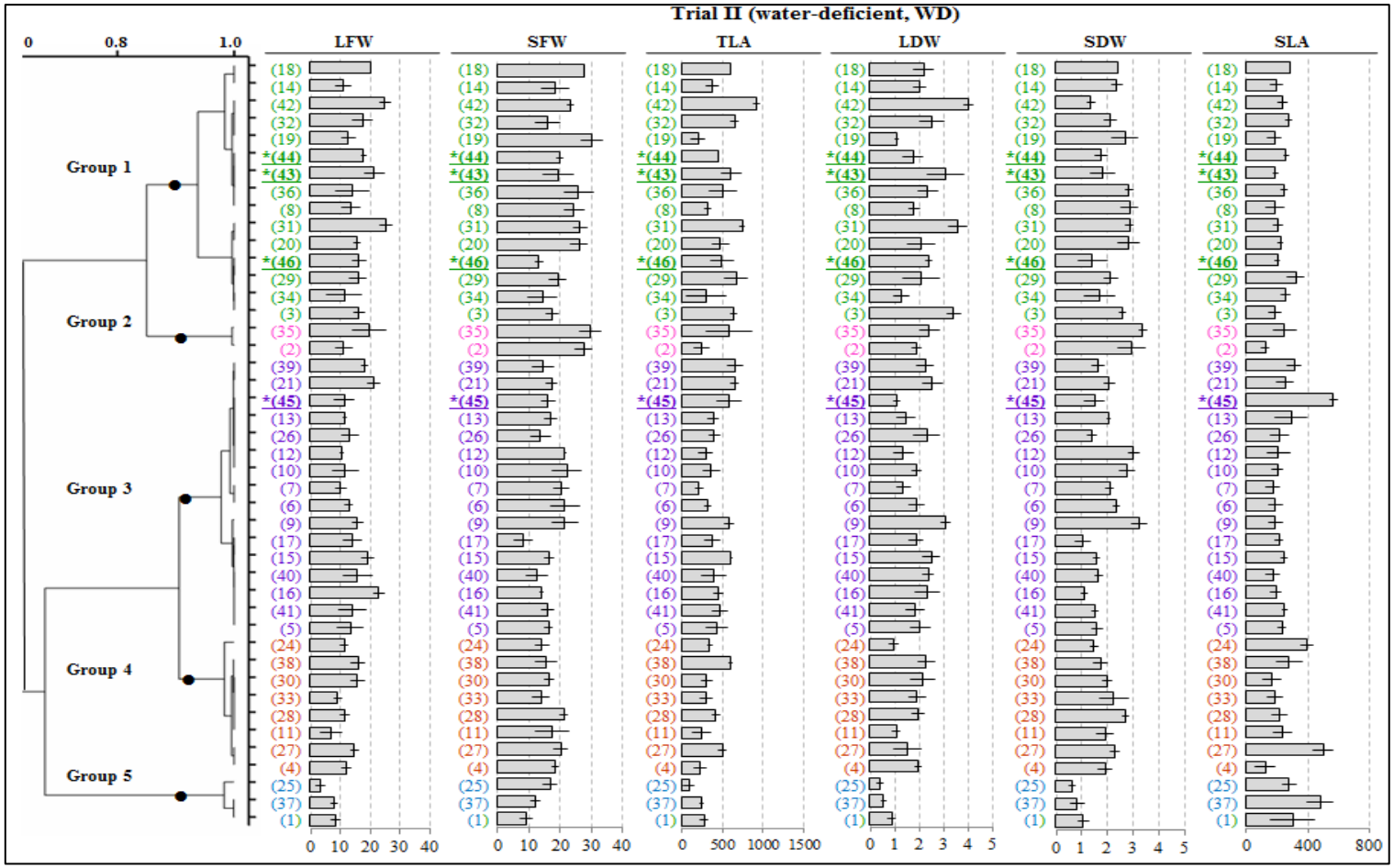


Figure 6.10: Biomass partitioning of 44 amaranth accessions under water-deficient (WD) conditions in their respective groups in Trial II. Data represents mean and error bar indicates standard error of mean (SE) for leaf fresh weight (LFW), stem fresh weight (SFW), total leaf area (TLA), leaf dry weight (LDW), stem dry weight (SDW) and specific leaf area (SLA). The underline accession is checks.

6.3.4 Genotypic variations in plant physiology in response to drought stress

6.3.4.1 Relative water content (RWC)

RWC of WD treatment was significantly reduced at further soil drying, i.e. 50% WHC and 20% WHC ($P<0.001$), and there was no significant differences exhibited among the groups (Table 6.5). However, significant interaction of WT*Accession was found for RWC at 20% WHC in Trial II when the data was analysed among accessions without grouping ($P<0.01$). In Trial I, RWC significantly reduced from 87% (0 DAT, 100% WHC) to 61% (6 DAT, 20% WHC) while in Trial II, the reductions of RWC were slightly lower, with the leaf water status declined from 91% at the beginning of drought stress (0 DAT, 100% WHC), then dropped to 83% at further soil drying (6 DAT, 50% WHC) and reached 76% at terminal drought stress (10 DAT, 20% WHC). The broad sense heritability showed low estimate values for RWC with ranged of 0.01 to 0.14, and extremely low at 0 DAT and 10 DAT under WS treatment (0.001, respectively).

6.3.4.2 Chlorophyll fluorescence

The efficiency of PSII activities under WS and WD treatment were reduced over time in both trials as the plants increased in size. Severe drought stress (20% WHC) significantly reduced quantum yield (QY) of photosystem II (PSII) photochemistry under light adaptation (*Fv'/Fm'*) in both Trials ($P<0.01$) and dark adaptation (*Fv/Fm*) in Trial II ($P<0.05$) (Table 6.6). It is interesting to note that, groups that had biomass partitioning mainly in stems rather than in leaf, which include the lowest category in Trial I (Group 2) and the highest and lowest category in Trial II (Group 2 and Group 5, respectively) exhibits high *Fv'/Fm'* at 20% WHC under WS treatment. This eventually contributed to higher relative reductions in *Fv'/Fm'* under severe drought stress compared with other groups. In comparison, Group 2 in Trial II also had high relative reduction in *Fv/Fm*, but Group 5 was able to retain high value of *Fv/Fm* under WS and WD treatment. The broad sense heritability showed low estimate values for *Fv'/Fm'* and *Fv/Fm* parameters recorded in both trial environments with ranged from 0.03 to 0.21, and exceptionally low for *Fv'/Fm'* at 6 DAT under WS treatment in Trial II (0.001).

Table 6.5: Effects of water treatment (WT: water-sufficient, WS and water-deficient, WD) on relative water content (RWC) in 44 vegetable amaranth accession evaluated in groups based on yield performance under control conditions in the two screening trials (Trial I and Trial II).

Relative water content (RWC)										
Trial I				**Trial II**					
	0 DAT	**6 DAT**			**0 DAT**	**6 DAT**		**20 DAT**	
		WS	**WD**			**WS**	**WD**	**WS**	**WD**
Mean	89.99	90.84	61.12	**Mean**	90.33	87.09	82.92	86.73	75.98
σ^2_G (F pr.)	2.43ns	4.15ns	7.10ns	**σ^2_G (F pr.)**	0.07ns	1.09ns	0.52ns	0.15ns	16.88ns
H_B	0.09	0.09	0.02	**H_B**	0.001	0.04	0.01	0.001	0.06
Group				**Group**					
1	88.32a	90.86a	57.77a	1	91.50a	87.98a	80.05a	87.96a	68.01a
2	90.55a	89.98a	66.55a	2	89.30a	86.57a	75.27a	83.19a	69.88a
3	88.46a	90.92a	62.36a	3	90.08a	87.01a	79.61a	85.98a	63.86a
WT		***		4	90.75a	86.02a	73.34a	87.51a	61.55a
Group		ns		5	90.04a	86.22a	84.34a	84.89a	65.34a
WT*G		ns		**WT**		***		***	
				Group		ns		ns	
				WT*G		ns		ns	

Trial I: 0 DAT is 100% WHC and 6 DAT is 20% WHC.

Trial II: 0 DAT is 100% WHC, 6 DAT is 50% WHC and 10 DAT is 20% WHC.

Data represents grand mean, variance with significance level among accessions (σ^2_G (F pr.)), broad-sense heritability (H_B), mean of grouping and significance level of ANOVA analysis. Probability significantly different at P<0.05 (*), P<0.01 (**), P<0.001 (***) or not significant (ns). Values in columns identified with the same letter are not statistically different among accessions based on Tukey's Pairwise method (P<0.05).

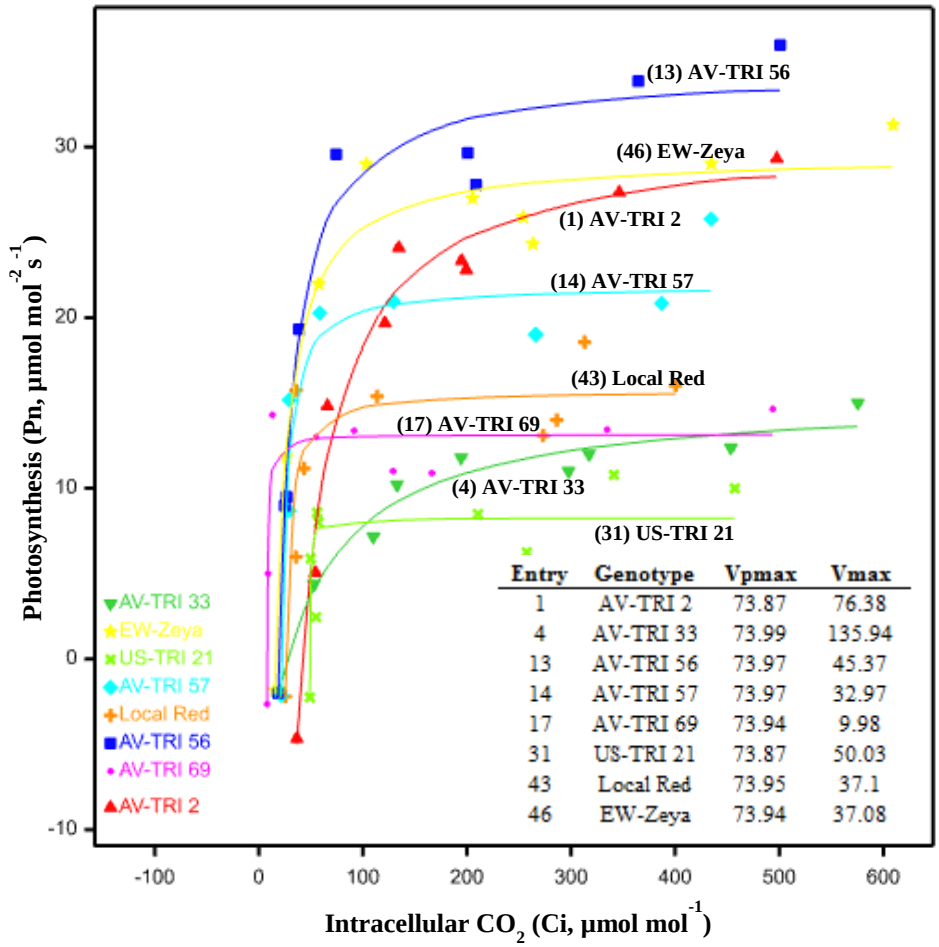
Table 6.6: Effects of water treatment (WT: water-sufficient, WS and water-deficient, WD) on light and dark adapted quantum yield of Photosystem II in 44 vegetable amaranth accessions evaluated in groups based on yield performance under control conditions in the two screening trials (Trial I and Trial II).

Chlorophyll fluorescence															
Light-adapted quantum yield (*Fv'/Fm'*)										**Dark-adapted quantum yield (*Fv/Fm*)**					
	Trial I			**Trial II**						**Trial II**					
	0 DAT	**6 DAT**			**0 DAT**	**6 DAT**		**10 DAT**			**0 DAT**	**6 DAT**		**10 DAT**	
		WS	**WD**			**WS**	**WD**	**WS**	**WD**			**WS**	**WD**	**WS**	**WD**
Mean	0.65	0.56	0.49	**Mean**	0.54	0.52	0.53	0.54	0.53	**Mean**	0.72	0.67	0.67	0.64	0.63
σ^2_G (F pr.)	0.0005ns	0.0003ns	0.0005ns	**σ^2_G (F pr.)**	0.0001ns	0.0001ns	0.0005ns	0.0003ns	0.0008ns	**σ^2_G (F pr.)**	0.0004ns	0.0003ns	0.0002ns	0.0008***	0.0006ns
H_B	0.21	0.2	0.04	**H_B**	0.03	0.001	0.01	0.06	0.09	**H_B**	0.06	0.09	0.04	0.21	0.05
Group				**Group**						**Group**					
1	0.66a	0.56a	0.49a	1	0.53a	0.51a	0.53a	0.55a	0.54a	1	0.73a	0.66a	0.66a	0.64bc	0.64a
2	0.65a	0.58a	0.48a	2	0.54a	0.55a	0.52a	0.57a	0.52a	2	0.71a	0.64a	0.63a	0.65ab	0.61a
3	0.65a	0.56a	0.48a	3	0.55a	0.52a	0.54a	0.54ab	0.51a	3	0.72a	0.67a	0.67a	0.64abc	0.62a
WT		***		4	0.54a	0.52a	0.51a	0.51b	0.50a	4	0.72a	0.69a	0.68a	0.60b	0.60b
Group		ns		5	0.53a	0.52a	0.51a	0.55a	0.55a	5	0.72a	0.72a	0.71a	0.68a	0.68a
WT*G		ns		**WT**		ns		**		**WT**		ns		*	
				Group		***		**		**Group**		ns		*	
				WT*G		ns		ns		**WT*G**		ns		ns	

Trial I: 0 DAT is 100% WHC and 6 DAT is 20% WHC.
Trial II: 0 DAT is 100% WHC, 6 DAT is 50% WHC and 10 DAT is 20% WHC.
Data represents grand mean, variance with significance level among accessions (σ^2_G (F pr.)), broad-sense heritability (H_B), mean of grouping and significance level of ANOVA analysis. Probability significantly different at P<0.05 (*), P<0.01 (**), P<0.001 (***) or not significant (ns). Values in columns identified with the same letter are not statistically different among accession based on Tukey's Pairwise method (P<0.05).

6.3.4.3 Photosynthetic characteristics

The Pn/Ci response curve of the eight amaranth accessions obtained at time 0 may predict the biochemical and biophysical components of photosynthesis in response to genotypic differences (Figure 6.12). It illustrates that the photosynthesis of the amaranth accessions was limited by similar PEP carboxylase capacity (Vpmax range at 73.0 µmol $mol^{-2}s^{-1}$) on the initial slope (Ci < 50 ppm). The photosynthesis increased rapidly then became stable when Ci reached approximately 100 µmol $mol^{-2}s^{-1}$. The saturated point of Ci (Vmax) could be varied among the amaranth accessions with range of 9.0 to 130.0 µmol $mol^{-2}s^{-1}$.



Entry	Genotype	Vpmax	Vmax
1	AV-TRI 2	73.87	76.38
4	AV-TRI 33	73.99	135.94
13	AV-TRI 56	73.97	45.37
14	AV-TRI 57	73.97	32.97
17	AV-TRI 69	73.94	9.98
31	US-TRI 21	73.87	50.03
43	Local Red	73.95	37.1
46	EW-Zeya	73.94	37.08

Figure 6.11: Summary Pn/Ci response curve for eight well-watered amaranth accessions before the onset of drought treatment. The shape of photosynthesis in response to Ci concentration was limited by PEP carboxylation (Vpmax) on the initial slope and the maximum CO_2 concentration point (Vmax) of the asymptote of non-horizontal line.

Moderate drought stress (50% WHC) had no effect on any of the photosynthetic parameters including Pn, Gs, Ci, E, WUE and Ls, with the exception of WUEi in Trial II (Table 6.7). Differences in photosynthetic parameters among groups was observed before the onset of drought stress although there was no significant differences was observed at 50% WHC in both WS and WD conditions. However, significant interactions between water treatment and accessions without grouping were observed for all photosynthetic parameters, which may imply that leaf gas exchange differed among amaranth accessions, not based on yield performance under the well-watered conditions grouping. As the water treatment progressed, Pn, Gs, Ci, E and WUE were significantly reduced while WUEi and Ls were significantly increased overtime as plants increased in size under both WS and WD treatments. At 0 DAT, the lowest category in Trial II (Group 5) had the highest Pn, WUE, WUEi and Ls but had the lowest Gs, E and Ci compared with other groups. Furthermore, the highest category in Trial II (Group 2) also exhibit the lowest E and the highest WUEi, probably related with biomass partitioning, similar patterns obtained in quantum yield of PSII. Moderate broad sense heritability estimates were found in most of the photosynthetic parameters (ranging from 0.30 to 0.55), however, the estimated values for photosynthetic parameters under WD treatment at 50% WHC were generally lower than WS treatment, and exceptionally lower for Gs (0.04), E (0.03) and WUEi (0.05).

Table 6.7: Effects of water treatment (WT: water-sufficient, WS and water-deficient, WD) on photosynthetic capacity in 44 vegetable amaranth accessions evaluated in groups based on yield performance under control conditions in the two screening trials (Trial I and Trial II).

Trial II	Photosynthesis ($\mu mol\ CO_2\ m^{-2}s^{-1}$)			Stomatal conductance ($mol\ H_2O\ m^{-2}s^{-1}$)			Transpiration ($mmol\ H_2O\ m^{-2}s^{-1}$)			Intracellular [CO2] ($\mu mol\ CO_2\ mol^{-1}$)		
	0 DAT	6 DAT		0 DAT	6 DAT		0 DAT	6 DAT		0 DAT	6 DAT	
		WS	WD		WS	WD		WS	WD		WS	WD
Mean	29.13	19.3	19.07	0.19	0.13	0.12	4.51	3.48	3.17	110.4	108.52	95.78
σ^2_G (F pr.)	51.05***	26.58***	11.45**	0.002***	0.02***	0.002ns	0.90***	1.33***	0.06ns	1439***	1741***	915***
H_B	0.5	0.34	0.16	0.48	0.36	0.04	0.4	0.42	0.03	0.51	0.35	0.21
Group												
1	27.58ab	19.13a	18.96a	0.18a	0.13a	0.12a	4.47ab	3.45a	3.19a	121.5a	107.6ab	90.22a
2	27.83ab	19.43a	16.36a	0.17ab	0.12a	0.09a	3.77b	3.55a	3.22a	104.4abc	117.7ab	73.22a
3	29.59ab	18.69a	18.67a	0.19a	0.13a	0.12a	4.64a	3.63a	3.08a	112.2ab	128.7a	101.62a
4	32.15a	19.83a	19.54a	0.20a	0.12a	0.12a	4.77a	3.11a	3.17a	97bc	83.8b	96.68a
5	27.26b	21.83a	22.37a	0.15b	0.12a	0.14a	3.80b	3.76a	3.49a	84.9c	73.3b	105.21a
WT		ns			ns			ns			ns	
Group		ns			ns			ns			ns	
WT*Group		ns			ns			ns			ns	

Trial II	Instantaneous WUE ($\mu mol\ mol^{-1}$)			Intrinsic WUE ($\mu mol\ mmol^{-1}$)			Stomatal limitation		
	0 DAT	6 DAT		0 DAT	6 DAT		0 DAT	6 DAT	
		WS	WD		WS	WD		WS	WD
Mean	6.65	6.13	6.35	163.3	170.25	176.45	0.71	0.72	0.75
σ^2_G (F pr.)	2.62***	2.06***	1.10***	705***	854***	127ns	0.01***	0.01***	0.006**
H_B	0.49	0.41	0.26	0.51	0.28	0.05	0.51	0.35	0.21
Group									
1	6.30c	6.38a	6.16a	155.4b	169.2ab	177.4	0.69c	0.73ab	0.77a
2	7.62a	5.42a	5.60a	164.7b	170.7ab	202.5a	0.73abc	0.70ab	0.81a
3	6.52bc	5.67a	6.61a	163.4b	156.4b	176.6a	0.70bc	0.67b	0.7a
4	6.99ab	6.77a	6.29a	167.1b	194a	172a	0.74ab	0.78a	0.75a
5	7.51a	6.07a	6.39a	191.4a	185.7b	165.4a	0.78a	0.81a	0.73a
WT		ns			ns			ns	
Group		ns			ns			ns	
WT*Group		ns			*			ns	

Trial II: 0 DAT is 100% WHC and 6 DAT is 50% WHC. Data represents grand mean, variance with significance level among accessions (σ^2_G (F pr.)), broad-sense heritability (H_B), mean of grouping and significance level of ANOVA analysis. Probability significantly different at P<0.05 (*), P<0.01 (**), P<0.001 (***) or not significant (ns). Values in columns identified with the same letter are not statistically different among accession based on Tukey's Pairwise method (P<0.05).

6.3.5 Genotypic variation in drought resistance, adaptability and recovery

There was a significant difference on DTF, DTW and DTR among the groups, with lowest category (Group 5) being the earliest to initiate panicle emergence at 1 DAT while the high and medium category (Group 1 and Group 3, respectively) only began to flower later at 4 DAT, and Group 5 also demonstrate slower responses to wilting (5 DAT) and to recover (4 DAT) while other groups response rapidly once soil water status started to change (Table 6.8). However, there was no significant difference in LWS and DS among groups, although DS-1R and DS-5R were differed among the accessions without grouping. Most of the accessions had leaves rolled into a V-shape or U-shape and showed symptoms such as slight tip drying in early response to drought stress. Upon re-watering, most of the accessions had more than 5 leaves showing senescence during the recovery process and grow more branches along the stem after few days of re-watering (Figure 6.13; Appendix 6.4). It is important to note that accession AV-TRI 26 and AV-TRI 54 were unable to recover after experiencing severe drought stress and re-watering, and accession AV-TRI 57, AV-TRI 58 and US-TRI 24 died after 5 days of re-watering. High broad-sense heritability occurred for recovery assessment, moderate for DTW and exceptionally low for DTW, LWS and DS.

Table 6.8: Effects of water-deficient (WD) on days to the early panicle flowering (DTF), days to wilting (DTW), leaf wilting scoring (LWS), drought stress symptoms scoring (DS) and effects of re-water on the days to recover (DTR) and DS at first and fifth day of recovery (DS-1R, DS-5R respectively) in 44 vegetable amaranth accessions.

	Drought stress				**Recovery**		
	DTF	**DTW**	**LWS**	**DS**	**DTR**	**DS-1R**	**DS-5R**
Range	0-4	1-8	0-5	0-7	1-6	0-9	0-9
Mean	3ns	3.48ns	3ns	2ns	2***	4***	5***
H_B	0.24	0.06	0.02	0.01	0.67	0.74	0.51
Group							
1	4a	4b	3a	2a	1b	3a	5a
2	3ab	3b	3a	2a	2b	5a	5a
3	4a	3b	3a	1a	1b	3a	5a
4	2b	3b	2a	2a	2b	3a	5a
5	1c	5a	2a	2a	4a	4a	2a

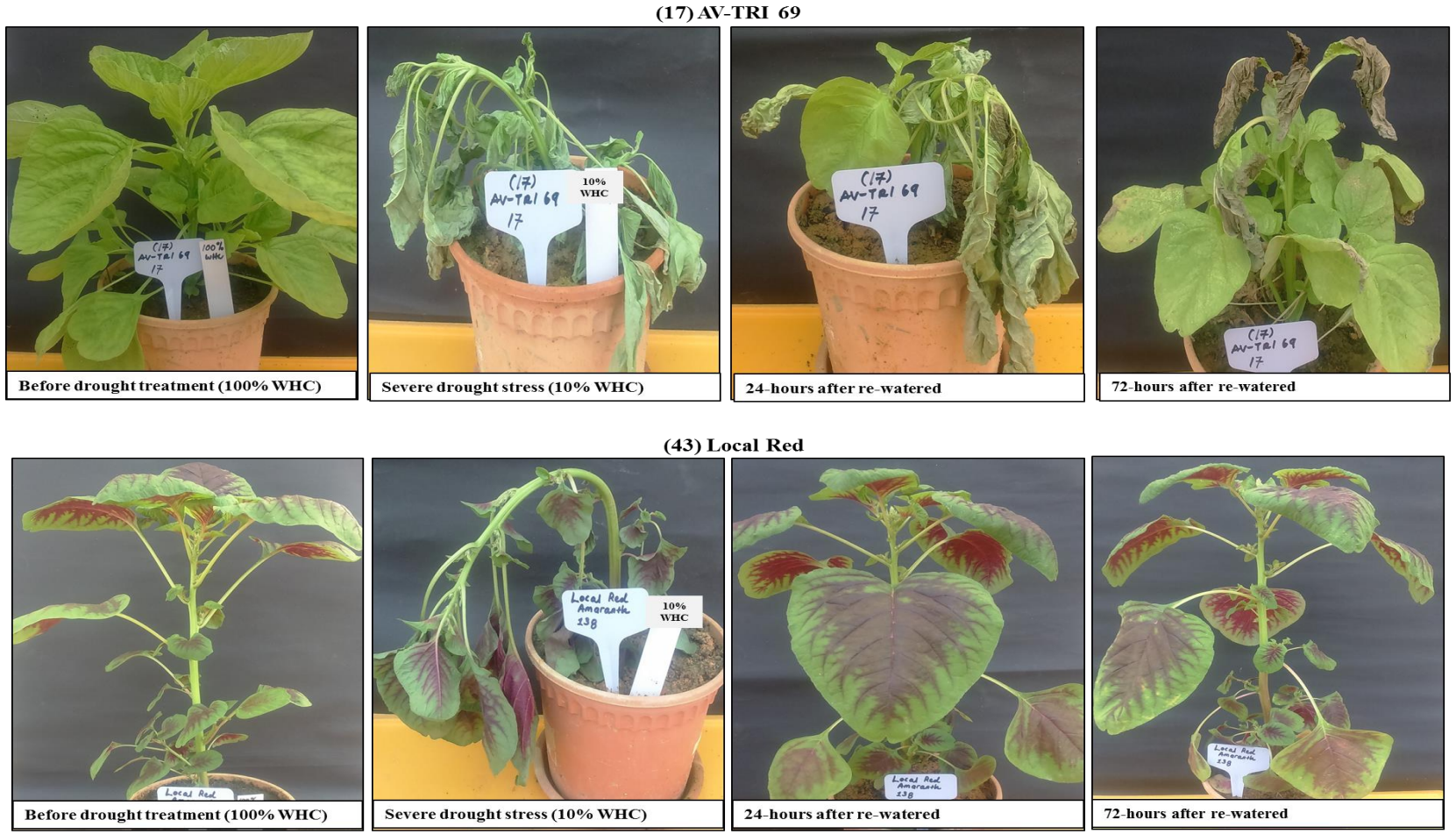


Figure 6.12: The examples of re-watering assessment on amaranth accessions. The images were captured (i) on a day before the imposition of drought stress (100% WHC), (ii) at terminal drought stress (10% WHC), (iii) after 24-hours of re-watered and (iv) after 72-hours of re-watered. More pictures of re-watered assessment are presented in Appendix 6.4

6.3.6 Correlation

Table 6.9 and Table 6.10 summarizes correlation coefficient (r) describing the degree of associations among the growth and physiological traits measured under WS and WD treatments in the two drought screening trials. In Trial I, under WS treatments, yield was positively correlated with *Fv'/Fm'* at 100%WHC (r=0.42, P<0.001), and negatively correlated with *Fv'/Fm'* at 20% WHC (r =-0.26, P<0.01) and SLA (r=-0.22, P<0.01). In contrast, under WD treatments, yield showed negative correlations with RWC at 20% WHC (r=-0.19, P<0.05). In Trial II, yield showed a negative correlation with *Fv/Fm* at 50% WHC (r>-0.34, P<0.01) in both WS and WD treatments. Further, under WS treatments, yield was also positively correlated with Ci at 100% WHC (r=0.27, P<0.001), *Fv'/Fm'* at 20% WHC (r= 0.28, P<0.001), RWC at 100% WHC (r=0.28, P<0.01) and negatively correlated with Ls at 100% WHC (r=-0.28, P<0.001), SLA (r=-0.33, P<0.001), WUE and WUEi at 100% WHC (r=0.21, P<0.01 and r=-0.26, P<0.001 respectively). Under WD treatments, yield was positively correlated with RWC at 20% WHC (r=0.47, P<0.0001).

The selection of traits are primarily emphasized with traits that were consistently associated in both water treatment and thus, it is important to note that RWC at 50% WHC was positively correlated with LDW (r>0.10, P<0.01) and TLA (r>0.17, P<0.001), while SLA was negatively correlated with only LDW (r>-0.50, P<0.001) observed in WS and WD conditions. Meanwhile, *Fv/Fm* at 50% WHC showed various association with other physiological traits under both water treatment which include positive correlation with *Fv/Fm* at 100% WHC and 20% WHC (r>0.34 and r>0.26, P<0.001 respectively), *Fv'/Fm'* at 100% WHC and 50% WHC (r >0.17, P<0.05 and r >0.19, P<0.01 respectively) and WUE at 100% WHC (r =0.21, P<0.05,), and negatively correlated with LFW (r<-0.20, P<0.001), TLA (r >-0.30, P<0.001), E and Gs at 100% WHC (r >-0.31, P<0.01 and r >0.21, P<0.05 respectively).

In the re-watering assessment, yield and leaf biomass traits (LDW, LFW and TLA) were positively correlated with DTF (r>0.20, P<0.05) and negatively correlated with DTR (r>-0.27, P<0.05), while SDW was negatively correlated with DTW (r=-0.21, P<0.05) and SFW was negatively correlated with DTR (r=-0.21, P<0.05) (Table 6.11). While DTF and DTR were mostly associated with yield and biomass partitioning, DTW was generally correlated with physiological traits

including E at 100% WHC (r=0.25, P<0.01), Gs at 50% WHC (r=0.22, P<0.01), RWC at 20% WHC (r=0.24, P<0.05), and negatively correlated with WUE at 100% WHC (r=-0.22, P<0.05). The drought symptom scoring after 5 days of recovery (DS-5R) showed positive correlation with DTF (r=0.18, P<0.05) and negative correlation with DTW (r=-0.25, P<0.01), while drought symptom scoring on the first day of recovering showed positive correlation with DTR (r=0.38, P<0.001) and DS-5R (r=0.52, P<0.001). The leaf wilting scoring was negatively correlated with DTR (r=-0.29, P<0.01).

Table 6.9: Correlation coefficients (r) for traits associated with water-sufficient (WS) in the bottom diagonal and water-deficient (WD) in the top diagonal for the 44 vegetable amaranth accessions in Trial I.

	LDW	LFW	Fv'/Fm'_20	Fv'/Fm'_100	RWC_20	RWC_100	SDW	SFW	SLA	TLA	Yield
LDW	-	0.73***	-0.05	-0.05	-0.43***	-0.13	0.30***	0.34***	-0.02	0.82***	0.60***
LFW	0.78**	-	0.03	0.05	-0.11	-0.1	0.14	0.38***	0.29***	0.77***	0.77***
Fv'/Fm'_20	-0.26**	-0.2*	-	0.52***	0.08	0.09	-0.1	-0.08**	0.33***	0.16	-0.04
Fv'/Fm'_100	0.39***	0.4***	0.1	-	0.07	0	-0.08	-0.09	0.42***	0.20*	-0.04
RWC_20	-0.13	-0.03	0.06	-0.15	-	0.22**	-0.30***	-0.20*	0.19*	-0.24**	-0.19*
RWC_100	0.01	0.01	0.03	0.11	0.05	-	-0.21**	-0.15	0.11	-0.07	-0.16
SDW	0.59***	0.50***	-0.26**	0.39***	-0.14	-0.05	-	0.82***	-0.14	0.16	0.64***
SFW	0.45***	0.53***	-0.26**	0.33***	-0.09	-0.02	0.87***	-	-0.09	0.27**	0.89***
SLA	-0.31***	-0.1	0.26**	-0.21**	0.04	-0.06	-0.29***	-0.3***	-	0.50***	0.08
TLA	0.81***	0.74***	-0.11	0.27**	-0.09	0.01	0.43***	0.3***	0.24**	-	0.57***
Yield	0.71***	0.89***	-0.26**	0.42***	-0.06	0.01	0.77***	0.86***	-0.22**	0.61***	-

LDW: leaf dry weight, LFW: leaf fresh weight, Fv'/Fm': light-adapted quantum yield at 20% or 100% WHC, RWC: relative water content at 20% or 100% WHC, SDW: stem dry weight, SFW: stem fresh weight, SLA: specific leaf area, TLA: total leaf area

Table 6.10: Correlation coefficients (r) for traits associated with water-sufficient (WS) in the bottom diagonal and water-deficient (WD) in the top diagonal for the 44 vegetable amaranth accessions in Trial II.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	-	0.04	0.38***	-0.12	0.42***	0	0.09	0.15	-1.00***	-0.03	-0.39***	-0.02	0.02	0	-0.03	-0.03
2	0.19**	-	0.01	0.45***	-0.01	0.44***	-0.05	0.02	-0.04	-1.00***	-0.08	0	0.04	-0.07	0.02	-0.20*
3	0.18*	-0.1	-	-0.1	0.87***	0.06	-0.01	0.1	-0.41***	-0.01	0.54***	0.05	-0.30***	-0.12	-0.09	-0.25**
4	0.05	0.51***	-0.13	-	-0.06	0.88***	-0.04	0.07	0.13	-0.46***	0.03	0.73***	0	-0.03	-0.06	0.02
5	0.23**	-0.11	0.91***	-0.09	-	0.04	-0.03	0	-0.45***	0.01	0.63***	0.04	-0.26**	-0.12	-0.09	-0.24*
6	0.07	0.49***	-0.06	0.92***	-0.04	-	-0.01	0.14	0.01	-0.46***	0.02	0.84***	-0.05	-0.04	-0.02	-0.05
7	0.17*	-0.03	0.1	-0.17*	0.07	-0.11	-	0.65***	-0.08	0.05	-0.12	0	0.12	0.27**	0.01	0.19*
8	0.16*	0.06	0.08	-0.15*	0.04	-0.11	0.81***	-	-0.14	-0.02	-0.15	0.11	0.01	0.19*	-0.06	0.14
9	-0.99***	-0.20**	-0.23**	-0.05	-0.28***	-0.07	-0.17*	-0.17*	-	0.03	0.35***	0.02	-0.01	0	0.03	0.04
10	-0.19**	-1.00***	0.1	-0.53***	0.11	-0.51***	0.04	-0.05	0.20**	-	0.08	-0.02	-0.04	0.07	-0.02	0.19*
11	-0.21**	-0.18*	0.81***	-0.1	0.88***	-0.07	-0.01	-0.03	0.16**	0.18*	-	0.07	-0.29***	-0.14	-0.04	-0.16
12	0	0	0	1.00***	0	1.00***	-0.13	-0.18**	0.05	-0.03	-0.03	-	-0.04	-0.04	-0.07	0.04
13	-0.1	-0.05	-0.03	-0.04	0.06	-0.08	0.01	0.04	0.09	0.05	0.13	-0.04	-	0.15	-0.09	0.18*
14	0	0	0	0	0	0	-0.08	0	0.02	-0.13	-0.03	0.07	-0.02	-	-0.47***	0.27**
15	-0.08	-0.05	0.03	-0.13	0.06	-0.15*	0.24**	0.30***	0.06	0.06	0.09	-0.14	0.32***	-0.30***	-	0.07
16	-0.14	-0.08	-0.21**	0	-0.15*	-0.05	0.07	0.11	0.14	0.08	-0.08	-0.08	0.20**	-0.17*	0.23**	-
17	-0.13	0.05	-0.24**	0.13	-0.15*	0.03	-0.20**	-0.25***	0.13	-0.06	-0.07	-0.02	0.19**	0.17*	-0.04	0.26***
18	0.02	0.02	-0.09	0.08	0	0.03	0.07	0.03	-0.02	-0.01	0	0.01	0.07	0.24**	-0.16*	0.12
19	0.06	-0.20**	0.07	-0.1	0.08	-0.08	0.14	0.07	-0.04	0.20**	0.05	0.04	-0.07	-0.09	-0.04	0.1
20	0.05	-0.15*	0.08	-0.09	0.06	-0.09	0.23**	0.13	-0.06	0.16*	0.04	-0.03	-0.1	0.18*	0.01	0.12
21	0.11	0.14	-0.01	0.06	-0.01	0.02	0	0.1	-0.15*	-0.14	-0.04	-0.09	0.03	-0.03	0.12	-0.01
22	0.20**	0.13	0.05	0.06	0.05	0.1	0.49***	0.33***	-0.19**	-0.12	-0.05	0.03	-0.03	-0.22**	0.13	-0.03
23	0.29***	0.13	0.06	0.14	0.09	0.12	0.41***	0.41***	-0.30***	-0.13	-0.03	0.05	-0.01	-0.14	0.17*	0
24	-0.22**	-0.09	-0.12	-0.05	-0.12	-0.1	-0.51***	-0.21**	0.22**	0.09	0	-0.04	0.07	0.03	0.01	0.19**
25	-0.02	-0.01	0.06	-0.19*	0.02	-0.13	0.73***	0.84***	0.02	0.03	0.02	-0.15*	0.05	-0.03	0.23**	0.12
26	-0.65***	0	-0.09	0	0.12	0	-0.21**	-0.14	0.64***	0.11	0.47***	0.01	0.28***	0.05	0.13	0.20**
27	-0.02	-0.69***	0.23**	-0.51***	0.15*	-0.32***	0.17*	0.07	0.03	0.69***	0.14	0.07	-0.05	-0.03	-0.02	-0.1
28	-0.90***	-0.09	-0.37***	-0.03	-0.39***	-0.06	-0.19**	-0.13	0.91***	0.09	0.04	0.01	0.13	0.03	0.05	0.18**
29	-0.08	-0.83***	0.11	-0.60***	0.1	-0.62***	0.06	-0.01	0.08	0.83***	0.12	-0.16*	0.06	-0.09	0.03	-0.01
30	0.27***	0.11	0.08	0	0.08	0.01	0.72***	0.82***	-0.28***	-0.11	-0.04	-0.08	0.02	-0.09	0.28***	0.06

Continue…

1: Intracellular CO_2 concentration (Ci) at 100% WHC, 2: Ci at 50% WHC, 3: Transpiration (E) at 100% WHC, 4: E at 50% WHC, 5: Stomatal conductance (Gs) at 100% WHC, 6: Gs at 50%WHC, 7: Leaf dry weight (LDW), 8: Leaf fresh weight (LFW), 9: Stomatal limitation (Ls) at 100% WHC, 10: Ls at 50% WHC, 11: Photosynthesis (Pn) at 100% WHC, 12: Pn at 50% WHC, 13: Light-adapted quantum yield (Fv'/Fm') at 50% WHC, 14: Fv'/Fm' at 100% WHC, 15: Fv'/Fm' at 20% WHC, 16:dark-adapted quantum yield (Fv/Fm) at 20% WHC, 17: Fv/Fm at 50% WHC, 18:Fv/Fm at 100% WHC, 19: relative water content (RWC) at 20% WHC, 20: RWC at 50% WHC, 21: RWC at 100% WHC, 22: Stem dry weight (SDW), 23: Stem fresh weight (SFW), 24: Specific leaf area (SLA), 25: Total leaf area (TLA), 26: Instantaneous water use efficiency (WUE) at 100% WHC, 27: WUE at 50% WHC, 28: Intrinsic water use efficiency (WUEi) at 100% WHC, 29: WUEi at 50% WHC, 30: Yield. *, **, ** significant at P<0.05, <0.01, <0.001, respectively.

Table 6.10: (Continued)

	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	-0.16	-0.07	0.06	0.04	-0.15	-0.04	-0.1	-0.07	0.17*	-0.76***	0.14	-0.90***	-0.05	0.03
2	-0.14	-0.24*	-0.02	-0.09	0.02	-0.02	-0.11	0.03	0.07	-0.08	-0.57***	0	-0.81***	-0.06
3	-0.31***	-0.07	0.29***	-0.03	-0.01	0.02	0.07	0.01	0.09	-0.58***	0.14	-0.51***	-0.08	0.1
4	-0.01	-0.16	-0.04	0	0.1	-0.13	-0.06	0.05	0.03	0.06	-0.44***	0.06	-0.55***	0
5	-0.21*	-0.06	0.11	-0.04	-0.05	0.12	0.07	0	0.03	-0.34***	0.08	-0.55***	-0.06	0.04
6	-0.03	-0.16	0.04	0.02	0.01	-0.14	-0.07	0.11	0.1	-0.11	-0.15	-0.06	-0.59***	0.04
7	-0.03	-0.06	0.03	0.27**	-0.08	0.30***	0.26**	-0.34***	0.62***	-0.12	0.07	-0.12	0.06	0.54***
8	-0.31***	-0.17	0.49***	0.21*	-0.05	0.17*	0.38***	0.02	0.77***	-0.27**	-0.02	-0.20**	-0.08	0.81***
9	0.16	0.07	-0.07	-0.04	0.15	0.04	0.09	0.07	-0.16	0.76***	-0.14	0.90***	0.05	-0.02
10	0.14	0.24**	0.02	0.09	-0.02	0.03	0.11	-0.03	-0.07	0.08	0.56***	0	0.82***	0.06
11	-0.06	0.04	0.05	-0.06	0.11	0.1	0.1	0.06	-0.14	0.34***	0.01	0.24**	0.04	-0.02
12	0.07	-0.03	0.03	0.04	0.03	-0.14	-0.05	0.12	0.03	-0.07	0.20*	-0.05	-0.15	0.03
13	0.31***	0.06	-0.24**	0	0.08	-0.04	-0.25**	-0.08	0	0.06	0.02	0.05	0.04	-0.15
14	0.40***	0.31***	-0.09	0.34***	0.03	-0.12	-0.04	-0.12	0.12	-0.03	0	0	0.02	0.09
15	-0.06	-0.04	0	-0.12	-0.05	0.01	-0.08	-0.11	-0.03	0.11	0.08	0.13	0.01	-0.09
16	0.32***	0.23**	0.04	0.20*	-0.01	-0.17*	-0.02	-0.06	0.12	0.1	0.04	0.09	0.19*	0.07
17	-	0.43***	-0.47***	0.09	0.04	-0.20*	-0.27**	-0.14	-0.34***	0.27**	0.13	0.21*	0.16	-0.35***
18	0.34***	-	-0.24**	0.04	0.01	-0.20*	-0.08	-0.14	-0.24**	0.11	0.16	0.1	0.21*	-0.14
19	0	-0.01	-	0.18*	0.13	-0.04	0.29***	0.27**	0.43***	-0.25**	0.05	-0.1	0	0.47***
20	-0.14	0.13	0.17*	-	0.07	-0.13	-0.06	-0.05	0.29***	-0.06	0.11	-0.03	0.09	0.08
21	0.01	-0.06	0.1	0.08	-	-0.02	-0.04	0.01	-0.07	0.14	-0.02	0.16	0.08	-0.06
22	-0.11	0.01	0.07	-0.05	0.08	-	0.61***	-0.07	0.12	0.13	-0.02	-0.01	0.07	0.48***
23	-0.08	0.08	0.01	0	0.26***	0.75***	-	-0.02	0.27**	0.04	-0.03	0.01	0.05	0.85***
24	0.07	-0.01	-0.03	-0.02	-0.03	-0.53***	-0.33***	-	0.16	0.02	0.08	0.05	-0.06	0
25	-0.22**	0.04	0.08	0.17*	-0.01	0.22**	0.22**	0.03	-	-0.25**	0.02	-0.22**	-0.09	0.62***
26	0.22**	0.1	-0.01	-0.04	-0.06	-0.20*	-0.20*	0.25***	-0.02	-	-0.13	0.81***	0.17*	-0.13
27	-0.25**	-0.04	0.23**	0.17*	-0.1	-0.09	-0.14	-0.04	0.13	-0.13	-	-0.09	0.66***	-0.03
28	0.16*	-0.02	-0.04	-0.02	-0.1	-0.21**	-0.30***	0.30***	0.04	0.70***	-0.06	-	0.1	-0.11
29	-0.1	-0.01	0.21**	0.17*	-0.09	-0.14	-0.14	0.06	0.03	0.01	0.76***	0	-	-0.01
30	-0.19**	0.07	0.04	0.07	0.22**	0.66***	0.85***	-0.33***	0.62***	-0.21**	-0.04	-0.26***	-0.09	-

1: Intracellular CO_2 concentration (Ci) at 100% WHC, 2: Ci at 50% WHC, 3: Transpiration (E) at 100% WHC, 4: E at 50% WHC, 5: Stomatal conductance (Gs) at 100% WHC, 6: Gs at 50%WHC, 7: Leaf dry weight (LDW), 8: Leaf fresh weight (LFW), 9: Stomatal limitation (Ls) at 100% WHC, 10: Ls at 50% WHC, 11: Photosynthesis (Pn) at 100% WHC, 12: Pn at 50% WHC, 13: Dark-adapted quantum yield (Fv/Fm) at 50% WHC, 14: Fv/Fm at 100% WHC, 15: Fv/Fm at 20% WHC, 16: light-adapted quantum yield (Fv'/Fm') at 20% WHC, 17: Fv'/Fm' at 50% WHC, 18:Fv'/Fm' at 100% WHC, 19: relative water content (RWC) at 20% WHC, 20: RWC at 50% WHC, 21: RWC at 100% WHC, 22: Stem dry weight (SDW), 23: Stem fresh weight (SFW), 24: Specific leaf area (SLA), 25: Total leaf area (TLA), 26: Instantaneous water use efficiency (WUE) at 100% WHC, 27: WUE at 50% WHC, 28: Intrinsic water use efficiency (WUEi) at 100% WHC, 29: WUEi at 50% WHC, 30: Yield, 31: Days to flowering (DTF), 32: Days to recover (DTR), 33: Days to wilting (DTW). *, **, ** significant at P<0.05, <0.01, <0.001, respectively.

Table 6.11: Correlation coefficient (r) between drought-adaptive capabilities and physiological responses.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Yield	30	0.05	-0.06	0.17	0.00	0.10	0.05	0.53***	0.81***	-0.05	0.06	0.01	0.03	-0.15	0.11	-0.08	0.10	-0.31***	-0.12
DTF	31	0.12	0.12	0.02	0.14	0.04	0.09	0.35***	0.27**	-0.11	-0.12	-0.08	0.02	0.14	0.07	0.11	0.03	-0.04	-0.07
DTR	32	-0.04	-0.03	-0.10	-0.09	-0.15	-0.08	-0.12	-0.21**	0.05	0.03	-0.03	-0.07	0.04	-0.03	0.05	0.07	0.07	0.09
DTW	33	0.09	0.11	0.25**	0.11	0.11	0.22**	-0.12	0.11	-0.09	-0.11	0.05	0.19	-0.06	-0.17	0.16	-0.01	-0.10	-0.08
DS-5R	34	-0.02	0.00	0.00	-0.01	0.04	-0.02	0.08	-0.09	0.03	-0.01	0.03	0.02	-0.04	0.02	0.00	-0.07	0.03	0.06
DS-1R	35	-0.09	0.05	-0.07	0.05	0.00	0.01	0.16	-0.07	0.09	-0.05	0.06	-0.03	-0.02	0.09	0.02	0.04	0.11	0.06
LWS	36	-0.07	0.07	0.03	-0.06	0.09	-0.05	-0.07	0.01	0.06	-0.07	0.12	0.00	-0.08	0.05	0.04	-0.01	0.10	0.19*

		19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
Yield	30	0.46***	0.08	-0.05	0.46***	0.84***	0.00	0.6***	-0.17*	-0.01	-0.14	-0.05						
DTF	31	-0.06	0.00	0.02	0.2*	0.03	-0.13	0.25**	-0.06	-0.12	-0.13	-0.07	0.18*					
DTR	32	0.01	0.02	0.14	-0.22**	-0.21*	0.01	-0.13	0.14	0.06	0.22**	0.12	-0.25**	-0.16				
DTW	33	0.24**	-0.02	0.03	-0.21*	-0.06	0.03	0.06	-0.22*	0.00	-0.08	-0.17	0.03	-0.10	0.15			
DS-5R	34	-0.17*	-0.18*	-0.02	0.26**	0.03	-0.13	-0.11	0.04	0.04	-0.03	0.05	-0.03	0.18*	-0.05	-0.25**		
DS-1R	35	-0.19*	-0.10	0.04	0.18*	0.12	-0.14	0.00	0.15	-0.07	0.09	-0.03	0.04	0.15	0.38***	-0.14	0.52***	
LWS	36	-0.06	-0.04	0.00	0.00	0.06	0.05	-0.03	0.12	0.01	0.02	-0.01	0.04	0.02	-0.29**	0.06	0.11	-0.11

1: Intracellular CO_2 concentration (Ci) at 100% WHC, 2: Ci at 50% WHC, 3: Transpiration (E) at 100% WHC, 4: E at 50% WHC, 5: Stomatal conductance (Gs) at 100% WHC, 6: Gs at 50%WHC, 7: Leaf dry weight (LDW), 8: Leaf fresh weight (LFW), 9: Stomatal limitation (Ls) at 100% WHC, 10: Ls at 50% WHC, 11: Photosynthesis (Pn) at 100% WHC, 12: Pn at 50% WHC, 13: Dark-adapted quantum yield (Fv/Fm) at 50% WHC, 14: Fv/Fm at 100% WHC, 15: Fv/Fm at 20% WHC, 16: light-adapted quantum yield (Fv'/Fm') at 20% WHC, 17: Fv'/Fm' at 50% WHC, 18:Fv'/Fm' at 100% WHC, 19: relative water content (RWC) at 20% WHC, 20: RWC at 50% WHC, 21: RWC at 100% WHC, 22: Stem dry weight (SDW), 23: Stem fresh weight (SFW), 24: Specific leaf area (SLA), 25: Total leaf area (TLA), 26: Instantaneous water use efficiency (WUE) at 100% WHC, 27: WUE at 50% WHC, 28: Intrinsic water use efficiency (WUEi) at 100% WHC, 29: WUEi at 50% WHC, 30: Yield, 31: Days to flowering (DTF), 32: Days to recover (DTR), 33: Days to wilting (DTW), 34: Drought symptoms scoring after 5 days of recovering (DS-5R), 35: Drought symptoms scoring at first day of recovering (DS-1R), LWS: Leaf wilting scoring. *, **, ** significant at P<0.05, <0.01, <0.001, respectively.

6.3.7 Comparison of yield and physiological responses based on drought tolerance indices

6.3.7.1 Analysis of drought tolerance indices procedures in two screening trials

Stress intensity of Trial I was significantly higher than Trial II (0.73 and 0.31 respectively). This demonstrated that drought stress imposed during Trial I was severe that the yield loss was higher than the moderate drought stress imposed during Trial II. Therefore, tolerance indices were performed separately for each trial (Table 6.12) and the mean comparisons between accessions was determined for each tolerance index (Appendix 6.5). Significant differences were only observed for GMP, MP and STI in both screening trials ($P<0.05$), which can be considered as a good indicator in discriminating tolerant/susceptible accessions.

There were also significant interactions between drought screening trials and accessions observed for GMP ($P<0.01$), MP ($P<0.001$) and STI ($P<0.05$), and significant differences examined for the main effects of trials on DI ($P<0.001$), TOL ($P<0.05$) and YSI ($P<0.01$), while the SSI index showed no effect for either screening trials (Table 6.12). This depicted that GMP, MP and STI were stable indices which can be used in either severe or moderate drought stress, while TOL, SSI and YSI may varied in different drought stress conditions.

6.3.7.2 Interrelationships among indices

To further verify the most suitable and stable screening criterion, the correlation coefficient between yield under stress condition (Ys), non-stress condition (Yp) and drought tolerance indices were calculated (Table 6.13). Yp had a very weak association with Ys in Trial I, depicting that high yielding accession under normal condition did not anticipate superior yield under severe drought stress condition. However, in Trial II, Yp was strongly associated with Ys ($P<0.01$), indicating that high yielding accessions under normal condition was also expected to have high yield under moderate drought stress.

Yp and Ys were positively correlated with GMP, MP and STI in both drought screening trials ($P<0.001$).This indicates that these indices were more effective in identifying high yielding accessions in normal condition as well as in moderate or severe drought stress. Therefore, accessions which possess high values of STI, MP and GMP can be considered superior.

Meanwhile, YSI was positively correlated with Ys but negatively correlated with Yp in both drought screening trials (P<0.01). This denotes that selection based on YSI should provide accessions with low yield in normal condition but high yield in moderate or severe drought stress condition. Other than that, selection based on DI also can be used to identify accession with low yield in normal condition but high yield in severe drought stress condition. In contrast, SSI was positively correlated with Yp but negatively correlated with Ys (P<0.001), indicating that SSI was suitable to identify accessions with high yield in normal condition but low yield in moderate or severe drought stress (larger reduction of yield). TOL also can be used to identify accessions with those criterions, but only in moderate drought stress.

Table 6.12: The analysis of variance for DI, GMP, MP, SSI, STI, TOL and YSI drought tolerance indices across the two drought screening trials (Trial I and Trial II).

SI: stress intensity, DI: drought resistance index, GMP: geometric mean productivity, MP: mean productivity, SSI: stress susceptibility index, STI: stress tolerance index, TOL: stress tolerance and YSI: yield stability index.

		DI	GMP	MP	SSI	STI	TOL	YSI
Trials	**SI**	**Mean**						
Trial I	0.73	0.36ns	7.88*	10*	0.91ns	0.27*	11.6ns	0.34ns
Trial II	0.31	0.78ns	39.4***	40.7***	0.87ns	0.72***	14.9ns	0.73ns
SOV	**d.f.**							
Trial	1	13.07***	74960***	70981***	0.09ns	15.90***	809.4*	11.7**
Accession	43	0.19ns	231**	229***	0.56ns	0.31***	172.6*	0.08ns
Trial*Accession	43	0.14ns	107**	105***	0.35ns	0.13*	98.7ns	0.06ns
Error	220	0.09	51	51.8	0.57	0.09	121	0.08

Data represents mean with level of significant difference among accessions within each drought screening trial. ANOVA table: mean square with level of significance difference at P<0.05, 0.01, 0.001, non-significant (*, **, ***, ns, respectively).

Table 6.13: Correlation coefficient between yield of vegetable amaranth accessions in with DI, GMP, MP, SSI, STI, TOL and YSI in the two drought screening trials.

		Yp	Ys	DI	GMP	MP	SSI	STI	TOL
Trial I	**Ys**	0.12							
	DI	-.479**	.605**						
	GMP	.791**	.682**	-0.052					
	MP	.977**	.330**	-.325**	.900**				
	SSI	.620**	-.289**	-.906**	.334**	.527**			
	STI	.774**	.681**	-0.012	.983**	.884**	.270**		
	TOL	.974**	-0.108	-.618**	.637**	.903**	.687**	.620**	
	YSI	-.620**	.289**	.906**	-.334**	-.527**	-1.00**	-.270**	-.687**
Trial II	**Ys**	.511**							
	DI	-0.01	.821**						
	GMP	.856**	.878**	.467**					
	MP	.903**	.831**	.404**	.993**				
	SSI	.444**	-.487**	-.862**	-0.034	0.044			
	STI	.839**	.856**	.448**	.978**	.971**	-0.029		
	TOL	.674**	-.291**	-.717**	.197**	.291**	.913**	.197**	
	YSI	-.444**	.487**	.862**	0.034	-0.044	-1.00**	0.03	-.913**

6.3.7.3 Drought tolerance ranking of the 44 amaranth accessions using biplot analysis and its association with physiological responses

Biplot analysis based on principle component analysis was used to identify the relationships between indices and to distinguish superior accessions (Farshadfar *et al*., 2012). Smaller angles between dimension vectors in the same direction indicate high correlation of the variable traits in terms of discriminating accessions (Mwadzingeni *et al*., 2016). These will distinguish amaranth accessions into four established group criterions (Group A, B, C and D), according to Fernandez (1992).

The PCAs axes clearly separate the indices into different groups. The first two PCAs accounted for 94.01% and 97.72% of total variations in Trial I and Trial II respectively (Figures 6.14a & 6.14b; Appendix 6.6). PC1 accounted for 64.11% variations in Trial I and 58.64% in Trial II with Yp, Ys, GMP, MP, STI, SSI and TOL. PC2 explained 29.90% of the total variation in Trial I and 39.08% in Trial II with Yp, Ys, DI, GMP, MP, STI and YSI. Therefore, selection of accessions with high PC1 and PC2 will be high yielding in both normal and stress condition (Group A); high PC1 and low PC2 will be high yielding in normal condition but low in stress condition (Group B); and low PC1 and high PC2 will be low yielding in normal condition but high in stress condition (Group C).

Most of the accessions in Trial I concentrated in the centre of the dimensions vector, which reflect a very weak association between Yp and Ys, and this lead to a weak discrimination of tolerant/susceptible accessions. Nevertheless, accession AV-TRI 44, AV-TRI 11, US-TRI 21 and US-TRI 51 were more inclined into MP, which can be considered superior accessions amongst the vegetable amaranth studied during Trial I. Meanwhile, in Trial II, accessions were scattered further in the direction of a particular vector and hence, tolerant/susceptible accessions can be easily distinguished. Accessions AV-TRI 3, US-TRI 39 and US-TRI 51 among others, were more inclined towards MP, GMP and STI, reveals that these accessions were superior. Overall, the GMP, MP and STI indices are a good criterion in determining drought/susceptible accessions and therefore, the ranking for tolerance was ascertained based on these indices in both drought screening trials (Figure 6.15).

The dendogram constructed based on GMP, MP and STI discriminates the 44 amaranth accessions into four clusters; high tolerance, moderate tolerance, low

tolerance and high susceptibility in two trials (Figures 6.15a-i & 6.15b-i). Accessions AV-TRI 18, AV-TRI 44, AV-TRI 3, AV-TRI 11, US-TRI 21, US-TRI 39, US-TRI 46, US-TRI 51, Local Red and Local PR were the most tolerant and had consistent drought tolerance performance across the two screening trials while accession AV-TRI 2, US-TRI 6 and US-TRI 47 were the most susceptible. The performance of the remaining accessions was varied and unstable, depending on the condition or stress intensity of the environements. For example, accession AV-TRI 53 was highly tolerant to drought stress during Trial I, but it turns out to be the most susceptible among the 44 amaranth accessions during Trial II. Other than that, the identification of tolerant/susceptible accessions could not be classified based on morphological characters as the distributions of drought tolerant accessions were varied within cluster that shared similar traits (Figures 6.15a-ii and 6.15b-ii).

Figures 6.16 and 6.17 summarize the association between tolerance grouping and all the physiological responses at once under WS and WD conditions for Trial I and Trial II respectively. In Trial I, the PCA revealed that the first two PCAs accounted for 65.66% and 66.40% of total variations in WS and WD conditions respectively (Appendix 6.6). High tolerance accessions were clearly discriminated from susceptible accessions in both WS and WD conditions although there was overlapping between high, moderate and low tolerance accessions in the directions of biomass partitioning. There was also no difference in the directions of dimension vectors (biomass partitioning, RWC and *Fv'/Fm'*) between the two water treatments. In contrast, in Trial II, the PCA revealed that the first two PCAs accounted for 57.26% and 48.31% of total variations on WS and WD conditions respectively (Appendix 6.6). Under WS condition, a clear demarcation was observed between highly tolerant and susceptible accessions, as highly tolerant accessions clustered together in the direction of yield biomass while susceptible accessions were more inclined towards SLA and *Fv/Fm*. Meanwhile, the moderate and less tolerance accessions were scattered in the centre of dimensions vector, with some of the accessions were more inclined towards the positive side of PC2 (Ci, Gs, E, Pn, WUE, WUEi, Ls, *Fv'/Fm'*). Under WD condition, the high and moderate tolerance accessions were clustered together towards the positive side of PC1 (SLA, E, Gs, RWC, biomass partitioning, Pn, WUE, WUEi, Ls, *Fv'/Fm'*), and could be clearly distinguished from high susceptibility accessions which were more inclined towards the negative side of PC1 (Ci and *Fv/Fm*).

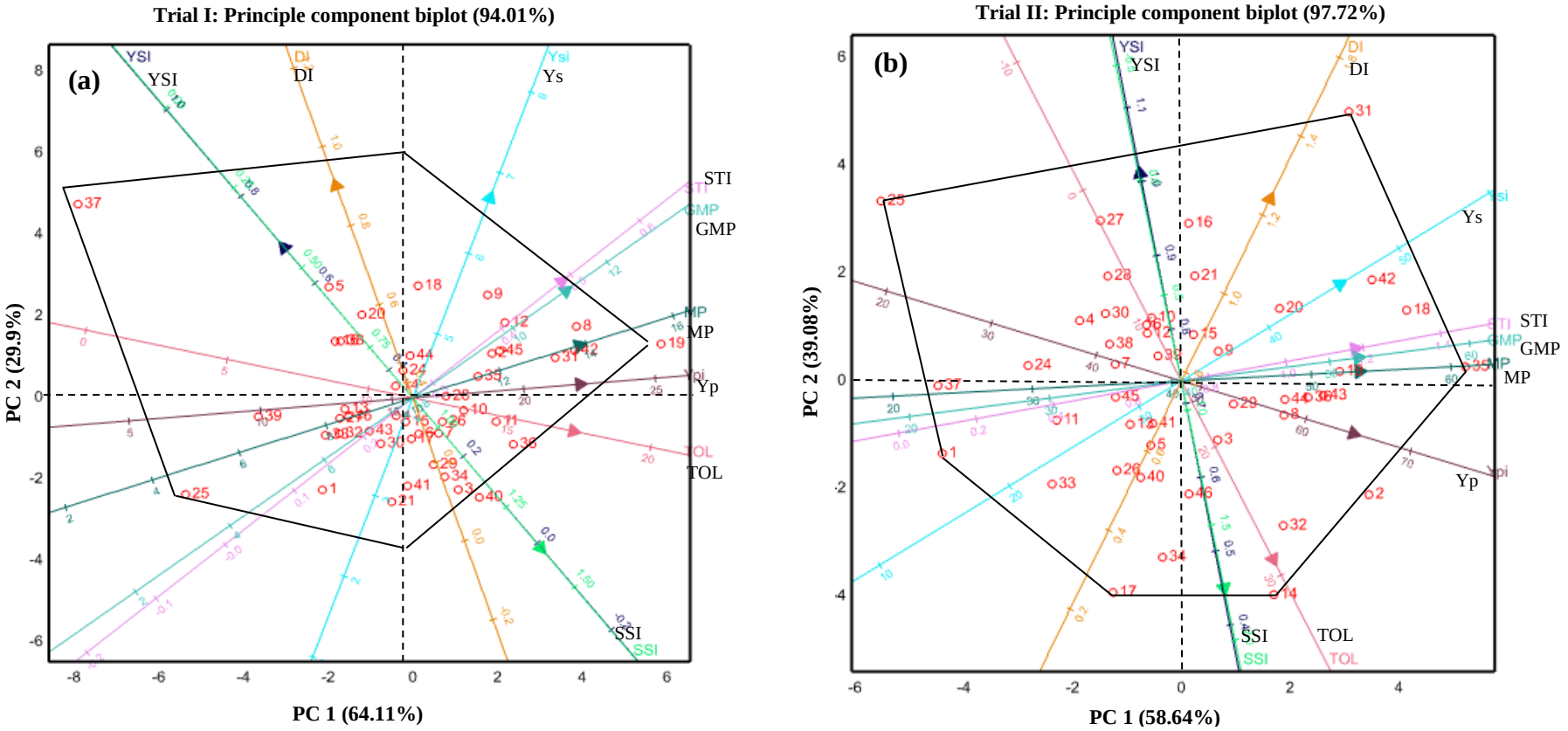


Figure 6.13: Principle component biplot grouping in (a) Trial I and (b) Trial II.
DI: drought resistance index, GMP: geometric mean productivity, MP: mean productivity, SSI: stress susceptibility index, STI: stress tolerance index, TOL: stress tolerance and YSI: yield stability index.

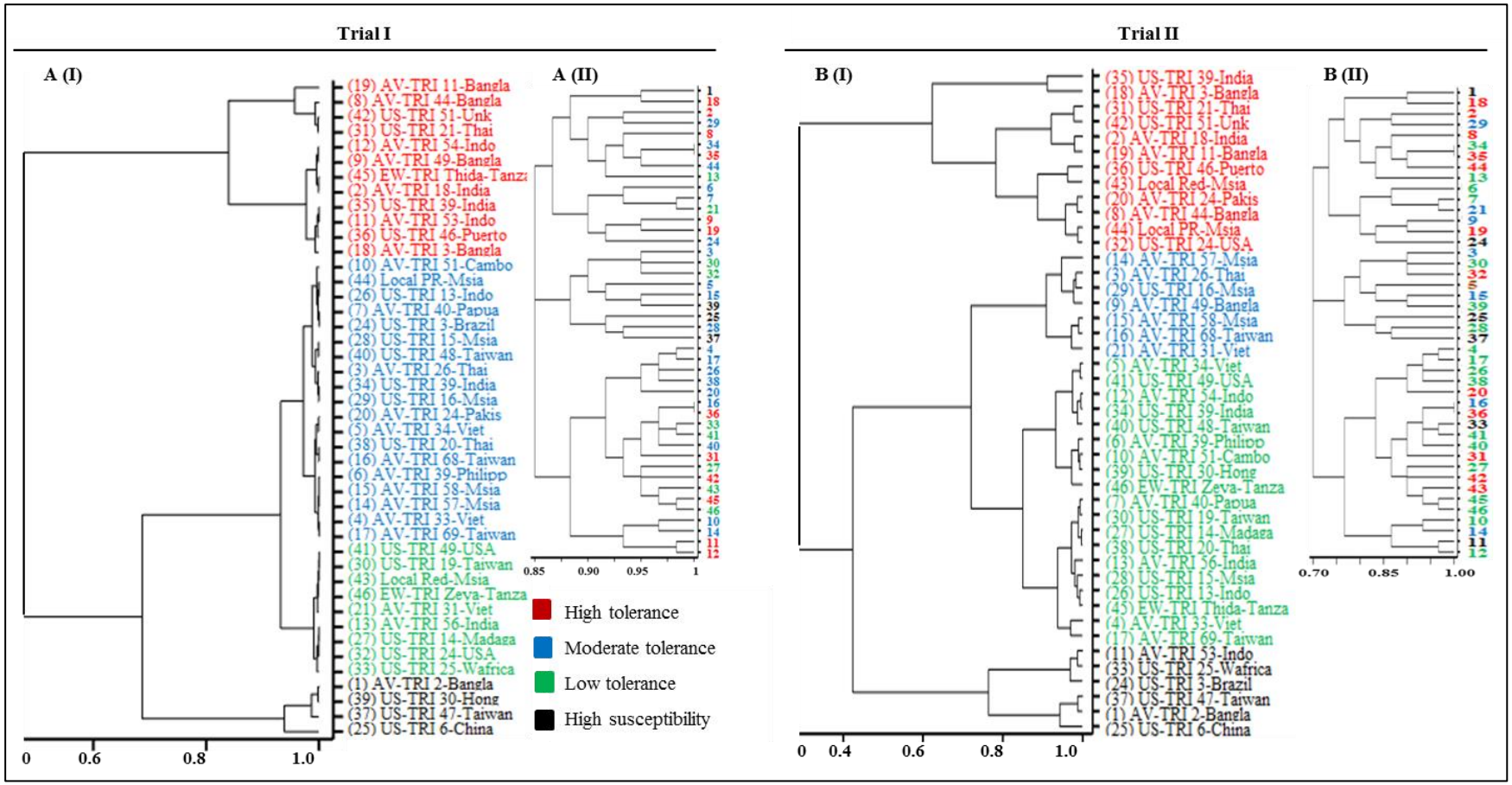


Figure 6.14: The drought tolerance ranking of the 44 amaranth accessions calculated based on the GMP, MP and STI values in two trials (Trial I and Trial II). A (I) and B (I) are dendogram developed using Manhattan-distances and discriminate accessions into four clusters of drought tolerance ranking (high, moderate and low tolerance, and high susceptibility). A (II) and B (II) are morphological-based dengdogram that shows the distribution of drought tolerant accessions within clusters in two trials.

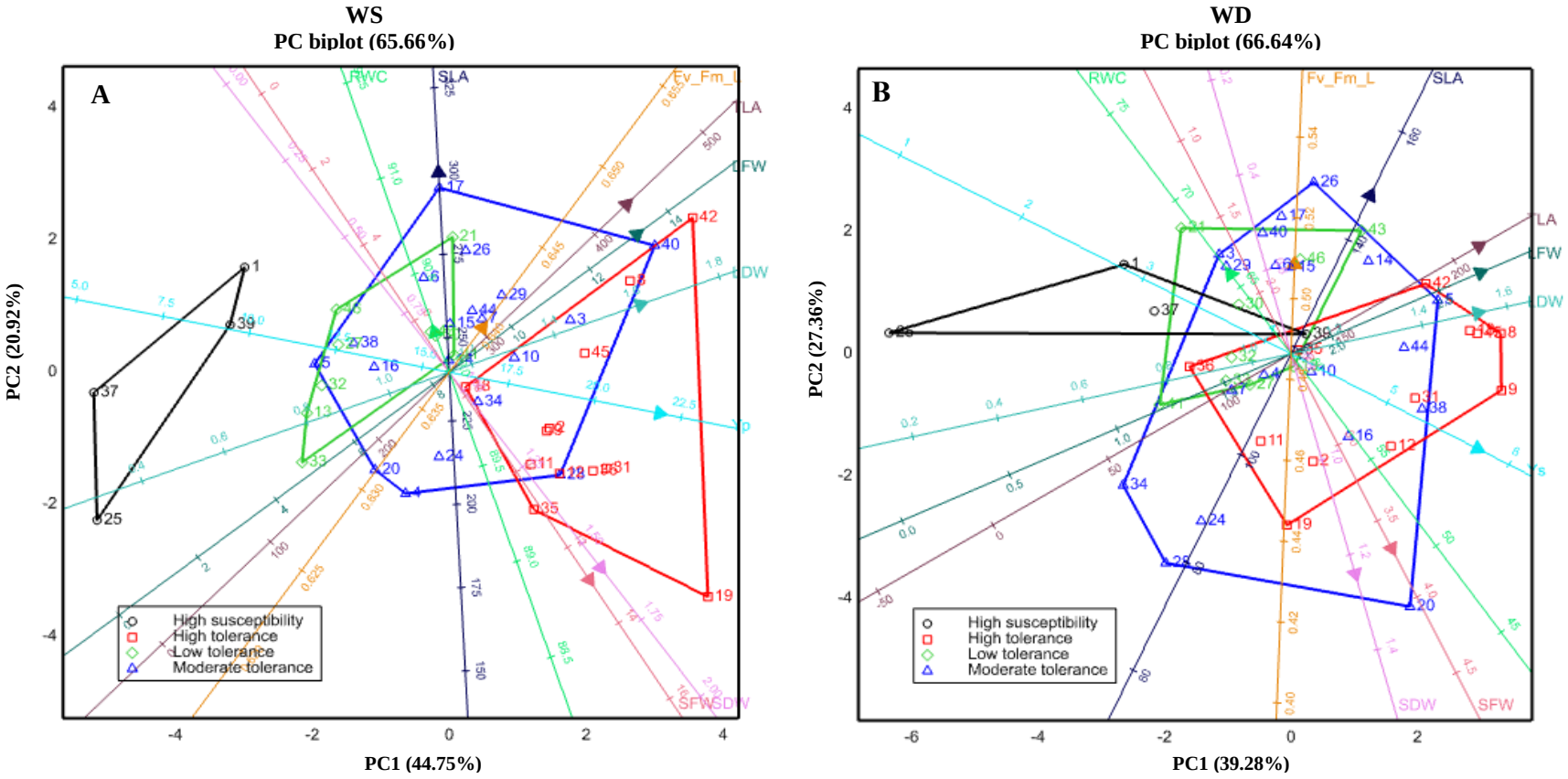


Figure 6.15: Principle component biplot grouping in association with physiological responses under water-sufficient (WS) and water-deficient (WD) conditions at 6 DAT in Trial I.

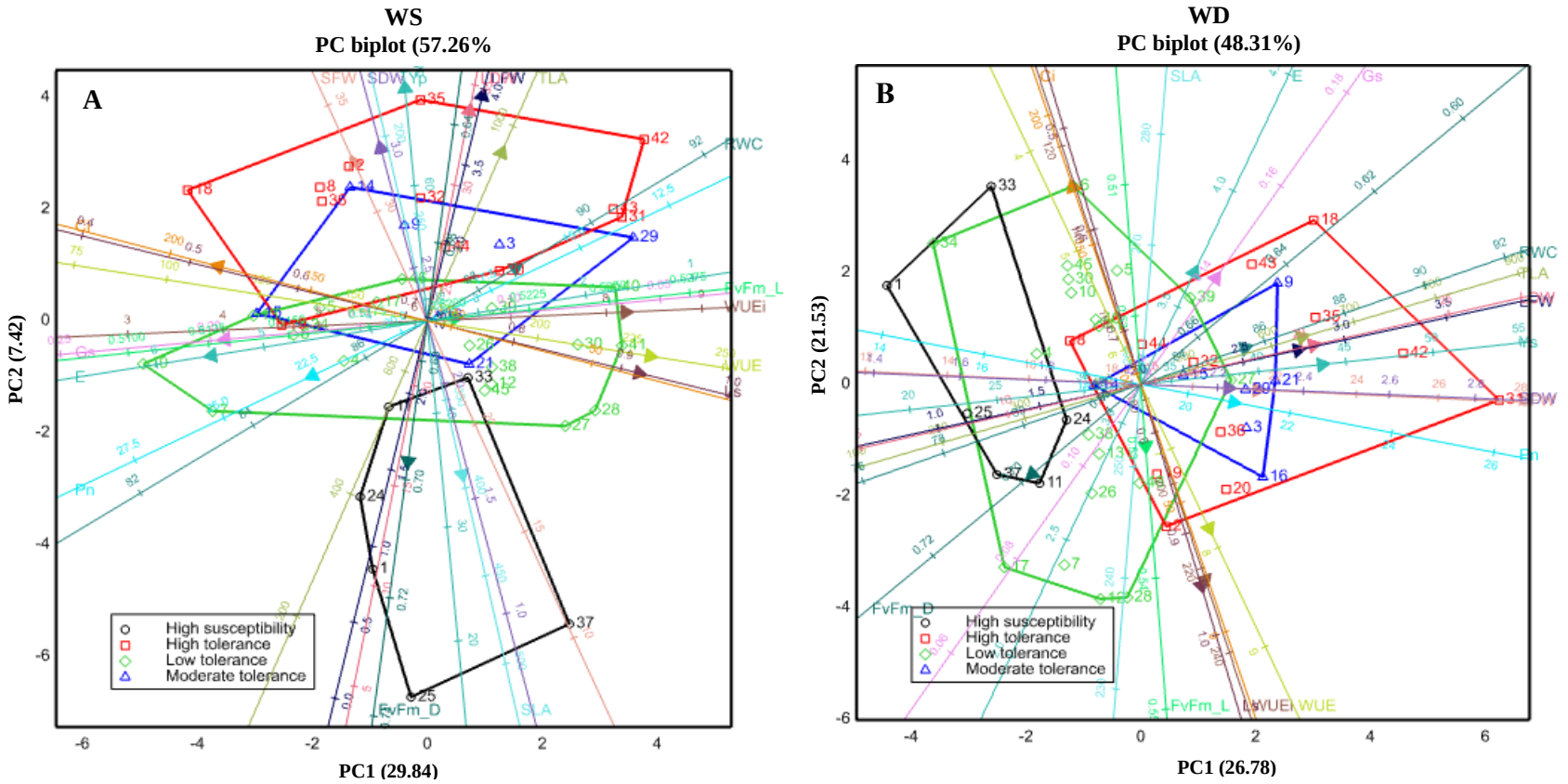


Figure 6.16: Principle component biplot grouping in association with physiological responses under water-sufficient (WS) and water-deficient (WD) conditions at 10 DAT in Trial II. Ci: Intracellular CO_2 concentration , E: Transpiration, Gs: Stomatal conductance, LDW: Leaf dry weight, LFW: Leaf fresh weight, Ls: Stomatal limitation, Pn: Photosynthesis, Fv/Fm: Dark-adapted quantum yield, Fv'Fm': Light-adapted quantum yield, RWC : Relative water content, SDW: Stem dry weight , SFW: Stem fresh weight, SLA: Specific leaf area, TLA: Total leaf area, WUE: Instantaneous water use efficiency, WUEi: Intrinsic water use efficiency and Ys/Yp: Yield under WS/WD conditions

6.3.8 Genome-wide association study for drought phenotypic traits

A total of 19 marker association traits (MTA) were observed in combined analysis of 11 drought traits, including yield, stem fresh weight, total leaf area, specific leaf area, days to flowering, days to re-cover, and intracellular CO_2, stomatal limitation, photosynthesis and intrinsic water use efficiency at 50% WHC, in 44 *A. tricolor* accessions (Table 6.14). 17 out of 19 SNP markers were associated with traits under drought condition (WD). Remarkably, the SNP marker for stem fresh weight was similar in normal (WS) and WD conditions, located in scaffold 3 at 6,349,480 of the genome. The chromosome and location of SNP marker associated with stem fresh weight had similar annotation functions with gene CAS: Exportin 2 of *Arabidopsis thaliana*, as shown in the Manhattan plots of –log(p-values) and the Q-Q (quantile-quantile) plots of expected vs observed p-values (Figure 6.17).

Table 6.14: List of 19 significant SNP markers associated with yield, stem fresh weight (SFW), total leaf area (TLA), specific leaf area (SLA), days to flowering (DTF), days to re-cover (DTR), and intracellular CO_2 (Ci50), stomatal limitation (Ls50), photosynthesis (Pn50) and intrinsic water use efficiency (WUEi) at 50% WHC of 44 *A. tricolor* accessions under water-sufficient (WS) and water-deficient (WD) conditions.

Trait	Water treatment	SNP Marker	Chr	Position	R^2
DTF		33436794	SCAFFOLD 1	37520113	0.23183
Ci50	WD	33421360	SCAFFOLD 1	28076692	0.33779
		33431188	SCAFFOLD 9	19506277	0.3896
DTR	WD	33402910	SCAFFOLD 1	1256803	0.30944
		33433567	SCAFFOLD 9	12010106	0.30944
Ls50	WD	33421360	SCAFFOLD 1	28076692	0.33548
		33431188	SCAFFOLD 9	19506277	0.3833
Pn50	WD	33438956	SCAFFOLD 2	35402231	0.24017
SFW	WD	33458985	SCAFFOLD 3	6349480	0.32933
SFW	WS	33458985	SCAFFOLD 3	6349480	0.26482
SLA	WD	33430580	SCAFFOLD 10	21287331	0.504
		33415113	SCAFFOLD 2	27277661	0.47847
		33436368	SCAFFOLD 2	19885054	0.49574
		33423475	SCAFFOLD 2	21841752	0.52945
		33438956	SCAFFOLD 2	35402231	0.53687
WUEi50	WD	33426688	SCAFFOLD 13	19820708	0.24836
		33414588	SCAFFOLD 5	22710040	0.1892
TLA	WS	33414740	SCAFFOLD 5	18976010	0.23287
Yield	WD	33458985	SCAFFOLD 3	6349480	0.17734

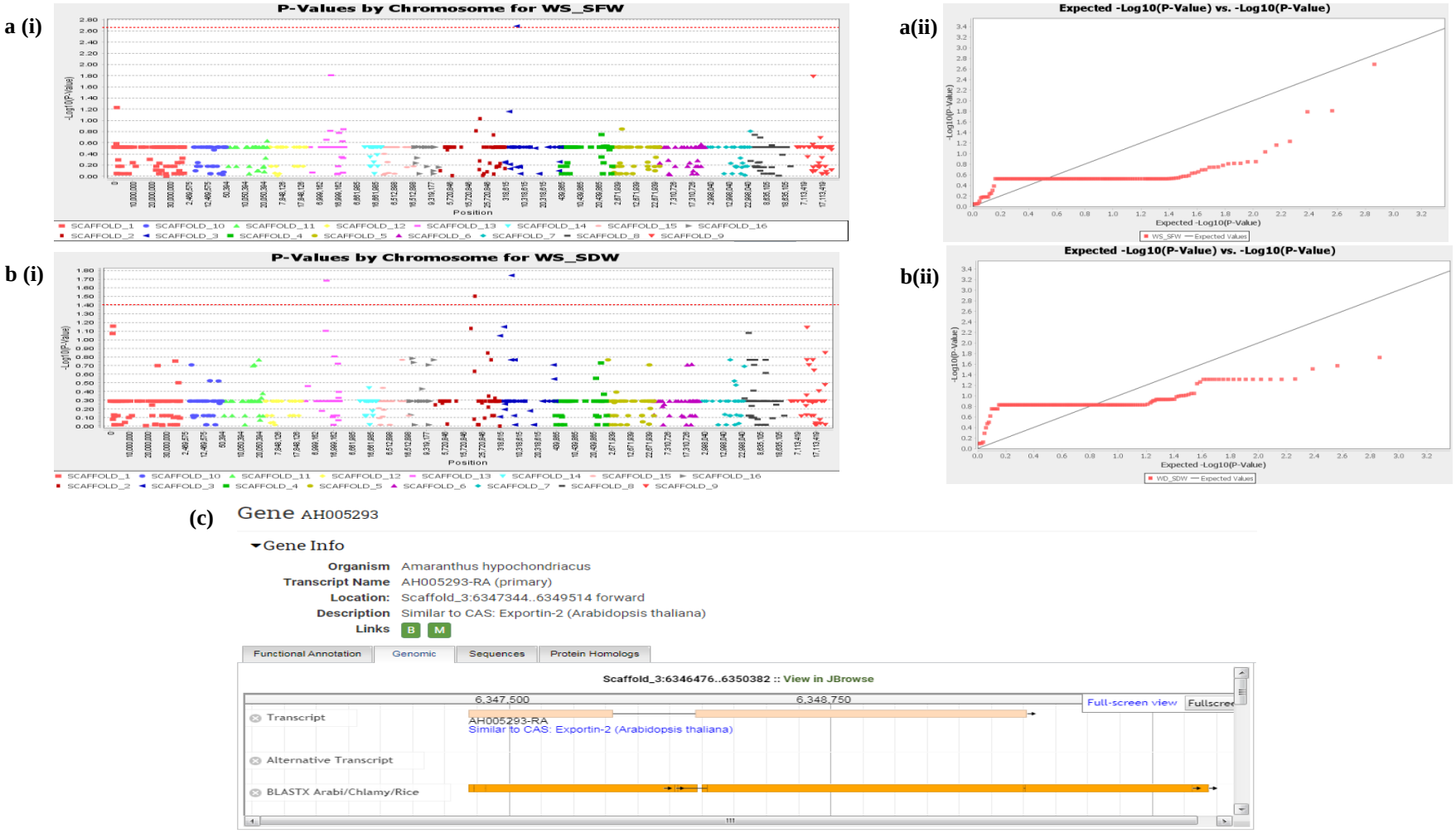


Figure 6.17: The Manhattan plots of –log(p-values) and the Q-Q (quantile-quantile) plots of expected vs observed p-values of stem fresh weigh (a) under water-sufficient and (b) water-deficient. (c) SNP marker similar annotation functions with gene CAS: Exportin 2 of *Arabidopsis thaliana*.

6.4 Discussion

6.4.1 Growth of amaranth is significantly influenced by environmental changes

In this study, the growth of amaranth in irrigated and water stress conditions was significantly affected by the environmental conditions. The reduction of yield loses in Trial I was substantially higher (73%) than in Trial II (31%) revealing that environmental stress intensity plays an important role in drought response and adaption of the amaranth accessions evaluated in this study. This result has also been observed in maize, grown in multiple environment conditions (Hao *et al*., 2011) and wheat grown, in rainfed and irrigated locations (Ali and El-Sadek, 2011). It is possible that the more severe drought stress imposed in Trial I caused a reduction in metablic activity in comparison to the moderate drought stress imposed in Trial II (Naya *et al*., 2007; Ma *et al*., 2006). Different stress adaptation mechanisms in amaranth might be determined by the capacity of accessions to adapt to different type of drought stress to enhance their growth and development (Fang and Xiong, 2015; Iseki *et al*., 2013).

This experiment was repeated twice at different time periods to ensure the repeatability of the study and hence to increase the accuracy in estimating trait heritability (Herzig *et al*., 2018; Mathew *et al*., 2018). Low to medium broad sense heritability (0.10-0.30) was observed in yield and most of the studied traits in Trial I while moderate to high broad sense heritability (0.40-0.60) was observed in yield, fresh and dry weight of leaf and stem, and leaf gas exchange parameters in Trial II. Nevertheless, the yield heritability was reduced under drought stress and this could be due to a complex trait and polygenic nature of yield (Turner *et al*., 2014). The reduction of yield heritability under drought stress was also found in in wheat (Mathew *et al*., 2018; Sanad *et al*., 2019), maize (Hao *et al*. 2011), rice (Kumar *et al*., 2014) and cassava (*Manihot esculenta* Crantz) (Oliveira *et al*., 2015).

Although the heritability of biomass partitioning into leaf and stem decreased under stress, moderate heritability was still found, in the presence of large genetic variability under drought stress. High heritability and genetic advances also have been estimated in *A. tricolor* for leaf yield, and a strong correlation with plant height, number of leaves and stem diameter in normal conditions (Sarker *et al*., 2014; Shukla *et al*., 2006). This leaf and stem traits with stable heritability confirmed that it was a genetic based trait, which was less

influenced by the environment and can be effectively selected for both normal and drought stress conditions (Dalal *et al*. 2017; Sanad *et al*., 2019).

6.4.2 Stem biomass is likely to influence genotypic variation in physiological activity and reveals the critical role of recovery in drought adaptation of amaranth

A. tricolor is also known as stem amaranth in Bangladesh (Akhter *et al*., 2013; Ahammed *et al*., 2013) due to its prominent divergence of stem weight in comparison to leaf weight (Akhter *et al*., 2013). The amaranth collections evaluated in this study had high variability, heritability and diversity index for stem traits, demonstrating that these traits are polymorphic (Chapter 3). Besides, Sarker *et al*., (2017), Andini *et al*., (2013) and Shukla *et al*., (2009) also found that an evaluation on plant height and stem diameter was helpful in identifying accessions with high protein content and other interesting vegetable production traits. In this study, the biomass partitioning into leaves and stem was significantly varied among amaranth accessions in normal conditions. The various above-ground biomasses partitioning in these amaranth accessions had differing responses to severe and moderate drought stress. For example, accessions that had high stem biomass accumulation compared to leaves performed well when stress intensity was high (Trial I) but when stress intensity was moderate (Trial II), these accessions ranked far below the rest of other accessions.

The difference in this feature among amaranth accessions may imply that their strategies for controlling water use are also varied. A study in sorghum by Perrier *et al*., (2017) demonstrates that the fraction of biomass into leaves and stem changed over time in normal conditions, and the reduction of stem biomass was less than leaf biomass under drought stress. This has also reported by Liu and Stützel (2002), whereby accessions that initially had high leaf biomass moved to high stem biomass over time in both normal and drought stress conditions. The reason behind this adaptation strategy to drought stress in amaranth to date, has never been studied. Although there was an obvious pattern between high stem biomass with particular traits such as high Fv'/Fm', WUE and Pn, the correlation of biomass stem with these traits were non-significant and weak. However, the high stem biomass with completed internode growth in sweet sorghum was reported to have an increased insoluble sugar accumulation under drought stress

(Ghate *et al*., 2017), and although the stem biomass decreased under drought stress, the stems largely recovered after re-watering (Perrier *et al*., 2017).

Re-watering may reveal the critical role of recovery in drought adaptation. Fast and efficient recovery from drought stress may be among the key determinants of plant drought adaptation (Chen *et al*., 2016). In this study, days to recovery after re-watering where significantly negatively associated with stem biomass, meaning amaranth accessions with high stem biomass recovered rapidly. This showed that stem biomass could possibly affect the physiological responses of amaranth genotypes under drought stress. This could be a strategy of amaranth to alter xylem sap in the stems during drought stress, such as to improve the chances of survival, save resources and serve as recovery after the stress phase (Shabala *et al*., 2016).

6.4.3 Low Fv/Fm value might be a potential surrogate trait for high yielding amaranth in normal and moderate drought stress conditions

Remarkably, significant negative correlations between Fv/Fm at 50% WHC and yield were consistently observed in WS and WD conditions during moderate drought stress (Trial II), implying the direct contribution of the trait with yield, which can be considered as an important target trait during selection (Mwadzingeni *et al*., 2016; Sareen *et al*., 2014). Initially, Fv/Fm was high during early vegetative phase (100% WHC) but then decreased as plant size increased over time (50% WHC). The Fv/Fm was only significantly reduced when leaf water status decreased to 60-70% under drought stress conditions (20% WHC), as also observed by Hura *et al*., (2007a) in *A. cruentus* under 30% field water capacity. However, the association of Fv/Fm with yield was only observed at 50% WHC, depicting that accession with low Fv/Fm at 50% WHC considered high yielding in normal condition and will anticipate superior yield under drought stress condition. Such genotypic differences of mechanisms in Fv/Fm at 50% WHC might be determined by the capacity of accessions to adapt to drought conditions (Iseki *et al*., 2013).

Fv/Fm provides a rapid way to assess plant health but caution should be used as Fv/Fm is often misinterpreted as specific indicator of PSII photoinhibition (decrease of CO_2 fixation) due to the damage of PSII core subunit D1 (Malnoë, 2018; Murchie and Lawson, 2013; Adams and Demmig-Adams, 2004). Rather, Fv/Fm represents quantum yield of PSII that will be low not only when the PSII is inactivated but also due to thermal dissipation (through slowly relaxing non-

photochemical quenching, NPQ) (Malonë, 2018). The used of Fv/Fm to evaluate the drought tolerance of crops is contradictory (Nemeskéri and Helyes, 2019). For example, Fv/Fm of pot-grown grapevines decreased when water potential dropped (Zulini *et al*., 2007), but no changes were observed in strawberries (Razavi *et al*., 2008) and soybean (Ohashi *et al*., 2006) grown under drought conditions. Meanwhile, Fv/Fm of drought tolerant barley (Li *et al*., 2006) and tomato varieties (Bahadur *et al*., 2010) were higher than the drought sensitive varieties.

In contrast, drought tolerant amaranth accessions in this study had lower Fv/Fm than the susceptible accessions, as also demonstrated by the biplot in which susceptible accessions were positively associated with Fv/Fm under drought stress. A low Fv/Fm in high yielding accessions in this study might indicate as a photoprotective role rather than photoinhibition. At 50% WHC, the mitigation of photoinhibition is likely an up-regulation mechanism to dissipate excess electrons (Iseki *et al*., 2013), as also observed in the *Arabidopsis thaliana* suppressor of quenching1 (*soq1*) mutant that exhibits lower Fv/Fm than wild type Col-0. The *soq1* mutant exhibits enhanced NPQ, a process of dissipating excess light energy, which plays an important role for photoprotection (Malnoë *et al*., 2018). Therefore, further study on NPQ or other electron dissipation mechanisms such as photorespiration and cyclic electron that are up-regulated under drought stress (Iseki *et al*., 2013; Kohzuma *et al*., 2009; Bartoli *et al*., 2005) are required to strengthen the use of Fv/Fm as ultimate surrogate traits for drought tolerance in amaranth. Besides, with this amaranth collection, direct selection of Fv/FM would be less effective to improve drought tolerance in amaranth because of its low heritability as it was highly influence by environmental variance.

6.4.4 GMP, MP and STI were the most stable indices to distinguish tolerant/susceptible amaranth germplasm in moderate and severe drought stress

Identification of drought tolerant amaranth is the ultimate goal of this research. Previous studies have concluded that the effectiveness of selection indices where dependent on the stress severity (Talebi *et al*., 2009; Panthuwan *et al*., 2002). Therefore, there is a need to determine whether to use severe or moderate drought stress to evaluate stress tolerance in amaranth germplasm (Ali and El-Sadek, 2016). In this study, under severe drought stress, yield in normal condition (Yp) were not

correlated with yield in stress condition (Ys), depicting that indirect selection for drought tolerant accessions based on the performance under irrigated conditions would not be effective if the stress condition was severe (Anwar *et al*., 2011; Gholipouri *et al*., 2009). Furthermore, the biplot revealed that in severe drought stress condition, stress indices were less discriminative the amaranth accessions than the moderate drought stress condition, suggesting that the finding of tolerant amaranth should be in moderate stress, similar results have been observed in wheat (Ali and El-Sadek, 2016).

Nevertheless, the evaluation of drought stress indices in amaranth accession at various level of stress can facilitate plant breeders to identify stable accessions in diverse environments. Accessions that show low fluctuations of yield under various levels of drought stress conditions can be considered drought tolerant and stable (Ali & El-Sadek, 2016). In this study, GMP, MP and STI were the best indicators of accessions stability and able to distinguish tolerant/susceptible amaranth accessions in both severe and moderate drought stress. The selection criterion based on high GMP, MP and STI will lead to the selection of accessions with high yield potential under both stress and non-stress conditions (Cabello *et al*., 2013). These indices were also useful to identify tolerant/susceptible accessions in safflower (*Carthamus oxyacanthus* Bieb.) (Majidi *et al*., 2011) and wheat (Pireivatlou *et al*., 2010).

Other than that, according to Fernandez (1992), Farshadfar and Sutka (2002) and Gholipouri *et al*., (2009), an index can be considered effective if accessions of Group A (high yielding in normal and stress condition) are clearly distinguished from the accessions from the other three groups. In this study, the biplot of moderate drought stress revealed that accessions were clearly discriminated into different group and showed that GMP, MP and STI were the best criterion to distinguish accessions of Group A from the others. Overall, drought stress significantly reduced yield of some accessions and some of them revealed tolerance to drought, which suggested the genetic variability for drought tolerance exist in this amaranth collection.

6.4.5 Genetic bases of drought tolerance traits in amaranth

Marker trait association is key to identifying genomic regions that are associated with phenotypic traits of breeding significance. This present study identified a total

of 19 highly significant marker trait associations under contrasting water regimes in 11 drought traits, including yield, stem fresh weight, total leaf area, specific leaf area, days to flowering, days to re-cover, and intracellular CO_2, stomatal limitation, photosynthesis and intrinsic water use efficiency at 50% WHC, in 44 *A. tricolor* accessions. The selection of MTAs detected in the MLM model had a lower threshold (−log(p−value)<4, similar to morphological traits (Chapter 4; subheading 4.3.3), and although the mixed model was superior, but it still leads to false negative and false positive.

Drought phenotyping traits are highly complex and often low heritability, and the MTAs found in this study could be located on regions that influence the respective traits directly or indirectly (Mwanzengini, et al., 2016). Remarkably, SNP marker associated with stem fresh weight was similar under normal and drought stress conditions. Ideally, the effects of such loci may not be influenced by the change in external environment (Matthews *et al*., 2008). Such genomic regions could be useful in marker-assisted selection or gene introgression when breeding for broad adaptation (Mwadzengeni et *al*., 2017).

6.5 Conclusion

The growth of amaranth in irrigated and water stress conditions was significantly influenced by environmental conditions. Moderate stress intensity maybe more suitable selection environment for the identification of drought tolerant amaranth and their possible surrogate traits. The study demonstrated that yield in amaranth is a complex trait, and that the differences of above-ground biomass partitioning into leaves and stems may be a compromise with other physiological traits such as chlorophyll fluorescence and photosynthesis. Strong negative correlations between stem biomass and days to recovery provide evidence that one of the possible strategies of amaranth is an increase in stem biomass to compensate plant growth after re-watering. Remarkably, significant negative correlations between Fv/Fm at 50% WHC and yield in normal and drought stress conditions may imply the direct contribution of the trait with yield, which might be considered as important target traits during selection. However, direct selection of Fv/Fm will be less effective to improve drought tolerance in amaranth because of its low heritability. Overall, drought stress significantly reduced the yield of some accessions and some of them revealed tolerance to drought, suggesting that the amaranth mini core collection

used in this study could be a rich source of genetic diversity for breeding purposes for drought tolerance traits. Further studies are required to quantify stem traits and chlorophyll fluorescence of diverse genotypes and this could be done using a pool of well characterized drought tolerant and a contrasting set of drought susceptible genotypes. This is a valuable preliminary data to initiate marker-assisted selection and trait introgression of amaranth under drought-stressed and non-stressed conditions.

CHAPTER 7

GENERAL DISCUSSION, LIMITATION AND CONCLUSION

7.1 Finding impact: A preliminary roadmap for breeding leafy vegetable amaranth (*A. tricolor*) with improved drought tolerance traits

Developing breeding programmes for underutilised crops begins with cultivar development based on consumer preference, adequate adaptation to various environmental conditions, long-shelf life, superior taste, high nutritional value and affordable food (Sogbohossou *et al*., 2014; Afari-Sefa *et al*., 2012). The identification of product targets requires proper strategy in collecting and characterizing germplasms, which is the primary step for the exploitation of genetic diversity and to screen desired traits, and genomic tool can accelerate the entire development of cultivars (Perez-Gonzalez, 2001). The overall aim of this thesis was to identify vegetable *A. tricolor* accessions with superior drought tolerance traits with the ultimate aim of developing climate resilient crops for future agriculture. This study provides preliminary efforts that can guide subsequent efforts in cultivar development with improved drought tolerance traits in amaranth. This include germplasm characterization (Chapter 3), genetic diversity analysis (Chapter 4) and screening drought tolerance traits (Chapters 5 and 6), and from this, the genetic basis for drought tolerance traits in amaranth has been elucidated.

7.1.2 Germplasm characterization

Amaranth has high phenotypic plasticity and a large amount of genetic diversity (Rastogi and Shukla, 2013) and therefore, it is important to characterize the amaranth germplasm. This is the preliminary requirement for the exploitation of useful traits in a breeding programme (Brandolini *et al*., 2000). Although *ex-situ* conservation of amaranth has been improved and genetic variability has been characterised recently, their utilisation and management mainly depends on resources available in the selected germplasm (Thapa and Blair, 2018; Gerrano *et al*., 2017; Sogbohossou *et al*., 2014; Andini *et al*., 2013). In this study, a larger collection of accessions from AVRDC Genebank and *A. tricolor* accessions from USDA Genebank were chosen to investigate the diversity panel of *A. tricolor*, from

a diverse origin including from Asia, Africa and America (Chapter 3). In AVRDC germplasm, the morphological database provides full lists of plant characters, while USDA germplasm was lacking in those characters. However, a seed obtained from a Genebank has a possibility that the morphological characters are different from database, as observed in this study. Therefore, characterization of traits should be repeated, especially traits that have high heritability (FAO, 2014).

To be able to study the diversity of *A. tricolor* panel in depth, 120 A. tricolor *accessions* were selected using standard stratification procedure (Dwivedi *et al*., 2005; Van Hintum, 1994), together with 68 accessions comprised of other 17 species. These accessions were planted in three replications, and morphological characterisation was obtained based on 10 qualitative characters (Chapter 3). This study showed that characterization of germplasm based on the 10 qualitative traits was less efficient in discriminating plant-type species (grain, vegetable and weed), species identification and geographical origin. This could be due to a small number of qualitative or quantitative traits being studied, similar to the findings reported by Thapa and Blair, (2018); Sarker *et al*., (2017); Andini *et al*., (2013) and Shukla *et al*., (2009), which are also used less than 15 traits to evaluate genetic diversity in amaranth. Owing to its plasticity and domestication history (Stetter and Schimdt, 2017), to date, classifying plant-type species in amaranth based on morphological characters can only be achieved through a large data set of both quantitative and qualitative traits, provided that the number of accessions in individual species are uniformed (Sogbohossou *et al*., 2014). Other than that, GBS platform suggested that the population structure analysis must be taken into account for the classification of amaranth based on geographical origin and morphological traits (Wu and Blair, 2017).

7.1.3 Population structure and genetic diversity

Vegetable species of amaranth have been less studied by molecular means than pseudo-cereal grain amaranths as well as weed species, especially when both are phylogenic related and the occurrence of domestication events between them were proven (Mallory *et al*., 2008; Khaing *et al*., 2014; Stetter *et al*., 2015, 2017). The rapid advance in NGS technology has reduced the genotyping prices and allows for a wide utilisation of GBS platform to genotype any crops including individual accession in genebanks collections (Elshire *et al*., 2011). In this study, population

structure based on DArTseq-SNPs revealed that amaranth diversity can be successfully grouped according to their sub-genera. One clade of *A. tricolor* accessions from Bangladesh was grouped into one individual cluster with distinct morphological characters (Chapter 4). This demonstrates that DArTseq genotyping was successful in creating a core collection that represents the diversity of a single species.

A genetic analysis of all these accessions would not only reveal duplicates and genetically closely related individuals, but also allow categorization of accessions into the correct species. In this study, two *A. tricolor* accessions (AV-TRI 20 and AV-TRI 28) from Asia deviated from *A. tricolor* clade and were grouped together with sub-genera *Amaranthus amaranthus,* which mainly belonged to grain and weed amaranths (Chapter 4). Two assumptions for this finding, either those two amaranths were wrongly identified as *A. tricolor* (Wu and Blair, 2017) or were originally a landrace that was grown in a region where grain amaranth was traditionally cultivated over a long time through seeds exchange (Das, 2016; Jimenez *et al*., 2013; Brenner *et al*., 2010). In a previous study, GBS accurately identified *A. caudatus* accession PI 490752, characterized as *A. hypochondriacus* by 11 SSR markers (Kietlinski *et al*., 2014), but it should be assigned into the *A. caudatus* group (Wu and Blair, 2017). Therefore, re-analysis should be carried out for these two *A. tricolor* accessions, with addition of larger morphological data, which could correct the possible misclassification.

However, population structure analyses of all 16 amaranth accessions showed that this GBS data has limited ability to resolve species level relationships. The population structure is often hierarchical, and the estimation on K-value strongly depends on sampling and genotyping efforts. The number of genetic groups detected by the ancestry estimation program does not necessarily correspond to the number of biologically meaningful populations in the sample (François and Duran, 2010). Other than different construction of SNP library used in this study, the bias number of accession per species could contribute to the lack discrimination of geographical origin and species level. This also observed in 3,431 DArSeq SNPs used to conduct genetic diversity in 89 safflower accessions (*Carthamus tinctorius* L.), in which the SNP showed weak correlation between safflower diversity pattern and origins, to be compared with to a larger SNP dataset (Hassani *et al*., 2020).

7.1.4 Phenotyping screening of drought tolerance traits

Drought tolerance is a complex biological process which involves interactions between morphological traits, physiological and biochemical processes, dependent on the level of drought severity, and timing in relation to the stage of crop development (Kumar *et al*., 2012; Bahadur *et al*., 2011; Kamoshita *et al*., 2008). The mechanisms of how plant might be adapted to drought strongly influence experimental design (Gilbert and Medina, 2016). In these screening trials, drought stress was imposed on the individual plant, so comparative physiological responses can be applied to any genotype presents in an environment (Gilbert and Medina, 2016). As it is challenging to identify a single drought stress indicator for crop plants, phenotyping screening for drought tolerance traits in this study were done at vegetative phase to prevent biased interpretation for the effects of drought stress on the entire plant (Bertolli *et al*. 2014). Screening were conducted in pots under the shade house condition to obtain better and controlled soil water deficit condition in each pot, which is difficult to achieve under field trials conditions. Besides, physiological responses such as osmotic adjustment and leaf senescence could be measured consistently in the shade house (Zhang *et al*., 2017). As this is the first study that screens a large collection of *A. tricolor* genotypes, and data on drought mechanism of amaranth was still unavailable, several phenotyping techniques have been employed to identify the strategy of drought resistance in amaranth. This includes transpiration efficiency, proline accumulation, chlorophyll content, photosynthetic capacity, root morphology, chlorophyll fluorescence and relative water content.

The results have showed that there were genotypic differences existed in amaranth lines evaluated for leaf yield potential. In this study, yield performance and physiological responses of amaranth genotypes was not affected under mild drought stress (70% WHC), and only started to affect plants at moderate drought stress (50% WHC). Obvious changes observed in a plant when subjected to drought stress are leaf wilting and senescence. This depicted that the alteration of biomass allocation mainly in stem rather than in leaves under drought stress was due to reduction in leaf area. This was a result of drought avoidance mechanisms of the plant to limit transpiration and stomatal conductance at early response of drought stress (Omami and Hammes 2006).

This finding also demonstrates that a change in stem biomass was probably the main mechanisms of drought tolerance in amaranth. Beside, genotypes that had high stem biomass with relatively high leaf biomass under control condition performed well under drought stress, and was identified as highly tolerant genotypes among others. Stem biomass was negatively correlated with PSII photochemistry, depicted that under drought stress, the higher the stem biomass, the lower the maximum efficiency at which light absorbed by PSII is used for reduction of Plastoquinone-A (*Fv/Fm*). This then prevents oxidative damage and photoinhibition to the plants, so that photosynthetic activity is retained, sufficient for leaf expansion and longevity (Hussain and Ali, 2015). The mechanism behind this finding is unclear, but highly likely due to osmotic adjustment in stem, as depicted by high positive correlation between stem biomass and leaf relative water content under drought stress. Relative water content and proline accumulation may serve as indicator for plant water status which was vital to ensuring an accurate assessment of the relative capacity for osmotic adjustment in plant (Chen *et al*., 2016; Sanders *et al*., 2012).

Osmotic adjustment is often associated with an accumulation of specific solutes with protective functions (Blum, 2017). Compatible solutes such as sugars, proline and glycine betaine can accumulate in the cytoplasm, and help to protect cellular proteins, enzymes and membranes against dehydration (Simova-Stoilova et al., 2016). A major function of the stem in annual crops is to transport those compatible solutes between the root system and the aerial parts (Shabala *et al*., 2016). Drought stress had shown to affect the xylem sap composition. An accumulation of solutes in the stem may be caused by the altered source/sink network under abiotic stress, which may be important for a subsequent recovery phase (Pinheiro *et al*., 2004). This may indicate that these amaranth genotypes aimed to store solutes such as amino acids and carbohydrates in stems rather than transport the nutrients to the active leaves during drought stress, which then use when water is available, as reported in Vargas-Ortiz *et al*., (2013). Costea and DeMason, (2006) also observed that stem length, diameter, orientation, branching pattern and colour were depending on environmental conditions, which give us insight knowledge to study more on stem morphology under drought stress.

However, this finding still requires further study to evaluate if the initial positive effects observed can be translated into improve crop yield under field

conditions. Lack of genotypic variability in transpiration efficiency and chlorophyll content in response to drought stress could be due to a small number of observations or the local environmental condition did not permit those responses as observed by previous study (Jomo *et al*., 2016). Nevertheless, these differences in performance of agronomic traits portray the potential success in future improvement work of amaranth for different purposes. It also can be used to pre-screen lines for further verification in the field.

7.1.5 Genetic basis for drought tolerance traits

Breeding programmes established for amaranth have just begun and need further assistance for increasing yield (Stetter *et al*., 2016; Alemayehu *et al*., 2015; Brenner *et al*., 2010). The genome of amaranth is relatively small (500 Mbp) and diploid, making it easy to study potential genetic constraints for domestication as well as drought tolerance traits (Stetter and Schimdt, 2017). Although *Amaranthus tricolor* genome has not yet been sequenced, genome-wide association study of candidate regions associated with quantitative traits can be carried out using reference genome of close related species, *Amaranthus hyponchondriacus* (Lightfoot *et al*., 2017).

The goal of GWAS is to discern genomic regions that could either be markers, genes or QTL associated with key agro-morphological traits for marker-assisted breeding, gene discovery or gene introgression (Edae *et al*., 2014). From the plant breeding perspective, the latter situation would often be desirable, if the response to selection for such a marker is desirable for all the associated traits. In the present study, out of 19 MTAs identified, one SNP was such, which was reliable, stable and was involved in MTAs associated with stem fresh weight under both control and drought conditions. This SNP with related traits may be due to the correlation among the traits or due to pleiotropic effect of specific genomic regions on more than one trait (Jabbari *et al*., 2018). The markers identified in this study are useful genomic resources to initiate marker-assisted selection and trait introgression of amaranth under control and drought stress conditions, and for fine mapping and cloning of the underlying genes and QTL.

7.2 Conclusion

This study lays a foundation for the improvement of the amaranth as a crop for the future to mitigate climate change and contribute to healthy diets. The study emphasised on an understanding the fundamentals of drought tolerance traits in amaranth. It was difficult to separate the 188 amaranth core collection based on the plant-type namely, grain, vegetable and weed along with geographical region. This could be due to intraspecific diversity of the collections and the small number of some of the accessions used per species. Phenotypic diversity study in amaranth can be improved with either larger data of qualitative and quantitative characters or with integration of GBS-SNP markers. The effectiveness of amaranth diversity panel for trait selection proved to be informative. A total of 25 MTAs associated with branching index, inflorescence color, terminal inflorescene shape, petiole pigmentation and terminal infloresce attitude. The highly significant genotypic differences observed in several physiological traits of 44 *A. tricolor* genotypes indicate that the amaranth panel used in this study could be a rich source of genetic diversity for breeding purposes for drought tolerance traits. Three drought tolerance indices, GMP, MP and STI were able to distinguish tolerant levels among amaranth genotypes based on yield performance under control and drought stress conditions. Stem biomass and Fv/Fm could be the possible surrogate traits for drought tolerance in amaranth. Two or more yield indicators should be emphasized at once in a larger sample size, in replication to prevent biased interpretation among amaranths, as a breeding target. Preliminary results on the genetic basis for drought tolerance traits in amaranth have been elucidated in this study. One reliable and stable SNP marker was involved in MTA for stem weight in control and drought conditions. Further studies are required to validate this significant marker using a larger population and in replication, to initiate marker-assisted selection, QTL and trait introgression of amaranth under control and drought stress conditions.

7.3 Limitation of the current study and recommendation for future amaranth research

(i) For seed characterization, 10 qualitative traits that were observed in this study were less effective in differentiating species into grain, vegetable and weed types. An experiment involving a large morphological data, both qualitative and quantitative traits should be conducted to identify amaranth

species as a preliminary selection for cultivars development. Evaluation on morphological traits is also necessary to assess consumer preferences, market value and nutritional value before evaluating the seeds further in breeding programme.

(ii) For screening of 44 amaranth genotypes in each trial, with three replications, one set of block/replication (n=88) was performed at a time and the next block/replications were performed at every 5 day interval. This is because screening practice for drought tolerance in shade-house/field requires considerable space, time and works which include planting and ascertained measurements at specific time. Although a robust REML analysis and ANOVA with split plot design (Genstat, 18th edition) were employed in the analysis, environmental condition in each replication varied (as seen in weather data in Chapter 6), and thus increased the experimental error. It is recommended that screening of a larger sample size, in replications at once, with only two or three traits assessed, may have the potential to identify drought tolerance surrogate traits in amaranth.

(iii) The findings of drought tolerance screening, colour pigmentation may influence the response of the plants subjected to drought stress. As amaranth has significant amount of betacyanin content in leaf and stem, further screening involving betacyanin accumulation under drought stress could assist in explaining the genotypic variation in leaf gas exchange, proline accumulation and chlorophyll fluorescence. This can verify the findings obtained in Chapter 5, where red amaranth has better adaptation to drought stress than green amaranth, due to the possibility of betacyanic accumulation in red leaf amaranth, which may have contributed to an increase in total photoprotective capacity (Nakashima *et al*., 2011).

(iv) Lastly, there is need for further research under field conditions. Data collected in this study and future studies may be of use to crop modellers. Such a future study would be useful as a tool for policy formulation and identification of future research areas on amaranth cultivars.

REFERENCE

1001 Genomes Consortium (2016). 1,135 genomes reveal the global pattern of polymorphism in *Arabidopsis thaliana*. *Cell*, *16* , 481–491.

Achigan-Dako, E., Sogbohossou, O. D. & Maundu, P. (2014). Current knowledge on *Amaranthus* spp.: Research avenues for improved nutritional value and yield in leafy amaranths in sub-Saharan Africa. *Euphytica*, *197*, 303–31.

Acquaah, G. (2007). *Principles of plant genetics and breeding*. Oxford. Blackwell Publishing Ltd.

Adams, W.W. III, Zarter, C.R., Ebbert, V. & Demmig-Adams, B. (2004). Photoprotective strategies of overwintering evergreens. *Bioscience*, *4*, 41–49.

Afari-Sefa, V., Tenkouano, A., Ojiewo, C. O., Keatinge, J., & Hughes, Jd. A. (2012). Vegetable breeding in Africa: constraints, complexity and contributions toward achieving food and nutritional security. *Food Sec*, *4*, 115–127.

Ahammed, A. U., Rahman, M. M., & Mian, M. A. K. (2013). Mutivariate analysis in stem amaranth (*Amaranthus tricolor*). *Bangladesh Journal of Plant Breeding and Genetics*, *26*(1), 11–17.

Ahmad, R., Anjum, M.A., & Malik, W. (2018). Characterization and evaluation of mango germplasm through morphological, biochemical, and molecular markers focusing on fruit production: An overview. *Mol Biotechnol*, *2*, 1–16.

Ahmed, C. B., Rouina, B. B., Sensoy, S., Boukhris, M., & Abdallah, F. B. (2009). Changes in gas exchange, proline accumulation and antioxidative enzyme activities in three olive cultivars under contrasting water availability regimes. *Environmental and Experimental Botany*, *67*, 345–352.

Akaneme, F. I. & Ani, G. O. (2013). Morphological assessment of genetic variability among accessions of *Amaranthus hybridus*. *World Applied Sciences Journal*, *28*(4), 568–577.

Akin-Idowu, P. E, Gbadegesin, M. A., Orkpeh, U., Ibitoye, D. O., Odunola, O. A. (2016). Characterization of grain amaranth (*Amaranthus* spp.) germplasm in South West Nigeria using morphological, nutritional, and random amplified polymorphic DNA (RAPD) analysis. *Resources*, *5*, 1-15.

Akther, C. A., Hassan M., Raihan, M. S., Hossain, M. M., & Mian, M. A. K. (2013). Genetic divergence in stem amaranth (*Amaranthus tricolor* L.) genotypes for yield and its component characters. T*he Agriculturist*, *11*(1), 82-88.

Alemayehu, F. R., Bendevis, M. A. & Jacobsen, S. E. (2014). The potential for utilizing the seed crop Amaranth (*Amaranthus* spp.) in East Africa as an alternative crop to support food security and climate change mitigation. *J Agron Crop Sci*, *201*, 321-329.

Alexander, D. H. & Lange, K. (2011). Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. *BMC Bioinformatics*, 12-246.

Ali, F., Kanwal, N., Ahsan, M., Ali, Q., Bibi, I., & Niazi, N. K. (2015). Multivariate analysis of grain yield and its attributing traits in different maize hybrids grown under heat and drought stress. *Scientifica*, 1-6.

Ali, M. B. & El-Sadek, A. N. (2016). Evaluation of drought tolerance indices for wheat (*Triticum aestivum* L .) under irrigated and rainfed conditions, *Communications in Biometry and Crop Science*, *11*(1), 77–89.

Ali, M. B., Khandaker, L., & Oba, S. (2009). Comparative study on functionalcomponenrs, antioxidant activity and color parameters of selected colored leafy vegetables as affected by photoperiods. *J. Food Agric. Environ*, *7*, 392–398.

Ali, S., Liu, Y., Ishaq, M., Shah, T., Abdullah, Ilyas, A. & Din, I. U. (2017). Climate change and its impact on the yield of major food crops: Evidence from Pakistan, *Foods*, *6*, 39.

Allemann, J., Van Den Heever, E., Viljoen, J. (1996). Evaluation of *Amaranthus* as a possible vegetable crop. *Applied Plant Science*, *10*, 1-4.

Alvares-Jubete, L., Holse, M., Hansen, A., Arendt, E. K. & Gallagher, E. (2009). Impact of baking on vitamin E content of pseudocereals amaranth, quinoa and buckwheat. *Cereal Chem*, *86*, 511-515.

Anajum, F., Yaseen, M., Rasul, E., Wahid, A., & Anjum, S. (2011). Water stress in barley *(Hordeum vulgare)*: Effects on morphological characters. *Pak J Agri Sci*, *40*, 43-44.

Andini, R., Yoshida, S., Yoshida, Y. & Ohsawa, R. (2013). *Amaranthus* genetic resources in Indonesia: Morphological and protein content assessment in comparison with worldwide amaranths. *Genetic Resources and Crop Evolution*, *60*, 2115–2128.

Andrews, K. R., Good, J. M., Miller, M. R., Luikart, G., Hohenlohe, P.A. (2016). Harnessing the power of RADseq for ecological and evolutionary genomics. *Nat. Rev. Genet*, *17*, 81–92.

Anwar, J., Subhani, G. M., Hussain, M., Ahmad, J., Hussain, M., & Munir, M. (2011). Drought tolerance indices and their correlation with yield in exotic wheat genotypes. *Pakistan Journal of Botany*, *43*(3), 1527–1530.

Archak, S., Tyagi, R. K., Harer, P. N., Mahase, L. B., Singh, N., Dahiya, O. P., … Bansal, K. C. (2016). Characterization of chickpea germplasm conserved in the Indian National Genebank and development of a core set using qualitative and quantitative trait data. *The Crop Journal*, *4*(5), 417–424.

Ashraf, M. & Foolad, M. R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany*, *59*, 206–216.

Babayev, H., Mehvaliyeva, U., Aliyeva, M., Feyziyev, Y. & Guliyev, N. (2014). The study of NAD-malic enzyme in *Amaranthus cruentus* L. under drought. *Plant Physiology and Biochemistry*, *81*, 84-89.

Babita M., Maheswari M., Rao L. M., Shanker A. K., & Rao D. G. (2010). Osmotic adjustment, drought tolerance and yield in castor (*Ricinus communis* L.) hybrids. *Environmental and Experimental Botany*, *69*, 243–249.

Bac-Molenaar, J. A., Granier, C., Keurentjes, J. J. B., & Vreugdenhil, D. (2016). Genome-wide association mapping of time-dependent growth responses to moderate drought stress in Arabidopsis: GWAS mapping of growth responses to moderate drought. *Plant Cell Environ*, *39*, 88–102.

Bagavathiannan, M. V., & Norsworthy, J. K. (2016). Multiple-herbicide resistance is widespread in roadside Palmer amaranth populations. *PLoS ONE*, *11*(4), 1–9.

Bahadur, A. Chattarjee, A., Kumar, R., Singh, M. & Naik, P.S. (2011). Physiological and biochemical basis of drought tolerance in vegetables. *Water*, *38*, 1-16.

Bahadur, A., Kumar, R., Mishra, U., Rai, A. & Singh, M. (2010). *Physiological approaches for screening of tomato genotypes for moisture stress tolerance*. In Proceedings of the National Conference of Plant Physiology (NCPP-2010) BHU, Varanasi, India. 25th–27th November 2010, p. 142.

Baker, N. R. & Rosenqvist, E. (2004). Applications of chlorophyll fluorescence can improve crop production strategies: An examination of future possibilities. *J Exp Bot, 55*, 1607-1621.

Balakrishnan, R., Nair, N. V., & Sreenivasan, T. V. (2000). A method for establishing a core collection of *Saccharum officinarum* L. Germplasm based on quantitative-morphological data. *Genetic Resources and Crop Evolution*, *47*(1), 1–9.

Baloch, F. S., Andeden, E. E., Alsaleh, A., Hatipoğlu, R., Nachit, M., O¨ zkan, H. (2016). High levels of segregation distortion in the molecular linkage map of bread wheat representing WANA (West Asia and North Africa) region. *Turkish Journal of Agriculture and Forestry*, *40*, 352–364.

Banerjee, P. P., Kole, P. C. (2009). Analysis of genetic architecture for some physiological characters in sesame (*Sesamum indicum* L.). *Euphytica, 168*, 11–22.

Bänziger, M., Setimela, P. S., Hodson, D., & Vivek, B. (2004). 'New direction for a diverse planet'. Proceedings of the 4th International Crop Science Congress. Breeding for improved drought tolerance in maize adapted to southern Africa.

Bänzinger, M., Edmeades, G.O., Beck, D. & Bellon, M. (2000). Breeding for drought and nitrogen stress tolerance in maize: From theory to practice. *Mexico D.F. CIMMYT*, 44–46.

Barilli, E., Cobos, M. J., Carrillo, E., Kilian, A., Carling, J., & Rubiales, D. (2018). A High-Density Integrated DArTseq SNP-Based Genetic Map of *Pisum fulvum* and Identification of QTLs Controlling Rust Resistance. *Frontiers in Plant Science*, *9*(2), 1–13.

Barrio, D. A. & Anon, M. C. (2010). Potential antitumor properties of a protein isolate obtained from the seeds of *Amaranthus mantegazzianus*. *Eur J Nutr, 49*, 73–82.

Bartlett, M. K., Scoffoni, C., & Sack, L. (2012). The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomass: A global meta-analysis. *Ecology Letters*, *15*, 393–405.

Bartoli, C.G., Gomez, F., Gergoff, G., Guiamet, J. J. & Puntarulo, S. (2005). Up-regulation of the mitochondrial alternative oxidase pathway enhances photosynthetic electron transport under drought conditions. *J. Exp. Bot*, 56, 1269-1276.

Bates, L. S., Waldren, R. P., & Teare, I. K. (1973). Rapid determination of free proline for water stress studies. *Plant and Soil*, *39*, 205–208.

Behera, B. & Patnaik, S. N. (1982). Genome analysis of Amaranthus dubius Mart. ex Thell. through the study of *Amaranthus spinosus* and *A. dubius* hybrids. *Cytologia, 47*, 379–389.

Bellasio C., Beerling D. J. & Griffiths H. (2015). An Excel tool for deriving key photosynthetic parameters from combined gas exchange and chlorophyll fluorescence: theory and practice. *Plant, Cell and Environment*.

Bellasio, C., Beerling, D. J., & Griffiths, H. (2016). Deriving C4 photosynthetic parameters from combined gas exchange and chlorophyll fluorescence using an Excel tool: Theory and practice. *Plant Cell and Environment*, *39*(6), 1164–1179.

Benjamin, J. G., Nielsen, D. C. (2006.) Water deficit effects on root distribution of soybean, field pea and chickpea. *Field Crops Research, 97*, 248–253.

Bennett, M. D. & Smith, J. B. (1991). Nuclear DNA amounts in angiosperms. *Philos. T. Roy. Soc. B, 334*, 309-345.

Bennetzen, J. L. (2000). Transposable element contributions to plant gene and genome evolution. *Plant Mol. Biol. 42*, 251–269.

Bertolli, S. C., Mazzafera, P. & Souza, G. M. (2014). Why is it so difficult to identify a single indicator of water stress in plants? A proposal for a multivariate analysis to assess emergent properties. *Plant Biology, 16(*3), 578–585.

Bhatnagar-Mathur, P., Devi, M. J., Reddy, S. D., Lavanya, M., Vadez, V., Serraj, R., Yamaguchi-Shinozaki, K. & Sharma, K. K. (2007). Stress inducible expression of At DREB1A in transgenic peanut (Arachis hypogea L.) increases transpiration efficiency under water-limiting conditions. *Plant Cell Rep, 26*, 2071–2082.

Blum A. (1998). Principal Estimates of Plant Water Status for Selection Work. AFRA Regional Training Course on Selection Methods for Drought Tolerance in Cereals and Legumes. South Africa. ARC-Roodeplaat, Pretoria.

Blum A. (2016). Stress, strain, signaling, and adaptation – not just a matter of definition. *Journal of Experimental Botany, 67*, 562–565.

Blum, A. (2004). *Sorghum physiology*. In: H. T. Nguyen, A. Blum (Eds.), *Physiology and Biotechnology Integration for Plant Breeding* (pp. 141-223).New York: Marcel Dekker.

Blum, A. (2010). *Plant breeding for water-limited environments*. London. Springer.

Blum, A. (2017). Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. *Plant Cell and Environment*, *40*, 4–10.

Blum, A., Golan, G., Mayer, J., Sinmena, B. (1997). The effect of dwarfing genes on sorghum grain filling from remobilized stem reserves under stress. *Field Crops Res, 52*, 43–54.

Bouslama, M., Schapaugh, W. T. (1984). Stress tolerance in soybean. Part 1: evaluation of three screening techniques for heat and drought tolerance. *Crop Sci*, *24*, 933-937.

Bowne, J. B., Erwin, T. A., Juttner, J., Schnurbusch, T., Langridge, P., Bacic, A., et al. (2012). Drought responses of leaf tissues from wheat cultivars of differing drought tolerance at the metabolite level. *Mol. Plant*. *5*, 418–429.

Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., Buckler, E. S. (2007). TASSEL: Software for association mapping of complex traits in diverse samples. *Bioinformatics, 23*, 2633-2635.

Bramley, H., Turner, N. C, Turner, D. W., Tyerman, S. D. (2009). Roles of morphology, anatomy, and aquaporins in determining contrasting hydraulic behavior of roots. *Plant Physiology*, *150*, 348–364.

Brandolini, A., Castagna, R. & Empilli. (2000). Morpho- agronomic variability of the diploid wheat *Triticum monococcum* L. *Plant Genet. Resour. Newsl*., 124, 36-40.

Brenner, D. M., Baltensperger, D. D., Kulakow, P. A., Lehmann, J. W., Myers, R. L., Slabbert, M. M. and Sleugh, B. B. (2000). *Genetic resources and breeding of Amaranths*. In: J. Janick (Eds.), *Plant Breeding Reviews* (pp. 227-285), New Jersey: John Wiley & Sons Inc.

Brenner, D. M., Widrlechner, M. P. (1998). *Amaranthus* seed regeneration in plastic tents in green houses. *Plant Genet Resour Newsl*, *116*, 1–4.

Brenner, D., Baltensperger, D., Kulakow, P., Lehmann, J., Myers, R., Slabbert, M. & Sleugh, B. (2010). Genetic resources and breeding of Amaranthus. *Plant Breed Rev, 19*, 227-285.

Brinez, B., Blair, M. W., Kilian, A., Carbonell, S. A. M, Chiorato, A. F., Rubiano, L. B. (2012). A whole genome DArT assay to assess germplasm collection diversity in common beans. *Molecular Breeding*, *30*, 181–193.

Bruinsma, J. (1963). The quantified analyses of chlorophyll a and b in plant extracts. *Photochem Photobiol*, *2*, 241-249.

Cabello, R., Monneveux, P., De Mendiburu, F., & Bonierbale, M. (2013). Comparison of yield based drought tolerance indices in improved varieties, genetic stocks and landraces of potato (Solanum tuberosum L.). *Euphytica*, *193*(2), 147–156.

Cai, Y. Z., Sun, M., Wu, M. X., Huang, R. & Corke, H. (1998). Characterization and quantification of betacyanin pigments from diverse *Amaranthus* species [J]. *J. Agr. Food Chem. 46*(6), 2063-2070.

Casique-Arroyo, G., Martínez-Gallardo, N., de la Vara, L. G. & Délano-Frier, J. P. (2014). Betacyanin biosynthetic genes and enzymes are differentially induced by (a) biotic stress in *Amaranthus hypochondriacus*. *PLoS ONE*, *9*(6), 1-18.

Cavanagh, C. R., Chao, S., Wang, S., Huang, B. E., Stephen, S., Kiani, S., et al. (2013). Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. *Proc. Natl. Acad. Sci. U.S.A. 110*, 8057–8062.

Chan, K. F. & Sun, M. (1997). Genetic diversity and relationships detected by isozyme and RAPD analysis of crop and wild species of *Amaranthus*. *Theor Appl Genet*, *95*, 865–873.

Chandi, A., Jordan, D. L., York, A. C., Burton, J., Milla-lewis, S. R., Spears, J., … Wells, R. (2013). Response of herbicide-resistant Palmer Amaranth (*Amaranthus palmeri*) accessions to drought stress, *International Journal of Agronomy*, *2013*, 1-8.

Chaney, L., Mangelson, R., Ramaraj, T., Jellen, E. N., Maughan, P. J. (2016). The complete chloroplast genome sequences for four *Amaranthus* species (Amaranthaceae), *Applications in Plant Sciences*, *4*(9), 1-6.

Chatterjee, A., & Solankey, S. S. (2015). Chapter 9: Functional physiology in drought tolerance of vegetable crops. *Climate Dynamics in Horticulture Science*, *1*, 150-171.

Chen, D., Wang, S., Cao, B., Cao, D., Leng, G., Li, H., Deng, X., et al. (2016). Genotypic variation in growth and physiological response to drought stress and re-watering reveals the critical role of recovery in drought adaptation in maize seedlings. *Frontiers in Plant Science*, *6*(1), 1–15.

Chivenge, P., Mabhaudhi, T., Modi, A. T., Mafongoya, P. (2015). The potential role of neglected and underutilised crop species as future crops under water scarce conditions in Sub-Saharan Africa. *Int. J. Environ. Res. Public Health 2015, 12*, 5685–5711.

Clouse, J. W., Adhikary, D., Page, J. T., Ramaraj, T., Deyholos, M. K., Udall, J. A., Fairbanks, D. J., Jellen, E. N. & Maughan, P. J. (2016). The Amaranth Genome: Genome, Transcriptome, and Physical Map Assembly. *Plant Genome*, *9*, 1-14.

Colom, M. R., Vazzana, C. (2003). Photosynthesis and PSII functionality of drought-resistant and drought-sensitive weeping lovegrass plants. *Environmental and Experimental Botany*, *49*, 135–144.

Condon, A. J., Richards, R. A., Rebetzke, G. J. & Farquhar, G. D. (2002). Improved intrinsic water use efficiency and crop yield. *Crop Sci*. *42*, 122-131.

Cooper, M., Gho, C., Leafgren, R., Tang T., & Messina C. (2014). Breeding drought-tolerant maize hybrids for the US corn-belt: discovery to product. *Journal of Experimental Botany*, *65*, 6191–6204.

Costea, M., Brenner, D. M., Tardif, F. J., Tan, Y. F., & Sun, M. (2006). Delimitation of *Amaranthus cruentus* L. and *Amaranthus caudatus* L. using micromorphology and

AFLP analysis: An application in germplasm identification. *Genetic Resources and Crop Evolution*, *53*(8), 1625–1633.

Costea, M., DeMason, D. A. (2001). Stem morphology and anatomy in *Amaranthus* L. (Amaranthacea*e) – taxonomic significance. Journal of the Torrey Botanical Society, 128*, 254–281.

Costea, M., Weaver, S. E. & Tardif, S. J. (2004). The biology of Canadian weeds. 130. *Amaranthus retroflexus* L., *A. powellii* S. Watson and *A. hybridus* L. *Can. J. Plant Sci*, *84*, 631-668.

Cruz, V. M. V, Kilian, A., Dierig, D. A. (2013). Development of DArT marker platforms and genetic diversity assessment of the US collection of the new oilseed crop lesquerella and related species, *PloS One*, *8*, e64062.

Dai, A. (2013). Increasing drought under global warming in observations and models. *Clim Change*, *3*, 52-58.

Dalal, A., Attia, Z. & Moshelion, M. (2017). To produce or to survive: how plastic is your crop stress physiology? *Front Plant Sci*, *8(2067)*, 1–6

Daryanto, S., Wang, L. & Jacinthe, P. A. (2016). Global synthesis of drought effects on cereal, legume, tuber and root crops production: A review. *Agric. Water Manage*. *1*, 18–33.

Das, S. (2012). Systematics and taxonomic delimitation of vegetable, grain and weed amaranths: A morphological and biochemical approach. *Genet. Resour. Crop Evol*, *59*, 289–303.

Das, S. (2016). *Infrageneric classification of amaranths*. In: S. Das (Eds.) *Amaranthus: A promising crop of future*. (pp. 49–56). Singapore: Springer.

Dawson, I. K., Powell, W., Hendre, P., Bančič, J., Hickey, J. M., Kindt, R., Hoad, S., Hale, I., & Jamnadass, R. (2019). Tansley review: The role of genetics in mainstreaming the production of new and orphan crops to diversify food systems and support human nutrition. *New Phytologist*, 1-18.

de Carvalho, K., de Campos, M. K. F., Domingues, D. S., Pereira, L. F. P. & Vieira, L. G. E. (2013). The accumulation of endogenous proline induces changes in gene expression of several antioxidant enzymes in leaves of transgenic *Swingle citrumelo. Mol. Biol. Rep*. *40*, 3269–3279.

De Datta, S. K., & Malabuyoc, J. A., Aragon, E. L. (1988). A field screening technique for evaluating rice germplasm for drought tolerance during the vegetative stage. *Field Crops Research*, *19*(2), 123-134.

De Ron, A. M., Sparvoli, F., Pueyo, J. J. & Bazile, D. (2017). Protein crops: Food and feed for the future. *Front. Plant Sci.*, 8, 1-4.

Delano-Frier, J.P., Aviles-Arnaut, H., Casarrubias-Castillo, K., Casique-Arroyo, G., Castrillon-Arbelaez, Vargas-Ortiz, E et al. (2011). Transcriptomic analysis of grain amaranth (*Amaranthus hypochondriacus*) using 454 pyrosequencing: Comparison with *A. tuberculatus*, expression profiling in stems and in response to biotic and abiotic stress. *BMC Genomics*, *12*(363), 1–18.

Devi, M. J., Sinclair, T. R., Vadez, V., Krishnamurthy, L. (2009). Peanut genotypic variation in transpiration efficiency and decreased transpiration during progressive soil drying. *Field Crop Res*, *114*, 280-285.

Doyle, J. J., & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus (San Francisco, Calif.)*, *12*, 13–15.

Dwivedi, S. L., Upadhayaya, H. D., & Hedge, D. M. (2005). Development of core collection using geographic information and morphological descriptors in

safflower (*Carthamus tinctorius* L.) germplasm. *Genetic Resources and Crop Evolution*, 52, 821-830.

Ebert, A. W. (2014). Potential of underutilized traditional vegetables and legume crops to contribute to food and nutritional security, income and more sustainable production systems. *Sustainability*, *6*, 319–335.

Edae, E. A., Byrne, P. F., Haley, S. D., Lopes, M. S., & Reynolds, M. P. (2014). Genome-wide association mapping of yield and yield components of spring wheat under contrasting moisture regimes. *Theor. Appl. Genet. 127*, 791–807.

Edwards, G. & Walker, D. (1983). C3, C4: Mechanisms, and cellular and environmental regulation of photosynthesis. Oxford. Blackwell Scientific Publications.

Ehdaie, B., Hall, A. E., Farquhar, G. D., Nguyen, H. T. & Waines, J. G. (1991). Water-use efficiency and carbon isotope discrimination in wheat. *Crop Sci*, *31*, 1282-1288.

Ehleringer, J. (1983). Ecophysiology of *Amaranthus palmeri*, a sonoran desert summer annual. *Oecologia*, *57*, 107-112.

Ehleringer, J. R., Cerling, T. E & Helliker, B. R. (1997). C4 photosynthesis, atmospheric CO_2 and climate, *Oecologia*, *112*, 285–299.

Ehleringer, J. R., Sage, R. F., Flanagan, L. B. & Pearcy, R. W. (1991). Climate change and the evolution of C4 photosynthesis. *Trends in Ecology & Evolution*, *6*, 95–99.

Ejieji CJ, Adeniran KA. 2010. Effects of water and fertilizer stress on the yield, fresh and dry matter production of grain Amaranth (Amaranthus cruentus). Australian Journal of Agricultural Engineering 1: 18-24.

El-Harty, E. H. (2016). Effect of water deficit on seed yield and proline content in some faba bean genotypes, *J. Plant Production, Mansoura Univ*., *7*(6): 653- 658.

Elliott, J., Deryng, D., Müller, C., Frieler, K., Konzmann, M., Gerten, D., et al. (2014). Constraints and potentials of future irrigation water availability on agricultural production under climate change. *PNAS*, *111*(9), 3239–44.

El-Sharkawy, M. A., Loomis, R. S, Williams, W. A. (1968). Photosynthetic and respiratory exchanges of carbon dioxide by leaves of the grain amaranth. *J. Appl. Ecol*. *15*, 243–251.

El-Sharkawy. (2016). Prospects of photosynthetic research for increasing agricultural productivity, with emphasis on the tropical C4 *Amaranthus* and the cassava C3-C4 crops. *Photosynthetica*, *54*(2), 161-184.

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE*, *6*, e19379.

Emokaro, C. O., Ekunwe, P. A. & Osifo, A. (2007). Profitability and production constraints in dry season amaranth production in Edo South, Nigeria. *J. Food Agric. Environ*, *5*, 281–283.

Erum, S., Naeemullah, M., Masood, S., Qayyum, A., & Rabbani, M. A. (2012). Genetic divergence in Amaranthus collected from Pakistan. *J. Anim. Plant Sci*., *22*, 653–658.

Espitia, E. (1992). Amaranth germplasm development and agronomic studies in Mexico. *Food Rev. Int*., *8*, 71–86.

Fahad, S., Bajwa, A. A., Nazir, U., Anjum, S. A., Farooq, A., Zohaib, A., … Huang, J. (2017). Crop Production under Drought and Heat Stress: Plant Responses and Management Options. *Frontiers in Plant Science*, *8*, 1–16.

Falush, D., Stephens, M., & Pritchard, J. K. (2007). Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Mol. Ecol. Notes*, *7*, 574–578.

Fang, Y., & Xiong, L. (2015). General mechanisms ofdrought response and their application in drought resistance improvement in plants. *Cell. Mol. Life Sci*. *72*, 673–689.

FAO, (2014). FAOSTAT. Food and Agriculture Organization of the United Nations. Rome, Italy. http://faostat.**fao**.org/default.aspx.

Farshadfar, E., J. Sutka, (2002). Multivariate analysis of drought tolerance in wheat substitution lines. *Cereal Res, Commun*, *31*, 33-39.

Fellers, J.P. (2008). Genome filtering using methylation-sensitive restriction enzymes with six base pair recognition sites. *Plant Gen*, *1*, 146–152.

Fen, L. L., Ismail, M. R., Zulkarami, B., Rahman, M. S. A., & Islam, M. R. (2015). Physiological and molecular characterization of drought responses and screening of drought tolerant rice varieties. *Bioscience Journal*, *31*(3), 709–718.

Fernandez, G. C. J., (1992). *Effective selection criteria for assessing stress tolerance*. In: C.G. Kuo (Ed.), *Proceedings of the International Symposium on Adaptation of Vegetables and Other Food Crops in Temperature and Water Stress* (pp. 257-270). Tainan, Taiwan.

Fischer, R. A. & Maurer, R. (1978). Drought resistance in spring wheat cultivars. I. Grain yield response. *Aust J Agr Res*, *29*, 897–907.

Franco, J. A., Arreola, J., Vicente, M. J., & Martínez-Sánchez, J. J. (2008). Nursery irrigation regimes affect the seedling characteristics of *Silene vulgaris* as they relate to potential performance following transplanting into semi-arid conditions. *J. Hortic. Sci. Biotechol*. *83*, 15–22.

Franco, J. A., Martínez-Sánchez, J. J., Fernández, J. A., & Bănón, S. (2006). Selection and nursery production of ornamental plants for landscaping and xerogardening in semi-arid environments. J. *Hortic. Sci. Biotechnol*. *81*, 3–17.

François, O. (2016). Running structure-like population genetic analyses with R, Tutorials in population genetics, *U. Grenoble-Alpes*, 1-9.

François, O., & Durand, E. (2010). Spatially explicity Bayesian clustering models in population genetics. *Molecular Ecology Resources*, *10*, 773–784.

Frankel, O. H., & Brown A. H. D. (1984). *Current plant genetic resources – a critical appraisal.* In: V. L. Chopra, B. C. Joshi B. C., R. P. Sharma, & H. C. Bansal (Eds). *Genetics: new frontiers Vol. IV* (pp. 1-13). New Delhi: Oxford & IBH Publ. Co.

Franks, P. J. & Farquhar, G. D. (2007). The mechanical diversity of stomata and its significance in gas-exchange control. *Plant Physiology*, *143*, 78–87.

Gamel, T. H., Mesallam, A. S., Damir, A. A., Shekib, L. A. & Linssen, J. P. (2007). Characterization of amaranth seed oils. *J. Food Lipids*, *14*, 323–334.

Gangopadhyay, K. K., Mahajan, R. K, Kumar, G., Yadav, S. K., Meena, B. L., Pandey, C., Bisht, I. S. (2010). Development of a core set in brinjal, *Crop Sci*, *50*, 755–762.

Garrec, J. P., & Letourneur, L. (1981). Fluoride absorption by the root and foliar tissue of the horse bean (*Viciafaha minor calcicole*) and lupine (*Lupinusluteus calcituge*). *Fluoride*, *14*, 30-38.

George, S., Parida, A., & Swanimathan, S. (2012). *Wheat and Rice Crops: "Omics" approaches for abiotic stress toleranc*e. In N. Tuteja, S. S. Gill, F. Tiburcio, R. Tuteja (Eds.), *Improving crop resistance to abiotic stress* (pp. 695-713). New Jersey, USA: Wiley-Blackwell.

Gerrano, A. S., van Rensburg, W. S. J., & Adebola, P. O. (2014). Agro-morphological variability of *Amaranthus* genotypes in South Africa. *Acta Horti*., *11*(1035): 183-187.

Gerrano, A. S., Willem S. J., Rensburg, V., & Adebola, P. O. (2006). Genetic diversity of *Amaranthus* species in South Africa, *Euphytica*, *151*, 103–110.

Gerrano, D. A., van Rensburg, W. S. J., Mavengahama, S., Bairu, M., Venter, S., Adebola, P. (2017). Qualitative morphological diversity of *Amaranthus* species. *Journal of Tropical Agriculture*. *55*, 12-20.

Ghannoum, O., 2009. C4 photosynthesis and water stress. *Annals of Botany*, *103*(4), 635-644.

Ghate, T., Deshpande, S. & Bhargava, S. (2017). Accumulation of stem sugar and its remobilisation in response to drought stress in a sweet sorghum genotype and its near-isogenic lines carrying different stay-green loci. *Plant Biol*, *19*, 396–405.

Ghiabi, S., Sharafi, S. & Talebi, R. (2013). Morpho-physiological and biochemical alternation responses in different chickpea (*Cicer arietinum* L.) genotypes under two constructing water regimes. *Int. J. Biosci*., 3(8):57-65.

Gholipoor, M. & Sinclair, T. R. (2012). Genotypic variation within sorghum for transpiration response to drying soil. *Plant Soil*, 357, 35-40.

Gholipouri, A., Sedghi, M. R., Sharifi, S. & Nazari, N. M. (2009). Evaluation of drought tolerance indices and their relationship with grain yield in wheat cultivars. *Recent Res. Sci. Technol*, *1*(4), 195-198.

Gilbert, M. E., & Medina, V. (2016). Drought adaptation mechanisms should guide experimental design. *Trends in Plant Science*, *21*(8), 639–647.

Gindaba, J., Rozanov, A., & Negash, L. (2004). Response of seedlings of two Euca- lyptus and three deciduous tree species from Ethiopia to severe water stress, *For. Ecol. Manage*, *201*, 119–129.

Gindaba, J., Rozanov, A., Negash, L., (2004). Response of seedlings of two Eucalyptus and three deciduous tree species from Ethiopia to severe water stress. *For. Ecol. Manage*. *201*, 119–129.

Godar, A. S., Varanasi, V. K., Nakka, S., Prasad, P. V. V., Thompson, C. R., & Mithila, J. (2015). Physiological and molecular mechanisms of differential sensitivity of Palmer amaranth (*Amaranthus palmeri*) to mesotrione at varying growth temperatures. *PLoS ONE*, *10*(5), 1–17.

Golabadi, M., Arzani, A., Maibody, S. A. M. (2006). Assessment of drought tolerance in segregating populations in durum wheat. *Afr. J. Agric. Res*, **5**, 162-171.

Govindaraj, M., Vetriventhan, M. & Srinivasan, M. (2015). Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. *Genetics Research International*, 1–14.

Grant, W.F. (1959). Cytogenetic studies in Amaranthus: Chromosome numbers and phylogenetic aspects. *Canadian Journal of Genetics and Cytology*, *1*, 313–328.

Greizerstein, E. J. & Poggio, L. (1994). Karyological studies in grain Amaranths. *Cytology*, *59*, 25-30.

Grubben, G. J. H. (2004). *Vegetables*, In G. J. H. Grubben, G.J.H. & O. A. Denton (Eds.). In *Plant resources of Tropical Africa* (pp.68). Netherlands: PROTA/ Foundation/Backhuys Publishers/CTA.

Gudu, S. & Gupta, V. K. (1988). Electrophoresis as an aid in the identification of various species and cultivars of grain amaranths. *Acta Horticulture*, *218*, 231–238.

Guillen-Portal, F. R., Baltensperger, D. D., & Nelson, L.A., (1999). *Plant population influence on yield and agronomic traits in 'Plainsman' grain amaranth*. In: J. Janick (Ed.). *perspectives on new crops and new uses* (pp. 190-193). Alexandria, VA: ASHS Press.

Guo, P., Li, M. (1996). Studies on photosynthetic characteristics in rice hybrid progenies and their parents. I. chlorophyll content, chlorophyll-protein complex and chlorophyll fluorescence kinetics. *Journal of Tropical and Subtropical Botany*, *4*, 60-65.

Gupta, V. K. & Gudu, S. (1991). Interspecific hybrids and possible phylogenetic relations in grain amaranths. *Euphytica*, *52*, 33-38.

Hakam, N. & Simeon, J. P. (1996). Effect of low temperature on the activities of oxygen scavenging enzymes in two populations of the C4 grass. *Echinochloa crusgalli*. *Physiologia Plantarum*, *97*, 209–216.

Hao, Z.-F., Li, X.-H., Su, Z.-J., Xie, C.-X., Li, M.-S., Liang, X.-L., … Zhang, S.-H. (2011). A proposed selection criterion for drought resistance across multiple environments in maize. *Breeding Science*, *61*(2), 101-108.

Hare, P. D., Cress, W. A. & Van Staden, J. (1998). Dissecting the roles of osmolyte accumulation during stress. *Plant Cell Environment*, *21*, 535–553.

Hassani, S. M. R., Talebi, R., Pourdad, S. S., Naji, A. M. & Fayaz, F. (2020). In-depth genome diversity, population structure and linkage disequilibrium analysis of worldwide diverse safflower (*Carthamus tinctorius* L.) accessions using NGS data generated by DArTseq technology. *Molecular Biology Reports*, *47*, 2123-2135.

Hassanzadeh, M., Ebadi, A., Panahyan-e-Kivi, M., Eshghi, A. G., Jamaati-e-Somarin, S., Saeidi, M., Zabihi-e-Mahmoodabad, R. (2009). Evaluation of drought stress on relative water content and chlorophyll content of sesame (*Sesamum indicum* L.) genotypes at early flowering stage. *Research Journal of Environmental Science*, *3*, 345–350.

Hatch, M. D. (1987). C4 photosynthesis: A unique end of modified biochemistry, anatomy and ultrastructure. *Biochima et Biophysica Acta*, *895*, 81-106.

Hattersley, P. (1992). *C4 photosynthetic pathway variation in grasses (Poaceae): Its significance for arid and semi-arid lands*. In: G. P. Chapman (Eds.), *Desertified grasslands: Their biology and management* (pp. 181-212). London: Academic Press.

Hauptli, H. & Jain S. (1984). Allozyme variation and evolutionary relationships of grain amaranths (*Amaranthus* spp.), *Theor. Appl. Genet*., *69*,153-165.

Hauptli, H. & Jain, S. (1985). Genetic variation in outcrossing rate and correlated floral traits in a population of grain amaranth (*Amaranthus cruentus* L.). *Genetica*, *66*, 21–27.

Haussmann, B. I. G., Mahalakshmi, V., Reddy, B. V. S., Seetharama N, Hash, C. T., Geiger, H. H. (2002). QTL mapping of stay-green in two sorghum recombinant inbred populations. *Theoretical and Applied Genetics*, *106*, 133-142.

Hayat, S., Hayat, Q., Alyemeni, M. N., Wani, A. S., Pichtel, J. & Ahmad, A. (2012). Role of proline under changing environments: A review. *Plant Signal. Behav*, *7*, 1456–1466.

Henry, A. Gowda, V. R. P., Torres, R.O., McNally, K. L., & Serraj, R. (2011). Variation in root system architecture and drought response in rice (*Oryza sativa*): Phenotyping of the *Oryza* SNP panel in rainfed low land fields. *Field Crops Res. 120*, 205–214.

Herzig, P., Maurer, A., Draba, V., Sharma, R., Draicchio, F., Bull, H., Milne, L., Thomas, W. T., Flavell, A. J. & Pillen, K. (2018). Contrasting genetic regulation of plant development in wild barley grown in two European environments revealed by nested association mapping, *J Exp Bot*, *69*(7), 1517–1531

Hlaváčová, M., Klem, K., Smutná, P., Škarpa, P., Hlavinka, P., Novotná, K., Papantová. P. & Trnka, M. (2017). Effect of heat stress at anthesis on yield formation in winter wheat. *Plant, Soil and Environment*, *63*(3), 139–144.

Ho, W. K., Chai, H. H., Kendabie, P., Ahmad, N. S., Jani, J., Massawe, F., … Mayes, S. (2017). Integrating genetic maps in bambara groundnut [*Vigna subterranea* (L) Verdc .] and their syntenic relationships among closely related legumes, *BMC Genomics*, *18*, 192.

Hossain, A. B. S., Sears, A. G., Cox, T. S, Paulsen, G. M.(1990). Desiccation tolerance and its relationship to assimilate partitioning in winter wheat. *Crop Sci*, *30*, 622-627.

Hoyos-Villegas, V., Song, Q., & Kelly, J. D. (2017). Genome-wide association analysis for drought tolerance and associated traits in common bean. *The Plant Genome*, *10*(1), 1-17.

Huerta-Ocampo, J. A., Briones-Cerecero, E. P., Mendoza-Hernández, G., De León-Rodríguez A. & de la Rosa, A. P. B. (2009). Proteomic Analysis of amaranth (*Amaranthus hypochondriacus* L.) leaves under drought stress. *Int J Plant Sci*, *170*, 990-998.

Hura, T., Grzesiak, S., Hura, K., Thiemt, E., Tokarz, K., and Wedzony, M. (2007a). Physiological and biochemical tools useful in drought tolerance detection in genotypes of winter *triticale*: Accumulation of ferulic acid correlates with drought tolerance. *Ann. Bot*., *100*, 767–775.

Hura, T., Hura, K., Grzesiak, M., & Rzepka, A. (2007b). Effect of long-term drought stress on leaf gas exchange and fluorescence parameters in C3 and C4 plants. *Acta Physiologiae Plantarum*, *29*,103-113.

Hussain, B., & Ali, B. (2015). leaf longevity in plants under water stress – A review. *Indian Journal of Plant Science*, *4*(4), 127–133.

IPCC. 2012. Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation. A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change [Field, C.B., V. Barros, T.F. Stocker, D. Qin, D.J. Dokken, K.L. Ebi, M.D. Mastrandrea, K.J. Mach, G.-K. Plattner, S.K. Allen, M. Tignor, and P.M. Midgley (Eds.), Cambridge University Press, Cambridge, UK, and New York, NY, USA, pp. 582.

Irving, L. (2015). Carbon assimilation, biomass partitioning and productivity in grasses. *Agriculture*, *5*(4), 1116–1134.

Iseki, K., Homma, K., Irie, T., Endo, T., Shiraiwa, T., Iseki, K., … Shiraiwa, T. (2013). The long-term changes in midday photoinhibition in rice (*Oryza sativa* L .) growing under fluctuating soil water conditions, Plant Production Science, *16*(4).

Iseki, K., Takahashi, Y., Muto, C., Naito, K., & Tomooka, N. (2016). Diversity of drought tolerance in the genus *Vigna*. *Frontiers in Plant Science*, *11*(10), 1–18.

Iturbide, G., A. & Gispert, M. (1994). *Grain amaranths (Amaranthus spp.)*. In: J.E. Hernandez-Bermejo & J. Leon (Eds.), *Neglected crops: 1492 from a different perspective* (pp. 93–101). ROME. FAO.

Jabbari, M., Fakheri, B. A., Aghnoum, R., Nezhad, N. M., & Ataei, R. (2018). GWAS analysis in spring barley (*Hordeum vulgare* L.) for morphological traits exposed to drought. *PLoS ONE*, *13(*9), 1–22.

Jacobsen, S. E. & Mujica, A. (2003). The genetic resources of Andean grain amaranths (*Amaranthus caudatus* L, *A. cruentus* and *A. hypochondriacus* L) in America. *Plant Genet. Resour. Newsl, 133*, 41–44.

Jagadev, P. N., Shamal, K. M., Lenka, L. (1991). Genetic divergence in rape mustard. *Indian J. Genet. Plant Breed*. *51*, 465-466.

Jain, S. K., Hauptli, H. & Vaidya, K. R. (1982). Outcrossing rate in grain amaranths. *Journal of Heredity*, 73, 71–72.

Jain, S. M. & Gupta, S. D. (2013). *Biotechnology of Neglected and Underutilized Crops*. Berlin, Germany. Springer.

Jaleel, C. A., Manivannan, P., Wahid, A., Farooq, M., Al-Juburi, H. J, Somasundaram. R., Panneerselvam, R. (2009). Drought stress in plants: A review on morphological characteristics and pigments composition. *Int J Agric Biol*, *11*(1): 100–105

Jamalluddin, N., Massawe, F. J., & Symonds, R. C. (2018). Transpiration efficiency of Amaranth (*Amaranthus* sp.) in response to drought stress. *The Journal of Horticultural Science and Biotechnology*, 1–12.

Jangpromma N., Songsri P., Thammasirirak S., J. P. (2010). Rapid assessment of chlorophyll content in sugarcane using SPAD chlorophyll meter across different water stress conditions. *Asian Journal of Plant Sciences*, *9* (6), 368–374.

Jimenez, F. R., Maughan, P. J., Alvarez, A., Kietlinski, K. D., Smith, S. M., Pratt, D. B., … Jellen, E. N. (2013). Assessment of genetic diversity in Peruvian Amaranth (*Amaranthus caudatus* and *A. hybridus*) germplasm using single nucleotide polymorphism markers. *Crop Science*, *53*(2), 532–541.

Jiménez-Aguilar, D. M. & Grusak, M. A. (2017). Minerals, vitamin C, phenolics, flavonoids and antioxidant activity of *Amaranthus* leafy vegetables. *Journal of Food Composition and Analysis*, 58, 33–39.

Jin, H., Xu, M., Chen, H., Zhang, S., Han, X. & Tang, Z. (2016). Comparative proteomic analysis of differentially expressed proteins in *Amaranthus hybridus* L. roots under cadmium stress. *Water Air Soil Pollut*, *227*, 1–12.

Johnson, D. A., & Asay, K. H. (1993). Viewpoint : Selection for improved response in cool-season grasses. *Journal of Range Management*, *46*(5), 194-202.

Johnson, D. M., Woodruff, D. R., McCulloh, K. A. & Meinzer, F.C. (2009). Leaf hydraulic conductance, measured in situ, declines and recovers daily: leaf hydraulics, water potential and stomatal conductance in four temperate and three tropical tree species. *Tree Physiol*. 29: 879–887.

Jombart, T. &Ahmed I. (2011). Adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatic*s.

Jomo, O. M., Netondo, G. W., & Musyimi, D. M. (2016). Drought inhibition of chlorophyll content among seven *Amaranthus* species. *IARJSET*, *3*, 1362-1371.

Judd, W. S., C. S., Kellog, E. A., Stevens, P. F., Donoghue, & Michael J. (2008). *Plant Systematics: A Phylogenetic Approach*. Sunderland, MA. Sinauer Associates, Inc.

Kadam, N. N., Xiao, G., Melgar R. J., Bahuguna, R. N., Quiñones, C., Tamilselavan, A., Prasad, V. V. & Jagadish, K. S. V. (2014). Agronomic and physiological responses to high temperature, drought, and elevated CO_2 interactions in cereals. *Advances in Agronomy*, *127*,111-156.

Kadereit, G., Borsch, T., Weising, K., Freitag, H. (2003). Phylogeny of Amaranthaceae and Chenopodiaceae and the evolution of C4 photosynthesis. *Int J Plant Sci*, *164*, 959–86.

Kamoshita, A., Babu, R. C., Boopathi, N. M., & Fukai, S. (2008). Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rainfed environments. *Field Crops Research*, 1*09*(1-3), 1-23.

Katiyar, R. S., Shukla S. & Rai, S. (2000).Varietal performance of grain amaranth (*A. hypochondriacus*) on sodic soil. *Proc Nat Acad Sci*, *70*, 185–187.

Kauffman, C. S., & Weber, L. E. (1990). *Grain amaranth*. In: J. Janick, & J. E. Simon (Eds.), *Advances in new crops* (pp. 127139). Portlandm:Timber Press.

Khaing A. A., Moe K. T, Chung J. W., Baek, H. J., & Park, Y. J. (2013). Genetic diversity and population structure of the selected core set in Amaranthus using SSR markers. *Plant Breeding*, *132*, 165-173.

Khanam, U. K. S. & Oba, S. (2014). Phenotypic plasticity of vegetable amaranth, *Amaranthus tricolor* L. under a natural climate, *Plant Prod. Sci*, *17*(2), 166-172.

Khandaker, L., Akond, A. S. M. G. M & Oba, S. (2009). Air temperature and sunlight intensity of different growing period affects the biomass, leaf color and betacyanin pigment accumulations in red amaranth (*Amaranthus tricolor* L.). *Journal of Central European Agriculture*, *10*, 439-448.

Kholova, J., Hash, C. T., Kakkera, A., Kocova, M. &Vadez, V. (2010). Constitutive water-conserving mechanisms are correlated with the terminal drought tolerance of pearl millet *Pennisetum glaucum* (L.), *R. Br. J. Exp Bot*, 61, 369–377.

Khoury, C. K., Bjorkman, A. D., Dempewolf, H., Ramirez-Villegas, J., Guarino, L., Jarvis, A., … Struik, P. C. (2014). Increasing homogeneity in global food supplies and the implications for food security. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, 4001–4006.

Kietlinski, K. D., Jimenez, F., Jellen, E. N., Maughan, P. J., Smith, S. M., & Pratt, D. B. (2014). Relationships between the weedy (Amaranthaceae) and the grain amaranths. *Crop Science*, *54*, 220-228.

Kilian, A., Wenzl, P., Huttner, E., Carling, J., Xia, L., Blois, H., et al. (2012). *Diversity Arrays Technology: A Generic Genome Profiling Technology on Open Platforms*. In: FPaA, Bonin (Eds.). *Data Production and Analysis in Population Genomics: Methods and protocol* (pp. 67–89). New York Heidelberg Dordrecht London: Springer.

Kissel, E., van Asten, P., Swennen, R., Lorenzen, J. & Carpentier, S. C. (2015). Transpiration efficiency versus growth: Exploring the banana biodiversity for drought tolerance. *Sci Hortic*. *185*, 175-182.

Koevoets, I. T., Venema, J. H., Elzenga, J. T. M., & Testerink, C. (2016). Roots withstanding their environment: Exploiting root system architecture responses to abiotic stress to improve crop tolerance. *Front. Plant Sci*. 7, 1335.

Kogan, F., Guo, W., & Yang, W. (2019). Drought and food security prediction from NOAA new generation of operational satellites. *Geomatics, Natural Hazards and Risk*, *10*(1), 651–666.

Kohzuma, K., Cruz, J. A., Akashi, K., Hoshiyasu, S., Munekage, N. Y., Yokota, A. & Kramer, D. M. (2009). The long-term responses of the photosynthetic proton circuit to drought. *Plant Cell Environ*, *32*, 209-219.

Köşkeroğlu, S. & Tuna, A. L. (2010). The investigation on accumulation levels of proline and stress parameters of the maize (Zea mays L.) plants under salt and water stress. *Acta Physiol Plant*, *35*, 541-549.

Kouzai, Y., Yamanaka, Y., Yamamoto, M., Toyoda, K., Watanabe, M., Matsui, H., … Noutoshi, Y. (2016). Expression profiling of marker genes responsive to the

defence-associated phytohormones salicylic acid, jasmonic acid and ethylene in *Brachypodium distachyon*. *BMC Plant Biology*, *16*(1), 1–11.

Krichen, L., Audergon, J. M., & Trifi-Farah, N. (2012). Relative efficiency of morphological characters and molecular markers in the establishment of an apricot core collection. *Hereditas*, *149*(5), 163–172.

Krishnamurthy, L., Vadez, V., Devi, M.J., Serraj, R., Nigam, S.N., Sheshshayee, M.S., Chandra, S. and Aruna, R. (2007). Variation in transpiration efficiency and its related traits in a groundnut (*Arachis hypogaea* L) mapping population. *Field Crop Res, 103*, 189-197.

Kuk, Y. I., Shin, J. S., Burgos, N. R., Hwang, T. E., Han, O., Cho, B. H., Jun, S. & Guh, J. O. (2003). Antioxidative enzymes offer protection from chilling damage in rice plants. *Crop Science*, *43*, 2109–2117.

Kulakow, A. P., Hauptli, H. (1994). *Genetic characterisation of grain amaranth*. In: Peredes-Lopez) (Eds.). *Amaranth Biology, Chemistry and Technology* (pp. 9-22). Boca Raton: CRC Press.

Kumar, A., Dixi, S., Ram, T., Yadaw, R. B, Mishra, K. K, Mandal, N. P. (2014). Breeding high-yielding drought-tolerant rice: Genetic variations and conventional and molecular approaches. *Journal of Experimental Botany 65*, 6265–6278.

Kumar, R., Solankey, S. & Singh, M. (2012). Breeding for drought tolerance in vegetables. *Vegetable Science*, *39*, 1-15.

Kumar, R., Solankey, S. S., & Singh, M. (2012). Breeding of drought tolerance in vegetables. *Vegetables Sciences*, 39, 1–15.

Kurukulasuriya, P., Ajwad, M. I. (2007). Application of the Ricardian technique to estimate the impact of climate change on smallholder farming in Sri Lanka. *Climate Change*, *81*, 39–59.

Laidò, G., Marone, D., Russo, M. A., Colecchia, S. A., Mastrangelo, A .M, De Vita P., et al. (2014). Linkage disequilibrium and genome-wide association mapping in tetraploid wheat (*Triticum turgidum* L.). *PloS one*, *9*(4): e95211.

Lal, A. & Edwards, G. E. (1996). Analysis of inhibition of photosynthesis under water stress in the C4 species *Amaranthus cruentus* and *Zea mays*: Electron transport, CO_2 fixation and carboxylation capacity. *Functional Plant Biology*, 23, 403–412.

Lamaoui, M., Jemo, M., Datla, R., & Bekkaoui, F. (2018). Heat and drought stresses in crops and approaches for their mitigation, *Frontiers in Chemistry*, *6*(2), 1–14.

Lambers, H., Atkin, O. K., Millenaar, F. F. (2002). *Respiratory patterns in roots in relation to their functioning*. In: Y. Waisel, A. Eshel, U. Kafkafi (Eds.). *Plant roots, the hidden half* (pp. 521-552). New York: Marcel Dekker.

Lan, J. (1998). Comparison of evaluating methods for agronomic drought resistance in crops. *Acta Agriculturae Boreali-occidentalis Sinica*, *7*, 85–87.

Lanoue, K. Z., Wolf, P. G., Browning, S., Hood, E. E. (1996). Phylogenetic analysis of restriction-site variation in wild and cultivated *Amaranthus* species (Amaranthaceae). *Theor Appl Genet*, *93*, 722-732.

Lawlor, D. W. & Cornic, G. (2002). Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell Environ*, *25*, 275–294.

Lee, J. R., Hong, G. Y., Dixit, A., Chung, J. W., Ma, K. H., … Park, Y.J. (2008). Characterization of microsatellite loci developed for *Amaranthus hypochondriacus* and their cross-amplifications in wild species. *Conservation Genetics*, *9*, 243-246.

Lehmann, J. W. (1996). Case history of grain amaranth as an alternative crop. *Cereal Foods World*, *41*, 399-409.

Li, D., Dossa, K., Zhang, Y., Wei, X., Wang, L., Zhang, Y., et al. (2018). GWAS uncovers differential genetic bases for drought and salt tolerances in sesame at the germination stage. *Genes*, *9*, 87.

Li, H. S., J., Bhavani, S., Vikram, P., Sehgal, D., Huerta-Espino, J., Kilian, A., … Singh, S. (2015). A high density GBS map of bread wheat and its application for dissecting complex disease resistance traits. *BMC Genomics*, *16*, 216.

Li, Q., Deng, M., Xiong, Y., Coombes, A., & Zhao, W. (2014). Morphological and photosynthetic response to high and low irradiance of *Aeschynanthus longicaulis*. *Scientific World Journal*, 1-8.

Li, R. H., Guo, P. G., Michael, B., Stefania, G., Salvatore, C. (2006). Evaluation of chlorophyll content and fluorescence parameters as indicators of drought tolerance in barley. *Agric. Sci. China*, *5*, 751-757.

Lightfoot, D. J., Jarvis, D. E., Ramaraj, T., Lee, R., Jellen, E. N., & Maughan, P. J. (2017). Single-molecule sequencing and Hi-C-based proximity-guided assembly of amaranth (*Amaranthus hypochondriacus*) chromosomes provide insights into genome evolution. *BMC Biology*, *15*(1), 1–15.

Lin, B. B. (2011). Resilience in Agriculture through Crop Diversification: Adaptive Management for Environmental Change. *BioScience*, *61*(3), 183–193.

Lin, Z. F. & Ehleringer, J. (1983). Photosynthetic characteristics of *Amaranthus tricolor*, a C4 tropical leafy vegetable. *Photosythesis Res*, *4*, 171-178.

Liu, C. C., Liu, Y. G., Guo, K., Fan, D. Y., Li, G. G.,.. Ynag, R. (2011). Effect of drought on pigments, osmotic adjustment and antioxidant enzymes in six woody plant species in karst habitats of southwestern China. *Environ. Exp. Bot*, *71*, 174-183.

Liu, F. & Stützel, H. (2002a). Leaf water relations of vegetable amaranth (*Amaranthus* spp) in response to soil drying. *European Journal of Argonomy*, *16*, 137-150.

Liu, F. & Stützel, H. (2002b). Leaf expansion, stomatal conductance and transpiration of vegetable amaranth (Amaranthus spp.) in response to soil drying. *Journal of the American Society for Horticultural Science*, 127: 878-883.

Liu, F. & Stützel, H. (2004). Biomass partitioning, specific leaf area and water use efficiency of vegetable amaranth (*Amaranthus* spp.) in response to drought stress. *Science Horticulture*, *102*, 15-27.

Liu, H. & Osborne, C. P. (2015). Water relations traits of C4 grasses depend on phylogenetic lineage, photosynthetic pathway, and habitat water availability. *Journal of Experimental Botany*, *66*(3), 761-773.

Liu, S. C., Kuang, H. Q. & Lai, Z. X. (2014). Transcriptome analysis by Illumina high throughout paired-end sequencing reveals the complexity of differential gene expression during *in vitro* plantlet growth and flowering in *Amaranthus tricolor* L. *PLOS One*, *9*(6), 1–17.

Liu, W., Shahid, M. Q., Li, Z., Chen, Y., Jiang, L., Diao, M., Liu, X., Lu, Y. (2015). Evaluation of genetic diversity and development of a core collection of wild rice (*Oryza rufipogon* Griff.) populations in China. *PLoS One*, 1-23.

Long, Q., Rabanal, F.A., Meng, D., Huber, C.D., Farlow, A., Platzer, A., Zhang, Q., Vilhjálmsson, B.J., Korte, A.,.. Nizhynska, V. (2013). Massive genomic variation and strong selection in *Arabidopsis thaliana* lines from Sweden. *Nat. Genet*. *45*, 884–890.

Luoh, J. W., Begg, C. B., Symonds, R. C., Ledesma, D., & Yang, R. (2014). Nutritional yield of African indigenous vegetables in water-deficient and water-sufficient conditions. *Food and Nutrition* Sciences, *5*, 812-822.

Ma, Q. Q., Wang, W., Li, Y. H., Li, D. Q. & Zou, Q. (2006). Alleviation of photoinhibition in drought-stressed wheat (Triticum aestivum) by foliar-applied glycinebetaine. *Journal of Plant Physiology*, *163*, 165–175.

Maatallah, S., Ghanem, M. E., Albouchi, A., Bizid, E., & Lutts, E. (2010). A greenhouse investigation of responses to different water stress regimes of *Laurus nobilis* trees from two climatic regions. *Journal of Arid Environments*, *74*, 327–337.

Mabhaudhi, T., Chibarabada, T., Modi, A. (2016). Water-Food-Nutrition-Health Nexus: Linking Water to Improving Food, Nutrition and Health in Sub-Saharan Africa. *Int J Environ Res Public Health*, *13* (1), 107-126.

Magombeyi, M. S., Taigbenu, A. E. (2008). Crop yield risk analysis and mitigation of smallholder farmers at quaternary catchment level: Case study of B72A in Olifants river basin, South Africa. *Physics and Chemistry of the Earth*, *33*, 744–756.

Majidi, M., Tavakoli, V., Mirlohi, A., Sabzalian, M. R. (2011). Wild safflower species (*Carthamus oxyacanthus* Bieb.): A possible source of drought tolerance for arid environments. *Aust. J. Crop Sci*, *5*(8), 1055-1063.

Malek, M. A., Rafii, M. Y., Shahida Sharmin Afroz, M., Nath, U. K. & Mondal, M. M. A. (2014). Morphological characterization and assessment of genetic variability, character association, and divergence in soybean mutants. *Scientific World Journal*.

Mallory, M. A., Hall, R. V., McNabb, A. P., Pratt, D. B., Jellen, E. N., Maughan, P. J. (2008). Development and characterization of microsatellite markers for the grain amaranths. *Crop Sci*, *48*, 1098–106.

Malnoë, A. (2018). Photoinhibition or photoprotection of photosynthesis? Update on the (newly termed) sustained quenching component qH. *Environmental and Experimental Botany*, *154*(2), 123–133.

Malnoë, A., Schultink, A., Shahrasbi, S., Rumeau, D., Havaux, M., & Niyogi, K. K. (2018). The plastid lipocalin LCNP is required for sustained photoprotective energy dissipation in Arabidopsis. *Plant Cell*, *30*(1), 196–208.

Malviya, N., Yadav, D., Sarangi, B. K. & Yadav, M. K. (2012). analysis of genetic diversity in cowpea (Vigna unguiculata L. Walp.) cultivars with random amplified polymorphic DNA markers. *Plant Systematics and Evolution*, *29*, 523-526.

Mandal, J. & Dhangrah, V. K. (2009). Studies on yield and its attributes in vegetable amaranth. *Veg. Sci.*, *36*(1),17-21.

Mandal, N., & Das, P. K. (2002). Intra-and interspecific genetic diversity in grain *Amaranthus* using random amplified polymorphic DNA Markers. *Plant Tissue Culture and Biotechnology*, *12*, 49-56.

Marees, A. T., de Kluiver, H., Stringer, S., Vorspan, F., Curis, E., Marie-Claire, C., & Derks, E. M. (2018). A tutorial on conducting genome-wide association studies: Quality control and statistical analysis. *International Journal of Methods in Psychiatric Research*, *27*(2), 1–10.

Marek, Ž., Jana, R., Katarína, O., & Brestiè, M. (2009). Osmotic adjustment in winterwheat varieties and its importance as a mechanismof drought tolerance. *Cereal Res. Commun*., *37*, 569–572.

Martin, F. W. & Telek, L. (1979). *Vegetables of hot humid tropics part 6: Amaranth and celosia* (pp. 1-21). New Orleans. U.S. Department of Agriculture.

Masih, I., Maskey, S., Mussá, F. E. F., & Trambauer, P. (2014). A review of droughts on the African continent: a geospatial and long-term perspective. *Hydrol. Earth Syst. Sci*., *18*, 3635–3649.

Massawe, F. J., Mwale, S. S, Roberts, J. A. (2005). Breeding in Bambara groundnut (*Vigna subterranea* (L.) Verdc.): Strategic considerations. *Afr J Biotechnol*, *4*, 463–471.

Massawe, F., Mayes, S., Cheng, A. (2016). Crop diversity: An unexploited treasure trove for food security, *Trends in Plant Science*, *21*, 365-368.

Mathew, I., Shimelis, H., Mwadzingeni, L., Zengeni, R., Mutema, M. & Chaplot, V. (2018). Variance components and heritability of traits related to root : shoot biomass allocation and drought tolerance in wheat, *Euphytica*, 214-225.

Maughan, P. J., Sisneros, N., Luo, M. Z., Kudrna, D., Ammiraju, J. S. S, Wing, R. A. (2008). Construction of an *Amaranthus hypochondriacus* bacterial artificial chromosome library and genomic sequencing of herbicide target genes. *Crop Sci*, *48*, 85–94.

Maughan, P. J., Smith, S. M., Fairbanks, D. J. & Jellen, E. N. (2011). Development, characterization, and linkage mapping of single nucleotide polymorphisms in the grain amaranths (*Amaranthus* sp.). *Plant Genome*, *4*, 92–101.

Maughan, P. J., Yourstone, S. M., Jellen, E. N, Udall, J. A. (2009). SNP discovery via genomic reduction, barcoding, and 454-pyrosequencing in amaranth. *Plant Genome. 2*, 260–70.

Maundu, P., E. Achigan-Dako & Morimoto, Y. (2009), *Biodiversity of African vegetables*. In: C. M. Shackleton, M. W. Pasquini & A. W. Drescher (Eds.). *African Indigenous Vegetables in Urban Agriculture* (pp. 65–104), London: Earthscan.

Maxwell, K. & Johnson, G. N. (2000). Chlorophyll fluorescence –a practical guide. *Journal of Experimental Botany*, *51*(345), 659–668.

Mayes, S., Massawe, F. J., Alderson, P. G., Roberts, J. A., Azam-Ali, S. N. & Hermann, M. (2011). The potential for underutilized crops to improve security of food production. *J Exp Bot*, *63*, 1075-1079.

Meng Ben, W. & Zhang, Q. (2009). Issues in using the WinRHIZO system to determine physical characteristics of plant fine roots. *Acta Ecologica Sinica*, *29*(2), 136–138.

Mhike, X., Okori, P., Magorokosho, C., & Ndlela, T. (2012). Validation of the use of secondary traits and selection indices for drought tolerance in tropical maize (*Zea mays* L.), *6*(2), 96–102.

Mitchell, J. H., Siamhan, D., Wamala, M. H., Risimeri, J. B., Chinyamakobvu, E., Henderson, S. A., Fukai, S., (1998). The use of seedling leaf death score for evaluation of drought resistance of rice. *Field Crops Res*. 55: 129–139.

Mitra, J. (2001). Genetics and genetic improvement of drought resistance in crop plants. *Current Science*, *80*(6), 758–763.

Mittler. R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci*, *7*, 405–410.

Mogga, M., Sibiya, J., Shimelis, H., Lamo, J., & Yao, N. (2018). Diversity analysis and genome-wide association studies of grain shape and eating quality traits in rice (*Oryza sativa* L .) using DArT markers, *PLoS ONE*, 1–19.

Moreno, M. V., Nishinakamasu, V., Loray, M. A., Alvarez, D., Gieco, J., Vicario, A., Hopp, H. E., Heinz, R. A., Paniego, N., & Lia, V. V. (2013). Genetic characterization of sunflower breeding resources from Argetina: Assessing diversity in key open-pollinated and composite populations. *Plant Genetic Resources*, *11*, 238–249.

Morison, J. I. L., Baker, N. R., Mullineaux, P. M., & Davies, W. J. (2008). Improving water use in crop production. *Philos. Trans. R. Soc. B Biol. Sci*, *363*, 639–658.

Mosyakin, S. L. & Robertson, K. R. (1996). New infrageneric taxa and combinations in *Amaranthus* (Amaranthaceae). *Ann. Bot. Fenn.*, *33*, 275–282.

Mosyakin, S. L. and K. R. Robertson. 2003. Amaranthus. Pp. 410–435 in Flora of North America North of Mexico, vol. 4, ed. Flora of North America Editorial Committee. New York: Oxford University Press.

Mujica, A. & Jacobsen, S. E. (2003). The genetic resources of Andean grain amaranths (*Amaranthus caudatus* L., *A. cruentus* L. and *A. hypohondriacus* L.) in America. *Plant Genet Resour Newsl*, *133*, 41-44.

Murchie, E. H., & Lawson, T. (2013). Chlorophyll fluorescence analysis: A guide to good practice and understanding some new applications. *Journal of Experimental Botany*, *64*(13), 3983–3998.

Murray, M. J. (1940). The genetics of sex determination in the family Amaranthaceae. *Genetics*, *25*, 409–431.

Mustafa, M. A., Mayes, S., Massawe, F. (2019). *Crop diversification through a wider use of underutilised crops: A strategy to ensure food and nutrition security in the face of climate change*. In: A. Sarkar. S. R. Sensarma, G. W. vanLoon, G. W (Eds.). *Sustainable, solutions for food security: Combating climate change by adaptation* (pp. 125-149). Cham, Switzerland: Springer Nature.

Mwadzingeni, L., Shimelis, H., Tesfay, S., & Tsilo, T. J. (2016). Screening of bread wheat genotypes for drought tolerance using phenotypic and proline analyses. *Frontiers in Plant Science*, *7*, 1-12.

Nadeem, M. A., Habyarimana, E., Çiftçi, V., Nawaz, M. A., Karaköy, T., Comertpay, G., … Baloch, F. S. (2018). Characterization of genetic diversity in Turkish common bean gene pool using phenotypic and whole-genome DArTseq-generated silicoDArT marker information. *PLoS ONE*, *13*(10), 1–28.

Nakashima, T., Araki, T. & Ueno, O. (2011). Photoprotective function of betacyanin in leaves of *Amaranthus cruentus* L. under water stress. *Photosynthetica*, *49*(4), 497-506.

Narayanan, S., Mohan, A., Gill, K. S., & Prasad, P. V. V. (2014). Variability of Root Traits in Spring Wheat Germplasm, *PLoS ONE*, 9(6): 1-15.

Nasir, F., Islam, S., Munna, G., Ray, S. & Awal, R. (2016). Effectiveness of Amaranthus gangeticus in arsenic extraction from soil. *J. Sci. Res.*8, 71–79.

Naya, L., Ladrera, R., Ramos, J., González, E. M., Arrese-Igor, C., Minchin, F. R. & Becana, M. (2007). The response of carbon metabolism and antioxidant defenses of alfalfa nodules to drought stress and to the subsequent recovery of plants. *Plant Physiology*, *144*, 1104–1114.

Neill, S. O., & Gould, K. S. (2003). Anthocyanins in leaves: Light attenuators or antioxidants?. *Funct. Plant Biol*., *30*, 865-873.

Neill, S., Desikan, R., Hancock, J. (2002). Hydrogen peroxide signalling. *Curr Opin Plant Biol*, *5*, 388–395.

Nemeskéri, E. & Helyes, J. (2019). Physiological Responses of Selected Vegetable Crop Species to Water Stress, *Agronomy*, 1-19.

Neugart, S., Baldermann, S., Ngwene, B., Wesong, J. & Schreiner, M. (2017). Indigenous leafy vegetables of Eastern Africa: A source of extraordinary secondary plant metabolites. *Food Research International*, *100*, 411–422.

Njeru, E. M. (2013). Crop diversification: A potential strategy to mitigate food insecurity by smallholders in sub-Saharan Africa. *Journal of Agriculture, Food Systems, and Community Development*, *3*(4), 63-69.

O'Neil, P. M., Shanahan, J., & Schepers, J. S. (2006). Use of chlorophyll fluorescence assessments to differentiate corn hybrid response to variable water conditions. *Crop Sci.* 46, 681-687.

Oboh, B. (2007). Multivariate analysis of the diversity among some Nigerian accessions of *Amaranthus hybridus*. *International Journal of Plant Breeding and Genetics*, *1*, 89-94.

Oduwaye, O. A., Barnek, M., Čechov, J., & Raddov, J. (2014). Reliability and comparison of the polymorphism revealed in amaranth by amplified fragment length polymorphisms (AFLPs) and inters simple sequence repeats (ISSRs). *Journal of Plant Breeding and Crop Science*, *6*(4), 48–56.

Ogbonnaya, C. I., Nwalozie, M. C., Roy-Macauley, H., Annerose, D. J. M. (1998). Growth and water relations of Kenaf (*Hibiscus cannabinus* L.) under water deficit on a sandy soil, *Ind. Crops Prod.* 8, 65–76.

Ohashi, Y., Nakayama, N., Saneoka, H. & Fujita, K. (2006). Effects of drought stress on photosynthetic gas exchange, chlorophyll fluorescence and stem diameter of soybean plants. *Biol. Plant*, *50*, 138-141.

Ojuederie, O. B., Balogun, M. O., Fawole, I., Igwe, D. O. & Olowolafe, M. O. (2014). Assessment of the genetic diversity of African yam bean (*Sphenostylis stenocarpa* Hochst ex. A Rich Hams) accessions using amplified fragment length polymorphism (AFLP) markers. *African Journal of Biotechnology*, *13*(18), 1850-1858.

Oliveira, M. R. G., van Noordwijk, M., Gaze, S. R., Brouwer, G., Bona, S., Mosca, G., Hairiah, K. (2000). *Auger sampling, ingrowth cores and pinboard methods.* In: A. L. Smit, A. G. Bengough, C. Engels, M. van Noordwijk, S. Pellerin, S. C. van de Geijn (Eds). *Root methods, A handbook* (pp. 175-201). Berlin, Germany: Springer Verlag.

Omami, E. N. & Hammes, P. S. (2006). Interactive effects of salinity and water stress on growth, leaf water relations, and gas exchange in amaranth (*Amaranthus* spp.). *New Zealand Journal of Crop and Horticultural Science*, *34*, 33-44.

Oo, W. H., & Park, Y.J. (2013). Analysis of the genetic diversity and population structure of amaranth accessions from South America using 14 SSR markers. *Korean Journal of Crop Science*, *58*(4), 336–346.

Ors, S., & Suarez, D. L. (2017). Spinach biomass yield and physiological response to interactive salinity and water stress. *Agricultural Water Management*, *190*, 31–41.

Ort, D. R. & Baker, N. R. (2002). A photoprotective role for O_2 as an alternative electron sink in photosynthesis, *Curr Opin Plant Biol*, *5*, 193–198.

Ortiz, R., E., Tapia, N. R.,& Sanchez, A. M. (1998). Sampling strategy for a core collection of Peruvian quinoa germplasm. *Theor. Appl. Genet*. *96*, 475-483.

Osmont, K. S., Sibout, R., Hardtke, C. S. (2007). Hidden branches: developments in root system architecture. *Annual Review of Plant Biology*, *58*, 93–113.

O'toole, J. C., & Cruz, R. T. (1979). Leaf rolling and traspiration. *Plant Science Letters*, 16(1), 111-114.

Oyelana, O. A., & Ugborogho, R. E. (1992). Meiosis, pollen morphology and perianth stomata of some taxa of *Amaranthus* L. (Amaranthaceae), *Nigeria Journal of Botanical Taxonomy and Geobotany*, *103*, 5-6.

Pace, J., Lee, N., Naik, H. S., Ganapathysubramanian, B., & Lübberstedt, T. (2014). Analysis of Maize (*Zea mays* L.) seedling roots with the high-throughput image analysis tool ARIA (Automatic Root Image Analysis). *PloS One*, *9*(9), e108255.

Padulosi, S., Hodgkin, T., Williams, J. T., Haq, N. (2002). *Underutilised crops: Trends, challenges and opportunities in the 21st century*. In: J. M. M. Engels, Ramanatha Rao V., A. H. D. Brown, M. T., Jackson (Eds.), *Managing plant genetic diversity, Vol. 30* (pp. 323-338). Rome: IPGRI.

Pailles, Y., Ho, S., Pires, I. S., Tester, M., & Negrão, S. (2017). Genetic diversity and population structure of two tomato species from the Galapagos Islands, *8*(2), 1–11.

Pal, M., & Khoshoo., T. N. (1973). Evolution and improvement of cultivated amaranths. VI. Cytogenetic relationship in grain types. *Theor. Appl. Genet*, *43*, 343–350.

Pandey, R. M. &Singh, R. (2011). Genetic studies for biochemical and quantitative characters in grain amaranth (*Amaranthus hypochondriacus* L.). *POJ*, *3*(4), 129-134.

Pandey, R. M. (2009). Genetic divergence of parents and F2 segregation in grain Amaranths. *Cien. Inv. Agr*, *36*(1), 77-84.

Panthuwan, G., S. Fokai, M. Cooper, S. Rajatasereekul, O'Toole, J. C. (2002). Yield response of rice genotypes to different types of drought under rainfed lowlands. Part 1: Grain yield and yield components. *Field Crop Res*, *41*, 45-54.

Park, Y-J., Nishikawa, T., Matsushima, K., Minami, M., & Nemoto, K. (2014). A rapid and reliable PCR-restriction fragment length polymorphism (RFLP) marker for the identification of *Amaranthus cruentus* species, *Breeding Science*, *64*, 422-426.

Parra-Cota, F. I., Penã-Cabriales, J. J., de los Santos-Villalobos, S., Martínez-Gallardo, N. A. & Délano-Frier, J. P. (2014). *Burkholderia ambifaria* and *B. caribensis* promote growth and increase yield in grain amaranth (*Amaranthus cruentus* and *A. hypochondriacus*) by improving plant nitrogen uptake. *PLoS One*, *9*, 1-14.

Passioura, J. B. (2012). Phenotyping for drought tolerance in grain crops: When is it useful to breeders?, *Functional Plant Biology*, *39*, 851–859.

Pedersen, B., Kalinowski, L. S. & Eggum, B. O. (1987). The nutritive value of amaranth grain (*Amaranthus caudatus*), *Plants Foods Hum. Nutr*. 36:309-324.

Pedersen, B., Kalinowski. L. S., Eggum, B. O. (1987). The nutritive value of amaranth grain (*Amaranthus caudatus*). *Plants Foods Hum. Nutr*. 36, 309-324.

Peek, M.S., Russek-Cohen, E., Wait, D. A., Forseth, I. N. (2002). Physiological response curve analysis using nonlinear mixed models, *Oecologia*, *132*, 175-180.

Perez-Gonzalez, S., Dennis, F., Mondragon, C. & Byrne, D. (2001). *The importance of germplasm preservation and use for temperate zone fruit production in the tropics and subtropics*. In: *VI International symposium on temperate fruit growing in the tropics and subtropics* (pp. 25-32). Mexico: Acta Hort (ISHS).

Pérez-Pérez, J. G., Syvertsen, J. P., Botía, P., & García-Sánchez, F. (2007). Leaf water relations and net gas exchange responses of salinized *Carrizo citrange* seedlings during drought stress and recovery. *Ann. Bot*, *100*, 335–345.

Perrier, L., Pot, D., Rouan, L., Jaffuel, S., Roques, S., Soutiras, A., … Clément-Vidal, A. (2017). Plasticity of sorghum stem biomass accumulation in response to water deficit: a multiscale analysis from internode tissue to plant level. *Frontiers in Plant Science*, *8*(9), 1-14.

Perry, M. C., & McIntosh, M. S. (1991). Geographical patterns of variation in the USDA Soyabean germplasm collection: I. Morphological traits. *Crop Sci*., *31*, 1350-1355.

Pinheiro, C.; Passarinho, J.A.; Ricardo, C.P. (2004). Effect of drought and rewatering on the metabolism of Lupinus albus organs. *J. Plant Physiol*, 161, 1203–1210.

Pireivatlou, A. S., Masjedlou, B. D. & Aliyev, R. T. (2010). Evaluation of yield potential and stress adaptive trait in wheat genotypes under post anthesis drought stress conditions. *Afric J of Agric Res*, *5*, 2829-2836.

Poland, J. A., Brown, P. J., Sorrells, M. E., Jannink, J. L. (2012). Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. *PLoS ONE*, *7*, e32253.

Popa, G., Cornea, C. P., Ciuca, M., Barbeanu, N., Popa, O., Marin, D. I. (2010). Studies on genetic diversity in *Amaranthus* species using the RAPD markers. *Analele Universitatii din Oradea, Fascicula Biologie*, *17*(2), 280-285.

Porra, R. J., Thompson, W. A., & Kriedemann, P. E. (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochim. Biophys. Acta*, *975*, 384-394.

Purohit, S. S., & Sharma, S. (1985). Fluoride effects on germination, growth and georeaction of *Brassica campestris*. *Comp. Physiol. Ecol*., *10*, 254-258.

Rao, I. M. (2002). *Role of physiology in improving crop adaptation to abiotic stresses in the tropics: The case of common bean and tropical forages*. New York. Marcel Dekker.

Rastogi, A. & Shukla, S. (2013). Amaranth: A New Millennium Crop of Nutraceutical Values. *Critical Reviews in Food Science and Nutrition*, *53*, 109-125.

Ratnakumar, P., Vadez, V., Nigam, S. N., & Krishnamurthy, L. (2009). Assessment of transpiration efficiency in pea- nut (*Arachis hypogaea* L.) under drought using a lysimeter system. *Plant Biology*, *1*, 124–130.

Rauf, S. & Sadaqat H. A. (2008). Identification of physiological traits and genotypes combined to high achene yield in sunflower (*Helianthus annuus* L.) under contrasting water regimes, *Australian Journal of Crop Science*, *1*, 23–30.

Raut, V. R., Dodake, S. S., & Chimote, V.P. (2014). Evaluation of genetic diversity in grain amaranth (*Amaranthus hypochondriacus*) at molecular level using ISSR markers. *Indian J Agric Biochem*, 27(1), 60–65.

Ray, D. R., & Sinclair, T. R. (1998). The effect of pot size on growth and transpiration of maize and soybean during growth and transpiration of maize and soybean during water deficit stress. *Journal of Experimental Botany*, *49*, 1381–1386.

Razavi, F., Pollet, B., Steppe, K., Van Labeke, M. C. & Labeke, M. C. (2008). Chlorophyll fluorescence as a tool for evaluation of drought stress in strawberry. *Photosynthetica*, *46*, 631–633.

Riggins, C. W., Peng, Y. H., Stewart, C. N. & Tranel, P. J. (2010). Characterization of de novo transcriptome for waterhemp (*Amaranthus tuberculatus*) using GS-FLX 454 pyrosequencing and its application for studies of herbicide target-site genes. *Pest Manag Sci.*, *66*(10), 1042–52.

Ritchie, J. T. (1981). *Climate and soil water*. In: L. S. Murphy, L. F. Welch, & E. C. Doll (Eds.), *Moving up the yield curve: Advances and obstacles* (pp. 1–24). Madison, WI: ASA Special Publication Number, 39.

Robert, Y. N., Hiroe, K., & Yotaro, K. (2008). Antioxidant activity of various extracts and fractions of *Chenopodium* quinoa and *Amaranthus* spp. seeds: Analytical, nutritional and clinical methods. *Food Chem*, *106*, 760–766.

Rong-hua L., Pei-guo G., Baum M., Grando S., & Ceccarelli S. (2006). Evaluation of chlorophyll content and fluorescence parameters as indicators of drought tolerance in barley. *Agricultural Sciences in China, 5*, 751-757.

Rosales, M. A., Cuellar-Ortiz, S. M., Arrieta-Montiel, M. P., Acosta-Gallegos, J., Covarrubias, A. A. (2011). Physiological traits related to terminal drought resistance in common bean (*Phaseolus vulgaris* L.): drought resistance-related traits in common bean. *J. Sci. Food Agric. 93*, 324–331.

Rout, N. P., Shaw, B. P. (2001). Salt tolerance in aquatic macrophytes: possible involvement of the antioxidative enzymes. *Plant Sci, 160*, 415–423.

Sack, L., & Holbrook, N. M . (2006). Leaf hydraulics. *Annual Review of Plant Biology, 57*, 361–381.

Sack, L., Scoffoni, C., John, G. P., Pooter, H., Mason, C. M., Mendez-Alonzo, R., Donovan, L. A. (2013). How do leaf veins influence the worldwide leaf economic spectrum? Review and synthesis. *Journal of Experimental Botany, 64*, 4053–4080.

Sadeghian, S. Y., Fazli, H., Mohammadian, R., Taleghani, D. F., Mesbah, M. (2000). Genetic variation for drought stress in sugar beet. *J Sugar Beet Res, 37*:55–77.

Sadok, W. & Sinclair, T. R. (2011). Crops yield increase under water limited conditions: review of recent physiological advances for soybean genetic improvement. *Adv.Agron.* 113, 313–337.

Sage, R. F. (2001). C4 plants. *Encyclopedia of Biodiversity, 1*, 575–598.

Sage, R. F. (2004). The evolution of C4 photosynthesis. *New Phytologist, 161*, 341-370.

Sammour, R. H, Radwan, S. A., & Mira, M. (2012). Genetic diversity in genus *Amaranthus*: From morphology to genomic DNA, *6*(11).

Sanad, M. N. M. E., Smertenko, A. & Garland-Campbell, K. (2019). Differential dynamic changes of reduced trait model for analyzing the plastic response to drought phases : a case study in spring, *Frontiers in Plant Science, 10*(4), 1–19.

Sánchez, F. J., Manzanares, M., de Andres E. F., Tenorio J. L. & Ayerbe, L. (1998). Turgor maintenance, osmotic adjustment and soluble sugar and proline accumulation in 49 pea cultivars in response to water stress. *Field Crops Research, 59*, 225–235.

Sanders, G. J., & Arndt, S. K. (2012). *Osmotic Adjustment under drought conditions*. In: R. Aroca (Eds). *Plant responses to drought stress*. Berlin, Heidelberg: Springer.

Saraswati, P. A., Basavaraja, H., Kunnal, L. B., Mahajanashetti, S. B., & Bhat, A. R. S. (2011). Crop diversification in Karnataka: An economic analysis. *Agricultural Economics Research Review*, 24, 351-357.

Sareen, S., Tyagi, B. S., Sarial, A. K., Tiwari, V., & Sharma, I. (2014). Trait analysis, diversity, and genotype x environment interaction in some wheat landraces evaluated under drought and heat stress conditions. *Chil. J. Agric. Res, 74*, 135–142.

Sarker, U. & Oba, S. (2018). Drought stress effects on growth, ROS markers, compatible solutes, phenolics, flavonoids, and antioxidant activity in *Amaranthus tricolor*. *Appl Biochem Biotech*, *186* (4), 999-1016.

Sarker, U., Islam, M. T., Rabbani, M. G. & Oba, S. (2018). Phenotypic divergence in vegetable amaranth for total antioxidant capacity, antioxidant profile, dietary fiber, nutritional and agronomic traits. *Acta Agriculturae Scandinavica, 68*, 67-76.

Sarker, U., Islam, M. T., Rabbani, M. G., & Oba, S. (2014). Genotypic variability for nutrient, antioxidant, yield and yield contributing traits in vegetable amaranth. *Journal of Food, Agriculture and Environment, 12*(3–4), 168–174.

Sarker, U., Islam, M. T., Rabbani, M. G., & Oba, S. (2015). Variability, heritability and genetic association in vegetable amaranth (*Amaranthus tricolor* L.). Spanish Journal of Agricultural Research, 13(2), 1–8.

Sarker, U., Islam, M. T., Rabbani, M. G., & Oba, S. (2017). Genotypic diversity in vegetable amaranth for antioxidant, nutrient and agronomic traits. *Indian Journal of Genetics and Plant Breeding*, 77(1), 173–176.

Saucedo, A. L., Hernández-Domínguez, E. E., de Luna-Valdez, L. A., Guevara-García, A. A., Escobedo-Moratilla, A. & Bojorquéz-Velázquez, E. (2017). Insights on structure and function of a late embryogenesis abundant protein from *Amaranthus cruentus*: An intrinsically disordered protein involved in protection against desiccation, oxidant conditions, and osmotic stress. *Front. Plant Sci., 8*, 497.

Sauer, J. D. (1950). The grain amaranths: A survey of their history and classification. *An Mo. Bot. Gard, 37*, 561-632.

Sauer, J. D. (1957). Recent migration and evolution of the dioecious amaranths. *Evolution, 11*, 11–31.

Sauer, J. D. (1967). The grain amaranths and their relatives: A revised taxonomic and geographic survey. *Ann. Mo. Bot. Gdn, 54*, 103–137.

Sauer, J. D. (1972). The dioecious amaranths: a new species name and major range extensions. *Madroño, 21*, 425–434.

Sauer, J. D. (1976). *Grain amaranths*. In: N.W. Simmonds (Eds.), *Evolution of crop plants* (pp.4-7). London. Longman Group.

Sauer, J. D. (1993). *Amaranthaceae: Amaranth family*. In: *Historical geography of crop plants: A select roster* (pp.9–14). Boca Raton, FL. CRC Press.

Savita, S. G. (2006). Diversity of linseed germplasm for yield and yield components. PhD Thesis, University of Agricultural Sciences, Dharwad, pp 73.

Schippers, R. R. (2004). Le´gumes africains indige`nes: pre ´sentation des espe`ces cultive´es. Margraf Publishers, Wuerzburg.

Schlemmer, M .R., Francis, D. D., Shanahan, J. F., & Schepers, J. S. (2005). Remotely measuring chlorophyll content in corn leaves with differing nitrogen levels and relative water content. *Agronomy Journal*, *97*, 106– 112.

Schnetzler, K. A., Breen, W. M. (1994). *Food Uses and Amaranth Product Research: A Comprehensive Review*. In: O., Paredes López (Eds.). *Amaranth. Biology, Chemistry and Technology* (pp. 155-184). Boca Raton, FL: CRC Press.

Schoenlechner, R., Siebenhandl, S., & Berghofer, D. E. (2008). *Pseudocereals*. In: E. Arendt, & F. Dal Bello (Eds.). *Gluten-free cereal products and beverages* (pp. 149–176). London: Academic Press.

Segura, V., Vilhjálmsson, B. J, Platt, A., Korte, A., Seren, Ű., Long, Q, et al. (2012). An efficient multi-locus mixed-model approach for genome-wide association studies in structured populations. *Nature genetics*, *44*(7), 825–30.

Selvan, R. K., Yassin, M. G., & Govindarasu, R. (2013). Studies on genetic paramteres in grain *Amaranthus* (*Amaranthus hypochondriacus* L.) as influenced by plant densities. *J. Plant Breed. Genet, 1*, 34-42.

Shabala, S. & Shabala, L. (2011). Ion transport and osmotic adjustment in plants and bacteria. *BioMolecular Concepts, 2*, 407–419.

Shabala, S., White, R. G., Djordjevic, M. A., Ruan, Y. L., Mathesius, U. (2016). Root-to-shoot signalling: Integration of diverse molecules, pathways and functions. *Funct. Plant Biol.*, 43, 87–104.

Shannon, C. E., & Weaver, W. (1949). *The mathematical theory of communication*. Urbana, IL. University of Illionis Press.

Shao, L., Chen, X., Chen, Y., Sun, B., Chow, W., & Peng, C. (2013). Differential responses of photosystem II activity to photooxidation in red and green tissues of *Amaranthus tricolor* leaves. *Pak. J. Bot*., *45*(6), 1905-1912.

Shiferaw, B., Prasanna, B. M., Hellin, J., & Bänziger, M. (2011). Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. *Food Security*, *3*(3), 307–327.

Shinwari, Z. K., Rehaman, H., & Rabbani, A. (2014). Morphological traits based genetic diversity in safflower (*Carthamus tinctorius* L.), *Pak. J. Bot*, *46*(4), 1389-1395.

Shukla, A., Srivastava, N., Suneja, P., Yadav, S. K., Hussain, Z., Rana, J. C, Yadav, S. (2018). Untapped amaranth (*Amaranthus* spp.) genetic diversity with potential for nutritional enhancement. *Genet Resour Crop Evol*, *65*, 243-253.

Shukla, S., Bhargava, A., Chatterjee, A. & Singh, S. P. (2006). Genotypic variability in vegetative amaranth (*Amaranthus tricolor* L.) for foliage yield and its contributing traits over successive cuttings and years. *Euphytica*, *151*, 103-110.

Shukla, S., Bhargava, A., Chatterjee, A., Pandey, A. C. & Mishra, B. K. (2010). Diversity in phenotypic and nutritional traits in vegetable amaranth (*Amaranthus tricolor*), a nutritionally underutilised crop. *J Sci Food Agric*, 90, 139-144.

Siddique, M. A., Khalequzzaman, M., Islam, M. Z. Baktiar, M. H. K., & Ahmed, M. S. (2016). Genetic diversity assessment of quantitative traits in landraces of *Aus* rice. *Bangladesh J. PI. Breed. Genet*, *29*(2), 17-24.

Silva, M. A., Jifon, J. L., Sharma, V., Da Silva, J. A. G, Caputo, M. M, Damaj, M. B, et al. (2011). Use of physiological parameters in screening drought tolerance in sugarcane genotypes. *Sugar Tech*., *13*, 178-184.

Simova-Stoilova, L., Vassileva, V., & Feller, U. (2016). Selection and Breeding of Suitable Crop Genotypes for Drought and Heat Periods in a Changing Climate: Which Morphological and Physiological Properties Should Be Considered? *Agriculture*, *6(*2), 26.

Sinclair, T. R., Tanner, C. B., & Bennett, J. M. (1984). Water use efficiency in crop production. *Bioscience*, 34, 40–60.

Singh, B., Reddy, K. R., Redoña, E. D., & Walker, T. (2017). Screening of rice cultivars for morpho-physiological responses to early-season soil moisture stress. *Rice Science*, *24*(6), 322–335.

Singh, S. S., & Singh, S. P. (2002). Varietal performance and foliage yield in vegetable amaranth. *South Indian Horticulture*, *50*(1/3), 241-244.

Siwar, C., F. Ahmed, A. Begum. (2013). Climate change, agriculture and food security issues: Malaysian perspective, *Journal of Food, Agriculture & Environment*, *11*(2), 1118-1123.

Slabbert, M. M. & den Heever, E. V. (2007). Selection of traditional crops for improved drought tolerance in leafy amaranth: moving towards sustainable food supply. *Acta Hortic*, *752*, 281-286.

Slabbert, M. M., de Ronde K., Caetano, T., Spreeth, M., van den Heever, E. (2004). *Development and evaluation of mutant germplasm of Amaranthus*. In: *Genetic improvement of under-utilized and neglected crops in low income food deficit countries through irradiation and related techniques* (pp. 13–23). Vienna: International Atomic Energy Agency: IAEA-TECDOC-1426.

Slabbert, R. & Krüger, G. H. J. (2011). Assessment of changes in photosystem II structure and function as affected by water deficit in *Amaranthus hypochondriacus* L. and *Amaranthus hybridus* L. *Plant physiology and biochemistry*. *49*, 978-998.

Slabbert, R. & Krüger, G. H. J. (2014). Antioxidant enzyme activity, proline accumulation, leaf area and cell membrane stability in water stressed Amaranthus leaves. *South African Journal of Botany*, *95*, 123-128.

Sneath, P. H. A. & Sokal, R. R. (1973). Numerical Taxonomy. WH Freeman. San Francisco.

Sogbohossou, E. O. D. & Achigan-Dako, E. G. (2014). Phenetic differentiation and usetype delimitation in *Amaranthus* spp. from worldwide origins. *Sci. Hortic.(Amst)*, *178*, 31–42.

Sogbohossou, E. O. D., Achigan-Dako, E. G., Maundu, P., Solberg, S., Deguenon, E. M. S., Mumm, R. H., Hale, L., Deynze & Schranz, M. E. (2018). A roadmap for breeding orphan leafy vegetable species: A case study of *Gynandropsis gynandra* (Cleomaceae). *Horticulture Research*, *5*(1), 1–15.

Soltys-kalina, D., & Marczewski, W. (2016). The effect of drought stress on the leaf relative water content and tuber yield of a half-sib family of 'Katahdin'-derived potato cultivars, *Breeding Science*, (66): 328–331.

Srivastava, R. (2011), Nutritional quality of some cultivated and wild species of *Amaranthus* L. *International Journal Pharmaceutical Sciences and Research*, *17*, 3152-3156.

Štefúnovà, V., Bežo, M., Labajovà, M., & Senkovà, S. (2014). Genetic analysis of three amaranth species using issr markers. *Emirates Journal of Food and Agriculture*, *26*(1), 35–43.

Stetter, M. G., & Schmid, K. J. (2017b). Analysis of phylogenetic relationships and genome size evolution of the Amaranthus genus using GBS indicates the ancestors of an ancient crop. *Molecular Phylogenetics and Evolution*, *109*, 80–92.

Stetter, M. G., Müller, T., & Schmid, K. J. (2017a). Genomic and phenotypic evidence for an incomplete domestication of South American grain amaranth (*Amaranthus caudatus*). *Molecular Ecology*, *26*(3), 871–886.

Stetter, M. G., Zeitler, L., Steinhaus, A., Kroener, K., Biljecki, M., & Schmid, K. J. (2016). Crossing methods and cultivation conditions for rapid production of segregating populations in three grain amaranth species. *Frontiers in Plant Science*, *7*, 1-15.

Strack, D., Vogt, T. & Schliemann, W. (2003). Recent advances in betalain research. *Phytochemistry*, *62*(3), 247-269.

Sunil, M., Hariharan, A. K., Nayak, S., Gupta, S., Nambisan, S. R., Gupta, R. P., Panda, B., Choudhary, B., Srinivasan, S. (2014). The draft genome and transcriptome of *Amaranthus hypochondriacus*: A C4 dicot producing high-lysine edible pseudo-cereal. *DNA Res*. *21*, 585–602.

Suresh, S., Chung, J. W., Cho, G. T., Sung, J. S., Park, J. H., Gwag, J. G., & Baek, H. J. (2014). Analysis of molecular genetic diversity and population structure in *Amaranthus* germplasm using SSR markers. *Plant Biosystems*, *148*(4), 635–644.

Tabatabaei, I., Pazouki, L., Bihamta, M. R., Mansoori, S., Javaran, M. J., Niinemets, Ü. (2011). Genetic variation among Iranian sesame (*Sesamum indicum* L.) accessions vis-à-vis exotic genotypes on the basis of morpho-physiological traits and RAPD markers. *Austr. J. Crop Sci*, *5*, 1396–1407.

Talebi, R., Fayaz, F., & Naji, A. M. (2009). Effective selection criteria for assessing drought stress tolerance in durum wheat (*Triticum durum* Desf.). *General and applied plant physiology*. 35, 64–74.

Taub, D. R. & Lerdau, M. T. (2000). Relationship between leaf nitrogen and photosynthetic rate for three NAD-ME and three NADP-ME C4 grasses. *Am. J. Bot*. *87*, 412–417.

Teutonico, R. A, & Knorr, D. (1985). Amaranth: composition properties and applications of a rediscovered food crop. *Food Technology*. *39*, 44-00.

Thapa, R., & Blair, M. (2018). Morphological assessment of cultivated and wild amaranth species diversity. *Agronomy*, *8*(11), 272.

Thevar, P. A., Kirkham, M. B., Aiken, R. M., Kofoid, K. D., & Xin, Z. (2010). *Optimizing water use with high transpiration efficiency plants* (pp. 29–31). Brisbane, Australia: World Congress of Soil Science, Soil Solutions for a Changing World 6.

Tinker, N. A., Kilian, A., Wight, C. P., Heller-Uszynska, K., Wenzl, P., Rines, H. W., Bjørnstad, A., Howarth, C. J., Jannink, J. L.,… Langdon, T. (2009). New DArT markers for oat provide enhanced map coverage and global germplasm characterization. *BMC Genomics*, *10*, 39.

Tipple, B. J., & Pagani, M. (2007). The early origins of terrestrial C4 photosynthesis. *Annual Review of Earth and Planetary Sciences*, *35*, 435–461.

Toscano, S., Farieri, E., Ferrante, A., & Romano, D. (2016). Physiological and Biochemical Responses in Two Ornamental Shrubs to Drought Stress. *Frontiers in Plant Science*, *6*(7), 1-12.

Transue, K., Fairbanks, D. J., Robison, L. R, Andersen, W. R. (1994). Species Identification by RAPD Analysis of Grain Amaranth Genetic Resources. *Crop Science*, *34*, 21-35.

Trucco, F., Tranel, P. J. (2011). *Amaranthus*. In: Kole C (Eds.). *Wild crop relatives: Genomic and breeding resources vegetables, Vol XXVI* (pp. 11–21). Heidelberg, Berlin: Springer-Verlag.

Tsutsumi, N., Tohya, M., Nakashima, T. & Ueno, U. (2017). Variations in structural, biochemical, and physiological traits of photosynthesis and resource use efficiency in *Amaranthus* species (NAD-ME-type C4). *Plant Production Science*, *20*(3), 300-312.

Tuberosa, R. (2012). Phenotyping for drought tolerance of crops in the genomics era. *Frontiers in Physiology*, *3*(9), 1–26.

Tuberosa, R., Turner, N. C., & Cakir, M. (2014). Preface (Two decades of inter-drought conferences: Are we bridging the genotype-to-phenotype gap?), *Journal of Experimental Botany*, *65*, 6137–6139.

Turner, N.C., Blum, A., Cakir, M., Steduto, P., Tuberosa, R. & Young, N. (2014). Strategies to increase yield and yield stability of crops under drought – are we making progress? *Functional Plant Biology*, *41*, 1199–1206.

Ueno, O. (2001). Ultrastructural localization of photosynthetic and photorespiratory enzymes in epidermal, mesophyll, bundle sheath, and vascular bundle cells of the C4 dicot *Amaranthus viridis*. *J. Exp. Bot*., *52*, 1003-1013.

Umar, M., & Siddiqui, Z. S. (2018). Physiological performance of sunflower genotypes under combined salt and drought stress environment. *Acta Botanica Croatica*, *77*(1), 36–44.

UN DESA. 2011. World Urbanization Prospects: The 2011 Revision. New York, United Nations.

Upadhyaya, H. D. (2003). Phenotypic diversity in groundnut (*Arachis hypogaea* L.) core collection assessed by morphological and agronomic evaluations, *Genetic Resources and Crop Evolutions*, *50*, 539-550.

Uzildaya, B., Turkana, I., Sekmena, A.H., Ozgura, R. & Karakayab, H. C. (2012). Comparison of ROS formation and antioxidant enzymes in Cleome gynandra (C4) and *Cleome spinosa* (C3) under drought stress. *Plant Science*, *182*, 59–70.

Vadez, V. (2014). Root hydraulics: The forgotten side of roots in drought adaptation. *Field Crops Research*, *165*, 15–24.

Valdes-Rodriguez, S., Segura-Nieto, M., Chagolla-Lopez, A., Vargas-Cortina, A.V.Y., Martinez-Gallardo, N. & Blanco-Labra., A. (1993). Purification, characterization, and complete amino acid sequence of a trypsin inhibitor from amaranth (*Amaranthus hypochondriacus*) seeds. *Plant Physiology*, *103*, 1407-1412.

Valdiani, A., Talei, D., Javanmard, A., Tan, S. G., Kadir, M. A., Maziah, M., Morpho-molecular analysis as a prognostic model for repulsive feedback of "*Andrographis paniculata*" to allogamy (2014). *Gene*, *542*, 156–167.

Valkoun, J. J. (2001). Wheat pre-breeding using wild progenitors. *Euphytica*, *119*, 17–23.

van Hintum, Th. J. L. (1994). *Hierarchical approaches to the analysis of genetic diversity in crop plants*. In: T. Hodgkin, A H. D. Brown, Th. J. L. van Hintum, & E. A. V. Morales (Eds). *Core collections of plant genetic resources* (pp. 23-34). UK: John Wiley and Sons.

Varalakshmi, B. (2004). Characterization and preliminary evaluation of vegetable amaranth (*Amaranthus* spp.) germplasm. *Plant Genet Res Newsl*., *137*, 55–57.

Vargas-Ortiz, E., Espitia-Rangel, E., Tiessen, A., & Délano-Frier, J. P. (2013). Grain amaranths are defoliation tolerant crop species capable of utilizing stem and root carbohydrate reserves to sustain vegetative and reproductive growth after leaf loss. *PLoS ONE*, *8*(7), 1–13.

Veasey, E. A., Borges, A., Rosa, M. S., Queiroz-Silva, J. R., Bressan, E. A. & Peroni, N. (2008). Genetic diversity in Brazilian sweet potato (*Ipomoea batatas* (L.) Lam., Solanales, Convolvulaceae) landraces assessed with microsatellite markers. *Genetics and Molecular Biology*, *31*(3), 725-733.

Venskutonis, P. R., & Kraujalis, P. (2013). Nutritional components of amaranth seeds and vegetables: A review on composition, properties, and uses. *Comprehensive Reviews in Food Science and Food Safety*, *12*(4), 381–412.

Verma, V., Foullces, M. J., Worland, A. J., Sylvester-Bradley, R., Caligari, P. D. S., Snape J. W. (2004). Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought-stressed environments. *Euphytica*, *135*, 255-263.

Vermeulen (a), S. J., Campbell, B. M. & Ingram, J. S. I. (2012). Climate change and food system. *Annual Review of Environment and Resources*, 37: 195-222.

Vermeulen (b), S. J., Aggrawal, P. K., Ainslie, A., Angelone, C., Campbell, B. M., Challinor, A. A., Hansen, J. W. Ingram, J. S. I., Jarvis, A., & Kristjanson, P. (2012). Options for support to agriculture and food security under climate change. *Environ. Sci. Policy*, *15*, 136–144.

von Caemmerer, S. & Furbank, R. T. (2016). Strategies for improving C4 photosynthesis. *Current Opinion in Plant Biology*, *31*, 125–134.

VSN International (2015). Genstat for Windows 18th Edition. VSN International, Hemel Hempstead, UK. Web page: Genstat.co.uk.

Wang, K., Zhang, L. X., Guo, M., Lv, L. X., Zhao, Y. G., Zhang, L. S. Li, B. Z., Han, M. Y. & Alva, A. (2013). Influence of salt stress on growth and antioxidant responses of two malus species at callus and plantlet stages. *Pak. J. Bot.*, *45*(2), 375-381.

Wang, M. Ben, & Zhang, Q. (2009). Issues in using the WinRHIZO system to determine physical characteristics of plant fine roots. *Acta Ecologica Sinica*, *29*(2), 136–138.

Wang, X. Q., & Park, Y. J. (2013). Comparison of genetic diversity among amaranth accessions from South and Southeast Asia using SSR markers. *Korean Journal of Medicinal Crop Science*, *21*(3), 220–228.

Wang, X., Wang, L., & Shangguan, Z. (2016). Leaf gas exchange and fluorescence of two winter wheat varieties in response to drought stress and nitrogen supply, *PLoS ONE*, *11*(1), 1–15.

Wang, Y. Bräutigam, A., Weber, A. P. & Zhu, X. G. (2014). Three distinct biochemical subtypes of C4 photosynthesis? A modelling analysis. *Journal of experimental botany*, *65*(13), 3567–3578.

Waselkov, K. E., Boleda, A. S., Olsen, K. M. (2018). A phylogeny of the genus *Amaranthus* (Amaranthaceae) based on several low-copy nuclear loci and chloroplast regions. *Systematic Botany*, *43*(2), 439-458.

Wassom, J. J. & Tranel, P. J. (2005). Amplified fragment length polymorphism-based genetic relationships among weedy Amaranthus species. *Journal of Heredity*, *96*, 410–416.

Wasson, A. P., Richards, R. A., Chatrath, R., Misra, S .C., Prasad, S.V.S., Rebetzke, G. J., Kirkegaard, J. A., Christopher J. & Watt, M. (2012). Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. *J. Exp.Bot. 63*, 3485–3498.

Weinstein, L. H. (1977). Fluoride and plant life. *J. Occup. Med*., 19, 49-78.

Whitehead, W. F., Carter, J. & Sigh, B. P. (2002). Effect of planting date on vegetable amaranth leaf yield, plant height and gas exchange. *HortScience*, *37*(5), 773-777.

Wilhite, D. A. 2005. *Drought and Water Crises: Science, Technology and management Issues*. Florida, USA. CRC Press.

Williams, M. H., Rosenqvist, E., Buchhave, M. (1999). Response of potted miniature roses (*Rosaxhybrida*) to reduced water availability during production. *J Hortic Sci Biotechnol*, *74*, 301–308.

Wu, H. X., & Corke, H. (1999). Genetic diversity in physical properties of starch from a world collection of *Amaranthus*. *Cereal Chem*. 76: 877–883.

Wu, H. X., Sun, M., Yue, S. X., Sun, H. L., Cai, Y., Huang, R. H., Brenner, D., & Corke, H. (2000).Field evaluation of an *Amaranthus* genetic resource collection in China. *Gene Resou Crop Evol*, *47*, 43–53.

Wu, X., & Blair, M. W. (2017). Diversity in grain amaranths and relatives distinguished by genotyping by sequencing (GBS). *Frontiers in Plant Science*, *8*, 1–12.

Xiurong, Z., Yingzhong, Z., Yong, C., Xiangyun, F., Qingyuan, G., Mingde, Z., & Hodgkin, T. (2000). Establishment of sesame germplasm core collection in China. *Genetic Resources and Crop Evolution*, *47*(3), 273–279.

Xu, F. & Sun, M. (2001). Comparative analysis of phylogenetic relationships of grain amaranths and their wild relatives (*Amaranthus*; Amaranthaceae) using internal transcribed spacer, amplified fragment length polymorphism, and double-primer fluorescent inter simple sequence repeat markers. *Molecular Phylogenetics and Evolution*, *21*, 372-3.

Yan, H., Gang, L. Z., Zhao, C. Y., Guo, W. Y. (2000). Effects of exogenous proline on the physiology of soybean plantlets regenerated from embryos in vitro and on the ultrastructure of their mitochondria under NaCl stress. *Soybean Science*, *19*, 314–319.

Yin, C., Berninger, F., Li, C. (2006). Photosynthetic responses of *Populus przewalski* subjected to drought stress. *Photosynthetica*, *44*(1):62–8.

Zadehbagheri, M., Azarpanah, A., & Javanmardi, S. (2014). Proline metabolites transport an efficient approach in corn yield improvement as response to drought conditions. *International Journal of Farming and Allied Sciences*, *3*(5), 453-461.

Zafar, N., Aziz, S., & Masood, S. (2006). Phenotypic divergence for agro-morphological traits among landrace genotypes of rice (*Oryza sativa* L.) from Pakistan. *Int. J. Agric. Biol*., *6*, 335-339.

Zdravkovic, J., Jovanovic, Z., Djordjevic, M., Girek, Z., Zdravkovic, M., & Stikic, R. (2013). Application of stress susceptibility index for drought tolerance screening of tomato populations. *Genetika*, *45*, 679–689.

Zegaoui, Z., Planchais, S., Cabassa, C., Djebbar, R., Abrous, O., & Carol, P. (2018). Variation in relative water content, proline accumulation and stress gene expression in two cowpea landraces under drought. *Journal of Plant Physiology*, *218*(6), 26–34.

Zeven, A. C., Waninge, J., Van Hintum, T., & Singh, S. P. (1999). Phenotypic variation in a core collection of common bean (*Phaseolus vulgaris* L.) in the Netherlands. *Euphytica*, *109*, 93-106.

Zhang, J., Jia, W., Yang, J., & Ismail, A. M. (2006). Role of ABA in integrating plant responses to drought and salt stresses. *Field Crops Research*, *97*, 111–119.

Zhang, L. J., Peng, T. T., Chen, X. H., Zhao, S. P., Zhang, S. D., Liu H. L., Dong, L., F., & Yu, S. X. (2014). Effect of drought stress on lipid peroxidation and proline content in cotton roots. *The J. of Animal and Plant Sci*., *24*(6), 1729-1736.

Zhang, M., Kimatu, J. N., Xu, K. & Liu, B. (2010). DNA cytosine methylation in plant development. *J Genet Genomics*, *37*, 1–12.

Zhou, H., Akçay, E., & Brent, R. H. (2019). Estimating C4 photosynthesis parameters by fitting intensive *A/Ci* curves. *Photosynthesis Research*, *41*(2), 181-194.

Zimisuhara, B., Valdiani, A., Shaharuddin, N. A., Qamaruzzaman, F., & Maziah, M. (2015). Structure and principal components analyses reveal an intervarietal fusion in Malaysian mistletoe fig (*Ficus deltoidea* Jack) populations. *International Journal of Molecular Sciences*, *16*(7), 14369–14394.

Zivcak, M., Brestic, M., & Sytar, O. (2016). *Osmotic adjustment and plant adaptation to drought stres*s. In: M. Hossain, S. Wani, S. Bhattacharjee, D. Burritt, L. S.Tran (Eds). *Drought Stress Tolerance in Plants* (Vol 1). Basel, New Zealand: Springer, Cham.

Zulini, L., Rubinigg, M., Zorer, R. & Bertamini, M. (2007). Effects of drought stress on chlorophyll fluorescence and photosynthetic pigments in grapevine leaves (*Vitis vinifera* cv. 'White Riesling'). *Acta Hortic*, *754*, 289–294.

APPENDIX

CHAPTER 3: CHARACTERIZATION OF AMARANTH GERMPLASM AND DEVELOPMENT OF A CORE SET USING QUALITATIVE DATA DERIVED FROM GENEBANK MORPHOLOGICAL DATABASE

Appendix 3.1: Lists of amaranth germplasms (a) from AVRDC Genebank and (b) USDA Genebank, and (c) commercial varieties.

Appendix 3.1a: An entire collection of 578 amaranth accessions conserved in The World Vegetable Center Genebank, Taiwan (AVRDC). The quality of passport data: (✓) is complete, (X) is not complete and (N/A) is not available.

No.	Label	Species	ID	Origin country	Quality of passport data
1	ATR 1	Atropurpureus	VI044435	Indonesia	X
2	BLI 1	Blitoides	VI036227	Hungary	✓
3	BLT 1	Blitum	VI044404	India	✓
4	BLT 2	Blitum	VI044405	India	✓
5	BLT 3	Blitum	VI044423	India	✓
6	BLT 4	Blitum	VI055068	Malaysia	✓
7	BLT 5	Blitum	VI055122	Malaysia	✓
8	BLT 6	Blitum	VI055123	Malaysia	✓
9	BLT 7	Blitum	VI049036	Thailand	✓
10	BLT 8	Blitum	VI050997	Kenya	✓
11	BLT 9	Blitum	VI056127	Cambodia	✓
12	BLT 29	Blitum	VI044447	Korea	X
13	BLT 10	Blitum	VI046137	Laos	✓
14	BLT 11	Blitum	VI055922	Laos	✓
15	BLT 12	Blitum	VI055755	Laos	✓
16	BLT 13	Blitum	VI055968	Laos	✓
17	BLT 15	Blitum	VI055121	Malaysia	✓
18	BLT 16	Blitum	VI044384	India	✓
19	BLT 17	Blitum	VI044431	Thailand	N/A
20	BLT 18	Blitum	VI058952	Unknown	N/A
21	BLT 19	Blitum	VI058953	Unknown	N/A
22	BLT 20	Blitum	VI058954	Unknown	N/A
23	BLT 21	Blitum	VI059038	Unknown	N/A
24	BLT 22	Blitum	VI059039	Unknown	N/A
25	BLT 23	Blitum	VI059041	Unknown	N/A
26	BLT 24	Blitum	VI059042	Unknown	N/A
27	BLT 25	Blitum	VI061495	Malawi	N/A
28	BLT 26	Blitum	VI061507	Malawi	N/A
29	BLT 27	Blitum	VI061508	Malawi	N/A
30	BLT 28	Blitum	VI061493	Madagascar	N/A

No.	Label	Species	ID	Origin country	Quality of passport data
31	BLT 14	Blitum	VI055988	Laos	✓
32	CRU 1	Cruentus	VI036230	Austria	✓
33	CRU 2	Cruentus	VI036231	Austria	✓
34	CRU 3	Cruentus	VI044457	Zimbabwe	✓
35	CRU 5	Cruentus	VI050455	Unknown	✓
36	CRU 6	Cruentus	VI059414	Tanzania	✓
37	CRU 7	Cruentus	VI033487	Malaysia	✓
38	CRU 8	Cruentus	VI044437-A	Malaysia	✓
39	CRU 9	Cruentus	VI044440-A	Nigeria	✓
40	CRU 10	Cruentus	VI036228	Hungary	✓
41	CRU 11	Cruentus	VI050473	Sudan	✓
42	CRU 12	Cruentus	VI044366	Ethiopia	✓
43	CRU 13	Cruentus	VI044376	Ghana	✓
44	CRU 14	Cruentus	VI051006	Zambia	✓
45	CRU 15	Cruentus	VI044449	Guatemala	✓
46	CRU 16	Cruentus	VI044453	Mexico	✓
47	CRU 17	Cruentus	VI060290	Zimbabwe	N/A
48	CRU 19	Cruentus	VI058950	Unknown	N/A
49	CRU 20	Cruentus	VI058951	Unknown	N/A
50	CRU 21	Cruentus	VI058955	Unknown	N/A
51	CRU 22	Cruentus	VI061503	Unknown	N/A
52	CRU 23	Cruentus	VI061505	Unknown	N/A
53	CRU 24	Cruentus	VI061494-A	Madagascar	N/A
54	CRU 25	Cruentus	VI061494-B	Madagascar	N/A
55	CRU 26	Cruentus	VI062429	Madagascar	N/A
56	CRU 27	Cruentus	VI062430	Madagascar	N/A
57	CRU 28	Cruentus	VI062431	Madagascar	N/A
58	CRU 29	Cruentus	VI041466	Philippines	N/A
59	CRU 30	Cruentus	VI054576	Philippines	N/A
60	CRU 31	Cruentus	VI054577	Philippines	N/A

Appendix 3.1a: (Continued)

No.	Label	Species	ID	Origin country	Quality of passport data
61	CRU 32	Cruentus	VI054583	Philippines	N/A
62	CRU 33	Cruentus	VI054579	Philippines	N/A
63	CRU 34	Cruentus	VI054580	Philippines	N/A
64	CRU 35	Cruentus	VI047484	Tanzania	N/A
65	CRU 36	Cruentus	VI060289	Tanzania	N/A
66	CRU 37	Cruentus	VI060470	Tanzania	N/A
67	CRU 38	Cruentus	VI063764	Nigeria	N/A
68	CRU 39	Cruentus	VI045973	Viet Nam	N/A
69	CRU 40	Cruentus	VI061504	Cameroon	N/A
70	CRU 41	Cruentus	VI061518-B	Sudan	N/A
71	CRU 42	Cruentus	VI042962	Indonesia	N/A
72	CRU 43	Cruentus	VI044456	Zimbabwe	X
73	CRU 4	Cruentus	VI033460	USA	✓
74	CRU 18	Cruentus	VI061487	USA	N/A
75	CRU 44	Cruentus	VI044469	USA	X
76	CRU 45	Cruentus	VI048583	USA	X
77	DUB 20	Dubius	VI046143	Laos	✓
78	DUB 21	Dubius	VI054800	Laos	✓
79	DUB 1	Dubius	VI047537	Viet Nam	✓
80	DUB 2	Dubius	VI047576	Viet Nam	✓
81	DUB 3	Dubius	VI047485	Tanzania	✓
82	DUB 4	Dubius	VI050448	Tanzania	✓
83	DUB 5	Dubius	VI050464	Tanzania	✓
84	DUB 6	Dubius	VI047748	Bangladesh	✓
85	DUB 7	Dubius	VI047799	Bangladesh	✓
86	DUB 8	Dubius	VI047911	Bangladesh	✓
87	DUB 9	Dubius	VI048009	Bangladesh	✓
88	DUB 10	Dubius	VI048676	Thailand	✓
89	DUB 11	Dubius	VI048724	Thailand	✓
90	DUB 12	Dubius	VI048926	Thailand	✓
91	DUB 13	Dubius	VI048976	Thailand	✓
92	DUB 14	Dubius	VI048985	Thailand	✓
93	DUB 15	Dubius	VI049094	Thailand	✓
94	DUB 16	Dubius	VI049896	Thailand	✓
95	DUB 17	Dubius	VI057074	Cambodia	✓
96	DUB 18	Dubius	VI057160	Cambodia	✓
97	DUB 19	Dubius	VI044377	Surinam	✓
98	DUB 22	Dubius	VI044439	Nigeria	✓

No.	Label	Species	ID	Origin country	Quality of passport data
99	DUB 23	Dubius	VI050158	Taiwan	✓
100	DUB 24	Dubius	VI051002	Kenya	✓
101	DUB 26	Dubius	VI050458	Unknown	✓
102	DUB 27	Dubius	VI050459	Unknown	✓
103	DUB 28	Dubius	VI050462	Unknown	✓
104	DUB 29	Dubius	VI050463	Unknown	✓
105	DUB 30	Dubius	VI050466	Unknown	✓
106	DUB 31	Dubius	VI058949	Unknown	✓
107	DUB 32	Dubius	VI046050	Viet Nam	N/A
108	DUB 33	Dubius	VI046051	Viet Nam	N/A
109	DUB 34	Dubius	VI046622	Viet Nam	N/A
110	DUB 35	Dubius	VI046240	Viet Nam	N/A
111	DUB 36	Dubius	VI050453	Tanzania	N/A
112	DUB 37	Dubius	VI061492	Tanzania	N/A
113	DUB 38	Dubius	VI061490-A	Uganda	N/A
114	DUB 39	Dubius	VI061490-B	Uganda	N/A
115	DUB 40	Dubius	VI061514	Uganda	N/A
116	DUB 41	Dubius	VI061515	Uganda	N/A
117	DUB 42	Dubius	VI042963	Indonesia	N/A
118	DUB 43	Dubius	VI042981	Indonesia	N/A
119	DUB 44	Dubius	VI061491	Rwanda	N/A
120	DUB 45	Dubius	VI061497	Malawi	N/A
121	DUB 46	Dubius	VI050461	Unknown	N/A
122	DUB 47	Dubius	VI058956	Unknown	N/A
123	DUB 48	Dubius	VI058957	Unknown	N/A
124	DUB 49	Dubius	VI058958	Unknown	N/A
125	DUB 50	Dubius	VI050612	Viet Nam	X
126	DUB 51	Dubius	VI050616	Viet Nam	X
127	DUB 52	Dubius	VI050617	Viet Nam	X
128	DUB 53	Dubius	VI050451	Tanzania	X
129	DUB 54	Dubius	VI050460	Tanzania	X
130	DUB 25	Dubius	VI048582	USA	✓
131	GRA 1	Gracilis	VI056002	Cambodia	✓
132	GRA 2	Gracilis	VI056015	Cambodia	✓
133	GRA 3	Gracilis	VI056019	Cambodia	✓
134	GRA 4	Gracilis	VI057220	Cambodia	✓
135	GRA 5	Gracilis	VI056235	Philippines	N/A
136	GRA 6	Gracilis	VI054569	Philippines	N/A

Appendix 3.1a: (Continued)

No.	Label	Species	ID	Origin country	Quality of passport data
137	GRA 7	Gracilis	VI054578	Philippines	N/A
138	GRA 8	Gracilis	VI054581	Philippines	N/A
139	GRZ 1	Graecizans	VI036225	Hungary	✓
140	GRZ 2	Graecizans	VI044403	India	✓
141	GRZ 3	Graecizans	VI044388	India	✓
142	HYB 3	Hybridus	VI051004	Kenya	✓
143	HYB 4	Hybridus	VI059040	Unknown	N/A
144	HYB 5	Hybridus	VI059043	Unknown	N/A
145	HYB 6	Hybridus	VI059044	Unknown	N/A
146	HYB 7	Hybridus	VI050445	Unknown	N/A
147	HYB 1	Hybridus	VI044419	USA	✓
148	HYB 2	Hybridus	VI044421	USA	✓
149	HYP 1	Hypochondriacus	VI033455-A	Peru	✓
150	HYP 2	Hypochondriacus	VI033455-B	Peru	✓
151	HYP 3	Hypochondriacus	VI033463	Peru	✓
152	HYP 4	Hypochondriacus	VI044394	India	✓
153	HYP 5	Hypochondriacus	VI044397	India	✓
154	HYP 6	Hypochondriacus	VI044399	India	✓
155	HYP 7	Hypochondriacus	VI044400-A	India	✓
156	HYP 8	Hypochondriacus	VI044414	India	✓
157	HYP 9	Hypochondriacus	VI046621	Viet Nam	✓
158	HYP 10	Hypochondriacus	VI047539	Viet Nam	✓
159	HYP 11	Hypochondriacus	VI047551	Viet Nam	✓
160	HYP 12	Hypochondriacus	VI047552	Viet Nam	✓
161	HYP 13	Hypochondriacus	VI047594	Viet Nam	✓
162	HYP 14	Hypochondriacus	VI050998	Cameroon	✓
163	HYP 15	Hypochondriacus	VI050999	Cameroon	✓
164	HYP 16	Hypochondriacus	VI051000	Cameroon	✓
165	HYP 17	Hypochondriacus	VI051001	Cameroon	✓
166	HYP 18	Hypochondriacus	VI044369	Ghana	✓
167	HYP 19	Hypochondriacus	VI044373	Ghana	✓
168	HYP 20	Hypochondriacus	VI044374	Ghana	✓
169	HYP 21	Hypochondriacus	VI044375-A	Ghana	✓
170	HYP 22	Hypochondriacus	VI044365-A	Ghana	✓
171	HYP 23	Hypochondriacus	VI050128	Kenya	✓
172	HYP 24	Hypochondriacus	VI051003	Kenya	✓
173	HYP 25	Hypochondriacus	VI033462-A	Ecuador	✓
174	HYP 26	Hypochondriacus	VI044395	Afghanistan	✓
175	HYP 28	Hypochondriacus	VI044454	Mexico	✓
176	HYP 29	Hypochondriacus	VI044479	Nepal	✓
177	HYP 30	Hypochondriacus	VI036229	Hungary	✓
178	HYP 31	Hypochondriacus	VI042947	Indonesia	N/A
179	HYP 32	Hypochondriacus	VI042948	Indonesia	N/A
180	HYP 33	Hypochondriacus	VI042949	Indonesia	N/A
181	HYP 34	Hypochondriacus	VI042950	Indonesia	N/A
182	HYP 35	Hypochondriacus	VI042956-A	Indonesia	N/A
183	HYP 36	Hypochondriacus	VI044408	India	N/A
184	HYP 37	Hypochondriacus	VI047483	Tanzania	N/A
185	HYP 38	Hypochondriacus	VI059412	Tanzania	N/A
186	HYP 39	Hypochondriacus	VI060291	Tanzania	N/A
187	HYP 40	Hypochondriacus	VI060293	Tanzania	N/A
188	HYP 41	Hypochondriacus	VI060466	Tanzania	N/A
189	HYP 42	Hypochondriacus	VI060468-A	Tanzania	N/A
190	HYP 43	Hypochondriacus	VI060468-B	Tanzania	N/A
191	HYP 44	Hypochondriacus	VI060469	Tanzania	N/A
192	HYP 45	Hypochondriacus	VI060471	Tanzania	N/A
193	HYP 46	Hypochondriacus	VI060472	Tanzania	N/A
194	HYP 47	Hypochondriacus	VI060473	Tanzania	N/A
195	HYP 48	Hypochondriacus	VI062427	Tanzania	N/A
196	HYP 49	Hypochondriacus	VI062428	Tanzania	N/A
197	HYP 50	Hypochondriacus	VI061506	Unknown	N/A
198	HYP 51	Hypochondriacus	VI062432	Unknown	N/A
199	HYP 52	Hypochondriacus	VI062433	Unknown	N/A
200	HYP 53	Hypochondriacus	VI062434	Unknown	N/A
201	HYP 54	Hypochondriacus	VI059037-A	Unknown	N/A
202	HYP 55	Hypochondriacus	VI059037-B	Unknown	N/A
203	HYP 56	Hypochondriacus	VI044441	Nigeria	N/A
204	HYP 57	Hypochondriacus	VI060467	Malawi	N/A
205	HYP 58	Hypochondriacus	VI061489	Madagascar	N/A
206	HYP 59	Hypochondriacus	VI050611-A	Viet Nam	X
207	HYP 60	Hypochondriacus	VI050449	Unknown	X
208	HYP 61	Hypochondriacus	VI050457	Unknown	X
209	HYP 62	Hypochondriacus	VI050446	Sudan	X
210	HYP 27	Hypochondriacus	VI044460-A	USA	✓
211	LEU 1	Leucocarpus	VI044445	India	✓
212	MAN 1	Mantegazzianus	VI044427	USA	✓

Appendix 3.1a: (Continued)

No.	Label	Species	ID	Origin country	Quality of passport data
213	PAL 1	Palmeri	VI044473	Senegal	✓
214	RET 1	Retroflexus	VI048310	Viet Nam	✓
215	RET 2	Retroflexus	VI048311	Viet Nam	✓
216	RET 3	Retroflexus	VI048391	Viet Nam	✓
217	RET 4	Retroflexus	VI033461	Venezuela	X
218	SP 20	Sp	VI054798	Laos	✓
219	SP 21	Sp	VI054799	Laos	✓
220	SP 1	Sp	VI033450	Malaysia	✓
221	SP 2	Sp	VI033471	Malaysia	✓
222	SP 3	Sp	VI033472	Malaysia	✓
223	SP 4	Sp	VI033477	Malaysia	✓
224	SP 5	Sp	VI033479	Malaysia	✓
225	SP 6	Sp	VI033481	Malaysia	✓
226	SP 7	Sp	VI038220	Bangladesh	✓
227	SP 8	Sp	VI056560	Bangladesh	✓
228	SP 9	Sp	VI056561	Bangladesh	✓
229	SP 10	Sp	VI056562	Bangladesh	✓
230	SP 11	Sp	VI056563	Bangladesh	✓
231	SP 12	Sp	VI056564	Bangladesh	✓
232	SP 13	Sp	VI056565	Bangladesh	✓
233	SP 14	Sp	VI056566	Bangladesh	✓
234	SP 15	Sp	VI049502	Thailand	✓
235	SP 16	Sp	VI049504	Thailand	✓
236	SP 17	Sp	VI049530	Thailand	✓
237	SP 18	Sp	VI048919	Thailand	✓
238	SP 19	Sp	VI049784	Thailand	✓
239	SP 22	Sp	VI044416	Unknown	✓
240	SP 23	Sp	VI050253	Taiwan	✓
241	SP 24	Sp	VI046233-A	Viet Nam	✓
242	SP 25	SP	VI044448	India	X
243	SPI 25	Spinosus	VI046123	Laos	✓
244	SPI 1	Spinosus	VI044410	Puerto Rico	✓
245	SPI 2	Spinosus	VI044411	Puerto Rico	✓
246	SPI 3	Spinosus	VI044412	Puerto Rico	✓
247	SPI 4	Spinosus	VI044436	Thailand	✓
248	SPI 5	Spinosus	VI048656	Thailand	✓
249	SPI 6	Spinosus	VI048723	Thailand	✓
250	SPI 7	Spinosus	VI040944	Thailand	✓

No.	Label	Species	ID	Origin country	Quality of passport data
251	SPI 8	Spinosus	VI049240	Thailand	✓
252	SPI 9	Spinosus	VI049248	Thailand	✓
253	SPI 10	Spinosus	VI049149	Thailand	✓
254	SPI 11	Spinosus	VI049533	Thailand	✓
255	SPI 12	Spinosus	VI049576	Thailand	✓
256	SPI 13	Spinosus	VI049584	Thailand	✓
257	SPI 14	Spinosus	VI049218	Thailand	✓
258	SPI 15	Spinosus	VI049405	Thailand	✓
259	SPI 16	Spinosus	VI049407	Thailand	✓
260	SPI 17	Spinosus	VI049484	Thailand	✓
261	SPI 18	Spinosus	VI049499	Thailand	✓
262	SPI 19	Spinosus	VI049811	Thailand	✓
263	SPI 20	Spinosus	VI049608	Thailand	✓
264	SPI 21	Spinosus	VI055072	Malaysia	✓
265	SPI 22	Spinosus	VI055082	Malaysia	✓
266	SPI 23	Spinosus	VI044413	Dominican Republic	✓
267	SPI 24	Spinosus	VI044415	India	✓
268	SPI 26	Spinosus	VI055129	Malaysia	N/A
269	SPI 27	Spinosus	VI055130	Malaysia	N/A
270	SPI 28	Spinosus	VI055131	Malaysia	N/A
271	SPI 29	Spinosus	VI055132	Malaysia	N/A
272	SPI 30	Spinosus	VI046232	Viet Nam	N/A
273	SPI 31	Spinosus	VI046294	Viet Nam	N/A
274	SPI 32	Spinosus	VI046297	Viet Nam	N/A
275	SPI 33	Spinosus	VI050502	Thailand	X
276	SPI 34	Spinosus	VI044428	Indonesia	X
277	SPI 35	Spinosus	VI044429	Indonesia	X
278	THU 1	Thunbergii	VI050467	Unknown	✓
279	THU 2	Thunbergii	VI050468	Unknown	✓
280	THU 3	Thunbergii	VI050454	Unknown	N/A
281	THU 4	Thunbergii	VI060475	Tanzania	N/A
282	THU 5	Thunbergii	VI050456	Unknown	X
283	TRI 86	Tricolor	VI055809	Laos	✓
284	TRI 1	Tricolor	VI038237	Bangladesh	✓
285	TRI 2	Tricolor	VI044476	Bangladesh	✓
286	TRI 3	Tricolor	VI047504	Bangladesh	✓
287	TRI 4	Tricolor	VI047505-A	Bangladesh	✓
288	TRI 5	Tricolor	VI047508	Bangladesh	✓

Appendix 3.1a: (Continued)

No.	Label	Species	ID	Origin country	Quality of passport data
289	TRI 6	Tricolor	VI047510	Bangladesh	✓
290	TRI 7	Tricolor	VI047517-A	Bangladesh	✓
291	TRI 8	Tricolor	VI047666	Bangladesh	✓
292	TRI 9	Tricolor	VI047667	Bangladesh	✓
293	TRI 10	Tricolor	VI047668	Bangladesh	✓
294	TRI 11	Tricolor	VI047675	Bangladesh	✓
295	TRI 12	Tricolor	VI047676	Bangladesh	✓
296	TRI 13	Tricolor	VI047681	Bangladesh	✓
297	TRI 14	Tricolor	VI047682	Bangladesh	✓
298	TRI 15	Tricolor	VI047699-A	Bangladesh	✓
299	TRI 16	Tricolor	VI047719	Bangladesh	✓
300	TRI 17	Tricolor	VI047746-A	Bangladesh	✓
301	TRI 18	Tricolor	VI047747	Bangladesh	✓
302	TRI 19	Tricolor	VI047772	Bangladesh	✓
303	TRI 20	Tricolor	VI047781	Bangladesh	✓
304	TRI 21	Tricolor	VI047790	Bangladesh	✓
305	TRI 22	Tricolor	VI047795	Bangladesh	✓
306	TRI 23	Tricolor	VI047800-A	Bangladesh	✓
307	TRI 24	Tricolor	VI047804	Bangladesh	✓
308	TRI 25	Tricolor	VI047829	Bangladesh	✓
309	TRI 26	Tricolor	VI047836	Bangladesh	✓
310	TRI 27	Tricolor	VI047838	Bangladesh	✓
311	TRI 28	Tricolor	VI047871	Bangladesh	✓
312	TRI 29	Tricolor	VI047872	Bangladesh	✓
313	TRI 30	Tricolor	VI047880	Bangladesh	✓
314	TRI 31	Tricolor	VI047928	Bangladesh	✓
315	TRI 32	Tricolor	VI047929	Bangladesh	✓
316	TRI 33	Tricolor	VI048021	Bangladesh	✓
317	TRI 34	Tricolor	VI048057	Bangladesh	✓
318	TRI 35	Tricolor	VI048076	Bangladesh	✓
319	TRI 36	Tricolor	VI048089	Bangladesh	✓
320	TRI 37	Tricolor	VI048109	Bangladesh	✓
321	TRI 38	Tricolor	VI048113	Bangladesh	✓
322	TRI 39	Tricolor	VI048145-A	Bangladesh	✓
323	TRI 40	Tricolor	VI048164	Bangladesh	✓
324	TRI 41	Tricolor	VI048170	Bangladesh	✓
325	TRI 42	Tricolor	VI048233-A	Bangladesh	✓
326	TRI 43	Tricolor	VI048269	Bangladesh	✓
327	TRI 44	Tricolor	VI048275	Bangladesh	✓
328	TRI 45	Tricolor	VI048286	Bangladesh	✓
329	TRI 46	Tricolor	VI048301	Bangladesh	✓
330	TRI 47	Tricolor	VI055346	Bangladesh	✓
331	TRI 48	Tricolor	VI055347	Bangladesh	✓
332	TRI 49	Tricolor	VI055348	Bangladesh	✓
333	TRI 50	Tricolor	VI055349	Bangladesh	✓
334	TRI 51	Tricolor	VI055350	Bangladesh	✓
335	TRI 52	Tricolor	VI055351	Bangladesh	✓
336	TRI 53	Tricolor	VI055352	Bangladesh	✓
337	TRI 54	Tricolor	VI055353	Bangladesh	✓
338	TRI 55	Tricolor	VI055354	Bangladesh	✓
339	TRI 56	Tricolor	VI055355	Bangladesh	✓
340	TRI 57	Tricolor	VI055356	Bangladesh	✓
341	TRI 58	Tricolor	VI056854	Bangladesh	✓
342	TRI 59	Tricolor	VI057136	Cambodia	✓
343	TRI 60	Tricolor	VI055441	Cambodia	✓
344	TRI 61	Tricolor	VI055442	Cambodia	✓
345	TRI 62	Tricolor	VI056122	Cambodia	✓
346	TRI 63	Tricolor	VI056168	Cambodia	✓
347	TRI 64	Tricolor	VI057270	Cambodia	✓
348	TRI 65	Tricolor	VI057299	Cambodia	✓
349	TRI 66	Tricolor	VI044420	China	✓
350	TRI 67	Tricolor	VI044444	India	✓
351	TRI 68	Tricolor	VI044446	India	✓
352	TRI 69	Tricolor	VI047439	India	✓
353	TRI 70	Tricolor	VI047441	India	✓
354	TRI 71	Tricolor	VI058498	India	✓
355	TRI 72	Tricolor	VI059413	India	✓
356	TRI 73	Tricolor	VI042954	Indonesia	✓
357	TRI 74	Tricolor	VI042958	Indonesia	✓
358	TRI 75	Tricolor	VI042960	Indonesia	✓
359	TRI 76	Tricolor	VI042961	Indonesia	✓
360	TRI 77	Tricolor	VI042966-A	Indonesia	✓
361	TRI 78	Tricolor	VI042969	Indonesia	✓
362	TRI 79	Tricolor	VI042971	Indonesia	✓
363	TRI 80	Tricolor	VI042972	Indonesia	✓
364	TRI 81	Tricolor	VI042975	Indonesia	✓

Appendix 3.1a: (Continued)

No.	Label	Species	ID	Origin country	Quality of passport data
365	TRI 82	Tricolor	VI042976	Indonesia	✓
366	TRI 83	Tricolor	VI042978	Indonesia	✓
367	TRI 84	Tricolor	VI042979	Indonesia	✓
368	TRI 85	Tricolor	VI042983	Indonesia	✓
369	TRI 87	Tricolor	VI043724	Malaysia	✓
370	TRI 88	Tricolor	VI043725	Malaysia	✓
371	TRI 89	Tricolor	VI033469	Malaysia	✓
372	TRI 90	Tricolor	VI033470	Malaysia	✓
373	TRI 91	Tricolor	VI033473	Malaysia	✓
374	TRI 92	Tricolor	VI033474	Malaysia	✓
375	TRI 93	Tricolor	VI033475	Malaysia	✓
376	TRI 94	Tricolor	VI033476	Malaysia	✓
377	TRI 95	Tricolor	VI033478	Malaysia	✓
378	TRI 96	Tricolor	VI033480	Malaysia	✓
379	TRI 97	Tricolor	VI033482	Malaysia	✓
380	TRI 98	Tricolor	VI033483	Malaysia	✓
381	TRI 99	Tricolor	VI033484	Malaysia	✓
382	TRI 100	Tricolor	VI033485	Malaysia	✓
383	TRI 101	Tricolor	VI033486	Malaysia	✓
384	TRI 102	Tricolor	VI033488	Malaysia	✓
385	TRI 103	Tricolor	VI033489	Malaysia	✓
386	TRI 104	Tricolor	VI033490	Malaysia	✓
387	TRI 105	Tricolor	VI055034	Malaysia	✓
388	TRI 106	Tricolor	VI055050	Malaysia	✓
389	TRI 107	Tricolor	VI055051	Malaysia	✓
390	TRI 108	Tricolor	VI055062	Malaysia	✓
391	TRI 109	Tricolor	VI055069	Malaysia	✓
392	TRI 110	Tricolor	VI055095	Malaysia	✓
393	TRI 111	Tricolor	VI055113	Malaysia	✓
394	TRI 112	Tricolor	VI055137	Malaysia	✓
395	TRI 113	Tricolor	VI055138	Malaysia	✓
396	TRI 114	Tricolor	VI055139	Malaysia	✓
397	TRI 115	Tricolor	VI044426	Malaysia	✓
398	TRI 116	Tricolor	VI044438-A	Nigeria	✓
399	TRI 117	Tricolor	VI044396-A	Pakistan	✓
400	TRI 118	Tricolor	VI044407	Papua New Guinea	✓
401	TRI 119	Tricolor	VI044450	Papua New Guinea	✓
402	TRI 120	Tricolor	VI054571	Philippines	✓
403	TRI 121	Tricolor	VI049430	Taiwan	✓
404	TRI 122	Tricolor	VI049431	Taiwan	✓
405	TRI 123	Tricolor	VI050111	Taiwan	✓
406	TRI 124	Tricolor	VI050214	Taiwan	✓
407	TRI 125	Tricolor	VI054536	Taiwan	✓
408	TRI 126	Tricolor	VI041062	Thailand	✓
409	TRI 127	Tricolor	VI049005	Thailand	✓
410	TRI 128	Tricolor	VI049006	Thailand	✓
411	TRI 129	Tricolor	VI049129	Thailand	✓
412	TRI 130	Tricolor	VI049787	Thailand	✓
413	TRI 131	Tricolor	VI044425	Thailand	✓
414	TRI 132	Tricolor	VI044389	Turkey	✓
415	TRI 136	Tricolor	VI044455	Unknown	✓
416	TRI 137	Tricolor	VI047387	Viet Nam	✓
417	TRI 138	Tricolor	VI047526-A	Viet Nam	✓
418	TRI 139	Tricolor	VI047555-A	Viet Nam	✓
419	TRI 140	Tricolor	VI047556-A	Viet Nam	✓
420	TRI 141	Tricolor	VI047577	Viet Nam	✓
421	TRI 142	Tricolor	VI047603	Viet Nam	✓
422	TRI 143	Tricolor	VI050615-A	Viet Nam	✓
423	TRI 144	Tricolor	VI050615-B	Viet Nam	✓
424	TRI 145	Tricolor	VI047669	Bangladesh	N/A
425	TRI 146	Tricolor	VI047680	Bangladesh	N/A
426	TRI 147	Tricolor	VI047692	Bangladesh	N/A
427	TRI 148	Tricolor	VI047693	Bangladesh	N/A
428	TRI 149	Tricolor	VI047777-A	Bangladesh	N/A
429	TRI 150	Tricolor	VI056853	Bangladesh	N/A
430	TRI 151	Tricolor	VI056855-A	Bangladesh	N/A
431	TRI 152	Tricolor	VI056855-B	Bangladesh	N/A
432	TRI 153	Tricolor	VI056856	Bangladesh	N/A
433	TRI 154	Tricolor	VI056142	Cambodia	N/A
434	TRI 155	Tricolor	VI057284	Cambodia	N/A
435	TRI 156	Tricolor	VI042943	Indonesia	N/A
436	TRI 157	Tricolor	VI042944	Indonesia	N/A
437	TRI 158	Tricolor	VI042945	Indonesia	N/A
438	TRI 159	Tricolor	VI042946	Indonesia	N/A
439	TRI 160	Tricolor	VI042951	Indonesia	N/A
440	TRI 161	Tricolor	VI042955	Indonesia	N/A

Appendix 3.1a: (Continued)

No.	Label	Species	ID	Origin country	Quality of passport data
441	TRI 162	Tricolor	VI042957	Indonesia	N/A
442	TRI 163	Tricolor	VI042964	Indonesia	N/A
443	TRI 164	Tricolor	VI042965	Indonesia	N/A
444	TRI 165	Tricolor	VI042967	Indonesia	N/A
445	TRI 166	Tricolor	VI042968	Indonesia	N/A
446	TRI 167	Tricolor	VI042970	Indonesia	N/A
447	TRI 168	Tricolor	VI042973	Indonesia	N/A
448	TRI 169	Tricolor	VI042974	Indonesia	N/A
449	TRI 170	Tricolor	VI042977	Indonesia	N/A
450	TRI 171	Tricolor	VI042980	Indonesia	N/A
451	TRI 172	Tricolor	VI042982	Indonesia	N/A
452	TRI 173	Tricolor	VI042984	Indonesia	N/A
453	TRI 174	Tricolor	VI042985	Indonesia	N/A
454	TRI 175	Tricolor	VI044434	Indonesia	N/A
455	TRI 176	Tricolor	VI033468	Malaysia	N/A
456	TRI 177	Tricolor	VI055133	Malaysia	N/A
457	TRI 178	Tricolor	VI055134	Malaysia	N/A
458	TRI 179	Tricolor	VI055140	Malaysia	N/A
459	TRI 180	Tricolor	VI054573	Philippines	N/A
460	TRI 181	Tricolor	VI044382	Taiwan	N/A
461	TRI 182	Tricolor	VI044383	Taiwan	N/A
462	TRI 183	Tricolor	VI047144	Taiwan	N/A
463	TRI 184	Tricolor	VI060474	Tanzania	N/A
464	TRI 185	Tricolor	VI060292	Tanzania	N/A
465	TRI 186	Tricolor	VI041041	Thailand	N/A
466	TRI 187	Tricolor	VI062426	Uganda	N/A
467	TRI 189	Tricolor	VI050447	Unknown	N/A
468	TRI 190	Tricolor	VI050450	Unknown	N/A
469	TRI 191	Tricolor	VI050465	Unknown	N/A
470	TRI 192	Tricolor	VI045972	Viet Nam	N/A
471	TRI 193	Tricolor	VI045975	Viet Nam	N/A
472	TRI 194	Tricolor	VI046046	Viet Nam	N/A
473	TRI 195	Tricolor	VI046231	Viet Nam	N/A
474	TRI 196	Tricolor	VI046234	Viet Nam	N/A
475	TRI 197	Tricolor	VI046235	Viet Nam	N/A
476	TRI 198	Tricolor	VI046236	Viet Nam	N/A
477	TRI 199	Tricolor	VI046237	Viet Nam	N/A
478	TRI 200	Tricolor	VI046238	Viet Nam	N/A
479	TRI 201	Tricolor	VI046241	Viet Nam	N/A
480	TRI 202	Tricolor	VI046291	Viet Nam	N/A
481	TRI 203	Tricolor	VI046295	Viet Nam	N/A
482	TRI 204	Tricolor	VI046299-A	Viet Nam	N/A
483	TRI 205	Tricolor	VI046619	Viet Nam	N/A
484	TRI 206	Tricolor	VI046749	Viet Nam	N/A
485	TRI 207	Tricolor	VI047366	Viet Nam	N/A
486	TRI 208	Tricolor	VI047381	Viet Nam	N/A
487	TRI 209	Tricolor	VI047382	Viet Nam	N/A
488	TRI 210	Tricolor	VI047501	Bangladesh	X
489	TRI 211	Tricolor	VI047764	Bangladesh	X
490	TRI 212	Tricolor	VI047847-A	Bangladesh	X
491	TRI 213	Tricolor	VI047848	Bangladesh	X
492	TRI 214	Tricolor	VI047870-A	Bangladesh	X
493	TRI 215	Tricolor	VI047897	Bangladesh	X
494	TRI 216	Tricolor	VI048052	Bangladesh	X
495	TRI 217	Tricolor	VI048103	Bangladesh	X
496	TRI 218	Tricolor	VI048146	Bangladesh	X
497	TRI 219	Tricolor	VI048198	Bangladesh	X
498	TRI 220	Tricolor	VI048200	Bangladesh	X
499	TRI 221	Tricolor	VI048201-A	Bangladesh	X
500	TRI 222	Tricolor	VI048257	Bangladesh	X
501	TRI 223	Tricolor	VI044443	India	X
502	TRI 224	Tricolor	VI048528	Japan	X
503	TRI 225	Tricolor	VI055029	Malaysia	X
504	TRI 226	Tricolor	VI054572	Philippines	X
505	TRI 227	Tricolor	VI049004	Thailand	X
506	TRI 228	Tricolor	VI050609-A	Viet Nam	X
507	TRI 229	Tricolor	VI050610-A	Viet Nam	X
508	TRI 230	Tricolor	VI050613	Viet Nam	X
509	TRI 231	Tricolor	VI050614-A	Viet Nam	X
510	TRI 133	Tricolor	VI044379-A	USA	✓
511	TRI 134	Tricolor	VI044381	USA	✓
512	TRI 135	Tricolor	VI044470	USA	✓
513	TRI 188	Tricolor	VI061488	USA	N/A
514	VIR 45	Viridis	VI055822	Laos	✓
515	VIR 46	Viridis	VI055959-A	Laos	✓
516	VIR 47	Viridis	VI055959-B	Laos	✓

Appendix 3.1a: (Continued)

No.	Label	Species	ID	Origin country	Quality of passport data
517	VIR 48	Viridis	VI054797	Laos	✓
518	VIR 49	Viridis	VI046116	Laos	✓
519	VIR 50	Viridis	VI046127	Laos	✓
520	VIR 1	Viridis	VI048627	Thailand	✓
521	VIR 2	Viridis	VI048640	Thailand	✓
522	VIR 3	Viridis	VI048697	Thailand	✓
523	VIR 4	Viridis	VI048700	Thailand	✓
524	VIR 5	Viridis	VI048714	Thailand	✓
525	VIR 6	Viridis	VI048794	Thailand	✓
526	VIR 7	Viridis	VI048822	Thailand	✓
527	VIR 8	Viridis	VI048826	Thailand	✓
528	VIR 9	Viridis	VI048840	Thailand	✓
529	VIR 10	Viridis	VI048851	Thailand	✓
530	VIR 11	Viridis	VI048873	Thailand	✓
531	VIR 12	Viridis	VI048883	Thailand	✓
532	VIR 13	Viridis	VI048964	Thailand	✓
533	VIR 14	Viridis	VI049001	Thailand	✓
534	VIR 15	Viridis	VI049054	Thailand	✓
535	VIR 16	Viridis	VI049131	Thailand	✓
536	VIR 17	Viridis	VI049158	Thailand	✓
537	VIR 18	Viridis	VI049168	Thailand	✓
538	VIR 19	Viridis	VI049171	Thailand	✓
539	VIR 20	Viridis	VI049199	Thailand	✓
540	VIR 21	Viridis	VI049202	Thailand	✓
541	VIR 22	Viridis	VI049209	Thailand	✓
542	VIR 23	Viridis	VI049216	Thailand	✓
543	VIR 24	Viridis	VI049219	Thailand	✓
544	VIR 25	Viridis	VI049224	Thailand	✓
545	VIR 26	Viridis	VI049250	Thailand	✓
546	VIR 27	Viridis	VI049402	Thailand	✓
547	VIR 28	Viridis	VI049403	Thailand	✓

No.	Label	Species	ID	Origin country	Quality of passport data
548	VIR 29	Viridis	VI049404	Thailand	✓
549	VIR 30	Viridis	VI049406	Thailand	✓
550	VIR 31	Viridis	VI049473	Thailand	✓
551	VIR 32	Viridis	VI049592	Thailand	✓
552	VIR 33	Viridis	VI049609	Thailand	✓
553	VIR 34	Viridis	VI049639	Thailand	✓
554	VIR 35	Viridis	VI049725	Thailand	✓
555	VIR 36	Viridis	VI049918	Thailand	✓
556	VIR 37	Viridis	VI049893	Thailand	✓
557	VIR 38	Viridis	VI049698	Thailand	✓
558	VIR 39	Viridis	VI056885	Philippines	✓
559	VIR 40	Viridis	VI054570	Philippines	✓
560	VIR 41	Viridis	VI054574	Philippines	✓
561	VIR 42	Viridis	VI055027	Malaysia	✓
562	VIR 43	Viridis	VI055097	Malaysia	✓
563	VIR 44	Viridis	VI055126	Malaysia	✓
564	VIR 51	Viridis	VI047528	Viet Nam	✓
565	VIR 52	Viridis	VI054535	Taiwan	✓
566	VIR 53	Viridis	VI044432	Indonesia	✓
567	VIR 54	Viridis	VI048811	Thailand	N/A
568	VIR 55	Viridis	VI056886	Philippines	N/A
569	VIR 56	Viridis	VI056887	Philippines	N/A
570	VIR 57	Viridis	VI055125	Malaysia	N/A
571	VIR 58	Viridis	VI055127	Malaysia	N/A
572	VIR 59	Viridis	VI055128	Malaysia	N/A
573	VIR 60	Viridis	VI055135	Malaysia	N/A
574	VIR 61	Viridis	VI046239	Viet Nam	N/A
575	VIR 62	Viridis	VI048809	Thailand	X
576	VIR 63	Viridis	VI048993	Thailand	X
577	VIR 64	Viridis	VI050516	Thailand	X
578	VIR 65	Viridis	VI050525	Thailand	X

Appendix 3.1b: 179 amaranth accessions obtained from United State Department of Agriculture (USDA) Genebank. The quality of passport data: (✓) is complete and (X) is not complete.

No.	Label	Species	ID	Country origin	Quality of passport data
1	HYB 2	Hybridus	Ames 26235	China	X
2	RET 1	Retroflexus	Ames 26236	China	X
3	Zaire_1	Tricolor	Ames 1980	Zaire	✓
4	India_1	Tricolor	Ames 1982	India	✓
5	India_2	Tricolor	Ames 1983	India	✓
6	Unknown_1	Tricolor	Ames 1988	Unknown	✓
7	Taiwan_1	Tricolor	Ames 1993	Taiwan	X
8	Taiwan_2	Tricolor	Ames 1998	Taiwan	✓
9	China_1	Tricolor	Ames 2017	China	✓
10	Thailand_1	Tricolor	Ames 2024	Thailand	X
11	Msia_1	Tricolor	Ames 2029	Malaysia	✓
12	Indonesia_1	Tricolor	Ames 2039	Indonesia	✓
13	India_3	Tricolor	Ames 2040	India	X
14	India_4	Tricolor	Ames 2051	India	✓
15	India_5	Tricolor	Ames 2091	India	X
16	India_6	Tricolor	Ames 2099	India	✓
17	India_7	Tricolor	Ames 2100	India	✓
18	India_8	Tricolor	Ames 2101	India	✓
19	India_9	Tricolor	Ames 2102	India	X
20	India_10	Tricolor	Ames 2103	India	X
21	India_11	Tricolor	Ames 2104	India	✓
22	India_12	Tricolor	Ames 2105	India	✓
23	India_13	Tricolor	Ames 2106	India	✓
24	India_14	Tricolor	Ames 2107	India	✓
25	India_15	Tricolor	Ames 2108	India	✓
26	India_16	Tricolor	Ames 2109	India	✓
27	India_17	Tricolor	Ames 2110	India	✓
28	India_18	Tricolor	Ames 2112	India	✓
29	India_19	Tricolor	Ames 2113	India	✓
30	India_20	Tricolor	Ames 2114	India	✓
31	India_21	Tricolor	Ames 2115	India	✓
32	India_22	Tricolor	Ames 2116	India	✓
33	India_23	Tricolor	Ames 2117	India	✓
34	India_24	Tricolor	Ames 2118	India	✓
35	India_25	Tricolor	Ames 2119	India	✓
36	India_26	Tricolor	Ames 2120	India	✓
37	India_27	Tricolor	Ames 2121	India	✓
38	India_28	Tricolor	Ames 2122	India	✓
39	India_29	Tricolor	Ames 2123	India	✓
40	India_30	Tricolor	Ames 2124	India	✓
41	India_31	Tricolor	Ames 2125	India	✓
42	India_32	Tricolor	Ames 2126	India	X
43	India_33	Tricolor	Ames 2127	India	✓
44	India_34	Tricolor	Ames 2128	India	✓
45	India_35	Tricolor	Ames 2129	India	✓
46	India_36	Tricolor	Ames 2130	India	✓
47	India_37	Tricolor	Ames 2131	India	✓
48	India_38	Tricolor	Ames 2132	India	✓
49	India_39	Tricolor	Ames 2134	India	X
50	India_40	Tricolor	Ames 2135	India	X
51	India_41	Tricolor	Ames 2138	India	✓
52	India_42	Tricolor	Ames 2139	India	✓
53	India_43	Tricolor	Ames 2140	India	✓
54	India_44	Tricolor	Ames 2141	India	✓
55	India_45	Tricolor	Ames 2142	India	X
56	India_46	Tricolor	Ames 2143	India	✓
57	India_47	Tricolor	Ames 2145	India	✓
58	India_48	Tricolor	Ames 2146	India	X
59	India_49	Tricolor	Ames 2147	India	✓
60	India_50	Tricolor	Ames 2148	India	✓

Appendix 3.1b: (Continued)

No.	Label	Species	ID	Country origin	Quality of passport data
61	India_51	Tricolor	Ames 2149	India	✓
62	HK_1	Tricolor	Ames 2196	Hong Kong	✓
63	HK_2	Tricolor	Ames 2197	Hong Kong	✓
64	HK_3	Tricolor	Ames 2198	Hong Kong	✓
65	Taiwan_3	Tricolor	Ames 2199	Taiwan	✓
66	HK_4	Tricolor	Ames 2202	Hong Kong	X
67	HK_5	Tricolor	Ames 2203	Hong Kong	✓
68	HK_6	Tricolor	Ames 2204	Hong Kong	✓
69	HK_7	Tricolor	Ames 2205	Hong Kong	X
70	HK_8	Tricolor	Ames 2207	Hong Kong	✓
71	HK_9	Tricolor	Ames 2209	Hong Kong	✓
72	USA_1	Tricolor	Ames 2214	USA	X
73	India_52	Tricolor	Ames 2221	India	✓
74	India_53	Tricolor	Ames 2222	India	✓
75	India_54	Tricolor	Ames 2223	India	✓
76	India_55	Tricolor	Ames 2224	India	✓
77	India_56	Tricolor	Ames 2225	India	✓
78	India_57	Tricolor	Ames 2226	India	✓
79	India_58	Tricolor	Ames 2227	India	✓
80	India_59	Tricolor	Ames 2228	India	X
81	India_60	Tricolor	Ames 2229	India	✓
82	India_61	Tricolor	Ames 2230	India	✓
83	HK_10	Tricolor	Ames 5099	Hong Kong	✓
84	HK_11	Tricolor	Ames 5100	Hong Kong	✓
85	HK_12	Tricolor	Ames 5101	Hong Kong	✓
86	HK_13	Tricolor	Ames 5102	Hong Kong	X
87	Unknown_2	Tricolor	Ames 5109	Unknown	X
88	WA_1	Tricolor	Ames 5110	West Africa	✓
89	PNG_1	Tricolor	Ames 5111	Papua New Guinea	✓
90	Taiwan_4	Tricolor	Ames 5113	Taiwan	✓
91	PR_1	Tricolor	Ames 5117	Puerto Rico	✓
92	PR_2	Tricolor	Ames 5118	Puerto Rico	✓
93	USA_2	Tricolor	Ames 5126	USA	✓
94	USA_3	Tricolor	Ames 5128	USA	✓
95	USA_4	Tricolor	Ames 5134	USA	✓
96	USA_5	Tricolor	Ames 5139	USA	✓

No.	Label	Species	ID	Country origin	Quality of passport data
97	Unknown_3	Tricolor	Ames 5161	Unknown	✓
98	Unknown_4	Tricolor	Ames 5162	Unknown	✓
99	Unknown_5	Tricolor	Ames 5163	Unknown	✓
100	USA_6	Tricolor	Ames 5303	USA	✓
101	India_62	Tricolor	Ames 5311	India	X
102	HK_14	Tricolor	Ames 5317	Hong Kong	✓
103	Madagascar_1	Tricolor	Ames 5354	Madagascar	✓
104	Bangladesh_1	Tricolor	Ames 5368	Bangladesh	✓
105	Unknown_6	Tricolor	Ames 5383	Unknown	✓
106	Unknown_7	Tricolor	Ames 15323	Unknown	✓
107	USA_7	Tricolor	Ames 15328	USA	X
108	Unknown_8	Tricolor	Ames 15329	Unknown	✓
109	China_2	Tricolor	Ames 15330	China	X
110	Unknown_9	Tricolor	Ames 15331	Unknown	✓
111	India_63	Tricolor	Ames 18049	India	✓
112	USA_8	Tricolor	Ames 25153	USA	X
113	China_3	Tricolor	Ames 26208	China	X
114	China_4	Tricolor	Ames 26209	China	X
115	China_5	Tricolor	Ames 26210	China	X
116	China_6	Tricolor	Ames 26211	China	X
117	China_7	Tricolor	Ames 26212	China	X
118	China_8	Tricolor	Ames 26213	China	X
119	China_9	Tricolor	Ames 26214	China	X
120	China_10	Tricolor	Ames 26215	China	X
121	China_11	Tricolor	Ames 26216	China	X
122	China_12	Tricolor	Ames 26217	China	X
123	China_13	Tricolor	Ames 26218	China	X
124	China_14	Tricolor	Ames 26219	China	X
125	China_15	Tricolor	Ames 26220	China	X
126	China_16	Tricolor	Ames 26221	China	X
127	China_17	Tricolor	Ames 26222	China	X
128	China_18	Tricolor	Ames 26223	China	X
129	China_19	Tricolor	Ames 26225	China	X
130	China_20	Tricolor	Ames 26226	China	X
131	China_21	Tricolor	Ames 26227	China	X
132	China_22	Tricolor	Ames 26228	China	X

Appendix 3.1b: (Continued)

No.	Label	Species	ID	Country origin	Quality of passport data
133	China_23	Tricolor	Ames 26229	China	X
134	China_24	Tricolor	Ames 26230	China	X
135	China_25	Tricolor	Ames 26231	China	X
136	Msia_2	Tricolor	Ames 29034	Malaysia	X
137	India_64	Tricolor	Ames 29035	India	✓
138	Brazil_1	Tricolor	Ames 29504	Brazil	X
139	Brazil_2	Tricolor	Ames 29505	Brazil	X
140	USA_9	Tricolor	NSL 6100	USA	X
141	India_65	Tricolor	PI 214036	India	✓
142	India_66	Tricolor	PI 277267	India	X
143	India_67	Tricolor	PI 277268	India	✓
144	India_68	Tricolor	PI 277269	India	X
145	PNG_2	Tricolor	PI 349553	Papua New Guinea	X
146	China_26	Tricolor	PI 419057	China	X
147	China_27	Tricolor	PI 419121	China	X
148	India_69	Tricolor	PI 462126	India	✓
149	India_70	Tricolor	PI 462127	India	X
150	India_71	Tricolor	PI 462128	India	X
151	India_72	Tricolor	PI 462129	India	✓
152	Unknown_10	Tricolor	PI 477918	Unknown	X
153	China_28	Tricolor	PI 478310	China	✓
154	China_29	Tricolor	PI 527321	China	✓
155	India_73	Tricolor	PI 566899	India	✓
156	India_74	Tricolor	PI 599683	India	X
157	USA_10	Tricolor	PI 603896	USA	X
158	USA_11	Tricolor	PI 603897	USA	X
159	USA_12	Tricolor	PI 603898	USA	X
160	USA_13	Tricolor	PI 603899	USA	X
161	Taiwan_5	Tricolor	PI 604668	Taiwan	X
162	Taiwan_6	Tricolor	PI 604669	Taiwan	✓
163	Thailand_2	Tricolor	PI 607446	Thailand	✓
164	India_75	Tricolor	PI 608761	India	X
165	India_76	Tricolor	PI 619252	India	X
166	USA_14	Tricolor	PI 632237	USA	X
167	Unknown_11	Tricolor	PI 633590	Unknown	X
168	Unknown_12	Tricolor	PI 633591	Unknown	X
169	Unknown_13	Tricolor	PI 633594	Unknown	X
170	Unknown_14	Tricolor	PI 633595	Unknown	X
171	Taiwan_7	Tricolor	PI 636179	Taiwan	X
172	India_77	Tricolor	PI 666331	India	✓
173	India_78	Tricolor	PI 667171	India	X
174	India_79	Tricolor	PI 667172	India	X
175	India_80	Tricolor	PI 669847	India	✓
176	HK_15	Tricolor	PI 674260	Hong Kong	✓
177	India_81	Tricolor	PI 674261	India	X
178	Unknown_15	Tricolor	PI 674262	Unknown	✓
179	Unknown_16	Tricolor	PI 674263	Unknown	X

Appendix 3.1c: Commercialised amaranth varieties

(i) From East-West Seed, Thailand, used as checks variety:

No.	Label	Variety name	Species	Commercialised region
1	EW-Thida	THIDA	*A. Tricolor*	Tanzania
2	EW-Zeya	ZEYA	*A. Tricolor*	Tanzania
3	EW-#20863	#20863	*A.cruentus*	Tanzania
4	EW-#20866	#20866	*A.cruentus*	Tanzania

(ii) Local Malaysian variety and Tanzanian landrace; used as checks variety, seed characterization, chlorophyll extraction and drought screening:

No.	Label	Source of seeds	Seeds characterization							Drought screening evaluation				
			Morphological assessment							Chapter 5 and 6 (Chlorophyll extraction)	Chapter 5		Chapter 6	
			Germination period (days)	Plant height (cm)	Plant diameter (cm)	No. of leaves	Growth habit	Stem pubescence	Leaf pubescence		Exp. I (Label)	Exp. II (Label)	Trial I (Label)	Trial II (Label)
Local Malaysian Variety *(Amaranthus tricolor)*														
1	Red Amaranth (1)	NING DE AGRICULTURAL & SEEDS LTD	6	17	2	22	5	No	No	✓	C2			
2	Red Amaranth (2)	BAJA SERBAJADI	6	11	2	19	5	No	No	✓	C3	RA	Local Red	Local Red
3	Red Amaranth (3)	SYARIKAT PERTANIAN KAGAYAKI SDN. BHD	6	17	2	19	6	No	No	✓				
4	Red Amaranth (4)	KNOWN-YOU SEED CO., LTD	3	25	3	31	5	No	No	✓				
5	Red Amaranth (5)	MINARA SEEDS PTY LTD	3	27	2	37	4	No	No	✓				
6	Round Leaf Green Amaranth (2)	NING DE AGRICULTURAL & SEEDS LTD	3	9	2	27	4	No	No	✓	D3			
7	Round Leaf Green Amaranth (3)	KNOWN-YOU SEED CO., LTD	6	21	2	20	5	No	No	✓	D2			
8	Perfect Red Amaranth (1)	GREEN WORLD	6	22	2	22	5	No	No	✓				
9	Perfect Red Amaranth (2)	BAJA SERBAJADI	6	13	3	23	5	No	No	✓	C1	PR	Local PR	Local PR
10	Pointed Leaf Green Amaranth (1)		6	8	2	11	4	No	No	✓				
11	Pointed Leaf Green Amaranth (2)	BAJA SERBAJADI	6	21	3	36	4	No	No	✓				
12	Pointed Leaf Green Amaranth (3)	GREEN WORLD	6	13	3	38	4	No	No	✓	D1			
Tanzanian Landrace (*Amaranthus cruentus*)														
13	Black-seeded amaranth		3	34	2	11	4	Yes	Yes	✓	B1			
14	White-seeded amaranth		3	31	2	13	4	Yes	Yes	✓	B2			
15	Mixed-seeded amaranth		3	28	2	13	4	Yes	Yes	✓	B3			

Appendix 3.2: Characterization traits on 32 morphological descriptors (22 qualitative and 10 quantitative data) provided through publicly available The World Vegetable Center Genebank passport and characterizations data (http://seed.worldveg.org/).

QUALITATIVE TRAITS			
Plant parts	**No.**	**Traits**	**Description**
Plant architecture	1	Branching index	1. Branching all along the stem; 2. Few branches (all near the base of the stem); 3. No branches
	2	Growth habit	1. Erect; 2. Prostrate
	3	Sex type	1. Monoecious; 2. Dioecious; 3. Polygamous
Leaf	4	Leaf margin	1. Crenate; 2. Entire; 3. Mixture (entire and undulate); 4. Undulate
	5	Leaf pigmentation	1. Basal area pigmented; 2. Central spot; 3. Dark green; 4. Entire lamina purple or pink; 5. Margin and vein pigmented; 6.Mixture; 7. Normal green;8. One pale green or chlorotic stripe on normal green; 9. One stripe (V-shaped); 10. Others; 11. Two-stripes
	6	Leaf pubescence	1. Conspicuous; 2. Low; 3. None
	7	Leaf shape	1. Cuneate; 2. Elliptical; 3. Lanceolate; 4. Mixture; 5. Obovate; 6. Other; 7. Oval; 8. Ovatainate; 9. Rhombic
	8	Petiole pigmentation	1. Dark green; 2. Dark purple; 3. Green; Mixture (green and purple); 4. Purple; 5. White
	9	Prominence of leaf veins	1. Rugose (veins prominent); 2. Smooth
	10	Spines in leaf axils	1. Absent; 2. Present
Stem	11	Stem pigmentation	1. Green; 2. Mixture (green and pink); 3. Purple or pink; 4. White
	12	Stem pubescence	1. Conspicuous; 2. Low; 3. None
Inflorescence	13	Inflorescence color	1. Green; 2. Mixture (green and pink); 3. Other; 4. Pink; 5. Red; 6. Yellow
	14	Inflorescence density index	1. Dense; 2. Intermediate; 3. Lax; 4. Mix (dense and intermediate)
	15	Presence of axillary inflorescence	1. Absent; 2. Present
	16	Terminal inflorescence attitude	1. Drooping; 2. Erect
	17	Terminal inflorescence shape	1. Club-shaped at tips; 2. Other; 3. Panicle with long branches; 4. Panicle with short branches; 5. Spike (dense)
	18	Seed coat type	1.Mixture; 2. Opaque 3. Translucent
	19	Seed color	1. Black; 2. Brown; 3. Mixture (pale yellow and black); 4. Mixture (pale yellow and pink); 5. Pale yellow
	20	Seed shape	1. Ellipsoid or ovoid; 2. Round
	21	Seed shattering	1. High (>50%); 2. Intermediate (10-50%); Low (<10%)
Vegetable production	22	Germination rate	1. Rapid (<2 days); 2. Slow (2-7 days); 3. Very slow (>7 days)
QUANTITATIVE TRAITS			
Plant architecture	1	Mean length of basal lateral branches (cm)	
	2	Mean length of top lateral branches (cm)	
	3	Plant height (cm)	At flowering stage
Leaf	4	Leaf length (cm)	On 6th or 8th leaf
	5	Leaf width (mm)	On 6th or 8th leaf
Inflorescence	6	Length of axillary inflorescence (cm)	
	7	Terminal inflorescence stalk length (cm)	
	8	Terminal inflorescence laterals length (cm)	
Vegetable production	10	Days to flowering	
	11	1000-seed weight (g)	

Appendix 3.3: Characterization traits for six qualitative descriptors provided through the publicly available USDA Genebank passport and characterizations data. (https://npgsweb.ars-grin.gov/gringlobal/search.aspx).

QUALITATIVE TRAITS	
Traits	**Descriptive**
Stem pigmentation	1. (GR) Green; 2. Mix; 3. (PS) Pink base and pink stem; 4: (RB) Green stem with red or darker base; 5. (RD) Red or darker stem with solid colouring, can have pink or red base; 6. (ST) Amaranthine stripes on stem, can have pink or red base
Leaf pigmentation	1. (BP) Basal area pigmented; 2. (CD) Cholorophyll deficient, pale marks, that can be white, yellow, orange, pink or red; 3. (CS) Central spot; 4. (GN) Normal green; 5. Mix; 6. (RD) Entire lamina amaranthine (purple to pink); 7. (RV) Margin and vein pigmented; 8. (SE) Sectoring, patches that radiate away from the midvein; 9. (SP) Speckled
Inflorescence shape	1. (AT) In leaf axils and terminal; 2. (AX) Mostly in leaf axils; 3. Mix
Seed colour	1. (BE) Brown edges with black sides; 2. (BL) Black seed coat; 3. (EQ) Utricle splits at the equator; 4. (GN) Green; 5: Other
Seed shape	1. (EB) Ellipsoid or ovoid with rounded bulging perisperm; 2. (RB) Round, with rounded bulging perisperm

Appendix 3.4: Selection of accessions of Group 9 (A. *tricolor* AVRDC Genebank) to be included in the core set. Accessions in bold were selected for core set and entry description is presented in Appendix 3.1a.

Cluster 1	Cluster 2	Cluster 3		Cluster 4
TRI 1-Bangla 1	TRI 2-Bangla 2	TRI 4-Bangla 5	TRI 59-Cambo 72	TRI 30-Bangla 35
TRI 75-Indo 89	TRI 15-Bangla 16	TRI 17-Bangla 18	TRI 61-Cambo 74	**TRI 143-Viet 165**
TRI 87-Msia 102	TRI 12-Bangla 13	TRI 16-Bangla 17	**TRI 63-Cambo 76**	**TRI 46-Bangla 59**
TRI 88-Msia 103	TRI 19-Bangla 21	TRI 34-Bangla 41	TRI 65-Cambo 78	**TRI 226-Philip 137**
	TRI 20-Bangla 22	TRI 35-Bangla 42	TRI 60-Cambo 73	**TRI 120-Philip 136**
	TRI 40-Bangla 49	**TRI 42-Bangla 54**	TRI 8-Bangla 9	TRI 47-Bangla 60
	TRI 31-Bangla 37	TRI 23-Bangla 25	TRI 9-Bangla 10	TRI 49-Bangla 62
	TRI 41-Bangla 50	TRI 214-Bangla 32	TRI 10-Bangla 11	**TRI 51-Bangla 64**
	TRI 3-Bangla 4	TRI 140-Viet 158	**TRI 43-Bangla 56**	TRI 50-Bangla 63
	TRI 21-Bangla 23	**TRI 224-Japan 100**	**TRI 66-China 79**	TRI 48-Bangla 61
	TRI 22-Bangla 24	TRI 144-Viet 166	**TRI 228-Viet 161**	TRI 56-Bangla 69
	TRI 33-Bangla 39	TRI 73-Indo 87	**TRI 71-India 85**	TRI 52-Bangla 65
	TRI 39-Bangla 47	TRI 76-Indo 90	TRI 90-Msia 105	TRI 53-Bangla 66
	TRI 24-Bangla 26	TRI 77-Indo 91	TRI 102-Msia 117	**TRI 54-Bangla 67**
	TRI 36-Bangla 43	**TRI 83-Indo 97**	TRI 136-Unk 154	TRI 55-Bangla 68
	TRI 37-Bangla 45	**TRI 84-Indo 98**	**TRI 91-Msia 106**	**TRI 57-Bangla 70**
	TRI 11-Bangla 12	TRI 131-Thai 149	TRI 98-Msia 113	**TRI 223-India 80**
	TRI 221-Bangla 53	TRI 79-Indo 93	TRI 103-Msia 118	
	TRI 211-Bangla 20	TRI 80-Indo 94	**TRI 104-Msia 119**	
	TRI 216-Bangla 40	TRI 81-Indo 95	**TRI 69-India 83**	
	TRI 32-Bangla 38	TRI 82-Indo 96	TRI 111-Msia 127	
	TRI 217-Bangla 44	**TRI 85-Indo 99**	TRI 62-Cambo 75	
	TRI 38-Bangla 46	**TRI 137-Viet 155**	**TRI 86-Laos 101**	
	TRI 218-Bangla 48	TRI 112-Msia 128	TRI 105-Msia 121	
	TRI 220-Bangla 52	**TRI 138-Viet 156**	TRI 110-Msia 126	
	TRI 28-Bangla 33	**TRI 123-Taiwan 140**	TRI 70-India 84	
	TRI 29-Bangla 34	TRI 130-Thai 148	TRI 113-Msia 129	
	TRI 219-Bangla 51	**TRI 229-Viet 162**	**TRI 114-Msia 130**	
	TRI 18-Bangla 19	TRI 67-India 81	**TRI 125-Taiwan 142**	
	TRI 74-Indo 88	**TRI 116-Nigeria 132**	**TRI 128-Thai 146**	
	TRI 142-Viet 160	**TRI 133-USA 151**	**TRI 129-Thai 147**	
	TRI 141-Viet 159	**TRI 115-Msia 131**		
	TRI 27-Bangla 29	TRI 89-Msia 104		
	TRI 222-Bangla 55	TRI 95-Msia 110		
	TRI 5-Bangla 6	**TRI 68-India 82**		
	TRI 6-Bangla 7	TRI 93-Msia 108		
	TRI 7-Bangla 8	TRI 94-Msia 109		
	TRI 25-Bangla 27	**TRI 135-USA 153**		
	TRI 212-Bangla 30	**TRI 96-Msia 111**		
	TRI 14-Bangla 15	**TRI 72-India 86**		
	TRI 26-Bangla 28	**TRI 92-Msia 107**		
	TRI 44-Bangla 57	TRI 100-Msia 115		
	TRI 124-Taiwan 141	TRI 101-Msia 116		
	TRI 132-Turkey 150	TRI 99-Msia 114		
	TRI 215-Bangla 36	TRI 97-Msia 112		
	TRI 106-Msia 122	**TRI 117-Pakistan 133**		
	TRI 107-Msia 123	**TRI 13-Bangla 14**		
	TRI 58-Bangla 71	**TRI 64-Cambo 77**		
	TRI 118-Papua 134	**TRI 108-Msia 124**		
	TRI 134-USA 152	**TRI 230-Viet 163**		
	TRI 210-Bangla 3	TRI 231-Viet 164		
	TRI 45-Bangla 58	TRI 109-Msia 125		
	TRI 78-Indo 92	**TRI 119-Papua 135**		
	TRI 139-Viet 157	**TRI 227-Thai 144**		
	TRI 213-Bangla 31	**TRI 127-Thai 145**		
	TRI 225-Msia 120	TRI 121-Taiwan 138		
	TRI 126-Thai 143	**TRI 122-Taiwan 139**		

Appendix 3.5: Selection of accessions of Group 10 (*A. tricolor* USDA Genebank) to be included in the core set. Accessions in bold were selected for core set and entry description is presented in Appendix 3.1b.

Cluster 1	Cluster 2		Cluster 3	Cluster 4			Cluster 5	Cluster 6
Bangladesh_1 1 ..	**Msia_1 131 ..))**	India_14 61 ..)))	**Brazil_1 2 ..)**	China_3 6 ..)))	HK_4 36 ..)))	**Madagascar_1 130 .....).....)..)**	Unknown_16 161 ..))	India_19 66 ..))
China_11 14 ..)	India_20 67 ..))	India_15 62 ..)))	**India_38 85 ..))**	China_6 9 ..))))	**India_55 102 ..)..))**	**China_1 4 ..)**	USA_6 167 ..))	India_48 95 ..)))
India_7 54 ..)	India_24 71 ..))	India_16 63 ..)))	India_45 92 ..))	China_7 10 ..))))	India_6 53 ..)))	China_29 32 ..))	USA_7 168 ..))	Taiwan_6 142 ..)))
India_17 64 ..)	**India_54 101 ..))**	India_18 65 ..)))	Taiwan_5 141 ..))	China_8 11 ..))))	India_10 57 ..))))	China_15 18 ..))	USA_9 170 ..))	**India_39 86 ..)))**
India_78 125 ..)	India_74 121 ..))	India_22 69 ..)))	**USA_11 172 ..))**	China_10 13 ..))))	India_11 58 ..))))	HK_1 33 ..))	China_20 23 ..))	**Taiwan_3 139 ..)..))**
India_80 127 ..).	USA_2 163 ..))	India_31 78 ..)))	India_66 113 ..))	China_12 15 ..))))	India_12 59 ..))))	HK_2 34 ..))	India_1 48 ..))	India_70 117 .....)..).....)…
India_76 123 .....)	USA_3 164 ..))	India_34 81 ..)))	Unknown_10 155 ..))	China_13 16 ..))))	India_21 68 ..))))	HK_3 35 ..))	India_3 50 ..))	
India_5 52 ..)	USA_5 166 ..))	**India_41 88 ..)))**	**USA_12 173 ..)..)**	China_14 17 ..))))	India_23 70 ..))))	HK_5 37 ..))	**Indonesia_1 129 ..))**	
	USA_13 174 ..).....)	India_43 90 ..)))	India_64 111 .....))	China_16 19 ..))))	**India_26 73 ..))))**	HK_8 40 ..))	Unknown_1 146 ..).....)	
	India_75 122 ..))	India_46 93 ..)))	China_9 12 .....))	China_19 22 ..))))	**India_28 75 ..))))**	HK_9 41 ..))	**HK_7 39 ..))**	
	USA_8 169 ..)))	India_50 97 ..)))	**Brazil_2 3 ..))**	China_22 25 ..))))	India_29 76 ..))))	HK_10 42 ..))	**India_40 87 ..)))**	
	PNG_2 134 ..)))	India_51 98 ..)))	China_5 8 ..)))	China_23 26 ..))))	India_30 77 ..))))	HK_12 44 ..))	Unknown_3 148 ..)))	
	USA_10 171 ..).....))	India_53 100 ..)))	China_26 29 ..)))	HK_11 43 ..))))	India_35 82 ..))))	**HK_14 46 ..))**	Unknown_11 156 ..)))	
	**India_9 56 ..))) **	India_56 103 ..)))	**China_28 31 ..)..)..)**	**HK_13 45 ..))))**	India_36 83 ..))))	India_4 51 ..))	Unknown_13 158 ..).....)	
	India_67 114 ..)))	India_57 104 ..)))	India_37 84 ..))	**HK_15 47 ..)..)))**	India_42 89 ..))))	**India_25 72 ..))**	China_25 28 ..))	
	India_69 116 ..)))	India_58 105 ..)))	India_79 126 ..)))	China_17 20 .....))))	India_49 96 ..))))	**India_27 74 ..))**	Unknown_9 154 ..).....)	
	India_72 119 ..)))	India_59 106 ..)))	Unknown_7 152 ..)..))	India_2 49 ..)))))	India_52 99 ..))))	**Taiwan_2 138 ..))**		
	India_81 128 ..)))	**PNG_1 133 ..)))**	India_65 112 .....)..)..)	India_71 118 ..)))))	India_61 108 ..))))	Taiwan_4 140 ..))		
	Taiwan_1 137 ..)))	**PR_2 136 ..)..))**	China_2 5 ..))	USA_1 162 ..)))))	India_62 109 ..))))	Unknown_2 147 ..))		
	Taiwan_7 143 ..)))	India_32 79 ..)))	China_27 30 ..)))	**Zaire_1 177 ..)..)..)))**	**PR_1 135 ..))))**	Unknown_4 149 ..))		
	Thailand_1 144 ..)))	**Msia_2 132 ..)..)))**	Unknown_8 153 ..)))	China_4 7 .....)))	Unknown_5 150 ..))))	Unknown_6 151 ..))		
	Thailand_2 145 ..)))	India_73 120 .....)..)..)..	Unknown_14 159 ..)))	**India_8 55 ..))) **	Unknown_15 160 ..))))	**USA_4 165 ..))**		
	Unknown_12 157 ..)))		**USA_14 175 ..)))**	India_33 80 ..))))	**WA_1 176 ..)..)))**	China_24 27 ..))		
	Unknown_12 157 ..)))		China_18 21 ..)))	**India_47 94 ..))))**	India_60 107 .....)))	India_44 91 ..))		
	India_13 60 ..)))		HK_6 38 ..).....))	India_77 124 ..).....)..))	India_63 110 .....)))	India_68 115 ..))		
				China_21 24 ..))				

Appendix 3.6: Frequency distribution (%) of 22 qualitative traits of individual amaranth species in AVRDC Genebank. Figures in parenthesis are the frequency distribution (%) of traits representing the core set. The comparison of characteristic variations (%) in whole set (Wset) and core set (Cset) for each morphological trait are also presented in these tables.

atr: A. atropurpureus, blt: A. blitoides, leu: A. leucorcapus, gra: A. gracilis, grz: A. graecizans, hyb: A. hybridus, man: A. mantegazzianus, pal: A. palmeri, ret: A. retroflexus, thu: A. thunbergii, bli: A. blitum, cru: A. cruentus, dub: A. dubius, hyp: A. hypochondriacus, sp: A.sp. spi: A. spinosus, vir: A. viridis, tri: A. tricolor

No.	PLANT ARCHITECTURE	*atr*	*blt*	*leu*	*gra*	*grz*	*hyb*	*man*	*pal*	*ret*	*thu*	*bli*	*cru*	*dub*	*hyp*	*sp*	*spi*	*vir*	*tri*	Wset	Cset
1	**Branching index**																				
	1. Along the stem	100		100	90	10	10	100		50	90	31 (13)	78 (33)	92 (14)	65 (9)	48 (8)	82 (7)	44 (5)	51 (24)	57	18
	2. Few branches		100				90		100	25	10	38 (13)	11 (6)		26 (12)		7	2 (2)	35 (13)	20	9
	3. Many branches				10	90				25		31 (13)	11 (6)	8 (3)	9 (3)	52 (20)	11 (7)	54 (4)	13 (6)	22	7
	4. No branches																		2 (1)	1	1
2	**Growth habit**																				
	1. Erect	100		100	100	100	90	100	100	100	100	82 (35)	100 (44)	100 (17)	100 (24)	100 (28)	96 (14)	32 (9)	98 (40)	88	32
	2. Prostate		100				10					18 (6)					4 (0)	68 (2)	2 (0)	12	1
3	**Sex type**																				
	1. Monoecious	100	100	100	100	100	100	100	100	100	100	94 (41)	89 (39)	97 (17)	100 (24)	88 (20)	93 (8)	96 (9)	98 (38)	96	31
	2. Polygamous											6 (0)	11 (6)	3 (0)		13 (4)	7 (8)	4 (2)	2 (2)	4	2

Appendix 3.6: (Continued)

No.	LEAF TRAITS	*atr*	*bli*	*leu*	*gra*	*grz*	*hyb*	*man*	*pal*	*ret*	*thu*	*blt*	*cru*	*dub*	*hyp*	*sp*	*spi*	*vir*	*tri*	Wset	Cset
4	**Leaf margin**																				
	1. Crenate														7 (0)			5 (0)	2 (2)	2	1
	2. Entire	100	100	100	100			90	10		100	65 (18)	50 (22)	71 (8)	25 (14)	68 (12)	28 (6)	67 (7)	62 (20)	57	17
	3. Mixture																		1 (1)	0	0
	4. Undulate						100	10	90	100		35 (24)	50 (22)	29 (17)	68 (14)	32 (6)	72 (6)	28 (4)	35 (17)	41	15
5	**Leaf pigmentation**																				
	1. Basal area pigmented														3 (0)				4 (1)	2	1
	2. Central spot									25			11 (6)	3 (3)	3 (3)	4 (4)			13 (7)	7	4
	3. Dark green													39 (6)		4 (4)		2 (0)	1 (0)	4	1
	4. Lamina purple or pink										90	6 (6)	6 (6)		9 (3)	29 (13)			25 (8)	14	5
	5. Margin, vein pigmented									25	10	6 (0)	17 (6)		21 (9)				9 (1)	7	1
	6. Mixture																7 (0)		4 (0)	2	0
	7. Normal green	100	100		100	100	100	100	100	25		71 (29)	61 (22)	58 (8)	59 (9)	50 (13)	86 (11)	98 (11)	30 (13)	55	10
	8. Chlorotic stripe			100						25		6 (6)	6 (6)		6 (0)				6 (0)	4	1
	9. One stripe (V-shaped)																7 (4)		7 (4)	4	2
	10. Others											12				13 (4)			1 (1)	2	1
	11. Two stripes (V-shaped)																		2 (2)	1	0
6	**Leaf pubescence**																				
	1. Conspicuous					33									6 (0)				1 (1)	1	0
	2. Low		100		10	33					10	35 (12)	33 (17)	17 (0)	12 (6)		11 (4)	7 (0)	19 (8)	16	6
	3. None	100		100	90	33	100	100	100	100	90	65 (29)	67 (28)	83 (17)	82 (18)	100 (28)	89 (11)	93 (11)	81 (31)	83	25
7	**Leaf shape**																				
	1. Cuneate		100			33						18 (6)				4 (4)			1 (1)	2	1
	2. Elliptical			100						25		18 (12)	33 (6)	36 (6)	15 (9)	33 (4)	7 (0)	34	16 (6)	21	5
	3. Lanceolate	100				33	10	100		25	33	12	44 (6)	14 (6)	71 (12)	17 (4)	82 (7)		10 (5)	22	5
	4. Mixture																		4 (2)	2	1
	5. Obovate					33						12 (6)			3 (0)				1 (1)	1	1
	6. Other												6 (0)			13 (4)			9 (4)	5	2
	7. Oval											6 (6)							3 (2)	2	3
	8. Ovatainate				90		90		100	25	33	35 (12)	17 (6)	44 (6)	12 (3)	4		57 (7)	19 (7)	26	6
	9. Rhombic				10					25	33			6 (0)		29 (8)	11 (7)	9 (4)	37 (11)	21	5

Appendix 3.6: (Continued)

No.	LEAF TRAITS	*atr*	*bli*	*leu*	*gra*	*grz*	*hyb*	*man*	*pal*	*ret*	*thu*	*blt*	*cru*	*dub*	*hyp*	*sp*	*spi*	*vir*	*tri*	Wset	Cset
8	**Petiole pigmentation**																				
	1. Dark green													3 (3)		25 (0)			8 (4)	5	1
	2. Dark purple											18 (6)								1	0
	3. Green	100	100	100	100		90	100	100	50		24 (12)	78 (22)	58 (3)	74 (12)	58 (4)	86 (14)	74 (7)	42 (19)	56	14
	4. Mixture					33						6 (6)							2 (1)	2	0
	5. Purple					33	10			50	100	29 (6)	22 (22)	39 (11)	26 (12)	17 (21)	14 (0)	26 (4)	45 (33)	34	8
	6. White					33						24 (12)							3 (2)	3	1
9	**Prominence of leaf veins**																				
	Rugose	100	100	100	100	100	100	100	100	100	100	100 (41)	100 (44)	100 (17)	100 (24)	100 (25)	100 (14)	95 (11)	100 (41)	99	33
	Smooth																	5 (0)		1	
10	**Spines in leaf axils**																				
	Absent		100		100	100	100	100	100	100	100	100 (41)	100 (44)	100 (17)	100 (24)	92 (28)	11 (4)	100 (11)	100 (43)	93	32
	Present	100														8 (0)	89 (11)			7	1
No.	**STEM TRAITS**																				
11	**Stem pigmentation**																				
	Green			100	100			100		25		24 (6)	56 (22)	58 (11)	56 (6)	40 (20)	54 (7)	9 (2)	39 (18)	38	1
	Mixture											6 (6)							2 (1)	1	3
	Purple or pink		100			100	100		100	75	100	65 (24)	44 (22)	42 (6)	44 (18)	60 (8)	46 (7)	91 (9)	60 (20)	60	5
	White											6 (6)								0	0
12	**Stem pubescence**																				
	Conspicuous						10						33 (17)		26 (6)				4 (2)	6	2
	Low		100		10	100	90	100				18 (6)	44 (11)	25 (6)	24 (6)	8 (4)	21 (0)	16 (2)	36 (16)	27	11
	None	100		100	90					100	100	82 (35)	22 (17)	75 (11)	50 (12)	92 (24)	79 (14)	84 (9)	61 (21)	67	20

Appendix 3.6: (Continued)

No.	INFLUORESCENCE TRAITS	*atr*	*bli*	*leu*	*gra*	*grz*	*hyb*	*man*	*pal*	*ret*	*thu*	*blt*	*cru*	*dub*	*hyp*	*sp*	*spi*	*vir*	*tri*	Wset	Cset
13	**Inflorescence colour**																				
	Green	100		100	10	10	90		100	50		53 (24)	50 (17)	89 (14)	59 (6)	45 (14)	96 (14)	12 (4)	67 (29)	59	20
	Mixture												17 (11)	3			4 (0)			1	1
	Other														3 (0)	18 (5)		16 (2)	4 (2)	5	1
	Pink					90	10				100	6 (0)	22 (11)	6 (3)	9 (6)	5 (0)		67 (5)	13 (1)	19	4
	Red		100							50		24 (12)			29 (12)	32 (5)			15 (7)	12	6
	Yellow				90			100				18 (6)	11 (6)	3 (0)		5 (5)		5 (0)	1 (1)	4	2
14	**Inflorescence density**																				
	Dense					100	100				90	65 (12)	78 (28)	36 (0)	35 (18)	38 (8)	75 (11)	68 (4)	37 (17)	48	9
	Intermediate	100		100	100					25	10	35 (29)	21 (11)	42 (8)	41 (0)	21 (8)	18 (4)	23 (7)	19 (10)	25	11
	Lax								100	75			33 (6)	22 (8)	24 (6)	42 (8)	7 (0)	9 (0)	44 (13)	27	11
	Mix (Dense, Intermediate)		100					100											1 (1)	0	0
15	**Axillary inflorescence**																				
	Absent		100		100	100			100	100	10	53 (29)	41 (18)	75 (11)	62 (12)	63 (13)	46 (11)	72 (5)	81 (32)	71	23
	Present			100				100			90	47 (12)	59 (29)	25 (6)	38 (12)	38 (13)	54 (4)	39 (5)	19 (9)	29	10
16	**Terminal inflorescence attitude**																				
	Drooping				10						10	24 (6)		31 (6)	12 (6)	25 (8)	64 (4)	4 (2)	15 (7)	18	6
	Erect	100	100	100	90			100	100	100	20	76 (35)	100 (47)	69 (11)	88 (18)	75 (17)	36 (11)	96 (9)	85 (34)	82	27
17	**Terminal inflorescence shape**																				
	Club-shaped at tips							100									4 (0)		3 (1)	1	1
	Other					33						6 (6)				13 (4)			5 (1)	3	1
	Panicle with long branches			100									29 (12)	8 (0)	32 (0)	4 (0)		2 (0)	5 (1)	8	3
	Panicle with short branches	100				33						24 (12)	53 (24)	56 (8)	38 (15)	42 (18)	79 (11)	89 (11)	7 (2)	37	7
	Spike (dense)		100			33			100	100	100	71 (24)	18 (12)	36 (8)	29 (9)	42 (13)	18 (4)	9 (0)	81 (34)	51	21

Appendix 3.6: (Continued)

No.	SEED TRAITS	*atr*	*bli*	*leu*	*gra*	*grz*	*hyb*	*man*	*pal*	*ret*	*thu*	*blt*	*cru*	*dub*	*hyp*	*sp*	*spi*	*vir*	*tri*	Wset	Cset
18	Seed coat type																				
	Mixture														3					0	0
	Opaque					10						31 (6)	74 (37)	13 (3)	17 (0)	20 (4)	15 (0)	80 (6)	22 (10)	31	8
	Translucent	100	100	100	100	90		100	100	100	100	69 (31)	26 (5)	87 (16)	80 (27)	80 (24)	85 (15)	20 (6)	78 (30)	69	26
19	Seed colour																				
	Black	100	100		10	100			100	34		94 (38)	11 (11)	90 (19)	30 (7)	84 (20)	89 (11)	78 (7)	80 (31)	74	24
	Brown				90							6 (0)	44 (6)	10 (0)	37 (10)	12 (8)	11 (4)	22 (4)	20 (9)	20	7
	Mixture (pale yellow, black)												6 (6)		3 (0)					1	0
	Mixture (pale yellow, pink)												6 (0)		3 (0)					1	0
	Pale yellow			100				100		66			33 (22)		27 (10)	4 (0)				5	3
20	Seed shape																				
	Ellipsoid or ovoid	100	100	100	100	100			100	100	100	100 (38)	100 (42)	94 (16)	83 (17)	68 (20)	93 (11)	100 (11)	92 (37)	92	31
	Round							100						6 (3)	17 (10)	32 (8)	7 (4)		8 (3)	8	3
21	Seed shattering																				
	High (>50%)		100		90	20					10	31 (13)	79 (37)	26 (6)	67 (20)		44 (0)	6 (0)	24 (8)	29	10
	Intermediate (10-50%)				10	80		100	100	100	90	44 (19)	11 (0)	68 (13)	27 (7)	80 (16)	22 (4)	56 (6)	66 (27)	55	18
	Low (<10%)	100		100								25 (6)	11 (5)	6 (0)	7 (0)	20 (12)	33 (11)	39 (6)	9 (4)	16	5
22	**VEGETABLE PRODUCTIONS**																				
	Germination rate																				
	Rapid					100								94 (14)	12 (0)	8 (4)		91 (7)	2 (1)	25	2
	Slow	100	100	100	100			100	100		100	100 (41)	100 (42)	6 (3)	88 (24)	84 (20)	100 (14)	9 (4)	98 (40)	75	30
	Very slow															8 (4)				0	1

Appendix 3.7: Mean and range of 10 quantitative traits of individual amaranth species in AVRDC Genebank and selected core set**.**

			Whole set		Core set					Whole set		Core set	
No.	**QUANTITATIVE TRAITS**	**Species**	**Mean**	**Range**	**Mean**	**Range**	**No.**	**QUANTITATIVE TRAITS**	**Species**	**Mean**		**Mean**	**Min**
1	Mean length of basal lateral branch (cm)	*atr*	45.5		45.5		3	Leaf length (cm)	*atr*	5.9		5.9	
		Blt	59		59				*Blt*	4.4		4.4	
		Leu	50.5		50.5				*Leu*	7.5		7.5	
		Gra	37.5		37.5				*Gra*	16.9	11.0 - 19.5	19.1	
		Grz	35.8	21.3 - 45.5	35.8	21.3 - 45.5			*Grz*	7.3	5.9 - 8.8	7.3	5.9 - 8.8
		Hyb	36.9	28.8 - 49.7	36.9	28.8 - 49.7			*Hyb*	7.0	5.5 - 9.4	7.0	5.5 - 9.4
		Man	20		20				*Man*	7.7		7.7	
		Pal	1.1		1.1				*Pal*	2.5		2.5	
		Ret	9.1	3.6 - 20.5	9.1	3.6 - 20.5			*Ret*	8.4	6.1 - 13.8	8.4	6.1 - 13.8
		Thu	36.2	21.0 - 66.0	36.2	21.0 - 66.0			*Thu*	11.4	7.3 - 15.8	11.4	7.3 - 15.8
		Bli	39.3	3.5 - 67.9	31.2	3.5 - 45.0			*Bli*	7.4	3.2 - 9.5	8.3	6.5 - 9.5
		Cru	27.6	0.6 - 79	20.8	0.9 - 59.1			*Cru*	18.3	7.5 - 39.1	15.7	7.5 - 21.4
		Dub	18	0.6 - 80.5	6.0	1 - 11.1			*Dub*	16.8	7.9 - 28.0	13.5	10.0 - 15.8
		Hyp	15.3	0.4 - 66.1	24.0	4.1 - 59.2			*Hyp*	17.4	7.7 - 34.5	13.5	9.3 - 21.4
		Sp	40	2.6 - 81	34.1	2.7 - 72.3			*Sp*	8.8	5.4 - 12.5	7.9	6.9 - 9.4
		Spi	21.7	2.0 - 69.0	23.9	4.1 - 44.2			*Spi*	12.6	5.0 - 23.6	12.6	5.0 - 21.7
		Vir	39.7	2.4 - 84.0	40.2	10.0 - 84.0			*Vir*	7.4	3.6 - 24.3	8.4	4.4 - 19.4
		Tri	15.9	0.6 - 73.4	20.3	1.0 - 73.4			*tri*	12.6	2.2 - 26.7	12.7	2.2 - 23.8
2	Mean length of top lateral branch (cm)	*atr*	2.4		2.4		4	Leaf width (cm)	*atr*	3.4		3.4	
		Blt	12.8		12.8				*blt*	1.6		1.6	
		Leu	29.8		29.8				*leu*	4.3		4.3	
		Gra	3.3	3.0 -3.8	3.8				*gra*	12.6	6.0 -16.0	14.9	
		Grz	3.1	2.6 - 3.5	3.1	2.6 - 3.5			*grz*	4.3	3.0 - 6.3	4.3	3.0 - 6.3
		Hyb	13.0	1.9 - 26.6	13.0	1.9 - 26.6			*hyb*	4.4	3.1 - 6.3	4.4	3.1 - 6.3
		man	11.9		11.9				*man*	4.3		4.3	
		pal	1		1				*pal*	1.6		1.6	
		ret	4.2	3.1 - 5.1	4.2	3.1 - 5.1			*ret*	4.6	3.1 - 7.8	4.6	3.1 - 7.8
		thu	6.1	5.3 - 7.5	6.1	5.3 - 7.5			*thu*	7.4	4.1 - 10.8	7.4	4.1 - 10.8
		bli	5.5	0.3 - 30.0	2.0	0.3 - 3.5			*bli*	5.3	3.2 - 8.8	6.0	3.9 - 8.8
		cru	14.4	0.8 - 33.8	11.7	2.9 - 33.8			*cru*	8.7	6.1 - 14.2	8.3	6.1 - 14.2
		dub	7.9	0.7 - 37.0	5.1	0.8 - 15.9			*dub*	11.8	5.9 - 18.8	9.8	7.3 - 10.8
		hyp	11.0	1.1 - 38.7	14.4	2.0 - 32.5			*hyp*	8.4	3.9 - 14.3	7.0	5.1 - 10.3
		sp	7.1	0.9 - 30.2	5.9	0.9 - 10.8			*sp*	4.7	2.9 -7.0	4.3	2.9 - 5.1
		spi	11.3	1.0 - 31.0	12.0	1.0 - 25.7			*spi*	8.6	3.3 - 15.9	8.7	3.3 - 15.9
		vir	9.3	0.8 - 27.7	10.5	2 - 20.5			*vir*	5.5	2.7 - 16.8	6.0	3.3 - 13.5
		tri	4.9	0.3 - 26.0	6.1	0.3 - 26.0			*tri*	8.5	1.3 - 17.3	8.5	1.6 - 14.2

Appendix 3.7: (Continued)

			Whole set		Core set					Whole set		Core set	
No.	**QUANTITATIVE TRAITS**	**Species**	**Mean**	**Range**	**Mean**	**Range**	**No.**	**QUANTITATIVE TRAITS**	**Species**	**Mean**		**Mean**	**Min**
5	Length of axillary inflorescence (cm)	*atr*	5.2		5.2		7	Terminal inflorescence laterals length (cm)	*atr*	12.7		12.7	
		blt							*blt*				
		leu	11.5		11.5				*leu*	38.3		38.3	
		gra							*gra*	7.5	1.6 - 12.0	10.0	
		grz							*grz*	0.7		0.7	
		hyb	8.2	3.1 - 13.3	8.2	3.1 - 13.3			*hyb*	4.6	1.4 - 10.0	4.6	1.4 - 10.0
		man	3.8		3.8				*man*	7.4		7.4	
		pal							*pal*	4.6		4.6	
		ret							*ret*	5.3	3.7 - 6.7	5.3	3.7 - 6.7
		thu	10.4	5.3 - 15.5	10.4	5.3 - 15.5			*thu*	3.7	1.5 - 5.0	3.7	1.5 - 5.0
		bli	6.7	4.2 - 14.0	9.9	5.7 - 14.0			*bli*	6.0	0.5 -25.0	7.4	1.1 - 25.0
		cru	8.2	1.5 - 10.1	7.4	1.5 - 10.1			*cru*	12.4	0.6 - 30.2	13.3	0.6 - 20.6
		dub	10.1	7.4 - 13.5	10.0	7.8 - 12.2			*dub*	16.1	0.8 - 39.1	11.5	0.8 - 33.2
		hyp	14.1	1.0 - 23.8	12.2	1.0 - 19.3			*hyp*	16.0	2.0 - 44.9	12.7	2.0 - 24.4
		sp	6.8	4.0 - 9.5	6.1	5.3 - 7.3			*sp*	9.4	0.6 - 28.4	7.6	3.0 - 10.5
		spi	9.7	2.6 - 24.9	6.2	2.9 - 10.3			*spi*	16.5	2.9 - 41.1	16.8	2.9 - 40.0
		vir	5.9	2.6 - 12.0	6.9	3.0 - 12.0			*vir*	7.0	0.6 - 26.9	8.9	2.8 - 17.0
		tri	8.6	0.9 - 20.1	8.5	1.2 - 17.5			*tri*	4.9	0.9 - 24.6	6.1	0.9 - 24.6
6	Terminal inflorescence stalk length (cm)	*atr*	0.9		0.9		8	Plant height (cm)	*atr*	46.2		46.2	
		blt							*blt*	76.5		76.5	
		leu	2.2		2.2				*leu*	40.9		40.9	
		gra	29.0	18.4 - 35.1	29.1				*gra*	121.5	50.5 - 159.5	159.5	
		grz	1.1		1.1				*grz*	67.5	58.9 - 75.8	67.5	58.9 - 75.8
		hyb	17.2	10.5 - 25.4	17.2	10.5 - 25.4			*hyb*	67.7	59.0 - 76.5	67.7	59.0 - 76.5
		man	18.5		18.5				*man*	79.2		79.2	
		pal	1		1				*pal*	7.2		7.2	
		ret	1.3	0.0 - 2.0	1.3	1.0 - 2.0			*ret*	27.6	16.4 - 47.9	27.6	16.4 - 47.9
		thu	19.1	12.5 - 29.0	19.1	12.5 - 29.0			*thu*	90.8	42.0 - 116.5	90.8	42.0 - 116.5
		bli	10.2	1.0 - 19.9	10.5	1.6 - 19.9			*bli*	61.1	23.0 - 116.0	56.5	24.9 - 96.8
		cru	32.3	12.7 - 56.5	33.7	12.7 - 56.5			*cru*	141.0	83.4 - 191.6	140.3	83.4 - 191.6
		dub	15.9	1.0 - 49.0	13.9	1.0 - 24.1			*dub*	89.0	13.9 - 160.8	56.3	13.9 - 114.0
		hyp	17.0	1.0 - 40.4	22.8	1.0 - 35.7			*hyp*	101.7	35.6 - 222.5	87.2	35.6 - 139.5
		sp	10.8	0.7 - 37.7	4.8	0.7 - 15.8			*sp*	86.9	27.6 - 137.3	64.8	27.6 - 98.0
		spi	3.3	1.2 - 14.8	3.2	1.2 - 7.6			*spi*	62.1	21.8 - 195.0	48.0	26.2 - 90.5
		vir	9.5	0.4 - 43.5	13.0	0.4 - 43.5			*vir*	53.7	18.1 - 155.4	51.5	21.2 - 81.5
		tri	8.0	0.0 - 29.5	8.9	0.9 - 29.5			*tri*	49.2	5.9 - 193.5	52.4	5.9 - 111.6

Appendix 3.7: (Continued)

			Whole set		Core set	
No.	**QUANTITATIVE TRAITS**	**Species**	**Mean**	**Range**	**Mean**	**Range**
9	Days to flowering	*atr*	56		56	
		blt	35		35	
		leu	46		46	
		gra	47.0	40.0 -49.0	49.0	
		grz	32.0	27.0 - 35.0	32.0	27.0 - 35.0
		hyb	26.0	25.0 - 27.0	26.3	25.0 - 27.0
		man	25		25	
		pal	24		24	
		ret	49.0	36.0 - 82.0	49.0	36.0 - 82.0
		thu	63.0	35.0 - 90.0	62.7	35.0 - 90.0
		bli	33.0	18.0 - 63.0	37.9	27.0 - 63.0
		cru	49.0	27.0 - 115.0	53.4	27.0 - 115.0
		dub	55.0	27.0 - 86.0	52.0	30.0 - 76.0
		hyp	53.0	21.0 - 115.0	42.0	21.0 - 91.0
		sp	51.0	28.0 - 101.0	57.5	28.0 - 82.0
		spi	47.0	31.0 - 89.0	49.8	31.0 -80.0
		vir	41.0	27.0 - 113.0	54.7	33.0 - 113.0
		tri	52.0	24.0 - 106.0	50.4	24.0 - 91.0
10	1000-seed weight (g)	*atr*	0.2		0.2	
		blt	0.1		0.1	
		leu	0.6		0.6	
		gra	0.3	0.2 - 0.7	0.2	
		grz	0.6	0.1 - 0.9	0.6	0.1 - 0.9
		hyb	0.3	0.1 - 0.4	0.3	0.1 - 0.4
		man	0.8		0.8	
		pal	0.6		0.6	
		ret	0.6	0.5 - 0.7	0.6	0.5 - 0.7
		thu	0.9	0.8 - 1.2	0.9	0.8 - 1.2
		bli	0.5	0.1 - 1.2	0.5	0.1 - 0.8
		cru	0.2	0.1 - 0.7	0.2	0.1 - 0.7
		dub	0.3	0.1 - 0.9	0.2	0.1 - 0.4
		hyp	0.4	0.1 - 1.4	0.4	0.1 - 0.9
		sp	0.3	0.0 - 1.3	0.5	0.1 - 1.3
		spi	0.4	0.0 - 1.2	0.5	0.1 - 0.9
		vir	0.4	0.0 - 0.8	0.6	0.4 - 0.8
		tri	0.7	0.0 - 1.7	0.7	0.1 - 1.3

Appendix 3.8: Frequency distribution (%) of five qualitative traits in A. tricolor accessions of USDA Genenbank by country of origin. The figures in the parenthesis are the values of representative of the core set.

Country of origin	NOB	Stem pigmentation					Leaf pigmentation							Inflorescence shape			Seed colour				Seed shape	
		GR	Mix	RB	RD	ST	BP	CD	CS	GN	Mix	RD	RV	AT	AX	Mix	BE	BL	GN	Other	EB	RB
Bangladesh	1				1							1		1			1				1	
Indonesia	1	1								1				1			1				1	
Madagascar	1		1				1							1				1			1	
West Africa	1		1							1				1				1			1	
Zaire	1	1								1				1			1				1	
Brazil	2					2						1	1	2								
Malaysia	2		1			1					2			1		1	1	1			2	
Papua New Guinea	2		1								2			1	1			1			1	
Puerto Rico	2		2							1	1			2				2			2	
Thailand	2		2								2			1			2				2	
Taiwan	7	4 (2)	2 (1)						1 (1)	2 (1)	3 (1)			5 (2)			1 (1)	4 (2)			5 (3)	
USA	14	5 (2)		2 (1)		6 (2)		1 (1)	1 (1)	4 (1)	6 (1)		1 (1)	8 (2)	1 (1)			5 (1)			4 (1)	1 (0)
Hong Kong	15	10 (2)	1 (0)	1 (1)		3 (2)			5 (3)	10 (2)				14 (4)				15 (5)			15 (5)	
Unknown	16	9	3 (1)	2		1			3 (0)	10 (0)	1 (1)			12 (1)		3 (0)	1	7	1		9	
China	29	6 (1)	1 (0)	4 (2)	2 (1)	16 (1)	2 (1)		18 (1)	5 (1)		4 (2)		25 (4)			3 (2)				1 (1)	2 (1)
India	81	11 (5)	45 (5)	4 (2)	5 (2)	11 (2)		1	2 (1)	31 (7)	32 (4)	4 (2)	1 (1)	71 (14)		1 (0)	7 (1)	60 (13)		1 (0)	69 (15)	1 (0)
%		27 (7)	36 (4)	8 (4)	5 (2)	24 (4)	2 (1)	1 (1)	18 (4)	40 (7)	30 (4)	6 (2)	2 (1)	95 (18)	1 (1)	3 (0)	15 (3)	82 (18)	1	1	94 (21)	4 (1)

NOB: Observation number, GR: Green, RB: Red or darker base but green stem, RD: Red or darker stem with solid colouring, can have pink or red base, ST: Amaranthine stripes on stem, can have pink or red base, BP: Basal area pigmented, CD: Chlorophyll deficient, pale marks, that can be white, yellow orange, pink or red, CS: Central spot, RV: Margin and vein pigmented, AT: In leaf axils and terminal, AX: Mostly in leaf axils; BE: Brown edges with black sides, BL: Black seed coat, EB: ellipsoid or ovoid with rounded bulging perisperm; RB: Brown edges with black sides

Appendix 3.9: Country representation of accessions in the core set of amaranth. The figure in the parenthesis represents the total accessions for each description.

Species	Country of origin	Species	Country of origin
***A. atropurperus* (1)**	Indonesia	***A. sp* (7)**	Malaysia (1)
***A. blitoides* (1)**	Hungary		India (1)
***A. blitum* (7)**	India (2)		Thailand (1)
	Korea (1)		Taiwan (1)
	Thailand (1)		Laos (1)
	Cambodia (1)		Bangladesh (2)
	Malaysia (1)	***A. spinosus* (4)**	Thailand (1)
	Laos (1)		Puerto Rico (1)
***A. cruentus* (8)**	Malaysia (1)		Laos (1)
	Austria (2)		Thailand (1)
	Ethiopia (1)	***A. thunbergii* (3)**	Unknown (3)
	Guatemala (1)	***A. tricolor* (120)**	Bangladesh (20)
	Mexico (1)		Brazil (2)
	Tanzania (1)		
	Zimbabwe (1)		Cambodia (2)
	Sudan (1)		China (6)
***A. dubius* (6)**	Surinam (1)		Hongkong (5)
	Viet Nam (2)		India (21)
	Thailand (1)		Indonesia (4)
	Tanzania (1)		Japan (1)
	Cambodia (1)		Laos (1)
***A. gracilis* (1)**	Cambodia		Madagascar (1)
***A. graecizans* (3)**	Hungary (1)		Malaysia (13)
	India (2)		Nigeria (1)
***A. hybridus* (3)**	USA (2)		Pakistan (1)
	Kenya (1)		Papua New Guinea (4)
***A. hypochondriacus* (8)**	Ecuador (1)		Philippines (2)
	Hungary (1)		Puerto Rico (2)
	Ghana (1)		Taiwan (7)
			Tanzania (2)
	Afghanistan (1)		Thailand (6)
	India (1)		Turkey (1)
	Mexico (1)		Unknown (5)
	Nepal (1)		USA (7)
	Viet Nam (1)		Vietnam (8)
***A. leucocarpus* (1)**	India		West Africa (1)
***A. mantegazzianus* (1)**	USA		Zaire (1)
***A. palmeri* (1)**	Senegal	***A. viridis* (6)**	Indonesia (1)
***A. retroflexus* (5)**	Venezuela (1)		Laos (1)
	Viet Nam (3)		Thailand (3)
	China (1)		Malaysia (1)

Appendix 3.10*:* The observed morphological traits of 188 amaranth core set.

Growth habit: erect, prostrate; Branching index: branches all along the stem, many or few branches at the base of the stem; Stem pigmentation: green, pink, pink/green, purple, purple/pink, white, others; Leaf pigmentation: green, dark green, basal area pigmented, central spot, entire lamina purple/pink, chlorotric strip on green leaf, one or two stripe (v-shaped), margin and vein pigmented, purple spotted on green leaf, mixture, other; Petiole pigmentation: green, pink, dark purple, pink/green, purple, purple/pink, white, others; Inflorescence color: green, red, green/red, pink, yellow, other; Leaf shape: cuneate, elliptical, lanceolate, mixture, obovate, other, oval, ovatainate, rhombic; Leaf margin:crenate, enrire, undulate, entire/uduate; Terminal inflorescence shape: club-shaped at tips, panicle with long or short branches, spike (dense): Terminal inflorescence attitude: drooping, erect

Entry	Genotype	Growth habit	Branching index	Stem pigmentation	Leaf pigmentation	Petiole pigmentation	Inflorescence color	Leaf shape	Leaf margin	Terminal inflorescence shape	Terminal inflorescence attitude
1	AV-ATR	Erect	Along the stem	Pink	Normal green	Green	Green	Lanceolate	Entire	Short branches	Erect
2	AV-GRA	Erect	Along the stem	Purple/Pink	Normal green	White	Pink	Lanceolate	Entire	Other	Erect
3	AV-GRA SIL	Erect	Many branches	Purple/Pink	Normal green	Pink/Green	Pink	Cuneate	Entire	Other	Erect
4	AV-GRA ASC	Erect	Many branches	Purple/Pink	Normal green	Pink/Green	Green	Obovate	Undulate	Other	Erect
5	AV-MAN	Erect	Few branches	Green	Normal green	Green	Yellow	Lanceolate	Entire	Club-shaped	Erect
6	AV-BLITO	Prostate	Many branches	Purple/Pink	Normal green	Green	Green	Cuneate	Entire	Other	Erect
7	AV-LEU	Erect	Along the stem	Green	Normal green	Green	Green	Elliptical	Entire	Spike (dense)	Erect
8	AV-PAL	Erect	Few branches	Purple/Pink	Normal green	Green	Green	Ovatainate	Entire	Other	Erect
9	AV-RET 1	Erect	Few branches	Purple/Pink	Margin/Vein	Purple	Red	Ovatainate	Entire	Spike (dense)	Erect
10	AV-RET 2	Erect	Along the stem	Green	Normal green	Green	Green	Lanceolate	Entire	Spike (dense)	Erect
11	AV-RET 3	Erect	Many branches	Purple/Pink	chlorotic stripe	Green	Green	Rhombic	Undulate	Spike (dense)	Erect
12	AV-RET 4	Erect	Along the stem	Purple/Pink	Central spot	Purple	Red	Rhombic	Entire	Spike (dense)	Erect
13	US-RET 1	Erect	Along the stem	Pink	Normal green	Green	Green	Elliptical	Entire	Long branches	Erect
14	AV-SPI 1	Erect	Along the stem	Purple/Pink	Normal green	Green	Green	Lanceolate	Entire	Short branches	Drooping
15	AV-SPI 4	Erect	Many branches	Green	Normal green	Green	Green	Rhombic	Entire	Short branches	Erect
16	AV-SPI 5	Erect	Along the stem	Green	One stripe	Green	Green	Rhombic	Undulate	Short branches	Erect
17	AV-SPI 6	Erect	Many branches	Purple/Pink	Normal green	Green	Green	Lanceolate	Undulate	Spike (dense)	Erect
18	AV-SP 1	Erect	Many branches	Green	Others	Green	Green	Rhombic	Undulate	Other	Erect
19	AV-SP 2	Erect	Along the stem	Purple/Pink	Normal green	Green	Other	Elliptical	Entire	Short branches	Erect
20	AV-SP 3	Erect	Along the stem	Green	Normal green	Green	Green	Cuneate	Undulate	Short branches	Erect
21	AV-SP 4	Erect	Many branches	Green	Normal green	Green	Green	Lanceolate	Entire	Other	Erect
22	AV-SP 5	Erect	Many branches	Purple	Margin/Vein	Purple	Green/Red	Rhombic	Undulate	Other	Erect
23	AV-SP 6	Erect	Many branches	Purple/Pink	Entire lamina	Dark purple	Red	Rhombic	Entire	Spike (dense)	Drooping
24	AV-SP 7	Erect	Many branches	Green	Dark green	Green	Green	Rhombic	Entire	Spike (Dense)	Drooping
25	AV-VIR 1	Prostate	Many branches	Purple/Pink	Normal green	Green	Pink	Rhombic	Entire	Short branches	Erect
26	AV-VIR 4	Erect	Along the stem	Purple/Pink	Normal green	Purple	Green	Cuneate	Entire	Short branches	Erect
27	AV-VIR 6	Erect	Many branches	Purple/Pink	Normal green	Green	Pink	Ovatainate	Entire	Short branches	Erect
28	AV-VIR 9	Erect	Few branches	Green	Normal green	Green	Green	Ovatainate	Undulate	Short branches	Erect

Appendix 3.10: (Continued)

Entry	Genotype	Growth habit	Branching index	Stem pigmentation	Leaf pigmentation	Petiole pigmentation	Inflorescence color	Leaf shape	Leaf margin	Terminal inflorescence shape	Terminal inflorescence attitude
29	AV-VIR 12	Erect	Along the stem	Purple/Pink	Normal green	Purple	Pink	Ovatainate	Entire	Short branches	Drooping
30	AV-VIR 14	Erect	Along the stem	Purple/Pink	Normal green	Green	Other	Rhombic	Entire	Short branches	Drooping
31	AV-CRU 1	Erect	Along the stem	Purple/Pink	Normal green	Pink	Green	Elliptical	Entire	Long branches	Erect
32	AV-CRU 2	Erect	Along the stem	Green	Normal green	Green	Green	Lanceolate	Undulate	Short branches	Erect
33	AV-CRU 3	Erect	Along the stem	Purple/Pink	Margin/Vein	Pink	Green/Red	Ovatainate	Entire	Short branches	Erect
34	AV-CRU 5	Erect	Few branches	Purple/Pink	chlorotic stripe	Green	Yellow	Ovatainate	Undulate	Club-shaped	Erect
35	AV-CRU 6	Erect	Many branches	Purple/Pink	Margin/Vein	Purple	Pink	Ovatainate	Entire	Short branches	Erect
36	AV-CRU 12	Erect	Along the stem	Green	Normal green	Green	Green	Lanceolate	Entire	Short branches	Erect
37	AV-CRU 14	Erect	Along the stem	Green	Central spot	Purple	Pink	Other	Undulate	Short branches	Erect
38	AV-CRU 15	Erect	Along the stem	Green	Normal green	Green	Green	Lanceolate	Undulate	Club-shaped	Erect
39	AV-HYB 1	Erect	Few branches	Purple/Pink	Normal green	Green	Green	Rhombic	Undulate	Spike (dense)	Erect
40	AV-HYB 2	Prostate	Few branches	Purple/Pink	Normal green	Green	Green	Cuneate	Undulate	Spike (dense)	Erect
41	AV-HYB 3	Erect	Along the stem	Purple/Pink	Normal green	Purple	Green	Lanceolate	Entire	Spike (dense)	Drooping
42	US-HYB 2	Erect	Along the stem	Purple	Entire lamina	Purple	Red	Elliptical	Entire	Spike (dense)	Erect
43	AV-GRA 1	Erect	Along the stem	Purple	Central spot	Green	Green	Ovatainate	Undulate	Spike (dense)	Drooping
44	EW-CRU #20866	Erect	Along the stem	Green	Normal green	Green	Green	Ovatainate	Undulate	Club-shaped	Erect
45	AV-HYP 2	Erect	Few branches	Purple/Pink	Normal green	Green	Green	Ovate	Entire	Long branches	Erect
46	AV-HYP 3	Erect	Few branches	Green	Normal green	Green	Pink	Elliptical	Undulate	Long branches	Erect
47	AV-HYP 5	Erect	Along the stem	Purple/Pink	Margin/Vein	Pink	Pink	Lanceolate	Entire	Long branches	Drooping
48	AV-HYP 6	Erect	Few branches	Purple/Pink	Central spot	Green	Red	Lanceolate	Undulate	Long branches	Erect
49	AV-HYP 10	Erect	Along the stem	Green	Normal green	Green	Green	Lanceolate	Entire	Spike (dense)	Erect
50	AV-HYP 13	Erect	Along the stem	Purple/Pink	Entire lamina	Dark purple	Red	Lanceolate	Entire	Spike (Dense)	Erect
51	AV-HYP 14	Erect	Few branches	Purple/Pink	Margin/Vein	Purple/Pink	Red	Elliptical	Undulate	Long branches	Drooping
52	AV-HYP 16	Erect	Many branches	Purple/Pink	Margin/Vein	Purple/Pink	Pink	Ovatainate	Entire	Spike (Dense)	Drooping
53	AV-BLI 1	Erect	Few branches	Purple/Pink	Normal green	Green	Green	Obovate	Undulate	Spike (dense)	Erect
54	AV-BLI 3	Erect	Along the stem	Green	Normal green	White	Green	Elliptical	Undulate	Spike (dense)	Erect
55	AV-BLI 4	Erect	Few branches	Purple/Pink	Normal green	Dark purple	Red	Elliptical	Entire	Spike (dense)	Erect
56	AV-BLI 7	Erect	Many branches	White	Normal green	Pink/Green	Green	Ovatainate	Undulate	Long branches	Erect
57	AV-BLI 10	Prostate	Along the stem	Purple/Pink	Central spot	Green	Green	Ovatainate	Entire	Short branches	Erect
58	AV-BLI 12	Erect	Many branches	Purple/Pink	Entire lamina	Purple	Red	Cuneate	Entire	Other	Erect
59	AV-BLI 13	Erect	Along the stem	White	chlorotic stripe	White	Green	Ovatainate	Undulate	Short branches	Drooping
60	AV-THU 1	Erect	Few branches	Purple/Pink	Entire lamina	Purple	Red	Rhombic	Undulate	Short branches	Erect
61	AV-THU 2	Erect	Along the stem	Purple/Pink	Entire lamina	Purple	Pink	Ovatainate	Undulate	Spike (dense)	Erect

Appendix 3.10: (Continued)

Entry	Genotype	Growth habit	Branching index	Stem pigmentation	Leaf pigmentation	Petiole pigmentation	Inflorescence color	Leaf shape	Leaf margin	Terminal inflorescence shape	Terminal inflorescence attitude
62	AV-THU 3	Erect	Along the stem	Purple/Pink	Margin/Vein	Purple	Green	Lanceolate	Undulate	Spike (dense)	Erect
63	AV-DUB 1	Erect	Along the stem	Green	Central spot	Green	Green	Ovatainate	Entire	Spike (Dense)	Erect
64	AV-DUB 2	Erect	Many branches	Pink/Green	Central spot	Pink/Green	Green	Ovatainate	Entire	Spike (Dense)	Erect
65	AV-DUB 6	Erect	Along the stem	Pink	Normal green	Pink	Pink	Cuneate	Undulate	Short branches	Erect
66	AV-DUB 7	Erect	Along the stem	Purple/Pink	Normal green	Purple	Green	Elliptical	Undulate	Spike (Dense)	Erect
67	AV-DUB 13	Erect	Along the stem	Pink	Normal green	Pink	Green	Ovatainate	Undulate	Spike (Dense)	Erect
68	AV-DUB 15	Erect	Along the stem	Purple/Pink	Central spot	Purple	Green	Lanceolate	Undulate	Spike (Dense)	Drooping
69	AV-TRI 1	Erect	Many branches	Purple/Pink	Entire lamina	Dark purple	Red	Rhombic	Undulate	Short branches	Erect
70	AV-TRI 2	Erect	No branches	Purple/Pink	chlorotic stripe	Purple	Green	Rhombic	Undulate	Short branches	Drooping
71	AV-TRI 3	Erect	Along the stem	Purple/Pink	chlorotic stripe	Purple	Green/Red	Elliptical	Undulate	Short branches	Drooping
72	AV-TRI 4	Erect	Many branches	Purple/Pink	Entire lamina	Purple	Red	Ovatainate	Undulate	Long branches	Erect
73	AV-TRI 5	Erect	Along the stem	Purple/Pink	Central spot	Purple	Red	Ovatainate	Undulate	Spike (dense)	Erect
74	AV-TRI 6	Erect	Along the stem	Green	Normal green	Green	Green	Ovatainate	Entire	Spike (dense)	Erect
75	AV-TRI 7	Erect	Along the stem	Purple/Pink	Entire lamina	Purple	Green/Red	Ovatainate	Entire	Short branches	Erect
76	AV-TRI 8	Erect	Along the stem	Purple/Pink	Entire lamina	Purple	Green/Red	Ovatainate	Entire	Short branches	Erect
77	AV-TRI 9	Erect	Along the stem	Purple/Pink	Entire lamina	Purple	Green/Red	Ovatainate	Entire	Spike (dense)	Erect
78	AV-TRI 10	Erect	Along the stem	Purple/Pink	Entire lamina	Purple	Green/Red	Ovatainate	Entire	Short branches	Erect
79	AV-TRI 11	Erect	Few branches	Purple/Pink	Entire lamina	Purple	Green/Red	Rhombic	Entire	Short branches	Erect
80	AV-TRI 12	Erect	Few branches	Purple/Pink	Central spot	Green	Green	Cuneate	Entire	Spike (dense)	Erect
81	EW-TRI Thida	Erect	Along the stem	Green	Normal green	Green	Green	Ovatainate	Entire	Spike (dense)	Erect
82	EW-TRI Zeya	Erect	Along the stem	Green	Central spot	Green	Green	Ovatainate	Entire	Spike (dense)	Erect
83	AV-TRI 15	Erect	Along the stem	Green	Normal green	Green	Green	Rhombic	Entire	Spike (dense)	Erect
84	AV-TRI 16	Erect	Along the stem	Purple/Pink	Central spot	Purple	Green	Ovatainate	Undulate	Other	Erect
85	AV-TRI 17	Erect	Many branches	Green	Others	Green	Green	Rhombic	Undulate	Short branches	Erect
86	AV-TRI 18	Erect	Along the stem	Green	Two stripes	Green	Green	Lanceolate	Undulate	Short branches	Erect
87	AV-TRI 19	Erect	Few branches	Green	Normal green	White	Yellow	Rhombic	Entire	Club-shaped	Erect
88	AV-TRI 20	Erect	Along the stem	Purple/Pink	Central spot	Purple	Red	Other	Undulate	Short branches	Erect
89	AV-TRI 21	Erect	Along the stem	Purple/Pink	Central spot	Purple	Green	Ovatainate	Entire	Long branches	Drooping
90	AV-TRI 22	Erect	Few branches	Green	Normal green	Green	Green	Rhombic	Undulate	Long branches	Erect
91	AV-TRI 23	Erect	Along the stem	Green	Central spot	Green	Green	Rhombic	Undulate	Long branches	Erect
92	AV-TRI 24	Erect	Along the stem	Green	Normal green	Green	Green	Mixture	Entire/Undulate	Long branches	Erect
93	AV-TRI 25	Erect	Along the stem	Purple/Pink	Central spot	Green	Green	Elliptical	Undulate	Long branches	Drooping
94	AV-TRI 26	Erect	Few branches	Purple/Pink	Central spot	Green	Green	Ovatainate	Entire	Long branches	Erect

Appendix 3.10: (Continued)

Entry	Genotype	Growth habit	Branching index	Stem pigmentation	Leaf pigmentation	Petiole pigmentation	Inflorescence color	Leaf shape	Leaf margin	Terminal inflorescence shape	Terminal inflorescence attitude
95	AV-TRI 27	Erect	Many branches	Green	Normal green	Green	Green	Lanceolate	Undulate	Short branches	Erect
96	AV-TRI 28	Erect	No branches	Purple/Pink	Normal green	Green	Green	Ovatainate	Entire	Spike (dense)	Erect
97	AV-TRI 29	Erect	Few branches	Green	Normal green	Green	Green	Lanceolate	Undulate	Spike (dense)	Erect
98	AV-TRI 30	Erect	Along the stem	Green	chlorotic stripe	Green	Green	Rhombic	Undulate	Spike (dense)	Drooping
99	AV-TRI 31	Erect	Along the stem	Purple/Pink	Entire lamina	Dark purple	Red	Ovatainate	Undulate	Spike (dense)	Erect
100	AV-TRI 32	Erect	Many branches	Green	Normal green	Green	Green	Ovatainate	Entire	Short branches	Erect
101	AV-TRI 33	Erect	Along the stem	Green	Normal green	Green	Green	Lanceolate	Undulate	Short branches	Erect
102	AV-TRI 34	Erect	Many branches	Purple/Pink	Central spot	Purple	Green	Rhombic	Entire	Short branches	Erect
103	AV-TRI 35	Erect	Along the stem	Purple/Pink	Entire lamina	Purple	Pink	Ovatainate	Entire	Spike (dense)	Erect
104	Local PR	Erect	Along the stem	Purple/Pink	Entire lamina	Purple	Red	Ovatainate	Entire	Short branches	Erect
105	AV-TRI 37	Erect	Few branches	Pink	Central spot	Green	Green	Ovatainate	Undulate	Long branches	Drooping
106	AV-TRI 38	Erect	Along the stem	Pink	Central spot	Green	Green	Elliptical	Entire	Long branches	Erect
107	AV-TRI 39	Erect	Many branches	Purple/Pink	Entire lamina	Dark purple	Red	Rhombic	Undulate	Long branches	Drooping
108	AV-TRI 40	Erect	Along the stem	Purple/Pink	Entire lamina	Dark purple	Red	Ovatainate	Undulate	Long branches	Erect
109	AV-TRI 41	Erect	Along the stem	Green	Spotted purple	Purple	Green	Lanceolate	Undulate	Other	Erect
110	AV-TRI 42	Erect	Along the stem	Green	Spotted purple	Purple	Green	Elliptical	Entire	Other	Other
111	AV-TRI 43	Erect	Along the stem	Purple	Entire lamina	Dark purple	Red	Ovatainate	Undulate	Short branches	Drooping
112	AV-TRI 44	Erect	Along the stem	Purple/Pink	Margin/Vein	Pink/Green	Other	Mixture	Entire	Short branches	Erect
113	AV-TRI 45	Erect	Few branches	Purple	Entire lamina	Purple	Red	Rhombic	Entire	Short branches	Drooping
114	AV-TRI 46	Erect	Along the stem	Purple/Pink	Entire lamina	Purple	Green/Red	Ovatainate	Undulate	Short branches	Erect
115	AV-TRI 47	Erect	Along the stem	Pink/Green	Margin/Vein	Green	Pink	Rhombic	Undulate	Spike (dense)	Drooping
116	AV-TRI 48	Erect	Few branches	Green	Normal green	Green	Green	Ovatainate	Undulate	Spike (dense)	Erect
117	AV-TRI 49	Erect	Few branches	Purple/Pink	Entire lamina	Dark purple	Red	Rhombic	Entire	Spike (dense)	Erect
118	AV-TRI 50	Erect	Few branches	Purple	Entire lamina	Purple	Red	Mixture	Entire		Erect
119	AV-TRI 51	Erect	Few branches	Green	Normal green	Green	Green	Rhombic	Entire	Short branches	Drooping
120	AV-TRI 52	Erect	Few branches	Purple/Pink	Normal green	Green	Green	Other	Undulate	Short branches	Erect
121	AV-TRI 53	Erect	Few branches	Green	Normal green	Green	Green	Ovate	Undulate	Spike (dense)	Drooping
122	AV-TRI 54	Erect	Few branches	Green	Normal green	Green	Green	Ovate	Undulate	Spike (dense)	Erect
123	AV-TRI 55	Erect	Along the stem	Green	Normal green	Green	Green	Rhombic	Undulate	Other	Erect
124	AV-TRI 56	Erect	Along the stem	Purple/Pink	Margin/Vein	Purple	Pink	Lanceolate	Undulate	Other	Erect
125	AV-TRI 57	Erect	Few branches	Green	Normal green	Green	Green	Rhombic	Undulate	Other	Drooping
126	AV-TRI 58	Erect	Few branches	Purple/Pink	Central spot	Purple	Green	Rhombic	Entire	Other	Drooping
127	AV-TRI 59	Erect	Along the stem	Green	chlorotic stripe	Green	Green	Lanceolate	Entire	Other	Erect

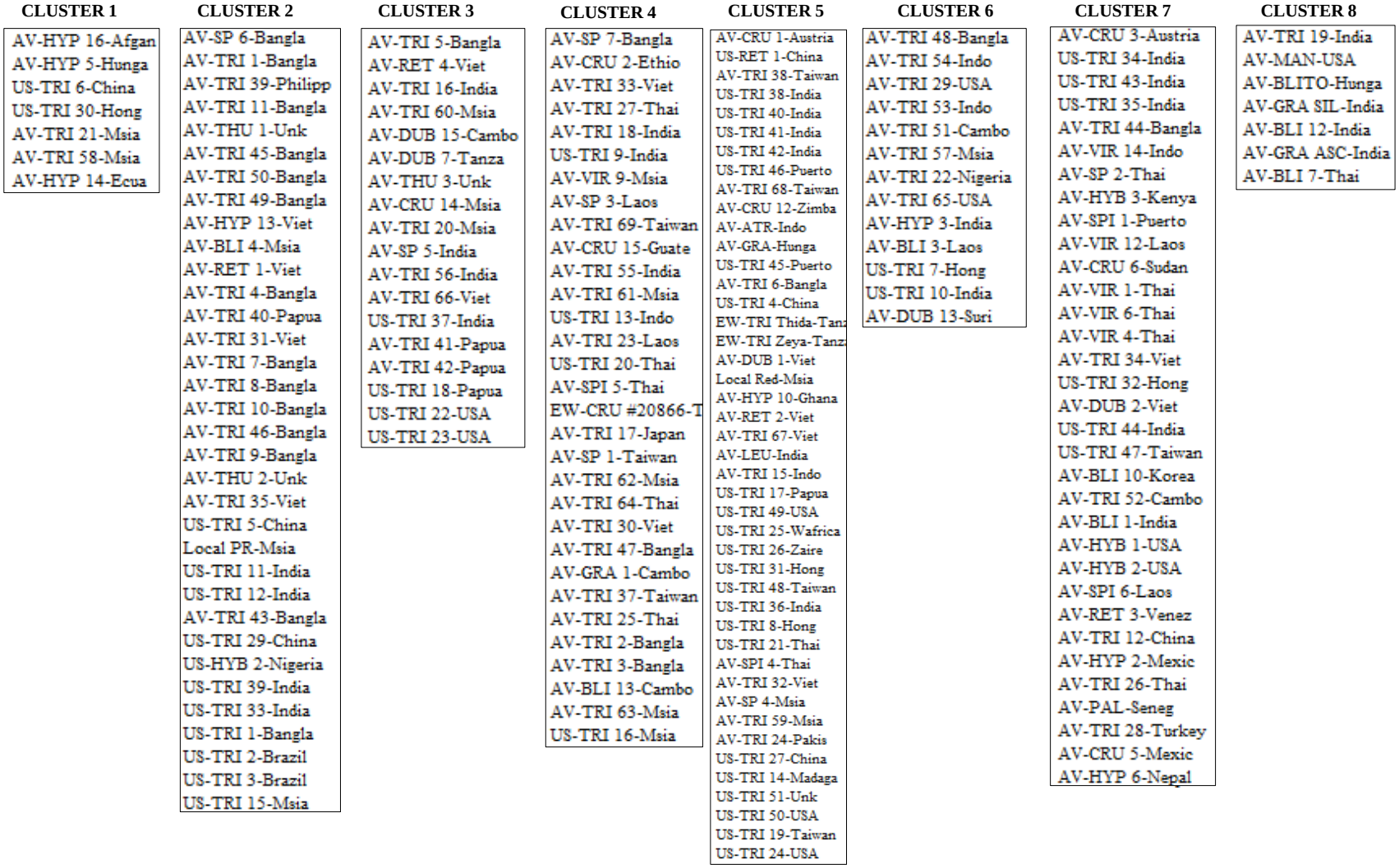
Appendix 3.10: (Continued)

Entry	Genotype	Growth habit	Branching index	Stem pigmentation	Leaf pigmentation	Petiole pigmentation	Inflorescence color	Leaf shape	Leaf margin	Terminal inflorescence shape	Terminal inflorescence attitude
128	AV-TRI 60	Erect	Along the stem	Purple/Pink	Central spot	Purple	Green	Rhombic	Undulate	Other	Erect
129	AV-TRI 61	Erect	Along the stem	Green	Normal green	Green	Green	Lanceolate	Undulate	Other	Erect
130	AV-TRI 62	Erect	Along the stem	Green	Others	Green	Green	Other	Undulate	Other	Erect
131	AV-TRI 63	Erect	Along the stem	Pink/Green	Central spot	Pink/Green	Green	Rhombic	Undulate	Short branches	Drooping
132	AV-TRI 64	Erect	Along the stem	Green	chlorotic stripe	Green	Green	Other	Undulate	Other	Drooping
133	AV-TRI 65	Erect	Few branches	Green	Normal green	Green	Green	Rhombic	Undulate	Other	Erect
134	AV-TRI 66	Erect	Along the stem	Purple/Pink	Margin/Vein	Purple	Pink	Rhombic	Entire	Other	Erect
135	AV-TRI 67	Erect	Along the stem	Green	Normal green	Green	Green	Lanceolate	Entire	Spike (dense)	Erect
136	AV-TRI 68	Erect	Along the stem	Green	Normal green	Green	Green	Lanceolate	Entire	Short branches	Erect
137	AV-TRI 69	Erect	Along the stem	Green	Normal green	Green	Green	Obovate	Undulate	Short branches	Erect
138	US-TRI 1	Erect	Along the stem	Pink	Entire lamina	Purple	Red	Elliptical	Entire	Long branches	Erect
139	US-TRI 2	Erect	Along the stem	Pink	Entire lamina	Purple	Red	Rhombic	Entire	Spike (dense)	Erect
140	US-TRI 3	Erect	Along the stem	Pink	Entire lamina	Purple	Red	Rhombic	Entire	Spike (dense)	Drooping
141	US-TRI 4	Erect	Along the stem	Green	Normal green	Green	Green	Ovatainate	Entire	Spike (dense)	Erect
142	US-TRI 5	Erect	Along the stem	Purple	Entire lamina	Purple	Red	Ovatainate	Entire	Short branches	Erect
143	US-TRI 6	Erect	Along the stem	Purple/Pink	Mixture	Pink/Green	Green/Red	Ovatainate	Entire	Spike (dense)	Drooping
144	US-TRI 7	Erect	Along the stem	Pink	Central spot	Green	Green	Ovatainate	Undulate	Short branches	Erect
145	US-TRI 8	Erect	Along the stem	Green	Normal green	Green	Green	Ovatainate	Entire	Short branches	Erect
146	US-TRI 9	Erect	Along the stem	Green	Normal green	Green	Green	Ovatainate	Undulate	Short branches	Erect
147	US-TRI 10	Erect	Along the stem	Pink	Normal green	Green	Green	Ovate	Undulate	Short branches	Erect
148	US-TRI 11	Erect	Along the stem	Purple	Entire lamina	Purple	Red	Ovatainate	Undulate	Short branches	Erect
149	US-TRI 12	Erect	Along the stem	Purple	Entire lamina	Purple	Red	Ovatainate	Undulate	Short branches	Erect
150	US-TRI 13	Erect	Along the stem	Green	Normal green	Green	Green	Rhombic	Crenate	Short branches	Erect
151	US-TRI 14	Erect	Along the stem	Green	Basal area	Green	Green	Ovate	Entire	Long branches	Erect
152	US-TRI 15	Erect	Along the stem	Pink	Central spot	Purple	Green/Red	Elliptical	Entire	Spike (dense)	Erect
153	US-TRI 16	Erect	Along the stem	Others	Others	Others	Green	Rhombic	Undulate	Short branches	Erect
154	US-TRI 17	Erect	Along the stem	Green	Normal green	Green	Green	Ovate	Entire	Spike (dense)	Erect
155	US-TRI 18	Erect	Along the stem	Green	Spotted purple	Purple	Green	Ovate	Crenate	Other	Other
156	US-TRI 19	Erect	Along the stem	Green	Central spot	Purple/Pink	Green	Ovatainate	Entire	Long branches	Erect
157	US-TRI 20	Erect	Along the stem	Green	Normal green	Green	Green	Rhombic	Undulate	Long branches	Erect
158	US-TRI 21	Erect	Along the stem	Green	Normal green	White	Green	Ovatainate	Entire	Short branches	Erect
159	US-TRI 22	Erect	Along the stem	Purple	Entire lamina	Purple	Red	Elliptical	Entire	Other	Other
160	US-TRI 23	Erect	Along the stem	Purple	Margin/Vein	Purple	Red	Elliptical	Crenate	Other	Other

Appendix 3.10: (Continued)

Entry	Genotype	Growth habit	Branching index	Stem pigmentation	Leaf pigmentation	Petiole pigmentation	Inflorescence color	Leaf shape	Leaf margin	Terminal inflorescence shape	Terminal inflorescence attitude
161	US-TRI 24	Erect	Along the stem	Purple	Central spot	Green	Green	Rhombic	Entire	Long branches	Erect
162	US-TRI 25	Erect	Along the stem	Green	Normal green	Green	Green	Elliptical	Entire	Long branches	Erect
163	US-TRI 26	Erect	Along the stem	Green	Normal green	Green	Green	Ovatainate	Entire	Long branches	Erect
164	US-TRI 27	Erect	Along the stem	Pink	Basal area	Green	Green	Rhombic	Entire	Spike (dense)	Erect
165	Local Red	Erect	Along the stem	Green	Central spot	Green	Green	Ovatainate	Entire	Short branches	Erect
166	US-TRI 29	Erect	Along the stem	Purple	Entire lamina	Purple	Red	Lanceolate	Entire	Spike (dense)	Erect
167	US-TRI 30	Erect	Along the stem	Purple/Pink	Central spot	Pink/Green	Green	Lanceolate	Entire	Other	Drooping
168	US-TRI 31	Erect	Along the stem	Green	Normal green	Green	Green	Other	Entire	Other	Erect
169	US-TRI 32	Erect	Along the stem	Pink/Green	Central spot	Green	Green	Other	Entire	Spike (dense)	Erect
170	US-TRI 33	Erect	Along the stem	Purple	Entire lamina	Purple/Pink	Red	Lanceolate	Undulate	Spike (dense)	Erect
171	US-TRI 34	Erect	Along the stem	Purple/Pink	Margin/Vein	Pink	Red	Lanceolate	Entire	Short branches	Erect
172	US-TRI 35	Erect	Along the stem	Purple/Pink	Margin/Vein	Purple/Pink	Red	Rhombic	Entire	Short branches	Erect
173	US-TRI 36	Erect	Along the stem	Green	Normal green	Green	Green	Ovatainate	Entire	Other	Erect
174	US-TRI 37	Erect	Along the stem	Pink/Green	Margin/Vein	Pink	Green	Other	Undulate	Other	Erect
175	US-TRI 38	Erect	Along the stem	Pink	Normal green	Green	Green	Elliptical	Entire	Short branches	Erect
176	US-TRI 39	Erect	Along the stem	Purple	Margin/Vein	Purple	Red	Lanceolate	Entire	Short branches	Erect
177	US-TRI 40	Erect	Along the stem	Green	Normal green	Green	Green	Lanceolate	Entire	Short branches	Erect
178	US-TRI 41	Erect	Along the stem	Green	Normal green	Green	Green	Lanceolate	Entire	Short branches	Erect
179	US-TRI 42	Erect	Along the stem	Green	Normal green	Green	Green	Lanceolate	Entire	Short branches	Erect
180	US-TRI 43	Erect	Along the stem	Purple/Pink	Margin/Vein	Pink	Green	Lanceolate	Entire	Short branches	Erect
181	US-TRI 44	Erect	Along the stem	Pink/Green	Central spot	Pink/Green		Lanceolate	Entire	Short branches	Erect
182	US-TRI 45	Erect	Along the stem	Pink	Normal green	Pink	Green	Lanceolate	Entire	Other	Erect
183	US-TRI 46	Erect	Along the stem	Green	Normal green	Green	Green	Lanceolate	Entire	Short branches	Erect
184	US-TRI 47	Erect	Along the stem	Pink/Green	Central spot	Green	Green/Red	Ovatainate	Entire	Short branches	Erect
185	US-TRI 48	Erect	Along the stem	Green	Normal green	Green	Green	Other	Entire	Other	Erect
186	US-TRI 49	Erect	Along the stem	Green	Normal green	Green	Green	Lanceolate	Entire	Long branches	Erect
187	US-TRI 50	Erect	Along the stem	Pink/Green	Basal area	Green	Green	Elliptical	Entire	Short branches	Erect
188	US-TRI 51	Erect	Along the stem	Pink/Green	Basal area	Green	Green	Ovatainate	Entire	Other	Erect

Appendix 3.11: A clear version of dendrogram (arranged in hierarchical similarity coefficient accordingly) developed for 188 amaranth mini core collections.

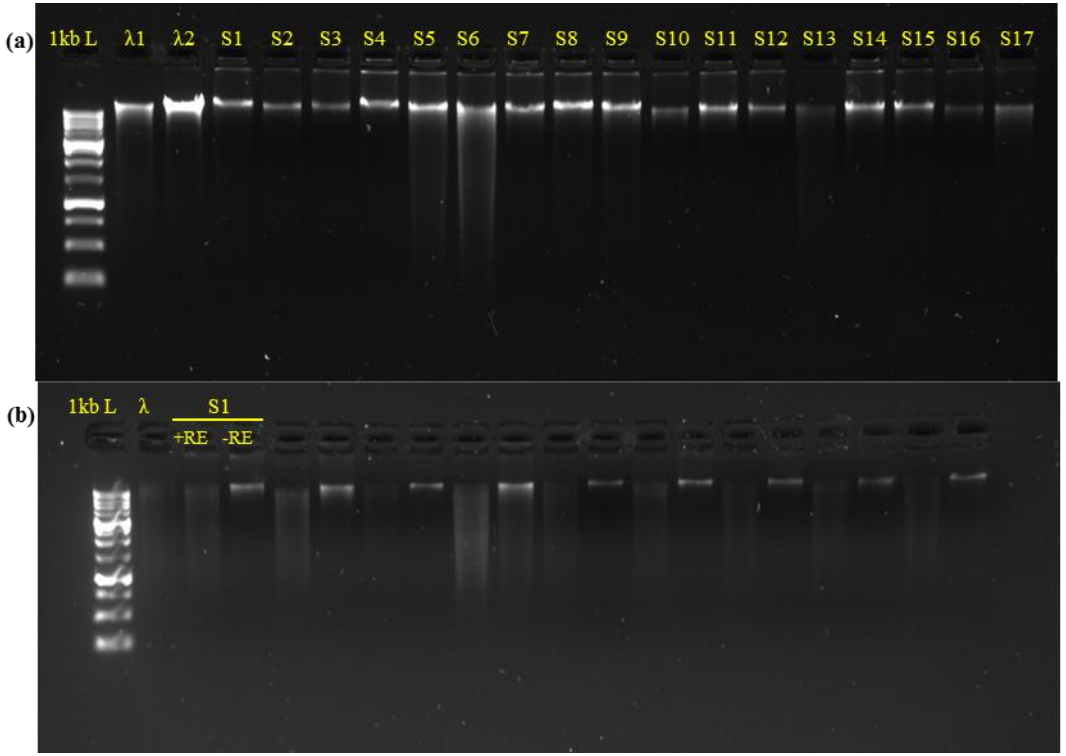


CHAPTER 4: CONSTRUCTION OF A HIGH-DENSITY DArTseq-SNPs BASED POPULATION STRUCTURE IN THE AMARANTH MINI CORE COLLECTION AND ITS ASSOCIATION WITH MORPHOLOGICAL TRAITS AND GEOGRAPHICAL ORIGIN

Appendix 4.1: Gel electrophoresis analysis

(a) An example of gel electrophoresis analysis to quantify the concentration of gDNA extraction for 10 samples (S1-S2), comparing with λ DNA intensity at 100ng (λ1) and 200ng (λ2) and 1kbL is kb ladder.

(b) An example of gel electrophoresis analysis for RE digestion of gDNA. +RE shows that the presence of RE in the sample and –RE shows no presence of RE in the sample (negative control).



Appendix 4.2: Characterization of morphological traits for each cluster developed from SNP marker-UPGMA based dendrogram in (a) 188 amaranth accessions and (b) 120 *A. tricolor*

Appendix 4.2a: 188 amaranth accessions comprised of 18 species.

Traits		**Clsuter I (n=118)**	**Cluster II (n=33)**	**Cluster III (n=37)**
Growth habit	Erect	118	30	36
	Prostate	0	3	1
Branching index	Along the stem	92	15	22
	Few branches	18	5	9
	Many branches	7	13	5
	No branches	1	0	1
Petiole pigmentation	Dark purple	6	2	1
	Green	62	16	21
	Others	1	0	0
	Pink	4	1	4
	Pink/Green	5	3	1
	Purple	35	9	7
	Purple/Pink	3	0	2
	White	2	2	1
Stem pigmentation	Green	52	6	13
	Others	1	0	0
	Pink	11	1	3
	Pink/Green	8	0	1
	Purple	12	3	0
	Purple/Pink	34	21	20
	White	0	2	0
Leaf pigmentation	Basal area	4	0	0
	Central spot	23	4	5
	chlorotic stripe	5	2	1
	Dark green	0	1	0
	Entire lamina	26	5	1
	Margin/Vein	10	2	6
	Mixture	1	0	0
	Normal green	42	18	23
	One stripe	0	0	1
	Others	3	1	0
	Spotted purple	3	0	0
	Two stripes	1	0	0

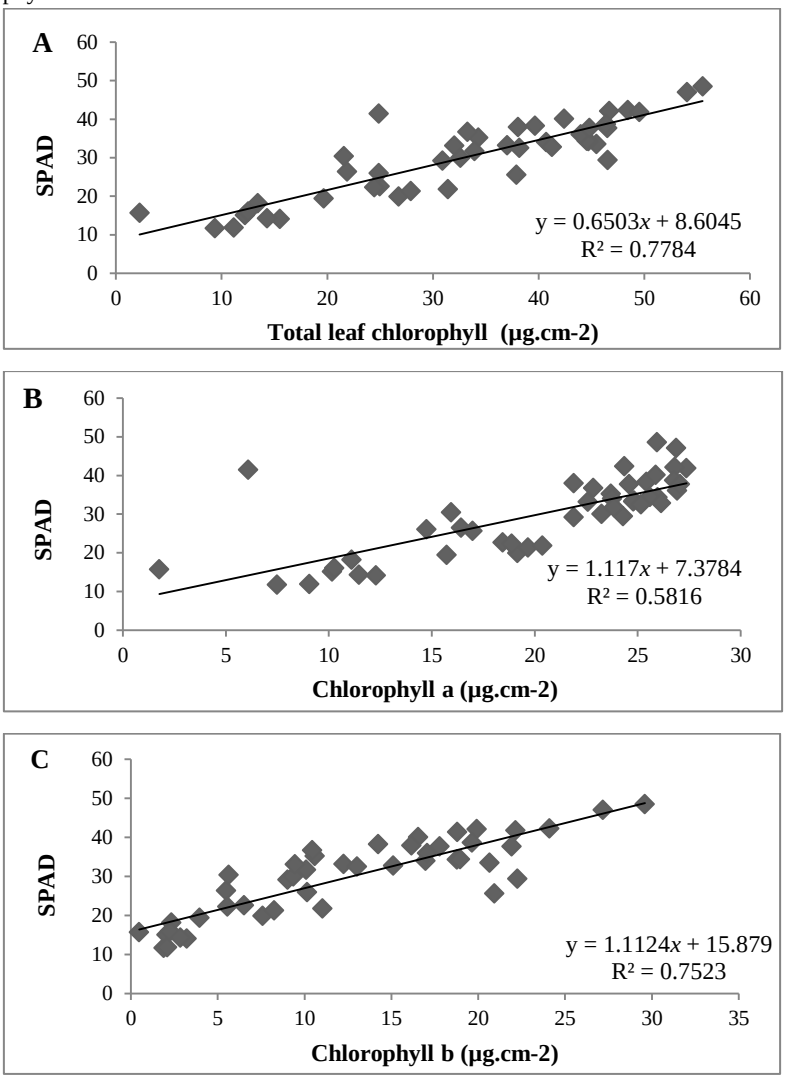
Traits		**Clsuter I (n=118)**	**Cluster II (n=33)**	**Cluster III (n=37)**
Leaf margin	Crenate	3	0	0
	Entire	68	20	19
	Entire/Undulate	1	0	0
	Undulate	46	13	18
Leaf shape	Cuneate	1	6	1
	Elliptical	11	3	7
	Lanceolate	23	4	13
	Mixture	3	0	0
	Obovate	1	2	0
	Other	7	0	2
	Ovatainate	36	9	10
	Ovate	6	0	1
	Rhombic	30	9	3
Inflorescence color	Green	77	17	24
	Green/Red	10	1	1
	Other	1	2	0
	Pink	4	6	6
	Red	24	6	5
	Yellow	1	1	1
Terminal inflorescence attitude	Drooping	19	6	6
	Erect	95	27	31
	Other	4	0	0
Terminal inflorescence shape	Club-shaped	1	1	3
	Long branches	19	1	7
	Other	24	9	0
	Short branches	46	11	11
	Spike (dense)	27	11	16

Appendix 4.2b: 120 *A. tricolor* accessions.

Traits		Cluster I-A (n=105)	Cluster I-B (n=7)	Cluster II-A (n=6)	Cluster II-B (n=8)
Branching index	Along the stem	80	7	5	1
	Few branches	17	0	1	0
	Many branches	7	0	0	0
	No branches	1	0	0	1
Growth habit	Erect	105	7	6	2
Petiole pigmentation	Dark purple	6	0	0	0
	Green	60	2	0	1
	Others	1	0	0	0
	Pink	4	0	0	0
	Pink/Green	5	0	0	0
	Purple	24	5	6	1
	Purple/Pink	3	0	0	0
	White	2	0	0	0
Stem pigmentation	Green	48	4	0	0
	Others	1	0	0	0
	Pink	11	0	0	0
	Pink/Green	7	1	0	0
	Purple	10	2	0	0
	Purple/Pink	28	0	6	2
Leaf pigmentation	Basal area	3	1	0	0
	Central spot	23	0	0	1
	chlorotic stripe	5	0	0	0
	Entire lamina	19	1	6	0
	Margin/Vein	9	1	0	0
	Mixture	1	0	0	0
	Normal green	41	1	0	1
	Others	3	0	0	0
	Spotted purple	0	3	0	0
	Two stripes	1	0	0	0

Traits		Cluster I-A (n=105)	Cluster I-B (n=7)	Cluster II-A (n=6)	Cluster II-B (n=8)
Leaf margin	Crenate	1	2	0	0
	Entire	59	4	5	1
	Entire/Undulate	1	0	0	0
	Undulate	44	1	1	1
Leaf shape	Cuneate	1	0	0	0
	Elliptical	7	4	0	0
	Lanceolate	21	2	0	0
	Mixture	3	0	0	0
	Obovate	1	0	0	0
	Other	7	0	0	1
	Ovatainate	31	0	5	1
	Ovate	5	1	0	0
	Rhombic	29	0	1	0
Inflorescence color	Green	72	5	0	1
	Green/Red	4	0	6	0
	Other	1	0	0	0
	Pink	4	0	0	0
	Red	22	2	0	1
	Yellow	1	0	0	0
Terminal inflorescence attitude	Drooping	19	0	0	0
	Erect	86	3	6	2
	Other	0	4	0	0
Terminal inflorescence shape	Club-shaped	1	0	0	0
	Long branches	18	1	0	0
	Other	19	5	0	0
	Short branches	40	1	5	1
	Spike (dense)	26	0	1	1

Appendix 5.1: Calibration of linear function of SPAD against (A) Total leaf chlorophyll, (B) Chlorophyll a and (C) Chlorophyll b.



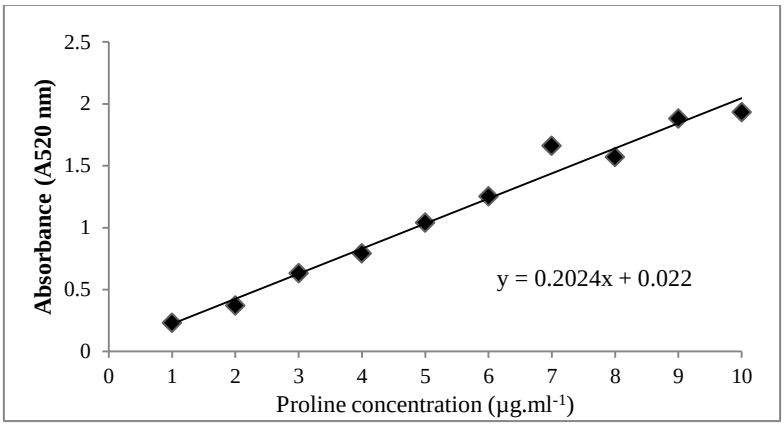
Appendix 5.2*:* Analysis of variance (ANOVA) of a split plot design for Experiment I (Transpiration efficiency)

(a) Biomass	Source of variation	df	m.s	F	P-value
Leaf fresh weight	Block	3	18.54	1.15	
	Treatment (T)	1	4440.59	274.55	<0.001**
	Error A	3	16.17		
	Genotype (G)	8	64.75	3.97	0.001*
	T x G	8	45.62	2.8	0.013*
	Error B	48	16.31		
Leaf dry weight	Block	3	0.3	0.29	
	Treatment (T)	1	37.76	111.73	0.002*
	Error A	3	0.36		
	Genotype (G)	8	5.84	0.37	<0.001**
	T x G	8	1.05	0.19	0.147
	Error B	48	0.65		
Root fresh weight	Block	3	9.79	0.29	
	Treatment (T)	1	3737.81	111.73	0.002*
	Error A	3	33.45		
	Genotype (G)	8	7.16	0.37	0.932
	T x G	8	3.64	0.19	0.992
	Error B	48	19.46		
Root dry weight	Block	3	0.52	0.42	
	Treatment (T)	1	26.18	21.34	0.019*
	Error A	3	1.23		
	Genotype (G)	8	2.52	5.76	<0.001**
	T x G	8	0.35	0.8	0.607
	Error B	48	0.44		
Stem fresh weight	Block	3	14.05	1.53	
	Treatment (T)	1	7266.95	791.63	<0.001**
	Error A	3	9.18		
	Genotype (G)	8	91.62	3.8	0.002*
	T x G	8	43.7	1.81	0.097
	Error B	48	24.08		
Stem dry weight	Block	3	0.31	0.85	
	Treatment (T)	1	80.35	106 .23	<0.001*
	Error A	3	0.45		
	Genotype (G)	8	15.1	8.97	<0.001**
	T x G	8	1.85	1.61	0.149
	Error B	48	1.15		
Root to shoot ratio	Block	3	0.01	0.35	
	Treatment (T)	1	0.08	2.21	0.234
	Error A	3	0.04		
	Genotype (G)	8	0.1	7.38	<0.001**
	T x G	8	0.02	1.53	0.174
	Error B	48	0.01		
Total leaf area	Block	3	14482	0.55	
	Treatment (T)	1	4122307	156.69	<0.001**
	Error A	3	26325		
	Genotype (G)	8	51438	2.06	0.058
	T x G	8	34983	1.4	0.22
	Error B	48	24933		
Specific leaf area	Block	3	2845	1.27	
	Treatment (T)	1	192490	85.78	0.003*
	Error A	3	2244		
	Genotype (G)	8	5695	1.34	0.249
	T x G	8	2371	0.56	0.808
	Error B	48	4263		
Yield	Block	3	42.98	1.23	
	Treatment (T)	1	23068	661.27	<0.001**
	Error A	3	34.89		
	Genotype (G)	8	250.82	4.32	<0.001**
	T x G	8	139.05		
	Error B	48	58.04		

Appendix 5.2: (Continue)

(b) Total chlorophyll content	Source of variation	df	m.s	F	P-value
2 DAT	Block	3	27.86	0.41	
	Treatment (T)	1	46.84	0.69	0.467
	Error A	3	67.79		
	Genotype (G)	8	52.24	1.5	0.182
	T x G	8	43.34	1.24	0.294
	Error B	48	34.81		
8 DAT	Block	3	150	1.22	
	Treatment (T)	1	21.1	0.17	0.707
	Error A	3	123.4		
	Genotype (G)	8	248.8	1.73	0.116
	T x G	8	67	0.46	0.875
	Error B	48	144.1		
14 DAT	Block	3	585.9	1.44	
	Treatment (T)	1	112	0.28	0.636
	Error A	3	406.4		
	Genotype (G)	8	468.8	2.4	0.029*
	T x G	8	275	1.41	0.218
	Error B	48	195.7		
(c) Transpiration	**Source of variation**	**df**	**m.s**	**F**	**P-value**
Total water transpired	Block	3	0.04	1.92	
	Treatment (T)	1	21.46	945.06	<0.001**
	Error A	3	0.02		
	Genotype (G)	8	0.06	1.5	0.184
	T x G	8	1.83	46.97	<0.001**
	Error B	48	0.04		
Transpiration efficiency	Block	3	0.55	0.71	
	Treatment (T)	1	28.62	37.16	0.009*
	Error A	3	0.77		
	Genotype (G)	8	16.61	17.76	<0.001**
	T x G	8	1.32	1.41	0.216
	Error B	48	0.94		
Soil water when FTSW=0	Genotype	8	0.0017	1.87	0.108
	Error	27	0.00092		
FTSW threshold decline	Genotype	8	0.024	1.06	0.42
	Error	27	0.022		
Days to wilting	Genotype	8	7.06	1.153	0.192
	Error	27	4.6		

Appendix 5.3: Calibration curve of the standard proline solutions used to calculate free proline content in the leaf sample.



Appendix 5.4: Analysis of variance (ANOVA) of a split plot design for Experiment II (Genotypic variation in growth, root morphology and plant physiology).

	Total yield		**LeafDW**		**LeafFW**		**StemDW**		**StemFW**		**RootDW**		**RootFW**	
Sourve of variation	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.
Block stratum	5	1808	5	3.408	5	736	5	22.24	5	4139.5	5	4139.5	5	18.42
Block.WP stratum														
WT	1	299435***	1	1213.92***	1	47282.2***	1	2945.06***	1	97093.4***	1	97093.4***	1	1507.56***
Residual	5	273	5	8.118	5	413.8	5	11.03	5	1424.7	5	1424.7	5	13.93
Block.WP.SP stratum														
H	3	16481***	3	190.89***	3	3572.7**	3	1785.47***	3	22468.7***	3	22468.7***	3	1683.56***
H*WT	3	20306***	3	47.121**	3	3734.3**	3	330.85	3	6602.1*	3	6602.1*	3	217.24***
Residual	30	1898	30	9.295	30	622.6	30	21.05	30	1934.7	30	1934.7	30	23.6
Block.WP.SP.SSP stratum														
G	3	781^{ns}	3	52.918***	3	1932.4***	3	135.47***	3	362^{ns}	3	362^{ns}	3	457.72***
G*WT	3	4104*	3	34.662**	3	497.1^{ns}	3	73.37**	3	888.2^{ns}	3	888.2^{ns}	3	77.84*
G*Harvest	9	2119^{ns}	9	47.468***	9	1022.7***	9	16.93^{ns}	9	983.6^{ns}	9	983.6^{ns}	9	94.9***
G*WT*Harvest	9	1988^{ns}	9	13.857*	9	433*	9	20.11^{ns}	9	1854***	9	1854*	9	35.61^{ns}
Residual	118	1094	112	6.203	114	196.1	116	14.16	116	651.3	116	651.3	115	21.8

	TLA		**SLA**		**R/S**		**RAD**		**RL**		**RLPV**		**RSA**		**RV**	
Sourve of variation	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.
Block stratum	5	703691	5	8239	5	0.0020	5	0.018	5	2.85E+06	5	5.98E+10	5	114290	5	33.59
Block.WP stratum																
WT	1	26331026***	1	40156^{ns}	1	0.039*	1	0.013^{ns}	1	1.52E+08***	1	4.16E+12**	1	5434925***	1	925.21**
Residual	5	299509	5	11550	5	0.003	5	0.014	5	3.02E+06	5	1.64E+11	5	46494	5	19.94
Block.WP.SP stratum																
H	3	18233921***	3	60162***	3	0.049***	3	0.043**	3	4.50E+07***	3	1.32E+12**	3	1797550***	3	482.4***
H*WT	3	3823882***	3	52655*	3	0.0057*	3	0.032*	3	5.22E+06^{ns}	3	4.56E+10ns	3	651544**	3	206.07**
Residual	30	449073	30	8343	30	0.0014	30	0.007	30	5.78E+06	30	2.23E+11	30	129138	30	37.28
Block.WP.SP.SSP stratum																
G	3	898788^{ns}	3	23026**	3	0.038***	3	0.03**	3	2.47E+06^{ns}	3	1.20E+10ns	3	487703**	3	149.76**
G*WT	3	442934^{ns}	3	5707^{ns}	3	0.0038**	3	0.015*	3	1.50E+07*	3	3.43E+11*	3	165257^{ns}	3	21.55^{ns}
G*Harvest	9	713976*	9	40010***	9	0.004***	9	0.011*	9	1.51E+07***	9	5.28E+11***	9	311819**	9	61.85*
G*WT*Harvest	9	1065879*	9	46092***	9	0.00069^{ns}	9	0.01^{ns}	9	2.18E+06^{ns}	9	6.84E+10ns	9	47046^{ns}	9	29.69^{ns}
Residual	119	347130	114	5843	116	0.00078	116	0.005	120	4.11E+06	117	1.00E+11	120	95823	118	27.89

Appendix 5.4: (Continue)

	Ci		Pn		Gs		E		Proline		TCC		RWC	
Source of variation	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.
Block stratum	3	5165	3	52.5	3	0.002	3	0.32	3	14.93	5	42.56	5	0.024
Block.WP stratum														
WT	1	5177^{ns}	1	25.29ns	1	0.001ns	1	0.04ns	1	204.46**	1	195.15^{ns}	1	0.28**
Residual	3	6907	3	11.05	3	0.002	3	1.17	3	1.47	5	125.93	5	0.01
Block.WP.SP stratum														
H	3	31452**	3	9230.86***	3	0.347***	3	216.06***	3	6.45^{ns}	3	839.85***	3	0.31***
H*WT	3	1605ns	3	ns	3	0.006ns	3	2.99*	3	60.52*	3	97.42**	3	0.1***
Residual	18	4521	18	25.87	18	0.002	18	0.84	18	17.05	30	18.48	30	0.01
Block.WP.SP.SSP stratum														
G	3	7909ns	3	48.72*	3	0.002*	3	3.05***	3	21.42**	3	44.66^{ns}	3	0.022^{ns}
G*WT	3	6124ns	3	63.18**	3	0.004**	3	1.38*	3	9.48ns	3	89.35^{ns}	3	0.033*
G*Harvest	9	4771ns	9	69.7***	9	0.003***	9	1.58***	9	17.34***	9	76.11*	9	0.029**
G*WT*Harvest	8	16203***	9	37.66*	9	0.005***	9	1.86***	9	14.59**	9	8.61^{ns}	9	0.028*
Residual	69	3738	72	14.09	72	0.001	72	0.37	69	4.326	120	33.85	117	0.01111

CHAPTER 6: ASSESSMENT OF DROUGHT TOLERANCE IN VEGETABLE AMARANTH (*AMARANTHUS TRICOLOR*) GERMPLASM ACCESSIONS: POTENTIAL FOR THE DEVELOPMENT OF IMPROVED DROUGHT TOLERANCE CULTIVARS

Appendix 6.1: Individual ANOVA table for yield and biomass partitioning, RWC, chlorophyll fluorescence and photosynthetic capacity in Trial I and Trial II.

Yield and biomass partitioning, Trial I

	Yield (g)		LFW (g)		SFW (g)		TLA (cm^2)		LDW (g)		SDW (g)		SLA (cm^2g^{-1})	
	WS	**WD**	**WS**	**WD**	**WS**	**WD**	**WS**	**WD**	**WS**	**WD**	**WS**	**WD**	**WS**	**WD**
Mean	15.78	4.21	8.42	1.81	7.37	2.4	265.49	129.29	1.15	1.09	0.94	0.78	239.62	118.78
SOV														
Block Error	562		147		136		82557		3.61		2.71		42305	
WT	8838*		2882*		1626*		12224197**		0.19ns		1.76ns		963703**	
WP Error	748		129		63.34		4152		0.13		0.57		7092	
G	28.88***		12.52***		13.07***		17104**		0.47***		0.32***		4008**	
WT*G	20.54***		8.28**		6.90***		7713**		0.15*		0.08ns		2807ns	
SP Error	9.4		3.25		2.54		3677		0.1		0.06		1515	

Yield and biomass partitioning, Trial II

	Yield (g)		LFW (g)		SFW (g)		TLA (cm^2)		LDW (g)		SDW (g)		SLA (cm^2g^{-1})	
	WS	**WD**	**WS**	**WD**	**WS**	**WD**	**WS**	**WD**	**WS**	**WD**	**WS**	**WD**	**WS**	**WD**
Mean	48.11	33.24	23.2	14.5	24.9	18.73	682.32	440.83	2.48	2	2.27	2.02	305.06	257.44
SOV														
Block Error	2224		676		454		713489		5.34		1.98		12471	
WT	19453***		6690**		3327*		5132472**		20.34**		5.48ns		199527*	
WP Error	466		154		113		213922		0.2		0.54		54610	
G	6673**		234.29***		289.25***		318207***		4.59***		3.42**		80039***	
WT*G	115.11ns		42.42*		41.01ns		46112*		0.41ns		0.36ns		41261ns	
SP Error	94.9		26.7		36.19		31761		0.49		0.39		33114	

Appendix 6.1: (Continue)

Relative water content (RWC)						Chlorophyll fluorescence							
						Light-adapted quantum yield (Fv'/Fm')					Dark-adapted quantum yield (Fv/Fm)		
	Trial I		Trial II			Trial I		Trial II			Trial II		
Mean	**0 DAT**	**6 DAT**	**0 DAT**	**6 DAT**	**10 DAT**	**0 DAT**	**6 DAT**	**0 DAT**	**6 DAT**	**10 DAT**	**0 DAT**	**6 DAT**	**10 DAT**
WS	89.99	90.84	90.33	87.09	86.73	0.65	0.56	0.54	0.52	0.54	0.72	0.67	0.64
WD		61.12		82.92	75.98		0.49		0.53	0.53		0.67	0.63
SOV	**d.f.**	**6 DAT**	**d.f.**	**6 DAT**	**10 DAT**	**d.f.**	**6 DAT**	**d.f.**	**6 DAT**	**10 DAT**	**d.f.**	**6 DAT**	**10 DAT**
Block Error	2	2343	3	3011	2765	2	0.14	3	0.16	0.02	3	0.06	0.1
WT	1	58296**	1	9124**	40687*	1	0.36ns	1	0.0002ns	0.03ns	1	0.02ns	0.03ns
Error	2	845.7	3	189.6	2788	2	0.07	3	0.008	0.01	3	0.03	0.06
G	43	199.30ns	43	226.4**	199.01***	43	0.01*	43	0.008***	0.01***	43	0.01*	0.01**
WT*G	43	229.20ns	43	121.50ns	181.64**	43	0.06ns	43	0.002ns	0.005ns	43	0.04ns	0.01ns
WP Error	172	209.3	258	129.6	94.61	172	0.06	258	0.003	0.006	258	0.004	0.01

	Photosynthesis (µmol CO_2 $m^{-2}s^{-1}$)		Stomatal conductance (mol H_2O $m^{-2}s^{-1}$)		Transpiration (mmol H_2O $m^{-2}s^{-1}$)		Intracellular [CO2] (µmol CO_2 mol^{-1})		Instantaneous WUE (µmol mol^{-1})		Intrinsic WUE (µmol $mmol^{-1}$)		Stomatal limitation	
Mean	**0 DAT**	**6 DAT**	**0 DAT**	**6 DAT**	**0 DAT**	**6 DAT**	**0 DAT**	**6 DAT**	**0 DAT**	**6 DAT**	**0 DAT**	**6 DAT**	**0 DAT**	**6 DAT**
WS	29.13	19.3	0.19	0.13	4.51	3.48	110.4	108.52	6.65	6.13	163.3	170.25	0.71	0.72
WD		19.07		0.12		3.17		95.78		6.35		176.45		0.75
SOV		**6 DAT**		**6 DAT**		**6 DAT**		**6 DAT**		**6 DAT**		**6 DAT**		**6 DAT**
Block Error		28		0.003		6.51		2060		3.67		746		0.02
WT		2.62ns		0.010ns		8.49ns		16542ns		5.20ns		4227ns		0.09ns
WP Error		67		0.003		1.77		9938		11.67		5490		0.06
G		138.83***		0.008***		4.82***		11560***		10.23***		4979***		0.08***
WT*G		119.82***		0.007***		4.40***		5523**		8.44***		3346*		0.04**
SP Error		55		0.003		1.86		3248		2.93		2179		0.02

Re-water assessment							
	DTF	**DTW**	**LWS**	**DS**	**DTR**	**DS-1R**	**DS-5R**
Mean	3	3.48	3	2	2*	4	5
SOV							
Genotype	2.67**	2.87ns	2.209ns	2.22ns	5.73***	16.24***	29.61***
Error	1.39	3.48	2.10	2.24	0.80	1.71	7.12

Appendix 6.2*:* ANOVA analysis for grouping for yield and biomass partitioning, RWC, chlorophyll fluorescence and photosynthetic capacity in Trial I and Trial II, as well as re-water assessment.

Trial II								
SV	**d.f.**	**Yield**	**LFW**	**SFW**	**TLA**	**LDW**	**SDW**	**SLA**
Trt	1	19452.8***	6690.16***	3326.97***	5132472***	20.3376***	5.344***	199527*
Cluster	4	5848.8***	1444.05***	1912.25***	1276531***	25.5529***	19.7638***	244752***
Trt.Cluster	4	606.8***	161.61**	171.55**	97878ns	1.1601ns	0.3599ns	72678ns
Error	342	114.6	43.56	49.43	60575	0.7351	0.5579	37107

Trial II							
SV	**d.f.**	**RWC_6DAT**	**RWC_10DAT**	**Fv'/Fm'_6DAT**	**Fv'/Fm'_10DAT**	**Fv/Fm_6DAT**	**Fv/Fm_10DAT**
Trt	1	6124.9***	40687***	0.000239ns	0.034603*	0.016914ns	0.033814*
Cluster	4	258.3ns	196.5ns	0.023373***	0.028135**	0.004012ns	0.019449*
Trt.Cluster	4	154.8ns	173.2ns	0.000536ns	0.004672ns	0.006493ns	0.001186ns
Error	342	164.7	163.6	0.004683	0.006492	0.005197	0.008073

Trial II								
SV	**d.f.**	**Pn_6DAT**	**Gs_6DAT**	**Ci_6DAT**	**E_6DAT**	**WUE_6DAT**	**WUEi_6DAT**	**Ls_6DAT**
Trt	1	4.3ns	0.009943ns	14265ns	8.505ns	4.418ns	3380ns	0.0928ns
Cluster	4	70.38ns	0.00207ns	9354ns	1.126ns	3.705ns	3735ns	0.06077ns
Trt.Cluster	4	9.39ns	0.00189ns	8341ns	1.047ns	8.035ns	6480*	0.05502ns
Error	342	74.33	0.004557	4523	2.652	4.595	2648	0.0298

Re-water assessment								
SV	**d.f.**	**DS**	**DS_1R**	**DS_5R**	**DTF**	**DTR**	**DTW**	**LWS**
Group	4	3.411ns	2.998ns	26.96ns	13.335***	12.552***	10.008*	1.949ns
Error	121	2.239	6.602	14.71	1.447	1.325	3.063	2.146

Appendix 6.3: Mean for each genotype in each water treatment (WS and WD) in Trial I and Trial II.

Entry	Genotype	Relative water content									
		Trial I				Trial II					
		WS		WD		WS			WD		
		0 DAT	6 DAT	0 DAT	6 DAT	0 DAT	6 DAT	10 DAT	0 DAT	6 DAT	10 DAT
1	AV-TRI 2	90.22	92.68	85.48	73.32	90.16	86.76	87.37	90.71	86.35	72.31
2	AV-TRI 18	83.38	92.15	89.07	70.07	88.31	81.84	79.57	88.28	73.58	69.1
3	AV-TRI 26	91.33	91.36	83.49	47.65	87.55	90.85	87.11	88.96	83.26	72.21
4	AV-TRI 33	88.78	92.36	83.98	68.19	90.19	76.13	83.93	91.32	75.52	70.94
5	AV-TRI 34	93	94.12	92.01	74.17	91.95	85.78	84.06	91.8	80.58	74.63
6	AV-TRI 39	91.66	87.13	86.96	53.41	89.96	88.88	86.19	92.96	83.92	74.23
7	AV-TRI 40	87.3	92.33	79.36	62.18	90.3	78.8	86.04	89.46	82.79	73.31
8	AV-TRI 44	95.57	95.12	85.34	59.65	92.09	86.07	88.74	90.31	75.3	71.24
9	AV-TRI 49	92.39	86.8	91.17	48.66	88.95	99.7	90.69	88.38	91.69	76.76
10	AV-TRI 51	82.9	86.75	80.68	52.97	91.01	85.97	86.1	86.82	78.08	70.3
11	AV-TRI 53	94.11	97.08	90.59	49.79	89.91	89.72	94.17	91.33	81.82	80.07
12	AV-TRI 54	90.14	87.48	89.67	60.46	90.5	85.74	88.98	89.05	83.87	76.31
13	AV-TRI 56	93.46	81.48	86.87	53.92	89.42	84.98	86.89	90.61	79.95	74.36
14	AV-TRI 57	94.92	90.27	95.36	59.87	90.95	80.89	83.53	91.05	80.39	72.36
15	AV-TRI 58	88.87	92.88	90.08	62.72	88.61	80.36	83.45	88.27	73.54	72.92
16	AV-TRI 68	86.96	96.96	86.93	73.23	85.96	94.25	83.78	88.42	86.21	81.67
17	AV-TRI 69	92.49	89.77	85.52	72.56	88.99	79.72	85.12	91.66	80.98	68.06
18	AV-TRI 3	93.97	94.04	89.27	56.82	100	93.74	88.56	101.92	89.83	81.15
19	AV-TRI 11	88.47	86.51	77.92	45.32	90	81.38	89.23	88.96	76.19	71.8
20	AV-TRI 24	85.8	92.58	81.89	43.29	89.77	84.56	90.13	89.44	79.12	77
21	AV-TRI 31	88.86	90.09	90.49	73.38	91.75	87.55	88.98	90.17	85.38	74.35
24	US-TRI 3	88.94	91.91	91.65	65.46	91.5	87.8	86.2	90.5	84.57	76.46
25	US-TRI 6	94.23	91.95	96.47	74.83	86.8	84.85	90.1	87.89	87.43	79.61
26	US-TRI 13	90.71	87.72	83.86	78.22	91.63	88.06	78.89	90.13	81.42	71.35
27	US-TRI 14	91.38	93.52	85.85	60.27	88.98	86.99	83.3	90.12	83.6	79.02
28	US-TRI 15	90.64	96.29	84.75	50.97	90.59	84.13	87.82	90.84	77.59	68.65
29	US-TRI 16	89.45	88.18	85.59	65.86	87.34	96.45	86.58	90.17	92.63	84.54
30	US-TRI 19	89.84	86.5	89.01	86.17	89.95	85.44	90.5	91.33	81.41	77.81
31	US-TRI 21	87.95	90.15	87.12	52.21	89.36	92.76	87.64	90.24	94	85.06
32	US-TRI 24	86.13	88.67	85.77	65.33	91.83	85.58	87.62	91.6	81.15	76.82
33	US-TRI 25	93.57	92.64	85.43	59.13	91.05	89.13	85.56	91.73	73.9	68.54
34	US-TRI 29	91.32	92.68	75.91	49.59	91.78	87.31	87.82	92.15	79.53	71.74
35	US-TRI 39	90.17	82.22	89.05	78.03	89.52	91.3	86.81	90.32	88.26	83.98
36	US-TRI 46	85.54	90.9	88.26	80.36	92.4	86.77	87.24	93.45	86.3	80.56
37	US-TRI 47	90.48	89.93	92.38	80.98	91.07	87.04	77.19	91.53	82.06	73.41
38	US-TRI 20	86.25	88.38	86.68	61.13	89.32	88.8	88.58	88.8	79.02	74.74
39	US-TRI 30	84.89	88.07	84.89	43.84	88.66	88.61	83.33	89.26	91.01	74.3
40	US-TRI 48	90.95	91.73	85.98	53.28	90.73	85.95	88.42	91.38	79.24	73.04
41	US-TRI 49	86.24	95.77	86.64	51.33	91.38	89.62	88.19	94.71	87.64	80.15
42	US-TRI 51	92.65	86.12	85.27	64.03	90.49	91.31	91.99	90.79	92.58	89.31
43	Local Red	87.9	91.42	89.03	46.53	90.44	91.34	89.63	89.81	85.59	81.25
44	Local PR	95.36	91.8	91.05	50.01	91.08	82.14	87.02	91.95	78.85	79.16
45	EW-Thida	86.87	96.61	89.89	60.02	91.7	88.28	86.54	88.18	86.69	82.93
46	EW-Zeya	93.45	93.75	91	49.98	90.74	88.56	86.64	91.65	85.5	75.62
	Mean	**89.99**	**90.84**	**87.13**	**61.12**	**90.33**	**87.09**	**86.73**	**90.65**	**82.92**	**75.98**

Appendix 6.3: (Continued)

Entry	Genotype	Photosynthesis (µmol CO_2 $m^{-2}s^{-1}$)				Stomatal conductance (mol H_2O $m^{-2}s^{-1}$)				Intercellular [CO_2] (µmol CO2 mol^{-1})				Transpiration rate (mmol H_2O $m^{-2}s^{-1}$)			
		WS		WD		WS		WD		WS		WD		WS		WD	
		0 DAT	6 DAT	0 DAT	6 DAT	0 DAT	6 DAT	0 DAT	6 DAT	0 DAT	6 DAT	0 DAT	6 DAT	0 DAT	6 DAT	0 DAT	6 DAT
1	AV-TRI 2	18.45	22.82	37.33	12.82	0.09	0.15	0.17	0.10	54.00	117.50	78.50	162.40	2.79	4.26	4.62	2.72
2	AV-TRI 18	21.73	25.16	37.81	14.50	0.19	0.15	0.20	0.07	180.70	111.40	53.80	35.60	4.23	4.21	4.51	2.43
3	AV-TRI 26	6.40	21.84	15.14	19.49	0.04	0.12	0.21	0.10	150.30	76.80	261.10	77.10	1.62	2.85	6.09	2.34
4	AV-TRI 33	20.61	13.97	39.51	17.91	0.11	0.11	0.23	0.11	74.50	151.10	78.70	104.10	3.51	2.93	5.43	3.53
5	AV-TRI 34	20.53	20.65	27.55	22.47	0.12	0.15	0.18	0.16	98.90	151.90	110.30	136.50	3.45	4.24	4.62	3.44
6	AV-TRI 39	18.43	12.28	33.75	12.82	0.12	0.13	0.16	0.15	133.40	202.80	49.60	223.50	3.61	3.54	4.01	3.36
7	AV-TRI 40	23.93	28.51	35.52	13.08	0.16	0.23	0.23	0.06	121.20	164.90	110.50	48.30	3.71	6.03	5.19	1.76
8	AV-TRI 44	13.83	10.27	20.45	13.37	0.15	0.10	0.20	0.08	220.20	181.20	187.70	136.90	3.44	2.97	4.53	2.20
9	AV-TRI 49	41.71	20.99	43.21	22.68	0.31	0.14	0.33	0.15	141.40	141.10	143.20	123.70	6.95	3.40	7.03	3.38
10	AV-TRI 51	28.24	35.66	35.60	14.90	0.18	0.30	0.29	0.12	114.80	168.10	123.00	138.60	5.11	7.32	5.69	3.11
11	AV-TRI 53	31.80	28.25	42.45	21.86	0.19	0.17	0.30	0.11	99.00	85.30	124.00	61.20	3.99	4.19	5.48	3.43
12	AV-TRI 54	44.56	15.71	29.67	14.16	0.29	0.08	0.22	0.07	108.10	67.60	139.90	40.60	5.77	2.36	5.46	1.70
13	AV-TRI 56	16.39	18.93	39.63	23.08	0.10	0.17	0.29	0.12	109.20	179.20	138.40	89.10	3.32	4.82	6.05	2.92
14	AV-TRI 57	18.18	16.29	34.25	18.22	0.14	0.12	0.24	0.10	139.70	138.00	133.90	95.50	4.07	3.66	5.56	2.99
15	AV-TRI 58	26.07	19.82	26.93	24.63	0.16	0.18	0.22	0.15	111.80	185.40	169.60	93.40	4.52	4.81	5.44	3.63
16	AV-TRI 68	8.79	28.02	35.72	26.51	0.05	0.16	0.23	0.14	81.30	87.20	106.10	40.80	1.72	4.24	5.24	3.06
17	AV-TRI 69	21.52	8.21	27.11	11.51	0.09	0.08	0.16	0.06	62.70	192.90	93.00	57.40	2.22	2.55	4.00	1.66
18	AV-TRI 3	12.37	19.03	37.53	21.38	0.14	0.21	0.28	0.20	231.50	216.70	137.70	99.70	3.63	5.91	6.06	4.45
19	AV-TRI 11	18.42	27.91	32.23	24.46	0.12	0.19	0.13	0.13	106.70	114.40	27.00	49.60	3.54	4.83	2.79	4.48
20	AV-TRI 24	23.30	17.98	44.62	19.35	0.15	0.10	0.27	0.10	110.20	69.10	89.00	57.70	4.10	3.02	5.52	2.48
21	AV-TRI 31	26.25	18.13	17.69	27.14	0.14	0.11	0.19	0.16	77.70	97.80	225.30	83.10	3.82	3.19	4.78	3.82
24	US-TRI 3	33.86	26.91	38.44	23.33	0.18	0.19	0.26	0.14	62.60	85.40	106.70	86.40	4.44	5.48	5.47	3.29
25	US-TRI 6	21.11	27.46	24.61	27.34	0.14	0.15	0.17	0.16	131.40	66.30	128.50	95.10	4.18	4.70	4.56	3.64
26	US-TRI 13	22.46	11.89	32.18	17.20	0.15	0.07	0.18	0.10	83.80	99.70	68.80	67.00	3.51	2.73	4.53	2.27
27	US-TRI 14	28.19	14.17	42.84	31.35	0.17	0.07	0.27	0.18	97.20	31.50	96.10	76.70	4.37	2.37	5.67	4.06
28	US-TRI 15	18.51	13.28	33.45	17.23	0.09	0.06	0.21	0.08	37.90	51.60	89.30	36.20	2.74	1.65	5.03	1.91
29	US-TRI 16	29.29	17.50	35.65	19.71	0.17	0.08	0.25	0.13	80.90	42.00	130.80	89.20	5.16	2.21	6.33	2.81
30	US-TRI 19	26.35	17.31	36.19	14.58	0.24	0.08	0.28	0.10	197.00	100.80	149.30	148.90	5.67	1.95	6.27	2.72
31	US-TRI 21	44.67	11.50	43.16	29.68	0.28	0.09	0.26	0.15	95.30	25.20	80.40	40.50	6.26	1.70	5.95	3.90
32	US-TRI 24	23.63	32.25	23.75	19.38	0.12	0.19	0.14	0.12	51.30	85.70	97.90	92.10	3.09	4.26	3.40	3.58
33	US-TRI 25	23.35	19.53	42.46	16.77	0.13	0.11	0.25	0.13	75.70	91.00	82.90	173.30	3.87	2.74	6.02	3.82
34	US-TRI 29	26.89	21.06	35.22	9.15	0.17	0.16	0.24	0.07	117.00	173.30	120.50	168.50	4.74	4.04	5.66	2.19
35	US-TRI 39	23.39	13.71	28.40	18.22	0.13	0.09	0.17	0.12	90.30	124.10	92.70	110.90	3.37	2.90	2.98	4.01
36	US-TRI 46	29.16	22.24	38.23	15.61	0.15	0.16	0.20	0.08	60.80	142.20	49.50	69.40	3.55	4.79	4.47	2.76
37	US-TRI 47	28.78	15.20	33.26	26.96	0.15	0.07	0.17	0.15	57.80	36.10	58.90	58.10	3.38	2.30	3.26	4.11
38	US-TRI 20	21.29	25.26	35.17	13.26	0.12	0.15	0.22	0.08	81.00	73.80	99.60	86.60	3.47	3.57	4.82	2.59
39	US-TRI 30	32.93	15.67	38.34	15.92	0.21	0.10	0.29	0.12	102.20	120.60	146.50	129.90	5.48	2.42	5.93	3.72
40	US-TRI 48	33.04	12.24	39.39	19.61	0.21	0.06	0.26	0.13	106.00	80.90	117.60	113.40	5.79	1.80	5.87	3.99
41	US-TRI 49	28.90	13.94	28.73	16.83	0.17	0.07	0.18	0.10	97.00	38.80	103.80	114.40	4.43	1.78	5.05	3.43
42	US-TRI 51	23.40	12.30	23.40	18.60	0.17	0.06	0.17	0.12	124.80	57.80	124.80	89.90	4.78	1.43	4.78	2.97
43	Local Red	21.67	15.81	22.24	20.58	0.16	0.08	0.17	0.14	154.10	65.00	155.10	131.30	4.61	1.65	4.72	3.37
44	Local PR	28.23	23.16	30.68	18.03	0.18	0.15	0.18	0.12	115.10	76.30	81.90	98.30	4.60	4.04	4.38	4.04
45	EW-Thida	21.79	18.34	23.25	16.17	0.12	0.10	0.14	0.12	77.90	80.50	95.80	126.00	2.81	2.88	3.45	3.97
46	EW-Zeya	30.32	17.90	31.06	17.41	0.20	0.13	0.16	0.09	126.20	126.00	55.70	57.60	3.74	4.32	2.89	3.31
	Mean	**24.61**	**19.30**	**33.04**	**19.07**	**0.16**	**0.13**	**0.22**	**0.12**	**107.97**	**108.52**	**111.67**	**95.78**	**4.03**	**3.48**	**4.99**	**3.17**

Appendix 6.3: (Continued)

Entry	Genotype	Instantaneous water use efficiency (WUE, µmol mol^{-1})				Intrinsic water use efficiency (WUEi, µmol mmol^{-1})				Stomatal limitation (Ls)			
		WS		WD		WS		WD		WS		WD	
		0 DAT	6 DAT	0 DAT	6 DAT	0 DAT	6 DAT	0 DAT	6 DAT	0 DAT	6 DAT	0 DAT	6 DAT
1	AV-TRI 2	6.91	5.77	8.70	4.87	217.60	157.60	231.80	133.00	0.86	0.69	0.79	0.58
2	AV-TRI 18	5.17	6.06	8.87	6.36	118.50	160.00	191.50	217.20	0.54	0.71	0.86	0.91
3	AV-TRI 26	4.24	7.66	2.48	8.33	156.30	185.60	71.70	190.50	0.63	0.80	0.36	0.80
4	AV-TRI 33	6.03	4.68	7.45	4.48	185.40	139.10	174.40	165.70	0.81	0.61	0.79	0.73
5	AV-TRI 34	5.78	5.04	6.04	6.37	169.80	136.40	160.20	146.60	0.74	0.61	0.71	0.64
6	AV-TRI 39	4.99	3.46	9.31	6.21	148.80	106.40	226.00	148.40	0.66	0.48	0.87	0.43
7	AV-TRI 40	6.03	4.75	6.83	7.68	154.30	124.40	156.40	206.80	0.68	0.57	0.71	0.88
8	AV-TRI 44	4.02	3.63	5.66	4.91	95.20	124.20	115.40	152.00	0.44	0.54	0.51	0.65
9	AV-TRI 49	6.01	6.30	6.15	6.76	132.50	148.10	132.00	154.80	0.61	0.64	0.61	0.68
10	AV-TRI 51	5.55	4.97	7.36	4.76	156.00	119.20	148.70	147.20	0.70	0.55	0.67	0.64
11	AV-TRI 53	8.11	6.67	8.88	6.67	173.10	175.20	145.10	193.30	0.76	0.77	0.67	0.84
12	AV-TRI 54	7.72	7.04	5.46	8.50	153.80	192.50	139.80	211.90	0.71	0.83	0.63	0.90
13	AV-TRI 56	4.93	4.27	6.75	7.90	164.70	119.80	136.90	199.70	0.72	0.54	0.63	0.77
14	AV-TRI 57	4.67	4.79	6.25	6.53	144.70	147.00	142.00	173.20	0.64	0.64	0.64	0.75
15	AV-TRI 58	5.82	4.38	4.79	7.02	159.20	115.80	122.90	172.60	0.71	0.52	0.56	0.76
16	AV-TRI 68	5.08	6.59	7.00	8.05	185.50	173.90	159.00	204.70	0.79	0.77	0.72	0.89
17	AV-TRI 69	9.90	3.46	7.37	7.00	247.20	114.80	171.80	197.40	0.84	0.51	0.76	0.88
18	AV-TRI 3	3.43	3.60	6.29	5.35	93.20	96.30	138.20	123.00	0.39	0.44	0.63	0.74
19	AV-TRI 11	5.60	5.77	11.85	5.51	166.10	156.70	249.00	198.20	0.72	0.70	0.93	0.87
20	AV-TRI 24	5.67	6.92	8.17	7.88	161.70	190.40	165.70	198.00	0.71	0.82	0.76	0.85
21	AV-TRI 31	7.07	5.66	3.46	7.54	180.80	171.40	92.30	178.00	0.80	0.75	0.43	0.78
24	US-TRI 3	7.63	5.07	7.52	7.58	186.60	182.10	157.60	176.50	0.84	0.77	0.71	0.78
25	US-TRI 6	5.17	5.94	5.53	7.66	148.90	186.90	149.50	170.60	0.66	0.83	0.67	0.75
26	US-TRI 13	6.41	4.51	7.38	8.16	163.90	173.20	190.50	193.50	0.74	0.75	0.82	0.83
27	US-TRI 14	6.41	5.80	7.91	8.24	167.60	215.80	162.50	180.40	0.74	0.92	0.74	0.80
28	US-TRI 15	6.76	8.03	7.28	8.73	215.30	226.10	170.60	214.60	0.90	0.87	0.76	0.91
29	US-TRI 16	5.68	8.69	5.65	7.45	175.70	231.20	142.00	196.70	0.79	0.90	0.67	0.77
30	US-TRI 19	4.33	9.26	5.98	5.10	105.40	253.10	131.40	141.00	0.49	0.74	0.60	0.62
31	US-TRI 21	7.24	7.29	7.51	7.28	161.60	196.70	171.90	210.80	0.74	0.94	0.78	0.86
32	US-TRI 24	7.69	7.40	7.02	5.81	199.30	174.10	170.30	174.80	0.87	0.78	0.74	0.76
33	US-TRI 25	6.36	6.90	7.26	3.83	183.40	176.20	170.10	123.60	0.80	0.77	0.78	0.56
34	US-TRI 29	5.71	5.15	6.51	3.67	155.70	129.10	149.90	128.60	0.69	0.55	0.68	0.57
35	US-TRI 39	6.94	4.78	9.52	4.84	175.50	181.30	173.20	187.80	0.77	0.68	0.76	0.71
36	US-TRI 46	7.96	4.66	8.86	5.70	189.90	141.90	192.60	190.40	0.84	0.64	0.87	0.82
37	US-TRI 47	8.47	6.51	10.28	6.64	199.40	212.60	201.50	192.70	0.85	0.91	0.85	0.85
38	US-TRI 20	6.11	7.75	7.76	5.71	180.70	184.50	163.80	181.00	0.79	0.81	0.73	0.78
39	US-TRI 30	6.12	6.93	6.54	5.97	162.00	178.70	132.50	187.80	0.73	0.69	0.61	0.66
40	US-TRI 48	5.86	8.85	6.87	5.00	159.20	230.30	150.00	160.70	0.72	0.79	0.68	0.71
41	US-TRI 49	6.58	8.38	5.69	5.18	167.70	212.80	162.70	161.30	0.74	0.90	0.73	0.71
42	US-TRI 51	4.71	8.58	4.71	7.56	151.00	200.80	151.00	183.90	0.67	0.85	0.67	0.77
43	Local Red	4.47	10.07	4.47	6.28	131.20	219.20	132.70	151.30	0.60	0.84	0.60	0.66
44	Local PR	6.10	7.05	7.20	4.73	156.50	183.80	176.50	172.70	0.70	0.80	0.78	0.75
45	EW-Thida	8.01	6.20	6.70	4.35	182.80	184.40	171.90	153.60	0.80	0.79	0.75	0.67
46	EW-Zeya	8.16	4.37	10.88	5.34	160.30	161.50	194.90	217.30	0.68	0.68	0.85	0.85
	Mean	**6.17**	**6.13**	**7.05**	**6.35**	**164.64**	**170.25**	**160.01**	**176.45**	**0.72**	**0.72**	**0.71**	**0.75**

Appendix 6.3: (Continued)

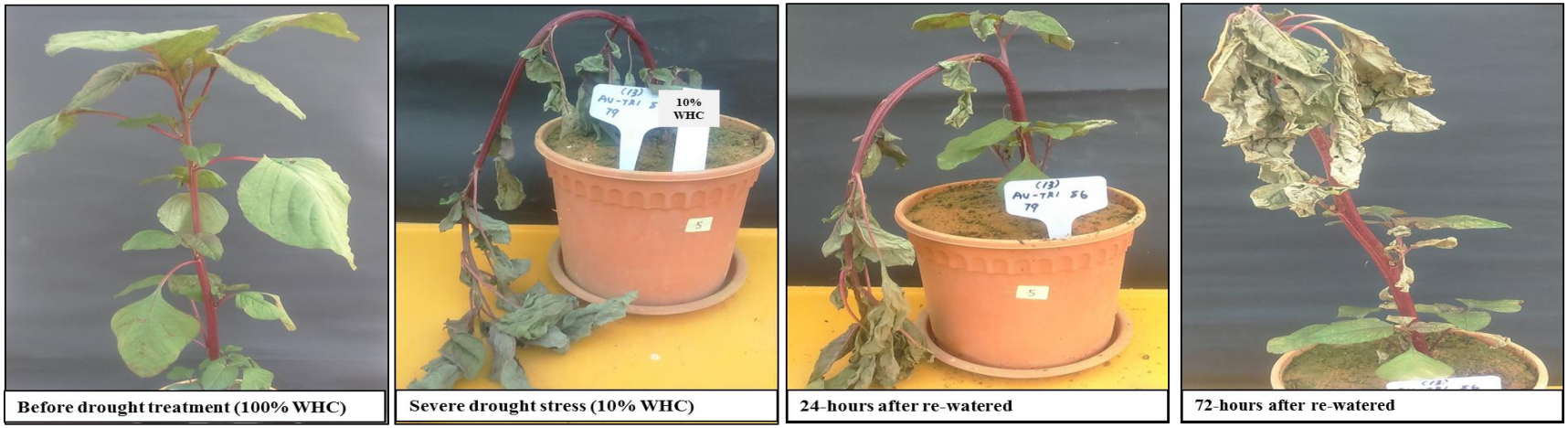
Entry	Genotype	Light-adapted quantum yield (*Fv'/Fm'*)										Dark-adapted quantum yield (*Fv/Fm*)					
		Trial I				Trial II						Trial II					
		WS		WD		WS			WD			WS			WD		
		0 DAT	6 DAT	0 DAT	6 DAT	0 DAT	6 DAT	10 DAT	0 DAT	6 DAT	10 DAT	0 DAT	6 DAT	10 DAT	0 DAT	6 DAT	10 DAT
1	AV-TRI 2	0.63	0.58	0.61	0.57	0.56	0.53	0.57	0.56	0.53	0.58	0.7	0.72	0.67	0.72	0.71	0.66
2	AV-TRI 18	0.65	0.54	0.61	0.38	0.58	0.56	0.53	0.57	0.51	0.5	0.73	0.61	0.61	0.71	0.61	0.61
3	AV-TRI 26	0.67	0.54	0.64	0.51	0.51	0.5	0.54	0.53	0.47	0.58	0.68	0.66	0.64	0.72	0.64	0.58
4	AV-TRI 33	0.67	0.54	0.63	0.45	0.55	0.49	0.53	0.55	0.49	0.52	0.7	0.69	0.56	0.73	0.7	0.58
5	AV-TRI 34	0.62	0.55	0.62	0.45	0.5	0.5	0.49	0.52	0.52	0.49	0.72	0.62	0.63	0.72	0.64	0.62
6	AV-TRI 39	0.62	0.53	0.61	0.55	0.54	0.54	0.49	0.54	0.54	0.52	0.7	0.69	0.64	0.72	0.67	0.56
7	AV-TRI 40	0.65	0.6	0.65	0.46	0.52	0.5	0.47	0.53	0.54	0.49	0.75	0.68	0.63	0.74	0.67	0.6
8	AV-TRI 44	0.66	0.53	0.66	0.55	0.55	0.48	0.51	0.54	0.53	0.52	0.74	0.65	0.62	0.75	0.68	0.63
9	AV-TRI 49	0.65	0.56	0.67	0.64	0.56	0.51	0.56	0.55	0.52	0.59	0.69	0.63	0.61	0.69	0.64	0.62
10	AV-TRI 51	0.66	0.6	0.65	0.41	0.49	0.52	0.53	0.53	0.51	0.52	0.71	0.67	0.61	0.71	0.69	0.59
11	AV-TRI 53	0.66	0.56	0.58	0.47	0.54	0.53	0.54	0.52	0.54	0.52	0.73	0.7	0.62	0.74	0.69	0.62
12	AV-TRI 54	0.66	0.53	0.61	0.49	0.56	0.51	0.52	0.55	0.54	0.5	0.76	0.72	0.63	0.75	0.71	0.64
13	AV-TRI 56	0.69	0.6	0.6	0.58	0.56	0.5	0.49	0.56	0.5	0.49	0.72	0.68	0.66	0.74	0.69	0.63
14	AV-TRI 57	0.65	0.56	0.63	0.5	0.56	0.42	0.49	0.55	0.46	0.51	0.75	0.68	0.62	0.73	0.67	0.55
15	AV-TRI 58	0.68	0.56	0.66	0.49	0.57	0.51	0.52	0.55	0.52	0.49	0.72	0.69	0.64	0.74	0.7	0.64
16	AV-TRI 68	0.6	0.57	0.62	0.41	0.56	0.53	0.54	0.55	0.55	0.54	0.71	0.62	0.7	0.71	0.64	0.68
17	AV-TRI 69	0.7	0.61	0.66	0.58	0.54	0.61	0.56	0.58	0.62	0.56	0.69	0.67	0.7	0.72	0.71	0.67
18	AV-TRI 3	0.64	0.55	0.67	0.47	0.52	0.51	0.59	0.54	0.52	0.54	0.7	0.67	0.63	0.71	0.65	0.65
19	AV-TRI 11	0.66	0.59	0.66	0.56	0.52	0.54	0.55	0.54	0.55	0.54	0.69	0.73	0.65	0.72	0.73	0.66
20	AV-TRI 24	0.63	0.51	0.65	0.43	0.54	0.53	0.54	0.56	0.51	0.53	0.71	0.66	0.61	0.74	0.67	0.62
21	AV-TRI 31	0.66	0.59	0.65	0.56	0.56	0.54	0.55	0.57	0.55	0.56	0.71	0.69	0.68	0.73	0.69	0.68
24	US-TRI 3	0.67	0.58	0.63	0.44	0.53	0.5	0.51	0.53	0.52	0.54	0.74	0.69	0.65	0.74	0.69	0.63
25	US-TRI 6	0.61	0.57	0.6	0.51	0.48	0.48	0.54	0.48	0.5	0.52	0.71	0.7	0.66	0.72	0.71	0.63
26	US-TRI 13	0.65	0.55	0.67	0.51	0.59	0.53	0.63	0.59	0.51	0.61	0.72	0.69	0.64	0.73	0.69	0.62
27	US-TRI 14	0.63	0.53	0.72	0.58	0.49	0.55	0.49	0.51	0.5	0.47	0.72	0.67	0.6	0.72	0.67	0.59
28	US-TRI 15	0.62	0.56	0.63	0.43	0.54	0.53	0.54	0.55	0.52	0.52	0.74	0.73	0.66	0.73	0.72	0.6
29	US-TRI 16	0.65	0.56	0.63	0.48	0.49	0.48	0.55	0.53	0.5	0.5	0.7	0.64	0.65	0.72	0.64	0.66
30	US-TRI 19	0.65	0.55	0.61	0.47	0.48	0.52	0.55	0.52	0.53	0.5	0.69	0.63	0.59	0.68	0.65	0.57
31	US-TRI 21	0.62	0.5	0.64	0.45	0.46	0.54	0.59	0.46	0.52	0.56	0.7	0.61	0.68	0.71	0.62	0.68
32	US-TRI 24	0.64	0.59	0.63	0.46	0.59	0.55	0.56	0.56	0.56	0.54	0.75	0.68	0.62	0.72	0.66	0.63
33	US-TRI 25	0.63	0.56	0.63	0.57	0.55	0.51	0.47	0.53	0.51	0.49	0.74	0.64	0.57	0.71	0.63	0.56
34	US-TRI 29	0.61	0.51	0.59	0.32	0.55	0.44	0.51	0.53	0.47	0.48	0.69	0.66	0.55	0.72	0.66	0.57
35	US-TRI 39	0.64	0.54	0.67	0.56	0.51	0.55	0.6	0.51	0.56	0.59	0.74	0.67	0.7	0.71	0.65	0.65
36	US-TRI 46	0.66	0.59	0.65	0.5	0.51	0.53	0.59	0.51	0.54	0.6	0.74	0.62	0.63	0.73	0.65	0.62
37	US-TRI 47	0.67	0.61	0.64	0.51	0.57	0.54	0.56	0.56	0.55	0.55	0.7	0.73	0.72	0.72	0.73	0.69
38	US-TRI 20	0.66	0.56	0.64	0.44	0.62	0.57	0.47	0.6	0.53	0.49	0.76	0.76	0.6	0.74	0.73	0.58
39	US-TRI 30	0.61	0.57	0.63	0.4	0.5	0.52	0.59	0.53	0.51	0.55	0.75	0.66	0.61	0.73	0.65	0.61
40	US-TRI 48	0.66	0.59	0.62	0.46	0.58	0.56	0.58	0.59	0.56	0.51	0.77	0.67	0.65	0.76	0.67	0.65
41	US-TRI 49	0.64	0.53	0.64	0.4	0.58	0.48	0.52	0.57	0.52	0.47	0.73	0.69	0.65	0.72	0.67	0.63
42	US-TRI 51	0.66	0.57	0.63	0.43	0.44	0.53	0.59	0.51	0.53	0.58	0.72	0.61	0.67	0.73	0.64	0.68
43	Local Red	0.67	0.56	0.65	0.45	0.55	0.46	0.53	0.55	0.51	0.56	0.75	0.66	0.66	0.73	0.65	0.66
44	Local PR	0.63	0.56	0.66	0.51	0.57	0.54	0.6	0.56	0.54	0.59	0.7	0.68	0.66	0.72	0.68	0.68
45	EW-Thida	0.64	0.52	0.64	0.44	0.53	0.52	0.6	0.53	0.51	0.54	0.71	0.7	0.63	0.66	0.66	0.64
46	EW-Zeya	0.68	0.61	0.65	0.57	0.53	0.56	0.6	0.55	0.59	0.56	0.79	0.7	0.69	0.76	0.69	0.71
	Mean	**0.65**	**0.56**	**0.64**	**0.49**	**0.54**	**0.52**	**0.54**	**0.54**	**0.53**	**0.53**	**0.72**	**0.67**	**0.64**	**0.72**	**0.67**	**0.63**

Appendix 6.3: (Continued)

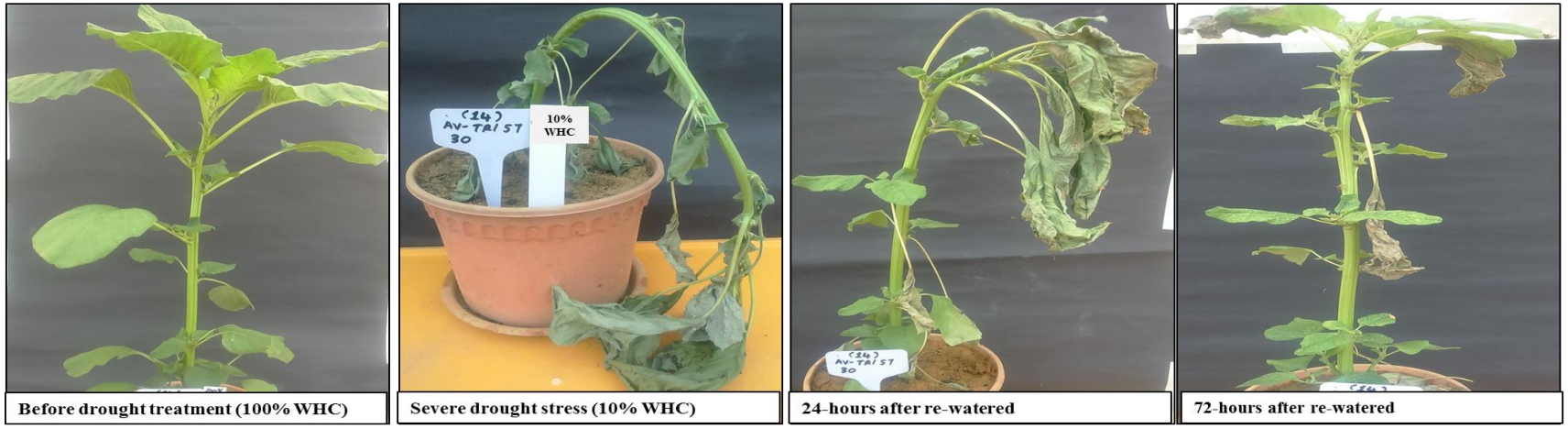
Entry	Genotype	Drought stress				Recovery		
		DTF	DTW	LWS	DS	DTR	DS-1R	DS-5R
1	AV-TRI 2	1	6	3	3	5	2	0
2	AV-TRI 18	3	2	3	1	2	5	9
3	AV-TRI 26	3	3	3	1	not recover	not recover	not recover
4	AV-TRI 33	3	1	1	1	1	6	6
5	AV-TRI 34	3	2	2	1	1	2	8
6	AV-TRI 39	4	3	3	2	3	4	7
7	AV-TRI 40	3	3	3	1	1	3	6
8	AV-TRI 44	4	3	3	1	2	6	5
9	AV-TRI 49	4	4	3	2	1	5	5
10	AV-TRI 51	3	2	3	1	1	5	9
11	AV-TRI 53	2	4	1	2	3	5	5
12	AV-TRI 54	2	3	4	2	not recover	not recover	not recover
13	AV-TRI 56	3	4	4	1	1	5	7
14	AV-TRI 57	4	3	3	2	1	5	not recover
15	AV-TRI 58	3	3	2	0	3	5	not recover
16	AV-TRI 68	3	4	2	1	1	0	0
17	AV-TRI 69	3	3	3	1	2	6	8
18	AV-TRI 3	3	5	0	2	3	5	0
19	AV-TRI 11	4	2	4	1	1	2	3
20	AV-TRI 24	1	3	4	4	1	3	6
21	AV-TRI 31	4	4	2	3	2	3	3
24	US-TRI 3	3	4	4	2	1	0	0
25	US-TRI 6	0	4	2	3	1	0	0
26	US-TRI 13	4	2	3	2	1	3	5
27	US-TRI 14	1	3	3	1	1	0	1
28	US-TRI 15	1	3	2	3	2	5	5
29	US-TRI 16	3	3	2	1	1	2	8
30	US-TRI 19	2	4	3	2	2	1	3
31	US-TRI 21	4	3	2	1	1	4	6
32	US-TRI 24	3	5	2	2	1	3	not recover
33	US-TRI 25	3	4	1	0	2	8	8
34	US-TRI 29	1	3	3	2	1	1	3
35	US-TRI 39	3	3	4	2	1	4	2
36	US-TRI 46	3	3	2	2	2	4	9
37	US-TRI 47	2	5	2	1	not recover	not recover	not recover
38	US-TRI 20	2	4	2	1	2	0	0
39	US-TRI 30	3	3	3	2	1	4	0
40	US-TRI 48	3	2	3	1	2	5	7
41	US-TRI 49	3	3	2	1	2	0	0
42	US-TRI 51	3	4	1	1	3	3	3
43	Local Red	4	4	4	3	1	0	0
44	Local PR	4	5	2	2	1	2	2
45	EW-Thida	3	2	2	1	1	3	3
46	EW-Zeya	4	4	4	2	1	3	4
	Mean	**3**	**3**	**3**	**2**	**2**	**3**	**4**

Appendix 6.4: Re-watered assessment.

(13) AV-TRI 56

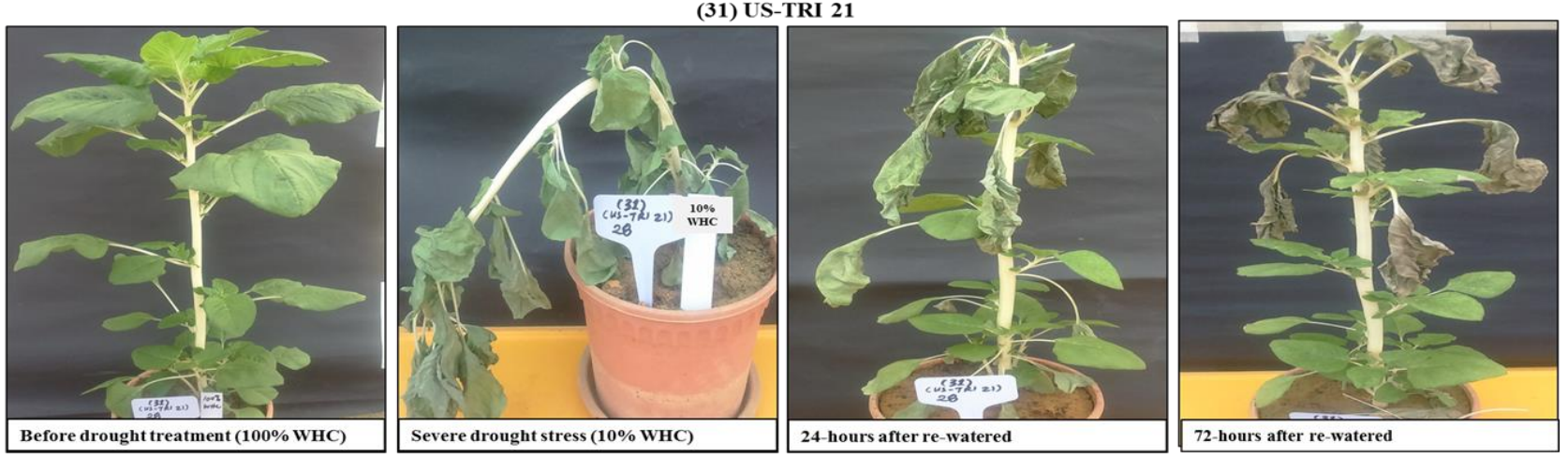


(14) AV-TRI 57



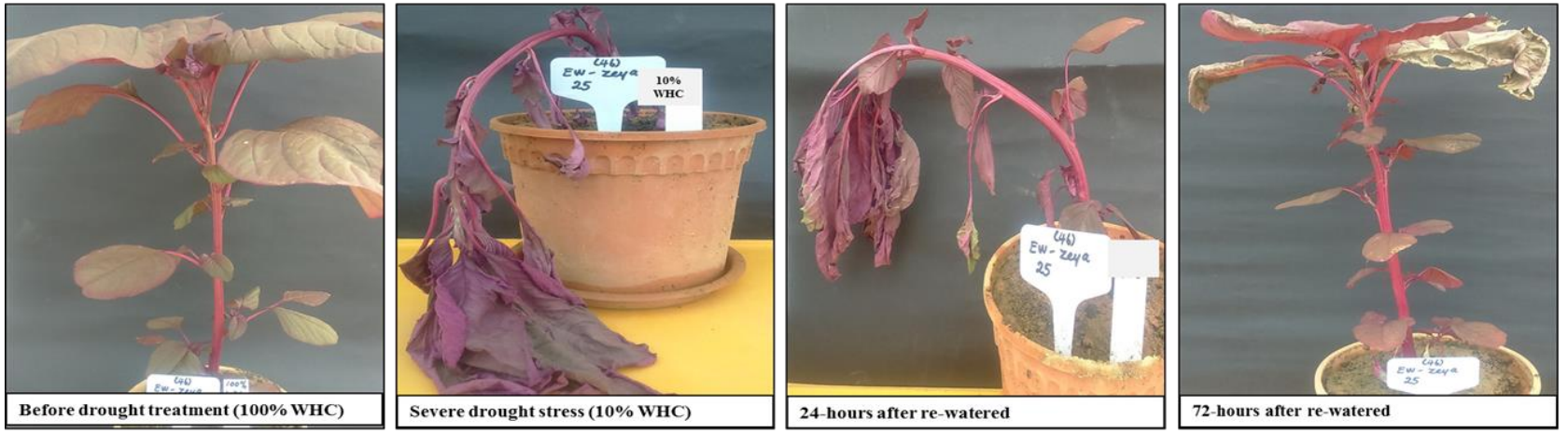
Appendix 6.4: (Continued)

(31) US-TRI 21



Before drought treatment (100% WHC) | Severe drought stress (10% WHC) | 24-hours after re-watered | 72-hours after re-watered

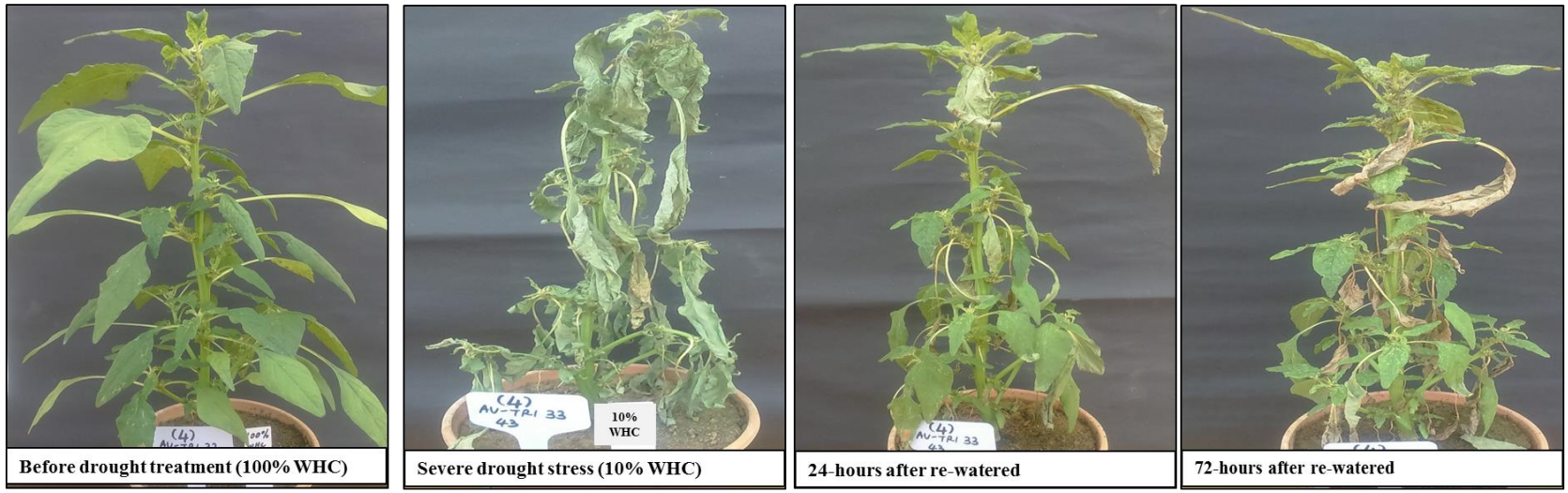
(46) EW-ZEYA



Before drought treatment (100% WHC) | Severe drought stress (10% WHC) | 24-hours after re-watered | 72-hours after re-watered

Appendix 6.4: (Continued)

(4) AV-TRI 33



Before drought treatment (100% WHC) | **Severe drought stress (10% WHC)** | **24-hours after re-watered** | **72-hours after re-watered**

Appendix 6.5: Mean and ranking for 44 amaranth genotypes based on drought tolerance indices (DI, GMP, MP, SSI, STI, TOL and YSI) across two trials of drought screening (Trial I and Trial II).

		Trial I									**Trial II**								
Entry	**Genotype**	**Yp**	**Ys**	**DI**	**GMP**	**MP**	**SSI**	**STI**	**TOL**	**YSI**	**Ypi**	**Ysi**	**DI**	**GMP**	**MP**	**SSI**	**STI**	**TOL**	**YSI**
1	AV-TRI 2	11.14a	3.09a	0.20a	5.86ab	7.11ab	0.98a	0.14ab	8.05a	0.28a	26.76def	***17.88d***	0.41a	21.49ef	22.32de	1.09a	0.22d	8.87a	0.66a
2	AV-TRI 18	18.44a	5.13a	0.38a	9.58ab	11.79ab	0.95a	0.38ab	13.31a	0.30a	68.80ab	38.85a-d	0.68a	51.49abc	53.82abc	1.4a	1.16a-d	29.95a	0.57a
3	AV-TRI 26	18.57a	3.05a	0.15a	7.30ab	10.81ab	1.09a	0.22ab	15.52a	0.20a	52.07a-f	33.60a-d	0.71a	41.31a-f	42.84a-e	1.21a	0.81a-d	18.47a	0.63a
4	AV-TRI 33	14.44a	4.15a	0.32a	7.60ab	9.29ab	0.95a	0.25ab	10.29a	0.31a	37.07b-f	30.46a-d	0.76a	33.59a-f	33.76b-e	-0.55	0.51bcd	6.62a	0.83a
5	AV-TRI 34	12.04a	5.43a	0.65a	7.99ab	8.73ab	0.62a	0.29ab	6.61a	0.55a	47.01a-f	29.87a-d	0.60a	37.27a-f	38.44a-e	1.13a	0.60a-d	17.14a	0.65a
6	AV-TRI 39	15.59a	3.89a	0.25a	7.71ab	9.74ab	0.98a	0.26ab	11.70a	0.28a	42.56a-f	34.37a-d	0.85a	38.16a-f	38.46a-e	0.67a	0.66a-d	8.19a	0.79a
7	AV-TRI 40	16.68a	3.94a	0.26a	7.94ab	10.31ab	1.01a	0.26ab	12.74a	0.26a	41.78a-f	30.32a-d	0.77a	34.97a-f	36.05a-e	0.77a	0.55a-d	11.46a	0.76a
8	AV-TRI 44	21.74a	5.60a	0.35a	**11.01a**	13.67ab	1.00a	0.50ab	16.14a	0.27a	58.23a-e	37.96a-d	0.76a	46.89a-e	48.10abc	1.06a	0.97a-d	20.27a	0.67a
9	AV-TRI 49	18.33a	5.79a	0.54a	10.07ab	12.06ab	0.86a	0.42ab	12.54a	0.37a	49.57a-f	37.32a-d	0.87a	42.88a-f	43.44a-e	0.84a	0.83a-d	12.25a	0.74a
10	AV-TRI 51	18.41a	4.15a	0.34a	8.31ab	11.28ab	1.00a	0.28ab	14.26a	0.27a	44.24a-f	33.86a-d	0.85a	38.29a-f	39.05a-e	0.55a	0.67a-d	10.38a	0.83a
11	AV-TRI 53	18.95a	4.21a	0.24a	8.80ab	11.58ab	1.06a	0.33ab	14.75a	0.22a	38.93a-f	24.53a-d	0.58a	30.05b-f	31.73b-e	0.90a	0.39cd	14.39a	0.72a
12	AV-TRI 54	19.76a	5.34a	0.48a	9.88ab	12.55ab	0.90a	0.4ab	14.42a	0.34a	44.77a-f	31.95a-d	0.99a	36.18a-f	38.36a-e	0.68a	0.58a-d	12.81a	0.79a
13	AV-TRI 56	12.09a	4.16a	0.41a	6.93ab	8.13ab	0.91a	0.21ab	7.93a	0.34a	45.12a-f	28.74a-d	0.69a	35.02a-f	36.93a-e	1.03a	0.54a-d	16.38a	0.68a
14	AV-TRI 57	15.9a	4.10a	0.42a	7.65ab	10.00ab	0.83a	0.25ab	11.80a	0.39a	64.25abc	29.60a-d	0.42a	43.44a-f	46.92a-d	1.71a	0.82a-d	34.65a	0.47a
15	AV-TRI 58	15.6a	3.81a	0.29a	7.50ab	9.7ab	0.91a	0.26ab	11.79a	0.33a	47.83a-f	35.62a-d	0.97a	40.34a-f	41.72a-e	0.76a	0.72a-d	12.21a	0.77a
16	AV-TRI 68	11.74a	4.98a	0.55a	7.56ab	8.36ab	0.77a	0.25ab	6.76a	0.44a	46.97a-f	36.95a-d	1.18a	40.27a-f	41.96a-e	0.11a	0.76a-d	10.02a	0.97a
17	AV-TRI 69	15.11a	3.86a	0.25a	7.57ab	9.48ab	0.99a	0.25ab	11.25a	0.27a	47.43a-f	22.05bcd	0.31a	32.26b-f	34.74b-e	1.74a	0.47bcd	25.38a	0.46a
18	AV-TRI 3	15.58a	5.79a	0.69a	9.13ab	10.68ab	0.81a	0.34ab	9.79a	0.41a	64.84abc	47.90abc	1.09a	55.59ab	56.37ab	0.75a	1.46ab	16.94a	0.77a
19	AV-TRI 11	26.47a	5.24a	0.29a	**11.45a**	**15.85a**	1.09a	**0.56a**	21.23a	0.20a	61.29abc	42.96a-d	0.91a	51.28abc	52.12abc	0.96a	1.14a-d	18.32a	0.70a
20	AV-TRI 24	12.64a	5.51a	0.58a	8.34ab	9.07ab	0.77a	0.28ab	7.13a	0.44a	54.51a-e	41.72a-d	0.99a	47.51a-d	48.11abc	0.65a	1.01a-d	12.80a	0.80a
21	AV-TRI 31	14.68a	2.98a	0.16a	6.55ab	8.83ab	1.08a	0.17ab	11.69a	0.21a	46.12a-f	38.40a-d	1.02a	41.75a-f	42.26a-e	0.49a	0.77a-d	7.72a	0.85a
24	US-TRI 3	16.34a	4.28a	0.44a	7.87ab	10.31ab	0.80a	0.27ab	12.06a	0.41a	33.58c-f	25.65a-d	0.65a	28.96c-f	29.61cde	0.72a	0.38cd	7.92a	0.78a
25	US-TRI 6	8.12a	1.72a	0.32a	***3.13b***	***4.92b***	0.67a	***0.04b***	6.40a	0.51a	***19.91f***	20.17bcd	0.70a	***19.71f***	***20.04e***	-0.43	***0.17d***	-0.26	1.13a
26	US-TRI 13	16.32a	4.22a	0.27a	8.23ab	10.27ab	1.02a	0.28ab	12.09a	0.26a	44.86a-f	26.86a-d	0.51a	34.47a-f	35.86a-e	1.19a	0.52bcd	18.00a	0.63a
27	US-TRI 14	11.95a	4.02a	0.35a	6.87ab	7.99ab	0.88a	0.2ab	7.93a	0.35a	36.94b-f	34.87a-d	1.01a	35.80a-f	35.91a-e	0.13a	0.56a-d	2.07a	0.96a
28	US-TRI 15	18.96a	4.04a	0.51a	7.50ab	11.50ab	0.97a	0.25ab	14.93a	0.29a	38.77a-f	32.58a-d	1.05a	34.00a-f	35.67a-e	0.46a	0.54a-d	6.19a	0.86a
29	US-TRI 16	16.54a	3.53a	0.21a	7.48ab	10.04ab	1.06a	0.23ab	13.00a	0.22a	53.22a-f	35.26a-d	0.87a	41.96a-f	44.24a-e	1.08a	0.81a-d	17.95a	0.67a
30	US-TRI 19	14.38a	3.62a	0.27a	6.99ab	9.00ab	0.94a	0.21ab	10.76a	0.31a	39.33a-f	32.17a-d	0.82a	35.42a-f	35.75a-e	0.55a	0.55a-d	7.17a	0.83a
31	US-TRI 21	21.14a	5.16a	0.31a	**10.40a**	13.15ab	1.02a	0.44ab	15.99a	0.25a	54.95a-e	**51.94a**	1.56a	53.02abc	53.45abc	0.05a	1.27abc	3.01a	1.02a
32	US-TRI 24	11.81a	3.82a	0.30a	6.69ab	7.82ab	0.90a	0.20ab	7.99a	0.34a	61.43abc	33.41a-d	0.56a	45.20a-f	47.42a-d	1.51a	0.93a-d	28.02a	0.53a
33	US-TRI 25	11.44a	3.78a	0.32a	6.52ab	7.61ab	0.89a	0.17ab	7.66a	0.35a	38.48a-f	23.04a-d	0.44a	29.59c-f	30.76cde	1.26a	0.39cd	15.44a	0.61a
34	US-TRI 29	17.24a	3.33a	0.17a	7.48ab	10.29ab	1.08a	0.23ab	13.91a	0.21a	51.84a-f	25.62a-d	0.42a	36.09a-f	38.73a-e	1.57a	0.58a-d	26.22a	0.51a
35	US-TRI 39	18.7a	4.54a	0.34a	8.98ab	11.62ab	0.92a	0.35ab	14.16a	0.32a	**72.01a**	49.15ab	1.03a	**59.35a**	**60.58a**	1.02a	**1.55a**	22.86a	0.69a
36	US-TRI 46	20.31a	3.84a	0.18a	8.77ab	12.07ab	1.09a	0.31ab	16.47a	0.20a	59.18a-e	40.23a-d	0.84a	48.65abc	49.71abc	1.05a	1.05a-d	18.94a	0.67a
37	US-TRI 47	7.11a	3.83a	1.07a	4.86ab	5.47b	0.15a	0.11ab	3.28a	1.11a	25.27ef	20.01cd	0.49a	22.38def	22.64de	0.74a	0.23d	5.26a	0.77a
38	US-TRI 20	12.02a	5.01a	0.52a	7.72ab	8.52ab	0.76	0.25ab	7.01a	0.45a	40.32a-f	31.42a-d	0.75a	35.49a-f	35.87a-e	0.69a	0.56a-d	8.89a	0.79a
39	US-TRI 30	9.02a	3.71a	0.41a	5.69ab	6.36ab	0.75	0.13ab	5.31a	0.45a	45.81a-f	33.00a-d	0.78a	38.46a-f	39.40a-e	0.73a	0.66a-d	12.81a	0.78a
40	US-TRI 48	19.07a	3.05a	0.12a	7.59ab	11.06ab	1.15	0.24ab	16.02a	0.16a	46.88a-f	28.21a-d	0.52a	36.27a-f	37.54a-e	1.26a	0.57a-d	18.67a	0.61a
41	US-TRI 49	15.07a	3.23a	0.16a	6.97ab	9.15ab	1.07	0.21ab	11.84a	0.22a	47.02a-f	30.27a-d	0.67a	37.08a-f	38.65a-e	1.02a	0.61a-d	16.76a	0.68a
42	US-TRI 51	22.28a	5.21a	0.32a	**10.61a**	13.75ab	1.02	0.46ab	17.06a	0.25a	60.94abc	48.22abc	1.15a	54.19abc	54.58abc	0.66a	1.28abc	12.72a	0.80a
43	Local Red	14.26a	3.65a	0.33a	6.91ab	8.96ab	0.90a	0.20ab	10.61a	0.34a	59.42a-d	40.89a-d	0.87a	49.1abc	50.16abc	1.10a	1.17a-d	18.53a	0.66a
44	Local PR	15.59a	4.79a	0.47a	8.36ab	10.19ab	0.85a	0.29ab	10.80a	0.38a	58.83a-e	37.62a-d	0.89a	45.81a-e	48.22abc	1.00a	0.93a-d	21.21a	0.69a
45	EW-Thida	19.45a	5.03a	0.41a	9.59ab	12.24ab	0.94a	0.37ab	14.41a	0.31a	44.96a-f	27.58a-d	0.69a	34.19a-f	36.27a-e	0.76a	0.51bcd	17.38a	0.76a
46	EW-Zeya	13.44a	3.68a	0.37a	6.68ab	8.56ab	0.84a	0.21ab	9.76a	0.39a	52.67a-f	29.46a-d	0.58a	38.53a-f	41.07a-e	1.34a	0.65a-d	23.21a	0.59a
LSD (5%)		10.00	2.29	0.57	3.45	5.06	0.63	0.23	10.30	0.50	14.25	16.72	0.68	12.67	12.50	1.29	0.50	18.45	0.40

Appendix 6.6: The PCA for tolerance indices for Trial I and Trial II, and PCA for physiological responses for WS and WD conditions in Trial I and Trial II.

Tolerance Index				
	Trial I		Trial II	
Principle component	**PC1**	**PC2**	**PC1**	**PC2**
Latent roots	5.77	2.69	5.28	3.52
Percentage (%) variation	64.11	29.9	58.64	39.08
Cumulative % variation	64.11	94.01	58.64	97.72
Latent vectors				
Yp	0.4	0.03	0.42	-0.13
Ys	0.19	0.5	0.39	0.24
DI	-0.18	0.53	0.22	0.45
GMP	0.37	0.27	0.43	0.06
MP	0.4	0.13	0.43	0.02
SSI	0.31	-0.38	0.1	-0.51
STI	0.36	0.29	0.42	0.08
TOL	0.39	-0.08	0.22	-0.44
YSI	-0.31	0.38	-0.1	0.51

TRIAL I				
	WS		WD	
Principle component	**PC1**	**PC2**	**PC1**	**PC2**
Latent roots	4.027	1.882	3.535	2.462
Percentage (%) variation	44.75	20.92	39.28	27.36
Cumulative % variation	44.75	65.67	39.28	66.64
Latent vectors				
Fv_Fm_L	0.08	0.11	0.01	0.23
LDW	0.44	0.16	0.46	0.10
LFW	0.43	0.33	0.47	0.21
RWC	-0.03	0.10	-0.15	0.20
SDW	0.35	-0.46	0.16	-0.56
SFW	0.32	-0.49	0.25	-0.49
SLA	-0.02	0.47	0.19	0.40
TLA	0.41	0.40	0.46	0.27
Yp	0.48	-0.10	0.46	-0.24

TRIAL II				
	WS		WD	
Principle component	**PC1**	**PC2**	**PC1**	**PC2**
Latent roots	5.07	4.66	4.55	3.66
Percentage (%) variation	29.84	27.42	26.78	21.53
Cumulative % variation	29.84	57.26	26.78	48.31
Latent vectors				
Ci	-0.38	0.10	-0.15	0.46
E	-0.39	-0.06	0.12	0.26
FvFm_D	-0.03	-0.26	-0.25	-0.21
FvFm_L	0.05	0.01	0.01	-0.14
Gs	-0.36	-0.03	0.17	0.25
LDW	0.08	0.39	0.39	0.09
LFW	0.10	0.42	0.36	0.08
Ls	0.38	-0.10	0.14	-0.47
Pn	-0.21	-0.10	0.21	-0.04
RWC	0.16	0.10	0.24	0.09
SDW	-0.08	0.32	0.20	-0.01
SFW	-0.13	0.29	0.27	-0.01
SLA	0.06	-0.29	0.00	0.04
TLA	0.13	0.31	0.37	0.11
WUE	0.40	-0.07	0.16	-0.34
WUEi	0.36	0.02	0.15	-0.46
Yp	-0.04	0.43	0.41	0.05