THE PHYSIOLOGICAL AND GENETIC BASES OF DROUGHT TOLERANCE IN BREAD WHEAT AND ANCESTRAL WHEAT SPECIES

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Dedicated to

The soul of my father, whose death came when I was still a little child

My lovely mother, who spent all her life for me

The most attentive and supportive, my lovely wife Sheelan

My beloved sons, Rama, Karo and Parsa

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%	Percent
$\Delta^{13}C$	Carbon isotope discrimination
µ	micron
µmol	micromol
‰	Parts per thousand
$^{13}C/^{12}C$	Carbon isotope ratio
A	Photosynthetic rate
AGDM	Above-ground dry matter
A_{max}	Maximum Photosynthetic rate
ANOVA	Analysis of variance
AW	Available water
AWFC	Available water at field capacity
BD	Soil bulk density
C_3	Carbon-3
C_4	Carbon-4
Ca	Partial pressure of CO_2 in air
chr	Chromosome
Ci	Intercellular CO^2 concentration
CIMMYT	Centro Internacional de Mejoramiento de Maiz y Trigo
cM	centi Morgan
cm	centimetre

CO_2	Carbon dioxide
CSIRO	Commonwealth Scientific and Industrial Research Organization
CT	Canopy temperature
d	Days
DAS	Days after sowing
DEFRA	Department for Environment, Food & Rural Affairs
DF	Degrees of freedom
DH	Doubled-haploid
E	Transpiration rate
FAOSTAT	Food and Agriculture Organization, Statistics Division
FC	Soil field capacity
FLA	Flag-leaf area
FLSW	Flag-leaf specific weight
Fm	Maximum chlorophyll fluorescence
Fv	Variable chlorophyll fluorescence
Fv/Fo	The ratio of variable to minimum chlorophyll fluorescence
g	gram
g m^{-2}	Gram per square metre
G x E	Genotype x environment interaction
g_s	Stomatal conductance
GS	Plant growth stage
h	hours
HGCA	Home Grown Cereals Authority

HI	Harvest index
I	litre
kg	kilogram
LI	Light intercepted
LOD	Logarithm of Odds
LTM	Long-term means
m	metre
MAS	Marker-assisted Selection
mm	millimeter
mmol	millimol
NDVI	Normalized Difference Vegetation Index
NILs	Near-isogenic lines
^{o}C	degrees Celsius
P	Probability
PGR	Plant growth regulator
PS II	Photosystem II
PVC	Polyvinyl chloride
QTL	Quantitative trait loci
r	Correlation coefficient
R^2	Coefficient of determination
RILs	Recombinant inbred lines
Rubisco	Ribulose-I 5-bisphosphate carboxylase-oxygenase
RUE	Radiation-use efficiency

SED	Standard error of difference
SEN_{END}	End of leaf senescence
SEN_{ONSET}	Onset of leaf senescence
SEN_{RATE}	Leaf senescence rate
SSI	Stress susceptibility index
SYN-DER	Synthetic derived wheat
t ha^{-1}	tonnes per hectare
TE	Transpiration efficiency
TGW	Thousand grain weight
UK	United Kingdom
WISP	Wheat Improvement Strategic Programme
WU	Water uptake
WUE	Water-use efficiency
$WUE_{biomass}$	Above-ground biomass per unit water uptake
YP	Yield potential
δ	Isotope composition

Abstract

Winter wheat (*Triticum aestivum* L.) is the major arable crop worldwide, with a total annual global production of about 716 million tonnes and annual UK production of about 16 million tonnes from about 1.8 million hectares of land. Currently in the UK, approximately 15-20% of annual wheat yield production is lost to drought (Foulkes *et al.*, 2002).

In the present study two field experiments in 2012-13 and 2013-14 were conducted characterising a doubled-haploid (DH) population of 94 lines derived from a cross between winter wheat Rialto and Savannah. Two glasshouse experiments were conducted using four DH lines from the Rialto x Savannah DH population and the two parents, three accessions of each of three parental wheat ancestral species (*T. bessarabicum, T. uratu* and *A. speltoides*), and seven amphidiploid lines derived from crosses between durum wheat cultivars and *T. bessarabicum*. Two irrigation treatments were applied in both field (fully irrigated and rain-fed) and glasshouse (well-watered and water-stressed) experiments at the University of Nottingham, School of Biosciences, Sutton Bonington Campus, UK (52^{o} 50' N, 1^{o} 15' W).

In each experiment, grain yield and above-ground dry matter partitioning were assessed at harvest, as well as a range of physiological traits at sequential assessments through the season. In the glasshouse experiments, water uptake and water-use efficiency (above-ground dry matter to total water uptake ratio) were also measured from the date of transplantation to harvest. Quantitative trait loci (QTL) analysis was carried out for the traits measured in the Rialto x Savannah DH population in the field experiments.

In the field experiments, drought reduced grain yield by 22% in 2013 and by 2% in 2014. In 2013, amongst the sub-set of six DH lines and the two parents, variation for grain $\Delta^{13}C$, leaf photosynthetic rate, stomatal conductance and transpiration efficiency was observed ($P<0.05$), and flag-leaf A_{max}, g_s and grain $\Delta^{13}C$ were positively associated with grain yield ($R^2=0.55$, $P<0.05$; $R^2=0.57$, $P<0.05$, and $R^2=0.47$, $P=0.06$, respectively). However, a negative relationship was found between TE and each of grain yield ($R^2=0.70$, $P<0.01$) and $\Delta^{13}C$ ($R^2=0.61$, $P<0.05$) for the cross-year mean, indicating that lower TE was based on high stomatal conductance.

Amongst the 94 R x S DH lines under drought, post-anthesis NDVI was positively correlated with grain yield, above-ground dry matter and TGW, and canopy

temperature was negatively associated with grain yield and TGW post-anthesis (P<0.05). Late onset and end of flag-leaf senescence were associated with greater grain yield, above-ground biomass and TGW under both irrigated and drought conditions in 2013. Therefore, overall present results suggested that genetic variation in maintaining grain yield was more related to water uptake rather than WUE under mild UK water stress.

In the glasshouse, the *T. bessarabicum* accessions had the highest flag-leaf photosynthetic rate, transpiration efficiency and SPAD associated with smaller leaf size and higher flag-leaf specific weight under drought compared with the amphidiploid lines and Rialto x Savannah DH lines. Individual amphidiploid lines had higher flag-leaf photosynthetic rate, leaf SPAD and later onset and end of flag-leaf senescence compared with the Rialto x Savannah DH lines associated with early flowering under droughted treatments.

For the QTL analysis in the Rialto x Savannah DH population, a number of QTLs clusters were identified for grain yield, yield components and physiological traits under irrigated and drought conditions. Co-located QTLs were identified on chr 3A for grain yield, above-ground dry matter, thousand grain weight, plant height, anthesis date and flag-leaf senescence duration, and for stay-green traits, thousand grain weight and grains per m^2. QTLs were also identified on chr 7D and 4A co-located with stay-green traits and anthesis date under irrigated and drought conditions.

Chapter 1 Introduction

1.1 Global wheat production

Bread wheat (*Triticum aestivum* L.) is an important crop worldwide, grown on about 219 million ha, with average total grain yield production of about 715.9 million tonnes (average on-farm yield 3.3 t ha^{-1}) in 2013 (FAOSTAT, 2015). In the UK, winter wheat is the most widely grown arable crop grown on 1,615 M ha in 2013, and approximately 30% of the area is on drought-prone soils (Foulkes *et al.*, 2001). The annual yield production is about 16 million tonnes with an average on-farm yield of around 8.0 t ha^{-1} (Foulkes *et al.*, 2001; Foulkes *et al.*, 2007c; DEFRA, 2014).

Average yields of wheat depends on country and region and ranged from more than 8.0 t ha^{-1} in N Western Europe to less than 1.0 t ha^{-1} in several countries in Central and West Asia and North Africa (Reynolds *et al.*, 2008). Increasing global population along with declining water resources and climate change associated with global warming are expected in the next 40-50 years, therefore raising food global demands by approximately 60% to feed around 10 billion people and leading to reduced food security (Reynolds *et al.*, 1999; G Fischer *et al.*, 2002; Rosegrant and Cline, 2003; Slafer *et al.*, 2005b; Reynolds and Borlaug, 2006; Lobell and Field, 2007).

In order to raise the wheat yield and minimize the effect of unfavourable environments, higher yield potential of cultivars under abiotic stress environments will be required whilst avoiding any proportional increase in the use of water or fertilizer (Hirel *et al.*, 2007; Cattivelli *et al.*, 2008; Foulkes *et al.*, 2009; Foulkes *et al.*, 2011). Wheat contributes, on average, 20% of the total calorific input of the world's population (Ortiz *et al.*, 2008). To preserve food security new cultivars with greater drought resistance must be developed (Aravinda Kumar *et al.*, 2011). Since about 32% of the cultivated wheat area in the world commonly face water limitation at key stages of wheat development (Chen *et al.*, 2012), the objective of many breeding programmes has become drought tolerance improvement (Kandic *et al.*, 2009).

1.2 Impacts of drought

Water stress is the main factor limiting wheat yield in rain-fed environments worldwide (Richards *et al.*, 2002). In these water-stress environments, yield genetic progress has been restricted as a result of genotype x environment interaction according to either site or season (Cooper *et al.*, 2001; Richards *et al.*, 2002; Christopher *et al.*, 2008). In crops including wheat, plant breeders and physiologists have classified the mechanisms of minimizing the effect of water limitation into (i) drought escape, (ii) drought avoidance (drought endurance with high internal water content) and (iii) drought tolerance (drought endurance with low internal water content) (May and Milthorpe, 1962). In this context, drought resistance has been considered to be a combination of drought avoidance and tolerance, but not drought escape (Fischer and Maurer, 1978).

Globally, drought restricts agricultural productivity more than any other abiotic stress, and many efforts have been carried out for improving crop yield as a major goal of plant breeding under water-stress environments (Tuberosa and Salvi, 2006; Cattivelli *et al.*, 2008; Mir *et al.*, 2012). In developing countries, around 50% of all wheat cultivated is grown under rain-fed conditions where annual rainfall is less than 600 mm (Allahdou, 2012). Drought affects 99 million ha of wheat cultivated in developing countries and about 60 million ha in developed countries, and can decrease average grain yield by 17-70% (Chen *et al.*, 2012). Under Mediterranean conditions, the environmental factor most limiting yield potential is drought stress related to high temperature, irradiance and low rainfall together during the most critical crop growth period of grain filling and grain formation (Araus *et al.*, 1998). Annual rainfall in this kind of environment is less than 1000 mm, and mostly concentrated in autumn and spring (Campiglia *et al.*, 2015), which can effectively cause major variation in crop yield performance (Rahimizadeh *et al.*, 2010), due to inadequate and irregular rainfall distribution and high temperatures during the grain filling stage (López-Bellido *et al.*, 1996).

In the UK, the annual yield loss to drought is around 20% (Foulkes *et al.*, 2002). These losses will be increased with climate change prediction and more frequent summer droughts (Foulkes *et al.*, 2007a). Therefore, identification of traits related to drought resistance without unfavorable effects on yield potential in high rainfall

years without drought could be the most effective strategy to raise yields on drought-prone soils in the UK (Aravinda Kumar *et al.*, 2011). Drought tolerant cultivars can effectively minimize the effect of droughts in wheat in the UK more than use of irrigation or early sowing associated with deeper roots because of the irrigation equipment's high cost and the rotational considerations of early sowing (Foulkes *et al.*, 2001).

Some improvements in wheat breeding in drought environments have been reported through drought escape (phenology modifications), selection for traits indirectly associated with water-use efficiency (e.g. resistance to soil toxicities or to root diseases) (Richards, 1996), and selection for physiological traits directly related to yield improvement under drought (Morgan, 1983; Loss and Siddique, 1994; Richards, 1996; Olivares-Villegas *et al.*, 2007). The benefit of drought escape partly depends on the timing of drought during the crop cycle (Richards, 1991; Loss and Siddique, 1994). As an example of drought escape, early flowering is considered to be a compromise, allowing the development of adequate dry matter without reducing soil water to a level that will limit reproductive growth after flowering in dry regions with predictable early-season rainfall (Foulkes *et al.*, 2002). A yield increase of 30 kg ha^{-1} has been reported by Fischer and Maurer (1978) for each day of flowering earliness in 34 spring wheat cultivars in northern Mexico.

However, late flowering cultivars maintained yield better than early flowering cultivars when drought occurred soon after sowing date and lasted until flowering date (Fischer *et al.*, 1977). Drought tolerance through improving photosynthetic and respiratory systems, assimilate partitioning and water-use efficiency increased yield maintenance under different environmental conditions (Olivares-Villegas *et al.*, 2007). The importance of traits associated with drought tolerance has been increasingly reported in recent years due to better understanding of their impact and associations with yield (Araus *et al.*, 2002; Rebetzke *et al.*, 2002; Olivares-Villegas *et al.*, 2007).

Some yield improvement studies in wheat in the past decades show the greater importance of biomass partitioning to grain yield rather than biomass itself (Austin *et al.*, 1980; Waddington *et al.*, 1986; Sayre *et al.*, 1997; Shearman *et al.*, 2005; Reynolds and Borlaug, 2006). Therefore, understanding the physiological and

genetic bases of biomass partitioning and radiation-use efficiency have become more important to increase harvest index towards the maximum HI estimated to be approximately 0.63 (Austin *et al.*, 1980; Reynolds and Borlaug, 2006). Grain yield potential was improved by approximately 30% through the best expression of harvest index, while the best expression of above-ground biomass can increase yield potential by approximately 34% above the best yielding CIMMYT spring wheat cultivars (Reynolds and Condon, 2007).

1.3 Evolution of bread wheat

About 10,000 years ago, wheat was cultivated for the first time as a part of the "Neolithic Revolution" (Shewry, 2009). The initially cultivated forms were einkorn (diploid AA) containing 14 chromosomes and emmer (tetraploid AABB) containing 28 chromosomes. Durum wheat (*Triticum turgidum* L. subsp *durum*), which is used today to make pasta, is also tetraploid (AABB; 28 chromosome), and the bread wheat (*Triticum aestivum* L.) used today to make dough and batter-based products is hexaploid (AABBDD; 42 chromosome) (Atwell *et al.*, 2001; Sramkova *et al.*, 2009).

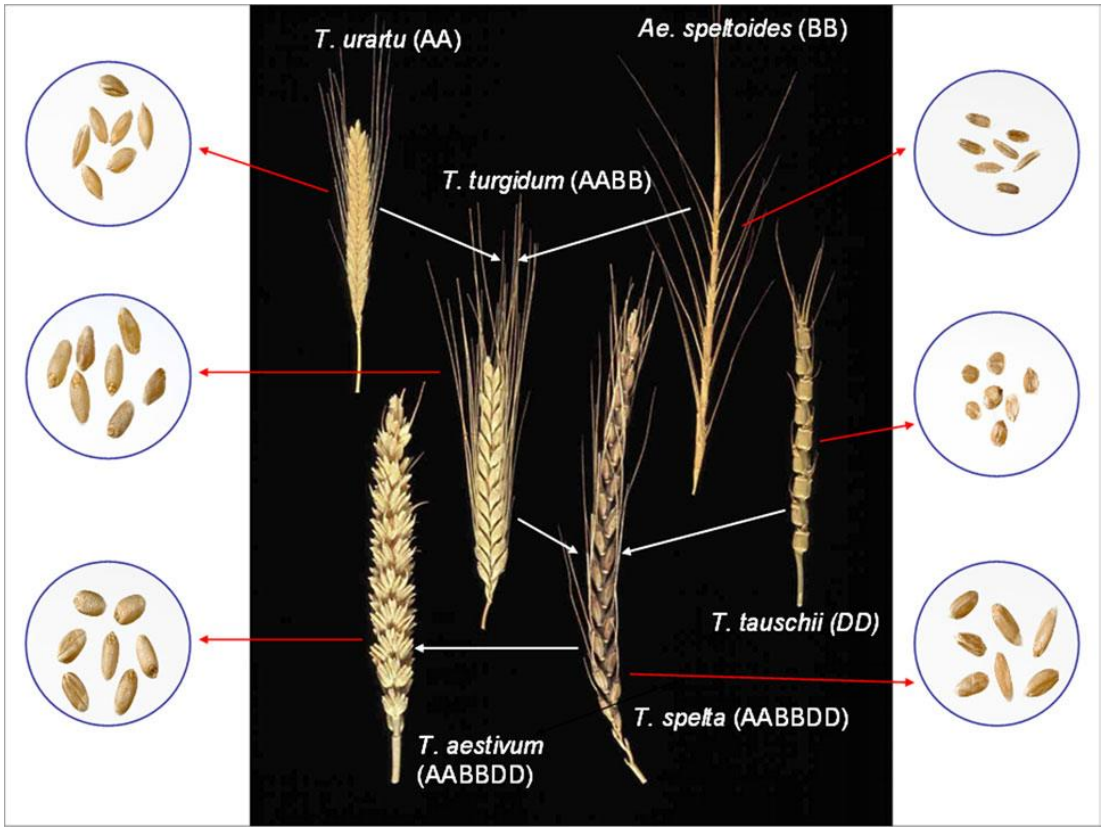


Plate 1.1 The evolutionary relationships between wild diploid grasses, durum and cultivated bread wheats; examples of spikes and grain. From (Shewry, 2009).

1.4 Trait-based breeding for drought resistance

High yield and yield stability under drought are associated with a combination of factors including drought escape and yield potential under optimal conditions, and/or drought resistance. The selection for drought resistance based on grain yield could be more difficult and less accurate for plant breeders than the mechanism of separating each factor (Fischer and Maurer, 1978). For instance, early flowering can benefit crops to escape from the effect of drought in a dry environment with early-season rainfall (Foulkes *et al.*, 2002). Grain yield improvement in a breeding programme could also be achieved genetically through a number of target traits related to drought resistance (Rebetzke *et al.*, 2002). The most important traits are those related to water uptake (e.g. canopy temperature), water-use efficiency (e.g. carbon isotope discrimination), and HI (e.g. stay-green and leaf senescence parameters) (Foulkes *et al.*, 2007b). The wheat breeder's main objective is usually grain yield genetic improvement under limited water availability (van Ginkel *et al.*, 1998; Richards *et al.*, 2002); however, selections based on higher grain yield alone are not consistently the best way of crop performance improvement under water stress, and efficiency of breeding can be increased by complementary selection for drought-based traits. (Cooper *et al.*, 1997).

Seasonal environmental changes such as rainfall affects genotype x environment interaction which makes genetic improvement progress slow as it reduces heritability of the target trait and limits its effectiveness as selection criterion (Feyerherm *et al.*, 1984; Calhoun *et al.*, 1994; Rebetzke *et al.*, 2006; Richards, 2006). A range of physiological traits have been recommended that could be utilized to increase selection efficiency and used as indirect selection criteria for improving yield under drought conditions (Reynolds and Tuberosa, 2008; Lonbani and Arzani, 2011). However, better understanding of the mechanism of a physiological trait's performance and proper evaluation methodologies are the major factors affecting success of the trait-based breeding under limited water availability (Richards, 1996; Araus *et al.*, 1998; Aparicio *et al.*, 2000).

Physiological trait-based breeding for grain yield potential (YP) focuses on improving all the three physiological components of yield potential which has been defined as a combination of the functions of the light intercepted (LI) and radiation-

use efficiency (RUE; above-ground dry matter per unit radiation interception) which produces above-ground biomass, and harvest index (HI) which is the partitioning of biomass to yield (Reynolds *et al.*, 2012):

YP = LI x RUE x HI Equation (2.1)

1.5 Marker-assisted selection

A variety of genomic and genetic techniques can currently be used by wheat breeders to identify gene locations controlling complex traits such as drought resistance through molecular genomic markers. These markers and associated genes can be introgressed into adapted cultivars through marker-assisted selection (MAS). Specific quantitative trait loci (QTL) can be identified by carrying out accurate phenotyping and developing genetic maps on particular genetic materials from bi-parental crosses, such as populations of recombinant inbred lines (RILs) or doubled-haploid lines developed using the maize pollination technique (Snape *et al.*, 2007). Association panels for accessions can also be used within genome wide association studies to identify genetic loci with allelic variations that control the phenotypic expression of the key traits (Horvath *et al.*, 2009; Rafalski, 2010).

For example, a genome-wide association study in Serbia reported 385 marker-trait associations for 20 morphological and agronomic traits including grain yield, biomass, harvest index, anthesis date and plant height using 96 accessions of winter wheat from 21 countries across five continents based on contrasting phenotypic expression for the breeding traits (Neumann *et al.*, 2011). Under rain-fed condition in France, a genome-wide association analysis also reported 62 single markers and 33 QTLs associated with early flowering traits using 227 genotypes of a wheat core collection highly contrasting for earliness (Le Gouis *et al.*, 2012).

To date, there are several studies reporting QTLs for drought tolerance traits in wheat. In the UK, for the Spark x Rialto DH winter wheat population, QTL analysis revealed a major effect of the 1BL/1RS interspecific chromosome translocation (Rialto 1B/1R, Spark, normal 1B) on stem water soluble carbohydrate amount (Foulkes *et al.*, 2006), in addition to detecting a number of QTL for this trait on chromosomes 2B, 4B, 3D and 7A (Snape *et al.*, 2007). Verma *et al.* (2004) also

reported a QTL on chromosome 2D for leaf area duration associated with grain yield under drought in a winter wheat Beaver x Soissons population in the UK.

Under rain-fed environments in Australia, using three wheat doubled-haploid mapping populations of Cranbrook x Halberd, Sunco x Tasman, and CD87 x Katepwa, 9 to 13 QTLs were identified in each population for leaf $\Delta^{13}C$ (Rebetzke *et al.*, 2008a), and 7 to 16 QTLs were identified for stem water-soluble carbohydrate amount in each population (Rebetzke *et al.*, 2008b). Pinto *et al.* (2010) detected two QTLs for grain yield on chromosome 3A and 4A under drought using the elite wheat Seri x Babax recombinant inbred line (RIL) population in NW Mexico.

1.6 Objectives

The objectives of this study were to identify novel variation for physiological traits determining water-use efficiency (WUE) and drought tolerance in wheat and to investigate underlying mechanisms and their genetic regulation. This was done by quantifying genetic variation in a UK winter wheat DH population derived from a cross between Rialto and Savannah in field experiments under irrigated and unirrigated conditions, and carrying out QTL analysis for grain yield and physiological traits. Both parents (Rialto and Savannah) are semi-dwarf (Rht-D1b) UK winter wheat and hard endosperm cultivars. Rialto is suitable for some bread-making processes, and first released in 1995. Savannah is a feed wheat cultivar with high yield potential, and first released in 1998. The performance of the most promising DH lines was then compared with that of a range of wheat amphidiploid lines and accessions of wild relatives of wheat in glasshouse experiments under well-watered and water stressed conditions to identify wide variation for key traits determining WUE and drought tolerance.

In the following chapter, the physiological and genetic bases of major traits associated with WUE improvement under drought stress in wheat are reviewed. The main hypotheses and thesis structure are then presented at the end of the literature review chapter.

Chapter 2 Literature Review

2.1 Definitions of water uptake and water-use efficiency

The grain yield potential under drought conditions is the product of three physiological crop components as described by Passioura (1977): (i) water uptake (WU), (ii) water-use efficiency ($WUE_{biomass}$; above-ground biomass per unit water uptake) and (iii) harvest index (HI) (Equ 2.1). Improvement in any of them should cause an increase in grain yield as they are mostly independent of each other, and their interaction (pleiotropic and epistatic effects) and over time (phenological stages and environmental changes) can be dissected (Reynolds and Tuberosa, 2008):

$$\text{Grain yield} = WU \times WUE_{biomass} \times HI \qquad \text{Equation (2.1)}$$

Where WU is the season-long water uptake, $WUE_{biomass}$ is the amount of above-ground dry matter per unit of water used and HI is the ratio of grain yield to total above-ground dry matter.

The water uptake under drought is highly associated with the depth of the root system, and can be affected by soil properties that limit root growth (Foulkes *et al.*, 2001; Richards *et al.*, 2001). Therefore, genetic improvement of the root system is essential particularly when available water remains after harvest, e.g. through later flowering genotypes or early sowing to extend the vegetative period of the crops in addition to early or later vigour and greater osmotic adjustment (Richards *et al.*, 2002).

In semi-dwarf bread wheat, more drought-tolerant genotypes tended to have more roots with greater root lengths in deeper soil profiles, while fewer roots with reduced root length were formed by non-tolerant genotypes in deep soil in an investigation carried out at the International Maize and Wheat Improvement Centre (CIMMYT) in NW Mexico (Manske *et al.*, 2000). Moreover, thin and deeper roots were observed under low soil water content, whereas thick and shorter roots were found under high soil water content (Li *et al.*, 2001). Significant genetic variation is reported for root traits in wheat and other cereals (O'toole and Bland, 1987). In a UK field experiment, for instance, differences were found in the root length per unit volume of soil at depth from 1-2 cm cm^{-3} between six modern winter wheat cultivars (Ford et al., 2002).

Bread wheat landraces have not yet been widely studied for the physiological bases of drought tolerance. However, selected landraces showed greater capability in water extraction from soil depth compared with check lines in an investigation screening over 2000 Mexican landraces for yield under drought (Reynolds *et al.*, 2007b).

Water is essential and required in the photosynthesis process for evapotranspiration and CO_2 assimilation in plants. Plants have different potential of water usage to assimilate CO_2 molecules and produce biomass; these differences relate to water-use efficiency (Condon *et al.*, 2002) which can be defined at either the photosynthetic organ scale or above-ground biomass and yield scales (Tambussi *et al.*, 2007). At the photosynthetic organ (e.g. leaf) level, water-use efficiency can be defined as the intrinsic water-use efficiency ($WUE_{intrinsic}$) which is the ratio of CO_2 assimilation rate (A) to transpiration rate (T) in the same period of time. The stomatal conductance (g) for CO_2 (g_c) and water vapour (g_w), and the difference between inside and outside the leaf of either CO_2 concentration ($C_a - C_i$) or water vapour concentration ($W_i - W_a$) are components of both A and T. Thus,

$$A = g_c (C_a - C_i) \;\; ; \;\; T = g_w (W_i - W_a) \qquad \text{Equation (2.2)}$$

$$W_{intrinsic} = A/T = [g_c (C_a - C_i)] / [g_w (W_i - W_a)] \qquad \text{Equation (2.3)}$$

$$W_{intrinsic} \approx 0.6\, C_a(1 - C_i/C_a)/(W_i - W_a) \qquad \text{Equation (2.4)}$$

Where the factor 0.6 refers to the relative diffusivities of CO_2 and water vapour in air (Condon *et al.*, 2002; Tambussi *et al.*, 2007).

However, crop breeders and physiologists also define water-use efficiency at the crop production level, i.e. the above-ground dry matter divided by water used over the period of crop life cycle which is the water-use efficiency for the above-ground dry matter (WUE_{AGDM}), and the grain divided by water used over the crop life cycle period which is the water-use efficiency for the grain (WUE_{grain}) (Abbate *et al.*, 2004; Tambussi *et al.*, 2007).

2.2 Wheat ancestral germplasm and genetic improvement

Genetic improvement of wheat depends on gene expression within a broad pool of functional genetic resources. Therefore, wild relatives of wheat and lines derived from them are sources of novel genetic variation for drought tolerance because of reducing genetic diversity during domestication (Dubcovsky and Dvorak, 2007). Allele transgressive segregations through crosses between conventional lines can also be an important source of new trait expression levels (Able *et al.*, 2007; Reynolds *et al.*, 2007c; Qi *et al.*, 2010).

The wide crossing technique identifies ancestral genetic variation and introgresses chromosome segments related to abiotic stress tolerance into adapted wheat cultivars (Chen, 2005). For example, octoploid amphidiploid (2n = 8x = 56 chromosome; AABBDDJJ) and hexaploid amphidiploid (2n = 4x = 42 chromosome; AABBJJ) material derived from crosses between bread and durum wheat (42 and 28 chromosomes, respectively) and either *Thinopyrum intermedium* or *Thinopyrum bessarabicum* (14 chromosomes; JJ or E^bE^b) are highly salt tolerant (Forster and Miller, 1985; King *et al.*, 1997; Hassani *et al.*, 2000; Fedak and Han, 2005; Qi *et al.*, 2010; Siahsar *et al.*, 2011).

The *Thinopyrum* genus is considered to be a valuable source of drought resistant novel genes to be introgressed into adapted wheat (King *et al.*, 1996; King *et al.*, 1997; Chen, 2005). Drought susceptibility has been evaluated through measuring drought tolerance indices under water stress compared to normal condition in *Tritipyrum* lines (Mitra, 2001; Allahdou, 2012). *Thinopyrum bessarabicum* is a perennial coastal wheat grass distributed in the Crimea (Dewey, 1984; Gorham *et al.*, 1986; King *et al.*, 1997; Qi *et al.*, 2010; Siahsar *et al.*, 2011), and able to grow in 350 mM of NaCl (King *et al.*, 1996). It is also Columbia root-knot nematode, *Melodogyne chitwodi* resistant, and tolerant of scab disease caused by *Fusarium graminearum* (Zhang *et al.*, 2002).

In the UK, the Wheat Improvement Strategic Programme (WISP) is a publicly funded pre-breeding programme which has been producing novel wheat germplasm aiming to identify new genetic variation and understand the genetic bases controlling traits limiting grain yield, such as drought tolerance (WISP, 2015). For example, the seven amphidiploid lines characterized in this study were provided by that project, and recently new amphidiploids have also been developed from crosses between

wheat and related species from the genera Aegilops, Secale, Thinopyrum, and Triticum to be phenotypically and genetically characterized for traits related to biotic and abiotic stresses (Nemeth *et al.*, 2015). In the present study, amphidiploids developed from crosses between durum wheat and *Thinopyrum bessarabicum* and accessions of *Thinopyrum bessarabicum*, *Aegilops speltoides* and *Triticum urartu* were investigated for novel variation in WUE and drought tolerance.

New genetic diversity is also being developed in CIMMYT from inter-specific crosses of wild relatives of bread wheat (those derived from crosses between durum wheat (tetraploid; AB genome) and the wild diploid ancestor *T. tauschii* (diploid; D genome) called synthetic or resynthesized hexaploid wheat (MujeebKazi *et al.*, 1996; Reynolds *et al.*, 2007b). Thus, synthetic-derived wheats were compared with their recurrent parents in an investigation at CIMMYT in Mexico, and relatively higher above-ground biomass was observed for synthetic lines with higher values for stem carbohydrates, water extraction parameters, and transpiration efficiency under drought than recurrent bread wheat parents (Reynolds *et al.*, 2007b). Drought-adapted lines have been developed through crosses between these synthetic wheat genotypes and elite wheat cultivars (Trethowan *et al.*, 2005; Reynolds *et al.*, 2007b), associated with root growth distributed relatively deeper in the soil (Reynolds *et al.*, 2001), and better maintenance of grain weight under water-stressed environments (Trethowan *et al.*, 2003).

2.3 Root growth and responses to drought

Above-ground vegetative growth is mainly affected by the capability of plants to extract water and nutrients through a better growth of root system in different soil profiles and environments (Richards *et al.*, 2001). In order to achieve better shoot growth and above-ground biomass, a better root system architecture and growth are required (Manske and Vlek, 2002). In water stress environments, a deep root system is associated with more water uptake from the deeper soil layers and enhanced drought tolerance (Richards *et al.*, 2001). In the UK, root structure of winter wheat typically consists of six seminal roots which arise from primordia in the embryo and 20 nodal roots which arise from the basal nodes of the main shoot and tillers (Gregory *et al.*, 1978). The wheat root system provides adaptability to a range of different environments; however, its structure importantly depends on the soil

properties, nutrients and water availability, and the plant growth environment (Vlek *et al.*, 1996).

The root to shoot ratio defines the distribution of dry matter between the root and the shoot (Hoad *et al.*, 2001). The translocation of a greater amount of the carbon assimilated to roots has been observed under water stress environments (Hamblin *et al.*, 1990; Gregory and Atwell, 1991; Palta and Gregory, 1997), and increased root to shoot ratio as a result of top-soil drying has also been reported in wheat (Blum and Sullivan, 1997). Similarly, Ehdaie *et al.* (2003) observed a greater root biomass under limited water availability compared with favourable conditions in the rye chromosome 1 (1RS) translocated bread wheat genotype under well-watered and drought conditions compared to the recurrent parent. Alternatively, a decrease of root to shoot ratio results in increasing WUE through increasing above-ground biomass. Therefore, optimizing this ratio according to the water availability in the environment is an important trait in a breeding programme to improve drought tolerance (Richards, 1987).

2.4 Canopy responses to drought

The effect of drought depends on the developmental stages of the crop life cycle when the drought occurs. The effect of drought incidence before anthesis on grain yield (Foulkes *et al.*, 2002) is usually through decreasing grains per m^2, and the grain weight and protein content may remain stable. In the case of severe reduction in grains per m^2, grain demand for N is no longer determined leaf senescence and grain N% may increase (Triboi and Triboi-Blondel, 2002). Drought incidence after anthesis affects CO_2 assimilation and grain weight (grain number per plant will remain stable). Nevertheless, the effect of drought typically has relatively greater effect on yield potential than high temperature post-anthesis in spite of undesirable direct effects of high temperature on duration of grain filling (Triboi and Triboi-Blondel, 2002).

Since drought can affect vegetative and reproductive stages, it is important to understand the crop response to water limitation at different developmental stages (Shi *et al.*, 2010). Early maturity, small plant size, and reduced leaf area have been reported to be associated with drought tolerance (Rizza *et al.*, 2004). Under drought

stress, the number of leaves per plant, leaf size, and leaf duration can be reduced by water stress (Shao *et al.*, 2008). Drought can also limit the leaf extension due to limited water availability for absorption by roots compared with the water status of plant tissues (Passioura, 1997), which can consequently reduce photosynthesis (Rucker *et al.*, 1995).

Under water deficit, physiologically improved crops can efficiently acquire available water in the soil. However, low water uptake potential greatly influences plant growth of critical developmental stages such as flowering time. At this stage, water shortage strongly affects the pollen growth stages and limits ear fertility and grain set in the spike and hence grain yield (Blum, 2005; Bots and Mariani, 2005; Lonbani and Arzani, 2011). More generally low leaf water potential limits the rate of photosynthetic activity due to reductions in chlorophyll content (Farzad *et al.*, 2007).

Since drought influences the wheat photosynthetic performance more through reductions in leaf area and light interception than through photosynthetic rate, drought has a large effect on radiation interception (Legg *et al.*, 1979). Therefore, the effect of soil moisture deficit at the onset of drought can be more specifically identified by tracking effects on green canopy area or light interception, than by its effects on biomass (Foulkes *et al.*, 2001). Jamieson *et al.* (1998) found the reduction in leaf area index with a soil moisture deficit of about 100 mm. In the UK, leaf expansion of wheat was restricted by a water deficit equivalent to 50% available water capacity (soil moisture deficit of 74 mm), and leaf senescence accelerated by 64% available water capacity (soil moisture deficit of 95 mm) (Foulkes *et al.*, 2001).

Above-ground biomass is reduced by drought through its effects on the crop growth rate and duration, through reduction of leaf area index and consequently light interception ability and CO_2 assimilation (Jamieson *et al.*, 1995b). Jamieson *et al.* (1995a) quantified how both a reduction in stomatal conductance and radiation interception results in transpiration reduction under water stress.

Grain yield is a complex trait and can be affected by various other traits positively or negatively (Iftikhar *et al.*, 2012). Although the heritability of yield and biomass are low, selection for above-ground dry matter can effectively contribute genetic

improvements of grain yield under water-stressed environments (Fischer and Wood, 1979; Rebetzke *et al.*, 2002; Rebetzke *et al.*, 2006).

Harvest index (HI) is the proportion of grain from above-ground biomass at harvest which is highly important to improve yield potential genetically under drought. HI under drought depends on the effect of water stress on pre-anthesis biomass partitioning to the structural organs and/or the accumulation and efficiency of utilization of carbohydrate reserves (Richards *et al.*, 2002).

2.5 Physiological traits related to drought resistance

It is clear that in wheat breeding some physiological and morphological traits related to drought tolerance can be used as selection criteria to improve grain yield as an alternative breeding approach (Aparicio *et al.*, 2000). The physiological traits should be highly heritable and amenable to high-throughput assessment in the field and/or marker-assisted selection (MAS) (Araus *et al.*, 1998). Important traits are those related to water uptake, stomatal function affecting water-use efficiency (as indicated by carbon isotope discrimination, see section 2.5.2) and senescence parameters (Foulkes *et al.*, 2007b; Lonbani and Arzani, 2011). Three basic sets of traits have been described in a physiological model based on their associations with drought resistance (Reynolds *et al.*, 2007c):

1) Traits associated with water uptake. Crops with high biomass at early growth stages (pre-anthesis) rapidly cover the ground surface, and hence reduce soil evaporation and reserve more water for use during later grain filling (Blum, 1998; Richards *et al.*, 2002). In addition, traits related to a cool canopy and/or crop water status can be indicators of root depth and ability to extract adequate water from deep soil profiles (Blum *et al.*, 1989; Reynolds *et al.*, 2005).

2) Traits associated with water-use efficiency (above-ground biomass per used water unit over the crop life cycle, assuming no water surface evaporations) include carbon isotope discrimination ($\Delta^{13}C$) of leaves or grains which indicates plant transpiration efficiency (assimilated CO_2 per unit water transpired) (Condon *et al.*, 2002), and osmotic adjustment which can sustain root growth despite water limitation and improve leaf water potential and WUE (Morgan and Condon, 1986; Bort *et al.*, 1996). In addition, traits related to radiation-use efficiency such as photoprotection

through pigment and antioxidant systems on which reduces incident radiation through leaf wax and leaf rolling may also enhance WUE (Havaux and Tardy, 1999; Niyogi, 1999; Richards *et al.*, 2002; Reynolds *et al.*, 2007c).

3) Harvest index (HI) and traits associated with HI. Stem water soluble carbohydrate reserves can increase HI through remobilizing CHO to grains under drought post-anthesis. In addition, stay-green traits such as later onset or reduced rate of leaf senescence are associated with HI during grain filling under water stress (Richards *et al.*, 2002).

2.5.1 Traits related to water uptake

Plant water and nitrogen uptake from deeper soil layers generally requires a deeper root system (Richard *et al.*, 2015). For instance, every 20 cm root depth can increase grain yield by up to 1 t ha^{-1} in wheat, in addition to increasing grain water-use efficiency associated with increased water availability during flowering and enhanced grain growth later in the season (Wasson *et al.*, 2014). Root architecture in wheat such as narrower lateral roots has also a great effect on improving water uptake through accessing more water from deeper soil profiles (Olivares-Villegas *et al.*, 2007; Christopher *et al.*, 2008). In Australia, a deeper and larger root in wheat was associated with a higher number and a more vertical angle of the seminal roots (Olivares-Villegas *et al.*, 2007; Manschadi *et al.*, 2010). Under controlled environment conditions, the root angle of Japanese winter wheat cultivars was related to the vertical distribution of roots in the field (Oyanagi *et al.*, 1993).

Although there is a wide range of genetic variation in root characteristics, direct selection in plant breeding programmes is currently impractical (Reynolds *et al.*, 2007b). However, traits related to stomatal conductance such as canopy temperature (CT) can be used as indirect indicator of the plant root capability to uptake water (Blum, 1988), and CT has been confirmed to be an indirect selection tool to increase breeding efficiency under drought (Brennan *et al.*, 2007). Reynolds *et al.* (2007b) reported positive association between CT and root length per cm^3, and about 50% of the variation in water uptake in deep soil layers was related to CT in synthetic derived (SYN-DER) wheat developed at CIMMYT using standard breeding procedures. Under drought environments, CT also accounted for 60% of variation in

grain yield in wheat recombinant inbred lines (RILs) (Olivares-Villegas *et al.*, 2007). Osmotic adjustment has also been associated with enhanced root growth under drought, although its genetic regulation seems to be simple, the benefits for yield are still debated (Serraj and Sinclair, 2002).

Under Mediterranean environments where early season rainfalls are typical, early vigour can increase water uptake through reducing the evaporation from the soil surface (Richards *et al.*, 2002). Although genetic variation of early vigour is generally low in modern cultivars due to the effect of *Rht1* and *Rht2* alleles that reduce cell wall length, a wheat-barley chromosome addition line in a wheat background covered the ground before the best wheat parent (Richards and Lukacs, 2002). Furthermore, Rebetzke *et al.* (2007) reported alternative dwarfing genes, e.g. *Rht8*, to reduce plant height without reducing early vigour and can be introgressed into wheat through marker assisted selection.

Coleoptile length in wheat has been associated with larger early leaves and more rapid rate of emergence which resulted in greater early growth and hence water uptake (Richards, 1992). Since coleoptile length can easily be measured for large numbers of genotypes, and has high heritability (Rebetzke *et al.*, 1999), longer coleoptiles can be combined with semi-dwarf stature in order to achieve better establishment and high yields (Richards and Lukacs, 2002). Better performance in grain yield and above-ground biomass than the recurrent parent was reported by Steele *et al.* (2006) for near isogenic lines (NILs) developed from introgressing four root length QTLs from Azucena into the upland rice variety Kalinga III through Marker-assisted backcrossing (MABC).

2.5.2 Traits related to WUE

2.5.2.1 Carbon isotope discrimination ($\Delta^{13}C$)

Carbon isotope discrimination has been used as a selection tool for detecting variation in transpiration efficiency (TE) at the leaf level and developing wheat varieties with improved WUE and drought tolerance; for example, CV. Drysdale was released in Australia in 2002 following backcross selection for $\Delta^{13}C$ and had higher grain yield under drought compared with the recurrent parental line Hartog (Richards, 2006). Wheat, a C3 plant, discriminates against the heavier stable carbon

isotope (^{13}C) in favour of the lighter isotope (^{12}C) and more abundant form in the atmosphere (99%) during photosynthetic carbon dioxide fixation; Rubisco has an intrinsic discrimination value of -30‰ against $^{13}CO_2$ (Pask *et al.*, 2012). However, the primary CO_2 fixation enzyme of C4 plants, Phospho-enol pyruvate (PEP) carboxylase, has much smaller discrimination (approximately -2 to 6‰), therefore a different C isotope ratio can be observed in C3 and C4 plants (Farquhar *et al.*, 1989).

The content of ^{13}C in plant materials can be quantified as carbon isotope composition ($\delta^{13}C$) which is the value of $^{13}C/^{12}C$ ratio in the plant samples compared to the value of the same ratio in "an accepted international standard, the limestone Pee Dee belemnite" (Condon *et al.*, 2002). Carbon isotope discrimination is the value of $^{13}C/^{12}C$ ratio in the plant samples compared to the value of the same ratio in the air (Farquhar and Richards, 1984). Thus,

$$\delta^{13}C\ (‰) = \left(\frac{R_P}{R_S} - 1\right) * 1000 \qquad \text{Equation (2.5)}$$

$$\Delta^{13}C = \frac{R_a}{R_P} - 1 \qquad \text{Equation (2.6)}$$

Where R_P is the $^{13}C/^{12}C$ ratio in the plant material; R_S is the $^{13}C/^{12}C$ ratio of the standard; ‰ is per mil; and R_a is the $^{13}C/^{12}C$ ratio of the atmosphere (Condon *et al.*, 2002).

$\Delta^{13}C$ measured on leaves during early canopy development (under favourable water conditions) can be related with TE at this stage of development, while grain $\Delta^{13}C$ at maturity (under water stressed condition) can be related to TE during the grain filling period (Pask *et al.*, 2012). In C3 plants, $\Delta^{13}C$ may practically contribute for selection in transpiration efficiency. Since $\Delta^{13}C$ is proportionate to the partial pressure of CO_2 in the intercellular air space of leaves (Ci), more discrimination occurs against ^{13}C with higher Ci (i.e. when stomata are open). Also with more closed stomata and lower Ci the gradient of CO_2 into the leaf relative to water vapour out of the leaf increases, i.e. TE increases. Thus, TE is negatively associated with discrimination against ^{13}C (Farquhar *et al.*, 1989). Although, measuring $\Delta^{13}C$ provides no information on whether the variation in TE is related to the variation in g or A, and the vapour pressure affecting TE at the leaf level, it can identify the relative

differences between genotypes within an experiment as a useful breeding tool (Farquhar *et al.*, 1989; Aravinda Kumar, 2006).

$\Delta^{13}C$ can be easily assessed with reasonable cost in wheat; therefore, it has become one of the most widely used traits to quantify genetic variation in TE under water limited environments (Condon *et al.*, 2002; Aravinda Kumar *et al.*, 2011). Interpreting $\Delta^{13}C$ values should take account of both the plant organ sampled (leaf and grain) and the degree of water stress in the experiment.

There is a positive relationship between this discrimination and Ci (Condon *et al.*, 2002; Monneveux *et al.*, 2006), and a negative relationship with leaf transpiration efficiency (TE) (Farquhar and Richards, 1984; Xue *et al.*, 2002; Pask *et al.*, 2012) as mentioned above; therefore, selecting low $\Delta^{13}C$ genotypes could increase biomass and grain yield under water stress (Farquhar and Richards, 1984; Monneveux *et al.*, 2006). Thus, in Australia, Rebetzke *et al.* (2002) reported increases of grain yield of 2-15% for low carbon isotope discrimination lines compared with high discrimination sister lines. Selection for low C isotope discrimination has been also proposed to improve grain yield in wheat under severe drought in Australia (Condon *et al.*, 2004; Richards, 2006; Reynolds *et al.*, 2009).

However, positive correlations have also been found between grain $\Delta^{13}C$ and intrinsic water-use efficiency under both favourable and limited water environments (Yasir *et al.*, 2013). Under rain-fed and irrigated conditions, positive correlations were also observed between both flag-leaf $\Delta^{13}C$ and grain $\Delta^{13}C$, and leaf CO_2 exchange rate, stomatal conductance and internal to ambient CO_2 concentration ratio (Ci/Ca) for five durum wheat (*Triticum durum* Desf.) cultivars (Monneveux *et al.*, 2006). Relationships between genetic variation in $\Delta^{13}C$ and photosynthesis rate (A_{max}) have been reported to be positive amongst 49 bread wheat genotypes under restricted water availability in China (Yasir *et al.*, 2013). Under post-anthesis water limited environments, a positive relationship between $\Delta^{13}C$ and grain yield was reported in various countries (Condon *et al.*, 1987; Ehdaie *et al.*, 1991; Morgan *et al.*, 1993; Araus *et al.*, 1998; Merah *et al.*, 2001b; Tsialtas *et al.*, 2001; Monneveux *et al.*, 2005; Monneveux *et al.*, 2006; Xu *et al.*, 2007; Yasir *et al.*, 2013). In the UK, a positive association was also found between grain $\Delta^{13}C$ and grain yield under both irrigated and unirrigated conditions in a doubled-haploid winter wheat population

derived from the cross between cultivars Beaver × Soissons (Aravinda Kumar *et al.*, 2011).

Most of the associations reported between genetic variation in $\Delta^{13}C$ and grain yield have been either positive or neutral where there has been strong dependence on "within-season" rainfall. In these environments, it seems there is a trade-off between higher TE and season-long water uptake with water uptake being the most driver for yield (Blum, 2005). In contrast, associations have tended to be either negative or neutral in stored moisture and severe drought environments (Condon *et al.*, 2002). Reynolds *et al.* (2007a) has proposed potential yield gains of about 9% above the best yielding cultivars for the best expression of carbon isotope discrimination under drought.

It is essential to understand the inheritance of $\Delta^{13}C$ in the development of strategies aimed for efficient selection for TE in breeding programme. Some reports stated that broad- and narrow-sense heritability for $\Delta^{13}C$ is high when expressed on a plot or entry-mean basis, and the nature and size of gene action of $\Delta^{13}C$ is under strong genetic control in bread wheat (Rebetzke *et al.*, 2006).

Rebetzke *et al.* (2002) found higher grain yield (between 2% and 5%) for selected low $\Delta^{13}C$ lines compared to high $\Delta^{13}C$ lines in a breeding programme at CSIRO in Canberra, Australia, after two backcrosses of a low $\Delta^{13}C$ cultivar Quarrion into variety Hartog. Moreover, low $\Delta^{13}C$ also increased above-ground biomass, harvest index and grain weight. In addition, higher heritability for $\Delta^{13}C$ ($h^2 = 0.93 \pm 0.16$) was reported than grain yield ($h^2 = 0.55 \pm 0.19$) and above-ground biomass ($h^2 = 0.34 \pm 0.20$), and significant genetic correlation was found between $\Delta^{13}C$ and grain yield, and $\Delta^{13}C$ and above-ground biomass (Richards *et al.*, 2002).

Therefore, it has been suggested that in severe water-stress environments in Australia selection for high biomass and grain yield indirectly through low $\Delta^{13}C$ can be more effective than direct selection of either biomass or yield in early generations of a breeding programme (Rebetzke *et al.*, 2002; Rebetzke *et al.*, 2006). It has been similarly reported that $\Delta^{13}C$ was positively associated with above-ground biomass and harvest index under severe water-stress environments in Australia (Condon *et al.*, 1987; Ehdaie *et al.*, 1991; Lopezcastaneda and Richards, 1994; Merah *et al.*,

2001a; Zhu *et al.*, 2010; Yasir *et al.*, 2013); this may be indirectly associated with a greater water uptake through a deeper rooting system.

2.5.2.2 Leaf photosynthetic traits

Chlorophyll molecules in a leaf absorb light energy which can be used to drive photosynthesis (photochemistry), providing the chemical (in the form of ATP and NADPH) for CO_2 fixation in the Calvin cycle, or it can be re-emitted as light chlorophyll fluorescence or dissipated as heat (Zhang *et al.*, 2010b). Photosynthesis is the initial process of producing assimilated CO_2 which contributes dry matter in higher plants. So, increasing photosynthetic potential in new cultivars is one of the most important approaches to raise above-ground dry matter and grain yield in wheat. Soil water stress can effectively reduce light-saturated leaf net CO_2 assimilation rate (A_{max}), stomatal conductance (g_s), transpiration rate (E), grain yield, and water-use efficiency (WUE) (Turner *et al.*, 1984, 1985; Shangguan *et al.*, 2000; Xue *et al.*, 2004). Shangguan *et al.* (1999) also reported the effect of gradual and fast water limitation in which photosynthetic capacity of plants was greater under gradual than under fast drying resulting in higher net photosynthetic gas-exchange.

In wheat, variation in gas-exchange parameters between different genotypes has been previously reported (Blum, 1990; Morgan and Lecain, 1991; Fischer *et al.*, 1998; Xue *et al.*, 2002). However, gas-exchange measurements from light-saturated leaves may not always scale to canopy photosynthesis, as a result of different leaf photosynthetic performances at different times, and differences in leaf photosynthesis between shaded and fully illuminated leaves in the canopy. Therefore, genetic variation in light saturated photosynthetic rate (A_{max}) is not always positively associated with grain yield response (Reynolds *et al.*, 2012). During the past decades, photosynthetic capacity has been improved by increasing leaf area index and radiation-use efficiency in wheat (Jiang *et al.*, 2000). The contribution of flag-leaf photosynthesis is estimated to be about 30–50% the assimilated CO_2 in grain filling in wheat (Sylvester-Bradley *et al.*, 1990; Foulkes *et al.*, 2007c).

In wheat, studies have previously reported a positive association amongst genotypes between grain yield and the CO_2 assimilation rate at light saturation and stomatal

conductance under irrigated conditions (Blum, 1990; Jiang *et al.*, 2000). Fischer *et al.* (1998) and Lu *et al.* (1998) also reported that higher grain yield was more strongly associated with higher g_s than with higher A_{max}. However, no correlations between CO_2 assimilation rate, stomatal conductance, WUE, and grain yield was found by Xue *et al.* (2002) under water stress in hard red winter wheat cultivars, or by Gent and Kiyomoto (1985) under irrigation in winter wheat. Techniques of leaf gas exchange can provide an assessment of the transpiration efficiency of the whole-crop level. Presently there are several commercial portable systems (e.g. LI-6400, Li-Cor Inc., Lincoln, Nebraska, USA) to measure leaf gas exchange parameters facilitated with options for controlling CO_2, humidity, temperature and light, and provide real-time measurements of CO_2 uptake (A), transpiration (E), leaf stomatal conductance (g), and the intercellular CO_2 mole fraction (Ci) as indicators of the photosynthesis activity occurring within the leaf (Long and Bernacchi, 2003).

In the case of high temperature and solar radiation, stomatal closure reduces intercellular CO_2 availability because of excessive light, and consequently an energetic/metabolic imbalance of photosynthesis occurs due to the effect of water and/or heat stress. This can be detected through chlorophyll fluorescence assessment technique (Araus *et al.*, 1998). Leaf chlorophyll fluorescence indicates the maximum rate of photosystem II is used to evaluate the effect of environmental stress on the photosynthetic efficiency. Lu and Zhang (1998) have shown in a study on wheat that in dark-adapted leaves, the photosystem II (PS II) photochemistry has not been affected by water stress whereas it modified PS II photochemistry in light-adapted leaves. Water stress did also not affect the efficiency of PS II for the youngest fully expanded winter wheat leaf and the efficiency of potential photosynthetic quantum conversion (Shangguan *et al.*, 2000).

Information about changes in the efficiency of photochemistry and heat dissipation can be obtained by measuring the amounts of chlorophyll fluorescence. Chlorophyll fluorescence is strongly related with the efficiency of PS II, which can reflect the photosynthesis efficiency in different plants. Chlorophyll fluorescence analysis has become one of the most influential techniques available to plant physiologists to determine leaf photosynthetic performance, and it has been used in the study of wheat water stress (Zhang *et al.*, 2010b). In winter wheat, drought reduced the

efficiency of the PS II (*Fv/Fo*; the ratio of variable to minimum chlorophyll fluorescence) and the efficiency of potential photosynthetic quantum conversion (*Fd/Fs*) of leaves under well-watered and drought conditions (Shangguan *et al.*, 2000). Under water stress, positive associations of *Fv* (variable chlorophyll fluorescence), *Fm* (maximum chlorophyll fluorescence) and dark-adapted *Fv/Fm* ratio were reported with grain yield in bread wheat (Farzad *et al.*, 2007). Rad *et al.* (2014) has also reported a strong association between *Fv/Fm* ratio and grain yield which explained about 57% of the variation in grain yield under drought.

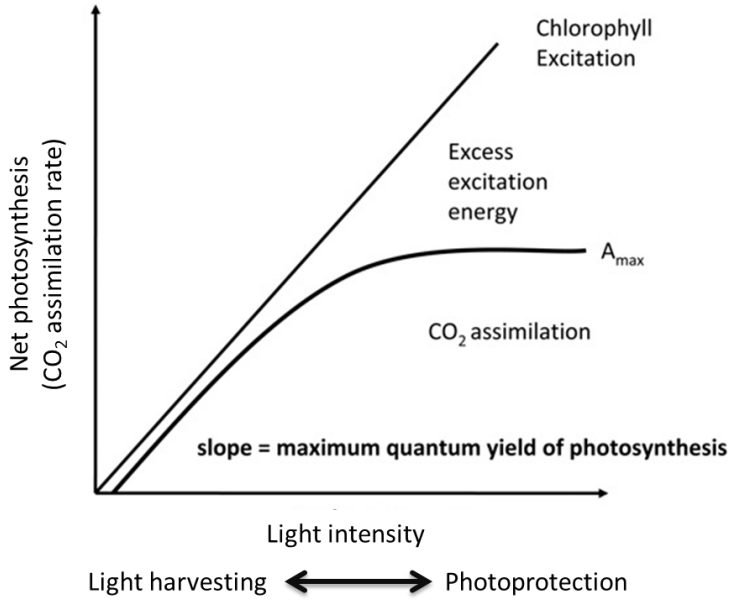


Figure 2.1 A schematic diagram showing the light response and the principle of photoprotection by differentiating the absorbed light used in plant leaf photosynthesis. From (Murchie and Niyogi, 2011).

2.5.3 Traits related to HI

2.5.3.1 Stay-green traits

'Stay-green' is delayed leaf senescence compared with a typical reference genotype and increases plant productivity through maintaining the active photosynthetic rate (Joshi *et al.*, 2007). The stay-green trait can be defined in different ways; such as: (i) delayed onset and normal rate, (ii) normal onset and slower rate and (iii) enhanced onset and rate of senescence which extend the photosynthesis process as a result of

genetic modifications defining the onset and rate of senescence (functional stay green) resulting in higher yield as a consequence. Compare this with non-functional types of stay green such as: (iv) normal onset and rate of senescence and (v) rapid tissue death, depend on chlorophyll maintenance due to lesions in its catabolism, and deficiency in photosynthetic capability at the same time (Thomas and Howarth, 2000; Jiang *et al.*, 2004; Joshi *et al.*, 2007).

Leaf senescence is considered to be a remobilization of nutrients from vegetative tissues to reproductive organs through a regulated process which is genetically and environmentally controlled (Lu *et al.*, 2001; Verma *et al.*, 2004; Lim *et al.*, 2007; Masclaux-Daubresse *et al.*, 2008; Guiboileau *et al.*, 2010; Bogard *et al.*, 2011). The major change in the chloroplasts during leaf senescence is the disassembling of the photosynthesis system and hence the photosynthetic activity decrease, which is more related to loss of Ribulose-bisphosphate carboxylase/oxygenase (Rubisco) rather than loss of thylakoid proteins which occurs during late leaf senescence (Woolhouse, 1984; Crafts-Brandner *et al.*, 1990; Okada *et al.*, 1992; Grover, 1993; Lu *et al.*, 2001).

During grain filling, there is a balance between nitrogen demand by and supply to the grain which leads to accelerated or delayed leaf senescence. Water deficit is one of the environmental factors affecting the availability of nitrogen, and accelerating the leaf senescence (Triboi and Triboi-Blondel, 2002). Drought resistance in wheat genotypes can be determined through flag-leaf senescence parameters such as onset and rate of senescence (Verma *et al.*, 2004). Grain filling period can be extended, and CO_2 assimilation maintained through delaying leaf senescence to ensure that maximum grain mass is attained (Verma *et al.*, 2004).

Increasing carbon fixation by 11% has been reported as a result of delaying the onset of senescence by 2 days in *Lolium temulentum* (Thomas and Howarth, 2000). This can be especially valuable under heat and/or drought environments where rapid and early leaf senescence are highly expected, and hence assimilate supply to the grain decreases (Spano *et al.*, 2003). In maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.), genetic variation to delay leaf senescence associated with extended grain filling duration has been reported (Russell, 1991; Borrell and Hammer, 2000), and similar genetic variation exists in other species (Richards *et al.*, 2002). Bogard *et al.*

(2011) reported a strong negative relationship between post-anthesis leaf senescence duration and anthesis date for a recombinant doubled haploid winter wheat population from the cross Toisondor x CF9107, and associations with grain protein content and grain yield under low N condition. Verma *et al.* (2004) reported significant and positive association between delayed flag-leaf senescence and grain yield in wheat amongst lines of a Beaver x Soissons DH population under drought in the UK.

A positive correlation between genetic variation in stay-green and grain yield in many crops has been reported under drought (Borrell and Hammer, 2000; Gorny and Garczynski, 2002; Campos *et al.*, 2004; Foulkes *et al.*, 2007c). This is mainly due to source limitation of grain yield under post-anthesis abiotic stress whereas grain growth is generally considered to be sink limited under favourable conditions (Bogard *et al.*, 2011). Christopher *et al.* (2008) found that the stay-green phenotype in spring wheat cultivar SeriM82 was associated with extraction of deep soil water. In durum wheat (*Triticum turgidum* L. subsp. *durum*), stay-green mutants have been identified with a higher rate and duration of grain filling (Spano *et al.*, 2003). Flag-leaf stay-green duration also showed a positive correlation with water-use efficiency during grain filling in pot-grown old and modern cultivars of winter wheat (Gorny and Garczynski, 2002). Jiang *et al.* (2004), however, found negative correlations between stay green traits and grain yield in rice.

2.5.3.2 Stem carbohydrate reserves

The stem water-soluble carbohydrates (WSC) include sugars such as fructans, sucrose, glucose and fructose which are accumulated as reserves. They may comprise more than 40% of total stem dry weight at anthesis in wheat which can be effectively remobilised and redistributed to the developing grains (Ruuska *et al.*, 2006). Stem WSC has an important role in buffering grain yield under post-anthesis water stress (Ruuska *et al.*, 2006; Saint Pierre *et al.*, 2010).

The contribution of stem reserves to grain yield may differ from 6 to 100% depending on environmental conditions and cultivars, and will generally be greater under post-anthesis water stress. Positive associations have been reported between stem WSC and each of harvest index and grain yield amongst modern UK cultivars

under both irrigation and drought conditions (Foulkes *et al.*, 2002; Foulkes *et al.*, 2007c). In Mexico, among 36 genetically diverse wheat lines, 32% of the total WSC variation was found due to genetic effects (Saint Pierre *et al.*, 2010). Therefore, selection for higher stem storage of water soluble carbohydrate (WSC) for improved grain-filling and increased grain yields in drought-prone environments has been recommended (Rebetzke *et al.*, 2007).

2.5.4 High-throughput phenomics

Although genomic and marker-assisted selection play an important role in breeding strategies to select based on genotypic information, phenotyping for target traits is still an essential requirement (Jannink *et al.*, 2010). In this context, high-throughput phenotyping has been advanced to facilitate low cost phenotyping in large mapping populations development and association panels (McMullen *et al.*, 2009).

In recent years, wheat breeding companies and public plant research institutions such as the Australian Plant Phenomics Facility at CSIRO, and institutes in the European Plant Phenotyping Network have established fully automated facilities in glasshouses and growth chambers by using robotics and remote sensing techniques to assess plant growth and performance under the accurately controlled environments (Pereyra-Irujo *et al.*, 2012). However, plants experience different conditions from the real field environmental conditions under controlled environments and results cannot consistently be generalized to the field (Passioura, 2012). Therefore, screening at different field locations for the target environment is important for reliable improvement (Cairns *et al.*, 2013).

Field-based remote sensing techniques include spectral reflectance indices and are indirect remote-sensing tools that can evaluate many genotypes easily and in a relatively short time for photosynthetic traits, canopy green area and radiation-use efficiency (RUE). The reflected spectra from crop canopies is measured at different wavelengths in the electromagnetic spectrum in the photosynthetically active radiation (PAR) and near-infrared radiation (NIR) regions (Aparicio *et al.*, 2000). The most common spectral vegetation indices are the normalized difference vegetative index (NDVI; $(R_{900}-R_{680})/(R_{900}+R_{680})$) (Gutiérrez-Rodríguez *et al.*, 2004), simple ratio (SR; (R_{900}/R_{680})) (Peñuelas *et al.*, 1997a), and photochemical reflectance

index (PRI; (R_{531}-R_{570})/ (R_{531}+R_{570})) (Penuelas *et al.*, 1995). The NDVI is positively associated with green area index (GAI) with the strongest correlations observed at lower values of GAI (GAI< 3), so NDVI is a good methodology for measuring senescence. In recent years, hyperspectral indices have been used to assess the plant content of photosynthetic pigments which are different between plants in spectral absorption characteristics, and different indices were developed for detecting and predicting chlorophyll status in plants (Bannari *et al.*, 2007; Lopes *et al.*, 2012).

The photochemical reflectance index (PRI) was associated with the xanthophyll cycle pigments and with the efficiency of the plant canopy's photosynthesis, and developed to estimate the canopy photosynthetic activity (Gamon *et al.*, 1992). In cereals, Filella *et al.* (1996) reported correlations between PRI and radiation-use efficiency. PRI has also been used to assess the photosynthesis at the leaf level in Mediterranean trees, *Quercus ilux* and *Phillyrea latifolia* (Peñuelas *et al.*, 1997b). Penuelas *et al.* (1993) suggested the simple ratio pigment index (SRPI) to measure the ratio of the carotenoid and Chl-a content, and found a positive relationship between SRPI and the carotenoid/Chl-a ratio in apple trees. In addition, the normalized difference pigment index (NDPI) has been used to assess the ratio of total pigments to Chl-a (Penuelas *et al.*, 1993). Gitelson *et al.* (1996) has proposed the green NDVI (GNDVI) for chlorophyll estimation at the leaf level.

Positive associations (either on a linear or a logarithmic basis) have been found between genetic variation in these indices and above-ground dry matter, leaf area index and green area index (Fernandez *et al.*, 1994; Bellairs *et al.*, 1996; Peñuelas *et al.*, 1997a). In wheat, NDVI was positively associated amongst genotypes with grain yield (Das *et al.*, 1993; Aparicio *et al.*, 2000; Serrano *et al.*, 2000; Reynolds *et al.*, 2007b). In bread wheat, the positive relationship between NDVI and grain yield was reported to be stronger under unirrigated conditions (r = 0.54) compared to that under irrigated conditions (r = 0.35) in Mexico (Gutiérrez-Rodríguez *et al.*, 2004).

Canopy temperature is associated with plant photosynthetic activity through plant water transpiration requirements, and hence CO_2 assimilation. It is a broadly used application in many heat and drought breeding programmes as it can effectively reduce the variation at the level of the experimental unit between plants and parts of individual plant. However, a particular environment with a clear sky and windless

conditions is required to measure CT (Pask *et al.*, 2012). Infrared imaging was reported for the first time by Blum and colleagues in Israel in 1982 to assess canopy temperature variations between wheat genotypes under water stress, and found to be associated with yield under favourable water conditions (Reynolds *et al.*, 1994; Reynolds *et al.*, 2012). Positive associations for cooler canopies with grain yield have been found, and suggested to be related to a deeper root system based on physiological evidence (Pinto *et al.*, 2010; Lopes *et al.*, 2012). Under drought, the most drought-adaptive trait of those investigated was found to be canopy temperature which was associated with yield phenotypically (r = −0.75, P<0.0001) and genetically (R (g) = −0.95, P<0.0001), and had high heritability (h^2 = 0.65, P<0.0001) amongst Seri/Babax recombinant inbred line population genotypes under different water regimes in Mexico and rain-fed conditions in Australia (Olivares-Villegas *et al.*, 2007).

2.6 Wheat genetic analysis for drought tolerance

Quantitative traits (complex traits) are those traits such as yield, grain quality and water-use efficiency that are controlled by many genes (Collard *et al.*, 2005). Owing to the involvement of many traits and their interaction with the environment, grain yield improvement in drought conditions is a challenge. Extensive knowledge on the molecular basis of abiotic-stress adaptation has been generated through transgenic and genomic technologies. While conventional plant breeding has achieved important yield gains in drought-prone environments, progress may be restricted by lack of variation within the gene pool of wheat breeding programmes for important traits. Three main approaches to broaden genetic diversity can be employed to enlarge gene pools: first, introgression from germplasm with compatible genomes; second, wide crosses involving inter-specific or inter-generic hybridization; and third, genetic transformation (Reynolds and Tuberosa, 2008).

A relatively smaller genetic improvement has been achieved in wheat yield under dryland environments compared with that in favourable water availability environments or where irrigation is applied, and high variation in seasonal rainfall results in high variation in yield from season to season. Small genetic gains in such environments are mainly a result of genotype x year and/or genotype x location and genotype x year x location interaction effects on yield (Calhoun *et al.*, 1994; van

Ginkel *et al.*, 1998; Richards *et al.*, 2002). Most breeders cannot quantify their genetic advances in yield in terms of the actions of known genes, except for major genes for qualitative traits, e.g. genes for disease resistance and major vernalization and photoperiod and dwarfing genes currently in use, in particular *Rht-B1* and *Rht-D1* (Snape *et al.*, 2007). Currently molecular marker techniques are primarily being used for selecting some disease resistances which are difficult to evaluate or for resistances to viruses and nematodes. However, they can have an important application on progressing crop breeding programmes in drought tolerance (Richards, 2006; Cattivelli *et al.*, 2008).

To reduce the gap between yield potential and actual yield in drought environments, several selection approaches can be exploited in wheat breeding: 1. new physiological understanding to identify traits and new high-throughput phenotyping tools to select for drought-tolerance genotypes. 2. Quantitative trait loci (QTL) discovery through analysis of bi-parental populations or marker trait association discoveries through analysis of association panels through genotyping and phenotyping of populations for drought tolerance traits. 3. Functional genomics approaches to identify candidate sequences to dissect QTLs to develop perfect markers for marker-assisted selection or for a transgenic approach. Therefore, new tools for breeding, such as QTLs for marker-assisted selection and single genes for plant transformation offer opportunities to improve the efficiency of wheat breeding programmes (Cattivelli *et al.*, 2008).

In bread wheat, most genetic improvements to date have achieved for the biotic stress resistance related to traits influenced by a few genes (Bonnett *et al.*, 2005; William *et al.*, 2007) rather than genetically complex traits due to high cost and lack of information on phenotypic responses of the crop genotypes to environments (Snape, 2004; Varshney *et al.*, 2005). However, physiological traits influenced by many genes can be enhanced by selection for genetic markers in wheat (Araus *et al.*, 2002; Condon *et al.*, 2002; Rebetzke *et al.*, 2002; Richards *et al.*, 2002; Condon *et al.*, 2004; Reynolds and Trethowan, 2007).

2.7 Quantitative trait loci (QTL)

2.7.1 QTL analysis

Many approaches have been developed in biotechnology for improving plant reproduction through genetic manipulations such as genome sequencing, gene expression analyses or gene transformation, and genetic marker systems. Genetic markers have advanced plant breeding strategy through identifying gene-based traits related to yield improvement and implementing these markers in plant breeding (Reynolds *et al.*, 2012). Three types of genetic marker have been developed: 1) morphological or visible markers which include phenotypic traits or characters; 2) biochemical markers, which are allelic variations of enzymes called isozymes; and 3) DNA or molecular markers, which show locations of variation in DNA (Winter and Kahl, 1995; Jones *et al.*, 1997; Collard *et al.*, 2005).

The most broadly used type of markers are DNA markers as a result of their abundance which cannot be affected by environmental factors and/or the developmental stage of the plant, unlike morphological and biochemical markers which are highly affected by both biotic and abiotic factors (Winter and Kahl, 1995; Paterson, 1996). There are, generally, three methods for detecting genetic markers; hybridization, polymerase chain reaction (PCR) and DNA sequencing, through techniques which can be either gel electrophoresis (chemically stained using ethidium bromide or silver) or colorimetric probes or radioactive (Winter and Kahl, 1995; Jones *et al.*, 1997; Gupta *et al.*, 1999; Joshi *et al.*, 1999; Collard *et al.*, 2005).

These markers can be divided into polymorphic markers which show and categorize differences between different species or individuals of the same species, and monomorphic markers which show similar sizes for the tested genotypes. Polymorphic markers, based on their ability to differentiate between heterozygotes and homozygotes, can be codominant markers which quantify size of the differences through perhaps many marker alleles (bands on the gel), or dominant markers which only show either present or absent through a pair of marker alleles (Collard *et al.*, 2005).

The position and relative genetic distances between markers along chromosomes are defined as a linkage map, which is used to identify chromosomal locations containing genes and quantitative trait loci (QTLs) associated with target traits; such maps are based on the principle that genes and markers segregate through chromosome recombination (crossing over) during sexual reproduction (meiosis)

(Paterson, 1996). The more tightly-linked genes or markers are together (the lower the frequency of recombination between two markers) the more closely they are situated on a chromosome, and vice versa (Collard *et al.*, 2005).

QTL analysis is done through two main steps: firstly, constructing the map of the markers, secondly, detecting the relationship between phenotypic data and the markers using statistical software (Kearsey and Farquhar, 1998). Various types of crop population are applicable to be analysed for QTL detections. However, those populations derived from a cross between two inbred lines (F2, backcross, recombinant inbred lines and doubled haploid lines) are the most beneficial as the marker-QTL linkages in the F_1 result in the derived F_2 populations being in linkage disequilibrium (Kearsey, 1998).

Quantitative trait loci are regions within genomes that contain genes associated with a particular quantitative trait. DNA markers are mainly used in agricultural research for linkage map construction for crop species. Linkage maps have been utilised for identifying chromosomal regions that contain genes controlling simple traits (controlled by a single gene) and quantitative traits using QTL analysis. This association with traits is known as QTL mapping. DNA markers may be used as molecular tools for marker-assisted selection (MAS) in plant breeding (Collard *et al.*, 2005). Estimation of the loci number controlling genetic variation in a segregating population and their map positions in the genome, gene action, phenotypic effects, pleiotropic effects, and epistatic interactions with other QTLs is done by using molecular linkage genetic maps and QTLs mapping technology (Zhang *et al.*, 2010a).

There are three broadly-used methods for identifying QTLs: 1) single-marker analysis, 2) simple interval mapping and 3) composite interval mapping (Tanksley, 1993; Liu, 1997). QTL mapping uses the linear model to describe the relationship between the phenotypic value and the putative QTL. The difficulty with the individual marker analysis occurs when a QTL is sited between two markers. In this situation, the QTL effect is absorbed by the markers, part in the left and other in the right. The right position of the QTL and its effect is never estimated exactly (Tanksley, 1993; Collard *et al.*, 2005). Interval mapping was firstly developed by Lander and Botstein (1989). It is designed for mapping a single QTL per

chromosome as the statistical model only has a single QTL. It can still detect multiple QTL if more than one QTL are present in a chromosome and they are not too close to each other, unless it may only show a single large peak in the test statistic profile. The estimated effects of QTL will be biased if multiple QTL exist in the same linkage groups. The best model to map multiple QTL is the multiple regression model (Hu and Xu, 2009).

2.7.2 QTL studies for drought tolerance traits in wheat

Drought tolerance is a complex trait which is regulated through the effect of multiple genes in a specific location on crop chromosomes, i.e. QTLs, which depend on the population, plant growth stage, environmental conditions and other factors (Kosova *et al.*, 2014). QTL mapping can explore the genetic variations (Ashraf *et al.*, 2008) through estimating the gene location, size and activity pattern for quantitative traits of a crop (Vinh and Paterson, 2005). Many studies have been conducted for QTL mapping for drought-tolerant traits in various crops such as wheat (Quarrie *et al.*, 1994), barley (Souyris *et al.*, 1997), maize (Sari-Gorla *et al.*, 1999), cotton (Saranga *et al.*, 2001), sorghum (Sanchez *et al.*, 2002) and rice (Bernier *et al.*, 2008).

Under water stress, three grain yield QTL clusters in wheat were detected associated with the semi-dwarfing gene *Rht-B1* on chromosome 4BS and with the vernalisation genes *Vrn-A1* on chromosome 5AL and *Vrn-D1* on chromosome 5DL, and the strongest effect of grain yield QTLs was located on chromosomes 7AL and 7BL in a hexaploid wheat doubled haploid population derived from crosses between Chinese Spring and SQ1 (a high ABA expressing breeding line) (Quarrie *et al.*, 2005). Kirigwi *et al.* (2007) found a QTL related to SSR locus Xwmc89 on chromosome 4AL affecting grain yield and yield components such as grain fill rate, spike density, biomass production, and drought susceptibility index in a mapping population derived from Mexican wheat cultivars.

In Australia, 7 QTLs across populations and 10 QTLs across environments were detected on chromosomes 1A, 1B, 2B, 2D, 4B, 7A and 7B due to large transgressive segregation for stem WSC, and heritability of 0.67 to 0.83 was also reported through characterizing three doubled-haploid bread wheat populations derived from crosses between Cranbrook and Halberd, Sunco and Tasman, and CD87 and Katepua

(Rebetzke *et al.*, 2007). Moreover, Rebetzke *et al.* (2008a) reported a number of QTLs affecting $\Delta^{13}C$ across different environments in Australia using three wheat DH mapping populations, and demonstrated the relationship between $\Delta^{13}C$ and grain yield through associations found between these QTLs. Peleg *et al.* (2009) demonstrated a total of 110 QTLs mapped for 11 growth and yield-related traits under drought in a RIL population derived from a cross between durum wheat cv. Langdon and wild emmer (*Triticum turgidum* ssp. dicoccoides); QTLs affecting yield on chr 2B, 4A, 5A and 7B were co-located with QTLs for $\Delta^{13}C$ and osmotic adjustment showing relationships between these traits and grain yield under water stress.

For early vigour associated with early ground cover and a longer coleoptile, a QTL was identified on chr 6A which contributed up to 8% of the variation in coleoptile length and 14% of seedling leaf width in the population derived from a cross between Chinese semi-dwarf wheat Chuan-Mai 18 and a tall breeding line Vigour 18 (Spielmeyer *et al.*, 2007). A QTL controlling the accumulation of abscisic acid (ABA) concentration was identified on chr 5A in a wheat population derived from a cross between a high-ABA- and low-ABA- producing genotype (Quarrie *et al.*, 1994). In winter wheat, two QTLs on chr 2B and 2D were identified under normal and water-stressed environments by Verma *et al.* (2004) influencing leaf senescence parameters for a DH population derived from a cross between Beaver and Soissons in the UK by using amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers. RAPD and ISSR markers have also been found to be associated with flag leaf senescence in wheat under drought stress (Milad *et al.*, 2011).

The genetic basis of stay-green has been studied in addition to the environmental effects in some crops. In soybean three stay-green mutants, and a cytoplasmic gene, cytG, two recessive alleles, d1 and d2, and a dominant gene, G, regulated the greenness in leaves, pod walls, seed coats and embryos (Jiang *et al.*, 2004). An allele of the *sid (senescence induced degradation)* locus has been observed in the grass *Festuca pratensis* which is inherited as a single recessive nuclear gene (Thomas, 1987). Though, this stay green mutant was termed a cosmetic stay-green for the reason that retaining chlorophyll for a long time in green tissues of individuals

homozygous for *sid*^{y} is not related to an extended period of photosynthetic CO_2 assimilation capacity (Thomas, 1982; Hauck *et al.*, 1997; Spano *et al.*, 2003).

In wheat, genetic variation in functional stay-green (delayed senescence associated with extended photosynthesis) lines has been reported (Silva *et al.*, 2001; Verma *et al.*, 2004). Silva *et al.* (2001) has proposed one gene with two alleles to control this trait in bread wheat which had a high heritability and showed partial dominance with an additive effect, based on analysis of three fixed (P_1, P_2 and F_1) and three segregating (F_2, BC_1F_1 and BC_2F_1) generations.

2.8 Hypotheses and Objectives of the Project

The present study is focused on identifying and understanding the physiological determinants of genetic variation in drought tolerance and their genetic control using a doubled-haploid Rialto x Savannah (94 lines) winter wheat population which was characterized in the field and using selected DH lines and wider amphidiploids and wheat wild relative genotypes which were characterized in the glasshouse under well-watered and unirrigated conditions.

The main objectives of this project were identifying novel traits determining WUE and drought tolerance in wheat and underlying mechanisms by comparing genetic variation between lines of the Rialto x Savannah DH population and between these lines and wheat amphidiploid lines (e.g. durum wheat x *Thinopyrum bessarabicum*), and ancestral wheat species. The genetic bases of the traits was investigated through identifying QTLs for traits associated with drought tolerance traits and grain yield under well-watered and unirrigated conditions.

The main hypotheses were:

1. There is variation in grain yield and above-ground DM and DM partitioning amongst the lines of the Rialto x Savannah DH population and there is genetic variation of grain yield, above-ground dry matter and HI in responses to water deficits amongst Rialto x Savannah DH lines under drought conditions.

2. Carbon isotope discrimination ($\Delta^{13}C$) of grain is positively related to above-ground dry matter (AGDM) and grain yield under UK drought conditions amongst lines of the Rialto x Savannah DH population.

3. Carbon isotope discrimination ($\Delta^{13}C$) of the grain is negatively related to flag-leaf transpiration efficiency (WUE) and positively related to photosynthetic rate and stomatal conductance amongst Rialto x Savannah DH lines under drought conditions.

4. Physiological traits can be identified associated with genetic variation in the Rialto x Savannah DH population in grain yield under irrigated and drought conditions and responses to restricted water availability.

5. There is genetic variation amongst Rialto x Savannah DH lines for flag-leaf transpiration efficiency, photosynthetic rate and stomatal conductance under UK drought conditions and this is correlated with $\Delta^{13}C$, grain yield and yield components under drought.

6. There are positive associations between NDVI post-anthesis, flag-leaf visual score senescence traits and flag-leaf chlorophyll fluorescence and grain yield and above-ground DM amongst the Rialto x Savannah DH lines under drought, and between these traits and biomass and yield responses to water restricted availability.

7. Wild relatives of hexaploid bread wheat have higher water-use efficiency and higher expression of drought-tolerance traits compared with the UK winter wheat Rialto x Savannah DH lines and the two parents.

8. Amphidiploid lines, derived from crosses between wheat wild relative species and bread wheat, have higher water-use efficiency and higher expression of drought-tolerance traits compared with the UK winter wheat Rialto x Savannah DH lines.

9. For Rialto x Savannah DH lines, stomatal aperture traits measured in the glasshouse consistent with the expression of these traits measured in the field.

10. Quantitative trait loci (QTLs) can be identified for grain yield and associated physiological traits explaining variations in WUE and drought tolerance under irrigated and unirrigated conditions in the winter wheat Rialto x Savannah DH population.
11. Co-located QTLs for target drought tolerance traits can be found in the Rialto x Savannah DH population under the two irrigation treatments in the two years.

In order to test these hypotheses, two field experiments examining a DH population of 94 lines derived from the F_1 between winter wheat cultivars Rialto and Savannah were carried out under well-watered and unirrigated conditions. In addition, two glasshouse experiments examining the two parental genotypes (Rialto and Savannah) and 4 Rialto x Savannah DH population lines, three accessions from each of three parental wild species (*T. bessarabicum*, *T. uratu* and *A. speltoides*), and seven amphidiploid lines from crosses between durum wheat and *Thinopyrum bessarabicum* were carried out under well-watered and unirrigated conditions. The experiments were conducted at the University of Nottingham farm and the glasshouses at Sutton Bonington campus, University of Nottingham, Leicestershire, UK.

2.9 Thesis structure

The materials and methods applied in the present study are described in Chapter 3. In the field experiments, 94 Rialto x Savannah DH lines and the two parents were screened for agronomic and physiological traits under irrigated and unirrigated conditions in 2013 and 2014. Harvest traits including grain yield, above-ground dry matter and grain carbon isotope discrimination ($\Delta^{13}C$) are described in Chapter 4. The physiological traits including gas-exchange and leaf senescence parameters and stay green traits in the field experiments and associations with grain yield are described in Chapter 5. In the glasshouse experiments, two parental genotypes (Rialto and Savannah) and four DH lines of the Rialto x Savannah DH population, three accessions of each of three parental wild wheat relatives (*T. bessarabicum*, *T. uratu* and *A. speltoides*), and seven amphidiploid lines from crosses between durum wheat and *Thinopyrum bessarabicum*, were characterized in 2013 and 2014. The results of the glasshouse experiments are described in Chapter 6. Chapter 7 describes

the genetic analysis of agronomic and physiological traits in the field experiments for the Rialto x Savannah DH population to identify QTL associated with grain yield, WUE and drought tolerance under irrigated and unirrigated conditions. The overall results of the present study are discussed in the general discussion in Chapter 8. Some suggestions and future work for wheat drought tolerance improvement in the UK and worldwide are presented at the end of this chapter.

Chapter 3 Materials and Methods

3.1 Field experiments

In this Chapter, the experimental design, plant materials and plot management for the field experiments are described as well as the details of the plant and crop measurement.

3.1.1 Experimental designs and plant materials

In this study, a doubled-haploid (DH) population of 94 lines, derived from the F_1 between UK winter wheat Rialto and Savannah was characterised in two field experiments in 2012-13 (referred hereafter as 2013) and 2013-14 (referred hereafter as 2014). Both parents (Rialto and Savannah) are semi-dwarf (Rht-D1b) UK winter wheat and hard endosperm cultivars. Rialto is suitable for some bread-making processes, and first released in 1995. Savannah is a feed wheat cultivar with high yield potential, and first released in 1998. Each experiment was carried out using a randomised block, split–plot design, in which two irrigation treatments (fully irrigated and unirrigated) were randomised on main-plots, and the DH lines and the two parents were randomised on sub-plots (1.62 m x 6 m) with two replicates.

3.1.2 Experimental site and plot management

The site was located at the University of Nottingham Farm, Leicestershire, UK (52^o 50' N, 1^o 15' W, 50 m above sea level). The soil type was a sandy loam to 80 cm over kyper marl clay Dunnington Heath Series (soil indices: P = 5, K = 3, Mg = 4, pH = 7.6 in 2013; P = 5, K = 2, Mg = 4, pH = 6.8 in 2014). A total of 180 kg N ha^{-1} in three splits (March, April (GS31) and May (GS37)) in the form of ammonium nitrate was applied in both years. In the irrigated main-plots, water was applied using a linear overhead irrigator to maintain soil moisture deficit to 50% available water capacity (180 mm). In 2013, a total of 168 mm of water was applied from early booting to late grain-filling stage (20 mm in May, 43 mm in June and 105 mm in July). In 2014, a total of 80 mm was applied (20 mm in May, 40 mm in June and 20 mm in July) from early booting to late grain-filling stage.

The previous crop was winter oats in 2013 and spring oil-seed rape in 2014, and the seeds were sown using a Wintersteiger drill on 10 October 2012 and 19 November 2013. Seed rate was adjusted by genotype according to 1000-grain weight to achieve

a target seed number of 350 m^{-2}. The metrological data are summarised and presented in Chapter 4.3.1 for air temperature, rainfall and solar radiation which were collected from the nearby meteorological station at Sutton Bonington Campus in each season, and the long-term data were compiled from the UK climate - Historic station data website (MetOffice, 2015).

A prophylactic programme of fungicides, herbicides and pesticides was applied to maintain undisturbed healthy crop growth in all experiments depending on prevalent problems and local conditions to control diseases, weeds and pests. The nitrogen, potassium and phosphorus fertilizer levels were maintained according to the standard recommended agronomic practice. A sub-set of six lines from the R x S DH population (with high/low TE based on grain $\Delta^{13}C$ from previous field results (Foulkes, personal communication)) and the two parents were selected to assess gas-exchange measurement in the field experiments. All other phenotypic and physiological measurements were carried out on all 94 DH lines and the two parents in both seasons.

Plate 3.1 Field experiment mid-July 2013 showing differences between irrigated (right side) and unirrigated (left side) treatments.

3.1.3 Plant and crop measurements

3.1.3.1 Crop developmental stages

The key developmental stages were measured based on the decimal code of growth stages (GS) described by Zadoks *et al.* (1974). Dates of growth stage 31 (GS31; onset of stem extension), GS39 (flag leaf emergence), GS61 (anthesis date) and GS89 (physiological maturity) were recorded in all sub-pots in both years. Five plants per sub-plot were pulled up and the stage of the main shoot was recorded for GS31. For GS39 and GS61, a visual assessment for the whole plant in each sub-plot was carried out, and a growth stage was taken when more than 50 % of the main shoots were at the specific stage. Physiological maturity (GS89) was assessed as the date when less than 25% of the stem area was remaining green.

3.1.3.2 Grain yield, above-ground DM and yield components

In both 2013 and 2014 experiments, samples of ca. 75 shoots per sub-plot were hand-harvested at ground level 1-2 days before combine harvest. In the laboratory, shoots were separated into fertile (those with an ear) and infertile shoots and counted. The fertile shoots were then separated into ears and straw. A 25% sub-sample of the straw was taken (by fresh weight) and weighed. The dry weight of the ears and the sub-sample of the straw were recorded after drying at 80^{o}C for 48 h. After threshing the ears using a Wintersteiger KG threshing machine (WINTERSTEIGER, Austria) (Plate 3.2a), the dry weight of all plant components (grain, chaff and straw) was separately weighed after drying for 48 h at 80^{o}C. Five hundred grains from a sub-sample of grain (ca. 20 g) were counted by a Contador seed counter (PFEUFFER, Germany) (Plate 3.2b) and weighed to obtain the 1000 grain weight. The sub-plots were machine-harvested on an area of at least 5 m^2 (calculated by measuring the actual plot length and width prior to combining), and grain yield then adjusted to 85% DM.

In 2013, ears per m^2 was measured a few weeks before harvest by counting the fertile shoots in a metre row length; this was done for three randomly selected 1 metre row lengths per sub-plot. In both years, ears per m^2 was also calculated by dividing the machine-harvested grain weight per fertile shoot from the hand-harvested sample. Above-ground dry matter per m^2 (AGDM) was calculated dividing the machine

harvested grain yield by the harvest index (ratio of grain dry weight to above-ground dry weight) in the hand-harvested sample. Grains per ear was calculated by dividing the grain weight per ear by the individual grain weight in the hand-harvested sample. In both years, plant height was measured 1-2 days before harvest from ground level to the tip of the ear for three randomly selected fertile shoots per sub-plot.

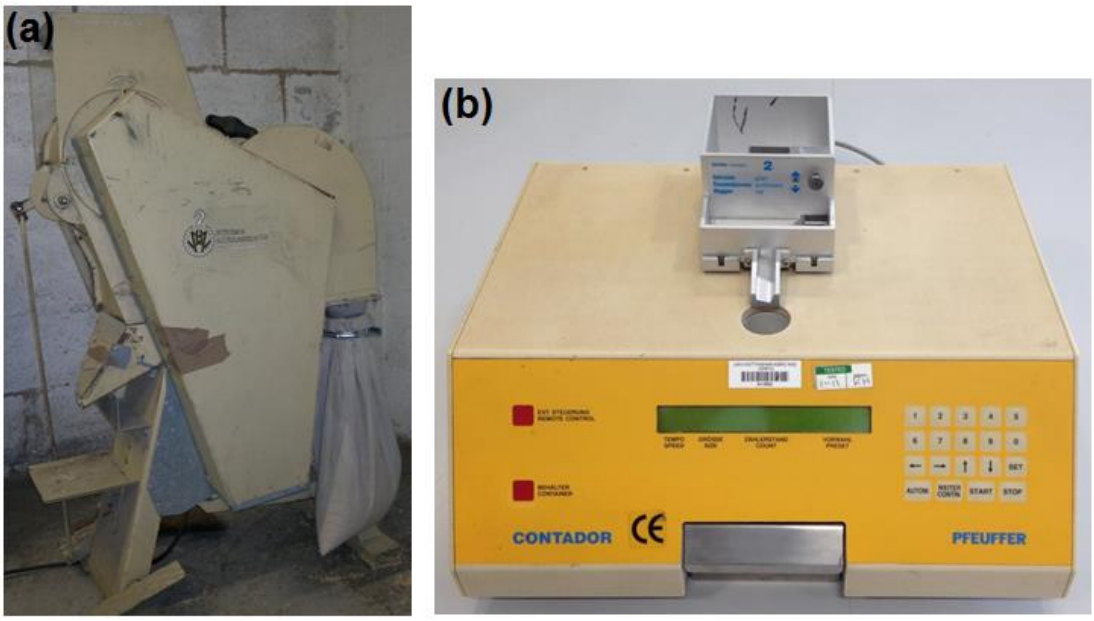


Plate 3.2 (a) F. Walter & H. Wintersteiger KG threshing machine and (b) Contador seed counter.

3.1.3.3 Stress susceptibility index

Stress susceptibility index (SSI) is used to compare genotypes drought susceptibility as it could be independent of the positive association between yield potential and absolute yield losses under drought. It has been suggested by Fischer and Maurer (1978) in order to evaluate the relative genotypic yield performance under stress and favourable water conditions (Aravinda Kumar *et al.*, 2011), as follow:

$$SSI = [1 - (Ys/Yp)]/ [1 - (Ys_m/Yp_m)] \quad \text{Equation (3.1)}$$

Where Ys and Yp are the yield of the individual cultivar under stress and irrigated conditions, respectively. Ys_m and Yp_m are the mean yields of all cultivars in the experiment under stress and non-stress conditions, respectively. Values less than 1.0

denote smaller yield reduction than the mean yield reduction of all genotypes under drought, and are considered to indicate drought resistant genotypes.

3.1.3.4 Carbon isotope discrimination ($\Delta^{13}C$)

In both field experiments, carbon isotope discrimination was measured in all sub-plots under droughted conditions by using a mass spectrometer for $\Delta^{13}C$ determination. Hand harvested grains were oven dried for 48 h at 80^{o}C, and milled to a fine powder using the Ultra Centrifugal Mill ZM 200 (Retsch, Germany) (Plate 3.3a).

In 2013, samples of 0.20 – 0.25 mg fine powder were weighed using a Mettler MT5 analytical microbalance (Mettler-Toledo Ltd, UK) (Plate 3.3b), and encapsulated in tin capsules. Each tin capsule containing the sample was crimped at the top of the capsule with a pair of straight forceps and folded over. The edge of the forceps and a flat spatula were used to gently compact the capsule into a small, tight cube. Each capsule was then dropped into the plastic tray containing series of wells numbered to identify the sample. The final weight to the nearest milligram (3 decimal places) of the capsule was recorded after it was sealed. The encapsulated samples were then sent to the James Hutton Isotope Laboratory, Dundee for the carbon isotope discrimination analysis. In 2014, samples were sent to OEA Laboratories Limited, Callington, Cornwall UK for the carbon isotope discrimination analysis. In both cases, the milled samples (1 mg) were then weighed analysed through an online system composed of an elemental analyser, a TripleTrap and a mass spectrometer (Carlo Erba 2100, Milan, Italy) to determine carbon isotope composition (Aravinda Kumar *et al.*, 2011). The EA interfaced with an isotope ratio mass spectrometer (IRMS; Thermo-Finnigan Deltaplus Advantage, Bremen, Germany) to analyse ^{13}C:^{12}C ratio of the plant material.

In both years, as described by Farquhar *et al.* (1989), the values of $^{13}C/^{12}C$ ratio (R) were expressed as carbon isotope composition ($\delta^{13}C$), calculated as:

$$\delta^{13}\mathrm{C}\ (‰) = \left[\frac{\mathrm{R\ sample}}{\mathrm{R\ standard}} - 1\right] \times 1000 \qquad \text{Equation (3.2)}$$

Where the standard used for calibration was Vienna Pee Dee Belemnite calcium carbonate (VPDB), and the analytical precision was ~0.1‰.

Then, $\delta^{13}C$ was converted to values of discrimination (Δ) against ^{13}C ($\Delta^{13}C$) which is calculated as:

$$\Delta^{13}C = \frac{\delta a - \delta p}{1 + \delta p} \qquad \text{Equation (3.3)}$$

Where 'a' refers to the C isotope composition of air and 'p' to plant, and the carbon isotope composition of air was taken as -8‰.

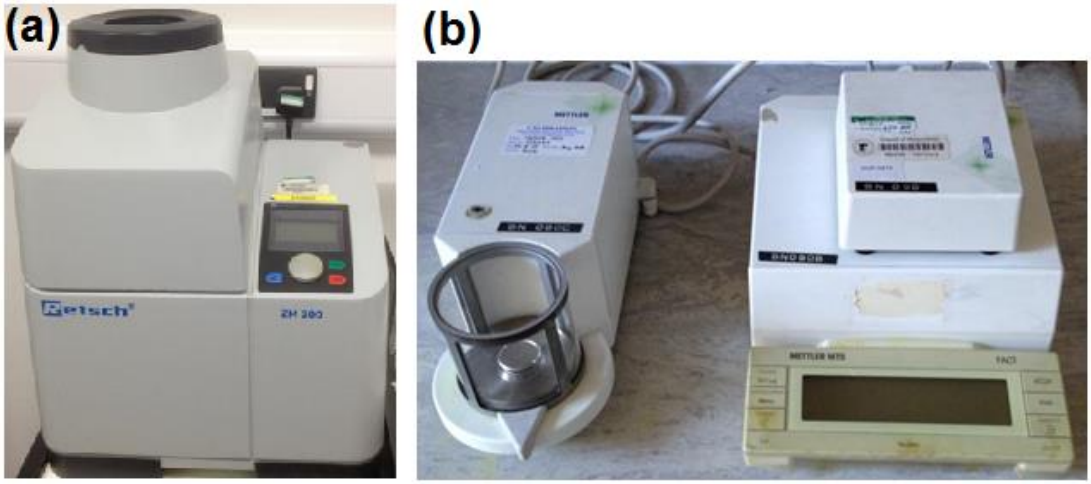


Plate 3.3 (a) Ultra Centrifugal Mill ZM 200 milling machine and (b) Mettler MT5 microbalance.

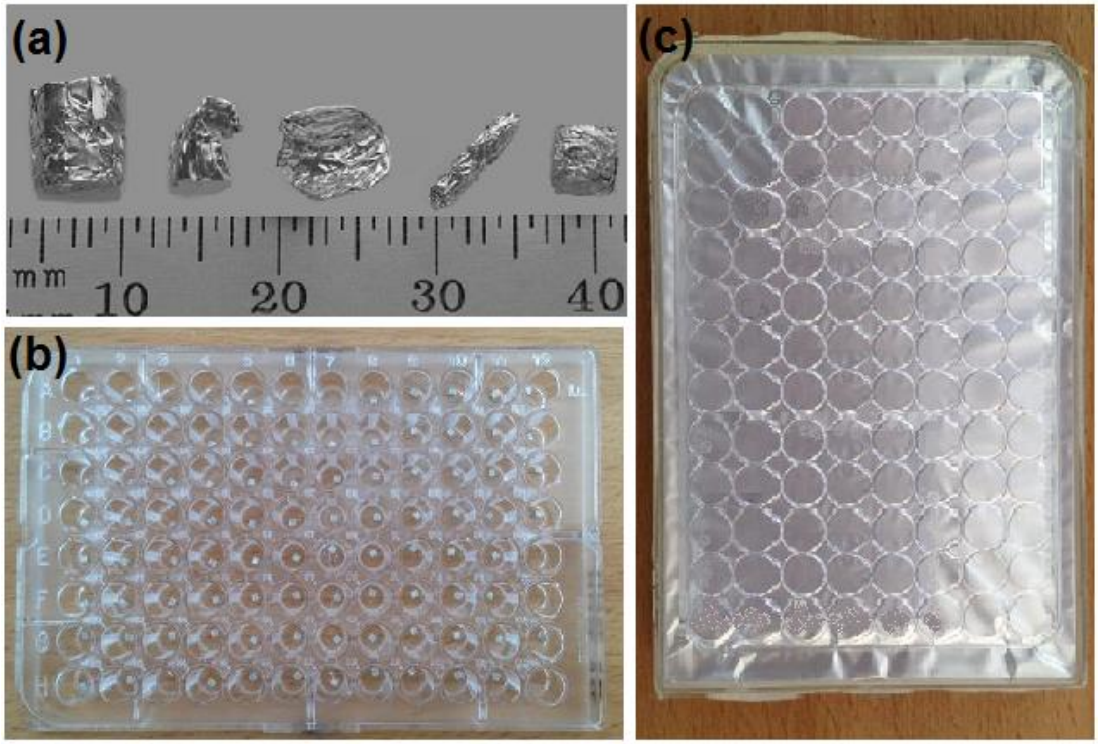


Plate 3.4 The process of encapsulation, (a) steps to encapsulate the samples, and (b) a plastic tray containing the tin capsules, and (c) the plastic tray was secured and ready for the $\Delta^{13}C$ analysis.

3.1.3.5 Normalized Difference Vegetative Index (NDVI)

The Normalized Difference Vegetative Index (NDVI) was measured using a Cropscan, Inc. spectral radiometer MSR16R model with 16 sensor bands in the 450 – 1750 nm region. Measurements (at approximately GS61+3 weeks, GS61+4 weeks and GS61+5 weeks) (on 9, 16 and 26 July in 2013, and on 26 June, 10 and 18 July in 2014) were taken in each sub-plot in the unirrigated treatment in each year in two replicates.

The spectroradiometer was held level to the crop canopy, and the sensor was placed approximately 50 cm above the crop. A reading was taken per sub-plot between 11.00h and 15.00h when the sky was clear and there was sufficient radiation (Pask *et al.*, 2012). NDVI was then calculated as Eq. 3.4 (Gutiérrez-Rodríguez *et al.*, 2004).

$$NDVI = (R_{900}-R_{680})/ (R_{900}+R_{680})) \quad \text{Equation (3.4)}$$

3.1.3.6 Canopy temperature

In both experiments, canopy temperature was measured for all genotypes in the unirrigated treatment approximately five weeks after anthesis (on 12 July in 2013, and on 17 July in 2014) using a handheld INF151 infrared thermometer (UEI Test Instruments, NJ, USA). Three readings per sub-plot were taken on a day with a clear sky with little wind between 11.00 h to 14.00 h when the temperature was above 25^{o}C and when the leaf surfaces were dry. The infrared thermometer was pointed to the plot with a constant distance and angle for all readings. The ambient air temperature was also recorded at the start of each set of five sub-plot measurements to calculate the canopy temperature depression.

3.1.3.7 Flag-leaf chlorophyll fluorescence

A hand-held chlorophyll fluorometer Fluorpen FP-100 (PSI, Brno, Czech Republic) was used to measure flag-leaf chlorophyll fluorescence (quantum yield; ΔF/Fm') at approximately GS61+3 weeks and GS61+4 weeks in each experiment (on 10 and 17 July in 2013, and on 8 and 16 July in 2014). Readings were taken for three randomly selected flag-leaves per sub-plot in the unirrigated treatment in two replicates in

cloudless conditions, when the leaves were well illuminated, and their surfaces were dry between 11.00h to 14.00h (Pask *et al.*, 2012).

3.1.3.8 Flag-leaf gas-exchange measurements

In both years, leaf gas-exchange measurements of light-saturated photosynthetic rate (A_{max}), stomatal conductance (g_s) and transpiration efficiency (TE) were taken weekly on flag leaves for a subset of six DH lines and the two parents under unirrigated conditions from approximately GS61-7 days to GS61+21 days in 2013 (on 19, 27 June, 4 and 15 July) and 2014 (on 6, 17, 24 June and 7 July) using a LiCor 6400-XT Photosynthesis system (LiCor NE, USA). Measurements were taken for three flag-leaves per sub-plot when the leaf surfaces were dry and well illuminated from 11.00h to 14.00h. The system was connected to a leaf chamber measuring cuvette with 2 cm^2 leaf surface area. Conditions in the leaf chamber were set as: cuvette temperature 20ºC, flow rate 500 µmol s^{-1}, CO_2 concentration 400 µmol mol^{-1} and artificial light supply (PAR) 2000 µmol m^{-2} s^{-1} (PQuantum 500 µm, 10% blue). Values were recorded for A_{max} (µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s; mmol H_2O m^{-2} s^{-1}) and transpiration efficiency (TE; µmol $mmol^{-1}$) after 2-5 minutes.

Plate 3.5 LiCor 6400-XT Photosynthesis system used for measuring leaf gas-exchange, leaf chamber clamped a wheat leaf at anthesis.

3.1.3.9 Flag-leaf senescence measurements

Flag-leaf senescence was measured from anthesis (GS61) to full senescence every 3-4 days using a visual senescence score chart ranging from 0 – 10 (0; fully green and 10; fully senesced). Visual assessments were carried out for each sub-plot in both the irrigated and unirrigated treatments, and the values were fitted against thermal time (GS61; base temperature 0^{o}C) applying a logistic regression equation using GenStat 15^{th} edition software package (Payne *et al.*, 2012) as:

$$Y = A + C / (1 + \exp(-B \times (X - M))) \qquad \text{Equation (3.5)}$$

Where Y is the visual senescence score; X is thermal time from GS61 (base temp. 0^{o}C); A is the lower asymptote; M is the thermal time for the point of inflection; B is the slope at the point of inflection taken as the rate of senescence; and A+C is the upper asymptote. The onset of leaf senescence (SEN_{ONSET}) was taken as the thermal time (base temp. 0^{o}C) post-anthesis (GS61) at leaf visual senescence score 2 and end of leaf senescence (SEN_{END}) as thermal time at leaf visual senescence score 9.5. Senescence rate (SEN_{RATE}; slope) were estimated as B. Values were calculated for each sub-plot and the fitted values subjected to ANOVA.

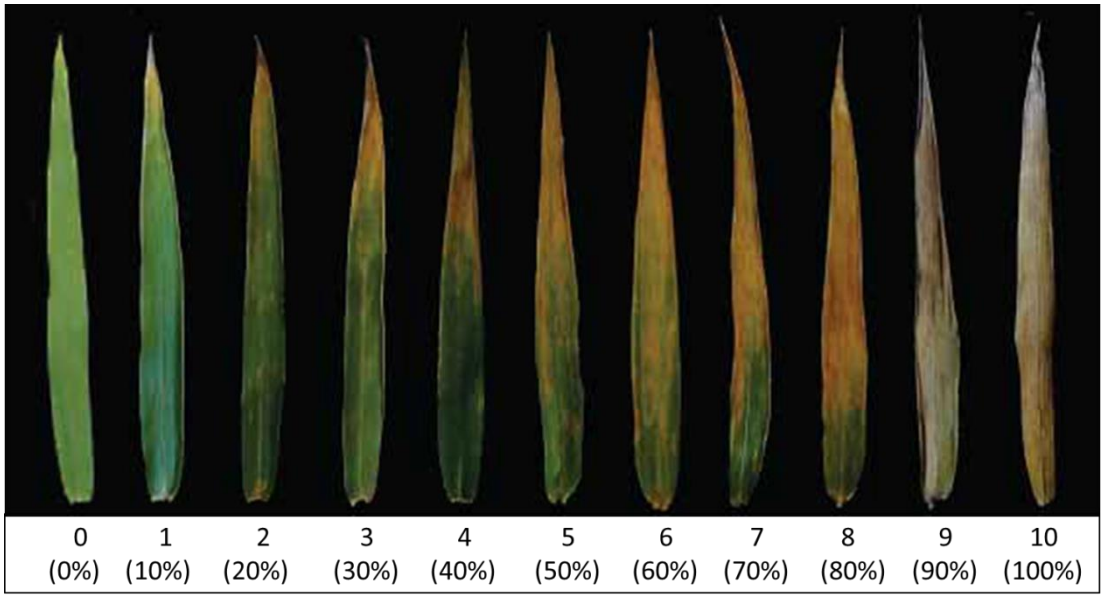


Plate 3.6 Flag-leaf senescence visual score scale indicating approximate % of leaf senescence. From (Pask *et al.*, 2012).

3.2 Glasshouse experiments

3.2.1 Experimental design and treatments

Two glasshouse experiments were conducted, one in each of 2012-13 (referred hereafter as 2013) and 2013-14 (referred hereafter as 2014) at the University of Nottingham, School of bioscience, Sutton Bonington Campus, UK (52^{o} 50' N, 1^{o} 15' W). Twenty two wheat genotypes were examined: the two parents (Rialto and Savannah) and four DH lines from the Rialto x Savannah DH population (lines 20, 25, 63 and 88), three accessions of each of three parental wild wheat relatives (*T. bessarabicum, T. uratu* and *A. speltoides*), and seven amphidiploid lines derived from crosses between durum wheat and *T. bessarabicum* (*T. bessarabicum* x cv Karim, *T. bessarabicum* x cv Stewart, *T. bessarabicum* x cv Langdon, *T. bessarabicum* x cv Macoun, *T. bessarabicum* x cv Creso, *T. bessarabicum* x cv Neodur and *T. bessarabicum* x cv Azaziah). The three accessions of *T. uratu* were not assessed in 2014 as they did not survive after transplanting them into the glasshouse.

A 'split-plot' randomized block design was adopted with two irrigation regimes (well-watered and water-stressed) and three replicates using PVC columns (15 cm x 50 cm). Seeds were sown (on 13 December 2012 and 20 November 2013) in a modular tray (308 cells) and initially grown under vernalizing temperatures at 6^{o}C with a photoperiod of 12 hours in a controlled-environment growth room. After the vernalization period (62 days in 2013 and 77 days in 2014 after sowing), the seedlings were transplanted into the columns (one plant per each column) in the glasshouse. The plants were transplanted into the soil columns after vernalization for each experiment. Plants were irrigated weekly to return columns to 90% of available water at field capacity (AWFC). Water stress was applied in the water-stressed treatment by returning the soil water content of the columns to 50% AWFC from GS39 to GS61; and 25% AWFC from GS61 to physiological maturity based on estimated evapotranspiration (gravimetric analysis). Two sets of 22 columns (one replicate for each irrigation treatment) were weighed every week using a digital scale (model STW-60 KE). The measurements were used to calculate the water uptake in each moisture regime for each genotype; and all experimental columns in respective moisture regimes received water inputs according to these values per genotype.

Plate 3.7 The glasshouse experiment at flowering in 2014.

3.2.2 Plant and soil column management

The PVC columns were 15 cm diameter and 50 cm in height, and the bottom was closed with MyPex polypropylene to allow drainage. Columns were filled with soil medium (50% sand and 50% top loam soil) to the top and irrigated to saturation while the soil had sunk, and the process repeated to the point that there was no more consolidation to achieve natural compaction of the soil. In both years, in order to measure bulk density and the amount of water at field capacity (FC), three columns were well irrigated (until the water came out from the drainage holes). After leaving the columns for draining to reach field capacity for 48 hours, a soil sample was extracted from each column and weighed before and after drying for 24 hours at 115 ^{o}C. Then, the gravimetric soil water content (WC %) (Eq. 3.6) and soil bulk density (BD) (Eq. 3.7) at field capacity (FC) were measured as described by Rowell (2014):

$$\text{WC } \% = \frac{\text{Wet soil (g)} - \text{Oven dried soil (g)}}{\text{Wet soil (g)}} \times 100 \qquad \text{Equation (3.6)}$$

$$\text{BD } (\text{g cm}^{-3}) = \frac{\text{Oven dried soil (g)}}{\text{Soil volume (cm}^3)} \qquad \text{Equation (3.7)}$$

The average of soil bulk density was 1.16 g cm^{-3} in 2013 and 1.33 g cm^{-3} in 2014. The average gravimetric water content (WC %) at field capacity was 17.4% in 2013 and 14.2% in 2014. Available water was considered to be half of the water content%

(Or and Wraith, 2002). The average volumetric available water at field capacity was 1.28 l per column in 2013 and 0.99 l per column in 2014.

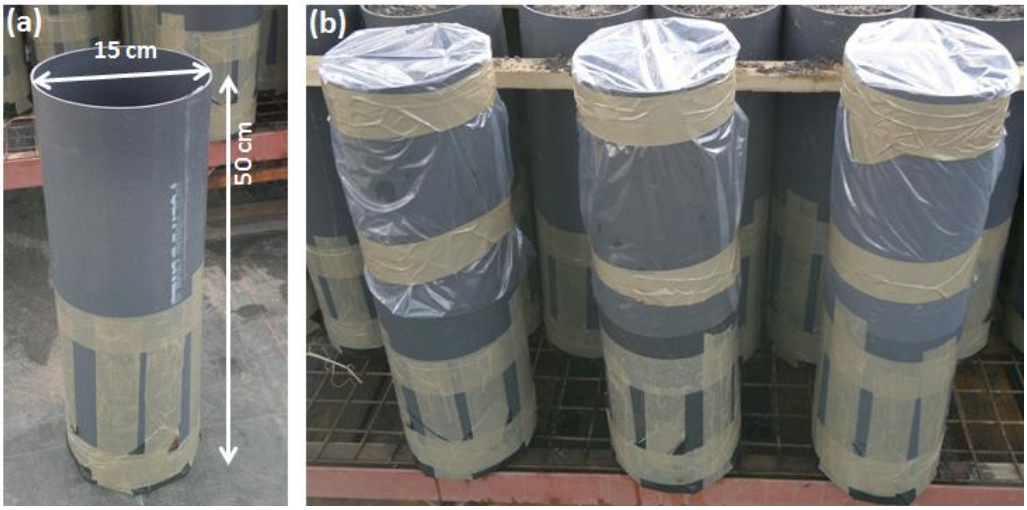


Plate 3.8 A sample of the plastic columns (a) and the three columns used to find bulk density and water content ratio (b).

In both experiments, plants were initially irrigated with VITAX nutrient solution (Vitax LTD, UK) diluted at a ratio of 1:200 during transplanting and after as necessary to avoid any major and minor nutrient deficiencies. Plants were sprayed with fungicide and insecticide as necessary to control fungal pathogens and aphids. The main shoot, first and second tillers were tagged with wire in different colours during tillering stage in order to be used for gas-exchange and growth analysis measurements. The glasshouse had vents on either side of the central ridge to control the temperature as close as possible to the outside temperature. There was supplementary heating to avoid frost and supplementary lighting during the experiments. The daily minimum and maximum temperature and humidity were recorded using a Tinytag Ultra 2 data logger (Gemini Data Loggers Ltd, UK) in both years.

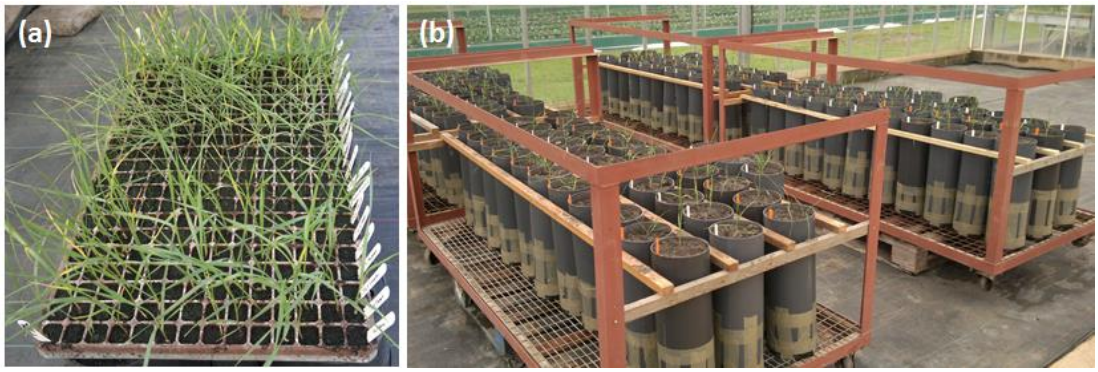


Plate 3.9 (a) plant seedlings in modular tray before transplanting and (b) in the columns after transplanting.

3.2.3 Plant measurements

3.2.3.1 Plant development

Developmental stages of GS31, GS39, GS61 and physiological maturity (GS89; take as stem 90% senesced) were recorded, and the visual assessment was carried out on the main shoots in all individual plants in both seasons, as detailed in 3.1.2.1.

3.2.3.2 Plant water uptake and water-use efficiency

In 2013, plant water uptake (WU; cumulative water used by the plant from transplanting to harvest) was calculated based on the weight of column (one replicate of each irrigation treatment) at transplanting ($_T$) and harvest ($_H$) taking into account the amount of water supplied to each column. In 2014, WU was based on the weight of each column in each replicate at transplanting and harvest in 2014, taking into account the amount of water supplied to each column. In both years it was assumed that drainage and soil evaporation were zero from transplanting to harvest (Eq. 3.7).

$$WU\ (l) = \text{Column weight } (kg)_T + \text{Irrigation } (l) - \text{Column weight } (kg)_H$$

Equation (3.8)

Water-use efficiency was calculated for the above-ground dry matter (WUE_{AGDM}; Eq. 3.8) and grain dry weight (WUE_{grain}; Eq. 3.9) as:

$$WUE_{AGDM}\ (g\ l^{-1}) = \text{AGDM at harvest } (g\ plant^{-1})/WU\ (l) \qquad \text{Equation (3.9)}$$

$$WUE_{grain}\ (g\ l^{-1}) = \text{Grain } (g\ plant^{-1})/WU\ (l) \qquad \text{Equation (3.10)}$$

3.2.3.3 Grain yield and yield components

At harvest, individual plants were hand-harvested at ground level in both 2013 and 2014 experiments. In 2013, the tagged main shoots were separated from the rest of the plant after sampling only for the Rialto x Savannah DH lines as the seeds for the rest of the plants were shed when placed into the sampling bags. For the other genotypes for all shoots any shed grain was collected and growth analysis was carried out on the basis of all ears (main shoots and other fertile ears combined per plant). In 2014, the tagged main shoots and the rest of plant were sampled separately for all genotypes. The main shoot was separated into ear and straw, and then rachis length was measured. Ear and straw dry weights were recorded after drying at 80^{o}C for 48 h.

In both years for the rest of the shoots per plant, fertile and infertile shoots were separated. The fertile shoots were then separated into ears and straw. The dry weight of the ears and straw were recorded after drying at 80^{o}C for 48 h. After hand-threshing the ears, the dry weight of all plant components (grain, chaff and straw) was recorded after drying for 48 h at 80^{o}C. 500 grains from each plant were counted by a seed counter and weighed to obtain the 1000-grain weight. HI was the ratio of grain dry weight per plant to total dry weight per plant. Grains per ear was calculated as the total number of grains per plant divided by the number of ears per plant. In both years, plant height was measured before harvest from the ground level to the tip of the ear for three randomly selected fertile shoots per plant.

3.2.3.4 Leaf gas-exchange measurements

In both years, flag-leaf gas-exchange of photosynthetic rate, stomatal conductance and transpiration efficiency was measured for all genotypes in irrigated and droughted treatments (in two replicates). Measurements were taken every two weeks from booting stage (GS41) to mid-to-late grain filling (GS61+28 days) in 2013 (3, 20 May, 6 and 11 June), and 2014 (1, 21 May, 10 and 19 June), using a LiCor 6400-XT Photosynthesis system (LiCor NE, USA). Measurements were taken for three flag-leaves per plant (main shoot and tillers 1 and 2), as detailed in sub-section 3.1.2.6.

Plate 3.10 LiCor 6400-XT Photosynthesis system used for measuring leaf gas-exchange, leaf chamber clamped on a wheat leaf at anthesis.

3.2.3.5 Leaf chlorophyll content (SPAD)

Leaf relative chlorophyll content (SPAD) was measured weekly for the newest fully emerged leaf on the main shoot from stem elongation (GS31) to mid-grain filling (GS61+4wks) in both 2013 (8, 15, 23 April, 10, 22, 31 May and 20 June) and 2014 (14, 25 March, 10, 23 April, 16, 28 May, 3, 10 and 19 June) using a chlorophyll meter (SPAD-502; Minolta, Osaka, Japan). Three readings were taken per plant (main shoot and tillers 1 and 2) in both well-watered and droughted treatments from 10.00h to 14.00h in a day when the sky was clear and the leaves were well illuminated and their surfaces were dry (Pask *et al.*, 2012).

3.2.3.6 Flag-leaf area (FLA)

In 2014, three flag-leaves were assessed at GS61 on the main, first and second tillers of each plant, and their length and width were recorded. At harvest, these three flag-leaves were weighed after drying for 48 h at 80^{o}C. The ratio of the weight at harvest to the leaf area at GS61 (flag-leaf specific weight; FLSW) was then calculated as:

$$\mathrm{FLSW}(\mathrm{g\,m^{-2}}) = \frac{\text{leaf dry weight (g)}}{(\text{leaf length (m)} \times \text{leaf width (m)} \times 0.83)} \qquad \text{Equation (3.11)}$$

3.2.3.7 Flag-leaf senescence measurements

Flag-leaf senescence was measured from anthesis (GS61) to full senescence twice a week using a visual senescence score chart ranging from 0 – 10 (0; fully green and 10; fully senesced). Visual assessments were carried out for each main shoot under both well-watered and droughted treatments. SEN_{ONSET}, SEN_{RATE} and SEN_{END} were calculated for each genotype, as described in sub-section 3.1.2.7.

3.3 Statistical analysis

For both field and glasshouse data, GenStat 15th Edition (VSN International, 2015) was used for statistical analysis of variance (ANOVA) by applying a split-plot design, and the least significant difference (LSD) test was used to compare the means between specific treatments. The data were tested for normality using Shapiro-Wilk test prior ANOVA analysis, and non-normally distributed data were then transformed by square root to improve the normality of the trait distribution, if necessary. For grain yield and yield component traits in the field experiments, a Restricted Maximum Likelihood (REML) variance-component model was used to analyse effects of season, irrigation and genotype and their interactions; this is an auto-regressive model fitted to the spatial correlations across the field. GenStat 15th Edition was also used for calculating Pearson's correlation coefficient, multiple linear regression analysis using the mean values in each year, and fitting the flag-leaf senescence score values against thermal time (GS61; base temperature 0^{o}C) using a logistic regression equation of senescence in both years and for the cross-year mean. The GraphPad Prism 6.00 software package was used for making graphs and linear regression curve fitting analysis to calculate the relationships between all phenotypic variables in both years and for the cross-year mean. META-R (Multi Environment Trail Analysis with R) software version 5.0 was used for Principal component analysis and calculating the broad sense heritability for yield and yield component traits in both years.

3.4 QTL analysis

The genetic map used for QTL analysis was constructed using JoinMap version 4 (Van Ooijen, 2006) using 578 SNP markers obtained from CerealsDB database

website based at the University of Bristol with support from BBSRC (Wilkinson *et al.*, 2012). First, the genotyped lines (124 lines) from Rialto x Savannah DH population and 5000 markers were entered into the software, and then all repeated and redundant markers were excluded after which 578 markers remained in order to obtain the linkage groups. The linkage groups were defined from the 'groupings (tree)' tab sheet based upon the test for linkage LOD 6 for each group, and then 22 linkage groups were mapped based on map distance calculation using the Haldane's mapping function (Haldane, 1919). In total, a map length of 2171 cM was distributed on the 21 chromosomes with the density of 3.8 cM per marker. There was relatively poorer coverage of markers on genome D and chromosomes 4B and 7B in genome B compared with genome A. The number of markers, and the length and density of each group for each chromosome are shown in Chapter 7.

QTL analysis was carried out using the MapQTL software package version 6 (Van Ooijen, 2009). First, interval mapping (IM) was carried out for QTL detection using 22 genetic linkage groups of 578 markers and phenotypic data of grain yield, yield component and physiological traits for 91 lines of Rialto x Savannah DH population used in the present study. Then, permutation test was carried out in order to determine the significance threshold of the LOD score (Churchill and Doerge, 1994) by taking the genome wide (including all groups) threshold with a P-value of 0.05 for each of the trait x irrigation x year combinations. Significant QTLs (LOD > genome wide LOD at 0.95 relative cumulative count) and putative QTLs (LOD > 2) for each trait are shown in Figures 7.1-7.3 using MapChart 2.3 software package (Voorrips, 2002).

Chapter 4 Harvest results of Rialto x Savannah DH population in field experiments

4.1 Introduction

This chapter describes the results for grain yield and yield components, biomass production, HI and grain $\Delta^{13}C$ at harvest for the 2013 and 2014 field experiments. The effects of irrigation and genotype on grain yield and yield components, above-ground DM (AGDM) and DM partitioning are quantified. Relationships between grain yield and yield components, AGDM and dry matter partitioning are described. Meteorological data for the two seasons are presented for the most important variables affecting development, crop growth and yield: mean air temperature, rainfall and solar radiation. Crop development dates for anthesis and physiological maturity and plant height are also presented.

Carbon isotope discrimination ($\Delta^{13}C$) has been used as a selection tool for detecting variation in transpiration efficiency (TE; above-ground biomass per unit crop transpiration) and developing wheat varieties with improved TE and drought tolerance (Richards, 2006). There is a positive relationship between $\Delta^{13}C$ and CO_2 level in the intercellular spaces of the leaf (Condon *et al.*, 2002), and a negative relationship with leaf transpiration efficiency (TE) (Farquhar and Richards, 1984). Therefore, selecting for lower $\Delta^{13}C$ could be an indicator for higher biomass and grain yield genotype (Monneveux *et al.*, 2006). Higher TE could theoretically increase grain yield under limited water availability, but the extent of the benefit depends on the extent of the water stress (Blum, 2005). In the present study, $\Delta^{13}C$ was measured on grains from hand harvested samples of all genotypes under unirrigated conditions in 2013 and 2014. Genotype effects on $\Delta^{13}C$ for all DH lines and the relationship between grain yield and grain $\Delta^{13}C$ for all the lines was examined.

The aim of this chapter is to describe results at harvest for genetic variation for grain yield and harvest traits and responses to irrigation in each season and across seasons. The physiological bases of the irrigation and genotype effects and their interaction for grain yield harvest traits will then be examined in more detail in Chapter 6.

The specific hypotheses tested in this chapter are:

1. There is variation in grain yield and above-ground DM and DM partitioning amongst the lines of the Rialto x Savannah DH population and there is genetic variation of grain yield, above-ground dry matter and HI in responses to water deficits amongst Rialto x Savannah DH lines under drought conditions.
2. Carbon isotope discrimination ($\Delta^{13}C$) of grain is positively related to above-ground dry matter (AGDM) and grain yield under UK drought conditions amongst lines of the Rialto x Savannah DH population.

4.2 Materials and Methods

A DH population of 94 lines was derived from the F_1 between winter wheat cultivars Rialto and Savannah. These DH lines with their parents were characterised in two field experiments in 2012-13 and 2013-14. The experiments were carried out using a randomised block, split-plot design, in which two irrigation treatments (fully irrigated and unirrigated) were randomised on main-plots, and the DH lines and the two parents were randomised on sub-plots (1.62 x 6 m) with two replicates. The experimental site was located at the University of Nottingham Farm, Leicestershire (52^o 50' N, 1^o 15' W).

In both 2013 and 2014 experiments, samples of ca. 75 fertile shoots were hand-harvested at ground level a 1-2 days before combine harvest and separated into ears and straw. The sub-plots were machine harvested on an area of at least 5 m^2 and grain weights adjusted to 85% DM. the grain yield, yield components, HI and above-ground dry matter were assessed as described in Chapter 3. Anthesis dates (GS61) and plant height were recorded in each sub-plot as described in Chapter 3.1.2.1 and 3.1.2.2, respectively.

For grain yield and yield components, a Restricted Maximum Likelihood (REML) mixed linear model was used to analyse effects of season, irrigation and genotype and their interactions; this is an auto-regressive model fitted to the spatial correlations across the field. SEDs from REML analysis are shown in the Tables 4.1, 4.2, 4.3, 4.4 and 4.5 along with means, maximum and minimum for each trait in both years and across experiments. The grain $\Delta^{13}C$ analysis for all plots in the unirrigated

treatments was carried out as described in Chapter 3. Full details of the methodology used in the analysis are given in Chapter 3.1.2.3.

A sub-set of six lines from the R x S DH population and the two parents were selected to assess leaf gas-exchange traits in the field experiments (see Chapter 5). The grain $\Delta^{13}C$ variation within this sub-set lines and its relationship with grain yield are also presented and discussed in this chapter to assist comparisons with the gas-exchange data sets in Chapter 5.

4.3 Results

4.3.1 Environmental conditions

Mean air temperature, rainfall and solar radiation are presented in Fig. 4.1 for both seasons and the long-term means (LTM) (MetOffice, 2015).

4.3.1.1 Rainfall

The total rainfall from sowing to harvest in 2012-13 was 601 mm slightly higher than the LTM (6.5% > LTM (1961-2014)); rainfall was above average from early to late tillering (October, November and December) (88.0 mm > LTM), and early to late booting (May) (24.4 mm > LTM) and during mid to late-grain filling (July) (27.5 mm > LTM). However, from early to late stem extension (January and February), rainfall was considerably lower than the LTM (35.1 mm < LTM), and from flowering to mid-grain filling (June) conditions were slightly drier than average (6.6 mm < LTM) (Fig. 4.1b).

In 2013-14, rainfall from sowing to harvest was 513.6 mm (11.7% > LTM (1961-2014)); 87.4mm less than 2012-13. Higher than average rainfall occurred from early tillering to mid-stem elongation (January and February) (53.9 mm > LTM), and early to late booting (May) (37.0 mm > LTM). However, crops received less rainfall than the LTM (37.4 mm < LTM) during grain filling (June and July) in 2013-14 (Fig. 4.1a).

4.3.1.2 Mean air temperature

In 2012-13, the mean air temperature from sowing (October) to harvest (August) was 8.9^{o}C (0.4^{o}C < LTM (1961-2014)). This was associated with overall lower temperatures from sowing (October) to the beginning of anthesis (June) (0.9^{o}C < LTM). However, during mid to late-grain filling (July) conditions were warmer than the LTM (2.1^{o}C > LTM).

In 2013-14, from sowing to harvest, mean air temperature was 10.4^{o}C, 0.6^{o}C warmer than the LTM; this was mainly due to higher temperatures from early tillering (December) to the end of grain filling (July) (1.6^{o}C > LTM) (Fig. 4.1c). Over the grain-filling period (June and July), mean air temperature was 16.6^{o}C, 1.3^{o}C warmer than the LTM.

4.3.1.3 Solar radiation

In 2012-13, the total incident solar radiation from sowing to harvest was 3329 MJ m^{-2} (2.4% > LTM (2000-2014)). This related to generally bright conditions from the end of stem elongation to harvest (April to August; 5.5% > LTM). However, conditions were duller than average from sowing to late stem elongation (November to March; 9.6% < LTM).

In 2013-14, the total incident solar radiation from sowing to harvest was also higher (3328 MJ m^{-2}) than the LTM (2000-2014) by 8.5%. Incident solar radiation was lower than the LTM by 2.8% from early stem elongation to early flowering (April to June), but generally higher than the LTM from early flowering to harvest by 18.8% (Fig. 4.1d).

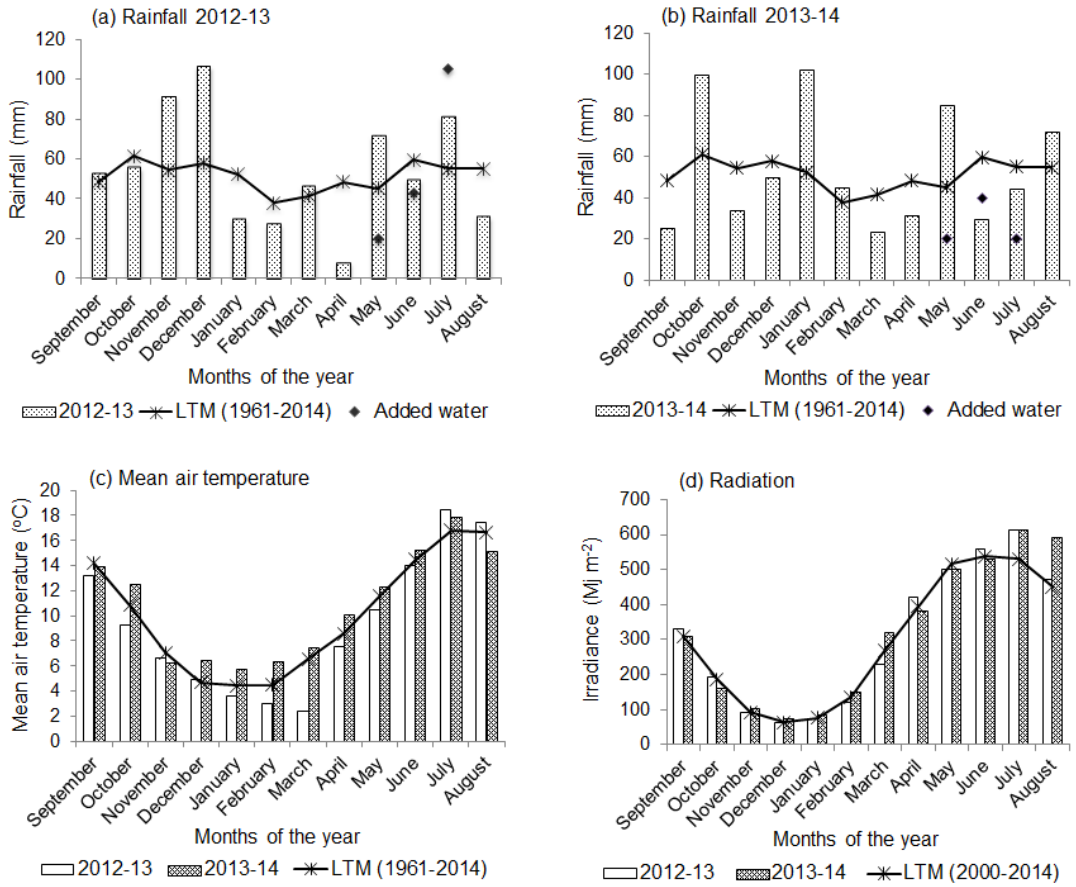


Figure 4.1 Mean monthly rainfall for 2012-13 (a), 2013-14 (b), monthly air temperature (c) and monthly solar radiation (d) compared to the long-term mean; 1961-2014 for (a), (b) and (c), and 2000-2014 for (d).

4.3.2 Plant development

In 2013, there was no effect of irrigation treatment (P=0.56) on anthesis date (GS61). Genotypes differed in the range 248.8 to 253.5 days after sowing (DAS; P<0.001). DH line 84 was the latest to reach anthesis in both irrigated and unirrigated conditions (253.5 and 254.2 DAS, respectively), and the earliest lines to reach anthesis were line 76 (248.8 DAS) under irrigated conditions, and line 43 (248.9 DAS) under unirrigated conditions. Regarding the parents, Rialto reached anthesis one day earlier than Savannah in both irrigation treatments (Fig. 4.2a and 4.2b). Date of physiological maturity also differed between genotypes from 297 (line 12) to 305 DAS (line 84) and 288 (line 51) to 296 DAS (line 84) under irrigated and unirrigated conditions, respectively (P<0.001). Overall, restricted water availability advanced physiological maturity (PM) by 10 days from sowing (P<0.001). The parents reached PM on the same day under irrigated conditions (Fig. 4.2c) while

Rialto reached PM four days earlier than Savannah under unirrigated conditions (Fig. 4.2d). There was no irrigation x genotype interaction for either date of anthesis (P=0.32) or PM (P=0.44; Table 4.2).

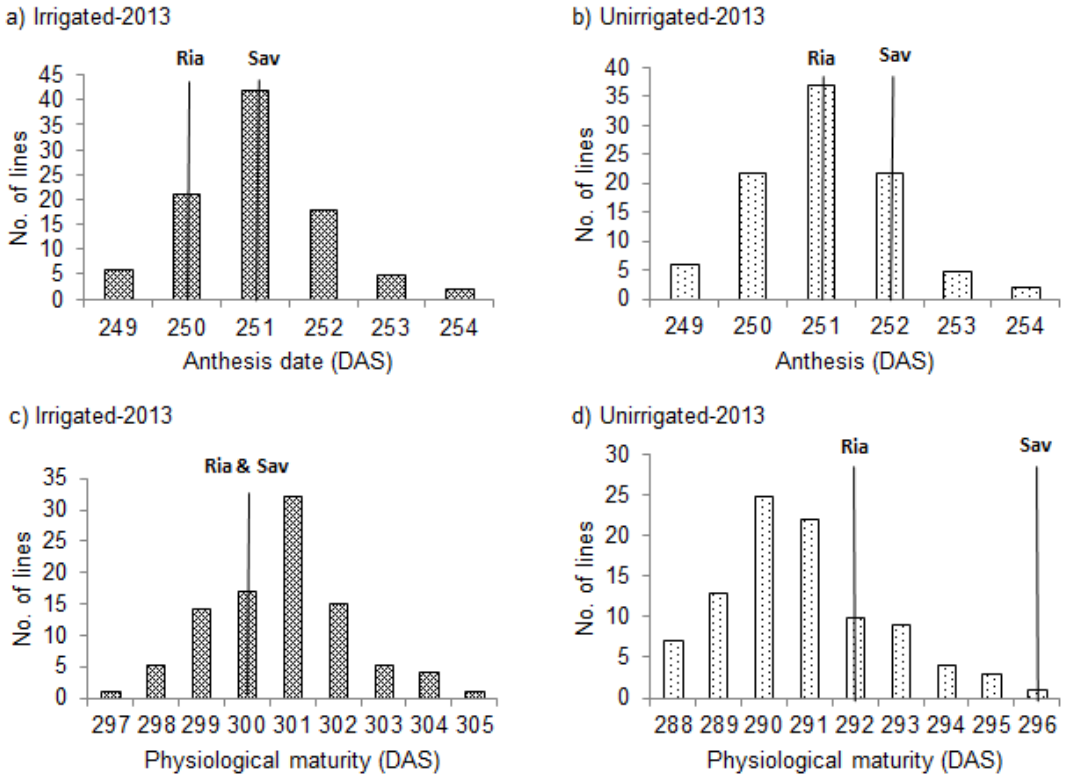


Figure 4.2 Frequency distributions of 94 Rialto x Savannah DH lines and the two parents for (a) anthesis date (GS61) under irrigated, (b) anthesis date under unirrigated, (c) physiological maturity date under irrigated and (d) physiological maturity date under unirrigated conditions in 2013 (DAS = days after sowing).

In 2014, anthesis date was affected by genotype (P<0.001), but not by irrigation (P=0.58) or the irrigation x genotype interaction (P=0.69). DH lines ranged in anthesis date (AD) from 203.1 (line 76) to 209.2 DAS (line 84), and from 203.2 (line 47) to 210.7 DAS (line 9) under irrigated and unirrigated conditions, respectively. Regarding the parents, Rialto reached anthesis one day and three days earlier than Savannah under irrigated and unirrigated conditions, respectively (Fig. 4.3a and 4.3b). Overall restricted water availability in the unirrigated treatment advanced PM by 4.6 days (P<0.001). DH Lines differed in date of PM from 253.4 (line 90) to 261.0 DAS (line 9) and 248.1 (line 56) to 255.4 DAS (line 84) under irrigated and unirrigated conditions, respectively. Rialto reached physiological maturity three and four days earlier than Savannah (Fig. 4.3c and 4.3d) under irrigated and unirrigated conditions, respectively. Across seasons, later sowing date and higher prevailing

temperature also affected anthesis date and shortened the period from sowing to anthesis by 44 days in 2014 compared to 2013.

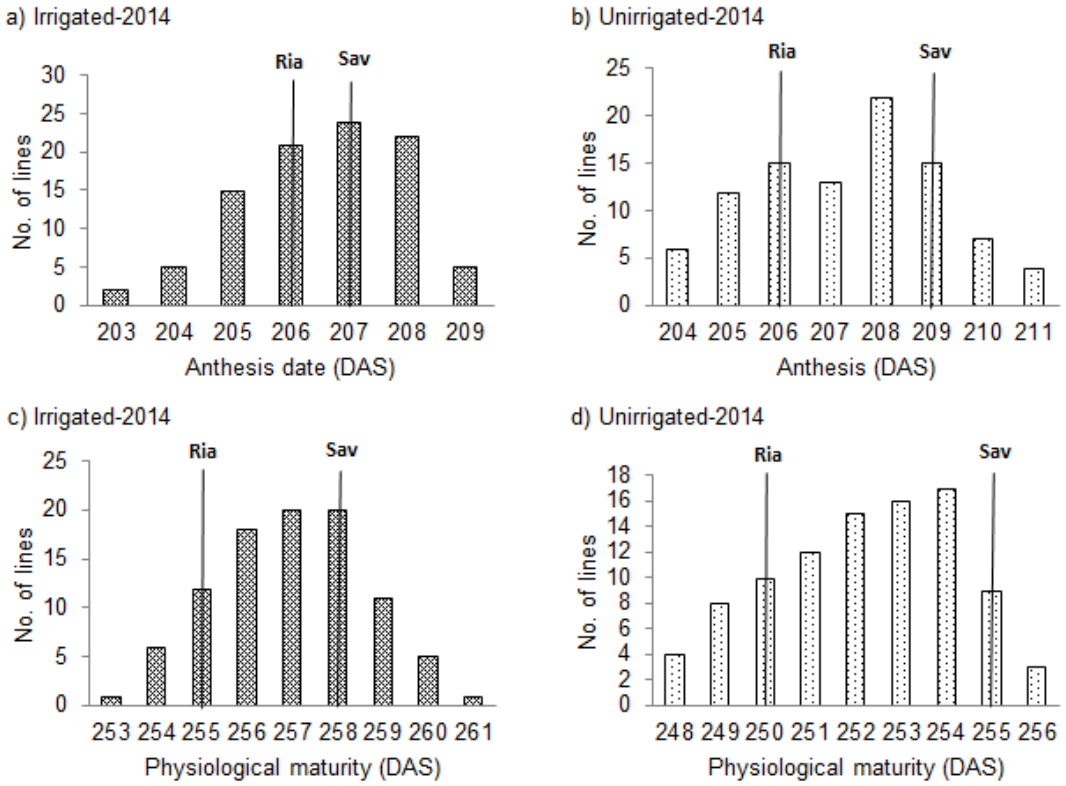


Figure 4.3 Frequency distributions of 94 Rialto x Savannah DH lines and the two parents for (a) anthesis date (GS61) under irrigated, (b) anthesis date under unirrigated, (c) physiological maturity date under irrigated and (d) physiological maturity date under unirrigated conditions in 2014 (DAS = days after sowing).

4.3.3 Effects of irrigation and genotype on grain yield and yield components

4.3.3.1 Grain yield

In 2013, restricted water availability in the unirrigated treatment reduced grain yield by 2.96 t ha^{-1} (P=0.04; Table 4.1). Grain yield ranged from 11.94 (line 62) to 14.45 t ha^{-1} (line 88), and 9.19 (line 62) to 11.37 t ha^{-1} (line 48) under irrigated and unirrigated conditions, respectively. The decrease under drought ranged amongst genotypes from 1.72 (line 54) to 4.03 (line 88) (P<0.001). Regarding the parents, Savannah (13.63 and 11.3 t ha^{-1}) yielded higher than Rialto (13.46 and 10.41 t ha^{-1}) under both irrigated and unirrigated treatments, respectively (Fig. 4.4a and 4.4b).

There was no transgressive segregation above the higher parent Savannah in the unirrigated treatment. However, DH lines 35 and 88 had higher grain yield than Savannah under irrigated conditions ($P<0.001$).

In 2014, grain yield was affected by irrigation ($P=0.05$) and genotype ($P=0.001$), while the irrigation x genotype interaction was not significant ($P=0.43$; Table 4.1). Restricted water availability in the unirrigated treatment reduced yield slightly by 0.2 t ha^{-1} ($P<0.05$; Table 4.1). The DH lines ranged from 10.02 (Line 57) to 14.85 t ha^{-1} (line 47) under irrigated conditions, and 10.45 (Line 21) to 13.62 t ha^{-1} (line 51) under unirrigated conditions. Under both irrigated and unirrigated treatments, Savannah (13.76 and 12.66 t ha^{-1}, respectively) had higher grain yield than Rialto (13.32 and 11.7 t ha^{-1}, respectively) (Fig. 4.4c and 4.4d). There was no transgressive segregation under either irrigated or unirrigated conditions.

Averaging across years, drought reduced grain yield by 1.56 t ha^{-1} (12.2%; $P<0.001$) which was mostly due to effects in 2013 rather than 2014. Overall genotypes differed in the range 11.48 (DH line 1) to 14.27 t ha^{-1} (DH line 20) and 10.24 (DH line 60) to 12.10 t ha^{-1} (DH line 82) under irrigated and unirrigated conditions, respectively. The irrigation x genotype interaction was not significant. The grain yield under unirrigated conditions correlated amongst DH lines with grain yield under irrigated conditions in 2013 ($P<0.001$; $R^2=0.18$; Fig. 4.5a), 2014 ($P=0.008$; $R^2=0.07$; Fig. 4.5b) and for the cross-year mean ($P<0.001$; $R^2=0.20$; Fig. 4.5c). There was no transgressive segregation above the higher parent under either irrigated or unirrigated conditions.

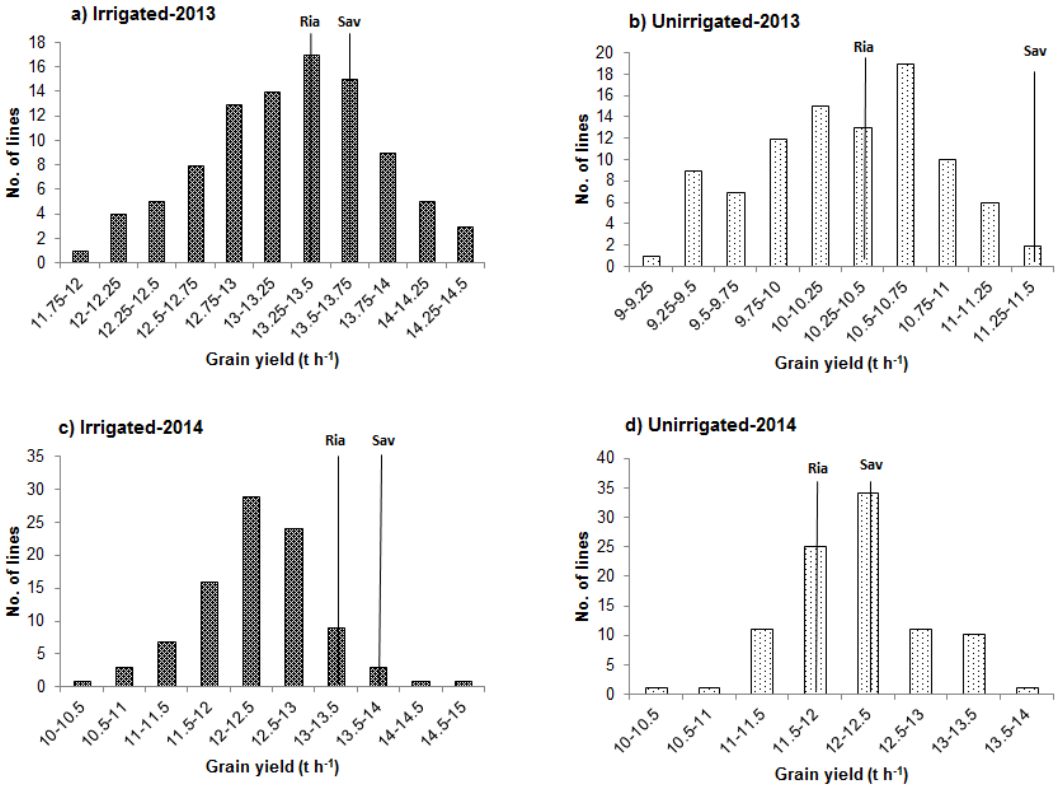


Figure 4.4 Frequency distributions for 94 Rialto x Savannah DH lines and the two parents for grain yield (85% DM) (a) under irrigated 2013, (b) unirrigated 2013, (c) irrigated 2014 and (d) unirrigated conditions 2014.

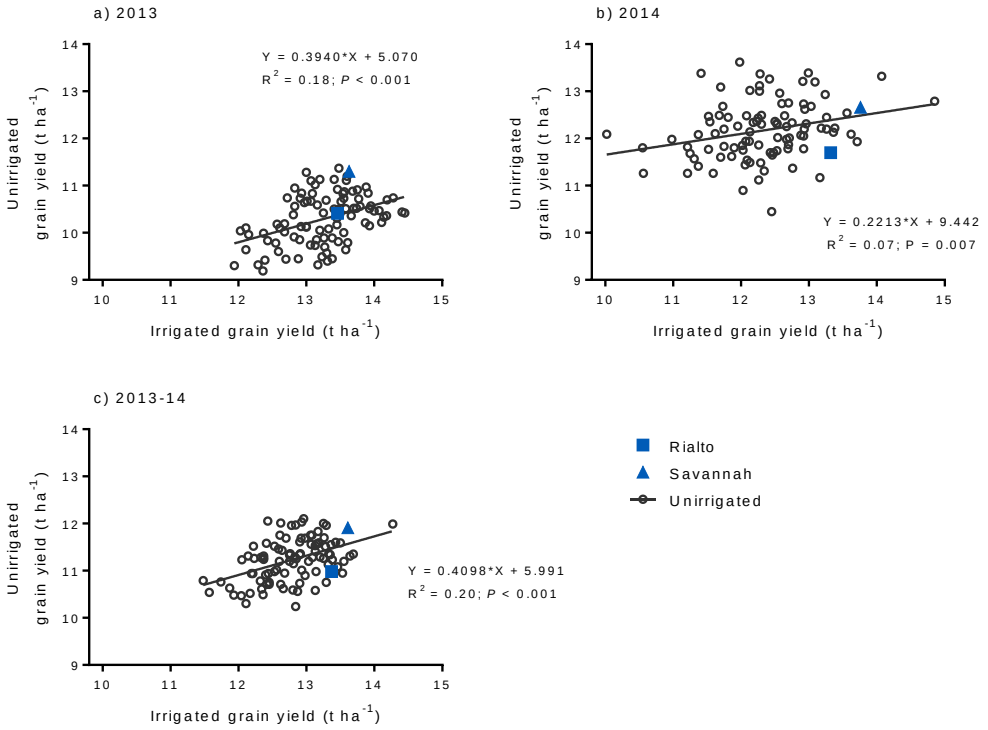


Figure 4.5 Linear regressions of grain yield t ha^{-1} (85% DM) under irrigated conditions on grain yield t ha^{-1} under unirrigated conditions for 94 Rialto x Savannah DH lines in (a) 2013, (b) 2014 and (c) cross-year mean. Rialto (■) and Savannah (▲) are also shown.

4.3.3.2 Harvest index

In 2013, overall HI was lower under drought (0.52) than under irrigated conditions (0.55) (P<0.001; Table 4.1). DH lines ranged from 0.53 (line 64) to 0.59 (line 46) under irrigated conditions, and from 0.48 (line 87) to 0.56 (line 63) under unirrigated conditions (P<0.001). The decrease in HI with restricted water availability ranged amongst DH lines from 0.0005 (Line 48) to 0.07 (Line 87) (P=0.04; Table 4.1). Savannah had higher HI under irrigated and unirrigated conditions (0.57 and 0.55, respectively) than Rialto (0.54 and 0.53, respectively) (Fig. 4.6a and 4.6b).

In 2014, overall HI was slightly lower under drought (0.51) than under irrigated conditions (0.52) (P<0.001; Table 4.1). DH lines differed in the range 0.46 (line 73) to 0.55 (Savannah) and 0.44 (line 79) to 0.55 (line 46) under irrigated and unirrigated conditions, respectively. The decrease with restricted water availability ranged amongst DH lines from -0.04 (Line 73) to +0.05 (Line 34) (P=0.12; Table 4.1).

Savannah had higher HI than Rialto in both irrigation treatments in each season (Fig. 4.6).

Averaged over years, drought reduced HI from 0.54 to 0.52 (P<0.001). Genotypes differed in the range 0.50 (line 86) to 0.57 (line 46) and 0.48 (line 97) to 0.55 (line 63) under the irrigated and drought conditions, respectively (P<0.001). The interactions between genotype and each of irrigation (P=0.004) and year (P<0.001) were significant (Table 4.1). With regard to the parents, Savannah had higher HI under both irrigated and unirrigated conditions (0.56 and 0.54, respectively) than Rialto (0.53 and 0.52, respectively). There was no transgressive segregation under either irrigated or unirrigated treatments.

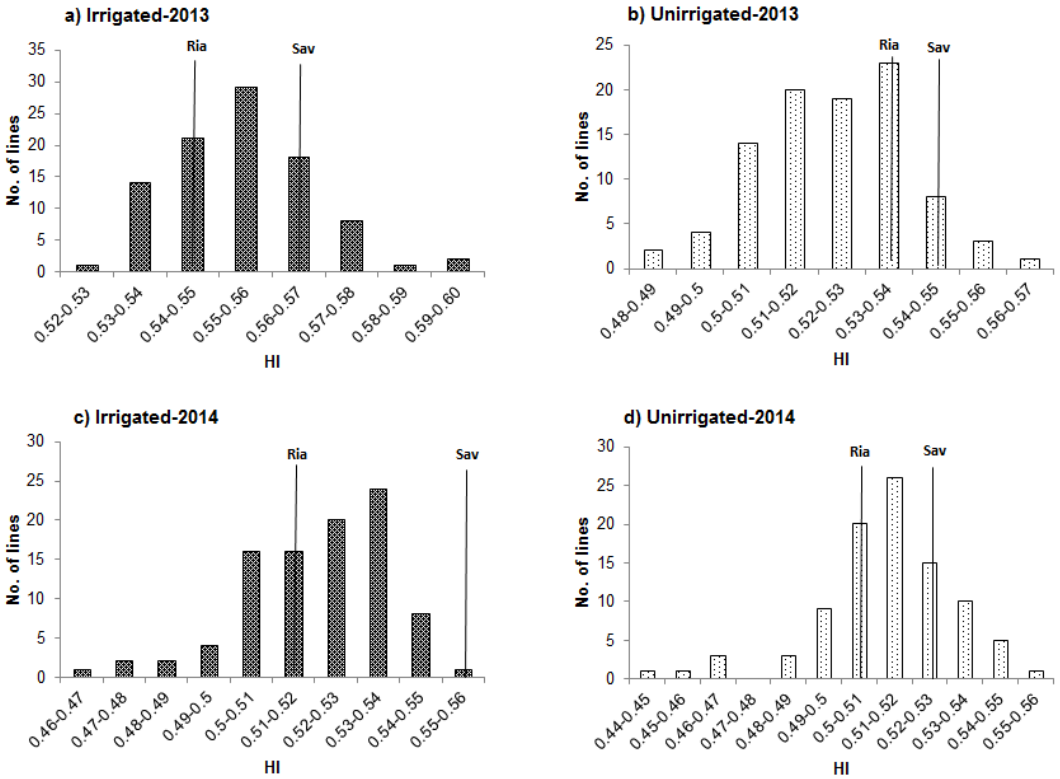


Figure 4.6 Frequency distributions for 94 Rialto x Savannah DH lines and the two parents for harvest index (a) under irrigated 2013, (b) unirrigated 2013, (c) irrigated 2014 and (d) unirrigated conditions 2014.

4.3.3.3 Above-ground dry matter

Above-ground dry matter (AGDM) was decreased by drought in 2013 from 2040 to 1673 g m^{-2} (P<0.001). Genotypes differed (P<0.001) in the range 1826 (line 16) to 2246 g m^{-2} (line 35); and 1462 (line 60) to 1853 g m^{-2} (line 12) under irrigated and unirrigated conditions, respectively. However, the irrigation x genotype interaction was not significant (P=0.14; Table 4.1). Rialto had higher biomass (2127 g m^{-2}) than Savannah (2052 g m^{-2}) under irrigated conditions, but lower biomass (1667 g m^{-2}) than Savannah (1740 g m^{-2}) under unirrigated conditions (Fig. 4.7a and 4.7b).

In 2014, there was no effect of irrigation on AGDM (P=0.98). DH lines differed in the range 1670 (line 1) to 2466 g m^{-2} (line 47) under irrigated conditions, and 1718 (line 24) to 2294 g m^{-2} (line 82) under unirrigated conditions (P=0.03). There was also no significant irrigation x genotype interaction (P=0.46; Table 4.1). Similarly to 2013, Rialto had higher biomass (2210 g m^{-2}) under irrigated conditions and lower biomass (1925 g m^{-2}) under unirrigated conditions than Savannah (2042 and 2012 g m^{-2}), respectively (Fig. 4.7c and 4.7d).

Averaging over years, there were effects of irrigation and genotype, but not for the irrigation x genotype interaction (P=0.39). DH lines differed in the range 1808 (line 1) to 2228 g m^{-2} (line 11) under irrigated conditions, and 1650 (line 60) to 1998 g m^{-2} (line 45) under unirrigated conditions. Regarding the parents, Rialto had higher biomass under irrigated conditions, and lower biomass under unirrigated conditions than Savannah (P<0.001).

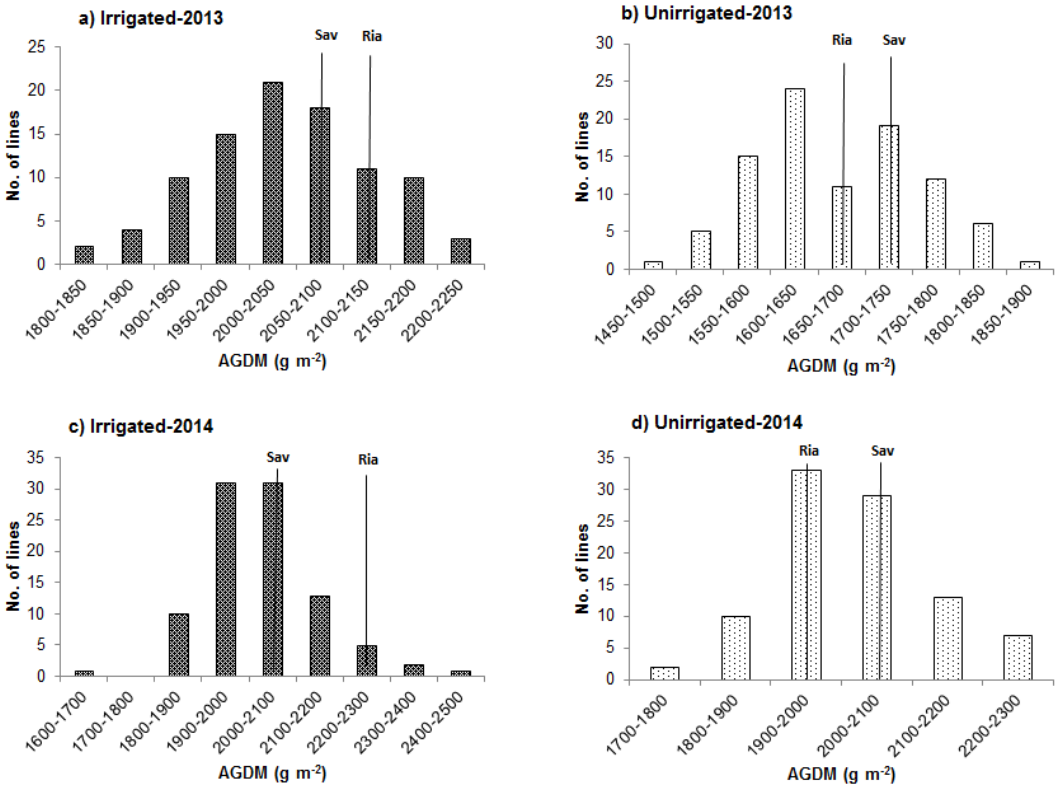


Figure 4.7 Frequency distributions for 94 Rialto x Savannah DH lines and the two parents for above-ground DM under (a) irrigated 2013, (b) unirrigated 2013, (c) irrigated 2014 and (d) unirrigated conditions (2014).

4.3.3.4 Plant height

In 2013, drought shortened the plant height by 2.2 cm (P<0.001). The DH lines ranged between 58.1 (line 62) to 86.0 cm (line 44) under irrigated conditions, and 54.4 (lines 62) to 85.8 cm (line 12) under unirrigated conditions (P<0.001). The irrigation x genotype interaction was not significant (P=0.36; Table 4.1). Savannah (72.1 and 71.0 cm) was slightly taller under both irrigated and unirrigated conditions, respectively, than Rialto (69.7 and 69.0 cm), respectively (Fig. 4.8a and b).

In 2014, there was no statistically significant effect of irrigation (P=0.21). Differences between genotypes were observed in the range 52.7 (line 62) to 84.0 cm (line 88) under irrigated conditions, and 55.9 (line 52) to 84.4 cm (line 12) under unirrigated conditions (P<0.001; Table 4.1). There was a trend for an irrigation x genotype interaction (P=0.06). Parents had similar heights (Savannah 71.4 cm and Rialto 70.7 cm) under irrigated conditions, while Savannah (74.0 cm) was taller than Rialto (69.6 cm) under unirrigated conditions (Fig. 4.8c and d).

Averaging over years, there were effects of irrigation and genotype (P<0.001). DH lines ranged from 56.2 (line 62) to 84.8 cm (line 88) under irrigated conditions, and from 56.7 (line 52) to 85.4 cm (line 12) under unirrigated conditions. However, the irrigation x genotype interaction was not significant (P=0.23; Table 4.1). Savannah (73.0 and 72.5 cm) was taller under both irrigated and unirrigated conditions than Rialto (69.9 and 69.3 cm), respectively.

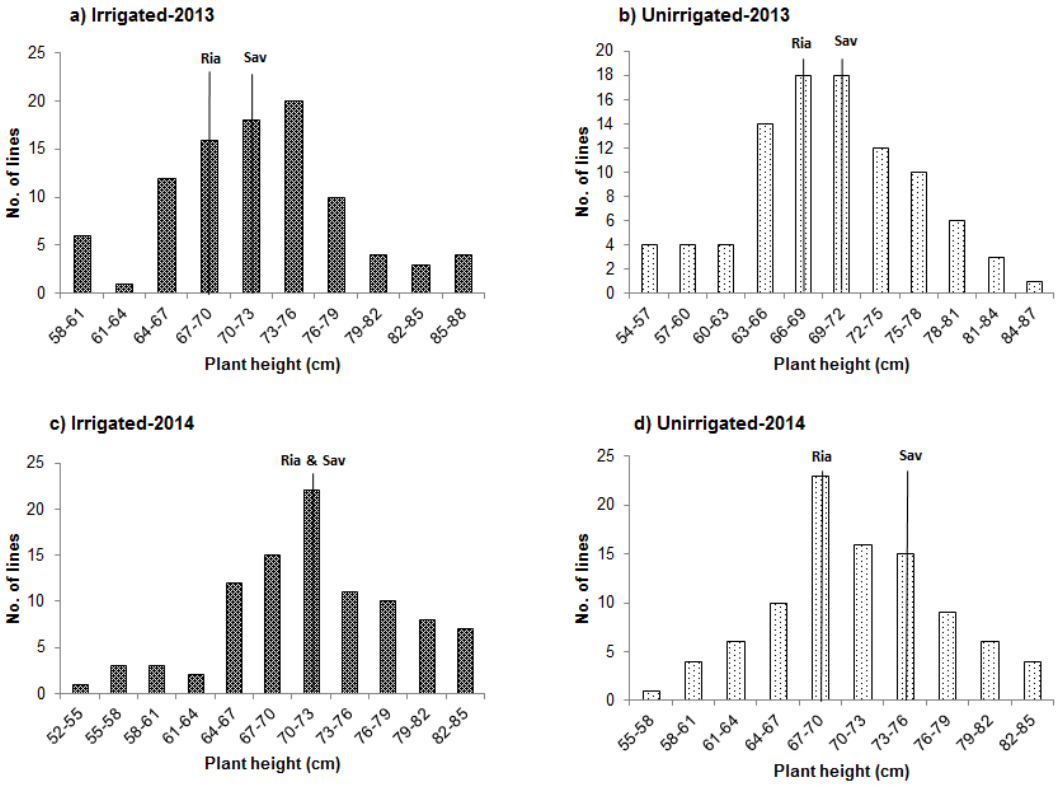


Figure 4.8 Frequency distributions for 94 Rialto x Savannah DH lines and the two parents for plant height (a) under irrigated 2013, (b) unirrigated 2013, (c) irrigated 2014 and (d) unirrigated conditions 2014.

Table 4.1 Mean, maximum, minimum, df and S.E.D. for grain yield (85% DM), HI and above-ground DM under irrigated and unirrigated treatments for 94 Rialto x Savannah DH lines and the two parents in 2013, 2014 and cross-year mean.

Traits	Grain yield (85% DM) t ha^{-1}		HI		Above ground DM (g m^{-2})		Plant height (cm)	
2013	**Irri.**	**Unirri.**	**Irri.**	**Unirri.**	**Irri.**	**Unirri.**	**Irri.**	**Unirri.**
Mean	13.3	10.3	0.55	0.52	2040	1673	72.0	69.8
Max.	14.5	11.4	0.59	0.56	2246	1853	86.0	85.8
Min.	11.9	9.2	0.53	0.48	1828	1462	58.1	54.4
Rialto	13.5	10.4	0.54	0.53	2127	1668	69.1	69.0
Savannah	13.6	11.3	0.56	0.55	2052	1740	72.1	71.0
SED (df)								
Irrigation (1)	*0.08****		*0.001****		*15.6****		*0.28****	
Genotype (95)	*0.35****		*0.008****		*59.0****		*1.72****	
Irri. x Gen (95)	*0. 50**		*0.012**		*84.0*^{ns}		*2.44*^{ns}	
2014								
Mean	12.3	12.2	0.52	0.51	2024	2022	71.6	71.0
Max.	14.9	13.6	0.55	0.55	2466	2294	84.0	84.4
Min.	10.0	10.5	0.46	0.44	1670	1718	52.7	55.9
Rialto	13.3	11.7	0.51	0.51	2210	1925	70.7	69.6
Savannah	13.8	12.7	0.55	0.53	2042	2012	71.4	74.0
SED (df)								
Irrigation (1)	*0.09**		*0.002****		*19.1*^{ns}		*0.44*^{ns}	
Genotype (95)	*0.58***		*0.010****		*106.7**		*2.29****	
Irri. x Gen (95)	*0.82*^{ns}		*0.020*^{ns}		*151.0*^{ns}		*3.25*^{ns}	
2013-14								
Mean	12.8	11.2	0.54	0.52	2032	1847	71.8	70.4
Max.	14.3	12.1	0.57	0.55	2228	1998	84.8	85.4
Min.	11.5	10.2	0.51	0.48	1808	1650	56.2	56.7
Rialto	13.4	11.0	0.53	0.52	2170	1788	69.9	69.3
Savannah	13.6	11.9	0.56	0.54	2038	1868	73.0	72.5
SED (df)								
Year (1)	*0.08****		*0.005*^{ns}		*13.4***		*0.64*^{ns}	
Irrigation (1)	*0.06****		*0.001****		*12.1****		*0.28****	
Genotype (95)	*0.35****		*0.007****		*61.8****		*1.50****	
Irri. x Gen (95)	*0.49*^{ns}		*0.010***		*87.6*^{ns}		*2.13*^{ns}	
Year x Gen (95)	*0.49****		*0.010****		*87.7**		*2.17**	

N.B: ***denotes P<0.001; **P<0.01 and *P<0.05 significance levels; ^{ns} = not significant.

4.3.3.5 Straw and chaff dry matter

Cross-year analysis showed effects of irrigation (P<0.001), year (P=0.02) and genotype (P<0.001) on straw (stem and leaf sheath) dry matter. The interaction between year and genotype (P=0.001) was also significant, and there was a trend for an interaction between irrigation and genotype (P=0.06; Table 4.2). Genotypes

differed in the range 601.5 (line 1) to 801.3 g m^{-2} (line 11), and 570.0 (line 71) to 764.1 g m^{-2} (line 10) under irrigated and unirrigated conditions, respectively. With regard to the parents, Rialto had higher straw DM than Savannah under irrigated (P<0.001).

For the chaff dry matter, cross-year analysis showed differences for the effect of irrigation (P<0.001), year (P=0.02) and genotype (P<0.001). The irrigation x genotype (P=0.004) and genotype x year (P<0.001) interactions were also significant (Table 4.2). Under irrigated conditions, lines ranged from 226.2 (line 51) to 316.3 g m^{-2} (line 73) and under unirrigated conditions, from 195.7 (line 50) to 320.1 g m^{-2} (line 79) (P<0.001). Rialto (267 and 215.1 g m^{-2}) had more chaff DM than Savannah (232.2 and 211.5 g m^{-2}) under both irrigated and unirrigated conditions, respectively (P<0.001).

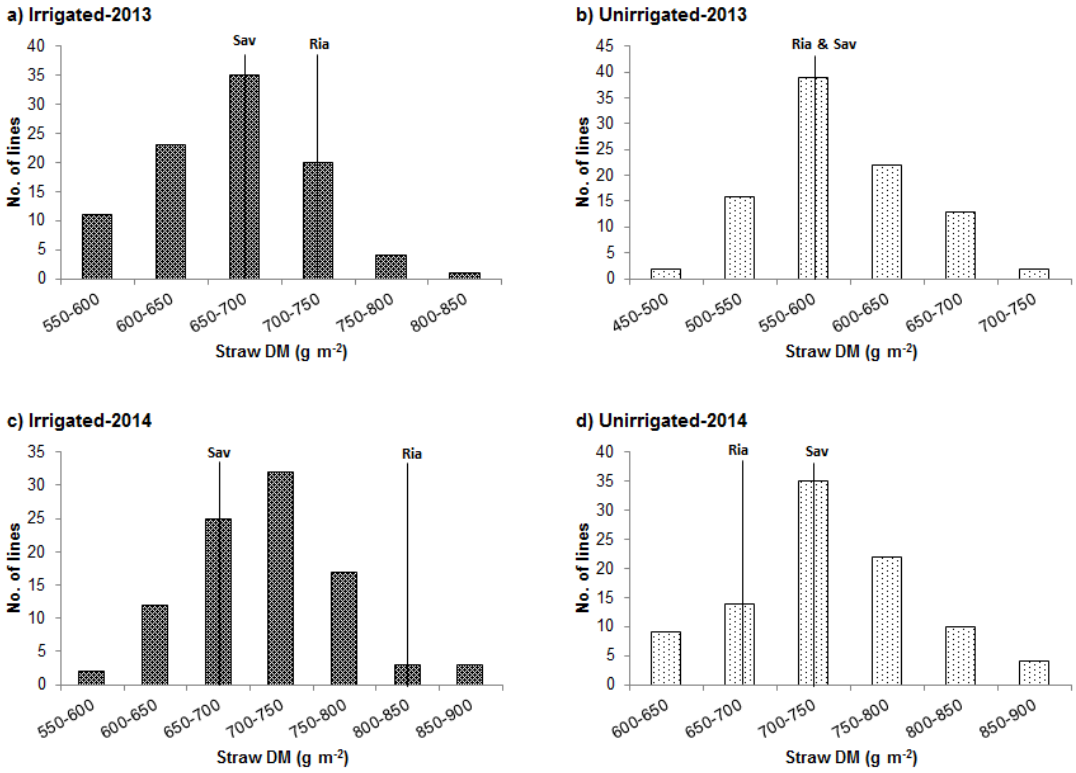


Figure 4.9 Frequency distributions for 94 Rialto x Savannah DH lines and the two parents for straw dry matter (a) under irrigated 2013, (b) unirrigated 2013, (c) irrigated 2014 and (d) unirrigated conditions 2014.

4.3.3.6 Ears m^{-2}

In 2013, drought overall decreased ears m^{-2} from 528.2 to 483.2 m^{-2} (P<0.001; Table 4.2). DH lines ranged from 453.6 (line 2) to 603.5 m^{-2} (line 90) under irrigated conditions, and 410.9 (line 1) to 562.9 m^{-2} (line 77) under unirrigated conditions (P=0.001). The irrigation x genotype interaction was not statistically significant (P=0.62). The parents were not significantly different in both irrigation treatments.

In 2014, drought decreased ears m^{-2} from 615.7 to 586.9 m^{-2} (P=0.009; Table 4.2). DH lines ranged from 425.8 (line 1) to 771.6 m^{-2} (line 47) under irrigated conditions, and 474.3 (line 24) to 805.2 m^{-2} (line 47) under unirrigated conditions (P<0.001). The parents were not significantly different under both irrigation treatments. The decrease with restricted water availability ranged from -196.8 (line 43) to +163.7 ears m^{-2} (line 90), respectively (P=0.05).

From the cross-year analysis, there were effects for ears m^{-2} of year (P=0.01) and irrigation x genotype interaction (P=0.01), but not for irrigation (P=0.11; Table 4.2). Genotypes differed in the range 463.1 (line 1) to 685.2 ears m^{-2} (line 90) and 459.4 (line 23) to 650.5 ears m^{-2} (line 43) under irrigated and unirrigated conditions, respectively.

Table 4.2 Mean, maximum, minimum, df and S.E.D. for chaff dry weight, straw dry weight, ears per m^2 and anthesis date under irrigated and unirrigated treatments for 94 Rialto x Savannah DH lines and the two parents in 2013, 2014 and cross-year mean.

Traits	Chaff DM ($g\ m^{-2}$)		Straw DM ($g\ m^{-2}$)		Ears m^{-2}		Anthesis date (DAS)	
2013	**Irri.**	**Unirri.**	**Irri.**	**Unirri.**	**Irri.**	**Unirri.**	**Irri.**	**Unirri.**
Mean	244.2	203.9	667.6	592.6	528.2	483.2	250.8	250.8
Max.	272.6	230.1	806.2	713.5	603.5	562.9	253.5	254.2
Min.	210.1	175.0	561.8	490.3	453.6	410.9	248.8	248.9
Rialto	260.5	198.6	713.2	584.0	539.4	503.1	250.0	251.0
Savannah	230.8	196.4	658.0	582.4	548.8	513.3	251.0	251.6
SED (df)								
Irrigation (1)	*1.90****		*6.14****		*6.27****		*0.07*^{ns}	
Genotype (95)	*8.60****		*29.85****		*20.41***		*0.45****	
Irri. x Gen (95)	*12.20*^{ns}		*42.32*^{ns}		*28.48*^{ns}		*0.63*^{ns}	
2014								
Mean	254.2	248.5	714.2	735.4	586.9	615.7	206.6	207.2
Max.	411.7	421.7	890.2	897.7	770.0	804.4	209.2	210.7
Min.	208.2	186.6	572.0	603.7	424.8	469.8	203.1	203.2
Rialto	275.7	231.9	805.5	697.5	636.8	570.3	206.4	205.7
Savannah	232.5	229.6	690.3	743.6	554.4	592.7	207.2	208.5
SED (df)								
Irrigation (1)	*3.05*^{ns}		*8.89**		*9.49***		*0.76*^{ns}	
Genotype (95)	*20.97****		*45.24****		*44.70****		*0.80****	
Irri. x Gen (95)	*29.66**		*64.13*^{ns}		*63.41**		*1.35*^{ns}	
2013-14								
Mean	249.2	226.2	691.0	663.9	557.9	549.4	228.7	229.0
Max.	316.3	320.1	801.3	764.1	685.2	650.5	231.3	232.3
Min.	226.2	195.7	601.5	570.0	463.1	459.4	226.1	226.1
Rialto	267.0	215.1	759.1	638.1	587.6	529.6	228.2	228.4
Savannah	232.2	211.5	674.2	659.9	556.3	557.1	229.1	230.0
SED (df)								
Year (1)	*4.07**		*13.24**		*12.25***		*0.31****	
Irrigation (1)	*1.91****		*5.45****		*5.05*^{ns}		*0.10***	
Genotype (95)	*11.50****		*27.40****		*26.65****		*0.46****	
Irri. x Gen (95)	*16.27***		*38.85*^{ns}		*37.76***		*0.65*^{ns}	
Year x Gen (95)	*16.47****		*39.76***		*38.57****		*0.69****	

N.B: *** denotes P<0.001; **P<0.01 and *P<0.05 significance levels; ^{ns} = not significant.

4.3.3.7 Grains m^{-2}

In 2013, drought overall decreased grains m^{-2} from 25,148 to 23,667 m^{-2} (P=0.003; Table 4.3). Doubled-haploid lines ranged from 19,764 (line 44) to 31,455 m^{-2} (line 70) under irrigated conditions, and 17,240 (line 40) to 29,734 m^{-2} (line 74) under unirrigated conditions (P<0.001). The irrigation x genotype interaction was not statistically significant. In both irrigated and unirrigated treatments Savannah (27,122 and 26,290 m^{-2}, respectively) had more grains m^{-2} than Rialto (24,277 and 25,777 m^{-2}, respectively) (Fig. 4.10a and b).

In 2014, effects of irrigation (P=0.42) and irrigation x genotype interaction (P=0.36) on grains m^{-2} were not significant (Table 4.3). DH lines ranged from 21,412 (line 1) to 33,977 m^{-2} (line 9) under irrigated conditions, and 21,957 (line 40) to 34,025 m^{-2} (line 9) under unirrigated conditions (P<0.001). In contrast to 2013, under irrigated and unirrigated treatments Savannah (26,948 and 25,745 m^{-2}, respectively) had slightly lower grain number per unit area than Rialto (28,756 and 26,013 m^{-2}, respectively) (Fig. 4.10c and d). Doubled-haploid lines 87, 46 and 9 in the range 32,865 to 33,977 m^{-2} showed transgressive segregation producing more grains m^{-2} than the higher parent under irrigated conditions and 12 lines showed transgressive segregation compared to the higher parent under unirrigated conditions.

From the cross-year analysis, there were effects of irrigation (P=0.005), year (P=0.028) and genotype (P<0.001), and a weak trend for the irrigation x genotype interaction (P=0.10; Table 4.3). Overall drought decreased grains m^{-2} from 25,713 to 25,065 m^{-2} (P=0.005). Genotypes differed in the range 21,117 (line 78) to 30,554 m^{-2} (line 87) and 20,123 (line 40) to 31,021 m^{-2} (line 9) under irrigated and unirrigated conditions, respectively.

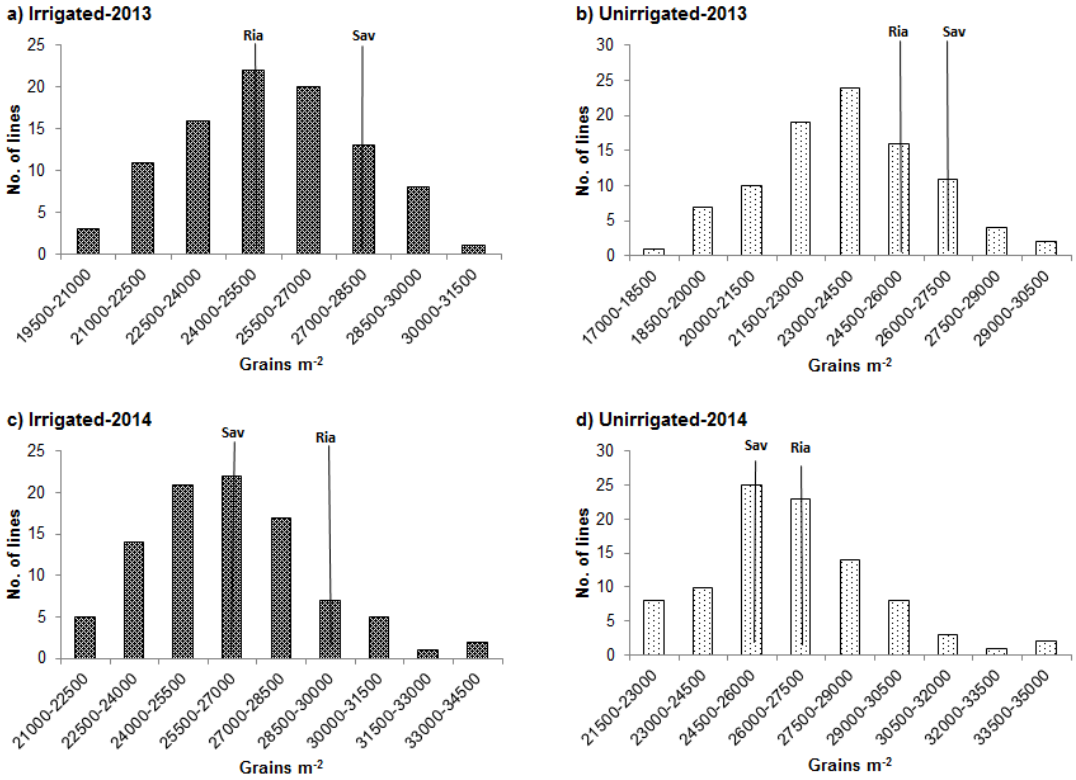


Figure 4.10 Frequency distributions for 94 Rialto x Savannah DH lines and the two parents for grains m^{-2} under (a) irrigated 2013, (b) unirrigated 2013, (c) irrigated 2014 and (d) unirrigated conditions 2014.

4.3.3.8 Grains per ear

In 2013, grains per ear was decreased from 49.0 in the irrigated to 47.7 in the unirrigated treatment (P=0.004). Doubled-haploid lines ranged from 37.8 (line 48) to 57.4 (Line 70) in the irrigated treatment, and 38.6 (line 78) to 57.6 (line 74) in the unirrigated treatment (P<0.001). The irrigation x genotype interaction was significant with decreases under drought in the range -9.3 (line 17) to +10.6 (line 32) (P=0.02; Table 4.3). Savannah (49.6) had more grains than Rialto (45.6) under irrigated conditions, but slightly fewer (Savannah 50.0; Rialto 51.4) under unirrigated conditions (P<0.001; Fig. 4.11a and b).

In 2014, drought decreased grains per ear slightly from 45.3 to 43.4 (P<0.001). Genotypes ranged from 35.4 (lines 79) to 60.1 (line 87) under irrigated conditions, and 33.5 (line 47) to 54.5 (line 24) under unirrigated conditions (P<0.001). The irrigation x genotype interaction was not significant (P=0.14; Table 4.3). Similarly, Savannah (48.8) had more grains ear^{-1} than Rialto (45.5) under irrigated conditions,

but slightly fewer (Savannah 45.4; Rialto 46.1) under unirrigated treatments (P< 0.05) (Fig. 4.11c and d).

For the cross-year analysis, there were effects on grains ear^{-1} of year (P=0.02) and genotype (P<0.001), and the irrigation x genotype (P=0.002) and genotype x year (P<0.001) interactions, but not of irrigation (P=0.41) (Table 4.3). DH lines ranged from 39.2 (line 48) to 58.0 (line 87) under irrigated conditions, and from 36.8 (line 47) to 53.9 (line 87) under unirrigated conditions.

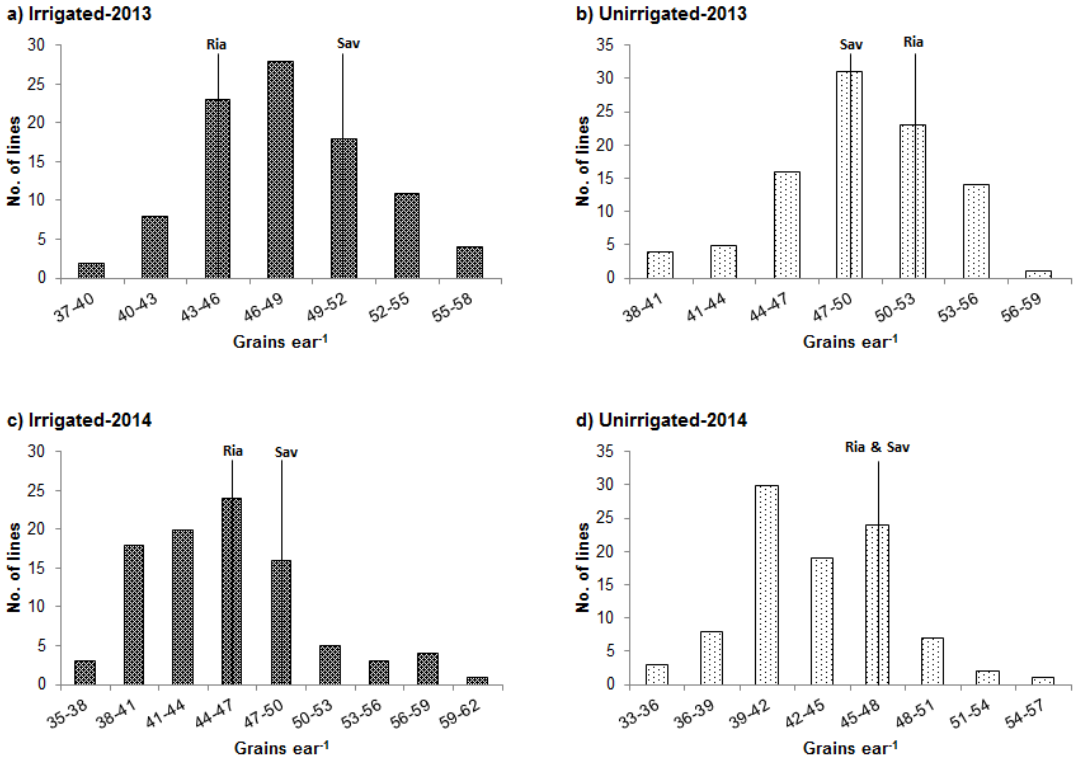


Figure 4.11 Frequency distributions for 94 Rialto x Savannah DH lines and the two parents for grains ear^{-1} under (a) irrigated 2013, (b) unirrigated 2013, (c) irrigated 2014 and (d) unirrigated conditions 2014.

4.3.3.9 Thousand grain weight

In 2013, drought decreased TGW from 41.3 to 34.0 g (P<0.001). DH lines ranged from 34.3 (line 9) to 49.0 g (line 78) under irrigated conditions, and 27.3 (line 72) to 42.9 g (line 76) under unirrigated conditions (P<0.001). The irrigation x genotype interaction was significant with decreases under drought amongst the genotypes in the range 3.5 (line 9) to 11.2 (line 99) (P=0.009; Table 4.3). Savannah (40.4 g) was

slightly higher than Rialto (38.7 g) under irrigated conditions, and slightly lower under unirrigated conditions (Savannah 34.8 g; Rialto 35.0 g) (Fig. 4.12a and b).

In 2014, drought reduced TGW slightly from 40.3 to 39.3 g (P<0.001). Doubled-haploid lines differed in the ranges 32.7 (line 89) to 49.7 g (line 78) under irrigated conditions, and 33.7 (line 9) to 45.7 (line 45) under unirrigated conditions (P<0.001). The irrigation x genotype interaction was not significant (P=0.61; Table 4.3). Savannah, under irrigated and unirrigated conditions, (43.0 and 41.9 g, respectively) had heavier grains than Rialto (39.2 and 38.1 g, respectively) (Fig. 4.12c and d).

From the cross-year analysis, there was no effect of year on TGW (P=0.15). Drought reduced TGW from 40.8 to 36.7 g (P<0.001). Genotypes differed in the range 34.0 (line 9) to 49.6 g (line 78) under irrigated conditions, and 30.8 (line 87) to 43.0 g (line 76) under unirrigated conditions (P<0.001). The year x genotype (P=0.01) and irrigation x year (P<0.001) interactions were significant, but there was no irrigation x genotype interaction (P=0.30; Table 4.3). With regard to the parents, Savannah, under irrigated and unirrigated conditions, (42.1 and 38.2 g, respectively) had heavier grains than Rialto (39.1 and 36.5 g, respectively) (P<0.001).

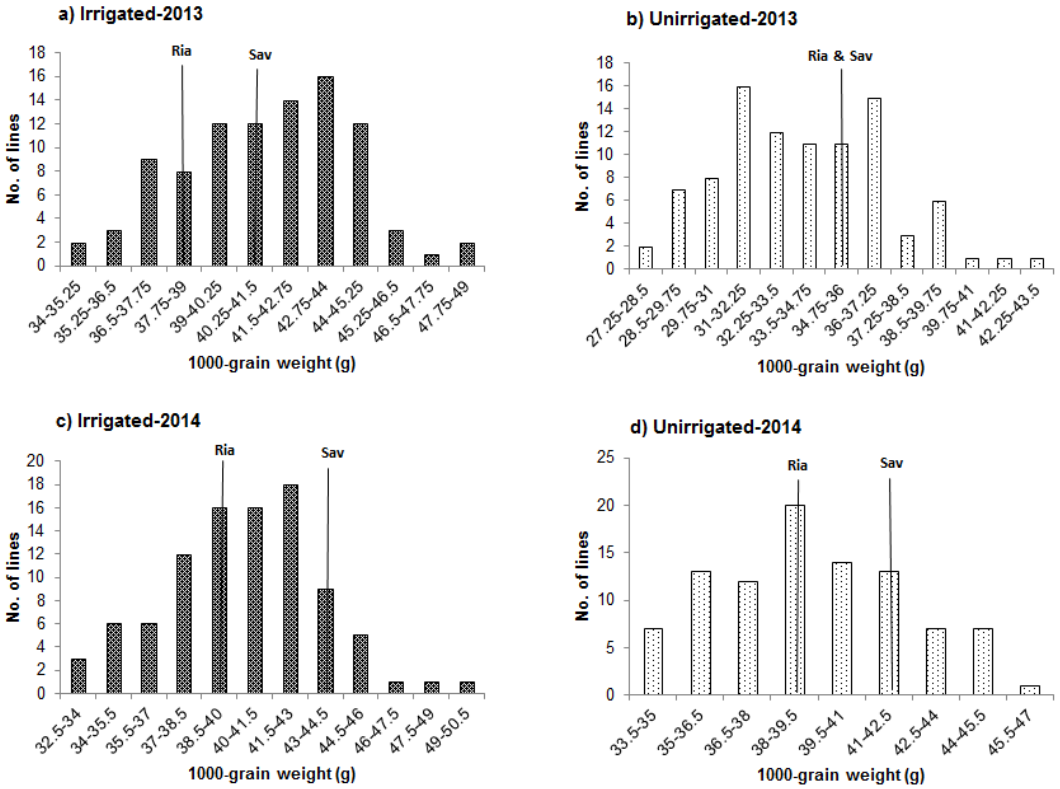


Figure 4.12 Frequency distributions for 94 Rialto x Savannah DH lines and the two parents for thousand grain weight under (a) irrigated 2013, (b) unirrigated 2013, (c) irrigated 2014 and (d) unirrigated conditions 2014.

4.3.3.10 Grain carbon isotope discrimination $\Delta^{13}C$

Carbon isotope discrimination of grains was assessed in the unirrigated treatment in 2013 and 2014. In 2013, DH lines differed in the range 18.74 (line 21) to 20.01 ‰ (line 56) (P=0.007; Table 4.3). Regarding the parents, Savannah (19.92 ‰) was higher than Rialto (19.30 ‰) (P<0.05; Fig. 4.13a). There was no transgressive segregation amongst genotypes.

In 2014, genotypes differed from 19.85 (line 18) to 21.59 ‰ (line 58) (P=0.015; Table 4.3). There was no transgressive segregation amongst genotypes. The parents also differed in grain $\Delta^{13}C$ from 20.12 (Rialto) to 21.19 ‰ (Savannah) (P=0.05; Table 4.3; Fig. 4.13b).

Averaging over years, DH lines differed in the range 19.58 (line 18) to 20.63 ‰ (line 58) (P<0.001; Table 4.3). Savannah (20.56 ‰) was higher than Rialto (19.71 ‰) (P<0.05). However, there was no statistically significant transgressive segregation amongst genotypes.

For the sub-set of six lines and the two parents, genotypes differed in grain $\Delta^{13}C$ in the range 19.3 (Rialto and line 88) to 19.9 ‰ (Savannah) in 2013 (P=0.007), and 20.1 (Rialto) to 21.2 ‰ (Savannah) in 2014 (P=0.015). Averaging over years, genotypes ranged from 19.7 (Rialto) to 20.6 ‰ (Savannah) (P<0.001; Table 4.4).

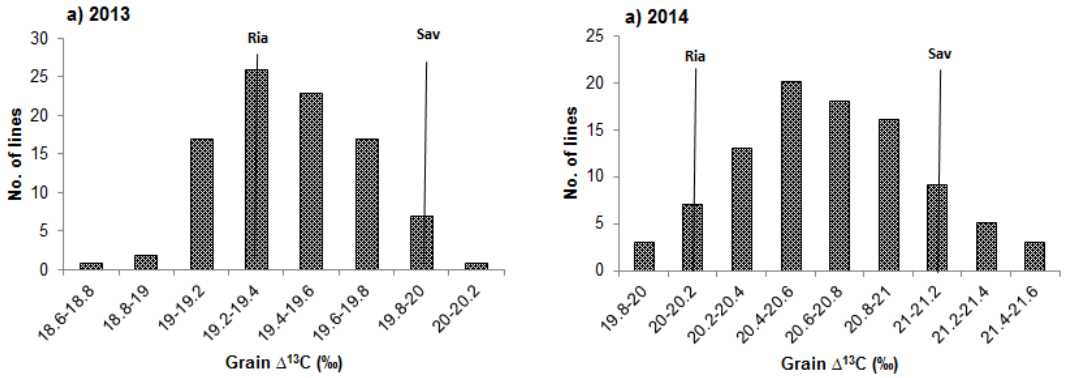


Figure 4.13 Frequency distributions for 94 Rialto x Savannah DH lines and the two parents for carbon isotope discrimination of grain in (a) 2013 and (b) 2014 under unirrigated conditions.

Table 4.3 Mean, maximum, minimum, df and S.E.D. for grains m^{-2}, grains ear^{-1} and thousand grain weight of each irrigated and unirrigated treatments; and grain carbon isotope discrimination of grain under unirrigated conditions for 94 Rialto x Savannah DH lines and the two parents in 2013, 2014 and cross-year mean.

Traits	Grains m^{-2}		Grains ear^{-1}		TGW (g)		Grain $\Delta^{13}C$ (‰)	
2013	**Irri.**	**Unirri.**	**Irri.**	**Unirri.**	**Irri.**	**Unirri.**	**Irri.**	**Unirri.**
Mean	25148	23667	47.7	49.0	41.3	34.0	-	19.4
Max.	31455	29734	57.4	57.6	49.0	42.9	-	20.0
Min.	19764	17240	37.8	38.6	34.3	27.3	-	18.7
Rialto	24277	25777	45.6	51.4	38.7	35.0	-	19.3
Savannah	27122	26290	49.6	50.0	40.4	34.8	-	19.9
SED (df)								
Irrigation (1)	*394.7***		*0.46***		*0.78****		-	
Genotype (95)	*1646.0****		*2.25****		*1.11****		*0.29***	
Irri. x Gen (95)	*2338.0*^{ns}		*3.19**		*1.37***		-	
2014								
Mean	26252	26452	45.3	43.4	40.3	39.3	-	20.7
Max.	33977	34025	60.1	54.5	49.7	45.7	-	21.6
Min.	21412	21957	35.4	33.5	32.7	33.7	-	19.9
Rialto	28756	26013	45.5	46.1	39.2	38.1	-	20.1
Savannah	26948	25745	48.8	45.4	43.0	41.9	-	21.2
SED (df)								
Irrigation (1)	*244.3*^{ns}		*0.44****		*0.20****		-	
Genotype (95)	*1607.0****		*2.35****		*1.50****		*0.42**	
Irri. x Gen (95)	*2273.0*^{ns}		*3.33*^{ns}		*2.10*^{ns}		-	
2013-14								
Mean	25713	25065	46.5	46.2	40.8	36.7	-	20.1
Max.	30554	31021	58.0	53.9	49.6	43.0	-	20.6
Min.	21117	20123	39.2	36.8	34.0	30.8	-	19.6
Rialto	26883	25885	45.6	48.7	39.1	36.5	-	19.7
Savannah	27394	26406	49.2	47.8	42.1	38.2	-	20.6
SED (df)								
Year (1)	*362.9**		*0.49**		*0.92*^{ns}		*0.13**	
Irrigation (1)	*209.6***		*0.32*^{ns}		*0.15****		-	
Genotype (95)	*1175.0****		*1.63****		*0.94****		*0.26****	
Irri. x Gen (95)	*1665.0*^{ns}		*2.31***		*1.34*^{ns}		-	
Year x Gen (95)	*1678.0***		*2.32****		*1.48***		*0.38*^{ns}	

N.B: ***denotes P<0.001; **P<0.01 and *P<0.05 significance levels; ^{ns} = not significant.

Table 4.4 Mean, maximum, minimum, df and S.E.D. for carbon isotope discrimination of grain under unirrigated conditions for subset of 6 Rialto x Savannah DH lines and the two parents in 2013, 2014 and cross-season selected for leaf gas exchange measurements (see chapter 5).

Genotype	2013	2014	Cross-year mean
Line 1	19.5	21.0	20.2
Line 20	19.8	20.8	20.3
Line 25	19.6	20.5	20.0
Line 63	19.8	20.5	20.1
Line 64	19.4	20.4	19.9
Line 88	19.3	20.4	19.8
Rialto	19.3	20.1	19.7
Savannah	19.9	21.2	20.6
Mean	19.6	20.6	20.1
Max	19.9	21.2	20.6
Min	19.3	20.1	19.7
SED (df)			
Year (1)	-	-	*0.09***
Genotype (7)	*0.18**	*0.26**	*0.16***
Gen. x Year (7)	-	-	*0.23*^{ns}

N.B: ***denotes P<0.001; **P<0.01 and *P<0.05 significance levels; ^{ns} = not significant.

4.3.4 Relationships between grain yield and harvest traits

4.3.4.1 Relationships between grain yield and biomass and harvest index

In 2013, there was a positive linear relationship amongst DH lines between HI and grain yield under both irrigated (R^2=0.05; *P*=0.03) and unirrigated (R^2=0.09; *P*=0.001; Fig. 4.14a) conditions. Similarly positive linear relationships were observed in 2014 under irrigated (R^2=0.08; *P*=0.004) and unirrigated (R^2=0.10; *P*=0.002; Fig. 4.14b) conditions. Averaging across years, a positive linear relationship between HI and grain yield was also found under both irrigated (R^2=0.11; *P*<0.001) and unirrigated (R^2=0.09; *P*=0.001; Fig. 4.14c) conditions.

In 2013, a positive linear relationship amongst DH lines was found between above-ground dry matter and grain yield under both irrigated (R^2=0.66; *P*<0.001) and unirrigated (R^2=0.70; *P*<0.001; Fig. 4.14d) conditions. Similarly a positive linear

relationship was observed in 2014 under irrigated (R^2=0.59; P<0.001) and unirrigated (R^2=0.50; P<0.001; Fig. 4.14e) conditions. Averaging across years, a linear relationship between AGDM and grain yield was also found under both irrigated (R^2=0.58; P<0.001) and unirrigated (R^2=0.55; P<0.001; Fig. 4.14f) conditions. The associations between grain yield and AGDM amongst DH lines were stronger than those between grain yield and HI under both irrigated and unirrigated conditions in each year.

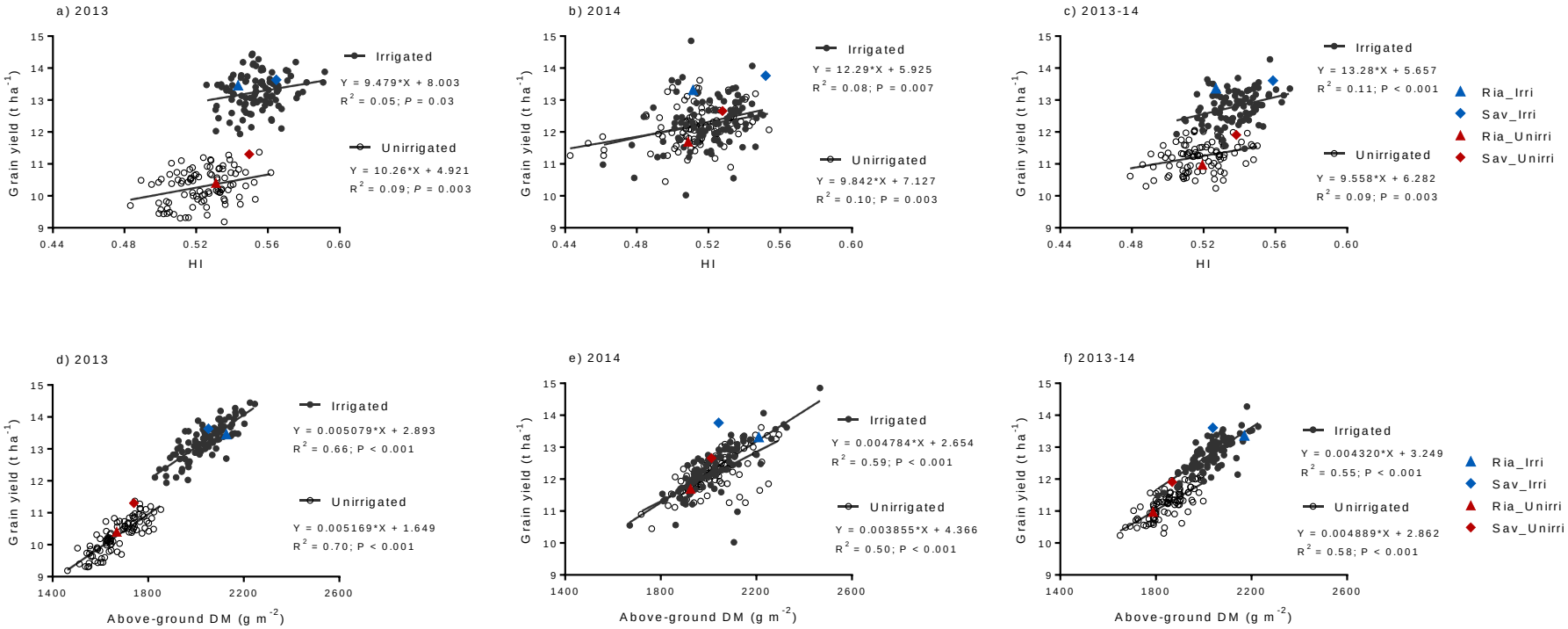


Figure 4.14 Linear regression of grain yield (85% DM) on HI in (a) 2013, (b) 2014 and (c) cross-year mean, and linear regression of grain yield (85% DM) on above-ground DM in (d) 2013, (e) 2014 and (f) cross-year mean under irrigated and unirrigated conditions for 94 Rialto x Savannah DH lines. Rialto under irrigated (▲) and unirrigated (Δ) conditions, and Savannah under irrigated (♦) and unirrigated (◊) conditions are also shown.

4.3.4.2 Relationships between grain yield, grains m^{-2} and TGW

In 2013, there was a trend for a positive linear relationship (R^2=0.04; P=0.06) between grain yield and grains m^{-2} amongst DH lines under irrigated conditions, while no association was found under unirrigated conditions (P=0.44; Fig. 4.15a). In 2014, a positive linear relationships between grain yield and grains m^{-2} amongst DH lines was found under both irrigated (R^2=0.27; P<0.001) and unirrigated (R^2=0.19; P<0.001; Fig. 4.15b) conditions. Averaging across years, a positive linear relationship was found between grain yield and grains m^{-2} under irrigated (R^2=0.13; P<0.001), but not under unirrigated conditions (P=0.16; Fig. 4.15c).

In 2013, for thousand grain weight, there was a positive linear relationship with grain yield amongst DH lines under unirrigated conditions (R^2=0.16; P<0.001). Under irrigation, the relationship was not significant (P=0.14; Fig. 4.15d). In 2014, there was no association either under irrigated (P=0.14) or unirrigated (P=0.13; Fig. 4.15e) conditions. Cross-year analysis showed a positive relationship between thousand grain weight and grain yield under unirrigated conditions (R^2=0.11; P<0.001), but no association under irrigated conditions (P=0.19; Fig. 4.15f).

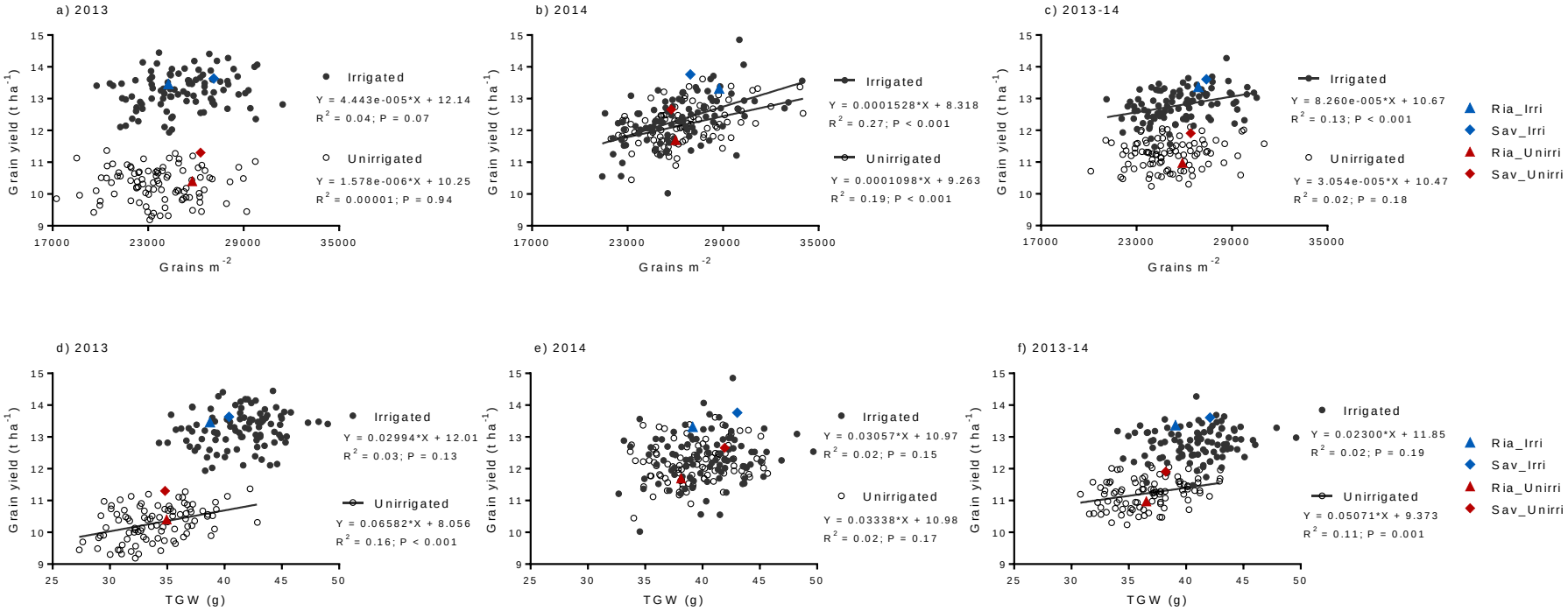


Figure 4.15 Linear regression of grain yield (85% DM) on grains m^{-2} in (a) 2013, (b) 2014 and (c) cross-year mean, and linear regression of grain yield (85% DM) on thousand grain weight (TGW) in (d) 2013, (e) 2014 and (f) cross-year mean under irrigated and unirrigated conditions for 94 Rialto x Savannah DH lines. Rialto under irrigated (▲) and unirrigated (Δ) conditions, and Savannah under irrigated (♦) and unirrigated (◊) conditions are also shown.

4.3.5 Relationships between grain yield, crop height and anthesis date

In 2013, a positive linear relationship amongst DH lines was found between grain yield and plant height under both irrigated (R^2=0.37; P<0.001) and unirrigated (R^2=0.25; P<0.001; Fig. 4.16a) conditions. However, no associations were found in 2014 under both irrigated (P=0.88) and unirrigated (P=0.33; Fig. 4.16b) conditions. Averaging across years, there was a positive linear relationship between grain yield and plant height under irrigated (R^2=0.11; P=0.001) and unirrigated (R^2=0.12; P<0.001; Fig. 4.16c) conditions.

In 2013, anthesis date showed no associations with grain yield amongst DH lines under either irrigated (P=0.11) or unirrigated conditions (P=0.14; Fig. 4.16d). In 2014, there was a negative linear relationship under irrigated conditions (R^2=0.07; P=0.009), but no association under unirrigated conditions (P=0.31; Fig. 4.16e). The cross-year regression showed negative linear relationships between anthesis date and grain yield under irrigated (R^2=0.11; P=0.002) and unirrigated (R^2=0.15; P<0.001; Fig. 4.16f) conditions.

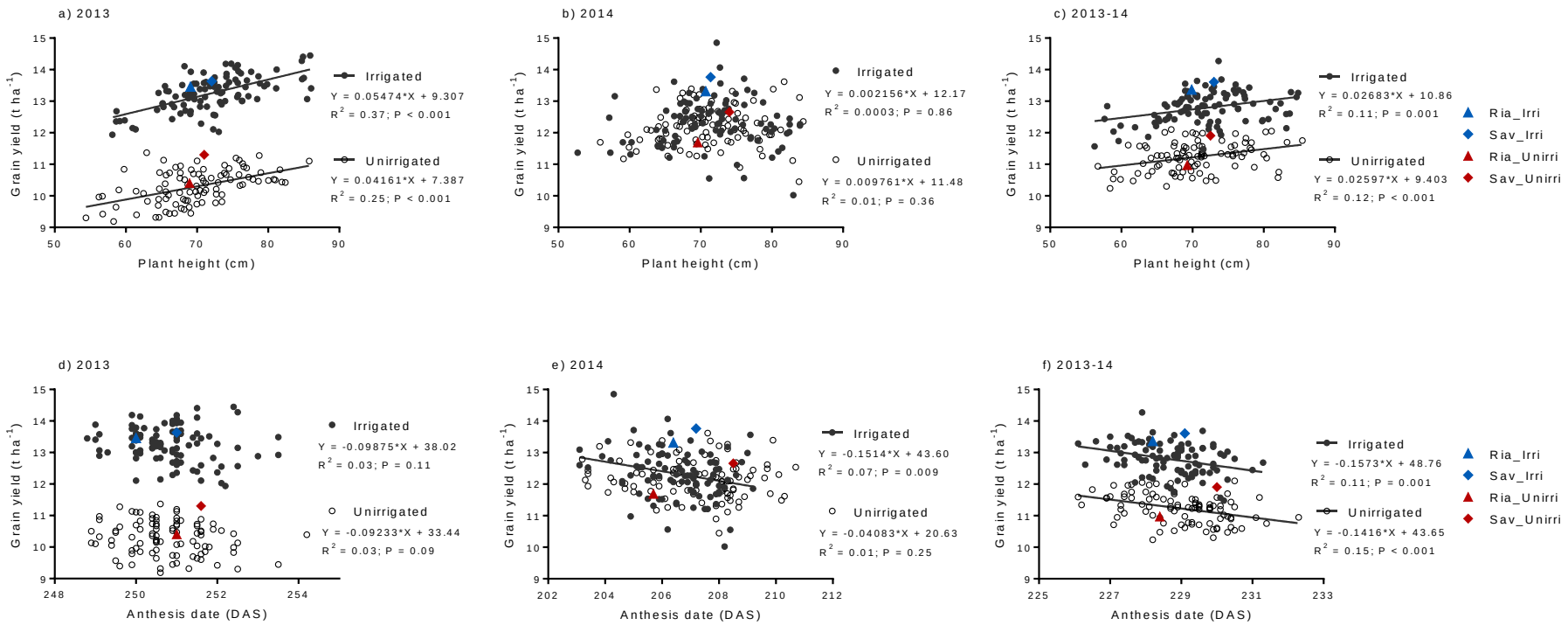


Figure 4.16 Linear regression of grain yield (85% DM) on plant height in (a) 2013, (b) 2014 and (c) cross-year mean, and linear regression of grain yield (85% DM) on anthesis date in (d) 2013, (e) 2014 and (f) cross-year mean under irrigated and unirrigated conditions for 94 Rialto x Savannah DH lines. Rialto under irrigated (▲) and unirrigated (Δ) conditions, and Savannah under irrigated (♦) and unirrigated (◊) conditions are also shown.

4.3.6 Relationships between above-ground DM, anthesis date and plant height

In 2013 and 2014, no associations amongst DH lines were found between above-ground DM and anthesis date under either irrigated (P=0.98 and P=0.22, respectively) or unirrigated (P=0.83 and P=0.20, respectively) (Fig. 4.17a and b) conditions. Averaging across years, similarly no significant linear relationships between AGDM and anthesis date were found under either irrigated (P=0.25) or unirrigated (P=0.51; Fig. 4.17c) conditions.

In 2013, a positive linear relationship amongst DH lines was found between AGDM and plant height under either irrigated (R^2=0.43; P<0.001) or unirrigated (R^2=0.44; P<0.001; Fig. 4.17d) conditions. In contrast, no significant relationships between AGDM and plant height were found in 2014 under irrigated (P=0.28) or unirrigated (P=0.45; Fig. 4.17e) conditions. Averaging across-years, there were positive linear relationships between AGDM and plant height under irrigated (R^2=0.22; P<0.001) and unirrigated (R^2=0.17; P<0.001; Fig. 4.17f) conditions.

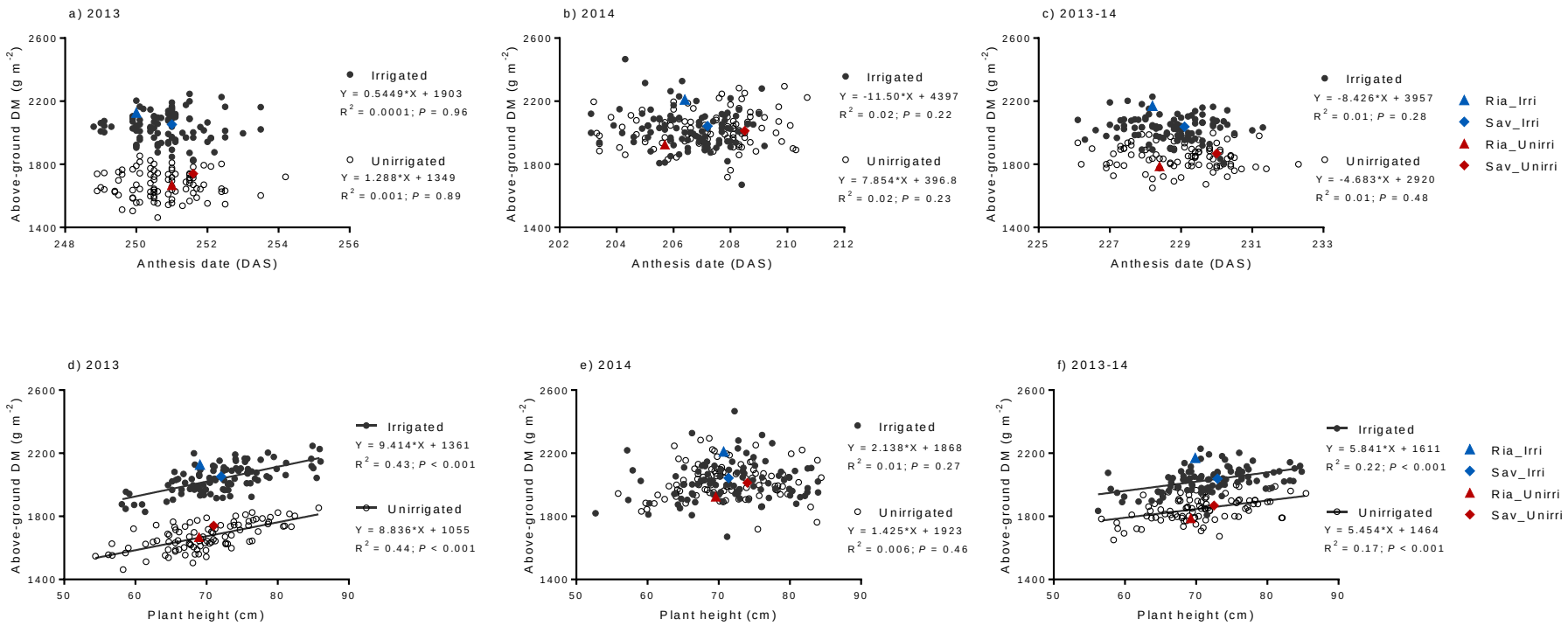


Figure 4.17 Linear regression of above-ground DM on anthesis date in (a) 2013, (b) 2014 and (c) cross-year mean, and linear regression of above-ground DM on plant height in (d) 2013, (e) 2014 and (f) cross-year mean under irrigated and unirrigated conditions for 94 Rialto x Savannah DH lines. Rialto under irrigated (▲) and unirrigated (Δ) conditions, and Savannah under irrigated (♦) and unirrigated (◊) conditions are also shown.

4.3.7 Relationship between grains m^{-2} and thousand grain weight

In 2013, a strong negative relationship amongst DH lines was found between grains m^{-2} and TGW under either irrigated (R^2=0.43; P<0.001) or unirrigated (R^2=0.30; P<0.001; Fig. 4.18a) conditions. Similarly in 2014, a negative linear relationship was observed under irrigated (R^2=0.56; P<0.001) and unirrigated (R^2=0.54; P<0.001; Fig. 4.18b) conditions. Averaging across years, a negative linear relationship between grains m^{-2} and TGW was found under both irrigated (R^2=0.63; P<0.001) and unirrigated (R^2=0.51; P<0.001; Fig. 4.18c) conditions.

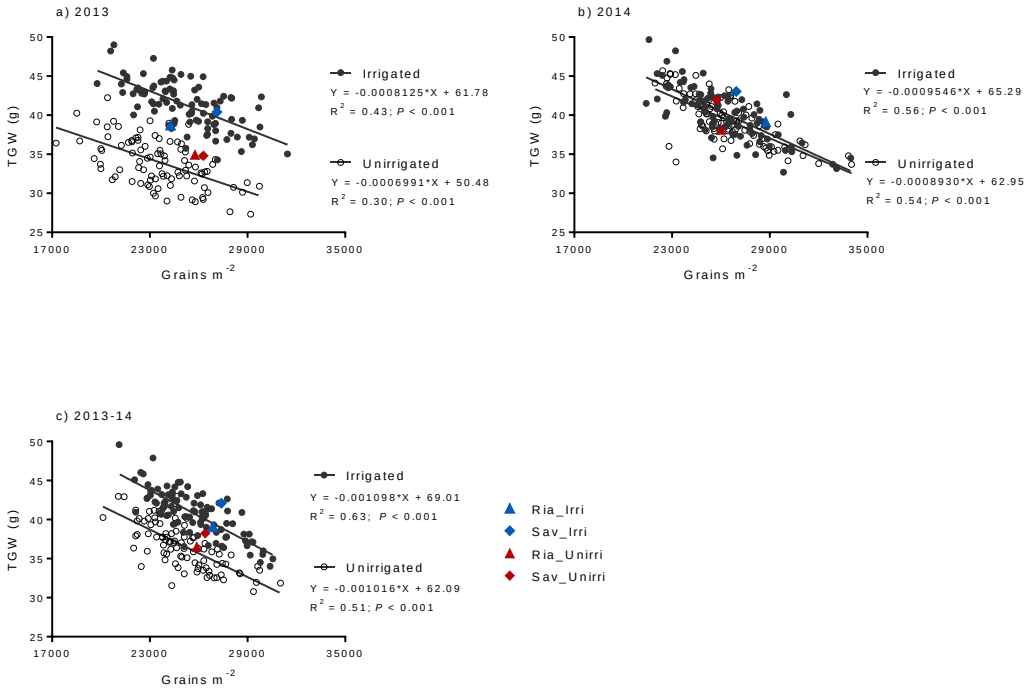


Figure 4.18 Linear regression of grains m^{-2} on thousand grain weight (TGW) in (a) 2013, (b) 2014 and (c) cross-year mean under irrigated and unirrigated conditions for 94 Rialto x Savannah DH lines. Rialto under irrigated (▲) and unirrigated (Δ) conditions, and Savannah under irrigated (♦) and unirrigated (◊) conditions are also shown.

4.3.8 Relationships between grain yield and grain $\Delta^{13}C$

Carbon isotope discrimination ($\Delta^{13}C$) of the grain was measured for all genotypes under unirrigated conditions in both years. In 2013, grain $\Delta^{13}C$ showed no overall association with grain yield amongst DH lines (P=0.22). However, there was a positive linear relationship between grain yield and grain $\Delta^{13}C$ for the sub-set of

eight genotypes selected for leaf gas exchange analysis (R^2=0.46; *P*=0.06; Fig. 4.19a, see Chapter 5). In 2014, there was no association between grain yield and grain $\Delta^{13}C$ amongst either DH lines (*P*=0.73) or the sub-set of lines (*P*=0.31; Fig. 4.19b). Cross-year analysis also showed no association between grain yield and grain $\Delta^{13}C$ amongst either DH lines (*P*=0.57) or the sub-set of lines (*P*=0.17; Fig. 4.19c). There was, however, a strong trend for positive relationship between grain $\Delta^{13}C$ measured in 2013 and grain $\Delta^{13}C$ measured in 2014 amongst DH lines (R^2=0.04; *P*=0.06) and statistically significant amongst the sub-set of lines in the unirrigated treatment in 2013 and 2014 (R^2=0.49; *P*=0.05; Fig. 4.19d).

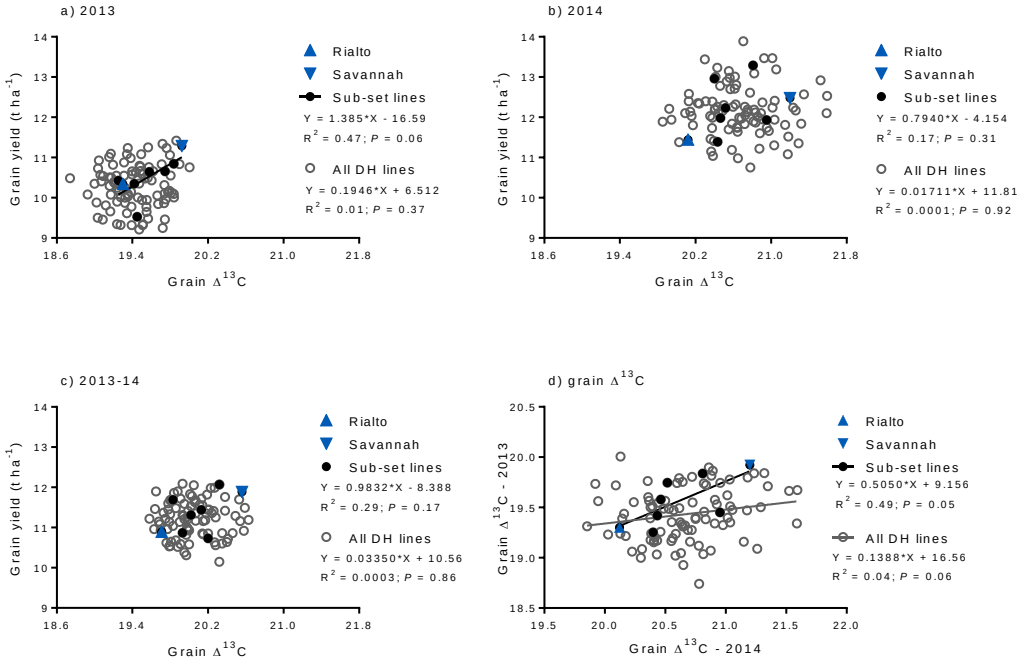


Figure 4.19 Linear regressions of grain yield on grain carbon isotope discrimination ($\Delta^{13}C$) in (a) 2013, (b) 2014 and (c) cross-year mean, and (d) linear regression of $\Delta^{13}C$ in 2013 on 2014 under unirrigated conditions for 94 Rialto x Savannah DH lines and the two parents, and for a sub-set of six DH lines and the two parents.

4.3.9 Associations among agronomic traits

Principal component analysis (PCA) was performed to identify the association between grain yield and traits under irrigated and drought conditions. PCA is a statistical method to interpret the effect of a number of variables to identify the principal directions in which the data varies through linear combinations of the data (groups of trait), and each linear combination will correspond to a principal component. In 2013, grain yield was positively correlated with AGDM and PH, and negatively with anthesis date under both irrigated and unirrigated conditions. AGDM was also positively associated with PH under both irrigated and unirrigated conditions (Fig. 4.20a and 4.20b). Under drought condition, grain $\Delta^{13}C$ was negatively associated with AGDM in 2013 (Fig. 4.20b).

In 2014, a positive relationship amongst DH lines confirmed between grain yield and each of HI and ears per m^2 under irrigated condition (Fig. 4.20c), and between grain yield and each of HI and TGW under unirrigated conditions (Fig. 4.20d). Grain yield was also negatively associated with anthesis date under both irrigated and unirrigated conditions (Fig. 4.20c and 4.20d).

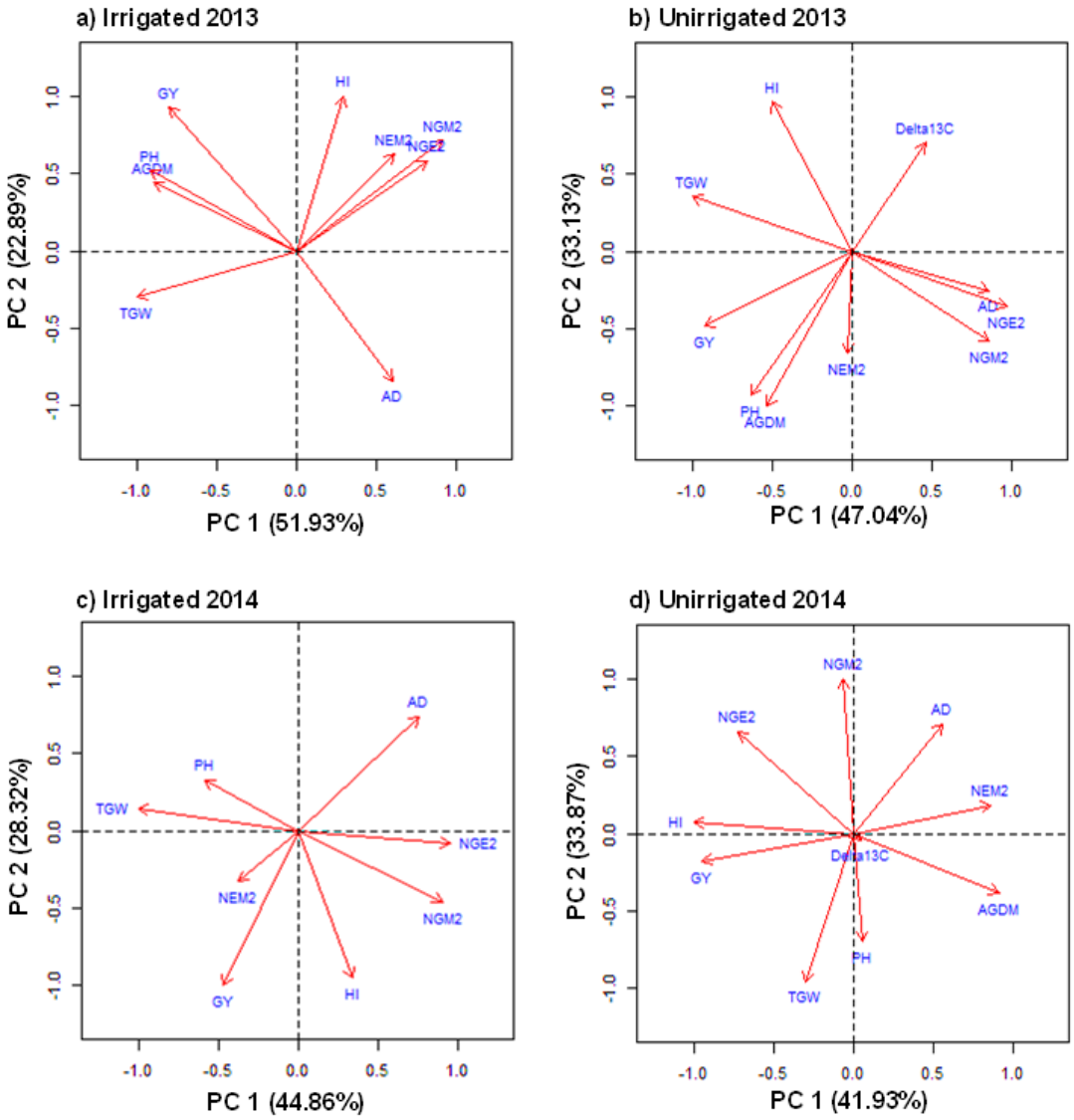


Figure 4.20 Principal component analysis among 94 DH lines of the Rialto x Savannah population for the performance of grain yield (GY; t ha^{-1}), harvest index (HI), above-ground dry matter (AGDM; g m^{-2}), plant height (PH; cm), grains m^{-2} (NGM2), grains ear^{-1} (NGE2), ears m^{-2} (NEM2), thousand grain weight (TGW; g), anthesis date (AD; day) and grain $\Delta^{13}C$ (Delta 13C) under (a) irrigated 2013, (b) unirrigated 2013, (c) irrigated 2014 (AGDM was not included due to low heritability) and (d) unirrigated 2014.

4.4 Discussion

First the genetic variation in crop development and plant height and relationships with grain yield are discussed, and then the relationships between grain yield and harvest traits including grain carbon isotope discrimination. The discussion will mainly focus on the results in 2013 as the drought effect was greater in this year than in 2014.

4.4.1 Crop growth responses to water availability

Yield components are determined at different crop developmental stages and have different sensitivity to drought stress (Cattivelli *et al.*, 2008). The impact of phenological development can help the crop avoid either direct effects of the stress or coincidence of the most sensitive phases with the most likely occurrence of the stress (Slafer *et al.*, 2005a). In the present study, although, parents and DH lines differed in their anthesis date in both seasons, drought only advanced anthesis date by 1-2 days, associated with mild water stress in the unirrigated treatment in the pre-anthesis period in both years. The present study showed that the earlier flowering was weakly associated with higher grain yield under irrigated and drought conditions across both years. However, no correlation was found between grain yield responses to drought and anthesis date under drought in this study.

Under the UK field environment, similar small advancements of anthesis date due to drought in winter wheat have been observed by Verma *et al.* (2004), and a small effect of drought was also found advancing anthesis date by 1-2 days by Foulkes *et al.* (2001) and Aravinda Kumar *et al.* (2011) in UK wheat field experiments. However, days to anthesis and to maturity were affected by drought stress, and reduced by about 10% within the Seri/Babax recombinant inbred line population under different water stress regimes in NW Mexico and under rain-fed conditions in Australia (Fischer and Maurer, 1978; Olivares-Villegas *et al.*, 2007).

The post-anthesis environmental conditions have also impact on the timing of leaf senescence and physiological maturity (Altenbach *et al.*, 2003). The effect of drought on maturity date has been reported in pot experiments for UK winter wheat (Gooding *et al.*, 2003), and spring wheat cultivars in USA (Altenbach *et al.*, 2003). In the present study, restricted water availability advanced physiological maturity (PM) by

10 days in 2013 and 5 days in 2014; water limitation occurred mainly over the second half of grain-filling period in 2013 but in 2014 mainly over the first half of grain-filling period. The duration from anthesis date to PM under drought was weakly negatively associated with yield loss to drought in 2013 ($R^2 = 0.05$; $P = 0.04$) indicating that longer grain-filling period under drought corresponds with higher grain yield. This might be explained by a stay-green effect and/or a drought escape effect with the timing of the onset of drought occurring relatively later in the crop life cycle for stay-green lines with extend durations for grain filling (Fischer and Maurer, 1978; Triboi and Triboi-Blondel, 2002).

The present study also showed that grain yield and above-ground dry matter were positively associated with plant height under irrigated and unirrigated treatments in 2013. Although, semi-dwarf genotypes have higher HI through reducing stem biomass partitioning, taller cultivars can have longer and wider leaves and potentially provide more CO_2 assimilates to the grain (Rebetzke *et al.*, 2004). It is unlikely that grain yield can be improved through further reducing plant height in the UK since plant height reduction below ca. 70 cm can reduce radiation-use efficiency and the above-ground biomass (Reynolds, 1996), as well as storage of water-soluble carbohydrates (WSC) in the stem which can directly influence HI especially under post-anthesis abiotic stresses (Reynolds and Tuberosa, 2008).

4.4.2 Grain yield and above-ground dry matter responses to drought

In the UK, drought typically occurs late in the season, and reduces yield in the range of 1-3 t ha^{-1} (Foulkes *et al.*, 2001, 2002). Genetic variation in grain yield reduction has been frequently reported due to water stress in wheat (Foulkes *et al.*, 2002; Olivares-Villegas *et al.*, 2007; Kandic *et al.*, 2009; Aravinda Kumar *et al.*, 2011). In the present study, the effect of drought on grain yield and yield components was much greater in 2013 compared to 2014, associated with drier conditions from mid-grains filling to harvest in 2013. In 2014, drought occurred mostly between ear emergence and mid-grain filling which restricted grains per m^2 more than grain weight (Foulkes *et al.*, 2002). In 2013, restricted water availability reduced grain yield by 22% while reduction was minimal at 2% in 2014. The yield reductions in 2013 were similar to those (*c.* 20-30%) reported by Foulkes *et al.* (2002)

investigating six winter wheat cultivars, and larger than those (*c.* 5-15%) reported by Aravinda Kumar *et al.* (2011) using a doubled-haploid (DH) winter wheat population in the UK. Harvest biomass and HI reductions with prolonged drought are frequently reported in the literature (Foulkes *et al.*, 2001, 2002; Kandic *et al.*, 2009; Aravinda Kumar *et al.*, 2011).

Savannah maintained grain yield under drought better compared to Rialto in both years. This was related to better maintenance of biomass in Savannah compared to Rialto. Among the DH lines, in 2013, reduction in grain yield ranged from -30% (line 99) to -15% (line 59) under drought mainly due to better maintenance of above-ground biomass rather than HI. This was supported by multiple regression analysis results showing that AGDM accounted for about 70% of the genetic variation, while HI accounted for only about 11% of the genetic variation in grain yield under drought conditions. The irrigation x genotype interaction in 2013 could be mainly explained by genotypes with higher yield under irrigated tending to lose more yield under drought since a positive association was found between yield loss and yield under irrigated condition. The relationship between yield potential and absolute yield loses under drought has been frequently reported (Fischer and Maurer, 1978; Foulkes *et al.*, 2007c).

Above-ground dry matter (AGDM) was reduced by drought by 18% on average in 2013, and apparently influenced by plant height in 2013 and for the cross-year mean. However, there was no irrigation x genotype interaction for the AGDM in both years. HI was also affected by drought in both 2013 (-5.4%) and 2014 (-1.5%). The irrigation x genotype interaction in HI was associated with the irrigation x genotype interaction in grain yield since HI response to drought was correlated with grain yield response to drought for the cross-year mean.

Among yield components, drought decreased TGW on average by 18% and grains per m^2 by 6% due to post-anthesis water stress in 2013. In addition, grains per m^2 was negatively associated with TGW under both irrigation treatments. The reduction in TGW indicates source limitation of grain growth under post-anthesis abiotic stress whereas yield is likely sink limited under favourable conditions (Bogard *et al.*, 2011). Averaging over years, a positive relationship was found between TGW and grain yield only under unirrigated conditions, and between grains per m^2 and yield

only under irrigated conditions which could be evidence for sink limitation of grain yield (Aravinda Kumar *et al.*, 2011). This is in agreement with the findings of Foulkes *et al.* (2002) and Aravinda Kumar *et al.* (2011) on drought responses in UK winter wheat. Therefore grain yield potential can be increased through improving the sink potential of grain number per unit area under favourable environments and source supply and grain size under post-anthesis water stress (Reynolds *et al.*, 2005; Acreche and Slafer, 2009).

4.4.3 Carbon isotope discrimination and yield relationship

In the present study, grain $\Delta^{13}C$ was 1.2% lower (i.e. higher TE) in 2013 compared with 2014 which may be associated with earlier onset of post-anthesis drought and a higher degree of water stress in 2013. Genetic variation was found in this study for grain $\Delta^{13}C$ between genotypes under drought conditions in both years. Grain $\Delta^{13}C$ values measured in 2013 were correlated with those measured in 2014 for the DH lines. Although, the association was statistically significant, it was relatively weak suggesting low heritability of grain $\Delta^{13}C$. Rebetzke *et al.* (2006) also reported relatively low heritability of grain $\Delta^{13}C$ expression.

In this study, a significant positive correlation between grain $\Delta^{13}C$ and grain yield was only found for the sub-set of eight genotypes in 2013, i.e. indicating a negative correlation between TE and grain yield. This could imply that the basis of the lower TE amongst genotypes was high stomatal conductance (Morgan *et al.*, 1993). The mechanisms underlying the generic variation in grain $\Delta^{13}C$ will be examined further in Chapter 5.

The genetic range in $\Delta^{13}C$ values in the present study was close to those reported by Aravinda Kumar *et al.* (2011) of 19.2 to 20.5‰ for a Beaver x Soissons winter wheat population in the UK. However, Monneveux *et al.* (2006) reported a lower range of values for grain $\Delta^{13}C$ (14.8 - 15.5 ‰) in durum wheat cultivars under rain-fed conditions in Montpellier, France. Previous investigations have found that grain $\Delta^{13}C$ variation in C_3 plants is related to the internal CO_2 concentration (Ci) in the intercellular spaces of the mesophyll and high $\Delta^{13}C$ values are associated with high Ci (related to high stomatal conductance and/or low photosynthetic activity) and hence low TE (Morgan *et al.*, 1993; Monneveux *et al.*, 2006; Rebetzke *et al.*, 2006).

Consistent with the present results on the subset of genotypes, positive relationships amongst genotypes between grain $\Delta^{13}C$ and grain yield have been found in UK rain-fed conditions by Aravinda Kumar *et al.* (2011). In rain-fed environments with mild water stress, there is typically a positive relationship between grain $\Delta^{13}C$ and Ci, and a negative relationship with water-use efficiency (TE) (Pask *et al.*, 2012). Thus, previous studies have examined the association between yield and $\Delta^{13}C$ in Mediterranean or similar environments, and most of the associations reported were either positive or neutral where there has been strong dependence on within-season rainfall (Condon *et al.*, 1987; Ehdaie *et al.*, 1991; Morgan *et al.*, 1993; Araus *et al.*, 1998; Merah *et al.*, 2001b; Tsialtas *et al.*, 2001; Monneveux *et al.*, 2005; Monneveux *et al.*, 2006; Xu *et al.*, 2007; Yasir *et al.*, 2013), while they have tended to be either negative or neutral in stored moisture and drier environments (current-rainfall) (Condon *et al.*, 2002; Rebetzke *et al.*, 2006).

Present results for the subset of genotypes therefore suggest that higher yield under mild UK water stress is related to water uptake rather than WUE, and that WUE decreased as a consequence of increasing water uptake. Thus, selection for high grain $\Delta^{13}C$ may be more effective for yield improvement than selection for lower grain $\Delta^{13}C$ and higher TE (Blum, 2005). The physiological basis of this apparent negative association between TE and grain yield is examined further at the leaf-scale by gas-exchange measurements for the subset of genotypes in Chapter 5.

4.5 Conclusion

The results in this Chapter allow the following conclusions to be made:

1. Genetic variation was found for grain yield and yield components in response to drought amongst Rialto x Savannah DH lines in 2013, and grain yield response to drought was associated with above-ground biomass and plant height more than HI under drought in 2013.

2. Grain yield was positively correlated with thousand grain weight under drought treatment, and with grains per m^2 under irrigated treatment amongst Rialto x Savannah DH lines for the cross-year mean.

3. In 2013, higher yield potential was associated with higher yield loss under drought.

4. Genetic variation for grain $\Delta^{13}C$ was found under drought amongst Rialto x Savannah DH lines, and higher grain $\Delta^{13}C$ (i.e. low WUE) was associated with higher grain yield under drought amongst the sub-set of Rialto x Savannah DH lines and the two parents.

The following chapter will investigate the effect of leaf photosynthetic and stay-green traits under drought associated with water-use efficiency and grain yield loss to drought amongst Rialto x Savannah DH lines.

Chapter 5 Physiological traits and relationships with biomass and grain yield in Rialto x Savannah DH population field experiments

5.1 Introduction

Techniques of leaf gas-exchange can provide an assessment of the transpiration efficiency (TE) at the leaf level. In C3 plants, $\Delta^{13}C$ variation is related to grain yield (GY) and TE as a result of the $\Delta^{13}C$ association with the internal CO_2 concentration (Ci) to ambient CO_2 concentration (Ca) ratio; and high Δ13C resulting from high Ci/Ca is associated with low TE (Morgan *et al.*, 1993; Monneveux *et al.*, 2006; Rebetzke *et al.*, 2006). In wheat, flag-leaf photosynthesis contributes about 30-50% of CO_2 assimilates for grain filling; therefore, leaf photosynthetic rate and the onset and rate of flag-leaf senescence are significant factors for determining yield potential (Verma *et al.*, 2004).

This chapter describes the relationships between physiological traits and grain yield and yield components at harvest under irrigated and unirrigated conditions for the 2012-13 and 2013-14 field experiments. Results are presented for a subset of eight genotypes for genetic variation in flag-leaf gas exchange (photosynthetic rate, stomatal conductance and transpiration efficiency) and for all genotypes for flag-leaf chlorophyll fluorescence, canopy photosynthesis (via Normalized Difference Vegetative Index (NDVI) and canopy temperature) and flag-leaf senescence (onset senescence, senescence rate and end of senescence) in each year and for the cross-year means. Genetic variation between DH lines is quantified, and mechanisms explaining trait relationships with grain yield and yield components are discussed. The overall aim of this chapter is to quantify genetic variation amongst the Rialto x Savannah DH lines and transgressive segregation compared to the parents for physiological traits and associations with grain yield under irrigated and unirrigated conditions and with responses to restricted water availability.

The specific hypotheses tested in this chapter are:

1. Carbon isotope discrimination ($\Delta^{13}C$) of the grain is negatively related to flag-leaf transpiration efficiency (WUE) and positively related to photosynthetic rate and stomatal conductance amongst Rialto x Savannah DH lines under drought conditions.

2. Physiological traits can be identified associated with genetic variation in the Rialto x Savannah DH population in grain yield under irrigated and drought conditions and responses to restricted water availability.
3. There is genetic variation amongst Rialto x Savannah DH lines for flag-leaf transpiration efficiency, photosynthetic rate and stomatal conductance under UK drought conditions and this is correlated with $\Delta^{13}C$, grain yield and yield components under drought.
4. There are positive associations between NDVI post-anthesis, flag-leaf visual score senescence traits and flag-leaf chlorophyll fluorescence and grain yield and above-ground DM amongst the Rialto x Savannah DH lines under drought, and between these traits and biomass and yield responses to water restricted availability.

5.2 Materials and Methods

A DH population of 94 lines was derived from the F_1 between winter wheat cultivars Rialto and Savannah. These DH lines with their parents were characterised in two field experiments in 2012-13 and 2013-14 at The University of Nottingham Farm, Leicestershire (52^o 50' N, 1^o 15' W, 50 m above sea level). The experimental design and plot management were described in Chapter 3.

In both years, leaf gas-exchange measurements of photosynthetic rate, stomatal conductance and transpiration efficiency were taken weekly on green flag leaves for the subset of six DH lines and the two parents under unirrigated conditions from GS61-7 days to GS61+35 days in 2013 (on 19, 27 June, 4 and 15 July) and 2014 (on 6, 17, 24 June and 7 July) using a LiCor 6400-XT Photosynthesis system (LiCor NE, USA). The gas exchange measurements were carried out as described in Chapter 3.

Normalized Difference Vegetative Index (NDVI) was measured using the Cropscan MSR-16 spectral radiometer in both years. Measurements (at approximately GS61+21 days, GS61+28 days and GS61+35 days) (on 9, 16 and 26 July in 2013, and on 26 June, 10 and 18 July in 2014) were taken in each sub-plot in the unirrigated treatment in each year in two replicates between 11.00 h and 15.00 h. The sensor was placed approximately 50 cm above the crop. Canopy temperature was measured in the unirrigated treatment approximately 35 days after anthesis in both

experiments (on 12 July in 2013, and on 17 July in 2014) using a handheld INF151 infrared thermometer (UEI Test Instruments, NJ, USA) in clear sunny conditions. A FP 100 Fluorometer (PSI, Brno, Czech Republic) was used to measure chlorophyll fluorescence (quantum yield) two times (at approximately GS61+21 days and GS61+28 days) in each experiment (on 10 and 17 July in 2013, and on 8 and 16 July in 2014). Readings were taken for three flag leaves per sub-plot avoiding outer rows in the unirrigated treatment in two replicates.

Visual assessments of flag-leaf senescence were carried out for each sub-plot under both irrigated and unirrigated treatments. Onset of leaf senescence (SEN_{ONSET}), senescence rate (SEN_{RATE}) and end of leaf senescence (SEN_{END}) were estimated as described in Chapter 3.

5.3 Results

5.3.1 Leaf gas-exchange measurements

Measurements were taken on calendar dates approximately coinciding with GS61-7d (i.e. one week before anthesis), GS61+7d, GS61+14d and GS61+21d. Post-anthesis mean values were calculated averaging over the 3 readings. Results for flag-leaf photosynthetic rate, stomatal conductance and transpiration efficiency are presented in this section.

5.3.1.1 Leaf photosynthetic rate (A_{max})

In 2013, pre-anthesis A_{max} differed amongst genotypes in the range 30.4 (line 1) to 33.7 µmol m^{-2} s^{-1} (line 63) (P=0.09). For post-anthesis A_{max}, genotypes differed in the range 15.1 (line 88) to 21.0 µmol m^{-2} s^{-1} (line 63) (P<0.05; Table 5.1). In 2014, there was a strong trend for differences in pre-anthesis A_{max} between genotypes in the range 31.2 (line 88) to 34.2 µmol m^{-2} s^{-1} (Savannah) (P=0.06). For post-anthesis A_{max}, genotypes differed in the range 17.9 (Rialto) to 23.8 µmol m^{-2} s^{-1} (Savannah) (P=0.07) (Table 5.1).

There was no effect of year on pre-anthesis A_{max} (P=0.87). Averaging over years, genotypes differed in the range 31.5 (line 88) to 33.8 µmol m^{-2} s^{-1} (Savannah) (P=0.02). The year x genotype interaction was significant with responses to year

(increases in 2013 compared to 2014) in the range -2.82 (line 1) to 1.86 µmol m^{-2} s^{-1} (line 63) (*P*=0.04). For post-anthesis A_{max}, there was no effect of year (*P*=0.39). Averaging over years, genotypes differed in the range 17.4 (line 88) to 22.2 µmol m^{-2} s^{-1} (Savannah) (*P*<0.01), and Savannah was higher than Rialto (17.5 µmol m^{-2} s^{-1}). There was no year x genotype interaction (*P*=0.26; Table 5.1).

In 2013, a positive linear relationship was found between pre-anthesis A_{max} and grain yield (R^2=0.55; *P*=0.04; Fig. 5.1a), but not in 2014 (*P*=0.88; Fig. 5.1b) or for the cross-year mean (*P*=0.48; Fig. 5.1c). Grain $\Delta^{13}C$ was positively linearly associated with pre-anthesis A_{max} in 2014 (R^2=0.68; *P*=0.01; Fig. 5.1e) and for the cross-year mean (R^2=0.46; *P*=0.07; Fig. 5.1f), but not in 2013 (*P*=0.17; Fig. 5.1d). No associations were found between grain yield and post-anthesis A_{max} in either year (2013 (*P*=0.55) and 2014 (*P*=0.90)) or for the cross-year mean (*P*=0.96) (Fig. 5.2a, 5.2b and 5.2c). Positive linear relationships were found between post-anthesis A_{max} and $\Delta^{13}C$ in both years (2013 (R^2=0.57; *P*=0.05) and 2014 (R^2=0.42; *P*=0.09)) and for the cross-year mean (R^2=0.55; *P*=0.04; Fig. 5.2d, 5.2e and 5.2f).

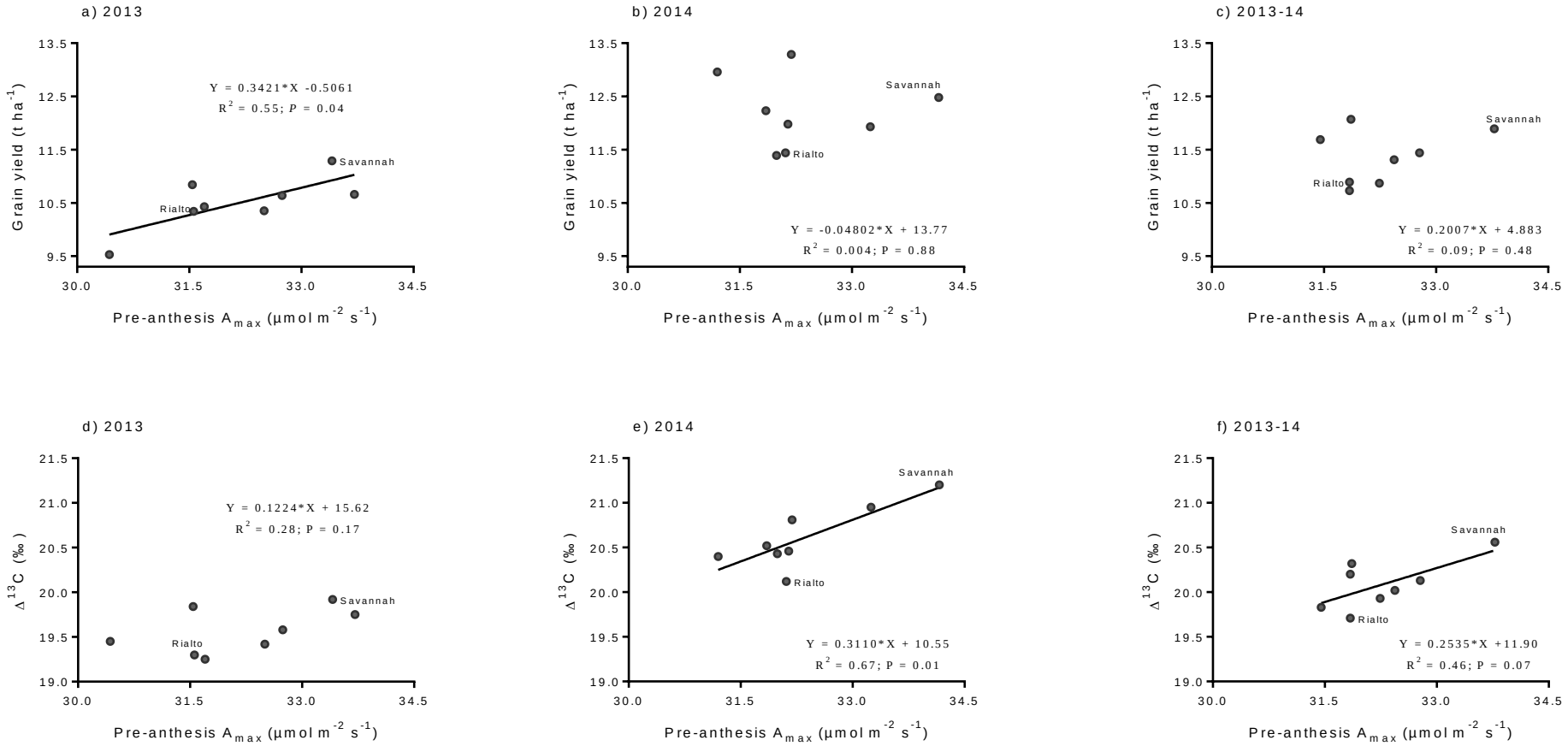


Figure 5.1 Linear regressions of pre-anthesis flag-leaf photosynthetic rate (A_{max}; µmol m^{-2} s^{-1}) on grain yield (85% DM; t ha^{-1}) in (a) 2013, (b) 2014 and (c) cross-year mean, and on grain $\Delta^{13}C$ (‰) in (d) 2013, (e) 2014 and (f) cross-year mean under unirrigated conditions for six Rialto x Savannah DH lines and the two parents.

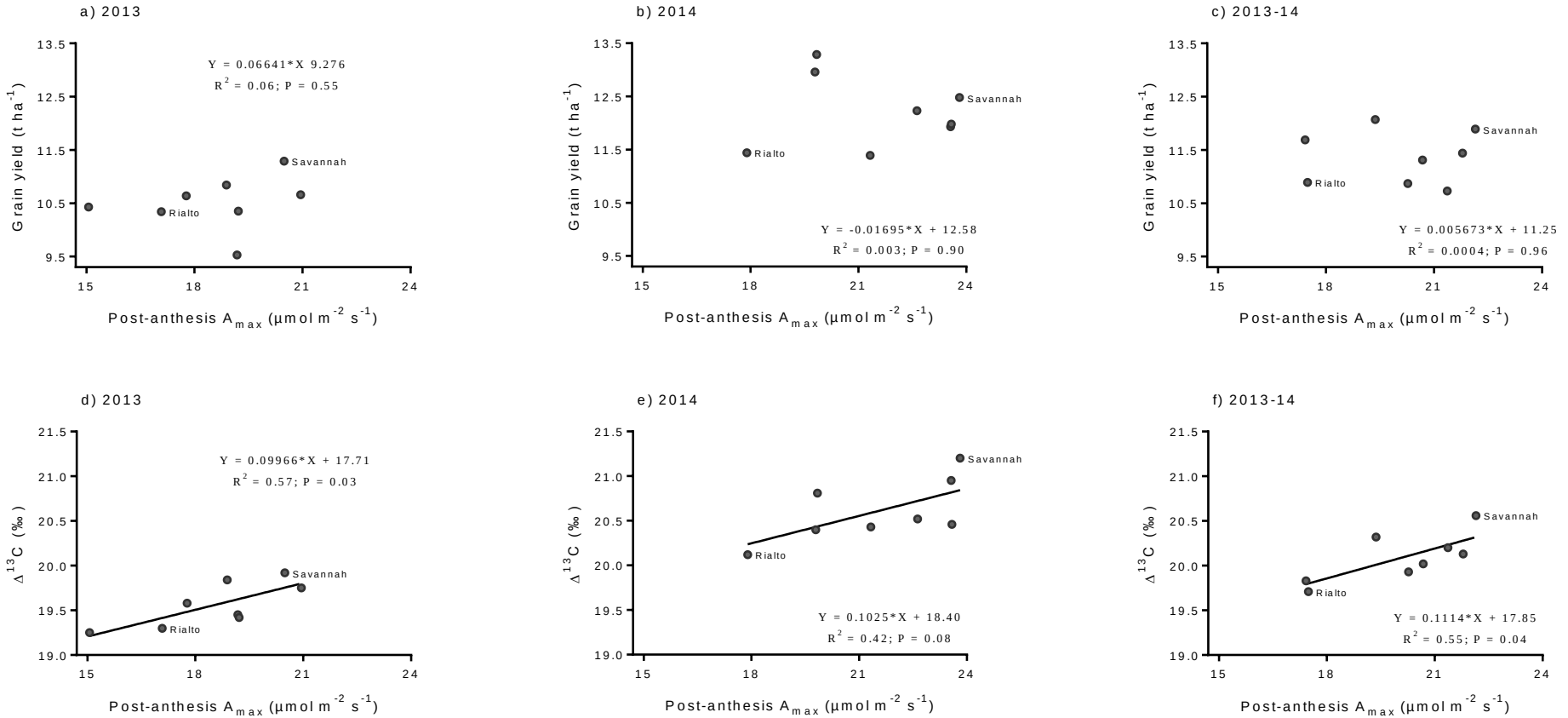


Figure 5.2 Linear regressions of post-anthesis flag-leaf photosynthetic rate (A_{max}; µmol m^{-2} s^{-1}) on grain yield (85% DM; t ha^{-1}) in (a) 2013, (b) 2014 and (c) cross-year mean, and on grain $\Delta^{13}C$ (‰) in (d) 2013, (e) 2014 and (f) cross-year mean under unirrigated conditions for six Rialto x Savannah DH lines and the two parents.

Table 5.1 Pre-anthesis and post-anthesis flag-leaf photosynthetic rate (A_{max}) in unirrigated treatment for six Rialto x Savannah DH lines and the two parents in 2013, 2014 and cross-year mean, and standard errors of the differences of the means (SED) and degrees of freedom (df).

Traits	Pre-anthesis A_{max} (µmol m^{-2} s^{-1})			Post-anthesis A_{max} (µmol m^{-2} s^{-1})		
Genotypes	2013	2014	2013-14	2013	2014	2013-14
Line 1	30.4	33.3	31.8	19.2	23.6	21.4
Line 20	31.5	32.2	31.9	18.9	19.8	19.4
Line 25	32.7	32.1	32.4	17.8	23.6	20.7
Line 63	33.7	31.9	32.8	21.0	22.6	21.8
Line 64	32.5	32.0	32.2	19.2	21.3	20.3
Line 88	31.7	31.2	31.5	15.1	19.8	17.4
Rialto	31.6	32.1	31.8	17.1	17.9	17.5
Savannah	33.4	34.2	33.8	20.5	23.8	22.2
Mean	32.2	32.4	32.3	18.6	21.6	20.1
SED (df)						
Year (2)	-	-	0.89^{ns}	-	-	2.74^{ns}
Gen (7)	$0.89^{(0.09)}$	$1.22^{(0.06)}$	0.56^{*}	1.34^{*}	$1.73^{(0.07)}$	1.09^{**}
Year x Gen (7)	-	-	1.16^{*}	-	-	3.09^{ns}

N.B: *** denotes P<0.001; **P<0.01 and *P<0.05 significance levels; ^{ns} = not significant.

5.3.1.2 Stomatal conductance (g_s)

In 2013, for pre-anthesis g_s, there was no effect of genotype (*P*=0.32). For post-anthesis g_s, genotypes differed in the range 0.17 (line 88) to 0.26 mol m^{-2} s^{-1} (Savannah) (*P*=0.006). In 2014, there was no statistically significant difference for either pre-anthesis or post-anthesis g_s between genotypes. From the cross-year ANOVA, pre-anthesis g_s was lower in 2013 than in 2014 by 45% (*P*=0.02). Genotype (*P*=0.21) and year x genotype (*P*=0.16) effects were not significant (Table 5.3). For post-anthesis g_s, genotypes differed in the range 0.27 (Rialto) to 0.37 mol m^{-2} s^{-1} (Savannah) (*P*=0.02). There was no effect of year (*P*=0.13) or year x genotype interaction (*P*=0.23; Table 5.3).

In 2013, in spite of the absence of statistically significant differences between the genotypes, there was a positive linear relationship between pre-anthesis g_s and grain yield (R^2=0.59; *P*=0.03 Fig. 5.3a), but not in 2014 (*P*=0.47; Fig. 5.3b) or for the cross-year mean (*P*=0.73; Fig. 5.3; Fig. 5.3c). No association was found between grain yield and post-anthesis g_s in 2013 (*P*=0.29) or 2014 (*P*=0.98) or for the cross-year mean (*P*=0.94; Fig. 5.4a, 5.4b and 5.4c). A positive linear relationship amongst

genotypes was found between post-anthesis g_s and grain $\Delta^{13}C$ in 2013 (R^2=0.63; *P*=0.02) and for the cross-year mean (R^2=0.47; *P*=0.06), but not in 2014 (*P*=0.14) (Fig. 5.4d, 5.4e and 5.4f).

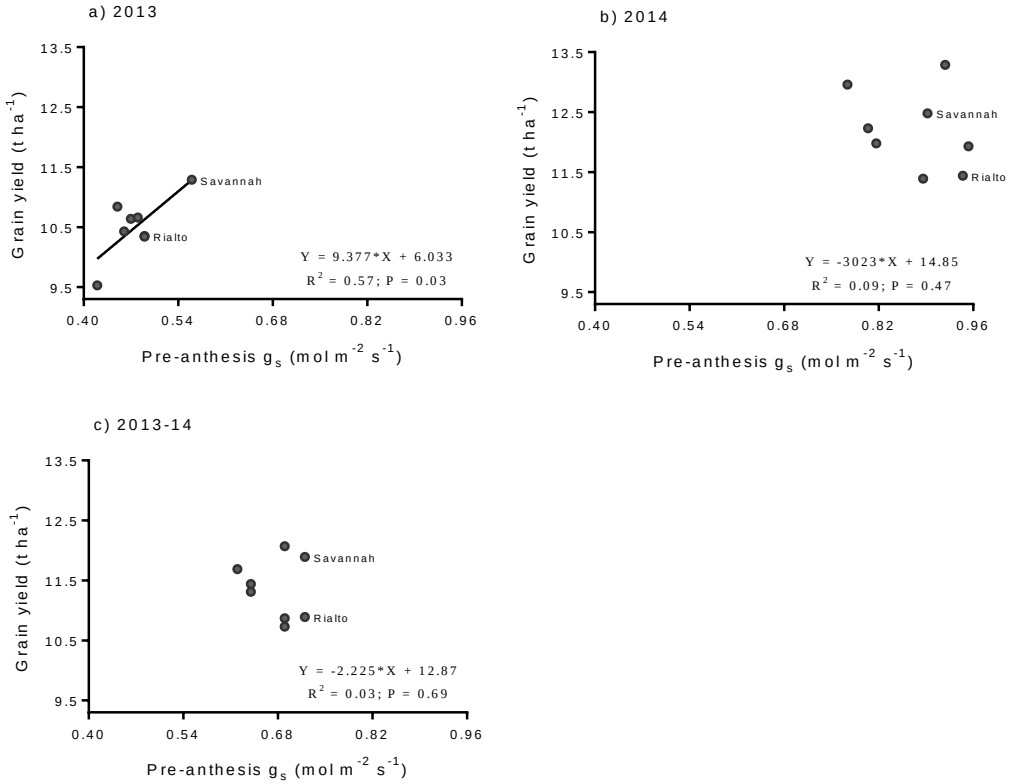


Figure 5.3 Linear regressions of pre-anthesis stomatal conductance (g_s; mol m^{-2} s^{-1}) on grain yield (85% DM; t ha^{-1}) in (a) 2013, (b) 2014 and (c) cross-year mean under unirrigated conditions for six Rialto x Savannah DH lines and the two parents.

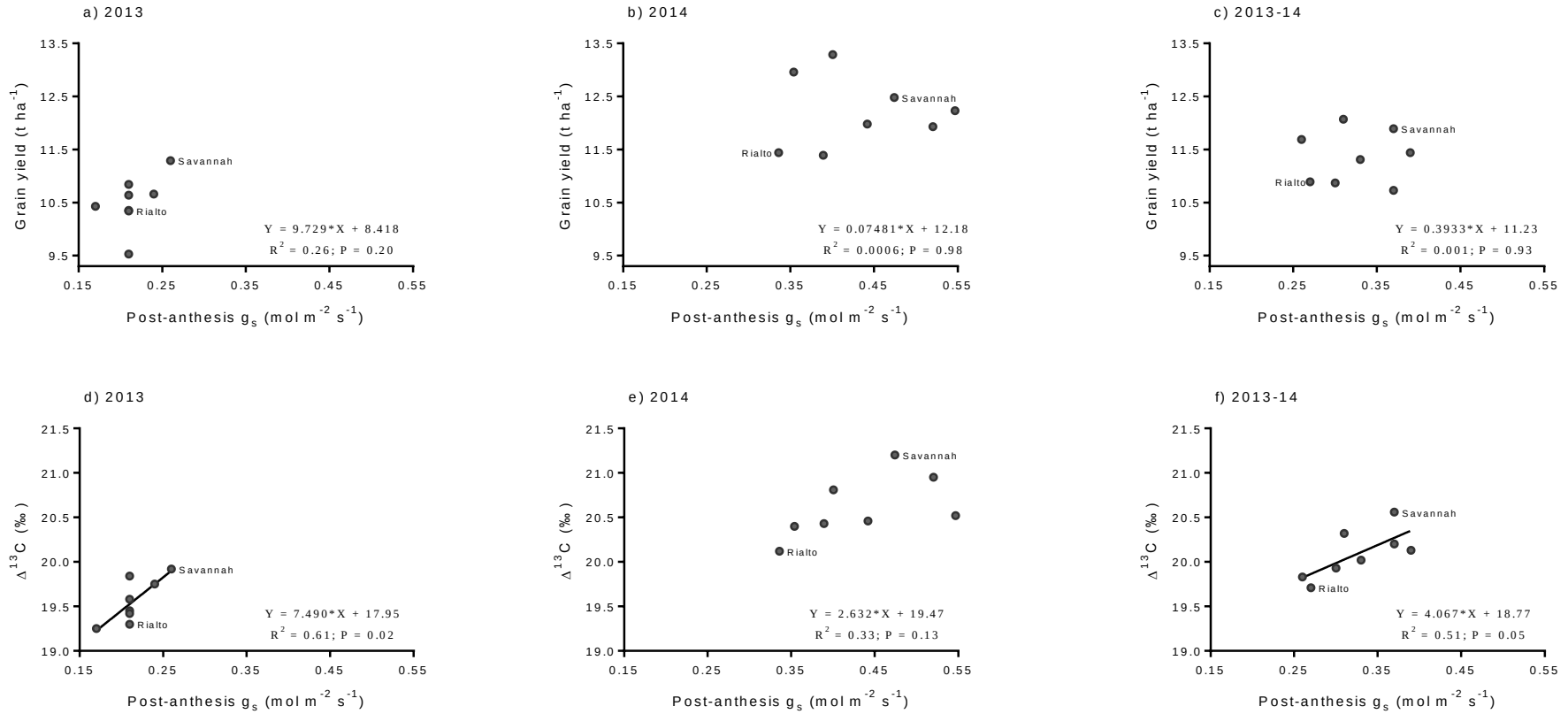


Figure 5.4 Linear regressions of post-anthesis flag-leaf stomatal conductance (g_s; mol m^{-2} s^{-1}) on grain yield (85% DM; t ha^{-1}) in (a) 2013, (b) 2014 and (c) cross-year mean, and on grain $\Delta^{13}C$ (‰) in (d) 2013, (e) 2014 and (f) cross-year mean under unirrigated conditions for six Rialto x Savannah DH lines and the two parents.

Table 5.2 Pre-anthesis and post-anthesis flag-leaf stomatal conductance (g_s) in unirrigated treatment for six Rialto x Savannah DH lines and the two parents in 2013, 2014 and cross-year mean, and standard errors of the differences of the means (SED) and degrees of freedom (df).

Traits	Pre-anthesis g_s (mol m^{-2} s^{-1})			Post-anthesis g_s (mol m^{-2} s^{-1})		
Genotypes	2013	2014	2013-14	2013	2014	2013-14
Line 1	0.42	0.95	0.69	0.21	0.52	0.37
Line 20	0.45	0.92	0.69	0.21	0.40	0.31
Line 25	0.47	0.82	0.64	0.21	0.44	0.33
Line 63	0.48	0.80	0.64	0.24	0.55	0.39
Line 64	0.49	0.89	0.69	0.21	0.39	0.30
Line 88	0.46	0.77	0.62	0.17	0.35	0.26
Rialto	0.49	0.94	0.72	0.21	0.34	0.27
Savannah	0.56	0.89	0.72	0.26	0.47	0.37
Mean	0.48	0.87	0.68	0.22	0.43	0.33
SED (df)						
Year (1)	-	-	0.060^{*}	-	-	0.086^{ns}
Gen (7)	0.046^{ns}	0.070^{ns}	0.042^{ns}	0.013^{**}	0.169^{ns}	0.036^{*}
Year x Gen (7)	-	-	0.082^{ns}	-	-	0.098^{ns}

N.B: *** denotes P<0.001; **P<0.01 and *P<0.05 significance levels; ^{ns} = not significant.

5.3.1.3 Transpiration efficiency (TE)

In 2013, there was no significant difference for either pre-anthesis or post-anthesis TE between genotypes. In 2014, for pre-anthesis TE, there was no effect of genotype (Table 5.3). For post-anthesis TE, genotypes differed in the range 4.65 (Savannah) to 5.55 µmol mol^{-1} (Rialto) (*P*=0.03). Averaging over years, for pre-anthesis TE there was no effect of genotype (*P*=0.69) or year x genotype interaction (*P*=0.80). However, for post-anthesis TE, genotypes differed in the range 4.64 (Savannah) to 5.27 µmol mol^{-1} (line 64) (*P*=0.03). The year x genotype interaction was not significant (*P*=0.07) (Table 5.3).

Post-anthesis TE was negatively linearly associated with grain yield in both years (2013 R^2=0.45; *P*=0.07; and 2014 R^2=0.39; *P*=0.01) and for the cross-year mean (R^2=0.69; *P*=0.01; Fig. 5.5a, 5.5b and 5.5c). In 2013, the relationship between TE and $\Delta^{13}C$ was not significant (*P*=0.32; Fig. 5.5d). However, a negative linear relationship was found in 2014 (R^2=0.73; *P*=0.007) and for the cross-year mean (R^2=0.61; *P*=0.02) (Fig. 5.5e and 5.5f).

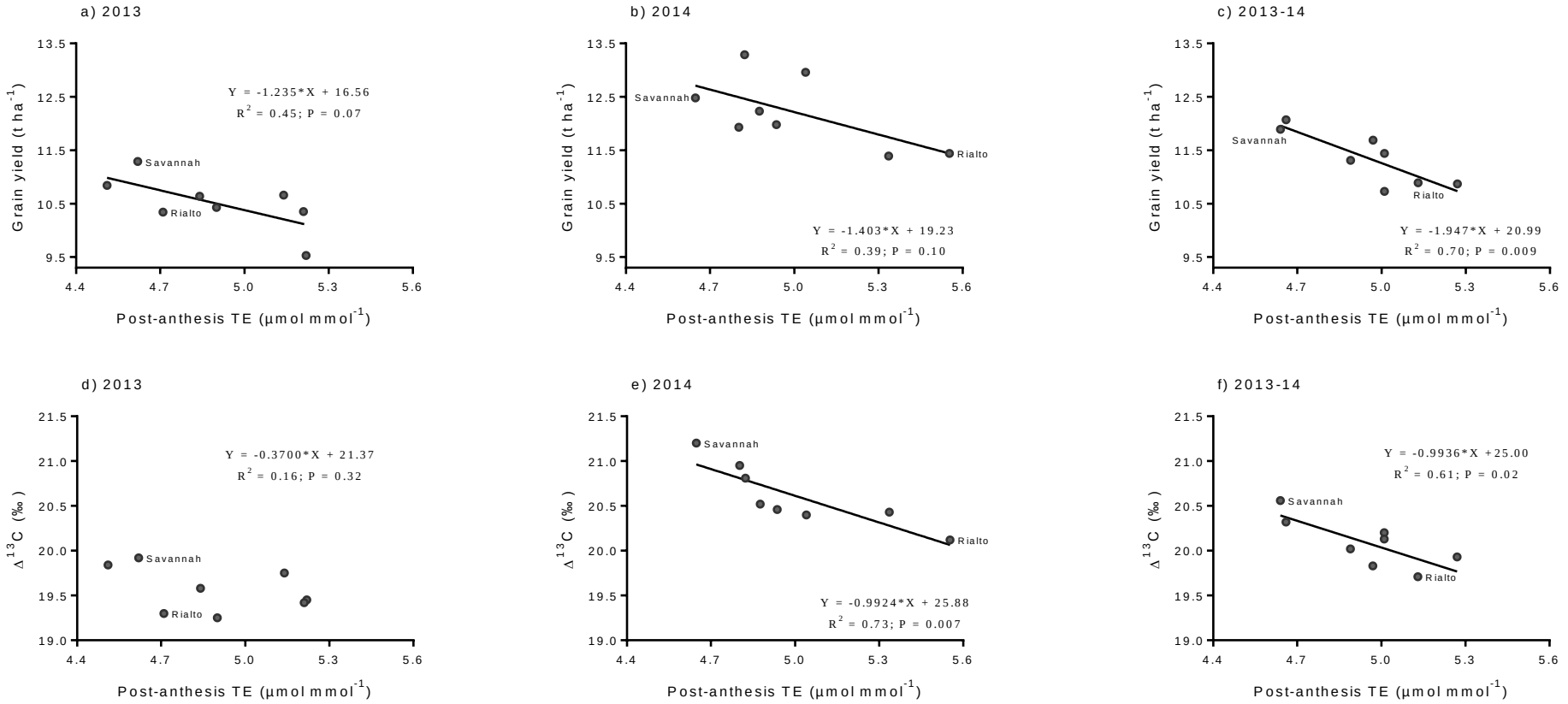


Figure 5.5 Linear regressions of post-anthesis flag-leaf transpiration efficiency (TE; µmol mol^{-1}) on grain yield (85% DM; t ha^{-1}) in (a) 2013, (b) 2014 and (c) cross-year mean, and on grain $\Delta^{13}C$ (‰) in (d) 2013, (e) 2014 and (f) cross-year mean under unirrigated conditions for six Rialto x Savannah DH lines and the two parents.

Table 5.3 Pre-anthesis and post-anthesis flag-leaf transpiration efficiency (TE) in unirrigated treatment for six Rialto x Savannah DH lines and the two parents in 2013, 2014 and cross-year mean, and standard errors of the differences of the means (SED) and degrees of freedom (df).

Traits	Pre-anthesis TE (µmol mmol^{-1})			Post-anthesis TE (µmol mmol^{-1})		
Genotypes	2013	2014	2013-14	2013	2014	2013-14
Line 1	4.81	5.53	5.17	5.22	4.80	5.01
Line 20	5.00	5.52	5.26	4.51	4.82	4.66
Line 25	5.02	5.33	5.17	4.84	4.94	4.89
Line 63	5.31	5.40	5.36	5.14	4.88	5.01
Line 64	4.73	5.34	5.04	5.21	5.34	5.27
Line 88	5.11	5.37	5.24	4.90	5.04	4.97
Rialto	4.88	5.18	5.03	4.71	5.55	5.13
Savannah	5.19	5.44	5.32	4.62	4.65	4.64
Mean	5.01	5.39	5.20	4.89	5.00	4.95
SED (df)						
Year (1)	-	-	*0.126^{ns}*	-	-	*0.266^{ns}*
Gen (7)	*0.312^{ns}*	*0.268^{ns}*	*0.205^{ns}*	*0.278^{ns}*	*0.199**	*0.171**
Year x Gen (7)	-	-	*0.299^{ns}*	-	-	*0.349^{ns}*

N.B: ***denotes P<0.001; **P<0.01 and *P<0.05 significance levels; ^{ns} = not significant.

Table 5.4 The phenotypic correlation (r) of pre-anthesis and post-anthesis photosynthetic rate (A_{max}; µmol m^{-2} s^{-1}), stomatal conductance (g_s; mol m^{-2} s^{-1}) and transpiration efficiency (TE; µmol mmol^{-1}) with grain yield (GY; t ha^{-1}), harvest index (HI), above-ground dry matter (AGDM; g m^{-2}), thousand grain weight (TGW; g), grains m^{-2}, grain Δ^{13}C (‰) and canopy temperature (CT; ^{o}C) among six Rialto x Savannah DH lines and the two parents in 2013, 2014 and cross-year mean.

	Traits	GY	HI	AGDM	TGW	Grains m^{-2}	Δ^{13}C	CT
2013								
Pre-anthesis	A_{max}	0.74*	0.53^{ns}	0.24^{ns}	0.41^{ns}	0.57^{ns}	0.53^{ns}	-0.56^{ns}
	g_s	0.77*	0.41^{ns}	0.40^{ns}	0.24^{ns}	0.75*	0.43^{ns}	-0.46^{ns}
	TE	0.63$^{0.09}$	0.74*	-0.15^{ns}	0.73*	0.03^{ns}	0.53^{ns}	-0.45^{ns}
Post-anthesis	A_{max}	0.25^{ns}	0.54^{ns}	-0.36^{ns}	0.16^{ns}	-0.15^{ns}	0.76*	0.29^{ns}
	g_s	0.50^{ns}	0.70*	-0.28^{ns}	0.37^{ns}	0.08^{ns}	0.79*	0.01^{ns}
	TE	-0.67^{ns}	-0.51^{ns}	-0.15^{ns}	-0.09^{ns}	-0.24^{ns}	-0.40^{ns}	0.07^{ns}
2014								
Pre-anthesis	A_{max}	-0.06^{ns}	0.35^{ns}	-0.22^{ns}	0.23^{ns}	-0.23^{ns}	0.82*	-0.21^{ns}
	g_s	-0.30^{ns}	-0.10^{ns}	-0.30^{ns}	-0.32^{ns}	0.02^{ns}	0.28^{ns}	0.31^{ns}
	TE	0.60^{ns}	0.48^{ns}	0.47^{ns}	0.08^{ns}	0.33^{ns}	0.83*	-0.30^{ns}
Post-anthesis	A_{max}	-0.05^{ns}	0.29^{ns}	-0.19^{ns}	0.48^{ns}	-0.38^{ns}	0.65$^{0.08}$	-0.62$^{0.10}$
	g_s	0.01^{ns}	0.64$^{0.08}$	-0.27^{ns}	0.69$^{0.06}$	-0.45^{ns}	0.58^{ns}	-0.17^{ns}
	TE	-0.83*	-0.62$^{0.10}$	-0.43^{ns}	-0.06^{ns}	-0.28^{ns}	-0.78*	-0.03^{ns}
2013-14								
Pre-anthesis	A_{max}	0.29^{ns}	0.58^{ns}	-0.17^{ns}	0.59^{ns}	-0.05^{ns}	0.68$^{0.07}$	-0.45^{ns}
	g_s	-0.15^{ns}	0.16^{ns}	-0.33^{ns}	-0.09^{ns}	-0.03^{ns}	0.35^{ns}	0.32^{ns}
	TE	0.74*	0.76*	0.20^{ns}	0.58^{ns}	-0.14^{ns}	0.66$^{0.07}$	-0.07^{ns}
Post-anthesis	A_{max}	0.02^{ns}	0.39^{ns}	-0.34^{ns}	0.46^{ns}	-0.48^{ns}	0.74*	-0.23^{ns}
	g_s	0.03^{ns}	0.58^{ns}	-0.50^{ns}	0.66$^{0.08}$	-0.68$^{0.06}$	0.69$^{0.06}$	-0.03^{ns}
	TE	-0.83*	-0.62$^{0.10}$	-0.43^{ns}	-0.06^{ns}	-0.28^{ns}	-0.78*	-0.03^{ns}

N.B: ***denotes P<0.001; **P<0.01 and *P<0.05 significance levels; ^{ns} = not significant.

5.3.2 Senescence traits

5.3.2.1 Post-anthesis Normalized Difference Vegetative Index (NDVI)

5.3.2.1.1 NDVI at GS61+21 days

In 2013, DH lines differed in the range 0.78 (line 95) to 0.90 (line 24) (P<.001), and in 2014 from 0.85 (line 56) to 0.90 (line 68) (P=0.02; Table 5.5). From the cross-year ANOVA, there was no effect of year (P=0.34). Genotypes differed in the range 0.82 (line 99) to 0.90 (line 24) (P<.001), and the year x genotype interaction was significant (P<.001; Table 5.5).

In 2013, NDVI under drought showed a positive linear relationship with grain yield amongst DH lines (R^2=0.25; *P*<.001; Fig. 5.6a). Averaging over years, there was also a positive relationship between NDVI and grain yield (R^2=0.04; *P*=0.03; Fig. 5.6c). A positive linear relationship between NDVI and AGDM at harvest was found amongst DH lines in 2013 (R^2=0.22; *P*<.001; Fig. 5.6d), and for the cross-year mean (R^2=0.05; *P*=0.03; Fig. 5.6f). Thousand grain weight was associated with NDVI in 2013 (R^2=0.08; *P*<.001; Fig. 5.6g) and for the cross-year mean (R^2=0.07; *P*=0.006; Fig. 5.6i).

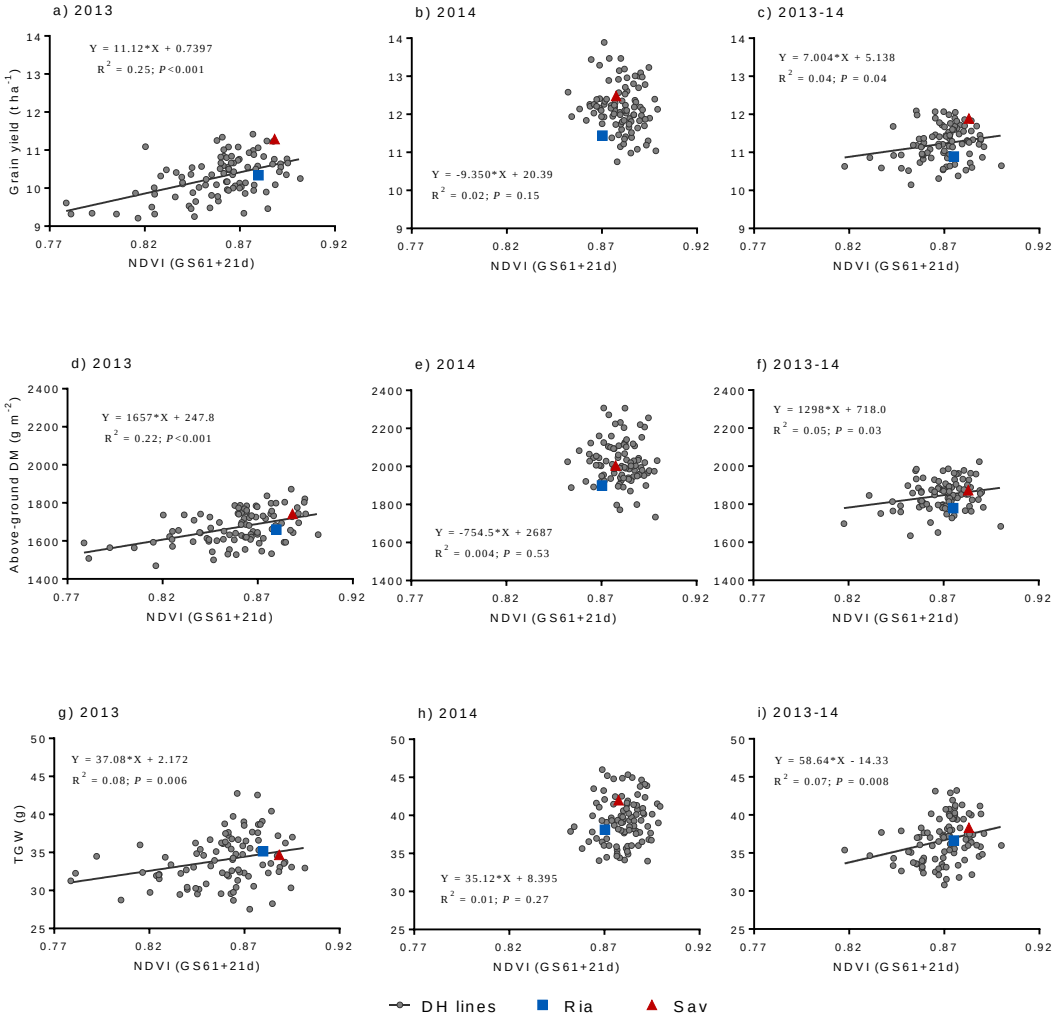


Figure 5.6 Linear regressions of NDVI (GS61+21d) on grain yield (85% DM; t ha^{-1}) in (a) 2013, (b) 2014 and (c) cross-year mean, on above-ground dry matter (AGDM; g m^{-2}) in (d) 2013, (e) 2014 and (f) cross-year mean, and on thousand grain weight (TGW; 100% DM; g) in (g) 2013, (h) 2014 and (i) cross-year mean under unirrigated conditions for 94 Rialto x Savannah DH lines. Rialto (■) and Savannah (▲) are also shown.

5.3.2.1.2 NDVI at GS61+28 days

Overall similar effects were observed at GS61+28 days as at GS61+21 days. In 2013, DH lines differed in the range 0.62 (line 99) to 0.79 (line 45) (P<.001), and in 2014 in the range 0.77 (line 99) to 0.88 (line 24) (P=0.04). Averaging over genotypes, NDVI in 2013 (0.72) was lower than 2014 (0.83) (P=0.03). Averaging over years, DH lines differed in the range 0.69 (line 99) to 0.82 (line 25) (P<.001); there was a year x genotype interaction (P<.001). There was no transgressive segregation amongst genotypes in both years or for the cross-year mean (Table 5.5).

A positive linear relationship with grain yield was found for NDVI at GS61+28 days in 2013 amongst DH lines (R^2=0.29; *P*<.001; Fig. 5.7a), and for the cross-year mean (R^2=0.03; *P*=0.05; Fig. 5.7c). A positive linear relationship between NDVI and AGDM was also found amongst DH lines in 2013 (R^2=0.24; *P*<.001; Fig. 5.7d), and for the cross-year mean (R^2=0.07; *P*=0.009; Fig. 5.7f). Thousand grain weight was similarly positively associated with NDVI in 2013 (R^2=0.07; *P*=0.009; Fig. 5.7g), and for the cross-year mean (R^2=0.04; *P*=0.05; Fig. 5.7i).

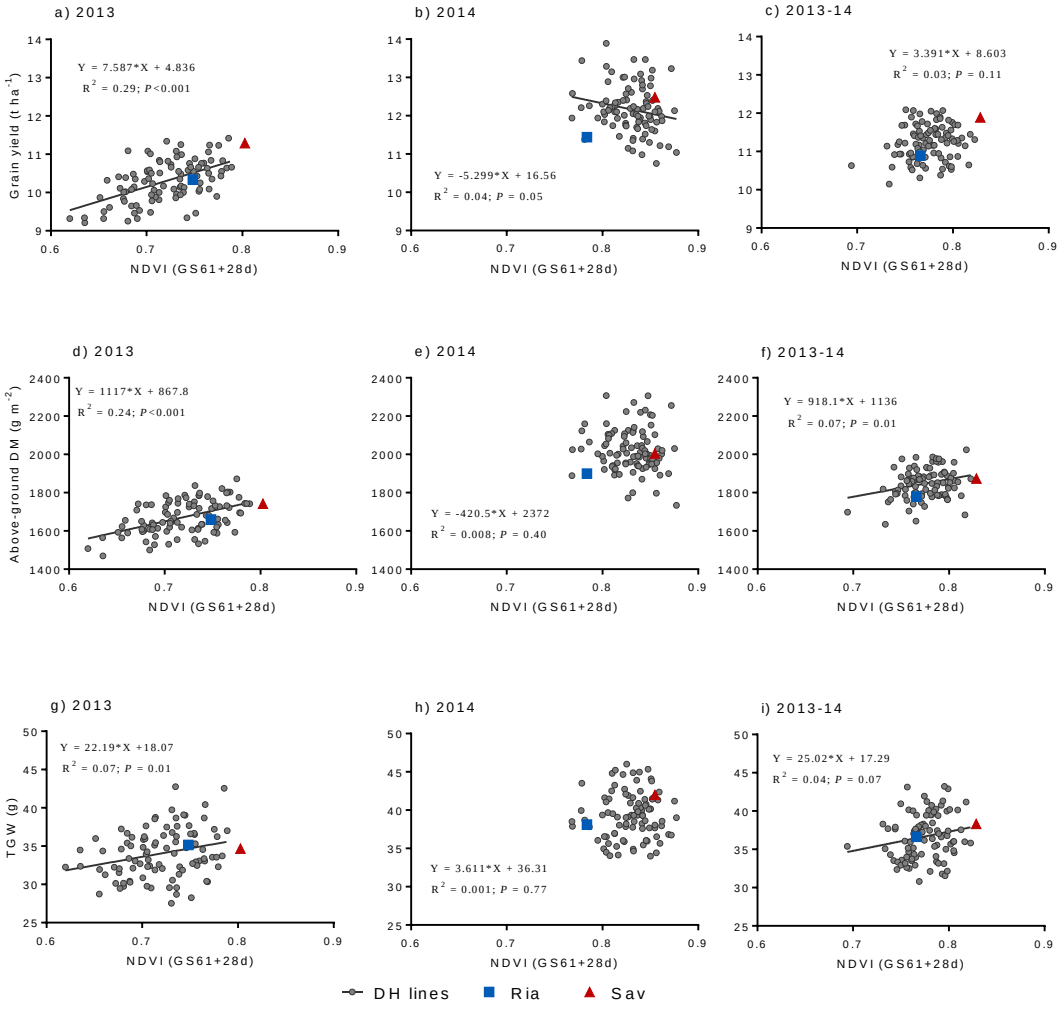


Figure 5.7 Linear regressions of NDVI (GS61+28d) on grain yield (85% DM; t ha^{-1}) in (a) 2013, (b) 2014 and (c) cross-year mean, on above-ground dry matter (AGDM; g m^{-2}) in (d) 2013, (e) 2014 and (f) cross-year mean, and on thousand grain weight (TGW; 100% DM; g) in (g) 2013, (h) 2014 and (i) cross-year mean under unirrigated conditions for 94 Rialto x Savannah DH lines. Rialto (■) and Savannah (▲) are also shown.

5.3.2.1.3 NDVI at GS61+35 days

Similar effects at GS61+35 days and associations with grain yield, biomass and grain weight were also observed to the previous assessments. Averaging over genotypes, NDVI in 2013 (0.36) was lower than 2014 (0.65) (P=0.002). Averaging over years, DH lines differed in the range 0.38 (line 99) to 0.61 (line 24) (P<.001), and the year x genotype interaction was significant (P=0.008; Table 5.5).

A positive linear relationship with grain yield was found for NDVI at GS61+35 days in 2013 amongst DH lines (R^2=0.08; *P*=0.002; Fig. 5.8a). However, a negative association was found in 2014 (R^2=0.07; *P*=0.03; Fig. 5.8b) and a trend for a negative association for the cross-year mean (*P*=0.06; Fig. 5.8c). Above-ground dry matter was also positively associated with NDVI in 2013 (R^2=0.18; *P*<.001; Fig. 5.8d), but not in 2014 (*P*=0.83; Fig. 5.8e) or for the cross-year mean (*P*=0.61; Fig. 5.8f). Thousand grain weight was not correlated with NDVI in either year (2013 *P*=0.75; and 2014 *P*=0.82) or for the cross-year mean (*P*=0.73; Fig. 5.8g, 5.8h and 5.8i). Overall, associations with grain yield, AGDM and TGW were strongest for NDVI at GS61+28 days followed by GS61+21 days and GS61+35 days.

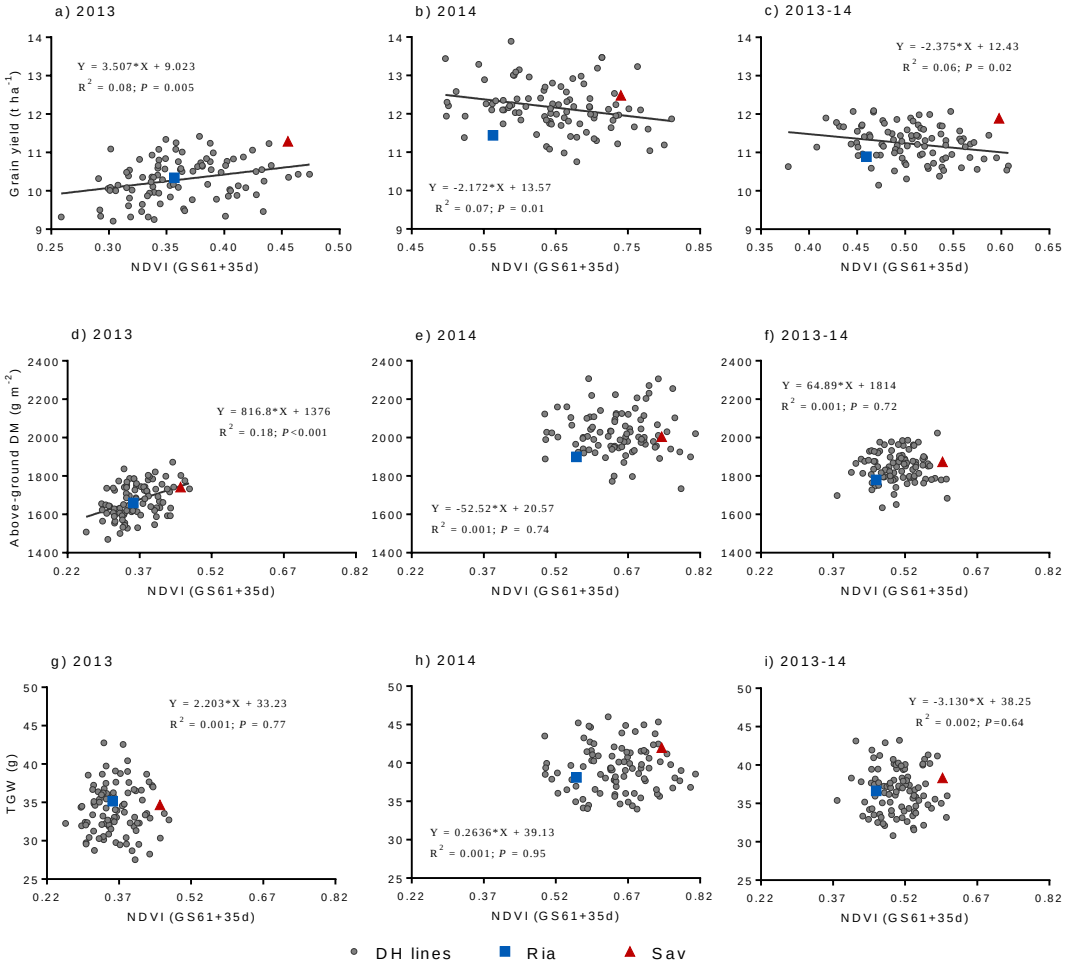


Figure 5.8 Linear regressions of NDVI (GS61+35d) on grain yield (85% DM; t ha^{-1}) in (a) 2013, (b) 2014 and (c) cross-year mean, on above-ground dry matter (AGDM; g m^{-2}) in (d) 2013, (e) 2014 and (f) cross-year mean, and on thousand grain weight (TGW; 100% DM; g) in (g) 2013, (h) 2014 and (i) cross-year mean under unirrigated conditions for 94 Rialto x Savannah DH lines. Rialto (■) and Savannah (▲) are also shown.

Table 5.5 Normalized Difference Vegetative Index (NDVI) in unirrigated treatment for 94 Rialto x Savannah DH lines and the two parents in 2013, 2014 and cross-year mean, and maximum, minimum, standard errors of the differences of the means (SED) and degrees of freedom (df).

Traits	NDVI (GS61+21d)	NDVI (GS61+28d)	NDVI (GS61+35d)
2013			
Mean	0.86	0.72	0.36
Max.	0.90	0.79	0.47
Min.	0.78	0.62	0.26
Rialto	0.88	0.75	0.36
Savannah	0.89	0.80	0.46
SED (df)			
Genotype (95)	*0.021****	*0.039****	*0.040****
2014			
Mean	0.88	0.83	0.65
Max.	0.90	0.88	0.81
Min.	0.85	0.77	0.50
Rialto	0.87	0.78	0.56
Savannah	0.88	0.85	0.74
SED (df)			
Genotype (95)	*0.011**	*0.028**	*0.080***
2013-14			
Mean	0.87	0.78	0.51
Max.	0.90	0.82	0.61
Min.	0.82	0.69	0.38
Rialto	0.88	0.77	0.46
Savannah	0.88	0.83	0.60
SED (df)			
Year (2)	*0.016*^{ns}	*0.018**	*0.014***
Genotype (95)	*0.012****	*0.024****	*0.045****
Year x Gen. (95)	*0.023****	*0.038****	*0.065***

N.B: ***denotes P<0.001; **P<0.01 and *P<0.05 significance levels; ^{ns} = not significant.

Table 5.6 The phenotypic correlation (r) of NDVI (GS61+21d), NDVI (GS61+28d) and NDVI (GS61+35d) with grain yield (GY; 85% DM; t ha^{-1}), above-ground dry matter (AGDM; g m^{-2}), thousand grain weight (TGW; g), harvest index (HI), grains m^{-2} and plant height (PH; cm) among 94 Rialto x Savannah DH lines in 2013, 2014 and cross-year mean.

Traits	GY	AGDM	TGW	HI	Grains m^{-2}	PH
2013						
NDVI (GS61+21d)	0.51***	0.47***	0.28**	0.12^{ns}	0.21*	0.33**
NDVI (GS61+28d)	0.55***	0.50***	0.26**	0.14^{ns}	0.28**	0.25*
NDVI (GS61+35d)	0.31**	0.43***	0.03^{ns}	-0.17^{ns}	0.26*	0.28**
2014						
NDVI (GS61+21d)	-0.14^{ns}	-0.05^{ns}	0.12^{ns}	-0.11^{ns}	-0.09^{ns}	0.08^{ns}
NDVI (GS61+28d)	-0.16^{ns}	-0.07^{ns}	0.05^{ns}	-0.12^{ns}	-0.10^{ns}	0.26**
NDVI (GS61+35d)	-0.23*	-0.02^{ns}	0.02^{ns}	-0.25*	-0.13^{ns}	0.16^{ns}
2013-14						
NDVI (GS61+21d)	0.22*	0.23*	0.28**	-0.03^{ns}	-0.04^{ns}	0.26*
NDVI (GS61+28d)	0.20*	0.27**	0.20*	-0.11^{ns}	0.03^{ns}	0.26**
NDVI (GS61+35d)	-0.19^{ns}	0.05^{ns}	-0.04^{ns}	-0.35^{ns}	-0.04^{ns}	0.18$^{(.07)}$

N.B: ***denotes P<0.001; **P<0.01 and *P<0.05 significance levels; ^{ns} = not significant.

5.3.2.2 Canopy temperature (CT)

Canopy temperature (CT) was measured in the unirrigated treatment approximately 35 days after anthesis in both 2013 and 2014. In 2013, DH lines differed in the range 21.8 (line 12) to 25.4 ^{o}C (line 92) (P=0.02); for the parents, there was no difference between Savannah (23.3 ^{o}C) and Rialto (23.2 ^{o}C) (Table 5.7). There was no transgressive segregation for lower CT. However, 6 DH lines showed higher CT than the highest parent in the range 24.8 to 25.4 ^{o}C.

In 2014, DH lines differed in the range 21.0 (line 58) to 24.0 ^{o}C (line 9) (P<0.05); for the parents, Savannah (21.1 ^{o}C) had a cooler canopy than Rialto (23.0 ^{o}C) (Table 5.7). There was no transgressive segregation amongst genotypes.

Averaging over years, DH lines differed in the range 22.1 (line 35) and 24.2 ^{o}C (line 35) (P<0.05). There was a weak trend for an effect of year (P=0.10), and there was a year x genotype interaction (P=0.004; Table 5.7). There was transgressive segregation amongst genotypes for line 60 (23.1 ^{o}C) showing a higher CT than the highest parent.

A negative linear relationship between grain yield and CT was found in 2013 amongst DH lines (R^2=0.16; P<.001; Fig. 5.9a), but not in 2014 (P=0.10; Fig. 5.9b) or for the cross-year mean (P=0.62; Fig. 5.9c). Thousand grain weight was also negatively associated with CT in 2013 (R^2=0.06; P=0.001; Fig. 5.9d), but not in 2014 (P=0.34; Fig. 5.9e). Cross-year analysis showed a weak negative linear relationship between TGW and CT (R^2=0.04; P=0.02; Fig. 5.9f).

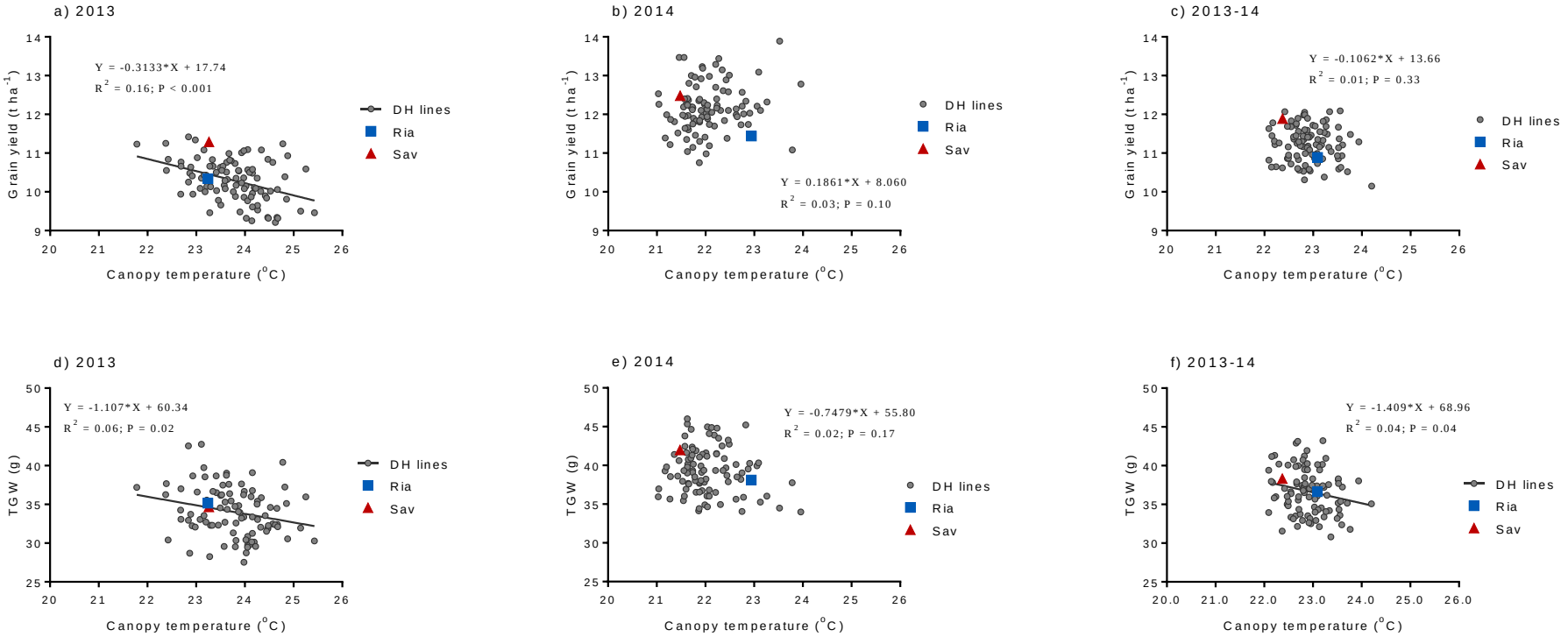


Figure 5.9 Linear regressions of canopy temperature (CT; ^{o}C) on grain yield (GY; 85% DM; t ha^{-1}) in (a) 2013, (b) 2014 and (c) cross-year mean, and on thousand grain weight (TGW; g) in (d) 2013, (e) 2014 and (f) cross-year mean under unirrigated conditions for 94 Rialto x Savannah DH lines. Rialto (■) and Savannah (▲) are also shown.

5.3.2.3 Flag-leaf chlorophyll fluorescence (QY)

Flag-leaf chlorophyll fluorescence (quantum yield; QY) was measured on calendar dates approximately coinciding with GS61+21 days and GS61+28 days in the unirrigated treatment in each season. Averaging over readings, in 2013 DH lines differed in post-anthesis QY in the range 0.71 (line 44) to 0.76 (line 37) (*P*=0.04). For the parents, there was no difference between Savannah (0.76) and Rialto (0.74) (Table 5.7), and no transgressive segregation amongst genotypes was found.

In 2014, DH lines differed in post-anthesis QY in the range 0.66 (line 23) to 0.75 (line 51) (*P*=0.03). For the parents, there was no difference between Savannah (0.74) and Rialto (0.71) (Table 5.7), and there was no transgressive segregation amongst genotypes.

Averaging over years, there were no effects of year (*P*=0.12) or year x genotype interaction (*P*=0.12). DH lines differed in the range 0.69 (line 44) to 0.75 (line 25) (*P*=0.001). Savannah (0.75) had higher QY than Rialto (0.72) (Table 5.7) and there was no transgressive segregation amongst genotypes.

In 2013, QY showed a weak positive linear relationship with grain yield amongst DH lines (R^2=0.04; *P*=0.06; Fig. 5.10a). In 2014, the relationship was not significant (*P*=0.49; Fig. 5.10b). Cross-year analysis also showed a weak trend for a positive association between QY and grain yield (R^2=0.02; *P*=0.08; Fig. 5.10c). Above-ground dry matter was also weakly associated amongst DH lines with QY in 2013 (R^2=0.05; *P*=0.02), 2014 (R^2=0.05; *P*=0.03) and for the cross-year mean (R^2=0.06; *P*=0.01; Fig. 5.10d, 5.10e and 5.10f).

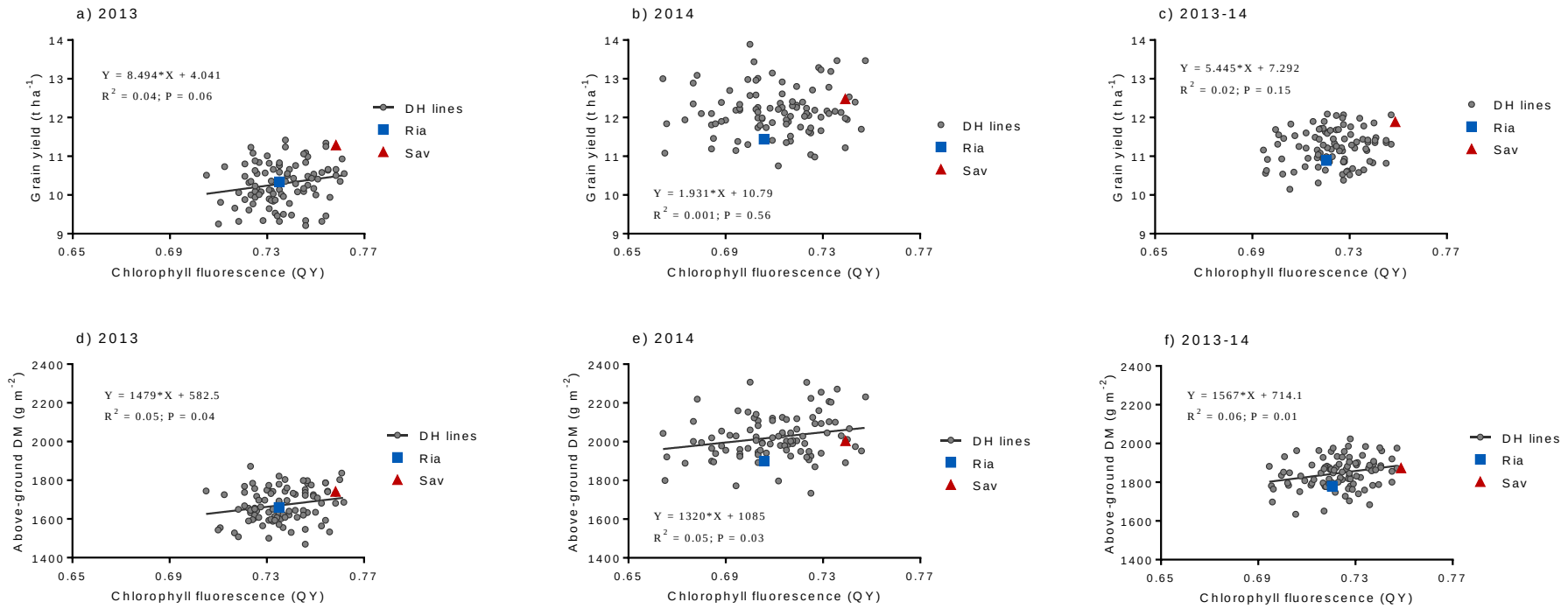


Figure 5.10 Linear regressions of flag-leaf chlorophyll fluorescence (QY) on grain yield (GY; 85% DM; t ha^{-1}) in (a) 2013, (b) 2014 and (c) cross-year mean, and on above-ground dry matter (AGDM; g m^{-2}) in (d) 2013, (e) 2014 and (f) cross-year mean under unirrigated conditions for 94 Rialto x Savannah DH lines. Rialto (■) and Savannah (▲) are also shown.

Table 5.7 Canopy temperature (CT; GS61+35d) and chlorophyll fluorescence (QY; GS61+35d) in unirrigated treatments for 94 Rialto x Savannah DH lines and the two parents in 2013, 2014 and cross-year mean, and maximum, minimum, standard errors of the differences of the means (SED) and degrees of freedom (df).

Traits	CT (^{o}C)			QY		
	2013	**2014**	**2013-14**	**2013**	**2014**	**2013-14**
Mean	23.8	22.1	22.9	0.74	0.71	0.72
Max.	25.4	24.0	24.2	0.76	0.75	0.75
Min.	21.8	21.0	22.1	0.71	0.66	0.69
Rialto	23.2	22.9	23.1	0.74	0.71	0.72
Savannah	23.3	21.5	22.4	0.76	0.74	0.75
SED (df)						
Year (2)			*0.59*^{ns}			*0.010*^{ns}
Genotype (95)	*0.78**	*0.68**	*0.52**	*0.016**	*0.023**	*0.014****
Year x Gen. (95)			*0.93***			*0.022*^{ns}

N.B: ***denotes P<0.001; **P<0.01 and *P<0.05 significance levels; ^{ns} = not significant.

5.3.3 Flag-leaf senescence rate and duration

Flag-leaf senescence was measured under both irrigated and unirrigated conditions. Averaging over genotypes in each year, onset of flag-leaf senescence occurred earlier and flag-leaf senescence rate was higher, and effects of drought were greater in 2013 compared to 2014 (Fig. 5.11).

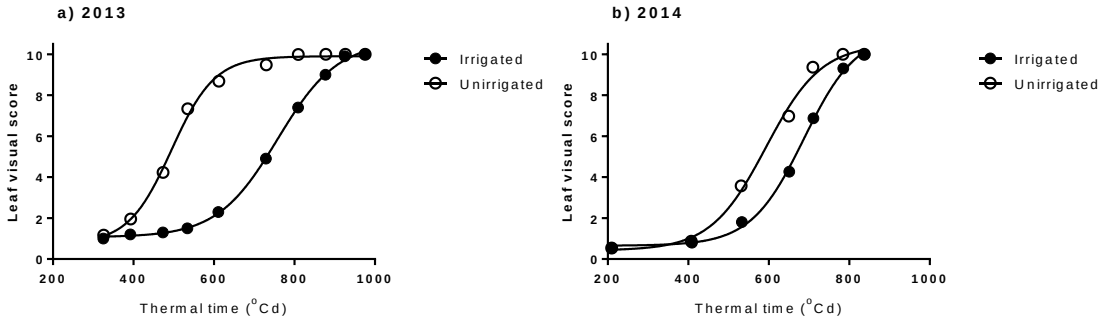


Figure 5.11 Flag-leaf visual senescence score on thermal time (^{o}Cd) post-GS61 (base temp. 0 ^{o}C) averaging over 94 Rialto x Savannah DH lines fitted with logistic regression equation under irrigated and unirrigated conditions in (a) 2013 and (b) 2014.

5.3.3.1 Onset of flag-leaf senescence (SEN_{ONSET})

In 2013, drought advanced onset of post-anthesis (GS61) flag-leaf senescence from 594.8 to 404.1 ^{o}Cd (P=0.02). DH lines ranged from 473.1 (line 64) to 707.7 ^{o}Cd (line 46) under irrigated conditions, and from 282.2 (line 87) to 491.7 ^{o}Cd (line 63) under unirrigated conditions (P<.001). The parents did not differ in the onset of senescence under either irrigated or unirrigated conditions. There was an irrigation x genotype interaction (P<.001) with the advancement of SEN_{ONSET} under drought ranging from 61.2 (line 31) to 314.7 ^{o}Cd (line 9) (Table 5.8).

In 2014, drought advanced SEN_{ONSET} from 560.6 to 490.1 ^{o}Cd (P=0.05). DH lines ranged from 437.9 (line 2) to 650.3 ^{o}Cd (line 46) under irrigated conditions, and 392.8 (line 79) to 565.9 ^{o}Cd (line 24) under unirrigated conditions (P<.001). The genotype x irrigation interaction was not significant (P=0.30). The parents did not differ under either irrigated or unirrigated conditions (Table 5.8).

Averaged over years, drought advanced SEN_{ONSET} from 577.7 to 447.1 ^{o}Cd (P<.001). The interaction between irrigation and genotype (P<.001) was significant. For the parents, Rialto (569.8 ^{o}Cd) started senescence earlier than Savannah (641.2 ^{o}Cd) under irrigated conditions, but not under unirrigated conditions (460.5 and 444.9 ^{o}Cd, respectively) (Table 5.8). No transgressive segregation, however, was observed under either irrigated or unirrigated conditions.

In 2013, there was a positive linear relationship amongst DH lines between SEN_{ONSET} and grain yield under irrigated (R^2=0.16; P<.001) and unirrigated (R^2=0.21; P<.001; Fig. 5.12a) conditions. However, no relationship was observed in 2014 under either irrigated (P=0.10) or unirrigated (P=0.37; Fig. 5.12b) conditions. Averaging across years, a positive linear relationship between SEN_{ONSET} and grain yield was found under both irrigated (R^2=0.13; P<.001) and unirrigated (R^2=0.19; P<.001; Fig. 5.12c) conditions. Similarly, a positive linear relationship amongst DH lines was found in 2013 between SEN_{ONSET} and AGDM under both irrigated (R^2=0.11; P=0.001) and unirrigated (R^2=0.08; P=0.004; Fig. 5.12d) conditions, and averaging across years, (R^2=0.13; P<.001 and R^2=0.06; P=0.02, respectively; Fig. 5.12f).

In 2013, grain weight was positively associated with SEN_{ONSET} under unirrigated (R^2=0.14; *P*<.001), and weakly negatively associated under irrigated (R^2=0.05; *P*=0.04; Fig. 5.13a) conditions. Positive linear relationships between SEN_{ONSET} and grain weight were also found amongst DH lines under unirrigated conditions in 2014 and for the cross-year mean (R^2=0.11; *P*<.001 and R^2=0.18; *P*<.001, respectively) (Fig. 5.13b and 5.13c).

Under unirrigated conditions, negative relationships were observed between anthesis date and SEN_{ONSET} in 2013, 2014 and for the cross-year mean (R^2=0.32; *P*<.001; R^2=0.18; *P*<.001 and R^2=0.32; *P*<.001, respectively). Under irrigated conditions, there was also a negative correlation in 2014 (R^2=0.06; *P*=0.02) (Fig. 5.13d, 5.13e and 5.13f).

5.3.3.2 End of flag-leaf senescence (SEN_{END})

In 2013, drought overall advanced the end of flag-leaf senescence from 901.2 to 656.2 ^{o}Cd (*P*=0.04). DH lines ranged from 831.9 (line 72) to 954.4 ^{o}Cd (line 64) under irrigated conditions, and from 517.8 (line 99) to 782.1 ^{o}Cd (line 84) under unirrigated conditions (*P*<.001). For the parents, Rialto (592.9 ^{o}Cd) reached end of senescence earlier than Savannah (730.5 ^{o}Cd) under unirrigated, but not under irrigated conditions (877.7 and 883.0 ^{o}Cd, respectively). The irrigation x genotype interaction was significant with advancement in SEN_{END} under drought ranging from 130.9 (line 88) to 368.3 ^{o}Cd (line 64) (P<.001; Table 5.8).

In 2014, drought advanced the end of senescence from 793.7 to 728.6 ^{o}Cd (*P*=0.04). DH lines differed in the range 735.5 (line 10) to 857.2 ^{o}Cd (line 33) and 612.0 (line 99) to 807.2 ^{o}Cd (line 81) under irrigated and unirrigated conditions, respectively (*P*<.001). The advancement under drought for SEN_{END} ranged from -11.1 (line 35) to 164.0 ^{o}Cd (line 100) (*P*=0.04). For the parents, Rialto (807.6 ^{o}Cd) reached end of senescence later than Savannah (749.8 ^{o}Cd) under irrigated conditions, but earlier (687.2 ^{o}Cd) than Savannah (715.7 ^{o}Cd) under unirrigated conditions (Table 5.8).

Averaging over seasons, DH lines ranged from 794.9 (line 72) to 900.3 ^{o}Cd (line 49) and from 595.8 (line 92) to 764.6 ^{o}Cd (line 91) under irrigated and drought conditions, respectively (*P*<.001). Drought advanced SEN_{END} overall from 847.4 in irrigated conditions to 692.4 ^{o}Cd (*P*=0.002). The advancement under drought for

SEN_{END} ranged amongst DH lines from 90.5 (line 63) to 225.3 ^{o}Cd (line 64) (P<0.001). For the parents, Rialto (640.0 ^{o}Cd) reached SEN_{END} earlier than Savannah (723.1 ^{o}Cd) under unirrigated, but not under irrigated conditions (813.8 and 845.3 ^{o}Cd, respectively) (Table 5.8). There was no transgressive segregation under either irrigated or unirrigated conditions.

SEN_{END} showed a weak positive linear relationship with grain yield amongst DH lines in 2013 under unirrigated conditions (R^2=0.04; P=0.03). Under irrigation, there was also a trend for a positive relationship (P=0.06; Fig. 5.14a). In 2013, SEN_{END} showed a weak positive linear relationship with grain weight amongst DH lines under unirrigated conditions (R^2=0.03; P=0.08). Positive linear relationships were also observed in 2014 under irrigated (R^2=0.13; P=0.001) and unirrigated (R^2=0.23; P<.001; Fig. 5.15b) conditions. Averaging across years, a positive linear relationship between SEN_{END} and grain weight was found under irrigated (R^2=0.05; P=0.03) and unirrigated (R^2=0.14; P<.001; Fig. 5.15c) conditions.

In 2013, there was a positive linear relationship amongst DH lines between SEN_{END} and anthesis date under unirrigated (R^2=0.18; P<.001), but not under irrigated (P=0.99; Fig. 5.15d) conditions. Negative relationships were found in 2014 under both irrigated (R^2=0.55; P<.001) and unirrigated (R^2=0.27; P<.001; Fig. 5.15e) conditions. Averaging across years, a negative linear relationship between SEN_{END} and anthesis date amongst DH lines was also found under irrigated (R^2=0.21; P<.001), but not under unirrigated (P=0.98; Fig. 5.15f) conditions.

Table 5.8 Onset of flag-leaf senescence (SEN_{ONSET}), flag-leaf senescence rate (SEN_{RATE}) and end of flag-leaf senescence (SEN_{END}) in irrigated and unirrigated treatments for 94 Rialto x Savannah DH lines and the two parents in 2013, 2014 and cross-year mean, and maximum, minimum, standard errors of the differences of the means (SED) and degrees of freedom (df).

Traits	SEN_{ONSET} (^oCd)		SEN_{RATE} ($^oCd^{-1}$)		SEN_{END} (^oCd)	
2013	Irrigated	Unirrigated	Irrigated	Unirrigated	Irrigated	Unirrigated
Mean	594.8	404.1	0.014	0.021	901.2	656.2
Max.	707.7	491.7	0.033	0.038	954.4	782.1
Min.	473.1	282.2	0.006	0.007	831.9	517.8
Rialto	544.0	418.2	0.012	0.029	883.0	592.9
Savannah	663.0	423.8	0.020	0.014	877.7	730.5
SED						
Irrigation (2)	*4.70**		*0.0012*^{ns}		*13.32**	
Genotype (95)	*23.15****		*0.0041****		*29.34****	
Irri. X Gen (95)	*32.91****		*0.0063****		*43.38****	
2014						
Mean	560.6	490.1	0.016	0.017	793.7	728.6
Max.	650.3	565.9	0.037	0.032	857.2	807.2
Min.	437.9	392.8	0.008	0.011	735.5	612.0
Rialto	595.6	471.6	0.015	0.021	807.6	687.2
Savannah	619.4	497.2	0.035	0.017	749.8	715.7
SED						
Irrigation (2)	*5.96**		*0.0017*^{ns}		*3.59**	
Genotype (95)	*28.01****		*0.0032****		*19.26****	
Irri. X Gen (95)	*39.86*^{ns}		*0.0050**		*27.34**	
2013-14						
Mean	577.7	447.1	0.015	0.019	847.4	692.4
Max.	664.2	507.3	0.032	0.032	900.3	764.6
Min.	455.5	360.2	0.008	0.012	794.9	595.8
Rialto	569.8	444.9	0.013	0.025	845.3	640.0
Savannah	641.2	460.5	0.028	0.016	813.8	723.1
SED						
Year (2)	*5.69**		*0.0005*^{ns}		*2.04**	
Irrigation (2)	*3.79****		*0.0008**		*6.90***	
Genotype (95)	*18.17****		*0.0027****		*17.55****	
Irri. X Gen (95)	*25.84****		*0.0039****		*25.64****	
Year x Gen (95)	*26.19****		*0.0038***		*24.78****	

N.B: *** denotes P<0.001; **P<0.01 and *P<0.05 significance levels; ^{ns} = not significant.

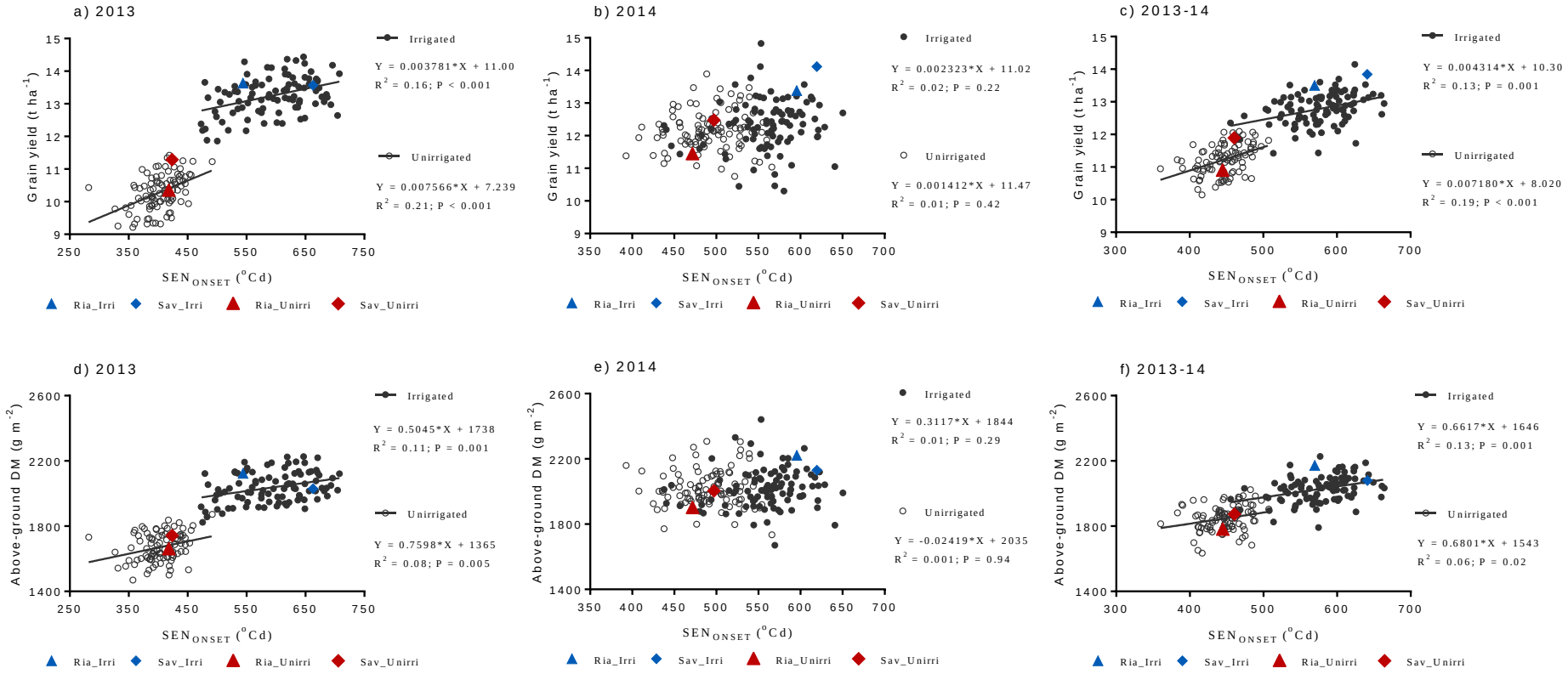


Figure 5.12 Linear regressions of flag-leaf onset of senescence (SEN_{ONSET}; ^{o}Cd post-anthesis) on grain yield (GY; 85% DM; t ha^{-1}) in (a) 2013, (b) 2014 and (c) cross-year mean, and on above-ground dry matter (AGDM; g m^{-2}) in (d) 2013, (e) 2014 and (f) cross-year mean, under irrigated and unirrigated conditions for 94 Rialto x Savannah DH lines. Rialto under irrigated (▲) and unirrigated (▲) conditions, and Savannah under irrigated (♦) and unirrigated (♦) conditions are also shown.

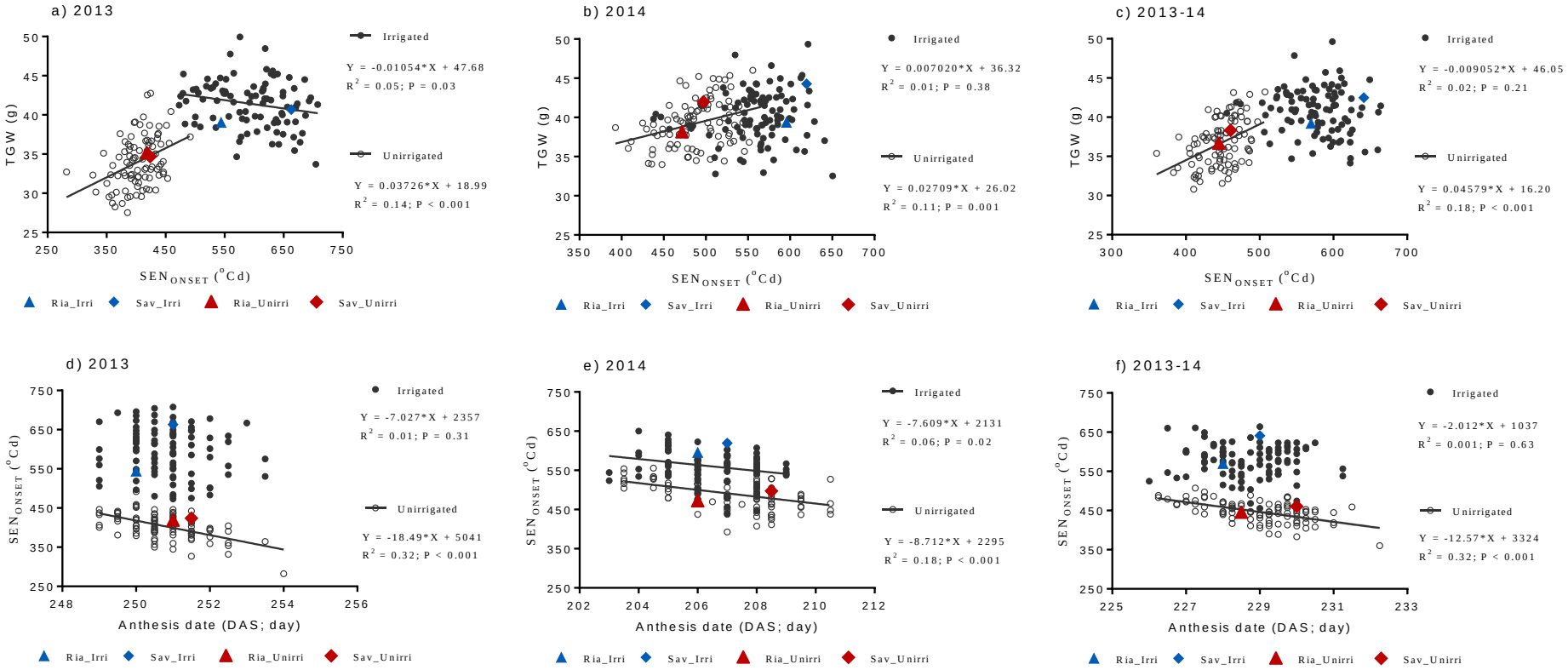


Figure 5.13 Linear regressions of flag-leaf onset of senescence (SEN_{ONSET}; ^{o}Cd post-anthesis) on thousand grain weight (TGW; g) in (a) 2013, (b) 2014 and (c) cross-year mean, and on anthesis date (GS61, DAS; day) in (d) 2013, (e) 2014 and (f) cross-year mean under irrigated and unirrigated conditions for 94 Rialto x Savannah DH lines. Rialto under irrigated (▲) and unirrigated (▲) conditions, and Savannah under irrigated (♦) and unirrigated (♦) conditions are also shown.

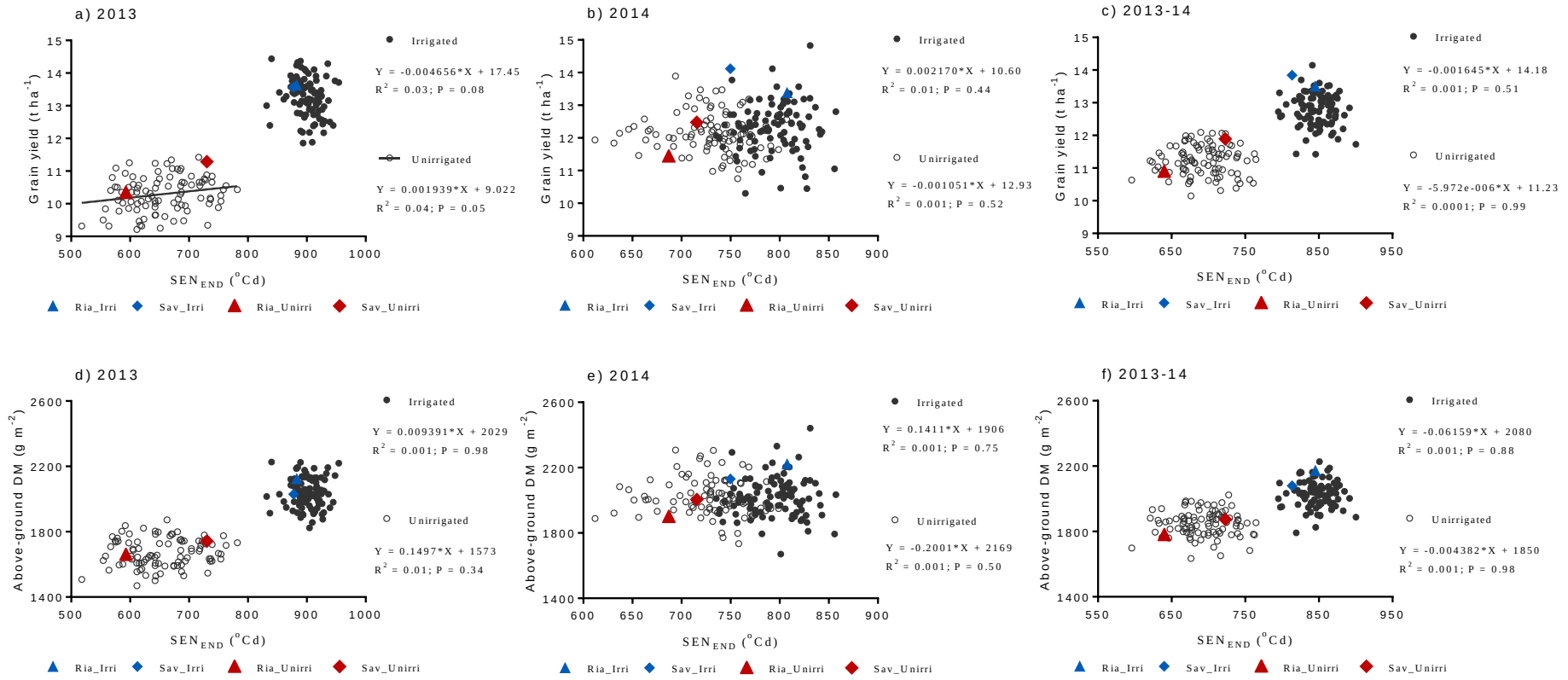


Figure 5.14 Linear regressions of end of flag-leaf senescence (SEN_{END}; ^{o}Cd post-anthesis) on grain yield (GY; 85% DM; t ha^{-1}) in (a) 2013, (b) 2014 and (c) cross-year mean, and on above-ground dry matter (AGDM; g m^{-2}) in (d) 2013, (e) 2014 and (f) cross-year mean, under irrigated and unirrigated conditions for 94 Rialto x Savannah DH lines. Rialto under irrigated (▲) and unirrigated (▲) conditions, and Savannah under irrigated (♦) and unirrigated (♦) conditions are also shown.

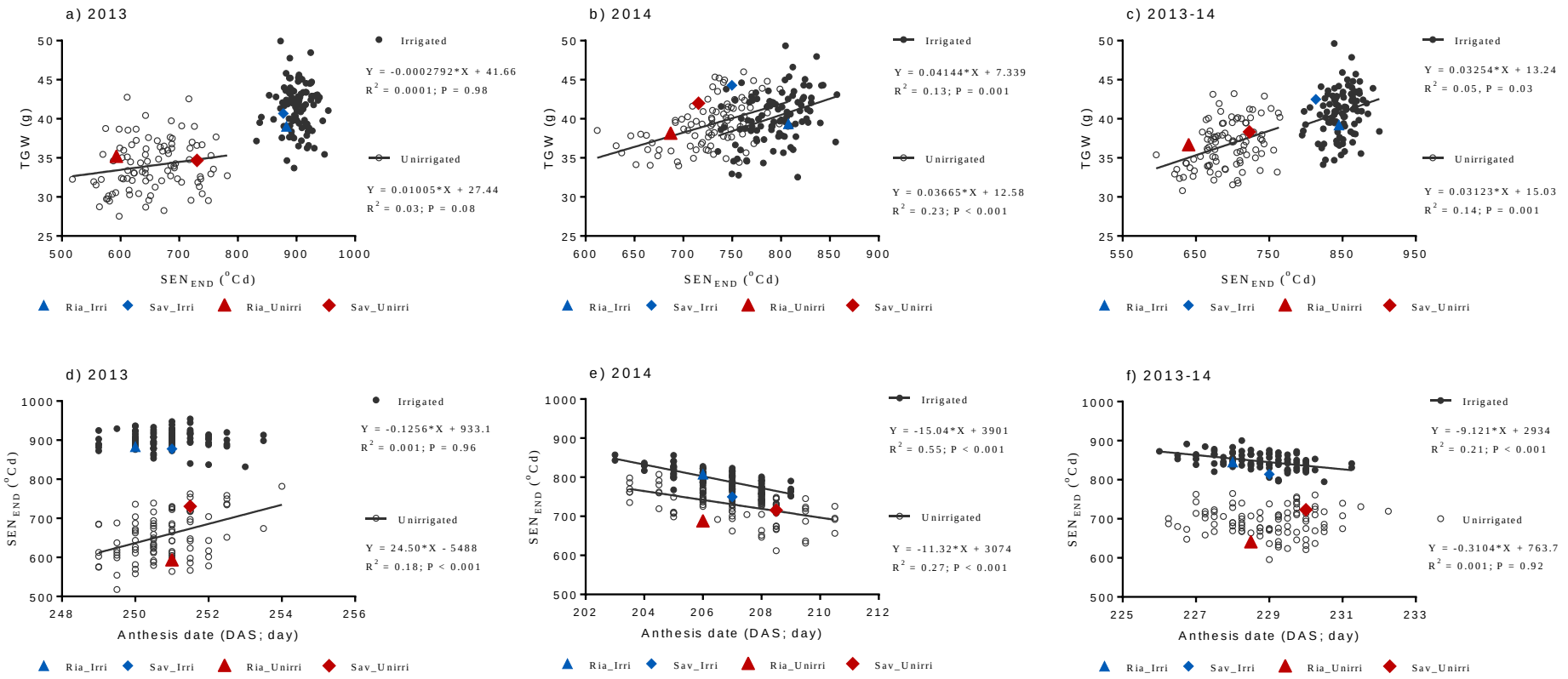


Figure 5.15 Linear regressions of end of flag-leaf senescence (SEN_{END}; ^{o}Cd post-anthesis) on thousand grain weight (TGW; g) in (a) 2013, (b) 2014 and (c) cross-year mean, and on anthesis date (GS61, DAS; day) in (d) 2013, (e) 2014 and (f) cross-year mean under irrigated and unirrigated conditions for 94 Rialto x Savannah DH lines. Rialto under irrigated (▲) and unirrigated (▲) conditions, and Savannah under irrigated (♦) and unirrigated (♦) conditions are also shown.

Table 5.9 The phenotypic correlation (r) of onset of flag-leaf senescence (SEN_{ONSET}), flag-leaf senescence rate (SEN_{RATE}) and end of flag-leaf senescence (SEN_{END}) with grain yield (GY; 85% DM), above-ground dry matter (AGDM), thousand grain weight (TGW), harvest index (HI), anthesis date (GS61, DAS) and grains m^{-2} among 94 Rialto x Savannah DH lines in 2013, 2014 and cross-year mean under irrigated and unirrigated conditions.

	Traits	GY (t ha^{-1})	AGDM (g m^{-2})	TGW (g)	HI	AD (DAS; day)	Grains m^{-2}
2013							
Irrigated	SEN_{ONSET} (^oCd)	0.40^{***}	0.32^{**}	-0.21^{*}	0.16^{ns}	-0.10^{ns}	0.32^{**}
	SEN_{RATE} ($^oCd^{-1}$)	0.41^{***}	0.27^{**}	-0.22^{*}	0.24^{*}	-0.10^{ns}	0.31^{**}
	SEN_{END} (^oCd)	-0.19^{ns}	0.00^{ns}	0.01^{ns}	-0.30^{**}	0.0003^{ns}	-0.12^{ns}
Drought	SEN_{ONSET} (^oCd)	0.46^{***}	0.29^{**}	0.38^{***}	0.32^{**}	-0.55^{***}	0.04^{ns}
	SEN_{RATE} ($^oCd^{-1}$)	0.04^{ns}	0.12^{ns}	0.03^{ns}	-0.11^{ns}	-0.52^{***}	0.05^{ns}
	SEN_{END} (^oCd)	0.22^{*}	0.11^{ns}	$0.18^{.08}$	0.20^{*}	0.42^{***}	-0.02^{ns}
2014							
Irrigated	SEN_{ONSET} (^oCd)	0.17^{ns}	0.13^{ns}	0.10^{ns}	0.10^{ns}	-0.24^{*}	0.04^{ns}
	SEN_{RATE} ($^oCd^{-1}$)	0.12^{ns}	0.03^{ns}	-0.12^{ns}	0.23^{*}	0.10^{ns}	0.21^{*}
	SEN_{END} (^oCd)	0.05^{ns}	0.03^{ns}	0.33^{**}	-0.01^{ns}	-0.74^{***}	-0.26^{*}
Drought	SEN_{ONSET} (^oCd)	0.09^{ns}	0.00^{ns}	0.34^{***}	0.13^{ns}	-0.41^{***}	-0.23^{*}
	SEN_{RATE} ($^oCd^{-1}$)	0.14^{ns}	0.05^{ns}	-0.23^{*}	0.12^{ns}	0.08^{ns}	0.17^{ns}
	SEN_{END} (^oCd)	-0.05^{ns}	-0.06^{ns}	0.47^{***}	0.01^{ns}	-0.51^{***}	-0.38^{***}
2013-14							
Irrigated	SEN_{ONSET} (^oCd)	0.38^{**}	0.35^{***}	-0.12^{ns}	0.10^{ns}	-0.04^{ns}	0.32^{**}
	SEN_{RATE} ($^oCd^{-1}$)	0.36^{***}	0.23^{*}	-0.21^{*}	0.25^{*}	0.08^{ns}	0.37^{***}
	SEN_{END} (^oCd)	-0.10^{ns}	-0.03^{ns}	0.22^{*}	-0.15^{ns}	-0.45^{***}	-0.21^{*}
Drought	SEN_{ONSET} (^oCd)	0.43^{***}	0.24^{*}	0.43^{***}	0.28^{**}	-0.55^{***}	-0.14^{ns}
	SEN_{RATE} ($^oCd^{-1}$)	0.20^{*}	0.13^{ns}	-0.04^{ns}	0.09^{ns}	-0.34^{***}	0.14^{ns}
	SEN_{END} (^oCd)	0.02^{ns}	0.01^{ns}	0.38^{***}	0.04^{ns}	0.003^{ns}	-0.31^{**}

N.B: ***denotes P<0.001; **P<0.01 and *P<0.05 significance levels; ^{ns} = not significant.

5.3.4 Grain yield associations with stay-green traits

In 2013, multiple linear regressions showed that each of post-anthesis NDVI and SEN_{ONSET} explained about 20% of grain yield phenotypic variation amongst DH lines under drought. CT explained 15.8%, and chlorophyll fluorescence 4.2% of the phenotypic variation in grain yield under drought. However, when using two traits in the linear regression, NDVI and SEN_{ONSET} explained about 36%; CT and SEN_{ONSET} explained 30%, and NDVI and CT explained 23% of the phenotypic variation amongst DH lines in grain yield under drought. In this multiple linear regression, using three traits did not explain any more variation than using two traits.

Table 5.10 Multiple linear regression analysis of grain yield (85% DM) t ha^{-1} with post-anthesis NDVI, canopy temperature (CT), flag-leaf chlorophyll fluorescence (QY) and SEN_{ONSET} for 94 R x S DH lines under unirrigated in 2013.

Best subsets	R^2	df	NDVI	CT	QY	SEN_{ONSET}
I trait	20.8	2	<0.001	-	-	-
	20.2	2	-	-	-	<0.001
	15.8	2	-	<0.001	-	-
	4.2	2	-	-	0.03	-
2 traits	35.9	3	<0.001	-	-	<0.001
	30.1	3	-	<0.001	-	<0.001
	23.1	3	0.002	0.05	-	-

5.4 Discussion

The discussion will consider the physiological basis of genotypic differences in gas-exchange, stay-green traits and leaf senescence parameters and associations with grain yield and drought tolerance. Since, the effect of drought on target traits was greater in 2013 compared to 2014, due to drier conditions during grain filling in 2013; the discussion will focus mainly on effects in 2013 and the cross-year mean.

5.4.1 Flag-leaf stomatal aperture traits and associations with yield

Water-use efficiency is an important attribute of crop growth in drought-prone environments which relates to transpiration efficiency at the leaf scale (Tamussi *et al.*, 2007). Information on leaf TE improves understanding of genetic variation in response to water stress underlying WUE (Yasir *et al.*, 2013).

Under mild drought in this study, variation for leaf photosynthetic rate, stomatal conductance and transpiration efficiency were observed amongst the subset of genotypes. Flag-leaf A_{max} and g_s were positively associated with grain yield and $\Delta^{13}C$, while leaf TE was negatively associated with grain yield and $\Delta^{13}C$. Genotypic variation in gas-exchange parameters (e.g. photosynthetic rate, stomatal conductance and transpiration efficiency) has been previously observed in wheat and associated with grain yield (Blum, 1990; Morgan and Lecain, 1991; Fischer *et al.*, 1998).

The positive association of flag-leaf A_{max} and g_s, and the negative association of TE with grain yield in the present study are consistent with previous evidence that genotypes with higher A_{max} and g_s had higher grain yield and lower WUE under water stress (Jiang *et al.*, 2000; Condon *et al.*, 2002; Monneveux *et al.*, 2006). However, Xue *et al.* (2002) found no correlations between CO_2 assimilation rate and stomatal conductance and grain yield under water stress in hard red winter wheat cultivars in USA. Under irrigated conditions, positive correlations between A_{max}, g_s, and grain yield have been reported in spring wheat (Blum, 1990; Fischer *et al.*, 1998; Lu *et al.*, 1998).

In the present study, the positive associations of A_{max} and g_s, and the negative association of TE with grain $\Delta^{13}C$ are consistent with findings of previous studies (Morgan *et al.*, 1993; Monneveux *et al.*, 2006; Rebetzke *et al.*, 2006) which found

that $\Delta^{13}C$ genetic variation in wheat is positively related to the internal CO_2 concentration (Ci), and negatively to TE; a high Ci could be related to high g_s and/or low photosynthetic capacity of leaf (i.e. lower TE is associated with higher grain yield). Present findings, therefore, suggest that higher grain yield under UK drought conditions could be driven by increased water uptake rather than WUE (Blum, 2005), and grain $\Delta^{13}C$ could therefore be a useful selection criterion for improving yield performance (as an indicator of water uptake) in breeding programmes under drought. Similarly, Aravinda Kumar *et al.* (2011), examining a winter wheat Beaver x Soissons DH population in the UK in field conditions, found a trade-off between TE and season-long water uptake under mild UK droughts.

5.4.2 Canopy temperature and chlorophyll fluorescence and associations with grain yield

Under mild drought in the present study, post-anthesis canopy temperature was negatively correlated with grain yield and TGW, and cooler canopies had a lower drought susceptibility index amongst the DH lines in 2013. This is consistent with previous findings that cooler canopies were associated with greater grain yield under drought in wheat (Pinto *et al.*, 2010; Lopes *et al.*, 2012). This association may be related to a deeper root system based on physiological evidence (Lopes and Reynolds, 2012). Therefore, grain yield association with canopy temperature supports water uptake as the main driver of yield under UK drought conditions.

Canopy temperature was the most drought-adaptive trait associated with grain yield amongst Seri/Babax recombinant inbred lines under drought in Mexico and rain-fed conditions in Australia (Olivares-Villegas *et al.*, 2007). Thus, canopy temperature is considered to be an important indicator of stress-adapted genotypes under drought conditions, and cooler canopies can be related to the water extract potential from deeper soil profiles and/or agronomic evapotranspiration efficiency of genotypes under water stress, and hence more CO_2 assimilates for grain formation.

Positive associations of leaf chlorophyll fluorescence (*Fv/Fm*) with grain yield were reported under water stress in wheat (Farzad *et al.*, 2007). Rad *et al.* (2014) also found an association between *Fv/Fm* ratio and grain which explained about 57% of the variation in grain yield under drought in bread wheat. In the present study, there

was a weak positive correlation between post-anthesis chlorophyll fluorescence and each of grain yield and TGW in both years. The association may have been relatively weak because leaves were not dark adapted.

In response to drought, the PS II activity can be affected. This may reflect the plant photosynthesis potential, and hence grain yield (Guan *et al.*, 2014). However, Lu and Zhang (1998) proposed that water stress decreases CO_2 assimilation more than the quantum yield of PS II electron, and found CO_2 assimilation decreased about 85% but ØPS II only by 30%. Although, chlorophyll fluorescence can be used for studying plant photosynthesis, and could relatively be used as a selection criterion to evaluate the grain yield under water stress (Yang *et al.*, 2007). Present results indicated that QY measured under ambient radiation conditions was not strongly correlated amongst the DH winter wheat genotypes under mild UK drought.

5.4.3 Post-anthesis canopy senescence

Greater yield production associated with longer green canopy area duration (stay-green) amongst genotypes has been reported in sorghum (Borrell and Hammer, 2000), maize (Campos *et al.*, 2004) and wheat (Gorny and Garczynski, 2002; Foulkes *et al.*, 2007c) under drought. Genotypes, generally, varied in their canopy greenness (NDVI) in 2013, and positive associations between NDVI measurements and grain yield, above-ground dry matter and TGW were observed under drought, and higher NDVI genotypes under drought had less drought susceptibility index for grain yield.

Positive correlations between grain yield and NDVI have been reported in previous investigations under water stress (Das *et al.*, 1993; Aparicio *et al.*, 2000; Serrano *et al.*, 2000; Gutiérrez-Rodríguez *et al.*, 2004; Reynolds *et al.*, 2007b; Reynolds *et al.*, 2007c; Lopes *et al.*, 2012). In the UK, a significantly positive correlation was found between flag-leaf green area remaining at 14 days and 35 days after anthesis and grain yield under drought for a DH winter wheat Beaver x Soissons population (Verma *et al.*, 2004). Similarly, in Australia, greater yield was found by Christopher *et al.* (2008) for stay-green lines which maintained green leaf area longer during the grain-filling period for two CIMMYT wheat lines SeriM82 and Hartog compared to check lines.

Regarding leaf senescence, there were differences between genotypes in flag-leaf senescence durations, and the effect of drought advanced SEN_{ONSET} by 13-47% and SEN_{END} by 14-39% between DH lines in 2013. Savannah had later SEN_{END} than Rialto under drought. In 2013, delaying SEN_{ONSET} was associated with greater grain yield. In 2013, SEN_{ONSET} explained variation in yield under drought, but not under irrigated. Thus, this trait could be a useful tool to predict drought response in breeding programme.

Thomas and Howarth (2000) reported the increase of carbon fixation by 11% as a result of delaying the onset of senescence by 2 days in *lolium temulentum*. Verma *et al.* (2004) also reported significant and positive association between delaying leaf senescence and grain yield in wheat under different environmental conditions in the UK. Positive correlations have also been found, in line with the present study, between longer flag-leaf duration and grain yield in durum wheat (Hafsi *et al.*, 2007; Gregersen *et al.*, 2008), and longer crop seasons with yield in spring wheat (Takahashi and Nakaseko, 1990).

Higher grain yield associated with longer stay-green is mainly due to yield source limitation under post-anthesis abiotic stress (Christopher *et al.*, 2008; Bogard *et al.*, 2011), and greener canopies maintain the active photosynthetic rate better (Joshi *et al.*, 2007). The mechanism associated with the genetic variation in stay-green cannot be certain from present results; it could be associated with increased potentials in water and N uptake with deeper roots and/or reduced post-anthesis N remobilization from leaves to grain.

Anthesis date was negatively associated with the rate and onset of flag-leaf senescence in this study under drought. The mechanism could be related to environmental changes such as water availability, temperature and N availability associated with anthesis date (Thomas and Stoddart, 1980; Lim *et al.*, 2007). Moreover, leaf senescence may be indirectly influenced by early flowering which increases indirectly post-anthesis N uptake as a result of reducing pre-anthesis N uptake and hence delaying N remobilization and prolonging leaf senescence duration (Bogard *et al.*, 2010; Bogard *et al.*, 2011).

Overall stronger correlations between stay-green traits and grain yield were found under drought than under irrigated conditions, but correlations were still significant under irrigated conditions. Therefore, grain yield may be limited by source and sink in modern UK cultivars in optimal conditions (Shearman *et al.*, 2005), and stay-green traits can be potentially an important selection criterion for stay-green lines under both drought and optimal environments in future UK breeding programs. Results showed that NDVI and leaf visual senescence score were valuable phenotyping methods for stay-green assessment under UK drought condition.

Chapter 6 Rialto x Savannah DH lines and ancestral wheat genotypes in glasshouse experiments

6.1 Introduction

This chapter describes the biomass, yield and yield components and flag-leaf gas exchange traits (photosynthetic rate, stomatal conductance and transpiration efficiency) for a subset of Rialto x Savannah DH lines and ancestral wheat species and derivatives in 2013 and 2014 in two glasshouse experiments. Genetic variation is quantified, and mechanisms explaining the relationships between traits and grain yield response to drought are discussed. Wheat ancestral species have not been widely characterised for drought tolerance and associated traits; therefore, assessments to identify novel genetic variation in physiological traits under targeted drought stress to identify the most drought tolerant genotypes is required (Reynolds *et al.*, 2007b). Genetic improvement of wheat depends on utilizing different gene expression within a broad pool of functional genetic resources. Therefore, wild relatives of wheat and lines derived from them are sources of novel genetic variation for drought tolerance because of reducing genetic diversity during domestication (Dubcovsky and Dvorak, 2007; Reynolds *et al.*, 2007c). Moreover, wheat wild relatives are generally grown in areas with high environmental stress where dry conditions prevail and are well adapted to these conditions since they are exposed to many years of natural selection and can survive the harsh climatic conditions of extreme years. Amphidiploid lines can be developed from crosses between wheat and related species from the genera *Aegilops*, *Secale*, *Thinopyrum*, and *Triticum* and phenotypically and genetically characterized for traits related to biotic and abiotic stresses (Nemeth *et al.*, 2015). The overall aim of this chapter is to quantify genetic variation for water-use efficiency and drought-tolerance traits between selected lines of the winter wheat Rialto x Savannah DH population representing the highest and lowest values of grain $\Delta^{13}C$ (i.e. highest and lowest values of transpiration efficiency in the population) and novel wheat amphidiploid lines (bread wheat x *Thinopyrun bessarabicum*) and accessions of ancestral wheat species (*T. bessarabicum*, *T. uratu* and *A. speltoides*), and to describe results for grain yield and physiological traits for genotypes and responses to irrigation in each experiment and across experiments.

The specific hypotheses tested in this chapter are:

1. Wild relatives of hexaploid bread wheat have higher water-use efficiency and higher expression of drought-tolerance traits compared with the UK winter wheat Rialto x Savannah DH lines and the two parents.
2. Amphidiploid lines, derived from crosses between wheat wild relative species and bread wheat, have higher water-use efficiency and higher expression of drought-tolerance traits compared with the UK winter wheat Rialto x Savannah DH lines.
3. For Rialto x Savannah DH lines, stomatal aperture traits measured in the glasshouse consistent with the expression of these traits measured in the field.

6.2 Materials and Methods

6.2.1 Experimental design and treatments

Two glasshouse experiments were conducted in 2012-13 and 2013-14 at the University of Nottingham, School of Bioscience, Sutton Bonington Campus, UK (52^{o} 50' N, 1^{o} 15' W). Twenty two genotypes were examined: two parental winter wheat genotypes (Rialto and Savannah) and four winter wheat DH lines from the Rialto x Savannah DH population (lines 20, 25, 63 and 88), three accessions of each three wheat wild relative species (*T. bessarabicum*, *T. uratu* and *A. speltoides*), and seven amphidiploid lines derived from crosses between durum wheat and *T. bessarabicum* (*T. bessarabicum* x cv. Karim, *T. bessarabicum* x cv. Stewart, *T. bessarabicum* x cv. Langdon, *T. bessarabicum* x cv. Macoun, *T. bessarabicum* x cv. Creso, *T. bessarabicum* x cv. Neodur and *T. bessarabicum* x cv. Azaziah). The three accessions of *T. uratu* were not assessed in 2014 as they did not survive after transplanting into the glasshouse. A split-plot randomized block design was adopted with two irrigation regimes (well-watered and water-stressed) and three replicates. Plants were grown in PVC columns which were 15 cm diameter and 50 cm in height, filled with soil medium (50% sand and 50% sandy loam top soil). After vernalization in a growth room (6 ^{o}C, 12 h photoperiod for 62 days in 2012-13 and 77 days in 2013-14 after sowing) in modules, the seedlings were transplanted into the columns (one plant per column) in the glasshouse.

6.2.2 Water uptake and water-use efficiency

Plants were irrigated weekly to return columns to 90% available water at field capacity (AWFC) in the irrigated treatment and in the water-stressed treatment up to GS39. Water was applied in the water-stressed treatment by returning the soil water content of the columns to 50% AWFC from GS39 to GS61, and 25% AWFC from GS61 to physiological maturity based on estimated evapotranspiration (gravimetric analysis) in two sets of 22 columns (one replicate for each irrigation treatment). Plant water uptake (WU) was calculated based on gravimetric analysis of soil columns as described in Chapter 3. Water-use efficiency (WUE) was then calculated based on dry weights at harvest as below:

$WU\ (l) = \text{Column weight } (kg)_T + \text{Irrigation } (l) - \text{Column weight } (kg)_H$

$WUE_{AGDM}\ (g\ l^{-1}) = AGDM\ (g\ plant^{-1})/WU\ (l)$

$WUE_{grain}\ (g\ l^{-1}) = Grain\ (g\ plant^{-1})/WU\ (l)$

6.2.3 Flag-leaf measurements and harvest analysis

In both years, flag-leaf gas-exchange was carried out to estimate photosynthetic rate, stomatal conductance and transpiration efficiency on green flag leaves for all genotypes in irrigated and droughted treatments. Measurements were taken every two weeks from booting stage (GS41) to mid-to-late grain filling (GS61+28 days) in both 2013 (3, 20 May, 6 and 11 June) and 2014 (1, 21 May, 10 and 19 June) from 10.00 h to 14.00 h, using a LiCor 6400-XT Photosynthesis system (LiCor NE, USA). The system was connected to a leaf chamber measuring cuvette with 2 cm^2 leaf surface. Conditions in the leaf chamber were set up as: cuvette temperature 20 ºC, flowrate to 500 µmols, 400 µmols mol^{-1} of CO_2 concentration and artificial light supply of 2000 PAR µmol m^{-2} s^{-1} (PQuantum 500, 10% blue). At the maximum photosynthetic rate (A_{max}), the values were recorded for A_{max} (µmol CO_2 m^{-2} s^{-1}), stomatal conductance (mmol H_2O m^{-2} s^{-1}) and transpiration efficiency (µmol $mmol^{-1}$).

Flag-leaf chlorophyll content (SPAD) was also measured weekly for the newest fully emerged leaf on the main shoot from stem elongation (GS31) to mid-grain filling

(GS61+28 days) in both 2013 (8, 15, 23 April, 10, 22, 31 May and 20 June) and 2014 (14, 25 March, 10, 23 April, 16, 28 May, 3, 10 and 19 June) from 10.00 h to 14.00 h using a chlorophyll meter (SPAD-502; Minolta, Osaka, Japan).

Flag-leaf senescence was measured from anthesis to full senescence every 3-4 days by using a visual senescence score chart ranging from 0-10 (0; fully green and 10; fully senesced), as described by Gaju *et al.* (2009). Visual assessments were carried out for each plant under both irrigated and droughted treatments. Onset of leaf senescence (SEN_{ONSET}; at leaf visual score 2), senescence rate (SEN_{RATE}; slope) and end of leaf senescence (SEN_{END}; at leaf visual score 9.5) were calculated for each genotype as described in Chapter 3.

At harvest, individual plants were hand-harvested (main shoot and rest of plant separately) at ground level and separated into their components ears and straw. All plant components were weighed after drying for 48 h at 80 ^{o}C, then ears were hand-threshed and grains were weighed after drying for 48 h at 80 ^{o}C. Full details of the growth analysis at harvest are given in Chapter 3.

6.2.4 Statistical analysis

GenStat 15th edition software package was used for ANOVA and regression analysis using the mean values for genotypes in response to irrigation treatments, and the graphs were made using GraphPad Prism version 6.00 Software, La Jolla California USA. The data were tested for normality using Shapiro-Wilk test prior ANOVA analysis. Data for grains ear^{-1} and TGW were transformed by square root in 2013, 2014 and for the cross-year mean.

6.3 Results

6.3.1 Environmental conditions

On average, the glasshouse daily temperature was higher than the outside temperature by 6 ^{o}C in both 2013 and 2014. Generally, from transplanting (February) to the end of tillering (March), average minimum and maximum temperatures were lower in 2013 (0.8 and 15 ^{o}C, respectively) than 2014 (5 and 24 ^{o}C, respectively). However, from stem elongation (April) to the end of grain filling (August) average

minimum and maximum temperatures were higher in 2013 (9 and 32 ^{o}C, respectively) than 2014 (11 and 29 ^{o}C, respectively). During grain filling, maximum values of minimum and maximum temperatures in 2013 were 18 and 43 ^{o}C and in 2014 19 and 41 ^{o}C, respectively. Solar radiation data collected from Sutton Bonington metrological station for 2013 and 2014 are presented in Figure 6.2. The total incident solar radiation from transplanting to harvest was 2440 in 2013 and 2416 MJ m^{-2} in 2014. Incident solar radiation from anthesis to late grain filling (June and July) was similar in 2013 (1173 MJ m^{-2}) and 2014 (1125 MJ m^{-2}).

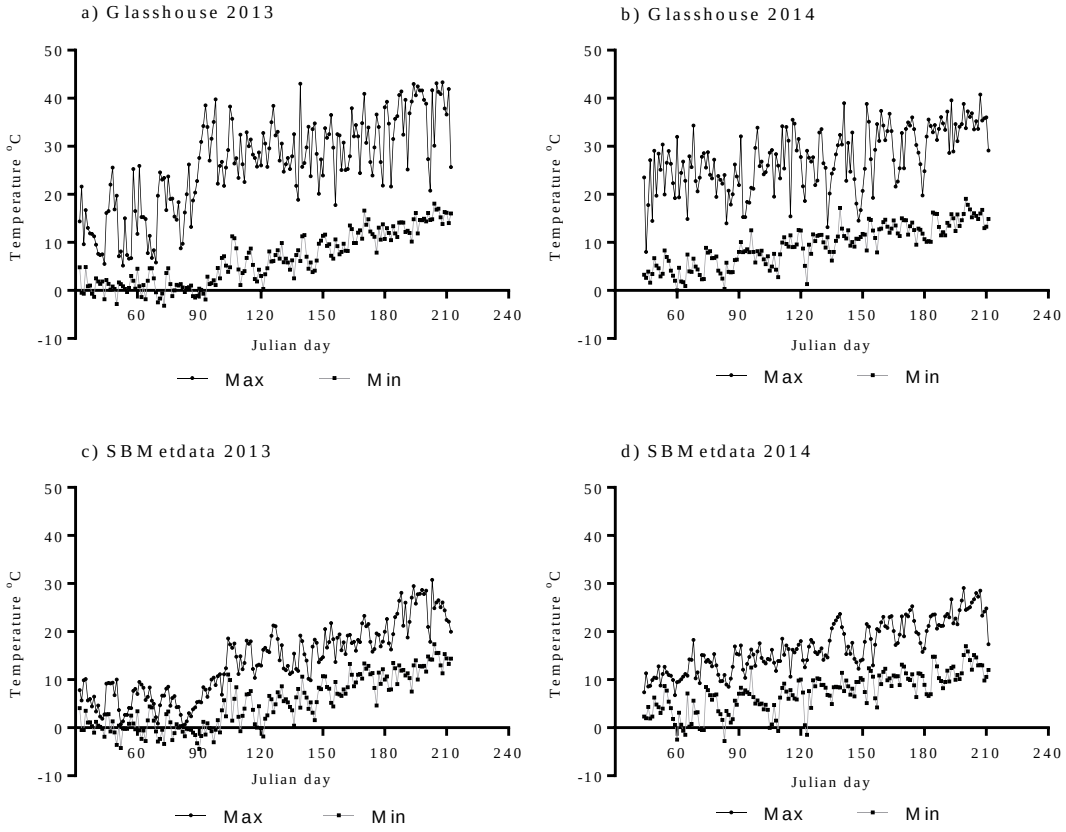


Figure 6.1 Maximum and minimum daily temperatures collected in the glasshouse in (a) 2013 and (b) 2014; and from Sutton Bonington meteorological station in (c) 2013 and (d) 2014.

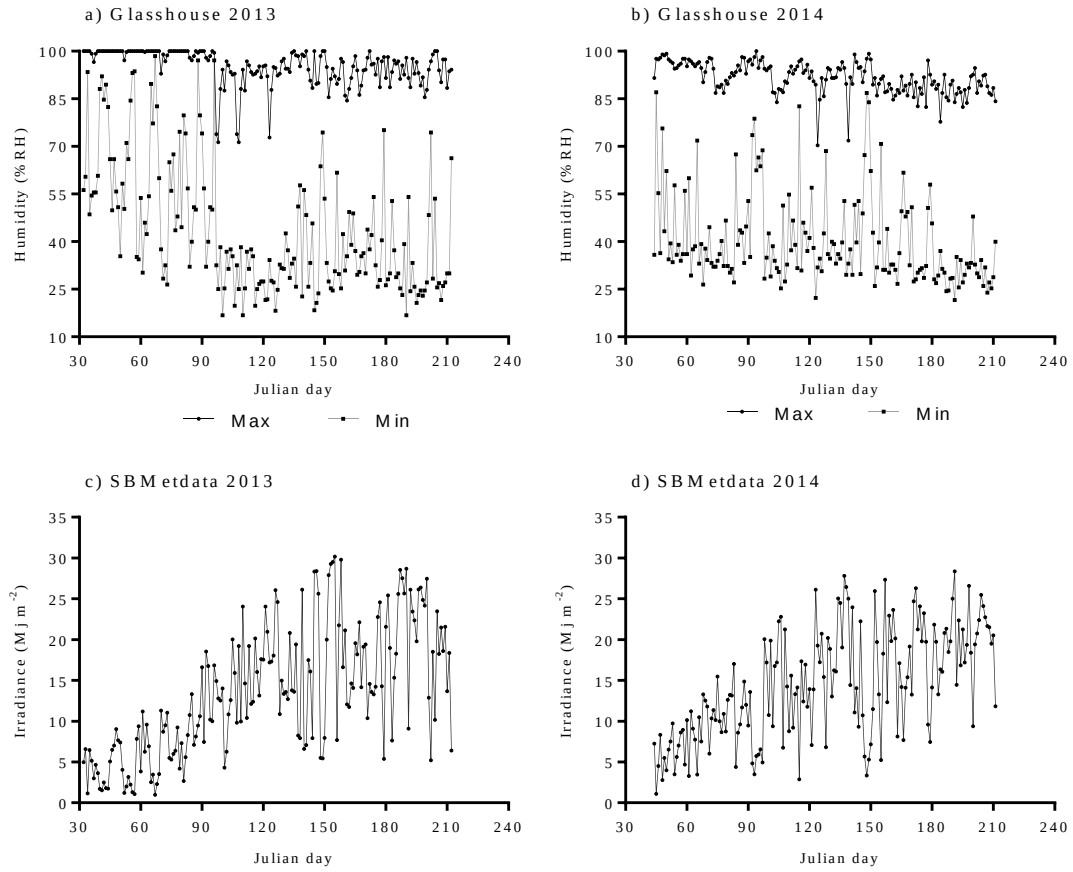


Figure 6.2 Maximum and minimum daily humidity collected in the glasshouse in (a) 2013 and (b) 2014; and daily solar radiations from Sutton Bonington meteorological station in (c) 2013 and (d) 2014.

6.3.2 Plant development

Averaging across genotypes, onset of stem elongation started earlier in 2014 by around 15 days than 2013, but the period from stem elongation (GS31) to anthesis (GS61) was longer by 28 days. Amphidiploid lines were the fastest developing genotypes followed by Rialto x Savannah DH lines, and ancestral wheat species were the latest developing genotypes. Amphidiploid line *T. bessarabicum* x Karim was the fastest developing genotype at all growth stages. Overall drought did not affect the development stages of the genotypes in either 2013 or 2014 ($P>0.05$; Table 6.1).

In 2013, genotypes differed at onset of stem elongation (GS31) in the range 114.3 (*T. bessarabicum* x Karim) to 184.0 DAS (DH line 25) under irrigated conditions, and 115.0 (*T. bessarabicum* x Karim) to 141.6 DAS (Savannah) under droughted conditions ($P<0.001$). Anthesis date ranged from 155.7 (*T. bessarabicum* x Karim) to 188.7 DAS (DH line 25) under irrigated conditions, and 159.3 (*T. bessarabicum* x Karim) to 189.0 DAS (*T. bessarabicum* 2) under droughted conditions ($P<0.001$). The advancement with restricted water availability for anthesis date ranged from 5.2 (Savannah) to 20.0 DAS (DH line 25) ($P<0.001$; Table 6.1).

In 2014, genotypes ranged in GS31 from 102.7 (*T. bessarabicum* x Karim) to 133.7 DAS (*T. bessarabicum* 2) under irrigated conditions, and from 101.3 (*T. bessarabicum* x Karim) to 130.0 DAS (*T. bessarabicum* 2) under droughted conditions ($P<0.001$). At GS61, genotypes ranged from 173.0 (*T. bessarabicum* x Karim) to 206.0 DAS (all *T. bessarabicum* accessions) under irrigated, and 173.0 (*T. bessarabicum* x Karim) to 207.0 DAS (*T. bessarabicum* 2) under droughted conditions ($P<0.001$), and the irrigation x genotype interaction was not significant ($P=0.87$; Table 6.1).

Table 6.1 Development stages (GS31, GS39 and GS61; days after sowing; DAS), and standard errors of the differences of the means (SED) and degrees of freedom (df) under irrigated and droughted treatments for 22 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) in 2013 and 19 genotypes in 2014.

	Genotypes	GS31 (DAS; day)				GS39 (DAS; day)				GS61 (DAS; day)			
		2013		2014		2013		2014		2013		2014	
		Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought
Amphidiploid lines	*T. bessarabicum* x Azaziah	124.0	124.0	107.3	106.0	139.0	139.0	150.3	149.0	160.0	161.0	177.7	175.7
	T. bessarabicum x Creso	126.0	126.0	108.3	107.0	140.0	140.0	153.0	155.0	164.0	164.0	179.0	178.0
	T. bessarabicum x Karim	114.3	115.0	102.7	101.3	126.7	133.7	130.3	128.0	155.7	159.3	173.0	173.0
	T. bessarabicum x Langdon	129.3	126.0	109.7	109.7	143.0	140.0	153.0	157.7	166.7	164.0	180.0	180.0
	T. bessarabicum x Macoun	122.3	130.0	111.0	112.0	139.5	140.0	153.0	153.0	162.2	164.0	179.0	179.0
	T. bessarabicum x Neodur	126.3	130.0	108.3	108.3	141.0	141.0	153.0	153.0	163.0	164.3	179.0	180.0
	T. bessarabicum x Stewart	122.3	124.3	108.3	108.3	141.3	141.0	153.0	155.0	164.0	164.0	180.0	180.0
R x S DH lines	Rialto	141.3	129.3	109.7	110.7	166.0	153.7	157.0	157.7	182.3	171.3	181.0	179.0
	Savannah	133.3	141.6	110.7	113.0	158.3	165.6	160.3	161.0	177.0	182.2	181.0	181.0
	Line 20	133.3	128.7	112.0	111.7	155.7	152.0	159.0	159.7	172.3	169.3	180.0	178.0
	Line 25	148.0	129.3	110.7	109.7	171.7	149.7	159.0	161.0	188.7	168.7	182.0	180.0
	Line 63	140.7	130.7	107.3	110.7	162.7	153.3	159.7	158.3	178.3	168.3	178.0	182.0
	Line 88	132.7	129.3	116.7	118.0	160.7	153.3	165.0	161.7	179.3	171.3	181.0	181.0
Ancestral wheat species	*T. bessarabicum* 1	130.7	134.0	131.0	130.0	150.7	155.0	177.0	175.7	185.3	188.3	206.0	203.7
	T. bessarabicum 2	137.7	136.7	133.7	131.0	153.7	155.7	179.0	177.0	188.3	189.0	206.0	207.0
	T. bessarabicum 3	130.3	134.0	129.0	129.0	151.0	153.1	174.3	174.3	184.3	186.3	200.0	204.7
	T. uratu 1	128.7	130.0	-	-	145.3	145.7	-	-	163.0	164.0	-	-
	T. uratu 2	130.7	130.0	-	-	144.7	143.3	-	-	167.0	162.0	-	-
	T. uratu 3	129.3	129.3	-	-	147.7	147.0	-	-	168.0	168.0	-	-
	A. speltoides 1	128.4	125.3	112.0	114.0	145.3	141.0	158.3	161.7	177.4	174.7	186.0	186.0
	A. speltoides 2	129.3	125.0	116.3	118.0	146.3	140.3	162.3	161.0	178.7	175.0	188.7	186.0
	A. speltoides 3	126.7	128.7	118.7	118.7	145.0	145.7	160.3	159.7	177.3	175.3	187.0	186.0
Mean		130.3	129.0	113.9	114.1	148.9	146.8	158.8	158.9	172.9	170.7	184.4	184.2
SED (df)													
	Irrigation	*1.21^{ns} (2)*		*1.21^{ns} (2)*		*0.62^{ns} (2)*		*0.49^{ns} (2)*		*0.80^{ns} (2)*		*0.63^{ns} (2)*	
	Genotype	*2.60*** (84)*		*1.89*** (72)*		*2.17*** (84)*		*1.48*** (72)*		*2.16*** (84)*		*1.75*** (72)*	
	Irri. x Gen.	*3.79*** (84)*		*2.88^{ns} (72)*		*3.06*** (84)*		*2.09^{ns} (72)*		*3.08*** (84)*		*2.49^{ns} (72)*	

N.B: ***denotes $P<0.001$; $^{**}P<0.01$ and $^{*}P<0.05$ significance levels; ^{ns} = not significant.

6.3.3 Above-ground dry matter, grain yield and yield components

6.3.3.1 Above-ground dry matter

Drought decreased AGDM from 44.8 to 32.4 g plant^{-1} in 2013 (P=0.003). Genotypes ranged from 10.2 (*T. bessarabicum* 2) to 79.1 g plant^{-1} (*T. bessarabicum* x Neodur); and from 7.1 (*T. bessarabicum* 1) to 53.3 g plant^{-1} (*T. bessarabicum* x Langdon) under irrigated and droughted conditions, respectively (P<0.001). The decrease under drought ranged from -6.4 (DH line 25) to 35.8 g plant^{-1} (*T. bessarabicum* x Neodur) (P<0.001; Table 6.2). Averaging over irrigation treatments, amphidiploid lines generally had the highest AGDM followed by R x S DH lines and then ancestral wheat species.

In 2014, there was a trend for the effect of drought (P=0.06). Genotypes differed in the range 5.90 to 49.42 g plant^{-1} under irrigated conditions, and 5.82 to 48.12 g plant^{-1} under droughted conditions, in each case ranging from *T. bessarabicum* 2 to *T. bessarabicum* x Neodur, respectively (P<0.001). There was no irrigation x genotype interaction (P=0.14; Table 6.2). Averaging over irrigation treatments, amphidiploid lines and R x S DH lines generally had higher AGDM than the ancestral wheat species.

Overall AGDM was higher in 2013 by 15% than in 2014 (P<0.001). Averaging over years, drought reduced AGDM form 42.2 to 32.5 g plant^{-1} (P<0.001). Genotypes differed in the range 8.0 to 64.3 g plant^{-1} under irrigated conditions, and 6.5 to 45.7 g plant^{-1} under droughted conditions, in each case ranging from *T. bessarabicum* 2 to *T. bessarabicum* x Neodur, respectively (P<0.001). The decrease in AGDM under drought was in the range 1.6 g plant^{-1} (*T. bessarabicum* 2) to 18.5 g plant^{-1} (*T. bessarabicum* x Neodur) (P<0.001; Table 6.2).

6.3.3.2 Grain yield

In 2013, drought reduced grain yield from 11.31 to 8.52 g plant^{-1} (P=0.01). Genotypes differed in the range 0.19 (*T. bessarabicum* 2) to 28.19 g plant^{-1} (DH line 63) under irrigated conditions, and 0.13 (*T. bessarabicum* 2) to 22.74 g plant^{-1} (DH line 63) under droughted conditions (P<0.001). The decrease under drought was in the range -4.63 (DH line 25) to 7.31 g plant^{-1} (*T. bessarabicum* x Neodur) (P<0.001;

Table 6.3). Averaging over irrigation treatments, R x S DH lines had the highest grain yield followed by amphidiploid lines and then the ancestral wheat species.

In 2014, there was a trend for drought to reduce grain yield (P=0.06). Genotypes differed in the range 0.04 (*T. bessarabicum* 2) to 18.37 g plant^{-1} (DH line 25) under irrigated conditions, and 0.06 (*T. bessarabicum* 2) to 13.16 g plant^{-1} (Rialto) under droughted conditions (P<0.001). Grain yield decrease under drought ranged from -0.50 (*A. speltoides* 3) to 7.42 g plant^{-1} (DH line 25) (P<0.001; Table 6.3). Averaging over years and genotypes, grain yield decreased with drought from 10.18 to 7.74 g plant^{-1} (P=0.001). The decrease in grain yield under drought ranged from -0.88 (*A. speltoides* 3) to 5.76 g plant^{-1} (DH line 63) (P<0.001; Table 6.3).

6.3.3.3 Harvest index

There was no effect of drought on HI in 2013 (P=0.27). Decreases under drought were in the range -0.09 (Savannah) to 0.05 (DH line 63) (P=0.009; Table 6.4). Averaging over irrigation treatments, R x S DH lines generally had highest HI followed by amphidiploid lines and then ancestral wheat species.

In 2014, HI was decreased by drought from 0.21 to 0.19 (P=0.04). Genotypes differed in the range 0.01 (*T. bessarabicum* 2) to 0.44 (DH line 63) under irrigated conditions, and 0.01 (*T. bessarabicum* 2) to 0.37 (Savannah) under droughted conditions (P<0.001). Decreases under drought ranged from -0.03 (*T. bessarabicum* x Karim) to 0.12 (*T. bessarabicum* x Langdon) (P=0.01; Table 6.4). Averaging over irrigation treatments, R x S DH lines had the highest HI followed by amphidiploid lines and then the ancestral wheat species.

6.3.3.4 Plant height

In 2013, there was no effect of irrigation on plant height (P=0.17). Genotypes differed in the range 54.3 (DH line 63) to 146.8 cm (*T. bessarabicum* x Neodur) under irrigated conditions, and 58.4 (DH line 63) to 127.2 cm (*T. bessarabicum* x Neodur) under droughted conditions (P<0.001). The decrease under drought was in the range -6.58 (*T. uratu* 1) to 19.56 cm (*T. bessarabicum* x Neodur) (P=0.009; Table 6.4). Averaging over irrigation treatments, amphidiploid lines were the tallest followed by ancestral wheat species and R x S DH lines.

In 2014, drought reduced plant height from 81.1 to 78.8 cm (P=0.05). Genotypes differed in the range 50.2 (DH line 63) to 112.7 cm (*T. bessarabicum* x Stewart) under irrigated conditions, and 46.9 (Savannah) to 113.9 cm (*T. bessarabicum* x Neodur) under droughted conditions (P<0.001). There was no irrigation x genotype interaction (P=0.99; Table 6.4). Averaging over irrigation treatments, amphidiploid lines were the tallest genotypes followed by the ancestral wheat species and the R x S DH lines.

Table 6.2 Above-ground dry matter per plant (AGDM) for 22 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) in 2013 and 19 genotypes in 2014 and cross-year mean; and above-ground dry matter per main shoot ($AGDM_m$) for 4 Rialto x Savannah DH lines and the parents in 2013, 2014 and cross-year mean; and AGDM per main shoot for 19 genotypes in 2014, and standard errors of the differences of the means (SED) and degrees of freedom (df) under irrigated and droughted treatments.

	Genotypes	AGDM (g plant^{-1})						$AGDM_m$ (g shoot^{-1})					
		2013		2014		2013-14		2013		2014		2013-14	
		Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought
Amphidiploid lines	*T. bessarabicum* x Azaziah	49.91	33.00	34.45	26.75	42.18	29.87	-	-	3.85	4.31	-	-
	T. bessarabicum x Creso	59.91	45.32	46.34	39.92	53.12	42.62	-	-	4.04	4.14	-	-
	T. bessarabicum x Karim	36.27	29.56	38.04	34.87	37.16	32.22	-	-	5.69	5.59	-	-
	T. bessarabicum x Langdon	66.67	53.28	41.05	30.53	53.86	41.90	-	-	4.63	3.04	-	-
	T. bessarabicum x Macoun	52.84	44.14	45.77	35.33	49.30	39.74	-	-	3.51	3.59	-	-
	T. bessarabicum x Neodur	79.09	43.33	49.42	48.12	64.25	45.72	-	-	4.25	5.22	-	-
	T. bessarabicum x Stewart	66.23	49.46	45.62	33.87	55.93	41.67	-	-	4.86	3.41	-	-
R x S DH lines	Rialto	45.76	40.02	43.72	38.12	44.74	39.07	4.65	5.06	5.87	6.00	5.56	5.77
	Savannah	36.16	21.80	31.91	27.67	34.04	24.74	4.41	5.18	4.35	4.23	4.43	4.20
	Line 20	42.45	35.26	42.66	34.54	42.55	34.90	3.22	5.16	5.18	5.05	4.99	5.08
	Line 25	31.30	37.67	46.45	32.20	38.87	34.94	3.28	4.88	5.28	4.44	4.77	4.62
	Line 63	47.02	41.17	38.46	31.71	42.74	36.44	4.69	5.20	4.47	4.10	4.17	4.30
	Line 88	43.28	40.82	41.63	37.38	42.46	39.10	4.65	4.10	4.70	5.02	4.70	5.06
Ancestral wheat species	*T. bessarabicum* 1	15.71	7.11	9.80	9.48	12.75	8.30	-	-	1.37	1.42	-	-
	T. bessarabicum 2	10.17	7.12	5.90	5.82	8.04	6.47	-	-	0.78	0.78	-	-
	T. bessarabicum 3	13.68	10.27	19.10	8.86	16.39	9.57	-	-	2.21	2.20	-	-
	T. uratu 1	27.99	20.21	-	-	-	-	-	-	-	-	-	-
	T. uratu 2	32.56	17.96	-	-	-	-	-	-	-	-	-	-
	T. uratu 3	38.45	23.69	-	-	-	-	-	-	-	-	-	-
	A. speltoides 1	67.93	35.28	47.38	31.99	57.66	33.64	-	-	1.03	1.12	-	-
	A. speltoides 2	67.71	40.06	44.97	38.49	56.34	39.27	-	-	0.83	0.97	-	-
	A. speltoides 3	54.57	36.21	45.56	39.70	50.06	37.96	-	-	0.83	0.84	-	-
Grand Mean		44.80	32.40	37.80	30.81	42.23	32.53	4.15	4.93	3.57	3.45	4.77	4.84
SED (df)	*Year*	-		-		*0.692**** *(4)*		-		-		*0.097*^{ns} *(4)*	
	Irrigation	*0.680*** *(2)*		*1.718*$^{(0.06)}$ *(2)*		*0.885**** *(4)*		*0.343*^{ns} *(2)*		*0.364*^{ns} *(2)*		*0.141*^{ns} *(4)*	
	Genotype	*2.531**** *(84)*		*2.559**** *(72)*		*1.793**** *(144)*		*0.495*^{ns} *(20)*		*0.473**** *(72)*		*0.296**** *(40)*	
	Irri. x Gen.	*3.562**** *(84)*		*3.919*^{ns} *(72)*		*2.622**** *(144)*		*0.725*^{ns} *(20)*		*0.745*^{ns} *(72)*		*0.408*^{ns} *(40)*	
	Year x Gen.	-		-		*2.563**** *(144)*		-		-		*0.394*^{ns} *(40)*	

N.B: ***denotes $P<0.001$; $^{**}P<0.01$ and $^{*}P<0.05$ significance levels; ^{ns} = not significant.

Table 6.3 Grain yield per plant (GY) for 22 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) in 2013 and 19 genotypes in 2014 and cross-year mean; and grain yield per main shoot (GY_m) for 4 Rialto x Savannah DH lines and the parents in 2013, 2014 and cross-year mean; and grain yield per main shoot for 19 genotypes in 2014, and standard errors of the differences of the means (SED) and degrees of freedom (df) under irrigated and droughted treatments.

	Genotypes	GY (g plant^{-1})						GY_m (g shoot^{-1})					
		2013		2014		2013-14		2013		2014		2013-14	
		Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought
Amphidiploid lines	*T. bessarabicum* x Azaziah	10.74	7.37	5.65	4.62	8.20	5.99	-	-	1.18	1.00	-	-
	T. bessarabicum x Creso	13.45	9.27	6.59	6.07	10.02	7.67	-	-	0.88	0.86	-	-
	T. bessarabicum x Karim	10.96	9.04	12.20	12.10	11.58	10.57	-	-	1.88	1.95	-	-
	T. bessarabicum x Langdon	9.56	6.03	7.10	1.71	8.33	3.87	-	-	1.27	0.40	-	-
	T. bessarabicum x Macoun	11.30	7.39	4.49	3.83	7.89	5.61	-	-	0.70	0.86	-	-
	T. bessarabicum x Neodur	16.00	8.69	8.82	7.72	12.41	8.20	-	-	1.26	1.36	-	-
	T. bessarabicum x Stewart	9.58	4.92	7.39	4.71	8.49	4.81	-	-	1.40	0.71	-	-
R x S DH lines	Rialto	23.88	17.48	17.19	13.16	20.54	15.32	2.44	2.38	2.62	2.67	2.57	2.60
	Savannah	17.05	12.32	11.24	10.19	14.15	11.26	2.64	2.39	2.07	2.04	2.21	2.13
	Line 20	23.09	18.23	16.57	12.31	19.83	15.27	2.44	2.82	2.39	2.32	2.40	2.45
	Line 25	15.02	19.65	18.37	10.95	16.69	15.30	1.90	2.76	2.31	1.90	2.21	2.12
	Line 63	28.19	22.74	17.04	10.97	22.61	16.86	1.84	2.82	2.06	1.83	2.01	2.08
	Line 88	22.89	20.80	15.51	12.11	19.20	16.45	2.59	2.77	2.18	2.21	2.28	2.35
Ancestral wheat species	*T. bessarabicum* 1	0.95	0.37	0.48	0.55	0.72	0.46	-	-	0.14	0.15	-	-
	T. bessarabicum 2	0.19	0.13	0.04	0.06	0.11	0.10	-	-	0.01	0.01	-	-
	T. bessarabicum 3	1.17	0.66	1.24	0.67	1.20	0.66	-	-	0.24	0.23	-	-
	T. uratu 1	8.06	5.63	-	-	-	-	-	-	-	-	-	-
	T. uratu 2	6.56	3.67	-	-	-	-	-	-	-	-	-	-
	T. uratu 3	10.10	5.53	-	-	-	-	-	-	-	-	-	-
	A. speltoides 1	5.76	2.70	7.08	3.00	6.42	2.85	-	-	0.18	0.16	-	-
	A. speltoides 2	2.68	1.96	2.43	2.63	2.55	2.29	-	-	0.09	0.11	-	-
	A. speltoides 3	1.64	2.89	3.49	4.00	2.57	3.45	-	-	0.15	0.13	-	-
Grand Mean		11.31	8.52	8.57	6.39	10.18	7.74	2.31	2.66	1.21	1.10	2.28	2.29
SED (df)	*Year*	-		-		0.495^{**} (4)		-		-		0.056^{ns} (4)	
	Irrigation	0.336^{*} (2)		$0.550^{(0.06)}$ (2)		0.306^{***} (4)		0.170^{ns} (2)		0.155^{ns} (2)		0.113^{ns} (4)	
	Genotype	1.077^{***} (84)		0.898^{***} (72)		0.728^{***} (144)		0.299^{ns} (20)		0.199^{***} (72)		0.163^{*} (40)	
	Irri. x Gen.	1.526^{***} (84)		1.353^{***} (72)		1.048^{***} (144)		0.422^{ns} (20)		0.314^{ns} (72)		0.238^{ns} (40)	
	Year x Gen.	-		-		1.118^{***} (144)		-		-		0.217^{ns} (40)	

N.B: ***denotes $P<0.001$; $^{**}P<0.01$ and $^{*}P<0.05$ significance levels; ^{ns} = not significant.

Table 6.4 Harvest index (HI) and plant height for 22 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) in 2013 and 19 genotypes in 2014 and cross-year mean, and standard errors of the differences of the means (SED) and degrees of freedom (df) under irrigated and droughted treatments.

	Genotypes	HI						Plant height (cm)					
		2013		2014		2013-14		2013		2014		2013-14	
		Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought
Amphidiploid lines	*T. bessarabicum* x Azaziah	0.22	0.22	0.16	0.17	0.19	0.20	103.0	102.9	98.4	98.6	100.7	100.7
	T. bessarabicum x Creso	0.23	0.20	0.14	0.15	0.18	0.18	100.8	82.6	82.3	75.5	91.5	79.0
	T. bessarabicum x Karim	0.30	0.30	0.32	0.35	0.31	0.33	71.3	74.1	66.9	67.8	69.1	71.0
	T. bessarabicum x Langdon	0.14	0.12	0.17	0.05	0.16	0.09	116.6	119.0	107.7	94.4	112.2	106.7
	T. bessarabicum x Macoun	0.21	0.17	0.10	0.11	0.15	0.14	114.2	104.8	92.7	86.7	103.5	95.8
	T. bessarabicum x Neodur	0.20	0.20	0.18	0.16	0.19	0.18	146.8	127.2	111.4	113.9	129.1	120.6
	T. bessarabicum x Stewart	0.14	0.10	0.16	0.14	0.15	0.12	139.2	126.2	112.7	110.2	126.0	118.2
R x S DH lines	Rialto	0.52	0.43	0.39	0.35	0.46	0.39	64.4	67.2	60.8	58.6	62.6	62.9
	Savannah	0.48	0.57	0.35	0.37	0.41	0.47	59.8	62.2	50.8	46.9	55.3	54.5
	Line 20	0.54	0.52	0.39	0.35	0.47	0.44	61.1	59.7	52.6	51.0	56.8	55.3
	Line 25	0.49	0.52	0.40	0.34	0.44	0.43	58.4	62.7	57.0	57.1	57.7	59.9
	Line 63	0.60	0.55	0.44	0.35	0.52	0.45	54.3	58.4	50.2	47.0	52.3	52.7
	Line 88	0.53	0.51	0.37	0.32	0.45	0.42	71.4	68.2	63.6	63.3	67.5	65.8
Ancestral wheat species	*T. bessarabicum* 1	0.06	0.05	0.04	0.05	0.05	0.05	91.4	90.2	76.8	77.0	84.1	83.6
	T. bessarabicum 2	0.02	0.02	0.01	0.01	0.01	0.01	82.6	73.1	68.6	68.2	75.6	70.6
	T. bessarabicum 3	0.08	0.06	0.07	0.07	0.08	0.07	96.6	100.3	88.0	83.7	92.3	92.0
	T. uratu 1	0.29	0.28	-	-	-	-	102.4	109.0	-	-	-	-
	T. uratu 2	0.20	0.20	-	-	-	-	101.9	100.0	-	-	-	-
	T. uratu 3	0.27	0.23	-	-	-	-	121.7	120.3	-	-	-	-
	A. speltoides 1	0.08	0.08	0.15	0.09	0.12	0.09	116.6	107.2	106.8	105.4	111.7	106.3
	A. speltoides 2	0.04	0.05	0.05	0.06	0.05	0.06	100.1	100.6	98.4	101.6	99.3	101.1
	A. speltoides 3	0.03	0.08	0.08	0.10	0.05	0.09	101.6	95.8	94.2	90.3	97.9	93.0
Grand Mean		0.26	0.25	0.21	0.19	0.23	0.22	94.4	91.4	81.1	78.8	86.6	83.7
SED (df)	*Year*	-		-		0.007^{**} *(4)*		-		-		3.16^{*} *(4)*	
	Irrigation	0.006^{ns} *(2)*		0.004^{*} *(2)*		0.004^{*} *(4)*		1.41^{ns} *(2)*		0.55^{*} *(2)*		0.59^{**} *(4)*	
	Genotype	0.018^{***} *(84)*		0.020^{***} *(72)*		0.010^{***} *(144)*		3.58^{***} *(84)*		4.43^{***} *(72)*		2.87^{***} *(144)*	
	Irri. x Gen.	0.026^{**} *(84)*		0.028^{**} *(72)*		0.019^{***} *(144)*		5.15^{**} *(84)*		6.12^{ns} *(72)*		3.99^{ns} *(144)*	
	Year x Gen.	-		-		0.020^{***} *(144)*		-		-		5.06^{***} *(144)*	

N.B: ***denotes $P<0.001$; $^{**}P<0.01$ and $^{*}P<0.05$ significance levels; ^{ns} = not significant.

6.3.3.5 Grains ear^{-1}

In 2013, there was no effect of drought (P=0.30). Genotypes differed in the range 2.1 (*A. speltoides* 3) to 51.8 grains ear^{-1} (DH line 63); and 2.5 (*T. bessarabicum* 2) to 46.8 grains ear^{-1} (DH line 88) under irrigated and droughted conditions, respectively (P<0.001). The decrease under drought was in the range -12.7 (*T. bessarabicum* x Karim) to 9.3 (DH line 20) (P=0.04; Table 6.5).

In 2014, there was again no effect of irrigation (P=0.64). Genotypes differed in the range 2.2 (*T. bessarabicum* 2) to 38.5 grains ear^{-1} (Rialto) under irrigated conditions, and 2.4 (*T. bessarabicum* 2) to 39.3 grains ear^{-1} (DH line 20) under droughted conditions (P<0.001). There was no irrigation x genotype interaction (P=0.59; Table 6.5).

Averaging over genotypes, there was no effect of year (P=0.18) or irrigation treatment (P=0.78). Genotypes differed in the range 2.7 (*A. speltoides* 2) to 44.8 grains ear^{-1} (DH line 63); and 2.4 (*T. bessarabicum* 2) to 40.7 grains ear^{-1} (DH line 20) under irrigated and droughted conditions, respectively (P<0.001). The irrigation x genotype interaction was not significant (P=0.20; Table 6.5). Averaging over years and irrigation treatments, R x S DH lines had more grains ear^{-1} than amphidiploid lines which, in turn, had more grains ear^{-1} than the ancestral wheat species.

6.3.3.6 Ears plant^{-1}

In 2013, drought reduced ears plant^{-1} from 24.5 to 37.6 (P=0.03). Genotypes differed in the range 9.0 (*T. bessarabicum* x Karim) to 143.3 (*A. speltoides* 3) ears plant^{-1} under irrigated conditions, and 5.33 (*T. bessarabicum* 1) to 105.7 (*A. speltoides* 3) ears plant^{-1} under droughted conditions (P<0.001). The reduction under drought was in the range -3.0 (*T. bessarabicum* x Macoun) to 63.7 ears plant^{-1} (*A. speltoides* 1) (P<0.001; Table 6.5).

In 2014, there was a trend for a reduction in ears plant^{-1} under drought from 32.3 to 23.0 ears plant^{-1} (P=0.06). Genotypes differed in the range 5.3 (*T. bessarabicum* 2) to 133.3 ears plant^{-1} (*A. speltoides* 3) under irrigated conditions, and 4.3 (*T. bessarabicum* 2) to 96.7 ears plant^{-1} (*A. speltoides* 2) under droughted conditions (P<0.001). Decreases in ears plant^{-1} under drought were in the range 1.0 (*T.*

bessarabicum x Langdon and *T. bessarabicum* 2) to 49.0 ears plant^{-1} (*A. speltoides* 1) (P<0.001; Table 6.5).

Averaging over years, ears plant^{-1} was decreased by drought from 34.2 to 24.0 ears plant^{-1} (P=0.003). Genotypes differed in the range 9.5 (*T. bessarabicum* 3) to 138.3 ears plant^{-1} (*A. speltoides* 3) under irrigated conditions, and 6.3 (*T. bessarabicum* 1) to 98.0 ears plant^{-1} (*A. speltoides* 3) under droughted conditions (P<0.001). The decrease under drought ranged from -0.2 (*T. bessarabicum* x Karim) to 56.0 (*A. speltoides* 1) ears plant^{-1} (P<0.001; Table 6.5). Averaging over years and irrigation treatments, *A. speltoides* accessions produced most ears plant^{-1} followed by R x S DH lines and amphidiploid lines, with the *T. bessarabicum* accessions having fewest ears per plant.

Table 6.5 Grains per ear and ears per plant for 22 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) in 2013 and 19 genotypes in 2014 and cross-year mean under irrigated and droughted treatments, and standard errors of the differences of the means (SED) and degrees of freedom (df) under irrigated and droughted treatments.

	Genotypes	Grains ear^{-1}						Ears plant^{-1}					
		2013		2014		2013-14		2013		2014		2013-14	
		Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought
Amphidiploid lines	*T. bessarabicum* x Azaziah	18.40	15.93	12.27	11.63	15.34	13.78	20.67	15.67	18.67	13.67	19.67	14.67
	T. bessarabicum x Creso	15.19	13.54	12.66	15.30	13.93	14.42	22.67	19.33	17.67	12.67	20.17	16.00
	T. bessarabicum x Karim	26.62	20.48	26.11	30.47	26.36	25.47	9.00	11.00	11.00	9.33	10.00	10.17
	T. bessarabicum x Langdon	11.62	9.37	14.57	3.62	13.10	6.49	23.00	19.00	16.00	15.00	19.50	17.00
	T. bessarabicum x Macoun	20.68	11.41	5.43	7.64	13.06	9.53	14.67	17.67	28.67	17.67	21.67	17.67
	T. bessarabicum x Neodur	20.20	15.32	13.78	18.19	16.99	16.76	21.33	15.33	23.33	17.33	22.33	16.33
	T. bessarabicum x Stewart	16.32	9.98	11.63	11.44	13.98	10.71	17.00	13.67	18.67	14.33	17.83	14.00
R x S DH lines	Rialto	39.62	35.32	38.50	35.56	39.06	35.44	20.00	11.67	12.33	10.67	16.17	11.17
	Savannah	28.93	32.98	24.72	30.04	26.83	31.51	17.67	11.00	11.67	10.00	14.67	10.50
	Line 20	36.09	42.08	32.83	39.26	34.46	40.67	20.67	10.00	15.33	11.67	18.00	10.83
	Line 25	28.19	40.86	32.91	26.99	30.55	33.92	18.67	10.00	14.33	11.67	16.50	10.83
	Line 63	51.77	44.81	37.87	36.20	44.82	40.50	15.33	14.00	14.00	11.33	14.67	12.67
	Line 88	37.84	46.79	27.43	25.49	32.64	36.14	16.67	10.00	14.00	12.67	15.33	11.33
Ancestral wheat species	*T. bessarabicum* 1	12.80	14.25	13.79	18.95	13.30	16.60	16.67	5.33	9.33	7.33	13.00	6.33
	T. bessarabicum 2	4.18	2.47	2.19	2.38	3.18	2.42	14.00	10.00	5.33	4.33	9.67	7.17
	T. bessarabicum 3	20.51	12.82	25.38	26.96	22.94	19.89	10.67	9.33	8.33	5.00	9.50	7.17
	T. uratu 1	23.02	26.06	-	-	-	-	41.00	20.33	-	-	-	-
	T. uratu 2	16.83	18.26	-	-	-	-	48.00	23.00	-	-	-	-
	T. uratu 3	23.07	24.29	-	-	-	-	51.00	21.67	-	-	-	-
	A. speltoides 1	6.84	7.60	8.75	8.80	7.80	8.20	136.33	72.67	113.67	64.67	125.00	68.67
	A. speltoides 2	2.89	3.35	2.45	4.49	2.67	3.92	129.33	92.33	127.67	96.67	128.50	94.50
	A. speltoides 3	2.10	3.97	4.21	8.30	3.16	6.13	143.33	105.67	133.33	90.33	138.33	98.00
Grand Mean		21.08	20.54	18.29	19.04	19.69	19.61	37.62	24.48	32.28	22.96	34.24	23.95
SED (df)	*Year*	-		-		1.230^{ns} *(4)*		-		-		1.435^{ns} *(4)*	
	Irrigation	0.652^{ns} *(2)*		0.932^{ns} *(2)*		0.666^{ns} *(4)*		2.321^{*} *(2)*		$2.466^{(0.06)}$ *(2)*		1.667^{**} *(4)*	
	Genotype	3.117^{***} *(84)*		3.884^{***} *(72)*		2.546^{***} *(144)*		4.894^{***} *(84)*		4.845^{***} *(72)*		3.507^{***} *(144)*	
	Irri. x Gen.	4.356^{*} *(84)*		5.438^{ns} *(72)*		3.567^{ns} *(144)*		7.149^{***} *(84)*		7.110^{***} *(72)*		5.107^{***} *(144)*	
	Year x Gen.	-		-		3.714^{***} *(144)*		-		-		5.036^{ns} *(144)*	

N.B: ***denotes $P<0.001$; $^{**}P<0.01$ and $^{*}P<0.05$ significance levels; ^{ns} = not significant. For grains ear^{-1} ANOVA was carried out using transformed data (squire root).

6.3.3.7 Thousand grain weight

In 2013, there was a trend for TGW to increase under drought (P=0.06). Genotypes differed in TGW in the range 3.68 (*T. bessarabicum* 2) to 45.26 g (*T. bessarabicum* x Karim) under irrigated conditions, and 5.40 (*T. bessarabicum* 2) to 49.23 g (DH line 25) under droughted conditions (P<0.001). The increase under drought was in the range -3.70 (*T. bessarabicum* x Creso) to 18.33 (DH line 25) (P<0.001; Table 6.6). Averaging over irrigation treatments, R x S DH lines and amphidiploid lines generally had higher grain weight than ancestral wheat species.

In 2014, there was no effect of irrigation (P=0.17). Genotypes differed in TGW in the range 3.64 (*T. bessarabicum* 1) to 43.1 g (*T. bessarabicum* x Karim) under irrigated conditions, and 3.8 (*T. bessarabicum* 1) to 42.6 g (*T. bessarabicum* x Karim) under droughted conditions (P<0.001). The decrease under drought was in the range -5.20 (*T. bessarabicum* x Azaziah) to 7.44 g (DH line 20) (P=0.02; Table 6.6). Averaging over irrigation treatments, R x S DH lines and amphidiploid lines had similarly higher grain weight than ancestral wheat species.

Averaging over genotypes, TGW was higher in 2013 by 9% than in 2014 (P<0.001). Grain weight was not affected by drought (P=0.17). Genotypes overall differed in the range 4.1 (*T. bessarabicum* 2) to 44.2 g (*T. bessarabicum* x Karim) under irrigated, and 5.6 (*A. speltoides* 1) to 42.9 g (*T. bessarabicum* x Karim) under droughted conditions (P<0.001). The reduction in TGW under drought ranged from -7.41 (DH line 25) to 2.03 g (*T. bessarabicum* x Macoun) (P<0.001; Table 6.6).

6.3.3.8 Spikelet number

Averaging over years, drought did not affect the number of spikelets per ear (P=0.18), and genotypes ranged from 8.78 (*T. bessarabicum* 2) to 20.28 (Rialto) under irrigated conditions, and 8.69 (*T. bessarabicum* 2) to 27.5 (Rialto) spikelet ear^{-1} under droughted conditions (P<0.001). There was no irrigation x genotype interaction (P=0.39; Table 6.6). Averaging over years and irrigation treatments, R x S DH lines had most spikelets per ear followed by amphidiploid lines, *A. speltoides* accessions and *T. bessarabicum* accessions.

Table 6.6 Thousand grain weight (TGW) and spikelets per ear for 22 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) in 2013 and 19 genotypes in 2014 and cross-year mean under irrigated and droughted treatments, and standard errors of the differences of the means (SED) and degrees of freedom (df).

	Genotypes	TGW (g)						Spikelet ear^{-1}					
		2013		2014		2013-14		2013		2014		2013-14	
		Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought
Amphidiploid lines	*T. bessarabicum* x Azaziah	30.50	29.56	24.92	30.11	27.71	29.84	12.67	14.33	14.67	19.11	13.67	16.72
	T. bessarabicum x Creso	39.62	35.92	31.85	33.25	35.74	34.59	12.33	12.33	18.56	18.67	15.44	15.50
	T. bessarabicum x Karim	45.26	43.22	43.11	42.58	44.18	42.90	11.00	9.00	16.44	18.44	13.72	13.72
	T. bessarabicum x Langdon	37.72	36.32	33.80	32.68	35.76	34.50	13.00	12.00	20.11	19.67	16.56	15.83
	T. bessarabicum x Macoun	37.78	36.58	31.59	28.72	34.68	32.65	12.01	12.67	20.67	21.00	16.34	16.83
	T. bessarabicum x Neodur	37.62	37.51	27.30	30.44	32.46	33.98	10.67	12.44	20.56	23.00	15.61	17.72
	T. bessarabicum x Stewart	34.65	37.48	34.22	32.88	34.44	35.18	11.33	10.11	19.22	20.56	15.28	15.33
R x S DH lines	Rialto	30.52	43.21	36.17	35.14	33.34	39.18	20.67	22.67	19.89	21.00	20.28	21.83
	Savannah	33.84	36.15	40.73	35.43	37.29	35.79	18.44	16.26	19.11	19.89	18.78	18.08
	Line 20	30.85	44.06	34.21	26.77	32.53	35.42	18.22	19.33	20.89	21.56	19.56	20.44
	Line 25	30.90	49.23	39.42	35.92	35.16	42.57	16.00	19.56	19.22	17.56	17.61	18.56
	Line 63	37.97	38.33	33.67	29.46	35.82	33.89	17.11	18.89	18.00	18.89	17.56	18.89
	Line 88	36.23	44.71	41.60	37.49	38.92	41.10	19.33	19.33	20.11	21.78	19.72	20.56
Ancestral wheat species	*T. bessarabicum* 1	4.53	4.83	3.64	3.86	4.09	4.35	9.33	8.33	10.44	9.78	9.89	9.06
	T. bessarabicum 2	3.68	5.40	4.43	5.90	4.06	5.65	9.00	9.38	8.56	8.00	8.78	8.69
	T. bessarabicum 3	5.61	5.87	5.69	5.50	5.65	5.68	9.33	9.67	9.33	10.22	9.33	9.94
	T. uratu 1	8.59	10.63	-	-	-	-	19.00	17.33	-	-	-	-
	T. uratu 2	8.19	9.12	-	-	-	-	17.67	17.00	-	-	-	-
	T. uratu 3	8.61	10.60	-	-	-	-	18.67	17.67	-	-	-	-
	A. speltoides 1	6.18	5.71	7.26	5.39	6.72	5.55	12.33	10.11	12.78	15.89	12.55	13.00
	A. speltoides 2	7.57	5.85	7.89	7.09	7.73	6.47	11.22	9.00	14.22	15.11	12.72	12.06
	A. speltoides 3	4.50	6.91	6.23	5.85	5.36	6.38	9.34	8.89	12.00	13.89	10.67	11.39
Grand Mean		23.68	26.24	25.67	24.44	25.88	26.61	14.03	13.92	16.57	17.58	14.95	15.48
SED (df)	*Year*	-		-		0.300^{***} *(4)*		-		-		0.146^{***} *(4)*	
	Irrigation	0.666^{ns} *(2)*		0.761^{ns} *(2)*		0.524^{ns} *(4)*		0.437^{ns} *(2)*		0.381^{ns} *(2)*		0.332^{ns} *(4)*	
	Genotype	1.561^{***} *(84)*		1.387^{***} *(72)*		1.089^{***} *(144)*		0.923^{***} *(84)*		1.039^{***} *(72)*		0.966^{***} *(144)*	
	Irri. x Gen.	2.258^{***} *(84)*		2.056^{**} *(72)*		1.588^{***} *(144)*		1.348^{ns} *(84)*		1.480^{ns} *(72)*		1.011^{ns} *(144)*	
	Year x Gen.	-		-		1.529^{***} *(144)*		-		-		0.966^{***} *(144)*	

N.B: ***denotes $P<0.001$; $^{**}P<0.01$ and $^{*}P<0.05$ significance levels; ^{ns} = not significant.
ANOVA was carried out using transformed data for TGW (squire root).

6.3.3.9 Flag-leaf area (FLA) and specific weight (FLSW)

Drought did not affect flag-leaf area at flowering (P=0.61), genotypes differed in the range 6.4 (*T. bessarabicum* 2) to 56.8 (line 20) cm^{-2} under irrigated conditions, and 6.0 (*T. bessarabicum* 2) to 59.3 (Rialto) cm^{-2} under droughted conditions ($P<0.001$). The irrigation x genotype interaction was not significant ($P=0.18$; Table 6.7). Averaging over genotypes, R x S DH lines and amphidiploid lines had larger flag-leaf area than the ancestral wheat genotypes.

For flag-leaf specific weight (FLSW), genotypes ranged from 24.2 (*A. speltoides* 3) to 62.1 (*T. bessarabicum* 3) g m^{-2} under irrigated conditions, and from 24.9 (*A. speltoides* 3) to 58.7 (*T. bessarabicum* 3) g m^{-2} under droughted conditions ($P<0.001$). There was no irrigation effect ($P=0.89$) or irrigation x genotype interaction ($P=0.36$; Table 6.7). Averaging over genotypes, *T. bessarabicum* accessions had highest FLSW, then amphidiploid lines and R x S DH lines; and *A. speltoides* accessions had the lowest FLSW.

Table 6.7 Flag-leaf area (FLA; at anthesis) and flag-leaf specific weight (FLSW; at anthesis) for 19 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) in 2014 under irrigated and droughted treatments, and standard errors of the differences of the means (SED) and degrees of freedom (df).

	Genotypes	FLA (cm^2)		FLSW ($g\ m^{-2}$)	
		2014		2014	
		Irrigated	Drought	Irrigated	Drought
Amphidiploid lines	*T. bessarabicum* x Azaziah	37.86	34.67	47.2	49.3
	T. bessarabicum x Creso	40.97	43.86	49.9	51.6
	T. bessarabicum x Karim	42.62	40.84	55.4	52.4
	T. bessarabicum x Langdon	41.18	36.49	43.2	47.8
	T. bessarabicum x Macoun	42.68	47.83	43.8	44.9
	T. bessarabicum x Neodur	45.99	45.31	48.0	51.5
	T. bessarabicum x Stewart	43.96	37.33	44.1	43.8
R x S DH lines	Rialto	51.49	59.26	49.2	46.7
	Savannah	49.40	51.21	45.1	43.2
	Line 20	56.81	53.86	44.1	42.9
	Line 25	47.58	46.38	45.0	41.5
	Line 63	44.48	56.00	47.5	40.9
	Line 88	55.85	57.97	40.8	41.1
Ancestral species	*T. bessarabicum* 1	8.18	7.63	52.3	53.8
	T. bessarabicum 2	6.42	5.97	43.9	44.5
	T. bessarabicum 3	11.91	10.46	62.1	58.7
	A. speltoides 1	13.94	13.47	25.0	26.0
	A. speltoides 2	10.55	11.76	25.4	25.0
	A. speltoides 3	9.67	9.91	24.2	24.9
Grand Mean		34.82	35.27	44.0	43.7
SED (df)					
	Irrigation	*0.772^{ns} (2)*		*0.381^{ns} (2)*	
	Genotype	*2.581*** (72)*		*1.039 *** (72)*	
	Irri. x Gen.	*3.635^{ns} (72)*		*1.480^{ns} (72)*	

N.B: ***denotes $P<0.001$; $^{**}P<0.01$ and $^{*}P<0.05$ significance levels; ^{ns} = not significant

6.3.4 Water uptake and water-use efficiency

6.3.4.1 Cumulative water uptake

In 2013, the water uptake from transplanting to harvest was 22.4 l $plant^{-1}$ in the irrigated treatment and 13.4 l $plant^{-1}$ in the droughted treatment ($P<0.05$). Genotypes differed in the range 12.3 (*T. bessarabicum* 2) to 37.5 l $plant^{-1}$ (DH line 88), and 7.6 (*T. bessarabicum* 1) to 16.7 (DH line 63) under irrigated and droughted conditions, respectively ($P<0.05$; Fig. 6.3a). Averaging over irrigation treatments, *A. speltoides* accessions used most water (20.5 l $plant^{-1}$) followed by R x S DH lines (19.6 l $plant^{-1}$), amphidiploids (18.9 l $plant^{-1}$), *T. uratu* accessions (17.2 l $plant^{-1}$) and *T. bessarabicum* accessions (10.5 l $plant^{-1}$) ($P<0.05$).

In 2014, plants used 22.6 l plant^{-1} in the irrigated treatment and 15.1 l plant^{-1} in the droughted treatment form transplanting to harvest (P<0.05). Genotypes ranged from 12.1 (*T. bessarabicum* 2) to 27.1 l plant^{-1} (*A. speltoides* 3) under irrigated, and 8.8 (*T. bessarabicum* 2) to 18.3 l plant^{-1} (*T. bessarabicum* x Neodur) under droughted conditions (P<0.05; Fig. 6.3b). Averaging over irrigation treatments, similarly *A. speltoides* accessions (21.0 l plant^{-1}) used the highest amount of water followed by R x S DH lines (20.3 l plant^{-1}), amphidiploids (19.8 l plant^{-1}) and *T. bessarabicum* accessions (11.6 l plant^{-1}) (P<0.05).

Averaging over years, genotypes ranged in water uptake from 12.2 (*T. bessarabicum* 2) to 31.5 l plant^{-1} (DH line 88) under irrigated and 8.2 (*T. bessarabicum* 2) to 16.7 l plant^{-1} (*T. bessarabicum* x Neodur and *T. bessarabicum* x Creso) under droughted conditions (P<0.05; Fig. 6.3c). Averaging over years and irrigation treatments, *A. speltoides* accessions (20.7 l plant^{-1}) again used most water followed by R x S DH lines (20.0 l plant^{-1}), amphidiploids (19.3 l plant^{-1}) and *T. bessarabicum* accessions (11.0 l plant^{-1}) (P<0.05).

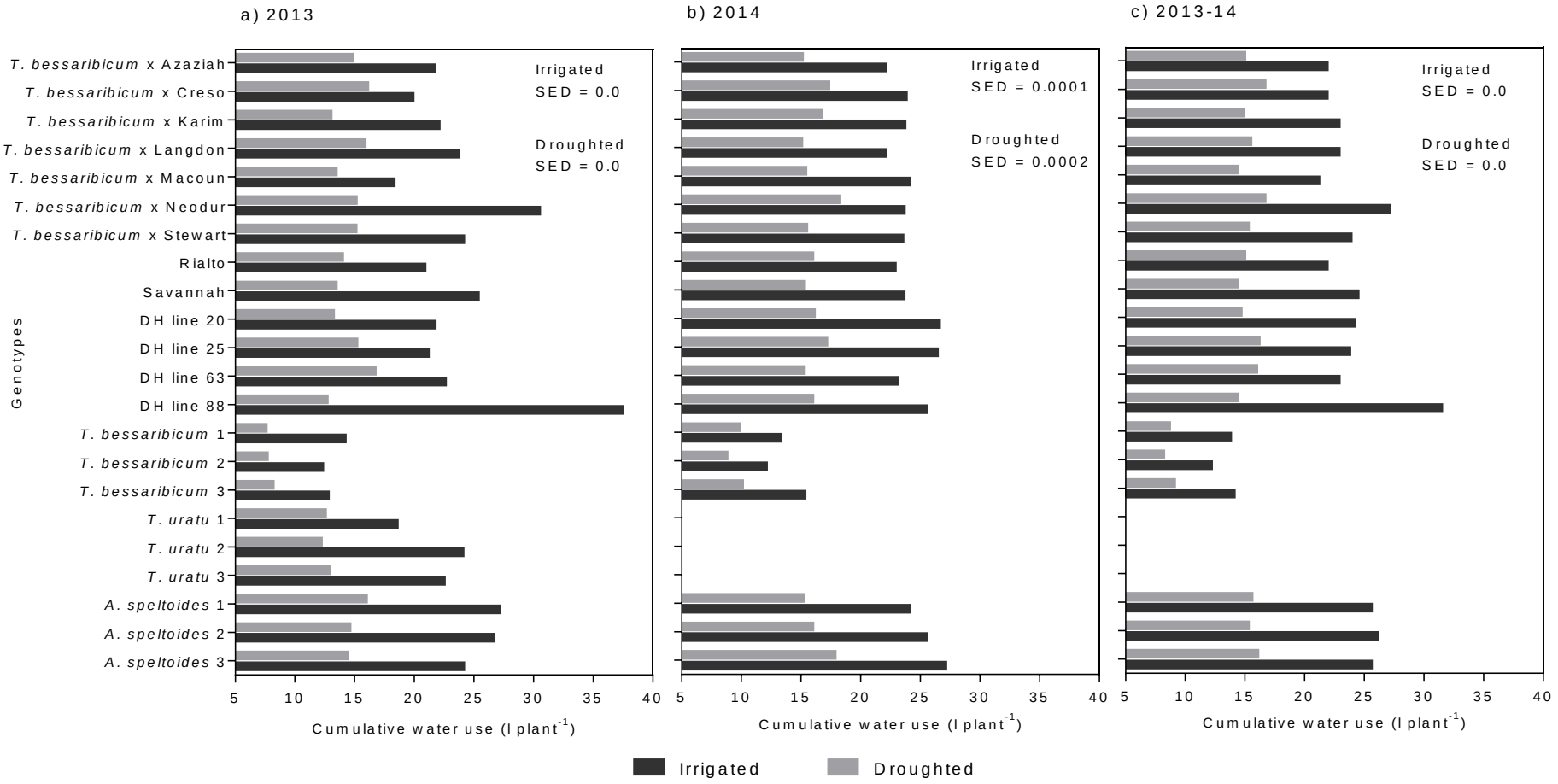


Figure 6.3 Cumulative water uptake per plant (WU) from transplanting to harvest for 22 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) in (a) 2013 and 19 genotypes in (b) 2014 and (c) cross-year mean in irrigated and droughted treatments.

6.3.4.2 Water-use efficiency

Drought overall increased WUE_{AGDM} from 1.97 to 2.31 g l^{-1} in 2013 (P=0.008). Genotypes differed in the range 0.83 (*T. bessarabicum* 2) to 3.01 g l^{-1} (*T. bessarabicum* x Creso); and 0.93 (*T. bessarabicum* 1 and 2) to 3.36 g l^{-1} (*T. bessarabicum* x Langdon) under irrigated and droughted conditions, respectively (P<0.001). The increase under drought ranged from -0.30 (*A. speltoides* 1) to 2.05 (DH line 88) g l^{-1} (P<0.001; Table 6.8). Averaging over irrigation treatments, amphidiploid lines had highest WUE_{AGDM} followed by R x S DH lines and then the ancestral wheat species.

In 2014, there was a trend for an increase in WUE_{AGDM} under drought (P=0.06). Genotypes differed in the range 0.49 (*T. bessarabicum* 2) to 2.09 g l^{-1} (*T. bessarabicum* x Neodur) under irrigated conditions, and 0.66 (*T. bessarabicum* 2) to 2.63 g l^{-1} (*T. bessarabicum* x Neodur) under droughted conditions (P<0.001). There was no irrigation x genotype interaction (P=0.13; Table 6.8). Averaging over irrigation treatments, amphidiploid lines and R x S DH lines had higher WUE_{AGDM} than ancestral wheat species.

Averaging over years, drought increased WUE_{AGDM} from 1.82 to 2.19 g l^{-1} (P=0.001). Genotypes differed in the range 0.66 (*T. bessarabicum* 2) to 9.48 g l^{-1} (*T. bessarabicum* x Creso) under irrigated conditions, and 0.79 (*T. bessarabicum* 2) to 2.78 g l^{-1} (*T. bessarabicum* x Macoun) under droughted conditions (P<0.001). Increases in WUE_{AGDM} under drought ranged from -0.09 (*T. bessarabicum* 3) to 1.38 (DH line 88) g l^{-1} (P<0.001; Table 6.8).

Table 6.8 Water-use efficiency from transplanting to harvest (WUE_{AGDM}) for 22 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) in 2013 and 19 genotypes in 2014 and cross-year mean under irrigated and droughted treatments, and standard errors of the differences of the means (SED) and degrees of freedom (df).

	Genotypes	WUE_{AGDM} (g l^{-1})					
		2013		2014		2013-14	
		Irrigated	Drought	Irrigated	Drought	Irrigated	Drought
Amphidiploid lines	T. *bessarabicum* x Azaziah	2.30	2.23	1.56	1.77	1.93	2.00
	T. *bessarabicum* x Creso	3.01	2.81	1.94	2.30	2.48	2.56
	T. *bessarabicum* x Karim	1.64	2.27	1.60	2.08	1.62	2.17
	T. *bessarabicum* x Langdon	2.81	3.36	1.86	2.02	2.33	2.69
	T. *bessarabicum* x Macoun	2.88	3.28	1.90	2.29	2.39	2.78
	T. *bessarabicum* x Neodur	2.59	2.86	2.09	2.63	2.34	2.75
	T. *bessarabicum* x Stewart	2.74	3.27	1.94	2.19	2.34	2.73
R x S Lines	Rialto	2.19	2.86	1.91	2.38	2.05	2.62
	Savannah	1.42	1.62	1.35	1.81	1.39	1.71
	Line 20	1.95	2.66	1.60	2.14	1.78	2.40
	Line 25	1.48	2.48	1.76	1.88	1.62	2.18
	Line 63	2.08	2.46	1.67	2.08	1.87	2.27
	Line 88	1.16	3.21	1.63	2.34	1.39	2.77
Ancestral wheat species	T. *bessarabicum* 1	1.10	0.93	0.74	0.96	0.92	0.95
	T. *bessarabicum* 2	0.83	0.93	0.49	0.66	0.66	0.79
	T. *bessarabicum* 3	1.07	1.26	1.25	0.88	1.16	1.07
	T. uratu 1	1.51	1.61	-	-	-	-
	T. uratu 2	1.35	1.47	-	-	-	-
	T. uratu 3	1.71	1.84	-	-	-	-
	A. speltoides 1	2.50	2.21	1.97	2.10	2.24	2.15
	A. speltoides 2	2.54	2.74	1.76	2.41	2.15	2.57
	A. speltoides 3	2.26	2.51	1.68	2.22	1.97	2.37
Grand Mean		1.97	2.31	1.61	1.95	1.82	2.19
SED (df)	*Year*	-		-		0.031^{***} *(4)*	
	Irrigation	0.031^{**} *(2)*		$0.088^{(0.06)}$ *(2)*		0.045^{***} *(4)*	
	Genotype	0.132^{***} *(84)*		0.144^{***} *(72)*		0.098^{***} *(144)*	
	Irri. x Gen.	0.185^{***} *(84)*		0.217^{ns} *(72)*		0.143^{***} *(144)*	
	Year x Gen.	-		-		0.139^{***} *(144)*	

N.B: ***denotes $P<0.001$; $^{**}P<0.01$ and $^{*}P<0.05$ significance levels; ^{ns} = not significant

6.3.5 Relationships between grain yield, above-ground DM and plant height

In 2013, a positive linear relationship was found between AGDM plant^{-1} and grain yield plant^{-1} amongst genotypes under droughted conditions (R^2=0.19; *P*=0.04), but not under irrigated conditions (*P*=0.31; Fig. 6.4a). A positive linear relationship was also observed in 2014 under irrigated (R^2=0.24; *P*=0.03) and droughted conditions (R^2=0.29; *P*=0.02; Fig. 6.4b); and averaging across years under droughted (R^2=0.20; *P*=0.06) conditions, but not irrigated conditions (*P*=0.21; Fig. 6.4c).

There was a positive linear relationship amongst genotypes between HI and grain yield plant^{-1} under both irrigated (R^2=0.86; *P*<0.001) and droughted conditions (R^2=0.86; *P*<0.001; Fig. 6.4d) in 2013 and 2014 (R^2=0.96; *P*<0.001and R^2=0.94; *P*<0.001; Fig. 6.4e, respectively), and averaging across years (R^2=0.94; *P*<0.001 and R^2=0.92; *P*<0.001; Fig. 6.4f, respectively) under both irrigated and droughted conditions. The associations between grain yield and HI amongst genotypes were stronger than those between AGDM and grain yield under both irrigated and droughted conditions in each year.

In 2013, a positive linear relationship amongst genotypes was found between AGDM plant^{-1} and plant height under irrigated conditions (R^2=0.26; *P*=0.02), but not droughted conditions (*P*=0.53). No statistically significant relationships were found in 2014 under either irrigated (*P*=0.32) or droughted conditions (*P*=0.47). Averaging across-years, there was positive linear relationship amongst genotypes between AGDM and plant height under irrigated conditions (R^2=0.23; *P*=0.04), but not under droughted conditions (*P*=0.34) (Table 6.10).

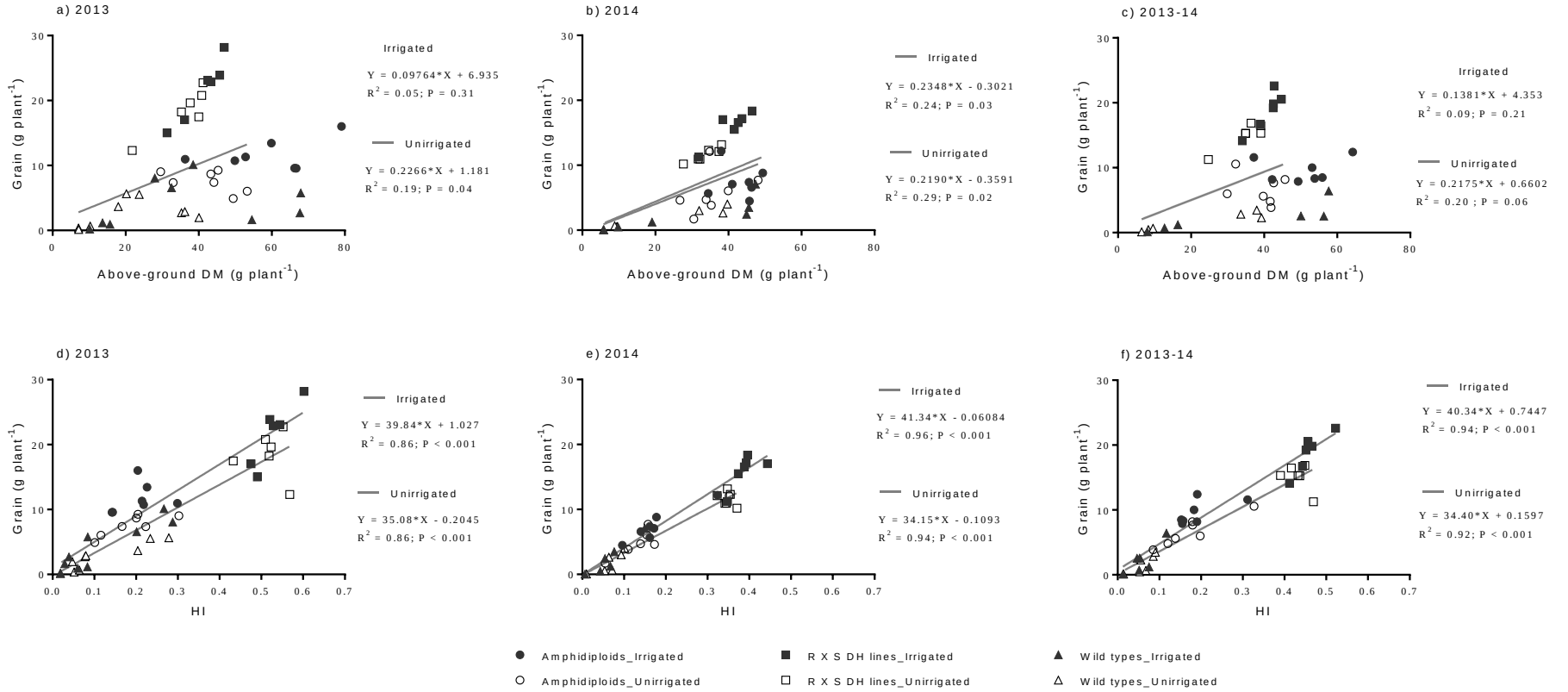


Figure 6.4 Linear regressions of grain yield (g plant^{-1}) on above-ground dry matter (AGDM; g plant^{-1}) under irrigated and droughted conditions in (a) 2013 for 22 genotypes, and in (b) 2014 and (c) cross-year mean for 19 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species); and grain yield (g plant^{-1}) on HI in (d) 2013 for 22 genotypes and in (e) 2014 and (f) cross-year mean for 19 genotypes under irrigated and droughted conditions.

Table 6.9 The phenotypic Pearson's correlation (r) for grain yield (GY), above-ground dry matter (AGDM), harvest index (HI), thousand grain weight (TGW), grains ear^{-1}, grains plant^{-1}, ear dry matter plant^{-1} (EDM), ears plant^{-1}, plant height (PH), anthesis date (AD; days after sowing, DAS) and water-use efficiency (WUE_{AGDM}) among 19 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) under irrigated (unshaded) and droughted (shaded) treatments. Correlations based on mean values in 2013 and 2104.

	Droughted										
Irrigated	GY (g plant^{-1})	AGDM (g plant^{-1})	HI	TGW (g)	Grains ear^{-1}	Grains plant^{-1}	EDM (g plant^{-1})	Ears plant^{-1}	PH (cm)	AD (DAS; day)	WUE_{AGDM} (g l^{-1})
GY	-	0.44^{ns}	0.96***	0.79***	0.90***	0.52*	0.93***	-0.35^{ns}	-0.67**	-0.55*	0.45*
AGDM	0.30^{ns}	-	0.28^{ns}	0.58**	0.09^{ns}	0.44^{ns}	0.62**	0.27^{ns}	0.23^{ns}	-0.85***	0.98***
HI	0.97***	0.11^{ns}	-	0.75***	0.93***	0.48*	0.85***	-0.39^{ns}	-0.75***	-0.45^{ns}	0.28^{ns}
TGW	0.77***	0.39^{ns}	0.73***	-	0.61**	0.07^{ns}	0.65**	-0.54*	-0.32^{ns}	-0.79***	0.59**
Grains ear^{-1}	0.88***	-0.08^{ns}	0.93***	0.62**	-	0.42^{ns}	0.75***	-0.48*	-0.77***	-0.24^{ns}	0.11^{ns}
Grains plant^{-1}	0.54*	0.44^{ns}	0.50*	0.08^{ns}	0.40^{ns}	-	0.76***	0.53*	-0.30^{ns}	-0.27^{ns}	0.40^{ns}
EDM	0.83***	0.62**	0.76***	0.53*	0.58**	0.78***	-	0.02^{ns}	-0.54*	-0.59**	0.62**
Ears plant^{-1}	-0.38^{ns}	0.42^{ns}	-0.41^{ns}	-0.55*	-0.55*	0.40^{ns}	0.18^{ns}	-	0.36^{ns}	0.06^{ns}	0.23^{ns}
PH	-0.56*	0.48*	-0.71***	-0.25^{ns}	-0.70***	-0.21^{ns}	-0.34 ^{ns}	0.33 ^{ns}	-	-0.08^{ns}	0.22^{ns}
AD	-0.39^{ns}	-0.70***	-0.27^{ns}	-0.75***	-0.13^{ns}	-0.09^{ns}	-0.40 ^{ns}	0.10 ^{ns}	-0.26 ^{ns}	-	-0.83***
WUE_{AGDM}	0.21^{ns}	0.94***	0.02^{ns}	0.38^{ns}	-0.11^{ns}	0.31^{ns}	0.46*	0.32^{ns}	0.53*	-0.72***	-

N.B: ***denotes $P<0.001$; $^{**}P<0.01$ and $^{*}P<0.05$ significance levels; ^{ns} = not significant.

Table 6.10 The phenotypic Pearson's correlation (r) between cumulative water uptake (WU), water-use efficiency (WUE_{AGDM}) and water-use efficiency for grain (WUE_{grain}) from transplanting to harvest, and each of grain yield (GY), above-ground dry matter (AGDM), harvest index (HI), thousand grain weight (TGW), plant height (PH) and anthesis date (AD; days after sowing, DAS) among 22 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) in 2013 and 19 genotypes in 2014 and for the cross-year mean (19 genotypes) under irrigated and droughted treatments.

	Traits	GY (g plant^{-1})	AGDM (g plant^{-1})	HI	TGW (g)	PH (cm)	AD (DAS; day)
2013							
Irrigated	WU (l)	0.44^{*}	0.61^{**}	0.32^{ns}	0.35^{ns}	0.09^{ns}	-0.19^{ns}
	WUE_{AGDM} (g l^{-1})	0.15^{ns}	0.88^{***}	-0.13^{ns}	0.42^{*}	0.50^{*}	-0.50^{*}
	WUE_{grain} (g l^{-1})	0.95^{***}	0.17^{ns}	0.90^{***}	0.70^{***}	-0.49^{*}	-0.06^{ns}
Droughted	WU (l)	0.44^{*}	0.86^{***}	0.31^{ns}	0.50^{*}	0.09^{ns}	-0.68^{***}
	WUE_{AGDM} (g l^{-1})	0.45^{*}	0.97^{***}	0.20^{ns}	0.66^{***}	0.12^{ns}	-0.60^{**}
	WUE_{grain} (g l^{-1})	0.99^{***}	0.39^{ns}	0.94^{***}	0.80^{***}	-0.66^{***}	-0.28^{ns}
2014							
Irrigated	WU (l)	0.58^{**}	0.93^{***}	0.51^{*}	0.54^{*}	0.01^{ns}	-0.82^{***}
	WUE_{AGDM} (g l^{-1})	0.41^{ns}	0.93^{***}	0.32^{ns}	0.53^{*}	0.34^{ns}	-0.82^{***}
	WUE_{grain} (g l^{-1})	0.97^{***}	0.46^{*}	0.97^{***}	0.85^{***}	-0.59^{**}	-0.70^{***}
Droughted	WU (l)	0.59^{**}	0.96^{***}	0.47^{*}	0.58^{**}	0.09^{ns}	-0.86^{***}
	WUE_{AGDM} (g l^{-1})	0.52^{*}	0.99^{***}	0.37^{ns}	0.52^{*}	0.18^{ns}	-0.83^{***}
	WUE_{grain} (g l^{-1})	1.00^{***}	0.50^{*}	0.98^{***}	0.72^{***}	-0.65^{**}	-0.66^{**}
2013-14							
Irrigated	WU (l)	0.53^{*}	0.79^{***}	0.44^{ns}	0.48^{*}	0.07^{ns}	-0.57^{*}
	WUE_{AGDM} (g l^{-1})	0.21^{ns}	0.94^{***}	0.02^{ns}	0.38^{ns}	0.53^{*}	-0.72^{***}
	WUE_{grain} (g l^{-1})	0.98^{**}	0.27^{ns}	0.95^{***}	0.77^{***}	-0.57^{*}	-0.40^{ns}
Droughted	WU (l)	0.50^{*}	0.93^{***}	0.39^{ns}	0.57^{*}	0.12^{ns}	-0.86^{***}
	WUE_{AGDM} (g l^{-1})	0.45^{*}	0.98^{***}	0.28^{ns}	0.59^{**}	0.22^{ns}	-0.83^{***}
	WUE_{grain} (g l^{-1})	0.99^{***}	0.41^{ns}	0.96^{***}	0.79^{***}	-0.68^{**}	-0.52^{*}

N.B: ***denotes $P<0.001$; $^{**}P<0.01$ and $^{*}P<0.05$ significance levels; ^{ns} = not significant.

6.3.6 Flag-leaf gas exchange traits

Measurements were taken every two weeks in both irrigated and droughted treatments from the booting stage (GS41) to mid-to-late grain gilling (GS61+28d) for 22 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) in 2013 and 19 genotypes in 2014.

6.3.6.1 Flag-leaf photosynthetic rate (A_{max})

In 2013, there was no effect of drought on pre-anthesis flag-leaf A_{max} (P=0.22). Genotypes differed in the range 14.1 (*T. uratu* 3) to 25.7 µmol m^{-2} s^{-1} (*T. bessarabicum* 1) under irrigated conditions, and 14.5 (*A. speltoides* 3) to 25.2 µmol m^{-2} s^{-1} (*T. uratu* 1) under droughted conditions (P=0.02). There was no irrigation x genotype interaction (P=0.28; Table 6.11). There was a weak trend for post-anthesis flag-leaf A_{max} to decrease under drought (P=0.10). Genotypes differed in the range 14.8 (*T. bessarabicum* 3) to 35.1 µmol m^{-2} s^{-1} (*T. bessarabicum* 1) and 12.3 (*T. uratu* 1) to 23.9 µmol m^{-2} s^{-1} (Savannah) under irrigated and droughted conditions, respectively (P<0.001). The decrease under drought ranged from -1.61 (*T. uratu* 2) to 15.74 (*T. bessarabicum* 1) µmol m^{-2} s^{-1} (P=0.06; Table 6.11).

In 2014, there was no effect of drought or irrigation x genotype interaction for flag-leaf A_{max} either pre-anthesis (P=0.16 and P=0.59, respectively) or post-anthesis (P=0.14 and P=0.59, respectively). Genotypes ranged in pre-anthesis A_{max} from 6.7 (*A. speltoides* 1) to 28.1 µmol m^{-2} s^{-1} (*T. bessarabicum* 1) under irrigated conditions, and 8.6 (*A. speltoides* 1) to 19.9 µmol m^{-2} s^{-1} (*T. bessarabicum* 3) under droughted conditions (P<0.001; Table 6.11). For post-anthesis A_{max}, genotypes differed in the range 8.0 (*A. speltoides* 2) to 22.6 (*T. bessarabicum* 1) µmol m^{-2} s^{-1} under irrigated conditions, and 4.1 (*A. speltoides* 2) to 26.7 (*T. bessarabicum* 1) µmol m^{-2} s^{-1} under droughted conditions (P<0.001; Table 6.11).

Averaging over years, flag-leaf A_{max} was higher in 2013 either pre-anthesis (P<0.001) or post-anthesis (P=0.01) than in 2014 by 30% and 41%, respectively. Drought reduced A_{max} from 18.6 to 16.2 µmol m^{-2} s^{-1} pre-anthesis (P=0.04), and 19.1 to 15.5 µmol m^{-2} s^{-1} post-anthesis (P=0.006). There was no irrigation x genotype interaction either pre-anthesis (P=0.58) or post-anthesis (P=0.08; Table 6.11). Pre-anthesis A_{max} ranged amongst genotypes from 13.8 (*A. speltoides* 3) to 26.9 µmol m^{-2}

s^{-1} (*T. bessarabicum* 1) under irrigated conditions, and 11.7 (*A. speltoides* 3) to 19.9 µmol m^{-2} s^{-1} (*T. bessarabicum* 2) under droughted conditions (P<0.001; Table 6.11). Post-anthesis genotypes ranged from 13.4 (*A. speltoides* 3) to 28.8 (*T. bessarabicum* 1) µmol m^{-2} s^{-1} under irrigated conditions, and 9.8 (*A. speltoides* 3) to 23.0 (*T. bessarabicum* 1) µmol m^{-2} s^{-1} under droughted conditions (P<0.001; Table 6.11). Averaging over years and irrigation treatments, *T. bessarabicum* accessions (21.7 and 21.4 µmol m^{-2} s^{-1}, respectively) had highest leaf A_{max} pre-anthesis and post-anthesis followed by amphidiploid lines (18.0 and 17.2 µmol m^{-2} s^{-1}, respectively), R x S DH lines (16.2 and 17.3 µmol m^{-2} s^{-1}, respectively) and *A. speltoides* accessions (14.0 and 13.2 µmol m^{-2} s^{-1}, respectively) (P<0.001).

6.3.6.2 Flag-leaf stomatal conductance (g_s)

In 2013, drought did not affect pre-anthesis flag-leaf g_s (P=0.16). Averaging over irrigation treatments, genotypes differed in the range 0.23 (DH line 88) to 0.45 mol m^{-2} s^{-1} (*T. bessarabicum* x Karim) (P=0.03). There was no irrigation x genotype interaction (P=0.63; Table 6.12). For post-anthesis g_s, drought decreased g_s from 0.40 to 0.27 mol m^{-2} s^{-1} (P=0.03). Genotypes differed in the range 0.16 (*T. bessarabicum* 3) to 0.49 mol m^{-2} s^{-1} (*T. bessarabicum* x Azaziah), and 0.17 (*T. uratu* 1) to 0.37 mol m^{-2} s^{-1} (Savannah) under irrigated and droughted conditions, respectively (P=0.007). The decrease under drought ranged from -0.12 (*T. bessarabicum* 3) to 0.29 mol m^{-2} s^{-1} (*T. bessarabicum* x Neodur) (P=0.02; Table 6.12).

In 2014, drought reduced pre-anthesis g_s from 0.48 to 0.36 mol m^{-2} s^{-1} (P=0.007). Genotypes ranged from 0.25 (*A. speltoides* 1) to 0.62 mol m^{-2} s^{-1} (*T. bessarabicum* x Azaziah) under irrigated conditions and 0.22 (*A. speltoides* 2) to 0.44 mol m^{-2} s^{-1} (*T. bessarabicum* x Azaziah) under droughted conditions (P=0.02; Table 6.12). The irrigation x genotype interaction was not significant (P=0.84). For post-anthesis g_s, there were no effect of irrigation (P=0.19) or irrigation x genotype interaction (P=0.23). Averaging over irrigation treatments, genotypes differed in the range 0.16 (*A. speltoides* 2) to 0.80 mol m^{-2} s^{-1} (Savannah) (P<0.001; Table 6.12).

There was no effect of year on flag-leaf g_s either pre-anthesis (P=0.19) or post-anthesis (P=0.28). Averaging over years, drought reduced g_s from 0.42 to 0.32 mol

m^{-2} s^{-1} pre-anthesis (P=0.009), and from 0.48 to 0.35 mol m^{-2} s^{-1} post-anthesis (P=0.03). For pre-anthesis g_s, genotypes ranged from 0.32 (DH line 25) to 0.52 mol m^{-2} s^{-1} (*T. bessarabicum* x Azaziah) under irrigated conditions and 0.20 (*A. speltoides* 3) to 0.45 mol m^{-2} s^{-1} (*T. bessarabicum* x Karim) under droughted conditions (P=0.004). There was no irrigation x genotype interaction (P=0.83; Table 6.12). Post-anthesis, genotypes ranged from 0.31 (*A. speltoides* 3) to 0.64 mol m^{-2} s^{-1} (Savannah) under irrigated conditions, and 0.19 (*A. speltoides* 1) to 0.56 mol m^{-2} s^{-1} (Savannah) under droughted conditions (P<0.001; Table 6.12). Decreases under drought ranged from -0.09 (*T. bessarabicum* 1) to 0.32 (*T. bessarabicum* x Azaziah) mol m^{-2} s^{-1} (P=0.01).

Averaging over years and irrigation treatments, amphidiploid lines (0.41 mol m^{-2} s^{-1}) had the highest pre-anthesis flag-leaf g_s followed by *T. bessarabicum* accessions (0.39 mol m^{-2} s^{-1}), R x S DH lines (0.36 mol m^{-2} s^{-1}) and *A. speltoides* accessions (0.31 mol m^{-2} s^{-1}). R x S DH lines (0.48 mol m^{-2} s^{-1}) had the highest post-anthesis g_s followed by *T. bessarabicum* accessions (0.43 mol m^{-2} s^{-1}), amphidiploid lines (0.42 mol m^{-2} s^{-1}) and *A. speltoides* accessions (0.27 mol m^{-2} s^{-1}) (P<0.001).

Table 6.11 Pre-anthesis and post-anthesis flag-leaf photosynthetic rate (A_{max}) for 22 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) in 2013 and 19 genotypes in 2014 and cross-year mean under irrigated and droughted treatments, and standard errors of the differences of the means (SED) and degrees of freedom (df).

	Genotypes	Pre-anthesis A_{max} (µmol m^{-2} s^{-1})						Post-anthesis A_{max} (µmol m^{-2} s^{-1})					
		2013		2014		2013-14		2013		2014		2013-14	
		Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought
Amphidiploid lines	*T. bessarabicum* x Azaziah	17.73	16.76	12.45	13.10	15.09	14.93	20.42	17.00	13.94	12.15	17.18	14.57
	T. bessarabicum x Creso	23.31	20.21	16.64	14.11	19.97	17.16	26.01	19.25	14.24	12.22	20.12	15.74
	T. bessarabicum x Karim	22.70	23.70	16.81	15.47	19.75	19.58	23.45	18.61	15.84	14.64	19.64	16.62
	T. bessarabicum x Langdon	18.08	21.57	14.82	12.91	16.45	17.24	25.95	18.32	13.68	12.86	19.82	15.59
	T. bessarabicum x Macoun	23.20	19.35	15.07	14.86	19.13	17.11	26.10	16.80	9.84	9.15	17.97	12.97
	T. bessarabicum x Neodur	22.45	22.55	16.59	15.98	19.52	19.27	27.87	16.39	12.55	9.47	20.21	12.93
	T. bessarabicum x Stewart	22.81	21.58	15.95	14.07	19.38	17.83	24.81	22.90	14.88	12.64	19.85	17.77
R x S DH lines	Rialto	18.69	20.06	15.60	13.83	17.15	16.94	27.27	19.45	13.94	10.60	20.61	15.03
	Savannah	23.42	14.79	12.53	9.51	17.97	12.15	24.73	23.88	10.54	11.10	17.64	17.49
	Line 20	21.78	17.73	16.01	12.50	18.90	15.11	25.73	19.29	12.68	11.14	19.21	15.21
	Line 25	20.32	18.81	13.80	9.32	17.06	14.06	26.26	21.52	13.11	9.42	19.69	15.47
	Line 63	25.31	19.75	11.18	10.47	18.24	15.11	24.86	22.51	8.82	9.37	16.84	15.94
	Line 88	22.01	15.29	13.10	11.75	17.56	13.52	28.37	19.70	11.89	9.76	20.13	14.73
Ancestral wheat species	*T. bessarabicum* 1	25.65	21.02	28.08	17.87	26.86	19.45	35.07	19.34	22.56	26.73	28.82	23.03
	T. bessarabicum 2	20.17	21.58	22.32	18.13	21.24	19.85	20.01	19.86	19.97	18.38	19.99	19.12
	T. bessarabicum 3	21.78	17.16	26.27	19.92	24.03	18.54	14.82	15.10	21.61	23.23	18.21	19.17
	T. uratu 1	23.07	25.16	-	-	-	-	24.18	12.34	-	-	-	-
	T. uratu 2	15.13	17.80	-	-	-	-	18.48	20.08	-	-	-	-
	T. uratu 3	14.06	16.87	-	-	-	-	16.83	15.54	-	-	-	-
	A. speltoides 1	22.61	17.75	6.72	8.64	14.66	13.20	22.86	17.44	8.97	5.40	15.91	11.42
	A. speltoides 2	24.60	16.96	8.87	11.04	16.74	14.00	25.44	20.02	8.01	4.13	16.72	12.08
	A. speltoides 3	17.71	14.50	9.82	8.97	13.76	11.74	17.65	12.70	9.09	6.85	13.37	9.78
Grand Mean		21.21	19.14	15.40	13.29	18.60	16.15	23.96	18.55	13.48	12.06	19.05	15.51
SED (df)	*Year*	-		-		0.143^{***} *(2)*		-		-		1.046^{*} *(2)*	
	Irrigation	0.754^{ns} *(2)*		0.536^{ns} *(2)*		0.510^{*} *(2)*		$0.833^{(0.10)}$ *(2)*		0.310^{ns} *(2)*		0.268^{**} *(2)*	
	Genotype	2.302^{*} *(42)*		2.152^{***} *(36)*		1.613^{***} *(72)*		2.332^{***} *(42)*		1.517^{***} *(36)*		1.188^{***} *(72)*	
	Irri. x Gen.	3.269^{ns} *(42)*		3.011^{ns} *(36)*		2.279^{ns} *(72)*		$3.328^{(0.06)}$ *(42)*		2.111^{ns} *(36)*		1.657^{ns} *(72)*	
	Year x Gen.	-		-		2.225^{***} *(72)*		-		-		1.941^{***} *(72)*	

N.B: ***denotes $P<0.001$; $^{**}P<0.01$ and $^{*}P<0.05$ significance levels; ^{ns} = not significant.

Table 6.12 Pre-anthesis and post-anthesis flag-leaf stomatal conductance (g_s) for 22 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) in 2013 and 19 genotypes in 2014 and cross-year mean under irrigated and droughted treatments, and standard errors of the differences of the means (SED) and degrees of freedom (df).

	Genotypes	Pre-anthesis g_s (mol m^{-2} s^{-1})						Post-anthesis g_s (mol m^{-2} s^{-1})					
		2013		2014		2013-14		2013		2014		2013-14	
		Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought
Amphidiploid lines	*T. bessarabicum* x Azaziah	0.42	0.32	0.62	0.44	0.52	0.38	0.49	0.27	0.90	0.48	0.69	0.37
	T. bessarabicum x Creso	0.45	0.32	0.49	0.36	0.47	0.34	0.40	0.26	0.57	0.30	0.49	0.28
	T. bessarabicum x Karim	0.44	0.47	0.57	0.42	0.50	0.45	0.39	0.27	0.85	0.56	0.62	0.41
	T. bessarabicum x Langdon	0.25	0.32	0.54	0.43	0.39	0.38	0.45	0.24	0.47	0.64	0.46	0.44
	T. bessarabicum x Macoun	0.38	0.27	0.44	0.33	0.41	0.30	0.45	0.21	0.33	0.23	0.39	0.22
	T. bessarabicum x Neodur	0.48	0.30	0.48	0.43	0.48	0.37	0.47	0.18	0.38	0.26	0.42	0.22
	T. bessarabicum x Stewart	0.35	0.30	0.44	0.35	0.39	0.33	0.42	0.32	0.56	0.30	0.49	0.31
R x S DH lines	Rialto	0.25	0.27	0.45	0.35	0.35	0.31	0.46	0.23	0.71	0.34	0.58	0.28
	Savannah	0.39	0.28	0.56	0.39	0.47	0.33	0.43	0.37	0.86	0.74	0.64	0.56
	Line 20	0.29	0.27	0.59	0.42	0.44	0.34	0.44	0.30	0.70	0.54	0.57	0.42
	Line 25	0.31	0.23	0.34	0.42	0.32	0.33	0.46	0.31	0.63	0.54	0.55	0.42
	Line 63	0.37	0.26	0.41	0.33	0.39	0.30	0.48	0.33	0.55	0.52	0.52	0.42
	Line 88	0.28	0.18	0.55	0.37	0.41	0.27	0.46	0.26	0.64	0.31	0.55	0.28
Ancestral wheat species	*T. bessarabicum* 1	0.30	0.25	0.54	0.27	0.42	0.26	0.21	0.21	0.55	0.73	0.38	0.47
	T. bessarabicum 2	0.43	0.41	0.55	0.40	0.49	0.40	0.30	0.31	0.63	0.72	0.47	0.52
	T. bessarabicum 3	0.30	0.26	0.60	0.34	0.45	0.30	0.16	0.27	0.54	0.50	0.35	0.39
	T. uratu 1	0.48	0.36	-	-	-	-	0.42	0.17	-	-	-	-
	T. uratu 2	0.43	0.32	-	-	-	-	0.46	0.32	-	-	-	-
	T. uratu 3	0.41	0.31	-	-	-	-	0.32	0.25	-	-	-	-
	A. speltoides 1	0.45	0.29	0.25	0.27	0.35	0.28	0.34	0.21	0.35	0.16	0.34	0.19
	A. speltoides 2	0.46	0.24	0.31	0.22	0.39	0.23	0.46	0.33	0.21	0.10	0.33	0.21
	A. speltoides 3	0.41	0.17	0.41	0.23	0.41	0.20	0.31	0.36	0.30	0.15	0.31	0.26
Grand Mean		0.38	0.29	0.48	0.36	0.42	0.32	0.40	0.27	0.56	0.43	0.48	0.35
SED (df)	*Year*	-		-		*0.047^{ns} (2)*		-		-		*0.109^{ns} (2)*	
	Irrigation	*0.022^{ns} (2)*		*0.001** (2)*		*0.010** (2)*		*0.007* (2)*		*0.042^{ns} (2)*		*0.021* (2)*	
	Genotype	*0.060* (42)*		*0.073* (36)*		*0.046** (72)*		*0.049** (42)*		*0.102*** (36)*		*0.056*** (72)*	
	Irri. x Gen.	*0.086^{ns} (42)*		*0.101^{ns} (36)*		*0.064^{ns} (72)*		*0.069* (42)*		*0.147^{ns} (36)*		*0.080* (72)*	
	Year x Gen.	-		-		*0.079* (72)*		-		-		*0.133*** (72)*	

N.B: ***denotes $P<0.001$; $^{**}P<0.01$ and $^{*}P<0.05$ significance levels; ^{ns} = not significant.

6.3.6.3 Transpiration efficiency (TE)

In 2013, there were no effects of irrigation and irrigation x genotype interaction for flag-leaf TE both pre-anthesis (P=0.49 and P=0.31, respectively) and post-anthesis (P=0.81 and P=0.30, respectively). Averaging over irrigation treatments, genotypes differed in the range 3.07 (*T. bessarabicum* 2) to 5.94 (*T. bessarabicum* x Macoun) pre-anthesis, and 2.81 (*A. speltoides* 3) to 6.45 µmol mol^{-1} (*T. bessarabicum* 1) post-anthesis (P=0.02; Table 6.13).

In 2014, for pre-anthesis flag-leaf TE, there was no effect of irrigation (P=0.42) or irrigation x genotype interaction (P=0.19). Averaging over irrigation treatments, genotypes differed in the range 2.15 (Savannah) to 4.67 µmol mol^{-1} (*T. bessarabicum* 1) (P<0.001; Table 6.13). For post-anthesis leaf TE, a trend was found for an increase in TE under drought (P=0.06). Genotypes differed in the range 1.67 (Savannah) to 4.42 µmol mol^{-1} (*T. bessarabicum* 3) under irrigated conditions, and 1.88 (Savannah) to 4.70 µmol mol^{-1} (*A. speltoides* 3) under droughted conditions (P=0.002). There was no irrigation x genotype interaction (P=0.90; Table 6.13).

Averaging over years, for pre-anthesis flag-leaf TE, there was no irrigation effect (P=0.40) or irrigation x genotype interaction (P=0.09). Averaging over irrigation treatments, genotypes ranged from 2.99 (*A. speltoides* 1) to 4.79 µmol mol^{-1} (*T. bessarabicum* 1) (P<0.001; Table 6.13). Flag-leaf post-anthesis TE slightly increased under drought from 3.48 to 3.58 µmol mol^{-1} (P=0.02). Genotypes differed in the range 2.56 (*T. bessarabicum* x Azaziah) to 5.97 µmol mol^{-1} (*T. bessarabicum* 1) under irrigated conditions, and 2.90 (*T. bessarabicum* 2) to 4.27 µmol mol^{-1} (*T. bessarabicum* x Stewart) under droughted conditions (P<0.001). The increase under drought ranged amongst genotypes from -0.98 (Rialto) to 1.95 µmol mol^{-1} (*T. bessarabicum* 1) (P=0.03; Table 6.13). Averaging over years and irrigation treatments, both pre-anthesis (P=0.001) and post-anthesis (P=0.006) *T. bessarabicum* accessions (4.06 and 3.94 µmol mol^{-1}, respectively) and amphidiploid lines (4.17 and 3.67 µmol mol^{-1}, respectively) had higher flag-leaf TE than R x S DH lines (3.73 and 3.19 µmol mol^{-1}, respectively) and *A. speltoides* accessions (3.34 and 3.49 µmol mol^{-1}, respectively).

Table 6.13 Pre-anthesis and post-anthesis flag-leaf transpiration efficiency (TE) for 22 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) in 2013 and 19 genotypes in 2014 and cross-year mean under irrigated and droughted treatments, and standard errors of the differences of the means (SED) and degrees of freedom (df).

	Genotypes	Pre-anthesis TE (µmol mol^{-1})						Post-anthesis TE (µmol mol^{-1})					
		2013		2014		2013-14		2013		2014		2013-14	
		Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought
Amphidiploid lines	*T. bessarabicum* x Azaziah	3.89	4.80	1.96	2.63	2.93	3.71	3.05	3.71	2.08	2.99	2.56	3.35
	T. bessarabicum x Creso	4.94	5.35	3.10	3.29	4.02	4.32	4.28	4.03	3.57	4.18	3.92	4.10
	T. bessarabicum x Karim	5.32	5.15	2.95	3.12	4.13	4.14	3.89	4.27	2.38	3.41	3.13	3.84
	T. bessarabicum x Langdon	5.89	5.99	2.57	2.67	4.23	4.33	3.78	3.99	3.09	2.79	3.44	3.39
	T. bessarabicum x Macoun	5.40	6.49	3.03	3.51	4.22	5.00	4.00	4.25	3.23	3.97	3.61	4.11
	T. bessarabicum x Neodur	4.81	5.05	3.20	3.38	4.01	4.21	3.98	4.54	3.65	3.46	3.81	4.00
	T. bessarabicum x Stewart	5.53	6.19	3.34	3.23	4.43	4.71	3.96	4.22	3.54	4.33	3.75	4.27
R x S DH lines	Rialto	4.41	5.26	3.21	3.03	3.81	4.14	4.01	4.67	2.39	3.70	3.20	4.18
	Savannah	5.38	3.26	2.16	2.14	3.77	2.70	3.92	4.05	1.67	1.88	2.80	2.97
	Line 20	4.95	4.45	2.76	2.58	3.85	3.52	3.67	3.81	2.24	2.44	2.96	3.12
	Line 25	4.61	4.83	4.03	1.97	4.32	3.40	3.63	4.18	2.48	1.94	3.06	3.06
	Line 63	4.88	4.80	2.42	2.61	3.65	3.70	3.68	4.10	1.76	2.49	2.72	3.29
	Line 88	5.36	5.48	2.24	2.61	3.80	4.04	3.92	4.37	2.26	3.26	3.09	3.82
Ancestral wheat species	*T. bessarabicum* 1	4.78	5.03	4.39	4.94	4.59	4.98	8.31	4.59	3.63	3.45	5.97	4.02
	T. bessarabicum 2	2.92	3.22	3.41	3.77	3.17	3.50	3.19	3.35	2.79	2.45	2.99	2.90
	T. bessarabicum 3	4.42	3.58	3.78	4.52	4.10	4.05	4.42	2.93	4.42	3.81	4.42	3.37
	T. uratu 1	3.45	4.65	-	-	-	-	3.54	4.82	-	-	-	-
	T. uratu 2	2.93	4.11	-	-	-	-	2.78	3.72	-	-	-	-
	T. uratu 3	3.34	5.03	-	-	-	-	3.17	3.55	-	-	-	-
	A. speltoides 1	3.39	3.99	2.15	2.41	2.77	3.20	3.64	3.68	3.20	2.87	3.42	3.27
	A. speltoides 2	3.75	4.45	2.39	3.83	3.07	4.14	3.28	3.32	4.02	3.56	3.65	3.44
	A. speltoides 3	3.16	5.00	1.94	3.63	2.55	4.32	3.42	2.20	3.98	4.70	3.70	3.45
Grand Mean		4.43	4.83	2.90	3.15	3.76	4.01	3.89	3.92	2.97	3.25	3.48	3.58
SED (df)	*Year*	-		-		*0.278* (2)*		-		-		*0.517^{ns} (2)*	
	Irrigation	*0.385^{ns} (2)*		*0.198^{ns} (2)*		*0.230^{ns} (2)*		*0.122^{ns} (2)*		*0.024$^{(0.06)}$ (2)*		*0.012* (2)*	
	Genotype	*0.560*** (42)*		*0.444*** (36)*		*0.359*** (72)*		*0.670* (42)*		*0.588** (36)*		*0.348*** (72)*	
	Irri. x Gen.	*0.865^{ns} (42)*		*0.643^{ns} (36)*		*0.546^{ns} (72)*		*0.934^{ns} (42)*		*0.810^{ns} (36)*		*0.479* (72)*	
	Year x Gen.	-		-		*0.567*** (72)*		-		-		*0.705*** (72)*	

N.B: ***denotes $P<0.001$; $^{**}P<0.01$ and $^{*}P<0.05$ significance levels; ^{ns} = not significant

6.3.6.4 Flag-leaf chlorophyll content (SPAD)

Flag-leaf chlorophyll content (SPAD) was measured weekly for the newest fully emerged leaf on the main shoot from stem elongation (GS31) to mid-grain filling (GS61+28d) for 22 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) in 2013 and 19 genotypes in 2014.

In 2013, drought reduced flag-leaf relative chlorophyll content slightly from 46.5 to 46.2 pre-anthesis (P=0.03), and from 50.4 to 47.2 post-anthesis (P=0.03). Pre-anthesis, genotypes differed in the range 32.9 (*A. speltoides* 3) to 57.9 (*T. bessarabicum* 1) under irrigated conditions, and 34.3 (*A. speltoides* 1) to 55.1 (*T. bessarabicum* x Karim) under droughted conditions (P<0.001). The decrease under drought ranged from -9.8 (DH line 25) to 8.9 (Rialto) (P<0.001; Table 6.14). Post-anthesis, drought reduced flag-leaf SPAD from 50.4 to 47.2; and genotypes ranged from 33.5 (*T. uratu* 3) to 63.4 (*T. bessarabicum* 1), and 29.7 (*T. uratu* 2) to 56.3 (*T. bessarabicum* 3) under irrigated and droughted conditions, respectively (P<0.001). There was no irrigation x genotype interaction (P=0.11; Table 6.14).

In 2014, pre-anthesis restricted water availability did not affect leaf SPAD (P=0.25). Averaging over irrigation treatments, genotypes ranged from 33.6 (*A. speltoides* 2) to 60.4 (*T. bessarabicum* 3) (P<0.001). The decrease under drought differed amongst genotypes from -3.7 (*A. speltoides* 1) to 6.8 (*T. bessarabicum* 1) (P=0.01; Table 6.14). Post-anthesis, drought decreased flag-leaf SPAD slightly from 41.4 to 40.4 (P=0.05). Genotypes differed in the range 24.9 (*A. speltoides* 3) to 62.6 (*T. bessarabicum* 3) under irrigated conditions, and 27.5 (*A. speltoides* 3) to 60.3 (*T. bessarabicum* 1) under droughted conditions (P<0.001). The irrigation x genotype interaction was not significant (P=0.47; Table 6.14).

Averaging over years, for pre-anthesis flag-leaf SPAD, there was a trend for drought to decrease leaf SPAD (P=0.06). Responses to drought ranged amongst genotypes from -3.8 to 5.7 for Rialto and Savannah, respectively (P=0.003). Post-anthesis, drought decreased flag-leaf SPAD from 47.1 to 44.9 (P=0.001), but the irrigation x genotype interaction was not significant (P=0.43; Table 6.14). Averaging over years and irrigation treatments, *T. bessarabicum* accessions had the highest flag-leaf SPAD either pre-anthesis or post-anthesis (53.4 and 56.7, respectively) followed by

amphidiploid lines (47.6 and 46.3, respectively), R x S DH lines (42.5 and 45.6, respectively) and *A. speltoides* accessions (36.1 and 35.1, respectively) ($P<0.001$).

Table 6.14 Pre-anthesis and post-anthesis flag-leaf chlorophyll content (SPAD) for 22 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) in 2013 and 19 genotypes in 2014 and cross-year mean under irrigated and droughted treatments, and standard errors of the differences of the means (SED) and degrees of freedom (df).

Genotypes		Pre-anthesis SPAD						Post-anthesis SPAD					
		2013		2014		2013-14		2013		2014		2013-14	
		Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought
Amphidiploid lines	*T. bessarabicum* x Azaziah	45.1	47.6	44.3	44.4	44.7	46.0	47.0	43.3	42.5	40.5	44.8	41.9
	T. bessarabicum x Creso	52.7	50.8	44.5	45.1	48.6	47.9	50.2	51.2	44.1	42.6	47.1	46.9
	T. bessarabicum x Karim	56.0	55.1	46.9	47.1	51.4	51.1	58.3	52.8	44.9	45.2	51.6	49.0
	T. bessarabicum x Langdon	48.3	50.5	40.0	40.6	44.2	45.5	49.5	48.0	39.8	39.5	44.7	43.8
	T. bessarabicum x Macoun	50.6	51.0	44.7	44.5	47.7	47.7	52.9	46.4	42.8	41.2	47.9	43.8
	T. bessarabicum x Neodur	54.2	50.8	43.6	46.3	48.9	48.5	54.4	50.3	40.8	43.3	47.6	46.8
	T. bessarabicum x Stewart	51.4	51.1	42.8	42.1	47.1	46.6	50.0	48.8	44.3	40.3	47.2	44.6
R x S DH lines	Rialto	42.1	49.5	41.3	41.5	41.7	45.5	57.7	52.0	40.9	37.8	49.3	44.9
	Savannah	45.4	36.5	41.4	39.0	43.4	37.8	53.5	52.7	36.5	36.0	45.0	44.4
	Line 20	44.9	44.3	40.7	39.9	42.8	42.1	52.5	48.6	37.0	35.9	44.8	42.3
	Line 25	39.9	49.7	42.9	39.6	41.4	44.7	55.8	53.7	40.6	34.3	48.2	44.0
	Line 63	46.7	48.0	40.8	40.2	43.7	44.1	57.1	55.2	39.3	39.1	48.2	47.2
	Line 88	42.3	45.0	39.9	40.3	41.1	42.7	54.4	51.5	37.4	36.7	45.9	44.1
Ancestral wheat species	*T. bessarabicum* 1	57.9	49.1	55.0	57.2	56.5	53.1	63.4	54.3	58.7	60.3	61.1	57.3
	T. bessarabicum 2	48.6	44.5	53.8	51.0	51.2	47.7	55.2	49.7	55.5	49.7	55.4	49.7
	T. bessarabicum 3	56.9	55.0	63.8	57.0	60.3	56.0	60.2	56.3	62.6	60.1	61.4	58.2
	T. uratu 1	47.1	45.3	-	-	-	-	36.5	34.7	-	-	-	-
	T. uratu 2	39.0	41.1	-	-	-	-	35.3	29.7	-	-	-	-
	T. uratu 3	41.2	43.7	-	-	-	-	33.5	36.1	-	-	-	-
	A. speltoides 1	42.2	34.3	34.3	38.0	38.2	36.2	42.7	42.3	28.1	28.8	35.4	35.6
	A. speltoides 2	37.2	38.3	35.3	32.0	36.2	35.1	45.8	42.2	25.4	28.7	35.6	35.4
	A. speltoides 3	32.9	36.0	35.8	33.9	34.4	35.0	42.5	39.3	24.9	27.5	33.7	33.4
Grand Mean		46.5	46.2	43.8	43.1	45.4	44.9	50.4	47.2	41.4	40.4	47.1	44.9
SED (df)	*Year*	-		-		*0.46^{**} (4)*		-		-		*0.85^{***} (4)*	
	Irrigation	*0.04^{*} (2)*		*0.41^{ns} (2)*		*$0.21^{(0.06)}$ (4)*		*0.54^{*} (2)*		*0.23^{*} (2)*		*0.28^{***} (4)*	
	Genotype	*1.88^{***} (84)*		*1.18^{***} (72)*		*1.13^{***} (144)*		*1.54^{***} (84)*		*1.86^{***} (72)*		*1.20^{***} (144)*	
	Irri. x Gen.	*2.60^{***} (84)*		*1.68^{*} (72)*		*1.57^{**} (144)*		*2.20^{ns} (84)*		*2.57^{ns} (72)*		*1.67^{ns} (144)*	
	Year x Gen.	-		-		*1.62^{***} (144)*		-		-		*1.86^{***} (144)*	

N.B: ***denotes $P<0.001$; $^{**}P<0.01$ and $^{*}P<0.05$ significance levels; ^{ns} = not significant.

6.3.7 Relationships between flag-leaf A_{max}, g_s and TE and AGDM and leaf specific weight, and leaf SPAD

In 2014, negative relationships were found between AGDM plant^{-1} and flag-leaf A_{max} both pre-anthesis under irrigated (R^2=0.56; P<0.001) and unirrigated (R^2=0.29; P=0.02) and post-anthesis under irrigated (R^2=0.61; P<0.001) and unirrigated (R^2=0.65; P<0.001) conditions, but not in 2013 (P>0.05). Averaging across years, AGDM plant^{-1} was negatively associated with pre-anthesis leaf A_{max} under irrigated (R^2=0.44; P=0.002), but not droughted (P=0.18) conditions; and AGDM was also negatively associated with post-anthesis flag-leaf A_{max} (R^2=0.19; P=0.06 and R^2=0.51; P<0.001, respectively) (Table 6.16).

In 2013, there was a positive linear association between AGDM and pre-anthesis flag-leaf TE under drought (R^2=0.49; P<0.001), but a negative association in 2014 (R^2=-0.23; P=0.05; Table 6.16). In 2013, post-anthesis flag-leaf g_s was positively associated with AGDM plant^{-1} under irrigated conditions (R^2=0.28; P=0.01). In 2014 (R^2=-0.44; P=0.002) there was a negative association between post-anthesis flag-leaf g_s and AGDM plant^{-1} and for the cross-year mean (R^2=-0.38; P=0.005) under droughted conditions.

In 2014, positive linear relationships were found between pre-anthesis flag-leaf A_{max} and flag-leaf specific weight under both irrigated (R^2=0.55; P<0.001) and droughted (R^2=0.59; P<0.001; Fig. 6.6a) conditions, and between post-anthesis A_{max} and FLSW (R^2=0.48; P=0.001 and R^2=0.55; P<0.001, respectively; Fig. 6.6b).

In 2013, a positive linear relationship amongst genotypes was found between pre-anthesis A_{max} and pre-anthesis leaf SPAD under both irrigated (R^2=0.23; P=0.02) and droughted conditions (R^2=0.33; P=0.005; Fig. 6.5a) and in 2014 (R^2=0.85; P<0.001 and R^2=0.80; P<0.001, respectively; Fig. 6.5b), and averaging across years (R^2=0.79; P<0.001 and R^2=0.74; P<0.001, respectively; Fig. 6.5c). Post-anthesis, there were positive linear relationships amongst genotypes between flag-leaf A_{max} and flag-leaf SPAD in 2013 under both irrigated (R^2=0.24; P=0.02) and droughted conditions (R^2=0.21; P=0.03; Fig. 6.5d). Similarly positive linear relationships between flag-leaf A_{max} and SPAD were observed in 2014 (R^2=0.85; P<0.001 and

R^2=0.89; P<0.001, respectively; Fig. 6.5e), and averaging across years (R^2=0.49; P=0.001 and R^2=0.75; P<0.001, respectively; Fig. 6.5f).

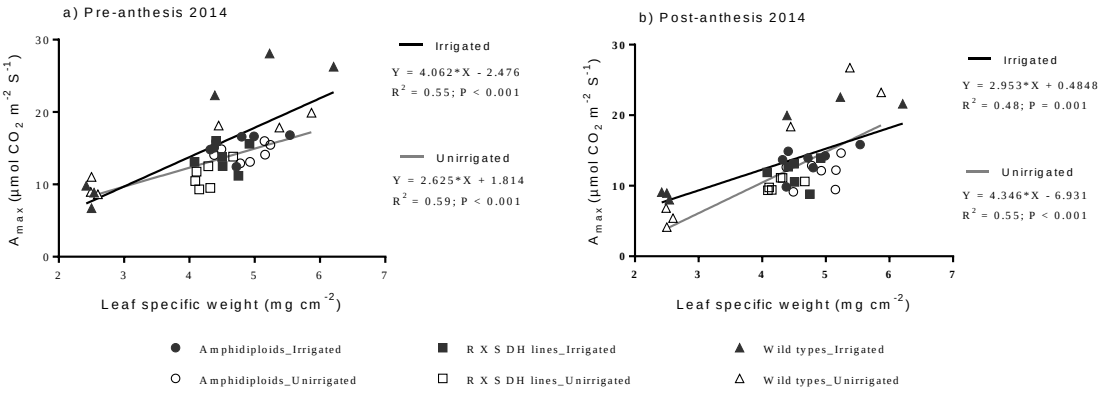


Figure 6.5 Linear regressions of pre-anthesis (a) and post-anthesis (b) flag-leaf A_{max} (µmol m^{-2} s^{-1}) on flag-leaf specific weight (FLSW; at anthesis) under irrigated and droughted conditions amongst 19 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) in 2014.

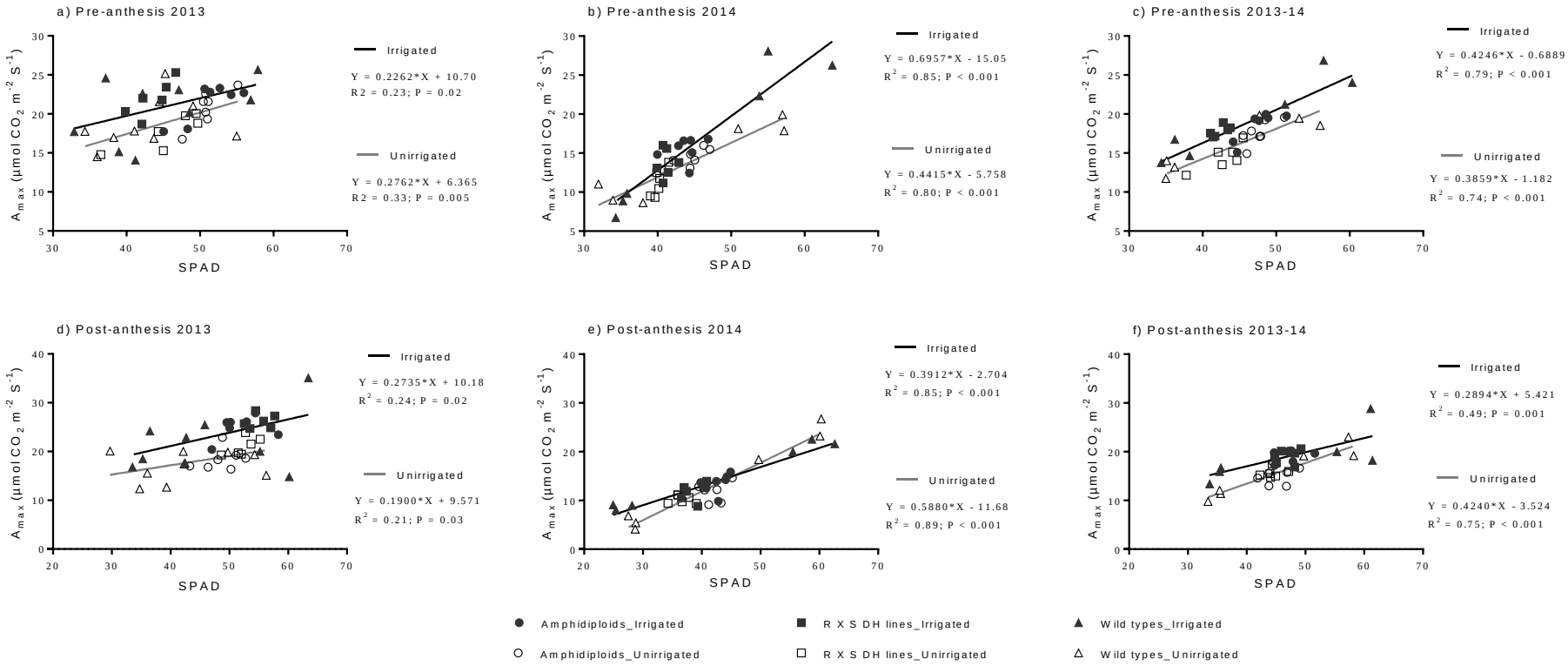


Figure 6.6 Linear regressions of pre-anthesis flag-leaf A_{max} (µmol m^{-2} s^{-1}) on pre-anthesis flag-leaf SPAD in (a) 2013 for 22 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) and in (b) 2014 and (c) cross-year mean for 19 genotypes, and linear regressions of post-anthesis flag-leaf A_{max} (µmol m^{-2} s^{-1}) on flag-leaf post-anthesis SPAD in (d) 2013 for 22 genotypes and in (e) 2014 and (f) cross-year mean for 19 genotypes under irrigated and droughted conditions.

Table 6.15 The phenotypic Pearson's correlation (r) between pre-anthesis and post-anthesis flag-leaf photosynthetic rate (A_{max}), stomatal conductance (g_s), transpiration efficiency (TE) and SPAD, and each of grain yield (GY), above-ground dry matter (AGDM), thousand grain weight (TGW), plant height (PH) and anthesis date (AD; DAS) among 22 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) in 2013 and 19 genotypes in 2014 and for the cross-year mean under irrigated and droughted treatments.

		GY (g plant^{-1})		AGDM (g plant^{-1})		TGW (g)		PH (cm)		AD (DAS; day)		WUE_{AGDM} (g l^{-1})	
	Traits	Irrigated	Droughted	Irrigated	Droughted	Irrigated	Droughted	Irrigated	Droughted	Irrigated	Droughted	Irrigated	Droughted
2013													
Pre-anthesis	A_{max} (µmol m^{-2} s^{-1})	0.12^{ns}	-0.12 ^{ns}	0.06 ^{ns}	0.02 ^{ns}	0.22 ^{ns}	0.12 ^{ns}	-0.18 ^{ns}	0.21 ^{ns}	0.15 ^{ns}	-0.29 ^{ns}	-0.06 ^{ns}	0.05 ^{ns}
	g_s (mol m^{-2} s^{-1})	-0.33 ^{ns}	-0.25 ^{ns}	0.21 ^{ns}	-0.23 ^{ns}	-0.22 ^{ns}	0.01 ^{ns}	0.38 ^{ns}	0.10 ^{ns}	-0.42*	-0.28 ^{ns}	0.29 ^{ns}	-0.20 ^{ns}
	TE (µmol mol^{-1})	0.49*	0.19 ^{ns}	0.26 ^{ns}	0.70***	0.81***	0.47*	-0.13 ^{ns}	0.32 ^{ns}	-0.20 ^{ns}	-0.60**	0.18 ^{ns}	0.77***
	SPAD	-0.08 ^{ns}	0.15 ^{ns}	-0.19 ^{ns}	0.13 ^{ns}	0.29 ^{ns}	0.45*	0.17 ^{ns}	0.05 ^{ns}	-0.23 ^{ns}	-0.27 ^{ns}	-0.12 ^{ns}	0.11 ^{ns}
Post-anthesis	A_{max} (µmol m^{-2} s^{-1})	0.36 ^{ns}	0.42*	0.19 ^{ns}	0.16 ^{ns}	0.41 ^{ns}	0.45*	-0.15 ^{ns}	-0.49*	0.08 ^{ns}	0.09 ^{ns}	0.08 ^{ns}	0.01 ^{ns}
	g_s (mol m^{-2} s^{-1})	0.64**	0.11 ^{ns}	0.53*	-0.04 ^{ns}	0.64**	0.01 ^{ns}	-0.11 ^{ns}	-0.41 ^{ns}	-0.37 ^{ns}	0.22 ^{ns}	0.49*	-0.15 ^{ns}
	TE (µmol mol^{-1})	-0.16 ^{ns}	0.42*	-0.27 ^{ns}	0.18 ^{ns}	-0.08 ^{ns}	0.53*	-0.04 ^{ns}	-0.09 ^{ns}	0.26 ^{ns}	-0.34 ^{ns}	-0.29 ^{ns}	0.26 ^{ns}
	SPAD	0.25 ^{ns}	0.40 ^{ns}	-0.23 ^{ns}	0.10 ^{ns}	0.40 ^{ns}	0.51*	-0.43*	-0.49*	0.39 ^{ns}	0.34 ^{ns}	-0.61**	-0.30 ^{ns}
2014													
Pre-anthesis	A_{max} (µmol m^{-2} s^{-1})	-0.36^{ns}	-0.39 ^{ns}	-0.75***	-0.54*	-0.25 ^{ns}	-0.18 ^{ns}	-0.11 ^{ns}	0.14 ^{ns}	0.64**	0.52*	-0.64**	-0.55*
	g_s (mol m^{-2} s^{-1})	-0.07 ^{ns}	0.37 ^{ns}	-0.51*	0.00 ^{ns}	0.20 ^{ns}	0.68**	-0.23 ^{ns}	-0.22 ^{ns}	0.11 ^{ns}	-0.36 ^{ns}	-0.46*	-0.04 ^{ns}
	TE (µmol mol^{-1})	-0.13 ^{ns}	-0.63**	-0.43 ^{ns}	-0.46*	-0.12 ^{ns}	-0.64**	-0.11 ^{ns}	0.32 ^{ns}	0.46*	0.71***	-0.35 ^{ns}	-0.46*
	SPAD	-0.41 ^{ns}	-0.34 ^{ns}	-0.78***	-0.69***	-0.29 ^{ns}	-0.19 ^{ns}	-0.11 ^{ns}	-0.02 ^{ns}	0.63**	0.57*	-0.66**	-0.71***
Post-anthesis	A_{max} (µmol m^{-2} s^{-1})	-0.37 ^{ns}	-0.36 ^{ns}	-0.78***	-0.81***	-0.29 ^{ns}	-0.25 ^{ns}	-0.06 ^{ns}	-0.14 ^{ns}	0.65**	0.65**	-0.68**	-0.82***
	g_s (mol m^{-2} s^{-1})	0.42 ^{ns}	0.02 ^{ns}	-0.28 ^{ns}	-0.66**	0.52*	0.12 ^{ns}	-0.57*	-0.55*	-0.22 ^{ns}	0.28 ^{ns}	-0.29 ^{ns}	-0.68**
	TE (µmol mol^{-1})	-0.71***	-0.29 ^{ns}	-0.03 ^{ns}	0.26 ^{ns}	-0.65**	-0.19 ^{ns}	0.68**	0.53*	0.47*	0.04 ^{ns}	0.05 ^{ns}	0.25 ^{ns}
	SPAD	-0.30 ^{ns}	-0.35 ^{ns}	-0.75***	-0.70***	-0.14 ^{ns}	-0.18 ^{ns}	-0.12 ^{ns}	-0.05 ^{ns}	0.54*	0.58**	-0.61**	-0.71***
2013-14													
Pre-anthesis	A_{max} (µmol m^{-2} s^{-1})	-0.29^{ns}	-0.31^{ns}	-0.66**	-0.32 ^{ns}	-0.20 ^{ns}	-0.01 ^{ns}	-0.08 ^{ns}	0.22 ^{ns}	0.43 ^{ns}	0.17 ^{ns}	-0.54*	-0.30 ^{ns}
	g_s (mol m^{-2} s^{-1})	-0.22 ^{ns}	0.13 ^{ns}	-0.28 ^{ns}	-0.08 ^{ns}	0.08 ^{ns}	0.48*	0.05 ^{ns}	-0.08 ^{ns}	-0.25 ^{ns}	-0.32 ^{ns}	-0.26 ^{ns}	-0.10 ^{ns}
	TE (µmol mol^{-1})	0.22 ^{ns}	-0.33 ^{ns}	-0.15 ^{ns}	0.17 ^{ns}	0.48*	-0.06 ^{ns}	-0.07 ^{ns}	0.47*	-0.12 ^{ns}	-0.07 ^{ns}	-0.04 ^{ns}	0.24 ^{ns}
	SPAD	-0.30 ^{ns}	-0.13 ^{ns}	-0.60**	-0.36 ^{ns}	-0.06 ^{ns}	0.13 ^{ns}	0.07 ^{ns}	0.07 ^{ns}	0.18 ^{ns}	0.11 ^{ns}	-0.44 ^{ns}	-0.37 ^{ns}
Post-anthesis	A_{max} (µmol m^{-2} s^{-1})	-0.04 ^{ns}	-0.14 ^{ns}	-0.44 ^{ns}	-0.71***	0.03 ^{ns}	-0.05 ^{ns}	-0.09 ^{ns}	-0.28 ^{ns}	0.26 ^{ns}	0.49*	-0.38 ^{ns}	-0.68**
	g_s (mol m^{-2} s^{-1})	0.63**	0.09 ^{ns}	-0.08 ^{ns}	-0.62**	0.70***	0.09 ^{ns}	-0.52*	-0.55*	-0.43 ^{ns}	0.33 ^{ns}	-0.11 ^{ns}	-0.63**
	TE (µmol mol^{-1})	-0.56*	-0.01 ^{ns}	-0.25 ^{ns}	0.38 ^{ns}	-0.49*	0.25 ^{ns}	0.35 ^{ns}	0.36 ^{ns}	0.40 ^{ns}	-0.32 ^{ns}	-0.16 ^{ns}	0.44 ^{ns}
	SPAD	-0.10 ^{ns}	-0.09 ^{ns}	-0.74***	-0.62**	0.00 ^{ns}	0.01 ^{ns}	-0.22 ^{ns}	-0.19 ^{ns}	0.36 ^{ns}	0.40 ^{ns}	-0.61**	-0.59**

N.B: ***denotes $P<0.001$; $^{**}P<0.01$ and $^{*}P<0.05$ significance levels; ^{ns} = not significant.

6.3.8 Relationships between post-anthesis flag-leaf A_{max}, g_s and TE under field conditions and glasshouse conditions.

Averaging over years, post-anthesis flag-leaf A_{max} measured in the field experiment was positively correlated with post-anthesis flag-leaf A_{max} measured in the glasshouse experiment under drought conditions (R^2 = 0.69; *P* = 0.04; Fig. 6.7a). There was also a positive association between post-anthesis g_s in the field experiment and post-anthesis g_s (R^2 = 0.67; *P* = 0.05; Fig. 6.7b) in the glasshouse experiment under drought conditions. In addition, a strong trend for a positive correlation between post-anthesis TE in the field and post-anthesis TE in the glasshouse was found (R^2 = 0.63; *P* = 0.06; Fig. 6.7c) under drought conditions.

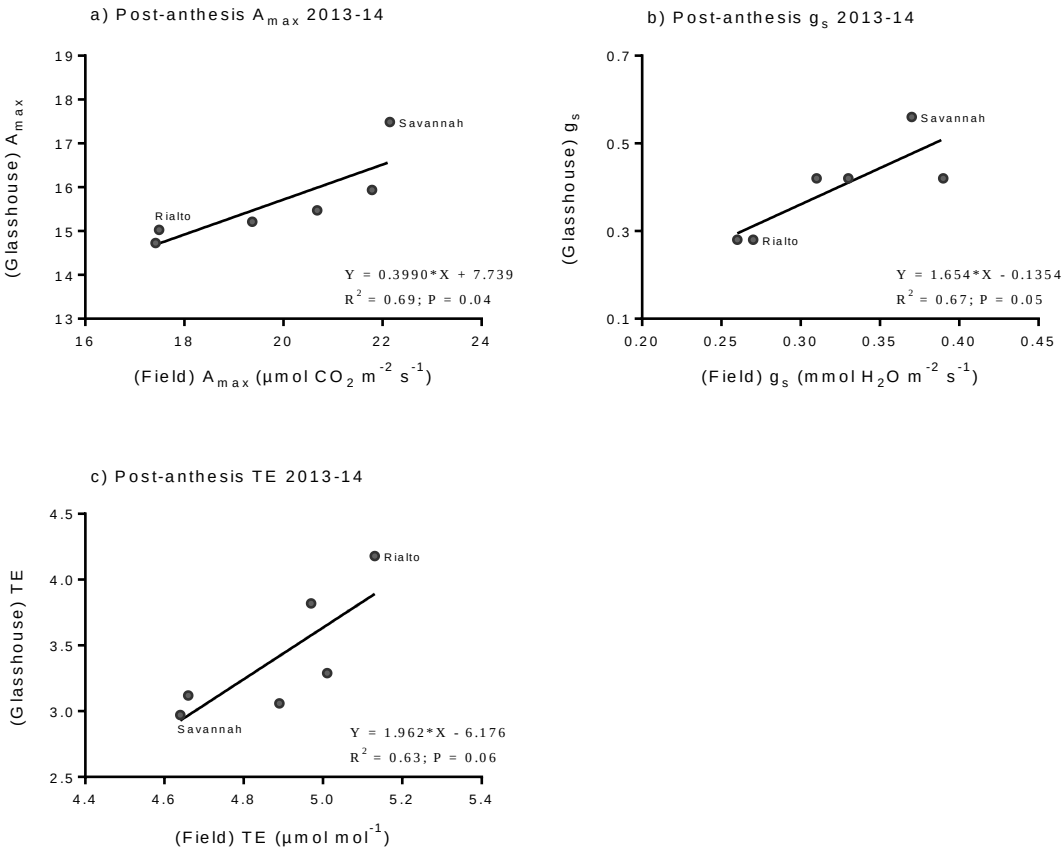


Figure 6.7 Linear regressions of (a) post-anthesis flag-leaf A_{max} (µmol m^{-2} s^{-1}), (b) g_s (mol m^{-2} s^{-1}) and (c) TE (µmol mol^{-1}) for the cross-year means (2013 and 2014) in the field on the cross-year means (2013 and 2014) in the glasshouse conditions for 4 R x S DH lines and the two parents under drought conditions.

6.3.9 Flag-leaf senescence

The temporal pattern of flag-leaf senescence for each group of germplasm (amphidiploid lines, R x S DH lines, *T. bessarabicum* accessions, *A. speltoides* accessions and *T. uratu* accessions) under irrigated and droughted treatments in 2013 and 2014 is presented in Figure 6.8. Amphidiploid lines had the latest onset and end of flag-leaf senescence followed by R x S DH lines, while ancestral wheat species (*T. bessarabicum*, *A. speltoides* and *T. uratu*) had earliest onset and end of flag-leaf senescence under both irrigated and unirrigated conditions in each year.

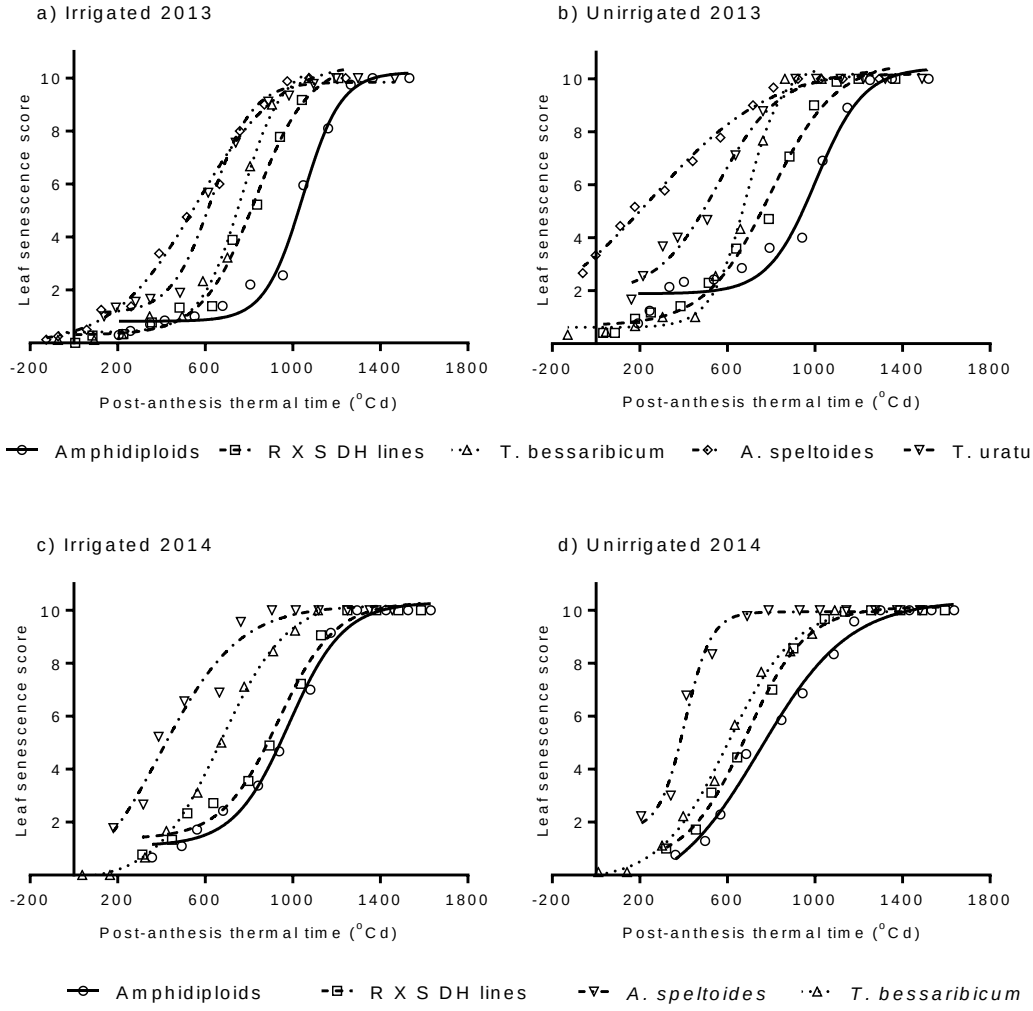


Figure 6.8 Regressions of visual flag-leaf senescence score on post-anthesis thermal time (^{o}Cd; 0 ^{o}C base temperature) for the groups of amphidiploid lines (7 lines), Rialto x Savannah DH lines (4 lines and parents), *T. bessarabicum* (3 accessions), *T. uratu* (3 accessions) and *A. speltoides* (3 accessions) under irrigated (a) and droughted (b) conditions in 2013, and under irrigated (c) and droughted (d) conditions in 2014.

6.3.9.1 Onset of Leaf senescence (SEN_{ONSET})

In 2013, there was no main effect of drought on SEN_{ONSET} (P=0.13). Averaging over irrigation treatments, genotypes differed in the range 29.4 (*A. speltoides* 1) to 908.1 ^{o}Cd (*T. bessarabicum* x Karim) (P<0.001). Responses to drought ranged amongst genotypes from -94.3 (DH line 25) to 539.4 ^{o}Cd (*A. speltoides* 2) (P=0.01; Table 6.16).

In 2014, drought advanced SEN_{ONSET} from 577.2 to 471.3 ^{o}Cd (P=0.006). Genotypes differed in the range 222.7 (*A. speltoides* 2) to 973.1 ^{o}Cd (*T. bessarabicum* x Karim) under irrigated conditions, and 282.6 (*A. speltoides* 3) to 732.8 ^{o}Cd (*T. bessarabicum* x Karim) under droughted conditions (P<0.001). There was a weak trend for an irrigation x genotype interaction (P=0.10; Table 6.16).

Averaging over years, drought advanced SEN_{ONSET} from 624.7 to 562.6 ^{o}Cd (P=0.02). Genotypes differed in the range 268.6 (*A. speltoides* 2) to 1009.9 (*T. bessarabicum* x Karim) ^{o}Cd under irrigated conditions, and 163.7 (*A. speltoides* 2) to 865.4 (*T. bessarabicum* x Karim) ^{o}Cd under droughted conditions (P<0.001). There was no irrigation x genotype interaction (P=0.21; Table 6.16). Averaging over years and irrigation treatments, amphidiploid lines (732.4 ^{o}Cd) were the latest to start leaf senescence followed by R x S DH lines (580.8 ^{o}Cd), *T. bessarabicum* accessions (510.2 ^{o}Cd) and *A. speltoides* accessions (193.7 ^{o}Cd) (P<0.001; Fig. 6.7).

6.3.9.2 End of leaf senescence (SEN_{END})

In 2013, drought advanced SEN_{END} from 1071.7 to 991.0 ^{o}Cd (P=0.008). Genotypes differed in the range 797.3 (*T. uratu* 3) to 1422.0 ^{o}Cd (*T. bessarabicum* x Karim) under irrigated conditions, and 565.6 (*A. speltoides* 2) to 1344.3 ^{o}Cd (*T. bessarabicum* x Karim) under droughted conditions (P<0.001). Advances in SEN_{END} under drought ranged from -35.2 (DH line 63) to 320.8 ^{o}Cd (*A. speltoides* 2) (P=0.02; Table 6.16).

In 2014, drought advanced SEN_{END} from 1131.9 to 989.1 ^{o}Cd (P=0.02). Genotypes differed in the range 701.0 (*A. speltoides* 2) to 1400.2 (*T. bessarabicum* x Karim) ^{o}Cd under irrigated conditions, and 530.3 (*A. speltoides* 2) to 1434.0 (*T.*

bessarabicum x Karim) ^{o}Cd under droughted conditions (P<0.001). There was no irrigation x genotype interaction (P=0.13; Table 6.16).

Averaging over years, drought advanced SEN_{END} from 1109.8 to 998.8 ^{o}Cd (P<0.001). Genotypes ranged from 793.7 (*A. speltoides* 2) to 1411.1 ^{o}Cd (*T. bessarabicum* x Karim) under irrigated conditions, and 547.9 (*A. speltoides* 2) to 1389.2 (*T. bessarabicum* x Karim) ^{o}Cd under droughted conditions (P<0.001). There was no irrigation x genotype interaction (P=0.12; Table 6.17). Averaging over years and irrigation treatments, amphidiploid lines (1215.3 ^{o}Cd) were the latest to reach end of leaf senescence followed by R x S DH lines (1069.7 ^{o}Cd), *T. bessarabicum* accessions (945.7 ^{o}Cd) and *A. speltoides* accessions (756.4 ^{o}Cd) (P<0.001; Fig. 6.7).

6.3.9.3 Leaf senescence rate (SEN_{RATE})

Averaging over years, drought accelerated the flag-leaf senescence rate from 0.009 to 0.012 $^{o}Cd^{-1}$ (P=0.01). Genotypes ranged from 0.006 (*T. bessarabicum* 2) to 0.013 $^{o}Cd^{-1}$ (*T. bessarabicum* x Stewart and Rialto) under irrigated conditions, and 0.007 (*T. bessarabicum* x Karim) to 0.020 $^{o}Cd^{-1}$ (*T. bessarabicum* x Stewart) under droughted conditions (P<0.001). Increases in senescence rate under drought amongst genotypes ranged from -0.012 to 0.004 $^{o}Cd^{-1}$ for *T. bessarabicum* x Neodur and *T. bessarabicum* x Karim, respectively (P<0.001; Table 6.17). Averaging over years and irrigation treatments, there was no difference in senescence rate between the groups (*T. bessarabicum* accessions (0.009 $^{o}Cd^{-1}$), amphidiploid lines (0.012 $^{o}Cd^{-1}$), R x S DH lines (0.010 $^{o}Cd^{-1}$) and *A. speltoides* accessions (0.011 $^{o}Cd^{-1}$)) (P=0.12; Fig. 6.8).

Table 6.16 Onset of post-anthesis flag-leaf senescence (SEN_{ONSET}) and end of flag-leaf senescence (SEN_{END}) for 22 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) in 2013 and 19 genotypes in 2014 and cross-year mean under irrigated and droughted treatments, and standard errors of the differences of the means (SED) and degrees of freedom (df) under irrigated and droughted treatments.

	Genotypes	SEN_{ONSET} (°Cd)						SEN_{END} (°Cd)					
		2013		2014		2013-14		2013		2014		2013-14	
		Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought
Amphidiploid lines	*T. bessarabicum* x Azaziah	797.0	746.8	878.8	692.8	837.9	778.2	1352.2	1216.4	1258.2	1245.0	1305.2	1230.7
	T. bessarabicum x Creso	871.8	840.8	742.4	496.6	807.1	712.0	1270.0	1237.6	1276.6	1181.8	1273.3	1209.7
	T. bessarabicum x Karim	1046.8	769.5	973.1	732.8	1009.9	865.4	1422.0	1344.3	1400.2	1434.0	1411.1	1389.2
	T. bessarabicum x Langdon	863.6	642.6	663.8	560.1	763.7	639.3	1134.4	1113.7	1239.2	1102.4	1186.8	1108.1
	T. bessarabicum x Macoun	806.1	839.6	638.4	478.6	722.3	685.4	1268.1	1188.5	1187.1	998.1	1227.6	1093.3
	T. bessarabicum x Neodur	785.0	720.2	579.6	464.7	682.3	623.4	1171.7	1109.0	1200.7	945.3	1186.2	1027.1
	T. bessarabicum x Stewart	904.4	735.2	724.3	511.4	814.3	724.1	1127.9	1153.1	1226.0	1225.3	1176.9	1189.2
R x S DH lines	Rialto	745.8	667.0	668.4	494.7	694.7	721.8	1008.0	957.1	1229.5	1098.0	1126.2	1048.4
	Savannah	599.5	332.9	643.7	549.0	634.0	494.2	1007.4	879.6	1244.4	1139.6	1118.5	988.8
	Line 20	745.2	573.5	487.6	518.3	616.4	583.3	1123.3	1120.5	1268.2	957.6	1195.8	1039.1
	Line 25	482.2	576.6	569.1	443.4	525.6	502.6	969.8	913.0	1124.5	961.2	1047.2	937.1
	Line 63	699.0	710.6	614.5	412.9	656.8	659.4	1119.5	1154.6	1222.2	968.4	1170.8	1061.5
	Line 88	664.8	714.4	555.6	470.8	610.2	590.9	1044.9	991.4	1216.2	954.8	1130.6	973.1
Ancestral wheat species	*T. bessarabicum* 1	639.6	550.7	477.4	446.4	558.5	494.5	937.8	844.1	951.9	874.8	944.9	859.4
	T. bessarabicum 2	530.8	529.9	414.0	391.9	472.4	470.2	967.5	831.2	1019.5	1030.4	993.5	930.8
	T. bessarabicum 3	637.8	573.7	539.3	390.4	588.6	540.1	976.6	895.7	1030.1	988.6	1003.3	942.2
	T. uratu 1	427.5	291.6	-	-	-	-	1072.9	925.5	-	-	-	-
	T. uratu 2	432.4	383.9	-	-	-	-	1039.0	889.0	-	-	-	-
	T. uratu 3	445.3	215.0	-	-	-	-	797.3	827.4	-	-	-	-
	A. speltoides 1	236.2	-177.4	308.8	293.7	272.5	248.2	896.4	792.2	847.0	589.2	871.7	690.7
	A. speltoides 2	314.5	-224.9	222.7	324.3	268.6	163.7	886.4	565.6	701.0	530.3	793.7	547.9
	A. speltoides 3	400.1	78.8	265.7	282.6	332.9	192.3	984.8	853.2	862.7	568.3	923.7	710.7
Grand Mean		639.8	504.1	577.2	471.3	624.7	562.6	1071.7	991.0	1131.9	989.1	1109.8	998.8
SED (df)	*Year*	-		-		$38.28^{(0.10)}$ *(4)*		-		-		7.95^{ns} *(4)*	
	Irrigation	54.00^{ns} *(2)*		7.98^{**} *(2)*		32.52^{*} *(4)*		7.32^{**} *(2)*		21.00^{*} *(2)*		11.87^{***} *(4)*	
	Genotype	77.80^{***} *(84)*		55.92^{***} *(72)*		46.99^{***} *(144)*		39.83^{***} *(84)*		64.00^{***} *(72)*		37.99^{***} *(144)*	
	Irri. x Gen.	120.40^{**} *(84)*		77.38^{ns} *(72)*		72.39^{ns} *(144)*		55.52^{*} *(84)*		90.60^{ns} *(72)*		53.62^{ns} *(144)*	
	Year x Gen.	-		-		75.16^{***} *(144)*		-		-		52.89^{***} *(144)*	

N.B: ***denotes $P<0.001$; $^{**}P<0.01$ and $^{*}P<0.05$ significance levels; ^{ns} = not significant.

Table 6.17 Post-anthesis flag-leaf senescence rate (SEN_{RATE}) for 22 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) in 2013 and 19 genotypes in 2014 and cross-year mean under irrigated and droughted treatments, and standard errors of the differences of the means (SED) and degrees of freedom (df) under irrigated and droughted treatments.

	Genotypes	SEN_{RATE} ($^oCd^{-1}$)					
		2013		2014		2013-14	
		Irrigated	Drought	Irrigated	Drought	Irrigated	Drought
Amphidiploid lines	*T. bessarabicum* x Azaziah	0.006	0.010	0.012	0.007	0.009	0.008
	T. bessarabicum x Creso	0.012	0.012	0.008	0.006	0.010	0.009
	T. bessarabicum x Karim	0.012	0.009	0.010	0.006	0.011	0.007
	T. bessarabicum x Langdon	0.017	0.019	0.007	0.011	0.012	0.015
	T. bessarabicum x Macoun	0.009	0.020	0.009	0.010	0.009	0.015
	T. bessarabicum x Neodur	0.009	0.029	0.007	0.011	0.008	0.020
	T. bessarabicum x Stewart	0.017	0.024	0.009	0.006	0.013	0.015
R x S DH lines	Rialto	0.017	0.012	0.009	0.007	0.013	0.010
	Savannah	0.007	0.010	0.009	0.008	0.008	0.008
	Line 20	0.011	0.010	0.008	0.012	0.009	0.011
	Line 25	0.006	0.013	0.010	0.011	0.008	0.012
	Line 63	0.009	0.011	0.008	0.008	0.009	0.009
	Line 88	0.010	0.018	0.008	0.009	0.009	0.014
Ancestral wheat species	*T. bessarabicum* 1	0.012	0.014	0.009	0.009	0.011	0.011
	T. bessarabicum 2	0.007	0.013	0.005	0.005	0.006	0.009
	T. bessarabicum 3	0.011	0.013	0.009	0.006	0.010	0.009
	T. uratu 1	0.011	0.008	-	-	-	-
	T. uratu 2	0.008	0.009	-	-	-	-
	T. uratu 3	0.013	0.007	-	-	-	-
	A. speltoides 1	0.006	0.005	0.008	0.018	0.007	0.011
	A. speltoides 2	0.007	0.008	0.015	0.026	0.011	0.017
	A. speltoides 3	0.007	0.007	0.007	0.019	0.007	0.013
Grand Mean		0.010	0.013	0.009	0.010	0.009	0.012
SED (df)	*Year*	-		-		*0.0008^{*} (4)*	
	Irrigation	*0.0010^{ns} (2)*		*0.0001^{**} (2)*		*0.0006^{*} (4)*	
	Genotype	*0.0022^{***} (84)*		*0.0025^{***} (72)*		*0.0017^{***} (144)*	
	Irri. x Gen.	*0.0032^{***} (84)*		*0.0035^{*} (72)*		*0.0024^{***} (144)*	
	Year x Gen.	-		-		*0.0024^{***} (144)*	

N.B: ***denotes $P<0.001$; $^{**}P<0.01$ and $^{*}P<0.05$ significance levels; ^{ns} = not significant.

6.3.9.4 Relationships between senescence parameters, anthesis date, TGW and grain yield

In 2013, a positive linear relationship amongst genotypes was found between SEN_{ONSET} and TGW under both irrigated (R^2=0.64; P<0.001) and droughted (R^2=0.46; P=0.001) conditions. Similar relationships were observed in 2014 under irrigated (R^2=0.48; P=0.001) and droughted (R^2=0.54; P<0.001) conditions, and averaging across years (R^2=0.56; P<0.001 and R^2=0.53; P<0.001, respectively; Table 6.18).

There were positive linear relationships amongst genotypes between SEN_{END} and TGW in 2013 under both irrigated (R^2=0.40; P=0.002) and droughted (R^2=0.74; P<0.001) conditions, and in 2014 (R^2=0.86; P<0.001 and R^2=0.93; P<0.001, respectively), and averaging across years (R^2=0.62; P<0.001 and R^2=0.87; P<0.001, respectively; Table 6.19).

In 2013, a negative linear relationship amongst genotypes was found between SEN_{ONSET} and anthesis date under irrigated conditions (R^2=0.23; P=0.02), but not under droughted conditions (P=0.14). Negative linear relationships were also observed under both irrigated (R^2=0.29; P=0.02) and droughted conditions (R^2=0.29; P=0.02) in 2014, and averaging across years (R^2=0.43; P=0.002 and R^2=0.28; P=0.02, respectively; Table 6.19).

There were negative linear relationships amongst genotypes between SEN_{END} and anthesis date in 2013 under both irrigated (R^2=0.48; P<0.001) and droughted conditions (R^2=0.40; P=0.002), and in 2014 (R^2=0.38; P=0.005 and P=0.14, respectively), and averaging across years (R^2=0.58; P<0.001 and R^2=0.32; P=0.01, respectively; Table 6.19).

Table 6.18 The phenotypic correlation (r) between onset of leaf senescence (SEN_{ONSET}), leaf senescence rate (SEN_{RATE}) and end of leaf senescence (SEN_{END}), and each of grain yield (GY), above-ground dry matter (AGDM), harvest index (HI), thousand grain weight (TGW), plant height (PH) and anthesis date (AD; DAS) among 22 genotypes (amphidiploid lines, R x S DH lines and ancestral wheat species) in 2013 and 19 genotypes in 2014 and cross-year mean under irrigated and droughted treatments.

	Traits	GY (g plant^{-1})	AGDM (g plant^{-1})	HI	TGW (g)	PH (cm)	AD (DAS; day)
2013							
Irrigated	SEN_{ONSET} (^{o}Cd)	0.36^{ns}	0.13^{ns}	0.23^{ns}	0.81***	-0.02^{ns}	-0.49*
	SEN_{RATE} (^{o}Cd^{-1})	0.08^{ns}	0.02^{ns}	0.06^{ns}	0.33^{ns}	0.15^{ns}	-0.31^{ns}
	SEN_{END} (^{o}Cd)	0.28^{ns}	0.23^{ns}	0.16^{ns}	0.73***	0.02^{ns}	-0.69***
Droughted	SEN_{ONSET} (^{o}Cd)	0.37^{ns}	0.14^{ns}	0.33^{ns}	0.66***	-0.20^{ns}	-0.26^{ns}
	SEN_{RATE} (^{o}Cd^{-1})	0.08^{ns}	0.39^{ns}	-0.07^{ns}	0.44*	0.33^{ns}	-0.16^{ns}
	SEN_{END} (^{o}Cd)	0.36^{ns}	0.41^{ns}	0.28^{ns}	0.69***	-0.06^{ns}	-0.61**
2014							
Irrigated	SEN_{ONSET} (^{o}Cd)	0.32 ^{ns}	0.07 ^{ns}	0.37 ^{ns}	0.69***	-0.12 ^{ns}	-0.54*
	SEN_{RATE} (^{o}Cd^{-1})	-0.01^{ns}	0.23 ^{ns}	0.01 ^{ns}	0.04 ^{ns}	0.13 ^{ns}	-0.23 ^{ns}
	SEN_{END} (^{o}Cd)	0.55*	0.16 ^{ns}	0.59**	0.85***	-0.32 ^{ns}	-0.62**
Droughted	SEN_{ONSET} (^{o}Cd)	0.38 ^{ns}	0.07 ^{ns}	0.42 ^{ns}	0.73***	-0.17 ^{ns}	-0.53*
	SEN_{RATE} (^{o}Cd^{-1})	-0.20^{ns}	0.37 ^{ns}	-0.26 ^{ns}	-0.46*	0.37 ^{ns}	-0.02 ^{ns}
	SEN_{END} (^{o}Cd)	0.33 ^{ns}	-0.08 ^{ns}	0.37 ^{ns}	0.72***	-0.26 ^{ns}	-0.36 ^{ns}
2013-14							
Irrigated	SEN_{ONSET} (^{o}Cd)	0.32^{ns}	0.02^{ns}	0.31^{ns}	0.75***	-0.03^{ns}	-0.66**
	SEN_{RATE} (^{o}Cd^{-1})	0.01^{ns}	0.10^{ns}	0.05^{ns}	0.39^{ns}	0.09^{ns}	-0.35^{ns}
	SEN_{END} (^{o}Cd)	0.47*	0.15^{ns}	0.43^{ns}	0.83***	-0.10^{ns}	-0.76***
Droughted	SEN_{ONSET} (^{o}Cd)	0.35^{ns}	0.07^{ns}	0.39^{ns}	0.71***	-0.16^{ns}	-0.48*
	SEN_{RATE} (^{o}Cd^{-1})	-0.17^{ns}	0.48*	-0.29^{ns}	-0.02^{ns}	0.62**	-0.15^{ns}
	SEN_{END} (^{o}Cd)	0.33^{ns}	0.15^{ns}	0.34^{ns}	0.72***	-0.13^{ns}	-0.55*

N.B: ***denotes $P<0.001$; $^{**}P<0.01$ and $^{*}P<0.05$ significance levels; ^{ns} = not significant.

6.4 Discussion

The discussion will focus on the novel genetic variation identified in the wider germplasm compared with the Rialto x Savannah DH lines and on the mechanisms underlying the genotypic differences in gas-exchange and leaf senescence parameters associated with drought tolerance and water-use efficiency.

6.4.1 Crop development, yield, biomass and plant height responses to drought

Generally above-ground dry matter per plant was lower in 2014 compared to 2013 associated with a trend for lower tiller number in 2014. This decrease in fertile-shoot plant in 2014 was probably caused by longer vernalization period by 15 days, and higher soil bulk density which might have caused root growth limitation than in 2013. Across both experiments and irrigation treatments, amphidiploid lines developed faster reaching anthesis 8 days earlier than Rialto x Savannah DH lines, and 25 days earlier than *T. bessarabicum* accessions. Both amphidiploids and R x S DH lines had a similar duration of the stem-elongation period from GS31 to GS61, but each was shorter in duration of stem-elongation than the ancestral wheat species.

Early flowering was associated with lower shoot number per plant, therefore the faster developing amphidiploid line (*T. bessarabicum* x Karim) had reduced shoot number compared to the slower developing genotypes, *A. speltoides* accessions and *T. bessarabicum* accessions. Developmental rate may therefore be partly associated with profuse tillering behaviour for the ancestral wheat genotypes. Wild relatives of wheat are very different to bread wheat, and grain yield and biomass were not expected to be comparable, but the wheat wild relatives were hypothesized to have high expression of WUE and drought tolerance and related traits. However, grain yield and biomass comparisons were relevant for amphidiploids compared to R x S DH lines. Overall the harvest index had more effect on the genetic variation in final grain weight than AGDM in these genotypes.

In both experiments, amphidiploid lines had lower grain yield and greater biomass compared to the adapted UK winter wheats (R x S DH lines). This is partly explained by increased plant height in the amphidiploids raising biomass but decreasing HI

compared to R x S DH lines. Thus, although amphidiploid lines produced a large biological yield, this was not reflected in the grain yield due to the lower harvest index compared to R x S DH lines.

Regarding plant height, various studies have shown that shorter wheat genotypes were associated with higher grain yields and increased HI (Fischer, 1985; Miralles and Slafer, 1997; González *et al.*, 2003; Bognár *et al.*, 2007; Toyota *et al.*, 2010).

In the present study, the percentage of grain yield and AGDM reduction under drought was generally higher for the amphidiploid lines than the R x S DH lines. However, DH line 25 and amphidiploid line *T. bessarabicum* x Karim had the lowest yield loss (8 and 9%, respectively) and biomass (10 and 13%, respectively) under drought. Ancestral wheat species had longer rachises but fewer spikelets per spike than R x S DH lines. However, amphidiploid lines had intermediate rachis length and spikelets per ear. In addition, R x S DH lines had heavier grain weights than amphidiploid lines and ancestral wheat species.

These results indicate that amphidiploid wheat genotypes had an ability to produce higher above-ground dry matter, but not to DM partition effectively to the grain yield. Therefore, higher biomass for amphidiploids is a promising trait of wheat breeders can increase HI in this material through improving traits related to biomass partitioning in the future breeding programmes.

6.4.2 Genetic variation in water uptake and water-use efficiency

In the present study, drought increased WUE_{AGDM} by 20% averaging over years and genotypes consistent with many previous investigations (Hubick and Farquhar, 1989; Condon *et al.*, 1990; Foulkes *et al.*, 2001; Akhter *et al.*, 2008). However, several investigations have reported a decrease of WUE_{AGDM} under drought (Pannu and Singh, 1993; Johnson and Henderson, 2002; Aravinda Kumar, 2006; Cabrera-Bosquet *et al.*, 2007).

Averaging over years, post-anthesis A_{max}, g_s and TE measured in the field for R x S DH lines and the parents were strongly correlated with those measured in the glasshouse. This increases the confidence in the present results measured in both

field and glasshouse experiments, and demonstrates measurements on the flag-leaf in glasshouse conditions can be representative of those in field grown conditions.

Leaf photosynthetic rates in the present study were generally lower in 2014 than those in 2013 for all genotypes (except for *T. bessarabicum* accessions), and genotypes differed in their photosynthetic efficiency under water stress in both years. Lower A_{max} in 2014 compared to those in 2013 may be associated with high soil bulk density and root growth limitation, and hence less water uptake and relatively higher water stress in 2014. High bulk density can effectively reduce root growth ability and above-ground growth as a consequence (Atwell, 1993; Bingham, 2001; Bingham and Bengough, 2003; Clark *et al.*, 2003).

Overall, there was a decrease in leaf photosynthetic rate (A_{max}) by 15% and stomatal conductance (g_s) by 25% whereas, leaf transpiration efficiency (TE) tended to be increased by 4% due to water stress. The decrease in A_{max} and g_s, and the increase in TE under drought are associated with increasing stomatal closure and low CO_2 concentration inside the leaf under water stress, and hence lower photosynthetic efficiency and relatively higher TE (Richards, 2000; Lawlor and Cornic, 2002; Lopes and Araus, 2006). Increasing WUE associated with low stomatal conductance has been previously reported by (Blum, 1990; Morgan and Lecain, 1991; Morgan *et al.*, 1993; vandenBoogaard *et al.*, 1997; Fischer *et al.*, 1998; Lu *et al.*, 1998).

Genotypes differed in the amount of water used under drought. *T. bessarabicum* accessions showed the smallest cumulative water (lowest shoot number) followed by amphidiploids and R x S DH lines, and *A. speltoides* accessions showed the greatest amount of WU (highest shoot number). Therefore, water uptake per plant was strongly related to the effect of shoot number per plant affecting plant demand for water. Amphidiploid lines had the highest water-use efficiency (WUE_{AGDM}) followed by UK adaptive winter wheat (R x S DH lines), and *T. bessarabicum* accessions had the lowest WUE_{AGDM} under irrigated and droughted treatments. These differences appeared to be associated more with the genetic variation in AGDM than the genetic variation in WU since the correlation between WUE and AGDM among genotypes was stronger than that with WU. Overall, higher WUE genotypes were associated with higher TE and lower g_s post-anthesis under drought.

Pooling over data across years, accessions of *T. bessarabicum* had the highest pre-anthesis and post-anthesis flag-leaf A_{max} amongst the genotype groups. The *T. bessarabicum* accessions also had the highest leaf SPAD amongst the genotype groups associated with smaller flag-leaf size and greater leaf specific weight. High leaf specific weight may be related to a higher amount of N and Rubisco per unit leaf area for *T. bessarabicum* accessions leading to reduced light saturation and higher photosynthetic capability per unit leaf area and hence higher A_{max} (Morgan *et al.*, 1990; Shearman *et al.*, 2005).

Although, relatively few differences in leaf A_{max} between R x S lines and amphidiploids were found, amphidiploid line *T. bessarabicum* x Karim had significantly higher A_{max} than the best R x S DH line, line 20 under drought. In wheat, genetic variation in A_{max} and g_s between different genotypes has been previously reported (Blum, 1990; Morgan and Lecain, 1991; Fischer *et al.*, 1998; Xue *et al.*, 2002). The high pre-anthesis A_{max} and SPAD by individual amphidiploid lines compared to R x S DH lines under drought was related to the flag-leaf specific weight, but not to a decrease in flag-leaf area. Thus, higher A_{max} for amphidiploid lines may be more useful than that for *T. bessarabicum* accessions since it appeared to be independent of leaf area.

In addition, pre-anthesis and post-anthesis flag-leaf chlorophyll content (SPAD) was positively associated with flag-leaf photosynthetic rate under both irrigated and droughted conditions. This variation related to flag-leaf specific weight since a strong positive association was found between SPAD and FLSW under both irrigated and droughted treatments. This indicates there is scope for using leaf SPAD as an indirect selection tool for higher photosynthetic rate.

The negative association between flag-leaf A_{max} and AGDM in the present study might be as a result of the wide variation in leaf morphology such as leaf area, specific weight and architecture between genotypes. Although, small leaf size increased flag-leaf A_{max}, it highly reduced AGDM per plant due to a decrease in light interception for the whole plant.

6.4.3 Effect of restricted water availability on genotype flag-leaf senescence

In the present study, early flowering genotypes were associated with later leaf senescence and a longer grain filling period. This association was consistent with the field experiment results in chapter 5 and those reported by Bogard *et al.* (2011) between post-anthesis leaf senescence duration and anthesis date. Leaf senescence during grain filling may be influenced by early flowering through indirectly increasing post-anthesis N uptake and reducing pre-anthesis N uptake and hence delaying N remobilization and resulting longer leaf senescence duration (Bogard *et al.*, 2010). Under drought, there may also have been drought escape effects with reducing the effect of drought with early flowering genotypes.

The onset and end of flag-leaf senescence were positively associated with thousand grain weight under both irrigated and droughted conditions. Negative correlations were found in previous investigations between rate of leaf senescence and thousand grain weight (Hafsi *et al.*, 2000), and positive correlations between flag-leaf duration and grain yield (Takahashi and Nakaseko, 1990; Alejar *et al.*, 1995; Hafsi *et al.*, 2007; Gregersen *et al.*, 2008).

Drought advanced onset and end of flag-leaf senescence by approximately 10%, and accelerated rate of flag-leaf senescence by 33%. Genotypes with longer leaf duration tended to have higher grain yield under irrigated conditions, while no correlation was found under drought conditions. Although AGDM per plant was also not correlated with flag-leaf senescence duration under both irrigated and droughted conditions, main shoot AGDM was strongly positively associated with both SEN_{ONSET} and SEN_{END} under both irrigated and droughted conditions. The absence of a correlation between senescence parameters and AGDM per plant may be partly due to wide variation in fertile shoots per plant amongst genotypes in this study.

Amphidiploid lines in this study were delayed in both onset and end of post-anthesis flag-leaf senescence compared with R x S DH lines, *T. bessarabicum* accessions and *A. speltoides* accessions under drought. Since delayed onset and end of leaf senescence under drought was associated with less biomass reduction in response to drought amongst genotypes, this delayed leaf senescence in amphidiploids might be

related to a deeper and/or longer root system resulting in more water and N uptake and prolonged green area compared to other genotypes (Sawhney and Singh, 2002; Kasim *et al.*, 2013; Guo *et al.*, 2015). This could partly explain the higher AGDM for amphidiploids compared with R x S DH lines under drought. The stay-green traits observed for the amphidiploid lines are encouraging and indicating scope for wheat breeders to introgress associated genes into adapted bread wheat to enhance drought tolerance in future breeding programmes.

6.5 Conclusions

The results in this Chapter allow the following conclusions to be made:

1. Amphidiploid lines developed faster and had higher above-ground biomass associated with taller plants, and lower grain yield due to low harvest index under drought compared with Rialto x Savannah DH lines and ancestral wheat species.
2. Accessions of *T. bessarabicum* had highest flag-leaf photosynthetic rate, leaf transpiration efficiency and leaf SPAD associated with smaller leaf size and higher flag-leaf specific weight under drought compared with the amphidiploid lines and Rialto x Savannah DH lines.
3. Amphidiploid lines had higher flag-leaf photosynthetic rate, leaf SPAD and water-use efficiency associated with higher flag-leaf specific weight under drought compared with the UK winter wheat Rialto x Savannah DH lines.
4. Amphidiploid lines had delayed onset and end of flag-leaf senescence compared to the Rialto x Savannah DH lines in part associated with early flowering under droughted treatments.

Chapter 7 QTL analysis in Rialto x Savannah DH population

7.1 Introduction

The results of the genetic analysis are described in this chapter carried out on the Rialto x Savannah DH population in field experiments in 2013 and 2014 under irrigated and unirrigated conditions at the University of Nottingham, Sutton Bonington campus. A genetic linkage map was first constructed for the Rialto x Savannah DH population using the software package JoinMap version 4 (Van Ooijen, 2006), and then the quantitative trait loci (QTL) analysis was performed separately for each of the irrigation treatments in the two years using the software package MapQTL version 6 (Van Ooijen, 2009) for agronomic and physiological traits associated with drought tolerance. Phenotypic data were assessed for harvest including grain yield, above-ground dry matter, harvest index, thousand grain weight, plant height, anthesis date and grain $\Delta^{13}C$, and traits related to canopy stay-green and photosynthesis, e.g. normalized difference vegetative index and canopy temperature, as described in Chapters 4 and 5. The results are presented for significant QTLs (LOD > genome wide permutation test threshold (LOD; GW) for each trait) and putative QTLs (LOD > 2.0) and their location on the chromosomes of interest.

In wheat, QTLs for drought tolerance traits have been reported in previous investigations (Verma *et al.*, 2004; Foulkes *et al.*, 2006; Snape *et al.*, 2007; Rebetzke *et al.*, 2008a; Peleg *et al.*, 2009; Pinto *et al.*, 2010). The overall objective of this chapter was to identify quantitative trait loci (QTL) for grain yield and yield components and physiological traits, and to understand the genetic bases of these traits under well water and unirrigated conditions, and to compare the identified QTLs with previous published QTLs in wheat.

The specific hypotheses tested in this chapter are:

1. Quantitative trait loci (QTLs) can be identified for grain yield and associated physiological traits explaining variation in WUE and drought tolerance traits under irrigated and unirrigated conditions in the Rialto x Savannah winter wheat DH population.

2. Co-located QTLs for target drought tolerance traits can be found in the Rialto x Savannah DH population under the two irrigation treatments in the two years.

7.2 Materials and Methods

7.2.1 Construction of the genetic linkage map

The genetic map used for QTL analysis was constructed using JoinMap version 4 (Van Ooijen, 2006) using 578 SNP markers obtained from CerealsDB website at the University of Bristol with support from BBSRC (Wilkinson *et al.*, 2012). First, the genotyped lines (124 lines) from the Rialto x Savannah DH population and 5,000 markers were entered into the software, and then all repeated and redundant markers were excluded after which 578 markers remained in order to obtain the linkage groups. The linkage groups were defined based upon the test for linkage LOD 6 for each group, and then 22 linkage groups were mapped based on map distance calculation using the Haldane's mapping function (Haldane, 1919). In total, a map length of 2171 cM was distributed on the 21 chromosomes with the density of 3.8 cM per marker. There was relatively poorer coverage of markers on genome D and chr 4B and 7B in genome B compared with genome A. The number of markers and the length and density of each group for each chromosome are shown in Table 7.1.

7.2.2 QTL analysis

QTL analysis was carried out using the MapQTL software package version 6 (Van Ooijen, 2009). First, interval mapping (IM) was carried out for the QTL detection using 22 genetic linkage groups of 578 markers and phenotypic data of harvest traits including grain yield, above-ground dry matter, harvest index, thousand grain weight, plant height, anthesis date and grain $\Delta^{13}C$, and traits related to canopy and leaf senescence for 91 lines of Rialto x Savannah DH population in the present study. Then, permutation test was carried out in order to determine the significance threshold of the LOD score (Churchill and Doerge, 1994) by taking the genome wide (including all groups) threshold with a P-value of 0.05 for each of the trait x irrigation x year combinations. Significant QTLs (LOD > genome wide LOD at 0.95 relative cumulative count) and putative QTLs (LOD > 2) for each trait are shown in

the result section in Tables 7.2-7.15, and also in Figures 7.1-7.3 using the MapChart 2.3 software package (Voorrips, 2002).

Table 7.1 Marker number, map length and density in each chromosome and genome for the Rialto x Savannah DH population genetic linkage map.

Chr	No. markers (M)	Map length (cM)	Map density (cM/M)
1A	41	95.7	2.3
1B	44	117.9	2.7
1D	15	70.9	4.7
2A	66	152.2	2.3
2B	44	115.5	2.6
2D	5	43.5	8.7
3A	40	177.4	4.4
3B	40	154.7	3.9
3D	9	62.5	6.9
4A1	15	84.0	5.6
4A2	11	56.3	5.1
4B	17	94.5	5.6
4D	13	28.6	2.2
5A	46	189.7	4.1
5B	20	130.4	6.5
5D	7	17.9	2.6
6A	37	142.4	3.8
6B	41	71.0	1.7
6D	9	52.7	5.9
7A	42	138.4	3.3
7B	9	90.1	10.0
7D	7	85.2	12.2
Genome			
A	298	1036.2	3.5
B	215	774.1	3.6
D	65	361.1	5.6
Total	578	2171.4	3.8

7.2.3 Broad sense heritability

Broad sense heritability was calculated for grain yield, above-ground dry matter, harvest index, thousand grain weight, plant height, anthesis date, grains per m^2 and $\Delta^{13}C$ for 94 DH lines of the Rialto x Savannah DH population under individual irrigation treatments (irrigated and unirrigated) in 2013, 2014 and for the cross-year mean using META-R (Multi Environment Trail Analysis with R) software version 5.0. The programme was developed by the International Maize and Wheat Improvement Centre (CIMMYT).

Broad-sense heritability (h^2) was calculated on a sub-plot basis as described by Rebetzke *et al.* (2002):

$$h^2 = \frac{\sigma^2_G}{(\sigma^2_G + \sigma^2_{G.E} + \sigma^2_{Residual})}$$

(Equation 7.1)

Where σ^2_G is genotypic variances, $\sigma^2_{G.E}$ is genotype x environment variance, and $\sigma^2_{Residual}$ is the residual variance.

7.3 Results

7.3.1 QTLs identified for grain yield and yield components

7.3.1.1 Grain yield

In 2013, a major QTL was found on chr 3A (LOD = 3.98) at 111.2 cM explaining 18.3% of the phenotypic variation coinciding with marker Excalibur_c2578_1966 under irrigated conditions. Under unirrigated conditions, the same QTL was found on chr 3A (LOD = 6.87) at almost the same position of 110.8 cM close to marker Excalibur_c2578_1966 explaining 19.4% of the variation in grain yield. Both QTLs indicated that grain yield was increased by the increase of the Rialto allele (Table 7.2; Fig. 7.1).

In 2014, no QTL with LOD > 2.0 was found under irrigated conditions; the highest LOD of 1.73 was found on chr 7A at 73.4 cM coinciding with markers BS00022145_51 and Excalibur_c97905_134 under irrigated conditions. However, a significant QTL on chr 4A1 (LOD = 3.32) at 16.4 cM close to marker BS00021738_51 explained 15.5% of the phenotypic variance under drought; yield increased with the presence of Savannah allele (Table 7.2; Fig. 7.1).

7.3.1.2 Harvest index

In 2013, two major QTLs were found under irrigated conditions, on chr 2A (LOD = 3.67) at 69.2 cM coinciding with marker BobWhite_c23953_312 explaining 17.0% of the phenotypic variation, and on chr 3B (LOD = 2.96) at 90.9 cM coinciding with marker BS00025679_51 explaining 13.9% of the phenotypic variation. For both QTLs higher HI was associated with the Savannah allele. Under unirrigated

conditions, a QTL was found on chr 4A (LOD = 3.65) at 28.4 cM coinciding with marker Excalibur_c7897_600 explaining 16.9% of the variation in HI; HI was increased by the presence of the Savannah allele (Table 7.2; Fig. 7.1).

In 2014, three putative QTLs were found under irrigated conditions on chr 3A (LOD = 2.15), 3B (LOD = 2.25) and 7A (LOD = 2.53) explaining 10.3, 10.8 and 12.0% of phenotypic variation, respectively. A significant QTL on chr 2A (LOD = 3.11) at 72.5 cM coinciding with marker BS00022241_51 was found under unirrigated conditions explaining 14.6% of the phenotypic variation with HI increasing with the Savannah allele (Table 7.2; Fig. 7.1).

7.3.1.3 Above-ground dry matter

In 2013, two putative QTLs for AGDM were found under irrigated conditions, on chr 3A (LOD = 2.40) at 111.2 cM explaining 11.4% of the phenotypic variation, and on chr 6A (LOD = 2.47) at 66.7 cM explaining 11.7% of the phenotypic variation. Under unirrigated conditions, a QTL was found on chr 3A (LOD = 5.01) at 82.0 cM close to marker Excalibur_c91154_164 explaining 22.4% of phenotypic variation, with AGDM increasing with the Rialto allele. Two putative QTLs were also found on chr 4D (LOD = 2.88) and 6A (LOD = 2.58) explaining 13.6 and 12.2% of the phenotypic variation, respectively (Table 7.2; Fig. 7.1).

In 2014, a QTL on chr 1D (LOD = 3.07) at 46.9 cM close to marker RFL_Contig1338_2646 was found under irrigated conditions explaining 14.4% of the phenotypic variation with AGDM increasing with the Rialto allele. Two putative QTLs on chr 3A (LOD = 2.04) and 7D (LOD = 2.21) under irrigated conditions, and one putative QTL on chr 6A (LOD = 2.70) under unirrigated conditions were found explaining 9.8, 10.6 and 12.8% of the phenotypic variation, respectively (Table 7.2; Fig. 7.1).

Table 7.2 QTLs identified using Interval Mapping (IM) for grain yield (GY), harvest index (HI) and above-ground dry matter (AGDM) under irrigated and unirrigated conditions for 91 DH lines of the Rialto x Savannah DH population in 2013 and 2014**.** Chr.: chromosome; LOD: log likelihood score; PT: permutation test threshold; GW: genome wide; Variance: residual variance after fitting the QTL; %Expl.: the percentage of variance explained by the QTL; Additive: (µA-µB)/2 (Savannah is A; Rialto is B).

Traits	Irrigation x year	Chr.	Position (cM)	LOD	LOD (PT; GW)	2 LOD range	Marker(s) at Locus	Closest marker(s)	Variance	% Expl.	Additive
GY	Irrigated-2013	3A	111.25	3.98	3.0	5.97	Excalibur_c2578_1966		0.27	18.30	-0.254
	Unirrigated-2013	3A	110.77	6.87	3.1	17.77		Excalibur_c2578_1966	0.21	29.40	-0.306
	Unirrigated-2014	4A1	16.37	3.32	3.1	32.27		BS00021738_51	0.31	15.50	0.246
HI	Irrigated-2013	2A	69.21	3.67	2.9	6.36	BobWhite_c23953_312		0.00	17.00	0.005
		3B	90.88	2.96	2.9	26.25	BS00025679_51		0.00	13.90	0.005
	Unirrigated-2013	4A2	28.37	3.65	3.1	18.26	Excalibur_c7897_600		0.00	16.90	0.006
	Irrigated-2014	3A	147.33	2.15	3.0	22.34		BS00049032_51	0.00	10.30	0.006
		3B	90.88	2.25	3.0	23.88	BS00025679_51		0.00	10.80	0.005
		7A	12.46	2.53	3.0	22.42		BS00079237_51, BS00077952_51	0.00	12.00	-0.006
	Unirrigated-2014	2A	72.51	3.11	2.9	25.49	BS00022241_51		0.00	14.60	0.007
AGDM	Irrigated-2013	3A	111.25	2.40	3.1	5.48	Excalibur_c2578_1966		7648.86	11.40	-32.268
		6A	66.67	2.47	3.1	30.76		CAP11_c1206_301, IaV8707 BS00096942_51, BS00074979_51 BS00022913_51	7621.73	11.70	34.957
	Unirrigated-2013	3A	81.95	5.01	3.0	19.29		Excalibur_c91154_164	5866.25	22.40	-44.515
		4D	28.56	2.88	3.0	23.56	IaV5607		6532.63	13.60	32.073
		6A	77.48	2.58	3.0	29.63	RAC875_rep_c107081_918		6634.73	12.20	30.405
	Irrigated-2014	1D	46.92	3.07	2.9	22.68		RFL_Contig1338_2646	12566.50	14.40	-54.174
		3A	90.81	2.04	2.9	26.82	BS00032025_51		13240.40	9.80	-39.368
		7D	55.79	2.21	2.9	52.30	BS00028760_51		13127.40	10.60	39.947
	Unirrigated-2014	6A	83.04	2.70	3.0	22.82		Excalibur_c9713_247 Excalibur_c53686_249	11066.80	12.80	42.030

7.3.1.4 Thousand grain weight

In 2013, two significant QTLs were found under irrigated conditions, one on chr 4A2 (LOD = 3.49) at 0.01 cM coinciding with markers Excalibur_c6749_694 and IaV8683 explaining 16.2% of the phenotypic variation, and the other on chr 5A (LOD = 3.29) at 123.7 cM coinciding with marker BS00047242_51 explaining 15.3% of the phenotypic variation. For both QTLs higher grain weight was observed with the Savannah allele. A putative QTL was also found on chr 3A (LOD = 2.05) explaining 9.9% of the phenotypic variation. Under unirrigated conditions, a major QTL was found on chr 3A (LOD = 3.97) at 114.7 cM coinciding with markers BS00036492_51 and BS00007502_51 explaining 18.2% of the phenotypic variation; grain weight was increased by the presence of the Rialto allele. Three putative QTLs were identified on chr 4A2 (LOD = 3.00), 5A (LOD = 2.12) and 6A (LOD = 2.11) explaining 14.1, 10.2 and 10.1% of the phenotypic variations, respectively (Table 7.3; Fig. 7.1).

In 2014, no QTL with LOD > 3.0 was found for TGW. However, five putative QTLs were found: on chr 1A (LOD = 2.33), 3A (LOD = 2.03) and 6A (LOD = 2.85) under irrigated conditions, and 3A (LOD = 2.60) and 4A (LOD = 2.43) under unirrigated conditions explaining 11.1, 9.8, 13.4, 12.3 and 11.6%, respectively, of the phenotypic variation (Table 7.3; Fig. 7.1).

7.3.1.5 Grains per m^2

In 2013, a major QTL was identified under irrigated conditions on chr 5A (LOD = 4.01) at 58.1 cM coinciding with marker Excalibur_c24051_502 explaining 18.4% of the phenotypic variation; higher grains m^{-2} was associated with the Rialto allele. In 2014, four putative QTLs were identified: on chr 3A (LOD = 2.13) and 6A (LOD = 2.27) under irrigated conditions, and 3A (LOD = 2.88) and 3B (LOD = 2.87) under drought explaining 10.2, 10.8, 13.5 and 13.5% of the phenotypic variation, respectively (Table 7.3; Fig. 7.1).

Table 7.3 QTLs identified using Interval Mapping (IM) for thousand grain weight (TGW) and grains per m^2 under irrigated and unirrigated conditions for 91 DH lines of the Rialto x Savannah DH population in 2013 and 2014. Chr.: chromosome; LOD: log likelihood score; PT: permutation test threshold; GW: genome wide; Variance: residual variance after fitting the QTL; %Expl.: the percentage of variance explained by the QTL; Additive: (µA-µB)/2 (Savannah is A; Rialto is B).

Trait	Irrigation x year	Chr.	Position (cM)	LOD	LOD (PT; GW)	2 LOD range	Marker at Locus	Closest marker	Variance	% Expl.	Additive
TGW	Irrigated-2013	3A	99.96	2.05	3.0	104.52		BS00027470_51	7.84	9.90	-0.953
		4A2	0.01	3.49	3.0	9.01	Excalibur_c6749_694, IaV8683		7.28	16.20	1.187
		5A	123.67	3.29	3.0	15.00	BS00047242_51		7.36	15.30	1.176
	Unirrigated-2013	3A	114.73	3.97	3.2	37.56	BS00036492_51, BS00007502_51		7.90	18.20	-1.356
		4A2	0.01	3.00	3.2	8.01	Excalibur_c6749_694, IaV8683		8.29	14.10	1.167
		5A	127.03	2.12	3.2	36.85	BS00021708_51		8.67	10.20	1.000
		6A	80.00	2.11	3.2	28.96	Excalibur_c9713_247 Excalibur_c53686_249		8.67	10.10	0.990
	Irrigated-2014	1A	44.63	2.33	3.0	19.06	BS00012210_51		9.19	11.10	1.084
		3A	141.33	2.03	3.0	48.54		BS00049032_51	9.33	9.80	-1.140
		6A	80.00	2.85	3.0	33.63	Excalibur_c9713_247		8.95	13.40	1.180
	Unirrigated-2014	3A	114.73	2.60	3.1	11.84	BS00036492_51, BS00007502_51		7.84	12.30	-1.074
		4A2	0.01	2.43	3.1	9.01	Excalibur_c6749_694, IaV8683		7.91	11.60	1.018
Grains m^{-2}	Irrigated-2013	5A	58.05	4.01	3.1	18.73	Excalibur_c24051_502		4778420	18.40	-1042.800
	Irrigated-2014	3A	160.67	2.13	3.0	41.86		CAP8_c665_409	5836280	10.20	962.051
		6A	64.67	2.27	3.0	39.93		CAP11_c1206_301 IaV8707 BS00096942_51 BS00074979_51 BS00022913_51	5795760	10.80	-935.609
	Unirrigated-2014	3A	141.33	2.88	3.0	27.54		BS00049032_51	5116840	13.50	1015.800
		3B	44.06	2.87	3.0	18.78		BobWhite_c22370_352	5118650	13.50	928.965

7.3.1.6 Ears per m^2

In 2013, two putative QTLs were found on chr 1B (LOD = 2.27) and 5A (LOD = 2.51) under irrigated conditions explaining 10.9 and 11.9%, respectively of the phenotypic variations. In 2014, three putative QTLs were also detected: on chr 2A (LOD = 2.07) under irrigated conditions, and 2A (LOD = 2.71) and 5A (LOD = 2.08) under unirrigated conditions explaining 9.9, 12.8 and 10.0%, respectively, of the phenotypic variation (Table 7.4; Fig. 7.1).

7.3.1.7 Grains per ear

In 2013, a QTL was identified under irrigated conditions on chr 5A (LOD = 5.09) at 58.1 cM coinciding with marker Excalibur_c24051_502 explaining 22.7% of the variation in grains per ear; grains per ear was increased by the presence of the Rialto allele. A putative QTL was also found on chr 2A (LOD = 2.95) explaining 12.1% of the phenotypic variation (Table 7.4; Fig. 7.1).

In 2014, a QTL on chr 2A (LOD = 5.09) at 72.5 cM coinciding with marker BS00022241_51 was found under unirrigated conditions explaining 12.2% of the phenotypic variation; the increase in grains per ear was associated with the Savannah allele. In addition, two putative QTLs on chr 3A (LOD = 2.80) under irrigated conditions, and 6A (LOD = 2.51) under unirrigated conditions were found explaining 13.2 and 11.9% of the phenotypic variation, respectively (Table 7.4; Fig. 7.1).

Table 7.4 QTLs identified using Interval Mapping (IM) for ears per m^2 and grains per ear under irrigated and unirrigated conditions for 91 DH lines of the Rialto x Savannah DH population in 2013 and 2014**.** Chr.: chromosome; LOD: log likelihood score; PT: permutation test threshold; GW: genome wide; Variance: residual variance after fitting the QTL; %Expl.: the percentage of variance explained by the QTL; Additive: (µA-µB)/2 (Savannah is A; Rialto is B).

Trait	Irrigation x year	Chr.	Position (cM)	LOD	LOD (PT; GW)	2 LOD range	Marker at Locus	Closest marker	Variance	% Expl.	Additive
Ears m^{-2}	Irrigated-2013	1B	8.91	2.27	3.0	26.28	Excalibur_c5888_169		1239.5	10.90	12.470
		5A	109.16	2.51	3.0	21.59	BS00004202_51		1224.9	11.90	-12.878
							RAC875_c79944_269				
							RAC875_c7186_1147				
							BobWhite_c40449_189				
	Irrigated-2014	2A	85.82	2.07	3.0	34.87		BS00080752_51	3956.2	9.90	-21.278
	Unirrigated-2014	2A	110.17	2.71	3.0	7.87	BS00003663_51		3643.0	12.80	-23.926
		5A	130.70	2.08	3.0	48.64		Excalibur_rep_c95828_165	3760.9	10.00	-21.154
Grains ear^{-1}	Irrigated-2013	2A	72.51	2.95	3.1	9.74	BS00022241_51		12.11	13.90	1.397
		5A	58.05	5.09	3.1	15.26	Excalibur_c24051_502		10.87	22.70	-1.797
	Irrigated-2014	3A	143.33	2.80	3.0	28.34		BS00049032_51	22.66	13.20	2.065
	Unirrigated-2014	2A	72.51	5.09	2.9	6.82	BS00022241_51		12.18	22.70	1.891
		6A	76.65	2.51	2.9	20.96	BS00109576_51		13.88	11.90	-1.370
							Ku_c1976_663				
							RAC875_c64852_655				
							CAP11_c862_116				

7.3.1.8 Plant height

In 2013, two QTLs were found under irrigated conditions, one on chr 3A (LOD = 7.46) at 111.3 cM coinciding with marker Excalibur_c2578_1966 explaining 31.5% of the phenotypic variation, the Rialto allele conferring taller plants; and the other on chr 6A (LOD = 4.69) at 106.3 cM close to markers BS00065076_51, BS00065500_51, BS00009331_51 and BS00096301_51 explaining 21.1% of the phenotypic variation, with the Savannah allele conferring taller plants. Under unirrigated conditions, three major QTLs were found, one on chr 1D (LOD = 3.33) at 10.7 cM close to markers BS00021851_51, BS00004145_51, BS00063146_51, BS00108305_51 and Excalibur_c3260_863 explaining 15.5% of the phenotypic variation; and the other two on chr 3A at 80.0 cM (LOD = 6.30; coinciding with marker Excalibur_c91154_164) and 110.8 cM (LOD = 7.14; close to marker Excalibur_c2578_1966) explaining 27.3 and 30.3% of the phenotypic variation, respectively; plant height was increased by the Rialto allele. A QTL under unirrigated conditions with increased height conferred by the Savannah allele was found on chr 6A (LOD = 4.68) at 74.2 cM coinciding with markers BS00027313_51, Ra_c14408_576 and BS00012297_51 explaining 21.1% of the phenotypic variation. In addition, two putative QTLs were found on chr 1D (LOD = 2.37) under irrigated conditions and 2A (LOD = 2.23) under unirrigated conditions explaining 11.3 and 10.7% of the phenotypic variation, respectively (Table 7.5; Fig. 7.1).

In 2014, four major QTLs similar to those in 2013 were found: two on chr 3A (LOD = 6.03 and LOD = 5.91) and one on 6A (LOD = 6.02) under irrigated conditions and 6A (LOD = 3.98) under unirrigated conditions explaining 26.3, 25.9, 26.3 and 18.2% of the phenotypic variation, respectively. In addition, four putative QTLs were detected: on chr 1D (LOD = 2.32) under irrigated, and 1D (LOD = 2.99), 2A (LOD = 2.27) and 5A (LOD = 2.25) under unirrigated conditions, explaining 11.1, 14.0, 10.9 and 10.7%, respectively, of the phenotypic variation (Table 7.5; Fig. 7.1).

Table 7.5 QTLs identified using Interval Mapping (IM) for plant height (PH) under irrigated and unirrigated conditions for 91 DH lines of the Rialto x Savannah DH population in 2013 and 2014**.** Chr.: chromosome; LOD: log likelihood score; PT: permutation test threshold; GW: genome wide; Variance: residual variance after fitting the QTL; %Expl.: the percentage of variance explained by the QTL; Additive: (µA-µB)/2 (Savannah is A; Rialto is B).

Trait	Irrigation x year	Chr.	Position (cM)	LOD	LOD (PT; GW)	2 LOD range	Marker at Locus	Closest marker	Variance	% Expl.	Additive
PH	Irrigated-2013	1D	14.72	2.37	3.0	41.92		BS00021851_51, BS00004145_51 BS00063146_51, BS00108305_51 Excalibur_c3260_863	36.33	11.30	-2.410
		3A	111.25	7.46	3.0	5.48	Excalibur_c2578_1966		28.07	31.50	-3.688
		6A	106.30	4.69	3.0	24.18		BS00065076_51, BS00065500_51 BS00009331_51, BS00096301_51	32.30	21.10	3.368
	Unirrigated-2013	1D	10.72	3.33	3.0	25.72		BS00021851_51, BS00004145_51 BS00063146_51, BS00108305_51 Excalibur_c3260_863	34.99	15.50	-2.691
		2A	136.17	2.23	3.0	25.84	BS00098033_51		37.00	10.70	2.125
		3A	79.95	6.30	3.0	10.44	Excalibur_c91154_164		30.12	27.30	-3.552
		3A	110.77	7.14	3.0	8.97		Excalibur_c2578_1966	28.86	30.30	-3.666
		6A	74.16	4.68	3.0	20.37	BS00027313_51 Ra_c14408_576 BS00012297_51		32.68	21.10	2.957
	Irrigated-2014	1D	3.00	2.32	2.9	25.72		BS00021702_51	37.15	11.10	-2.225
		3A	79.95	6.03	2.9	13.44	Excalibur_c91154_164		30.79	26.30	-3.501
		3A	110.77	5.91	2.9	8.97		Excalibur_c2578_1966	30.97	25.90	-3.400
		6A	74.16	6.02	2.9	14.33	BS00027313_51 Ra_c14408_576 BS00012297_51		30.80	26.30	3.314
	Unirrigated-2014	1D	22.72	2.99	3.0	53.92		D_GBF1XID01C7T2Q_63 Excalibur_c44711_453 BS00030948_51	30.37	14.00	-2.544
		2A	136.17	2.27	3.0	28.53	BS00098033_51		31.49	10.90	1.978
		5A	61.83	2.25	3.0	6.88	Kukri_c28077_282		31.53	10.70	-1.950
		6A	102.30	3.98	3.0	26.63		BS00065076_51, BS00065500_51 BS00009331_51, BS00096301_51	28.88	18.20	2.996

7.3.1.9 Anthesis date

In 2013, a major QTL was identified under both irrigated and unirrigated conditions on chr 7D at 0.0 cM coinciding with marker Ra_c6672_2576 explaining 18.4% of the phenotypic variation with later flowering associated with the Rialto allele. Two putative QTLs were also found on chr 3A (LOD = 2.00) under irrigated conditions and 4A (LOD = 2.53) under drought conditions explaining 9.6 and 12.0%, respectively, of the phenotypic variation (Table 7.6; Fig. 7.1).

In 2014, two QTLs in similar locations to the putative QTLs found in 2013 were identified on chr 3A (LOD = 2.98 and LOD = 2.86 coinciding with markers BS00036492_51 and BS00007502_51) and on 4A (LOD = 3.33 and LOD = 3.05 coinciding with markers Excalibur_c6749_694 and IaV8683) under both irrigated and unirrigated conditions, respectively. The QTL on 3A explained 14% of the phenotypic variation with later flowering associated with the Savannah allele, while the QTL on 4A explained 15.5% of the phenotypic variation and later flowering was associated with the Rialto allele. A putative QTL was also found under drought conditions on chr 5A (LOD = 2.32) explaining 11.1% of the phenotypic variation (Table 7.6; Fig. 7.1).

7.3.1.10 Grain $\Delta^{13}C$

In 2013, three putative QTLs for grain $\Delta^{13}C$ were identified under unirrigated conditions on chr 2A (LOD = 2.25) with increased $\Delta^{13}C$ associated with Rialto allele, on chr 3B (LOD = 2.11) with increased $\Delta^{13}C$ increased with Savannah allele, and on chr 5B (LOD = 1.91) with increased $\Delta^{13}C$ associated with Rialto allele; explaining 10.7, 10.1 and 9.2% of the phenotypic variation in grain $\Delta^{13}C$, respectively (Table 7.6; Fig. 7.1). In 2014, no QTLs for grain $\Delta^{13}C$ were found.

Table 7.6 QTLs identified using Interval Mapping (IM) for anthesis date (AD) and grain $\Delta^{13}C$ under irrigated and unirrigated conditions for 91 DH lines of the Rialto x Savannah DH population in 2013 and 2014. Chr.: chromosome; LOD: log likelihood score; PT: permutation test threshold; GW: genome wide; Variance: residual variance after fitting the QTL; %Expl.: the percentage of variance explained by the QTL; Additive: (µA-µB)/2 (Savannah is A; Rialto is B).

Trait	Irrigation x year	Chr.	Position (cM)	LOD	LOD (PT; GW)	2 LOD range	Marker at Locus	Closest marker	Variance	% Expl.	Additive
AD	Irrigated-2013	3A	114.73	2.00	3.1	22.17	BS00036492_51, BS00007502_51		0.78	9.60	0.30
		4A2	0.01	2.01	3.1	13.23	Excalibur_c6749_694, IaV8683		0.78	9.70	-0.29
		7D	0.00	4.02	3.1	18.76	Ra_c6672_2576		0.71	18.40	-0.41
	Unirrigated-2013	4A2	0.01	2.53	3.0	9.01	Excalibur_c6749_694		0.86	12.00	-0.34
		7D	0.00	4.02	3.0	7.76	Ra_c6672_2576		0.80	18.40	-0.44
	Irrigated-2014	3A	114.73	2.98	3.0	24.05	BS00036492_51, BS00007502_51		1.65	14.00	0.53
		4A2	0.01	3.33	3.0	12.23	Excalibur_c6749_694, IaV8683		1.62	15.50	-0.54
		5A	189.72	2.32	3.0	14.18	BS00037427_51 Kukri_rep_c95804_119		1.70	11.10	0.46
	Unirrigated-2014	3A	114.73	2.86	3.0	19.08	BS00036492_51, BS00007502_51		2.69	13.50	0.66
		4A2	0.01	3.05	3.0	7.01	Excalibur_c6749_694, IaV8683		2.66	14.30	-0.67
$\Delta^{13}C$	Unirrigated-2013	2A	144.36	2.25	3.1	15.13	BS00010664_51		0.06	10.70	-0.086
		3B	60.62	2.11	3.1	54.32		BobWhite_c828_329	0.06	10.10	0.084
		5B	44.24	1.91	3.1	43.19	BS00003655_51		0.06	9.20	-0.080

7.3.2 QTLs identified for stay-green and leaf senescence traits

7.3.2.1 Post-anthesis NDVI

7.3.2.1.1 NDVI (GS61+21 days)

In 2013, a major QTL was found under unirrigated conditions on chr 3A (LOD = 3.84) at 127.1 cM close to markers BS00065956_51 and BS00048757_51 explaining 17.7% of the phenotypic variation with increasing NDVI associated with the Rialto allele. Three putative QTLs under unirrigated conditions were detected on chr 5A (LOD = 2.26) in 2013, and on chr 2B (LOD = 2.10) and 7D (LOD = 2.96) in 2014, explaining 10.8, 10.1 and 13.9%, respectively, of the phenotypic variation (Table 7.7; Fig. 7.1).

7.3.2.1.2 NDVI (GS61+28 days)

Two significant QTLs were found under unirrigated conditions, one in 2013 on chr 3A (LOD = 3.58) at 127.3 cM coinciding with markers BS00065956_51 and BS00048757_51 explaining 16.6% of the phenotypic variation, and the other on chr 4A (LOD = 3.04) in 2014 at 5.0 cM close to markers BS00066809_51 and BS00066810_51 explaining 14.2% of the phenotypic variation. For both QTLs increasing NDVI was associated with the Rialto allele. In addition, two putative QTLs under unirrigated conditions were detected on chr 6D (LOD = 2.13) in 2013 and 3B (LOD = 2.59) in 2014, explaining 10.2 and 12.3% of the phenotypic variation, respectively (Table 7.7; Fig. 7.1).

7.3.2.1.3 NDVI (GS61+35 days)

In 2013, two significant QTLs were found under unirrigated conditions, one on chr 7A (LOD = 3.40) at 95.6 cM coinciding with marker BS00022202_51 explaining 15.8% of the phenotypic variation with increasing NDVI associated with the Savannah allele, and the other on chr 4A (LOD = 3.45) at 28.4 cM coinciding with marker Excalibur_c7897_600 explaining 16.0% of the phenotypic variation with the increase of NDVI associated with the Rialto allele. Moreover, two putative QTLs under unirrigated conditions were identified on chr 2A (LOD = 2.78) and 3B (LOD =

2.02) in 2013, explaining 13.1 and 9.7% of the phenotypic variation, respectively (Table 7.7; Fig. 7.1).

7.3.2.2 Canopy temperature (GS61+35 days)

In 2013, a QTL was identified for post-anthesis canopy temperature under unirrigated conditions on chr 3A (LOD = 3.07) at 103.3 cM coinciding with marker BS00065956_51 and BS00048757_51 explaining 14.4% of the phenotypic variation with increasing canopy temperature associated with the Savannah allele. Two putative QTLs were also identified for canopy temperature depression post-anthesis under unirrigated conditions on chr 1A (LOD = 2.21) and 6D (LOD = 2.06) in 2013, explaining 10.6 and 9.9%, respectively, of the phenotypic variation (Table 7.7; Fig. 7.1).

7.3.2.3 Flag-leaf chlorophyll fluorescence (GS61+35 days)

In 2013, a QTL for flag-leaf chlorophyll fluorescence was detected under unirrigated conditions on chr 4A (LOD = 3.71) at 26.9 cM coinciding with marker BS00004170_51 explaining 17.1% of the phenotypic variation with increasing chlorophyll fluorescence associated with the Savannah allele. A putative QTL was found in 2014 on chr 4A (LOD = 2.20) explaining 10.5% of the phenotypic variation (Table 7.7; Fig. 7.1).

Table 7.7 QTLs identified using Interval Mapping (IM) for post-anthesis NDVI (NDVI21; GS61+21d, NDVI28; GS61+28d, NDVI35; GS61+35d), post-anthesis chlorophyll fluorescence (PCF), post-anthesis canopy temperature (PCT) and canopy temperature depression (PCTD) under unirrigated conditions for 91 DH lines of the Rialto x Savannah DH population in 2013 and 2014**.** Chr.: chromosome; LOD: log likelihood score; PT: permutation test threshold; GW: genome wide; Variance: residual variance after fitting the QTL; %Expl.: the percentage of variance explained by the QTL; Additive: (µA-µB)/2 (Savannah is A; Rialto is B).

Trait	Irrigation x year	Group	Position	LOD	LOD (PT; GW)	2 LOD range	Marker at Locus	Closest marker	Variance	% Expl.	Additive
NDVI21	Unirrigated-2013	3A	127.13	3.84	3.0	22.60		BS00065956_51 BS00048757_51	0.00	17.70	-0.010
		5A	72.69	2.26	3.0	26.50		BS00009369_51	0.00	10.80	0.009
	Unirrigated-2014	2B	75.01	2.10	3.1	41.94	Excalibur_c30167_243 BobWhite_c19945_341		0.00	10.10	0.003
		7D	5.76	2.96	3.1	23.76		IaV8204	0.00	13.90	-0.004
NDVI28	Unirrigated-2013	3A	127.33	3.58	3.0	20.21	BS00065956_51, BS00048757_51		0.00	16.60	-0.016
		6D	0.00	2.13	3.0	13.23	BS00049818_51		0.00	10.20	-0.012
	Unirrigated-2014	3B	62.92	2.59	3.1	15.86	BobWhite_c828_329		0.00	12.30	-0.008
		4A2	5.01	3.04	3.1	21.11		BS00066809_51 BS00066810_51	0.00	14.20	-0.009
NDVI35	Unirrigated-2013	2A	14.92	2.78	3.1	20.70		BobWhite_c19433_185	0.00	13.10	-0.017
		7A	95.58	3.40	3.1	15.31	BS00022202_51		0.00	15.80	0.018
		3B	62.92	2.02	3.0	18.14	BobWhite_c828_329		0.00	9.70	-0.022
		4A2	28.37	3.45	3.0	28.33	Excalibur_c7897_600		0.00	16.00	-0.029
PCF	Unirrigated-2013	4A1	26.90	3.71	3.0	26.61	BS00004170_51		0.00	17.10	0.005
	Unirrigated-2014	4A2	28.37	2.20	3.1	30.33	Excalibur_c7897_600		0.00	10.50	-0.006
PCT	Unirrigated-2013	3A	130.33	3.07	3.0	26.60		BS00065956_51 BS00048757_51	0.40	14.40	0.280
PCTD	Unirrigated-2013	1A	20.91	2.21	2.9	15.93	RAC875_c29540_1109 BS00022432_51, BS00022173_51		0.77	10.60	0.302
		6D	41.06	2.06	2.9	36.38	D_contig26362_1277 IACX11026, BS00003568_51		0.77	9.90	-0.295

7.3.2.4 Onset of flag-leaf senescence (SEN_{ONSET})

In 2013, a QTL for SEN_{ONSET} was found under irrigated conditions on chr 7D (LOD = 5.13) at 3.76 cM coinciding with marker IaV8204 explaining 22.9% of the phenotypic variation, with later leaf senescence associated with the Savannah allele. Five putative QTLs were also found: on chr 5A (LOD = 2.84) and 6B (LOD = 2.11) under irrigated conditions, and 3D (LOD = 2.59), 4A (LOD = 2.23) and 7D (LOD = 2.89) under unirrigated conditions explaining 13.4, 10.1, 12.3, 10.7 and 13.3% of the phenotypic variation, respectively (Table 7.8; Fig. 7.1).

In 2014, no QTL with LOD > 3.0 was found. Four putative QTLs were detected on chr 2A (LOD = 2.81), 3A (LOD = 2.43) and 5B (LOD = 2.96) and 6D (LOD = 2.96) under irrigated conditions explaining 13.2, 11.6, 13.9 and 13.9%, respectively, of the phenotypic variation (Table 7.8; Fig. 7.1).

7.3.2.5 Rate of flag-leaf senescence (SEN_{RATE})

In 2013, a QTL was identified under irrigated conditions on chr 7D (LOD = 4.30) at 3.76 cM coinciding with marker IaV8204 explaining 19.6% of the phenotypic variation with leaf senescence rate increasing with the Savannah allele. Four putative QTLs were detected: on chr 2B (LOD = 2.05) and 5A (LOD = 2.38) in 2013 under unirrigated conditions and on 5A (LOD = 2.22) and 1A (LOD = 2.17) in 2014 under irrigated and unirrigated conditions, respectively, explaining 9.8, 11.4, 10.6 and 10.4% of the phenotypic variation, respectively (Table 7.8; Fig. 7.1).

7.3.2.6 End of flag-leaf senescence (SEN_{END})

In 2013, a QTL for SEN_{END} was identified under unirrigated conditions on chr 5A (LOD = 4.88) at 65.7 cM coinciding with marker BS00074301_51 explaining 21.9% of the phenotypic variation, with longer duration of leaf senescence associated with the Savannah allele (Table 7.9; Fig. 7.1).

In 2014, two QTLs were found under irrigated conditions, one on chr 3A (LOD = 3.24) at 114.7 cM coinciding with markers BS00036492_51 and BS00007502_51 explaining 15.1% of the phenotypic variation, and the other on chr 5A (LOD = 3.36) at 187.5 cM close to markers BS00037427_51 and Kukri_rep_c95804_119

explaining 15.6% of the phenotypic variation. Under drought, a similar QTL was also detected on chr 5A (LOD = 3.17) explaining 14.8% of the phenotypic variation. For these QTLs longer duration of leaf senescence was associated with the of Rialto allele. Six putative QTLs were also found on chr 1D (LOD = 2.24) and 5B (LOD = 2.09) under irrigated conditions, and 1A (LOD = 2.54), 1D (LOD = 2.84), 5B (LOD = 2.40) and 7D (LOD = 2.10) under unirrigated conditions explaining 10.7, 10.0, 12.1, 13.4, 11.5 and 10.1% of the phenotypic variation, respectively (Table 7.9; Fig. 7.1).

Table 7.8 QTLs identified using Interval Mapping (IM) for onset of leaf senescence (SEN_{ONSET}) and rate of leaf senescence (SEN_{RATE}) under irrigated and unirrigated conditions for 91 DH lines of the Rialto x Savannah DH population in 2013 and 2014. Chr.: chromosome; LOD: log likelihood score; PT: permutation test threshold; GW: genome wide; Variance: residual variance after fitting the QTL; %Expl.: the percentage of variance explained by the QTL; Additive: (µA-µB)/2 (Savannah is A; Rialto is B).

Trait	Irrigation x year	Chr.	Position (cM)	LOD	LOD (PT; GW)	2 LOD range	Marker at Locus	Closest marker	Variance	% Expl.	Additive
SEN_{ONSET}	Irrigated-2013	5A	182.54	2.84	3.1	22.36		BS00037427_51 Kukri_rep_c95804_119	3359.24	13.40	24.952
		6B	31.24	2.11	3.1	20.99	BS00066236_51		3485.57	10.10	19.848
		7D	3.76	5.13	3.1	7.76	IaV8204		2990.39	22.90	29.908
	Unirrigated-2013	3D	60.00	2.59	2.9	9.36	IaV1270		934.51	12.30	-11.468
		4A2	0.01	2.23	2.9	8.01	Excalibur_c6749_694 IaV8683		951.71	10.70	10.657
		7D	0.00	2.83	2.9	14.76	Ra_c6672_2576		923.34	13.30	12.207
	Irrigated-2014	2A	142.03	2.81	3.1	15.99		BS00010664_51	1585.86	13.20	16.353
		3A	88.95	2.43	3.1	28.13		BS00032025_51	1616.72	11.60	-15.520
		5B	44.24	2.96	3.1	10.34	BS00003655_51		1573.39	13.90	16.239
		6D	22.32	2.96	3.1	39.47		BS00036102_51	1573.64	13.90	-17.187
SEN_{RATE}	Irrigated-2013	7D	3.76	4.30	3.0	10.76	IaV8204		0.00	19.60	0.002
	Unirrigated-2013	2B	80.92	2.05	3.0	20.86	Excalibur_c42248_211 BS00028167_51 Excalibur_c29417_499		0.00	9.80	-0.002
		5A	65.69	2.38	3.0	24.86	BS00074301_51		0.00	11.40	-0.002
	Irrigated-2014	5A	42.80	2.22	2.9	41.01		BS00085711_51	0.00	10.60	-0.002
	Unirrigated-2014	1A	77.60	2.17	2.9	37.89		BS00064608_51	0.00	10.40	-0.001

Table 7.9 QTLs identified using Interval Mapping (IM) for end of leaf senescence (SEN_{END}) under irrigated and unirrigated conditions for 91 DH lines of the Rialto x Savannah DH population in 2013 and 2014. Chr.: chromosome; LOD: log likelihood score; PT: permutation test threshold; GW: genome wide; Variance: residual variance after fitting the QTL; %Expl.: the percentage of variance explained by the QTL; Additive: (µA-µB)/2 (Savannah is A; Rialto is B).

Trait	Irrigation x year	Group	Position	LOD	LOD (PT; GW)	2 LOD range	Marker at Locus	Closest marker	Variance	% Expl.	Additive
SEN_{END}	Unirrigated-2013	5A	65.69	4.88	3.1	5.76	BS00074301_51		2626.84	21.90	27.373
	Irrigated-2014	1D	7.67	2.24	3.1	18.72	BS00021851_51, BS00004145_51 BS00063146_51, BS00108305_51 Excalibur_c3260_863		711.17	10.70	-9.274
		3A	114.73	3.24	3.1	6.87	BS00036492_51, BS00007502_51		676.15	15.10	-11.226
		5A	187.54	3.36	3.1	14.18		BS00037427_51 Kukri_rep_c95804_119	672.24	15.60	-11.641
		5B	44.24	2.09	3.1	45.27	BS00003655_51		716.82	10.00	9.096
	Unirrigated-2014	1A	77.60	2.54	3.1	26.62		BS00064608_51	1375.40	12.10	14.566
		1D	8.72	2.84	3.1	24.72		BS00021851_51 BS00004145_51 BS00063146_51 BS00108305_51 Excalibur_c3260_863	1354.86	13.40	-14.809
		5A	189.72	3.17	3.1	12.18	BS00037427_51 Kukri_rep_c95804_119		1332.37	14.80	-15.269
		5B	130.37	2.40	3.1	5.15	BS00023161_51		1385.02	11.50	13.771
		7A	113.62	2.10	3.1	30.98	Ex_c61895_1195		1406.21	10.10	12.665

7.3.3 Co-located QTLs identified for agronomic and physiological traits

7.3.3.1 Genome A

On chr 1A, a co-located QTL was found at 77.6 cM close to marker BS00064608_51 under unirrigated conditions in 2014 for SEN_{RATE}, SEN_{IP} and SEN_{END} explaining 9.9 to 12.1% of the phenotypic variation (Fig. 7.1). On chr 2A, two pairs of co-located QTLs were identified, one at 143.2 cM close to marker BS00010664_51 under unirrigated in 2013 for $\Delta^{13}C$ and under irrigated conditions in 2014 for SEN_{ONSET} explaining 10.7 to 13.2% of the phenotypic variation; and the other at 72.5 cM coinciding with marker BS00022241_51 under irrigated conditions in 2013 and under unirrigated conditions in 2014 for grains per ear and unirrigated conditions in 2014 for HI explaining 13.9 to 22.7% of the phenotypic variation (Fig. 7.1).

On chr 3A, four co-located QTLs were identified: the first was at 81.3 cM close to marker Excalibur_c91154_164 under unirrigated conditions in 2013 for AGDM, under unirrigated conditions in 2013 and irrigated conditions in 2014 for PH, and under irrigated conditions in 2014 for SEN_{IP} explaining 10.1 to 27.3% of the phenotypic variation, the second was at 89.9 cM close to marker BS00032025_51 under irrigated conditions in 2014 for both AGDM and SEN_{ONSET} explaining 9.8 to 11.6% of the phenotypic variation, the third was at 112.9 cM close to markers Excalibur_c2578_1966, BS00036492_51 and BS00007502_51 under irrigated and unirrigated conditions in 2013 for GY, irrigated conditions in 2013 for AGDM, irrigated and unirrigated conditions in 2013 and irrigated conditions in 2014 for PH, irrigated conditions in 2013, irrigated and unirrigated conditions in 2014 for AD, unirrigated conditions in 2013 and 2014 for TGW and irrigated conditions in 2014 for SEN_{END} explaining 9.6 to 31.5% of the phenotypic variation; and the fourth was at 135.0 cM close to markers BS00065956_51, BS00048757_51 and BS00049032_51 under unirrigated conditions in 2013 for post-anthesis NDVI, canopy temperature and SEN_{IP}, under irrigated conditions in 2014 for HI, TGW and grains per ear and unirrigated conditions in 2014 for grains per m^2 explaining 9.8 to 17.7% of the phenotypic variation (Fig. 7.1).

On chr 4A, two co-located QTLs were found: the first was at 0.01 cM close to markers Excalibur_c6749_694 and IaV8683 under irrigated and unirrigated conditions in 2013, and unirrigated conditions in 2014 for TGW, irrigated conditions in 2013 and 2014 for maturity (MD), unirrigated in 2013 for SEN_{ONSET}, and irrigated and unirrigated conditions in both 2013 and 2014 for AD explaining 9.7 to 16.7% of the phenotypic variation. The second was at 28.4 cM close to marker Excalibur_c7897_600 under unirrigated conditions in 2013 for HI and unirrigated conditions in 2014 for MD, NDVI and chlorophyll fluorescence explaining 10.5 to 16.9% of the phenotypic variation (Fig. 7.1).

On chr 5A, a co-located QTL was found at 187.9 close to markers BS00037427_51 and Kukri_rep_c95804_119 under irrigated conditions in 2013 for SEN_{ONSET}, unirrigated conditions in 2014 for SEN_{IP}, irrigated and unirrigated conditions in 2014 for SEN_{END} and irrigated conditions in 2014 for AD explaining 11.1 to 15.6% of the phenotypic variation (Fig. 7.1). On chr 6A, a co-located QTL was identified at 81.0 cM close to markers Excalibur_c9713_247 and Excalibur_c53686_249 under unirrigated conditions in 2013 and irrigated conditions in 2014 for TGW, and under unirrigated conditions in 2014 for AGDM explaining 10.1 to 13.4% of the phenotypic variation (Fig. 7.1).

7.3.3.2 Genome B

On chr 3B, a co-located QTL was found at 62.2 cM close to marker BobWhite_c828_329 under unirrigated conditions in 2013 for $\Delta^{13}C$ and unirrigated conditions in 2014 for NDVI explaining 9.7 to 12.3% of the phenotypic variation (Fig. 7.1). On chr 5B at 44.2 cM, a co-located QTL was identified under unirrigated conditions in 2013 for $\Delta^{13}C$ and irrigated conditions in 2014 for SEN_{ONSET} and SEN_{END} explaining 9.2 to 13.9% of the phenotypic variation (Fig. 7.1).

7.3.3.3 Genome D

On chr 1D, a co-located QTL was found at 9.9 cM close to markers BS00021851_51, BS00004145_51, BS00063146_51, BS00108305_51 and Excalibur_c3260_863 under irrigated and unirrigated conditions in 2013 for PH, and irrigated and unirrigated conditions in 2014 for SEN_{END} and SEN_{IP} explaining 9.7 to 15.5% of the phenotypic variation (Fig. 7.1).

On chr 7D, two co-located QTLs were found: the first was at 2.2 cM close to markers Ra_c6672_2576 and IaV8204 under irrigated and unirrigated conditions in 2013 for AD and SEN_{ONSET}, irrigated conditions in 2013 for SEN_{RATE} and unirrigated conditions in 2014 for NDVI explaining 13.3 to 22.9% of the phenotypic variation. The second was at 44.6 cM close to markers BS00035732_51 and BS00028760_51 under irrigated conditions in 2014 and unirrigated conditions in 2013 for SEN_{IP}, irrigated conditions in 2013 for MD and irrigated conditions in 2014 for AGDM explaining 9.9 to 18.2% of the phenotypic variation (Fig. 7.1).

Table 7.10 Summary of co-located QTLs identified for 91 DH lines of the Rialto x Savannah DH population in 4 environments (1; Irrigated 2013, 2; Unirrigated 2013, 3; Irrigated 2014, 4; Unirrigated 2014) for grain yield (GY), harvest index (HI), thousand grain weight (TGW), grains ear^{-1}, plant height (PH), days to anthesis (AD), days to maturity (MD) and end of flag-leaf senescence (SEN_{END}); the chromosome, environment, the minimum and maximum percentage values of phenotypic variance explained by individual QTL and additive effect of the Savannah parent, the mean position, and the closest marker to the stable QTL are displayed for each trait.

Traits	Chr.	Environments	Position (cM)	% Expl.	Additive	Closest marker to locus
GY	3A	1, 2	111.01	18.3-29.4	(-)	Excalibur_c2578_1966
HI	3B	1, 3	91.88	10.8-13.9	(+)	BS00025679_51
TGW	3A	2, 4	114.73	12.3-18.2	(-)	BS00036492_51
						BS00007502_51
	4A2	1, 2, 4	0.005	11.6-16.2	(+)	Excalibur_c6749_694
						IaV8683
	6A	2, 3	80.00	10.1-13.4	(+)	Excalibur_c9713_247
						Excalibur_c53686_249
PH	1D	1, 2	12.72	11.3-15.5	(-)	BS00021851_51
						BS00004145_51
						BS00063146_51
						BS00108305_51
						Excalibur_c3260_863
	3A	1, 2, 3	110.93	25.9-31.5	(-)	Excalibur_c2578_1966
	3A	2, 3	79.95	26.3-27.3	(-)	Excalibur_c91154_164
	6A	2, 3	74.16	21.1-26.3	(+)	BS00027313_51
						Ra_c14408_576
						BS00012297_51
AD	3A	1, 3, 4	114.73	9.6-14	(+)	BS00036492_51
						BS00007502_51
	4A2	1, 2, 3, 4	0.01	9.7-15.5	(-)	Excalibur_c6749_694
						IaV8683
	7D	1, 2	0.00	18.4	(-)	Ra_c6672_2576
MD	4A2	1, 3	2.51	13-16.7	(-)	Excalibur_c6749_694
						IaV8683
Grains ear^{-1}	2A	1, 4	72.51	13.9-22.7	(+)	BS00022241_51
SEN_{END}	1D	3, 4	8.19	10.7-13.4	(-)	BS00021851_51
						BS00004145_51
						BS00063146_51
						BS00108305_51
						Excalibur_c3260_863
	5A	3, 4	188.63	14.8-15.6	(-)	BS00037427_51
						Kukri_rep_c95804_119

Chr.: chromosome; %Expl.: the percentage of variance explained for by the QTL; Additive: (µA-µB)/2 (Savannah is A; Rialto is B).

Table 7.11 Heritability for grain yield (GY), above-ground dry matter (AGDM), harvest index (HI), thousand grain weight (TGW), plant height (PH), days to anthesis (AD), grains per m^2 and $\Delta^{13}C$ amongst 94 DH lines of Rialto x Savannah DH population under irrigated and unirrigated conditions in 2013, 2014 and the cross-year mean.

Trait	Treatment	Heritability		
		2013	2014	2013-14
GY	Irrigated	0.60	0.13	0.14
	Unirrigated	0.63	0.18	0.37
	Irri. x Gen.	0.60	0.39	-
AGDM	Irrigated	0.46	0.03	-
	Unirrigated	0.67	0.05	0.34
	Irri. x Gen.	0.66	-	-
HI	Irrigated	0.65	0.55	0.58
	Unirrigated	0.63	0.61	0.57
	Irri. x Gen.	0.72	0.69	0.50
TGW	Irrigated	0.86	0.82	0.87
	Unirrigated	0.86	0.73	0.88
	Irri. x Gen.	0.91	0.88	0.70
PH	Irrigated	0.90	0.92	0.93
	Unirrigated	0.93	0.69	0.89
	Irri. x Gen.	0.96	0.87	0.91
AD	Irrigated	0.77	0.66	0.72
	Unirrigated	0.80	0.81	0.63
	Irri. x Gen.	0.87	0.86	0.65
Grains m^{-2}	Irrigated	0.49	0.64	0.66
	Unirrigated	0.50	0.45	0.54
	Irri. x Gen.	0.59	0.72	0.56
$\Delta^{13}C$	Unirrigated	0.37	0.33	0.31

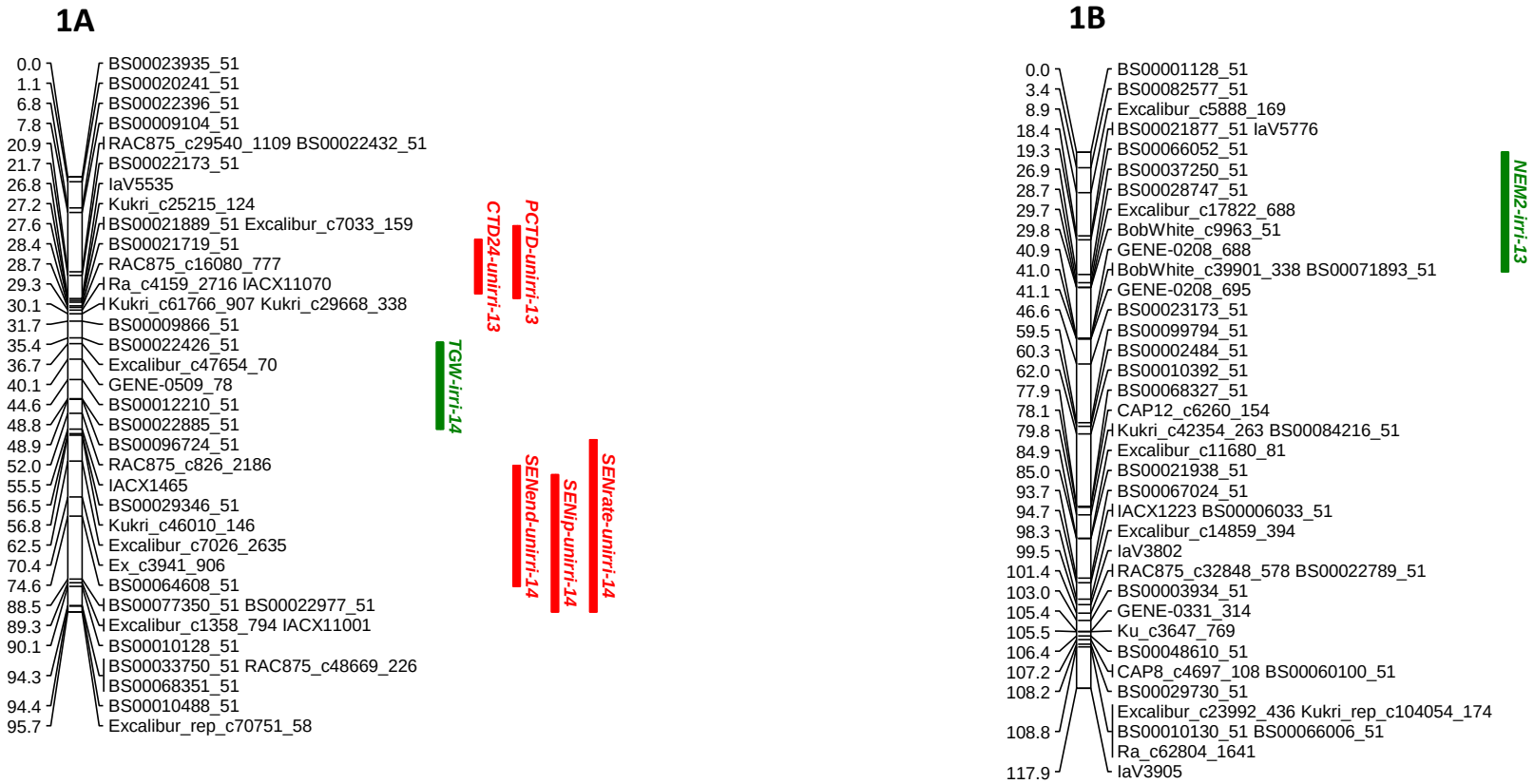


Figure 7.1 QTLs using Interval Mapping (IM) methods for 91 DH lines of the Rialto x Savannah DH population for grain yield (GY), harvest index (HI), above-ground dry matter (AGDM), thousand grain weight (TGW), grains m^{-2} (NGM2), grains ear^{-1} (NGE2), ears m^{-2} (NEM2), plant height (PH), days to anthesis (AD), days to maturity (MD), grain $\Delta^{13}C$, canopy temperature (PCT; post-anthesis CT, CT24; GS61+24d, CT29; GS61+29d), canopy temperature depression (PCTD; post-anthesis CTD, CTD24; GS61+24d, CTD29; GS61+29d), post-anthesis NDVI (NDVI21; GS61+21d, NDVI28; GS61+28d, NDVI35; GS61+35d), post-anthesis chlorophyll fluorescence (PCF), Onset of leaf senescence (SEN_{ONSET}), end of leaf senescence (SEN_{END}), leaf senescence inflection point (SEN_{IP}) and leaf senescence rate (SEN_{RATE}). Green-filled rectangle (-irri13; irrigated 2013, -irri14; irrigated 2014), red-filled rectangle (-unirri13; unirrigated 2013, -unirri14; unirrigated 2014). Error bar = 2 x LOD of the QTL.

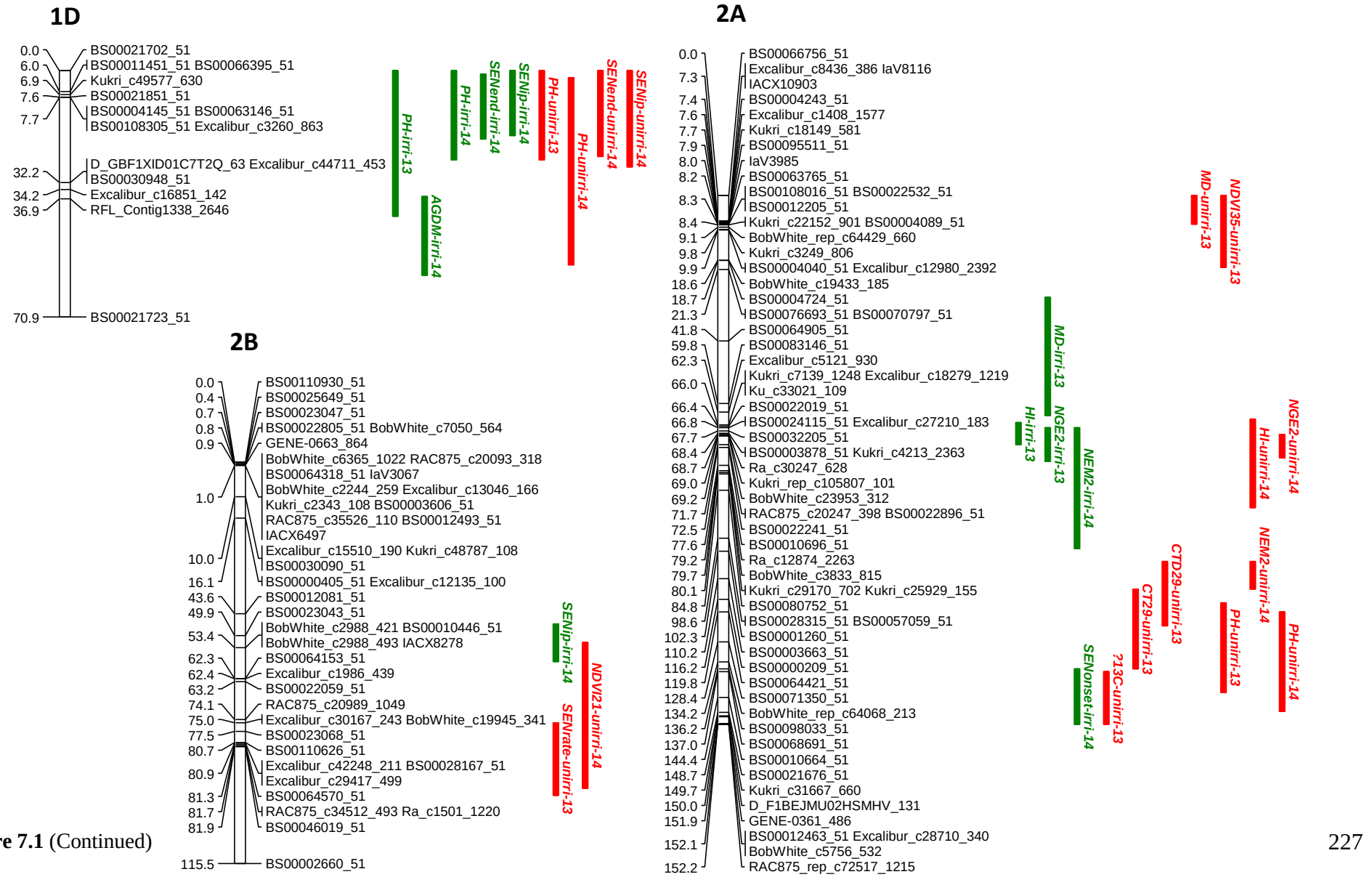
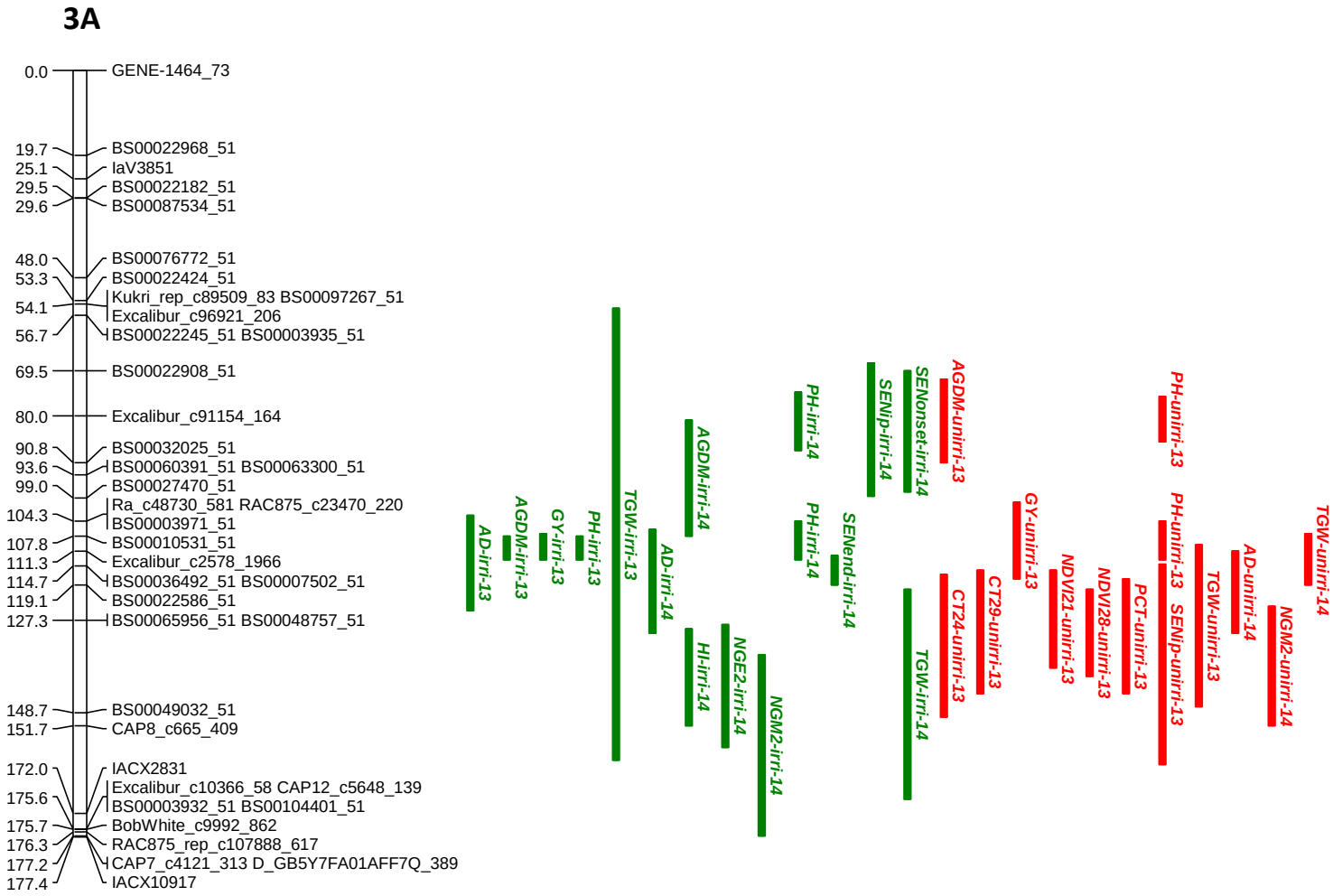


Figure 7.1 (Continued)



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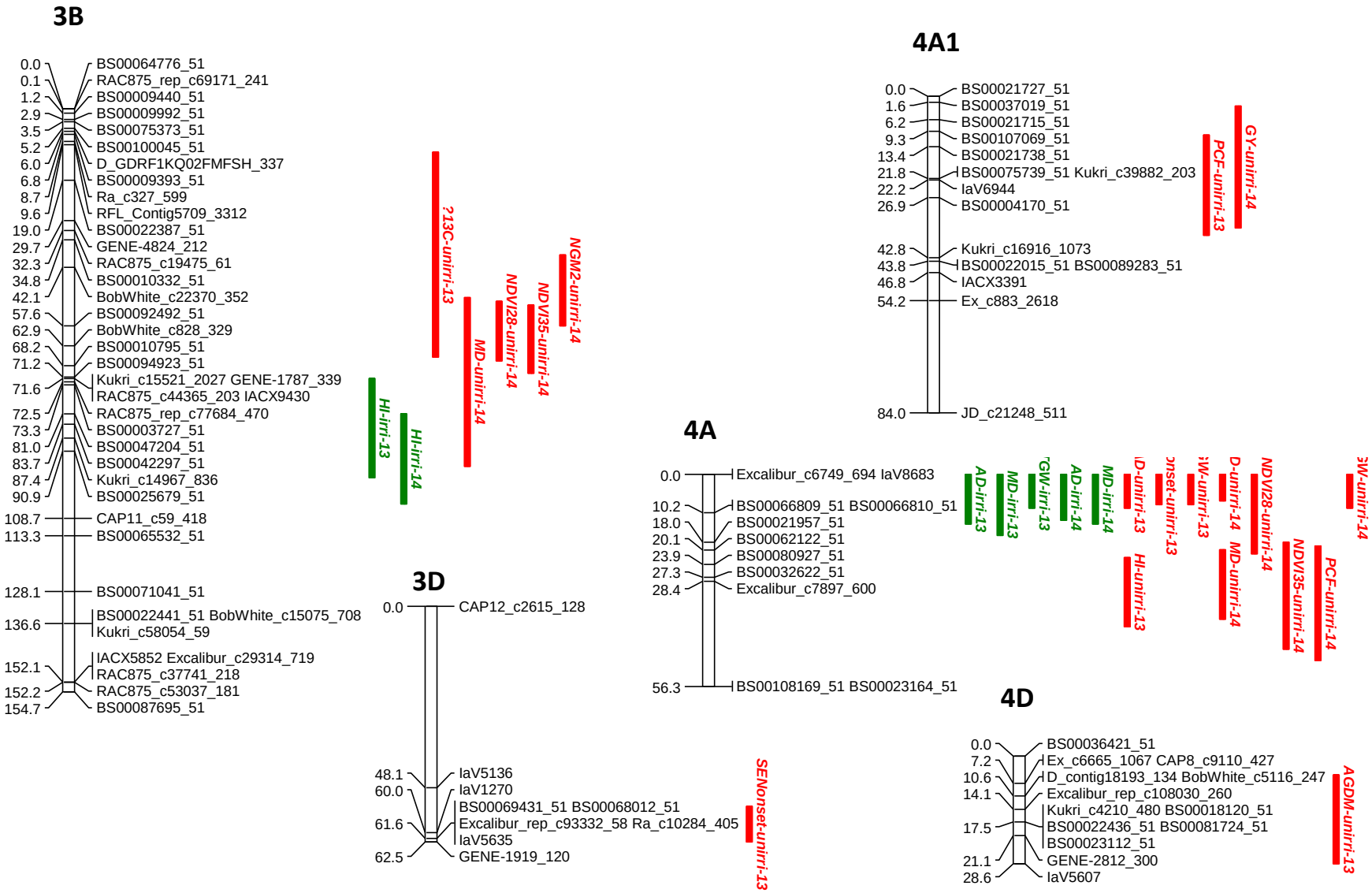

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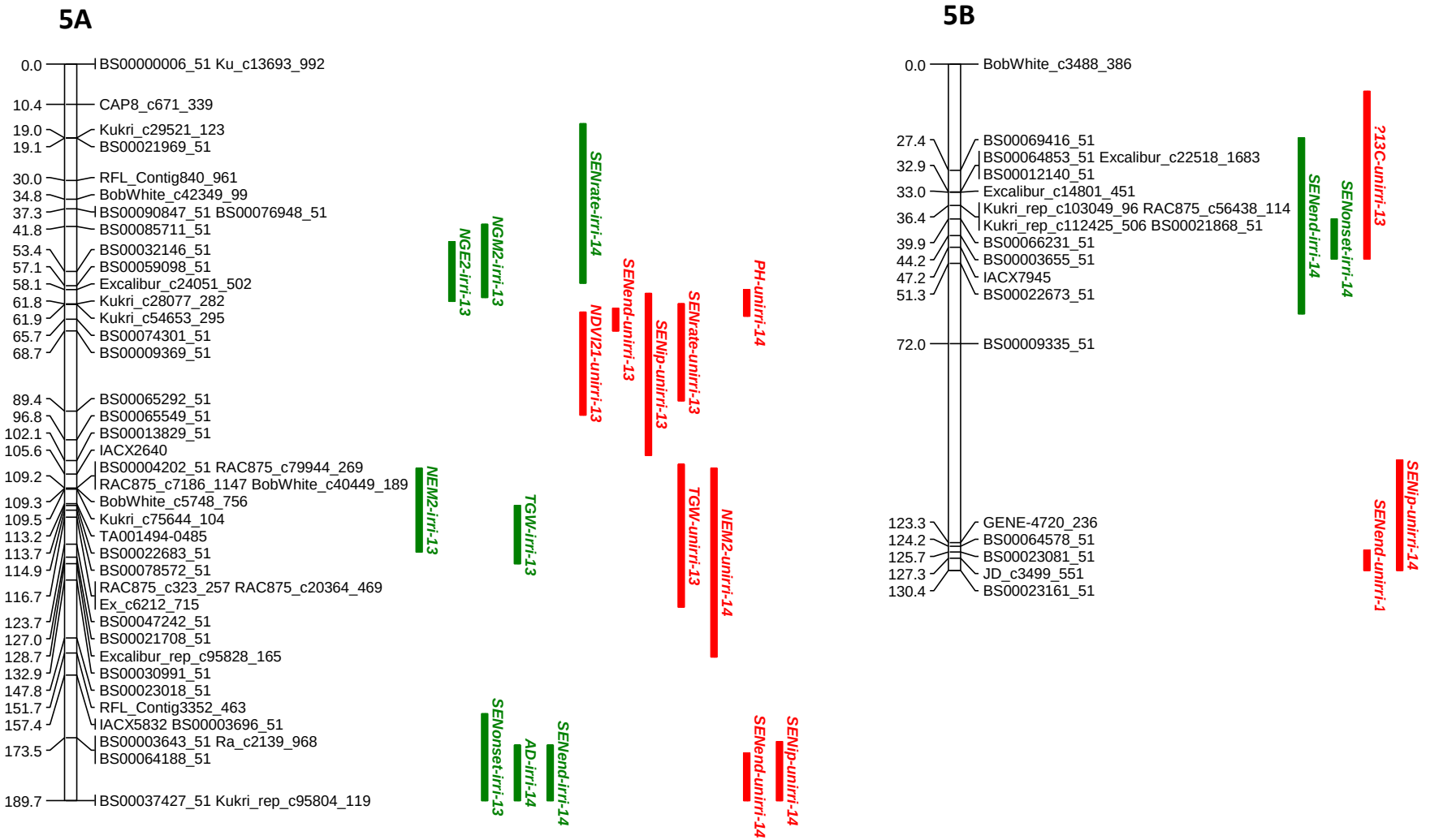


Figure 7.1 (Continued)

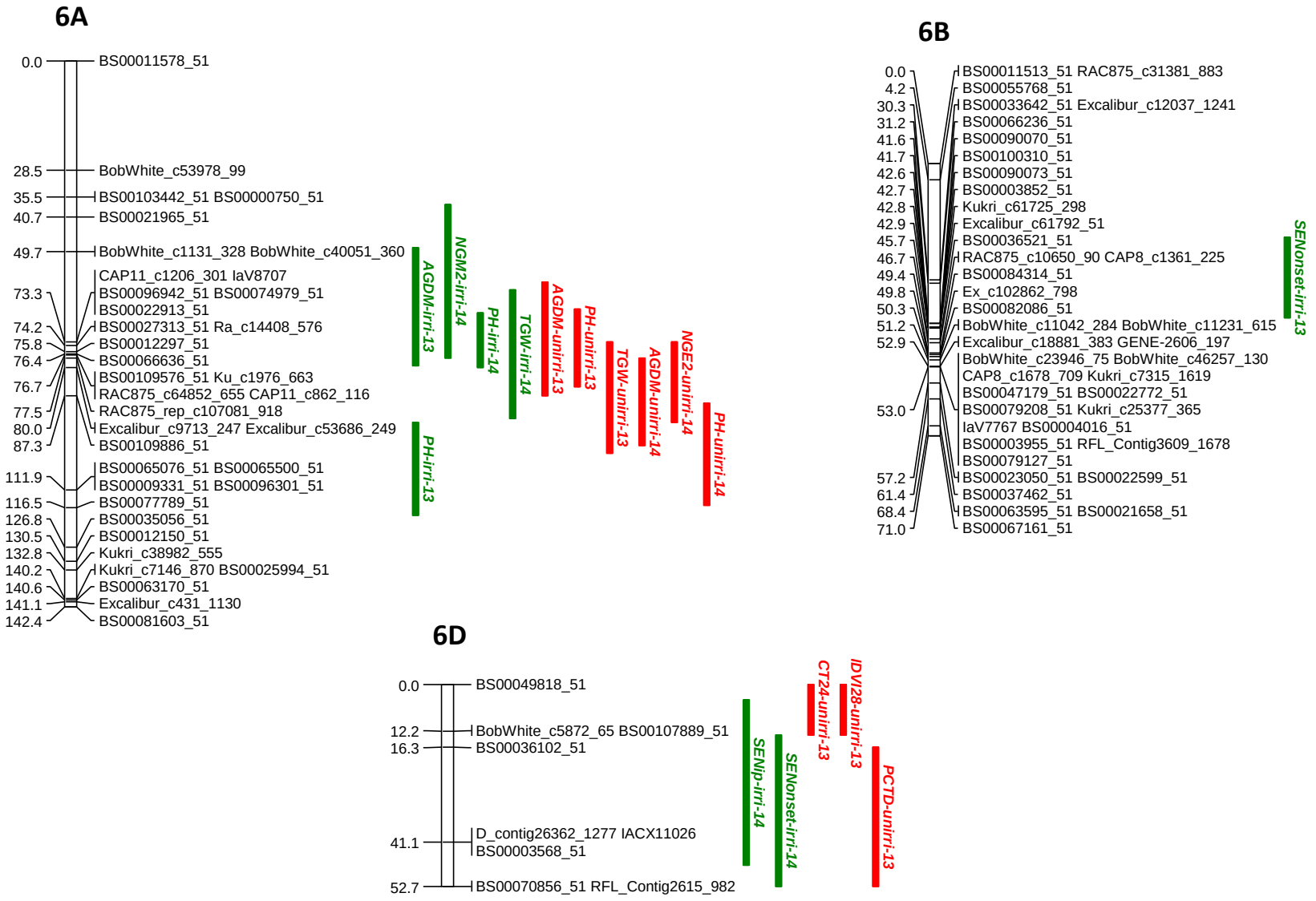
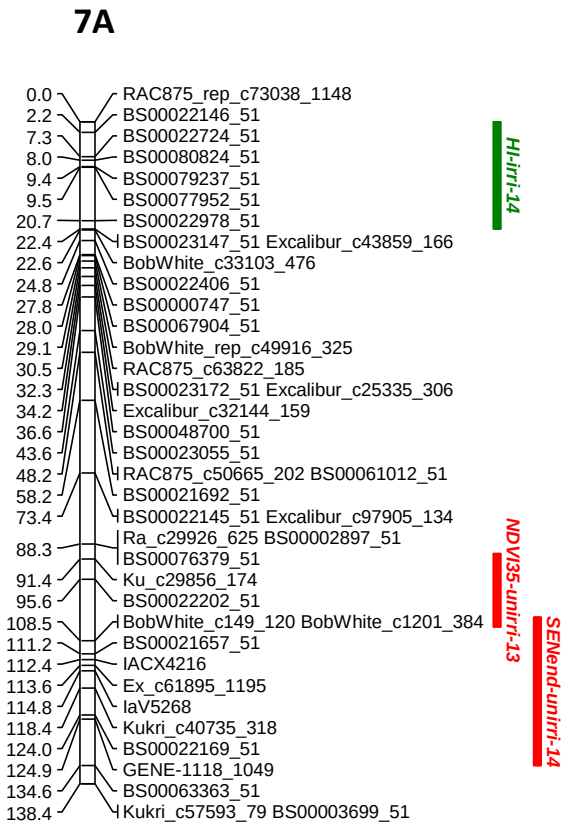


Figure 7.1 (Continued)



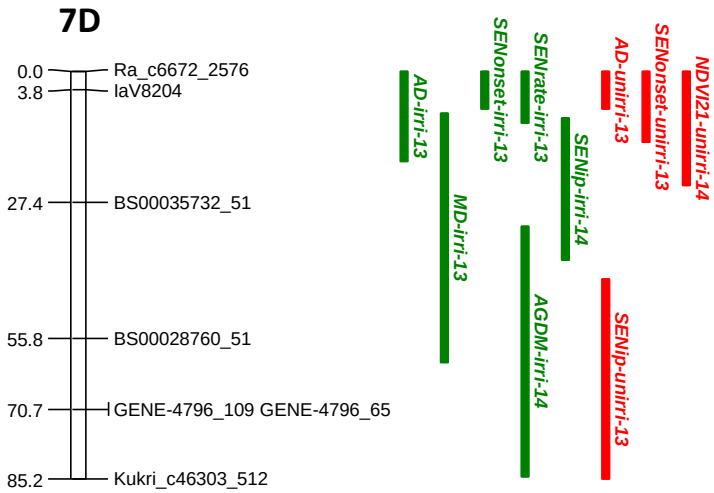


Figure 7.1 (Continued)

7.4 Discussion

Drought tolerance is regulated through the effects of multiple genes in a specific location, i.e. QTL, which depend on the population, plant growth stage, environmental conditions and other factors (Kosova *et al.*, 2014). QTL mapping can explore the genetic variation (Ashraf *et al.*, 2008) through estimating the gene location, size and activity pattern of a quantitative trait (Vinh and Paterson, 2005). In recent years, many studies have been conducted for QTL mapping for drought tolerance traits in wheat (Quarrie *et al.*, 1994; Marza *et al.*, 2006; Snape *et al.*, 2007; Rebetzke *et al.*, 2008a), barley (Souyris *et al.*, 1997; Comadran *et al.*, 2008), maize (Sari-Gorla *et al.*, 1999), cotton (Saranga *et al.*, 2001), sorghum (Sanchez *et al.*, 2002) and rice (Bernier *et al.*, 2008). The results of QTL analysis in the present study are discussed here.

7.4.1 QTLs identified for harvest traits

7.4.1.1 Grain yield

In a meta-QTL analysis reviewed by Zhang et al (2010), approximately 33% of the total of 541 QTLs reported in 59 publications was for grain yield distributed throughout the whole genome of wheat. Approximately 12% of the QTLs were mapped on genome A, 10% on genome B and 11% on genome D.

In the present study, two major QTL clusters on genome A were identified, one on chr 3A under both irrigated and unirrigated conditions in 2013, and the other on chr 4A under unirrigated conditions in 2014. The co-located QTL on chr 3A accounted for average of 18.9% of grain yield phenotypic variation. However, the significant genotype x irrigation and genotype x year interactions (discussed in chapter 4) and the relatively low heritability for grain yield showed that grain yield was also influenced by the environmental factors.

The QTL on chr 3A for grain yield was co-located with QTLs for AGDM, plant height and TGW with similar allelic effect of the parent Rialto. However, this QTL was also co-located with a QTL for anthesis date with earlier anthesis date associated with the Rialto allele consistent with the observation that early flowering was associated with higher grain yield, AGDM and TGW (as discussed in chapter 4). In a

previous study, using the same Rialto x Savannah DH population lines, six grain yield QTLs were detected including QTLs on chr 3A found under low and high nitrogen in three different field locations including the University of Nottingham, Sutton Bonington (Atkinson *et al.*, 2015).

Pinto *et al.* (2010) reported two QTLs for grain yield on chr 3A at different positions to the QTL on chr 3A in the present study, and one on chr 4A at similar position to the present study under drought using the elite wheat Seri x Babax recombinant inbred line (RIL) population. Three QTLs on chromosome 3A for grain yield were also identified by Campbell *et al.* (2003) consistent with the present study using a population of 98 chromosome 3A recombinant inbred lines (RICLs) between two winter wheat cultivars Wichita and Cheyenne.

QTLs on chr 4A for grain yield were also previously identified using two recombinant inbred line (RIL) populations derived from crosses between WL711 x PH132 and Opata85 x W7984 in different field locations in India (Kumar *et al.*, 2007), using a recombinant inbred line (RILs) population derived from a cross between Ning7840 spring wheat and Clark winter wheat under three different field conditions in Oklahoma, USA (Marza *et al.*, 2006), and using single-chromosome recombinant substitution lines derived from a cross between two Chinese spring wheats Kanto107 4A and Kanto107 4A (null Wx-B1b allele) under field conditions in Japan (Araki *et al.*, 1999).

The presently detected QTL on chr 3A may represent a novel gene for improving grain yield under mild drought, and could potentially be used to enhance yield by selection through developing a marker for this QTL for marker-assisted selection in future work.

7.4.1.2 Grain $\Delta^{13}C$

In the present study, three putative QTLs were identified for grain $\Delta^{13}C$ on chr 2A, 3B and 5B under mild drought conditions in 2013. The influence of parental alleles for these QTLs for the expression of $\Delta^{13}C$ was, however, different. The QTL on chr 3B, for which $\Delta^{13}C$ was increased (or TE decreased) by the Savannah allele, explained 10.1% of the phenotypic variation for grain $\Delta^{13}C$. However, for the other QTLs found on chr 2A and 5B explaining 10.7% and 9.2% of phenotypic variation,

respectively, $\Delta^{13}C$ was increased by the Rialto allele. Thus, a range of differential variation was found in the expression of $\Delta^{13}C$ in relation to the parental alleles in this study. The putative QTLs for $\Delta^{13}C$ in this study were not co-located with any of the QTLs identified for grain yield and TGW which may indicate that the inheritance of $\Delta^{13}C$ was not associated with that of yield.

The QTLs on chr 2A and 3B for grain $\Delta^{13}C$ were co-located with a QTL on chr 2A for SEN_{ONSET} and chr 3B for post-anthesis NDVI with different parental allelic effects suggesting higher $\Delta^{13}C$ (higher g_s) and stay-green genotypes may be associated with the ability to extract greater amount of water from the deep soil profiles. Similar to the present study, Rebetzke *et al.* (2008a) reported two QTL on chr 2A and 3B in two wheat DH populations (Cranbrook x Halberd and Sunco x Tasman) under several irrigated environments in Australia; and alleles for increased $\Delta^{13}C$ were contributed by either parent. The QTL on chr 3B in the present study, coincided with that found by Aravinda Kumar (2006) for the $\Delta^{13}C$ on chromosome 3B under irrigated and unirrigated conditions in the winter wheat Beaver x Soissons DH population.

The presently identified QTLs may potentially be used to develop markers for marker assist selection in wheat breeding programmes by selecting for high $\Delta^{13}C$ genotype (low TE) as an indirect way of selecting for greater seasonal water uptake under post-anthesis drought in the UK. However, further investigations can identify more stable QTLs for grain $\Delta^{13}C$ under UK drought conditions.

7.4.1.3 Plant height

Three major QTLs were identified under all environments on chr 3A (except for unirrigated 2014), 6A and 1D. A putative QTL on chr 2A was also found under unirrigated conditions in 2013 and 2014. The two QTLs on chr 3A for plant height (at 112.9 and 81.3 cM) were co-located with grain yield and AGDM with similar allelic effects of the Rialto on increasing biomass. QTLs for plant height were also identified in the Rialto x Savannah DH population on chr 2A, 3A, 6A and 1D by Atkinson *et al.* (2015) with Rialto allele increasing plant height under low and high N in three different field locations including Sutton Bonington.

QTLs on chr 1D, 3A and 6A were also identified for plant height using 111 lines of a BC_2F_1 population derived from a cross between the German winter wheat variety Flair and the synthetic wheat line XX86 developed in Japan (Huang *et al.*, 2004). A QTL on chr 3A was identified by Campbell *et al.* (2003), and a QTL on chr 2A by Yao *et al.* (2009) for plant height in winter wheat. In addition, QTLs for plant height have been identified on chr 2D, 3D and 4D by Wang *et al.* (2009) using winter wheat recombinant inbred lines (RILs) derived from the cross Heshangmai x Yu8679.

7.4.1.4 Anthesis date

Three significant QTLs were detected for anthesis date on chr 3A (in all environments except for unirrigated 2013), 4A (in all environments) and 7D (irrigated and unirrigated conditions in 2013). QTLs on chr 4A and 7D for anthesis date were co-located with QTLs for SEN_{ONSET} with early flowering and stay-green associated with Savannah allele indicating that early flowering was associated with longer flag-leaf green area duration (as discussed in Chapter 5). The QTL on chr 4A was co-located with a QTL for TGW with earlier flowering and higher grain weight associated with the Savannah allele, indicating early flowering was associated with higher grain weight as discussed in Chapter 4. Consistent with the present study, on chr 7D, co-location of QTLs for anthesis date, grain yield and leaf senescence duration was identified by Bogard *et al.* (2011) in winter wheat Toisondor x CF9107 population in the UK and France.

Similar to the present results, QTLs have been detected on chr 3A, 4A and 7D for anthesis date in previous investigations. Bogard *et al.* (2011) reported QTLs on chr 4A and 7D for anthesis date using winter wheat DH population from the cross between Toisondor and CF9107. The stable QTL for anthesis date on chr 7A was possibly linked with the Vrn-D3 gene on this chromosome (Bonnin *et al.*, 2008). A QTL on chr 3A under heat stress conditions at a different position, and one on chr 4A under irrigated conditions at the same position compared to present study were identified by Pinto *et al.* (2010) for anthesis date. In addition, QTLs for anthesis date were reported on chromosomes 5D and 6D (along with 1B, 2B and 3B) using winter wheat recombinant inbred lines (RILs) derived from the cross Heshangmai x Yu8679 (Wang *et al.*, 2009).

The QTLs presently identified on chr 4A and 7A suggest anthesis date is controlled by genes of major-effect in these regions in the Rialto x Savannah DH population, which can be used in marker-assist selection breeding programmes to optimise phenology for drought resistance in wheat.

7.4.2 QTLs identified for stay-green and leaf senescence traits

Several QTLs were identified for stay-green traits in the present study such as NDVI and CT under unirrigated condition. Three QTLs on chr 3A (unirrigated 2013), 4A (unirrigated 2013 and 2014) and 7D (unirrigated 2014) were identified for post-anthesis NDVI. The QTLs on chr 3A for post-anthesis NDVI was co-located with a QTL for post-anthesis CT, and the QTL on chr 7D was co-located with SEN_{RATE} and SEN_{ONSET} indicating genetic associations between these stay-green traits.

For leaf senescence, a range of QTLs were detected under irrigated and drought conditions in the present study. A significant QTL on chr 7D (irrigated and unirrigated 2013) was found for the SEN_{ONSET}. For the SEN_{RATE}, a major QTL on chr 7D (irrigated 2013) was found. Two significant QTLs were detected for the SEN_{END} on chr 3A (irrigated 2014) and 5A (irrigated 2014, unirrigated 2013 and 2014). The QTL on chr 7D for SEN_{ONSET} was co-located with the QTL identified for anthesis date as discussed above.

In a previous root study on Rialto x Savannah DH population, a pleiotropic QTL was identified on chr 7D for grain yield and root system traits with the effect of the Savannah allele conferring a larger root system providing possible evidence for a genetic relationship between stay-green and root system traits as discussed in Chapter 5 (Atkinson *et al.*, 2015). Similar to the present study, QTLs on chr 2A, 3A, 4A and 5A for post-anthesis NDVI, and on 3A for pre-anthesis CT were identified under drought, heat and irrigated conditions using the elite wheat Seri x Babax recombinant inbred line (RIL) population (Pinto *et al.*, 2010). On chr 2A, 3B and 4A, several QTLs for NDVI, and one on 3A for CT were also identified under drought and heat stress conditions using the RAC875 x Kukri DH population (Bennett *et al.*, 2012).

For the leaf senescence, Wang *et al.* (2015) also reported a QTL on chr 7D under irrigated conditions for the onset of leaf senescence, a QTL on chr 5A under drought

for the time to maximum leaf senescence rate, and QTLs on chr 1A and 5A under drought for the end of leaf senescence using a DH population derived from two winter wheat varieties Hanxuan10 x Lumai14. In addition, on chr 7D, Bogard *et al.* (2011) reported a co-located QTL for SEN_{ONSET}, grain yield and anthesis date as described above.

QTLs on chr 2A and 3A for flag-leaf 75% greenness under optimal and high temperature conditions, and two QTLs on chr 5A for the maximum rate of leaf senescence and on chr 7D for the time to maximum rate of leaf senescence under optimum temperature were reported by Vijayalakshmi *et al.* (2010) using a population of RILs from a cross between winter wheat cultivars Ventnor and Karl 92. In addition, coinciding with the present QTLs on chr 7D for the onset and rate of leaf senescence, and 1A for the end of leaf senescence; Kumar *et al.* (2010) identified three QTLs for green leaf area on chr 1A, 3B and 7D using Chirya x Sonalika RILs population.

The present results suggest that there are major gene-effects regulating the stay-green traits on chr 3A and 7D in the Rialto x Savannah DH population, which can be used to develop markers for MAS in future breeding programmes in order to improve drought tolerance in wheat. High heritability for the stay-green traits observed in the present study increases confidence in the ability to select among progeny for optimum expression of stay-green traits.

7.5 Conclusions

The results in this Chapter allow the following conclusions to be made:

1. A Co-located QTL on chr 3A for grain yield, above-ground dry matter, thousand grain weight, plant height, anthesis date and flag-leaf senescence duration was identified in the Rialto x Savannah DH population under irrigated and drought conditions.
2. On chr 3A, a co-located QTL was also identified for thousand grain weight, grains per m^2, NDVI, canopy temperature and flag-leaf senescence in the Rialto x Savannah DH population under irrigated and drought conditions.

3. A QTL for anthesis date on chr 7D was co-located with QTLs for NDVI and onset of flag-leaf senescence in the Rialto x Savannah DH population under irrigated and drought conditions.

Chapter 8 General Discussion

8.1 Introduction

This project investigated novel physiological traits determining WUE and drought tolerance and their genetic control in a wheat Rialto x Savannah DH population, and the underlying mechanisms. The project also focused on comparing genetic variation for WUE and drought-tolerance traits in UK adapted winter wheat R x S DH lines with wheat amphidiploid lines (durum wheat x *Thinopyrum bessarabicum*) and ancestral wheat species in the glasshouse experiments. The genetic bases of the traits for the R x S DH lines were investigated through QTL analysis. The main findings in the present study are discussed in this Chapter.

8.2 Genetic variation in TE and drought resistance in UK winter wheat

Variation for flag-leaf photosynthetic rate, stomatal conductance and transpiration efficiency was found amongst the sub-set of genotypes in the field experiments under drought. Genotypic variation for these flag-leaf traits has previously been reported in wheat (Blum, 1990; Morgan and Lecain, 1991; Fischer *et al.*, 1998; Xue *et al.*, 2002). The grain $\Delta^{13}C$ also differed between genotypes under drought conditions in both years. A lower range of grain $\Delta^{13}C$ under rain-fed condition was reported by Monneveux *et al.* (2006) than in the present study, and Aravinda Kumar *et al.* (2011) reported a genetic range of $\Delta^{13}C$ similar to present study for the UK winter wheat.

Amongst the sub-set lines in 2013, higher grain $\Delta^{13}C$ (i.e. lower TE) was associated with higher grain yield. The negative correlation between TE and grain yield was also confirmed by a negative correlation between flag-leaf TE measured directly through gas-exchange and each of grain yield and grain $\Delta^{13}C$. High $\Delta^{13}C$ values resulting from high Ci/Ca (and associated with high stomatal conductance) have been shown to lead to high CO_2 assimilation and hence higher grain yield, but low TE (Morgan *et al.*, 1993; Monneveux *et al.*, 2006; Rebetzke *et al.*, 2006). Higher grain $\Delta^{13}C$ associated with higher grain yield has been frequently reported in previous investigations under field drought conditions (Ehdaie *et al.*, 1991; Morgan *et al.*, 1993; Araus *et al.*, 1998; Merah *et al.*, 2001b; Tsialtas *et al.*, 2001; Condon *et al.*, 2002; Monneveux *et al.*, 2005; Xu *et al.*, 2007; Aravinda Kumar *et al.*, 2011). Under moderate drought conditions, higher grain yield could be driven by higher water

uptake rather than higher WUE (Blum, 2005), and there a trade-off between TE and season-long water uptake was reported under UK drought conditions (Aravinda Kumar *et al.*, 2011). Thus findings in the present study suggest that grain $\Delta^{13}C$ could be a useful selection criterion for improving yield performance in breeding programmes, and that selection for high grain $\Delta^{13}C$ may be effective for yield improvement under UK drought as an indirect indicator of water uptake rather than WUE.

Although, flag-leaf A_{max} and g_s were correlated with grain yield amongst the sub-set lines in the field experiments, in the glasshouse experiments a negative association between flag-leaf A_{max} and AGDM was found under drought. This result might have been due to an association between leaf morphology (leaf area, leaf specific weight) and leaf photosynthetic performance (Reynolds *et al.*, 2012); i.e. smaller leaves could be associated with higher flag-leaf A_{max}, but reduced light interception and biomass in the glasshouse experiments. Under water stress, higher A_{max} and g_s genotypes were shown to have higher grain yield and lower WUE in previous studies on wheat (Jiang *et al.*, 2000; Monneveux *et al.*, 2006; Yasir *et al.*, 2013).

8.3 Associations of yield loss with yield potential, anthesis date and plant height under UK drought

In the present field experiments, there was only a meaningful drought in 2013; therefore, the discussion will focus mainly on the effects in 2013 and the cross year mean. In 2013, the grain yield decrease under drought ranged from 15% to 30% between genotypes. Similar to the present study, 20 to 30% grain yield reductions were reported by Foulkes *et al.* (2002) investigating six winter wheat cultivars. However, smaller grain yield reductions of 5 to 15% were reported by Aravinda Kumar *et al.* (2011) using a doubled-haploid (DH) winter wheat Beaver x Soissons population in the UK.

In 2013, higher grain yield under irrigated conditions was associated with greater yield loss under drought indicating genetic variation in yield potential might be related to drought tolerance. However, there were some departure from this relationship; e.g. Savannah and DH line 48 yielded above the mean amongst genotypes under irrigated conditions with relatively low yield losses under drought.

A positive relationship between yield potential and absolute yield loss has been reported previously under drought in wheat (Fischer and Maurer, 1978; Foulkes *et al.*, 2007c).

Above-ground dry matter was reduced by drought on average by 18%, and higher AGDM was strongly associated with taller plants. However, plant height was not correlated with yield loss under drought or SSI. This is consistent with the observation that semi-dwarf isogenic lines do not consistently show drought tolerance compared to tall check cultivars under drought across environments (Foulkes *et al.*, 2002). Above-ground biomass reduction under drought is frequently reported in the literature (Foulkes *et al.*, 2001, 2002; Kandic *et al.*, 2009; Aravinda Kumar *et al.*, 2011), and various studies have demonstrated positive associations between plant height and AGDM amongst wheat genotypes (Fischer, 1985; Miralles and Slafer, 1997; González *et al.*, 2003; Bognár *et al.*, 2007; Toyota *et al.*, 2010; Aisawi *et al.*, 2015).

The present field experiments also showed that early flowering was weakly associated with higher grain yield under irrigated and drought conditions in both years. However, yield loss and SSI were not associated with anthesis date under drought. Traits associated with water uptake such as flowering time can lead to a better maintenance of grain yield under drought through drought escape redistributing water uptake favourably with regard to post-anthesis for crop growth. In the UK, no correlation between anthesis date and grain yield under UK drought was observed by Foulkes *et al.* (2001), who suggested that the benefit of any drought escape effect with early flowering might be cancelled out by a shorter period for root growth up to anthesis date and reduced season-long water uptake.

In the glasshouse experiments, early flowering genotypes were associated with higher grain yield and biomass, and showed greater long-season water uptake. Accessions of *T. bessarabicum* were the latest flowering genotypes associated with the lowest HI followed by R x S DH lines and amphidiploid lines under both irrigated and droughted treatments. However, the lower grain yield for accessions of *T. bessarabicum* may compared to R x S lines have been more associated with their low spikelet number and HI hence post-anthesis DM growth rather than their late anthesis date per se in the present study.

8.4 Drought resistant plant ideotype in UK winter wheat

Higher post-anthesis NDVI was associated with higher grain yield, AGDM and TGW under drought in the field experiments. Lower stress susceptibility index (SSI) was also strongly correlated with higher NDVI. In previous investigations, positive correlations between grain yield and NDVI amongst wheat genotypes have been frequently reported under rain-fed environments (Das *et al.*, 1993; Aparicio *et al.*, 2000; Serrano *et al.*, 2000; Gutiérrez-Rodríguez *et al.*, 2004; Reynolds *et al.*, 2007b; Reynolds *et al.*, 2007c).

In the field experiment in 2013, lower post-anthesis canopy temperature was associated with higher grain yield and lower SSI. Similar relationships have previously been found (Pinto *et al.*, 2010; Lopes *et al.*, 2012), and may be related to an association between a cooler canopy and a deeper root system and water extraction potential from deeper soil profiles under drought.

Post-anthesis flag-leaf chlorophyll fluorescence was weakly positively associated with grain yield and TGW, possibly in part due to the relatively mild drought in 2013, and was also positively associated with lower SSI. In wheat, positive associations of leaf *Fv/Fm* and grain yield under water stress were previously reported (Farzad *et al.*, 2007; Rad *et al.*, 2014). The physiological mechanism is presumably related to higher efficiency of PS II which can regulate the photosynthesis process and maximize the plant photosynthesis potential, and hence improve grain yield (Guan *et al.*, 2014). Leaf chlorophyll fluorescence, however, may not be a useful selection criterion for grain yield in wheat under UK water stress based on the presently relatively weak correlations observed under drought.

Savannah had higher post-anthesis NDVI and chlorophyll fluorescence than Rialto, but Savannah and Rialto had similar canopy temperature under drought conditions. Although, there was no transgressive segregation for these traits above the higher parent under unirrigated conditions, the parent Savannah and DH lines 12, 77 and 54 showed delayed onset of senescence and lower canopy temperature under drought than other genotypes associated with lower yield reduction under drought, indirectly indicating the drought tolerance and delayed senescence could be partly related to a deeper root system for these lines.

In the field experiment in 2013, genotypes with later onset of leaf senescence had lower SSI and less grain yield loss under drought. Similar associations between leaf senescence duration and each of AGDM and TGW loss under drought conditions were also found. Associations between genetic variation in leaf senescence duration and grain yield in wheat under drought have been reported in previous studies (Thomas and Howarth, 2000; Spano *et al.*, 2003; Verma *et al.*, 2004; Hafsi *et al.*, 2007; Gregersen *et al.*, 2008). The effect of stay-green on grain yield under drought is likely associated with source limitation of grain growth under post-anthesis abiotic stress (Christopher *et al.*, 2008; Bogard *et al.*, 2011), with canopies with prolonged green area maintaining active photosynthesis better (Joshi *et al.*, 2007).

Overall among R x S DH population lines, lower grain yield reduction and SSI under drought conditions appeared to be associated with higher NDVI, a cooler canopy, and longer flag-leaf green area duration (Table 8.1). The parent Savannah and DH lines 12 and 59 in this population had the lowest yield reduction and SSI associated with higher stay-green traits such as NDVI, CT and leaf senescence. Therefore, present results indicate stay-green traits could be important selection criteria for grain yield under drought in future breeding programmes.

Table 8.1 The phenotypic correlation (r) between actual grain yield loss (t ha^{-1}), yield loss (%) and stress susceptibility index (SSI), and each of plant height (PH), anthesis date (AD; days after sowing, DAS), post-anthesis NDVI, CT, CT depletion, QY, $\Delta^{13}C$, SEN_{ONSET}, SEN_{END} and SEN_{TOTAL} among 94 R x S DH lines, and post-anthesis A_{max} and g_s among 6 R x S DH lines and the two parents under drought in 2013.

Traits	Actual yield loss (t ha^{-1})	Yield loss (%)	Stress susceptibility index (SSI)
PH	0.03^{ns}	-0.09^{ns}	-0.09^{ns}
AD	-0.02^{ns}	0.02^{ns}	0.02^{ns}
Post-anthesis NDVI	-0.49^{***}	-0.53^{***}	-0.53^{***}
Post-anthesis CT	0.41^{***}	0.45^{***}	0.45^{***}
Post-anthesis ΔCT	-0.23^{*}	-0.23^{*}	-0.23^{*}
Post-anthesis QY	-0.16^{ns}	-0.20^{*}	-0.20^{*}
$\Delta^{13}C$	-0.15^{ns}	-0.15^{ns}	-0.15^{ns}
SEN_{ONSET}	-0.28^{**}	-0.35^{***}	-0.35^{***}
SEN_{END}	-0.41^{***}	-0.40^{***}	-0.40^{***}
SEN_{TOTAL}	-0.20^{*}	-0.16^{ns}	-0.16^{ns}
Post-anthesis A_{max}	-0.82^{*}	-0.83^{*}	-0.83^{*}
Post-anthesis g_s	-0.89^{**}	-0.95^{***}	-0.95^{***}

8.5 Novel variation for drought tolerance traits from wider wheat germplasm

In both glasshouse experiments, the amphidiploid lines had lower grain yield per plant compared to R x S DH lines due to lower HI, but greater biomass per plant due in part to increased plant height. However, the ancestral wheat species produced the least biomass associated with fewer tillers per plant in spite of their relatively high plant height.

The amphidiploid wheat genotypes produced higher AGDM than R x S lines, but did not efficiently partition DM to the grain. Higher AGDM for the amphidiploid lines was associated with taller plants and greater shoot number per plant. It was also related to delayed senescence and higher photosynthetic rate for amphidiploid lines observed under both irrigated and droughted conditions compared to R x S DH lines.

Lower HI for ancestral wheat species was associated with lower spikelets per ear and individual grain weight; and these traits were intermediate for amphidiploid lines compared to R x S DH lines. For these wider wheat genotypes, therefore, generally lower grain sink size and HI had more effect on the final grain yield than AGDM. Although, reductions in grain yield and AGDM per plant by drought were generally higher for amphidiploid lines than for the R x S DH lines, the effect of drought on one amphidiploid line Karim x *T. bessarabicum* was similar to the most drought tolerant of the R x S DH lines, line 25.

Grain yield reduction under water stress in the glasshouse experiments was generally associated with HI and spikelets per ear and grain weight. AGDM reduction under drought was strongly affected by shoot number per plant. In wheat, correlations amongst wheat genotypes between tiller survival and biomass under water stress have been reported in previous studies (Brisson *et al.*, 2001; Pandey *et al.*, 2001; Carvalho, 2009). However, AGDM reduction under drought amongst genotypes was not associated with reductions in shoot number per unit area in the field experiment in 2013.

Although amphidiploid lines were the earliest developing genotypes and *T. bessarabicum* accessions the latest, *T. bessarabicum* accessions used the smallest amount of water (associated with the lowest shoot number and a low HI) followed by

amphidiploids, R x S DH lines, and *A. speltoides* accessions with the greatest WU (associated with the highest shoot number). Therefore variation in shoot number per plant affected water uptake amongst genotypes more than differences in phenology in the present study. Amphidiploid lines had the highest water-use efficiency (WUE_{AGDM}) followed by R x S DH lines and *T. bessarabicum* accessions under drought. Overall, higher WUE_{AGDM} genotypes were associated with higher TE and lower g_s post-anthesis under drought.

Averaging over years, *T. bessarabicum* accessions and amphidiploid line *T. bessarabicum* x Karim had significantly higher flag-leaf A_{max}, g_s and TE than the highest R x S DH line 20. In addition, *T. bessarabicum* accessions had higher flag-leaf chlorophyll content (SPAD) than amphidiploid and R x S DH lines, and higher SPAD was associated with higher A_{max} amongst genotypes. The high values of flag-leaf A_{max} and SPAD for *T. bessarabicum* accessions were associated with smaller flag-leaf size and greater leaf specific weight. However, high pre-anthesis A_{max} and SPAD for the amphidiploid lines were related to the higher flag-leaf specific weight, but not smaller leaf size compared to the best check R x S DH lines. These effects could possibly be related to the amount of Rubisco per unit leaf area for *T. bessarabicum* accessions and amphidiploid lines since high photosynthesis per unit leaf area may be related to high leaf specific weight hence increased RUE (Morgan *et al.*, 1990; Shearman *et al.*, 2005). Thus, higher A_{max} for amphidiploid lines independent of leaf size effects may represent more useful variation for A_{max} than that for *T. bessarabicum* accessions. Therefore, results of this study suggest that there is potential for high expression of TE and A_{max} in amphidiploid lines to be introgressed into UK wheat cultivars through breeding programmes to enhance drought tolerance.

Averaging over years, genotype rankings for post-anthesis A_{max}, g_s and TE measured in the field for R x S DH lines and the parents were consistent with those measured in the glasshouse. This increases the confidence in the present results measured in both field and glasshouse experiments, and indicates the genetic variation for leaf A_{max}, g_s and TE of plants grown in glasshouse conditions was representative of that in field-grown plants.

8.6 Genetic regulation of drought resistance in UK winter wheat

Wheat breeders can currently identify the gene locations controlling quantitative traits such as drought tolerance and associated traits through molecular genomic markers (Snape *et al.*, 2007). To date, relatively slower genetic progress has been achieved in wheat yield under drought compared with favourable environments, mainly due to the large effects of genotype x year and/or genotype x location interactions on grain yield (Calhoun *et al.*, 1994; van Ginkel *et al.*, 1998; Richards *et al.*, 2002).

In the present study, four QTL clusters were identified; two of them located on chr 3A, and the other two on chr 4A and 7D. Under both irrigated and drought conditions, a QTL on chr 3A for grain yield was co-located with QTLs for AGDM, plant height, SEN_{END} and TGW with similar allelic effect of the parent Rialto, explaining on average 20% of phenotypic variation in grain yield. This suggested that genetic variation in grain yield was dependent more on biomass than HI which was supported by the stronger positive phenotypic association of grain yield with AGDM than with HI (Chapter 4). In addition, this QTL was co-located with a QTL for anthesis date, and the Rialto allele advanced the flowering time confirming the positive associations between early flowering and each of grain yield, AGDM, plant height and TGW.

Using the same Rialto x Savannah DH population, QTLs for grain yield and plant height were previously detected on chr 3A, with the allele increasing effect coming from Rialto under low N conditions in a field experiment at Sutton Bonington (Atkinson *et al.*, 2015). These effects for the QTL on chr 3A, therefore, could be pleiotropic and the QTL may represent a novel gene for improving grain yield under optimal and UK mild drought conditions, and could be used to enhance yield by developing a marker for this gene for application in marker-assisted selection in wheat breeding programmes.

The putative QTLs for $\Delta^{13}C$ in this study were not co-located with any of the QTLs identified for grain yield. However, two QTLs explaining approximately 10% of phenotypic variation for grain $\Delta^{13}C$ were co-located with QTLs for SEN_{ONSET} on chr 2A and post-anthesis NDVI on chr 3B. There were different parental allelic effect for

increasing grain $\Delta^{13}C$, the Savannah allele increased $\Delta^{13}C$ for the 3B QTL but decreased $\Delta^{13}C$ for the 2A QTL. In both cases the allele increasing $\Delta^{13}C$ (i.e. increasing WU also decreased stay-green). This suggested the co-location for each of these QTLs may be an effect of two tightly linked genes and further investigation is needed to identify the QTLs through fine mapping and associations with physiological traits related to drought resistance under UK drought conditions

For the stay-green traits, a major QTL on chr 3A explaining 15% of phenotypic variation for post-anthesis NDVI was co-located with post-anthesis CT, with the effect of the Rialto allele to increase NDVI and decrease canopy temperature which indicates a genetic association between cooler canopy and higher NDVI for these genotypes.

For the SEN_{ONSET}, two stable QTLs on chr 4A and 7D were co-located with QTLs for anthesis date. The Savannah allele delayed the onset of leaf senescence and advanced anthesis date. In agreement with the present results, on chr 7D, co-location of QTLs for AD, GY, and total leaf senescence duration was identified by Bogard *et al.* (2011), and on chr 4A a QTL for anthesis date was identified at a similar position to the present study by Pinto *et al.* (2010). The QTL on chr 7D for anthesis date may be related to the *VRN-D3* flowering gene which is known to accelerate plant development on this chromosome (Bonnin *et al.*, 2008). These effects, related to the QTLs on chr 4A and 7A explaining 13 and 16% of phenotypic variation in grain yield, respectively, might be pleiotropic and suggest that anthesis date and senescence are in part controlled by major effects of genes in these regions in the Rialto x Savannah DH population; and markers could be developed for these genes for application in marker-assist selection in breeding programmes to improve drought resistance in wheat.

8.7 Application of physiological traits and identified QTLs in breeding programme to improve drought tolerance

Winter wheat is the major arable crop in the UK, approximately 30% of UK wheat is grown on drought-prone lands (Foulkes *et al.*, 2001), and approximately 15-20% of annual wheat yield production is lost to drought (Foulkes *et al.*, 2002). Therefore, target physiological traits associated with drought resistance are needed to improve

selection of genotypes in wheat cultivar testing and breeding under UK drought conditions.

Carbon isotope discrimination provides an integrative measure of water-use efficiency (Morgan *et al.*, 1993). In the present study, higher grain $\Delta^{13}C$ was associated with higher photosynthetic rate and stomatal conductance, indicating that water uptake is the main driver for higher grain yield under UK drought conditions (Blum, 2005). Therefore grain $\Delta^{13}C$ could be a useful selection criterion for improving yield performance (as an indicator of water uptake) in breeding programmes under UK mild drought. Furthermore, present results show that canopy temperature has potential as an indirect indicator for deeper root system and higher water uptake and yield under UK drought condition.

Present findings on the amphidiploid lines also suggest that there is potential to select for higher leaf specific weight effects for high expression of TE and A_{max} whilst maintaining leaf area and light interception under UK drought condition. Stay-green traits, therefore, could be important selection criteria for improving grain yield under UK drought in future breeding programmes. These traits could be used to identify suitability to different soil types in rain fed UK conditions, e.g. sandy loam (draught prone) and silt loam (not drought prone) in the UK.

In plant breeding programmes there is scope to augment phenotypic selection with marker-assisted selection for drought tolerance traits. The present study identified promising QTLs on 3A for grain yield and plant height, 4A and 7D for development of precise markers through further fine mapping for application in wheat breeding programmes.

8.8 Future work

The results for grain $\Delta^{13}C$ and leaf photosynthetic traits in the field experiments suggested that water uptake is the main driver for improving yield under drought in the UK environment rather than water-use efficiency likely associated with a deeper root system and extracting more water from the deep soil layers. These leaf traits can therefore provide a useful indirect measure of genetic variation in water uptake. However, further studies are justified to identify direct genetic variation in root system architecture traits including root length density through high-throughput

phenotyping methods. In addition, the methodology of gas-exchange measurement needs to be developed for faster high-throughput screening (e.g. using multispectral cameras (VIS) and thermal imaging cameras) to estimate photosynthetic traits for larger number of genotypes in breeding programmes.

Although, an estimation of stomatal conductance and root depth variation can be provided through measuring canopy temperature, its use may be limited in the UK environment due to its windy and relatively cool climate conditions. Moreover, in the UK, there is no regular summer drought due to unpredictability of the rainfall in the summer. Therefore, rain-out shelters may be possibly used to phenotype selected near-isogenic lines and/or mapping populations. In addition, drought can be also imposed at any specific stage of crop development with the use of rain-out shelters.

Although, gas-exchange measurements in the glasshouse on single plants were related to these traits in the field experiments, it is still difficult to compare single plant measurements to the field (plot) scale on account of the variable influence of canopy architecture and environmental conditions. Thus, increasing the number of plants per column and column sizes could increase the reliability of these measurements in relation to the field conditions.

The present study identified a QTL on chr 3A controlling grain yield, yield components, and anthesis date under drought conditions, and confirmed that this grain yield QTL was also associated with flag-leaf senescence duration. Thus, chr 3A seems to carry an important gene for grain yield under mild UK drought, and fine mapping of this region to determine the molecular basis of this gene and associated traits is likely to improve understanding of the mechanisms determining drought tolerance in UK winter wheat. Although, grain $\Delta^{13}C$ QTLs were not associated with any grain yield QTLs in this study, a grain $\Delta^{13}C$ QTL on chromosome 3B was associated with stay-green traits. Therefore, further fine mapping for the 3B chromosome in the region of this QTL will improve understanding the physiological and genetic bases of grain $\Delta^{13}C$ under UK mild drought environments.

In addition, the two QTLs affecting post-anthesis flag-leaf senescence on chr 4A and 7D appeared to coincide with stable QTLs for anthesis date reflecting the impact of anthesis date on leaf senescence during grain filling under mild drought in the UK. Thus, these QTLs may be candidates for wheat breeders for marker developments for

marker-assisted selection to optimize anthesis date and leaf senescence for improved drought tolerance in breeding programmes.

Further genetic analysis of the amphidiploid lines showing novel variation for key target traits should be carried out with the development of genetic marker maps for relevant biparental populations derived from crosses between amphidiploid lines and hexaploid bread wheat genotypes. This will allow identification of novel QTL derived from the amphidiploids together with the development of backcross lines for target QTLs and fine mapping of QTLs to precise locations on the chromosomes. These QTLs can then be developed as targets for marker-assisted selection for drought tolerance traits, and candidate genes established.

8.9 Conclusions

Drought can decrease average grain yield by 17-70% worldwide (Chen *et al.*, 2012). In the UK, the annual yield loss to drought is around 15-20% (Foulkes *et al.*, 2002), and expected to be increased with climate change prediction and more frequent summer droughts (Foulkes *et al.*, 2007a). The most effective progress to raise yields on drought-prone soils in the UK can be the identification of traits and molecular markers associated with drought resistance for application in plant breeding (Aravinda Kumar *et al.*, 2011). Therefore, the present study investigated the physiological and genetic bases of novel variation in water-use efficiency and drought tolerance in UK winter wheat and a range of wheat amphidiploid lines and accessions of wild wheat ancestors. The following conclusions can be drawn based on the results of this study:

1. There was genetic variation for grain yield and above-ground DM and DM partitioning under irrigation and drought and for responses to drought amongst Rialto x Savannah DH lines. The genetic variation in grain yield appeared to be mainly explained by the ability to maintain water uptake and biomass under drought rather than WUE and HI amongst UK winter wheat genotypes.
2. Genotypic variation was found for the grain C isotope discrimination ($\Delta^{13}C$) in the field, and the parent Savannah and DH line 12 had high grain $\Delta^{13}C$ (i.e. low WUE) associated with high grain yield. Although there were significant

associations between grain $\Delta^{13}C$ and grain yield in the subset of lines, over all 94 DH lines the association between grain $\Delta^{13}C$ and grain yield was weak.

3. There was genetic variation flag-leaf transpiration efficiency, photosynthetic rate and stomatal conductance amongst Rialto x Savannah DH lines under drought conditions; there were associations between grain yield and A_{max}, g_s and TE under drought. The parent Savannah and DH line 20 had low TE, high A_{max} and high g_s associated with high grain yield under drought conditions.
4. Post-anthesis NDVI, canopy temperature, flag-leaf senescence duration and flag-leaf chlorophyll fluorescence were positively associated with grain yield and above-ground DM amongst the Rialto x Savannah DH lines and the two parents under drought.
5. There was genetic variation between Rialto x Savannah DH lines, amphidiploid lines and wild ancestral wheat genotypes for grain yield above-ground DM and DM partitioning response to restricted water availability. Amphidiploid line Karim x *T. bessarabicum* had higher biomass than the best of R x S DH lines and the two parents, but not higher grain yield due to lower HI.
6. The *T. bessarabicum* accessions and amphidiploid line Karim x *T. bessarabicum* had higher expression of flag-leaf TE, A_{max}, g_s and chlorophyll content (SPAD) compared with the highest of the UK winter wheat Rialto x Savannah DH lines and the two parents.
7. The QTL on chr 3A identified for grain yield under drought was co-located with thousand grain weight, plant height, anthesis date, NDVI and canopy temperature, explaining variations in drought tolerance. These QTLs may represent pleiotropic effects of one QTL on 3A for drought tolerance in the winter wheat Rialto x Savannah DH population.
8. The QTLs on chromosomes 4A and 7D identified for anthesis date were associated with onset of flag-leaf senescence explaining the genetic correlation between flowering time and leaf senescence under irrigated and unirrigated conditions in the Rialto x Savannah DH population winter wheat. A QTL on chr 7D was previously reported at different location on chr 7D; therefore the presently reported QTL on chr 7D appeared to be a novel QTL for anthesis date and onset of flag-leaf senescence.

9. Post-anthesis A_{max}, g_s and TE in the glasshouse experiments were well correlated with these traits measured in the field experiments amongst four R x S DH lines and the two parents under drought conditions indicating that genetic variation for flag-leaf photosynthesis rate in wheat may be reliably phenotyped under glasshouse conditions.

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