THE PHYSIOLOGICAL AND GENETIC BASES OF WATER-USE EFFICIENCY IN WINTER WHEAT

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Dedication

I dedicate this thesis to my wife, Mamata, for all her love, support and enduring patience with me during this period of my life and for allowing me to accomplish the goals I had for my professional life. Also to my son, Amit, for continually reminding me of the joy of discovering something new.

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ABSTRACT

Winter wheat (*Triticum aestivum* L.) is the most extensive arable crop in the UK grown on about 2 M ha p.a. There is a need to identify traits to ameliorate yield losses to drought which are on average about 15% per year. These losses will be exacerbated with predicted climate change. The overall objectives of the present study were to investigate the physiological and genetic bases of water-use efficiency (ratio of above-ground dry matter production to evapotranspiration; WUE) in winter wheat grown in UK conditions and to quantify relationships between WUE and yield performance under drought.

The present study used a doubled haploid (DH) population of 33 lines derived from a cross between Beaver and Soissons, known from previous work to contrast for WUE. Two glasshouse experiments (2002/3 and 2003/4) and two field experiments (one at ADAS Gleadthorpe, Nottinghamshire in 2002/3 and the other at Sutton Bonington, University of Nottingham in 2004/5) were conducted. In the glasshouse experiments, two irrigation treatments (with and without irrigation) were applied to four genotypes (two parents and two DH lines), and in the field two irrigation treatments (rainfed and fully irrigated) were applied to the two parents and the 33 DH lines. A range of physiological traits was measured, including developmental stages, carbon isotope discrimination ($\Delta^{13}C$), leaf gas-exchange variables, green areas and biomass at sequential samplings, and these traits were related to grain yield. Transpiration efficiency (ratio of above-ground dry matter production to transpiration; TE) was assessed using the established inverse relationship between TE and $\Delta^{13}C$.

In the glasshouse, WUE measured as the regression slope of dry matter on water use, did not differ amongst genotypes in 2003, but did in 2004. Soissons showed higher WUE than other genotypes under irrigation, and also higher WUE than Beaver under drought. For measurements of TE according to $\Delta^{13}C$, Soissons and line 134G showed lower $\Delta^{13}C$ values (higher TE) than line 134E and Beaver ($P<0.05$) in 2004 under both irrigation and drought. Soissons and line 134G showed consistently higher TE on account of lower stomatal conductance (g_s) and sub-stomatal CO_2 concentration (C_i) values. The early developing Soissons and line 134G exhibited greater flag-leaf green area persistence under drought than the late developing Beaver. Beaver tended to use

more water than Soissons under both irrigation and drought, but reductions in water use under drought were similar amongst genotypes. Lower seasonal water use for Soissons than Beaver was associated with a smaller root system. There was a tendency for dry matter of Beaver to be more depressed under drought than Soissons in both the years. Overall, it was not possible to detect significant differences in biomass responses to drought amongst the genotypes, but there were consistent genetic differences in WUE and TE observed under both irrigated and droughted conditions.

In the field experiments, the onset of drought coincided broadly with anthesis. The average grain yield losses under drought were 0.5 t ha^{-1} in 2003 and 1.6 t ha^{-1} in 2005. Averaging across site/seasons, $\Delta^{13}C$ correlated positively with grain yield amongst the 35 genotypes under irrigation ($r=0.35$; $P<0.05$) and under drought ($r=0.54$; $P<0.01$), indicating a negative trade off between TE and yield. $\Delta^{13}C$ decreased under drought and a higher TE was associated with a reduction in average flag-leaf g_s measured from flag leaf emergence to anthesis + 4weeks. Stomatal conductance was measured for eight of the 33 DH lines including the parents, and there was a trend for lower $\Delta^{13}C$ (higher TE) to be associated with lower g_s. The genetic differences in g_s were generally associated with corresponding decreases in C_i and net photosynthetic rate (A). Therefore results suggested that the negative relationship between TE, as indicated by $\Delta^{13}C$ and yield was associated with corresponding reductions in seasonal water use. There was a non-significant irrigation x genotype interaction at Gleadthorpe in 2003 and Sutton Bonington in 2005 for $\Delta^{13}C$ indicating that this trait was of high heritability. There was an irrigation x genotype interaction for grain yield ($P<0.05$). A small number of genotypes showed higher yield associated with low $\Delta^{13}C$ and these outlier lines could potentially be identified for breaking the negative linkage between yield and delta. In summary, WUE was negatively correlated with yield under drought in this population; and season-long water use appeared to be the most important component affecting yield levels under drought. It is suggested that selecting genotypes indirectly for high $\Delta^{13}C$ (low WUE) may be a strategy to improve grain yield under drought.

In the quantitative genetic analysis, the putative QTLs identified for target physiological traits were generally different at Gleadthorpe in 2003 and Sutton Bonington in 2005. The most confident putative QTLs for $\Delta^{13}C$ were mapped on chromosomes 3B

(LOD=2.32) and 2D (LOD=1.43). The identification of QTLs as potential candidate genes on these chromosomes may be associated directly with WUE in the Beaver x Soissons DH mapping population. The $\Delta^{13}C$ QTL on chromosome 3B was detected commonly in both the irrigation environments and the direction of allelic effects was consistent with the parental differences in $\Delta^{13}C$. This QTL may therefore represent a novel gene for optimising WUE. It is suggested that breeders could optimise TE by selection according to a marker for this gene involving further fine-mapping to identify a marker tightly linked to the gene. Such a marker would also provide a target for gene discovery in future work.

The results suggest that water use is the most important component of Passioura's yield model for yield improvement under UK conditions. Nevertheless, WUE and harvest index and their responses under drought will also likely play a role in yield improvement through breeding in the UK targeted at drought-prone environments in future years.

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GENERAL ABBREVIATIONS AND ACRONYMS

%	percent
‰	parts per thousand
µ	micron
µmol	micromol
$^{13}C/^{12}C$	Carbon isotope ratio
1B/1R	Chromosome translocation of the short arm of rye-chromosome 1R into wheat chromosome 1B
A	Photosynthetic rate
ABA	Abscisic acid
ADAS	Agricultural Development and Advisory Services
AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
APSIM	Agricultural Production Systems sIMulator
Ar	Aerodynamic resistance
AW	Available water
C_3	Carbon-3
C_4	Carbon-4
C_a	Partial pressure of CO_2 in air
Ci	Intercellular CO_2 concentration
CIMMYT	Centro Internacional de Mejoramiento de Maiz y Trigo
cm	centimetre
cM	centi Morgan
CO_2	Carbon dioxide
CSIRO	Commonwealth Scientific and Industrial Research Organization
d	Days
D.F	Degrees of freedom
DAS	Days after sowing
DH	Doubled-haploid lines
DNA	Deoxyribo nucleic acid
E	Transpiration rate
g	gram
g m^{-2}	Gram per square metre
G x E	Genotype x environment interaction
GAI	Green area index
g_s	Stomatal conductance
h	hours
HGCA	Home Grown Cereals Authority

HI	Harvest index
ICARDA	International Center for Agricultural Research in the Dry Areas
ITE	Instantaneous transpiration efficiency
ITMI	International Triticeae Mapping Initiative
JIC	John Innes Center
K*	Kruskal-Wallis test static
kg	kilogram
l	litre
LAI	Leaf area index
LOD	Logarithm of Odds
m	metre
MAFF	Ministry of Agriculture, Fisheries and Food
mm	millimeter
mmol	millimol
NILs	Near-isogenic lines
^{o}C	degrees Celsius
P	Probability
Ppd	Photoperiod insensitive gene
PVC	Polyvinyl chloride
QTL	Quantitative trait loci
r	Correlation coefficient
R^2	Coefficient of determination
Rht	Semi-dwarfing genes
RILs	Recombinant inbred lines
RLD	Root length density
Rubisco	Ribulose-1,5-bisphosphate carboxylase-oxygenase
RWC	Relative water content
RWD	Root weight density
s	second
s.e	standard error
S.E.D	Standard error of difference
SLA	Specific leaf area
SLN	Specific leaf nitrogen
SPAD	Soil-Plant Analysis Development system
SRL	Specific root length
SSR	Simple sequence repeats
t ha^{-1}	tonnes per hectare
TE	Transpiration efficiency

TGW	Thousand grain weight
TRSA	Total root surface area
UK	United Kingdom
VPD	Vapour pressure deficit
WUE	Water-use efficiency
δ	isotope composition
Δ or $\Delta^{13}C$	Carbon isotope discrimination value

CHAPTER1: INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important cereal crop of the word. Globally, more land is used to produce wheat than any other commodity (FAO, 1996; Jason, 2004). Most countries attempt to produce enough wheat to meet internal demand, to be self-sufficient in the world's most basic foodstuff. The world demand for wheat is growing at approximately 2% per year (Rosegrant *et al.*, 1995), while genetic gains in yield potential of irrigated wheat stand at less than 1% (Sayre *et al.*, 1997). The total area under wheat in the world is 216 million ha producing 626 million tonnes annually (FAO, 2005) with an average productivity of 2.8 t ha^{-1}. Wheat is produced under diverse environmental conditions ranging from well-irrigated to water-stress situations, and wheat yields are reduced by 50-90% of their irrigated potential by drought on at least 60 million ha in the developing world (Skovmand *et al.*, 2001).

Globally, CIMMYT (International Center for Maize and Wheat Improvement) recognizes 12 distinct mega-environments where wheat is produced which range from the Mediterranean climate of west Asia to most other parts of the globe. The Mediterranean-type climates (southern Europe, west Asia, north Africa, South Africa, southern Australia, and in southwest North and South America) account for about 10-15% of world's wheat production where the period of crop growth is usually restricted by lack of rainfall, water deficits, and high temperatures at the start and end of the growing season (Loss and Siddique, 1994). Thus, water stress is a major limitation to wheat growth and yield in these environments.

Winter wheat is the most widely grown arable crop in the UK accounting for over 40 per cent of tilled land in eastern counties of England (Foulkes *et al.*, 2001). Of the 21 million tonnes of total cereals production in UK, wheat alone contributes 15 million tonnes (DEFRA, 2006) and the average productivity is 8 t ha^{-1}. Of the 2 million ha of UK wheat grown annually, about 700,000 ha are grown on drought-prone soils (Foulkes *et al.*, 2001). On these drought-prone soils yield loss can range between 0-4 t ha^{-1} in a given year.

In the UK such water deficits can commonly limit wheat yield in some years (Foulkes and Scott, 1998) and drought typically occurs late in the season, with onset of stress

broadly co-incident with flowering (Foulkes *et al.*, 2001). Typically onset of drought is post-anthesis and losses are in the region of 1-2 t ha^{-1} (Foulkes *et al.*, 2002).

Extreme meteorological events, such as spells of high temperature, heavy storms, or droughts, disrupt crop production. Frequent droughts not only reduce water supplies but also increase the amount of water needed for plant transpiration. It is obvious that any significant change in climate on a global scale will have a strong impact on local agriculture, and therefore affect the world's food supply. Several uncertainties are related to the degree of temperature increase and to the concomitant changes in the precipitation patterns that determine the water supply to crops, and to the evaporative demand imposed on crops by climate change. With predicted climate change (Marsh, 1996) the frequency of dry years, and therefore of droughts, will increase in eastern counties of England where most UK wheat is grown. Climate change is predicted to lead to drier summers. The South East of England accounts for more than half the country's wheat area. Wassenaar *et al.* (1999) demonstrated in a modelling study in France that a strong relation between soil water holding capacity and yield of wheat under climate change in areas subject to summer droughts. When the variability of the UK climate is taken into account in estimates of the range of water-limited yield under climate change some studies suggest no change under current CO_2 levels and increases in yields under higher CO_2 levels (Harrison *et al.*, 2000). However, such modelling studies may not fully account for yield potential gains occurring through biomass production. In addition there will still be problems with regard to water availability patterns in lighter soils.

Since seasonal drought can also be a problem in favourable environments, physiological traits to minimise effects of drought stress, e.g. improving water-use efficiency (ratio of aerial biomass to evapotranspiration; WUE), may also influence yield in high yielding, rainfed environments over a run of years. Similarly, improving water-use efficiency could also be important in irrigated environments if it results in less water being used to achieve higher yields. There is evidence that recent genetic gains in yield of wheat in the UK are driven by above-ground biomass production, so there may be an increase in demand for water if there is no change in WUE (Foulkes *et al.*, 2001).

With regard to applying irrigation water, in the UK growers give priority to crops such as potatoes and sugar beet rather than winter wheat, as these realise better financial

returns to irrigation (Bailey, 1990). Choice of drought–resistant cultivars is the best way to minimise adverse effects of uncertain droughts. So future research on physiological traits to increase the yield per unit crop evapotranspiration under drought by genetic means to underpin breeding is required. Early sowing may be a useful option for winter wheat as it results in larger and deeper root systems (Barraclough and Leigh, 1984), but problems may occur in some cases related to increased incidence of take-all disease and/ or risks of frost damage to shoot apices in the spring.

Most currently used commercial UK wheat varieties apparently have similar WUE, although there is some evidence that WUE may have decreased with breeding. For example, the old tall winter wheat cultivar Maris Huntsman had higher WUE than other five semi-dwarf modern wheats (Foulkes *et al.*, 2001). There is therefore a need to identify sources of variation in WUE for breeders to exploit and to identify the main determinants associated with WUE in UK environments. Improvements in WUE have been a long-standing goal of plant scientists. If genetic improvements could be made in the amount of dry matter produced for a given amount of water transpired, improvements in drought resistance or irrigation efficiency would be expected (Richard, 1994). Improving WUE is generally thought to be of the most value in environments subject to severe water limitation, but higher WUE could potentially have value over a range of environments including those subject to more moderate water stress. There have been relatively few experimental studies relating the physiology and genetics of WUE of wheat under contrasting soil moisture situations in the UK conditions or relating WUE to stomatal aperture traits measured at the canopy or leaf level.

A combination of physiology and genetics may improve basic understanding of the complex trait of WUE and predictions of genotype × environment interactions, offering new avenues for optimizing WUE through breeding for specific environments.

The majority of economic traits in crop plants are quantitative in nature, each controlled by many genes or gene complexes that are described as quantitative trait loci (QTL). Genetic mapping of these QTL has been greatly facilitated in recent years due to two important developments, the availability of molecular markers, and the development of a variety of powerful and improved statistical methods. Using genetic mapping to dissect the inheritance of different complex traits in the same segregating population can

be a powerful means to distinguish common heredity from casual associations between such traits (Paterson *et al*., 1988).

In several cereal species, genetic maps have been used to identify chromosomal regions controlling traits related to drought stress response. Different segregating populations from maize, sorghum, rice, wheat, barley have been studied for quantitative characters, such as phenology, root characters, canopy architecture and growth, abscisic acid accumulation, photosynthetic parameters, chlorophyll amount or "stay green", water status and osmotic adjustment parameters, water-use efficiency and carbon isotope discrimination (This *et al*., 1999). Carbon isotope discrimination ($\Delta^{13}C$) has been used to select commercial wheat varieties recently by CSIRO (Commonwealth Scientific and Industrial Research Organisation) in Australia. The two wheat cultivars 'Drysdale' and 'Rees' have been bred and released for commercial cultivation using scientific gene selection criteria based on $\Delta^{13}C$. There is a negative relationship between $\Delta^{13}C$ and WUE (Farquhar and Richards, 1984). In this context, the present study used $\Delta^{13}C$ for assessing the relationships between WUE and genotype performance.

The overall objective of the present study was to investigate the physiological and genetic bases of water-use efficiency in winter wheat grown under UK conditions with the aim of identifying physiological criteria and associated QTLs that can be used as selection criteria in breeding programmes. The aim was to quantify the relationship between WUE and grain yield under well-watered and droughted environments as well as improved understanding of WUE.

The study was based on glasshouse and field experiments under well-watered and droughted conditions. The main objective was to identify the physiological and genetic bases of variation in WUE in winter wheat in an existing doubled-haploid mapping population derived from a cross between Beaver and Soissons.

An extensive review on the key determinant physiological processes and the genetic bases of WUE in relation to wheat is presented in the following chapter.

CHAPTER 2: REVIEW OF LITERATURE

The main aim of this literature review is to abstract and synthesize the existing knowledge on water-use efficiency (WUE) particularly with regard to physiological mechanisms associated with genetic variation with potential application for breeding. In addition, this review will provide evidence for different hypotheses to be tested in the glasshouse and field experiments. This review has been organised into three main parts. The first part deals with understanding the physiological basis of WUE and associated CO_2 and water vapour gas exchange processes. The major focus is on the mechanisms underlying processes associated with either carbon uptake or water loss that bring about changes in WUE. The second part deals with the use of the well established technique of carbon isotope discrimination ($\Delta^{13}C$) for estimating WUE, describing various interrelationships between $\Delta^{13}C$ and WUE and grain yield. Major attention has been placed on various environmental factors that are responsible for variation in $\Delta^{13}C$ across wide range of environments. The third part deals with the genetic basis of variation in WUE and determinant traits. The aim is to review associated quantitative trait loci for drought tolerance traits in wheat and other cereals. At the end of this chapter, a summary of the various hypotheses to be tested in this study is presented.

Water availability is expected to be the most prominent constraint to increasing agriculture production in the future. In many parts of the world, rainfed agriculture is increasingly becoming unproductive due to prolonged drought. The frequency of dry years and summer droughts is likely to increase as a result of predicted climate change (Marsh, 1996). In one estimate, wheat yields are reduced by 50-90% of their irrigated potential by drought on at least 60 million ha in the developing world (Skovmand *et al.*, 2001). On the other hand, global demand for wheat is growing at approximately 2% per year (Rosegrant *et al.*, 1995), while the genetic gains in yield potential of irrigated wheat stand at slightly less than 1% (Sayre *et al.*, 1997). Major research in this context is focused on improving the drought-resistance traits in crop plants, wheat in particular. Among a number of traits, improving the WUE is perhaps receiving the major attention among the crop researchers. Breeding for specific physiological traits that are expected to impart a yield advantage in dry environments has been difficult and associated with slow progress in genetic gains in yield. Increases in wheat yields in rainfed

environments have been achieved during most of this century through the use of conventional empirical breeding approaches. However, the effectiveness of conventional breeding will depend on non-genetic factors associated with the conduct of experiments (Richards *et al.,* 2002). In this context, physiological approaches can complement empirical breeding. Improved understanding of the factors regulating wheat production and precise targeting of physiological traits to reduce the impact of drought would aid rates of genetic progress in wheat improvement. The application of physiological approaches to breeding has been extensively reviewed by Blum (1988) for abiotic stresses, by Evans (1993) for yield potential, by Loss and Siddique (1994) for wheat under drought and by Chaves *et al.* (2002) for photosynthesis and growth in relation to water stress in the field.

In the UK wheat is the most important arable crop grown on an area of about 2 million ha annually. About 30% of this crop acreage is under drought-prone soils (MAFF, 1999). In the UK water deficits can commonly limit wheat yield in some years on light soils (Foulkes and Scott, 1998) and onset of moisture stress typically coincides with flowering (Foulkes *et al.,* 2001). The drought under UK conditions occurs in a less severe form than that observed in Mediterranean or dry-land areas of the world. However, with predicted climate change the frequency of dry years or water availability for irrigation, and therefore of droughts, seems likely to increase. So quantification of the effects of drought in these more marginal conditions by establishing the value of traits for drought resistance is important (Foulkes *et al*., 2001).

For any trait to be included in crop improvement programmes as a selection criterion, it should have sufficient genetic variability, a low genotype x environment interaction effect and be heritable. Existence of genetic variability in water-use efficiency has been known for almost a century (Briggs and Shantz, 1914). The framework proposed by Passioura (1977; 1983) has proved very useful for identifying important traits underlying yield of cereal crops growing in water-limited environments. This framework is based on grain yield alone, and not on drought protection or on survival under drought which were popular concepts in the past but which have been largely unsuccessful as selection criteria (Richards *et al.,* 2002).

Passioura proposed that, when water is limiting, the grain yield is a function of: (i) the amount of water used by the crop, (ii) how efficiently the crop uses this water for biomass growth (i.e., the water-use efficiency as above-ground biomass/ evapotranspiration), and (iii) the harvest index, (i.e., the ratio of grain yield to above-ground biomass). This framework is shown below.

Grain yield	=	***Crop water use***	X	***Water-use efficiency***	X	***Harvest index***

Since each of these components is likely to be largely independent of the others, an improvement in any one of them should result in an increase in yield. The review structure will follow this framework.

2.1 CROP WATER USE

2.1.1 Root size and morphology

About 30% of UK wheat crops encounter drought. So improved rooting could enhance additional capture of water to increase yields of droughted crops. Roots support the above-ground portion of a plant and supply water and nutrients by exploring a large soil volume. The ability of a plant to grow its root system and its capability to extract water and nutrients in different soil environments have profound effects on the above-ground growth as well as on the water and nutrient balance in the soil. Wheat exhibits remarkable plasticity in root growth, which adjusts to soil nutrient and water status (Vlek *et al.*, 1996). Good root growth is a prerequisite for improved shoot growth and higher yields, especially in marginal environments (Manske and Vlek, 2002) and a deep root system is often synonymous with drought resistance and with more water uptake from the soil (Richards *et al.*, 2001).

The size of a wheat plant's complete root system depends on the environment. Root systems consist of 'seminal' roots that arise from primordia in the embryo and 'nodal' roots, also referred to as 'crown' or 'adventitious' roots that arise from the basal nodes of the main shoot and tillers. Winter cereals in the UK typically produce about 6 seminal axes and about 20 nodal axes (Gregory *et al.*, 1978). Root and shoot production are

synchronised, with nodal axis appearance linearly related to leaf production in wheat (Klepper *et al.*, 1984; Vincent and Gregory, 1989) and barley (Wahbi and Gregory, 1995). The first nodal axes typically appear when about 3 main shoot leaves have emerged, and axes continue to be produced until stem elongation carries the nodes above the soil surface (Klepper *et al.*, 1984). Extension of both the seminal and nodal roots usually continues to flowering (Belford *et al.*, 1987).

The horizontal spread of wheat roots is usually 30-60 cm (Russell, 1977); and roots grow to a depth of up to 200 cm (Gregory *et al.*, 1978; Barraclough and Weir, 1988; Ford *et al.*, 2002). However, about 70% of total root length is found within top 0-30 cm of soil. This is because of proliferation of lateral axes and because roots grow towards areas of higher nutrient and water concentrations, where rooting can take place. In wheat, root dry matter growth attains its maximum value at anthesis and net translocation of carbon to the roots stops after anthesis (Gregory, 1994). Three root characteristics are considered critical to moisture extraction: (i) the rate at which the root system descends in the soil, (ii) the maximum rooting depth, and (iii) the root length density (the root length per unit volume of soil; RLD) (Azam-Ali and Squire, 2002).

Mineral nutrients and water availability are considered together in the majority of root studies. Experiments conducted on a silty clay loam soil at Rothamsted revealed that drought reduced the amount of root growth in the top soil although there was some compensatory growth in the subsoil as long as there was available nitrogen (Barraclough and Leigh, 1984). Roots also acquire a range of other nutrients like phosphorous which are less mobile in the soil than N. Theoretical estimates by van Noordwijk (1983) for P transport in soil and root uptake suggest that ten times more roots are required to capture all the P in soil solution compared with water. So a reduction in the root length in the surface layers is likely to have some effect on the ability of the crop to take up P. Reductions in root length due to soil drying have been reported by Blum and Sullivan (1997). They examined the effect of plant size (plant height and shoot biomass) on plant performance under the effect of various agents of stress and top-root drying using *Rht* (tall), *Rht1* and *Rht2* (semi-dwarf) and *Rht3* (dwarf) isogenic lines of spring wheat cv. Bersee. Top-soil drying resulted in decreased shoot biomass but increased total root length. Stomatal closure was the primary response to top soil drying. The dwarf plants

had relatively higher grain yields under stress indicating their resistance to top-root drying while tall plants showed no variation. Other experiments showed that barley is able to tolerate extreme drying of the top-soil without affecting photosynthesis providing that at least 50% of the root system is well supplied with water (i.e. soil moisture contents close to field capacity) (Bingham and McCabe, 2003).

Most genetic comparisons of root traits have focused on differences between old long-strawed and modern short-strawed cultivars. MacKey (1973), examining near-isogenic lines in wheat, found a positive correlation between plant height and rooting depth. Some cultivar comparisons support this result (Gupta and Virmani, 1973; Brown *et al.*, 1987) whereas the majority of reports indicate no consistent difference between semi-dwarfs and talls (e.g., Welbank *et al.*, 1974; Cholick *et al.*, 1977; Bingham *et al.*, 2002). Other studies have even shown that semi-dwarfs have larger root systems (Siddique *et al.*, 1990; Haberle *et al.*, 1995). In summary, there appears to be no effect of the semi-dwarf genes on relationships between above- and below-ground parts of the crop that has been consistently observed across environments.

A wide range of literature on varietal differences in rooting depth of wheat in response to moisture stress (e.g. Hurd 1968; Gregory *et al.,* 1978; Hoad *et al.*, 2001) is available. The effects of genotypic variations on root characteristics of crop plants were reviewed by O'Toole and Bland (1987). Li *et al.* (2001) reported that under lower soil water content thin roots extend greatly, but in well-watered treatments roots were short and thicker. They showed irrigation promotes greater xylem vessel diameter development which would promote faster and greater soil water uptake. Richards *et al.* (2001) emphasized the importance of a deep root system in extracting more water from soil for greater drought resistance. Improvements in rooting attributes can be brought about by increasing the rooting depth and optimizing the root distribution with depth by an increase in the duration of the vegetative period (Brown *et al.*, 1987; Wahbi & Gregory, 1989; Siddique *et al.*, 1990; Miralles *et al.*, 1997). The developmental variation amongst genotypes has a large influence on genetic variation in rooting traits. A genetic comparison of six modern UK cultivars in the field detected a two-fold difference in RLD at 80 cm depth, from about 1-2 cm cm^{-3}, with Shamrock exhibiting greater RLD compared to the other five varieties (Ford *et al.*, 2002). During drought, soil water

becomes less than required for potential transpiration by the plant. Drying of the soil surface may inhibit normal development of the nodal root system (Gregory *et al.*, 1978; Gregory, 1994). Barraclough and Weir (1988) found root and shoot growth of winter wheat were reduced by the imposition of an artificial drought even though the water use was not affected until after anthesis. Wheat cultivars with highest rates of root growth tend to have the lowest rates of transpiration per unit root mass and this tends to decrease the rate of water uptake per unit root length (van den Boogaard *et al.*, 1996). In a study conducted at CIMMYT, most drought-tolerant semi-dwarf bread wheats formed more roots in deeper soil layers, whereas the non-tolerant checks had fewer roots in deep soil (Manske *et al.*, 2000).

Improved root biomass has been associated with the 1BL.1RS wheat-rye translocation in spring wheat in California in pot-grown experiments (Ehdaie *et al.*, 2003). However, the effect of 1BL.1RS wheat-rye translocation on root dry weight was neutral in this investigation. Genetic differences in root dry matter may result from the extent to which genotypes differ in the degree of adjustment to the onset of nutrient stress, i.e., the functional equilibrium between root and shoot (Brouwer, 1963; van Noordwijk and de Willegen, 1987). The extent of genetic diversity in this adjustment is largely unknown.

The development of synthetic hexaploid wheat, product of crossing between durum wheat and wild (D genome) diploids has enabled new genetic diversity to be introduced into the bread wheat gene pool (Villareal *et al.*, 1995; CIMMYT, 1996). Wide crossing can be used to introduce stress adaptive genes from diploid and tetraploid genomes, for example through the production of synthetic hexaploids, i.e. by crossing modern tetraploid durum wheats with *Aegilops tauschii*, the ancestral donor of the D genome in hexaploid wheat. Wide-crossing has contributed significantly to the drought adaptation of CIMMYT wheat germplasm (Mujeeb-Kazi *et al.*, 1998; Reynolds *et al.*, 2005). The synthetic derived wheat lines have improved ability to extract water at intermediate (30-90 cm) rooting depths (Copland *et al.*, 2002).

The ratio between the amount of root dry mass and shoot dry mass is a measure of the allocation of resources between different plant components (Hoad *et al.*, 2001). When WUE is expressed as the ratio of above-ground biomass to water used, then the genotype that partitioned less to the roots would not only have a higher WUE but also

earlier canopy closure, there by reducing evaporation. This reduction in root/shoot ratio may be very important in superficially increasing WUE in Mediterranean-type environments (Richards, 1987). However, studies have shown that the response of wheat plants to limitations of soil water is to allocate a greater amount of the carbon assimilated to roots (Hamblin *et al.,* 1990; Gregory and Atwell, 1991; Palta and Gregory, 1997). Such partitioning of assimilates between roots and shoots appears to be highly dependent on genotype (Sadhu and Bhaduri, 1984). Some experiments showed that reducing the carbohydrate supplies to the roots tend to decrease the rate of extension of seminal axes and first order lateral roots (Bingham and Stevenson, 1993; Bingham *et al.*, 1996). On the other hand, a wheat ideotype should react positively to drought by producing a larger root biomass under droughted compared to favourable conditions (Ehdaie *et al.*, 2003). Many studies have demonstrated that top-soil drying resulted in increased root/shoot ratio in wheat (for e.g. Blum and Sullivan, 1997). Modifying root dry matter distribution may increase plant reproductive allocations, which may be favourable to increasing grain yields (Weiner, 1990). Hoad *et al.* (2001) reviewed on the management of cereal root systems and pointed out that the genetic variation in rooting characteristics could be taken advantage of in breeding programmes where lodging and drought are problems.

The synthesis and accumulation of chemical signals such as abscisic acid (ABA) are a natural drought avoidance mechanism in most plants (Turner, 1986). ABA production by dehydrating roots is also known to cause variations in stomatal responses, whilst leaf water status is kept constant (Gowing *et al.*, 1990; Davis and Zhang, 1991). These investigations provide an understanding of root-to-shoot signalling through the hormone ABA under water stress conditions.

2.1.2 Relationship between root length density and resource capture

Greater root length density has been associated with higher water absorption from the soil and subsequent improved performance of cereal crops (Passioura, 1983; Hamblin and Tennant, 1987; Gregory *et al.*, 1992; Hoad *et al.*, 2001). In rainfed wheat, root length densities are much higher in drier years (Hamblin *et al.,* 1990) and wheat grown under residual moisture depends on deeper roots to access moisture from deeper soil layers (Jordan *et al.*, 1983; Mian *et al.*, 1993). Studies at Rothamsted, UK have

demonstrated that drought increased the depth of rooting from 140 cm to 160 cm although the RLD was less than 0.1 cm cm^{-3} compared to the RLD of 5-10 cm cm^{-3} in the top soil (Barraclough, 1984). In droughted conditions and with an adequate supply of nitrogen, RLD of 1 cm cm^{-3} was found sufficient to allow all the available water to be extracted; below 80 cm depth water uptake was limited by root growth as RLD was below 1 cm cm^{-3}. Barraclough (1989) found that irrigated wheat used no water from below 80 cm whereas droughted plants removed water from the entire soil profile where roots were found. Root diameter is also important because small diameters with high resistance may limit the rate of transport of water and solutes towards the shoot. Calculations by McCoy *et al.* (1984) and Passioura (1983) for theoretical situations in which RLD, initial water status and root diameter were varied suggested that RLD values <0.1 cm cm^{-3} would require between 12 and 20 days to deplete various soils of available water. In another study in barley, it was reported that a RLD of about 1 cm cm^{-3} is required for extraction of 90% of the available water, and about 2 cm cm^{-3} for complete extraction as field-based estimate (Gregory and Brown, 1989 cited in Bingham *et al.*, 2002). This estimate was found to be similar with that predicted from theoretical considerations of water uptake by single roots (Van Noordwijk, 1983). Theoretically, the RLD of cereals in surface layers of most soils are adequate to access most of the available soil water and densities more than 1.0 cm cm^{-3} are associated with only small increases in the total amount of water taken up during yield forming period (Gregory *et al.*, 1978; King *et al.* 2003).

Wang and Smith (2004) reviewed simulation modelling studies of the above-ground growth, crop yield, below-ground root system formation, and root function for optimally supplying water and nutrients to the plant. Modelling root growth and function has been simplified in most crop simulation models due to limited root data. There are studies in UK winter wheat on root-modelling for simulating the root system functionality in terms of nutrient and water resource uptake and interaction with the above-ground organs (for e.g. King *et al.*, 2003; Bingham *et al.*, 2002). In this context, King *et al.* (2003) working in UK on cereal root modelling emphasized that a more uniform distribution of the roots with depth gave a greater economic return through improved water and nutrient capture. However, there are relatively few examples of breeders selecting for improved drought resistance according to rooting traits.

Since root studies are very labour-intensive most breeding programmes have largely avoided using root traits as selection criteria. The methods used in root research are (i) descriptive (e.g. core break method, mesh bag method, electrical capacitance method, rhizotrons, wax layer method, root angle method or assessing root pulling strength) and (ii) quantitative (manual sampling in the field and measuring root parameters). However, for screening large numbers of wheat genotypes indirect and practically feasible methods are required for use in selection programmes (Manske *et al.*, 2001). Hence, there is a need to develop precise, high-throughput screens for roots in future studies.

2.2 WATER-USE EFFICIENCY

2.2.1 Definitions

The term water-use efficiency (WUE) is generally used to express the ratio of aerial biomass to evapotranspiration. It can be expressed as [Equation 2.1]:

WUE (biomass) = TE/ [1+ (E_s/T)]..... ...Equation 2.1

Where TE is the transpiration efficiency (above-ground dry weight/transpiration), E_s is the water lost by evaporation from the soil surface, and T is water lost through transpiration by the crop (Richards, 1991). An increase in transpiration efficiency and/or a reduction in soil evaporation will increase WUE. Increasing TE and decreasing soil evaporation can be achieved through both management and genetic improvement.

A number of selection criteria or screening tools have been developed for fast reliable screening of TE and its determinants in breeding programmes, e.g. the $^{13}C/^{12}C$ isotope discrimination technique (Farquhar and Richards, 1984) for estimating TE and the viscous-flow porometer for estimating stomatal conductance (Richards *et al.*, 2002).

In this thesis, the gravimetric calculation of above-ground biomass WUE (g dry matter litre water^{-1}) has been standardized as the ratio of cumulative above-ground dry matter to cumulative water use [Equation 2.2]

WUE (above-ground biomass) = (W_2-W_1)/ (C_2-C_1).....Equation 2.2

Where, W_1= total crop dry matter (g plant^{-1}) at sampling time 1

W_2= total crop dry matter (g plant^{-1}) at sampling time 2

C_1= cumulative water use (l) at sampling time 1

C_2= cumulative water use (l) at sampling time 2

With regard to gas-exchange,

TE (instantaneous) = ITE_{ins}= Net photosynthetic rate/ Transpiration .....Equation 2.3

TE (intrinsic) = ITE_{int}= Net photosynthetic rate/ Stomatal conductance.....Equation 2.4

2.2.2 Determinants of WUE in crop plants

It is well known that the process of photosynthesis involves two separate steps: diffusion or CO_2 input through the stomata, and carboxylation. The CO_2 gradient from atmosphere to sub-stomatal cavity is mainly a function of plant biological processes affecting C_i (partial pressure of CO_2 inside the sub-stomatal cavity), since C_a (partial pressure of CO_2 in the air surrounding the leaf) is relatively stable. In contrast, the water gradient is mainly a function of weather variables and leaf surface temperature. The principal processes involved in determining C_i are carboxylation as influenced by the content and activity of Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase) enzyme; and the stomatal conductance (g_s), as regulated by stomata. There are therefore several biochemical and physiological processes influencing C_i, hence the CO_2 gradient from atmosphere to sub-stomatal cavity, by wheat canopies.

2.2.2.1 Stomatal conductance and Rubisco content

The rate of CO_2 uptake per unit leaf area relates directly to stomatal conductance, which, in turn, is controlled by stomatal regulatory processes. As stomata close, C_i decreases and TE at the level of a single leaf should increase. The canopy conductance should broadly follow a similar pattern. In addition, C_i is influenced by the carboxylation capacity of the plant, higher Rubisco concentration decreasing C_i hence increasing TE. In cereals, there are several reports of specific leaf nitrogen content (ratio of nitrogen (N) content to leaf area; SLN) correlating positively with TE, e.g. Borrell *et al.* (2000) in sorghum, presumably through effects on Rubisco content. Genetic variation in Rubisco content has been shown to correlate positively with CO_2 exchange rate in rice (Sarker *et al.*, 2000; Sivasankar *et al.*, 1998; Xu *et al.*, 1997) and wheat (Julian *et al.*, 1998), with light-saturated rate of photosynthesis in rice (Makino *et al.*, 2000) and with grain yield

in maize (Martinez-Barajas *et al.*, 1999). Varying the Rubisco content is suggested as a strategy to ensure adequate N reserves for grain filling in rice (Horton and Murchie, 2000). However, there are relatively few reports of genetic variation in Rubisco content or activity in hexaploid wheat; one recent investigation was reported by Xiao *et al.* (1998).

2.2.2.1.a Rubisco activity in relation to drought stress

There is evidence that the decrease in CO_2 assimilation rates found in drought-stressed leaves cannot be simply reversed by increasing the external CO_2 supply, showing that drought stress must also affect mesophyll metabolism (Lawlor, 1995; 2002 and Tang *et al.,* 2002). This mesophyll response becomes progressively more important with increasing water deficiency (Tezara and Lawlor, 1995; Udayakumar *et al.*, 1998). Several studies have suggested that decreased photosynthetic capacity results from impaired regeneration of ribulose-1, 5-bisphosphate (RuBP) (e.g. Gimenez *et al.*, 1992). Increased severity and duration of drought stress is known to decrease Rubisco activity and protein content in wheat (Kicheva *et al.,* 1994) and in some other species (Majumdar *et al.*, 1991). Rubisco activase expression is increased under heat stress in wheat (Law and Crafts-Brandner, 2001). The total Rubisco activity of wheat flag leaves was decreased when drought stress was imposed at anthesis; this decrease was accompanied by a decrease in both soluble protein and chlorophyll (Holaday *et al.,* 1992; Parry *et al.,* 2002). However, in wheat Rubisco activities were not correlated with leaf relative water content (RWC) (Parry *et al.*, 2002) suggesting a decrease in its contribution to the total amount of soluble protein. Udayakumar *et al.* (1998) demonstrated an inverse relationship between Rubisco content and $\Delta^{13}C$ among groundnut genotypes, suggesting that the variability in $\Delta^{13}C$ is dependent on mesophyll capacity rather than g_s; and that therefore mesophyll efficiency is important in determining $\Delta^{13}C$ and WUE.

2.2.2.1.b Stomatal conductance

Stomatal aperture density and leaf area, which are the main biological determinants of plant transpiration, are also the main determinants of carbon accumulation by plants because CO_2 flows to photosynthetic sites via the stomata. This leads to well

documented relationships between light interception, transpiration, photosynthesis, and biomass production as 'water for carbon' and 'water for temperature' trade-offs (Monteith 1993; Tardieu, 2005).

Stomatal conductance relates to how easily CO_2 can be moved into the leaf and is controlled by stomatal regulatory processes. Conductance to CO_2 is directly convertible to conductance to H_2O i.e. g_c, carbon = (g_s, water/ 1.6), where 1.6 is the ratio of diffusivities of H_2O and CO_2. H_2O is lighter and diffuses faster than CO_2. In general, TE of crops will be increased by low stomatal conductance. Measurement of g_s can give an insight into the transpiration of a crop canopy, which is closely related to biomass production. The link between g_s and transpiration is so close that transpiration could reliably be estimated from measurements of g_s (Azam-Ali, 1983). Besides this, evaporative demand on the leaf is a major environmental determinant of transpiration rate, which is most precisely measured as the vapour pressure gradient between the leaf and the air (Monteith, 1995).

Assuming the water vapour concentration gradient (W_i-W_a) is an independent environmental variable then TE at a given site is a negative function of the ratio C_i/C_a. For non-stressed C_3 plants, the value of C_i/C_a is around 0.7 and is determined by the balance between g_s and photosynthetic rate (A) (Condon *et al.*, 2002). If C_i/C_a ratio is lower as a result of increased photosynthetic capacity then there will be an increase in A per unit leaf area. However, if C_i/C_a is lower as result of lower g_s then there will be a reduction in A.

2.2.2.2 Genetic variation in WUE and determinant plant and canopy processes

Genotypic variation in WUE determined by both photosynthetic capacity and stomatal conductance has been reported in wheat (Condon *et al.,* 1990; Blum, 1990; Morgan and Le Cain 1991; Condon and Richards, 1993; Morgan *et al.,* 1993; Fischer *et al.,* 1998). Some of these studies are reviewed here.

Morgan and Le Cain (1991) in Colorado, USA investigated the genetic variation in 15 field-grown winter wheat genotypes under irrigated conditions and found greater WUE in genotypes with leaves of lower g_s. They found no significant date x cultivar interactions for A, g_s, C_i and C_i/C_a, indicating that similar week-to-week variations in

gas exchange were experienced by most genotypes. Blum (1990) in Israel found that high grain yield in spring wheat under drought was positively associated with the CO_2 assimilation rate at light saturation (A_{max}) and g_s; newer cultivars had significantly higher A_{max} and g_s than the older cultivars. In winter wheat, Gent and Kiyomoto (1985) did not observe a positive relation between grain yield and canopy and leaf CO_2 assimilation rates; and higher grain yield was related to a higher HI. Ashraf and Bashir (2003) in Pakistan demonstrated in an irrigated pot study that higher photosynthetic capacity, measured as CO_2 exchange rate, at the vegetative stage in spring wheat cv. Inqlab-91 resulted in higher grain yield. Whereas, a higher post-anthesis assimilation rate in cv. Barani-83 resulted in higher grain yield and WUE. Stomatal conductance was the primary factor affecting photosynthesis in Inqlab-91 at the vegetative stage but not in Barani-83. Thus, the cultivars appeared to have used different temporal patterns of photosynthesis for enhanced grain production.

Ritchie *et al.* (1990) in Texas observed in winter wheat that the most drought-resistant genotypes (e.g. cv. TAM W-101) had a higher photosynthetic capacity and greater g_s in water-stress conditions than the more susceptible genotypes (e.g. cv. Sturdy). TAM W-101 tended to have higher WUE than Sturdy under moderate to severe stress conditions, but not under well-watered conditions. High leaf RWC, A, and photosynthetic capacity traits conferred better drought resistance in TAM W-101. These two genotypes have been the subject of several investigations into the physiological nature of their respective responses to water-deficit stress (Schonfeld *et al.*, 1988; Ritchie *et al.*, 1990; Morgan *et al.*, 1993). Wheat research at CSIRO, Canberra, Australia showed that a decrease in stomatal conductance improved TE among wheat genotypes in greenhouse and field studies (e.g. Condon *et al.*, 1990; Condon and Richards, 1992) and the genetic variation in stomatal conductance unassociated with ABA responses has also been reported in spring wheat (Condon *et al.*, 1990). On the other hand various experiments have shown that stomatal responses are often more closely linked to soil moisture content than to leaf water status. This suggests that stomata are responding to chemical signals such as ABA produced by dehydrating roots even when the leaf water status is kept constant (Gowing *et al.*, 1990; Davis and Zhang, 1991). The regulation of gas exchange may not be a simple response to a dose of ABA coming from the root, but that the cells of the leaf will process information received by both the roots and the shoot. A long-distance

signal from the root and a locally generated signal within the leaf may both operate within the leaf at the same time (Davis *et al.*, 2002).

In cereals, for example rice, there are reports demonstrating genetic variation in photosynthetic capacity (Murchie *et al.*, 1999; Horton and Murchie, 2000) and are related to mesophyll conductance (Price *et al.*, 2002) as a key limitation to productivity under irrigation. Although many studies have correlated leaf gas–exchange results with WUE, often the relationship between short-term gas-exchange variables and the actual water-use efficiency for the whole growing season is poor, because there are a number of factors affecting dry matter accumulation but not leaf gas-exchange (Boyer, 1996). For example, the biomass production of a plant is not only determined by net photosynthesis during the day but also by respiratory losses at night. Gas exchange determination for short times during the day does not inform on dark respiration losses, which are influenced by temperature and the molecular composition of the dry matter. Therefore, although rapid and convenient, gas exchange measurements may not be totally reliable to assess differences in WUE at the whole crop level.

2.2.2.3 Canopy architecture and aerodynamic resistance

Independently of leaf and canopy conductance responses working through stomatal conductance, the canopy affects the rate of evapotranspiration according to its aerodynamic resistance to water vapour and heat transfer to the atmosphere (*Ar*) (Monteith, 1981). Crop height and surface roughness and the wind speed influence this. In general, taller crops with rougher surfaces have reduced *Ar* hence lower WUE. Under drought, where the green canopy area is usually small, effects of *Ar* on evapotranspiration are thought to be minor compared to those of canopy conductance (Squire, 1990). For wheat cultivars, differences in surface roughness are thought to have relatively little impact on WUE (Sylvester-Bradley *et al.*, 1990). The presence of *Rht* semi-dwarf genes may however have an effect on canopy conductance via *Ar*. In this regard, variation in crop height has been shown to influence WUE in studies on isogenic lines, e.g. shorter stature with the *Rht-D1* semi-dwarf gene conferring decreases in the region of 10% (Richards, 1992; Ehdaie and Waines, 1994; Foulkes *et al.*, 2001). However, this variation would not be associated with differences in *Ar*, since shorter stature would increase *Ar* hence WUE.

2.2.3 The physiological basis of the relationship between carbon isotope discrimination and TE

Atmospheric CO_2 contains the naturally occurring carbon isotopes ^{12}C, ^{13}C, and ^{14}C in the proportions 98.9%, 1.1%, and 10^{-10}%, respectively. $^{14}CO_2$ is present in such small quantities that it has no physiological relevance, but $^{13}CO_2$ is different. The chemical properties of $^{13}CO_2$ are identical to those of $^{12}CO_2$, but because of the slight difference in mass (2.3%) most plants assimilate less $^{13}CO_2$ than $^{12}CO_2$. Since $^{12}CO_2$ is lighter than $^{13}CO_2$, it diffuses slightly faster toward the carboxylation site within the chloroplast. For reasons not yet understood, Rubisco has an intrinsic discrimination value of –30‰ against $^{13}CO_2$ (Farquhar *et al.,* 1989). By contrast, Phospho-enol pyruvate (PEP) carboxylase, the primary CO_2 fixation enzyme of C_4 plants, has a much smaller isotope discrimination effect (about –2 to 6‰). Thus, the inherent difference between the discrimination effects of the two carboxylating enzymes causes the different isotope compositions observed in C_3 and C_4 plants (Farquhar *et al.,* 1989).

Carbon isotope composition is measured by use of a mass spectrometer, which yields the following ratio [Equation. 2.5]

$[R= {}^{13}CO_2 / {}^{12}CO_2]$.....Equation 2.5

$^{13}C/ {}^{12}C$ ratio values are expressed as carbon isotope composition ($\delta^{13}C$) values [Equation.2.6]. This is quantified on a "parts per mil" (= x 10^{-3} or ‰) basis; ‰ is not a unit and δ is dimensionless (Farquhar and Lloyd, 1993)

$\delta^{13}C\ (‰) = \{(^{13}C/ {}^{12}C_{\ sample})/ (^{13}C/ {}^{12}C_{\ standard}) - 1\} \times 1000$.....Equation 2.6

The standard represents the carbon isotopes contained in the Pee Dee limestone formation from a fossil belemnite. Carbon isotope discrimination is calculated with respect to the carbon isotopic ratio of air [Equation 2.7]

$\Delta^{13}C\ (‰) = \{(^{13}C/ {}^{12}C_{\ air}) / (^{13}C/ {}^{12}C_{\ plant}) - 1\} \times 1000$.....Equation 2.7

The value of the discrimination ($\Delta^{13}C$) against ^{13}C is calculated from δ_a and δ_p [Equation 2.8], where 'a' refers to air and 'p' to plant (Farquhar *et al*., 1989):

$$\Delta^{13}C = (\delta_a - \delta_p / (1+\delta_p) \quad \text{Equation 2.8}$$

On the PDB scale, free atmospheric CO_2 has a current deviation, $\delta^{13}C_a$, of approximately -8.0‰ (Farquhar *et al.*, 1989).

C_3 plants have typical values for isotope composition ($\delta^{13}C$) of about –28‰; C_4 plants have an average value of –14‰ (O'Leary, 1988). The negative sign indicates that both C_3 and C_4 plants have less ^{13}C than the isotope standard. Since the 'per mil' calculation involves multiplying by 1000, the actual isotope discrimination is small. Nonetheless, $\Delta^{13}C$ can be used as a reliable indirect measurement of TE. Most importantly, it has the capacity to integrate effects across time and across leaf layers within the canopy.

2.2.3.1 Carbon isotope composition in relation to the physiological behaviour of plants

In C_3 plants, $\Delta^{13}C$ is proportional to the partial pressure of CO_2 in the intercellular air space of leaves (C_i). More discrimination occurs when C_i is high, when stomata are open. Open stomata also facilitate water loss. Thus, TE is negatively associated with discrimination against ^{13}C (Farquhar *et al.*, 1989). In some circumstances the relationship can vary and the causes of this variation will be discussed later. Despite its apparent utility, the measurement of $\Delta^{13}C$ has several short comings such as it provides no information on the magnitudes of either A or T or whether variation in $\Delta^{13}C$ is being driven by variation in g_s or A. Furthermore, $\Delta^{13}C$ reveals no information on the impact of the vapour pressure gradient on the TE at the leaf level measured by gas-exchange. Hence, $\Delta^{13}C$ has limited application for comparing absolute values between experiments in different environments. Nevertheless, within an experiment it serves to identify relative differences between genotypes making it a potentially useful as a breeding tool (Hall *et al.*, 1994). Most importantly, $\Delta^{13}C$ is a lot easier and faster to measure than TE itself. Several reports confirm that $\Delta^{13}C$ of plant material is closely related to TE integrated over the duration of the growth period of the plant material sampled (Farquhar and Richards, 1984; Condon *et al.*, 1992).

2.2.4 Relationship between TE and WUE and $\Delta^{13}C$

Transpiration efficiency is the preferred measure for examining potential genetic variation in crop WUE. If the component of soil evaporation is minimised then an increase in TE will also increase WUE (Condon *et al.*, 2002). Information on the use of $\Delta^{13}C$ and stomatal aperture traits as indicators of TE under drought in the UK as whole is not available and there are no previous studies of application of these techniques in winter wheat in the UK climate.

TE and $\Delta^{13}C$ are linearly negatively related and $\Delta^{13}C$ values may be used to assess WUE among wheat cultivars (Farquhar and Richards, 1984; Condon *et al.*, 1987). In C_3 plants, $\Delta^{13}C$ is largely determined by of C_i/C_a which is related to the balance between g_s and carboxylation capacity. Higher $\Delta^{13}C$ may be related to a higher ratio of C_i/C_a due to greater g_s (Condon *et al.*, 1987; Morgan *et al.*, 1993), which leads to higher photosynthetic rates and, hence, higher yields even in the absence of stress. Alternatively, higher carboxylation capacity determined by non-stomatal factors, mainly the amount and activity of enzymes in the chloroplast (Condon *et al.*, 1987), may lead to a higher ratio of C_i/C_a hence higher $\Delta^{13}C$. The ratio of A to C_i is often considered for estimating carboxylation capacity (Rebetzke *et al.*, 2002).

2.2.5 Genetic correlations between $\Delta^{13}C$ and grain yield

In wheat, positive correlations between yield and $\Delta^{13}C$ have been reported (Condon *et al.*, 1987; Araus *et al.*, 1993, 1997; Morgan *et al.*, 1993; Fischer *et al.*, 1998; Rebetzke *et al.*, 2002). Selecting for high $\Delta^{13}C$ at an early stage of the breeding programme under low or high stress environments (Sayre *et al.*, 1995) or under well watered conditions (Morgan *et al.*, 1993; Condon *et al.*, 1990) has been reported. Fischer *et al.* (1998) showed a positive correlation between $\Delta^{13}C$ and yield among eight CIMMYT spring wheat varieties in Mexico under irrigated conditions and $\Delta^{13}C$ was in the range 18.4-19.4‰. A higher $\Delta^{13}C$ was associated with higher C_i, A and g_s. While, Araus *et al.* (1997) reported that $\Delta^{13}C$ ranged from 14‰ under rainfed conditions to 16.7‰ under irrigated conditions for a set of 144 wheat genotypes and that the genotypic variation in $\Delta^{13}C$ was due to variation in both g_s and A, with each contributing approximately in

equal amounts. A similar observation was also made in other studies (Condon *et al.*, 1990; Condon and Richards, 1993). In another study, selecting for a low $\Delta^{13}C$ has been associated with high TE for genetic improvement in grain yield under low rainfall environments in Australia (Rebetzke *et al.*, 2002). Alternatively, several pot studies have indicated a negative correlation between $\Delta^{13}C$ and shoot dry matter (Condon *et al.*, 1990; Ehdaie *et al.*, 1991; Ehdaie and Waines, 1994; Ehdaie 1995). Similarly, a negative correlation between $\Delta^{13}C$ and TE has been observed (Farquhar *et al.*, 1982; Farquhar and Richards, 1984; Ehdaie *et al.*, 1991; Johnson *et al.*, 1995; Matus *et al.*, 1996; Ehdaie and Waines 1996; Monneveux *et al.*, 2006) and these negative correlations were often inconsistent across water regimes and years.

The application of $\Delta^{13}C$ as a potential tool for wheat improvement has been practically possible. In this direction, concerted efforts have been made to complement traditional breeding methods with physiological selection criteria in Australian wheat improvement programmes by CSIRO. For example, backcrossing of an old Australian wheat cv. 'Quarrion' (low $\Delta^{13}C$ and high TE), into the cv. Hartog (Richards, 2004). Hartog (intermediate to high $\Delta^{13}C$ and low TE) has robust adaptation, good disease resistance and excellent grain quality. Two backcrosses were made to Hartog following selection for $\Delta^{13}C$ in the field (Richards, 2004). A yield advantage in wheat between 2 and 15% of the back-cross lines with low $\Delta^{13}C$ at yield levels from 5 t ha^{-1} to 1 t ha^{-1} was reported (Rebetzke *et al.*, 2002) when compared with high $\Delta^{13}C$ sister lines. Based on selection for $\Delta^{13}C$, the variety 'Drysdale' was released for southern New South Wales, Australia, in 2002 and 'Rees' for the northern Australian cropping region in 2003. This is the first example of the application of $\Delta^{13}C$ in selection for wheat breeding improvement.

Genetic variation for WUE and $\Delta^{13}C$ among semi-dwarf and dwarf modern wheat cultivars has been reported (Siddique *et al.*, 1990; Ehdaie *et al.*, 1991; Ehdaie, 1995), but the associated genetic gain in yield in these studies was relatively small.

2.2.5.1 Genetic correlations of $\Delta^{13}C$ with TE and yield under well-watered conditions

The association of $\Delta^{13}C$ with TE has been reported to be either positive or negative depending upon the soil moisture conditions. Most experiments show grain yield of irrigated cereals was positively associated with $\Delta^{13}C$ of the peduncle (Acevedo, 1993),

grain (Teulat *et al.*, 2001) or flag leaf (Monneveux *et al.* 2005; Merah *et al.*, 2002). Monneveux *et al.* (2006) in France reported a negative correlation between grain $\Delta^{13}C$ and TE (measured as CER/g_s) under rainfed conditions on account of low g_s, and a positive correlation under irrigated conditions on account of higher leaf CO_2 exchange rate (CER). The range of $\Delta^{13}C$ was 14.6-16.1‰ under rainfed conditions and 16.1-17.6‰ under irrigation. The leaf CO_2 exchange rate was positively correlated to g_s under irrigated conditions indicated that CER was driven by stomatal limitations. They concluded that breeding for high grain $\Delta^{13}C$ would lead, under Mediterranean drought conditions, to higher g_s, lower TE and higher grain yield. Blum (1996) pointed out that if selection for high photosynthetic capacity or higher crop productivity brings about an increase in stomatal conductance, then a concomitant increase in $\Delta^{13}C$ or a decrease in crop WUE is expected.

In another study, Royo *et al.* (2002) in Spain evaluated 25 durum wheat cultivars for $\Delta^{13}C$ in two contrasting water regimes and found a positive genetic correlation between $\Delta^{13}C$ and grain yield. $\Delta^{13}C$ was higher for the irrigated regime (17.4‰) than the rainfed regime (15.4‰). They concluded that selecting genotypes for yield based on high $\Delta^{13}C$ would be advantageous under well-watered conditions.

Condon *et al.* (1987) reported in a field study in Australia that under well-watered conditions $\Delta^{13}C$ measured in the plant stem and grain was positively correlated, but $\Delta^{13}C$ and TE were not negatively related; biological yield was strongly influenced by g_s. However, Condon *et al.* (1990) demonstrated in a glasshouse study a negative relationship between flag leaf $\Delta^{13}C$ and WUE under well-watered conditions and suggested the use of $\Delta^{13}C$ for yield selection under well-watered conditions. In another study, Ehdaie *et al.* (1991) in California reported a strong negative correlation between $\Delta^{13}C$ and WUE in spring wheat cultivars in both glasshouse and field experiments. A negative correlation was also observed between $\Delta^{13}C$ and total dry matter (TDM); and TDM was more strongly influenced by photosynthetic rate rather than the g_s. However, such negative correlations are not always observed. For example, Morgan *et al.* (1993) found a positive relationship between $\Delta^{13}C$ and WUE in irrigated field-grown winter wheat plants. Morgan *et al.* (1993) found a positive relationship between $\Delta^{13}C$ and WUE in the irrigated field grown winter wheat. Intrinsically high g_s of high-$\Delta^{13}C$ lines

can apparently result in moderate leaf desiccation by mid-day, which in turn can lower g_s relative to that of low-$\Delta^{13}C$ lines. These leaf gas-exchange data did not explain the positive relationship between $\Delta^{13}C$ and productivity arising out of differences in g_s or CER in this study. While Matus *et al.* (1996) found no significant correlation between $\Delta^{13}C$ and TE; and an inconsistent correlation between $\Delta^{13}C$ and above-ground dry matter at flowering of wheat, lentil and canola.

Ehdaie *et al.* (1991) observing average WUE of landraces and modern wheat genotypes in pot and field experiments concluded that selection for high $\Delta^{13}C$, under either wet or dry conditions, can improve WUE in wheat. In another pot study, however, on seven bread wheat cultivars grown under well-watered conditions, $\Delta^{13}C$ was negatively correlated with WUE, but positively with HI (Ehdaie and Waines, 1993). Matus *et al.* (1996) reported the existence of genetic variability for $\Delta^{13}C$ in spring bread wheat and durum wheat. The range of $\Delta^{13}C$ was large for wheat under well-watered conditions (21.9-22.9‰) as compared to drought (20.3-21.2‰), but they found no correlation between $\Delta^{13}C$ and TE. They concluded $\Delta^{13}C$ cannot be used for selection for TE. Thus, it is evident from these studies that causal relation between $\Delta^{13}C$ and WUE may change depending on the availability of soil water and genotypic performance. In general, under well-watered conditions most studies have reported positive genetic correlations between $\Delta^{13}C$ and TE, although there are several reports of neutral or negative relationships.

2.2.5.2 Genetic correlations of $\Delta^{13}C$ with TE and yield under drought

Under drought-stressed conditions, the value of $\Delta^{13}C$ tends to be lower (Condon *et al.*, 1990; Morgan *et al.*, 1993; Ehdaie, 1995). This is possibly because of a reduction in stomatal conductance improving TE (Farquhar and Richards, 1984). In a field experiment conducted at Montepellier, France, Merah *et al.* (2001) demonstrated a positive correlation between $\Delta^{13}C$ and yield of 144 durum wheat accessions (comprising landraces, improved cultivars and advanced breeding lines). The drought in this study was terminal occurring as early, mild and intermittent water stress. They reported that a higher $\Delta^{13}C$ was related to a higher C_i/C_a ratio due to a greater g_s leading to higher photosynthetic rates and higher yield; and high $\Delta^{13}C$ can be used for selecting for yield under drought.

Craufurd *et al.* (1991) demonstrated that early maturing barley cultivars escaped drought showing high $\Delta^{13}C$ and yield. Alternatively, Sayre *et al.* (1995) reported in a CIMMYT/ ICARDA based trial that under low and high-stressed conditions, $\Delta^{13}C$ may be potentially used to augment selection for high yield based rather than replace selection for yield itself. This would increase the rate of genetic gain in yield of spring wheat in water-limited environments. Thus, a high $\Delta^{13}C$ can be used for breeding programmes during early growth stages under water-stress.

In environments where crop growth is heavily dependent on moisture stored in the soil profile from rain that falls outside the main crop growth phase, selection for low $\Delta^{13}C$ is likely to be effective for yield improvement. In these environments, where transpiration makes up a high proportion of total crop water use, high TE promotes conservation of soil water, which sustains yield determining processes in the period leading up to and after anthesis. In stored-moisture environments, profligate high $\Delta^{13}C$ genotypes are more likely to exhaust the soil water supply before this critical phase (Richards *et al.,* 2001). The value of improved TE via reduced stomatal conductance may depend on available water (AW), e.g. higher TE is beneficial with low AW where all soil water is still used with higher TE. However, there may be a trade-off between higher TE and lower seasonal water use in environments where there is more AW in the soil profile (Araus *et al.*, 2002).

From a physiological point of view, several reasons could potentially explain a positive relationship between $\Delta^{13}C$ and yield. If low Δ is mediated by low g_s, plants with low $\Delta^{13}C$ may not use all available soil water, particularly in the range of moderate drought to well irrigated environments. Such an explanation is consistent with the variation in g_s rather than in intrinsic photosynthetic capacity amongst genotypes (Romagosa and Araus, 1991; Condon *et al.,* 1992).The inconsistency in relationships of $\Delta^{13}C$ and shoot dry matter or grain yield could be due to several genotypic and environmental factors that might have greater influence on plant growth under field than glasshouse environments (Ehdaie and Waines, 1996).

Thus, under relatively severe drought when crop growth is entirely dependent on stored soil moisture, a reduction in g_s would lead to higher WUE and so increase the yield.

Araus *et al.* (2001) summarized the research work done under Mediterranean environments showing that high $\Delta^{13}C$ genotypes, which sustain greater g_s and transpiration losses during grain filling, can provide higher yield in a wide range of environments with intermediate to low levels of drought stress. Thus the utility of $\Delta^{13}C$ in wheat breeding is likely to vary depending on the extent to which yield is limited by water supply. Richards *et al.* (2001) argue that in wheat, $\Delta^{13}C$ is a 'conservative' trait associated with somewhat slower water use and possibly slower growth rate. Consequently in environments where water is largely non-limiting, low $\Delta^{13}C$ (high TE) has been associated with relatively low yield potential in wheat (Condon *et al.,* 1987). The implication is that selection for high $\Delta^{13}C$ in these environments may prove useful in identifying lines with high yield potential. Selection for high $\Delta^{13}C$ may also be effective for yield improvement in rainfed environments where crop growth to anthesis is sustained by current rainfall or where drought is relieved well before anthesis. In these environments high $\Delta^{13}C$ should be associated with more dry matter at maturity (Richards *et al.,* 2001). Blum (2005) reviewed extensively that genotypic variations in WUE were driven mainly by variations in water use rather than by variations in plant dry matter production. It is therefore not surprising that selection for high WUE by using carbon isotope discrimination has tended to result in smaller or earlier flowering plants that use less water over the growing season (e.g. Condon *et al.* 2002). Thus selecting for low $\Delta^{13}C$ is beneficial under severe drought conditions. This has been demonstrated in barley (e.g. Craufurd *et al.*, 1991), in wheat (e.g. Rebetzke *et al.*, 2002).

2.2.5.3 Relationship between $\Delta^{13}C$, TE and yield under the influence of the aerial environment

The variations in canopy morphology, leaf size and orientation, and in stomatal conductance (g_s), may affect leaf energy balance and therefore the leaf-to-air vapour pressure gradient. This in turn may alter the actual flux of water vapour corresponding to a given value of $\Delta^{13}C$. Thus, even in genotypes of the same species growing in the same environment, the relative ranking with respect to $\Delta^{13}C$ may not reflect the actual ranking in terms of TE (Meinzer *et al.*, 1992). Increases in leaf temperature either because of radiation load or indirectly because of reduced water uptake may affect relationships between Δ and TE. A high heat stress might reduce WUE through deleterious effects on carboxylation/respiration and increase water use via the effects of

increased vapour-pressure deficit (VPD) associated with a higher temperature and leaf-to-air temperature difference. Thus the relationships between $\Delta^{13}C$ and TE or yield are often modified by the prevailing radiation and temperature (Bowling *et al.*, 2002).

Kemanian *et al.* (2005) reviewed that carbon flux varies in response to physiological and environmental factors. Among these factors, it is well documented that C_i/C_a decreases as water stress increases in field-grown potatoes (Vos and Groenwold, 1989), wheat (e.g. Whitfield, 1990), and sorghum (Williams *et al.*, 2001). Indirect evidence of that response to water deficit and associated decrease in stomatal conductance is the decrease in $\Delta^{13}C$ in water stressed plants (e.g. Hubick and Farquhar, 1989; Condon *et al.*, 1992). A few reports evidenced that the ratio C_i/C_a decreases with increasing leaf-to-air VPD (Condon *et al.*, 1992; Kemanian *et al.*, 2005), and this could be explained by a reduction in the stomatal conductance at increased leaf-to-air VPD (Mott and Parkhurst, 1991). Perhaps for this reason in many studies the correlation between $\Delta^{13}C$ and grain yield of wheat was not consistently positive across different environments (Ehdaie *et al.*, 1991; Condon *et al.*, 1992; Matus *et al.*, 1997). This inconsistency has been well-documented for a large number of studies investigating cereal genotypes grown in rainfed and irrigated environments in Australia (Condon *et al.*, 1987, 1993 and 2002; Condon and Richards, 1993; Condon and Hall, 1997), the Mediterranean region (Voltas *et al.*, 1999; Merah *et al.*, 2001; Royo *et al.*, 2002; Araus *et al.*, 2003) and elsewhere (Sayre *et al.*, 1995; Fischer *et al.*, 1998); and the relationships reported between grain yield and $\Delta^{13}C$ have only infrequently been negative. Much more often these relationships have been either 'positive' or 'neutral'. Such inconsistencies reflect that $\Delta^{13}C$ is under the influence of plant available soil moisture and prevailing weather at a given growth stage of the crop. The situation with positive or no correlation between $\Delta^{13}C$ and WUE could be related to the environmental conditions such as water availability and VPD during the sampling period and factors affecting variation in $\Delta^{13}C$ such as assimilation rate and stomatal conductance (Morgan *et al.*, 1993; Leidi *et al.*, 1999).

Condon and Richards (1992) demonstrated genotypic variations for $\Delta^{13}C$ in bread wheat in a field experiment in Australia and that the G x E interaction was small in relation to genetic variation in $\Delta^{13}C$ and this interaction was principally due to variation in the rate of water use among genotypes. In addition, there may have been differences in

genotypic rankings across environments according to the response of stomata to soil water depletion and/or to increasing VPD. Similarly in barley (Romagosa and Araus, 1991; Acevedo, 1993) and durum wheat (Araus *et al.*, 1993; Monneveux *et al.*, 2006), $\Delta^{13}C$ was found to vary genetically but environmental factors may cause even larger changes in the value of $\Delta^{13}C$, which could compromise the effective use of $\Delta^{13}C$ in breeding programmes.

Ehdaie and Waines (1996) opined that a higher stomatal conductance due to higher mean temperature and relative humidity of air in the glasshouse could result in higher $\Delta^{13}C$ than in field conditions. For wheat grown under non-irrigated conditions, grain yield is dependent largely on the plant available soil water content (Condon *et al.,* 1992; Sadras and Milroy 1996). The inherent variation in soil water content and soil nutrients in the field also may cause variation in $\Delta^{13}C$ (Hall *et al.,* 1994). In addition to grain yield, WUE is an important parameter under non-irrigated conditions since WUE is considered a component of adaptation to water stress (Ehdaie *et al.,* 1991; Morgan *et al.,* 1993; van den Boogaard *et al.*, 1997).

2.2.6 Variation in $\Delta^{13}C$ among different plant organs

Many studies have reported the use of various plant organs such as the flag leaf, peduncle, stem and grain for estimation of $\Delta^{13}C$. Condon and Richards (1992) found variation among genotypes in $\Delta^{13}C$ of different plant parts (flag leaf, stem, peduncle, grains and rachis) was always significant, and was typically 1.8‰. They reported that across several environments, mean $\Delta^{13}C$ ranged from 18‰ to 21.0‰ for early-formed dry matter and from 13.4‰ to 16‰ for grain. The genotype ranking for $\Delta^{13}C$ changed with different plant parts. Genotypic differences in grain $\Delta^{13}C$ were difficult to interpret because of the likelihood of some changes in genotype ranking resulting from differences in the degree of water stress encountered during grain filling. In addition, the contribution of remobilized carbon to grain $\Delta^{13}C$ may vary between environments and genotypes. Merah *et al.* (2001) studied the use of flag leaf, awns and grain for estimation of $\Delta^{13}C$ in a field experiment conducted under rainfed conditions in Montpellier, France which included 144 durum wheat accessions. The range of flag leaf $\Delta^{13}C$ (18.2 – 20.3‰) was higher than for grain $\Delta^{13}C$ (15.7-18.3 ‰). The higher values for flag leaf $\Delta^{13}C$ were accompanied by favourable conditions without water stress and could be due

to higher photosynthetic rate, while higher stomatal limitations on transpiration during grain yield formation led to lower $\Delta^{13}C$. In another study, peduncle $\Delta^{13}C$ in bread wheat showed a low G x E interaction indicating the relative ranking of genotypes remained consistent across environments (Ehdaie *et al.*, 1991). In summary, it is evident that grain samples are preferable for $\Delta^{13}C$ measurements under conditions of mild water stress, as they integrate effects over the time of grain yield formation.

2.3 HARVEST INDEX

Harvest index (ratio of grain to total above-ground dry matter at harvest; HI) is easier to measure than water use and WUE. Genotype ranking for HI is generally relatively stable across optimal environments (Gallagher and Biscoe, 1978). Like water use and WUE, the genetic manipulation of HI is complex under variable rainfed environments. Traits like early flowering, shorter height and peduncle length, tiller inhibition, stay-green and greater utilization of stem reserves and assimilate retranslocation may increase the HI under drought. Thus, incorporating genes that contribute to height reduction and to early flowering is an effective way to increase HI as they result in less growth of vegetative organs. In particular manipulating pre- and post-anthesis water use in favour of the grain filling period is one way to increase HI under drought. If post-anthesis water use as a proportion of total water use is large, HI will be large.

Substantial genetic variation exists in wheat for the physiological traits mentioned above (Reynolds *et al.*, 2001; Foulkes *et al.*, 2004). However, making genetic gains in yield under drought is not simple because of the unpredictability of drought and seasonal variation. In this context, the use of physiological approaches to complement empirical breeding is important. Several studies in winter wheat have quantified the reductions in HI due to drought; and the differences in HI contributed to the differences in yield responses to drought (Fischer and Wood, 1979; Innes *et al.*, 1981 & 1984; Innes and Blackwell, 1983; Foulkes *et al.*, 2002).

Reynolds *et al.* (2005) mentioned that genes affecting a greater relative partitioning of assimilates to the sink, resulting in a higher HI, may improve yield under drought since this will not be at the expense of water required for generating additional biomass. In this context, Richards *et al.* (2002) suggested that a high HI under drought may be either

constitutive (e.g. associated with *Rht* genes) or adaptive. The former is associated with increased WUE (where WUE is defined in terms of increased grain yield rather than biomass). However, in this case increased HI would be considered a pleiotropic effect of traits associated with improvement in grain-filling, such as remobilisation of stem reserves, increased access to water, etc.

At CIMMYT research is being focussed on improving drought tolerance by introgressing stress adaptive traits into empirically selected drought tolerant germplasm. In this regard, Reynolds *et al.* (1999; 2005) proposed a conceptual model of a drought resistant wheat cultivar, which should encompass high expression of physiological traits such as seed size, coleoptile length, early ground cover, pre-anthesis biomass, stem reserves/remobilization, spike photosynthesis, stomatal conductance, osmotic adjustment, accumulation of abscisic acid, heat tolerance, leaf anatomical traits (e.g. glaucousness, pubescence, rolling, thickness), high tiller survival, and stay-green. Among these, Richards *et al.* (2001) suggested the traits such as phenology or flowering habit, tiller initiation, xylem vessel diameter, leaf conductance, stay-green/ leaf rolling and assimilate retranslocation are proposed to be associated with HI under drought.

Foulkes *et al.* (2002) demonstrated differences in six winter wheat cultivars in stem reserve accumulation contributed to the ability to maintain HI hence grain yield under post-anthesis drought in UK. In another field study in spring wheat a positive linear relationship between HI and evapotranspiration was reported and this was attributed to different sink capacities established in irrigated and unirrigated treatments during the pre-anthesis phase coupled with greater pre-anthesis assimilate mobilization in unirrigated plants (Aggarwal *et al.*, 1986). This indicated that HI and season-long water use were not completely independent. Thus, it is evident that traits that influence HI could be genetically manipulated to some extent provided there is a clear physiological understanding of the mechanisms involved.

2.4 USE OF CROP SIMULATION MODELS TO IDENTIFY DROUGHT-RESISTANCE TRAITS

In addition to conceptual models (e.g. Reynolds *et al.*, 1999; 2005) crop simulation models may be used for yield prediction in stress environments. The modelling

approach can help to prioritise traits in crop ideotypes and can predict G x E interaction for optimising the trait selection in different climates.

Reports from CSIRO, Australia, indicated that an increased TE was associated with increased yields under drought in wheat (Richards *et al.,* 2002) in eastern Australia and yields of wheat genotypes with high TE were up to 10% higher than those with low TE; and in a Mediterranean-type climate increased TE did not reduce yields (Condon *et al.*, 2002). Simulation analysis of TE indicated benefits in a medium-rainfall of *ca.* 320 mm Mediterranean environment, particularly when sufficient nitrogen is supplied to the crop (Asseng *et al.,* 2002). In another simulation study, Asseng & van Herwaarden, (2003) showed that increasing the ability to accumulate stem carbohydrates for later remobilisation to the grain by 20% increased yields between 1.5 and 4 t ha^{-1} under moderate levels of water shortage. In favourable seasons, benefits were negligible due to high photosynthetic rates during grain filling and in very dry seasons also negligible due to low assimilate storage and poor sink demand.

The prediction of optimized phenological development for improving wheat yield under drought has been reported using the SIRIUS wheat model (Jamieson *et al.*, 1998). The accurate prediction of phenological development and LAI is more important for grain yield prediction than are the components of grain yield. SIRIUS model predicted the influence of water stress on partitioning of biomass to ear during pre-anthesis ear growth was more important in determining grain number than the effect on biomass accumulation during the same growth stage. The development of the Agricultural Production Systems sIMulator (APSIM) model (McGown *et al.,* 1996) for wheat and its calibration for a Mediterranean-type climate (Asseng *et al.,* 1998) has enabled the management factors affecting wheat yields to be analysed more comprehensively and for a range of soil types and historical weather records. As the model incorporates routines for water and nitrogen movement in the soil and the influence of water-logging events on the yield of wheat, it can be used to determine the potential yield in an environment. The APSIM model, used to simulate shoot and root growth, grain yield, water and nitrogen uptake, predicted final root depth and time course of root development as the key processes that regulate water and N uptake pattern for yield gain in Australian environments. In another study, a hypothetical genotype was simulated,

assuming faster and deeper root growth without requiring more assimilates from above ground, by restructuring the root system to sacrifice shallow roots in favour of an extended root profile. Thus increasing the root depth and water uptake could largely reduce deep drainage losses and resulted in a grain yield advantage of 27% (Asseng *et al.*, 2001). In a sensitivity analysis, Asseng *et al.* (1997) simulated root dynamics of wheat and predicted an advantage of larger root densities deeper in the soil profile when water and N deficiency occurs in soil. Alternatively, increasing the specific leaf area (ratio of leaf area to leaf weight; SLA) is most effective on low water holding sandy soils for increased yields in Mediterranean conditions, provided soil N supply is matched with plant N demand. However, higher SLA reduced grain yields on loamy and clay-loamy soils (Asseng *et al.*, 2003). On the sandy soils, the yields of genotypes with normal vigour were often limited due to poor leaf area development and biomass accumulation before anthesis (Asseng *et al.*, 2001). However, on clay soils, the crops grew more vigorously before anthesis and grain yields were restricted by the limited water available from the shallow wetting depth (Asseng *et al.*, 2001). Therefore, increased SLA was more effective in increasing grain yields on the low water holding sand than on the high water holding clay soils.

As reviewed earlier in section 2.1, crop simulation modelling can be used to assess the likely impact on water-use efficiency and yield of changing the expression of specific traits, e.g. root distribution (King *et al.*, 2003; Bingham *et al.*, 2002). Results of such simulations indicate that greater progress may be achieved by jointly optimising traits so that potential negative trade-offs between individual traits are minimised.

2.5 GENETICS OF WATER-USE EFFICIENCY

For a breeding programme to be successful in improving TE in bread wheat, it requires knowledge of the inheritance of $\Delta^{13}C$ and associated growth characteristics. There are two general approaches to identifying genes controlling WUE: (i) examination of near isogenic lines (NILs) and (ii) identification of QTLs in mapping populations. In this regard, $\Delta^{13}C$ has been reported to be both quite variable amongst genotypes and highly heritable evidencing strong genetic control with high broad sense heritability in bread wheat (Condon and Richards, 1992; Ehdaie *et al.*, 1991); in durum wheat (Araus *et al.* 1998; Merah *et al.*, 2001); in peanut (Hubick *et al.*, 1988); in alfalfa (Johnson and

Rumbaugh, 1995) or a narrow sense heritability of this trait in bread wheat (Ehdaie and Waines, 1994; Rebetzke *et al.*, 2002). This indicates the possibility of using delta for breeding for improved water-use efficiency or indirectly for ability to capture more water.

2.5.1 Studies on near isogenic lines

Ehdaie and Waines (1996) reported in pot and field studies the dwarfing genes *Rht1, Rht2 and Rht3* in NILs of spring wheats of Maringa and Nainari 60 to increase the harvest index, but to have relatively smaller effects on root dry matter. The *Rht2* gene depressed WUE by 15% only in Maringa background. In another study in well-watered field conditions, $\Delta^{13}C$ was negatively associated with grain yield and shoot dry matter in Maringa isogenic lines (Ehdaie and Waines, 1994). The dwarfing genes, in either genetic background in wet and dry field conditions, did not show changes in $\Delta^{13}C$ except for *Rht3* in Maringa background. In another study, Richards (1992) used NILs of bread wheat cultivars of Kalyansona x Chenab70 and Arz x Pato Argentino backgrounds. These NILs of spring wheat for the dwarfing genes *Rhtl*, *Rht2*, *Rht3* and *Rhtl+Rht2* as well as tall (*rht*) lines were grown in dry land field environments; and in pot and glasshouse experiments. In the field, tall wheats had a slightly higher $\Delta^{13}C$ relating to faster canopy growth. However, TE (above-ground dry weight/water transpired) and WUE (above-ground dry weight/evapotranspiration) declined with plant height in both glasshouse and field experiments. There was no evidence that tall wheats had a greater capacity for storage and remobilization of photosynthate in the stems than shorter wheats. The reasons for higher $\Delta^{13}C$ in tall lines were that tall lines had the faster expanding leaf canopies, the lowest photosynthetic capacity per unit area and higher stomatal conductances. However, Ehdaie and Waines (1996) reported the presence of dwarfing genes did not bring about significant changes in $\Delta^{13}C$ of 'Maringa' and 'Nainari60' spring wheat background in wet and dry field conditions. The $\Delta^{13}C$ ranged from 16 to 21‰ under well-watered and from 17.3 to 20.‰ under droughted field conditions. Many such studies using the NILs indicated that a lower TE is associated with the short lines, and this was mainly due to lower shoot dry matter production (Ehadaie and Waines, 1994; Ehadaie and Waines, 1996; Siddique *et al.*, 1990; Richards, 1992).

Ehdaie and Waines (1994) developed F_1, F_2, BC_1 and BC_2 populations from a cross between Chinese Spring (low $\Delta^{13}C$) and Yecora Rojo (high $\Delta^{13}C$) for genetic analysis of $\Delta^{13}C$ and found $\Delta^{13}C$ varied significantly only in well-watered treatments. In another study, Ehdaie *et al.* (2003) reported the yield advantage of wheat-rye translocation lines under droughted conditions was due to their greater WUE or rooting ability compared to their non-translocation counterparts. The effects of short arm translocations of rye chromosome 1 (1RS) on root biomass, WUE and agronomic performance in spring bread wheat cv. 'Pavon' were studied in pot experiments; total and root dry matter increased in 1RS.1AL and 1RS.1DL translocation lines in well-watered and in 1RS.1AL lines in droughted conditions. The 1RS translocations also exhibited increased water use as they increased transpiration rate. In the field experiment, similar positive effects of 1RS were found in wet and dry conditions.

Mohammady (2005) used F_2 backcross reciprocal monosomic crosses between wheat varieties Falchetto (low $\Delta^{13}C$) and 18 monosomic lines of Oxley (high $\Delta^{13}C$) to identify chromosomal location responsible for allelic variation in $\Delta^{13}C$. In this pot based study, chromosome 1D from Falchetto increased WUE and reduced $\Delta^{13}C$ over reciprocal lines. This suggested that the evolutionary source of the D genome (*Triticum tauschii*) and its relatives are potential sources of variation in WUE for breeding programmes.

Masle *et al.* (2005) reported in recombinant inbred lines (RILs) of Columbia (Col-4) and Landsberg *erecta* (*er*11) cross in *Arabidopsis* that the *ERECTA* gene, a putative leucine-rich repeat receptor-like kinase, regulates plant TE. Expression of this gene with higher photosynthetic rate and low stomatal conductance resulted in higher WUE irrespective of water availability. This gene was identified as a major locus for a QTL for $\Delta^{13}C$ on chromosome 2 that explained 21–64% phenotypic variance.

2.5.2 Quantitative trait loci (QTLs) detection using mapping populations

Several different types of populations may be utilized for mapping within a given plant species. F_2 populations, derived from F_1 hybrids, and backcross (BC) populations, derived by crossing the F_1 hybrid to one of the parents, are the simplest types of mapping populations developed for self-pollinating species. Inbreeding of individual F_2 plants allows the construction of RILs, which consist of a series of homozygous lines,

each containing a unique combination of chromosomal segments from the original parents (Collard *et al.,* 2005). However, for producing RI populations usually six to eight generations are required. Doubled haploid (DH) populations may be produced by regenerating plants by the induction of chromosome doubling from (i) pollen cells using micro-spore culture or (ii) from haploid embryos (produced by crossing F_1 plants with maize pollen) by treating with colchicine. However, the production of DH populations is only possible in species that are amenable to tissue culture e.g. cereal species such as rice, barley and wheat. The major advantages of RILs and DH populations are that they produce homozygous or true-breeding lines that can be multiplied and reproduced without genetic change occurring. This allows for the implementation of replicated trials across different locations and years (Collard *et al.,* 2005).

Linkage analysis with molecular genetic markers is a very powerful tool in the genetic analysis of quantitative traits (van Ooijen, 1999) such as WUE. These traits are often called complex traits in contrast to Mendelian traits and the relation between genotype and phenotype cannot be observed directly. The regions within genomes that contain genes associated with a particular quantitative trait are known as quantitative trait loci (QTLs). QTL analysis is usually carried out using interval mapping (Lander and Botstein, 1989). Linkage maps are constructed from the analysis of many segregating markers. 'QTL mapping' is based on the principle that genes and markers segregate via chromosome recombination (called crossing-over) during meiosis (i.e. sexual reproduction), thus allowing their analysis in the progeny (Paterson, 1996). Collard *et al.* (2005) reviewed the three main steps affecting construction of a linkage map: (1) *Size of the population*: Population sizes generally ranging from 50-250 individuals or even larger are required for high resolution mapping and this population must be phenotypically evaluated for quantitative traits before QTL detection. (2) *Identification of polymorphism*: It is critical that sufficient polymorphism (the nucleotide sequence of genomic DNA) exists between parents in order to construct a linkage map. This involves identification of DNA markers that reveal differences between parents followed by screening of the entire mapping population. (3) *Linkage analysis of markers*: This involves coding of data for each marker on each individual of a population and conducting linkage analysis using computer software programs. Linkage between markers is calculated as LOD score (logarithm of odds; likelihood ratio statistic). The

density of markers depends to some extent on the genome size and a higher density would give more precisely positioned markers on a linkage map. These positions or map distances are calculated using the 'Kosambi' mapping function (Kearsey and Pooni, 1996).

Essentially statistical procedures are used to assess the correlation between phenotypic values of different genotypes within the segregating population and the allelic composition at each locus used to produce a marker map. Regions of the genome that show significant LOD values are assumed to contain a QTL (van Ooijen, 1999). After construction of a linkage map the next step is detection of QTLs. This is done by QTL analysis using single marker analysis, simple interval mapping and composite interval mapping (Liu, 1998; Tanksley, 1993).

Phenotyping the population for the target trait is important and the laboratory marker data has to be correlated with the field data for QTL identification. In this regard, the need for developing high-throughput precise phenotyping screens for the target traits is very essential. Phenotypic correlations are commonly used to associate markers with traits and to genetically dissect complex traits into Mendelian factors. Once markers that are tightly linked to genes or QTLs of interest have been identified, breeders may use these markers as diagnostic tools to identify lines carrying the genes or QTLs in a marker-assisted selection (MAS) programme. The selected QTLs should account for the largest proportion of the phenotypic variance for the target trait. Thus, the main aim of QTL mapping is to produce a skeletal framework that covers all chromosomes evenly in order to identify markers flanking those QTLs that control target trait. Sometimes the closest markers flanking a QTL may not be tightly linked to a gene of interest, which means that recombination can occur between a marker and QTL, thus reducing the reliability and usefulness of the marker. So by using larger population sizes and a greater number of markers, more tightly-linked markers can be identified in high-resolution mapping. Therefore, high-resolution mapping of QTLs may be used to develop reliable markers for MAS (at least <5 cM but ideally <1 cM from the gene) and also to discriminate between a single gene and several linked genes (Michelmore, 1995; Mohan *et al.*, 1997).

The QTL analysis of quantitative traits in bread wheat has in part been hampered by its large genome size (Bennett *et al.*, 1982; cited in Quarrie *et al.*, 2005) with the majority of wheat DNA being repetitive sequences. The relatively recent origin of the hexaploid wheat (Huang *et al.*, 2002) and low levels of polymorphism (Chao *et al.*, 1989) makes it difficult to achieve detailed genetic maps of the whole genome as compared to other crop species (Quarrie *et al.*, 2005).

In spite of the difficulties in working with the large genome size of wheat, several studies show identification of QTLs associated with WUE and yield in wheat. Quarrie *et al.* (2005) reported fewer QTLs on the D genome than the A and B genomes, owing to the much lower density of markers obtained for the D genome of Chinese spring x SQ1 cross. Group 5 chromosomes gave the largest number of yield QTLs, and the group 6 chromosomes the least. Many studies revealed that group 5 chromosomes have been regarded to carry genes for abiotic stress resistance (Sutka and Snape, 1989; Manyowa and Miller, 1991; Cativelli *et al.*, 2002; Forster *et al.*, 1988, Mahmood and Quarrie, 1993).

Zhang and Ping (2002) mentioned in a review that a RFLP genetic linkage map of wheat RILs derived from W7984 x Opata was used to study QTL of 33 traits associated with WUE. The centromeric region of 1A and 1B were closely linked with QTLs for photosynthetic and root traits. The QTLs controlling WUE, plant height and growth rate were located on the group 2 chromosomes; root traits were located on the 6A and 6B chromosomes. A large gene cluster made up of seven QTLs controlling WUE of wheat was found near the centromeric region of 6D chromosome.

Teulat *et al.* (2002) studied the map generated from 167 RILs of barley from a cross between Tadmor and Er/Apm in three Mediterranean field environments differing in water availability. Ten QTLs for $\Delta^{13}C$ were identified: six of which presented main effects across three or two environments. Seasonal rainfall and the ratio of rainfall to evapotranspiration made large contributions to the environmental effect, but their influence on G x E for $\Delta^{13}C$ was weak. Eight QTLs for $\Delta^{13}C$ co-located with QTLs for physiological traits related to plant water status and/or osmotic adjustment, and/or for agronomic traits measured on this population.

2.5.2.1 Synteny among cereals for comparative mapping of QTLs

Molecular linkage maps with several major QTLs for $\Delta^{13}C$ in rice were recently published by Price *et al.* (2002) and Laza *et al.* (2006), so any QTL candidate gene regions identified in wheat could be compared with the rice genetic map to provide comparative information on possible homologue location in rice. Some examples of synteny are reviewed below.

The genome size of bread wheat is much larger than for durum wheat, barley, rice, maize and sorghum. This makes much more complex the use of molecular markers for breeding and selection in wheat. Bread and durum wheat have a lower level of polymorphism than barley or other cereals (Chao *et al.,* 1989; Devos *et al.,* 1995) and the level of polymorphism is not consistent across genomes or crosses (Langridge *et al.,* 2001).

Cereal annuals are known to share some level of homology which enables one to compare syntenic regions putatively linked to drought-tolerance traits. For example, rice, wheat and maize share extensive homologies in a number of regions in their genomes, e.g. chromosome 4, chromosome 2 and chromosome 2S respectively (Ahn *et al.,* 1993). The dwarfing alleles *Rht-B1* and *Rht-D1* in wheat are also the orthologues of the maize allele *dwarf-8.*

A conserved orthologous sequence of genes within a novel colinear region in wheat chromosome 1AS and rice chromosome 5S has been reported (Guyot *et al.,* 2004). Morgan and Tan (1996) identified a major gene controlling osmoregulation on the homoeologous arm in wheat (chromosome 7A), but this gene was probably at a more distal position, corresponding by synteny to a rice chromosome 6 segment. In various studies, group 5 chromosomes of wheat are reported to carry genes controlling a range of abiotic stress responses such as tolerance to freezing, drought, osmotic stress, and high temperatures (Snape *et al.,* 2001; Cattivelli *et al.,* 2002).

Chromosomes of homoeologous group 2 of wheat are orthologous to chromosome F of sorghum and chromosome 10 of maize (Gale and Devos, 1998) and chromosomes 7 and 4 of rice (Sorrells *et al.*, 2003) where QTLs for stay green have been reported in

sorghum (Tuinstra *et al.,* 1997; Kebede *et al.,* 2001), in maize (Bertin and Gallais, 2001), in rice (Jiang *et al*., 2004) and in wheat (Verma *et al*., 2004).

In barley, QTLs for $\Delta^{13}C$ on chromosomes 2H and 6H showed associations with TGW and on chromosome 6H between $\Delta^{13}C$ and (Teulat *et al.* 2002). One of the target loci on chromosome 7H of barley, where QTLs for osmotic potential ($\psi\pi$), RWC and some other traits have been collocated, was shown to be co-linear with a region of rice chromosome 8 (Teulat *et al.* 1998), where a QTL for osmotic adjustment at 70% of RWC was also found by Lilley *et al.* (1996). The anchor probe was mapped at the same homologous region of chromosome 8 of rice and chromosome 7 of wheat, but only on chromosome 1H of barley (Teulat *et al.* 1998). Laza *et al*. (2006) in Japan studied 101 RILs derived from an *indica* x *japonica* rice cross under flooded conditions and reported putative QTLs for $\Delta^{13}C$ on chromosomes 2, 4, 8, 9, 11 and 12.

QTLs for traits involved in drought resistance have been reported in several crops. For example, QTLs for osmotic adjustment were identified in rice (Lilley *et al.* 1996; Zhang *et al.* 2001), barley (Teulat *et al.* 1998, 2001) and wheat (Morgan and Tan 1996). Similarly QTLs have been identified for the anthesis silking interval in maize (Ribaut *et al.* 1996) and QTLs for stay green in sorghum (Tuinstra *et al.* 1997; Xu *et al.* 2000; Kebede *et al.* 2001) and wheat (Verma *et al.* 2004). However, there are only a few reports on QTLs for carbon isotope discrimination in crop plants. QTLs for $\Delta^{13}C$ have been reported in barley (Teulat *et al.* 2002; Forster *et al.* 2004), Arabidopsis (Hausmann *et al.* 2004; Juenger *et al.* 2005; Masle *et al.* 2005), tomato (Martin *et al.* 1989), soybean (Specht *et al.* 2001), rice (Price *et al.* 1997 and 2002; Laza *et al*., 2006) and brassica (Hall *et al.* 2005). To date there are no reported investigations available which have identified QTLs for $\Delta^{13}C$ in wheat.

Previous UK-based work analysed the genetic control of drought resistance utilising F_1 derived double-haploid (DH) mapping populations that were produced from crosses between Beaver (UK feed wheat variety) and Soissons (French bread wheat variety). These two cultivars are known to contrast for expression of target traits affecting performance under drought. Beaver (late flowering/poor canopy persistence) and Soissons (early flowering/ good canopy persistence) and their DH populations were utilised for further studies to quantify the associations between target drought resistance

traits like stem-soluble carbohydrate (SSC) reserves, flowering date, flag leaf green area (FLGA) persistence and yield response to drought (DEFRA, 2001; Verma *et al.*, 2004). Some of the significant findings were: (i) SSC reserves had neutral effects on absolute yield loss to drought, (ii) early flowering had neutral effects on absolute yield loss to drought (iii) 'Stay-green' measured as percent flag leaf remaining green at GS61+5 weeks was positively correlated with yield under drought, but not under irrigation. (iv) new QTLs for % FLGA on chromosome 2D associated directly with stay green effects (Verma *et al.*, 2004). However, this study did not investigate WUE.

Thus evidence from comparative mapping among other cereal species may help in identification of candidate genes in wheat associated directly with higher water-use efficiency. These QTLs may represent novel sources of improved WUE for breeders to exploit. In addition the information generated will be of value to breeders for further fine-mapping to identify markers tightly linked to genes controlling the trait, and for gene discovery.

2.6 HYPOTHESES AND OBJECTIVES

The present study is focused on improving understanding of the physiological determinants of water-use efficiency and their genetic control using doubled-haploid lines derived from a cross between winter wheat varieties Beaver (high $\Delta^{13}C$) and Soissons (low $\Delta^{13}C$), contrasting for carbon isotope discrimination and traits hypothesized to be determinants of WUE. Lines of this population were tested under irrigated and water-stressed regimes, in both glasshouse and field conditions.

Specific hypotheses were:

1. The genetic variation in TE in the DH population is due to differences in stomatal conductance, net photosynthetic rate and intercellular CO_2 concentrations of the flag leaf.

2. A decreased stomatal conductance or an increased net photosynthetic rate will reduce intercellular CO_2 concentration and increase TE.

3. A negative genetic correlation exists between carbon isotope discrimination and TE or WUE in the UK environment.

4. $\Delta^{13}C$ is associated with ratio intercellular CO_2 to air CO_2 (C_i / C_a). $\Delta^{13}C$ increases as C_i remains high and thus they are linearly related. Plants discriminating heavily against ^{13}C have low WUE and able to keep a relatively high C_i.

5. Quantitative trait loci (QTLs) for carbon isotope discrimination can be identified explaining variation in WUE in UK wheat.

6. There is a positive genetic correlation between TE or WUE and grain yield amongst lines of DH population under drought in the UK.

To test these hypotheses, two glasshouse experiments (at Sutton Bonington) involving 2 DH lines and 2 parental lines and three field experiments (at Gleadthorpe and Sutton Bonington, Nottinghamshire, UK) involving 33 DH lines and 2 parental lines (Beaver and Soissons), were carried out.

The present study had the following objectives.

Firstly, to quantify the relative contributions of genotype and environment (water availability) and to phenotypic variation in WUE in winter wheat. Secondly, to identify the physiological determinants of genetic and environmental variation in WUE in UK wheat with particular focus on stomatal aperture traits. Thirdly, to quantify the physiological interrelationships between expression of WUE and grain yield under irrigation and drought. Lastly, to identify quantitative trait loci (QTLs) for WUE and its determinant traits by characterizing doubled haploid lines of Beaver and Soissons and associating data with an existing micro-satellite marker map.

2.7 THESIS STRUCTURE

The materials used and methodologies adopted in the present investigation are described in chapter 3. In the glasshouse experiments, a subset of 4 genotypes differing in morpho-physiological traits was studied which allowed detailed observations of gravimetric estimation of WUE, gas-exchange measurements, water use, dry matter production at

frequent samplings in irrigated and droughted treatments for two years. The glasshouse experiments are described in chapter 4. The main aim was to identify processes underlying relationships between WUE and yield. In the field experiments, 33 DH genotypes and 2 parents were screened under irrigated and droughted treatments in the field at Gleadthorpe (in 2003) and at Sutton Bonington (in 2005); and 8 lines were selected for intensive study of physiological parameters. The field experiments are described in chapter 5. The main was to identify genetic correlations between WUE and sub-traits and between WUE and yield under irrigation and drought. Based on the phenotypic quantitative data collected for $\Delta^{13}C$ and other traits, genetic analysis was done to identify chromosomal regions and putative QTLs associated with WUE. The genetic analysis is described in Chapter 6. The overall outcome of the present study is discussed in chapter 7 with regard to improvement in water-use efficiency in wheat grown in the UK. This chapter will discuss of various target traits and mechanisms; and their relevance to physiological and genetic basis of water-use efficiency in UK winter wheat. The implications for future wheat breeding improvement in the UK are outlined. Some recommendations for future work and summary are set out at the end of chapter 7.

CHAPTER 3: MATERIALS AND METHODS

Two glasshouse experiments and two field experiments were conducted during the course of the investigation. Experimental design and treatments, plot or soil column management, crop or plant measurements and environmental measurements in each experiment are described in this chapter.

3.1 GLASSHOUSE EXPERIMENTS

3.1.1 Experimental design and treatments

One PVC column experiment was conducted in each of 2003 and 2004. A completely randomised design in 2003 and a randomised block design in 2004 were adopted. In each experiment there were two moisture regimes (well-watered and droughted regimes) in each of which four winter wheat genotypes were examined. There were four replicates. Within each replicate, there were six sub-replicate columns to allow for six destructive growth-analysis samplings through the growing cycle. A total of 192 PVC columns were therefore prepared with a view to six sequential growth-analysis samplings, with 32 columns per sampling (Plate 3.1). The PVC columns (root tubes) which were used were 0.1 m in diameter, and filled to a depth of 1.0 m with John Innes Compost No. 2 as growth medium. Tubes were cut longitudinally to facilitate removal of intact root systems, and were fastened together with celluloid tape. The bottom of the columns was covered with polythene in which 4-5 drainage holes were made.

3.1.1.1 Moisture regime treatment

Two treatments were imposed as 'well-watered' and 'droughted' and regimes when the plants were at 50 days after sowing (DAS) in 2003; and 75 DAS in 2004. Well watered treatments were irrigated to return columns to 90% field capacity (FC) by irrigating approximately every 7 d. The droughted treatments received the same amounts of water as the well watered treatment up to flag leaf emergence (GS39) (Tottman and Broad, 1987), after which genotypes in droughted columns received different quantities of water as per gravimetric estimation approximately every 7 d. Each genotype within an individual irrigation treatment received the same absolute amount of water throughout the experiment. Shortly before plantlets were transplanted into soil columns, the

columns were weighed 48 hours after saturation to obtain a mean column weight at FC. Soil moisture in columns was then maintained to the relevant %FC throughout the experiment by weighing the columns and adding sufficient water to equal the required weight of the column equating to the relevant %FC (Carman and Briske, 1982). The moisture content of John Innes compost No. 2 at FC is 33.5 % soil volumetric content. The columns were watered by hand from above. The sampling-replicate of 32 columns (2 moisture regimes x 4 genotypes x 4 replicates) designated for harvest growth analysis was weighed every week to the nearest gram using a digital weighing scale (model STW-60 KE). The measurements were used to calculate the average water use in each moisture regime; and all experimental columns in respective moisture regimes received water inputs according to these average values. The amount of irrigation water applied was 15.4 kg in the well-watered treatment and 6.7 kg in the droughted treatment in 2003; and 10.1 kg and 2.7 kg, respectively, in 2004. The soil surface of all the columns was filled with vermiculite to a depth of 2 cm (weighing 25 g) to minimise surface evaporation losses. In 2004, in each replicate, four soil columns containing no plants were included to estimate evaporation losses; the average water loss from these bare columns was used to correct the water use data across the moisture regimes. In the event, however, negligible soil evaporation occurred from the bare columns.

3.1.1.2 Genotype treatment

Four winter wheat genotypes were used in each experiment. These were the two parents and two DH lines from the population derived from Beaver and Soissons. The parents were known to contrast for transpiration efficiency as determined by the $^{13}C/^{12}C$ ratio, as well as several sub-traits associated with WUE, namely: specific leaf nitrogen content of flag leaves (SLN), flag leaf size, crop height (*Rht* genes) and presence of awns (Table 3. 1). Thus, previous work had indicated TE for Soissons was greater than for Beaver. The four genotypes used in each experiment were:

1. Beaver
2. Soissons
3. DH line 134G
4. DH line 134E.

The DH lines were chosen on the basis of contrasts in canopy architecture since no previous data were available on WUE or TE. Thus, within the available 60 DH lines, line 134G had a large flag leaf size but few shoots per plant and line 134E had a small flag leaf size and more shoots per plant. These differences in canopy architecture were hypothesised to have a significant bearing on their water saving behaviour under different water regimes. A higher flag leaf size would improve photosynthate remobilisation to grains during the post-anthesis stress and a reduced tillering would avoid wasteful use of water in growth of tillers that are destined to die. These differences were proposed to affect the WUE in the selected DH genotypes.

Table 3.1 Determinants of WUE for which Beaver and Soissons are known to contrast from previous work.

Character	**Parental expression**		**Candidate WUE determinant**
	Beaver	***Soissons***	
[1]Carbon isotope discrimination values (‰)	19.51	18.76	Transpiration efficiency
[2]Specific canopy N g m^{-2}	3.62	2.72	Rubisco content
Rht genes (height)	*Rht-D1*	*Rht-B1*	Aerodynamic resistance
+/- awns (surface roughness)	Unawned	Awned	Aerodynamic resistance

[1] Measured at ADAS Gleadthorpe 2001 (LSD=0.636; P=0.05); [2] Foulkes *et al.* (1998b)

Plate 3.1 Experimental layout in the glasshouse in 2004.

3.1.2 Management of plants in soil columns

3.1.2.1 Sowing and transplanting

Plants were sown on 7 March 2003 and 19 February 2004. In each experiment, plants (3 plants per 10 cm diameter plastic pot) were grown initially in a controlled-environment growth room under vernalising temperatures at 6 °C with a photoperiod of 12 hours for 35 d in 2003 and for 55 d in 2004 (Plate 3.2). Thus, the wheat seedlings were transplanted into soil columns in the glasshouse at 35 d in the first year and at 55 d in the second year. Plants were transplanted at 3 plants per column in 2003 and one plant per column in 2004 in the glasshouse. The glasshouses had vents on either side of the central ridge. An electric motor connected auto vent was operated manually to vary temperatures in the glasshouse to avoid frost risk. The glasshouses contained heating facilities through low pressure hot water circulated in pipes around each house and the air was warmed by the heat from hot interior surfaces and retained in the building by the roof and glass wall. There was no supplementary lighting during the experimentation.

Plate 3.2 Wheat genotypes grown in growth rooms and later transplanted into PVC columns in glasshouse.

The soil columns were filled with John Innes Compost No.2, which is a blend of loam (7 parts), sphagnum moss peat (3 parts), coarse sand or grits (2 parts) and fertilisers. For each m^3 of mix it also contains 0.6 kg ground lime stone, 2.4 kg hoof and horn meal, 2.4 kg super phosphate and 1.2 kg potassium sulphate. Each column was packed to a bulk density of approximately 0.97 g cm^{-3}. To ensure nitrogen was not limiting, 2 g N per column as NH_4NO_3 (34.5 % N) was added when the crop was 50 d (in 2003) and 67 d

(in 2004) old. A diluted soluble fertiliser solution containing N, P_2O_5 and K_2O (3:1:3) with Mg and trace elements was added to all the columns during transplanting and 10 d after transplanting to avoid any major and minor nutrient deficiencies. Plants were sprayed with fungicide and insecticide as necessary to control the powdery mildew and aphids, respectively.

3.1.3. Plant measurements

3.1.3.1 Developmental stages and growth analysis

Dates of onset of stem extension (GS31; growth stage 31), flag leaf emergence (GS39) and flowering (GS61) (Tottman, 1987) were recorded on the main stems of the plants in the sampling-replicate (32 columns; 2 moisture regimes x 4 genotypes x 4 replicates) designated for harvest growth analysis.

3.1.3.2 Shoot number, dry matter and green area per plant

The plant growth was quantified by sequential destructive samplings once every 2-3 weeks after imposing the moisture treatments. A total of six samplings in each year were carried out. Three plants per column in 2003 and one plant per column in 2004 were analysed at each sampling (32 columns). Shoots were removed from columns by cutting at soil level and separated into three categories: main shoots, tillers 1–3 and tillers 4+. For each shoot category, the shoot number was counted and material separated into: dead lamina, green lamina, non-green stem (plus attached leaf sheath), green stem (plus attached leaf sheath), green ear and non-green ear. Projected green areas were then recorded separately for: (i) leaf lamina, (ii) stem (plus attached leaf sheath) and (iii) ear using a leaf area meter (Li-Cor 3100, LI-COR inc. Lincoln, Nebraska).

3.1.3.3 Plant height

At harvest, crop height to the nearest cm was measured for main shoots from base level to the tip of the ear.

3.1.3.4 Root dry matter and root length density

The analyses for root parameters were done only for the sampling coinciding with GS61 in both years. For this sampling, after the plants were sampled the soil column was

separated into 0-20, 20-40, 40-60, 60-80 and 80-100 cm horizons. The soil samples were stored for 3-4 weeks in a cold room at 4°C prior to root washing. For the soil from each 20 cm horizon, roots were removed by washing by hand under a water jet and the organic debris was separated using forceps following the general procedure described by Manske *et al.* (2001). The washed root samples were placed in water-containing plastic jars and stored in cold room at 4°C until they were scanned. Before root image analysis, washed roots were placed in scanner trays the same size as the rectangular scan area in the root scanner computer system (Regent STD 1600+ which uses a winRHIZO™ 2002 software programme; Regent Instruments, UK). The roots were immersed in water and spread evenly in the scanner trays. The scanner trays had a scanning area of 22 x 30 cm. The roots were first digitized with an image acquisition device in the WinRHIZO system with a desktop optical scanner as the image acquisition device. Root analyses to estimate the root length density (cm cm^{-3}), average diameter (mm), root volume (cm^3), root surface area (cm^2), distribution and diameter classes were carried out. The scanned root samples were dried at 80°C for 48 h for estimation of root dry matter.

3.1.3.5 Plant water use and water-use efficiency

At each sampling, the columns were weighed to determine changes in water use through time by gravimetric analysis. The total amount of water used was calculated as the difference between the final and initial weight of columns taking into account the amount of water supplied to each column. The cumulative water-use efficiency for each irrigation x genotype treatment combination was calculated as the slope of the linear regression of above-ground dry matter on cumulative water use when forced through the origin. It was assumed that drainage and soil evaporation were zero from the time of the first growth analysis through to harvest.

3.1.3.6 Leaf relative water content (RWC)

In the 2004 experiment, flag leaves from each irrigation x genotype treatment combination (4 replications) were used for determination of RWC. The sampling was carried out in the morning hours in the first four sampling stages. The measurements were based on a procedure widely used in previous studies (e.g. Equiza *et al.*, 2001). Sampled leaves were sealed in plastic bags, placed above ice in a cool-box, and transported immediately to the laboratory. Leaf discs of 1 cm diameter were bored from

the sampled leaves using a steel borer and the fresh weight of discs determined using a Precisa 125A precision balance (Precisa Instruments AG, Switzerland). Leaf discs were then floated in a Petri dish containing distilled water for 24 h at room temperature in order to saturate them to full turgidity. The discs were then removed, blotted dry on paper towels and their saturated weights recorded. Discs were then dried at 80 °C for 48 h and the weight recorded. The RWC was calculated as [Equation 3.1]:

RWC= [(fresh weight-dry weight)/ (saturated weight-dry weight)] x 100....Equation 3.1

3.1.3.7 SPAD measurements

Chlorophyll content was determined non-destructively using a SPAD-502 meter (Minolta, Japan). The measurements were made on the flag leaf of main shoots from each irrigation x genotype combination in four replications, by clamping the SPAD sensor over the leaf lamina. The veins of the leaf were avoided as they could affect the results due to thickness and paleness. This instrument uses measurement of transmitted radiation in the red and near infrared wavelengths to provide numerical values related to leaf chlorophyll content. The SPAD meter normally gives values for wheat between 35 and 50 reflectance units. A close linear correlation between SPAD values and extractable chlorophyll content has been reported for a wide range species (Marquard and Tipton 2004; Finnan *et al*. 2004).

3.1.3.8 Gas exchange measurements

Leaf gas exchange was measured using a portable Infra-Red Gas Analysis (IRGA) system (CIRAS-1, PP-Systems, UK) in conjunction with a plant leaf cuvette having an area of 2.5 cm^2 of leaf surface (Plate 3.3). CIRAS-1 measures CO_2 concentration and water vapour pressure changes in the leaf cuvette, from which calculations for various gas-exchange parameters are performed. In both years, from stem extension (GS39) to mid grain filling (GS61+4 weeks) gas-exchange was assessed once or twice a week on the flag leaf of main shoots between 10.00 and 15.30 h. Before using the instrument the influx of CO_2 into the system was adjusted to ambient CO_2 concentration of 365 ppm. After clamping the flag leaf into the leaf cuvette attached to CIRAS it was then equilibrated for CO_2 exchange rate, stomatal conductance and transpiration. The steady state of these parameters was achieved after about 15 minutes. Three measurements per

column were taken for each genotype x irrigation combination in each replicate; and a total of either 96 or 72 readings were taken each time. Each measurement took about 90-120 s for recording an equilibrated value. Gas-exchange measurements were taken on days with sufficient sunlight and no artificial light source was used for illumination. This provided estimates of:

1. stomatal conductance (g_s) - mmol $m^{-2}s^{-1}$
2. assimilation rate (A) - μmol $m^{-2}s^{-1}$
3. sub-stomatal CO_2 concentration (C_i) - mmol CO_2 mol^{-1} air
4. transpiration rate (E) – mol $m^{-2}s^{-1}$

Plate 3.3: Portable photosynthetic system CIRAS-I (PP Systems, UK) used for measuring leaf gas-exchange variables. System console and leaf chamber mounted on a stand with wheat leaf clamped into.

3.1.3.9 Carbon isotope composition analysis

In 2003, flag leaf samples collected at anthesis (GS61) were dried for 48 h at 80°C. These samples were then ground to a fine powder (<200 μ particle diameter) using a Cyclotec 1093 sample mill (Foss Tecator Co.) for carbon isotope analysis using a mass spectrometer. In 2004, in addition to leaf samples at GS61 mature grain samples collected at harvest were ground to a fine powder (<250 μ particle diameter; Plate 3.4) after drying for 48 h at 80°C, for determination of the $^{12}C/^{13}C$ isotope ratio.

The carbon isotope discrimination analysis was performed at the British Geological Survey (BGS) Laboratory, Keyworth, Nottinghamshire, UK. $^{13}C/^{12}C$ ratios of bulk

organic matter were analysed in an online system comprising a Carlo Erba NA-1500 series elemental analyzer, a VG TripleTrap, and a VG Optima mass spectrometer (GV Instruments, Manchester, UK).

Sample preparation

Samples containing 1-2 mg C were loaded 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 flat spatula were used to gently compact the capsule into a small, tight cube or ball. Care was taken not to puncture the capsule. Each capsule was then dropped into the plastic block containing series of wells which were numbered to identify the sample. The final weight to the nearest microgram (3 decimal places) of the capsule was recorded after it was sealed. Care was taken to avoid and discard cross-contaminated samples. The lid of the culture tray was secured with laboratory tape and each tray was labelled.

Sample analysis

The tray containing the tin capsules was placed in the carousel of the Carlo Erba elemental analyser (to combust solid samples). The samples were sequentially introduced, in a continuous flow of helium carrier gas, into a 1020°C furnace. A pulse of oxygen gas promoted an exothermal flash oxidation of the tin, ensuring full combustion of the sample, and the product gases were further oxidised by chromium and cobaltous oxides in the lower part of the furnace. After removal of water and excess oxygen (by passage through magnesium perchlorate and hot copper), nitrogen and carbon dioxide were separated through a gas chromatography column before passing to the TripleTrap. In the TripleTrap, helium carrier gas sweeps the sample gases through a trap at –90°C (for complete removal of water), a trap at -196°C (for trapping of carbon dioxide), and a trap containing zeolite at -196°C (for optional trapping of nitrogen), before helium venting to atmosphere. The TripleTrap was then evacuated before warming the -196°C trap and expanding the sample CO_2 into the inlet of the Optima mass spectrometer. The Optima mass spectrometer has triple collectors allowing simultaneously monitoring of CO_2 ion beams at m/e = 44, 45 and 46; and a dual-inlet allowing rapid comparison of sample CO_2 compared with a reference CO_2. 45/44 ratios are converted to $^{13}C/^{12}C$ ratios after correction for common ion effects ('Craig' correction). The system performs an

automated run on up to 50 samples at a time. In each run two samples of a laboratory standard were analysed after every ten unknown leaf or grain samples. From knowledge of the laboratory standard's $\delta^{13}C$ value versus VPDB (Vienna Pee Dee belemnite; derived from regular comparison with international calibration and reference materials NBS-19 and NBS-22), the $^{13}C/^{12}C$ ratios of the unknown samples were converted to carbon isotope discrimination ($\delta^{13}C$) values versus VPDB (Tim Heaton, *personal communication*). Standards of broccoli plant material were calibrated against National Bureau of Standards NBS-19 and NBS-22. Replicate analysis of well-mixed samples indicated a precision of $\pm$ <0.1‰ (1 standard deviation). The resulting $\delta^{13}C$ values were used to calculate carbon isotope discrimination ($\Delta^{13}C$), which has a negative linear relationship with transpiration efficiency (Farquhar and Richards, 1984).

Therefore, $^{13}C/^{12}C$ ratio values were expressed as carbon isotope composition ($\delta^{13}C$) values [Equation 3.2], where

$$\delta^{13}C\ (‰) = [(R_{sample} / R_{standard})-1] \times 1000 \quad \text{.....Equation 3.2}$$

R is the $^{13}C/^{12}C$ ratio. The standard for comparison was a broccoli standard calibrated against Pee Dee belemnite (PDB) carbonate. The value of discrimination (Δ) against ^{13}C is calculated from δ_a and δ_p [Equation 3.3], where 'a' refers to air and 'p' to plant. The carbon isotope composition of air was taken as -8‰ (Farquhar *et al.*, 1989):

$$\Delta = (\delta_a - \delta_p \Delta / (1 + \delta_p) \text{.....Equation 3.3}$$

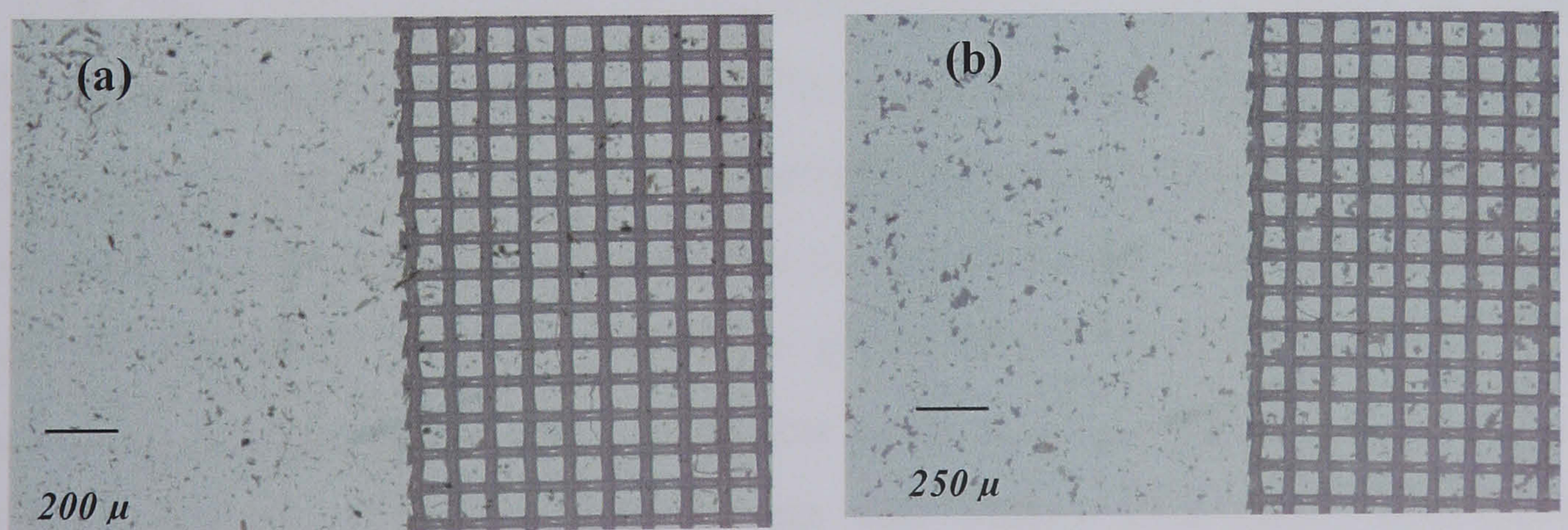


Plate 3.4 Scanned images of (a) leaf samples and (b) grain samples used for C^{13}/ C^{12} analysis, taken using Nikon digital net camera Dn 100 and SMZ 1500 microscope.

3.1.4 Environmental measurements

The glasshouse experiments were conducted under natural conditions without any control of radiation levels, relative humidity and temperature. However, the minimum temperature of the glasshouse was regulated to avoid risk of frost early in the growing season. The daily measurements of minimum and maximum temperature were recorded by a data logger (Campbell Scientific CR10). Only in 2004, four tube solarimeters were placed at a height of 2 m from the ground to measure the incident radiation in the glasshouse.

3.2 FIELD EXPERIMENTS

The experimental design and plot management for the field experiments are described in this section as well as crop measurements taken at various growth stages.

3.2.1 Experimental sites and plot management

Three field experiments were carried out. One field experiment was conducted in 2002/3 at the drought-prone site of ADAS Gleadthorpe, Nottinghamshire (55°13' N, 1° 6'; loamy sand to 35 cm over medium sand; Cuckney Series). The experiment was sown as a first wheat on 19 October 2002; the previous crop was main crop potatoes. A second field experiment was sown at University of Nottingham farm, Sutton Bonington (52° 50'N, 1° 15'W; stony medium loam to 80 cm over clay; Dunnigton Heath Series) in 2004/5 (Plate 3.5). This experiment was sown as a first wheat on 8 October 2004; the previous crop was winter oats. A third field experiment in 2003/4 had been previously sown at University of Nottingham farm, Sutton Bonington (stony medium loam to 80 cm over clay; Dunnigton Heath Series). This experiment was sown on 30 September 2003; the previous crop was winter oats. Due to severe lodging associated with July and August rainfall significantly above the long-term mean, however, the data from the experiment in 2003/4 at Sutton Bonington are not discussed in the main section of this thesis. A Summary of these results is presented in Appendices III-VI. In each experiment, an Oyjard drill was used to sow seeds at 325 seeds m^{-2} into 12 rows of 12 and 13.5 cm row width at Gleadthorpe and Sutton Bonington, respectively. A prophylactic programme of fungicides at GS31, GS39 and GS61 was applied in all experiments, and herbicides and pesticides were applied depending on prevalent

problems and local conditions to control diseases, weeds and pests to minimum levels. Nitrogen fertilizer was applied as ammonium nitrate prill to ensure that N was not limiting. No plant growth regulator (PGR) was applied at Gleadthorpe. At the more lodging-prone site of Sutton Bonington, PGR as chlormequat was applied at GS31. Full details of crop husbandry and the calendar date of operations are presented in Appendix I.

Plate 3.5. An aerial view of the field experiment at Sutton Bonington in 2004/5 in mid July showing treatment differences between the irrigated and unirrigated plots.

3.2.2 Experimental design and treatments

The experiments at Gleadthorpe and Sutton Bonington used a randomised split-plot design, with three replicates. The irrigation treatments were randomised on main plots, and within these, genotypes were fully randomised on sub-plots. Experimental plans are given in Appendix II.

3.2.2.1 Experimental treatments

Irrigation treatments (main plots)

Two irrigation treatments: (i) fully irrigated from onset of stem extension (GS31) to harvest and (ii) unirrigated were randomised on main plots. At Gleadthorpe, water was applied using a linear overhead irrigator and at Sutton Bonington using a trickle irrigation system. At both sites, water was applied in the irrigated treatment to maintain

soil moisture deficit, calculated using the ADAS Irriguide model (Bailey and Spackman, 1996), to <50 % available water (AW) up to GS61 + 4 weeks and 75 % AW thereafter. Irrigation was discontinued completely at full canopy senescence. Unirrigated treatments in both years did not receive any irrigation. At both sites there were 8 m discard areas between irrigation main plots.

Genotypes (sub-plots)

Thirty three DH lines and their parents, Beaver and Soissons, were randomised in sub-plots at Sutton Bonington. At Gleadthorpe an additional 13 DH lines were included, so 46 DH lines and their parents, Beaver and Soissons were randomised on sub-plots. The size of the sub-plot was 1.40 m x 10 m at Gleadthorpe and 1.62 x 12 m at Sutton Bonington. Parental differences for key physiological traits are given in section 3.1.1.2. This DH population was physiologically characterised for selected traits and yield response to drought at ADAS Gleadthorpe in a previous investigation in 1999/2000 and 2001/2 at Gleadthorpe (Foulkes *et al.*, 2002). Based on this information, a subset of six DH lines and the two parents was selected for intensive study according to values for key morphological traits (plant height, flag leaf size, presence/ absence of awns and ear size) (Plate 3.6). The morphological traits based on which of the eight genotypes were selected are presented in Table 3.2.

Table 3.2 Contrasting morphological traits used for selecting the eight wheat genotypes for intensive measurements.

Genotype	Plant height	Flag leaf size	Presence of awns	Ear size
134G	Tall	Large	Awned	Large
134E	Tall	Intermediate	Awned	Intermediate
21B	Semi-dwarf	Large	Unawned	Intermediate
22B	Tall	Large	Unawned	Intermediate
35A	Tall	Intermediate	Awned	Large
76A	Double-dwarf	Large	Awned	Large
Beaver	Semi-dwarf	Large	Unawned	Large
Soissons	Semi-dwarf	Intermediate	Awned	Intermediate

3.2.3 Crop measurements

Measurements taken in experiments at Gleadthorpe and Sutton Bonington are described in this section. The experimental years in the text are designated by their harvest years: i.e. 2002/3 is referred to as 2003.

3.2.3.1 Crop development

Dates of reaching different growth stages were based on the decimal code of growth stages (GS) explained by Zadoks *et al.* (1974) as revised by Tottman and Broad (1987). At Gleadthorpe, date of GS61 was assessed in all sub-plots. At Sutton Bonington, the dates of GS30, GS31 and GS39 were assessed only in sub-plots of four genotypes in two replicates and the date of GS61 in all sub-plots. For GS30 and GS31, five whole plants per sub-plot were pulled up and the stage of the main shoot was recorded. For GS39 and GS61, visual assessment of all shoots was carried out. A growth stage was assigned when more than 50 % of the main shoots were at the particular stage. Crop maturity was taken as the date when all the green lamina area had senesced and < 25% of the stem area was remaining green.

3.2.3.2 Crop height

A few days prior to harvest, crop height to the nearest cm from ground level to the tip of the ear was measured for five randomly selected fertile shoots per sub-plot.

3.2.3.3 Shoot number, green area and crop dry matter

<u>Gleadthorpe 2003</u>

A few days prior to harvest, approximately 100 shoots were selected at random from each sub-plot cutting off at ground level. The outer row on either side of the sub-plot was not sampled and samples were at least 50 cm in from the ends of plots. For the whole of the sample, ears were cut off at the collar, counted and then threshed. The weight of the grain and chaff was recorded after drying for 48 h at 80°C. The fresh weight of the total straw was recorded. A 20% sub-sample of straw was taken (by fresh weight) and the weight recorded after drying at 80°C for 48 h.

<u>Sutton Bonington 2005</u>

At growth stages GS31 and GS61, the quadrat samples were taken for the four selected genotypes studied in the glasshouse experiments. At harvest, the quadrat samples from each sub-plot were taken for the eight selected genotypes. The quadrat size was 0.72 m^2 (1.2 x 0.6 m). All plant material was sampled within one quadrat per sub-plot, oriented so that rows ran parallel with the 0.6 m sides of the quadrat. The sample size included

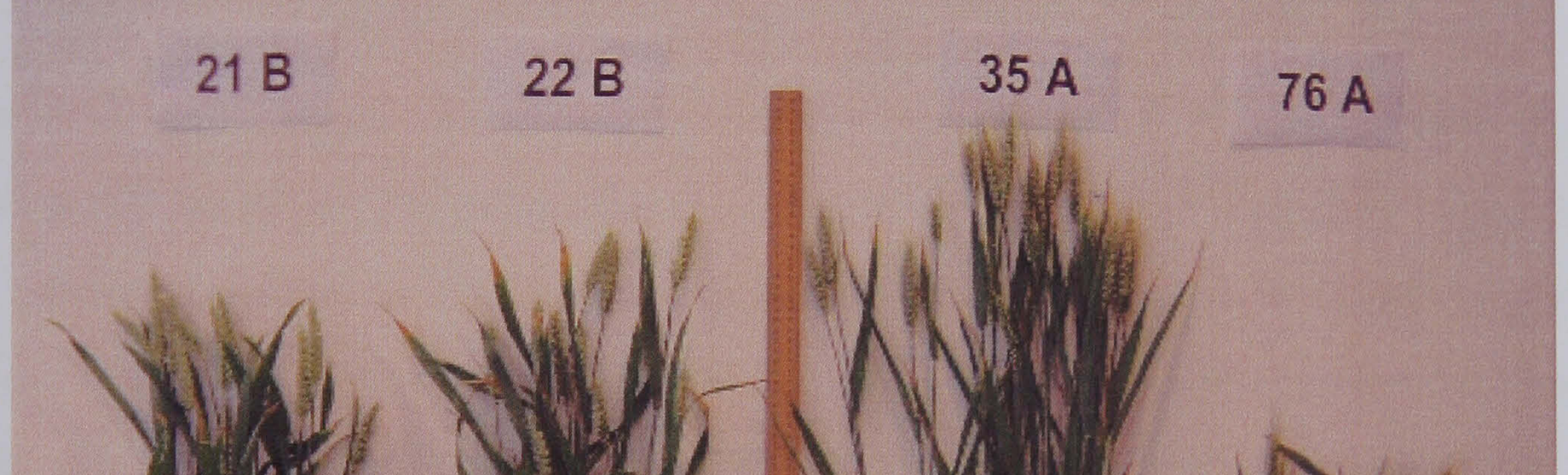


Plate 3.6. Six doubled haploid lines and two parents selected for intensive study contrasting for their physiological and morphological features.

eight adjacent rows in the sub-plot and the outer two rows of the sub-plot were avoided. Thus, the sub-plot sample size was 0.63 m^2 (8 x 0.132 x 0.6). To avoid 'near-neighbour'effects, the quadrats were placed at least 0.5 m apart and at least two rows in from the edges of the sub-plot and 0.5 m from their ends. At GS31 plants were dug up with their roots, which were later cut off in the laboratory at 'ground level' and discarded. At later growth stages, plants were cut at the ground level in the field. All the samples were stored in a cold room at 4 °C and growth analysis was carried out within 4 d of sampling.

At GS31 and GS61, the total fresh weight of all the above-ground plant material was recorded. An approximately 10% sub-sample by fresh weight (SS1) was then randomly taken, and kept for further analysis. At GS31, the weight of the remaining plant material (90%) was recorded after drying at 80 °C for 48 hours. At GS61, a further sub-sample (SS2) in addition to the SS1 sub-sample was taken as an approximate 20 % sub-sample by fresh weight and weighed after drying at 80 °C for 48 h. After the SS1 and SS2 sub-samples had been taken the remainder of the original sample was discarded. Above-ground dry weight m^{-2} was calculated on the basis of the 90% sub-sample at GS31 and the SS2 sub-sample at GS61.

The SS1 sub-sample was split into three categories of shoots defined as: (a) potentially fertile shoots (when the shoot showed no signs of yellowing at the tips of the youngest leaves at GS31, and when the shoot had an ear at GS61), (b) dying shoots (when the newest expanding leaf had begun to turn yellow at the tip), and (c) dead shoots (when the shoot had no green material). The shoot number for each category was recorded. The dry weight of the dying and dead shoots was recorded separately after drying at 80°C for 48 h. For the potentially fertile shoots, the material was separated into: (a) green leaf lamina (severed at ligule), (b) green true stem with attached leaf sheath, (c) non-green stem with attached leaf sheath, (d) dead leaf lamina (i.e. non-green – severed at ligule), (e) green ears (cut at the collar) and non-green ears. The dry weight of each component recorded after drying at 80 °C for 48 h. The projected areas of green lamina, green stem with attached leaf sheath and green ear components were measured using a Li-Cor 3100 leaf area meter; and then the samples were then weighed after drying at 80 °C for 48 h. The green area index (GAI,

defined as the green canopy area per unit ground area) was calculated after summing up the green areas of the lamina, stem and ear fractions.

At the sampling at harvest, shoots from the whole 0.63 m^2 sub-plot sample were counted in two categories: (a) fertile shoots (those with an ear), (b) infertile shoots (those without an ear). The infertile shoots were weighed after drying at 80 °C for 48 h. The ears from the fertile shoots were cut off and the fresh weight of straw was recorded. A 25 % sub-sample by fresh weight was taken, and the material separated into (a) leaf lamina, (b) stem and leaf sheath and (c) ear. The components were weighed after drying for 48 h at 80°C. The ears were threshed and the grain and chaff collected and weighed after drying at 80 °C for 48 h. A 40 g sub-sample of dried grain was taken and cleaned by removing the broken grains. The grains were then counted using a Contador seed counter (Pfeuffer inc., Germany) and the dry weight of the sample recorded. From this, the 1000-grain weight was recorded.

3.2.3.4 Lodging assessment

When lodging was observed, assessments of its incidence were made. A visual assessment of the percentage area of the crop which was lodged (lodging defined as when crop lodges between 45° and 90° from the vertical) was made of the whole sub-plot, including its edges, by walking around the plot perimeter. Two assessments of crop lodging were carried out, on 21 June (GS61+3 weeks) and 15 September 2004 (harvest). The results for percentage plot lodged at the time of harvest are given in Appendix V.

3.2.3.5 Combine grain yield and thousand grain weight at harvest

Prior to harvesting, tram-lines were cut-out. The sub-plot lengths of the resultant combine areas at Gleadthorpe (1.40 m by approximately 5 m) and Sutton Bonington (1.62 m by approximately 10 m) were recorded accurately. Combine grain yield was measured for all sub-plots. All combined grain from each sub-plot was weighed on the combine. Immediately after harvest, a 250 g sub-sample of grain was accurately weighed and then reweighed after drying at 80°C for 48 h, allowing sub-plot yields to be expressed as tonnes per hectare (t ha^{-1}) at 85 % dry matter. A further 75 g sub-

sample of grain was taken from each combine sample and cleaned by removing all broken grain. The number of grains and the weight of each sample after drying at 80°C for 48 h were recorded. The thousand grain weight was then calculated.

3.2.3.6 Gas-exchange measurements

In each experiment, gas-exchange measurements of photosynthetic rate, stomatal conductance and inter-cellular CO_2 concentration were recorded weekly at Gleadthorpe in 2003 and twice weekly at Sutton Bonington in 2004 and 2005 (from GS39 to GS61+ 5 weeks) on the eight selected genotypes for all sub-plots as detailed in sub-section 3.1.7.

3.2.3.7 Carbon isotope composition analysis

In 2003 and 2005, for each sub-plot samples from grain from the combine were oven-dried for 48 h at 80 ° C. These samples were ground to a fine powder and then submitted to analysis using the mass spectrometer for $\Delta^{13}C$ determination as described in sub-section 3.1.3.8.

3.3 DATA HANDLING AND STATISTICAL ANALYSIS

Data were entered into EXCEL 2003 (Microsoft Corporation) spreadsheets and analysed using GENSTAT 8.1 (Lawes Agricultural Trust, Rothamsted Experimental Station). Standard analysis of variance procedures for a two-way ANOVA (no blocking) or a two-way ANOVA in randomised blocks for glasshouse data and a split-plot design in randomised blocks for field data were used to calculate treatment means, standard errors and significant differences between treatment means. Replications were regarded as random effects, while irrigation treatment and genotype were fixed effects. For ANOVAs across seasons, Bartlett's test (P = 0.05) was used to test for the homogeneity of variances, and site/seasons were regarded as random effects. The mean square for the year or site/season effect was tested against an error mean square representing the variation between blocks within site/seasons. The mean square of the genotype and year or site/season x cultivar effects was tested against an error B mean square representing residual variation. Treatment means were compared using the least significant difference of the means of Fisher,

calculated from standard errors of the difference of the means using appropriate degrees of freedom, when the ANOVA indicated significant differences. In glasshouse experiments, standard linear regression analysis was performed to determine the water-use efficiency of irrigation x genotype treatment combinations as the slopes of the respective regressions of cumulative dry matter on cumulative water use. Parallel regression analysis (simple linear regression with groups) was used to test for differences between treatment combinations in each year separately. Pooled analysis of data across site/seasons was carried out wherever it was appropriate. Genetic correlations between traits and between respective traits and grain yield were calculated using the genotype means. The effects of presence/ absence of 1BL/1RS or of *Rht* genes were tested as a contrast between two levels of the main effects of DH lines. Treatment means for 1BL/1RS or 1B or for the *Rht1, Rht2, Rht1+Rht2* and *rht* groups of lines were compared using the variance ratio since there were only two levels for the comparison. The classification of 1BL/1RS and *Rht* lines were done at JIC (DEFRA, 2001).

3.4 GENETIC ANALYSIS

The target traits such as $\Delta^{13}C$, grain yield and thousand grain weight were selected for phenotypic evaluation. The phenotype data of these target traits and genotype data of B x S mapping population were analysed using the computer software package MapQTL version 5 (van Ooijen, 2004). The QTL analysis was done separately for Gleadthrope (2002/3) and Sutton Bonington (2004/5) experimental sites. The procedures involved in the QTL analysis are presented here.

3.4.1 Construction of genetic map

Initial mapping work at John Innes Centre, Norwich, in collaboration with University of Nottingham and ADAS on the original 49 lines of the Beaver x Soissons mapping population was carried out in a DEFRA Project OC9602 'Maintaining wheat performance through improved resistance to drought' using simple sequence repeats (SSR), amplified fragment length polymorphisms (AFLP), storage protein (Glutenin) loci and the major dwarfing genes *Rht-B1* and *Rht-D1* (Verma *et al.*, 2004). In a further collaborative DEFRA project 'Physiological traits influencing hardness and vitreosity in wheat grain' this skeletal map was expanded at the John Innes Centre

using further SSR markers to give a more comprehensive coverage (DEFRA, 2004). This included extending the SSR marker coverage to 65 lines to develop a more accurate map. For SSR analysis, the sets of publicly available GWM (Roder *et al.*, 1998), PSP (Bryan *et al.*, 1997), wheat microsatellite consortium, and BARC (Somers *et al.*, 2004) marker primer pairs were tested on the parents for polymorphism. From this parental screen, markers were chosen for mapping which gave easily scored polymorphisms. Fragments amplified by PCR (polymerase chain reaction) for all the DH lines and parents were separated on 5% polyacrylamide gels at 90 W for 1.5 h. The amplified fragments were visualized by silver staining.

From the characterized genotype data, map construction was carried out using JoinMap version 2.0 (Stam, 1993). The Kosambi mapping function (Kosambi, 1944) was used to calculate the centiMorgan (cM) distances. In total, 107 SSR markers mapped into 33 linkage groups using JoinMap and an extra 19 markers were incorporated into the map by calculating recombination distances to the nearest linked marker using consensus maps.

3.4.2 QTL Analysis

The approach used in the present work, where the population size is relatively small, was first to search for QTLs by applying analyses of variance (ANOVA) to partition the variation within and between genotypes for each marker in turn on each linkage group. Thus, for each marker the population was characterized into the two homozygous classes and an ANOVA carried out to detect the additive effect of each locus. If a significant difference was found, then a QTL linked to the marker locus was indicated. The ANOVA tests carried out on the target traits confirmed normal distribution of data.

More sophisticated analyses were then carried out to more accurately locate QTLs onto specific regions of the genome using least squares techniques for flanking marker models. Two separate statistical procedures were applied, interval mapping (Haley and Knott, 1992) and marker regression (Kearsey and Hyne, 1994). Only the interval mapping results are presented on account of their accuracy over simple marker regression analysis. For analysing the data for two environments

(Gleadthorpe 2003 and Sutton Bonington 2005) MapQTL version 5 (van Ooijen, 2004) was used. The results obtained with MapQTL 5.0 software were used for interpretation of putative QTLs and their location in the chromosomes of interest. The details of the analyses carried out to detect putative QTLs using the MapQTL software are presented below.

3.4.2.1 Construction of data files

MapQTL uses plain text files made with any text editor programme to load the data for analysis. Three data files were prepared. Firstly, a *locus genotype file* (*loc-file*) containing the genotype codes for the loci of DH mapping population was prepared. The loc-file had the actual genotype information for each locus and for all DH lines. Secondly, a *map file* was prepared which contained the map positions of all loci. Thirdly, the *quantitative data file* (*qua-file*) containing the data of the quantitative traits of all DH lines was prepared. These three data files were loaded into MapQTL software for QTL analysis. The details of the analyses carried out are described below.

3.4.2.2 Kruskal-Wallis analysis for non-parametric mapping

This analysis is based on the ranking of genotype means and has the advantage that the trait does not need to be normally distributed. The test was performed on each locus (marker) separately. No use was made of the linkage map other than for sorting the loci; and the test ranked all individuals according to the quantitative trait, while classifying them according to their marker genotype. A segregating QTL linked closely to the tested marker results in large differences in the average rank of the marker genotype classes. A test statistic (K*) based on the ranks in the genotype classes was calculated. The K* statistic is distributed approximately as a chi-square distribution. Since this test is performed on many loci, a significance level of 0.005 is recommended (van Ooijen, 2004).

3.4.2.3 Interval mapping

This method developed by Lander and Botstein (1989) is also referred to as single-QTL model. In this approach for each position on the genome at every centiMorgan

(cM) distance, the likelihood for the presence of a segregating QTL is determined (the likelihood under alternative hypothesis: H1). At the same time the genetic effects of the QTL and the residual variance are calculated. The likelihood under H1 is compared to the likelihood for the situation when a locus with no genetic effect would segregate, i.e. there is no segregating QTL (null hypothesis: H0). This comparison is done with a likelihood ratio statistic called the LOD, which is the 10-base logarithmic of the quotient of the two respective likelihoods. When the LOD score exceeds the significance threshold on a linkage group, a segregating QTL is declared; the position with the largest LOD on the linkage group is the estimated position of the QTL on the map. The results of QTL analysis were interpreted considering both significant values of the Kruskal-Wallis test statistic and LOD scores for each marker.

Calculation and interpretation of LOD threshold levels for QTLs

In QTL analysis the probability of arriving at wrong conclusions or errors are of two types. These are (a) a segregating QTL is predicted when in reality there is not (false positive: Type I) and (b) not detecting a QTL which actually is present (false negative: Type II). Usually an experiment-wise significance level of 5% is desired in crop or plant research. In the QTL analysis this is equivalent to the 'genome-wide' significance (i.e. probability) of obtaining a LOD score above the threshold somewhere on the whole genome by chance is 5%.

(i) Calculation of LOD score using cumulative distribution tables:

Suppose the required genome-wide significance level is α_g and the corresponding chromosome-wide significance level is α_c; the number of chromosome pairs is '*n*' and the average chromosome length '*l*' (cM), then $1-\alpha_c = \sqrt[n]{(1-\alpha_g)}$. For the present DH mapping population with 21 pairs of chromosomes, a genome-wide false positives rate of 5% would be $\sqrt[21]{(1-0.05)} = 0.9976$. For an average chromosome length of 77.5 cM, the table maximum LOD (van Ooijen, 1999) would be between 3.0 and 3.3. By interpolation a LOD score of 3.15 would be around the desired 5% genome-wide significance threshold in the present QTL analysis. However, a LOD threshold significance of 2.0 was used to detect 'putative QTLs' in this analysis.

(ii) Calculation of LOD score using a permutation test:

The estimation of LOD threshold significance by the table method was refined more accurately by permutation tests. This test was performed for interval mapping. Over a set of permutations the frequency distribution of the maximum LOD score was determined. In each iteration, the quantitative data were permuted over the individuals while the marker data remain fixed. Subsequently interval mapping was done on the obtained data set. The number of permutations was set to 10,000 iterations and the frequency distributions were obtained for each linkage group and genome-wide as a whole. A threshold significance with a P-value 0.05 was used to find the interval (as LOD score) in the results of permutation test where the 'relative cumulative count' (cumulative count ÷ total number of permutations) was about 1-0.05=0.95 or the first higher value. By this method, the genome-wide significance of 5% again indicated threshold significance for LOD for different loci which ranged from 3.1-3.4.

3.4.2.4 MQM mapping

This is based on multiple QTL models developed by Jansen (1993) and Jansen and Stam (1994). This approach involves use of significant markers as cofactors on the other chromosomes, with additive and dominant gene action only. This method utilizes approaches (i) to look for putative QTLs by interval mapping (ii) close to detected QTLs markers are selected as cofactors to take over the role of the nearby QTLs in the approximate multiple QTL models used in the subsequent MQM mapping. Fitting the selected cofactors will reduce the residual variance. If a QTL explained larger total variance, then the linked marker was used as a cofactor in subsequent MQM mapping enhance the power of search for other segregating QTLs. In the present analysis only for a few putative QTLs MQM mapping was applied to sort the segregating QTLs.

CHAPTER 4: WATER USE AND WATER-USE EFFICIENCY OF WHEAT GENOTYPES IN GLASSHOUSE EXPERIMENTS

This chapter describes results from two glasshouse experiments conducted during 2003 and 2004. The differences between four winter wheat genotypes, contrasting for morpho-physiological characteristics, on water uptake, water-use efficiency and dry matter growth were examined.

4.1 RESULTS

4.1.1 Weather

The temperature data collected for glasshouse experiments in 2003 and 2004 are presented in Figure 4.1 (a - b), along with development data for the wheat genotypes. In 2003, both minimum temperature and maximum temperature increased during the season reaching maximum values of 23.4 °C and 40.5 °C respectively on 9 August corresponding to the late grain filling stage. Unusually high temperatures after booting and during grain filling phase (Figure 4.1 (a)) resulted in poor spikelet fertility and grain filling in 2003. In 2004, the maximum temperature exceeded 30 °C one week before flag leaf emergence and 4-5 days before anthesis. However, temperatures tended to remain stable in the range of 20-25 °C during most of the crop cycle (Figure 4.1 (b)).

4.1.2 Plant development

Soissons was the fastest developing genotype to all stages followed by lines 134G, 134E and then Beaver (Table 4.1). Soissons flowered 9 and 7 days earlier than Beaver in 2003 and 2004, respectively, with intermediate values for the other two lines. Similar differences were observed earlier in the season at onset of stem extension (GS31). Line 134G flowered one day earlier than 134E (Table 4.1). Averaging across genotypes, the period of stem extension from GS31 to GS61 was 46 days in 2003 and 54 in 2004. In 2003, Beaver was relatively later at all the growth stages compared to 2004 probably owing to lack of vernalization temperatures during early growth. Irrigation delayed flowering in the range 1-5 days in 2003 and 1-2 days in 2004.

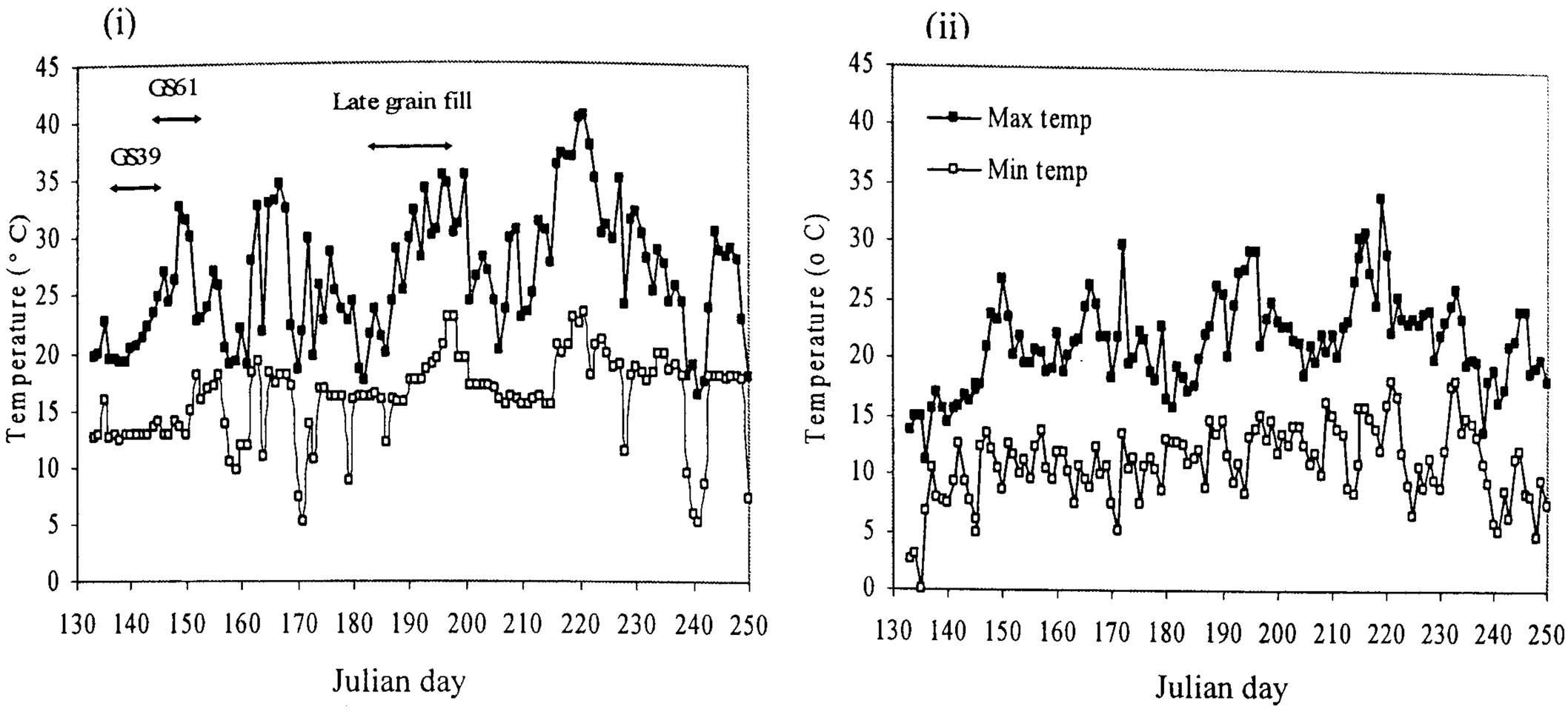


Figure 4.1.a Maximum and minimum temperatures collected from (i) glasshouse experiment - 2003 and (ii) Sutton Bonington meteorological station - 2003.

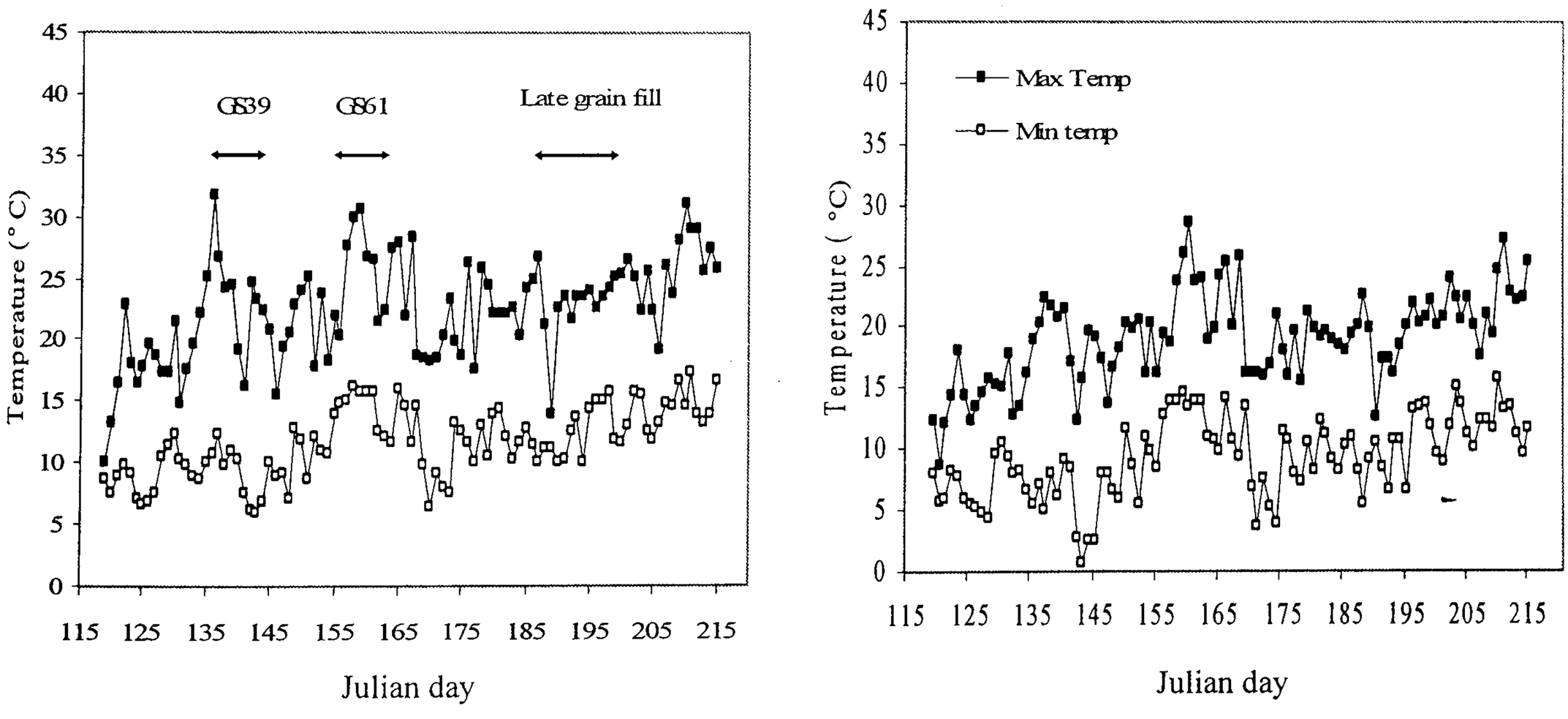


Figure 4.1.b Maximum and minimum temperatures collected from (i) glasshouse experiment - 2004 and (ii) Sutton Bonington meteorological station - 2004.

Table 4.1 Dates of different growth stages recorded on four wheat genotypes in irrigated and unirrigated treatments in 2003 and 2004.

Genotype	GS31						GS39						GS61					
	2003			*2004*			*2003*			*2004*			*2003*			*2004*		
	Irri	Unirri	Mean	Irri	Unirri	Mean	Irri	Unirri	Mean	Irri	Unirri	Mean	Irri	Unirr	Mean	Irri	Unirr	Mean
Beaver	27 April	23 April	25 April	16 April	16 April	16 April	28 May	25 May	26 May	20 May	18 May	19 May	13 June	8 June	10 June	07 June	05 June	06 June
Soissons	19 April	16 April	17 April	11 April	9 April	10 April	18 May	18 May	18 May	11 May	11 May	11 May	02 June	01 June	01 June	01 June	30 May	31 May
134G	21 April	18 April	19 April	11 April	10 April	11 April	21 May	20 May	20 May	14 May	11 May	12 May	05 June	03 June	04 June	04 June	03 June	03 June
134E	24 April	19 April	21 April	11 April	12 April	12 April	22 May	21 May	21 May	14 May	13 May	13 May	07 June	03 June	05 June	05 June	04 June	04 June

4.1.2.1 Shoot production and survival

2003

The latest genotype to reach GS31, Beaver, produced more total shoots plant^{-1} (22) at this stage than other genotypes in the range 10-16 ($P<0.001$) (data not shown). Averaging across irrigation treatments, Beaver and line 134E lost 34 and 40% of shoots between GS31 and GS61, whereas, Soissons and 134G lost only 21 and 20%, respectively. Post-flowering, at 101, 116 and 133 DAS, Beaver and line 134E maintained more fertile shoots plant^{-1} than line 134G and Soissons ($P<0.001$; Fig. 4.2). At harvest, Beaver (10) maintained more fertile shoots plant^{-1} than line 134E, Soissons and line 134G in the range 4-6 ($P<0.001$). Averaging across genotypes, drought reduced fertile shoots plant^{-1} by 2-3 at all stages after 101 DAS and significantly so at harvest ($P=0.014$). Drought reduced the total shoot number from 16 to 12 at 133 DAS ($P<0.001$).

At harvest, Beaver (13) and line 134E (10) had more infertile shoots than Soissons (6) and 134G (5) ($P<0.001$; Fig 4.4). The genotype x irrigation interaction for fertile shoot number plant^{-1} was not significant at harvest.

2004

Averaging across irrigation treatments, fertile shoots peaked at 109 DAS (Fig.4.3) where Beaver (26) and line 134E (21) produced more shoots plant^{-1} ($P<0.01$) than Soissons (19) and line 134G (16). Genotype differences were not significantly different after 109 DAS. At harvest, differences were not significantly different in the range 11-13 shoots plant^{-1}. The genotype x irrigation interaction effect was also not significant at harvest.

As in 2003, Beaver had more non-surviving shoots plant^{-1} at harvest (33) than by 134G (17) or Soissons (9), indicating poor tiller survival for Beaver compared to other genotypes (Fig. 4.5).

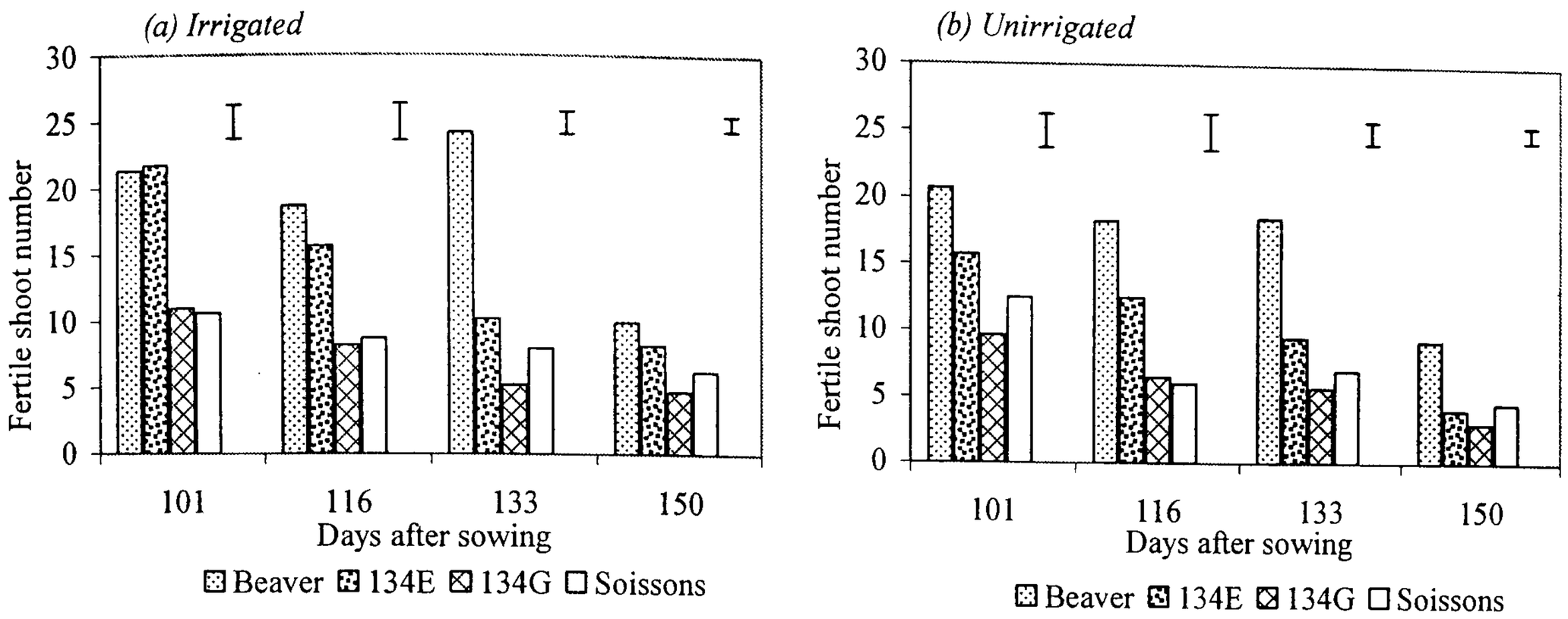


Figure 4.2 Fertile shoot number plant^{-1} of four wheat genotypes in the (a) irrigated and (b) unirrigated treatments in 2003. Error bars show the S.E.D of means (D.F=24) for the genotype x irrigation interaction effect.

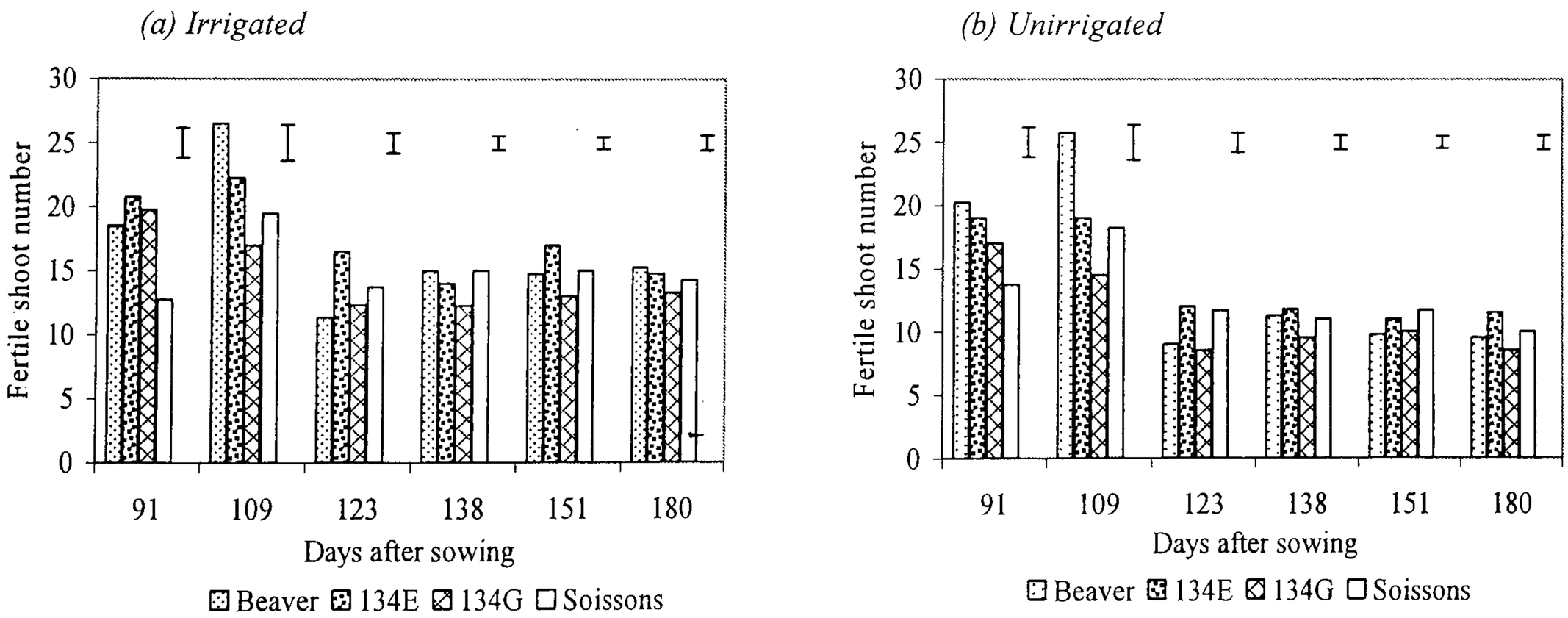


Figure 4.3 Fertile shoot number plant^{-1} of four wheat genotypes in the (a) irrigated and (b) unirrigated treatments in 2004. Error bars show the S.E.D of means (D.F=24) for the genotype x irrigation interaction effect.

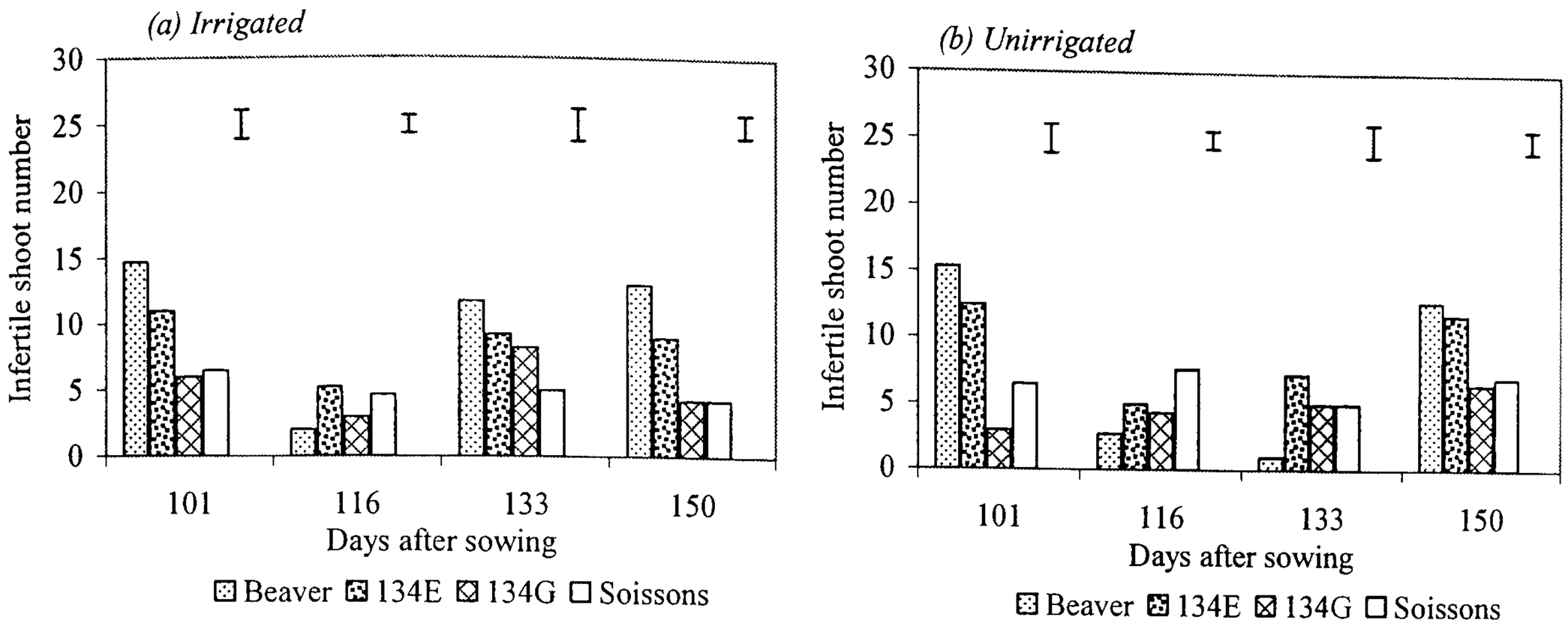


Figure 4.4 Infertile shoot number plant^{-1} of four wheat genotypes in the (a) irrigated and (b) unirrigated treatments in 2003. Error bars show the S.E.D of means (D.F=24) for the genotype x irrigation interaction effect.

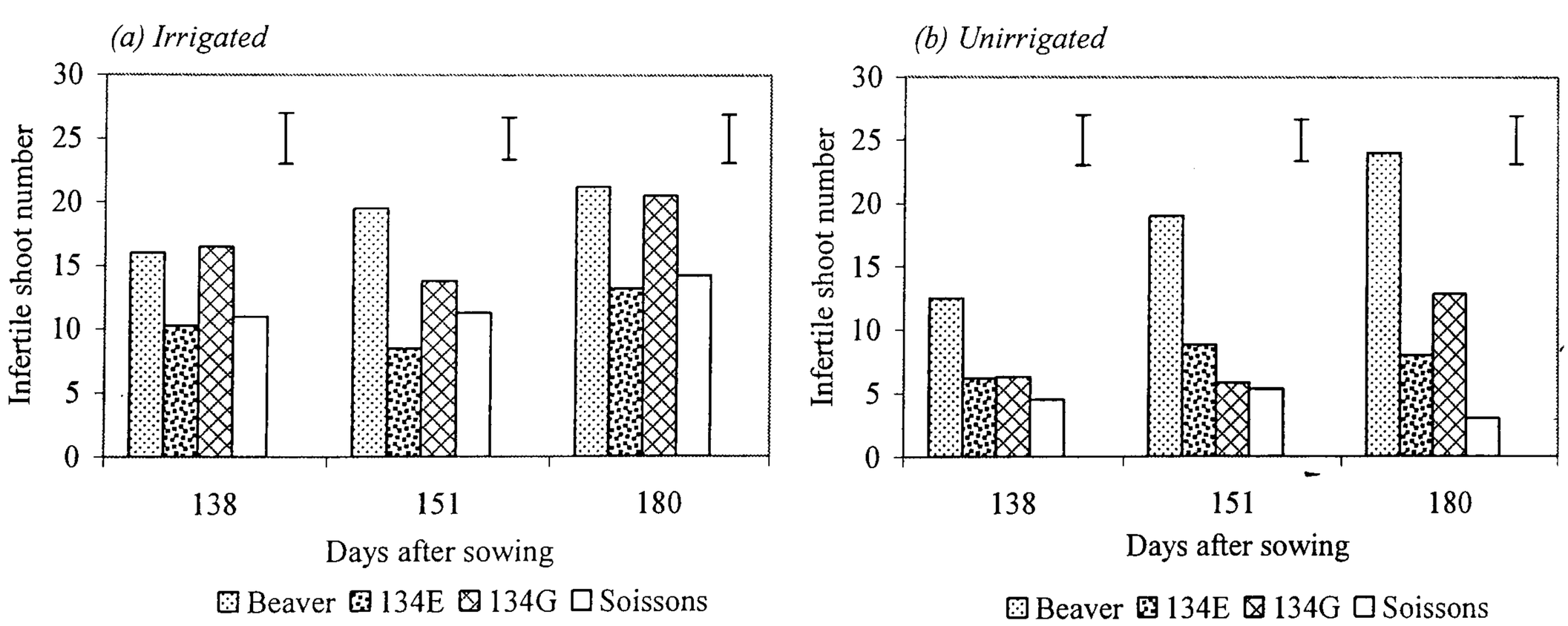


Figure 4.5 Infertile shoot number plant^{-1} of four wheat genotypes in the (a) irrigated and (b) unirrigated treatments in 2004. Error bars show the S.E.D of means (D.F=24) for the genotype x irrigation interaction effect.

4.1.3 Above-ground dry matter

2003

Averaging across irrigation treatments, dry matter plant^{-1} differed between genotypes at 82, 101 DAS and at harvest ($P<0.01$). At harvest, line 134G (9.7g), Soissons (9.1g) and line 134E (8.9g) accumulated more dry matter than Beaver (6.8 g) ($P<0.05$; Fig 4.6a). At 116 DAS, dry matter accumulation reached the maximum. Dry matter growth may have been restricted due to sink limitation associated with heat stress during the pre-anthesis in this experiment. Averaging across genotypes, dry matter was reduced in the unirrigated treatment ($P<0.01$) at all the samplings except at 82 DAS. At harvest, drought reduced mean shoot dry matter from 10.9 to 6.3 g plant^{-1} ($P<0.01$). The reduction ranged from 3.3 to 7.0 g plant^{-1} amongst genotypes, but the genotype x irrigation interaction effect was not significant at harvest.

2004

Averaging across the irrigation treatments, the dry matter plant^{-1} peaked at 151 DAS (GS61). Line 134G accumulated more dry matter than other genotypes at harvest ($P<0.05$; Fig. 4.6 b). Drought reduced the dry matter accumulation between 123 DAS (GS61+3 weeks) and harvest ($P<0.001$; Fig 4.5 b), with a 26.8 g plant^{-1} reduction at harvest ($P<0.001$). The genotype x irrigation interaction was significant at harvest ($P<0.05$) with Soissons maintaining dry matter better than Beaver; the reductions due to drought were 29 and 32 g plant^{-1}, respectively. Line 134E maintained dry matter best amongst all genotypes with a reduction of only 19 g plant^{-1} ($P<0.02$), while the reduction was 31 g plant^{-1} in line 134G.

In summary, at a given growth stage, there was a general trend for dry matter of Beaver to be more depressed under drought than Soissons in both the years. However, this general trend did not usually realise statistical significance. There were 3 plants per column in 2003 and one in 2004, so absolute values for dry matter were greater in 2004.

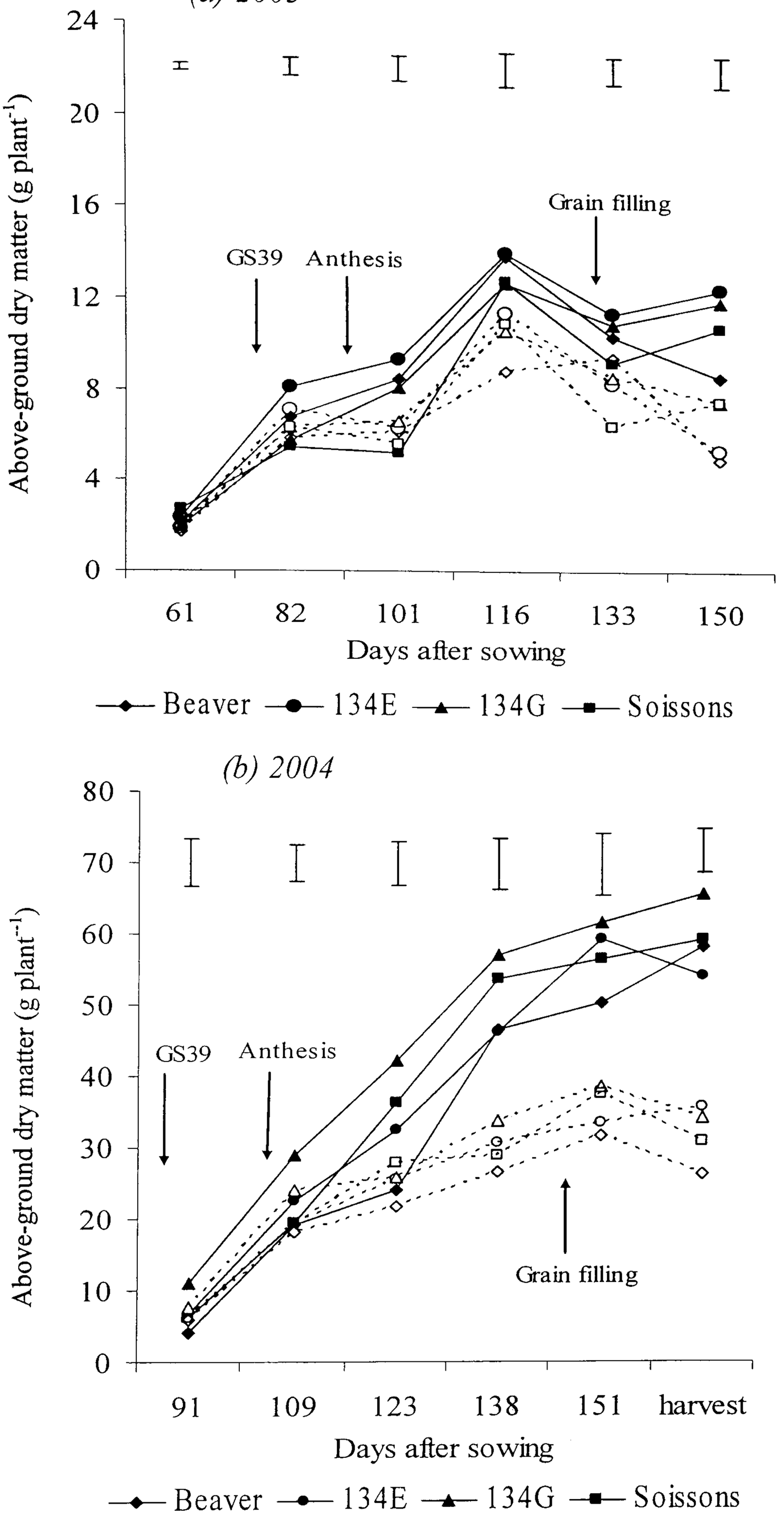


Figure 4.6 Above-ground dry matter accumulation plant^{-1} of four wheat genotypes in the irrigated (closed symbols, solid lines) and unirrigated conditions (open symbols, dotted lines). Error bars show S.E.D of mean (D.F=20) for the irrigation x genotype interaction effect.

4.1.3.1 Dry matter growth of leaves, stems and ears

4.1.3.1.1 Leaf dry matter

In 2003, drought decreased the dry matter accumulation in leaf laminae from 116 DAS (3 weeks after anthesis) ($P<0.01$). Genotypes showed differences in leaf dry matter at all the samplings ($P<0.01$; Fig.4.7). Beaver had higher leaf dry matter plant^{-1} at all samplings ($P<0.01$) with a higher proportion of dry matter in leaves at harvest (0.56g; $P<0.001$) followed by line 134E (0.41g), and Soissons and line 134G (both 0.28g). At harvest, leaf dry matter was higher for Beaver and line 134E (3.5 and 3.6g respectively; $P<0.01$) than for Soissons and line 134G (both 2.6 g). The genotype x irrigation interaction effect was not significant.

In 2004, drought decreased leaf dry matter at harvest from 6.6 to 4.7g ($P<0.01$). A similar trend was noticed at 123, 138 and 151 DAS ($P<0.002$; Fig. 4.7). However, the proportion of biomass in leaves at 123, 151 DAS and at harvest was more under drought ($P<0.05$); and was higher for Beaver than other genotypes ($P<0.05$) at all sampling stages. The genotype x irrigation interaction effect was not significant.

4.1.3.1.2 Stem dry matter

In 2003, drought decreased the partitioning of dry matter to stems at 61, 133 DAS and at maturity ($P<0.01$; Fig.4.7). At harvest, drought reduced stem dry matter from 4.0 to 2.4g ($P<0.001$). The DH lines accumulated more stem dry matter accumulation compared to their parents. At harvest 134E and 134G had a higher proportion of biomass as stem (0.4 each; $P<0.02$) than Soissons and Beaver (0.34 each).

In 2004, drought reduced stem dry matter at all growth stages except 138 and 151 DAS ($P<0.01$); at harvest the reduction was from 13.5 to 9.2g ($P<0.001$). Lines 134E and 134G accumulated more stem dry matter than Soissons or Beaver ($P<0.001$; Fig.4.7). At 151 DAS and harvest, drought reduced the proportion of biomass in the stem ($P<0.05$). The interaction effect was significant at harvest, with Beaver and 134E showing a greater reduction in stem partitioning due to drought ($P=0.005$).

4.1.3.1.3 Ear dry matter

The effects of drought on ear dry matter were diminished in 2003 due to poor overall grain growth which was probably due to combination of a lack of vernalization of

seedlings in the early growth stages and heat stress during grain filling. So the ear partitioning and ear dry matter in 2003 at harvest is not presented here on account of poor post-anthesis ear growth (see Fig.4.7). In 2004, drought reduced ear dry matter at harvest (36.1 cf. 15.4 g; $P<0.001$). Averaging across irrigation treatments, Soissons and 134G accumulated more ear dry matter at 109, 123 and 151 DAS than Beaver or 134E ($P<0.03$; Fig.4.7). At harvest, drought reduced the ear index from 0.64 to 0.50 ($P<0.001$). The proportion of ear dry matter varied among genotypes from 0.63 in Soissons to 0.52 in Beaver ($P<0.05$). The decrease in ear index due to drought was statistically significant in Beaver and line 134G ($P<0.04$), while this was not in Soissons and line 134E.

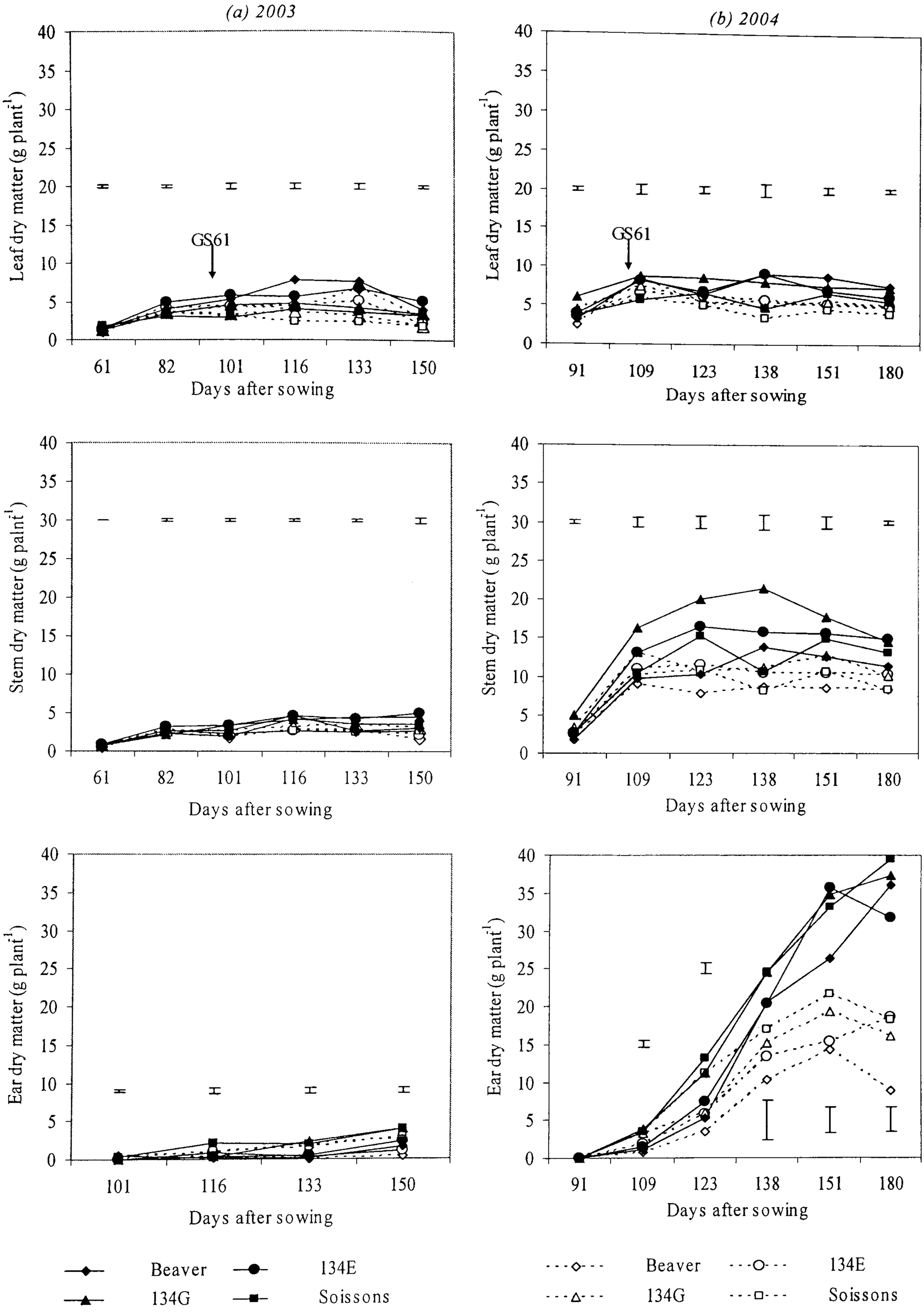


Figure 4.7 Leaf, stem plus leaf sheath and ear dry matter (g plant^{-1}) of four wheat genotypes in irrigated (closed symbols) and unirrigated (open symbols) treatments in (a) 2003 and (b) 2004. Error bars show the S.E.D. of mean (D.F=24) for genotype x irrigation interaction.

4.1.4 Root dry matter and root length density at harvest

2003

The root weight (root weight per unit volume; g m^{-3}) was calculated using a square root transformation as the data were not normally distributed. This was calculated for the 0-20, 40-60 and 80-100 cm depths. Averaging across irrigation treatments, genotypes differed in root biomass at depths to 60 cm depth ($P<0.02$; Fig.4.8). Irrigation improved root biomass in the 0-20 cm depth compared to the unirrigated treatment ($P=0.09$). While drought increased root biomass at deeper depths of 80-100 cm ($P=0.08$). Under irrigation, at 0-20 cm depth line 134E accumulated more root biomass 27.6g m^{-3} ($P=0.02$) and differed significantly over the other genotypes. Beaver showed a trend for greater root dry matter than Soissons at the 0-20 and 40-60 cm depths but the differences were not statistically significant (Figure 4.8). None of the genotype x irrigation interactions was significant.

Root length densities (RLD; root length per unit volume; cm cm^{-3}) were greater in the unirrigated treatment and differences were statistically significant at the 80-100 cm depth ($P<0.01$). Genotypic differences were weakly significant at 0-20 ($P=0.06$) and 40-60 cm ($P=0.09$) depths. Under irrigation, line 134E had higher RLD than other genotypes associated with its higher mean root weight (Fig. 4.10). At 80-100 cm depth, drought increased RLD ($P<0.01$) and genotype differences were not statistically significant. The interaction effect was weakly significant with Soissons having a greater increase in RLD at 80-100 cm depth than other genotypes under drought ($P=0.07$). The magnitude of the increase in RLD at 80-100 cm depth was in the sequence Soissons > Beaver > 134G > 134E.

2004

Root dry weight was higher in the irrigated treatment at all depths ($P<0.01$). Under irrigation, line 134G accumulated more root dry matter than the other genotypes at all soil depths ($P<0.01$) (Fig. 4.9 a & b). In addition, there was a trend for Soissons to have lower root biomass than Beaver at all the depths. At depths 60-80 cm and 80-100 cm, DH lines had higher root biomass compared to their parents and significantly so for line 134G. At 80-100 cm depth, the interaction effect was significant ($P=0.02$) with line 134G losing more root dry matter due to drought than line 134E, Soissons and Beaver.

RLD was greater under irrigation than under drought at all depths to 60 cm ($P<0.01$) and at 80-100 cm ($P=0.09$). Under irrigation, RLD was greater for Beaver than for Soissons ($P<0.01$; Fig. 4.11), except at 80-100 cm where both had similar values. Under irrigation, line 134G showed a trend for greater RLD than 134E at most depths (Fig. 4.11). A significant interaction was found at 80-100 cm depth, where Beaver increased RLD under drought compared to decreases for other genotypes ($P<0.04$).

In summary it is evident that root biomass and RLD increased under irrigation at deeper depths. Irrigated DH lines had greater root dry matter, e.g. 134G. Beaver had a greater RLD than Soissons in 2004 or Soissons had a greater RLD than Beaver in 2003, but only at 80-100 cm.

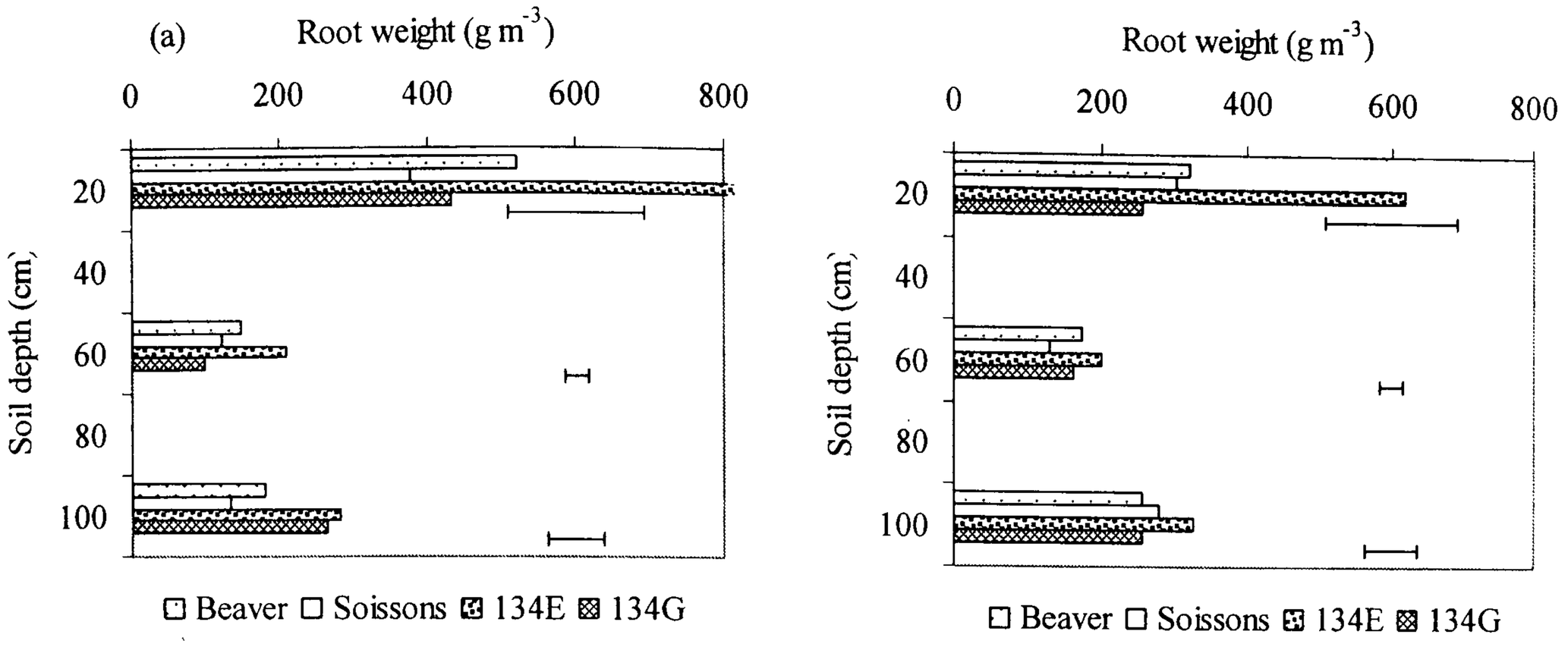


Figure 4.8 Root weight (g m^{-3}) at anthesis for the *(a)* irrigated and *(b)* unirrigated treatments for four wheat genotypes measured in 0-20, 40-60 and 80-100 cm depth soil horizons in 2003. Errors bars show S.E.D of mean of genotype x irrigation interaction (D.F=24). Data are square root transformed.

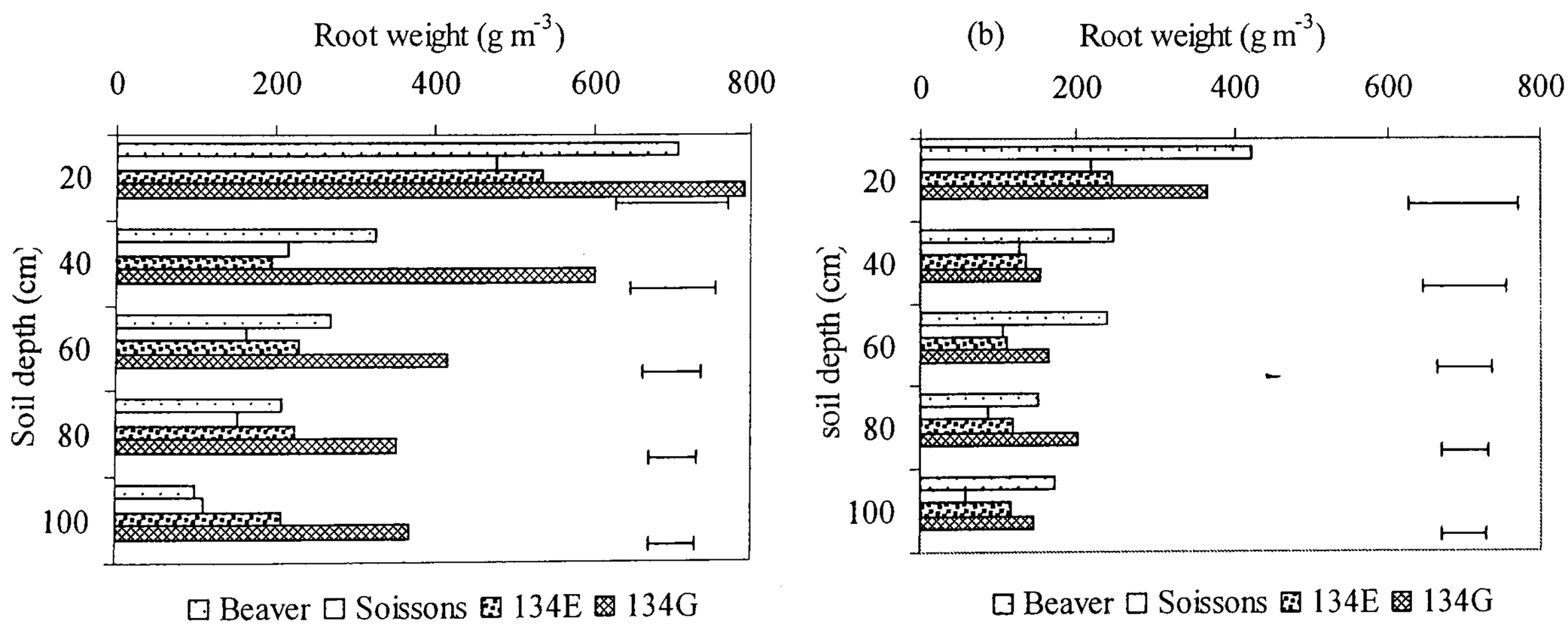


Figure 4.9 Root weight (g m^{-3}) at anthesis for the *(a)* irrigated and *(b)* unirrigated treatments for four wheat genotypes measured in 0-20,20-40, 40-60, 60-80 and 80-100 cm depth soil horizons in 2004. Errors bars show S.E.D of mean of genotype x irrigation interaction (D.F=16). Data are square root transformed.

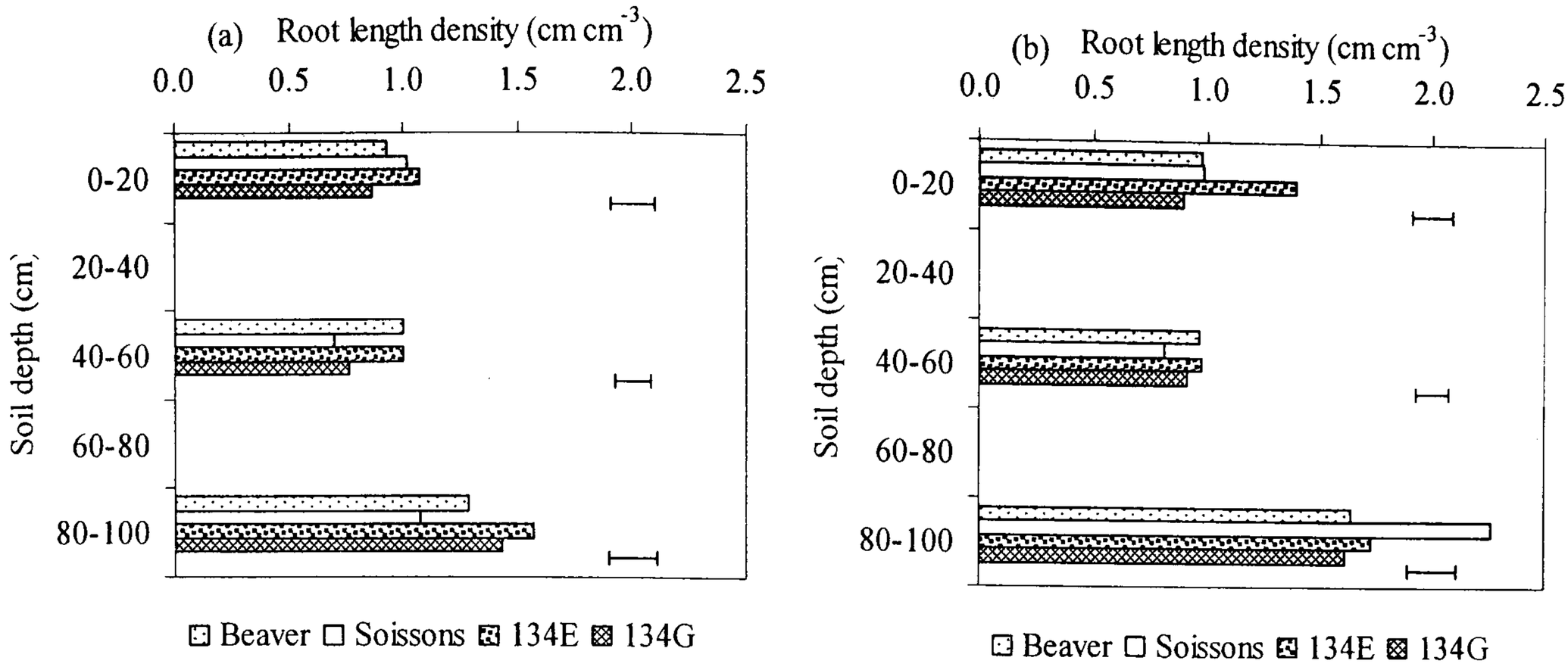


Figure 4.10 Profiles of root length densities (cm cm^{-3}) at anthesis for the *(a)* irrigated and *(b)* unirrigated treatments for four wheat genotypes measured in 0-20, 40-60 and 80-100 cm depth soil horizons in 2003. Errors bars show S.E.D. of mean of genotype x irrigation interaction (D.F=24).

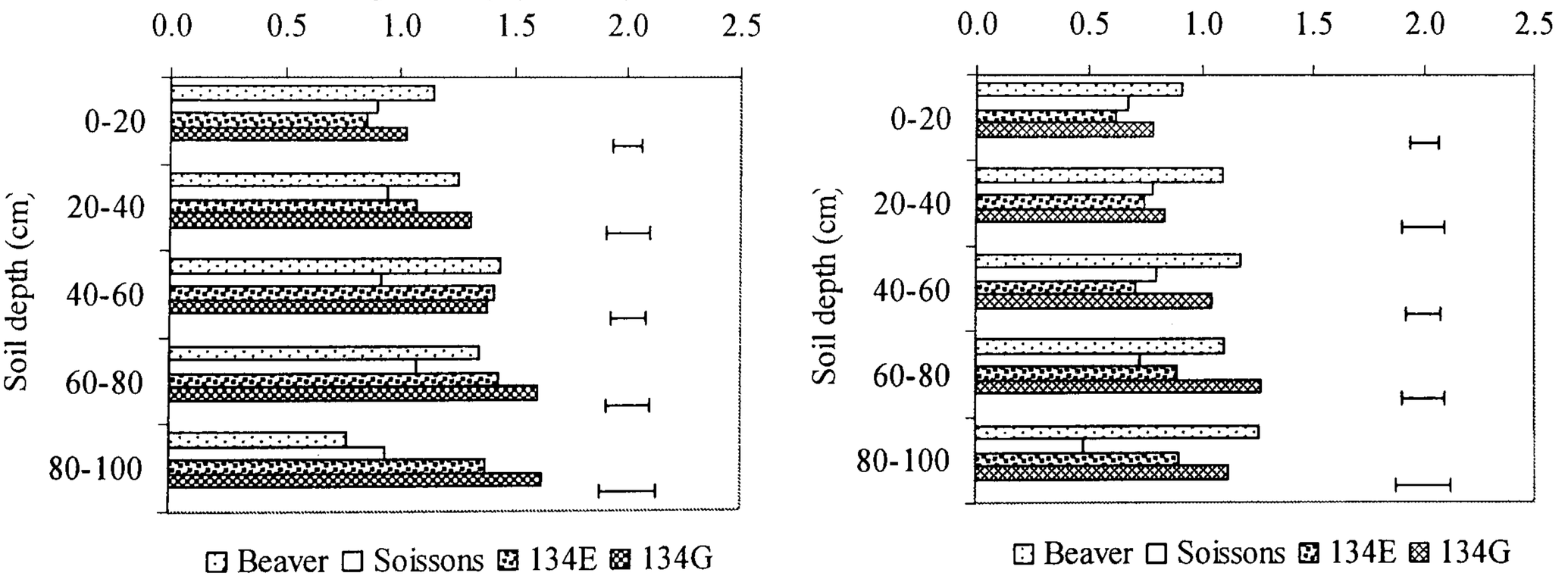


Figure 4.11 Profiles of root length densities (cm cm^{-3}) at anthesis for the *(a)* irrigated and *(b)* unirrigated treatments for four wheat genotypes measured in 0-20,20-40, 40-60, 60-80 and 80-100 cm depth soil horizons in 2004. Errors bars show S.E.D. of mean of genotype x irrigation interaction (D.F=16).

4.1.4.1 Specific root length (m g^{-1}), root diameter (mm) and surface area (cm^2)

The data on specific root length, root diameter and surface area are shown in Appendix VII and the genetic differences of the four wheat genotypes are described here.

2003

The specific root length (SRL; m g^{-1}) was analysed using a logarithmic transformation of data for the 0-20, 40-60 and 80-100 cm depths. Drought increased SRL significantly below the top 20 cm depth ($P<0.01$). Under irrigation, Soissons had significantly higher SRL at 0-20 cm depth than line 134E ($P=0.01$) and at 80-100 cm depth than DH lines ($P<0.04$). However, both the parents behaved similarly at all depths. Interaction effects were not significant.

Thicker roots (i.e. larger root diameter) were found in irrigated treatments at 0-20 cm depth ($P<0.03$) and in droughted treatments at 80-100 cm ($P<0.001$). Effects of genotype and interaction were not evident for root diameter ($P>0.2$). However, under droughted conditions, at 80-100 cm depth Beaver had thicker roots (0.66 mm) than Soissons (0.51) and each of the DH lines (0.55 mm).

Effects for the total root surface area (TRSA) in the soil profile showed trends similar to root diameter. TRSA was greater in irrigated treatments at 0-20 cm depth ($P<0.05$) and in unirrigated treatments at 80-100 cm depth ($P<0.001$). Beaver and line 134E had higher TRSA than Soissons and line 134G, respectively.

2004

SRL values tended to increase under droughted conditions, significantly so at 0-20, 20-40 and 60-80 cm depths ($P<0.05$). Under droughted conditions, Soissons exhibited greater SRL than Beaver and line 134G at depths 0-20 and 20-40 cm ($P<0.01$). At 80-100 cm depth, Soissons had greatest SRL followed by line 134E, 134G and Beaver, but the differences were not statistically significant. The interaction effect was weakly significant at 20-40 cm depth, wherein Soissons and line 134G increased SRL values under droughted conditions more than the other two genotypes ($P= 0.052$).

Drought significantly reduced the mean root diameter at all depths ($P<0.01$). Under irrigation, line 134G had thickest roots at 0-20, 20-40 and 60-80 cm depths followed by

Beaver and line 134E with Soissons having finer roots at all depths (P<0.03). Genotype x irrigation interaction was weakly significant at 0-20 cm (P= 0.054) and 80-100 cm (P= 0.057) depths with line 134G having more reduced root diameter under droughted conditions than other genotypes.

Irrigation resulted in higher TRSA at all soil depths (P< 0.03). The values were higher at 60-80 cm depth in irrigated than in unirrigated treatments (435 c.f. 267 cm^2; P<0.001). Under irrigation, among genotypes, 134G followed by Beaver had significantly higher TRSA over Line 134E and Soissons above 40 cm depth (P<0.05). At 40-60 cm depth, Soissons had significantly the least TRSA than rest of the genotypes (P=0.03). At 60-80 cm depth, 134G had significantly higher TRSA (575 cm^2; P<0.001). At 80-100 cm depth, line 134G and 134E had significantly higher TRSA than Beaver and Soissons (P=0.02). The genotype x irrigation effect was significant at the 80-100 cm depth; with line 134G showing decreased TRSA under drought (588 c.f. 297 cm^2; P=0.034) than other genotypes.

4.1.5 Green area (cm^2 $plant^{-1}$)

2003

Drought significantly decreased green area at all the samplings (P<0.01; Fig.4.12a). Under irrigation, green area was greater for Beaver than for Soissons at 133 DAS (GS61+4wks) and at harvest (P<0.03). At 101 DAS, there was a trend for line 134G and Beaver to lose more green area under drought than Soissons and line 134E (P=0.07). Under irrigation, Soissons and line 134G produced the least green area at 133 DAS (173 and 149 cm^2 $plant^{-1}$, respectively); and at harvest (42 and 33 cm^2 $plant^{-1}$, respectively). The differences in the development of genotypes resulted in variations in the production of green area at a given sampling. Although, the genotype x irrigation interaction was generally not statistically significant, there was a consistent trend for Beaver to lose more green area under drought than other genotypes.

2004

Green area decreased after 109 DAS (one week after anthesis) due to drought (P <0.001). Genotypic differences were significant at all the samplings (P<0.05). At 91 DAS, under irrigation, line 134G exhibited greatest green area (1088 cm^2 $plant^{-1}$) with other

genotypes in the range 610-779 cm^2 (Fig. 4.12b). At 109 DAS, 134E had maximum green area (1743 cm^2; P = 0.05) under irrigation followed by line 134G, while parents had the least. At 123 DAS, three weeks after anthesis, Beaver showed an increase in green area over line 134G (P = 0.043), but did not differ with line 134E and Soissons. At 138 DAS, line 134G and Beaver still showed greater green area than line 134E and Soissons (P = 0.026). However, a significant genotype x irrigation interaction was found (P = 0.033), wherein, with line 134G and Soissons lost more green area under drought as compared to 134E and Beaver. At 151 DAS Beaver which maintained significantly more green area in irrigated conditions (1071 cm^2; P = 0.002) than other genotypes. It is probable that genotype differences at any given sampling were partly as a result of developmental differences. For example, fast developing Soissons reached maturity at least ten days earlier than Beaver. Similarly, line 134G also reached maturity earlier than line 134E.

4.1.5.1 Leaf chlorophyll concentration estimate (SPAD units)

In 2003, the main effect of drought was not significant for leaf chlorophyll concentration. Under irrigation, genotypes differed at 68 DAS (GS39+1week). Beaver and Soissons exhibited higher chlorophyll content (45.59 and 44.67 units respectively) than lines 134E and 134G (41.19 and 36.52 units, respectively; P<0.001). Similar genotype differences were observed at GS61+3 weeks (P<0.001; Table 4.2). The main effects of irrigation and genotypes were not significantly different at GS61+4 weeks and GS61+5weeks. The irrigation interaction effect was not significant at any sampling. Average across all measurements, the genotype difference was weakly significant with Beaver and Soissons showing higher SPAD values than the two DH lines (P=0.055).

In 2004, the leaf chlorophyll concentration was only measured at GS61 and GS61+3weeks and differences among genotypes, irrigation or for their interactions were not statistically significant.

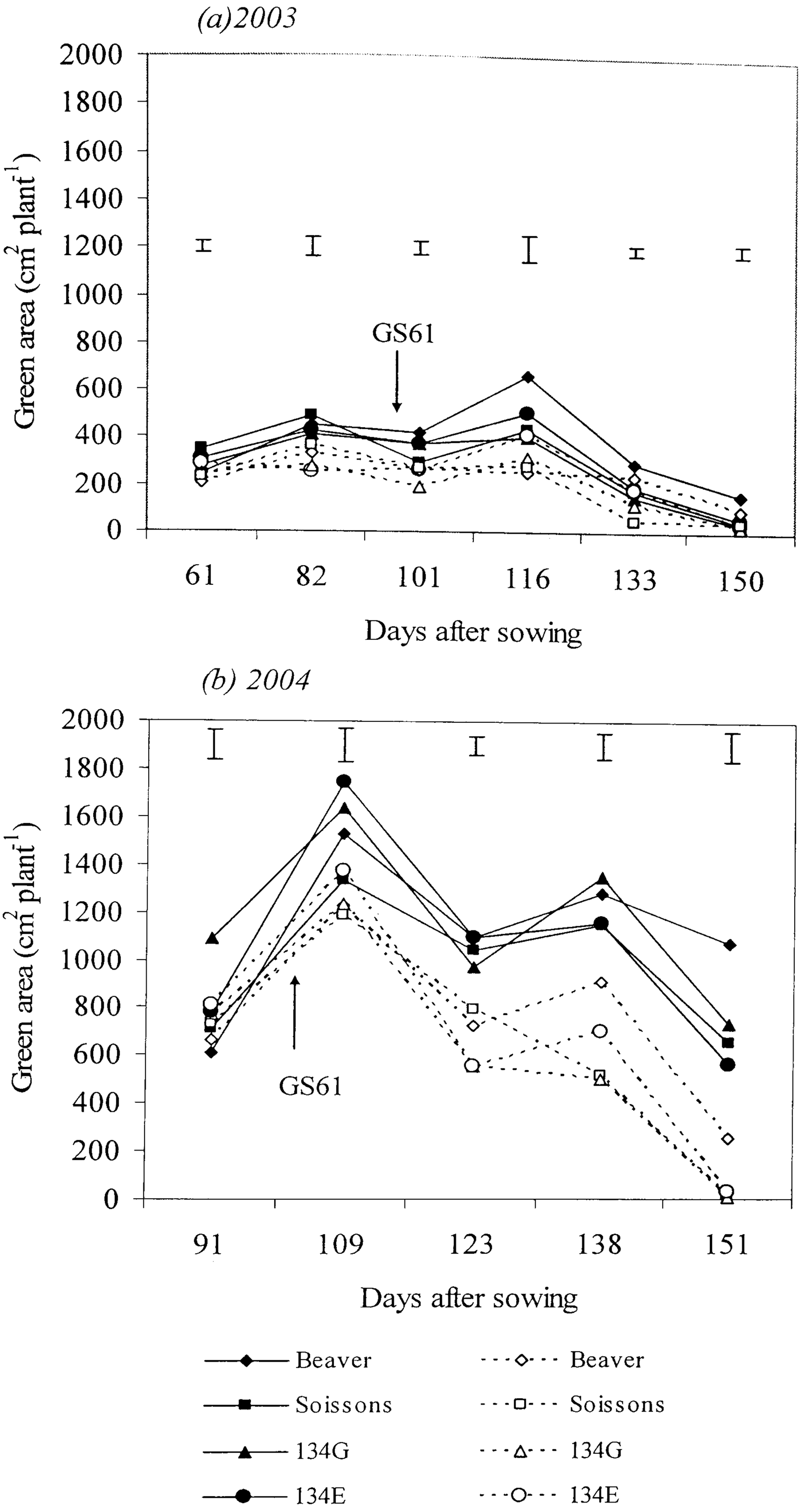


Figure 4.12 Green area (cm^2 $plant^{-1}$) of four wheat genotypes grown under irrigated (closed symbols) and unirrigated (open symbols) treatments in (a) 2003 and (b) 2004 glasshouse experiments. Error bars are S.E.D of mean (D.F=24) for genotype x irrigation interaction effects.

4.1.5.2 Leaf rolling

Leaf rolling percentage (difference between actual and rolled width; Begg, 1980) of the main shoot flag leaf was only measured in the droughted treatment in 2004. Line 134G exhibited a tendency for a higher degree of leaf rolling than other genotypes in the range 31.4-58.4%, but differences were not statistically significant (Table 4.2). Percent leaf rolling measured on secondary and tertiary tillers was not consistent.

Table 4.2 SPAD units and percent leaf rolling of four wheat genotypes in irrigated and unirrigated conditions.

	SPAD units on main shoot flag leaf					% leaf rolling on main shoot flag leaf
	2003				2004	2004
Genotypes	GS39+1wk	GS61+3wk	GS61+4wk	GS61+5wk	GS61+3wk	GS61+2wk
Irrigated						
Beaver	44.7	46.6	53.3	48.2	57.4	-
Soissons	45.6	47.6	50.0	47.8	52.9	-
134G	41.2	43.1	55.0	46.8	54.8	-
134E	36.5	38.1	51.1	47.5	53.9	-
Mean	42.0	43.9	52.4	47.6	54.8	-
Unirrigated						
Beaver	45.0	47.0	54.1	44.7	56.7	36
Soissons	45.5	47.5	51.3	46.1	54.2	53
134G	37.9	39.8	52.4	47.9	58.7	58
134E	40.5	42.1	51.3	50.3	58.6	51
Mean	42.2	44.1	52.3	47.2	57.4	50
S.E.D. (df)						
Irrigation (24)	1.11	1.13	1.37	1.52	1.20	-
Genotype (24)	1.57	1.60	1.93	2.15	1.69	12.2 (12)
Ir x Gen. (24)	2.22	2.25	2.73	3.05	2.39	-

4.1.5.3 Leaf relative water content (RWC)

Relative water content of the flag leaf of the main shoot was only measured in 2004. Averaging across irrigation treatments, Beaver and Soissons invariably maintained higher RWC than lines 134E and 134G throughout the growing season. Averaging across genotypes, drought reduced RWC at all the measurement dates (Fig.4.13; $P<0.05$), on average by 4.4%. Irrespective of the sampling time the RWC of all the genotypes was maintained at more than 80% under irrigated conditions between 99 DAS

and 138 DAS. A reduction of 3-5% was observed both in irrigated and droughted conditions after anthesis. The ranking of the genotypes varied little in both the irrigated and unirrigated treatments. This ranking was in the order of Beaver > Soissons > 134E > 134G. The genotype x irrigation interaction was not statistically significant.

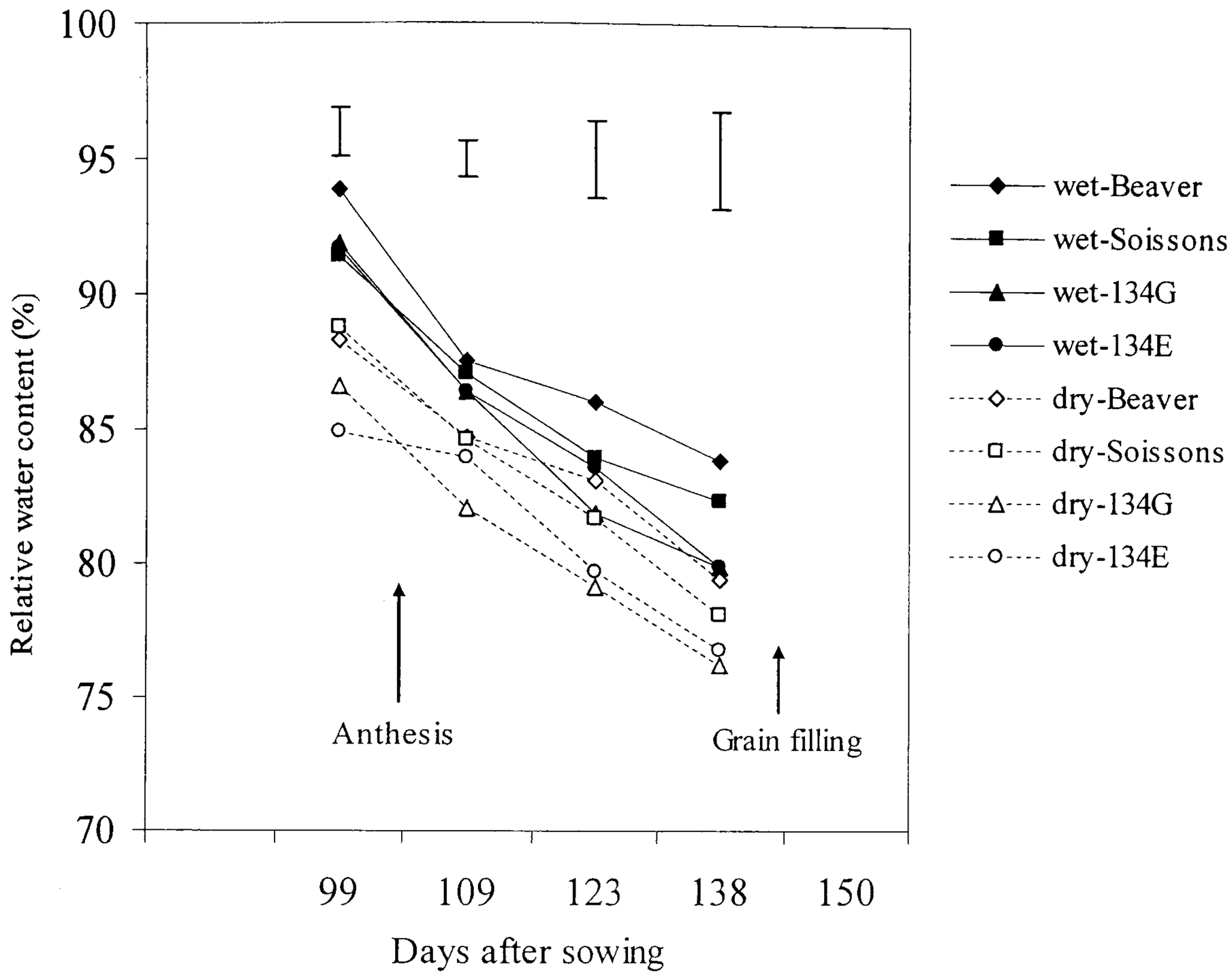


Figure 4.13 Relative water content (RWC) of the flag leaf of the main shoot of four wheat genotypes at different growth stages in irrigated and unirrigated treatments in 2004. Errors bars show S.E.D for the genotype x irrigation interaction effect.

4.1.6 Plant water use

4.1.6.1 Cumulative water use

The time-course changes in water use of the four genotypes in 2003 and 2004 are presented in Figure 4.14 (a) and (b). In 2003, the amount of water applied to columns during the experiment was 6.7 l in the unirrigated treatment and 15.4 l in the irrigated treatment (Fig. 4.14 a). The pattern of water use differed between genotypes and irrigation treatments ($P<0.001$). The water use by Soissons was smaller throughout the growing period compared to Beaver, both in the irrigated and unirrigated treatments ($P<0.05$). Line 134E used more water than 134G; these two lines were intermediate in their water use. The interaction effect for water use was not statistically significant.

In 2004, the amount of water applied to columns in the droughted and irrigated treatments were 2.7 and 10.1 l respectively (Fig. 4.14 b). Between the initial and final samplings, Line 134G and Beaver used more water (both 9 l) than line 134E and Soissons ($P=0.01$). The season-long water use by the genotypes was related to their development and maturity. Soissons, on account of its faster development than Beaver used less water, whilst line 134G used more water than 134E. The relative abilities of genotypes to maintain water use under irrigation appear to correspond to differences in rooting traits. Line 134G and Soissons exhibited a trend to maintain water uptake than line 134E and Beaver.

In 2003, Beaver and line 134E had higher total root surface area and root diameter, particularly in the droughted treatments. In addition a larger root volume was present in these genotypes. In 2004, total root surface area of line 134G and Beaver was more in the top 60 cm soil horizon, but at deeper profiles DH lines had higher root surface area and root volume than the parental lines ($P=0.02$). Beaver and 134G had greater RLD. On the other hand, Soissons always exhibited the smallest root diameter, root surface area and root volume and had greater specific root length (SRL; root length/ root weight), a measure of fineness of roots which resulted in effective capture of moisture in the soil profile.

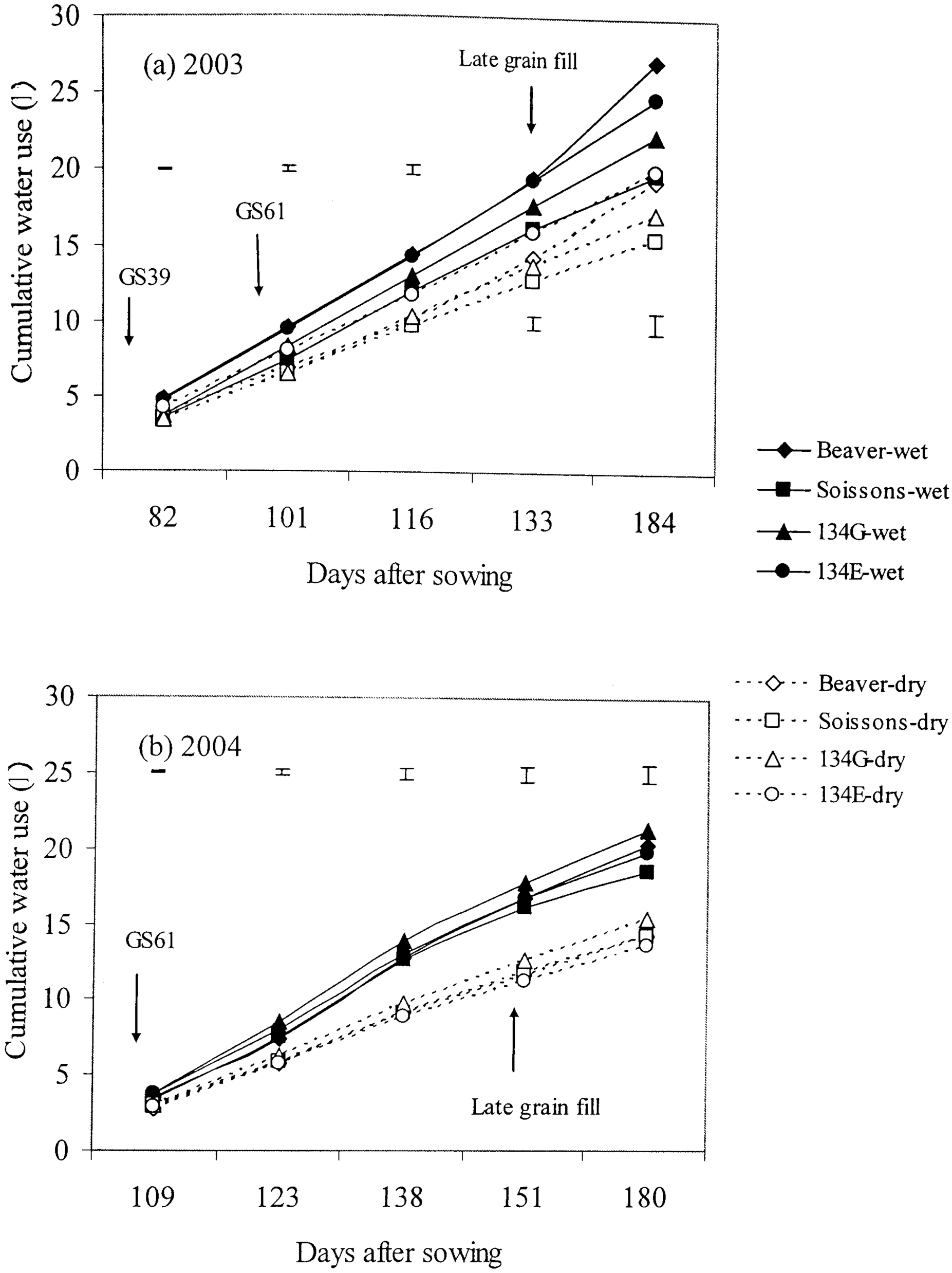


Figure 4.14 Cumulative water use (l) between samplings per column of four wheat genotypes in irrigated and unirrigated treatments in (a) 2003 and (b) 2004. Error bars show S.E.D. of mean of genotypes.

4.1.7 Season-long water use efficiency (WUE) (g dry matter kg^{-1} H_2O)

The relationship between total cumulative (from initial sampling to early grain filling in 2003 or to maturity in 2004) dry matter production and water use for irrigated and unirrigated plants is shown in Figures 4.15 and 4.16. To avoid the influence of heat stress, WUE was calculated only up to 116 DAS coinciding with GS61+4weeks in 2003. The dry matter to transpired water ratio (g kg^{-1}) for the irrigated and droughted plants was estimated from the slopes of the respective regression lines of cumulative dry matter against cumulative water use for the sequential samplings. To calculate WUE, a simple linear model fitted through the origin was applied to data for each irrigation /genotype combination in each year. These are presented in Figures 4.15 and 4.16. The mean slopes of genotypes in the irrigated and unirrigated treatments were compared using a t-test (paired two samples for means).

In 2003, a higher slope was found for the irrigated plants (Fig. 4.15). WUE ranged between 2.75 and 3.32 g kg^{-1} under irrigated conditions and 2.49 and 3.18 g kg^{-1} under droughted conditions (R^2 values > 0.8; $P<0.05$). A similar effect to that in 2003 was also observed in 2004, where WUE ranged between 2.90 and 3.43 g kg^{-1} under irrigated conditions (R^2 values > 0.8; $P<0.001$) and between 2.47 and 2.77 g kg^{-1} under droughted conditions ($P< 0.001$; Fig.4.16).

Soissons had a higher slope under irrigated conditions in both the years (3.23 and 3.43 respectively) as compared to Beaver (2.76 and 2.90 g kg^{-1} respectively). Among DH lines, line 134E had higher slopes in 2003 in both the irrigation treatments (3.32 and 3.18 g kg^{-1} respectively) than line 134G (2.75 and 2.66 g kg^{-1} respectively). However, in 2004, line 134E had a higher slope only under droughted conditions (2.75) compared to line 134G.

The irrigated and unirrigated data of each year were separately subjected to parallel regression analysis with the GENSTAT 8.1 statistical package to test for differences amongst genotypes.

In 2003, parallel regression analysis showed that under irrigation the variance accounted for by fitting the linear model to all data was 80.0%. Varying the slope by genotype did

not significantly improve the fit (P<0.65; accounting for 93.4% variance, estimated s.e = 4.52). Similarly the slopes for genotypes under drought were not significantly different.

In 2004, genotypes within individual irrigation treatments differed (P<0.01) on the basis of parallel regression analysis. In a separate analysis for the irrigated treatments, WUE was greater for Soissons (P<0.01) than other genotypes. Whilst under unirrigated conditions, a similar tend was observed. Further, pooled analysis of both irrigated and unirrigated treatment groups showed differences between the genotypes (P<0.001) explaining 91.2 % variance.

Applying parallel regression analysis to all data in 2003 and 2004 in irrigated and unirrigated conditions, the variance accounted for by fitting the linear model to all data was 89.2% (P< 0.01). Varying the slope by genotype did not significantly improve the fit (P<0.4, s.e = 5.60). However, parallel regression analysis showed that varying the slope by irrigation treatment did significantly improve the fit, WUE was greater under irrigated than under unirrigated conditions.

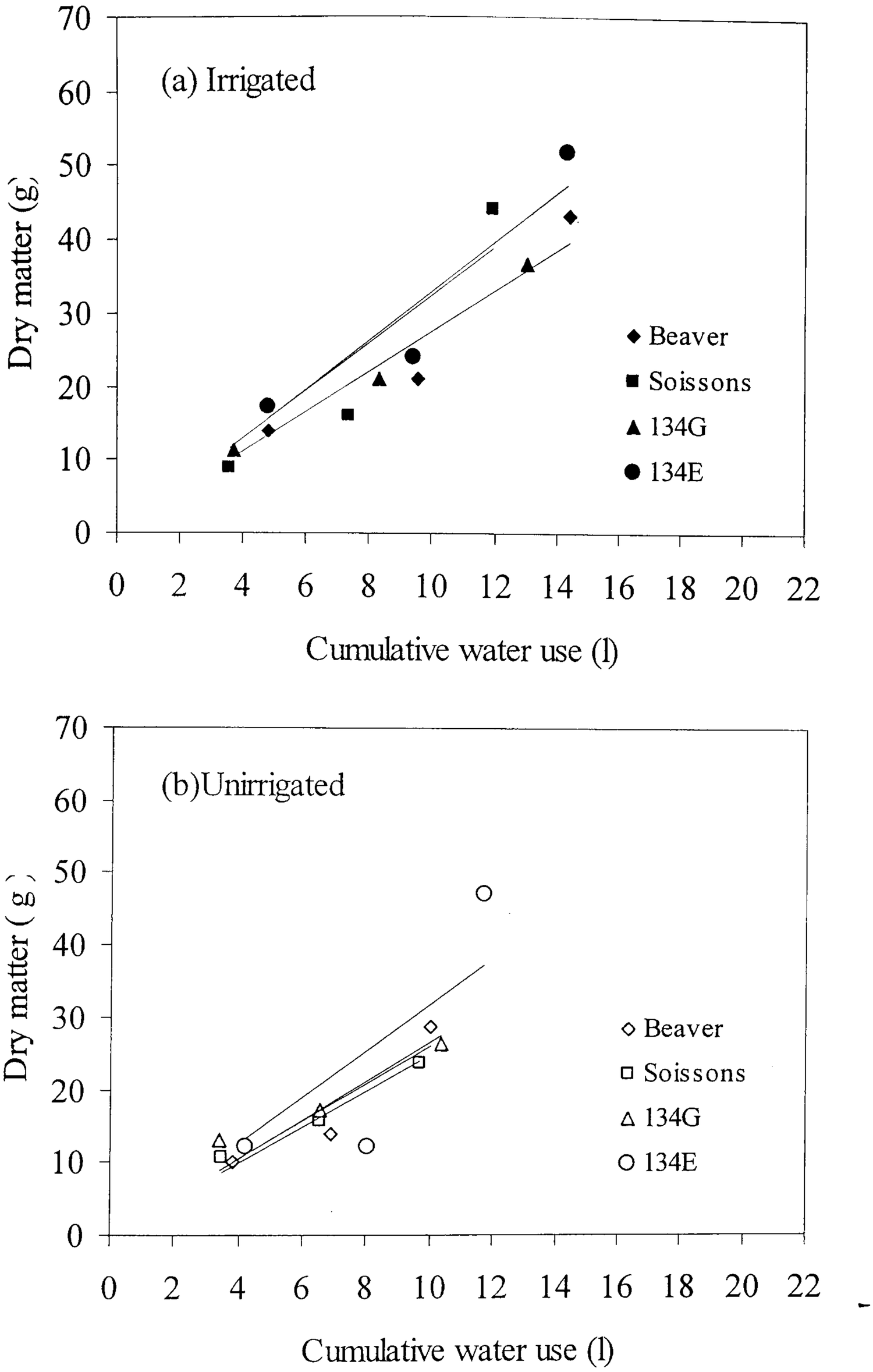


Figure 4.15 Regression of above-ground dry matter (g) on cumulative water use (l) for four wheat genotypes under (a) Irrigated (*Beaver* $y=2.76x$ ($R^2=0.91$); *Soissons* $y=3.23x$ ($R^2=0.86$); *134G* $y=2.75x$ ($R^2=0.99$); *134E* $y=3.32x$ ($R^2= 0.89$)) and (b) Unirrigated (*Beaver* $y=2.60x$ ($R^2=0.88$); *Soissons* $y=2.49x$ ($R^2=0.95$); *134G* $y=2.66x$ ($R^2=0.80$); *134E* $y=3.18x$ ($R^2= 0.66$)) treatments in 2003 (n=16).

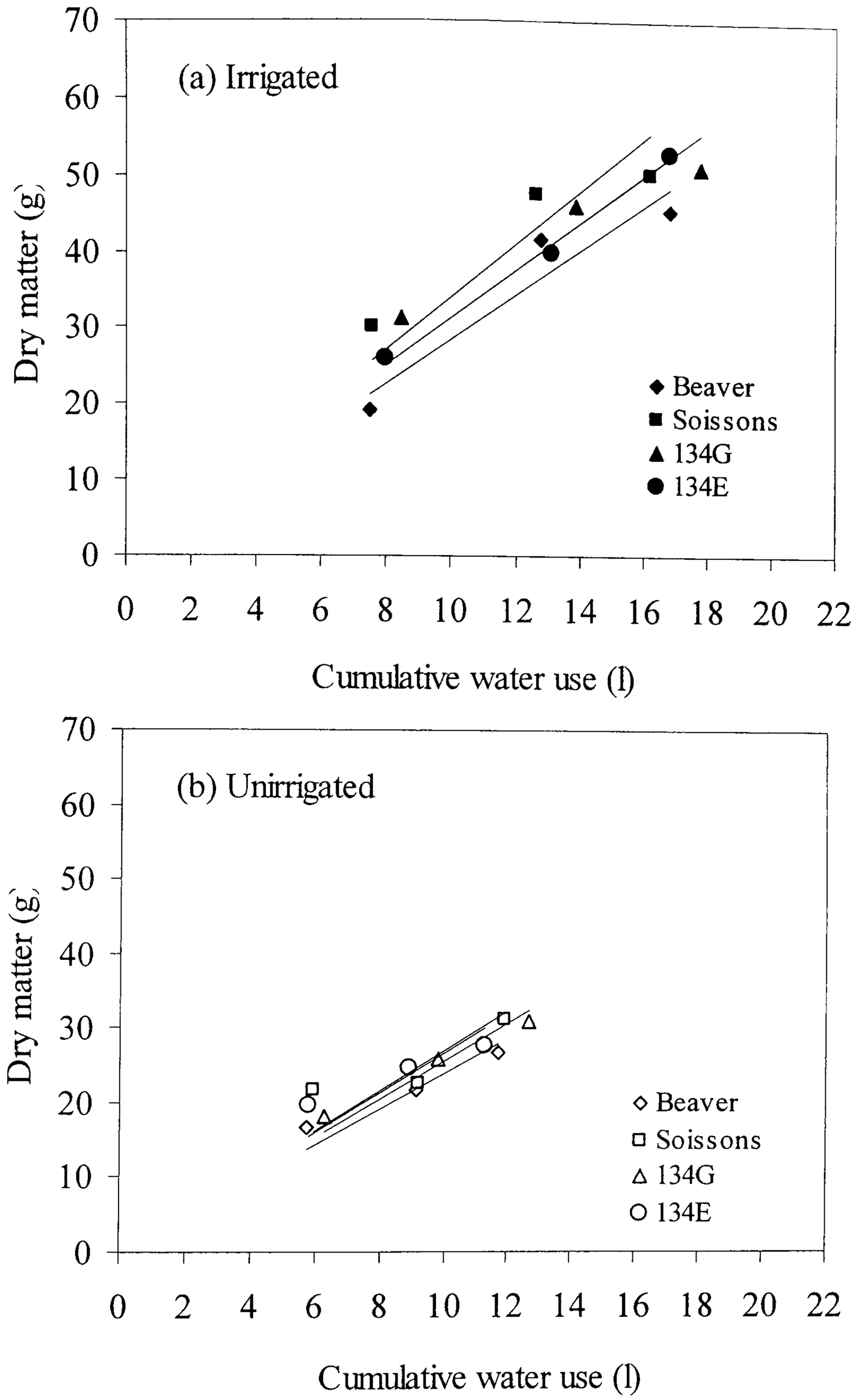


Figure 4.16 Regression of above-ground dry matter (g) on cumulative water use (l) for four wheat genotypes under (a) Irrigated (*Beaver* $y=2.90x$ ($R^2=0.93$); *Soissons* $y=3.43x$ ($R^2=0.92$); *134G* $y=3.16x$ ($R^2=0.86$); *134E* $y=3.15x$ ($R^2= 0.97$)) and (b) Unirrigated (*Beaver* $y=2.47x$ ($R^2=-0.48$); *Soissons* $y=2.77x$ ($R^2=0.63$); *134G* $y=2.65x$ ($R^2=0.47$); *134E* $y=2.75x$ ($R^2=0.52$)) treatments in 2004 (n=16).

4.1.8 Grain yield, harvest biomass and harvest index

In 2003, the grain growth was detrimentally affected due to lack of vernalization and heat stress in the glasshouse. So the differences amongst the genotypes and their responses to irrigation treatments for grain dry matter are of limited value. However, these problems did not occur in 2004 and good grain growth was maintained.

In 2003, averaging across irrigation treatments, grain yield of wheat genotypes ranged from 0.82 to 1.82 g plant^{-1} (P=0.03). These amounts of grain growth are unusually low, e.g. as compared to potential grain growth of 1.66 g ear^{-1} (HGCA wheat growth guide, 1998). Averaging across genotypes, drought reduced yield from 1.68 to 1.18 g plant^{-1} (P=0.09; Table 4.3). Soissons yielded more than Beaver, on average, by 0.68 g plant^{-1}. Soissons lost more yield (0.59 g) under drought than Beaver (0.14 g). Beaver also maintained yield better than line 134G (0.74 g) and 134E (0.54 g) respectively.

In 2004, irrigation increased the grain dry matter plant^{-1} by 17 g plant^{-1} (P<0.001). Averaged across irrigation treatments, Soissons produced a higher grain DM plant^{-1} (23.5 g) than Beaver (16.9 g) with intermediate values for lines 134E (19.9 g) and 134G (19.4 g; P<0.02). Under irrigation, Soissons produced higher grain yield (32.5; P=0.02) than Beaver (28.8g), line 134G (27.0g) or 134E (25.5g). The genotype x irrigation effect was significant (P<0.05) with Beaver showing a much larger depression in yield due to drought than other genotypes (P<0.03).

Harvest biomass decreased significantly due to drought in 2004 (P<0.001). Under irrigation, line 134G produced highest biomass (65.8 g plant^{-1}; P<0.02) with other genotypes in the range 54.3-59.5 g plant^{-1}. However, a larger reduction in biomass was observed with Beaver and line 134G than Soissons and line 134E under droughted conditions (P<0.03). The yield interaction though was mainly accounted for by the HI interaction, with Beaver exhibiting a significantly larger decrease in HI under drought than other genotypes. The two DH lines and Soissons maintained yield well in droughted conditions therefore on account of better maintenance of HI under droughted conditions (P<0.03; Table 4.3).

Drought affected the production of above-ground biomass in both the years (P<0.01). Cross-year analysis indicated that harvest biomass decreased from 38 g to 22 g plant^{-1}

under droughted conditions ($P<0.001$; Table 4.3). Beaver produced more harvest biomass than Soissons under irrigated conditions but lost more of its harvest biomass under unirrigated conditions. With respect to the DH lines, irrigated line 134G had consistently more biomass than line 134E. Soissons had a higher value for HI than other genotypes in both irrigated and droughted conditions in both the years ($P<0.01$). HI decreased under unirrigated conditions (0.28 cf. 0.21). Soissons had greater HI under irrigated (0.33) and unirrigated (0.28) than rest of the genotypes. The interaction effect was significant wherein Beaver decreased HI more under drought than other genotypes ($P<0.05$).

4.1.9 Thousand grain weight, ear number plant^{-1}, grains ear^{-1}

The data for yield components for 2004 are presented in Table 4.4. Drought decreased ear number plant^{-1} and grains ear^{-1}, and thousand grain weight (TGW) ($P<0.05$). With restricted water availability, TGW decreased by 7.5 g ($P<0.01$). Under irrigation, line 134G had significantly a higher TGW (55.3 g; $P<0.02$) than line 134E and Soissons (48 g each) or Beaver (45 g). The irrigation x genotype interaction was not significant for this yield component.

Although, ear number plant^{-1} and grains ear^{-1} decreased due to drought ($P<0.001$) there were no statistically significant differences amongst genotypes. Soissons had a trend for more grains ear^{-1} under irrigation than Beaver or the two DH lines ($P=0.09$). The interaction effects for ear plant^{-1} and grains ear^{-1} were not significant.

Table 4.3 Grain yield (g plant^{-1}), harvest biomass (g plant^{-1}) and harvest index of four wheat genotypes in irrigated and unirrigated treatments in 2003 and 2004.

	Yield (g plant^{-1})			Harvest Biomass (g plant^{-1})			HI		
	2003	2004	Cross-year mean	2003	2004	Cross-year mean	2003	2004	Cross-year mean
Irrigated									
Beaver	1.20	28.81	14.86	16.14	58.50	37.32	0.05	0.49	0.27
134E	1.09	25.46	13.28	19.40	54.32	36.86	0.06	0.47	0.26
134G	2.33	26.96	14.65	17.19	65.77	41.48	0.12	0.41	0.27
Soissons	2.11	32.46	17.28	13.75	59.47	36.61	0.12	0.55	0.33
Mean	1.68	28.42	15.02	16.62	59.52	38.07	0.09	0.48	0.28
Unirrigated									
Beaver	1.06	4.95	2.75	11.09	26.51	18.80	0.04	0.18	0.11
134E	0.55	14.41	7.48	12.36	35.78	24.07	0.04	0.40	0.22
134G	1.59	11.82	6.71	12.82	34.45	23.64	0.11	0.34	0.22
Soissons	1.52	14.61	8.06	10.49	30.90	20.69	0.11	0.46	0.28
Mean	1.18	11.45	6.25	11.69	31.91	21.80	0.08	0.35	0.21
S.E.D. (df)									
Irrigation (24)	0.28^{ns}	1.38^{***}	0.64^{***}	0.62^{***}	1.57^{***}	0.77^{***}	0.02^{ns}	0.03^{***}	0.15^{***}
Genotype (24)	0.40^{*}	1.96^{*}	0.91^{**}	0.88^{**}	2.22^{*}	1.09^{***}	0.02^{**}	0.04^{**}	0.02^{***}
Interaction (24)	0.57^{ns}	2.77^{*}	1.29^{**}	1.24^{ns}	3.14^{*}	1.54^{ns}	0.03^{ns}	0.06^{**}	0.03^{*}

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

Table 4.4 Thousand grain weight, number of ears plant^{-1} and grains ear^{-1} of four wheat genotypes in irrigated and unirrigated treatments in 2003 and 2004.

	Thousand grain weight (g)			Ears plant^{-1}			Grains ear^{-1}		
	2003	2004	Cross-year mean	2003	2004	Cross-year mean	2003	2004	Cross-year mean
Irrigated									
Beaver	25.8	45.0	35.4	2.67	15.25	8.96	12.6	43.3	27.9
134E	16.9	48.6	32.8	6.96	14.75	10.85	12.9	37.8	25.3
134G	31.0	55.3	43.2	4.67	13.25	8.96	18.5	37.0	27.7
Soissons	24.0	48.2	36.1	6.33	14.25	10.29	14.8	47.4	31.1
Mean	24.4	49.3	36.9	5.16	14.38	9.77	14.7	41.4	28.0
Unirrigated									
Beaver	24.8	40.6	32.7	0.42	9.50	5.17	15.6	17.3	16.4
134E	20.0	39.8	29.9	3.12	11.50	7.31	8.7	32.0	20.4
134G	33.9	49.0	41.5	3.33	8.50	5.92	17.8	27.9	22.8
Soissons	25.1	37.8	31.4	4.50	10.00	7.25	16.9	38.6	27.7
Mean	25.9	41.8	33.9	2.84	9.88	6.41	14.8	28.9	21.9
S.E.D (df)									
Irrigation (24)	1.80^{ns}	2.15^{**}	1.42^{*}	0.48^{**}	0.81^{***}	0.45^{***}	2.73^{ns}	3.57^{**}	2.13^{**}
Genotype (24)	2.55^{***}	3.04^{*}	2.01^{***}	0.68^{**}	1.15^{ns}	0.64^{**}	3.86^{ns}	5.04^{ns}	3.02^{ns}
Interaction (24)	3.60^{ns}	4.30^{ns}	2.85^{ns}	0.96^{ns}	1.62^{ns}	0.90^{ns}	5.45^{ns}	7.13^{ns}	4.27^{ns}

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

4.1.10 Gas-exchange measurements

The net photosynthetic rate (*A*), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and transpiration rate (E) were measured on the flag leaf of main shoots once or twice a week under natural sunlight from GS39 to GS61+ 4 weeks. From these measurements carboxylation capacity and instantaneous transpiration efficiency (ITE) were calculated as the ratios between A/C_i and A/E respectively. Data from a set of four measurement dates in 2003 and five measurement dates in 2004 for the above parameters were analysed (for all data see Appendix); the mean values across all measurement dates in 2003 and 2004 are presented in Tables 4.5 and 4.6, respectively.

4.1.10.1 Net photosynthetic rate (A; μmol $m^{-2}s^{-1}$)

In 2003, averaging across genotypes, there was a marginal trend for irrigation to improve *A* (P=0.13; Table 4.5). Averaging across irrigation treatments, line 134G (5.1 μmol $m^{-2}s^{-1}$) had higher net photosynthetic rate (P< 0.05) than line 134E (2.9 μmol $m^{-2}s^{-1}$). The mean values of *A* for Beaver and Soissons were similar (4.46 and 4.32 μmol $m^{-2}s^{-1}$, respectively). A similar trend was observed for genotype performance under irrigation, except Soissons showed a slight increase in *A* values. Under drought, line 134G and Soissons showed higher *A* values than Beaver and line 134E. However, the genotypes x irrigation interaction effects were not statistically significant.

In 2004, *A* was much higher than in 2003 probably on account of differences in the development as gas exchange measurements were concentrated in the active growth phase between GS39 to GS61. In addition, there was one plant per column rather than three. Again *A* was higher under irrigated than under unirrigated conditions (4.6 c.f 3.8 μmol $m^{-2}s^{-1}$), but not significantly so. Genotype differences were significant with Soissons having greater assimilation rates than other genotypes and lines 134G and 134E having greater rates than Beaver (P=0.032). The interaction effect was not significant.

Averaging across years, drought reduced *A* by 0.94 μmol $m^{-2}s^{-1}$ (P <0.05; Table 4.5). Soissons had higher *A* than other genotypes (P <0.01) and lines 134G and 134E showed

greater *A* values than Beaver. The genotype x irrigation interaction however was not significant.

4.1.10.2 Stomatal conductance (g_s; mmol $m^{-2}s^{-1}$)

In 2003, irrigation increased stomatal conductance (P<0.01). Averaging across irrigation treatments, line 134G (137 mmol $m^{-2}s^{-1}$) had higher g_s than 134E (73 mmol $m^{-2}s^{-1}$; P <0.05). Among parents, Soissons had higher g_s (120 mmol $m^{-2}s^{-1}$) than Beaver (112 mmol $m^{-2}s^{-1}$). Under irrigation, line 134G had significantly higher g_s rates than other genotypes and Soissons and Beaver had higher g_s rates than line 134E (P=0.013). The interaction effect was not significant.

The conductance values were higher in 2004 than in 2003. A higher stomatal conductance was found under irrigation (556 mmol $m^{-2}s^{-1}$) than under drought (377 mmol $m^{-2}s^{-1}$; P=0.002). Averaging across irrigation regimes, genotypes did not differ (P=0.31). Under irrigation, Soissons had higher g_s values (737 mmol m^{-2} s^{-1}) than other genotypes; and 134G and 134E had higher values than Beaver. Although, the interaction effects were not significant (P=0.16), Soissons and line 134G showed much larger reductions in g_s than Beaver and line 134E.

Cross-year analysis indicated g_s was reduced by 228 mmol $m^{-2}s^{-1}$ due to drought (P <0.01; Table 4.5).

4.1.10.3 Intercellular CO_2 concentration (C_i; mmol CO_2 mol^{-1}air)

In 2003, drought increased the mean C_i from 253 to 287 mmol CO_2 mol^{-1}air (P <0.02). Although genotype differences were not consistent across reading dates, on average line 134G and Soissons had lower C_i than line 134E or Beaver. The interaction effect was significant, with Beaver and line 134E showing a larger increase in C_i under drought than Soissons and line 134G (P <0.05; Table 4.5).

In 2004, drought decreased C_i values significantly from 475 to 460 mmol CO_2 mol^{-1}air (P = 0.037) and genotype effects were not statistically significant in the range 460 (134E) to 480 (Beaver) mmol CO_2 mol^{-1}air. The irrigation x genotype interaction effect was, however, significant (P<0.001). C_i was reduced by drought in Beaver and line 134G, there was effect on Soissons and was increased in line 134E.

Cross-year analysis showed a significant irrigation x genotype interaction, with Beaver and line 134E showing a larger increase in C_i under drought (P<0.02; Table 4.5) while, Soissons and line 134G showed reduced C_i.

4.1.10.4 Carboxylation capacity (A/C_i ratio)

The ratio of assimilation rate to intercellular CO_2 concentration was used to determine the carboxylation capacity (Table 4.6). In 2003, irrigation increased A/C_i ratio (18.6 cf. 13.9; P <0.05). Among genotypes, under irrigation, line 134G and Beaver had higher ratios (21.7 and 21.1, respectively) than Soissons and line 134E (16.2 and 15.6, respectively; P=0.08). Although, the ratio decreased under drought in general the irrigation x genotype interaction was not statistically significant.

In 2004, the carboxylation capacity was similar under irrigated and unirrigated conditions. Under irrigation, Soissons (33.3) and line 134E (27.7) had a higher A/C_i ratio than Beaver (18.8; P< 0.03). The interaction effect was significant (P< 0.05); with line 134G and Beaver increasing the ratio under drought in contrast to Soissons and line 134E which showed decreases under drought.

When averaged across the two years, a higher A/C_i ratio was found under irrigation (P<0.03), and the carboxylation capacity of Soissons (24.8) was higher than other genotypes in the range 21.7-19.9 (P=0.01). There was a trend for an interaction with line 134G and Beaver increasing the ratio under drought in contrast to Soissons and line 134E which showed decreased ratios under drought.

4.1.10.5 Transpiration rate (E; mmol $m^{-2}s^{-1}$)

In 2003, drought reduced mean transpiration rates (E) from 2.1 to 1.8 mmol $m^{-2}s^{-1}$ (P=0.13). Averaging across irrigation regimes, E for 134G (2.4 mmol $m^{-2}s^{-1}$) was higher than for 134E (1.3; P=0.012). Under irrigation, Soissons (2.3 mmol $m^{-2}s^{-1}$) had a higher transpiration rate than Beaver (1.8 mmol m^{-2} s^{-1}; P<0.02). The interaction effect was not consistent across measurement dates and the interaction effect for the mean value across readings was not statistically significant (Table 4.6). The values in 2004 were generally higher than in 2003. In 2004, irrigation and genotype effects and their interaction were

not significant. Genotypes values were similar in the range 4.46–4.79 mmol $m^{-2}s^{-1}$. However, there was a trend for drought to reduce transpiration rates from 5.1 to 4.2 mmol $m^{-2}s^{-1}$.

Cross-year analysis revealed the irrigated treatment had higher rates of transpiration than the unirrigated treatment ($P<0.001$), but there were no significant differences among the genotypes. The genotype x irrigation interaction was significant ($P=0.014$) and showed that overall the reduction in *E* under drought was greater in Soissons and line 134G than in Beaver ($P<0.02$).

4.1.10.6 Instantaneous transpiration efficiency (ITE; A/E; mmol CO_2 mol^{-1} H_2O)

Instantaneous transpiration efficiency was calculated as the ratio between net photosynthetic rate (*A*) and transpiration rate (*E*). In 2003, irrigation and genotype effects and their interaction effect were not statistically significant (Table 4.6). All the genotypes had a similar ITE in the range of 2.15–2.48. In 2004, ITE increased under droughted conditions by 0.37 mmol mol^{-1}($P=0.1$). Soissons had a higher ITE (2.95) than other genotypes in the range 2.30-2.74 mmol mol^{-1}. There was a significant interaction with line 134G showing a more increased ITE under droughted conditions ($P=0.002$) than other genotypes. Cross-year analysis showed that ITE increased under drought (2.64 cf. 2.32; $P<0.05$). Although the genotype differences were not statistically significant, the irrigation x genotype interaction was, with ITE under drought increasing more for line 134G than for other genotypes, and more for Soissons than for Beaver and line 134E ($P<0.05$).

Table 4.5 Mean values of net photosynthetic rate (A), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) for four wheat genotypes in irrigated and unirrigated treatments in 2003 and 2004.

	Net photosynthetic rate (μmol $m^{-2}s^{-1}$)			Stomatal conductance (mmol m^{-2} s^{-1})			Intercellular CO_2 (mmol CO_2 mol^{-1} air)		
	2003	2004	Cross-year mean	2003	2004	Cross-year mean	2003	2004	Cross-year mean
Irrigated									
Beaver	4.93	9.29	7.11	121.6	463	292	237.2	493.3	365.2
134E	3.66	12.35	8.00	95.4	481	288	254.5	444.1	349.3
134G	5.56	10.60	8.08	169.0	543	356	252.3	495.0	373.6
Soissons	4.13	15.54	9.83	140.3	737	439	266.6	467.3	366.9
Mean	4.57	11.94	8.26	131.5	556	344	252.7	474.9	363.8
Unirrigated									
Beaver	3.99	8.98	6.48	102.7	381	242	335.7	467.4	401.5
134E	2.10	10.29	6.20	51.1	404	228	290.1	475.1	382.6
134G	4.68	12.73	8.71	105.2	361	233	265.5	429.2	347.4
Soissons	4.52	11.24	7.88	99.6	362	231	256.9	465.2	361.0
Mean	3.82	10.81	7.32	89.6	377	234	287.1	459.2	373.1
S.E.D(df)									
Irrigation (24)	0.47^{ns}	0.94^{ns}	0.42^{*}	12.85^{**}	50.6^{*}	52.7^{**}	13.28^{*}	7.12^{ns}	7.64^{ns}
Genotypes (24)	0.67^{*}	1.33^{*}	0.60^{**}	18.18^{*}	71.6^{ns}	74.5^{ns}	18.78^{ns}	10.07^{ns}	10.81^{ns}
Interaction (24)	0.95^{ns}	1.88^{ns}	0.85^{ns}	25.71^{ns}	101.3^{ns}	106.1^{ns}	26.56^{*}	14.24^{*}	15.28^{*}

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

Table 4.6 Mean values of transpiration rate (*E*), carboxylation capacity and instantaneous transpiration efficiency for four wheat genotypes in irrigated and unirrigated treatments in 2003 and 2004.

	Transpiration rate (mmol $m^{-2}s^{-1}$)			Carboxylation capacity (mmol $m^{-2}s^{-1}$)			ITE (mol mol^{-1})		
	2003	2004	Cross-year mean	2003	2004	Cross-year mean	2003	2004	Cross-year mean
Irrigated									
Beaver	1.77	4.37	3.07	21.1	18.8	19.95	2.78	2.31	2.54
134E	1.58	5.06	3.32	15.6	27.7	21.67	2.30	2.43	2.37
134G	2.63	5.63	4.13	21.7	21.6	21.67	2.12	1.94	2.03
Soissons	2.31	5.41	3.86	16.2	33.3	24.75	1.73	2.95	2.34
Mean	2.07	5.12	3.60	18.6	25.4	22.01	2.23	2.41	2.32
Unirrigated									
Beaver	2.04	4.55	3.30	11.9	19.3	15.63	2.19	2.29	2.24
134E	1.05	4.52	2.79	7.2	21.7	14.47	2.00	2.36	2.18
134G	2.06	3.59	2.83	18.6	29.7	24.18	2.27	3.54	2.91
Soissons	1.85	4.05	2.95	18.0	24.3	21.13	2.72	2.94	2.83
Mean	1.75	4.18	2.97	13.9	23.8	18.85	2.29	2.78	2.54
S.E.D. (df)									
Irrigation (24)	0.21^{ns}	0.58^{ns}	0.17^{**}	2.26^{*}	2.15^{ns}	1.40^{*}	0.21^{ns}	0.34^{ns}	0.11^{*}
Genotypes (24)	0.29^{*}	0.83^{ns}	0.23^{ns}	3.19^{ns}	3.03^{*}	1.98^{**}	0.29^{ns}	0.49^{ns}	0.16^{ns}
Interaction (24)	0.41^{ns}	1.17^{ns}	0.33^{*}	4.51^{ns}	4.29^{*}	2.79^{ns}	0.42^{ns}	0.68^{*}	0.22^{**}

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

4.1.11 Carbon isotope discrimination analysis (‰; $\Delta^{13}C$)

2003

Leaf lamina samples at anthesis were analysed for $\Delta^{13}C$ and indicated that effects of irrigation, genotype and their interaction were not significant. The coefficient of variation associated with this trait was about 5%. The effects of irrigation or genotypes and/or their interactions were not statistically significant. However, averaging across genotypes, there was a trend for drought to reduce the $\Delta^{13}C$ values considerably. The reductions in $\Delta^{13}C$ under drought were apparently greater for line 134G and Soissons than the two other genotypes (Fig.4.17 a).

2004

$\Delta^{13}C$ was analysed on mature grains. Drought in the unirrigated treatment reduced delta by *c.* 1.40 ‰ ($P<0.001$; Fig. 4.17 b). The coefficient of variation was 3.2%. Averaging across irrigation regimes, line 134G had smaller $\Delta^{13}C$ (16.25 ‰) than other genotypes in the range 16.89-17.60 ‰ ($P<0.001$). However, there was no statistically significant difference for $\Delta^{13}C$ between Beaver and Soissons. The interaction effect was significant with line 134G and Beaver showing larger decreases in $\Delta^{13}C$ under drought than other genotypes. Beaver had a higher $\Delta^{13}C$ value than Soissons under irrigated conditions (18.04 and 17.90 ‰ respectively). However, the reverse was true under droughted conditions but these differences were not statistically significant. As compared to all the genotypes, line 134G showed lower $\Delta^{13}C$ values both in wet and dry stands (17.16 and 15.33 ‰ respectively; $P<0.02$). The magnitude of reduction in $\Delta^{13}C$ was higher in Beaver compared to Soissons to an extent of 2.30 ‰ ($P<0.05$). Under drought, the final grain yield of Beaver was lower and the grains were not properly developed compared to other genotypes. The data was found to vary as there was a G x E interaction for the grain $\Delta^{13}C$. So a separate analysis for leaf $\Delta^{13}C$ was done.

In contrast to grain, leaf $\Delta^{13}C$ values at anthesis showed a trend similar to that in 2003 and indicated that drought reduced $\Delta^{13}C$ values by *c.* 1.39 ‰ ($P=0.002$; Fig.4.17 c). Line 134G and Soissons showed lower $\Delta^{13}C$ (18.10 & 19.49 ‰, respectively) in the unirrigated treatment compared to line 134E and Beaver ($P<0.05$). This is consistent with higher ITE observed for line 134G and Soissons in the droughted treatment compared to Beaver and line 134E. The interaction effect was not significant indicting

that $\Delta^{13}C$ is highly heritable among the genotypes. The coefficient of variation was 4.4 %.

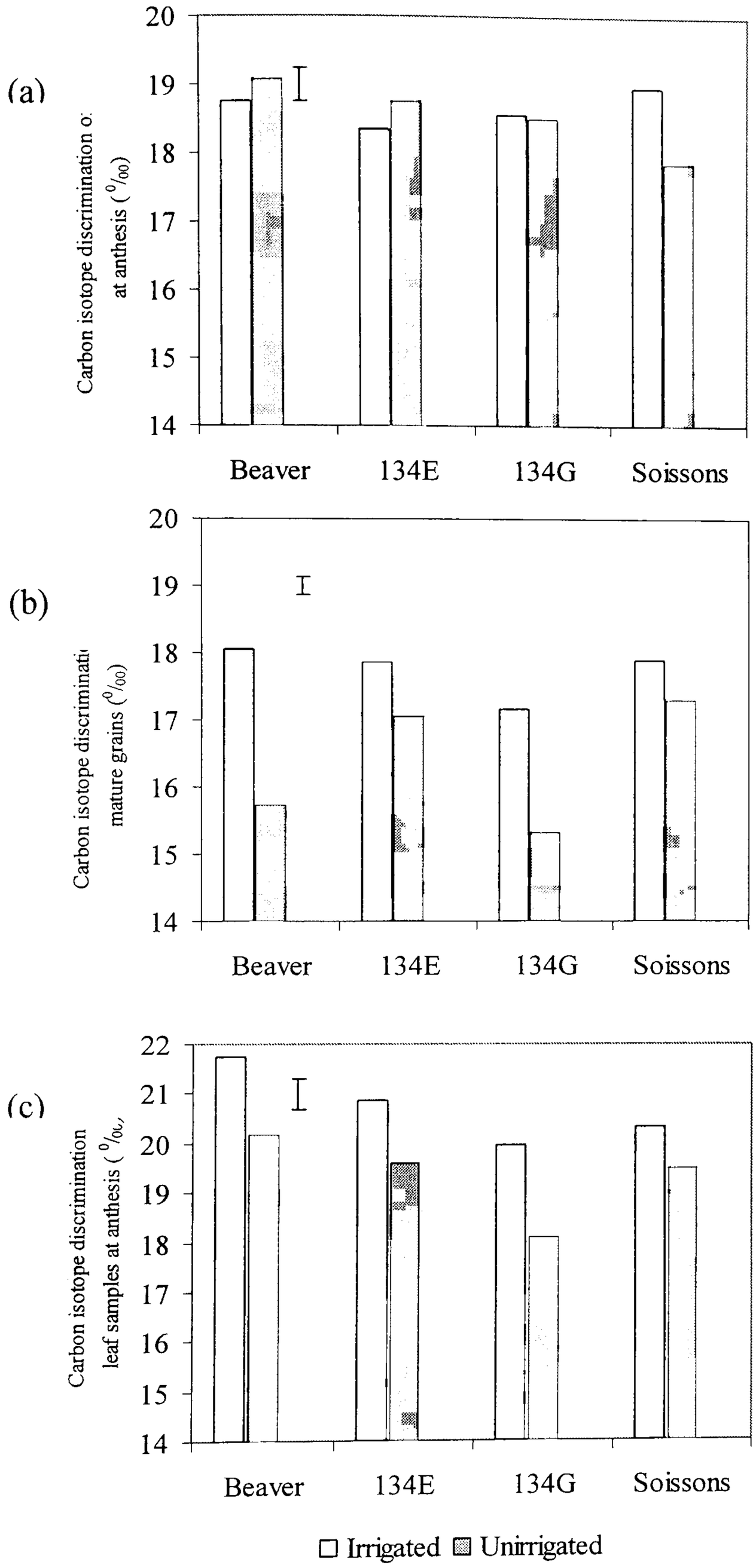


Figure 4.17 Carbon isotope discrimination ($\Delta^{13}C$) for (a) leaves at anthesis in 2003 (b) mature grains in 2004 and (c) leaves at anthesis in 2004 for four wheat genotypes in the glasshouse experiments. Errors bars show S.E.D. (D.F=24) for genotypes x irrigation interaction.

4.2 DISCUSSION

Water-use efficiency has often been discussed in relation to plant performance when water is limiting. Plants adapt to limited water availability through various morphological and physiological mechanisms and this requires an understanding of the various processes regulating the efficient use of water within a plant species. About 20% of the UK winter wheat yield potential is lost annually to drought (Foulkes *et al.*, 2002). The improvement of WUE of wheat has important implications for improving yield under drought and conserving water in the absence of drought for both economic and environmental benefits. The present work investigated genotypes which contrasted for several traits (flowering time, flag leaf size and presence of awns) and major agronomic genes (+1B/1R, -1B/1R, *Rht1* and *Rht2*); The results of analyses on the physiological basis of WUE in these genotypes are discussed in this section.

The framework proposed by Passioura (1977), as outlined in chapter 2, serves as the most appropriate way to identify traits that may limit wheat yields in water-limited environments. According to this, yield may be increased by an increase in water use or water-use efficiency and/or HI, provided that there would not be any significant negative relationships among the three variables. The inter-relationships among these variables are discussed in this section with a view to identifying mechanisms to improve performance under drought, and quantifying their genetic variability.

4.2.1 Root growth and water use

The pattern of water use by genotypes differed over the growing period, with early developing Soissons showing the smallest total water use and Beaver the greatest, whereas water use by the DH lines was similar. The effects of genotypic differences in root traits in relation to water use are discussed here.

Root traits may have a significant role to play in the development of new wheat genotypes with improved adaptability in water-limited conditions. Root length density is one important trait and the distribution of root length density with depth may be important. In the present study, the plant roots from columns were sampled at anthesis. It was observed that roots extended well below 100 cm by anthesis. Studies in the UK (Gregory *et al.*, 1978; Barraclough and Leigh, 1984), Australia (Hamblin *et al.*, 1990;

and Siddique *et al.*, 1990) and elsewhere (Boehm, 1978; Kaetterer *et al.*, 1993; Robertson *et al.*, 1993) have also reported a peak of root growth around anthesis, with a decline after anthesis when most assimilates are used for reproductive growth and only a small proportion for root growth (Gregory and Atwell, 1991). In addition, the wheat genotypes differ in depth of rooting under drought (Hurd, 1968; Gregory *et al.*, 1978) and differ for root dry weight with depth (Brown *et al.*, 1987; Siddique *et al.*, 1990; Miralles et al., 1997).

In this study, RLD for all the genotypes across moisture regimes at all depths exceeded 0.5 cm cm^{-3}. It has been reported that RLD of UK winter cereals typically varies with depth from about 5-10 cm cm^{-3} at 0-20 cm to 0.5-1.0 cm cm^{-3} at 80-100 cm (Barraclough *et al.*, 1989). Theoretically, the mean RLD of cereals in top layers of most soils are adequate to access most of the available soil water since densities more than 1 cm cm^{-3} are associated with only small increases in the total amount of water taken up during yield forming period (King *et al.* 2003). Theoretical calculations predict a critical RLD of about 1.0 cm cm^{-3} for nitrate uptake in cereals, above which further increases in RLD equate to excessive roots which do not influence N uptake (van Noordwijk, 1983). This range concurs with the results for uptake of all available water with a RLD of about 1.0 cm cm^{-3} in spring barley (Gregory and Brown, 1989) and winter wheat roots (Barraclough *et al.*, 1989). In the present study, genotypes showed some consistency in RLD across years. Beaver maintained a higher RLD than Soissons. However, line 134G had low RLD compared to 134E in 2003 and vice versa in 2004. These differences were associated with the observed genotypic differences in water uptake. The RLD values in the present study are in general agreement with ranges reported for winter wheat (Gregory *et al.*, 1978; Barraclough, 1984). RLD is usually highest in upper horizons, however, in this study root distribution with depth showed slightly higher values below 80 cm. In 2003, the pre-anthesis water use under drought was reduced by 30-50% and the post-anthesis water use by 44-46% in all the genotypes. Later flowering cultivars, Beaver and line 134E had a higher pre- to post-anthesis water use ratio (0.33 and 0.36 respectively) than 134G (0.30) and Soissons (0.22). Thus, post-anthesis water use was greater in Soissons and 134G. In 2004, water use by genotypes in the droughted treatment was reduced in the range 55-57% during the pre-anthesis period and 21-24% during the post-anthesis period. A smaller pre- to post-anthesis water use ratio was also

found for Soissons (0.46) over other genotypes (0.59 each) owing to differences in development. Soissons carries the *Ppd-D1a* allele for the photoperiod insensitivity which under UK conditions typically advances flowering by about 8 days (Worland *et al.*, 1998; Foulkes *et al.*, 2004). In this study, evaluating RLD as a factor is complicated by uncertainties about the fraction of total root length that is functional with respect to water uptake (Passioura 1983).

The percent reduction in the total RLD across soil horizons was significant ($P<0.05$) at about 25% under drought; and that of Soissons and 134G (27%) was lower in 2004 as compared to Beaver (7%) and 134E (37%). Previous studies have demonstrated that rooting densities of cereal crops in general are not considered a limiting factor for moisture uptake (Gregory *et al.,* 1978; Smucker, 1984). Water uptake is limited by the water potential in the rooted soil volume rather than by root density. However, there can be limitations in the soil profile due to rooting when RLD is less than 1 cm cm^{-3} (Barraclough *et al.*, 1989). Gregory and Brown (1989), Barraclough *et al.* (1989) and Robinson (1994) have shown that water uptake may be altered by RLD distribution with depth at anthesis. On the other hand, Hamblin and Tennant (1987) studied the root growth of wheat, lupins and peas in dryland areas of Western Australia and found no correlation between root length density and water uptake. Siddique *et al.* (1990) in Australia showed that old varieties had a higher RLD and dry matter than new cultivars in the top 40 cm of soil, where most roots occur. The total water use by old and modern cultivars was found to be similar.

Higher RLD for Beaver than Soissons may be attributed to a higher mean root dry weight for Beaver. The root dry matter accumulation was greatest in the 0-20 cm soil layer in all the genotypes. However, roots in the 0-20 cm horizon had high root specific weight so RLD was not greater than in deeper soil horizons. This is different to results of previous field investigations which reported exponential decreases in RLD with depth (Barraclough, 1984; Gregory *et al.* 1992).

In the present study, root distribution with depth differed for the genotypes in the different water regimes. Beaver and line 134G tended to use more water on account of greater RLD and a greater root dry matter partitioning. Root dry matter is typically 10% of total dry matter after flowering in C_3 cereals in temperate regions (e.g. Gregory *et al.*,

1978), but may be as high as 30% in Mediterranean environments (Siddique *et al.*, 1990). However, in this study the mean root partitioning was about 5% of total dry matter irrespective of the soil water supply. Several studies showed an increment in root dry matter under drought in winter wheat (Blum *et al.*, 1983; Barraclough, 1984; Palta and Gregory, 1997), as opposed to the decreased root dry weight found under droughted conditions in the present study. Genetic variation in the size of the root system of wheat cultivars has been reported (Siddique *et al.*, 1990; McCaig and Morgan, 1993; Miralles *et al.*, 1997), but the effect on growth of water stress can vary. Siddique *et al.* (1990) reported the yield improvement in modern cultivars was associated with reduced root dry matter. Improved root biomass has been associated with the 1BL/1RS wheat-rye translocation in spring wheat (Ehdaie *et al.*, 2003). It is interesting to note that in this study the highest root dry matter was found in Beaver, a 1B/1R translocation cultivar. However, the genetic differences in root dry matter presently reported may result from the extent to which genotypes differ in the degree of adjustment in root partitioning in response equally to the onset of stress, i.e., the functional equilibrium between root and shoot (Brouwer, 1963; van Noordwijk and de Willegen, 1987).

Soil and climatic conditions were found to have more influence on rooting depth and distribution than the genetic make-up of cultivars of winter wheat in England (Lupton *et al* 1974; Wilhelm *et al.,* 1982). In the present study, the smaller root system of Soissons can be ascribed to its faster development with fewer calendar days for root growth up to anthesis.

SRL (specific root length; m g^{-1}) is a measure of the fineness of the root system (Wilhelm *et al.*, 1982). Soissons had a significantly larger SRL (thicker roots) at most soil depths compared to Beaver in both years. DH lines showed differences only in 2003, but in general line 134E had finer roots. RLD of Soissons was always lower on account of developmental difference and reduced root length per soil volume, but Soissons had SRL compared to Beaver. Line 134G had always lower SRL and higher RLD than 134E. However, these contrasting genotypic differences in RLD in the present study do not appear to be a major mechanism for improving water uptake because most genotypes had their RLD exceeding 0.5 cm cm^{-3} by anthesis. It should be noted that with regard to nutrient uptake theoretically higher root densities are needed to access phosphorous in

soil solution than those needed to capture water and nitrogen in the upper surface horizon (Van Noordwijk, 1983).

The presence of the *Ppd-D1* gene which confers photoperiod insensitivity and early flowering (Worland, 1996) in Soissons increased its post-anthesis water use. This is in line with findings of Foulkes *et al.*, (2004) who demonstrated that early flowering with *Ppd-D1* isogenics (photoperiod-insensitive) in Mercia decreased pre-flowering water uptake under unirrigated conditions, but increased post-flowering uptake as compared to *ppd-D1* (photoperiod-sensitive) isogenic lines.

4.2.2 Water-use efficiency and dry matter production

Many have reported about the linear relationship between water use and dry matter production (de Wit, 1958; Hanks in Taylor *et al.*, 1983; Condon *et al.*, 2002; Foulkes *et al.*, 2002). This linearity is explained by the diffusion link between photosynthesis and transpiration. In 2003, although a linear relationship was found between the dry matter and the amount of water used by genotypes, parallel regression analysis revealed no significant differences for the slopes (WUE) among genotypes both under irrigated and unirrigated conditions. WUE was not significantly different when plants were well-watered or stressed.

Irrigated treatments showed higher WUE in both years. In 2004, averaging across irrigation treatments, a significant difference in slopes of genotypes was observed through parallel regression analysis. Soissons had higher WUE than Beaver. Similarly the two DH lines showed higher WUE than Beaver. When pooling data across seasons, effects of genotype and the genotype x irrigation interaction were not statistically significant. There are a few reports that drought stress decreases the dry matter to transpired water ratio (Johnson and Henderson, 2002; Pannu and Singh, 1993; Foulkes *et al.*, 2001). On the other hand, most of the results based on dry matter harvests of plants in controlled environments point to small increases in WUE with drought stress in C_3 plants (e.g. Fisher and Turner, 1978). In wheat many investigations (Aggarwal *et al.*, 1986; Hubick & Farquhar, 1989; Condon *et al.*, 1990; Li *et al.*, 2001; Foulkes *et al.*, 2001) have reported significant increases in WUE in response to drought. Present work

showed a slight decrease in WUE of some genotypes under water stress, but the irrigation x genotype interaction was not significant. One possible reason for lower WUE in droughted columns could be that the vermiculite was not completely effective in reducing evaporation from the soil surface and possibly some proportion of water might have been used to wet up the vermiculite before reaching the soil. Proportionally more water evaporation could have occurred from the surface in the droughted treatment than in the irrigated treatment and a greater proportion of it could have been used to wet up the vermiculite before it reached the soil.

4.2.2.1 Plant growth responses

Parental genotypes and DH lines differed in their developmental rate. Soissons developed faster than Beaver reaching anthesis a week earlier and line 134G 1-2 days earlier than 134E within this range. The slower developing cultivars, Beaver and line 134E had the maximum shoot number, but had greater tiller death between GS31 and GS61. The production of non-surviving shoots may be detrimental to crop performance when water is limited because the carbon within dying shoots is not completely re-distributed to fertile shoots (Donald, 1968; Thorne and Wood, 1987; Berry *et al.*, 2003). At maturity, slow developing Beaver and 134E had highest final ear number. This may be partly due to their profuse tillering behaviour. Under water-limited conditions, cereal crops with limited tillering have generally been shown to yield better than profusely tillering crops (Jones and Kirby, 1977; Duggan, *et al.*, 2000; Somersalo *et al.*, 1998; Brisson *et al.*, 2001). In addition, profuse tillering usually results in many non-surviving tillers which transpire most water in soil without contributing effectively to yield.

The water use pattern of parents differed with Soissons using most water in the irrigated and droughted treatments post-anthesis in both years on account of its early flowering. There was a trend for dry matter plant^{-1} of Beaver to be more depressed under drought than for Soissons, although Beaver produced more shoots. Ability to maintain ear-bearing tillers and ear dry matter partitioning was reduced under drought in the present study as evident with Beaver having more non-surviving shoots under drought which did not contribute to ear dry matter. Several studies have indicated that non-surviving shoots potentially did not contribute more dry matter than they contribute from their

own carbon fixation (Berry *et al.*, 2003; Simons, 1982). The proportion of shoots dying from early stem extension to anthesis under drought ranged amongst genotypes from 21 to 25% in 2003 and from 24 to 31% in 2004 across the genotypes. Genotypes responded differently. Beaver and line 134G had more shoot death compared to Soissons and 134E. Shorter growth duration (as indicated by early anthesis) constitutes an important attribute of drought escape, especially with regard to late-season water stress. Drought escape was observed with Soissons which tended to complete the sensitive tiller survival stage of development when most water was available. This was supported by relatively fewer aborted shoots in this genotype.

Slower developing Beaver and line 134E showed later senescence relative to flowering time in both the wet and dry moisture regimes. The loss of green area due to water stress increased from 35 to 85% from flag leaf emergence to visible maturity (when ears started ripening) in 2003; and from 20-90% in 2004. The response of wheat genotypes to drought in respect of leaf area has been well documented (Condon & Richards, 1992; Foulkes *et al.*, 2001). In this study, under irrigation Beaver and line 134E had more green area than Soissons and line 134G. While in 2003, genotype differences in green area plant^{-1} were not consistent. Maximum green area under irrigation did not predict pre-anthesis water use and biomass response to drought amongst genotypes in this study. Foulkes *et al.* (1993 and 2001) also found that cultivar differences in maximum green area under irrigation did not correlate well with differences in biomass response to drought; and maximum green area index was poor guide to seasonal growth, or water use during the growing season and a poor predictor of biomass response to drought in the UK's temperate climate. In the present study, the reduction in green area under drought was more for Beaver than for Soissons. In 2004, at 138 DAS (GS61+6wks in Soissons; GS61+5week in lines 134G and 134E; GS61+4weeks in Beaver) green area of Soissons was maintained well under drought compared to Beaver. Since genotype differences at any given sampling time in the present study are confounded by development, faster development of Soissons and or 134G could have resulted in these differences in green area responses through a 'drought escape' effect. The interaction effect was not evident in 2003.

The drought tolerance of a crop is sometimes achieved by maintaining a higher plant water status during a period of increasing water deficit (Turner, 1979). The possible options are (a) stomatal regulation of transpiration, (b) maintaining or increasing the water uptake from roots, (c) reduction in evaporative canopy area surfaces, or (d) reduction in radiation load absorbed by the canopy. It is reported that a higher flag leaf RWC in well-watered treatments could maintain vigorous assimilation, favourable for achieving higher grain yield (Li *et al.*, 2001). In the present study, genotypic differences for relative water content (RWC) under drought were not evident ($P<0.3$). An average reduction of about 4% in RWC was observed under droughted conditions. Thus, differences in RWC could not explain the differences in water uptake and crop growth under drought observed in the present study.

Reduction in transpiring surfaces by leaf rolling is another drought-avoidance strategy in plants (Richards *et al.*, 2002). Leaf rolling is a concomitant response to decreasing leaf water potential. In the present study, Soissons showed higher percent of leaf rolling under drought over Beaver and the DH lines. This leaf characteristic might reduce transpiration losses and improve water status.

4.2.2.2 Yield response to water availability

The effects of water stress on the yield reduction of crops have been well documented (Turner 1986; Blum *et al.*, 1990; Taylor *et al.*, 1996). Grain growth was lower in 2003 than in 2004 due to lack of vernalization and/or heat stress at late booting around the time of meiosis and which restricted grain number. High temperatures in 2003 also occurred between anthesis and maturity which would mainly affect assimilate deposition in the developing grain resulting in lower grain weight. Over the range of 12 °C to 26 °C grain weight is reduced at a rate of 4 to 8% per °C increase (Wardlaw *et al.*, 1980; Wiegand and Cuellar, 1981; Acevedo *et al.*, 1991). In the present study, the grain yield ranged from 0.82 to 1.82 g plant^{-1} and these amounts were unusually low. The mean coefficient of variation for grain yield in 2003 was about 56%. Such large variations in grain growth may be attributed to differential responses to high temperatures. Thus, data in 2003 provide limited information on yield responses and WUE was calculated only over the pre-anthesis period during this year. However, the yield responses depend on the timing of drought. Plaut *et al.* (2003) reported water deficits did not reduce kernel

number, but affected 1000-kernel weight in wheat. Blum *et al.* (1990) reported in spring wheat that the highest grain yield plant^{-1} was usually from the cultivar that excelled in kernels ear^{-1}, even though it was lowest in potential tillering. In the present study, in 2004 Soissons, line 134E and line 134G maintained grain yield plant^{-1} under drought better compared to Beaver. The reasons can be ascribed to better maintenance of harvest biomass under drought in Soissons and line 134E compared to Beaver. Soissons and lines 134E or 134G maintained HI better than Beaver. The susceptibility of Beaver to drought can be linked to more restricted water uptake and lower WUE under drought compared to Soissons. Foulkes *et al.* (2002) demonstrated decreases in individual ear weight at flowering under drought were associated with fewer grains ear^{-1} when the onset of drought occurred shortly before flowering. In the present study similar effects were found on grain ear^{-1} and this confirms floret survival is linked to assimilate supply to the ear (Slafer, 1996; Foulkes *et al.*, 2002).

The yield responses of the four genotypes to restricted water availability in 2004 differed significantly. Both harvest biomass (R^2=0.86) and HI (R^2=0.70) were correlated with yield responses. The faster developing Soissons maintained harvest biomass better than Beaver; and line 134E better than line 134G. Among yield components, in 2004, ear number plant^{-1} decreased on average by 31% and the decrease ranged from 22-38% among the genotypes. Grain yield plant^{-1}(60%), grains ear^{-1} (30%) and 1000-grain weight (15%) were also decreased due to water stress. This is in line with several reports on effects of pre-anthesis water stress on plant height, leaf area, grain number, grain weight and grain yield of wheat (e.g. Gupta *et al.,* 2001; Foulkes *et al.*, 2002). In the present study, Beaver lost more grain yield (82%) under drought as compared to Soissons (54%). Among the DH lines, 134G suffered a reduction in grain yield by 54% as compared to 43% for line 134E under drought. In this study, the comparative yield response to drought for Beaver and Soissons indicated that Beaver lost more grain yield than Soissons. The WUE of Beaver under drought was lower (2.47g DM kg^{-1}water; R^2=0.48) compared to Soissons (2.77 g DM kg^{-1}water; R^2=0.63).

These effects perhaps reflected the extent of drought incurred by plants during the period from ear emergence to the end of flowering, with larger deficits and reductions in grains ear^{-1} and ear number^{-1} plant for the later developing genotypes. G x E interactions for yield components were not evident, but the pattern of depression for yield

components among genotypes was consistent with Soissons escaping the drought early. In the present study, under irrigation Soissons (*Ppd-D1*) produced fewer ears plant^{-1} but this was compensated by more grains ear^{-1}, as was found by Worland (1996) and Foulkes *et al.* (2004).

4.2.3 Contribution of harvest index responses to drought resistance

Harvest index has been reported to be a relatively stable feature for a given cultivar in the absence of severe stress (Gallagher & Biscoe, 1978), but effects are commonly reported with drought (Innes *et al.,* 1984; Ehdaie, 1995; Foulkes *et al.,* 2002). In the present study, the average decrease from 0.48 to 0.35 in 2004 under drought was consistent with these findings. A larger reduction in HI of 0.31 for Beaver compared with 0.09 for Soissons ($P<0.05$) contributed to the differences in yield responses to drought. Significantly different reductions in harvest biomass under drought were also found with Beaver and line 134G showing larger decreases than Soissons and line 134E. The positive effects of *Ppd-D1* in improving the HI have been previously reported for UK wheat (Foulkes *et al.*, 2004) and in Soissons a decreased crop water use in the pre-anthesis period compared to *ppd-D1* UK bred varieties (Foulkes *et al.*, 2001).

The grain sink size is determined progressively through sequential formation of tillers, spikelets, florets and the grain with overlap between stages of growth. Soissons partitioned more dry matter into ears (63%) than Beaver (52%). DH lines had about 57% ear dry matter each. Reduction in HI under drought has been reported in UK wheat (Innes *et al.,* 1984; Innes and Blackwell, 1983; Foulkes *et al.*, 2002). Large reductions in HI are common under drought, given that HI only shows conservatism with environment under optimal growing conditions and can be reduced if a crop is grown under resource-limited conditions (Azam-Ali and Squire, 2002). Soissons maintained HI better compared to other genotypes associated with early flowering as described above.

Lines 134E and 134G are tall (*rht*) whereas Beaver (*Rht-D1b*, formerly *Rht2*) and Soissons (*Rht-B1b* formerly *Rht1*) are semi-dwarfs. DH lines (taller) and Soissons (*Rht1*) maintained better HI with smaller reductions under drought compared to Beaver. Greater reduction in grain yield and associated yield components under droughted conditions in this study could be as a result of poorer accumulation and/or translocation

and stem carbohydrate remobilisation during the period of water stress. It has been shown in previous studies that UK cultivars accumulate amounts of stem-soluble carbohydrate at flowering in the range 2-4 t ha^{-1} and these differences were associated with ability to maintain HI (Foulkes *et al.*, 1998 and 2002). However, stem soluble carbohydrate accumulation was not measured in the present study.

4.2.4 Leaf level transpiration efficiency

A quantitative analysis at the leaf scale by gas-exchange for estimating net photosynthetic rate (A) and transpiration (E) and stomatal conductance (g_s) would provide a better understanding of differences in genotypic responses in contrasting water regimes underlying WUE at the whole crop level. In this context, the analysis of gas exchange traits is discussed here. Stomatal opening is regulated according to soil water status (Gowing *et al.*, 1990; Davis and Zhang, 1991; Chaves and Oliveira, 2004); and at the cellular level stomatal regulation occurs during responses to vapour pressure deficit. Regulation of stomatal conductance enables the plant to modify gas exchange with the atmosphere reversibly, on a time scale of only a few minutes.

It is well established that photosynthesis is closely related to dry matter production in most crops, and plant responses and adaptation to biotic and abiotic stresses are reflected in changes in their photosynthetic rates (Richards *et al.*, 2002; Chaves and Oliveira 2004). Stomatal closure or a decrease in stomatal conductance leads to a decrease in CO_2 intake, decreased intercellular CO_2 partial pressure, and thereby decreased CO_2 assimilation and net photosynthesis (Farquhar and Sharkey, 1982). Soissons and line 134G showed significantly higher photosynthetic rates (A) in general across the readings between GS39 and GS61+4 weeks. Under well-watered conditions, Soissons and line 134G transpired more water, but they reduced transpiration (E) under water stress conditions more than Beaver and line 134E. The differences in the development stages of these genotypes might have also contributed to differences in their response to water stress.

Over all there was a decrease in net photosynthesis (A) under water stress by 11%, but the genotypes did not respond under drought significantly differently. Beaver had lower A than Soissons under irrigated and droughted conditions. There was an interaction

between date of measurement and genotype (P=0.04), with Soissons and line 134G having relatively higher values on 6 May (GS31+3weeks) and 7 July (GS61+3weeks) in 2004. Possibly this was due to development differences amongst genotypes.

Averaging across years, g_s reduced by 46% due to drought with no significant differences amongst genotypes. However, under drought, there was a trend for a greater reduction in g_s from GS 39 to GS61+4 weeks in 134G (58%) and Soissons (51%) compared to Beaver (38%) and 134E (7%). Lower g_s values are associated with slower crop growth rates and slower depletion of soil water from the start of stem elongation (Richards *et al.,* 2000). Thus, Beaver showed low mean stomatal conductance under drought, at a given growth stage, consistent with its slower growth rate compared to Soissons and line 134G. Stomatal conductance results were also consistent with a larger depression of dry matter for Beaver under drought than for Soissons. The variability of the gas exchange data on individual assessment dates reported here was relatively high, in particular for stomatal conductance. Nevertheless, in the present study there was a general trend for Soissons and line 134G to show decreased g_s supporting the hypothesis that a lower stomatal conductance would favour higher WUE of genotypes. Condon *et al*. (2002) pointed out that in cereals high TE is associated with conservative water use but also conservative growth in the absence of water stress. However, in drier 'stored-moisture' environments, the conservative gas-exchange associated with low g_s and high TE can result in a considerable yield gain

Pooling data across years, ITE_{ins} (calculated as *A/E* ratio) increased under drought by 14%. Line 134G and Soissons showed a trend for increased ITE_{ins} under drought by 64% and 21% with little changes observed in Beaver and 134E. These responses could be attributed to reduced g_s under water-stress for line 134G and Soissons. This is consistent with the previous work where greater reduction in g_s under water stress caused an increased WUE (Morgan *et al*., 1993; Van den Boogaard *et al*., 1997).

As stomata close, the CO_2 concentration inside the leaf initially declines with increasing stress and then increases as drought becomes more severe (Lawlor, 1995). Line 134G and Soissons showed 7% and 2% reductions in C_i under drought. C_i tended to decrease initially and thereafter increased progressively as the crop suffered more severe drought. Soissons had lower C_i values than Beaver in both the water regimes. This could have

contributed to higher TE in Soissons than in Beaver. A similar pattern for lower C_i associated with higher WUE was observed in line 134G compared to 134E. The interaction effect was significant with relatively higher C_i for Beaver under drought. Pooled data of all dates of measurement revealed a significantly lower C_i for Soissons than Beaver (data not shown) and is consistent with a higher ITE or WUE observed for Soissons. There is evidence from previous studies that lower mean C_i results in increase in transpiration efficiency (Farquhar and Richards, 1984; Condon *et al.*, 1990).

A reduction in C_i under drought was evident from the cross-year analysis for Soissons and line 134G (P=0.01). Genotypic variation in leaf size and stomatal distribution can affect stomatal aperture traits in plants, and Beaver and line 134E had relatively smaller leaf sizes. Genotypes with smaller leaf size tend to have increased stomatal density (Austin *et al.*, 1982; Dunstone *et al.*, 1973), a feature that could result in decreased WUE. These two genotypes showed no reductions in C_i. In theory, it is expected that a positive relationship exists between C_i/C_a and TE under water limited conditions (Farquhar and Richards, 1984; Richards *et al.*, 2001; Condon *et al.*, 2002). Thus, the observed lower C_i values in Soissons and line 134G might have resulted in their higher ITE_{ins}. Soissons showed a greater degree of stomatal closure under droughted conditions and maintained a relatively lower C_i relative to that in the well-watered treatment compared to other genotypes, thus optimising carbon gain with respect to water loss (Cowan, 1982). Similar contrasts were observed with respect to line 134G versus line 134E.

Ritchie (1974) demonstrated that, provided the soil surface is dry, once the leaf area index decreases below 3, transpiration decreases approximately linearly with leaf area to give zero transpiration at zero leaf area. In both years, the seasonal average value of green area was reduced by about 35% under drought. The proportional reduction in conductance under drought (46%) was greater than the reduction in green area. According to Bradford and Hsiao (1982), canopy size is generally more sensitive to water stress than stomatal conductance for many species. There was a significant reduction in the green area (P<0.05) but only a strong trend for a reduction in g_s (P<0.10) under water stress conditions.

Many researchers have demonstrated that an increase in WUE could be attributed to a reduction in stomatal conductance and/or increased assimilation rates (Blum, 1990; Morgan and LeCain, 1991; Van den Boogaard *et al.*, 1997; Fischer *et al.*, 1998; Lu *et al.*, 1998). Under drought, Soissons did differ consistently in g_s compared to Beaver but also showed significantly increased ITE_{ins} on account of increased net photosynthesis. ITE_{ins} was also improved for line 134G under drought as compared to 134E and Beaver.

In several studies high yield under drought in wheat lines was associated with higher net photosynthesis, lower stomatal conductance and lower sub-stomatal CO_2 concentration (Blum 1990; Fischer *et al.,* 1998; Lu *et al.,* 1998; Reynolds *et al.,* 2000). This is in agreement with the general findings of the present study that the performance of wheat genotypes like Soissons and line 134G was associated with higher values for net photosynthesis and lower stomatal conductance and C_i.

Gas-exchange studies give an estimate of the instantaneous transpiration efficiency for a specific leaf, while the isotopic ($^{13}C/^{12}C$) discrimination methods give an integrated value over time and leaf layers. It is rather difficult to relate instantaneous measures to values over longer periods, as diurnal changes in stomatal conductance and environmental conditions mean that the long-term average WUE may only loosely be related to measured instantaneous values (Jones, 2004). Hence in the present study, the carbon isotope discrimination technique was used to estimate the variation in intrinsic WUE among the genotypes over time.

It is evident from the present investigation that a higher A, lower g_s and C_i in both Soissons and line 134G resulted in higher TE at the leaf scale. This was also supported by gravimetric estimation of WUE which showed apparently higher values for these genotypes.

Many researchers have shown the robust negative association of $\Delta^{13}C$ and TE in C_3 plants like bread wheat (Condon *et al.,* 1990; Ehdaie *et al.,* 1991; Condon & Richards 1992) and barley (Hubick and Farquhar, 1989). In the present study, this relationship was not strongly evident in 2003. This may be due to the variation in the pattern of dry matter accumulation as a result of the crop being subjected to cyclic phases of water availability. In addition, treatment differences were difficult to quantify precisely due to

spatial variation in the glasshouse associated with heat stress. However, the regression of ITE (measured at leaf scale) versus leaf $\Delta^{13}C$ was negative and weakly significant (P =0.13) under droughted conditions. In 2003, leaf $\Delta^{13}C$ did not differ statistically significantly amongst genotypes, but Soissons exhibited a lower value under irrigation by 0.49‰ compared to Beaver and by 1.11‰ under drought. In 2004, green lamina at anthesis and mature grains at harvest were analysed for $\Delta^{13}C$ separately. Leaf $\Delta^{13}C$ for Soissons was lower than Beaver (P=0.002), consistent with estimates of more efficient water use by this cultivar. For example, lower g_s and lower C_i in Soissons and also lower WUE according to the gravimetric estimation. A non-significant G x E interaction indicated that $\Delta^{13}C$ is heritable in these genotypes. Previous studies in wheat showed the existence of a low or non-significant G x E interaction for $\Delta^{13}C$ indicating its high heritability (Condon *et al.*, 1987; Condon and Richards, 1992; Matus *et al.*, 1996). The droughted treatments had lower leaf $\Delta^{13}C$. Grain $\Delta^{13}C$ also differed similarly between the parents.

The genotypic range in $\Delta^{13}C$ in the present study (0.49‰ in 2003; and 1.93‰ in 2004) is in general agreement with those reported in previous studies in wheat (1.1-2.6‰ by Condon *et al.*, 1987; 2.8‰ by Ehdaie *et al.*, 1991; 1.1-1.8‰ by Condon *et al.*, 1992; 1.0-1.4‰ by Morgan *et al.*, 1993 and 0.32-0.62‰ by Xue *et al.*, 2002). In 2004, the range of leaf $\Delta^{13}C$ was higher (19.3-20.7‰) than for grains $\Delta^{13}C$ (16.4-17.7‰). This is also consistent with previous reports in wheat which showed higher mean $\Delta^{13}C$ in early-formed dry matter than for grains (Condon and Richards, 1992; Condon *et al.*, 1992; Merah *et al.*, 2001). In the present study, relatively lower g_s and significantly higher carboxylation capacity (A/C_i ratio) for Soissons and 134G may have resulted in higher TE and/or WUE in these genotypes. In 2003, leaf $\Delta^{13}C$ at anthesis did not vary much among the genotypes and across water regimes. Nevertheless, Soissons still expressed lower $\Delta^{13}C$ values than Beaver. In both the years $\Delta^{13}C$ values were low under water-limiting conditions (P<0.001).

Baker (1988) envisaged that a significant genotype-by-water regime interaction for $\Delta^{13}C$ may be of two types: non-crossover and crossover interactions. In 2004, crossover interactions were apparent for grain $\Delta^{13}C$ which showed different ranking for Beaver being the highest in irrigated and lowest in droughted treatment. Differences in the rate

and extent of soil water extraction among wheat genotypes have been shown to be associated with differences in $\Delta^{13}C$ particularly if working through g_s (Condon *et al.*, 1992 and 2002). Condon and Richards (1992) suggested that for genotypic comparisons of $\Delta^{13}C$ plants should be sampled early in development under well-watered conditions to avoid confounding effects related to differences in mid and late season water stress development. Based on analysis of leaf samples at anthesis, lower $\Delta^{13}C$ values for Soissons and line 134G compared to Beaver and line 134E were confirmed, consistent with the results that lower delta values resulted in improved WUE or TE. Both gravimetric estimation and gas-exchange measurements broadly confirmed these responses.

In summary, it is evident that earliness could favour post-anthesis water use (e.g. Soissons). It is also possible that genetic variation in root traits may correlate with season-long water use, especially when onset of drought coincides with anthesis. WUE measured as the regression slope of dry matter on water use was higher for Soissons and the two DH lines compared to Beaver. Later developing genotypes (Beaver and line 134E) had more non-surviving tillers hence they did not partition dry matter to ears better resulting in lower grain DM. A higher grain DM as a result of higher *A* rates was realised in Soissons and line 134G. The differences in green area production under well-watered conditions did not relate to water use amongst genotypes. However, late developing Beaver showed greater senescence under drought compared to Soissons. This indicated scope for improving flag-leaf green area persistence for drought resistance. Soissons and the two DH lines maintained HI better under drought compared to Beaver. Under non-limited water conditions Soissons and line 134G tended to use less water, but reductions in transpiration and stomatal conductance under drought were smaller for these genotypes. Thus, Soissons and line 134G showed higher ITE_{ins} on account of lower g_s and C_i values which was consistent with lower $\Delta^{13}C$ or higher WUE for these genotypes. However, the genotype responses to measured gas-exchange parameters need critical consideration in further studies to see whether a lower g_s and C_i results in a higher WUE in the UK grown wheat.

CHAPTER 5: WATER-USE EFFICIENCY OF WHEAT GENOTYPES IN FIELD EXPERIMENTS

The results of the field experiments carried out during the years 2002/03, 2003/04 and 2004/05 are presented in this chapter. The experiments were conducted in 2002/03 at ADAS Gleadthorpe, Nottinghamshire (55° 13'N, 1° 6'W) and in 2003/04 & 2004/05 at University of Nottingham farm, Sutton Bonington (52° 50'N, 1° 15'W). The experimental years are referred to hereafter by their harvest years: i.e. 2002/03 is referred to as 2003 etc.

5.1 RESULTS

In 2004, above average summer rainfall occurred leading to absence of drought in the unirrigated treatment. The soil moisture deficit was small and in the irrigated crop severe lodging at maturity occurred associated with August rainfall more than 300% greater than the long-term mean. The mean percentage of lodging was about 43% in unirrigated plots (see Appendix V) and the grain yield did not differ between the irrigation regimes. Due to lodging in 2004, data for this season are considered to be unreliable and use of these data has been restricted. However, the measurements taken during 2004 early in the growing season before lodging are used to support analysis of genetic differences in physiological traits underlying WUE, where appropriate.

5.1.1 Weather patterns and soil moisture deficits

Post-anthesis drought developed in the unirrigated treatments in 2003 and 2005. The soil moisture deficits (SMDs) in the irrigated and unirrigated treatments were predicted according to the ADAS irriguide model (Bailey and Spackman, 1996). In 2003, the available water (AW) to 1.2 m rooting depth at Gleadthorpe (loamy sand to 0.3 m over medium sand) was 105 mm. Previous work at this location has shown that the limiting deficit at which onset of drought occurs is 50% AW (i.e. 53 mm) pre-flowering and 65% AW (i.e. 68 mm) post-flowering. In 2003, onset of drought coincided with flag-leaf emergence, and in the irrigated treatment 175 mm of irrigation water was applied (Figure 5.1). However, drought was only sustained till mid grain filling, due to exceptionally high rainfall on 30 June (54 mm).

In 2005, at Sutton Bonington, the AW to 1.2 m rooting depth was 186 mm. SMD exceeded 92 mm (50% AW) on 30 May and limiting deficits were maintained until physiological maturity (Figure 5.1). A total 171 mm of irrigation water was applied in the irrigated treatment.

5.1.1.1 Experimental conditions and weather patterns

The 2002/3 season at Gleadthorpe was characterized by a cold December and January, followed a warm spring and then slightly above average temperatures until harvest, (Table 5.1). Mean air temperature exceeded 16 ^{o}C from June onwards. The rainfall below long-term mean was experienced between January and April. In the 2004/5 season at Sutton Bonington average rainfall was experienced from sowing until February. March and May were drier than average. The mean air temperature, rainfall and radiation levels were generally similar to the long-term mean.

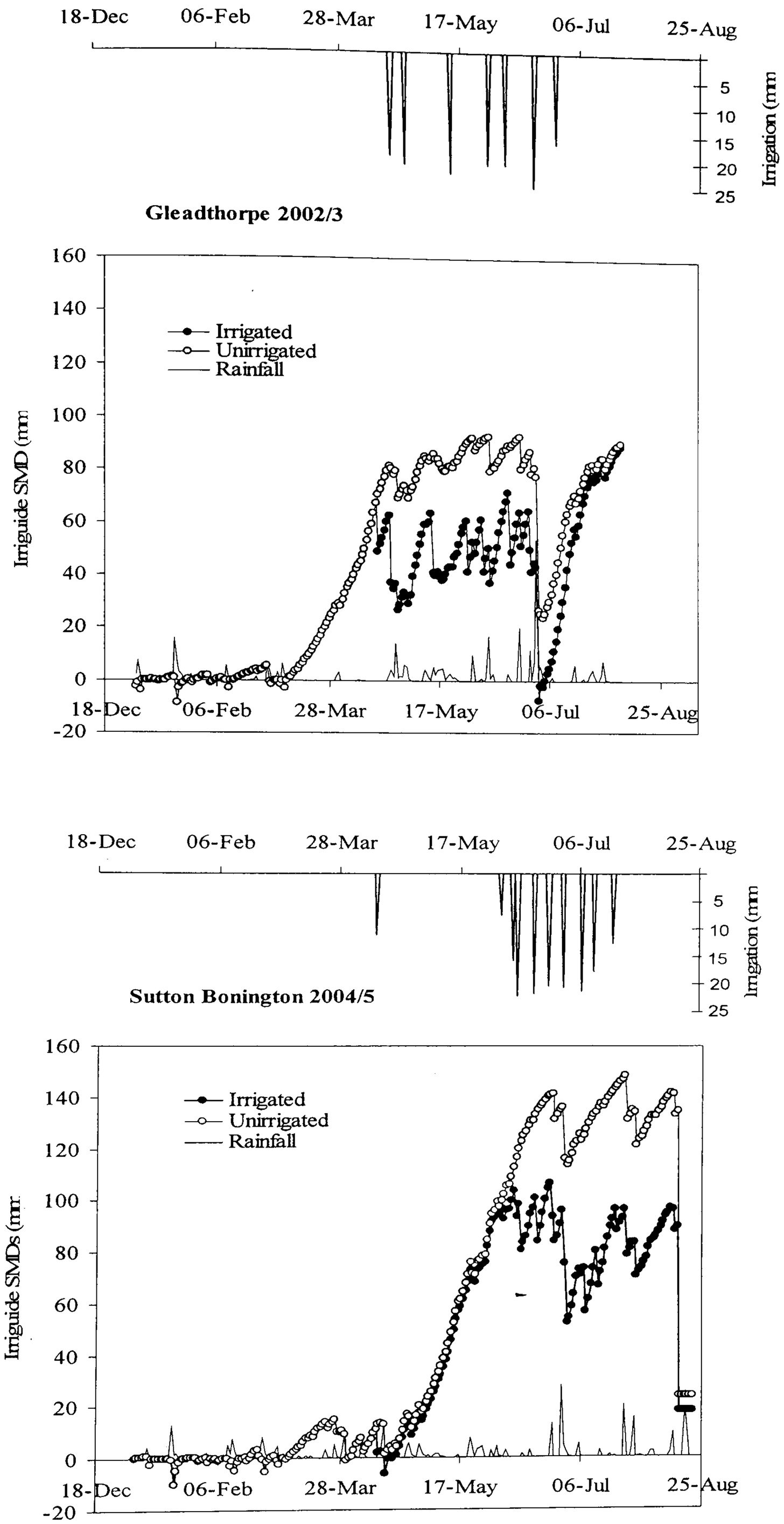


Figure 5.1 Soil moisture deficits in irrigated and unirrigated treatments in 2002/3 (Gleadthorpe) and 2004/5 (Sutton Bonington), daily rainfall and irrigation applied.

Table 5.1 Mean monthly weather data for Gleadthorpe and Sutton Bonington in experimental season and for the 30 year long-term mean (LTM) for Gleadthorpe (1975-2004) and Sutton Bonington (1975-2003).

	Mean air temperature (°C)					Rainfall (mm)					Solar radiation (MJ m^{-2})			
	Gleadthorpe		Sutton Bonington			Gleadthorpe		Sutton Bonington			Gleadthorpe	Sutton Bonington		
	2002/3	LTM of G.T	2003/4	2004/5	LTM of S.B	2002/3	LTM of G.T	2003/4	2004/5	LTM of S.B	2002/3	2003/4	2004/5	LTM of S.B
September	13.6	13.4	14.1	14.7	13.8	30	54	26	41	51	382	339	294	293
October	9.1	9.9	8.8	10.4	12.1	92	63	43	117	58	252	231	159	173
November	7.5	6.5	7.9	7.6	6.9	89	53	47	34	54	92	90	78	80
December	5.1	4.3	4.2	5.5	4.6	94	65	69	22	62	68	58	69	55
January	4.5	3.7	5.4	6.2	4.0	48	55	76	30	54	78	73	72	66
February	3.6	4.0	5.6	4.4	4.2	20	44	34	43	44	164	141	120	133
March	7.6	6.1	6.5	7.2	6.4	18	47	38	37	46	347	252	193	225
April	9.9	7.8	9.5	8.6	8.1	30	56	85	52	46	459	368	382	335
May	12.5	10.9	11.9	10.5	11.2	50	48	42	39	44	573	511	525	470
June	16.5	13.8	15.2	15.1	14.2	128	61	54	61	58	658	515	538	495
July	17.7	16.1	15.6	16.7	16.5	37	42	63	51	46	574	504	485	488
August	20.3	16.0	17.3	16.1	16.5	8	52	193	72	48	528	450	471	410

5.1.2 Effects of irrigation and genotype on grain yield and carbon isotope discrimination of 33 DH genotypes and 2 parents.

An overview of the results obtained from the analysis of combine grain yield and carbon isotope discrimination values for the 33 DH lines and the two parents Beaver and Soissons, is presented in the following sub-sections.

5.1.2.1 Grain Yield

5.1.2.1.1 2003

Averaging across genotypes, drought decreased grain yield from 8.52 to 8.03 t ha^{-1} (P=0.39; Table 5.2). Genotypes differed significantly in the range 10.2 to 6.4 t ha^{-1} (P<0.001). Yield losses due to drought ranged from 0.12 to 3.02 t ha^{-1} amongst the lines, and the irrigation x genotype interaction was significant (P<0.001). With regard to the parents, Beaver yielded higher under irrigation, and apparently lost more grain yield under drought compared to Soissons.

5.1.2.1.2 2005

Restricted water availability reduced average grain yields by 1.58 t ha^{-1}(P=0.047). Under irrigation, lines yielded in the range 12.38 t ha^{-1} to 8.97 t ha^{-1} (P<0.001). Reductions in grain yield under drought ranged from 0.57 to 3.84 t ha^{-1} (P=0.02). The genotype x irrigation interaction effect was significant with lines 14E and 19B losing grain yield in the range of 3.39 to 3.84 t ha^{-1}. Beaver again yielded higher than Soissons under irrigation and the reduction in their grain yield due to drought was greater for Beaver (1.91 t ha^{-1}) than Soissons (1.45 t ha^{-1}) (P=0.02; Table 5.2).

5.1.2.1.3 Cross site/season mean

Averaging across the two site-seasons, drought reduced grain yield from 9.5 to 8.4 t ha^{-1} (P<0.03; Table 5.2). Genotypes differed significantly in the range 8.2 to 10.9 t ha^{-1}. The yield responses to drought among all DH lines ranged from 0.11 to 2.46 t ha^{-1} (P<0.02). High yielding Beaver lost about 1.3 t ha^{-1} (P=0.02) compared to 1.0 t ha^{-1} for Soissons. The interaction effect for site/season x genotype was significant (P<0.001), with the parents and 24 lines showing relatively greater yield in 2005 compared to 2003.

Table 5.2 Combine grain yield (85%DM) for 33 doubled haploid lines and 2 parents in the population derived from Beaver x Soissons in 2003 (Gleadthorpe) and 2005 (Sutton Bonington) in irrigated and unirrigated treatments.

	Grain yield (85%DM)								
	2003			2005			Cross-year mean		
Genotype	*irri*	*unirri*	*mean*	*irri*	*unirri*	*mean*	*irri*	*unirri*	*mean*
118A	7.94	8.17	8.05	11.31	9.41	10.36	9.63	8.79	9.21
120B	8.66	7.32	7.99	10.40	9.26	9.83	9.53	8.29	8.91
121D	8.26	7.07	7.66	9.14	7.92	8.53	8.70	7.50	8.10
122I	8.93	7.81	8.37	10.91	9.29	10.10	9.92	8.55	9.23
133A	8.47	6.14	7.30	10.18	8.10	9.14	9.32	7.12	8.22
134C	10.15	8.40	9.27	9.80	7.88	8.84	9.97	8.14	9.06
134D	8.01	7.60	7.80	9.53	8.15	8.84	8.77	7.88	8.32
134E	8.34	8.68	8.51	9.60	8.72	9.16	8.97	8.70	8.83
134G	8.58	8.02	8.30	9.43	7.75	8.59	9.01	7.88	8.45
135B	7.15	7.62	7.39	10.53	8.66	9.59	8.84	8.14	8.49
137C	8.37	7.52	7.94	9.64	8.13	8.88	9.01	7.82	8.41
14A	9.43	8.33	8.88	10.00	8.44	9.22	9.71	8.38	9.05
14E	9.08	9.54	9.31	12.38	8.99	10.68	10.73	9.26	10.00
168D	8.63	8.77	8.70	10.76	8.71	9.74	9.69	8.74	9.22
17B	8.39	7.81	8.10	10.68	8.50	9.59	9.53	8.16	8.84
19B	8.78	7.71	8.25	12.01	8.16	10.08	10.39	7.94	9.17
1B	8.53	8.41	8.47	10.48	9.25	9.87	9.50	8.83	9.17
20A	6.99	7.05	7.02	9.35	8.28	8.82	8.17	7.67	7.92
21A	8.57	9.04	8.80	10.32	9.62	9.97	9.45	9.33	9.39
21B	9.51	9.09	9.30	10.53	9.07	9.80	10.02	9.08	9.55
22B	10.17	7.39	8.78	11.26	9.25	10.25	10.72	8.32	9.52
22C	8.85	8.56	8.70	11.14	9.34	10.24	9.99	8.95	9.47
33A	9.29	7.89	8.59	9.32	8.60	8.96	9.30	8.24	8.77
33C	8.85	8.33	8.59	10.64	9.21	9.93	9.75	8.77	9.26
35A	7.61	8.26	7.94	9.75	8.84	9.29	8.68	8.55	8.62
39C	10.04	7.02	8.53	9.97	10.04	10.01	10.01	8.53	9.27
48A	8.01	7.23	7.62	8.97	8.40	8.69	8.49	7.82	8.15
48B	7.93	6.80	7.36	10.37	8.84	9.61	9.15	7.82	8.49
48D	8.39	8.53	8.46	11.64	10.09	10.86	10.02	9.31	9.66
4F	7.62	8.30	7.96	9.15	7.62	8.39	8.39	7.96	8.17
5G	7.93	9.26	8.59	11.55	9.40	10.47	9.74	9.33	9.53
6E	8.02	8.07	8.05	11.52	10.31	10.92	9.77	9.19	9.48
76A	6.41	8.10	7.25	10.21	8.30	9.25	8.31	8.20	8.25
Beaver	9.70	9.05	9.38	12.05	10.15	11.10	10.88	9.60	10.24
Soissons	8.60	8.06	8.33	10.82	9.37	10.10	9.71	8.71	9.21
Mean	8.52	8.03	8.27	10.44	8.86	9.65	9.48	8.44	8.96

SED (df)					
				Year (2)	0.137
Irrigation(2)	0.468	Irr (2)	0.354	Irri (4)	0.293
Genotype(131)	0.485	Gen(133)	0.388	Gen.(264)	0.310
Ir x G (131)	0.822	IrxG(133)	0.646	Ir x G (264)	0.522
				Yr x Irr (4)	0.324
				Y x G (264)	0.453
				YxIxG(264)	0.739

5.1.2.2 Carbon isotope discrimination values (Delta; $\Delta^{13}C$; ‰)

5.1.2.2.1 2003

Drought decreased the grain delta from 20.28 to 19.37 ‰ (P=0.09). The genotypes differed significantly and under irrigation, delta ranged from 19.54 ‰ (line 135B) to 20.99 ‰ (line 22B) (P<0.001). The parents differed in $\Delta^{13}C$ with Soissons showing a lower value than Beaver both under irrigated and unirrigated conditions (P<0.001; Table 5.3). Under irrigation, the $\Delta^{13}C$ for Beaver and Soissons were 20.68 and 20.19 ‰ respectively. The reduction in delta under drought ranged from 0.35 to 1.34 ‰ amongst lines, but the genotype x irrigation interaction was not significant (P=0.12).

5.1.2.2.2 2005

Drought significantly reduced grain $\Delta^{13}C$ from 20.57 ‰ to 19.08 ‰ (P<0.005; Table 5.3). Under irrigation delta ranged from 20.0 ‰ (line 135B) to 21.23 ‰ (line 22B). Parents differed significantly with Soissons again showing lower values in both irrigated and unirrigated conditions than Beaver (P<0.001). Under irrigated conditions, the delta values for Beaver and Soissons were 20.91 and 20.37 ‰ respectively (P<0.001; Table 5.3). The irrigation x genotype interaction was not significant (P=0.1), and drought reduced grain $\Delta^{13}C$ in the range of 1.17 to 1.97 ‰ amongst genotypes.

5.1.2.2.3 Cross-site/season mean

Averaging across the two seasons, drought reduced grain $\Delta^{13}C$ by 1.20 ‰ (P=0.001). Under irrigation, $\Delta^{13}C$ ranged from 19.77 ‰ (line 135B) to 21.11‰ (line 22B) (P<0.001). The two parents differed consistently, in the two years as described above, under unirrigated conditions in the range of 19.09 ‰ (Soissons) and 19.49 ‰ (Beaver). The irrigation x line interaction was not significant and drought decreased $\Delta^{13}C$ in the range 0.86 ‰ (line 135B) to 1.49 ‰ (line 133A) (P=0.054; Table 5.3). The lack of a significant interaction is consistent with the literature demonstrating that $\Delta^{13}C$ appears to be highly heritable among the genotypes in the B x S population. Neither of the interaction effects was significant for site/season x irrigation (P=0.13) and site/season x irrigation x line (P=0.15). However, the site/season x line interaction was significant (P<0.001) indicated that the performance of genotypes was different in each site/season.

Averaging across 2003 and 2005, mean $\Delta^{13}C$ ranged from 19.34 ‰ (line 135B) to 20.46 ‰ (line 22B). Of the eight genotypes most intensively studied, lines 134G and 22B had minimum and maximum $\Delta^{13}C$ values (19.42 and 20.46 ‰ respectively). The ranking of the genotypes was in the order of 134G <Soissons <21B <35A <76A <Beaver <134E <22B. Under unirrigated conditions, in both years, line 134G and Soissons always had low delta values indicating high WUE. Whereas, lines 134E, 22B and Beaver always had high delta values indicating low WUE.

5.1.3 Relationship between $\Delta^{13}C$ and grain yield

Carbon isotope discrimination was measured on grains of all 35 genotypes obtained from combine harvest in 2003 and 2005. The grain $\Delta^{13}C$ was positively correlated with grain yield in each of the two site/seasons (Figure 5.2).

In 2003, $\Delta^{13}C$ showed a positive linear relationship with yield amongst genotypes (R^2=0.17; P=0.013) under drought. Under irrigation, the relationship was not significant (P=0.87). A similar positive linear relationship was observed in 2005 under drought (R^2=0.17; P=0.015) with no significant relationship under irrigated conditions (P=0.12). For individual irrigation treatments, mean values over two years showed a significant positive relationship between $\Delta^{13}C$ and grain yield (P<0.05). Coefficients of determination (R^2) were 0.29 under drought (P<0.001) and 0.12 under irrigation (P<0.04). The genotype x irrigation interaction effect just failed to realise significance (P=0.054). Thus, the genotype performance was apparently stable in each of the experiments.

In theory, carbon isotope discrimination values are negatively correlated with TE (Farquhar and Richards, 1984). Since WUE was not measured directly in the field, TE and $\Delta^{13}C$ are not always negatively correlated. So the present results indicate a negative relationship between TE and grain yield. More detailed measurements of growth and gas-exchange parameters were carried out in each year to examine processes underlying the differences in $\Delta^{13}C$ for eight selected genotypes. The following sections describe the growth and yield performance of the six DH lines and their parents for these stomatal aperture traits.

Table 5.3 Grain carbon isotope discrimination values (‰) for 33 doubled haploid lines and 2 parents in population derived from Beaver x Soissons in 2003 (Gleadthorpe) and 2005 (Sutton Bonington) in irrigated and unirrigated treatments.

	Grain delta (‰)								
	2003			2005			Cross-year		
Genotype	*irri*	*unirri*	*mean*	*irri*	*unirri*	*mean*	*irri*	*unirri*	*mean*
118A	20.67	19.33	20.00	20.89	19.49	20.19	20.78	19.41	20.09
120B	19.80	19.32	19.56	20.03	18.70	19.36	19.92	19.01	19.46
121D	20.63	19.46	20.04	20.77	19.19	19.98	20.70	19.32	20.01
122I	20.07	19.31	19.69	20.61	18.89	19.75	20.34	19.10	19.72
133A	20.25	19.10	19.68	20.70	18.86	19.78	20.47	18.98	19.73
134C	20.12	18.85	19.48	20.35	18.83	19.59	20.23	18.84	19.54
134D	19.85	19.23	19.54	20.51	19.03	19.77	20.18	19.13	19.66
134E	20.55	19.81	20.18	20.85	19.64	20.24	20.70	19.72	20.21
134G	20.14	19.02	19.58	20.07	18.44	19.25	20.11	18.73	19.42
135B	19.54	19.02	19.28	20.00	18.80	19.40	19.77	18.91	19.34
137C	19.93	18.90	19.41	20.50	19.24	19.87	20.21	19.07	19.64
14A	20.04	19.29	19.67	20.73	19.26	20.00	20.39	19.28	19.83
14E	20.69	19.64	20.17	20.74	19.37	20.06	20.72	19.51	20.11
168D	19.88	18.88	19.38	20.29	18.44	19.36	20.08	18.66	19.37
17B	19.99	19.14	19.57	20.11	18.93	19.52	20.05	19.04	19.54
19B	20.74	19.46	20.10	20.63	19.00	19.82	20.68	19.23	19.96
1B	20.35	19.36	19.85	20.72	19.37	20.05	20.53	19.37	19.95
20A	19.66	18.50	19.08	20.67	18.87	19.77	20.17	18.69	19.43
21A	20.78	19.73	20.26	21.10	19.75	20.42	20.94	19.74	20.34
21B	20.18	19.71	19.95	20.76	18.79	19.77	20.47	19.25	19.86
22B	20.99	19.92	20.46	21.23	19.69	20.46	21.11	19.80	20.46
22C	20.51	19.64	20.07	20.59	19.38	19.98	20.55	19.51	20.03
33A	20.04	18.82	19.43	20.07	18.78	19.42	20.06	18.80	19.43
33C	19.98	19.30	19.64	20.38	18.90	19.64	20.18	19.10	19.64
35A	20.40	19.76	20.08	20.27	19.10	19.68	20.33	19.43	19.88
39C	20.12	19.39	19.76	20.64	19.04	19.84	20.38	19.21	19.80
48A	20.05	19.08	19.57	20.73	18.85	19.79	20.39	18.96	19.68
48B	20.40	19.67	20.04	20.71	19.14	19.92	20.56	19.40	19.98
48D	20.23	19.58	19.91	20.37	19.18	19.77	20.30	19.38	19.84
4F	20.49	19.30	19.90	20.45	19.02	19.74	20.47	19.16	19.82
5G	20.59	20.24	20.42	21.04	19.39	20.21	20.81	19.81	20.31
6E	20.52	19.51	20.01	20.67	19.27	19.97	20.59	19.39	19.99
76A	20.61	19.89	20.25	20.60	18.97	19.78	20.60	19.43	20.02
Beaver	20.68	19.77	20.22	20.91	19.21	20.06	20.79	19.49	20.14
Soissons	20.19	19.08	19.64	20.37	19.10	19.74	20.28	19.09	19.69
Mean	20.28	19.37	19.82	20.57	19.08	19.83	20.42	19.23	19.83

SED (df)				Year (2)	0.0652
Irrigation (2)	0.293	Irri (2)	0.090	Irri (4)	0.1530
Genotype (132)	0.162	Geno.(130)	0.133	Gen.(262)	0.1048
Ir x G (132)	0.369	Irx G (130)	0.206	Ir x G (262)	0.2116
				Yr x Irr (4)	0.1663
				Yr x G (262)	0.1600
				YxIrxG(262)	0.2653

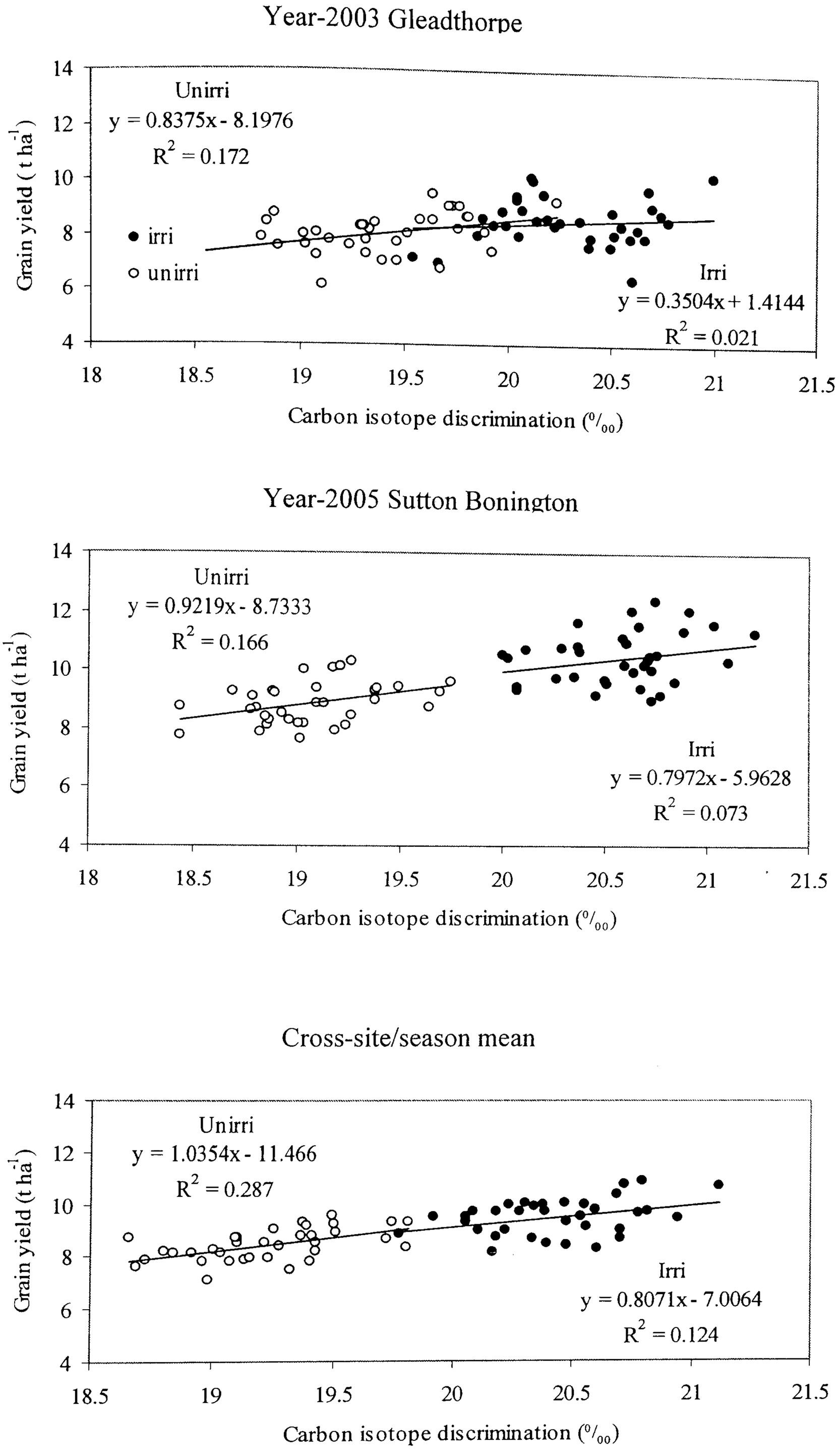


Figure 5.2 Relationships between combine grain yield (85% DM) and carbon isotope discrimination (‰) measured in mature grains for a set of 33 doubled haploid lines and 2 parents in the population derived from Beaver x Soissons grown at Gleadthorpe (2003) and Sutton Bonington (2005) in irrigated and unirrigated conditions.

5.1.4 Effects of genotypes and irrigation on yield and yield components

The responses to irrigation of eight wheat genotypes contrasting for morpho-physiological characteristics were examined. The contrasting differences for the basis of selection of these genotypes are presented in Table 3.2 and described in chapter 3. These genotypes differed for the key morphological traits like plant height, flag leaf size, presence/ absence of awns and ear size.

5.1.4.1 Grain yield

The grain yield responses to drought are shown in Table 5.2. In summary, averaging across the two years and across the 8 genotypes, drought reduced grain yield from 9.48 to 8.44 t ha^{-1} ($P<0.03$). Averaging across years, line 22B and Beaver produced highest grain yields under irrigation in the range 10.7-10.9 t ha^{-1} ($P<0.001$; Table 5.2). The interaction showing larger decreases of irrigation x genotype was significant ($P<0.02$) with line 22B (2.4 t ha^{-1}) and Beaver (1.3 t ha^{-1}), lines 134G and Soissons showing losses of about 1.0 t ha^{-1}, and lines 76A, 35A and 134E showed smallest reductions in grain yield in the range 0.1-0.3 t ha^{-1} due to drought (Table 5.2).

5.1.4.2 Above-ground dry matter production (AGDM)

5.1.4.2.1 2003

Drought reduced AGDM from 1811 g m^{-2} to 1699 g m^{-2}, but not significantly so. Under irrigation, the genotypes differed significantly in the range 1305-2118 g m^{-2} ($P<0.001$; Table 5.4). The genotype x irrigation interaction was weakly significant with line 22B and Beaver losing more AGDM due to drought than other genotypes ($P=0.07$) in the range 191-408 g m^{-2}.

5.1.4.2.2 2005

Drought reduced AGDM by about 209 g m^{-2}, but not significantly so. Genotype differences were evident with lines Beaver, 22B and 35A producing more AGDM under irrigation ($P<0.001$) than other genotypes. The interaction effect was not significant.

5.1.4.2.3 Cross-site/season mean

The reduction in AGDM was on average 161 g m^{-2} (P=0.12). Genotypic differences were significant with line 22B and Beaver producing more AGDM under irrigation (2210 and 2134 g m^{-2}, respectively; P<0.001) than other genotypes in the range 1614 to 2088 g m^{-2}. Lowest AGDM was observed for line 76A. The genotype x irrigation interaction was not significant (P=0.08). However, there was a trend for line 22B (382 g m^{-2}) to lose more biomass under drought than line 76A (20g m^{-2}).

5.1.4.3 Ear number m^{-2}

5.1.4.3.1 2003

Restricted water availability decreased ear number m^{-2} by 74 (P=0.032). Lines 22B, 35A and 134E produced more ears under irrigation than line 134G (P<0.001; Table 5.6). The interaction effect was not significant.

5.1.4.3.2 2005

The effect of drought was not significant (P=0.07). Under irrigation, Soissons (617 m^{-2}) and lines 134E and 35A (both 593 ears m^{-2}) produced more ears m^{-2} than line 134G (394 m^{-2}) (P<0.001). The irrigation x genotype interaction effect was not significant.

5.1.4.3.3 Cross-site/season mean

Drought resulted in fewer ears (P=0.07). Under irrigation, lines 22B, 35A and 134E had more ears than other genotypes in the range 615-443 m^{-2} (P<0.001). Soissons differed significantly compared to Beaver (590 c.f. 540; P<0.001). The irrigation x genotype interaction was not significant.

5.1.4.4 Thousand grain weight

Thousand grain weight (TGW) was measured on all 35 genotypes in contrast to the other yield components that were only measured on the eight selected genotypes.

5.1.4.4.1 2003

Unirrigated treatments showed larger differences (P<0.01; Table 5.5) on account of more grain weight. Under irrigation line 134G had a higher TGW (49.8 g; P<0.001) than 11 other genotypes in the range of 44.6-47.8g. Beaver and Soissons differed significantly in grain weight under irrigation (P<0.001). The interaction effect was significant with line 76A showing greater grain weight increase (P<0.001) under drought than lines 35A, 6E, 135B, Soissons and 4F.

5.1.4.4.2 2005

Drought decreased TGW significantly by 3.42 g (P=0.005). Lines 134G, 76A and Beaver had the greatest grain weight in the range of 46.5-51.1 g (P<0.001) under irrigation, but these lines lost most grain weight under drought (P<0.001). Soissons had the smallest grain weight (41 g; P<0.001) under irrigation. The largest reduction was found for line 76A (9g) followed by Beaver and 134E (4g each). Overall 28 lines of the 35 showed significant reductions in TGW due to drought (P<0.001).

5.1.4.4.3 Cross-site/season mean

Averaging across years drought reduced TGW from 43.9 to 42.7 (P<0.02; Table 5.5). Under irrigation, line 134G had a larger TGW (50.5 g; P<0.001) than other lines in the range of 49.3-38.9 g. Beaver differed significantly compared to Soissons, with greater TGW under irrigation (43.52 c.f. 40.44g; P<0.001). The irrigation x genotype interaction effect was significant with 12 lines showing a significant decrease in TGW due to drought (P<0.001). However, the response of parents was not significantly different.

5.1.4.5 Grain number ear^{-1}

5.1.4.5.1 2003

The reductions in grain number due to drought was weakly significant (P=0.053). In irrigated conditions, it ranged from 28 to 43 grains ear^{-1}. Lines 76A, and 134G had significantly more grains (P<0.001; Table 5.6). The response of lines to drought was not significantly different.

5.1.4.5.2 2005

Drought reduced grains ear^{-1} by 3.1 (P=0.053). Lines differed significantly under irrigation with 76A and Beaver having more fertile ears than line 134E in the range 38.7-23.7 grains ear^{-1} (25; P<0.001). The irrigation x genotype interaction was not significant.

5.1.4.5.3 Cross-site/season mean

Overall drought apparently reduced number of grains ear^{-1} by 0.3. Genotypes differed with lines 76A and 21B producing more grains ear^{-1} (38-41) under irrigation (P<0.001) than lines 35A and 134E (about 28) (P<0.001; Table 5.6). The irrigation x genotype interaction effect was not significant.

5.1.4.6 Plant height

5.1.4.6.1 2003

Drought did not reduce the plant height (Table 5.6). However, genotypes differed significantly with in the range of 94–58 cm (P<0.001). Soissons was slightly taller than Beaver (83 cm c.f. 78 cm; P<0.001). The irrigation x genotype interaction was not significant.

5.1.4.6.2 2005

Drought reduced height by 3 cm (P<0.013; Table 5.6). Under irrigation, line 134G (87 cm; P<0.02) was taller than other genotypes in the range 62-85 cm. The genotype x irrigation interaction was significant with Beaver and lines 21B, 35A and 76A showing greater reductions in plant height under drought than other genotypes (P<0.023).

5.1.4.6.3 Cross-site/season mean

Drought decreased plant height from 80 cm to 77 cm (P<0.01; Table 5.6). Under irrigation genotypes differed from 90 to 59 cm (P<0.01), but Beaver and Soissons were similar of about 80 cm. The irrigation x genotype interaction effect was not significant. However, there was a trend for Beaver to reduce plant height by about 5 cm more under drought than Soissons.

Table 5.4 Above-ground dry matter (g m^{-2}) and harvest index for eight wheat genotypes in irrigated and unirrigated treatments.

	Above-ground biomass (g m^{-2})			Harvest index		
Genotypes	*2003*	*2005*	*Cross-site/season mean*	*2003*	*2005*	*Cross-site/season mean*
Irrigated						
134E	1755	2103	1929	0.48	0.46	0.47
134G	1807	2083	1945	0.47	0.45	0.46
21B	1873	2042	1958	0.51	0.52	0.51
22B	2118	2303	2210	0.49	0.49	0.49
35A	1924	2253	2088	0.40	0.43	0.42
76A	1305	1923	1614	0.49	0.51	0.51
Beaver	1933	2335	2134	0.50	0.52	0.51
Soissons	1773	2271	2022	0.49	0.48	0.48
Mean	1811	2164	1988	0.48	0.48	0.48
Unirrigated						
134E	1738	1874	1806	0.49	0.47	0.48
134G	1664	1822	1743	0.48	0.43	0.46
21B	1714	1948	1831	0.53	0.47	0.50
22B	1710	1945	1828	0.52	0.48	0.50
35A	1828	2182	2005	0.44	0.41	0.42
76A	1571	1698	1634	0.52	0.49	0.50
Beaver	1742	2160	1951	0.52	0.47	0.50
Soissons	1624	2015	1819	0.50	0.47	0.48
Mean	1699	1955	1827	0.50	0.46	0.48
S.E.D. (df)						
- Year			6.1 (2)			0.002 (2)
Irrigation	121.1(2)	112.1(2)	82.5 (4)	0.005 (2)	0.004 (2)	0.003 (4)
Genotype	89.8(26)	75.5(28)	58.5 (54)	0.009(27)	0.010 (28)	0.007 (55)
Irr x Genotype	169.6(26)	150.1(28)	113.1(54)	0.014(27)	0.014 (28)	0.010 (55)

Table 5.5 Thousand grain weight (g) for 33 doubled haploid lines and the 2 parents in the population derived from Beaver x Soissons in 2003 (Gleadthorpe) and 2005 (Sutton Bonington) in irrigated and unirrigated treatments.

	1000-grain weight (g)								
	2003			2005			Cross-site/season mean		
Genotype	*irri*	*unirri*	*mean*	*irri*	*unirri*	*mean*	*irri*	*unirri*	*mean*
118A	47.8	45.9	46.8	49.3	44.2	46.7	48.5	45.0	46.8
120B	40.3	41.7	41.0	42.3	40.7	41.5	41.3	41.2	41.3
121D	40.6	41.0	40.8	42.9	40.4	41.6	41.8	40.7	41.2
122I	42.6	43.2	42.9	44.5	40.4	42.4	43.5	41.8	42.7
133A	45.9	44.6	45.3	45.2	43.1	44.1	45.6	43.8	44.7
134C	47.7	47.7	47.7	47.3	45.8	46.5	47.5	46.7	47.1
134D	48.8	48.3	48.6	47.2	45.2	46.2	48.0	46.8	47.4
134E	45.5	45.3	45.4	46.4	43.4	44.9	45.9	44.3	45.1
134G	49.8	50.4	50.1	51.1	47.1	49.1	50.5	48.8	49.6
135B	35.1	37.8	36.5	42.4	37.3	39.8	38.7	37.5	38.1
137C	44.1	43.2	43.6	42.9	41.8	42.3	43.5	42.5	43.0
14A	45.2	45.7	45.4	44.7	39.4	42.0	44.9	42.5	43.7
14E	44.9	45.1	45.0	47.8	43.0	45.4	46.4	44.0	45.2
168D	44.8	46.0	45.4	46.0	42.1	44.0	45.4	44.0	44.7
17B	43.6	45.9	44.8	45.9	42.6	44.2	44.7	44.3	44.5
19B	42.7	42.3	42.5	43.5	40.8	42.1	43.1	41.5	42.3
1B	40.9	42.1	41.5	47.1	42.9	45.0	44.0	42.5	43.2
20A	42.3	43.2	42.8	42.8	40.5	41.6	42.6	41.9	42.2
21A	42.1	42.4	42.3	46.7	42.1	44.4	44.4	42.3	43.3
21B	39.0	40.9	40.0	42.1	40.2	41.1	40.6	40.6	40.6
22B	43.4	45.1	44.3	45.9	44.1	45.0	44.7	44.6	44.6
22C	42.3	44.2	43.3	46.4	42.1	44.3	44.4	43.2	43.8
33A	45.5	44.7	45.1	47.2	44.7	45.9	46.3	44.7	45.5
33C	44.6	44.8	44.7	47.8	42.7	45.2	46.2	43.8	45.0
35A	35.1	39.9	37.5	42.3	41.6	42.0	38.7	40.7	39.7
39C	45.6	45.6	45.6	45.7	42.6	44.2	45.6	44.1	44.9
48A	45.9	44.9	45.4	44.5	43.7	44.1	45.2	44.3	44.7
48B	37.6	38.0	37.8	43.0	37.1	40.0	40.3	37.5	38.9
48D	41.6	43.9	42.7	42.1	38.3	40.2	41.9	41.1	41.5
4F	38.8	41.5	40.1	42.6	41.9	42.3	40.7	41.7	41.2
5G	45.3	47.1	46.2	53.4	49.3	51.3	49.3	48.2	48.8
6E	33.9	37.8	35.8	42.7	35.9	39.3	38.3	36.8	37.6
76A	32.3	38.7	35.5	45.5	36.4	41.0	38.9	37.6	38.2
Beaver	40.5	42.8	41.7	46.5	43.4	45.0	43.5	43.1	43.3
Soissons	39.9	42.6	41.2	41.0	38.6	39.8	40.4	40.6	40.5
Mean	42.5	43.5	43.0	45.3	41.9	43.6	43.9	42.7	43.3

SED (df)					
Irrigation(2)	0.56	Irr (2)	0.26	Year (2)	0.22
Genotype(131)	0.82	Gen(132)	0.67	Irr (4)	0.30
Irr x G (131)	1.27	IrxG(132)	0.97	Gen (263)	0.54
				Ir x G (263)	0.37
				Yr x Irr (4)	0.79
				Y x G (263)	0.81
				YxIxG(263)	1.13

Table 5.6 Ears m^{-2} and grains ear^{-1} and plant height for six DH lines and the 2 parents of the Beaver x Soissons population in irrigated and unirrigated treatments in 2003 and 2005.

		Ears m^{-2}			Grains ear^{-1}			Plant height (cm)		
Genotypes		*2003*	*2005*	*Cross-site /season mean*	*2003*	*2005*	*Cross-site /season mean*	*2003*	*2005*	*Cross-site /season mean*
Irrigated	134E	621	597	609	30.37	23.69	27.03	88.1	78.5	84.2
	134G	492	394	443	40.51	34.36	37.43	94.1	86.9	90.2
	21B	551	490	521	39.20	37.24	38.22	78.1	74.7	76.8
	22B	641	589	615	33.68	30.33	32.01	84.6	74.7	80.7
	35A	627	601	614	28.46	27.62	28.04	88.7	84.9	87.3
	76A	561	436	498	43.02	38.67	40.85	58.3	61.7	59.0
	Beaver	574	505	540	34.24	38.09	36.17	78.6	82.3	80.5
	Soissons	563	617	590	36.17	32.74	34.45	82.4	79.8	81.7
	Mean	579	528	554	35.71	32.84	34.27	81.6	77.9	80.0
Unirrigated	134E	597	657	627	32.53	25.57	29.05	89.7	75.2	81.5
	134G	382	415	399	40.11	31.11	35.61	91.9	83.6	88.1
	21B	466	544	505	42.40	30.16	36.28	78.1	64.3	70.8
	22B	533	609	571	39.93	28.23	34.08	86.3	74.2	79.1
	35A	626	600	613	34.08	24.11	29.10	88.4	78.3	82.9
	76A	464	494	479	40.62	37.35	38.99	56.9	53.4	56.2
	Beaver	447	514	480	40.77	32.42	36.60	77.8	74.1	75.9
	Soissons	528	652	590	35.48	28.82	32.15	83.2	78.9	80.4
	Mean	505	561	533	38.24	29.72	33.98	81.5	72.7	76.9
S.E.D. (df)	Year			12.88 (2)			0.76 (2)			0.53 (2)
	Irrigation	13.53 (2)	9.23 (2)	8.24 (4)	0.61 (2)	0.75 (2)	0.48 (4)	0.51 (2)	0.60 (2)	0.52 (4)
	Genotype	31.08(25)	31.71(28)	22.21(53)	2.55(25)	1.50(28)	1.46(53)	1.46 (28)	1.53 (25)	1.04 (53)
	Irr x Genotype	43.29(25)	42.95(28)	30.51(53)	3.42(25)	2.12(28)	1.99(53)	2.00 (30)	2.12 (25)	1.47 (53)

5.1.4.7 Carbon isotope discrimination and yield relationship in eight selected genotypes

As previously described a significantly positive relationship was observed between $\Delta^{13}C$ and grain yield for the 33 DH lines and 2 parents in both the years of the study. The range of $\Delta^{13}C$ values for the eight selected genotypes was representative of 33 DH lines and the 2 parents. Averaged across the 2 site/seasons when analysed on eight genotypes separately, positive genetic correlation between $\Delta^{13}C$ and grain yield was only weakly significant under drought (r=0.29) but was significant under irrigation (r=0.55; P<0.05). To explain the variations in carbon isotope discrimination values among genotypes detailed measurements of gas-exchange were carried on these eight genotypes, and the details of the various stomatal aperture traits measured are presented in the following section.

5.1.5 Gas-exchange measurements

The gas-exchange parameters (as described in the section 3.2.3.6) for eight genotypes were assessed under natural day-light on calendar dates approximately coinciding with flag leaf emergence, GS61 and GS61+1 week in 2003. In 2005, measurements were carried out on four dates approximately coinciding with GS39, GS39+2 weeks, GS61 and GS61+4 weeks. The measurements made in each of the growth stages and their means are presented in this section.

5.1.5.1 Photosynthetic rate (A; µmol m^{-2} s^{-1})

5.1.5.1.1 2003

Averaging over readings net photosynthetic rate declined from 13.4 to 12.0 µmol m^{-2} s^{-1} under drought. The genotypic difference was weakly significant (P=0.052) with line 22B showing higher *A* values (16.53) under irrigation. The interaction responses were not statistically significant (P=0.12), although lines 134G and Soissons showed a trend for a greater reduction under drought (Table 5.7).

5.1.5.1.2 2005

As expected, drought on average reduced *A* from 20.9 to 14.1 µmol m^{-2} s^{-1} (P<0.001). Genotypes differed in the range 18.1 to 23.1, with lines 76A and 134G showing higher *A*

values than Soissons and line 134E under irrigation (P<0.01). Both parents significantly showed reductions in net photosynthetic rate under drought in the range 3.7 to 5.2 μmol m^{-2} s^{-1} (P=0.022). Lines 76A, 134G and 134E showed larger reductions in the range 11-8 μmol m^{-2} s^{-1} than other DH lines in the range 5-6 μmol m^{-2} s^{-1} (P<0.05). The interaction of measurement date x irrigation x genotype was significant (P=0.04) (Table 5.7).

5.1.5.2 Stomatal conductance (g_s; mmol m^{-2} s^{-1})

5.1.5.2.1 2003

Averaging across genotypes, drought reduced stomatal conductance (P=0.063; Table 5.8). Genotypes differed under irrigation in the range 292-409 mmol m^{-2} s^{-1} with Soissons showing smaller values than most other genotypes (P=0.02). Although the interaction effect was not statistically significant, there was a trend for Soissons and line 134G to show larger decreases in g_s under unirrigated conditions than other genotypes.

5.1.5.2.2 2005

As expected, genotypes showed lower g_s values in the unirrigated treatment (P<0.01). The other effects were inconsistent. Stomatal conductance across all dates did not differ among parents and DH lines (P=0.23). The irrigation x genotype interaction was not significant.

5.1.5.3 Intercellular CO_2 concentration (C_i; mmol CO_2 mol^{-1} air)

5.1.5.3.1 2003

Drought had no detectable effect (P=0.80). The main effect of genotype and the genotype x irrigation interaction were not significant. However, line 35A and Soissons tended to have low C_i values and Soissons tended to show a reduction in C_i values in unirrigated conditions (Table 5.9).

5.1.5.3.2 2005

Drought reduced mean C_i values significantly from 271 to 231 mmol CO_2 mol^{-1} (P<0.01). Genotypes showed reduced C_i under drought (P<0.001). Genotypes differed in

the range 211.3-327.6 mmol CO_2 mol^{-1}, air with line 76A showing higher C_i than most other genotypes ($P<0.001$). Overall the irrigation x genotype interaction indicated Soissons and line 76A showed a larger reduction in C_i under drought than other genotypes ($P=0.06$). Significant interactions were found with C_i decreasing more under drought on date3 ($P=0.04$) than other measurement dates. Line 76A showed a relatively greater decrease in C_i than other genotypes on date2 (GS39+2wk).

Table 5.7 Photosynthetic rates (μmol m^{-2} s^{-1}) of eight wheat genotypes in irrigated and unirrigated treatments.

	Photosynthetic rate (μmol m^{-2} s^{-1})											
	2003 (Gleadthorpe)		2005 (Sutton Bonington)									
	GS61		GS39		GS39+2wk		GS61		GS61+4wk		Mean	
Genotypes	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*
134E	12.77	10.10	14.98	11.58	22.41	17.69	26.60	6.34	11.75	7.95	21.17	13.12
134G	14.56	6.10	16.71	11.33	20.28	14.33	33.96	7.11	12.23	10.20	23.03	12.98
21B	9.90	13.33	19.43	12.13	16.83	17.92	24.40	11.04	14.62	13.93	21.05	15.98
22B	16.53	13.07	13.05	7.69	19.46	18.29	22.80	10.74	13.08	10.87	19.33	14.13
35A	13.77	15.17	12.49	5.94	14.47	13.16	24.26	8.31	12.32	9.92	18.12	11.56
76A	14.47	16.73	15.25	8.82	20.01	17.76	37.79	4.94	10.48	10.05	23.11	12.62
Beaver	13.89	14.68	15.40	13.91	17.64	10.88	23.76	11.67	14.27	13.92	20.00	14.82
Soissons	11.58	7.05	16.92	16.92	21.39	18.68	24.06	12.67	13.38	12.93	21.17	17.50
Mean	13.43	12.03	15.53	11.02	19.06	16.09	27.20	9.10	12.77	11.22	20.87	14.09
S.E.D.(df)												
Date												3.12(2)
Irrigation	0.44 (1)		1.76(1)		0.78		-		0.94		0.57 (4)	
Genotype	2.0 (14)		2.76(14)		2.19		-		1.45		1.10 (56)	
Irri x Gen	2.67(14)		4.06(14)		3.00		-		2.13		1.56 (56)	
Date x Irri												3.22 (56)
Date x Gen												3.74 (56)
D x I x G												4.40 (56)

Table 5.8 Stomatal conductance (mmol m^{-1} s^{-1}) of eight wheat genotypes in irrigated and unirrigated treatments in 2003 and 2005.

	Stomatal conductance (mmol m^{-1} s^{-1})											
	2003 (Gleadthorpe)				2005 (Sutton Bonington)							
	GS61		GS39		GS39+2wk		GS61		GS61+4wk		Mean	
Genotypes	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*
134E	323.7	153.5	230.8	209.7	212.3	178.4	188.9	43.0	355.5	322.0	236.7	178.2
134G	393.4	217.3	268.0	252.3	161.7	147.4	106.3	41.4	399.5	366.3	223.7	191.7
21B	291.8	193.8	231.4	202.8	143.9	162.5	116.7	35.4	427.7	383.2	219.8	185.8
22B	254.7	238.7	163.6	172.7	165.7	141.6	156.6	52.7	413.8	369.0	214.8	173.8
35A	304.2	234.8	424.7	254.1	147.4	195.6	202.3	41.7	369.3	311.0	275.8	190.4
76A	408.8	305.3	228.5	167.4	154.6	126.3	97.7	32.4	440.5	406.8	220.2	173.1
Beaver	370.9	269.0	220.9	170.0	181.3	161.3	147.6	46.7	438.8	390.3	237.0	181.9
Soissons	305.0	174.3	318.8	213.4	185.4	152.8	98.7	44.4	393.7	356.3	239.0	181.6
Mean	344.1	223.4	260.8	205.3	169.0	158.2	139.3	42.2	404.9	363.1	233.4	182.1
S.E.D.(df)												
Date												71.76 (2)
Irrigation		12.08 (1)		40.54		4.10		-		1.99 (1)		10.25 (4)
Genotype		32.05 (14)		43.98		18.94		-		24.34 (14)		14.24 (56)
Irr x Gen		44.09 (14)		70.91		25.40		-		32.26 (14)		21.45 (56)
Date x Irr												73.21 (56)
Date x Gen												76.55 (56)
D x I x G												82.34 (56)

Table 5.9 Intercellular CO_2 concentration (mmol CO_2 mol^{-1} air) of eight wheat genotypes in irrigated and unirrigated treatments.

	Intercellular CO_2 concentration (mmol CO_2 mol^{-1} air)											
	2003 (Gleadthorpe)		2005 (Sutton Bonington)									
	GS61		GS39		GS39+2wk		GS61		GS61+4wk		Mean	
Genotypes	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*
134E	223.3	224.8	245.3	256.2	237.3	177.7	308.6	263.6	273.2	248.3	284.2	254.5
134G	233.2	271.7	271.3	264.6	153.8	207.7	286.9	203.1	263.7	253.2	262.1	250.2
21B	230.5	216.0	192.3	263.2	179.1	130.7	157.8	31.0	243.8	233.3	211.3	182.6
22B	202.0	221.2	234.8	279.4	163.6	141.8	268.6	264.3	259.3	248.2	249.6	251.5
35A	197.2	194.7	263.6	298.4	228.4	222.3	294.6	200.1	266.0	229.2	281.2	255.5
76A	221.3	221.0	281.3	275.0	217.2	78.6	469.7	307.2	269.7	265.5	327.6	249.6
Beaver	232.4	223.8	261.8	204.6	239.8	153.4	295.6	194.1	245.5	244.0	278.7	217.1
Soissons	246.1	187.5	277.8	199.8	146.7	383.9	269.0	96.2	242.3	234.7	271.2	187.4
Mean	223.3	220.1	253.6	255.1	205.3	157.4	293.8	194.9	257.9	244.5	270.7	231.0
S.E.D.(df)												
Date												85.1(2)
Irrigation	8.3(1)		12.43	21.22		-		0.15				8.1(4)
Genotype	18.6(14)		26.79	38.49		-		10.20				15.4(56)
Irr x Gen	25.9(14)		37.55	55.17		-		13.49				21.9(56)
Date x Irr												85.9(4)
Date x Gen												89.9(56)
D x I x G												95.0(56)

5.1.6 Effects of genotypes and irrigation on growth and partitioning

In the experiments located at Sutton Bonington, detailed growth analysis was carried out on four genotypes contrasting for differences in morphological and physiological characteristics which included two parents, Beaver and Soissons, and two DH lines: 134G and 134E. Samplings for these genotypes were taken at GS31 and GS61 in years 2004 and 2005; and at GS39 in 2004. The results obtained on these genotypes are presented in this section.

5.1.6.1 Date of anthesis

Drought advanced flowering date on average by 1 day in each year. Averaging across irrigation treatments, genotypes ranged in flowering from 28 May to 7 June in 2003, from 31 May to 9 June in 2004 and from 7 to 15 June in 2005, with Soissons reaching anthesis about a week earlier than Beaver (Table 5.10).

5.1.6.2 Green area index

From the sequential growth analysis it was evident that the maximum green area index was reached at GS39 in 2004 (see Appendix III for pre-anthesis growth analysis). The results of analyses at GS61 are presented here. In 2004, genotypic differences were significant with Beaver showing the largest GAI of 9.52 under irrigation compared to other genotypes in the range 6.3-7.7 ($P<0.01$; Table 5.11). The main effects of irrigation and the interaction with genotype were not statistically significant.

In 2005, genotypes behaved similarly with Beaver again producing the largest green area index of 9.33 ($P<0.06$) compared to other genotypes in the range 6.3-6.6 under irrigation. The irrigation x genotype interaction was significant with Beaver showing a greater reduction in green area index from 9.33 to 4.58 under drought than other genotypes ($P<0.002$). Soissons maintained green area index better (Table 5.11).

Table 5.10 Dates of GS61 for 33 doubled haploid lines and the 2 parents in the population of Beaver x Soissons in irrigated and unirrigated treatments.

	2003		*2004*		*2005*	
Genotype	*Irrigated*	*Unirrigated*	*Irrigated*	*Unirrigated*	*Irrigated*	*Unirrigated*
118A	01-Jun	30-May	04-Jun	04-Jun	08-Jun	07-Jun
120B	02-Jun	02-Jun	07-Jun	01-Jun	13-Jun	14-Jun
121D	06-Jun	03-Jun	08-Jun	05-Jun	15-Jun	15-Jun
122I	01-Jun	31-May	03-Jun	03-Jun	08-Jun	06-Jun
133A	03-Jun	01-Jun	09-Jun	03-Jun	15-Jun	14-Jun
134C	05-Jun	03-Jun	08-Jun	06-Jun	14-Jun	14-Jun
134D	28-May	29-May	31-May	03-Jun	07-Jun	07-Jun
134E	01-Jun	02-Jun	05-Jun	05-Jun	09-Jun	07-Jun
134G	31-May	30-May	04-Jun	04-Jun	07-Jun	07-Jun
135B	29-May	29-May	04-Jun	03-Jun	08-Jun	08-Jun
137C	01-Jun	03-Jun	01-Jun	05-Jun	08-Jun	07-Jun
14A	01-Jun	31-May	06-Jun	05-Jun	13-Jun	10-Jun
14E	31-May	03-Jun	31-May	03-Jun	08-Jun	08-Jun
168D	01-Jun	01-Jun	07-Jun	07-Jun	11-Jun	13-Jun
17B	30-May	28-May	01-Jun	01-Jun	08-Jun	07-Jun
19B	06-Jun	02-Jun	09-Jun	05-Jun	14-Jun	12-Jun
1B	05-Jun	02-Jun	09-Jun	07-Jun	16-Jun	14-Jun
20A	02-Jun	01-Jun	07-Jun	01-Jun	11-Jun	11-Jun
21A	06-Jun	04-Jun	08-Jun	03-Jun	15-Jun	14-Jun
21B	03-Jun	04-Jun	09-Jun	03-Jun	12-Jun	14-Jun
22B	01-Jun	03-Jun	07-Jun	05-Jun	08-Jun	08-Jun
22C	28-May	27-May	01-Jun	01-Jun	08-Jun	06-Jun
33A	01-Jun	01-Jun	07-Jun	05-Jun	12-Jun	14-Jun
33C	29-May	28-May	31-May	01-Jun	10-Jun	07-Jun
35A	08-Jun	06-Jun	09-Jun	06-Jun	09-Jun	09-Jun
39C	06-Jun	03-Jun	08-Jun	05-Jun	14-Jun	15-Jun
48A	31-May	31-May	05-Jun	01-Jun	08-Jun	07-Jun
48B	03-Jun	01-Jun	07-Jun	05-Jun	13-Jun	10-Jun
48D	01-Jun	31-May	05-Jun	05-Jun	08-Jun	08-Jun
4F	05-Jun	04-Jun	07-Jun	05-Jun	15-Jun	11-Jun
5G	07-Jun	03-Jun	09-Jun	09-Jun	16-Jun	13-Jun
6E	03-Jun	01-Jun	07-Jun	05-Jun	09-Jun	08-Jun
76A	03-Jun	02-Jun	07-Jun	05-Jun	14-Jun	11-Jun
BEAVER	06-Jun	04-Jun	09-Jun	05-Jun	16-Jun	14-Jun
SOISSONS	29-May	28-May	01-Jun	01-Jun	07-Jun	07-Jun
Mean	02-Jun	01-Jun	05-Jun	04-Jun	11-Jun	10-Jun

5.1.6.3 Flag leaf area index

Flag leaf area index showed an apparent reduction under drought in 2005, but not in 2004. In 2004, Beaver had a higher flag leaf area index than Soissons (2.22 c.f. 1.84; P<0.01), while lines 134E and 134G recorded 1.44 and 1.30, respectively (Table 5.11).

In 2005, although, the main effects of genotype and irrigation were not significant, the interaction was significant and drought reduced flag leaf area index of Beaver from 1.94 to 0.89 (P<0.01); whereas the other genotypes did not show any significant reductions in flag leaf green area.

Table 5.11 Flag leaf area index, green area index and above-ground dry matter at GS61 of four wheat genotypes in irrigated and unirrigated conditions at Sutton Bonington in 2004 and 2005.

	Flag leaf area index		Green area Index		Above-ground DM ($g\ m^{-2}$)	
	2004	*2005*	*2004*	*2005*	*2004*	*2005*
Irrigated						
134E	1.44	1.37	6.27	6.64	1307	1375
134G	1.29	1.36	6.35	6.33	1170	1314
Beaver	2.22	1.94	9.52	9.33	1593	1717
Soissons	1.84	1.38	7.65	6.25	1296	1445
Mean	1.70	1.51	7.45	7.14	1341	1463
Unirrigated						
134E	1.42	1.38	4.88	5.71	1345	1310
134G	1.31	1.10	5.87	4.89	1275	1247
Beaver	2.07	0.89	8.26	4.58	1551	1607
Soissons	1.87	1.34	10.71	5.69	1564	1410
Mean	1.67	1.18	7.43	5.22	1434	1394
S.E.D (df)						
Irrigation (2)	0.08	0.17	0.55	0.84	14.3	7.4
Genotype (12)	0.22	0.13	1.05	0.45	75.6	50.9
Irr x Gen. (12)	0.28	0.23	1.39	0.98	93.7	62.7

5.1.6.4 Above-ground biomass production

The integrated effects of growth and development occurring in the pre- and post-flowering phases were ultimately expressed as the harvest biomass and the harvest index. In 2004, as previously described some of the sub-plots were lodged at anthesis. The unirrigated treatments showed higher biomass probably on account of greater incidence of lodging in the irrigated than in the unirrigated sub-plots. However, genotypes differed significantly in irrigated conditions with biomass production at anthesis ranging from 1170 g m^{-2} (line134G) to 1593 g m^{-2} (Beaver) ($P<0.01$; Table 5.11).

A similar trend was observed in 2005 under irrigated conditions, in the range of 1314 to 1717 g m^{-2} for line134G and Beaver, respectively ($P<0.001$) and drought decreased biomass on average by 69 g m^{-2} ($P<0.01$). The irrigation x genotype interaction was not significant and genotypes ranked similarly with or without irrigation. Nevertheless, the reduction in biomass was more with Beaver and least in Soissons following the pattern of reductions in GAI.

Drought decreased leaf lamina dry weight from 199 to 175 g m^{-2} ($P=0.17$) in 2005. Under irrigated conditions significantly higher leaf lamina weight was found with Beaver in 2004 (264 g m^{-2}; $P<0.001$), but in 2005 the genotype differences were not statistically significant (269 g m^{-2}; $P=0.10$). The interaction effect was not significant (Table 5.12).

In both years, restricted water availability did not reduce stem-and-sheath dry matter ($P=0.15$). The effect of genotype and the interaction with irrigation were also not significant (Table 5.12).

In 2005, drought decreased stem partitioning from 0.61 to 0.57 ($P=0.02$) and stem-and-sheath biomass at anthesis from 808 to 676 g m^{-2} ($P=0.11$; Table 5.12). Stem dry matter was depressed most in Beaver (208 g m^{-2}) and least in Soissons (20 g m^{-2}). A similar trend was found for leaf lamina dry matter production.

The ear partitioning did not vary much between irrigation treatments and remained constant ($P=0.2$). However, Beaver partitioned a higher proportion of biomass to the ear in both years ($P<0.001$) than Soissons, with intermediate values for lines 134G and 134E (Table 5.12).

5.1.6.5 Effects on biomass partitioning at harvest

The effect of drought on biomass growth was evident at GS61 in 2005 for four lines. With more prolonged drought in this season drought decreased biomass by 69 g m^{-2} (P=0.01; Table 5.11). There were consistent differences among genotypes in both the years in crop dry weight at harvest (P<0.01), with values averaged over two site/seasons, ranging from 1614 g m^{-2} (line 76A) to 2210 g m^{-2} (line 22B). The biomass depression was more for line 22B (382 g m^{-2}) than for line 134G (202 g m^{-2}) (P=0.08). Thus, the grain yield sensitivity of line 22B to drought derived, in part, from a sensitivity of above-ground biomass to drought (P=0.06). The yield response of Beaver and Soissons to drought was 1.3 and 1.0 t ha^{-1} respectively. Averaged over the site/seasons, there was no change in HI in the irrigation regimes (Table 5.4). However, HI decreased with restricted water supply in 2005 from 0.48 to 0.46 (P=0.022). HI also differed averaged across site/seasons amongst the genotypes from 0.42 (line 35A) to 0.51 (line 76A) (P<0.001; Table 5.4). Higher HI in line 76A did not translate into a higher yield because of its low biomass production (1634 g m^{-2}), but it maintained yield better under drought than other genotypes. The converse was the case for line 22B and Beaver which did not maintain grain yield well under unirrigated conditions on account of pronounced reductions in biomass growth. Averaging across sites/seasons, line 21B and Soissons maintained HI better than other genotypes (Table 5.4).

Table 5.12 Leaf lamina dry matter, stem-and-sheath dry matter and ear dry matter at GS61 of the four wheat genotypes in irrigated and unirrigated treatments.

	Leaf lamina DM ($g\ m^{-2}$)		Stem-and-sheath DM ($g\ m^{-2}$)		Ear DM ($g\ m^{-2}$)	
	2004	*2005*	*2004*	*2005*	*2004*	*2005*
Irrigated						
134E	186	130	813	831	212	188
134G	182	201	667	780	164	176
Beaver	264	269	767	871	250	316
Soissons	238	198	795	750	227	210
Mean	217	199	760	808	213	222
Unirrigated						
134E	146	176	709	665	184	170
134G	153	160	801	646	178	158
Beaver	255	180	828	663	297	305
Soissons	234	187	949	730	255	230
Mean	197	175	822	676	229	216
S.E.D (df)						
Irrigation (2)	6.96	11.37	27.0	48.8	10.9	6.3
Genotype (12)	10.47	25.50	49.9	92.6	15.0	28.7
Irr x Gen. (12)	14.59	33.24	66.9	123.4	21.4	35.7

5.1.7 Effects of the 1B/1R translocation on $\Delta^{13}C$ and grain yield

The groups of DH lines with or without 1B/1R translocation were analysed to examine the effect of 1B/1R on grain $\Delta^{13}C$ and grain yield (Table 5.13). The effect of the 1B/1R translocation on grain yield was not statistically significant in both years under irrigated and unirrigated conditions. However, 1B/1R was found to increase $\Delta^{13}C$ in 2003 (P=0.07) and in 2005 (P<0.001).

Table 5.13 Carbon isotope discrimination (‰) and grain yield (85 % DM; t ha^{-1}) for groups of 1BL/1RS and 1B doubled-haploid lines from the Beaver x Soissons population.

	2003					2005				
	Irrigated		Unirrigated			Irrigated		Unirrigated		
	1B/1R	*1B*	*1B/1R*	*1B*	Prob.	*1B/1R*	*1B*	*1B/1R*	*1B*	Prob.
$\Delta^{13}C$	20.44	20.12	19.49	19.25	0.07	20.76	20.41	19.30	18.90	<0.001
Grain yield	8.46	8.52	7.98	8.00	0.71	10.32	10.42	8.80	8.81	0.37

N.B: Number of DH lines having 1B/1R=15 and 1B=18

5.1.8 Effects of *Rht* genes on $\Delta^{13}C$ and grain yield

The analysis of variance including contrast factors for comparing the *Rht1, Rht2, Rht1+Rht2* (double-dwarf) and *rht* (tall) genes of groups of DH lines indicated no significant differences between *Rht1* and *rht* lines in $\Delta^{13}C$ and grain yield (Table 5.14). Tall lines (*rht*) had significantly lower grain yield than *Rht2* lines in both years in the irrigated and unirrigated treatments (P<0.01). However, $\Delta^{13}C$ of tall lines was significantly lower than *Rht2* lines in 2003 (P<0.001), but not in 2005 (P=0.11). The effect of dwarfing (*Rht1+Rht2*) in reducing $\Delta^{13}C$ was weak (P=0.06) in 2003, but was significant in 2005 (P=0.04). *Rht1+Rht2* improved grain yield (P<0.05) compared to tall lines in 2005, but the reverse was the case in 2003. *Rht1* decreased $\Delta^{13}C$ significantly compared to *Rht2* in 2003 (P=0.06) and in 2005 (P=0.02). However *Rht2* improved grain yield significantly in both years compared to *Rht1* under irrigated and unirrigated conditions.

Table 5.14 Carbon isotope discrimination (‰) and grain yield (85% DM; t ha^{-1}) for groups of *Rht1, Rht2, Rht1+Rht2* and *rht* doubled haploid lines from the Beaver x Soissons population.

	2003					2005				
	Irrigated		Unirrigated		Contrasts	Irrigated		Unirrigated		Contrasts
Rht1 vs rht	***Rht1***	***rht***	***Rht1***	***rht***	***Prob***	***Rht1***	***rht***	***Rht1***	***rht***	***Prob***
$\Delta^{13}C$	20.27	20.24	19.37	19.18	1.00	20.52	20.59	19.03	19.08	0.29
Grain yield	10.15	10.01	8.79	8.45	0.46	10.02	10.01	8.67	8.45	0.47
Rht2 vs rht	***Rht2***	***Rht***	***Rht2***	***rht***	***Prob***	***Rht2***	***rht***	***Rht2***	***rht***	***Prob***
$\Delta^{13}C$	20.33	20.24	19.57	19.19	<0.001	20.67	20.59	19.14	19.08	0.11
Grain yield	11.1	10.01	9.46	8.45	<0.001	11	10.01	9.39	8.45	<0.001
Rht1+Rht2 vs rht	***Rht1+Rht2***	***rht***	***Rht1+Rht2***	***rht***	***Prob***	***Rht1+Rht2***	***rht***	***Rht1+Rht2***	***rht***	***Prob***
$\Delta^{13}C$	20.21	20.24	19.46	19.19	0.06	20.42	20.59	19.02	19.08	0.04
Grain yield	7.58	8.59	7.68	7.79	0.005	10.66	10.01	8.92	8.45	<0.001
Rht1 vs Rht2	***Rht1***	***Rht2***	***Rht1***	***Rht2***	***Prob***	***Rht1***	***Rht2***	***Rht1***	***Rht2***	***Prob***
$\Delta^{13}C$	20.27	20.33	19.37	19.57	0.06	20.52	20.67	19.03	19.14	0.02
Grain yield	8.48	8.87	7.65	8.68	0.002	10.02	11.01	8.67	9.39	<0.001

N.B: a) Number of DH lines possessing *Rht* genes: *Rht1*=5; *Rht2*=9; *Rht1+Rht2*=5 and *rht*=14;
b) *Rht-B1b* (formerly *Rht1*); *Rht-D1b* (formerly *Rht2*); *Rht1+Rht2*=double-dwarf; *rht*=tall

Table 5.13 Genetic correlation coefficients of parameters measured on the eight lines of Beaver x Soissons DH population in irrigated and unirrigated conditions in 2003.

Irrigated 2003	$\Delta^{13}C$	A	g_s	C_i	A/g_s	A/Ci	ITE	SLA	SLN	Grain ear^{-1}	Ear m^{-2}	Plant Ht	AGDM	HI
$\Delta^{13}C$	1													
A	**0.70**	1												
g_s	-0.19	0.15	1											
C_i	-0.57	-0.56	0.37	1										
A/g_s	**0.71**	0.67	-0.63	-0.69	1									
A/Ci	**0.74**	**0.95**	-0.07	**-0.79**	**0.79**	1								
ITE	**0.74**	0.59	-0.10	**-0.70**	0.51	0.69	1							
SLA	-0.69	-0.20	0.39	0.57	-0.43	-0.36	-0.63	1						
SLN	-0.17	0.21	-0.47	-0.32	0.50	0.31	-0.19	0.27	1					
Grain ear^{-1}	-0.29	-0.09	0.55	0.50	-0.43	-0.26	-0.58	0.69	-0.17	1				
Ear m^{-2}	0.69	0.27	-0.64	**-0.71**	0.68	0.49	0.63	**-0.90**	0.13	**-0.78**	1			
Plant Ht	-0.24	0.05	-0.37	-0.09	0.27	0.09	0.09	0.03	0.40	-0.55	0.06	1		
AGDM	0.20	0.12	**-0.70**	-0.29	0.60	-0.38	0.41	-0.15	0.34	-0.59	0.37	**0.70**	1	
HI	0.10	-0.20	0.14	0.62	-0.21	0.44	-0.32	0.20	-0.54	0.60	-0.37	-0.42	-0.15	1
Yield	0.22	-0.02	-0.56	0.09	0.42	-0.02	0.20	-0.02	0.00	-0.22	0.12	0.44	**0.84**	0.41

Unrrigated 2003	$\Delta^{13}C$	A	g_s	C_i	A/g_s	A/Ci	ITE	SLA	SLN	Grain ear^{-1}	Ear m^{-2}	Plant Ht	AGDM	HI
$\Delta^{13}C$	1													
A	**0.86**	1												
g_s	0.39	0.69	1											
C_i	-0.25	-0.33	0.12	1										
A/g_s	**0.83**	0.69	-0.05	-0.48	1									
A/Ci	**0.81**	**0.98**	0.61	-0.50	**0.72**	1								
ITE	**0.81**	**0.72**	0.05	-0.69	**0.93**	**0.77**	1							
SLA	-0.43	-0.47	-0.53	-0.53	-0.18	-0.31	-0.10	1						
SLN	-0.08	-0.25	-0.07	**0.85**	-0.18	-0.43	-0.43	-0.48	1					
Grain ear^{-1}	0.07	0.26	0.54	0.41	-0.16	0.12	-0.20	-0.59	0.53	1				
Ear m^{-2}	0.40	0.20	-0.35	-0.69	0.59	0.35	0.63	0.45	-0.66	**-0.81**	1			
Plant Ht	-0.38	-0.62	-0.68	0.17	-0.19	-0.56	-0.27	0.15	0.01	-0.47	0.29	1		
AGDM	0.32	0.21	-0.22	-0.18	0.51	0.28	0.43	-0.29	-0.27	-0.36	0.54	0.60	1	
HI	0.22	0.19	0.22	0.13	0.07	0.06	0.12	-0.33	0.48	**0.75**	-0.56	-0.54	-0.50	1
Yield	0.49	0.34	0.01	0.01	0.49	0.26	0.49	-0.60	0.31	0.50	-0.16	-0.06	0.29	0.68

N.B: The values in bold letters indicate significance at P=0.05

Table 5.16 Genetic correlation coefficients of parameters measured on the eight lines of Beaver x Soissons DH population in irrigated and unirrigated conditions in 2005.

Irrigated	$\Delta^{13}C$	A	g_s	C_i	A/g_s	A/Ci	SLA	Grain	Ear m^{-2}	Plant Ht	AGDM	HI
$\Delta^{13}C$	1											
A	-0.32	1										
g_s	**-0.71**	-0.26	1									
C_i	-0.12	0.23	0.19	1								
A/g_s	0.19	**0.83**	**-0.76**	0.06	1							
A/Ci	-0.07	0.39	-0.33	**-0.80**	0.44	1						
SLA	0.51	-0.31	-0.30	-0.51	-0.03	0.35	1					
Grain ear^{-1}	-0.04	0.44	-0.08	-0.02	0.32	0.33	-0.26	1				
Ear m^{-2}	0.31	**-0.72**	0.23	-0.16	-0.62	-0.31	0.49	-0.70	1			
Plant Ht	-0.41	0.37	0.41	-0.36	-0.50	0.05	-0.34	-0.36	0.18	1		
AGDM	0.24	**-0.75**	0.22	-0.27	-0.66	0.27	-0.09	-0.26	0.62	0.57	1	
HI	0.56	0.26	-0.47	-0.08	0.43	0.28	0.21	**0.77**	-0.33	-0.63	-0.18	1
Yield	0.62	-0.33	-0.20	-0.19	-0.14	-0.03	0.05	0.45	0.17	-0.12	0.59	0.69

Unirrigated	$\Delta^{13}C$	A	g_s	C_i	A/g_s	A/Ci	SLA	Grain	Ear m^{-2}	Plant Ht	AGDM	HI
$\Delta^{13}C$	1											
A	-0.02	1										
g_s	**-0.76**	-0.14	1									
C_i	0.30	-0.52	-0.23	1								
A/g_s	0.19	**0.97**	-0.39	-0.41	1							
A/Ci	-0.19	**0.85**	0.08	**-0.89**	**0.76**	1						
SLA	-0.10	-0.06	0.19	-0.03	-0.12	0.05	1					
Grain ear^{-1}	-0.41	0.09	-0.24	-0.23	0.14	0.12	-0.29	1				
Ear m^{-2}	**0.76**	0.27	-0.40	0.20	0.36	0.05	0.35	-0.67	1			
Plant Ht	0.01	0.05	0.40	0.32	-0.05	-0.18	-0.30	-0.66	0.15	1		
AGDM	0.20	0.17	0.28	-0.19	0.09	0.19	-0.28	-0.54	0.34	0.48	1	
HI	0.38	0.43	**-0.82**	-0.24	0.60	0.36	-0.16	0.58	0.12	-0.64	-0.42	1
Yield	0.49	0.54	-0.33	-0.40	0.60	0.51	-0.43	-0.12	0.44	0.04	**0.71**	0.34

N.B: The values in bold letters indicate significance at P=0.05

5.2 DISCUSSION

The results of the field study are discussed in this chapter through an analysis of various yield-forming traits and estimates of water-use efficiency to explain the relative performance of genotypes under irrigated and unirrigated field conditions. The issues pertaining to gas-exchange measurements and their relationships with carbon isotope discrimination will be dealt with in detail to elucidate the physiological mechanisms and their genetic variation regulating water-use efficiency in UK wheat cultivars. The present study used a doubled-haploid mapping population derived from a cross between Beaver (UK-bred, photoperiod sensitive) and Soissons (French-bred, photoperiod insensitive). These parents also contrast for several morpho-physiological traits which are detailed in section 3.2.2.1.

5.2.1 The impact of drought and weather conditions

For any crop, moisture availability is, principally, a function of the rainfall and the available water capacity (AWC) of the soil within the rooting zone. The major variation in AWC is due to soil texture and depth. However, there are also effects of soil structural condition and whether a soil is a topsoil or subsoil (Bailey, 1990). Lighter soils with poor structure and shallow topsoils have less AW. In the UK, AWC for winter wheat ranges from about 120 mm on light sandy soils to more than 250 mm on some deep silty clay loam soils, assuming a rooting depth of 1.5 m (Foulkes *et al.*, 1994).

In 2003, at Gleadthorpe, the onset of drought coincided with flag leaf emergence, and the irrigated treatment received 175 mm of water. In this year, soil moisture deficit (SMD; the depletion of the available water below field capacity) exceeded 80 mm for the period between 8 May and 29 June although the drought was only sustained till mid grain filling due to exceptionally high rainfall on 30 June (54 mm). In 2005, at Sutton Bonington the onset of drought coincided with ear emergence and the irrigated treatment received 171 mm of water. SMD exceeded 100 mm post-anthesis under unirrigated conditions after 4 June and the drought was sustained until physiological maturity with SMD raising to 135 mm. Thus, the post-anthesis drought was relatively severe in Sutton Bonington in 2005 as compared to Gleadthorpe in 2003.

In 2003, the available water of the loam medium sand (Cuckney soil series) at ADAS Gleadthorpe was 108 mm, of which the limiting deficit (LD) was 50% (54 mm) (Foulkes *et al.*, 2002). SMD up to 80 mm was sustained till 29 June. The sandy loam soil (Dunnington Heath series) at Sutton Bonington had an AW of 186 mm (Reeves, 1975), and yield-limiting SMDs were therefore those greater than 93 mm (50% AW, Bailey, 1990). Thus, limiting SMDs exceeded 100 mm from the beginning of anthesis to complete canopy senescence.

In this study to relate the effects of limited water availability on WUE for lines of Beaver x Soissons mapping population the popular technique of carbon isotope discrimination has been used. Carbon isotope discrimination (delta; $\Delta^{13}C$) reflects a measure of the extent to which photosynthesis (A) is maintained while stomatal conductance (g_s) decreases. During the photosynthetic processes, C_3 plants discriminate against the heavier carbon isotope ^{13}C. Transpiration efficiency as a process is dependent on the ratio of intercellular to atmospheric CO_2 partial pressure, a higher $\Delta^{13}C$ resulting from higher C_i/C_a, for example, due to higher stomatal conductance. Thus, TE (net photosynthesis/transpiration) is negatively associated with both C_i/C_a and $\Delta^{13}C$ (Farquhar & Richards, 1984; Ehleringer, 1990).

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

As per 'Passioura's yield model', yield may be increased by an increase in water use, water-use efficiency or harvest index. Here an attempt has been made to explain the variations in WUE of wheat genotypes based on $\Delta^{13}C$ and to interpret this within the framework proposed by Passioura (1977; 1996). It is well established that among several approaches, carbon isotope discrimination ($\Delta^{13}C$) is the best-suited technique for rapid and precise assessment of the genetic variability in WUE (Farquhar & Richards, 1984; Condon *et al.*, 1987; Ehleringer, 1990; Ebdon *et al.*, 1998 and Richards *et al.*, 2002) and as a predictor of yield performance under drought (Acevedo, 1993; Morgan *et al.*, 1993; Merah *et al.*, 1999). As predicted by ^{13}C theory, TE and $\Delta^{13}C$ are inversely related (Farquhar *et al.*, 1989) and such a relationship has been confirmed in several crop plants including cereals and legumes (Condon & Richards, 1993; Wright *et al.*, 1988). Thus, WUE is negatively associated with lower $\Delta^{13}C$. In the present study, regression analysis revealed that $\Delta^{13}C$ was positively correlated with grain yield under

unirrigated conditions amongst genotypes (P <0.02) in both years. The analysis of data pooled across two site/seasons also revealed a positive association in irrigated (P<0.05) and unirrigated conditions (P<0.001) amongst genotypes. Such a positive association of $\Delta^{13}C$ with grain yield has been reported for a range of C_3 cereals including bread wheat (Condon *et al.*, 1987; Ehdaie *et al.*, 1991; Morgan *et al.*, 1993; Ehdaie & Waines, 1996), durum wheat (Kirda *et al.*, 1992; Merah *et al.*, 2001; Monneveux *et al.*, 2006) and barley (Craufurd *et al.*, 1991; Acevedo, 1993). Similar associations have been reported for biomass production and $\Delta^{13}C$ (Condon *et al.*, 1987; Ehdaie, 1991; Condon & Richards, 1993; Condon *et al.*, 1993). These studies were conducted in Mediterranean or similar environments in which the crop depended on seasonal rainfall or stored moisture. The extent of drought stress in these studies was usually observed as relatively mild to severe terminal stress imposed at around flowering. On the other hand, several pot studies have also reported a negative correlation between $\Delta^{13}C$ and grain yield and above-ground dry matter in wheat (Condon *et al.*, 1990; Ehdaie *et al.*, 1991; Ehdaie and Waines, 1994; Ehdaie, 1995), in groundnut (Hubick *et al.*, 1988) or no correlation in spring wheat (Matus *et al.*, 1996).

In this study, a highly significant difference of 1.43‰ in $\Delta^{13}C$ was observed between the site/seasons. Such difference in $\Delta^{13}C$ may be attributable to the water regimes. In the unirrigated treatment, $\Delta^{13}C$ values were lower by 0.91‰ in 2003 (P=0.09) and 1.49‰ in 2005 (P<0.05) compared to the irrigated treatment. This clearly indicated a greater impact of post-anthesis drought in reducing $\Delta^{13}C$ values in 2005 than in 2003. Several studies have reported a significant decrease in $\Delta^{13}C$ with a decrease in soil water availability and/ or an increase in vapour pressure deficit (Condon *et al.*, 1987; Acevedo, 1993; Merah *et al.*, 1999; Merah *et al.*, 2001). In the present study, the mean vapour pressure deficit (VPD) during the crop season was about 0.5 kPa at Sutton Bonington and <0.30 kPa at Gleadthorpe in 2003. So the larger differences in $\Delta^{13}C$ at Sutton Bonington were mainly attributed to the differences in VPD compared to Gleadthorpe.

The $\Delta^{13}C$ of genotypes in the droughted treatment ranged from 18.5-20.2‰ in 2003, 18.4-19.8‰ in 2005 and 18.5-20.0‰ across site/seasons, while in the irrigated treatments $\Delta^{13}C$ was significantly higher. This is in agreement with previous reports

indicating a reduction in $\Delta^{13}C$ under water-limited conditions (e.g. Farquhar & Richards, 1984; Condon *et al.*, 1987; Richards *et al.*, 2002).

Averaging across both irrigation treatments, genotypes differed in their mean $\Delta^{13}C$ in the range of 19.0-20.6‰ in 2003, 19.2-20.5‰ in 2005, and 19.2-20.6‰ across site/seasons. These genetic ranges are in general agreement with previous studies in wheat (Condon & Richards, 1992; Morgan *et al.*, 1993; Merah *et al.*, 2001). Ehdaie *et al.* (1991) reported $\Delta^{13}C$ ranges of up to 2.8 ‰ among wheat genotypes although much of the variability was associated with considerable genotypic differences in development. Similarly variation in $\Delta^{13}C$ in the range of up to 3.7‰ for 144 accessions of durum wheat has been reported (Merah *et al.*, 2001). Previous work reported that differences in the rate and extent of soil water extraction among wheat genotypes were associated with differences in $\Delta^{13}C$ (Condon *et al.*, 1992). In the present study, the positive association between $\Delta^{13}C$ and grain yield indicated higher WUE was associated with lower grain yield. The physiological basis of this association was examined further at the leaf-scale by gas-exchange measurements for interpreting the field results of TE.

5.2.3 Relationships between $\Delta^{13}C$ and stomatal aperture traits

It is reported that the positive correlation between $\Delta^{13}C$ and grain yield suggests the dependence of genetic variation in $\Delta^{13}C$ on stomatal conductance (Condon *et al.*, 1987; Ehleringer, 1990; Acevedo, 1993; Morgan *et al.*, 1993; Merah *et al.*, 1999; Merah *et al.*, 2001). This is in general agreement with the observed positive relationship between $\Delta^{13}C$ and yield arising out of decreased stomatal conductance in the present study. Stomatal conductance values were decreased by 35% in 2003 (at GS61) and 22% in 2005 (between GS39 and GS61+4 weeks) due to water stress. Soissons exhibited higher g_s values than Beaver in the pre-anthesis period, perhaps on account of its faster development. However, at or after anthesis g_s values for Soissons were apparently lower than for Beaver in both years. The six DH lines also showed a trend for reduction in g_s values under drought. A significant interaction of genotypes with measurement date for g_s in 2005 showed some variability in the response of genotypes and that g_s ranking was certainly under the influence of the prevailing environmental conditions when the gas-exchange was measured. However, the reduction in g_s was weak for correlating the differences amongst the genotypes and the formulated hypothesis that decreased g_s

would favour an increase in TE was not supported in the present study. Further studies are required to examine whether reductions in gs favour increase in TE in the UK environment.

The genetic correlation of g_s with intrinsic TE (calculated as A/g_s ratio) in 2003 was negative under irrigated (r=-0.63) conditions with no significant correlation under drought. A similar trend was also observed in 2005 under irrigated (r=-0.76; P<0.05) and unirrigated (r=-0.39) conditions. Photosynthetic capacity (calculated as a ratio of net photosynthetic rate to intercellular CO_2 concentration; A/C_i) is another important gas-exchange trait to quantify the relationships between yield and $\Delta^{13}C$. If genotypic differences in $\Delta^{13}C$ were due to variation in photosynthetic capacity, then the relationships between $\Delta^{13}C$ and dry matter production would be expected to be negative, not positive (Condon *et al.*, 1987). In the present study, the small number of gas-exchange measurement dates makes it difficult to identify consistent relationships between leaf photosynthetic capacity and $\Delta^{13}C$. However, the correlation of A/C_i with $\Delta^{13}C$ was significant in irrigated (r=0.74; P<0.05) and unirrigated (r=0.81; P<0.05) treatments in 2003. This implies that higher photosynthetic capacity was associated with lower TE, which does not seem logical. These effects were not confirmed to be statistically significant in 2005. Soissons had a consistently higher A/C_i ratio than Beaver in 2005. Farquhar and Sharkey (1982) argued that internal CO_2 response curves are needed to separate stomatal from non-stomatal (mesophyll) effects in leaf photosynthesis in comparisons where genotypic differences in leaf conductance are evident. It is possible that in this study such effects, acting on leaf photosynthesis or else phenological differences, were associated with the differences observed among the genotypes.

When parental lines were examined, Beaver and Soissons showed significant differences (P<0.05) in their mean $\Delta^{13}C$ values across irrigation treatments in 2003 (20.2 and 19.6‰, respectively) and in 2005 (20.1 and 19.7‰, respectively). Both parents also showed consistent decreases in $\Delta^{13}C$ under drought. The greater reduction in $\Delta^{13}C$ in Soissons can be possibly related to effects of stomatal conductance. Soissons despite having higher g_s in the pre-anthesis period showed greater reduction in g_s under droughted conditions in the post-anthesis period compared to Beaver in both years. This

could possibly in part explain differences in $\Delta^{13}C$ of Soissons and Beaver arising out of variations in g_s. However, this needs further examination to see whether g_s and TE are negatively correlated in field crops in the UK environment.

Previous studies in winter wheat showed existence of a significant positive correlation between CO_2 exchange rate (CER or A) and g_s (Johnson *et al.*, 1987; Morgan *et al.*, 1990; Morgan & Le Cain, 1991; Monneveux *et al.*, 2006). This positive correlation between g_s and CO_2 exchange rate (A) precludes the establishment of simple relationships between TE and either g_s or CER because a higher g_s with higher A would certainly lead to a loss of water from the canopy resulting into a reduced TE. In the present study, such a positive relationship between g_s and A was observed for gas exchange in 2003 (measured at GS61), but was not statistically significant. A negative non-significant relationship between CER (or A) and g_s was found in 2005 (measured from GS39 to GS61+4weeks). This negative association between A and g_s might have resulted in a highly significant ($P<0.05$) positive correlations between A and ITE (ratio of A/g_s) in the irrigated ($r=0.83$) and unirrigated ($r=0.97$) treatments in 2005, implying that under the conditions when gas-exchange was measured a higher A with lower g_s favoured higher TE in general. The relationship between g_s and ITE (A/g_s ratio) is straight forward as they are inversely related. In this study, in general a greater WUE was realized in leaves with lower g_s in both years, but more significantly so in the irrigated treatment in 2005 ($r=-0.76$) than in other environments.

The correlation of $\Delta^{13}C$ with C_i under unirrigated conditions, appeared negative in 2003 ($r=-0.25$) and positive in 2005 ($r=0.30$), but these effects were not statistically significant. In theory, it is expected that a positive relationship exists between C_i/C_a and $\Delta^{13}C$ under water limited conditions (Farquhar and Richards, 1984; Richards *et al.*, 2001; Condon *et al.*, 2002). For the eight selected genotypes for intensive study of stomatal aperture traits, there was a general trend in reduction for A, g_s and C_i under drought in both years. However, the hypothesis that the genetic variation in TE in the DH population is due to differences in g_s, A and C_i of the flag leaf was partially supported in the present study owing to the variability of stomatal aperture traits on specific measurement dates (Tables 5.7-5.9). The genotype x irrigation interaction effects were not significant in 2003, but were in 2005. For example, Soissons under

drought showed larger reductions for these gas-exchange parameters compared to Beaver.

Different genetic rankings for these stomatal aperture traits on sequential measurement dates was evident and the interaction of measurement date x genotype was significant. So on specific dates these relationships showed variable genetic behaviour despite the positive correlation between *A* and TE. Thus, it is difficult to relate instantaneous measurements of gas-exchange to TE with certainty. Difficulty in relating variable measurements of leaf gas-exchange to genotype differences in $\Delta^{13}C$ was evident in the present study. This is possibly because of significant interactions found for measurement date x genotype in 2005. However, failure of gas-exchange data to clearly explain positive association between $\Delta^{13}C$ and grain yield, i.e. through detecting a positive relationship between g_s and *A*, may also indicate that the relationship was influenced by a combination of factors in most of the genotypes, e.g. g_s and *A*, plus the confounding effects of developmental differences amongst the genotypes.

Stomatal responses to environment vary within stands of a single species; measurements made over few days on a small part of a canopy are likely to be subject to confounding effects as found in the present study. Atmospheric factors like relative humidity, radiation, temperature or rainfall are likely to influence the values of gas exchange traits (Squire, 1990). There are also diurnal fluctuations in these atmospheric factors to consider. In addition, boundary layer effects need consideration when scaling from the effect of stomata operating at the leaf level to canopy gas exchange, because the leaf-to-air vapour pressure difference is likely to have an influence on leaves used in the gas-exchange cuvette. Thus, the 'scaling up' problem is associated with impacts of unstirred boundary layers around both the leaves and canopy as a whole (Jones, 1976; Cowan, 1988; Farquhar *et al.*, 1988). The vapour pressure deficit during the crop season at Sutton Bonington peaked in August at 0.48 kPa. However, there were large daily fluctuations. A vapour pressure deficit of *c.*1.02 kPa was observed on 22 June in this year. The experimental site at ADAS Gleadthorpe is about 40 miles within the radius of Nottinghamshire, UK and the VPD calculated for crop season at Sutton Bonington in 2003 was assumed to have similar effects in this site. However, the VPD was below

0.30 kPa during the crop season and increased only later in the season in August coinciding with grain filling.

Richards *et al.* (2002) indicated that measuring $\Delta^{13}C$ provides no information on the magnitude of either *A* or transpiration (*T*) or whether variation in $\Delta^{13}C$ is being driven by variation in stomatal conductance or photosynthetic capacity. Both seasonal and diurnal variation in the water vapour gradient may have more influence on WUE at the crop scale than genetic variation in TE (Tanner & Sinclair, 1983). Genotype responses may also vary in different environments. Condon *et al.* (2002) reported that the lower g_s of cultivar 'Quarrion', in two environments Wagga Wagga and Condobolin in eastern Australia, was associated with slower crop growth rates and slower soil water depletion during the middle part of the growing season from about the start of stem elongation. They also reported that this cultivar, despite having higher TE and low g_s behaved differently at sites with respect to biomass production and grain yield being higher at Condobolin and lower in Wagga Wagga. However, in the present study, such variation for TE between the sites/seasons among genotypes was not evident. The cross-site/season ANOVA indicated that the G x E interaction (P=0.054) for $\Delta^{13}C$ just failed to realise significance supporting the conclusion that $\Delta^{13}C$ was highly heritable in this population. This is in agreement with previous reports that either a small or non-significant G x E interaction effects indicates high heritability of $\Delta^{13}C$ in wheat (Condon *et al.*, 1987; Condon and Richards, 1992; Matus *et al.*, 1996) and in peanut (Hubick *et al.*, 1988; Wright *et al.*, 1988).

Wild diploid wheat species have higher photosynthetic rates than cultivated types (Evans and Dunstone, 1970) and the highest photosynthetic rates tend to occur in small leaved primitive diploid species (Dunstone *et al.,* 1973). Thus, an increase in leaf photosynthetic capacity is most often achieved in cereals by the concentration of leaf nitrogen into smaller leaves with low specific leaf area (SLA; $cm^2\ g^{-1}$) resulting from reduction in the rate of leaf area expansion during early crop growth. This results in less light interception, lower canopy photosynthesis and slower dry matter production (Condon *et al.*, 1993; Lopez-Casteneda *et al.*, 1995). Thus, if low $\Delta^{13}C$ is due to greater photosynthetic capacity per unit leaf and full light interception is maintained for only a relatively short period, the outcome may be a negative association between leaf level *A*

and crop biomass, and a positive association between biomass production and $\Delta^{13}C$. This could theoretically explain why in the present study a negative association of *A* and AGDM was found in the irrigated treatments in 2005 ($r=-0.75$; $P<0.05$).

It has been previously reported in sorghum that a negative relationship exists between TE and SLA with a tendency for specific leaf nitrogen (SLN; ratio of leaf nitrogen content to leaf area) to increase as SLA decreases (Hammer *et al.*, 1997). This suggests that regulation of conductance rather than assimilation capacity may be responsible for genetic variation observed in TE. In the present study, SLA decreased under drought in 2003. Soissons had a significantly higher SLA at GS61 than Beaver in both years. SLN showed a positive correlation with $\Delta^{13}C$ only under unirrigated conditions ($r=0.40$; $P<0.05$) for the 35 genotypes in 2003. Thus, present results did not confirm the published negative relationship of SLA and TE. In cereals, previous reports indicated that SLN correlates positively with WUE, e.g., in sorghum Borrell *et al.* 2000; Hammer *et al.* 1997), presumably through effects on Rubisco content. Genetic variation in Rubisco content (Julian *et al.* 1998) and Rubisco activity (Xiao *et al.* 1998) has been shown to correlate positively with photosynthesis rate in wheat.

Several studies have reported that the percent increase in WUE of wheat in dry compared to wet environments ranges 17-32 (Kimball *et al.*, 1995) or 40-46 (Chaudhuri *et al.*, 1990). Such a large variation depends on the method of calculation of WUE either based on grain mass or gas exchange. In the present study, at anthesis (GS61) intrinsic WUE (calculated as ratio of A/g_s) at leaf level showed a 37 % (in 2003) and 11% (in 2005) increase in WUE in unirrigated treatment.

5.2.4 Drought effects on growth processes

The extensive information collected on selected DH lines allows, the effects of drought on growth processes and associated component traits to be analysed to explain the physiological basis for WUE in the Beaver x Soissons population.

5.2.4.1 Evidence for post-anthesis drought and yield responses

In the UK drought typically occurs post-anthesis. Several UK studies related genotypic response to drought for season-long water uptake, harvest biomass and grain yield using

isogenic lines differing in traits such as crop height (Innes *et al*., 1985), ear population (Innes *et al*., 1981), date of ear emergence (Innes *et al*, 1985), leaf posture (Innes & Blackwell, 1983), other investigations examined canopy characteristics such as maximum GAI and stem soluble reserves or flowering time and their relationship with grain yield under drought for sets of cultivars (Foulkes *et al*., 1993, 2001 & 2002). $\Delta^{13}C$ as a candidate selection tool to quantify drought resistance in genotypes has been evaluated here. The yield response to irrigation in 2003 and 2005 ranged from 0.5 to 1.6 t ha^{-1} and was smaller than those reported previously for UK wheat of about 2 t ha^{-1} (Innes *et al*., 1985; Bailey, 1990; Foulkes *et al*., 2002). The range of yield differences among the genotypes was also higher on account of a strong G x E interaction observed. The range of flowering date of genotypes was wide at about 9 days, with regard to French-bred photoperiod insensitive bread wheat Soissons (Worland *et al*., 1994; Foulkes *et al*., 2002) and the late developing photoperiod sensitive UK-bred feed wheat Beaver. These differences in flowering date can be related to effects in water use pattern. Foulkes *et al*. (1993) suggested that under irrigated conditions, early flowering would lead to early maturity and hence to smaller biomass; while under drought conditions, there would be less time for growth hence less reduction in biomass. However, in a further study, Foulkes *et al.* (2004) examined the effects of the *Ppd-D1* allele in near-isogenic lines of Mercia and Cappelle-Desprez winter wheats and found no differences in yield responses to drought for early and late flowering cultivars. The effects of *Ppd-D1* on drought resistance traits such as early flowering, high stem reserves, deep rooting and WUE were found overall to be neutral on late-season drought resistance in the UK (Foulkes *et al*. 2004). In the present study, the regression of flowering date on the grain yield of genotypes was not statistically significant in each of the irrigation treatments. This was also the case for harvest biomass. However, fast developing Soissons produced small biomass at harvest with or without irrigation. These findings are in general agreement with the findings of Foulkes *et al*. (2001; 2004) in relation to flowering and drought resistance.

The variation in the range of flowering date of B x S mapping population may be associated with the *Ppd-D1* or *ppd-D1* alleles on chromosome 2D controlling photoperiod response (Verma *et al*., 2004). However, regression of flowering date on (i) irrigated yield (ii) unirrigated yield and yield loss under drought were all non significant

in both years ($r<0.2$). This indicates that early flowering did not lead to better maintenance of yield under drought amongst lines of Beaver x Soissons population in the present study. It is possible that the extended period of flowering may favour the development of a larger root more extensive system in photoperiod sensitive genotypes, allowing the crop to maintain water uptake during grain filling despite their water consumption earlier in the season (Foulkes *et al.*, 2002). In the present study, the high yielding genotypes tended to lose more grain yield under drought consistent with reports of a positive association between yield potential and absolute yield loss under drought, e.g. Fischer and Maurer (1978).

The maximum GAI at GS61 ranged from 6.3-9.5 under irrigation for the genotypes and was broadly representative of the range for maximum GAI present in UK wheat cultivars (Austin *et al.*, 1980; Foulkes *et al.*, 2001). To link maximum GAI with biomass production response of cultivars to drought, Foulkes *et al.* (1993) reported that the genetic variation in maximum GAI must be independent of variation in seasonal fractional interception of radiation (which depends on LAI and extinction coefficient k). However, such responses were not found on account of differences in thickness and transmissivity of leaves in canopies (Foulkes *et al.* 2001); and indicated that GAI was a poor guide to seasonal growth, or water use during the growing season and poor predictor of biomass response to drought in the UK's temperate climate. Similar effects could account for the lack of a correlation between maximum GAI and yield response to drought in the present study.

In the present study, the decreases in HI under drought was about 0.01 which is far less than those reported in previous UK studies (Innes *et al.*, 1981; 1984; Innes & Blackwell, 1983; Fischer & Wood, 1979; Foulkes *et al.*, 2002). The HI of lines 134E, 134G and 35A was generally above the range of mean HI for parents. The improvement of HI through UK breeding has been well documented (Austin *et al.*, 1989; Foulkes *et al.*, 2002) which mainly lies with the inclusion of semi-dwarf *Rht* genes. In the present study, the selected genotypes Beaver, Soissons, line 21B and 76A possessed *Rht1* or *Rht2* semi-dwarfing genes while lines 134G, 134E, 35A, 22B had *rht* genes. Under irrigated conditions, Beaver and line 21B had higher yields on account of having more grains ear^{-1}. Both these genotypes have the *Rht2* gene and this is in agreement with the

previous reports of improved spikelet fertility conferred by the *Rht2* gene (Gale & Youseffian, 1985). However, lower grains ear^{-1} was observed with line 76A, a double-dwarf genotype possessing *Rht1+Rht2* genes, compared to the tall lines.

Most genotypes, including Beaver, did not maintain grain yield under drought mainly because of reduction in biomass growth. The exception to this was observed in line 21B and Soissons which maintained HI better with or without irrigation.

5.2.4.2 Effects of the 1BL/1RS translocations

The 1BL/1RS wheat (*Triticum aestivum* L.) - rye (*Secale cereale* L.) centric translocation, involving the short arm of rye chromosome 1R, has been extensively used in wheat breeding programmes worldwide in recent decades (Lukaszewski, 1990; Rajaram *et al.*, 1990). These translocations have been reported to improve yield potential, stress tolerance and adaptation in bread wheat (Rajaram *et al.*, 1983; Villareal *et al.*, 1998; Ehdaie *et al.*, 2003). The presence of 1BL/1RS in some hard red winter wheats was associated with higher grain yield, aerial biomass, grain weight, and spikelet fertility (Carver and Rayburn, 1994; Foulkes *et al.*, 2001). However, the reported effects are generally either positive or neutral on grain yield (DEFRA, 2004). In the present study, the above-ground biomass at harvest was raised by 1BL/1RS in both years, but this was not realised as an increase in grain yield or as a reduction in yield loss under drought. Interestingly, the 1BL/1RS translocation was associated with higher $\Delta^{13}C$ in 2003 ($P=0.07$) and in 2005 ($P<0.001$). Lines with 1BL/1RS increased $\Delta^{13}C$ both under irrigated and unirrigated field conditions compared to their non-translocation counterparts. Thus, it is evident that modern wheat yield improvement in UK associated with the introduction of the 1BL/1RS translocation is more linked to greater biomass at harvest than to water-use efficiency. Previous reports of 1BL/1RS conferring greater biomass in modern wheat UK genotypes (Foulkes *et al.*, 2001; Shearman *et al.*, 2005) indicated that achieving greater yield potential in modern UK wheats may be associated with a decrease in WUE. This was also observed in the present study in that $\Delta^{13}C$ and grain yield of all 35 genotypes showed positive correlations under irrigated and unirrigated field conditions, indicating that genotypes with lower delta or higher WUE had reduced grain yields (Fig. 5.2).

5.2.4.3 Effects of Rht genes

The Norin-10 dwarfing genes *Rht1* and *Rht2* are located at homoeologous positions on the short arms of chromosome 4B and 4D, respectively. In addition to their primary effect in reduction in plant height, these genes have been associated with lodging resistance and greater response to nitrogen than the tall cultivars (Donald, 1968). An association of drought susceptibility and effects of *Rht* genes have been reported (Fischer and Wood, 1979; Duwayri, 1984; Ehdaie and Waines, 1996). In the present study, paired contrasts for comparing *Rht1, Rht2, Rht1+Rht2* and *rht* genes in groups of the 33 DH lines indicated no significant differences between *Rht1* and *rht* lines in $\Delta^{13}C$ and grain yield. Tall lines (*rht*) had significantly lower $\Delta^{13}C$ (higher WUE) and lower grain yield compared to *Rht2* lines in both years in irrigated and unirrigated treatments ($P<0.01$). This is in agreement with the findings of previously reported pot experiments that tall wheats had greater total dry matter and TE than semi-dwarf and dwarf wheats (Ehdaie *et al.*, 1991; Ehdaie and Waines, 1993). Richards (1992) in Australia and Ehdaie and Waines (1994) in California working on near isogenic lines of bread wheat for *Rht1, Rht2* and *Rht3* have also found that TE or WUE for total dry matter declined with plant height. The effects of double-dwarf genotypes (*Rht1+Rht2*) were inconsistent in 2003, but in 2005 they showed lower $\Delta^{13}C$ and higher grain yield ($P<0.05$) over tall lines. The differences between *Rht1* and *Rht2* were also significant for $\Delta^{13}C$ and grain yield. Genotypes with *Rht1* showed lower $\Delta^{13}C$ and lower grain yields in both years under irrigated and unirrigated treatments compared to *Rht2* genotypes. Thus, in the present study it seems that lower $\Delta^{13}C$ associated with *Rht1* under UK conditions may not be associated with yield improvement.

5.2.5 Relationships between $\Delta^{13}C$ and yield traits

In 2003, flowering time of 35 genotypes was positively correlated with $\Delta^{13}C$ under irrigated ($r=0.40$; $P<0.05$) and unirrigated ($r=0.42$; $P<0.05$) conditions. This implied that early flowering may lead to higher WUE. This can be ascribed to the effects of *Ppd-D1* allele in advancing flowering time of genotypes thus leading to drought escape. However, such effects were not confirmed in 2005 where $\Delta^{13}C$ correlated weakly with flowering time only under irrigated conditions ($r=0.32$; $P<0.10$) with no consistent effects under drought. These results are in agreement with a previous report on selection

for low $\Delta^{13}C$ to be associated with earlier anthesis date (Rebetzke *et al.*, 2002). However, previous findings in wheat showed strong negative associations between $\Delta^{13}C$ and days to heading or anthesis in Mediterranean cereals under terminal drought, with low $\Delta^{13}C$ genotypes flowering many days later than high $\Delta^{13}C$ genotypes (Ehdaie *et al.*, 1991; Craufurd *et al.*, 1991; Acevedo, 1993; Sayre *et al.*, 1995) while in some studies there has been little variation in flowering date (Condon *et al.*, 1987; Morgan *et al.*, 1993; Acevedo, 1993).

A negative correlation between $\Delta^{13}C$ and crop height was found in both years in (r=-0.40) but these associations were not statistically significant. However, tall lines generally had lower delta. There are also reports indicating no correlation between lower delta and increased height (Donovan and Ehleringer, 1994; Rebetzke *et al.*, 2002).The association between $\Delta^{13}C$ and AGDM was positive but weakly correlated. There are studies which reported either positive or neutral effects between $\Delta^{13}C$ and biomass production in wheat (Condon *et al.*, 1987; Ehdaie, 1991; Condon & Richards, 1993; Condon *et al.*, 1993; Richards, 1994). The factors influencing positive or negative genetic correlations between $\Delta^{13}C$ and yield will be discussed in more detail in chapters 6 and 7.

In summary, it is evident that under UK conditions the ability to maintain yield under drought is mainly associated with improvement in water use rather than WUE, i.e. it seems that the present wheat population may require ability to capture more water to fulfill their yield potential. The formulated hypothesis that there is a positive genetic correlation between TE or WUE and grain yield amongst lines of DH population under drought in the UK environment needs further consideration as some genotypes showed a trend for higher grain yield with higher $\Delta^{13}C$ (lower TE). There is apparently a trade-off between TE and seasonal water use in the UK environment. So further studies related to rooting in relation to effective water capture in new cultivars seem justified. The use of carbon isotope discrimination could serve the purpose of identifying genotypes with improved drought resistance but perhaps only as an indirect indicator of season-long water use.

CHAPTER 6: QUANTITATIVE GENETIC ANALYSIS

This chapter describes the results of the genetic analysis carried out on the Beaver x Soissons mapping population in field experiments conducted during the years 2002/03 (Gleadthorpe) and 2004/05 (Sutton Bonington). The phenotypic evaluation was done for grain yield and the physiological target traits such as grain yield, $\Delta^{13}C$ and thousand grain weight. The quantitative trait loci (QTL) analysis was performed separately for both the site/seasons using the software package MapQTL version 5 (van Ooijen, 2004). The results obtained were used for interpretation of putative QTLs and their location on the chromosomes of interest. The details of the procedures of the QTL analyses are presented in section 3.4.2.

6.1 RESULTS OF QTL ANALYSIS

The Beaver x Soissons DH mapping population containing 65 lines was developed originally by John Innes Centre (JIC), the University of Nottingham and ADAS, UK. A genetic map of this population was developed by JIC, consisting of SSR markers, AFLP markers and 7 major genes (*Glu-A1, Glu-B1, Glu-D1, Rht-B1, Rht-D1, B1, Ha*), giving a total of 222 markers, organized into 35 linkage groups (and 31 unlinked markers) which could be overlaid on the 21 wheat chromosomes. This gave polymorphic genome coverage of 51% (1628 cM, Table 6.1). There was relatively poor coverage of markers on chromosomes 4B, 4D and 7D. These marker data were used for QTL analysis of selected quantitative traits using 33 DH lines of the population in the present study. The target traits of grain yield, carbon isotope discrimination and thousand grain weight were studied to identify putative QTLs in irrigated and unirrigated conditions in the experiments carried out in 2003 and 2005. The full set of putative QTLs is presented in Tables 6.2-6.6. The most relevant QTLs are discussed in this chapter.

Table 6.1 Marker coverage for the Beaver x Soissons genetic map.

	1A	**1B**	**1D**	**2A**	**2B**	**2D**	**3A**	**3B**	**3D**	**4A**	**4B**
Coverage (cM)	82	69	53	65	88	140	45	83	99	68	31
Coverage (%)	55	46	35	43	59	93	30	55	66	45	21

	4D	**5A**	**5B**	**5D**	**6A**	**6B**	**6D**	**7A**	**7B**	**7D**	**Total**
Coverage (cM)	44	103	80	184	65	84	55	97	53	40	**1628**
Coverage (%)	29	69	53	100	43	56	37	65	35	27	**51**

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

6.1.1.1 2003

Under irrigated conditions, a putative QTL for $\Delta^{13}C$ was found on chromosome 7BL with marker *wmc70* at 0 cM position (K*=4.21; P=0.05; LOD=1.07). This QTL explained 14.7% of the phenotypic variance. The Soissons allele increased $\Delta^{13}C$. The information on other genomic regions is less conclusive and most of the LOD scores determined were well below the threshold level of significance (Table 6.2).

Under droughted conditions, a putative QTL was found on chromosome 5A at 59 cM with marker *gwm304* (K*=4.70; P=0.05; LOD=1.21) explaining 15.5% of the phenotypic variance and at 45 cM with marker *gwm205* (LOD=1.12) explaining 14.5% of the phenotypic variance. For these QTLs, $\Delta^{13}C$ was increased by the presence of the Soissons allele (Table 6.2) and these may potentially represent a single QTL. The high K* value (8.51) for marker *1D/barc149* on chromosome 1B is due to an effect of missing marker data in a small population. In summary, no QTLs of LOD> 2.0 were identified. However, several weakly significantly QTLs were identified that would be of potential importance if they could be confirmed in other experiments, with greater numbers of DH lines.

6.1.1.2 2005

Under irrigated conditions, a putative QTL of high significance for $\Delta^{13}C$ was found on chromosome 3B at 26 cM distance with marker *psp3144* (K*=8.78; P=0.005; LOD=2.32). This QTL alone explained 27.7% of phenotypic variance (Table 6.3) and $\Delta^{13}C$ was increased by the presence of Beaver allele consistent with the high $\Delta^{13}C$ (low water-use efficiency) of this parent. Other markers in this linkage group 15 were also associated with $\Delta^{13}C$, most prominently *barc229a* (LOD=1.95) and *wmc56* (LOD=1.5). For all these markers the additive effects were positive indicating that $\Delta^{13}C$ was increased by the presence of the Beaver allele. The automatic cofactor selection of markers in the QTL analysis also confirmed that this QTL was significantly associated with marker *psp3144* on chromosome 3B, after removing all other non-significant markers at a probability of 0.02. The effects of markers associations scattered on chromosomes 4D and 7BL were negligible.

Under droughted conditions, a putative QTL explaining 18.2% of phenotypic variation for $\Delta^{13}C$ was found on chromosome 2D with marker *2D3A/gwm30* at 93 cM position (K*=4.6; P=0.05; LOD=1.43). Another QTL of interest was found on chromosome 3B with marker *barc77* at 51 cM position (LOD=1.08) explaining 14% of the phenotypic variation (Table 6.3). This putative QTL also appeared under irrigated conditions explaining about 10% of trait variation.

Table 6.2 Summary of QTL analysis for carbon isotope discrimination under irrigated and unirrigated treatments in 2003 (Gleadthorpe) using Kruskal-Wallis (KW) non-parametric test and interval mapping.

$\Delta^{13}C$ 2003 (Irrigated)				KW test		Interval mapping			
Chr.	Group	cM	Locus	K*	Signif.	LOD	Variance	%Expl.	Additive
1B	2	11	1D/barc149	6.36	**	0.52	0.11	7.1	0.09
2A	6	29	s14/m11.11	4.17	**	0.64	0.11	8.5	0.10
3A	12	22	s14/m12.1	3.00	*	0.75	0.11	10.0	-0.11
3B	15	26	3B/psp3144	3.68	*	0.86	0.11	11.8	0.12
4B	19	0	Rht-B1b	3.89	**	1.03	0.10	13.4	0.13
4B	19	31	s13/m23.6	3.56	*	0.95	0.10	13.2	0.15
4D	20	3	4ABD/gwm192	4.24	**	0.89	0.11	11.6	0.12
4D	20	3	4ABD/gwm165	4.24	**	0.89	0.11	11.6	0.12
5A	22	23	s15/m46.3	4.48	**	0.23	0.12	3.7	0.07
6D	28	0	6D/p3200.2	2.95	*	0.59	0.11	7.9	-0.10
7BL	34	0	7BL/wmc70	4.21	**	1.07	0.10	14.7	-0.13
7BL	34	28	7BL/barc182	4.30	**	0.78	0.11	11.2	-0.12

$\Delta^{13}C$ 2003 (Unirrigated)									
Chr.	Group	cM	Locus	K*	Signif.	LOD	Variance	%Expl.	Additive
1B	2	7	1B/wmc85(rpt)	3.29	*	0.49	0.13	7.0	0.10
1B	2	10	3D/barc8	3.41	*	0.68	0.12	9.0	0.11
1B	2	11	1D/barc149	8.51	****	0.76	0.12	11.1	0.12
2D	11	0	s13/m20.2	3.38	*	0.59	0.13	8.5	0.11
3A	12	22	s14/m12.1	3.65	*	0.97	0.12	12.7	-0.13
3A	13	23	3A/gwm480	3.64	*	0.88	0.12	11.6	0.13
4A	17	18	s14/m20.5	3.12	*	0.47	0.13	6.5	-0.10
4A	17	24	4A/wmc491	2.87	*	0.1	0.14	1.5	-0.05
5A	21	45	5A/gwm205	3.14	*	1.12	0.12	14.5	-0.14
5A	21	59	5A/gwm304	4.70	**	1.21	0.12	15.5	-0.15
5A	21	65	5A/gwm415	2.85	*	0.77	0.12	10.2	-0.13
5A	22	23	s15/m46.3	3.28	*	0.4	0.13	6.3	0.09
6A	26	65	6A/gwm570	3.93	**	0.67	0.12	9.0	0.11
6B	27	27	s13/m36.1	2.81	*	0.44	0.13	6.2	0.09

Significance levels: *:0.1 **:0.05 ***:0.01 ****:0.005 *****:0.001; LOD=log likelihood score; Variance=residual variance after fitting the QTL; %Expl=the percentage of variance explained for by the QTL: 100*(H0:var-var)/population variance; Additive= estimated additive effect (μA{0}- μB{0})/2.

Table 6.3 Summary of QTL analysis for carbon isotope discrimination under irrigated and unirrigated conditions in 2005 (Sutton Bonington) using Kruskal-Wallis non-parametric test and interval mapping.

$\Delta^{13}C$ 2005 (Irrigated)				KW test		Interval mapping			
Chr	Group	cM	Locus	K*	Signif.	LOD	Variance	%Expl.	Additive
1B	2	11	1D/barc149	2.78	*	0.72	0.08	10.0	0.10
2D	10	0	2D/wmc41	3.40	*	0.46	0.09	6.5	0.08
3A	12	0	3AD/gwm2.2	3.68	*	0.65	0.08	9.3	-0.09
3B	15	0	3D/wmc54	3.96	**	0.95	0.08	12.4	0.11
3B	15	14	Gwm107.2	4.25	**	0.71	0.08	9.5	0.09
3B	15	16	3B/gwm285	2.99	*	0.81	0.08	10.6	0.10
3B	15	18	3B/psp3030.2	2.83	*	0.91	0.08	11.9	0.10
3B	15	26	3B/psp3144	8.78	****	2.32	0.07	27.7	0.16
3B	15	33	3B/wmc56	6.10	**	1.5	0.07	18.9	0.13
3B	15	37	1D/barc229a	7.50	***	1.95	0.07	24.6	0.15
3B	15	47	s14/m15.7	3.33	*	0.53	0.08	7.1	0.08
3B	15	48	s13/m20.9	2.97	*	0.49	0.09	6.8	0.08
3B	15	51	3A/barc77	3.64	*	0.75	0.08	10.0	0.10
4A	17	55	s12/m19.3	2.83	*	0.46	0.09	6.2	-0.08
4D	20	3	4ABD/gwm192	4.24	**	0.96	0.08	12.6	0.11
4D	20	3	4ABD/gwm165	4.24	**	0.96	0.08	12.6	0.11
4D	20	8	s14/M12.12	2.72	*	0.82	0.08	10.8	0.10
4D	20	11	4D/wmc457	2.79	*	0.39	0.09	5.3	0.07
6D	28	0	6D/p3200.2	3.34	*	0.63	0.08	8.5	-0.09
7BL	34	0	7BL/wmc70	3.68	*	0.69	0.08	9.2	-0.09
7BL	34	35	7BL/gwm344	3.94	**	0.71	0.08	9.4	-0.10
$\Delta^{13}C$ 2005 (Unirrigated)									
Chr	Group	cM	Locus	K*	Signif.	LOD	Variance	%Expl.	Additive
1D	4	0	s16/m51.4	3.50	*	0.83	0.09	10.9	0.11
1D	4	0	s15/m34.8	3.64	*	0.83	0.09	10.9	0.11
2B	7	40	2B5A/gwm410	3.33	*	0.78	0.09	11.8	0.11
2D	9	93	2D3A/gwm30	4.60	**	1.43	0.08	18.2	0.14
3B	15	51	3A/barc77	3.15	*	1.08	0.09	14.0	0.12
4A	17	24	4A/wmc491	4.10	**	0.42	0.09	5.7	-0.08
4A	18	0	4A7A7D/gwm350	4.25	**	0.73	0.09	9.7	0.10
5A	21	0	5A/barc10	3.57	*	0.63	0.09	8.4	0.09
5BL	24	0	5BL/Gwm371	2.99	*	0.68	0.09	10.6	-0.10
5D	25	184	5D/wmc443.2	4.00	**	0.82	0.09	10.8	-0.10
6D	29	16	6D/gdm127	3.64	*	0.89	0.09	12.6	0.11

Significance levels: *:0.1 **:0.05 ***:0.01 ****:0.005 *****:0.001; LOD=log likelihood score; Variance=residual variance after fitting the QTL; %Expl=the percentage of variance explained for by the QTL:100*(H0:var-var)/population variance; Additive=estimated additive effect (μA{0}- μB{0})/2.

6.1.2 Grain yield

6.1.2.1 2003

Under irrigated conditions in 2003, putative QTLs for grain yield were found on chromosome 6B with LOD scores ranging from 1.09 to 1.68. The K* statistic using the Kruskal-Wallis (KW) test indicated significant values (P< 0.01) for the markers *s12/m11.6* at 62 cM (K*=7.23; LOD=1.68) and *Gwm816* at 66 cM (K*=7.55; LOD=1.57). The percentage phenotypic variance explained by the putative QTL on 6B amounted to 21.4%. Presence of QTLs of greater effects were also noticed on chromosome 3B with markers *wmc56* and *barc229a* at 33 cM (K*=6.10; LOD=1.65) and 37 cM (K*=3.86; LOD=1.18), respectively, explained a maximum of about 21% of the variance in grain yield. The positive additive effects revealed that yield was increased by the presence of the Beaver allele for these QTLs. Minor effects for other markers on chromosomes 4A, 2D and 7D were apparent but did not realize statistical significance accepted in this study (Table 6.4).

Under unirrigated conditions in 2003, the QTL effects were different with a putative QTL on chromosome 3A located at 22cM with marker *s14/m12.1* (K*=8.02; P=0.005; LOD=2.15). This alone explained 26.5% of the phenotypic variation for grain yield under drought. Another putative QTL of lesser significance was found on chromosome 2A at 33 cM with marker *2A/gwm312* (k*=5.31; P=0.005; LOD= 1.97), which explained 24.5% of the phenotypic variation for the grain yield. The presence of the Soissons allele improved grain yield (Table 6.4).

Table 6.4 Summary of QTL analyses for grain yield under irrigated and unirrigated treatments in 2003 (Gleadthorpe) using Kruskal-Wallis (KW) non-parametric test and interval mapping.

Grain yield 2003 (Irrigated)				KW test		Interval mapping			
Chr.	Group	cM	Locus	K*	Signif.	LOD	Variance	%Expl.	Additive
2D	10	0	2D/wmc41	3.91	**	0.85	0.62	11.2	0.28
3B	15	33	3B/wmc56	6.10	**	1.65	0.56	20.6	0.39
3B	15	37	1D/barc229a	3.86	**	1.18	0.59	15.2	0.33
4A	17	0	s14/m20.6	4.66	**	1.01	0.58	17.4	0.35
4A	17	18	s14/m20.5	3.04	*	0.32	0.67	4.7	0.19
4A	17	55	s14/m11.10	2.82	*	0.55	0.65	7.4	0.23
5A	21	65	5A/gwm415	3.63	*	0.64	0.64	8.6	-0.26
6A	26	65	6A/gwm570	2.95	*	0.44	0.66	6.1	-0.21
6B	27	40	s14/m13.9	6.13	**	1.18	0.59	15.5	0.34
6B	27	40	s14/m11.4	3.68	*	1.18	0.59	15.5	0.34
6B	27	43	s16/m55.5	7.44	***	1.24	0.59	15.9	0.34
6B	27	54	s13/m23.7	3.96	**	1.27	0.59	16.3	0.34
6B	27	60	s14/m12.6	4.68	**	1.31	0.58	16.7	0.34
6B	27	60	s12/m18.4	6.23	**	1.31	0.58	16.7	0.34
6B	27	62	s12/m11.6	7.23	***	1.68	0.55	21.4	0.39
6B	27	63	s12/m13.4	5.33	**	1.09	0.60	14.1	0.32
6B	27	66	Gwm816	7.55	***	1.57	0.56	19.7	0.37
7D	35	0	7D/Gwm295	4.66	**	0.84	0.62	11.7	0.32
Grain yield 2003 (Unirrigated)									
Chr.	Group	cM	Locus	K*	Signif.	LOD	Variance	%Expl.	Additive
1B	3	14	1B/Gwm140	3.26	*	0.32	0.53	4.6	-0.16
1B	3	31	s23/m17.3	6.97	***	1.07	0.47	14.8	-0.29
2A	5	25	2A/gwm312	7.99	****	1.94	0.42	23.7	-0.36
2A	5	33	2A/gwm294	5.31	**	1.97	0.42	24.5	-0.37
2D	10	25	2D/gwm539	2.95	*	0.92	0.49	12.0	-0.26
3A	12	22	s14/m12.1	8.02	****	2.15	0.41	26.5	-0.39
3B	14	29	s15/m34.3	3.63	*	0.3	0.53	4.5	-0.16
3B	15	51	3A/barc77	3.09	*	0.37	0.53	5.1	0.17
3B	15	54	s13/m23.5	2.79	*	0.18	0.54	2.6	0.12
4A	17	46	s13/m17.3	4.76	**	0.54	0.52	7.2	0.20
4A	17	48	7AD/gwm635	2.90	*	0.57	0.51	7.7	0.21
4A	17	50	s13/m41.5	2.90	*	0.71	0.50	9.4	0.23
7A	31	15	7A/barc108	3.50	*	0.94	0.49	12.3	0.28
7A	31	15	7A/psp3050	4.23	**	0.94	0.49	12.3	0.28
7A	31	17	Gwm631	3.29	*	0.94	0.49	12.3	0.28
7A	31	21	s12/m25.2	3.94	**	1.02	0.48	13.3	0.29
7A	31	26	s12/m25.1	7.18	***	1.27	0.47	16.3	0.32
7A	31	46	s12/m15.2	2.95	*	0.79	0.50	10.4	0.24

Significance levels: *:0.1 **:0.05 ***:0.01 ****:0.005 *****:0.001; LOD=log likelihood score; Variance=residual variance after fitting the QTL; %Expl=the percentage of variance explained by the QTL: 100*(H0:var-var)/population variance; Additive=the estimated additive effect (μA{0}- μB{0})/2.

6.1.2.2 2005

In the irrigated environment, a putative QTL was found on chromosome 7A (linkage group 30) with marker *gwm60* at 42 cM (K8*=8.41; P=0.005; LOD=2.18). This QTL explained 26.2 % of phenotypic variance for grain yield. Another putative QTL was found on 5D (linkage group 25) at 184 cM with marker *wmc443.2* (k*=9.05; P=0.005; LOD=2.07) explaining 25.1% of phenotypic variation (Fig. 6.1). It was interesting to note that for these QTLs on chromosomes 7A and 5D yield was being increased by the presence of the Soissons allele. However, other putative QTLs of lesser significance were found on chromosome 3A with marker *gwm480* (K*=7.17; LOD=1.88) and 1B with *barc149* (K*=8.00; LOD=1.79) where the Beaver allele, explaining 23.2 and 22.9 % phenotypic variance, respectively, increased yield (Table 6.5).

In the droughted environment, a putative QTL for yield was found on chromosome 3A (linkage group 13) with marker *gwm480* at 23cM distance (K*=10.14; P=0.005; LOD=2.51). For this marker the presence of the Beaver allele improved yield. Another QTL on 5D was found with marker *wmc443.2* at 25 cM (K*5.07; P=0.05; LOD=1.12) explaining 14.5% of the phenotypic variance. This QTL under the influence of Soissons allele interestingly also improved the yield under irrigated conditions at the same marker locus explaining 25% of the phenotypic variance. The effects of markers *barc149* (chr 1B), *2ABDGwm382* (chr 2A), for which the Beaver allele increased yield, and *gwm304* (chr 5A), for which the Soissons allele increased yield, were found in both irrigated and unirrigated conditions, although the effects in one of the two treatments were low.

Table 6.5 Summary of QTL analyses for grain yield under irrigated and unirrigated conditions in 2005 (Sutton Bonington) using Kruskal-Wallis (KW) non-parametric test and interval mapping.

Grain yield 2005 (Irrigated)				KW test		Interval mapping			
Chr.	Group	cM	Locus	K*	Signif.	LOD	Variance	%Expl.	Additive
1A	1	0	s12/m19.4	3.36	*	0.61	0.69	8.1	0.25
1B	2	7	1B/wmc85(rpt)	5.48	**	1.54	0.60	20.4	0.39
1B	2	10	3D/barc8	6.16	**	1.8	0.59	22.2	0.41
1B	2	11	1D/barc149	8.00	****	1.79	0.58	22.9	0.42
1B	2	13	1B/GWM11	4.29	**	1.18	0.64	15.2	0.34
2A	6	0	2A/gwm356	3.18	*	0.99	0.66	13.1	0.32
2A	6	25	Gwm382.2D	3.49	*	1.18	0.64	15.3	0.34
2D	9	116	2D/wmc18	3.09	*	0.56	0.69	8.0	-0.25
3A	13	8	s13/m11.6	3.47	*	0.92	0.66	12.1	0.30
3A	13	23	3A/gwm480	7.17	***	1.88	0.58	23.2	0.43
4D	20	11	4D/psp3007	3.33	*	0.87	0.67	11.5	-0.30
4D	20	11	4D/wmc457	2.85	*	0.87	0.67	11.5	-0.30
4D	20	12	4D/psp3103	3.33	*	0.87	0.67	11.5	-0.30
4D	20	16	s13/M23.2	2.91	*	0.82	0.67	11.3	-0.29
5A	21	59	5A/gwm304	2.81	*	0.66	0.69	8.8	-0.26
5A	21	65	5A/gwm415	3.26	*	0.81	0.67	10.7	-0.30
5BL	24	0	5BL/Gwm371	4.59	**	0.88	0.66	13.0	-0.31
5D	25	184	5D/wmc443.2	9.05	****	2.07	0.56	25.1	-0.44
7A	30	42	7A/gwm60	8.41	****	2.18	0.56	26.2	-0.44
7A	30	46	7A/barc127	4.39	**	1.36	0.62	17.4	-0.36
7BL	34	0	7BL/wmc70	2.73	*	0.63	0.69	8.7	-0.26
Grain yield 2005 (Unirrigated)									
Chr.	Group	cM	Locus	K*	Signif.	LOD	Variance	%Expl.	Additive
1B	2	11	1D/barc149	4.43	**	0.81	0.41	10.9	0.22
1B	3	14	1B/Gwm140	4.13	**	0.66	0.42	8.8	-0.20
2A	5	0	2A2D/wmc177	2.95	*	0.89	0.40	12.6	-0.24
2A	5	25	2A/gwm312	3.91	**	0.48	0.43	6.9	-0.18
2A	6	25	Gwm382.2D	3.41	*	0.77	0.41	10.3	0.22
2A	6	32	s16/m43.1	2.83	*	0.45	0.43	6.1	0.17
3A	13	8	s13/m11.6	2.85	*	0.67	0.42	9.0	0.20
3A	13	23	3A/gwm480	10.14	****	2.51	0.30	34.0	0.40
4D	20	16	s13/M23.2	3.38	*	0.8	0.41	10.8	-0.22
5A	21	59	5A/gwm304	3.52	*	0.76	0.41	10.0	-0.22
5D	25	184	5D/wmc443.2	5.07	**	1.12	0.39	14.5	-0.26
6D	28	27	6D/barc173	3.45	*	1.05	0.40	13.6	0.25
6D	28	39	6D/cfd42	3.51	*	0.92	0.40	12.0	0.24

Significance levels: *:0.1 **:0.05 ***:0.01 ****:0.005 *****:0.001; LOD=log likelihood score; Variance=residual variance after fitting the QTL; %Expl=the percentage of variance explained for by the QTL: 100*(H0:var-var)/population variance; Additive=the estimated additive effect (μA{0}- μB{0})/2.

6.1.3 Thousand grain weight

6.1.3.1 2003

Under irrigated conditions, putative QTLs on chromosomes 1A (K*=3.94; LOD=1.18) and 2A (K*=5.0; LOD=1.08) each explained about 15% of the phenotypic variation for TGW. Thus, on chromosome 1A, for the marker locus *P3027* the presence of the Soissons allele increased TGW. On chromosome 2A, a QTL was found associated with marker *2A2D/wmc177* (K*=5.57; P=0.05) with a LOD value of 1.10 and TGW was increased by the presence of Beaver allele (Table 6.6).

Under unirrigated conditions, a QTL was associated with marker *2A2D/wmc177* (K*=7.08; P=0.01) on chromosome 2A with a LOD of 1.67 which explained 23% of the phenotypic variation, with the Soissons allele increasing TGW. Most of the other QTL effects of lesser significance were found scattered along linkage group 31 of chromosome 7A. A putative QTL of lesser significance on chromosome 1A was associated with marker locus *barc148* at 22 cM (LOD=0.81). Interestingly, this QTL was also associated with TGW with Soissons allele increasing TGW in irrigated conditions. This QTL explained more than 11% of the phenotypic variance for TGW (Table 6.6).

6.1.3.2 2005

Under irrigated conditions, on chromosome 7A marker *s13/m25.5* at 32 cM (k*=8.29; P=0.005; LOD=2.37), and marker *s12/m15.2* at 46 cM (K*=6.69; P=0.01; LOD=1.58) explained 31% and 20% of phenotypic variation, respectively. On chromosome 2A, marker *2A2D/wmc177* (K*=9.01; P=0.005) with a LOD value of 2.10 explained 27.6% of the phenotypic variation, where the Beaver allele increased TGW (Table 6.7).

Under drought, two putative QTLs were found on chromosome 7A with markers *gwm282* and *gwm332* (LOD=2.09 each) at 51 cM. They explained 25.3% of phenotypic variation for TGW. Three minor putative QTLs for TGW were found on chromosomes: (i) 2A with marker *2A2D/wmc177* (K*=8.57; P=0.005; LOD=1.65) with TGW being increased by the presence of the Beaver allele, (ii) 1B with *Glu-B1* at 24cM (K*=6.18; P=0.05; LOD=1.64) with TGW being increased by the presence of Soissons allele and

(iii) 7BL with marker *barc182* at 28 cM (K*=7.64; P=0.01; LOD=1.37) with TGW being increased by the presence of Soissons allele (Table 6.7).

Table 6.6 Summary of QTL analysis for 1000-grain weight under irrigated and unirrigated conditions in 2003 (Gleadthorpe) using Kruskal-Wallis non-parametric test and interval mapping.

1000-grain weight-2003 (Irrigated)				KW test		Interval mapping			
Chr.	Group	cM	Locus	K*	Signif.	LOD	Variance	%Expl.	Additive
1A	1	16	1A/P3027	3.941	**	1.18	15.02	15.2	-1.70
1A	1	22	1A/barc148	5.009	**	1.08	14.99	15.3	-1.75
1B	2	24	Glu-B1	3.244	*	0.73	16.00	9.6	-1.31
2A	5	0	2A2D/wmc177	5.57	**	1.10	14.97	15.5	1.66
4A	17	0	s14/m20.6	2.793	*	0.55	16.06	9.3	1.29
4B	19	0	Rht-B1b	4.41	**	0.60	16.29	8.0	-1.22
7BS	32	5	7BS/gwm297	3.048	*	0.47	16.54	6.6	1.08

1000-grain weight-2003 (Unirrigated)									
Chr.	Group	cM	Locus	K*	Signif.	LOD	Variance	%Expl.	Additive
1A	1	22	1A/barc148	5.349	**	0.81	8.05	11.2	-1.06
2A	5	0	2A2D/wmc177	7.088	***	1.67	6.99	22.9	1.45
4B	19	0	Rht-B1b	2.999	*	0.43	8.53	5.9	-0.75
7A	31	0	s13/m41.2	3.484	*	0.83	8.06	11.0	1.22
7A	31	4	s14/m13.6	4.321	**	0.95	7.93	12.5	1.30
7A	31	15	7A/barc108	3.414	*	0.52	8.42	7.0	0.87
7A	31	15	7A/psp3050	2.707	*	0.52	8.42	7.0	0.87
7A	31	32	s13/m25.5	2.867	*	0.47	8.44	6.8	0.80
7A	31	51	7A/gwm282	5.211	**	0.88	8.01	11.5	1.06
7A	31	51	7A/gwm332	4.62	**	0.88	8.01	11.5	1.06

Significance levels: *:0.1 **:0.05 ***:0.01 ****:0.005 *****:0.001; LOD=log likelihood score; Variance=residual variance after fitting the QTL; %Expl=the percentage of variance explained for by the QTL:100*(H0:var-var)/population variance; Additive=estimated additive effect (μA{0}-μB{0})/2.

Table 6.7 Summary of QTL analysis for 1000-grain weight under irrigated and unirrigated conditions in 2005 (Sutton Bonington) using Kruskal-Wallis non-parametric test and interval mapping.

1000-grain weight-2005 (Irrigated)				KW test		Interval mapping			
Chr.	Group	cM	Locus	K*	Signif.	LOD	Variance	%Expl.	Additive
1B	2	24	Glu-B1	2.87	*	0.93	6.28	12.2	-0.94
2A	5	0	2A2D/wmc177	9.01	****	2.10	5.18	27.6	1.41
4A	18	0	4A7A7D/gwm350	3.09	*	0.69	6.50	9.1	0.86
4A	18	13	4A/barc184	2.97	*	0.26	6.91	3.5	0.59
4D	20	0	Rht-D1b	2.93	*	0.80	6.41	10.5	0.88
4D	20	11	4D/psp3007	2.87	*	0.68	6.51	9.1	0.82
4D	20	12	4D/psp3103	2.87	*	0.68	6.51	9.1	0.82
5D	25	184	5D/wmc443.2	3.65	*	0.89	6.32	11.6	-0.91
7A	31	0	s13/m41.2	4.74	**	0.63	6.53	8.7	0.97
7A	31	15	7A/barc108	4.84	**	1.01	6.21	13.2	1.06
7A	31	15	7A/psp3050	4.64	**	1.01	6.21	13.2	1.06
7A	31	17	Gwm631	4.26	**	1.01	6.21	13.2	1.06
7A	31	21	s12/m25.2	2.98	*	0.59	6.60	7.8	0.79
7A	31	32	s13/m25.5	8.29	****	2.37	4.91	31.4	1.53
7A	31	46	s12/m15.2	6.69	***	1.58	5.73	19.9	1.19
7A	31	51	7A/gwm282	5.56	**	1.29	5.98	16.5	1.13
7A	31	51	7A/gwm332	4.62	**	1.29	5.98	16.5	1.13
7BS	32	5	7BS/gwm297	3.98	**	0.85	6.34	11.4	0.91
7BL	34	28	7BL/barc182	4.71	**	1.09	6.08	15.0	-1.07

1000-grain weight-2005 (Unirrigated)									
Chr.	Group	cM	Locus	K*	Signif.	LOD	Variance	%Expl.	Additive
1B	2	24	Glu-B1	6.18	**	1.64	6.74	20.4	-1.32
1B	2	38	1B/barc61	2.87	*	0.70	7.69	9.3	-0.89
2A	5	0	2A2D/wmc177	8.57	****	1.65	6.59	22.2	1.38
2B	8	0	2B/Gwm619.1	3.40	*	0.88	7.48	11.7	-1.06
4A	18	0	4A7A7D/gwm350	3.50	*	0.73	7.65	9.7	0.96
4B	19	0	Rht-B1b	3.53	*	0.57	7.83	7.6	-0.82
7A	31	0	s13/m41.2	5.20	**	0.43	7.97	6.0	0.87
7A	31	32	s13/m25.5	4.66	**	0.98	7.38	13.0	1.07
7A	31	46	s12/m15.2	4.55	**	0.95	7.43	12.4	1.03
7A	31	51	7A/gwm282	8.52	****	2.09	6.33	25.3	1.52
7A	31	51	7A/gwm332	7.16	***	2.09	6.33	25.3	1.52
7BL	34	28	7BL/barc182	7.64	***	1.37	6.94	18.1	-1.28
7BL	34	35	7BL/gwm344	3.35	*	0.67	7.72	8.9	-0.90

Significance levels: *:0.1 **:0.05 ***:0.01 ****:0.005 *****:0.001; LOD=log likelihood score; Variance=residual variance after fitting the QTL; %Expl=the percentage of variance explained for by the QTL:100*(H0:var-var)/population variance; Additive=estimated additive effect (μA{0}- μB{0})/2.

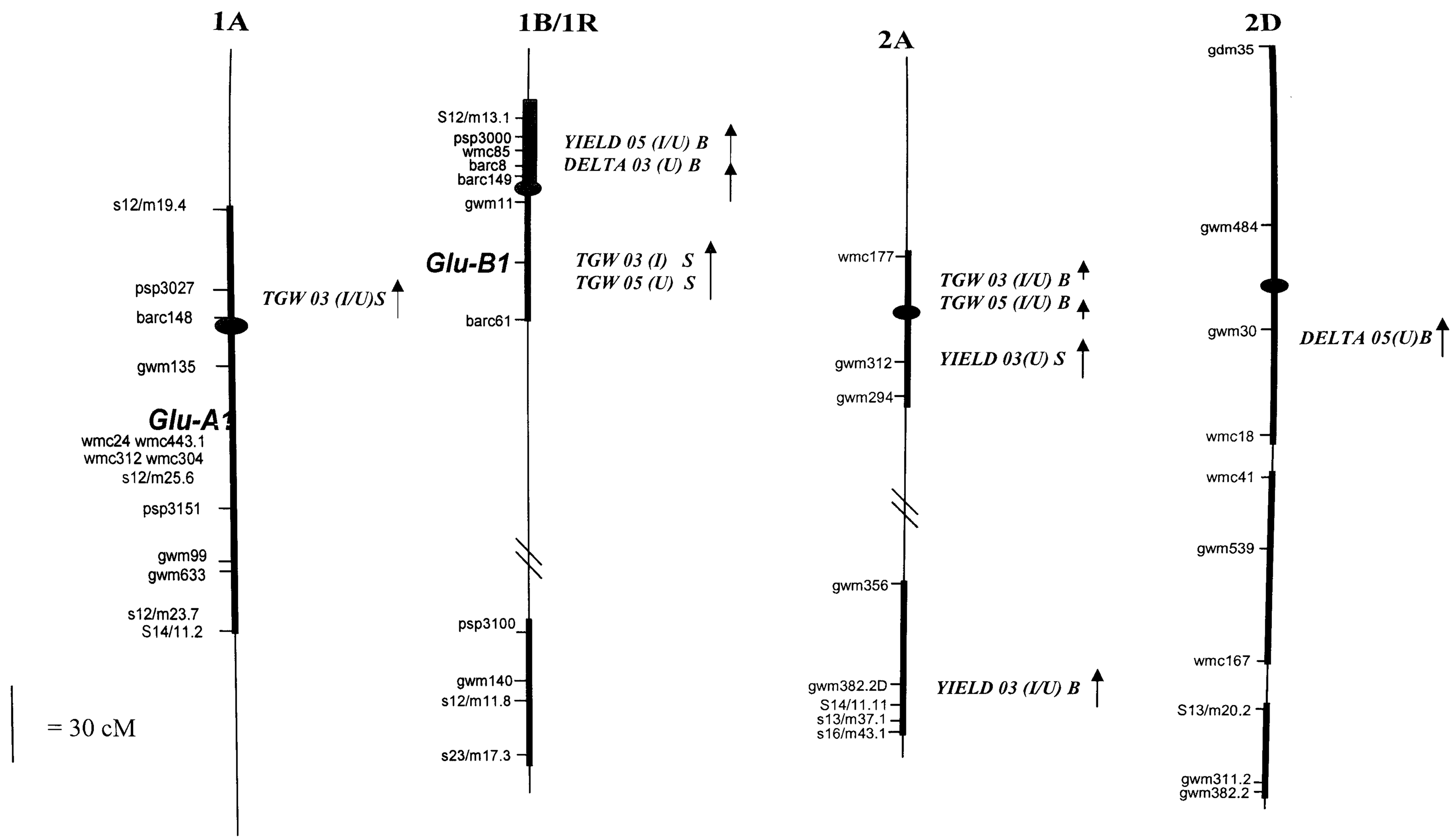
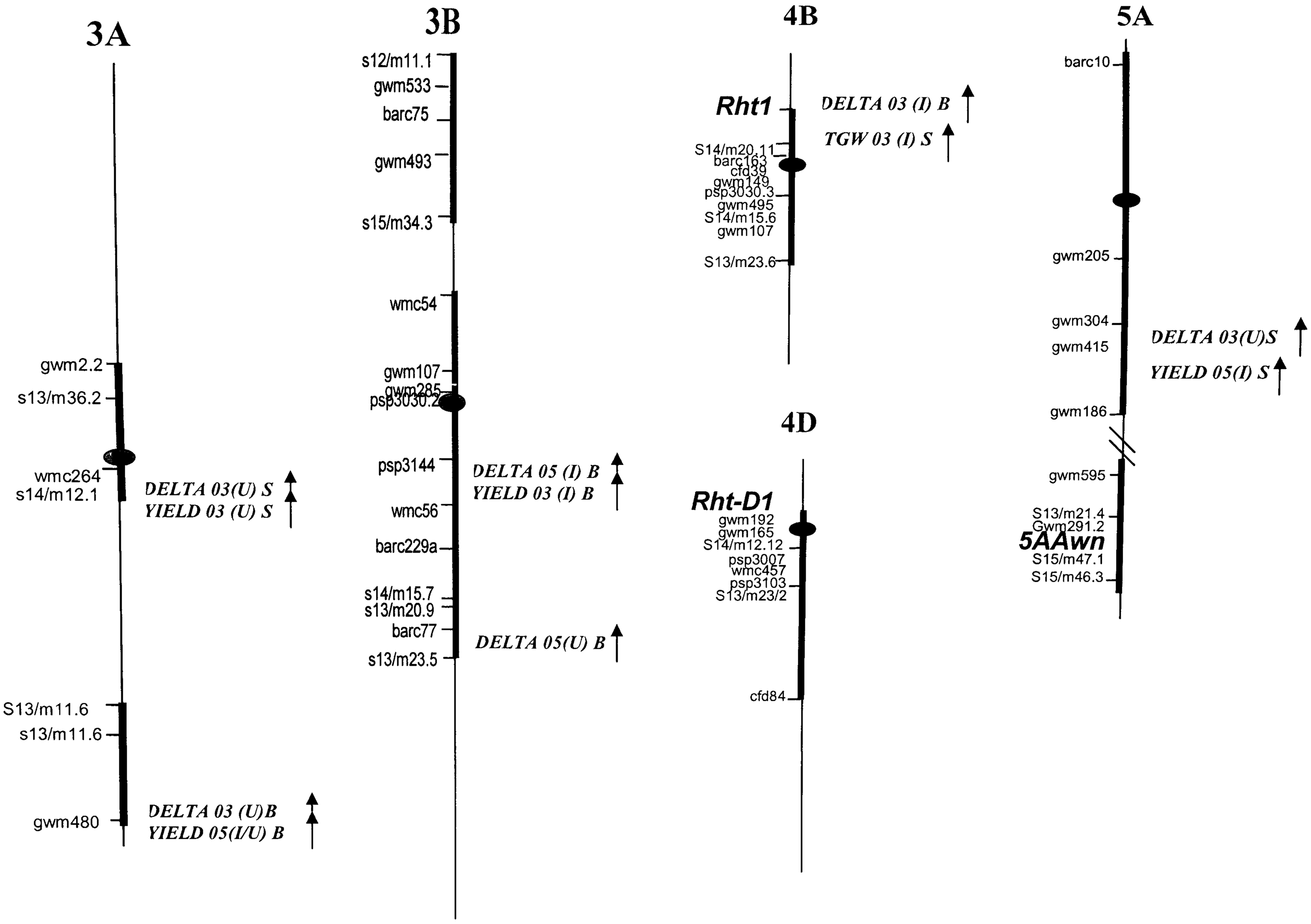


Figure 6.1 Summary of locations of QTL for traits on the Beaver x Soissons DH mapping population with the positions of marker loci on chromosomes of interest. *TGW*= thousand grain weight; *DELTA*=carbon isotope discrimination; *YIELD*=Grain yield; 03=2003 (Gleadthorpe); 05=2005 (Sutton Bonington); *I*=irrigated; *U*=unirrigated. Arrows and letter represent the parental allele increasing expression of trait. Values of K* test and LOD scores of interval mapping for putative QTLs are given in Tables 6.2-6.7.

Figure 6.1 continued. Chromosomes of interest.



Chromosomes of interest.

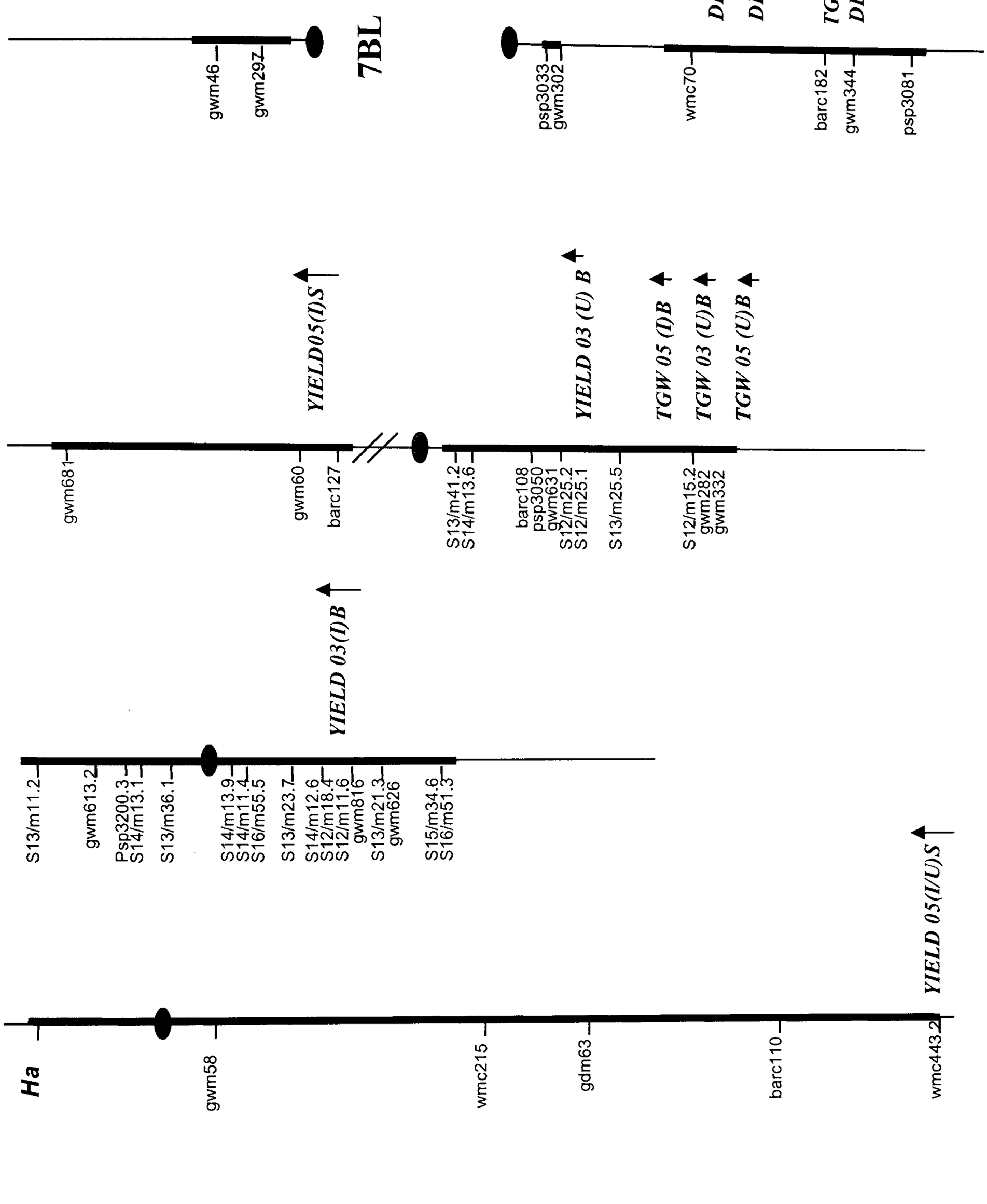


Figure 6.1 continued.

6.2 DISCUSSION

Most of the agriculturally important traits such as grain yield, yield components and quality are controlled by many genes and are known as 'quantitative' traits. The regions within genomes that contain genes associated with a particular quantitative trait are known as quantitative trait loci (QTL). Identification of these QTLs by conventional phenotypic evaluation, generating a genetic linkage map and applying QTL analysis for identifying chromosomal regions offers valuable scope for identifying markers for use in marker-assisted selection in breeding. In the present study, an attempt was made to investigate the genetic basis of water-use efficiency by identifying QTLs for carbon isotope discrimination ($\Delta^{13}C$) and quantifying co-linearity with QTLs for grain yield and thousand grain weight in the DH mapping population of Beaver x Soissons.

6.2.1 Mapping population of Beaver x Soissons

Population sizes used in genetic mapping studies generally range from 50 to 250 individuals (Young 1994; Mohan *et al.* 1997). However, larger populations are required for high-resolution mapping. The entire mapping population must be evaluated phenotypically for quantitative traits. Although the Beaver x Soissons mapping population contained 65 DH lines, the present study utilized only 33 lines because of difficulty in labour availability and the high cost of isotope analysis. The traits were phenotyped in three replicates, so the precision of measurements to some extent compensated for fewer DH lines. In addition, replication in each site/season ensured the analysis was sufficiently powerful to identify putative QTLs for target traits. The target traits were evaluated for all 33 DH lines and the two parents for QTL mapping for identification of associated chromosome regions. The results of QTL analysis are discussed here.

6.2.2 Mapping of carbon isotope discrimination ($\Delta^{13}C$)

QTLs for traits involved in drought resistance have been reported in several crops. For example, QTLs for osmotic adjustment were identified in rice (Lilley *et al.* 1996; Zhang *et al.* 2001), barley (Teulat *et al.* 1998, 2001) and wheat (Morgan and Tan, 1996). Similarly QTLs have been identified for the anthesis silking interval in maize (Ribaut *et*

al., 1996) as well as QTLs for stay green in sorghum (Tuinstra *et al.*, 1997; Xu *et al.*, 2000; Kebede *et al.*, 2001) and wheat (Verma *et al.*, 2004).

However, there are only a few reports on QTLs for carbon isotope discrimination in crop plants. QTLs for $\Delta^{13}C$ have been reported in barley (Teulat *et al.*, 2002; Forster *et al.*, 2004), Arabidopsis (Hausmann *et al.*, 2004; Juenger *et al.*, 2005; Masle *et al.*, 2005), tomato (Martin *et al.*, 1989), soybean (Specht *et al.*, 2001), rice (Price *et al.*, 1997 and 2002; Laza *et al.*, 2006) and brassica (Hall *et al.*, 2005). To date there are no reported investigations available which have identified QTLs for $\Delta^{13}C$ in wheat, although research is underway in CSIRO, Australia (R.A. Richards, personal communication.).

In the present study, the putative QTLs for $\Delta^{13}C$ obtained on the basis of interval mapping and the Kruskal-Wallis non-parametric test are discussed here. At Sutton Bonington in 2005, one putative QTL of higher significance for $\Delta^{13}C$ was found on the chromosome 3B (irrigated in 2005), $\Delta^{13}C$ being increased (or WUE decreased) by the Beaver allele which explained about 28% of the phenotypic variance for $\Delta^{13}C$. Other QTLs for delta under unirrigated conditions were also found on chromosome 2D and 3B (in 2005) which explained 18.2% and 14% of phenotypic variance, respectively, $\Delta^{13}C$ again being increased by the Beaver allele. Thus, in this study the presence of putative QTL for $\Delta^{13}C$ was evident on chromosome 3B. At Gleadthorpe in 2003, two putative QTLs for $\Delta^{13}C$ were found on chromosomes 7BL and 4B explaining more than 13% of the phenotypic variance (irrigated in 2003). The influence of parental alleles for these QTLs for the expression of $\Delta^{13}C$ was, however, different. The Soissons allele on 7BL increased $\Delta^{13}C$ and the Beaver allele on chromosome 4B decreased $\Delta^{13}C$. The putative QTLs found on chromosome 5A and 3A (unirrigated in 2003) explained more than 13% of the phenotypic variance and for these QTLs, $\Delta^{13}C$ was increased by the Soissons allele. The QTL analysis for Gleadthorpe data did not show QTLs of LOD> 2.0. Nevertheless, several putative QTLs identified in this study would be of potential importance if they could be confirmed in other experiments.

Thus, in this study a range of variation was found in the expression of $\Delta^{13}C$ under the influence of either of the parental alleles. This can be due to differences in genotypic performances specific to the environment. However, the putative QTL detected on 3B was of higher significance and this agrees with the parental characterization for delta.

There are only a few studies that relate genetic analysis of $\Delta^{13}C$ in the mapping population of plant species. In *Arabidopsis* Juenger *et al.* (2005) found significant genetic variation for $\delta^{13}C$ in the Landsberg *erecta* (L*er*) x Cape Verde Islands (Cvi) mapping population across 10 genomic regions. Both the parental ecotypes contained plus and minus alleles for $\delta^{13}C$ which explained the observed transgressive segregation in the RIL population. Similar genetic effects were also evident in the current Beaver x Soissons DH population with Beaver and Soissons possessing plus and minus alleles for variation in $\Delta^{13}C$.

Masle *et al.* (2005) assessed the phenotype of a population of RILs derived from a cross between Columbia (Col-4) and Landsberg *erecta* (*er*11) ecotypes of *Arabidopsis* for variation in transpiration efficiency and identified a significant QTL for $\Delta^{13}C$ on chromosome 2, which explained 21–64% of the total phenotypic variation in $\Delta^{13}C$. They reported the isolation of a gene that regulates transpiration efficiency, *ERECTA*, a putative leucine-rich repeat receptor-like kinase (LRR-RLK) known for its effects on inflorescence development. This is proposed as a major contributor to the locus for $\Delta^{13}C$ on *Arabidopsis* chromosome 2. Teulat *et al.* (2002) reported 10 QTLs for $\Delta^{13}C$ in barley with major QTLs on chromosome 2H and 4H and these were co-localised with other drought tolerant traits such as osmotic potential and relative water content.

Previous research investigated the genetic variation in $\Delta^{13}C$ between two wheat varieties Oxley (high $\Delta^{13}C$) and Falchetto (low $\Delta^{13}C$) (Mohammady-D, 2005). F_2 backcross reciprocal monosomic crosses between Falchetto and 18 monosomic lines of Oxley were used to identify chromosomal locations of the genes responsible for variation in $\Delta^{13}C$. Allelic variations were found on chromosome 1D for WUE and $\Delta^{13}C$ suggesting that the evolutionary source of the D genome (*Triticum tauschii*) and its relatives are potential variation sources for breeding programmes (Mohammady-D, 2005). In this pot-based study water stress was imposed by withholding water for 7 days at the meiosis stage of development and reciprocal families belonging to chromosome 1D were assessed for WUE at the F_3 generation stage. Chromosome 1D of Falchetto decreased $\Delta^{13}C$. In the present study, one putative QTL for $\Delta^{13}C$ was found on chromosome 2D under unirrigated conditions in 2005. So the importance of D genome as indicated by

the presence of a putative QTL for $\Delta^{13}C$ in the present study would relate to the importance of D genome from *Triticum tauschii* in further breeding programmes.

A QTL of lesser significance on chromosomes 4B near the *Rht-B1* locus (irrigated treatment in 2003) explained 13% phenotypic variance, with $\Delta^{13}C$ increased by the presence of Beaver semi-dwarf allele (decreasing WUE). *Rht* genes present on chromosome 4 modulate the morphology and physiology of wheat in a manner that involves compensation among several physiological processes. For instance, *Rht* genes decrease leaf area, but photosynthesis per unit leaf area increases (e.g. Richards *et al.* 2002). Although *Rht* genes increase leaf permeability to water vapour, plant water status changes in compensation to minimize differences in WUE. The results of previous work on near isogenic lines of spring wheat are contradictory. One study in Australia indicated that WUE declined with plant height and tall lines had a significantly higher $\Delta^{13}C$ (Richards, 1991), while another in California indicated the dwarfing genes depressed WUE by 15% and $\Delta^{13}C$ was negatively associated with total dry matter (Ehdaie and Waines, 1996). In winter wheat, a previous UK field study on cv. Mercia *Rht* NILs found that the *Rht-D1b* semi-dwarf allele decreased WUE compared to the tall control (Foulkes *et al.*, 2001) and therefore supported the present findings. In general, previous investigations therefore suggest that semi-dwarfing has decreased WUE consistent with the present findings for $\Delta^{13}C$.

6.2.3 Mapping of yield and yield components

6.2.3.1 Grain yield

Putative QTLs for grain yield were found on chromosome 6B and 3B (irrigated in 2003), 3A (unirrigated in 2003; irrigated & unirrigated in 2005), 2A (unirrigated in 2003), 7A (unirrigated in 2003 and irrigated in 2005) and on 5D (irrigated and unirrigated 2005). The QTLs on 5D explained 25.1% and 14.5% of the phenotypic variance for grain yield under irrigated and unirrigated treatments in 2005, respectively. Some of these QTLs coincided with yield or yield-related QTLs previously identified in wheat. Chromosome 5D is homoeologous with other group 5 chromosomes which are known to carry genes for abiotic stress resistance (Sutka and Snape, 1989; Manyowa and Miller, 1991; Cattivelli *et al.*, 2002; Quarrie *et al.*, 2005). In addition, QTLs for

yield also have previously been identified on chromosome 5B of bread wheat (Byrne *et al.*, 2002).

The putative QTL for yield found on chromosome 6B in the present study was in a homoelogous position to a yield QTL region identified in previous work in spring wheat (Blanco *et al.*, 2002; Quarrie *et al.*, 2005). The putative yield QTL on chromosome 6B explained 21.4 % of the phenotypic variance (irrigated in 2003) and is co-linear with the QTL for yield in the centromeric region of 6B in the ITMI (*International Triticeae Mapping Initiative*) population (Ayala *et al.* 2002).

The putative yield QTL clusters found on chromosome 3B and 7A in this study matched the yield QTL clusters found on these chromosomes for the Chinese Spring x SQ1 population (Quarrie *et al.*, 2005). QTLs on 3B explained 35.8 % of the phenotypic variance for yield and on 7A explained 43.6% (irrigated in 2005) and 16.3 % (unirrigated 2003) of the phenotypic variance for yield. Therefore several yield QTLs in the present study were relatively close to those reported in the previous literature.

6.2.3.2 Thousand grain weight(TGW)

Putative QTLs for TGW were found on chromosomes 7A (unirrigated in 2003; irrigated and unirrigated in 2005), 2A (irrigated and unirrigated in 2003 and 2005), 1A (irrigated and unirrigated in 2003) and the long arm of 7B (irrigated and unirrigated in 2005). The QTL on chromosome 2A is consistent with previous mapping work under irrigated conditions in 2002 at Gleadthorpe on the Beaver x Soissons population (DEFRA, 2004). In the present study, yield QTLs were coincident with TGW QTLs on chromosome 7A in the irrigated treatment in 2005. Earlier work on an ITMI population found a QTL on 7AL for spikelets per ear (Li *et al.*, 2002), and it is possible that the present QTL for TGW may represent a pleiotropic effect of a QTL for grains per ear. In other field studies, yield QTL clusters were coincident with significant effects for grains per ear and TGW on a Chinese spring x SQ1 wheat cross examined in a wide range of geographical locations (Quarrie *et al.*, 2005). Effects for TGW have also been associated with the 7DL.7Ag translocation in a study using near isogenic lines (Singh *et al.*, 1998).

6.2.4 Stability of QTLs - implications for use in breeding

$\Delta^{13}C$ provides an integrative measure of transpiration efficiency, mainly regulated by stomatal-aperture traits (Morgan *et al.* 1993; Reynolds *et al.* 2000); strong variations in climatic conditions are expected to have a significant influence on $\Delta^{13}C$ values (Merah *et al.* 2001). The effect of post-anthesis drought on $\Delta^{13}C$ at Sutton Bonington in 2005 was more severe than the mild drought at Gleadthorpe in 2003. The statistically non significant genotype x irrigation interaction found for $\Delta^{13}C$ from the cross site/season analysis of variance revealed a stable performance of genotypes across irrigation regimes.

The positive correlation between $\Delta^{13}C$ and grain yield suggested that the physiological basis of genetic differences in $\Delta^{13}C$ depended on stomatal conductance (Condon *et al.,* 1987; Acevedo, 1993; Morgan *et al.,* 1993; Merah *et al.,* 2001). Thus, gas exchange measurements at or after anthesis showed that g_s values for Soissons (low $\Delta^{13}C$) were apparently lower than for Beaver (high $\Delta^{13}C$) in both years.

The putative QTLs identified in irrigated and unirrigated environments were generally different, and also differed for a given irrigation treatment across site/seasons. This revealed that QTLs for $\Delta^{13}C$ were apparently not stable and were under the influence of prevailing environmental conditions. For instance, in 2003 (Gleadthrope), 13% and 15% phenotypic variance was accounted for by the $\Delta^{13}C$ QTLs on chromosomes 4B and 7BL under irrigation, respectively, while variation was mainly accounted for by QTLs on chromosomes 5A (15%) and 3A (13%) under drought. On the other hand, in 2005 (Sutton Bonington), 28% (irrigated) and 14% (unirrigated) of the total phenotypic variance was accounted for by the QTLs on chromosome 3B; and 18.2% for the QTL on chromosome 2D under drought.

Evidence of a non significant genotype x environment interaction for $\Delta^{13}C$ in each year or across years (P=0.06) in the general analysis of variance (as discussed in chapter 5) suggests that $\Delta^{13}C$ is heritable. However, the significant genotype x environment interaction for grain yield indicated the absolute yield loss under drought differed amongst genotypes. This was also supported by a significantly positive genetic correlation between delta and grain yield which suggested that selecting a genotype with

low Δ (higher WUE) may result in lower grain yield under drought. Nevertheless, the QTLs accounting for higher phenotypic variance identified in both environments may potentially serve as selection criteria for use in wheat breeding programmes. However, some of these QTLs may not be useful as they are not stable across seasons, but the QTL on 3B at 26 cM (marker *psp 3144*) could be of use as its detection was confirmed under irrigation in two site/seasons (although only weakly significant in 2005).

In addition, the present results as discussed in Chapter 5 suggest that water use rather than WUE is the main driver of genetic performance under mild UK droughts as compared to severe droughts in drier Mediterranean-type environments. The positive association between grain yield and $\Delta^{13}C$ found in both wet and dry environments can be linked to an existence of a trade-off between higher WUE (via lower stomatal conductance) and lower season-long water use and hence yield. It follows that QTLs for low $\Delta^{13}C$ (or high WUE) may be used as an indirect way of selecting for greater seasonal water use under post-anthesis drought in the UK

In this study, the putative QTLs for $\Delta^{13}C$ were not confidently co-located with any of the chromosomal regions for the putative QTLs detected for grain yield and TGW. It is apparent in this study that there is a genetic basis for inheritance of $\Delta^{13}C$, which may not be related to yield improvement in the UK conditions; further studies to determine whether genetic improvement in grain yield and higher WUE in winter wheat are independent in the UK needs to be ascertained.

In the following section, the associations of these QTLs for $\Delta^{13}C$ are compared with QTLs for drought resistance traits found in previous studies involving the B x S DH population in the UK.

6.2.5 Comparison of present results with previous work on B x S population

Initial QTL studies Verma *et al.* (2004) evaluated 49 lines of this Beaver x Soissons DH population at the Gleadthorpe site in 2000 and 2001 and identified a strong QTL for 'stay green' (% green flag leaf area remaining green at GS61+35d) on chromosome 2B under irrigation and on long arm of chromosome 2D under drought. The QTL for stay green on 2D was associated with a positive effect of the Soissons allele. In the present

work, a major putative QTL for $\Delta^{13}C$ was found at *gwm30* locus on the long arm of chromosome 2D (unirrigated in 2005) and explained about 18% of phenotypic variance, but the location was different to the QTL for 'stay-green' described in previous work on chromosome 2D.

The significance of the photo-insensitivity genes for early flowering has been reported in previous studies, wherein Soissons is known to carry a dominant *Ppd-D1a* allele for photoperiod insensitivity on the short arm of chromosome 2D (Verma *et al.*, 2004; Foulkes *et al.*, 2004). This genetic effect in Soissons was evidenced in its early flowering behaviour as compared to Beaver. A putative QTL was also identified on chromosome 2D for $\Delta^{13}C$ with the Soissons allele increasing $\Delta^{13}C$ and it can be speculated that *Ppd-D1a* conferring early flowering was associate with higher $\Delta^{13}C$ in the present study. This could also be related to a possible association between early flowering and low $\Delta^{13}C$, for improved WUE in the UK environment.

It is also interesting to note that some of the QTLs for $\Delta^{13}C$ detected in this study appear to be co-located to QTLs for drought resistance traits identified in previous work (DEFRA, 2004). This may include the QTL on chromosome 4B (irrigated in 2003) close to the centromeric position which is apparently co-located with a QTL for stem water soluble carbohydrates (irrigated and unirrigated in 2002) and percent flag leaf green area (irrigated in 2002) within 10 cM on linkage group 19 (DEFRA, 2004). Both these effects may be pleiotropic effects of the semi-dwarfing gene *Rht-D1b*. The positional correspondence in terms of markers appearing within 15 cM may imply the conserved organization of the genes in the genome and also the functions of the genes underlying the drought resistance traits. However, further genetic and physiological understanding is necessary for making use of these traits.

6.2.5.1 Co-location of QTLs for target traits in the present study

In this study, only at the Gleadthorpe site were QTLs for delta and grain yield found to be associated with the same marker *s14/m12.1* at 22 cM on chromosome 3A, with the Soissons allele increasing the expression of these traits under drought. However, the co-localisation of putative QTLs for delta, grain yield and TGW were different for different markers and linkage groups.

6.2.6 Synteny among cereals for QTLs for drought resistance

Although no formal syntenic analyses were carried out in this study, it was possible to make broad comparisons with other cereal species. The genome size of bread wheat is much larger (16.5 Gb) than for durum wheat (13 Gb), barley (4.5 Gb), rice (400 Mb), maize (2.5 Gb) and sorghum (730 Mb) making the identification and use of molecular markers for breeding and selection in wheat more complex. Bread and durum wheat have a lower level of polymorphism than barley or other cereals (Chao *et al.*, 1989; Devos *et al.*, 1995) and the level of polymorphism is not consistent across genomes or crosses (Langridge *et al.*, 2001).

The first grass consensus map aligning the genomes of seven different grass species using rice as a reference genome was published by Moore *et al.* (1995), and modified as 'concentric circle diagram' by Gale and Devos (1998) who suggested that the syntenic relationships among cereal annuals will be useful for predicting the loci of homologous major genes. This concept can be extended to a large number of QTL analyses for detecting the QTL shared among cereals as they share some level of homology. For example rice, wheat and maize share extensive homoelogies in a number of regions in their genomes, e.g. chromosome 4 in rice compared with chromosomes 2 and 2S in wheat and maize, respectively (Ahn *et al.*, 1993; Sorrells *et al.*, 2003). The dwarfing alleles *Rht-B1* and *Rht-D1* in wheat are also the orthologues of the maize *dwarf-8* gene and *Arabidopsis gibberellin insensitive* genes (Peng *et al.*, 1999).

A conserved orthologous sequence of genes within a novel colinear region in wheat chromosome 1AS and rice chromosome 5S has been reported (Sorrells *et al.*, 2003; Guyot *et al.* 2004). Morgan and Tan (1996) identified a major QTL controlling osmoregulation on the homoeologous arm in wheat (chromosome 7A), but this was probably at a more distal position on the chromosome, corresponding by synteny to a rice chromosome 6 segment. In the present study, a grain yield QTL was identified under drought (in 2003) on the same long arm of chromosome 7A, and it may be worth further study to see if any QTLs for osmoregulation are co-linear with this QTL.

Homoeologous group 5 chromosome of wheat is reported to carry genes controlling a range of abiotic stress responses such as tolerance to freezing, drought, osmotic stress,

and high temperatures (Snape *et al.*, 2001; Cattivelli *et al.*, 2002). QTL clusters for plant height, tiller number, spikelet number on chromosome 5A (Snape *et al.*, 1985), yield components (Araki *et al.*, 1999) and for grain yield (Kato *et al.*, 2000) have been reported. The accumulation of hormone abscisic acid (ABA) by plants in response to drought, and many other stresses is well known. In wheat, a highly significant QTL for ABA accumulation in droughted leaves was found on the long arm of chromosome 5A, coincident for the RFLP marker *Xpsr575* with the major vernalization responsive gene *Vrn1* (Quarrie *et al.,* 1994). The significance of chromosome 5A is in concurrence with the present findings that a putative QTL for delta was detected on the long arm of 5A in 2003 under drought (LOD=1.21). However, the association of marker *gwm304* on chromosome 5AL in the present study with ABA accumulation needs confirmation in further investigation.

Chromosomes of homoelogous group 2 of wheat are orthologous to chromosome F of sorghum, chromosome 10 of maize (Gale and Devos, 1998) and chromosomes 7 and 4 of rice (Sorrells *et al.*, 2003) where QTLs for stay green have been reported in sorghum (Tuinstra *et al.*, 1997; Kebede *et al.,* 2001), in maize (Bertin and Gallais, 2001), in rice (Jiang *et al.*, 2004) and in wheat (Verma *et al.*, 2004). In the present study, a putative QTL for $\Delta^{13}C$ was found on the long arm of chromosome 2D (LOD=1.43) with the presence of the Soissons allele decreasing $\Delta^{13}C$ (or increasing WUE). In maize, genomic regions significantly affecting tolerance to drought were found on chromosomes 1,3,5,6, and 8. For yield, a total of 50% of the phenotypic variance was explained by five putative QTLs (Agrama and Moussa, 1996). Group 3 chromosome of maize is orthologous to group 3 chromosome of wheat (as per map alignment from Gale and Devos, 1998). In this study, therefore potential synteny with maize was noticed by the presence of the putative QTL (LOD=2.32) for $\Delta^{13}C$ on chromosome 3B (irrigated in 2005), where Soissons allele increased WUE.

By synteny chromosome 2 of wheat is orthologous to chromosome 7 and 4 of rice (Sorrells *et al.*, 2003). QTLs for $\Delta^{13}C$ on chromosomes 2 and 7 were identified in rice (Price *et al.*, 2002; Laza *et al.*, 2006). So the QTL on group 7 of rice could potentially be syntenous with the QTL for delta in the present study on 2D. However, this may need further consideration because this QTL was not detected at Gleadthorpe in 2005. Price *et*

al. (2002) reported differential expression of QTLs for $\Delta^{13}C$ in upland rice experiments conducted at WARDA and IRRI. Such instability of QTLs in this study is in agreement where QTLs for $\Delta^{13}C$ were generally different at Gleadthorpe and Sutton Bonington.

There are studies in barley that can be easily extended to wheat. Barley like wild and cultivated wheat and rye possesses the basic seven chromosomes of the *Triticeae*. Hexaploid wheat (*Triticum aestivum* L.) contains the A, B, and D genomes and barley (*Hordeum vulgare* L.) contains the H genome (Campbell and Close, 1997). In crop plants, *dehydrin* proteins are produced in response to environmental stimuli with a dehydrative component, including drought, low temperature and salinity. *Dehydrin* loci located within QTL intervals are known to control important phenotypic traits including winter hardiness in barley (*Hordeum vulgare* L.) and anthesis-silking interval in maize (*Zea mays* L.). Map positions of some *dehydrin* genes in barley and wheat have been reported (Campbell and Close, 1997). Loci detected by *dhn1* and *dhn2* on linkage group 5H of barley were within position of a QTL contributing to winter-hardiness, photoperiod response, and heading date in a number of studies (Pan *et al*., 1994). This region of the group 5 chromosomes also houses the barley *Sh2* locus, which is a determinant of winter or spring growth habit, and the homoalleles of *Sh2*, *Vrn1* of wheat (chromosome 5A) and *Sp1* of rye (Galiba *et al*., 1995; Fowler *et al*., 1996). It is possible that the dehydrin loci on groups 5 of barley may be related to a $\Delta^{13}C$ QTL cluster, found in the present study, on chromosome 5A which explained 15% of the phenotypic variance (unirrigated in 2003).

In barley, QTLs for $\Delta^{13}C$ on chromosomes 2H and 6H showed associations with TGW and on chromosome 6H between Δ and several yield components, including TGW, relative water content (RWC), osmotic potential ($\psi\pi$), and osmotic potential at full turgor as well as several water soluble carbohydrates under drought (Teulat *et al.* 2002); One of the target loci on chromosome 7H, where QTLs for $\psi\pi$, RWC and some other traits have been co-located, was shown to be co-linear with a region of rice chromosome 8 (Teulat *et al.* 1998), where a QTL for osmotic adjustment at 70% of RWC was also found by Lilley *et al.* (1996). These drought tolerance traits can potentially be related to those QTLs found for Δ on chromosomes 7BL and 2D and for TGW on 7A and 2A in this study and further work seems justified to see whether this is the case.

Present results showed the detection of a total of nine putative QTLs for $\Delta^{13}C$: on chromosomes 7BL and 4B (irrigated in 2003), 5A, 3A, 1B (unirrigated in 2003), 3B and 4D (irrigated in 2005); and 2D and 3B (unirrigated in 2005) indicating a complex mechanism controlling expression of this trait. Hence, with the exception of the $\Delta^{13}C$ QTL on 3B in irrigated (LOD=2.32) and unirrigated (LOD=1.08) conditions at Sutton Bonington, QTLs found in the present study could be specific to one environment and were different between the two water treatments in each environment.

In summary, the putative QTLs of highest statistical significance were mapped on chromosomes 3B (LOD=2.32) and 2D (LOD=1.43). There is some evidence from comparative mapping in other species that may support the identification of QTLs on these chromosomes in wheat associated directly with water-use efficiency. Thus the hypothesis formulated for identification of QTLs for $\Delta^{13}C$ was supported by the present findings (Tables 6.2 & 6.3). These QTLs may therefore represent novel sources for improving WUE, for breeders to exploit. This information will be of value to breeders for further fine-mapping to identify markers tightly linked to genes controlling the trait, and for gene discovery.

The theory underpinning quantitative trait loci hypothesizes that specific chromosomal regions contain genes which would make a significant contribution to the expression of a complex trait. Identification of QTLs by comparing the linkage of polymorphic molecular markers and phenotypic trait measurements aid in the dissection of complex traits. This potentially enables the identification of the actual genes involved in the trait and understanding of the cellular roles and functions of these genes. Hence, the accuracy and precision of locating QTLs depends, in part, on the density of the linkage map created. The higher the density of the map, the more precise will be the location of the putative QTL. However, the most limiting factor for accurate location is number of meiotic break points. The density of the genetic map with a total of 222 markers was sufficient to carryout the QTL mapping in this study. These QTLs by other methods, such as positional cloning, can be used effectively to isolate specific genes. QTLs often interact in complex ways and their expression may be influenced by non-genetic factors. For example, QTLs for $\Delta^{13}C$ may be related to stomatal aperture traits and may be environmental specific as evidenced by specific detection either at Gleadthorpe or

Sutton Bonington, but much more data across a range of environments may be needed in future to relate these functions. In this study the QTLs for $\Delta^{13}C$, grain yield and TGW were different in both site/seasons. Some QTLs detected for grain yield and TGW were on the different linkage groups of the chromosome, e.g. 2A and 7A. Nevertheless, identification and functional analysis of genes affecting the phenotype offers great scope for developing markers for application in breeding programmes. If a known gene corresponds to the same region as a QTL for a quantitative trait, it is a candidate gene for that trait, although the phenotypic plasticity may often affect quantitative traits that are expressed differently in different environments (e.g. Kearsey and Pooni, 1996; Collard *et al.*, 2005). Thus further studies may be worth considering for relating genotypic plasticity and the genotype x environment interaction.

Improvements in abiotic stress tolerance such as drought can be achieved by genetic engineering. Transcription factors (proteins that bind to regulatory regions and control gene expression) have been shown to produce multiple phenotypic alterations, many of which are involved in stress responses. Research work at CIMMYT utilised *DREB1-A*, a transcription factor that recognizes dehydration response elements has been shown in *Arabidopsis thaliana* to play a crucial role in promoting the expression of drought-tolerance genes. Dehydration-Responsive Element Binding gene 1 (*DREB1*) and *DREB2* are transcription factors that bind to the promoter of genes such as *rd29A*, thereby inducing expression in response to drought, salt, and cold. The *A. thaliana DREB1A* gene with a stress-inducible promoter from the *rd29A* gene (stress-induced genes, such as *rd29A* in *A. thaliana*, are induced through the ABA-independent pathway) was transferred via biolistic transformation into bread wheat. Expression of the *DREB1A* gene in wheat demonstrated substantial resistance to water stress in comparison with checks under experimental greenhouse conditions, manifested by a 10-day delay in wilting when water was withheld (Pellegrineschi *et al.*, 2004). Therefore, if the genetic systems based on transgenes can be demonstrated to be effective, they will provide an attractive and complementary option for improving a plant's performance under stress conditions. CIMMYT is considering testing the *DREB1A* gene in the drought-tolerant wheat, to see if the resulting plants can use water even more efficiently. This may have major implications for its use in other cereal crops, such as rice, maize, and barley.

Nonetheless, if candidate genes for the putative QTLs in the present study are identified through further fine mapping, and if their function is proved through deletion 'knock out' techniques, then it may enable their transfer into other cereal species through transformation methods. Besides this, sequencing the gene and developing perfect markers offers a greater scope for combining marker assisted breeding into transgenic breeding for development of new cultivars not only in UK but across the wheat growing areas in the world.

CHAPTER 7: GENERAL DISCUSSION

In most areas and years in the UK the soil is returned to field capacity by rainfall during the autumn and winter. The replenishment to field capacity depends upon the maximum soil moisture deficit reached in the summer which in turn depends on the crop, soil and the summer rainfall pattern. In the UK, for winter wheat production there is a shortfall of about 130 mm between average rainfall and average evapotranspiration during spring and summer (Jones and Thomasson, 1985). Deep soils of fine texture usually hold ample moisture to meet the crop's demand for water after accounting for this shortfall (Gales and Wilson, 1981). Of the 2 million ha of wheat grown annually, 250 000 ha are on shallow soils and 220,000 ha are on sandy soils (MAFF, 1999), so a significant acreage of wheat land is likely to encounter drought in an average year (Innes *et al.*, 1985; Bailey 1990). Much of the UK wheat production area on these soil types can potentially suffer yield reductions due to droughts under present climatic conditions (Weir, 1988). Currently the wheat and arable area is concentrated in the South and East of England (MAFF, 2002); and soil moisture deficit in East Anglia, the East Midlands and the South-East parts of UK is the biggest current threat to production and these regions account in total for more than half the country's wheat area.

In the UK typically drought occurs late in the season, with onset of stress broadly co-incident with flowering (Foulkes *et al.*, 2001) and yield losses are in the region of 1-2 t ha^{-1} (Foulkes *et al.*, 2002). However, on drought-prone soils yield loss in wheat can range between 0-4 t ha^{-1} in a given year, (i.e. if onset of drought occurs earlier than flowering). Water availability could thus limit yield potential even on soil of moderate to high water availability in future years because of climate change. Climate change predicts greater frequency of drier summers in the south and east of England. Thus, there is considerable interest in increasing WUE to conserve soil water and improve yield under drought. Modern UK wheat cultivars have larger harvest above-ground biomass than their predecessors (Shearman *et al.*, 2005), so may have greater demand for water. Most currently commercial varieties apparently have similar WUE (Foulkes *et al.*, 2001) and WUE may even have decreased with breeding. There is evidence that changes in breeding in recent decades may have increased the demand of wheat crops for water in non-droughted environments. For example, the *Rht2* semi-dwarf gene

introduced in the mid 1970's has been found to be associated with lower WUE (Foulkes *et al.*, 1998a) and a higher WUE of an old tall cv. Maris Huntsman compared to five modern semi-dwarf cultivars was reported (Foulkes *et al.*, 2001). The 1BL/1RS translocation introduced in the mid 1980's associated with greater biomass production at harvest was also associated with greater requirement for water (Foulkes *et al.*, 1998b). These studies have provided possible opportunities for exploiting genetic diversity in modern wheat cultivars for an improved understanding of mechanisms originating either through WUE or enhanced water uptake for realising the attainable yield under drought. Hence, this study was aimed at identifying and quantifying sources of genetic variation in WUE for breeders to exploit and identifying the main determinants of this variation in WUE and understanding any genotype x environment interactions for WUE in the UK environment. In addition, identification of sub-traits for maintenance of crop production under drought is also important as these sub-traits can potentially be used as selection criteria in breeding programmes. Previous studies have targeted selection methods for drought-resistant traits, specifically: early flowering date, greater accumulation of stem carbohydrate reserves and better canopy persistence (DEFRA, 2002). Apart from identifying the target physiological traits underlying WUE and water use, the present study also investigated inter-relationships between water use and WUE and HI in relation to final yield performance.

The project was focussed on improving understanding of the physiological determinants of water-use efficiency and their genetic control using doubled-haploid lines derived from a cross between UK-grown winter wheats Beaver (UK feed wheat) and Soissons (French bread wheat). Previous evidence showed that the parents contrasted for *Rht* and *Ppd* genes and the presence or absence of the1BL/1RS translocation, and also differed for $\Delta^{13}C$ values (Foulkes, M.J., Personal communication). Lines of this population and the two parents were examined under irrigated and unirrigated conditions, in both glasshouse and field experiments. This chapter deals with how the present study was able to provide improved understanding of the physiological and genetic bases of WUE in UK winter wheat. The findings discussed are described in three broad sections. The first section deals with the physiological understanding of genetic variation with regard to water use, WUE and biomass partitioning to grains. The second section deals with the

genetic basis of drought resistance through identification of putative QTLs. The third section deals with the proposed direction of future work and overall conclusions.

7.1 PHYSIOLOGICAL BASIS OF GENETIC VARIATION IN DROUGHT PERFORMANCE

There should be significant genetic variation for a target physiological trait and the trait should be of high heritability for breeding to achieve results more quickly and efficiently than selecting progeny for yield performance alone. The genetic progress in yield in drought environments has been quite limited in most crop improvement programmes (Reynolds *et al.*, 2001; Slafer *et al.*, 2005). This may be in part because physiological studies that have focussed on plant responses to water stresses have in general occurred in parallel to breeding and largely the two have existed in separate arenas.

The magnitude of the yield reductions under UK drought has been reported to be about 2 t ha^{-1} (Innes *et al.*, 1985; Foulkes *et al.*, 2002). However, in this study, the yield reductions under drought ranged from 0.5 t ha^{-1} (in 2003; $P = 0.40$) to 1.6 t ha^{-1} (in 2005; $P<0.05$) and the yielding ability of genotypes was strongly influenced by the G x E interaction ($P<0.03$). So this indicated genetic variation in yielding ability under drought existed and could potentially be related to the performance of physiological traits. Traits putatively related to yield under drought conditions must positively affect water use, WUE or biomass partitioning to the grain (Passioura, 1977, 1996; Richards, 1996, 2000; Araus *et al.*, 2002).

7.1.1 Water use

The phenology of the crop has been considered to be one of the most important attributes for conferring the ability to perform better under stressful conditions in Australia (e.g. Passioura, 1996, 2002; Richards, 1996) and Spain (Villegas *et al.*, 2000). The impact and extent of droughts in these Mediterranean regions are more severe than in the UK. However, drought occurring during a particular growth stage of the crop cycle may result in severe yield reductions. So any changes in phenology should allow the crop to escape water stress by avoiding the coincidence of water stress during critical stages of development. Thus traits related to improved water use such as flowering time

become relevant when crops do not completely use the water potentially available for growth. The inclusion of the photoperiod insensitive, early-flowering cv. Soissons and a photoperiod sensitive, late-flowering cv. Beaver as parents resulted in differences in days-to-flowering of about 9 d. In the present field studies, flowering time did not correlate with yielding ability of genotypes under drought (r=0.2), indicating that early flowering did not lead to a better maintenance of grain yield under drought and the effect of flowering date on grain yield was neutral. However, in the glasshouse studies, there was a trend for the early flowering genotypes (for e.g. Soissons) to express 'drought escape' on account of relatively greater post-anthesis water use, but they had a lower overall water uptake. With regard to the field results, it is possible that later flowering might have favoured development of larger root system allowing the crop to maintain water uptake during grain filling, despite using more water in the pre-flowering period. More generally, photoperiod insensitivity in genotypes may theoretically have drought escape of more severe pre- and post-anthesis droughts than encountered in the present study. However, selection for early flowering especially from early sowings (i.e. sowing before late September) might lead to an increased risk of frost. Thus, selection for time of anthesis may need a careful consideration by combining frost tolerance into these genotypes to make potential use of available soil moisture later in the crop season.

The traits related to WUE and partitioning become more important when available water is already depleted in a given environment (see Slafer *et al.*, 2005). The breeding strategies may differ depending upon the climatic conditions, e.g. Mediterranean-type environments compared to the less severe droughts occurring in UK environments. In Mediterranean conditions, terminal drought develops increasingly throughout the late vegetative and grain filling phases (Loss and Siddique, 1994), while in UK drought coincides with post-anthesis development of crops and physiological maturity is only advanced by about 4-7 days (Foulkes *et al.*, 2002). In Mediterranean regions, like Western Australia or Spain, time to anthesis has tended to be reduced systematically as new cultivars have been released (e.g. Siddique *et al.*, 1989; Slafer *et al.*, 2005). So when the drought is expected to be terminal, selection for earliness increases 'drought escape' and improves the partitioning of the total water use by the crop to the grain filling period. In this study, flowering time (GS61) of the genotypes was not associated with the absolute grain yield loss of the genotypes under drought (r=0.33) which is in

support of previous studies in the UK (Foulkes *et al.*, 2001). However, a positive correlation between flowering date and yield under both irrigated and droughted conditions indicated higher yield is associated with late flowering, e.g. yield for Beaver was higher than Soissons in the field studies. Nevertheless, such differences were not evident in the glasshouse studies, possibly on account of a smaller number of comparisons amongst genotypes and that fact that the measurements were based on single plants. However, there was a correlation between flowering time and $\Delta^{13}C$ in one of the two field experiments, at Gleadthorpe in 2003, where earlier flowering was associated with higher grain $\Delta^{13}C$ ($P<0.05$). This suggests that a low Δ (high WUE) in early-flowering genotypes could possibly be due to a drought-escape effect during the grain-filling period. Alternatively early flowering with *Ppd-D1* may be associated with smaller leaves of higher specific leaf weight and net photosynthesis rate.

On the other hand the association of flowering time and grain $\Delta^{13}C$ was not confirmed statistically at Sutton Bonington in 2005 although the post-anthesis drought was even more severe. So the observed effect of 'drought escape' cannot be taken as certain with regard to $\Delta^{13}C$. Moreover, the non-significant regression of flowering date on grain yield in both site/seasons showed that early flowering did not lead to better maintenance of grain yield under drought. Thus, earliness is probably not very relevant as a major prospective trait according to results in this population for future breeding. On the other hand, 'drought escape' in the glasshouse experiments was observed in early flowering genotypes. That is, Soissons showed least seasonal-water use and relatively higher post-anthesis water use compared to Beaver, while DH lines 134G and 134E were intermediate in their responses. However, these contrasts did not provide evidence for large differences in post-anthesis water uptake, so there may be a trade-off between earlier flowering and the development of a less extensive rooting system in these early genotypes. It should be noted that net root growth normally ceases at anthesis.

Roots traits relating to a deeper and more extensive root system confer the ability to the crop canopy for capturing more water. Although they are difficult to measure, they are important to characterise as they are determinants of whether genotypes extract all the available soil water in a season under drought. A deep root system allows the crop to access water in deeper soil layers, and deeper rooting may be associated with higher

stomatal conductance under drought (Blum, 1993, 1996). In this study, root traits were measured in the glasshouse experiments on four genotypes. The differences in RLD for these genotypes indicated the existence of genetic variation for root distribution with depth in UK-adapted winter wheat. Beaver had greater RLD in each depth and total RLD across all depths. Regardless of RLD, higher root weight across all depths was evident in Beaver and line 134G. These observed genetic differences in root dry weight may be due to differences in the degree of functional equilibrium between root and shoot (Brouwer, 1963, van Noordwijk and de Willegen, 1987).

RLD was greater than the critical RLD of about 1.0 cm cm^{-3} for effective water capture (Barraclough *et al.*, 1989) at anthesis in Beaver and line 134G at depths below 40 cm in 2004, while in 2003 the RLD of all the four genotypes exceeded 1.0 cm cm^{-3} below 80 cm depth. The genotypic differences in absolute water use in the post-anthesis period were evident with early flowering cultivars using most water during this period. However, the genetic variation in root dry matter of genotypes relating to the observed differences in RLD for effective water uptake could not be linked to season-long water-use under drought in the glasshouse study. This needs to be further characterised along with post-anthesis water use in other early and late flowering genotypes. The concept of decreased root partitioning with breeding in recent decades in UK wheats has been suggested (Foulkes *et al.*, 1998; Shearman, 2001). But introduction of 1BL/1RS translocation may increase root biomass (Ehdaie *et al.*, 2003). In the glasshouse study, the drought susceptibility of Beaver in terms of its inability to maintain harvest biomass under drought was evident on account of restricted water uptake especially at later stages of development. This was evident by a significant G x E interaction ($P<0.03$). Soissons maintained biomass and yield better under drought. As this study quantified differences for only four genotypes for root traits, it is possible that an evaluation over a wider range of genotypes of root traits would reveal more useful variation in root traits in terms of their related differences in maintenance of biomass under drought. In any case, the incorporation of root traits into UK varieties will require the allocation of significant resources and a specific breeding effort including the development of high throughput, precise phenotyping root screens (Bingham and McCabe, 2004).

Baseline physiological studies have been carried out (Foulkes *et al.*, 2001; Shearman *et al.*, 2005) on set of historic UK varieties to establish which physiological traits are likely to be contributing to changes in variety performance over years. For example, such experiments have shown that harvest index is approaching its theoretical maximum of about 0.60, so that future gains in yield may increasingly depend upon achieving greater harvest biomass, whilst maintaining HI. This implies that future genetic gains in yield under UK drought conditions may require increases in both source and sink related traits. It is considered that the rate of photosynthesis during grain filling, the amount of assimilates available for remobilisation, root:shoot partitioning and the flag leaf area persistence during grain filling are key above-ground traits. In addition, genetic differences in the pattern of RLD distribution with depth and specific root weight may be key below-ground traits for increasing season-long water capture to underpin increases in biomass.

7.1.2 Water-use efficiency

Carbon isotope discrimination ($\Delta^{13}C$) is a promising trait for assessing genetic variation in WUE (Farquhar and Richards, 1984; Richards *et al.*, 2002) and also provides an indirect indication of water extracted by different genotypes. As previously discussed (chapters 4 & 5), the physiological basis of variation in $\Delta^{13}C$ in C_3 species is related to variation in C_i, i.e. when C_i is lower, Δ is lower and WUE (TE) is higher (Farquhar and Richards, 1984; Condon *et al.*, 1987). As $\Delta^{13}C$ correlates positively with g_s, it indicates the water status of the plant, especially under water-limited conditions. For example, higher delta may indicate an improved ability to access water due to differences in rooting ability. A lower delta relates to higher A/g_s, i.e. TE as measured by gas-exchange at the leaf level. Higher TE (lower delta) can result from either lower g_s or higher A associated with higher photosynthetic capacity. In this study, genetic differences in delta were found in the field of up to 1.14 % (droughted) and 1.34 ‰ (irrigated) averaged across site/seasons ($P<0.05$) while, in the glasshouse study, genotypes differed by 1.93 ‰ (in 2004; $P<0.01$). In both of these experiments no G x E interaction was found. This is consistent with observed trends in TE measured by gas-exchange in this study in the field and glasshouse experiments. There was a general trend for a negative genetic correlation between $\Delta^{13}C$ and gas-exchange TE and a positive genetic correlation with C_i in both glasshouse and field conditions. Since a higher TE amongst genotypes was

associated with generally lower A and lower g_s, it is possible that selection for a higher TE could lead to lower dry matter production under drought in the UK environment, i.e. the reduction in g_s was relatively more than the reduction in A. The observations that the genetic correlation between yield and $\Delta^{13}C$ was always positive ($P < 0.02$) in the present study suggesting decreased biomass productivity with higher WUE in the B x S DH population. Nevertheless, the positive correlation of delta and grain yield confirmed the theoretical physiological basis (Farquhar and Richards, 1984) for variation in $\Delta^{13}C$ to account for genetic differences in WUE in B x S mapping population. The reduction in $\Delta^{13}C$ in droughted compared to irrigated treatments both in glasshouse and field experiments reflected a decrease in C_i as result of reduced stomatal conductance in water-stressed plants. Similar effects of drought have been commonly observed in C3 cereals worldwide (Evans *et al.*, 1986; Richards *et al.*, 2001; Condon *et al.*, 2002). Small changes in $\Delta^{13}C$ under drought suggest poor stomatal control and profligate water use; conversely, large changes in $\Delta^{13}C$ suggested tight stomatal control and conservative water use. However, the hypothesis [(1) section 2.6; P40] that the genetic variation in TE in the DH population is due to differences in g_s or A or C_i of the flag leaf could not be confirmed by the gas-exchange measurements on account of a high variability associated with stomatal aperture traits across the measurement dates (Tables 5.7-5.9). With regard to genetic differences, a larger $\Delta^{13}C$ in Beaver and 134E than Soissons and 134G may imply less tight stomatal control of water loss for these genotypes i.e. higher g_s. The relatively lower g_s values associated with a conservative water use in Soissons and line 134G were confirmed in both glasshouse and field studies. In the present study, there were associated difficulties with gas-exchange measurements in the field which did not show statistically significant associations with grain $\Delta^{13}C$. There was no control over temperature or light in the field during gas-exchange measurement. Besides this the measurements were a series of spot readings made on single leaves hence the convincing evidence of a link between carboxylation capacity or stomatal conductance and $\Delta^{13}C$ was not found (Tables 5.15 & 5.16). So it is not possible to conclude unequivocally from these data the underlying cause of the variation in $\Delta^{13}C$ is stomatal conductance. However, from this study there was an indirect evidence of a link between g_s and $\Delta^{13}C$ since there was a positive genetic correlation between $\Delta^{13}C$ and yield. If $\Delta^{13}C$ was correlated with gs, then $\Delta^{13}C$ would provide an indirect measure of water use of the genotypes integrated over the growing period of the particular organ sampled.

Further work seems justified to test whether this is the case. The possible application of these findings in UK breeding programmes would be to select indirectly for delta (i.e. high $\Delta^{13}C$, low WUE) presumably associated with greater water use under drought in the UK.

The present study could not establish a clear negative relationship between $\Delta^{13}C$ and WUE on account of associated difficulties in relating the gas-exchange measurements obtained as a series of spot readings made on single leaves to $\Delta^{13}C$ (an indicator of transpiration efficiency integrated over leaf layers and time). The genotypic differences were apparent in the gas-exchange data, but data did not show a significant positive genetic correlation between gs and $\Delta^{13}C$. Therefore, further work would be needed first to establish that there is a negative relationship between TE and $\Delta^{13}C$ in field crops in the UK environment, before $\Delta^{13}C$ could be used for informing on water extraction amongst genotypes.

The ongoing breeding programmes in Australia have incorporated selection of genotypes based on $\Delta^{13}C$. 'Drysdale' and 'Rees' were the first commercial wheat varieties bred using $\Delta^{13}C$ as selection criteria. These varieties have a yield advantage in dry situations for crops growing on stored soil water. At CIMMYT also attempts are underway to improve drought tolerance by introgressing stress adaptive traits into empirically selected drought tolerant germplasm. CIMMYT's germplasm collection is being screened for high expression of many of the physiological traits that encompasses the conceptual model of a drought resistant wheat cultivar (Richards *et al.*, 2001; Skovmand *et al.*, 2001). Currently CIMMYT are considering use of $\Delta^{13}C$ but only in the same sense as in the present study, since high $\Delta^{13}C$ has been associated with high yield in both their irrigated and droughted mega-environments (Matthew Reynolds, personal communication). However, the use of delta for selection of varieties in UK environments needs some further careful consideration. Interestingly, in this study some of the lines showed a trend for low delta (high WUE) to be associated with higher grain yield. This was confirmed by the outlier lines for the relationship between delta and grain yield calculated at 95 % confidence interval across years. The values for delta and grain yield appearing above or below the threshold mean indicated that for some genotypes (e.g. 122I, 168D, 33C, Soissons, 39C and 48D) a higher grain yield was associated with lower delta or higher WUE. So the hypothesis [(3) section 2.6; P40] that

a negative genetic correlation exists between $\Delta^{13}C$ and TE was indirectly confirmed by a positive correlation between $\Delta^{13}C$ and grain yield in some of the genotypes. However, this requires further intensive physiological investigations in these genotypes to ascertain what mechanisms may be responsible for this apparent breakage of the negative linkage between WUE and grain yield.

Genetic variation in stomatal conductance partly explained the genetic variation in TE in the present study. Although genetic correlations were generally not statistically significant on individual assessment dates, there was a general trend in reductions in g_s across all data. Despite the variability in the gas-exchange data over the dates of measurement in this study, there was a general trend for reductions in g_s, A and C_i to be associated with higher TE and lower delta amongst genotypes under drought. The experimental variability may in part be an artefact of the temporal variation arising out of phenological differences among genotypes. Increased A could apparently not explain or provide a possible mechanism for an increased TE amongst genotypes in this study. So the hypothesis [(2) section 2.6; P40] that a decreased stomatal conductance or an increased net photosynthetic rate would reduce C_i and increase TE could not be confirmed by the present findings owing to the difficulties associated with environment conditions under which gas-exchange measurements were done and also in scaling up the data from the single leaf to the canopy scale. Further work involving more frequent gas-exchange measurements made using supplementary light to standardize the light environment are required to test this hypothesis. Nevertheless, there was some indirect evidence of a link between g_s and TE through the negative relationship between $\Delta^{13}C$ and yield. The hypothesis [(4) section 2.6; P41] that $\Delta^{13}C$ increases as C_i remains high could not be confirmed for the same reasons as mentioned above. In summary, it is more evident that minimising g_s favoured higher TE. However, this may not be a realistic breeding policy because in UK drought over years is not predictable, so a drought resistant variety must also perform well in the absence of drought. Thus there is a need to search for new sources of genetic variation in photosynthetic traits to increase WUE via increases in A.

This study focussed only on one mapping population derived from a Beaver x Soissons cross and it is possible that it may not be a representative of responses in other UK

germplasm. In this regard there is a need to search for novel sources of variation in WUE in germplasm unadapted to the UK environments. It is often found that photosynthetic rate per unit area is similar, or possibly less, in modern high yielding cereals than in their predecessors. This is certainly true of cultivated bread wheat compared with its wild diploid ancestors. The wild diploid wheats have a higher light saturated photosynthetic rate (Austin, 1988). Evans (1980) and Austin (1985) confirmed that hexaploid bread wheat has leaves of larger size and greater persistence than its ancestor, but 25-30% lower light-saturated photosynthesis rate (Austin *et al.*, 1987; Austin, 1988). This reflects on the scope for improvement in photosynthetic machinery in bread wheat genotypes introgressing genes from wild diploids, although it will be important to take account of any negative trade-offs with leaf size hence seasonal radiation interception.

Based on the resistance analogue for CO_2 and H_2O vapour fluxes into and out of individual leaves exposed to saturating light intensity, WUE is maximal when the rate of net photosynthesis is less than half its unstressed rate. For crop canopies the rate of *A* at which WUE is maximum is influenced by additional factors which may be susceptible to genetic modification. The resistance of the crop boundary layer to CO_2 and H_2O vapour transport affects transpiration more than assimilation and may vary with leaf size, leaf position and canopy architecture. Increases in this resistance will tend to increase WUE (Austin, 1987). However, these effects are likely to be small compared with effects of gas-exchange traits operating at the leaf level particularly in the UK conditions where drought is usually not very severe.

Hammer *et al.* (1997) demonstrated in sorghum a negative relationship between TE and specific leaf area, thus a higher SLA would relate to lower WUE. This study showed no relationship between SLA and TE. Indeed the SLA of Beaver was lower as a consequence of thicker leaves. The reason why a high SLA in Soissons resulted in a high WUE (low $\Delta^{13}C$) needs to be investigated further. Flag-leaf rolling under water stress indicates plant water status which is more often linked to deeper rooting as an adaptive feature to avoid leaf senescence and maintain green area (Reynolds *et al.*, 2001). Greater flag-leaf rolling was evident in Soissons which demonstrated a greater persistence of flag leaf area under drought as compared to Beaver. Soissons and

line134G sustained green area or leaf production during drought even under more severe water stress and possessed lower $\Delta^{13}C$ as consequence. This could possibly explain in part differences amongst genotypes in WUE arising out of differences in this self-defence strategy.

In this study, interestingly 1BL/1RS lines showed higher $\Delta^{13}C$. For example, Beaver possessing this translocation had higher $\Delta^{13}C$. This is possibly because of a positive association between $\Delta^{13}C$ and grain yield, with the higher yield being associated with higher g_s.

7.1.3 Harvest index

Most yield improvements in drought environments have come by increasing the transpirational component of season-long evapotranspiration through management and breeding, and by increasing HI through breeding in wheat. The genetic gains in yield in modern UK winter wheat cultivars have been mostly attributed to an increase in HI, but since the late 1980's there is evidence of increases in the above-ground biomass, in addition to the increase in HI. Austin (1980) predicted a theoretical limit to HI of 0.62 for winter wheat grown in NW Europe due to the biomass needed for physical support of reproductive structures and assimilate production. Shearman *et al.* (2005) reported that since about 1985 most yield genetic gains have been associated with increases in biomass, but still stated there was some scope for further increases in HI in UK wheat varieties in future years. An increase in HI with the semi-dwarf wheats introduced worldwide in the 1960s and 1970s has been associated with increased partitioning to the ear in the pre-anthesis period (Fischer, 1983). In the present study HI ranged from 0.45 to 0.53 amongst the DH lines under drought, and the genotype x drought interaction for yield was explained most by reduction in biomass. This was evident in Beaver which did not maintain grain yield under drought because of reduction in biomass growth. In contrast, line 76A which despite having maintained HI well under drought did not yield better because of poor biomass growth. Soissons which sustained yield under drought maintained both above-ground biomass and HI. Thus, the genetic differences in ability to maintain HI under water stress were associated with genetic differences in ability to maintain yield. Hence, it is evident in this study that improvement in HI under drought through breeding can be achieved to realise the attainable yield under drought. One

possible way to alleviate source limitation under drought stress lies in improved post-anthesis stem reserve mobilisation into grains. Higher HI under post-anthesis stress can be realised by improvement in late-season water use of these genotypes under droughted conditions, e.g. through better rooting or maintaining green leaf area under drought through 'stay-green' traits.

The 'stay-green' trait could be associated with favourable moisture acquisition since it may be related to deeper rooting and may indicate the ability of the green area of genotypes to persist green area for additional water extraction (Reynolds *et al.*, 2001; Verma *et al.*, 2004). In this study, Soissons (early developing) showed greater persistence of canopy under drought as compared to Beaver. A greater reduction in green area index of Beaver (late developing) under drought was found. Thus, these results showed that early flowering might have helped redistribute water uptake more favourably towards the grain filling phase. This indicates the ability of early genotypes (e.g. Soissons) to possibly acquire water use from deeper soil horizons even during later stages of grain filling. However, this needs to be characterised in further studies, and the trade-offs between flowering date and rooting need to be quantified.

In the glasshouse study, drought developed progressively from flag leaf emergence (GS39) and was sustained until full canopy senescence. This study showed evidence for genetic variation in maintenance of yield under drought which may reflect on the translocation of stem reserves to grain during grain filling effectively in genotypes, e.g. better maintenance of yield for Soissons and 134G, although stem reserves were not measured in this study. It would be desirable to increase biomass production concurrently with HI in order to further increase yield under drought, as direct selection for HI alone has been shown in some cases to result in a reduction in total biomass with no associated increase in yield (Sharma and Smith, 1986; Austin, 1994). Innes *et al.* (1984) showed in spring wheat that HI for high ABA genotypes (0.49) was marginally greater compared to low ABA genotypes (0.48), under early drought (water withheld for 4 weeks preceding anthesis) and ranged from 0.46 to 0.44 under late drought (no water between anthesis and maturity) conditions. The greater WUE of high ABA lines under late drought was associated with maintenance of biomass and reduced water use.

The effect of the 1BL/1RS translocation on grain yield and $\Delta^{13}C$ examined in this study revealed that 1B/1R translocation lines showed increase in $\Delta^{13}C$ in 2003 ($P=0.07$) and in 2005 ($P<0.001$), but not in grain yield as compared to non-translocation lines. Although there was a trend for increased harvest biomass with the1BL/1RS translocation in the 2003 field experiment. Austin (1999) and Foulkes *et al.* (1998) showed that 1BL/1RS rye translocation lines yielded more than their near contemporaries which do not carry translocation. The1BL/1RS translocation was associated with greater accumulation of stem reserves and conferring greater biomass at harvest in modern adapted UK wheat genotypes (Foulkes *et al.*, 2001; DEFRA, 2004).

The tall lines (*rht*) possessed low $\Delta^{13}C$ (high WUE) and had decreased grain yield under drought compared with *Rht2* lines, more significantly so in 2003 ($P<0.001$) than in 2005 ($P<0.10$). A low grain yield was observed in tall lines due to low HI. The observation of higher $\Delta^{13}C$ in tall lines has been reported in earlier studies by Richards (1992) and Ehdaie and Waines (1994). The current findings broadly agreed with these reports. *Rht* genes decrease available stem sink and hence anthesis biomass, but *Rht* genes as a consequence, increase ear fertility and HI. The increase in HI is proportionately greater than the decrease in harvest biomass (DEFRA, 2004). A higher biomass production in tall lines did not result in higher grain yields due to lower HI. So this overall suggests that breeding may have inadvertently decreased WUE. Thus the implications for greater 'water demand' of modern cultivars for yield improvement under drought needs to be reconsidered by improving assimilate remobilisation in the grain filling period. The positive association between HI and $\Delta^{13}C$ indicates as WUE increased partitioning of dry matter to grain decreased. This antagonism between HI and $\Delta^{13}C$ could make realising increases in grain yield difficult when selecting for high WUE. On the other hand, low WUE during the grain filling period or a higher water use might improve grain yield associated with a higher HI as observed in this study.

The principle constraint lies in the successful use of $\Delta^{13}C$ as a candidate character will still depend on determining those circumstances and crops in which Δ is lowered for a higher WUE without compromising grain yields. The high WUE of Soissons observed in this study may be ascribed to its inherently rapid development with production of most of biomass before drought becomes severe coinciding with post-anthesis. The

observed drought escape associated with higher WUE of Soissons could be ascribed to effective use of water during sensitive stages of development. Besides it may be more profligate in water use in the post-anthesis period thus be able to escape drought effects before grain filling.

7.1.4 Joint optimization of water use and WUE for drought resistance in the UK environment

For temperate cereals, including wheat, the utility of $\Delta^{13}C$ in breeding is likely to vary depending on the extent to which yield is limited by water supply and also at what stage of the crop cycle water stress occurs (Richards *et al.*, 2001). In wheat, $\Delta^{13}C$ is a conservative trait associated with somewhat slower water use and possibly slower growth rate. Consequently in environments subject to terminal (late-season) water stress (like in UK), it is not surprising that low $\Delta^{13}C$ (high WUE) was associated with relatively low yield potential in wheat. The implication is that selection for high $\Delta^{13}C$ in these environments may prove successful in identifying lines with high attainable yield under post-anthesis drought. Thus, reasons for why some genotypes with low $\Delta^{13}C$ showed a trend for high grain yield under drought, i.e. breaking the positive association between $\Delta^{13}C$ and yield, needs to be considered in further studies. So the hypothesis [(6) section 2.6; P41] that there is a positive genetic correlation between TE and grain yield under drought was indirectly disproved in the present study because of the positive genetic correlation between $\Delta^{13}C$ and grain yield (Fig. 5.2). Further studies to examine the apparent negative linkage between TE and grain yield in the UK environment are required. In environments where crop growth is heavily dependent on moisture stored in the soil profile from rain that falls outside the main crop growth phase, selection for low $\Delta^{13}C$ is likely to be effective for yield improvement, e.g. New South Wales (Condon *et al.*, 1987). Profligate high $\Delta^{13}C$ genotypes are more likely to exhaust the soil water supply before the critical grain filling phase (Richards *et al.*, 2002). This seems unlikely in the UK environment. Thus, it can be concluded from the observed relations under field conditions that: firstly, a strong negative relation exists between WUE and $\Delta^{13}C$; secondly, a strong positive relationship exists between $\Delta^{13}C$ and yield. The possible options of potentially breaking this negative linkage between lower WUE and higher yield in the UK include improving photosynthetic traits. Substantial genetic gains in yield may be achieved if breeders are able to produce cultivars with faster growth rates

and greater biomass at harvest. For example, Austin (1999) and Reynolds *et al.* (1999) suggested that modifying beneficially the CO_2 fixing photosynthetic enzyme Rubisco by reducing its oxygenase activity for improved biomass growth.

7.2 GENETIC BASIS OF WATER-USE EFFICIENCY

Breeding for productivity improvement needs to be more efficient than has been in the past. World demand for wheat is growing at approximately 2% per annum (Rosegrant *et al.*, 1995). The increasing world demand for food by population pressure and the risks associated with future climate change prompts for urgent need for research break-throughs, particularly targeted at drought resistance. Contributions from molecular biology for improved yield potential and drought resistance seem to depend upon an improved knowledge of yield and resource-use efficiency physiology and the ability to phenotype precisely for key target traits. Hence for a successful marker-assisted selection (MAS, selection based on the presence of a few genes or quantitative trait loci) programme, the value of a physiological trait in terms of its heritability and correlation with grain yield needs to be established first and then it may be useful to determine its genetic basis, such as the number and location of genes/ QTLs involved in its expression. In this context, phenotyping of physiological traits and further application in MAS for productivity improvement under water limited environment remains challenging for the crop scientists.

A combination of the application of physiology and genetics may improve basic understanding of complex genotype × environment interactions, such as plant response to drought conditions, offering new avenues for crop improvement. Using genetic mapping to dissect the inheritance of different complex traits in the same segregating population can be a powerful means to distinguish common heredity from casual associations between such traits (Paterson *et al.* 1988).

As carbon isotope discrimination provides an integrative measure of photosynthetic activity, mainly influenced by stomatal aperture opening (Morgan *et al.*, 1993; Reynolds *et al.*, 2000), variations in prevailing climatic conditions are likely to have a significant influence on $\Delta^{13}C$ values (Merah *et al.*, 2001). This can be related to the present study where drought was more severe in Sutton Bonington compared to Gleadthorpe; and the

drought at Gleadthorpe was sustained only up to mid-grain filling. This difference between these two site/seasons influenced the QTLs detected in each case. So it is important to consider the influence of climatic factors on $\Delta^{13}C$ under UK drought in further research to attribute correctly the causes of genetic variation in expression of $\Delta^{13}C$.

In this study, expression of $\Delta^{13}C$ under the influence of either of the parental alleles varied, and explained that genetic expression of $\Delta^{13}C$ may be specific to the environment. At Sutton Bonington in 2005, a putative QTL for $\Delta^{13}C$ on chromosome 3B in the irrigated environment explained 27.7% of the phenotypic variance. A QTL on chromosome 3B under drought was also identified which explained 14% of the phenotypic variance. Another putative QTL found on chromosome 2D also explained 18.2% of the phenotypic variance under drought. At Gleadthorpe in 2003, a putative QTL on 4B in the irrigated environment explained 13.4% phenotypic variance and on 7BL explained 14.7% variance; and under drought a putative QTL on chromosome 5A explained about 15.5% variance for $\Delta^{13}C$.

The importance and potential of chromosome 3B in carrying the allelic variation for delta was encouraging in this study as the expression of QTL was common to both water conditions with higher LOD scores in the irrigated (2.32) and unirrigated (1.08) conditions at Sutton Bonington. Importantly the alleles, which were responsible for decreased $\Delta^{13}C$, were from Soissons.

There is no reported evidence of QTLs for $\Delta^{13}C$ in bread wheat, although work on identifying QTLs in different spring wheat mapping populations is ongoing at CSIRO, Australia (Richards, R.A. Personal communication); and the present finding of putative QTL for delta on chromosome 3B is original in its context. Thus, the potential of the B genome needs a critical consideration in UK wheat breeding programmes for introgressing traits like $\Delta^{13}C$ and/or its association with other physiological traits related to drought resistance. The QTLs for other physiological traits such as plant water status or osmotic adjustment and/or yield components potentially co-localising with the QTL on chromosome 3B need further investigation to provide a strong physiological and genetic basis to this trait-marker association.

The importance and potential of the D genome in carrying allelic variation for $\Delta^{13}C$ in wheat has been reported (Mohammady, 2005). In this study, a putative QTL for $\Delta^{13}C$ was found on chromosome 2D with a high LOD score of 1.43 under unirrigated conditions. In a previous study on the Beaver x Soissons mapping population a QTL for stay green on the long arm of chromosome 2D was detected by Verma *et al.* (2004), but this was not linked to the QTL detected at the marker position of 2D in this study. Nevertheless, the evolutionary source of D genome- *Triticum tauschii* and its relatives - may provide a potential source for delta in order to improve WUE in the wheat cultivars. The possible association of this chromosome in influencing drought resistant traits such as expression of low $\Delta^{13}C$ or stay green needs further consideration in future breeding programmes.

For high resolution mapping generally larger populations comprising 50 to 250 individuals are required (Mohan *et al.*, 1997). In this study the number of DH genotypes used was low being 33. In addition, the power of detecting a QTL depends on the number of markers and the genome size of a species (see review by Collard *et al.*, 2005). Preliminary genetic mapping studies generally require 100 to 200 markers (Mohan *et al.*, 1997) and the power of detecting a QTL could be virtually the same for a marker spacing of 10 cM as for an infinite number of markers, and may slightly decrease for marker spacing of 20 or even 50 cM (Darvasi *et al.*, 1993). In this study, the detection of putative QTLs using genotype data with 222 molecular markers was able to provide information on the genetic basis of allelic variation among the target traits measured. The relatively higher percentage of variance associated with the QTL detected for $\Delta^{13}C$ on chromosome 3B suggests it would be reasonable to consider backcrossing followed by fine mapping in this case potentially to develop a marker for use in future breeding programmes.

$\Delta^{13}C$ results indicated stable ranking of genotypes across irrigation regimes as revealed by a statistically non-significant genotype x irrigation interaction. The physiological basis of genetic differences in $\Delta^{13}C$ appeared to depend on stomatal conductance. This was indicated by a positive correlation between $\Delta^{13}C$ and grain yield; similar findings were also reported in several previous studies in Mediterranean-type environments (Condon *et al.*, 1987; Acevedo, 1993; Morgan *et al.*, 1993; Merah *et al.*, 2001).

Associated reductions in stomatal conductance lead to a conservative water use, as indicated by apparently lower g_s values for Soissons (low $\Delta^{13}C$) than for Beaver (high $\Delta^{13}C$) in both years. On the other hand, yielding ability of genotypes differed across water regimes was evident by a significant genotype x environment interaction for grain yield. The positive correlation of delta and grain yield suggested that selecting a genotype with low Δ (higher WUE) may result in lower grain yield of wheat in UK environment. Nevertheless, the QTLs for delta accounting for a higher percentage of phenotypic variance identified in both environments may potentially serve as candidates for the development of markers for use in selection programmes. In addition, it seems that water use rather than WUE is the main driver of performance under mild UK droughts as compared to more severe droughts in 'stored water' environments. Thus, using QTLs for low $\Delta^{13}C$ (or high WUE) may serve as an indirect way of selecting for greater seasonal water use hence yield under drought in the UK.

7.2.1 Syntenic associations of the present findings in other cereals

Assessment of genetic diversity, new gene discovery, genetic dissection of complex traits and MAS of useful traits are some key areas of wheat improvement. The understanding about expression and inheritance of complex traits is progressing rapidly through positional cloning of QTL. Technological breakthroughs in genomics promise to provide a comprehensive picture of the various plant growth and developmental responses to ensure real gains in improving and stabilizing crop productivity. High-throughput genomics tools, coupled with innovative bioinformatics techniques, offer new avenues to exploit model systems to make rapid advances.

Hexaploid wheat consists of three closely related genomes designated A, B and D which are derived from three progenitor species. Gene redundancy is therefore the norm, with at least a triplicate homoeoallelic set for most of the genes. Unlike the smaller genome size of 130-140 Mb in *Arabidopsis thaliana* (model experimental plant) or 400 Mb for rice (model cereal), wheat has a large genome size estimated at 16,000 Mb (Arumuganathan and Earle, 1991). However, there seems to be a limitation in using information from rice genomics where information related to bread making and pasta quality, winter hardiness, vernalization etc. is required in wheat (Lagudah *et al.*, 2001). A high level of intra- and inter-chromosomal gene duplication in wheat maps compared

to rice maps (Dubcovsky *et al.*, 1996) on account of differences in their evolutionary divergence may lead to hybridisation in non-colinear regions in wheat and rice. Nevertheless, the use of rice genomics for positional cloning in wheat is still promising. Wheat being a hexaploid species tolerates chromosome deletion and aneuploidy better than any diploid crop species like rice. Despite these facts any syntenous relationship between rice and wheat (e.g. QTLs for $\Delta^{13}C$), especially for drought, would provide a basis for understanding the physiological and genetic mechanisms underlying this trait. Moore *et al.* (1995) suggested that the syntenic relationships among cereals will be useful for predicting the loci of homologous major genes. This concept can be extended to the QTL shared among cereals. A large number of QTL analyses will provide many instances of homologous QTLs among crop plants. Furthermore, most wheat chromosomes were shown to be syntenic with their rice (Kurata *et al.*, 1994) and maize counterparts (Ahn *et al.*, 1993). Linkage maps in wheat have confirmed evolutionary chromosomal translocation rearrangements involving chromosomes 2B, 4A, 5A, 6B and 7B, which were based on cytological evidence, and have established synteny among closely related grass species such as rice, maize, oats and wheat (Ahn *et al.*, 1993; Devos *et al.*, 1994; Borner *et al.*, 1998).

Laza *et al.* (2006) reported putative QTLs for $\delta^{13}C$ for RILs of flooded rice (*indica* x *japonica* cross) on chromosomes 2, 4, 8, 9, 11 and 12 across plant parts, growth stages and seasons. Chromosomes 2 and 9 decreased WUE (low $\delta^{13}C$). They indicated that differential expression of QTL for $\delta^{13}C$ was attributed to differential function of each QTL. These QTLs were associated with a few co-located QTLs for leaf traits like leaf N and leaf thickness informing on their physiological and genetic associations with leaf traits. In upland rice genotypes, QTLs for $\Delta^{13}C$ were found on chromosomes 1, 2, 3, 4, 6, 7, 10 and 12 (Price *et al.*, 2002), but these were different in each year at two locations tested at IRRI and WARDA. Nevertheless, the possible syntenic association of rice chromosomes 2, 4 and 12 with wheat requires further studies.

In maize, five putative QTLs significantly affecting tolerance to drought were found on chromosomes 1,3,5,6 and 8 (Agrama and Moussa, 1996). As per map alignment (Gale and Devos, 1998) group 3 chromosome of maize is orthologous to group 3 chromosome

of wheat. In this study, a potential synteny with maize was evident by a putative QTL (LOD=2.32) for $\Delta^{13}C$ on chromosome 3B for WUE.

In this study several putative QTLs for grain yield were found on chromosomes 3B, 5D, 6B and 7A under irrigation which each explained more than 20% of the phenotypic variance. QTLs on chromosomes 2A, 3A, 5D and 7A each explained more than 14% of the phenotypic variance under drought. Occurrence of yield QTLs on chromosomes 3A, 5D and 7A were common in both the irrigation treatments. The putative yield QTL clusters found on chromosome 3B and 7A in this study are in agreement with the yield QTL clusters found on these chromosomes for the Chinese spring x SQ1 population (Quarrie *et al.*, 2005). The yield QTL found on chromosomes 3A under drought was also in agreement with the previous work in Beaver x Soissons mapping population (DEFRA, 2004).

The importance of group 5 chromosomes to carry genes for abiotic stress resistance is well known (Forster *et al.*, 1988; Sutka and Snape, 1989; Cattivelli *et al.*, 2002; Quarrie *et al.*, 2005). The QTL identified on 5D in this study explained more than 14.0% of the phenotypic variance for grain yield under drought and coincided with yield or yield components QTLs previously identified in wheat (Snape *et al.*, 2001).

The putative QTL for yield found on chromosome 6B in the present study was also in a homoeologous position to a yield QTL region identified in previous work (Blanco *et al.*, 2002; Quarrie *et al.*, 2005). The putative yield QTL on chromosome 6B explained 21.4% of the phenotypic variance (irrigated in 2003) in the present study and is coincident with the QTL for yield in the centromeric region of 6B in the ITMI (International Triticeae Mapping Initiative) population (Ayala *et al.* 2002).

The putative QTL for TGW detected on chromosomes 2A was common to both the site/seasons under different irrigation regimes and explained phenotypic variance of greater than 15% in 2003 and 20% in 2005. This is in agreement with previous findings in this mapping population wherein a QTL for TGW on chromosome 2A appeared common to both the irrigation regimes in 2002 (DEFRA, 2004). Other QTLs on chromosomes 1A (in 2003) under irrigated conditions explained about 15% of the

phenotypic variance; and on 7A (in 2005), common to both irrigation regimes, explained more than 25% of the phenotypic variance for TGW.

In this study, a yield QTL was coincident with a QTL for TGW on chromosome 7A in the irrigated treatment in 2005. Research on an ITMI population showed that the long arm of 7A also harbours QTLs for spikelets per ear (Li *et al.*, 2002). The pleiotropic effect of other traits like grains/ear may be associated with QTLs for TGW and yield. So research focused on fine mapping for other physiological traits in this mapping population needs further attention. In other field studies, yield QTL clusters were coincident with significant effects for grains/ear and TGW on wheat crosses examined world wide (Quarrie *et al.*, 2005) and positive effects for yield and TGW were associated with the 7DL.7Ag translocation (Singh *et al.*, 1998). In this study, it was also observed that co-localisation of putative QTLs for yield and TGW was within 25 cM distance on chromosome 7A in 2003. At present the available information on co-localization of QTLs for traits in a specific mapping population and their synteny with other cereal crops is limited. However, from the information generated in the present findings, it seems that alleles for QTLs that increase water-use efficiency (decrease $\Delta^{13}C$) are not likely to improve grain yield in the UK environment. This provided a strong genetic basis for inheritance of $\Delta^{13}C$ suggesting a negative genetic correlation with yield in UK conditions. In the event if these two trait expressions are mutually exclusive then future breeding strategies should aim at breeding cultivars with high attainable yield under drought through maintaining the water use.

The present identification of a putative QTL for $\Delta^{13}C$ on chromosome 3B in wheat may be associated with water-use efficiency in UK wheats. This supports the hypothesis [(5) section 2.6; P41] that QTLs for $\Delta^{13}C$ can be identified for explaining the genetic variation in WUE in UK winter wheat (Tables 6.2 & 6.3). This QTL may represent a means of identifying novel sources of alleles for improving WUE for breeders world wide to exploit and the information derived from this study will be of value to breeders in countries with more severe droughts than in the UK for further fine-mapping to identify markers tightly linked to genes controlling the trait, and for gene discovery.

The cost of phenotyping for delta in breeding programmes is probably prohibitive; hence there is a need to identify markers. Although the success of use of delta in

developing new wheat cultivars has been demonstrated in Australia with the release of cultivars 'Drysdale' and 'Rees' the need for selecting wheat varieties under UK conditions based on carbon isotope signature still needs further consideration because a variety selected for drought must also perform as well as contemporary varieties under optimal conditions.

7.2.2 Potential use of QTLs in breeding programmes

Research on developing molecular markers for traits associated with drought tolerance in wheat is underway at CIMMYT. RILs populations are being used to identify genomic regions associated with a range of physiological parameters controlling drought tolerance (Ribaut *et al.*, 2001). Molecular markers can be used for tracing a favourable allele across generations and for identifying the most suitable line among the progeny. It is very important to have molecular markers linking with genes of interest for tracing favourable alleles. So developing a molecular marker within genes that are sequenced would aid in tracing favourable alleles. Microsatellite sequences can be identified within the gene, and primers to amplify the microsatellites can be designed. So if a marker is located within the gene sequence itself, it co-segregates with the target gene, and therefore the favourable alleles can be exactly traced. However, for polygenic traits, target genes have not been characterized at the molecular level. The selected genomic regions involved in a MAS experiment are chromosomal segments or QTLs. When a QTL represents a large chromosome segment of more than 10 cM, there should be a marker within the QTL to eliminate genotypes presenting a double recombination between the two flanking markers. A favourable allele can be introgressed using the marker in target germplasm through backcrossing or can be selected in a segregating population.

The MAS step, conducted only once, is based on the use of reliable PCR-based markers on large segregating populations derived from crosses between elite parental lines, which contrast for the target trait and allelic complementarity. So any information generated in tracing favourable alleles should be relevant for pyramiding favourable alleles at cloned genes or major QTLs in new germplasm.

Due to cost of the utilizing several QTLs, only markers that are tightly linked to no more than three QTLs are typically used (See review by Collard *et al.*, 2005). These QTLs for target traits can be linked directly to conceptual physiological models provided there is adequate genotyping and phenotyping data. Besides this, the development of mapping software and the use of more powerful statistical methods for an optimised MAS approach needs consideration. New developments and improvements in marker technology, integration of functional genomics (e.g. use of express sequence tag, single nucleotide polymorphisms and micro-array analysis to develop markers from genes as 'candidate genes') with QTL mapping and the availability of more saturated maps in future would improve efficiency of MAS. The development of high resolution maps will also facilitate the isolation of actual genes via 'positional cloning'. Positional cloning involves the use of tightly linked markers to isolate target genes by using the markers as a probe. The knowledge of the DNA sequence of the gene enables the design of perfect markers which are located within the actual gene sequence, thus eliminating the possibility of recombination between marker and gene (Ellis *et al.*, 2002).

7.3 FUTURE WORK

The main finding of this study showed that water use emerges as an important component of genotype performance under drought in the UK environment. $\Delta^{13}C$ provides a useful indirect measure of water use. Further studies are needed to identify genetic differences in root length density and root: shoot ratios of the genotypes for water uptake. Measurements of root traits across sampling stages were beyond the scope of the present study, as other above-ground traits were intensively assessed in the glasshouse and field experiments across samplings. There is a need to develop faster root screens for measurements of roots which remain time consuming and challenging for physiologists. Since water-use may be the most important component underlying yield response to drought in the UK, studies on identifying genetic diversity in rooting traits and QTLs for root traits would be worthy of further investigation.

Methodological considerations in imposing drought treatments in the glasshouse and in the field need to be revisited. This study highlighted the difficulty in relating measurements in the glasshouse on single plants to the field 'plot' scale on account of the variable influence of environmental conditions. Although, the gravimetric estimation

of water uptake in soil columns used in this study could provide an appropriate measure of WUE, it is very important that the growing conditions and timing of drought are properly defined because any small variation in these conditions would increase the residual variance and result in lower experimental precision. The main control of duration of water stress is the size of columns relative to plant size and this might sometimes lead to difficulty in achieving drought stress when the growth stage of the plants is advanced at transplantation. This study used plants which were grown in the growth rooms to undergo vernalization and later transplanted to the columns. So estimation of WUE or water use from the start of experiment could not be quantified. Ideally plant growth and water use would be monitored right from the beginning of germination in soil columns. Furthermore, use of rain-out shelters in the field experiments would provide an improved estimate of the influence of drought on crop performance because in the UK rainfall is not predictable; and the occurrence of summer rains may limit the water stress which can be imposed on the crop in drought research. Also droughts can be tailored to specific phenophases with the use of rain-out shelters.

Gas-exchange measurements for experiments involving a large number of genotypes is time consuming and difficult to measure. There is a need to develop faster screening methods to estimate stomatal conductance when phenotyping for larger populations. This is important to see whether there is a negative relationship between $\Delta^{13}C$ and TE in winter wheat grown in the UK. Some progress has been made with the development of the viscous-flow porometer at CSIRO, Australia in recent years. Although measurements of canopy temperature depression (CTD) provide an estimate of stomatal conductance, its use in UK environment is limited because of cooler climate condition and the temperature differences will be meagre and cumbersome even to detect small differences.

There is evidence that Soissons has higher flag leaf area persistence and maintains green area better under water stress conditions. Further investigations seem justified to identify QTLs associated with this stay-green mechanism. It seems that there is a negative trade-off between WUE and seasonal-water use in this study as indicated by a positive correlation between $\Delta^{13}C$ and grain yield. On the other hand, some genotypes with low

$\Delta^{13}C$ also showed a trend for high grain yield under drought. The reasons for this response need to be investigated further. Identification of fast, reliable and low-cost physiological surrogates in place of carbon isotope discrimination to screen large numbers of genotypes seems justified. It is also important to consider the influence of climatic factors on $\Delta^{13}C$ under UK drought in further research to attribute the causes of phenotypic variation in expression of $\Delta^{13}C$.

The possible association of the 3B chromosome in influencing expression of low $\Delta^{13}C$ was evident in this study. Hence the potential of the B genome needs a critical consideration in UK wheat breeding programmes for introgressing traits like $\Delta^{13}C$ and/or its association with other physiological traits related to drought resistance. The putative QTLs for delta of higher phenotypic variance identified in irrigated and droughted environments may serve potentially as candidates for the development of markers for wheat breeders to use in future selection programmes. Besides this the possible syntenic association of other cereal chromosomes with wheat with regard to $\Delta^{13}C$ needs further studies. Further fine mapping of the QTL for $\Delta^{13}C$ identified on chromosome 3B seems justified.

The recent international conference on drought (Interdrought-II: 2005, Rome, Italy) made several recommendations to address the future challenges in drought research. The findings of the present study would be in agreement with the following of these recommendations. (i) Plant biotechnology research aimed at enhancing plant production under limited water supply should assure effective collaboration with whole plant and field research. (ii) The seriousness of the problem, i.e. water scarcity, which is often used to justify biotechnology research, cannot allow biotechnology research to be continued within its own domain. (iii) Marker-assisted selection offers a great potential for plant breeding under water-limited conditions. (iv) Accurate phenotyping at the initial molecular mapping exercise is the most important prerequisite for success when complex, low heritable traits are to be mapped. (v) Considerable effort is needed towards understanding better the physiological processes that determine yield in any stress environment and their genetic control.

Thus, the present study provided improved understanding to integrate the physiology and genetic domains of research for underpinning various mechanisms explaining the

genetic variation in yield under water-limiting situations, particularly with regard to post-anthesis drought in the UK environment.

7.4 CONCLUSIONS

Post-anthesis drought under UK conditions is likely to have a major threat on winter wheat production as result of climate change. The frequency of dry years and droughts will increase with predicted climate change (Marsh,1996). According to the results of climate change models, there is a strong relationship between soil water availability and yield of wheat in areas subject to summer droughts (Wassenaar *et al.*, 1999). Thus, most of the wheat hectarage in the UK under drought-prone soils (Foulkes & Scott, 1998) may suffer from significant yield losses in future years. Some of these effects may be offset by the introduction of new cultivars by suitable genetic intervention strategies, so additional research and investment in genetics, physiology and breeding is critical to future. In this context, any improvement in the performance of a crop under water shortage can arise from identifying target physiological traits and making genetic changes to the crop that enable it to perform better in drought-prone environments (Turner, 1997; Richards *et al.*, 2002). The present study investigated the physiological and genetic bases of variation in WUE in UK winter wheat. Based on the results of this study, the following conclusions can be drawn:

1) Genotypic differences found in the field for $\Delta^{13}C$ were generally in agreement with measured transpiration efficiency.

2) Carbon isotope discrimination analysis revealed Soissons and line 134G had low $\Delta^{13}C$ values (high WUE) bõth in the glasshouse as well as in the field experiments compared to Beaver.

3) Delta ($\Delta^{13}C$) correlated positively with grain yield of field-grown wheat genotypes under drought and frequent gas-exchange measurements are needed to explain the variation associated with differences in stomatal conductance and C_i.

4) WUE can be improved by selecting lines for target traits like grain $\Delta^{13}C$. However, there may be an inverse relationship between WUE and grain yield in the UK

environment. Nevertheless, $\Delta^{13}C$ can be used as an indirect indicator of yield under drought.

5) The 1BL/1RS translocation increased $\Delta^{13}C$ both under irrigated and unirrigated conditions. Thus, a higher $\Delta^{13}C$ in 1BL/1RS lines was associated with reduced WUE. So yield improvement in UK 1BL/1RS wheats arises from greater water use associated with greater biomass production rather than improved water-use efficiency. In general, present results indicated in the UK conditions water use appears to be the major component underpinning yield improvement under drought.

6) There was a neutral effect of flowering on yield responses to drought. Early flowering may be linked with smaller biomass at flowering which would potentially favour post-flowering growth and hence maintenance of HI under drought. However, there may be a trade-off between early flowering and the development of a smaller less extensive root system in photoperiod insensitive genotypes, largely cancelling out the benefit of their smaller water consumption earlier in the season. So that post-flowering water uptake is only marginally increased in early flowering types.

7) The most confident putative QTLs were identified for $\Delta^{13}C$ on chromosomes 3B (in irrigated) and 2D (in unirrigated) in 2005. For the QTL on chromosome 3B, the Beaver allele increased $\Delta^{13}C$ consistent with the observed parental differences. If this trait-marker association could be confirmed in further work, fine mapping would be justified for marker development for this QTL.

8) There was a significant irrigation x genotype for grain yield but not for $\Delta^{13}C$ in the field experiments. The yield interaction was explained by differential ability amongst the genotypes to maintain water use, hence biomass, and HI. Of the components of Passioura's framework for yield improvement, in the UK the capacity of genotypes to maintain water use under drought seems to be the most important for maintenance of grain yield under drought amongst modern UK genotypes.

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APPENDIX I

Experiment	**2002/03 Gleadthorpe**
Field Name	YAO603
Field altitude	
Soil texture &series	Cuckney Series, loam medium sand to 35 cm over medium sand
Drainage	Good
Previous crop	Potato
Cultivations	Chisel Ploughed 11/09/02 Spring tined 28/09/02 Ploughed and furrow pressed 02/10/02 Tined and rolled 18/10/02
Sowing	19/10/02
Seed rate	250 seeds m^{-2}
Drill type & width	Oyjard, 2m
Row width	13.20 cm
Plot length	12 m
Plot width	1.61m Drilled / 2.00m centre to centre
Fertiliser	216 kg ha^{-1} Triple Super Phosphate (GS22) 06/02/03 164 kg ha^{-1} Muriate of Potash (GS 22/23) 13/02/03 135 kg ha^{-1} Ammonium Sulphate as Sulphur Gold (20/02/03) 5 kg Manganese Sulphate (GS22/23) 27/02/03 145 kg ha^{-1} Ammonium Nitrate (19/03/03) 237 kg ha^{-1} Ammonium Nitrate (14/04/03) 150 kg ha^{-1} Ammonium Nitrate (12/05/03)
Herbicide	1.25 l ha^{-1} Fenpath Ipex (difluefenican+isoproturan) 19/02/02 20 g ha^{-1} Ally (metsulfuron-methyl)23/04/03 1.75 l ha^{-1} Isoproturon 19/12/02 0.80 l ha^{-1} Starane 2 (fluroxypyr)23/04/03
Fungicide	0.4 l ha^{-1} Corbel 24/04/03 0.15 l ha^{-1} Fortress 24/04/03 0.75 l ha^{-1} Opus + 0.6 kg ha^{-1} Unix 02/05/03 1.50 l ha^{-1} Eclipse 10/05/03
	1.00 l ha^{-1} Silvacur 03/06/03
Insecticide	0.25 l ha^{-1} Cypermethrin 19/12/02 1.50 l ha^{-1} Dursban 24/01/03
Harvest date	14/08/2003

Experiment	**2003/04 Sutton Bonington**
Field Name	S24
Field altitude	50 m
Soil texture &series	Dunnigton Heath Series, medium stony loam to 80 cm over clay
Drainage	Good
Previous crop	Winter Oats
Soil Indices	P: 4, K: 3, Mg: 4, pH: 7.4
Cultivations	Ploughed 11/09/03 Cambridge rolled 16/09/03 Power Harrowed 30/09/03 Cambridge rolled 01/10/03
Sowing	30/09/03
Seed rate	325 seeds m^{-2}
Drill type & width	Oyjard, 2m
Row width	13.20 cm
Plot length	12 m
Plot width	1.61m Drilled / 2.00m centre to centre
Fertiliser	1.0 l ha^{-1} manganese 07/11/03 138 kg ha^{-1} N29%, SO_4 7.5% (40 kg ha^{-1} N) all plots 17/03/04 345 kg ha^{-1} N29%, SO_4 7.5% (100 kg ha^{-1} N) all plots 08/04/04 276 kg ha^{-1} N29%, SO_4 7.5% (80 kg ha^{-1} N) all plots 19/04/04 2.0 l ha^{-1} manganese 23/04/04
Herbicide	3.0 l ha^{-1} Isoproturon & 2.0 l ha^{-1} Trifluralin 07/11/03 25 g ha^{-1} Ally 23/04/04 0.75 l ha^{-1} Starane 23/04/04 0.125 l ha^{-1} Topik23/04/04
Plant growth regulators	2.5 l ha^{-1} New 5C Cycocel 23/04/04
Fungicide	0.5 l ha^{-1} Landmark 23/04/04 1.0 l ha^{-1} Bravo 23/04/04 0.75 l ha^{-1} Opus + 0.5 l ha^{-1} Acanto 25/05/04 0.35 l ha^{-1} Folicur + 0.5 l ha^{-1} Tern 14/06/04
Insecticide	0.25 l ha^{-1} Cypermethrin 07/11/03 0.7 l ha^{-1} Dursban 14/06/04
Miscellaneous	2.0 l ha^{-1} Cropspray (oil) 23/04/04
Harvest date	31/08/2004

Experiment	**2004/05 Sutton Bonington**
Field Name	S24
Field altitude	50 m
Soil texture &series	Dunnigton Heath Series, medium stony loam to 80 cm over clay
Drainage	Good
Previous crop	Winter Oats
Soil Indices	P: 5, K: 3, Mg: 5, pH: 7.5
Cultivations	Ploughed 24/09/04 Power Harrowed 26/09/04 Cambridge rolled 09/10/04
Sowing	08/10/04
Seed rate	325 seeds m^{-2}
Drill type & width	Oyjard, 2m
Row width	13.2 cm
Plot length	12 m
Plot width	1.625m Drilled / 2.00m centre to centre
Fertiliser	2.5 l ha^{-1} manganese 10/12/04 138 kg ha^{-1} N29%, SO_4 7.5% (40 kg ha^{-1} N) all plots 16/03/05 3.0 l ha^{-1} manganese 23/03/05 276 kg ha^{-1} N29%, SO_4 7.5% (80 kg ha^{-1} N) all plots 04/04/05 3.0 l ha^{-1} manganese 14/04/05 276 kg ha^{-1} N29%, SO_4 7.5% (80 kg ha^{-1} N) all plots 27/04/05 1.0 l ha^{-1} manganese 29/04/05
Herbicide	1.0 l ha^{-1} Round-up 10/10/04- to combat volunteer oats 2.3 l ha^{-1} Treflan 12/10/04 4.0 l ha^{-1} Isotron + Panther 0.8 l ha^{-1} 14/11/04 1.0 l ha^{-1} Starane XL 21/04/05
	0.125 l ha^{-1} Topic 21/04/05
Plant growth regulators	1.75 l ha^{-1} New 5C Cycocel 05/04/05 0.75 l ha^{-1} New 5C Cycocel 21/04/05 1.00 l ha^{-1} Terpal 17/05/05
Fungicide	1.0 l ha^{-1} Bravo 14/04/05 1.0 l ha^{-1} Bravo 29/04/05 0.5 l ha^{-1} Landmark 29/04/05 0.75 l ha^{-1} Opus + Corbel 0.33 l ha^{-1}+ Amistar 0.25 l ha^{-1} 31/05/05 0.35 l ha^{-1} Folicur + 0.5 l ha^{-1} Tern 20/06/05
Insecticide	0.25 l ha^{-1} Toppel 14/11/04 0.8 l ha^{-1} Dursban 20/06/05
Miscellaneous	1.0 l ha^{-1} Headland Fortune 21/04/05 0.16 l ha^{-1} Activator 90 17/05/05
Harvest date	15/08/2005

APPENDIX II
EXPERIMENTAL PLANS GLEADTHORPE 2002/3

N

REPLICATE 1

Irrigation	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*
Line trt no.	10	20	27	17	12	45	43	29	15	48	36	8	37	1	18	24
Plot No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Irrigation	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*
Line trt no.	34	50	47	36	32	41	7	30	38	46	44	40	9	13	11	16
Plot No.	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Irrigation	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*		*1*	*1*	*1*	*1*	*1*	*1*	*1*
Line trt no.	22	33	31	3	25	26	5	23	35	14	39	35	49	21	4	19
Plot No.	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
Irrigation	*1*	*1*	*1*	*1*												
Line trt no.	28	2	42	6												
Plot No.	49	50	51	52												
Irrigation	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Line trt no.	30	38	16	29	44	42	9	47	28	48	17	4	21	2	40	49
Plot No.	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68
Irrigation	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Line trt no.	36	5	3	33	41	1	31	25	11	15	43	12	34	18	6	20
Plot No.	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84
Irrigation	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Line trt no.	13	24	35	32	45	50	14	19	39	7	46	35	10	27	26	36
Plot No.	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
Irrigation	2	2	2	2												
Line trt no.	37	23	8	22												
Plot No.	101	102	103	104												

REPLICATE 2

	2	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*
	40	19	49	42	15	48	45	17	2	7	24	18	16	20	36	14
	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
	2	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*
	50	29	30	6	43	1	9	44	36	13	4	10	33	31	23	12
	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136
	2	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*
	35	11	41	39	28	47	21	34	48	22	9	25	8	5	26	35
	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152
	2	*2*	*2*	*2*												
	37	38	32	27												
	153	154	155	156												
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	41	13	40	4	7	12	30	43	20	14	15	50	11	37	19	31
	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1	47	42	8	36	10	6	35	17	35	46	49	3	21	33	45
	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	23	18	9	29	32	44	26	48	2	16	39	25	28	5	24	34
	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204
	2	2	2	2												
	22	36	38	27												
	205	206	207	208												

REPLICATE3

1	*1*	*1*	*1*	*1*	*1*	*1*	*1*		*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*
11	10	14	36	35	48	16	21	44	5	2	13	12	26	7	29	
209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	
1	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
18	4	9	40	31	29	24	20	34	6	43	30	45	33	47	41	
225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	
1	*1*	*1*	*1*	*1*	*1*	*1*	*1*		*1*	*1*	*1*	*1*	*1*	*1*	*1*	
42	15	19	50	25	17	49	22	35	32	29	23	1	36	38	27	
241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	
1	*1*	*1*	*1*													
37	9	8	46													
257	258	259	260													
2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
9	17	10	47	20	31	16	49	34	35	19	15	4	43	41	27	
261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	
2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
28	7	23	45	13	30	6	42	3	11	21	36	1	14	39	40	
277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	
2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
8	50	32	22	36	44	5	29	12	46	48	35	38	18	33	26	
293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	
2	2	2	2													
37	24	25	2													
309	310	311	312													

Trt no.	Genotype	Trt no.	Genotype	Trt no.	Genotype	Trt no.	Genotype	Trt no.	Genotype
1	134D	**11**	137C	**21**	48B	**31**	48A	**41**	177A
2	121D	**12**	134E	**22**	134C	**32**	22B	**42**	22D
3	19B	**13**	48D	**23**	39C	**33**	4F	**43**	148F
4	135 B	**14**	133A	**24**	Discard	**34**	14A	**44**	25A
5	1B	**15**	5G	**25**	21B	**35**	Beaver	**45**	15C
6	134G	**16**	120B	**26**	118A	**36**	Soissons	**46**	1A
7	175B	**17**	168D	**27**	35A	**37**	114A	**47**	121F
8	6E	**18**	17B	**28**	14E	**38**	2D	**48**	1D
9	122I	**19**	20A	29	22C	**39**	76 A	**49**	10F
10	21A	**20**	33C	30	33A	**40**	123C	**50**	5D

Irrigation **1 irrigated plots**
2 non-irrigated plots

EXPERIMENTAL PLANS SUTTON BONINGTON 2004/5

N

Main Road to Kingston

REPLICATE 1

Irrigation	Discard	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Discard	Discard
Line trt no.		18	2	31	9	1	6	35	29	32	34	16	30	33	15	8	19	17	24	20	14	34	10	26	23	33	28	21	13	4	12	25	5	27	22	7	11	3		
Plot No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37		

REPLICATE 2

Irrigation	Discard	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	Discard	Discard
Line trt no.		20	33	29	32	21	33	1	26	23	13	5	9	2	4	34	16	35	7	18	34	10	25	6	22	15	3	19	11	30	8	17	27	12	31	28	14	24		
Plot No.		75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111		

REPLICATE 3

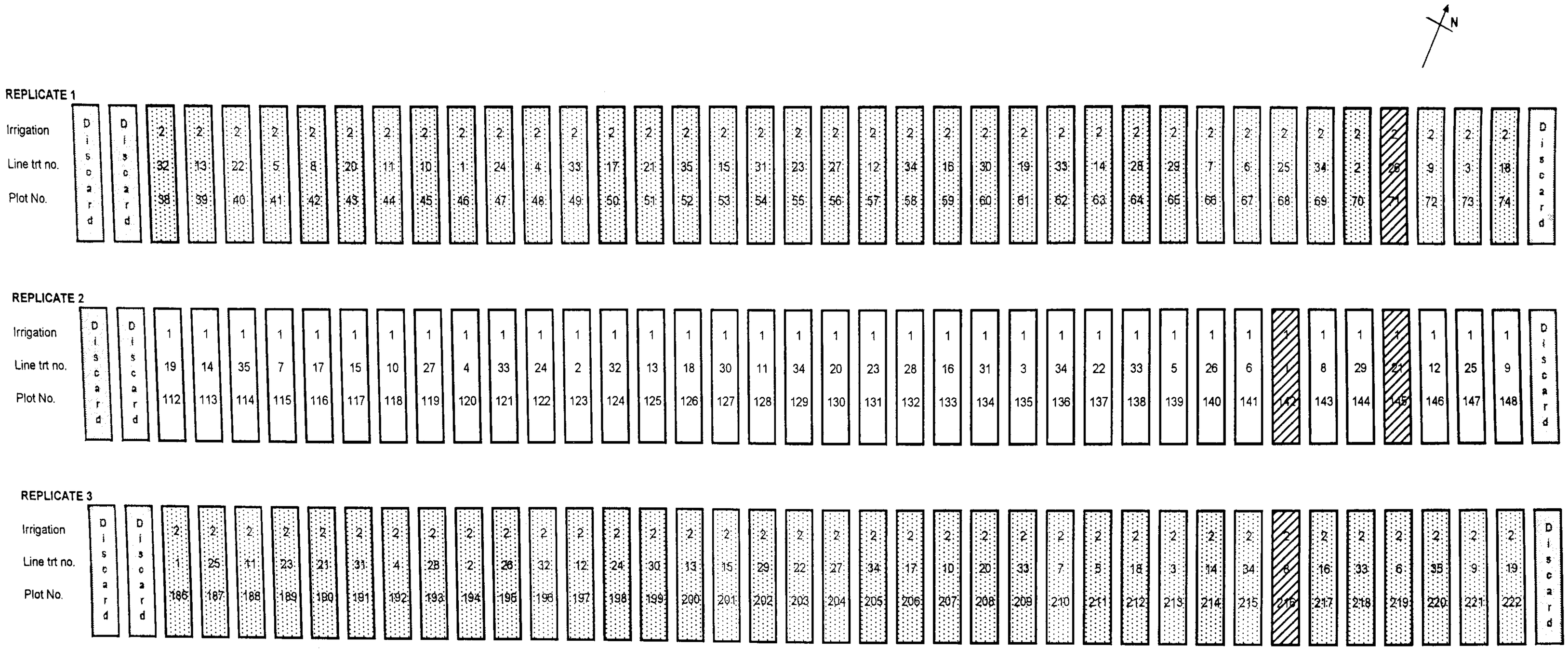
Irrigation	Discard	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Discard	Discard
Line trt no.		31	5	25	34	7	33	28	1	22	26	4	24	2	16	33	6	21	13	34	15	9	18	3	23	32	11	20	8	27	14	29	17	12	35	10	30	19		
Plot No.		149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185		

Trt no.	Line No.	Genotype	Trt no.	Line No.	Genotype
1	Line 1	134D	**19**	Line 20	33C
2	Line 2	121D	**20**	Line 21	48B
3	Line 3	19B	**21**	Line 22	134C
4	Line 4	135 B	**22**	Line 23	39C
5	Line 5	1B	**23**	Line 25	21B
6	Line 6	134G	**24**	Line 26	118A
7	Line 8	6E	**25**	Line 27	35A
8	Line 9	122I	**26**	Line 28	14E
9	Line 10	21A	**27**	Line 29	22C
10	Line 11	137C	**28**	Line 30	33A
11	Line 12	134E	**29**	Line 31	48A
12	Line 13	48D	**30**	Line 32	22B
13	Line 14	133A	**31**	Line 33	4F
14	Line 15	5G	**32**	Line 34	14A
15	Line 16	120B	**33**	Beaver	Beaver
16	Line 17	168D	**34**	Soissons	Soissons
17	Line 18	17B	**35**	Line 39	76 A
18	Line 19	20A			

Irrigation
1 Non-irrigated plots
2 Irrigated plots

Drill malfunction

EXPERIEMENTAL PLANS SUTTON BONINGTON 2004/5 (contd.)



Trt no.	Line No.	Genotype	Trt no.	Line No.	Genotype
1	Line 1	134D	19	Line 20	33C
2	Line 2	121D	20	Line 21	48B
3	Line 3	19B	21	Line 22	134C
4	Line 4	135 B	22	Line 23	39C
5	Line 5	1B	23	Line 25	21B
6	Line 6	134G	24	Line 26	118A
7	Line 8	6E	25	Line 27	35A
8	Line 9	122I	26	Line 28	14E
9	Line 10	21A	27	Line 29	22C
10	Line 11	137C	28	Line 30	33A
11	Line 12	134E	29	Line 31	48A
12	Line 13	48D	30	Line 32	22B
13	Line 14	133A	31	Line 33	4F
14	Line 15	5G	32	Line 34	14A
15	Line 16	120B	33	Beaver	Beaver
16	Line 17	168D	34	Soissons	Soissons
17	Line 18	17B	35	Line 39	76 A
18	Line 19	20A			

Irrigation
1 Non-irrigated plots
2 Irrigated plots

Drill malfunction

APPENDIX III

GROWTH ANALYSES IN 2004 AND 2005 FIELD EXPERIMENTS

Table III. a. Green area index, leaf lamina dry matter (g m^{-2}) and stem-and-sheath dry matter (g m^{-2}) of four wheat genotypes at GS31.

	Green area index		Leaf lamina dry matter (g m^{-2})		Stem-and-sheath dry matter (g m^{-2})	
	2004	2005	2004	2005	2004	2005
Irrigated						
134E	1.95	1.58	132.1	90.5	88.5	61.3
134G	1.77	1.13	109.3	70.5	76.0	41.1
Beaver	1.94	2.29	148.7	125.5	102.5	73.6
Soissons	2.19	1.65	129.9	90.6	99.9	60.5
Mean	*1.96*	*1.67*	*130.0*	*94.2*	*91.7*	*59.1*
Unirrigated						
134E	2.09	1.80	137.9	96.0	104.9	64.5
134G	2.14	1.16	113.4	67.2	92.6	41.7
Beaver	2.12	2.28	140.1	141.3	100.7	91.8
Soissons	2.16	1.91	131.4	108.9	101.1	79.0
Mean	*2.13*	*1.79*	*130.7*	*103.3*	*99.8*	*69.3*
SED (df)						
Irrigation (2)	0.26	0.01	13.8	1.8	12.9	2.72
Genotype (12)	0.24	0.13	8.0	7.7	6.1	5.8
Interaction (12)	0.39	0.16	16.9	9.6	14.8	7.6

Table III. b. Flag leaf green area (cm^2), total green area (cm^2), shoot number m^{-2}, leaf lamina dry matter (g) and stem-and-sheath dry matter (g) of four wheat genotypes at GS39 in 2004.

	Flag leaf green area (cm^2)	Total green area (cm^2)	Shoot number m^{-2}	Leaf lamina DM (g)	Stem-and-sheath DM (g)
Irrigated					
134E	221.4	1209	867	3.72	13.41
134G	319.3	1779	687	5.11	19.93
Beaver	349.0	1577	1069	5.15	18.68
Soissons	241.3	1299	860	4.11	16.32
Mean	*282.8*	*1466*	*871*	*4.52*	*17.08*
Unirrigated					
134E	206.5	1097	968	3.21	12.38
134G	304.2	1660	695	5.39	20.63
Beaver	373.9	1639	1048	5.45	18.85
Soissons	273.1	1382	848	3.98	16.66
Mean	*289.4*	*1444*	*890*	*4.51*	*17.13*
SED (df)					
Irrigation (2)	3.61	26.2	4.5	0.01	0.27
Genotype (11)	12.18	67.7	46.5	0.21	0.65
Interaction (11)	15.34	86.9	57.1	0.25	0.84

APPENDIX IV

GAS EXCHANGE ANALYSES

Table IV. c Net photosynthetic rate, stomatal conductance and inter-cellular CO_2 concentrations for eight wheat genotypes measured after anthesis in 2004 field experiment.

	Photosynthetic rate (µmol $m^{-2}s^{-1}$)				Stomatal conductance (mmol m^{-2} s^{-1})				Intercellular CO_2 concentration (mmol CO_2 mol^{-1} air)			
	GS61+4wk		GS61+6wk		GS61+4wk		GS61+6wk		GS61+4wk		GS61+6wk	
Genotypes	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*
134E	21.79	22.92	6.51	6.29	191.1	187.2	186.5	127.9	553.0	534.0	228.9	212.0
134G	23.55	12.35	4.16	4.09	201.5	116.2	131.5	120.7	551.9	655.5	209.7	265.4
21B	11.90	20.57	7.02	5.47	226.1	138.2	221.7	152.4	708.3	520.7	237.5	235.0
22B	22.20	17.47	5.84	2.51	254.1	167.9	169.4	122.4	578.8	580.1	215.0	274.5
35A	21.33	17.54	4.62	4.87	193.6	147.6	172.3	174.8	574.7	538.0	221.4	249.2
76A	22.60	20.24	6.47	4.17	179.4	166.7	170.7	118.7	544.2	560.8	200.2	242.5
Beaver	20.12	14.80	8.77	4.74	197.4	156.1	201.7	126.0	588.6	610.1	198.0	242.0
Soissons	19.85	18.67	4.89	7.00	256.2	140.6	195.2	132.7	619.3	540.3	229.0	200.0
Mean	20.42	18.07	6.04	4.89	212.4	152.6	181.1	134.4	589.9	567.4	217.5	240.1
SED (df)												
Irrigation (2)				1.967				14.80				32.97
Genotype (28)				1.843				16.01				37.01
Irri x Genotype(28)				3.133				25.83				59.03
Date x Irri (28)				3.686				32.01				74.03
Date x Genotype (28)				4.431				36.53				83.48

Table IV. d Gas-exchange parameters for four wheat genotypes in 2004.

	Photosynthetic rate (µmol $m^{-2}s^{-1}$)									
	GS39		GS39+1week		GS39+2weeks		GS61+4weeks		GS61+6weeks	
Genotypes	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*
134E	44.69	61.18	22.69	24.32	20.30	19.87	20.72	21.85	7.83	7.61
134G	22.91	65.55	28.71	29.69	22.55	23.13	22.49	11.29	5.48	5.41
Beaver	42.64	56.73	25.81	17.20	31.07	32.10	19.05	13.74	10.10	6.06
Soissons	49.50	51.17	21.48	27.49	15.24	15.44	18.79	17.60	6.21	8.32
Mean	*39.93*	*58.66*	*24.67*	*24.67*	*22.29*	*22.63*	*20.26*	*16.12*	*7.41*	*6.85*
SED(df)										
Irrigation (2)	1.778									
Genotype(12)	0.940									
IrxGen(12)	2.119									
Datex Ir(12)	3.686									
DatexG(12)	3.002									
Dx Ix G(12)	4.50									

	Stomatal conductance (m mol m^{-2} s^{-1})									
	GS39		GS39+1week		GS39+2weeks		GS61+4weeks		GS61+6weeks	
Genotypes	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*
134E	366.4	502.0	248.3	272.7	248.3	272.7	183.3	179.5	195.3	136.6
134G	271.6	560.5	201.9	266.1	201.9	266.1	193.8	108.5	140.3	129.4
Beaver	324.3	498.2	241.4	260.4	241.4	260.4	189.7	148.3	210.4	134.8
Soissons	496.8	474.2	316.3	249.5	316.3	249.5	248.5	132.8	203.9	141.4
Mean	*364.8*	*508.8*	*252.0*	*262.2*	*252.0*	*262.2*	*203.8*	*142.3*	*187.5*	*135.6*
SED (df)										
Irrigation (2)	9.56									
Genotype(12)	8.58									
IrxGen(12)	14.20									
Date x Ir(12)	27.12									
Date x G(12)	31.75									
D x Ir x G(12)	59.03									

	Intercellular CO_2 concentration (m mol CO_2 mol^{-1} air)									
	GS39		GS39+1week		GS39+2weeks		GS61+4weeks		GS61+6weeks	
Genotypes	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*	*Irri*	*Unirri*
134E	132.3	193.0	546.9	484.7	331.4	323.4	614.6	595.6	166.9	150.0
134G	136.1	246.6	386.7	444.6	277.4	345.7	613.5	717.1	147.7	203.3
Beaver	151.1	160.8	517.4	501.9	321.5	323.1	650.2	671.8	136.0	180.0
Soissons	171.2	132.0	471.7	491.4	391.8	346.7	680.9	602.0	167.0	137.9
Mean	*147.7*	*183.0*	*480.7*	*480.7*	*330.5*	*334.7*	*639.8*	*646.6*	*154.4*	*167.8*
SED (df)										
Irrigation (2)	28.68									
Genotype(12)	18.51									
IrxGen(12)	36.56									
Date x Ir(12)	111.9									
DatexG(12)	108.4									
D x Irx G(12)	122.8									

APPENDIX V

LODGING AND GRAIN YIELD IN 2004 EXPERIMENT

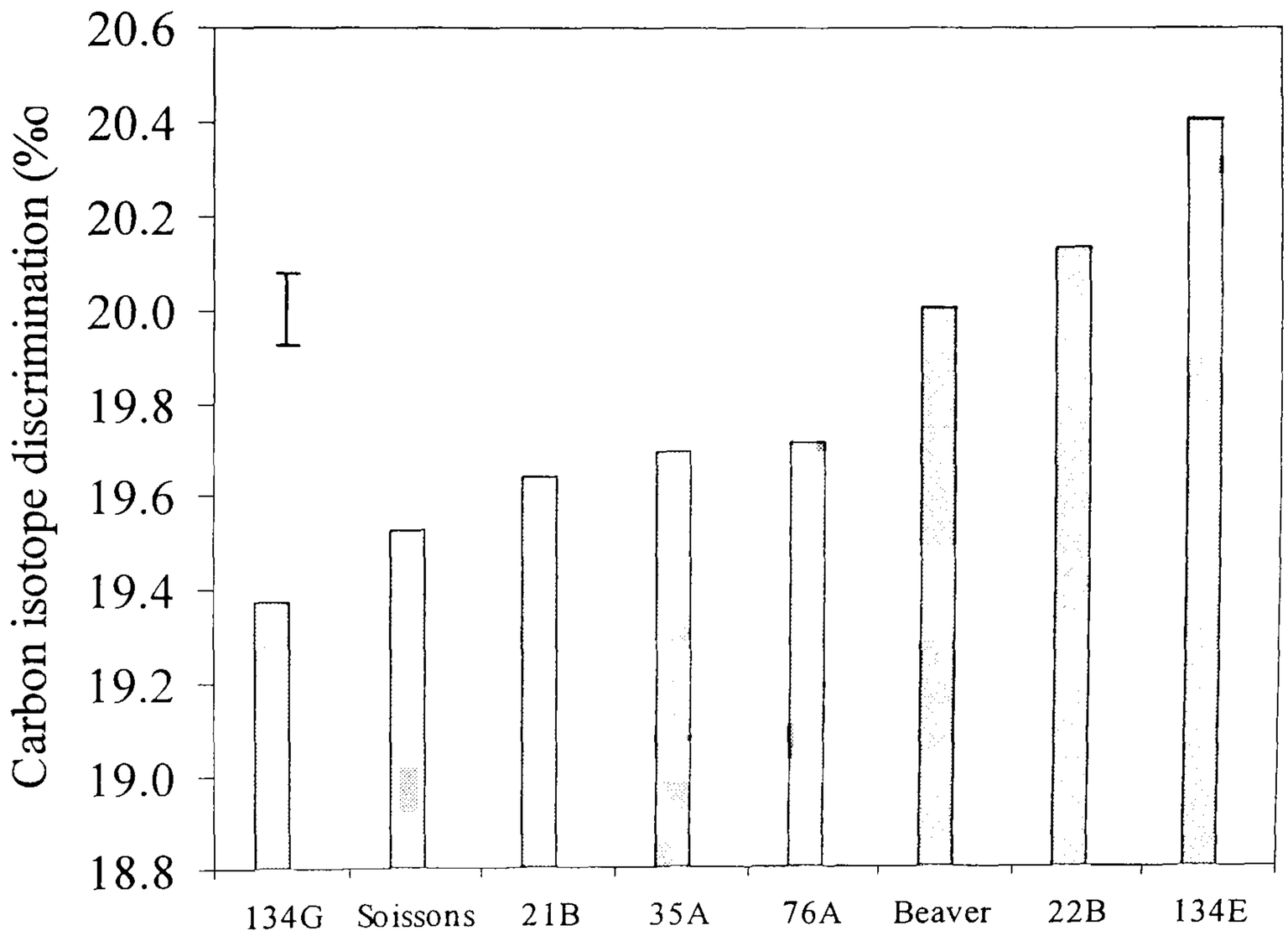
Table V. a. Hand-harvested grain yield (85% DM) and % plot lodged on 15 September 2004 for 35 doubled haploid lines in mapping population of Beaver x Soissons at Sutton Bonington 2004 in unirrigated treatments.

Sl. No	Line	Grain yield (85% DM)	Percentage plot lodged (15 Sept 2004)
1	134D	5.22	81.7
2	121D	7.66	13.0
3	19B	8.58	36.7
4	135B	9.10	34.0
5	1B	11.29	11.3
6	134G	7.39	76.7
7	6E	11.13	6.3
8	122I	7.86	81.7
9	21A	10.04	7.3
10	137C	3.52	100.0
11	134E	7.05	100.0
12	48D	10.17	10.7
13	133A	6.34	70.0
14	5G	8.92	3.3
15	120B	7.39	53.3
16	168D	8.50	35.0
17	17B	10.67	5.7
18	20A	6.90	98.3
19	33C	8.63	5.0
20	48B	11.18	5.7
21	134C	8.22	98.3
22	39C	8.59	38.3
23	21B	9.38	10.0
24	118A	9.46	32.5
25	35A	8.82	10.7
26	14E	9.48	51.7
27	22C	10.16	7.3
28	33A	8.04	76.7
29	48A	6.42	100.0
30	22B	8.64	36.7
31	4F	8.32	24.0
32	14A	9.27	100.0
33	BEAVER	10.56	4.8
34	SOISSONS	8.43	70.8
35	76A	9.21	3.0
	Mean	*8.59*	*43.0*

APPENDIX VI

CARBON ISOTOPE DISCRIMINATION (‰) IN 2004

Figure VI.a Carbon isotope discrimination values of eight wheat genotypes under unirrigated conditions at Sutton Bonington 2004. Error bars show S.E.D of means of genotypes.



APPENDIX VII

Table.VII a. Specific root length (m g^{-1}) and root diameter (mm) at anthesis for the irrigated and unirrigated treatments for four wheat genotypes measured in different soil horizon depths in glasshouse in 2003 and 2004.

Specific root length (m g^{-1})								
	2003			2004				
	0-20 cm	40-60 cm	80-100 cm	0-20 cm	20-40 cm	40-60 cm	60-80 cm	80-100 cm
Irrigated								
Beaver	3.05	4.21	4.25	1.19	1.58	1.75	1.81	1.90
Soissons	3.33	4.00	4.39	1.27	1.67	1.78	1.86	1.95
134G	3.03	4.33	4.07	1.40	1.37	1.54	1.69	1.65
134E	2.64	3.84	4.03	1.20	1.74	1.78	1.79	1.82
Mean	3.01	4.10	4.19	1.20	1.59	1.71	1.79	1.83
Unirrigated								
Beaver	3.41	4.03	4.20	1.35	1.65	1.71	1.86	1.87
Soissons	3.47	4.11	4.38	1.49	1.78	1.87	1.92	1.92
134G	3.53	4.09	4.19	1.32	1.72	1.79	1.80	1.89
134E	3.11	3.85	3.97	1.42	1.74	1.80	1.87	1.89
Mean	3.38	4.02	4.18	1.40	1.72	1.79	1.86	1.89
SED (df)								
Irrigation(24)	0.11*	0.11	0.09	0.04*	0.04*	0.05	0.04*	0.04
Genotype(24)	0.15*	0.15	0.12*	0.06	0.06*	0.07	0.05	0.06
Ir x G (24)	0.21	0.21	0.18	0.08	0.09	0.10	0.07	0.08

Root diameter (mm)								
	2003			*2004*				
	0-20 cm	*40-60 cm*	*80-100cm*	*0-20 cm*	*20-40 cm*	*40-60 cm*	*60-80 cm*	*80-100 cm*
Irrigated								
Beaver	0.73	0.50	0.44	0.77	0.82	0.65	0.62	0.56
Soissons	0.58	0.45	0.42	0.58	0.67	0.59	0.57	0.57
134G	0.63	0.47	0.49	0.86	0.81	0.81	0.71	0.73
134E	0.82	0.48	0.49	0.59	0.70	0.63	0.64	0.63
Mean	0.69	0.47	0.46	0.70	0.75	0.67	0.63	0.62
Unirrigated								
Beaver	0.55	0.50	0.66	0.67	0.80	0.64	0.57	0.58
Soissons	0.54	0.48	0.51	0.53	0.60	0.52	0.51	0.54
134G	0.52	0.48	0.55	0.51	0.73	0.54	0.59	0.53
134E	0.59	0.49	0.55	0.52	0.57	0.49	0.47	0.52
Mean	0.55	0.49	0.57	0.56	0.67	0.55	0.53	0.54
SED (df)								
Irrigation(24)	0.06	0.02	0.02	0.04*	0.05	0.05*	0.02*	0.03*
Genotype(24)	0.08	0.02	0.03	0.06*	0.07*	0.07	0.03*	0.04
Ir x G (24)	0.11	0.03	0.05	0.08	0.09	0.10	0.05	0.06

* denotes significance level P=0.05

Table.VII b. Total root surface area (cm^2) at anthesis for the irrigated and unirrigated treatments for four wheat genotypes measured in different soil horizon depths in glasshouse in 2003 and 2004.

	Total root surface area (cm^2)							
	2003			2004				
	0-20 cm	40-60 cm	80-100 cm	0-20 cm	20-40 cm	40-60 cm	60-80 cm	80-100 cm
Irrigated								
Beaver	269	132	139	468	481	470	411	216
Soissons	147	79	120	296	294	278	304	264
134G	188	87	179	423	564	544	575	588
134E	347	129	176	296	309	432	449	439
Mean	237	107	153	371	412	431	435	377
Unirrigated								
Beaver	114	90	351	371	360	372	312	365
Soissons	138	93	287	201	203	206	182	128
134G	116	81	271	283	211	284	367	297
134E	200	127	273	178	193	171	208	235
Mean	142	98	295	258	242	258	267	256
SED (df)								
Irrigation(24)	42*	13	29*	44*	49*	44*	39*	50*
Genotype(24)	60	19	41	62*	70*	62*	55*	70*
Ir x G (24)	84	27	59	87	98	88	78	99*

* denotes significance level P=0.05