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Improving water use efficiency and drought tolerance in UK winter wheats

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1. ABSTRACT

Wheat yields in the UK are often limited by water deficit during critical stages of crop development. The aim of this project was to provide information that could help guide the identification and development of varieties with greater drought tolerance (DT). A genetically diverse set of 120 wheat varieties was evaluated under irrigated and managed drought conditions in the field. There was significant variation for DT index (DTI), water use efficiency (WUE), yield potential and yield stability. Although the ranking of varieties differed with each year, several varieties showed consistently better than average DTI, and another set poorer than average. There was significant variation between varieties for anatomical and physiological traits associated with performance under water-limited conditions. Each trait, however, explained only a small proportion of the variance in DT or WUE. The combination of traits that describes a superior water-efficient variety, which blends resilience with yield potential, could aid breeding by focusing selection for secondary (proxy) traits, or via molecular markers linked to these traits.

The project also examined the relative ranking of 64 varieties tested across a range of sites that differed in soil water holding capacity. Unfortunately, insufficient drought developed on enough sites to adequately determine consistent differences in the performance of varieties for dry conditions. However, the approach has merit if data on varieties are accumulated across sites and years.

To help understand the genetic control of DT, WUE and component traits, 135 lines were genotyped using 11 diagnostic markers plus seven markers based on published QTL data. A subset of 94 lines was genotyped using 2499 DArT biallelic dominant markers. These genotypic data, in conjunction with the phenotypic data collected from the field experiments, could be used in future association studies to discover the genetic basis of traits controlling DT and WUE.

These results provide a comprehensive and quantitative description of UK winter wheat lines for DT, WUE and traits associated with improved yields under dry conditions. However, the ranking of varieties depended on which measure of DT was used, and the timing and severity of the water deficit. The data gathered in this study confirms previous findings, suggests possible new avenues for genetic improvement, and provides a foundation for further work.

2. SUMMARY

2.1. Introduction

Globally, drought causes more yield losses than any other single biotic or abiotic factor and remains one of the largest threats to food security. In the UK, it is estimated that approximately 30% of the wheat acreage is grown on drought-prone soils and at least 10% of the wheat yield is lost each year due to insufficient soil moisture, with greater losses in very dry years. Therefore, ‘drought’ in the UK is not an extreme phenomenon that occurs rarely; rather, crops frequently fail to attain their potential output because water supply cannot keep pace with demand, often during critical stages of yield formation. Development of varieties with increased drought tolerance and water use efficiency is therefore crucial to improving the productivity and sustainability of the wheat crop in the UK. Growers, however, have little guidance on which varieties are best suited for dry conditions, and breeders have little quantitative information on which lines are superior, or which traits would be beneficial when there is a shortage of rainfall. The aim of this work was to enable the identification and development of improved wheat varieties for water-limited conditions in the UK.

Drought tolerance can be defined in different ways. The absolute yield under droughted conditions is of primary importance to the grower, and in general, high yielding varieties do well across a range of conditions. However, some varieties are more sensitive to drought than others, so a highly ranked variety in the absence of stress may yield considerably less than other varieties when water is limiting. Therefore, a second useful measure is an indicator of yield stability, or more appropriately, stability in combination with yield potential (the best yield attainable in the absence of stress). Because climatic conditions in the UK can vary each year, it is not possible to measure yield in every possible environment. Therefore, a third measure of performance is the relative sensitivity of a variety to drought: the drought tolerance index (DTI) quantifies the proportion of yield potential that plants manage to maintain under drought, normalised by the intensity of the drought. Thus, a variety can be described by: 1) its yield potential; 2) actual yield under at least one drought scenario; 3) yield stability; 4) drought tolerance *per se* (the DTI). All these measures are inter-related and quantify different aspects of variety performance. In all cases, because in the UK there are frequently seasons and sites with little or no moisture deficit, yield potential of a variety always must be taken into account.

The concept of water use efficiency (WUE) is important when water is a limited and costly resource. WUE is calculated as the ratio of yield to the quantity of water used by the crop. High WUE can be achieved by increasing yield, decreasing water use, or a combination of both. In most circumstances for a rainfed crop, high WUE at the expense of yield would have little benefit, whereas varieties that make the most effective use of water should be at an advantage under any conditions. With little information on how UK varieties differ in WUE or which traits confer more effective use of water, this project aimed to fill some of this knowledge gap.

2.2. Materials and methods

The relative performance of wheat varieties in response to water availability was assessed by:

- Testing 120 wheat lines (including Recommended List (RL) varieties, elite breeding lines, old varieties and several French varieties) under irrigated and managed drought conditions in the field in 2007 and 2008, and a subset of 66 varieties in 2009. Drought was imposed approximately two weeks prior to flowering by covering plots with large polythene tunnel rainout shelters.
- Testing 64 varieties on eight breeders' sites over three years under natural rainfed conditions.
- Comparing current RL varieties across 13 official test locations comprising contrasting soil types over three years.

In addition, 135 lines were genotyped using 11 diagnostic markers plus seven markers based on published QTL data. A subset of 94 lines was chosen for genotyping using 2499 DArT biallelic dominant markers.

2.3. Results

2.3.1. Varieties

Results showed that significant genotypic diversity for drought tolerance and WUE exists within the wheat germplasm pool used by plant breeders to create new UK varieties. Thus, there is potential to improve the performance of future varieties for dry years and on drought-prone land.

Although the ranking of varieties changed in each trial, several varieties showed consistent contrasts in DTI, determined by measuring yield in test plots grown under fully irrigated conditions and under rainout shelters. For instance, Hobbit and Andalou had significantly better DTI scores than Gatsby and Dover, averaged across three years. The older, tall variety Cappelle Desprez also showed good DTI, but another older, tall variety Maris Widgeon, was poor. Both varieties had low relative yields, but certain characteristics of Cappelle Desprez allowed it to maintain a greater proportion of its yield potential, suggesting some intrinsic capacity to better withstand drought.

Relative comparison of varieties across a range of RL test locations that differed in soil water holding capacity showed only small differences between varieties in 2009. In 2007 and 2008 there was little stress due to sufficient rainfall. In 2006 there was enough water deficit on some sites such that Sahara showed a significant positive slope (by regressing relative yield against site drought stress index), indicating relatively better performance as conditions become drier. In breeders' trials (seven in the UK, one in France) in 2009, varieties showed significant yield responses to the range of water availabilities represented in these trials. For instance, Hobbit had a positive slope, while Exotic showed a negative slope.

2.3.2. Morphological and physiological traits

There was highly significant variation between lines for nearly all measured traits. A number of traits showed significant correlation with various measures of variety performance, such as irrigated yield potential, yield under droughted conditions, DTI and water use efficiency. However, as expected for complex traits, each single character explained only a small proportion of the variance in yield or drought tolerance. Traits that positively correlated with DTI were: maintenance of green canopy, stem dry mass at anthesis, soluble stem carbohydrates, leaf thickness, stem height and water use from deep soil layers (Table 1). Carbon isotope discrimination ratio (Delta) in leaf and grain was negatively associated with DTI but positively associated with yield. Leaf rolling and waxy bloom were positively correlated with droughted yields. Many varieties expressed combinations of both positive and negative traits, making it difficult to assess the value of one trait independently of others. A composite trait score that combined the effects of several key traits was devised as a quantitative description of a drought tolerant or water efficient type that could aid breeding for improved varieties.

Table 1. Relationships between traits and yield or drought tolerance. Positive signs and minuses indicate positive and negative phenotypic correlations, respectively; blanks indicate a neutral effect.

	Drought tolerance index[7]	Irrigated yield	Droughted yield
Maintenance of green canopy[1]	+	+	
Stem dry mass at anthesis[2]	+	-	
Stem carbohydrate concentration[3]	+	+	
Carbon isotope discrimination ratio[4]	-	+	+
Water extraction from deep soil layers	+	-	
Stem height	+	-	
Flag leaf thickness	+	+	
Flag leaf area		-	
Flag Leaf rolling			+
Flag leaf photosynthetic efficiency[5]		+	
Waxiness[6]			+

[1]Maintenance of green canopy cover is the proportion of canopy cover at anthesis under irrigated conditions that was maintained under droughted conditions)
[2]Stem dry mass (or also the ratio of ear:stem dry matter) measured at anthesis
[3]Concentration of water-soluble carbohydrates (WSC) in stems sampled at anthesis
[4]Measured in flag leaves at anthesis or grain at maturity
[5]Measured using chlorophyll fluorescence techniques
[6]Appearance of waxy bloom on leaves and stems
[7]The proportion of unstressed yield potential maintained under droughted conditions

There was good correlation between droughted and irrigated yields, indicating that yield potential was important in ensuring good yields when water was limiting. However, some high-yielding varieties were also unstable, losing rank position under droughted conditions. In breeding programmes, selection against traits that increase susceptibility to yield losses under dry conditions while simultaneously selecting for good yield potential may increase both yields and yield stability.

2.3.3. Water use efficiency

Water use efficiency (WUE) in a subset of 21 varieties was calculated on the basis of grain or total biomass yields and estimated water use, derived from calculations of crop evapotranspiration and measurements of soil water extraction in droughted plots.

- Significant genotypic variation was observed for WUE, which was affected largely by differences in yield.
- There were small but significant genotypic differences in water use and patterns of soil water extraction: some varieties such as Xi19 removed more water from deep soil layers (80-110 cm from the soil surface) than others such as Spark. Xi19 also showed greater drought tolerance than Spark.
- There was a slight negative correlation between yield potential and water extracted from deep soil layers, indicating a potential cost to greater root activity at depth. However, within a high-yielding group of varieties, a difference of 10 mm extra water extracted can translate to 0.2 t grain/ha at a typical WUE of 0.2 t/ha/mm.
- There was good correlation between WUE, droughted yields and drought tolerance *per se*, indicating that improvements in one trait will benefit the others.

2.4. Discussion

Improvements in wheat yields under water-limited conditions are a global challenge and pressing need. In arid areas, development of varieties that yield well when water is limiting is done empirically by direct selection for yield under these conditions. However, this is not practical in the UK or other regions that have periods of dry weather that are unpredictable in timing and severity. There has been considerable interest in secondary traits associated with drought tolerance and WUE as criteria for indirect selection for these targets. Findings from this project reveal that a drought-tolerant UK wheat phenotype (ideotype) is comprised of several characters, each of which individually makes a partial contribution to success under soil water deficit. The challenge is to quantify this ideotype to enable phenotypic selection on a large scale in breeding nurseries, or to pyramid a sufficient number of molecular markers.

Results also showed that one ideotype is not sufficient for the UK environment: varieties that are most successful during a water deficit prior to flowering, but with recovery of stress during grain filling, are different than those that yield relatively better when deficits develop only after flowering.

It is impossible for breeders to know which kind of drought predominates, so focus on traits such as maintenance of green canopy, which is important regardless of the timing of deficits, could be more fruitful than attempting to match the timing of sensitive developmental phases with potentially stress-free periods. Measurement of WUE in a range of varieties under dry field conditions revealed that the effective use of water can be improved mostly by increasing grain yields.

Evaluation of multi-location variety trial data to gain much-needed information on suitability of current varieties for drought-prone areas adds value to data already gathered in these trials. The approach used in this project could be applied to each new set of RL results, accumulating data on varieties during their lifetime on the List.

Novel contributions and outcomes from this project that can benefit the UK and international wheat community include:

- A comprehensive and quantitative description of a large set of UK winter wheat lines for DT, WUE and traits associated with improved yields under dry conditions. These lines can be used in selective crosses to create new varieties, and can be crossed to make bi-parental mapping populations in order to study the genetic control of traits more closely. Phenotypic datasets can be used for mining further information about trait x variety x environment interactions.
- Sets of varieties that show consistent contrasts in stem carbohydrates, carbon isotope discrimination ratio (extreme lines identified here already have been crossed in a WGIN-sponsored project), leaf morphology, etc.
- Methods that have been adapted to measure morpho-physiological traits rapidly and inexpensively on large numbers of varieties grown under field conditions.
- A cost-effective protocol to evaluate current RL varieties for drought tolerance and yield stability using multi-location RL trial data, including a database of soil, weather and variety data that can be augmented each year to increase the ability to differentiate between varieties according to water availability.
- A database of genotypic and phenotypic data that can be analysed for marker-trait associations.

Data sharing and collaboration agreements have been established with ADAS (ERYCC LINK project [LK0992]), Nottingham University (WGIN2 Drought work package), Triticarte Pty, Ltd (association studies) to make further use of the phenotype and marker data collected in this project.

3. TECHNICAL DETAIL

3.1. Introduction

Globally, drought causes more yield losses than any other single biotic or abiotic factor (Boyer, 1982). In the UK, insufficient soil moisture frequently limits crop productivity because rainfall often does not occur when crops need it most. The UK is one of the world's most efficient producers of arable crops, yet approximately 30% of the current wheat area is grown on drought-prone land and drought losses are on average 1-2 t ha^{-1} (Foulkes *et al*., 2007), which costs >£72M per year. As the wheat area expands onto less suitable land, it is likely that more wheat crops will experience drought, particularly in the main wheat growing areas in eastern England. Furthermore, climate change models predict that summers will become hotter and drier, exacerbating the problem and intensifying competition between agriculture, urban needs and environmentally-sensitive areas for limited water resources (Gornall e*t al*., 2010; Semenov, 2009). A stressed crop uses resources inefficiently and returns on inputs are poor. There is also evidence that a large component of yield instability (yield variation from site to site and year to year) is due to soil water availability (Dodig *et al*., 2008).

Ameliorating drought losses through increased irrigation of cereals in the UK is an unlikely prospect, though feasible in some areas (Dodd *et al*., 2011). Therefore, sustainable production of food and biofuels on drought-susceptible land depends on the development of varieties with improved water use efficiency (WUE) and drought tolerance. WUE is the yield produced per unit water consumed, and drought tolerance *per se* is the proportion of stress-free yield potential maintained when water is limiting (see other definitions of drought tolerance in the Summary). These are multiple-character traits, and genetic improvements are not easily accomplished; new varieties will not appear over-night. Thus, it is imperative to begin work now. Indeed, there has been and there continues to be a large international research effort. There have been numerous studies of drought tolerance of UK wheat lines, going back to the 1940s (Bingham, 1966; Innes *et al*., 1981; Austin, 1987). More recently, quantitative trait loci (QTL) controlling aspects of drought tolerance and WUE have been described (Foulkes *et al*., 2002; Verma *et al*., 2004; Foulkes *et al*., 2007; Kumar *et al*., 2010). Despite these advances in biology, drought tolerance remains a difficult and elusive target for practical breeding.

Currently, breeders are not equipped to make selections for improved drought tolerance because information is lacking on: 1) extent of variation for drought tolerance within elite germplasm; 2) key physiological processes and morphological characters that contribute to drought tolerance and WUE; 3) genetic factors that control these traits; 4) empirical and molecular tools to allow the selection of superior germplasm in breeding programmes. The aim of this project was to build a foundation of knowledge in order to deliver the tools that will enable breeders to develop improved varieties for water-limited conditions.

3.1.1. Untapped value of variety trial data

To quantify the relative drought tolerance of many different wheat varieties, and to locate the few with superior characteristics, large scale yield trials under varying environmental conditions would have to be conducted. Fortunately, this costly exercise is already conducted in the form of the HGCA Recommended List (RL) variety trials, which are conducted on a range of soil types across years under differing amounts of rainfall. Thus some sites in nearly every year develop some degree of drought stress. A drought stress index (DSI) can be derived to quantify the level of stress for each trial. The approach we used here employed the Sirius wheat growth simulation model that was run on the actual weather and soil variables from each trial site and year. The relative performances of varieties were then judged across a range of increasing DSI. The accuracy of the computed DSI depends on soil and rainfall data that are specific to each location, and use of a crop growth model that estimates actual water use. This approach has been successful in identifying drought tolerant types in sugar beet (Pidgeon *et al*., 2006), wheat (Chenu *et al*., 2011), barley (Rizza *et al*., 2004) and sorghum (Chapman *et al*., 2002).

3.1.2. Objectives

- Evaluation of the relative drought tolerance of current varieties using multi-location variety trial data (breeders' trials and Recommended List Official variety trials).
- Assessment of genetic diversity for drought tolerance, WUE and drought-related physiological and morphological traits in a panel of diverse UK lines grown under controlled field drought conditions.
- Genotype a subset of lines that contrast in responsiveness to water supply using markers for key targets that may affect drought tolerance and WUE.

3.2. Materials and methods

3.2.1. Evaluation of multi-location variety trial data

Two sets of multi-location variety trial data were used in this study: eight trials conducted by the partner breeders, and 13 official RL sites conducted mostly in the East of the UK (Appendix, Tables 1-6). Each trial or test environment was characterised by a drought stress index (DSI). This index quantifies the stress experienced by the crop by combining soil and weather data to compute how much water deficit accumulated over the course of the growing season. On sites and years with deep water retentive soil and sufficient rainfall, the DSI was low; on lighter soils with inadequate rainfall, DSI values increased. The drought tolerance or susceptibility of an individual variety can be illustrated by examining its performance relative to the trial mean as water becomes more limiting (along a range of increasing DSI). There are various ways to derive a DSI (Pidgeon *et al*., 2006), but we used the Sirius wheat growth model (Lawless *et al*., 2005) to simulate yields of a variety given the actual conditions of the trial, and yields in the absence of any water limitation. The ratio of these two yields provided a robust index describing the environment, regardless of whether or not the calibration variety was actually included in the trial, and was independent of how well the model simulated the observed yields. Calibration varieties are those varieties that were grown and measured extensively to build the parameters of the model. The DSI is a function of the modelled yield (Y') of a calibration variety (e.g. Mercia) using actual weather and soil conditions (Y'a) for each environment (a year, trial site combination), and the modelled yield of the calibration variety using weather inputs that eliminate any stress by supplying as much water as the crop demanded (the yield potential, Y'p; Eqn. 1). A related index is the TSI (transpiration-based stress index), which uses the accumulated ratio of the simulated crop evapotranspiration (ET'_a) to potential evapotranspiration in the absence of any stress (ET'_p; Eqn 2). The DSI and TSI are directly related (Appendix, Fig. 1). However, the indices differ slightly in that the DSI, being yield-based, is affected more by the biomass partitioning characteristics of the calibration variety. To express the performance of a variety, we calculated its relative yield (RY), which is the actual yield of a given genotype (Ya) divided by the trial mean yield averaged across all varieties tested (Eqn. 3). Other denominators could be used (e.g. Y'p), but with greater than 30 entries in a trial, there is little chance that the performance of an individual variety in a particular trial would have a significant influence on the value of the denominator.

1) $$DSI = 100 \times \left(1 - \frac{Y'_a}{Y'_p}\right)$$

2) $$TSI = \sum \left(1 - \frac{ET'_a}{ET'_p}\right)$$

3) $$RY = \frac{Ya}{\overline{Ya}}$$

For each variety, RY was plotted against DSI to obtain a slope and intercept, using a simple Finlay-Wilkinson regression approach (Finlay and Wilkinson, 1963). A negative slope indicates drought susceptibility; i.e., the relative performance of a variety decreases as conditions become drier, while a positive slope indicates relative drought tolerance. The intercept indicates yield potential. Fig. 1 illustrates two varieties with similar yield potential but with contrasting sensitivities to water availability, based on a preliminary analysis of historical RL data.

In order to calculate the DSI for the trial sets in this study, soil samples were taken at each trial site location. Where subsequent trials were located within a short distance of each other, and it was deemed that soil texture and depth was uniform throughout the area, one set of physical soil samples was used to characterise these neighbouring sets of trials. Soil samples were taken from the field in which trials were located. Soils were sampled at four horizons: 0-25 cm, 25-50 cm, 50-70 cm and 70+ cm. Four replicate sets were taken and bulked together for each depth. The samples were air-dried and then milled in a rotary grinder and the weights of dried soil and stones were recorded. Soft chalk in the samples milled down but harder chalks remained and were treated as stone. All samples were then finger-textured over a period of a few days by one, trained person using comparison soil standards (National Soil Resources Institute, Silsoe, UK) to determine the soil particle size class. Stone content was calculated by volume, assuming a standard soil bulk density of 1.40g ml^{-1} and a stone density 2.65 g ml^{-1}. Using the standard tables for available water (Hall *et al.,* 1977), and correcting for stone content, the available water capacity of soil at each site was determined and used as an input for the Sirius growth simulation model. A shallow rooting depth was used in the model when soil samples indicated physical barriers to root penetration at a given depth.

For the RL trial dataset, yields were obtained from the HGCA website; 13 fungicide-treated, first wheat sites with contrasting soil types were selected mainly from the East of the country. Weather data were obtained from the nearest MET Office site. Weather parameters and rainfall in particular were not recorded at any of the RL trial sites, or at least these data were not available. The collaborating breeders also established eight separate trials on sites contrasting in soil type and geographical location. In 2007, a set of 49 varieties were tested at each of the locations. In 2008 and 2009, an additional 15 varieties were tested to comprise a ‘core set’ of 64 varieties. This was to enable correspondence between the current project and another HGCA-sponsored LINK project managed by ADAS (ERYCC, LK0992).

For the breeders’ trials dataset, yields were obtained from the collaborating breeders. In a few cases, rainfall was measured on the trial site and these data were used to supplement MET Office data obtained from the station nearest the trial site. Soils were sampled on the actual trial fields for the RL and breeders sites as described above. A DSI value was assigned to each RL and breeder trial in each year. All site-specific soil, weather and yield data are held in a relational database for future access and data mining.

3.2.2. Drought tolerance, WUE and morpho-physiological traits

Plant husbandry and experimental set up

A series of trials were conducted at Broom’s Barn under managed drought and irrigated/rainfed conditions from 2007-2009. The soil was a sandy loam over chalky rubble in the Ashley variant series. Drought was imposed using large polythene tunnels as rainout shelters (Appendix, Photo 1). Further description of the polytunnel system and the effects of crop microenvironment have been described previously (Rajabi *et al*., 2009). Briefly, air temperatures in covered plots were on average 1°C greater than outside the polytunnels, and windspeed was reduced by approximately 50%, with the net result that evapotranspiration rates were 20% less under the polytunnels than outside. Thus, the effects on crop microclimate were minor, and conditions in covered plots were not unlike the environment typically experienced by a crop in a normal field.

In the first two years, 120 genotypes were sown in mini-plots 1.5 m long by 1.2 (6 rows) wide. The managed drought experiment was designed as a complete randomised block with three replicates for each genotype. In 2007, six polytunnel bays were constructed on the existing barley stubble, and cultivations were performed within the metal tunnel framework. Treated seed were sown using a Hege plot drill at a density of 200 seeds m^{-2}. Within each tunnel bay, three parallel strips of plots were sown, and two adjacent tunnel bays comprised one block. Standard agronomical practices were followed, with a crop protection programme performed according to guidelines established for official UK winter wheat variety trials (HGCA Recommended List Protocols; www.hgca.com). However, no growth regulators were used in order to allow full expression of genotypic differences in stem height. Plots of tall varieties were staked for support. In 2009, 66 genotypes were compared, but using larger (1.2 x 3 m) plots and four replications. Genotypes were selected by the consortium of breeders to represent the diversity of germplasm accessed by breeders to develop new varieties. The set also included 10 French varieties for contrast to the largely UK material. The set comprised new RL varieties, old varieties and elite breeding lines. During the selection of entries, attention was paid to pedigree, flowering time, height, 1 R/S translocation, and morphological characters such as awns and leaf waxiness.

Yield components and drought tolerance indices

In order to determine total above-ground biomass, plots were harvested by hand sickle in 2007, and with a self-propelled scythe mower (Al-KO, Ltd) in 2008 and 2009. Harvested plants were collected in bins or large bags, weighed, then a random subsample of approximately 12 plants was taken for measurement of yield components. The remaining plot sample was threshed using a stationary combine/thresher (Wintersteiger) with drum settings optimised to capture fine or shriveled grains without crushing larger grain. Threshed grain was sifted to remove extra trash, weighed, and then measured for grain moisture and specific grain weight using a laboratory grain moisture meter (Model AP6060, Sinar, Ltd.). Yields were adjusted to 85% dry matter content. Harvest index was the ratio of total above-ground plot biomass (minus stubble) to grain mass. Ears were removed from harvest subsamples counted, weighed, and then threshed using a floor-mounted thresher (Wintersteiger). Airflow settings were optimised to retain small and shrivelled grain. Threshed grain from the subsample was weighed to compute grain mass per ear. A further subsample of threshed grain was placed on a digital grain analyser (Marvin) that counted and weighed grain to determine mass per grain (thousand grain weight) and estimate number of grains per ear; it was also used to determine grain dimensions.

Drought tolerance was expressed on the basis of grain yield and total biomass using two indices (Ober *et al*. 2004). The drought tolerance index (DTI) is the inverse of the susceptibility index described by Fischer and Maurer (1978), which is the proportion of irrigated yield potential maintained under drought, normalised by the drought intensity of the trial.

4)
$$DTI = \frac{Y_D/Y_I}{\bar{Y}_D/\bar{Y}_I}$$

where Y_D is the yield under drought and Y_I is the genotype mean yield under irrigation. The denominator is the drought intensity based on the mean droughted and irrigated yields across all genotypes within a trial.

The yield/tolerance index (YTI) was described by Fernandez (1992), which combines relative stressed yield, relative yield potential, and drought intensity of the trial (Ehdaie *et al*., 2003).
5)
$$YTI = \left(\frac{Y_D}{\bar{Y}_D}\right) \cdot \left(\frac{Y_I}{\bar{Y}_I}\right) \cdot \left(\frac{\bar{Y}_D}{\bar{Y}_I}\right) = \frac{Y_D \cdot Y_I}{\left(\bar{Y}_I\right)^2}$$

Morpho-physiological measurements

Prior to harvest, the stem height of three to five random plants per plot were measured from soil surface to ear tip. The number of fertile main tillers was counted in an 80 cm section of an inner plot row to compute ears m^{-2}. In 2007, secondary tillers were also counted because an April drought caused an unusual level of secondary tillering.

A battery of morpho-physiological measurements was made during the course of the growing season, some of which have been described previously (Ober *et al*., 2005). Visual scores were made of leaf rolling (on a scale from 0, no rolling, to 3, midrib fully hidden); leaf epicuticular wax (on a scale from 0, ‘glossy’ to 5, complete coverage of leaf abaxial surface with blue/white bloom). The degree of flag leaf senescence during grain filling was scored on a scale from 0 (>90% of leaf area green) to 4 (≥ 75% leaf area yellow or brown). Senescence scores were also done for whole culm (upper three leaves). In 2009, there was pronounced appearance of drought-induced leaf necrotic spots, so this was scored on the basis of percentage leaf area affected.

Green canopy cover, expressed as a percentage of ground coverage, was determined using a spectral ratio meter (Spectrosense2+, Skye Instruments, Ltd) with sensors filtered to capture red and far red incoming (upward facing) and reflected (downward facing) radiation to compute a normalised reflectance ratio (NRR), similar in principle to the normalised difference vegetation index (NDVI). NRR values were converted to percentage canopy cover using a calibration curve created by comparing natural log-transformed NRR values and canopy cover derived from digital infrared photographs of plots over a range of canopy expansion as the crop developed. The photochemical reflective index (PRI) was measured similarly to NDVI, except using the spectral bands centred on the wavelengths 529 and 569 nm (Gamon *et al*., 1997). Leaf chlorophyll content was measured on flag leaves using a SPAD meter (Minolta) fitted with an RS232 interface communicating data directly to an Excel spreadsheet on a PDA palm computer.

Leaf morphology was described by measuring leaf thickness using a digital thickness gauge; leaf mass area ratio (LMA) and succulence index by measuring flag leaf areas with a belt conveyor planimeter (Delta-T, U.K.) and weighing leaf fresh and dry mass. Stomatal conductance was measured using a steady-state porometer (SC-1, Decagon Devices), or estimated using a viscous flow porometer (Thermoline, Ltd; Rebetzke *et al*., 1998). An RS232 port was added to the viscous flow porometer and an interface unit was constructed (R. Lefevre, Rothamsted) to allow data capture using the PDA. After determining LMA on the flag leaves, these samples were used for carbon isotope discrimination ratio measurements (below).

Water-soluble stem carbohydrates were measured using the anthrone method, scaled down for microtitre plate assays (Laurentin and Edwards, 2003) using fructose standards. Three random main stem tillers were harvested from plots within 3 days post anthesis. Ears were removed at the peduncle junction and leaves were stripped from culms, leaving stems and leaf sheaths. Stems were cut into 30 cm sections and flash-dried at 85°C, weighed, then ground with a tissue mill to pass a 1 mm screen. Fifty ± 20 mg of ground sample was placed in a 15 mL tube and extracted in 3.7 mL deionised water at 70°C for 30 min with shaking. Diluted aliquots were analysed in triplicate on each plate. Ears from these samples were also dried and weighed to determine ear dry mass near anthesis.

The accumulation of compounds resulting from stress-induced membrane degradation was assayed in flag leaf tissue in 2008. These lipid oxidation by-products react with thiobarbituric acid (TBA), hence are called TBA-reactive substances (TBARS; Hodges *et al*., 1999). The reaction product, when heated in acid, forms a stable pink colour. Malonyldialdehyde (MDA) is very similar to these degradation products and therefore was used as a standard for the assay, which was conducted using microtitre plates. Each sample was assayed using triplicate aliquots.

Water use efficiency

Water use during the period plots were covered was estimated by measuring changes in soil moisture content using a capacitance-type soil moisture probe that operates on the principle of frequency domain reflectrometry (Diviner2000, Sentek, Ltd), as described previously (Ober *et al*., 2005). Briefly, polyvinylchloride access tubes were installed in plots to a depth of 1.3 m in early spring by forming a slightly undersized hole using a hardened steel reamer driven by a hydraulic hammer. Tubes were pounded into place to ensure good contact between the tube and the surrounding soil, and then a water-tight seal was installed in the bottom of the tubes. Measurements were made on a subset of 21 genotypes, selected by breeders with knowledge of the genotypes and best guesses which would provide contrasts in water use and drought tolerance. Crop water use prior to covering plots was estimated based on potential evapotranspiration rates adjusted by a wheat crop coefficient (Howell *et al*., 1995) and percentage crop cover, determined as described above.

Carbon isotope discrimination ratio (Delta) was determined on flag leaf samples harvested at anthesis for LMA (above) in irrigated plots in all three years, while Delta from grain samples was determined on a subset of 33 genotypes only in 2009. Dried leaves were ground using an electric coffee grinding mill. The find-ground sample, including the dust, was transferred to a 2 mL tube for storage. A 1 ± 0.2 mg subsample was taken by stabbing the tip of a glass pipette into the sample. Pipettes were prepared in advance by bending the tip at 90° in a flame so that the sample could be transferred easily to the tin sample cups (Elemental Microanalysis Ltd). The cup plus sample was weighed on a microbalance (Mettler) to obtain the sample weight to ensure that it was within the defined tolerances. The cup was rolled into a ball and placed into a labelled 1.5 microfuge tube. All preparation was done on a static-free work surface, and precautions were taken to avoid build up of static charges that prevented working with small powder samples. Samples were shipped to SCRI (Dundee, UK) for analysis of [$^{15}N/^{14}N$] and [$^{13}C/^{12}C$] isotope ratios using an ANCA GSL elemental analyser (EA) coupled to a 20/20 isotope ratio mass spectrometer (both SerCon Ltd, Crewe, UK). Isotope analyses were done in collaboration with Dr. Wolfram Augenstein, SCRI.

To gain a more accurate measure of WUE than could be obtained based on estimates of crop water use in the field, three varieties were sown in large weighing lysimeters so that water use could be measured gravimetrically (by periodically weighing the lysimeters). There were three replicate bins of each variety, arranged in a complete randomised block design. The three varieties (Alchemy, Consort, Zebedee) were chosen based on contrasts for leaf Delta. The 90 L (0.8 x 0.8 x 1.2 m) capacity plastic bins used as lysimeters were filled with a loam/compost mix, used for a previous experiment on sugar beet, and then sown with wheat in three rows on 3 Nov., 2008. In the centre of each lysimeter an access tube for the Diviner soil moisture probe was installed in order to compare gravimetric measurements of plant water use with those derived from measurements of changes in soil moisture content. Plants were thinned to 5 cm spacing and received standard husbandry. Lysimeters were covered by a tall polythene rainout shelter similar to those used for the field experiments on 14 May to facilitate control over water inputs, which were applied to lysimeters in measured amounts to maintain soil near field capacity. Lysimeters (approx. 250 kg each) were weighed fortnightly with a telescopic tractor lift and crane load cell. A battery of morpho-physiological measurements were made on the plants at anthesis.

Statistics

The effects of water treatment and genotype on the measured traits were assessed using a two-way analysis of variance in a complete block design (Genstat 10.0, VSN International, Ltd., Oxford, UK). Water treatments and genotypes were fixed-effect factors. The effects of replications were random. Where appropriate, early and later-maturing (GS 61 > Julian Day 158) genotypes were analysed separately. When ANOVA showed significant effects for factors and interactions, the least significant difference (LSD) test ($P \leq 0.05$) was used to separate means for comparison. The relationships between traits were determined using Pearson's correlation test.

Genotype and genotype x trait (GGT) biplots (Yan and Kang, 2003), which graphically represent genotype main effects plus genotype x trait interactions, were created from a two-way matrix of genotypes vs. standardised trait values from the 2008 experiment using commercial software (GGEBiplot.com) that calculated the principle components. For further explanation of GGT biplots, see Ober *et al*. (2005).

3.2.3. Genotyping lines evaluated for drought tolerance and WUE

In total 135 lines were genotyped by RAGT, which was above and beyond the original number of lines set out in the proposal. The additional lines were added so that genotypic information would be available for the lines also included in the ERYCC Link project (LK0992). Markers for *Rht1, Rht2, Rht8, Ppd1, Ppd2, Pch1, Lr37, Vi*r, 1RS translocation and *Sm1* were used. In total 11 diagnostic or 'perfect' markers were used. In addition, seven markers based on published QTL data were used, comprising four yield QTLS and 3 flag leaf senescence QTLs. Standard procedures were followed for DNA extraction from grain samples of each line. Prior to DNA extraction, the purity of each genotype sample was double-checked.

A subset of 94 lines was chosen for genotyping using DArT markers, which are biallelic dominant markers developed by Triticarte, Ltd (Australia). The 94 lines comprised the core 64 variety set, plus an additional 30 lines chosen on the basis of contrasts for important traits in this study. The new, high density array used by Triticarte had many new markers from the D genome, and therefore improved coverage compared with older DArT arrays. Thirty and 334 markers also came from rye and triticale libraries, respectively. A proportion of the markers (1,529) were tentatively assigned to chromosomes based on well-curated genetic maps developed by Triticarte.

3.3. Results

3.3.1. Evaluation of multi-location variety trial data

Only small soil moisture deficits (SMD) developed in 2007 and 2008 because rainfall kept pace with evapotranspiration. The very dry April in 2007 (Appendix, Fig. 2) affected crop development, particularly the formation of secondary tillers, but did not appear to affect final yield to a great extent, based on DSI calculated for selected RL trial and breeders' sites (Appendix, Tables 1-6). In 2009, small SMD developed on some sites, which affected the yield and performance of varieties, but only in one variety (Scout) did DSI explain a statistically significant proportion of the variation in relative yield across a range of sites (Fig. 6; Appendix, Table 8). In the breeders' trials, however, six varieties showed significant slopes (Fig. 4; Appendix, Table 9). Interestingly, the differences in yield potential (the intercept) amongst these six varieties were generally small, and on this basis it would be difficult to distinguish between them. Only by examining the response to water availability across a range of sites (the slope) is it clear that these varieties show very different responses to water.

To extend the dataset, we also examined RL trial results from 2006, even though this crop year preceded the project. In 2006 sufficient SMD developed to allow differentiation of varietal performance, but again, only one variety showed a significant slope (Sahara; Appendix, Table 7). The yield potential of this variety was less than average, but yielded better than average at the sites with the greatest stress (Fig. 3). Although the responses of several other varieties did not reach statistical significance, it can be seen that varieties differ in yield trends over an environmental gradient across sites differing in water availability. By combining slope, intercept and scatter (yield variability), varieties can be roughly classified into those with 1) good yield potential combined with some measure of drought tolerance (positive slope; e.g. Ambrosia); 2) good yield potential but with drought susceptibility (negative slope; e.g. Oakley); 3) good yield potential combined with environmental stability (little slope and little scatter; e.g. Humber, Benedict).

The ability to discriminate varietal differences in responsiveness to available water was limited by the number of sites and the range of stress encountered. By combining data sets across years and sites, a better picture of each genotype could emerge. Data from the eight breeders' sites and the 13 RL sites was combined in 2009, but unfortunately the regressions were still limited by the small number of sites that had larger DSI values. There were no significant regressions for any varieties that were represented in the combined data sets (Appendix, Table 10).

These multi-environmental variety trial data can be mined for information in many ways. In addition to yield potential, it is important for farmers to know which varieties are the most stable across a range of conditions. When diseases are controlled well, water availability contributes greatly to the site to site and year to year variability in yields. Currently, information on yield stability is not published as part of the RL recommendation, but can be derived easily from these data. There are many indices that have been used to describe yield stability (e.g. Baxevanos, *et al*., 2008; Flores *et al*., 1998; Ober *et al.*, 2004). However, there is often a trade-off between yield stability and yield potential: a low-yielding variety frequently is able to maintain that yield under most conditions (Hohls, 2001). This is of limited value. It is more helpful to combine the yield potential and stability. Two related indications of this measure of performance that can be derived from GGE biplots (Yan and Kang, 2003) are the instability index and Yan's yield stability index. The former is a stability index similar to Shukla's index or the Eberhart and Russel index, which measure stability independently of yield. The latter index, derived from the GGE biplot distance (the distance between individual markers and the marker for the ideal genotype), is strongly correlated with the univariate Kang's YSi yield stability index (Alwala *et al*., 2010; Fan *et al*., 2007), which combines Shukla's environmental stability index with the rank value of genotypes based on yield. GGE biplot-derived yield stability was found to be the most reliable indicator of yield and stability across years (Baxevanos *et al*., 2008). The results show that varieties differ in these measures of stability (Fig. 4). Note that, for example, Oakley was mid-ranked for instability (considering environmental stability independently of yield potential), but because of its high yield potential, was top-ranked using the yield stability index (Fig. 4).

3.3.2. Drought tolerance, WUE and morpho-physiological traits

A significant level of water deficit was imposed in each trial from 2007 to 2009 using the managed drought facility (Table 2). Without these large rainout shelters, two of the years would have been wasted as little natural drought developed (Appendix, Fig. 2). It is valuable to judge genotypic performance based on results accumulated across sites and years. However, initially it is important to review the results from each individual trial, as each year was a unique environment.

Yield and drought tolerance

In 2007, grain yields varied from 5 t ha^{-1} to 12 t ha^{-1} under rainfed conditions (no supplemental irrigation was applied in 2007), and under droughted conditions grain yield was decreased by 14% overall (Table 2). There was a positive relationship (r = 0.47***) between rainfed and droughted yields, indicating general yield stability amongst UK wheat lines. The implication is that by selection for high yield potential under non-stressed conditions (the main driver of UK wheat breeding), there is good probability that those lines will also show good yields under stressed conditions.

The differential sensitivity of genotypes to water availability is also shown by the significant genotype x water interaction term in the ANOVA (Table 3). Drought tolerance *per se* was quantified by computing a drought tolerance index (DTI) for each genotype based on the ratio of droughted to well-watered yields, normalised for the stress intensity of the trial (Ober *et al*., 2004; Equation 4).

An index that reports a slightly different aspect of drought responsiveness is the yield/tolerance index (YTI) that combines relative yield performance under drought with unstressed relative yield potential (Ober *et al*., 2004; Equation 5). The distribution of genotypes (Fig. 8) shows that there were significant differences between genotypes in susceptibility to drought, with several genotypes showing significantly better DTI than the trial mean (DTI = 1). The yields and drought indices of all 120 genotypes are listed in the supplemental data.

In 2008, the same genotypes were tested (with a few entry replacements), but the intensity of the applied water deficit was greater than in 2007, causing an overall yield loss of 45% (Table 2). There was a positive relationship between droughted and irrigated yields, although with a large degree of scatter (r = 0.4***). Genotypic differences in the sensitivity to water availability are shown by their 'crossover' behaviour (Fig. 9; Sneller and Dombek, 1997) and by significant genotype x water regime interactions (Table 3). Greater levels of drought tolerance were more strongly associated with better yields under droughted conditions than decreased yields under irrigated conditions. As with all performance measures derived from ratios, both the nominator (droughted yield) and denominator (irrigated yield) need to be considered. The data show that some genotypes were better than others at combining both drought tolerance *per se* (DTI) with good yield potential (Fig 10, box). It is important to note that it was not just the most modern varieties that fell in this category, indicating that new varieties can be further improved by incorporating alleles that control the characteristics of these superior lines.

In 2009, a subset of 66 lines was tested, using larger plots and more replication to increase the accuracy of the experiment. This set comprised a 64-variety 'core' set also used in the ERYCC (Earliness and Resilience for Yield in a Changed Climate) project run in parallel by ADAS (LK0992), and the variety set used in the breeders' multi-location trials. As in previous years, there was a positive relationship relationship between irrigated and droughted yields (Fig. 11). A number of genotypes fell below the regression line, indicating poorer yields under droughted than irrigated conditions compared with the average response. Likewise, some genotypes fell above the regression line, indicating superior performance. Crossover types were clearly identified, indicating that Gatsby, for example, lost much of its yield potential under droughted conditions, compared with Andalou, which managed to maintain a greater proportion (Fig. 12). Crossover behaviour points to genotypic differences in sensitivity to water availability, which was corroborated by significant genotype x water regime interactions for grain yield (Table 3), and genotypic differences in DTI and YTI (Table 3, Figs. 13-14). Genotypes with greater YTI scores indicate good combination of droughted yield and yield potential, which can translate to good across-environment yield stability. In certain varieties, the DTI and YTI were greater on the basis of total above-ground biomass than on a grain yield basis. This indicates that the biomass production of that variety was less affected than grain yield; i.e., harvest index was affected by drought to a greater extent than other lines. Thus, in those varieties with much smaller DTI on a grain yield basis than total biomass basis, some stage or stages of grain formation were more sensitive to drought than growth in general. There was a slight negative correlation between DTI and well-watered yield potential, indicating some cost to drought tolerance, although in this trial the scatter was too large to indicate any strong trade-offs, particularly without the three very low yielding varieties (Maris Widgeon, Cappelle Desprez, Bacanora) that skewed the data (Fig 14).

The drought tolerance indices depend on an accurate measure of the yield potential in the absence of stress. It is possible that small, experimental plots do not provide the best picture of a variety's performance, and may introduce error into the derived DTI or YTI. Hence, values of DTI and YTI were also calculated for each variety using as a denominator in these ratios the mean yield obtained across the sites used in the breeders' trials (see section 3.3.1, above). This could be done because little stress developed on breeders' sites in each year of the project. In each year, the DTI and YTI derived from droughted and irrigated plot yields at Broom's Barn showed high correlation with DTI and YTI values derived from droughted yields at Broom's Barn and yield potential values from the breeders' trials (Fig. 16). Hence, irrigated yields obtained in experimental plots at Broom's Barn accurately reflected the yield potential measured in larger plots across a range of sites, and the DTI and YTI values derived from these experiments were robust.

There was a general positive association between DTI assessed under managed drought conditions, and drought tolerance assessed by evaluating performance across a range of sites differing in water availability (Fig. 17). Using these two different approaches that show similar assessments, there is increased confidence in the characterisation of varieties.

The ranking of genotypes differed with each year as environmental conditions varied, but a small set of varieties showed consistently better than average drought tolerance and YTI, and another set poorer than average (Fig 18). None of these, however, were current RL varieties, but these contrasting types are useful tools to dissect mechanisms of drought tolerance.

Where significant genotypic differences in yields were observed, it is instructive to see what yield components contributed to those differences. This can be done using path analysis (e.g. Ehdaie, 1995), or by simply comparing correlation coefficients as done here (full correlation matrices are available in the Supplemental Data). Grain yield is a function of the numbers of ears per m^2 and the mass of grain per ear. The latter in turn, is a function of the number of grains per ear and the mass of individual grains (thousand grain weight, TGW). For all yield components, there was highly significant genotypic variation under droughted and irrigated conditions, and strong effects of the water regime (Table 3). However, the genotype x water regime interaction term from ANOVA was significant only for grain yield and TGW. This suggests that the principle factor that altered the ranking of genotype performance between irrigated and droughted conditions was the differential ability to fill grains. Under droughted conditions, the number of main spike ears per m^2 was not associated with grain yield; grain mass per ear was the dominant factor, which was correlated with grain number per ear and TGW. Determination of grain number per ear has been associated with most increases in grain yield (Dolferus *et al*., 2011). The DTI, similarly, was positively associated with grain number per ear. Under irrigated conditions, grain yield was associated with mass per ear but not with main spike ears per m^2 or TGW. Therefore, most of the genotypic variation in yield was related to the number of grains formed per ear. The contribution of ears on secondary tillers to yield cannot be discounted, but in these trials information on yield components was gathered only for the main spike. In other work, the major effect on yield of dry conditions in spring was primarily through decreased fertility of secondary ears (Fischer, 2010; E Ober, unpublished). This may have been due to increased levels of stress by the time secondary tillers were forming ears, whereas main stem ears developed slightly in advance of critical stress levels.

Morphological and physiological traits

An extensive suite of traits was measured on genotypes grown under irrigated and droughted conditions at Broom's Barn (see Supplemental Data for the extended list). Many of these traits have been shown to be associated with drought tolerance in other studies; therefore, their role was also investigated in these experiments. Emphasis was placed on traits that can be measured quickly and cheaply, because on a breeding scale, if these traits are used to screen large numbers of lines, such considerations are important. Furthermore, the phenotype of individual traits may be controlled by a smaller number of major genes compared with other complex, multigenic traits such as yield. Therefore, the likelihood of developing selectable markers for morpho-physiological traits is greater than discovering useable markers for drought tolerance *per se*. Emphasis was also placed on measurement of traits under irrigated conditions rather than under droughted conditions, although not exclusively. This was for several reasons: 1) in practice, breeders will likely not be able to apply managed drought conditions in the field as done in this experimental work; 2) constitutively expressed characters, rather than drought-induced traits, should show greater heritability because of smaller influence of environmental conditions on their expression; 3) for many traits, uniform, good growing conditions should maximise genotypic variation and minimise other sources of variation that contribute to error variance. Certain traits were also measured under droughted conditions because inducible traits may confer advantages under drought conditions, but without the potential yield-offsetting costs of maintaining these traits when they are not needed under non-stressed conditions.

There was significant genotypic variation for nearly all morpho-physiological traits measured. This reflects the genetic diversity of the germplasm tested in these experiments. In this highly diverse set of genotypes, if genotypic variation had not been observed for a particular trait, it would suggest little likelihood of genetic improvement for that trait. The diversity of phenotypic expression that we observed in these materials indicates that 1) any trait that proves useful should be amenable to selection and improvement; 2) lines with high and low expression have been identified so that future bi-parental mapping populations can be created to study the genetic basis of these traits.

Trait associations are summarised in Table 1, and complete correlation matrices are in the Supplemental data. Correlations were performed with the entire set of lines, and also with subsets of lines separated by flowering time (Appendix, Fig. 3). Traits that positively correlated with DTI were: maintenance of green canopy cover, stem dry mass at anthesis, stem water-soluble carbohydrates (WSC), leaf thickness, stem height and water use from deep soil layers. Carbon isotope discrimination ratio (Delta) was negatively associated with DTI. Traits positively associated with irrigated grain yields were: stem WSC, Delta, photosynthetic efficiency, leaf chlorophyll content, green canopy cover and leaf thickness. Traits that showed negative associations with irrigated yield were: stem dry mass at anthesis, stem height, leaf senescence, flag leaf size. Trait associations with droughted grain yields were similar, including waxiness and leaf rolling, which showed positive correlations with yield. The strength of correlations between flag leaf size, stem height and yield were smaller in a subset of only the most recent varieties, suggesting that much of the breeding progress in these traits has already occurred. Ear dry mass at anthesis was positively associated with yield under droughted conditions, but was not significant under irrigated conditions.

Associations with YTI were similar to those observed with irrigated yield (since irrigated yields have a large influence on YTI). Trait and DTI associations were less pronounced in the 2007 and 2008 trials, but there was general agreement with the findings in the 2009 dataset. Correlations were examined across all genotypes, and also within subsets of genotypes separated by flowering time. Removing early-maturing genotypes from the analysis did not produce a radically different picture of trait-genotype associations.

There were several traits that had opposite correlations with DTI and grain yield. For example, maintenance of green canopy was positively associated with DTI, but negatively with irrigated grain yield. Similarly opposing effects were observed with irrigated stem dry mass at anthesis (positive with DTI, but negative with irrigated yield).

A large number of traits were assessed that showed highly significant genotypic variation, but showed little correlation with drought tolerance or yield. For example, the level of thiobarbituric acid reactive substances (TBARS) in droughted flag leaves varied between genotypes, but showed little relationship with performance measures such as DTI or droughted yield. This was surprising since elevated levels of TBARS, which reflects stress-induced damage to lipid membranes, has been associated with susceptibility to drought (HongBo, 2005; Guo *et al*., 2006).

Large matrices of phenotypic correlation coefficients can summarise the relationships between traits, but with large data sets, this is cumbersome, and does not include any information about the relationships between traits and genotypes. Multivariate statistical approaches such as genotype genotype x trait (GGT) biplots are powerful tools to summarise and condense these complex datasets, so that relationships between genotypes, traits and G x T interactions can be visualised in one plot (Yan and Kang, 2003; Ober *et al*., 2005). The biplot condenses the information into a small number of unique principle components that represent a greater proportion of the variance in the entire dataset. The advantage of the biplot is that correlations between individual traits and genotypes forming complex, multiple relationships can be visualized in one plot. A GGT biplot created from phenotypic data from droughted and irrigated experiments in 2008 revealed interesting relationships between genotypes and traits (Fig. 19). Varieties with good DT, such as Hobbit, Andalou and Solstice were clustered together, and at 180° opposite to varieties Mendel, Gladiator and Ambrosia. The trait DTI was clustered with maintenance of green canopy (because of positive correlation) and opposite to Delta (due to negative correlation).

The ideotype concept has been useful to guide physiology-based breeding, but provides only a qualitative description of trait combinations that should produce a superior genotype. A quantitative measure of the ideotype could be more powerful (Ober *et al*., 2010; Foulkes *et al*., 2011). The composite trait score combines trait values that show strong positive or negative associations with the target trait. The composite trait score computed for observations made in 2009 explained 48% of the variation in DTI (Fig. 20). The broad utility of the composite score will be proven if it describes well the DT of other populations in other environments.

Roots

Measurement of changes in soil moisture content in droughted plots allowed inferences to be made about the placement and activity of roots without the difficult physical work of excavating roots (Figs. 21, 22). There were significant differences between varieties in patterns of water uptake throughout the soil profile (Figs. 23), indicating differences in the placement and activity of roots. This is one of the few reports showing that UK wheat varieties differ in rooting patterns and in the ability to mine water from deep soil layers. It is interesting that there was a positive correlation between water extraction from depth and DT (Fig. 24). For instance, Xi19 had greater rates of water use at depth than Spark, which was also less drought tolerant than Xi19 (Fig. 25). Spark may invest more C into roots in shallow soil layers, which may benefit P uptake and perhaps provide better lodging resistance, but may be a disadvantage in dry conditions. A negative correlation with irrigated grain yields indicates a yield penalty associated with deep roots (Fig. 26).

Nevertheless, varieties with good yield potential show variation in water acquisition: an additional 10 mm water mined from deep soil layers can translate to 0.2 t ha^{-1} grain (assuming an average WUE of 0.02 t/ha/mm). The genotypic variation in root system architecture and patterns of soil water use suggest that there is sufficient useful genetic variation that progress can be made in improving these traits. The perennial challenge with all below-ground traits, however, is to develop screening methods that can be employed on a breeding scale.

Delta

Carbon isotope discrimination ratio (Delta) has been considered extensively as an indirect selection criterion for WUE. However, the relationship between yield, WUE and Delta depend a great deal on the evaporative demand and water supply of the environment in which the material is tested. In the Broom's Barn experiments, there was strong positive association between Delta measured in grain and yield (Fig. 27), and a negative correlation with DT (Fig. 28). Tested over three years, several varieties shown consistent contrasts for leaf and grain Delta (Fig. 29). Based on these data, a new mapping population (Garcia x Paragon) is being developed in order to map QTLs for Delta.

Water use efficiency

Water use efficiency (WUE) was estimated for a subset of 21 genotypes in 2007; however, because the trial was not drilled according to the plot plan, some of the soil moisture access tubes were placed in incorrect plots. Thus, some of the 21 genotypes did not have data from all three replicates, and additional genotypes outside this set were measured instead. Nevertheless, a set of genotypes were replicated, and differences in the computed WUE were observed (Fig. 30). Similar results were obtained in 2008 and 2009 (Fig. 31), although variety rankings changed from year to year. Across the three years, varietal differences were apparent, although not statistically significantly different (Fig. 32). The WUE was dominated by differences in yield, as differences in biomass and hence water use between the varieties were small. Hence, harvest index had the largest influence on varietal differences in WUE.

The estimated WUE derived from plot water use and yield produced values greater than other published values, and indeed greater than the general WUE expected from a C3 crop. In the literature, WUE reported for wheat range from 0.007 to 0.03 t grain ha^{-1} mm^{-1} or 0.7 to 3 g kg^{-1} H_2O (e.g. Misra *et al*., 2010). Estimates of WUE were derived from plants grown in large, in-field weighing lysimeters (the most accurate method) or derived from measurements of soil moisture extraction and crop-adjusted reference evapotranspiration rates, as done here. Low values of WUE are observed in low-yielding crops grown under high evaporative demand. WUE values also can be smaller for well-irrigated plants that consume larger amounts of water or in cases when evaporation or drainage from wet soil is underestimated (and considered instead to be water loss from the crop). In our experiments, once plots were covered, the soil surface dried quickly and thus soil evaporation and drainage were negligible. Thus, high values of estimated WUE are probably due to a combination of high yields (typical of small plot data) and underestimated water use in droughted plots. These possible sources of error notwithstanding, the WUE values are valuable as a relative comparison of variety differences in WUE.

There was good positive agreement between DTI and water use efficiency (WUE), indicating that these two targets can be improved simultaneously. Traits that showed the strongest association with WUE were flag leaf senescence (r = -0.72***), pre-anthesis green canopy cover (r = 0.69***) and stem WSC (r=0.57**). These associations also reflect the dominant influence of grain yield on WUE.

Three varieties (Alchemy, Consort, Zebedee) were grown in large (250 kg) weighing lysimeters to accurately determine WUE. There were no significant differences between varieties for WUE, yield, or any of the morpho-physiological traits. However, associations between the traits, measured on individual lysimeters, showed interesting relationships. For example, a negative correlation (as predicted from theory [Hall *et al*., 1994]) between WUE and Delta measured on flag leaves was shown (Appendix, Fig. 4). Values of crop water use over the growing season derived from soil moisture probe (Diviner) measurements compared favourably with those obtained gravimetrically (Appendix, Fig. 5). This result confirms that the Diviner probe can accurately estimate crop water use and corroborates its use in field measurements for estimating WUE.

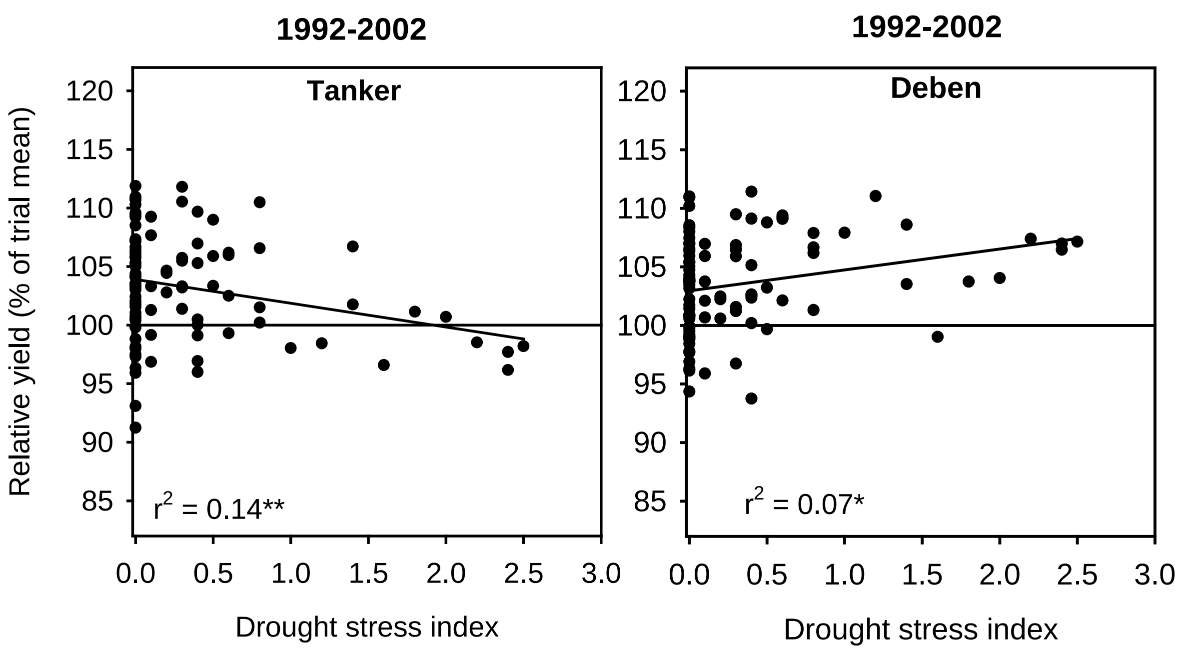


Fig. 1. Comparison of wheat variety yield performance across a range of environments increasing in drought stress. Data are from the HGCA RL Plus database; each point represents a trial between 1992-2002. The index was computed on the basis of rough approximations of soil type and interpolated weather data for the

region (Welham *et al*., 2005). A positive slope indicates yields better than average under drier conditions. A negative slope indicates greater drought susceptibility. Eight varieties (of 53) had significant slopes. Deben also showed greater stem WSC accumulation than Tanker. *,** = P < 0.05, 0.01 respectively.

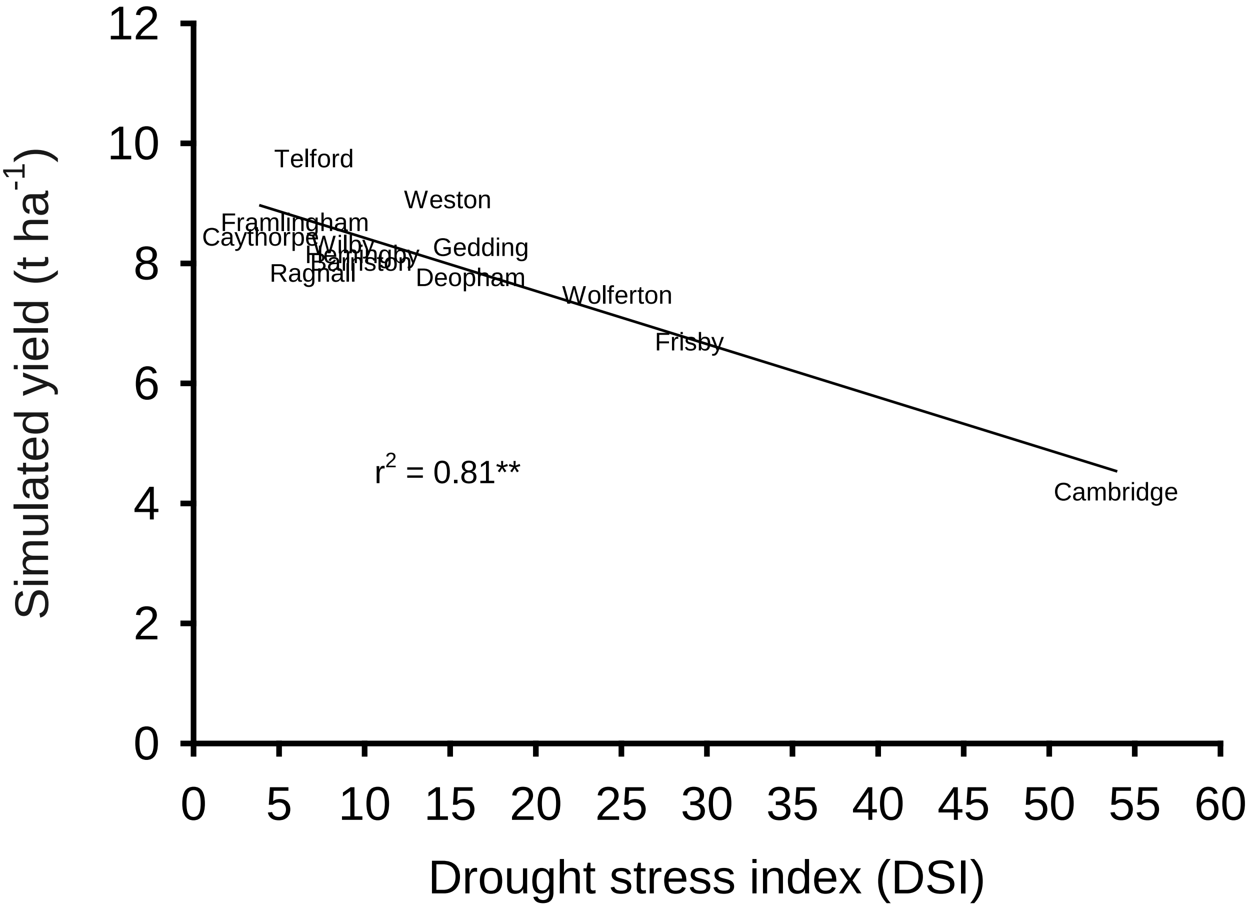


Fig. 2. Relationship between the simulated yield and the drought stress index (DSI) at selected RL sites in 2006 using Mercia as the modelled variety.

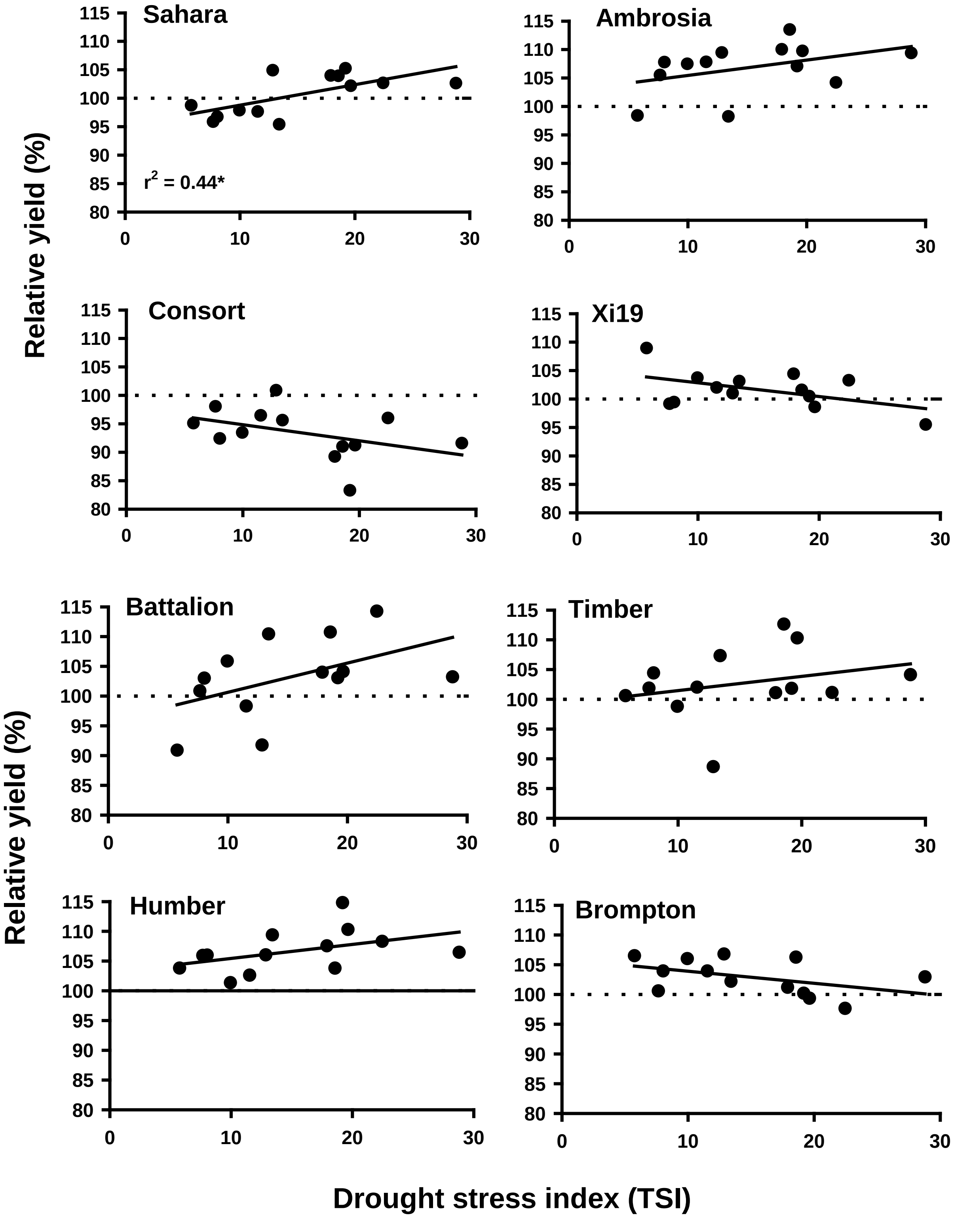


Fig. 3. Finlay-Wilkinson regressions of variety performance vs. the transpiration-based drought stress index based on RL trials in 2006. Only Sahara showed a statistically significant slope.

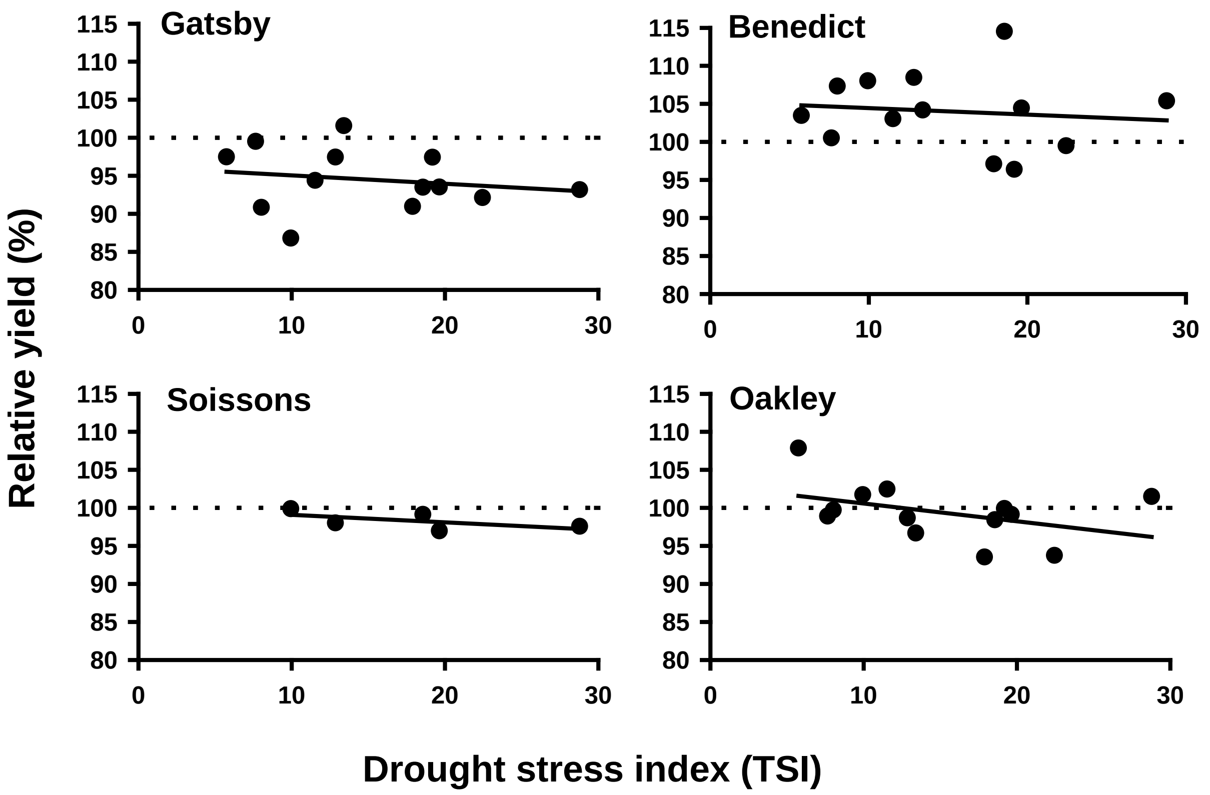


Fig. 3. continued. Finlay-Wilkinson regressions of variety performance vs. the transpiration-based drought stress index in the 2006 RL trials.

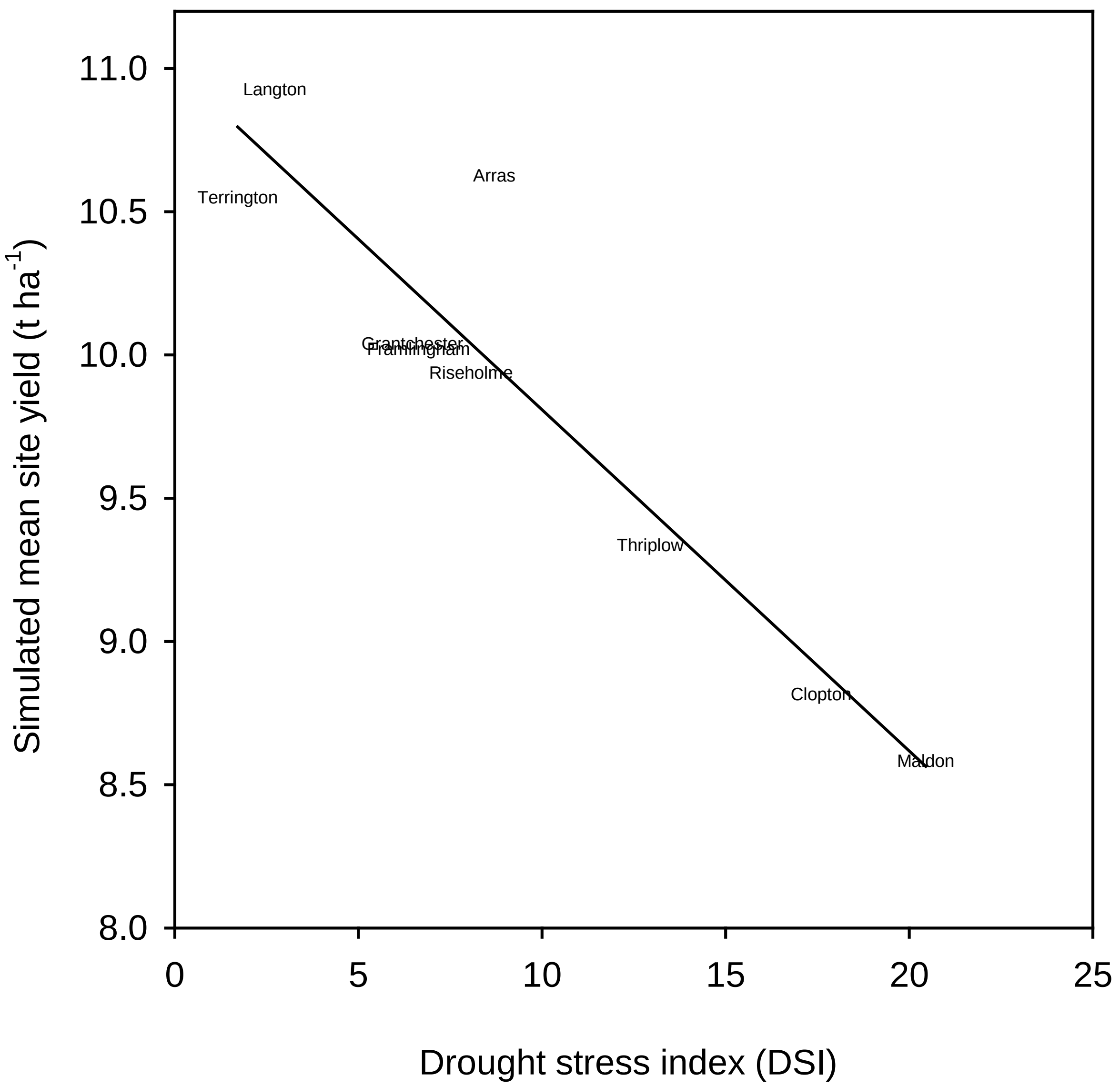
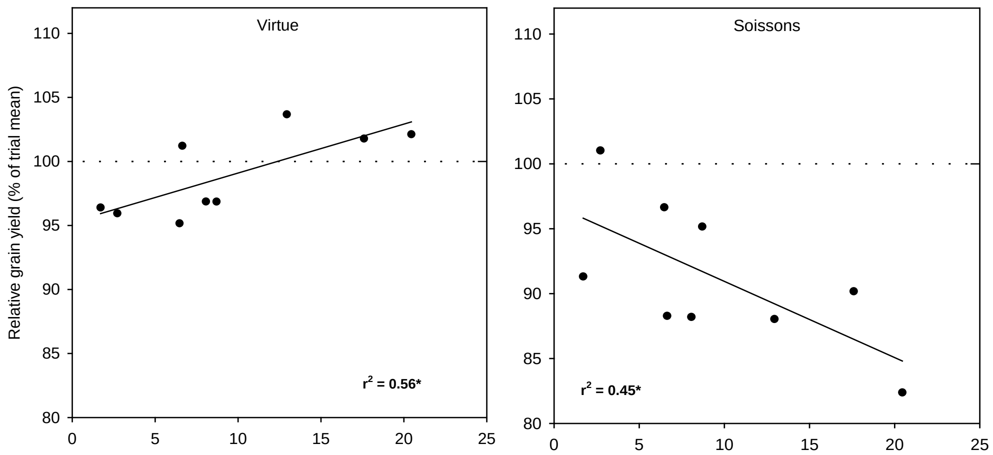


Fig. 4. Drought stress index and simulated yields of Mercia based on output from the Sirius wheat growth model for eight breeders' sites in 2009. Site-specific soil water holding capacity and rainfall (or from the nearest weather station) were used in the calculations.



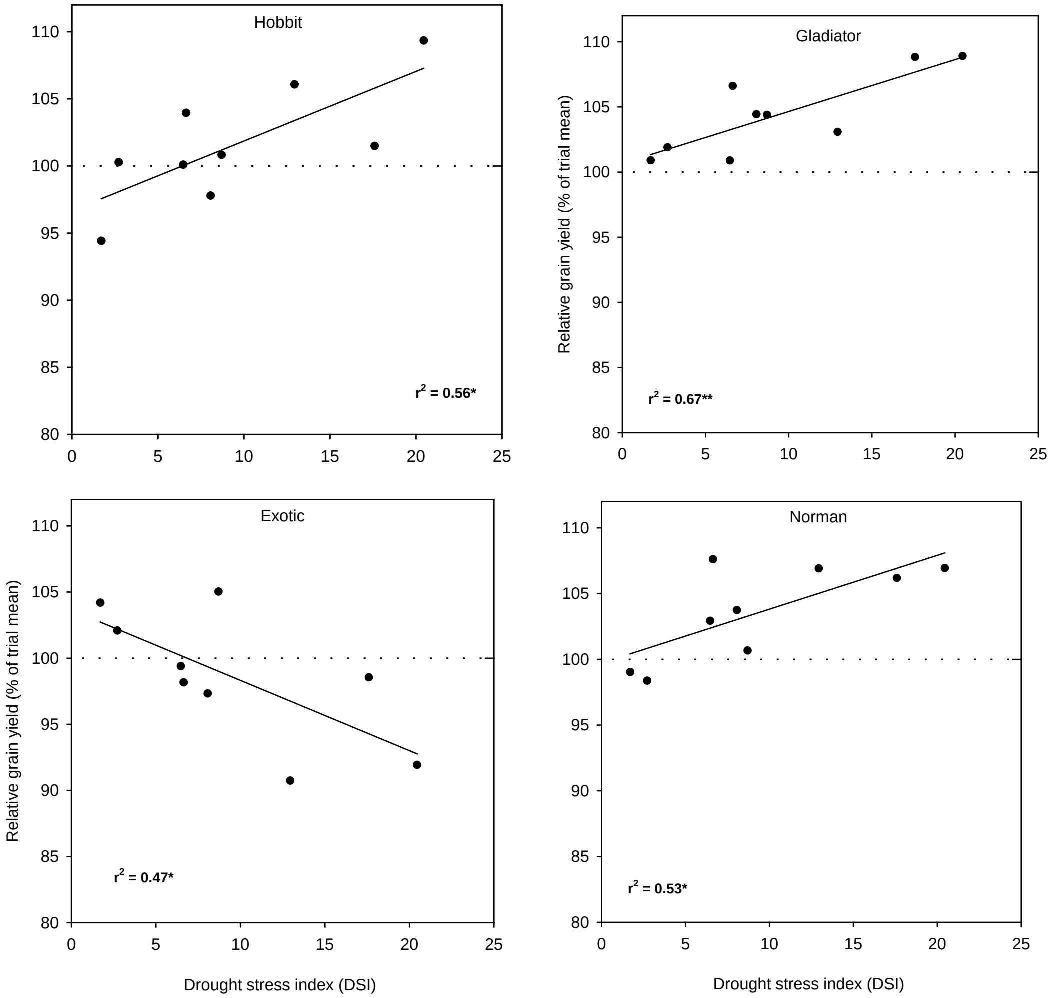


Fig. 5. Finlay-Wilkinson regressions of variety performance vs. the yield-based drought stress index of eight breeders' sites in 2009. Of 64 varieties tested, these six showed statistically significant slopes.

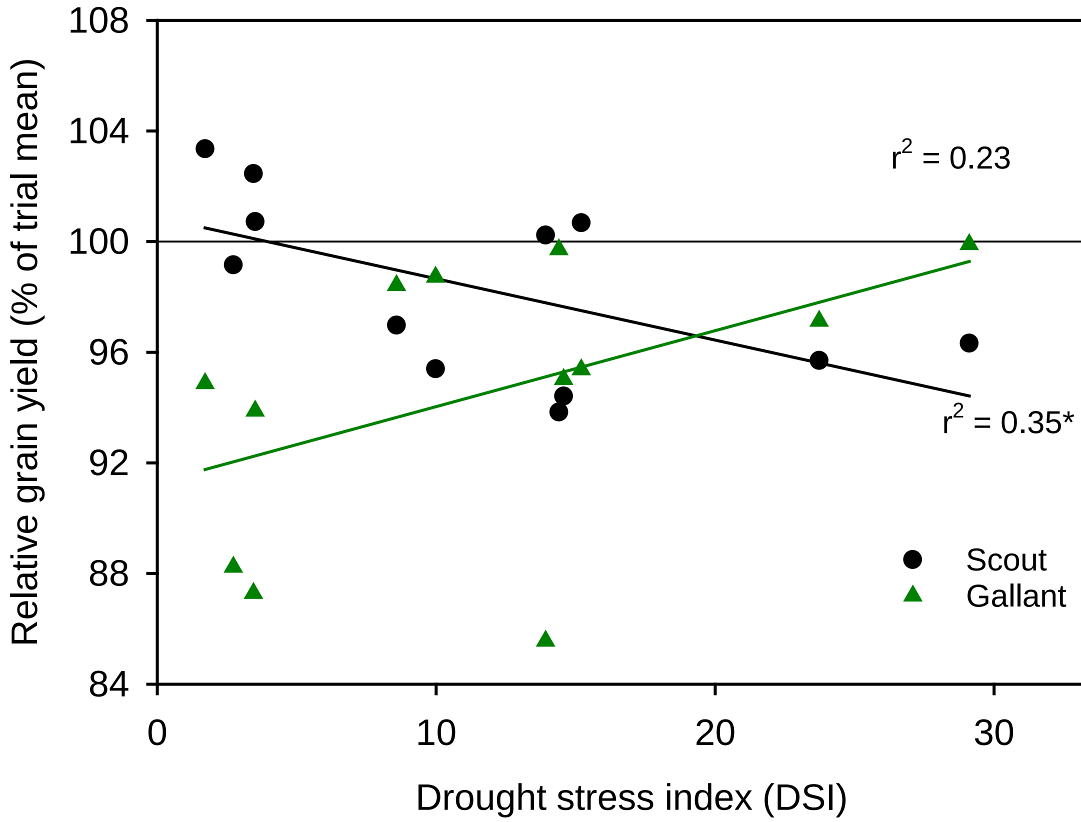
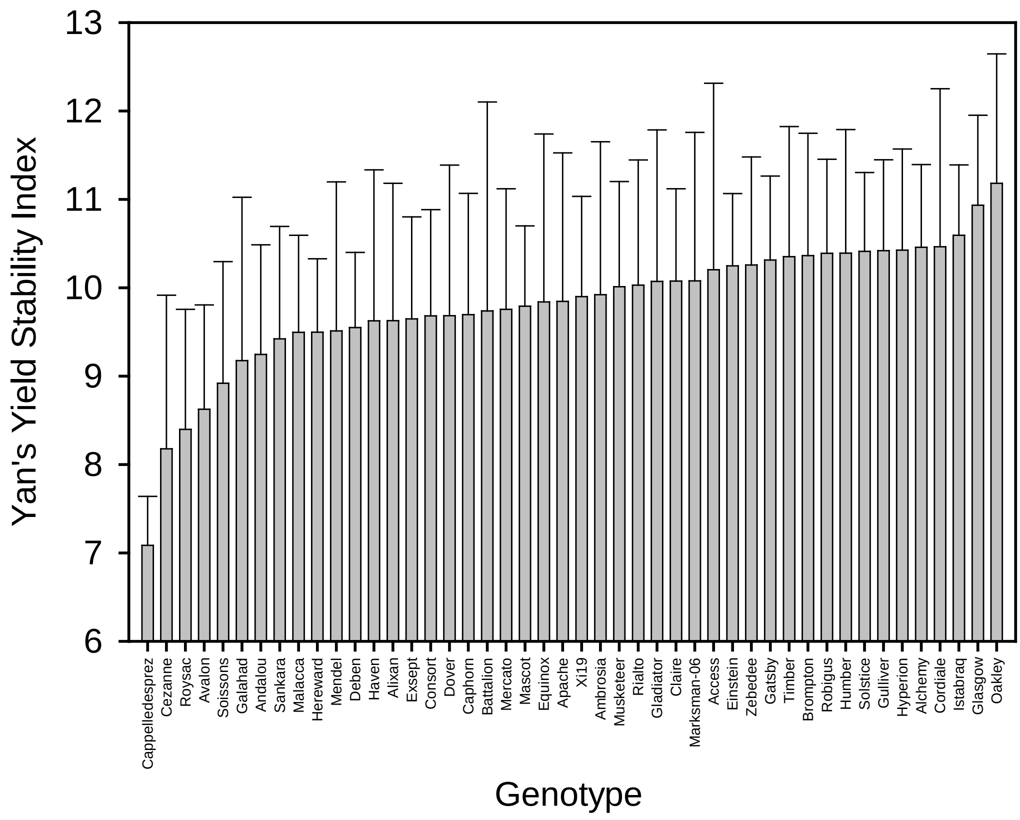


Fig. 6. Finlay-Wilkinson regressions of variety performance vs. the yield-based drought stress index of 12 RL sites in 2009. Of 33 varieties tested, only Scout showed a statistically significant slope. In contrast, Gallant showed a slight positive slope, but this was not statistically significant.



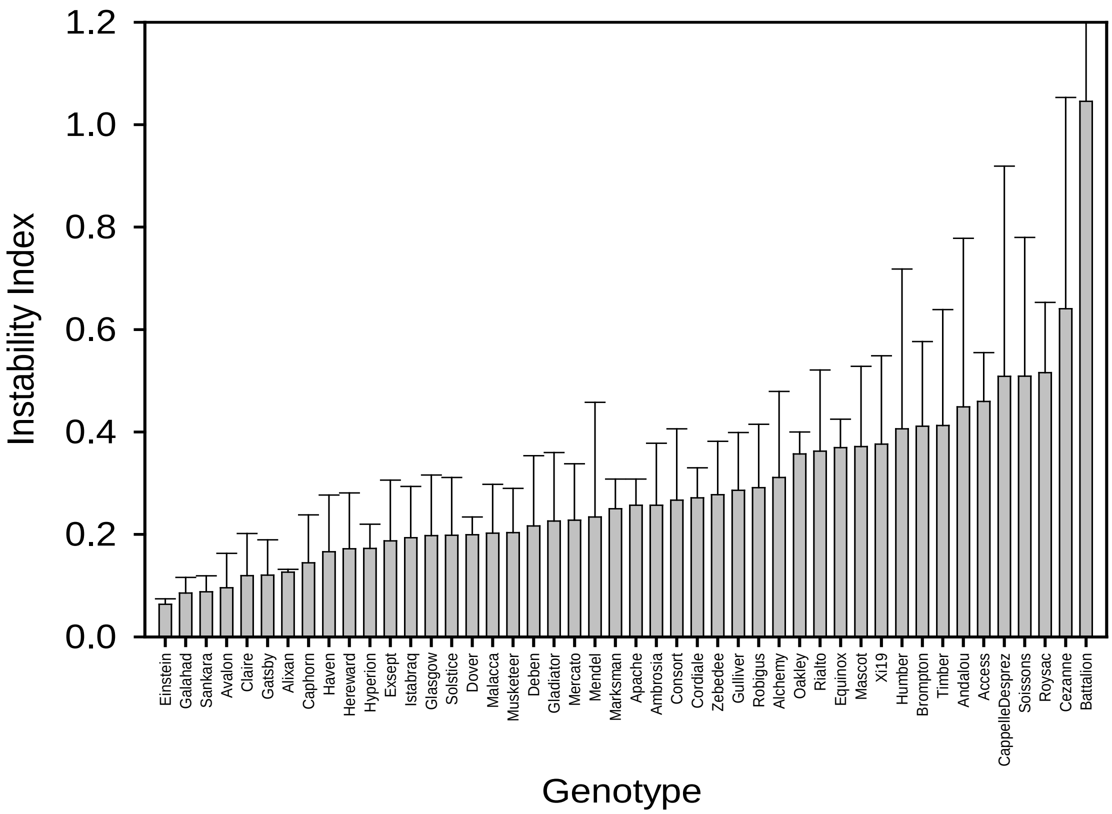


Fig. 7. Yield stability scores for varieties tested across eight breeders' sites computed for the years 2006-2008. Yan's stability index (top panel) combines yield stability and yield based on the GGE biplot distance for individual varieties. The instability index is related to stability alone (see text). Bars are mean ± se (n = 2-3; some varieties were not tested in 2006).

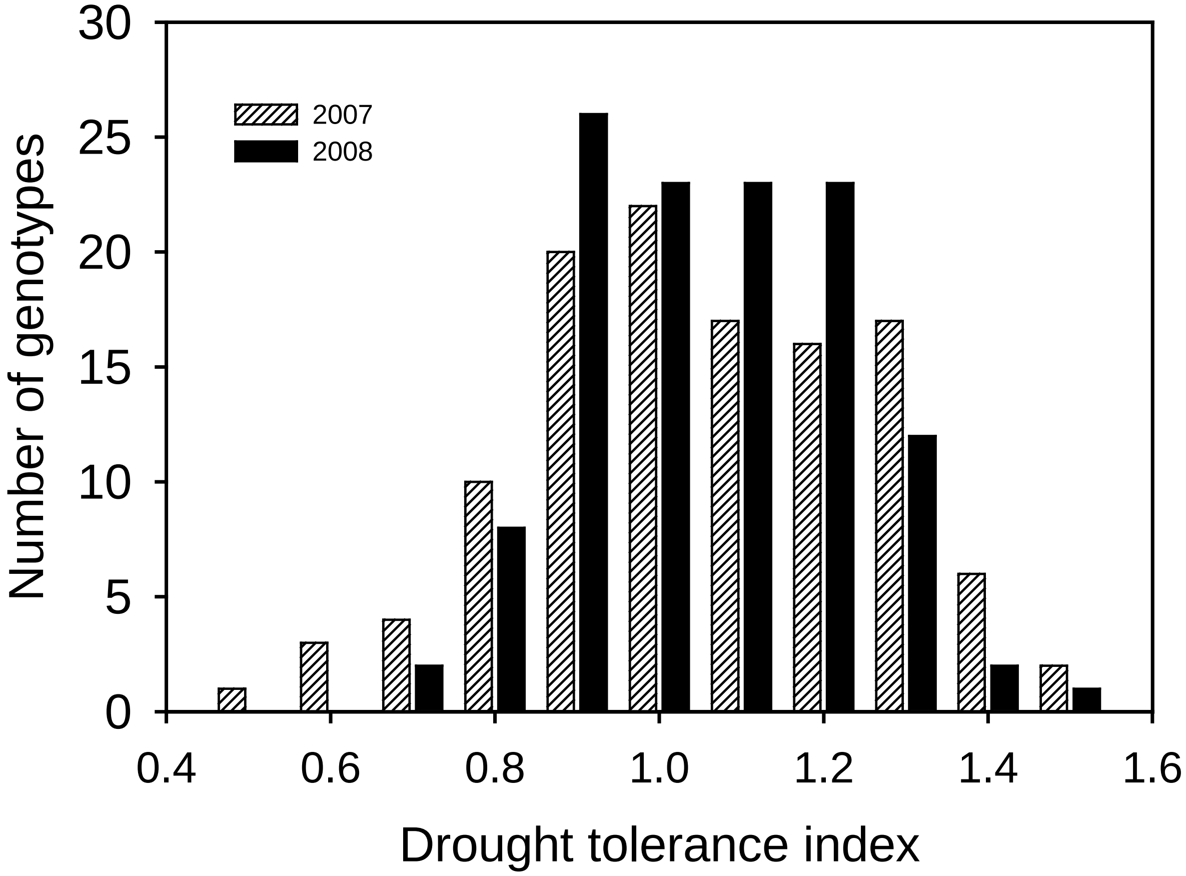


Fig. 8. Distribution of responses to drought among 120 wheat lines tested in 2007 and again in 2008 under managed drought and irrigated conditions in the field in at Broom's Barn ($LSD_{0.05}$ = 0.3). Drought tolerance indices greater than 1 indicate relatively greater drought tolerance than the trial mean.

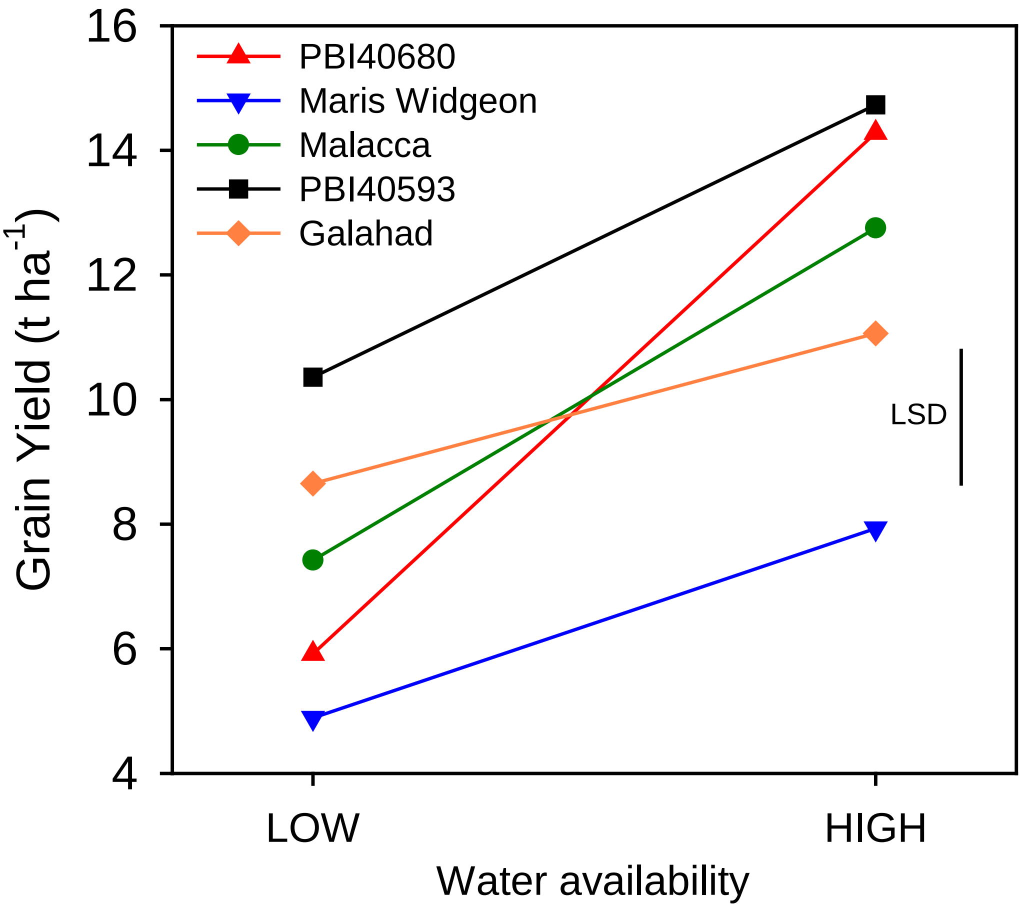


Fig. 9. Selected genotypes that illustrate crossover behaviour from the 2008 Broom's Barn experiments. The LSD bar represents the genotype x water regime interaction term from ANOVA. Genotypes that change rank *and* differ significantly at both water regimes is a rigorous test of crossover pairs (Sneller and Dombek, 1997; e.g. Galahad vs. PBI40680).

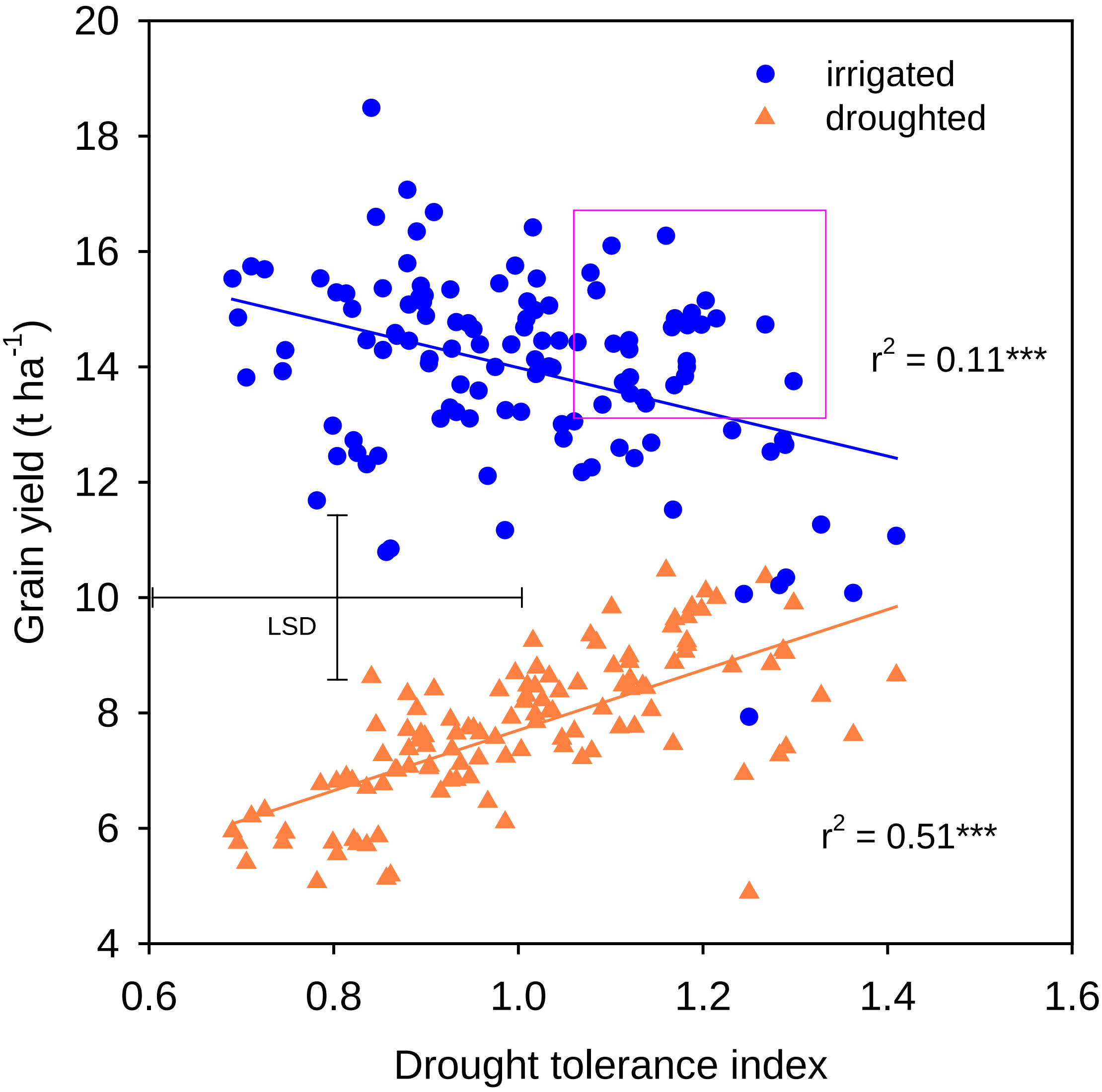


Fig. 10. Relationship between yields under irrigated and droughted conditions at Broom's Barn in 2008 and drought tolerance index (DTI). The LSD (P = 0.05) for the genotype x water regime interaction term is shown. The boxed area indicates genotypes with good yield potential plus better than average drought tolerance. Entries that fall above the regression lines are generally superior to those that fall below the regression lines.

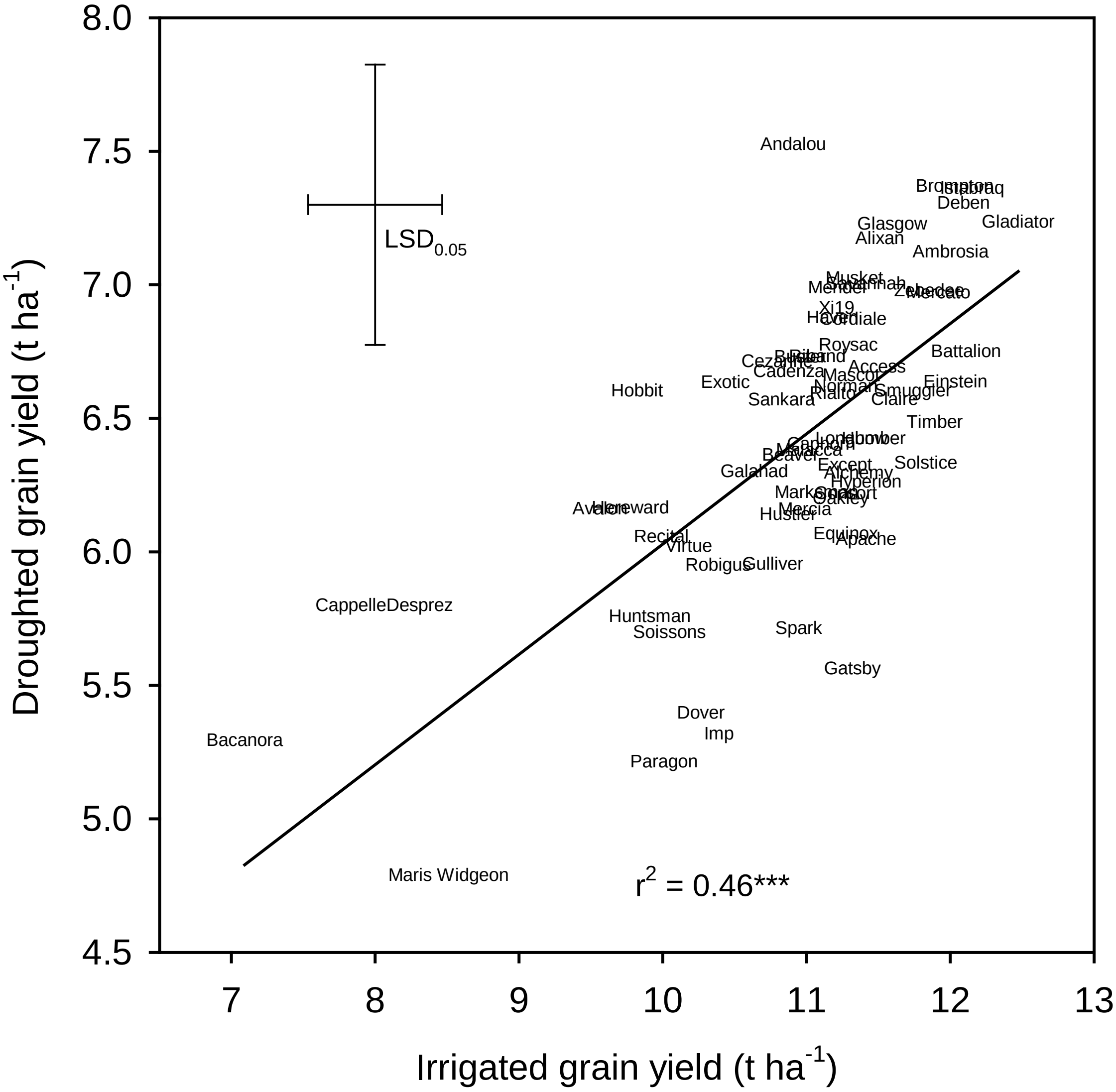


Fig. 11. Relationship between yields under irrigated and droughted conditions at Broom's Barn in 2009. The LSD (P = 0.05) is shown for both variates. Even though the correlation was significant, there was a large range in droughted yields (the vertical spread) of genotypes with above-average yield potential.

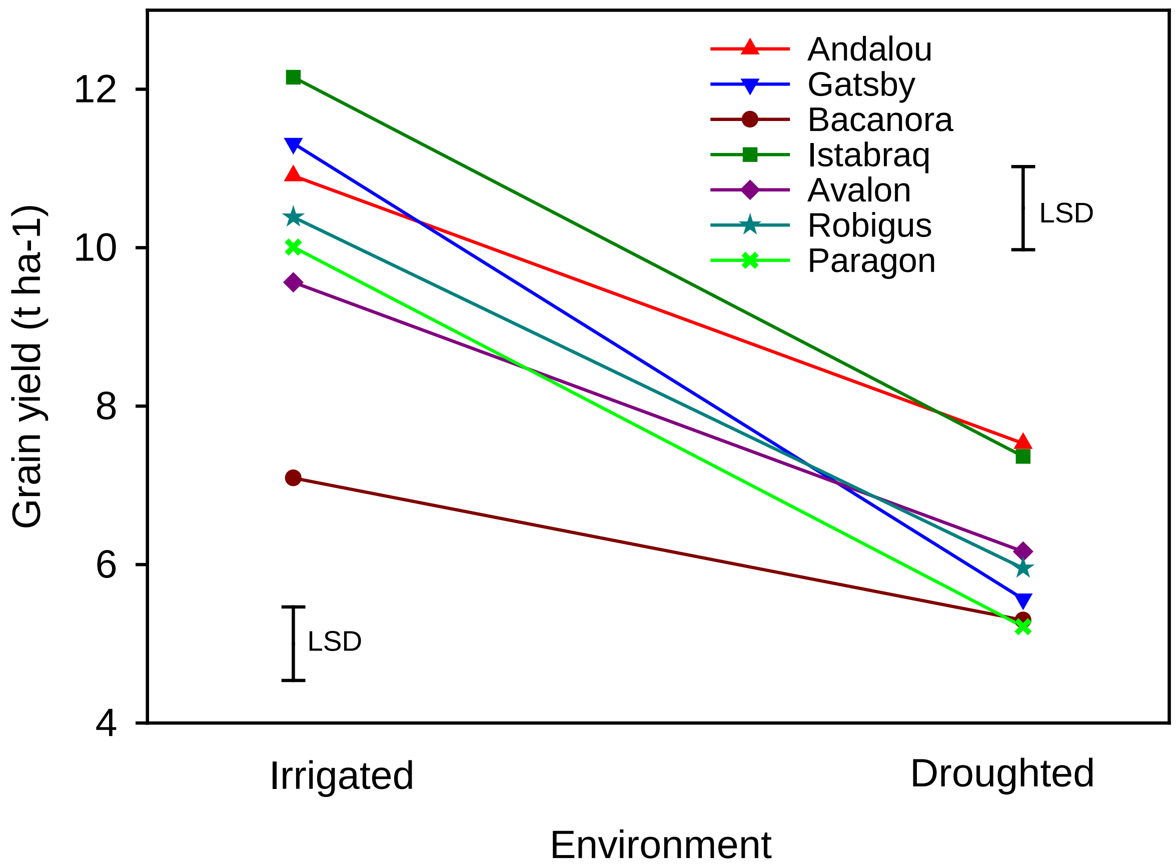


Fig. 12. Selected genotypes that illustrate crossover behaviour from the 2009 Broom's Barn experiments. The LSD (P = 0.05) from ANOVA are shown for each water regime. Note the change in ranking of Gatsby between environments: yield instability and drought susceptibility would not be known just by observing the high yield potential under optimum conditions. This contrasts with Istabraq, which was able to maintain a good ranking across environments, and thus was more stable. The shallow slope (yield stability) and drought tolerance of Bacanora is of little commercial interest because yields were low in both environments.

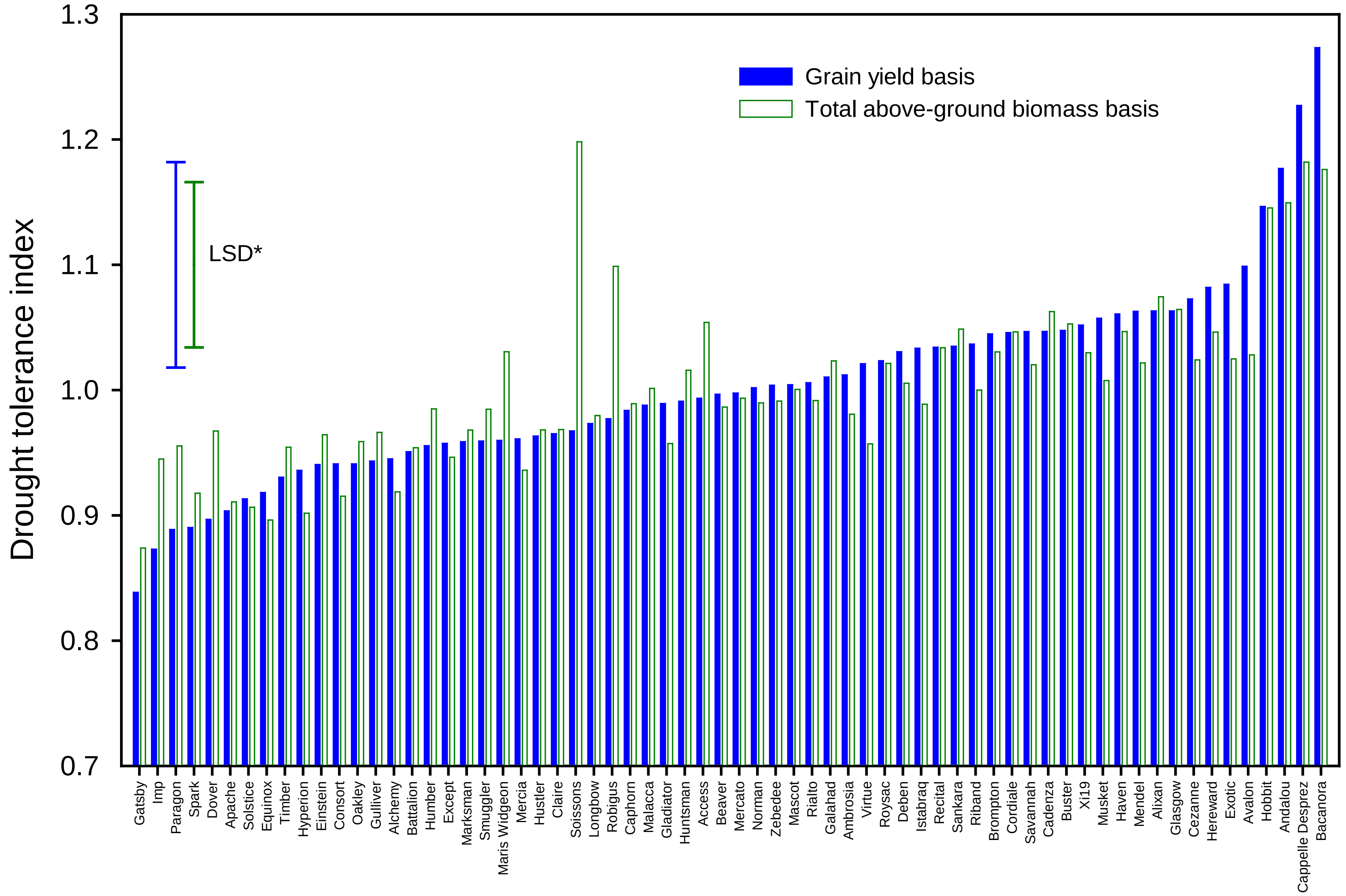


Fig. 13. Drought tolerance index (DTI) expressed on the basis of grain yield or above-ground biomass for lines tested at Broom's Barn in 2009. Note that in this experiment, certain lines were better able to maintain biomass under drought than grain yield, pointing to a greater sensitivity of grain formation to drought than biomass accumulation (e.g., Soissons had high ear number m^{-2}, but low grain mass per ear).

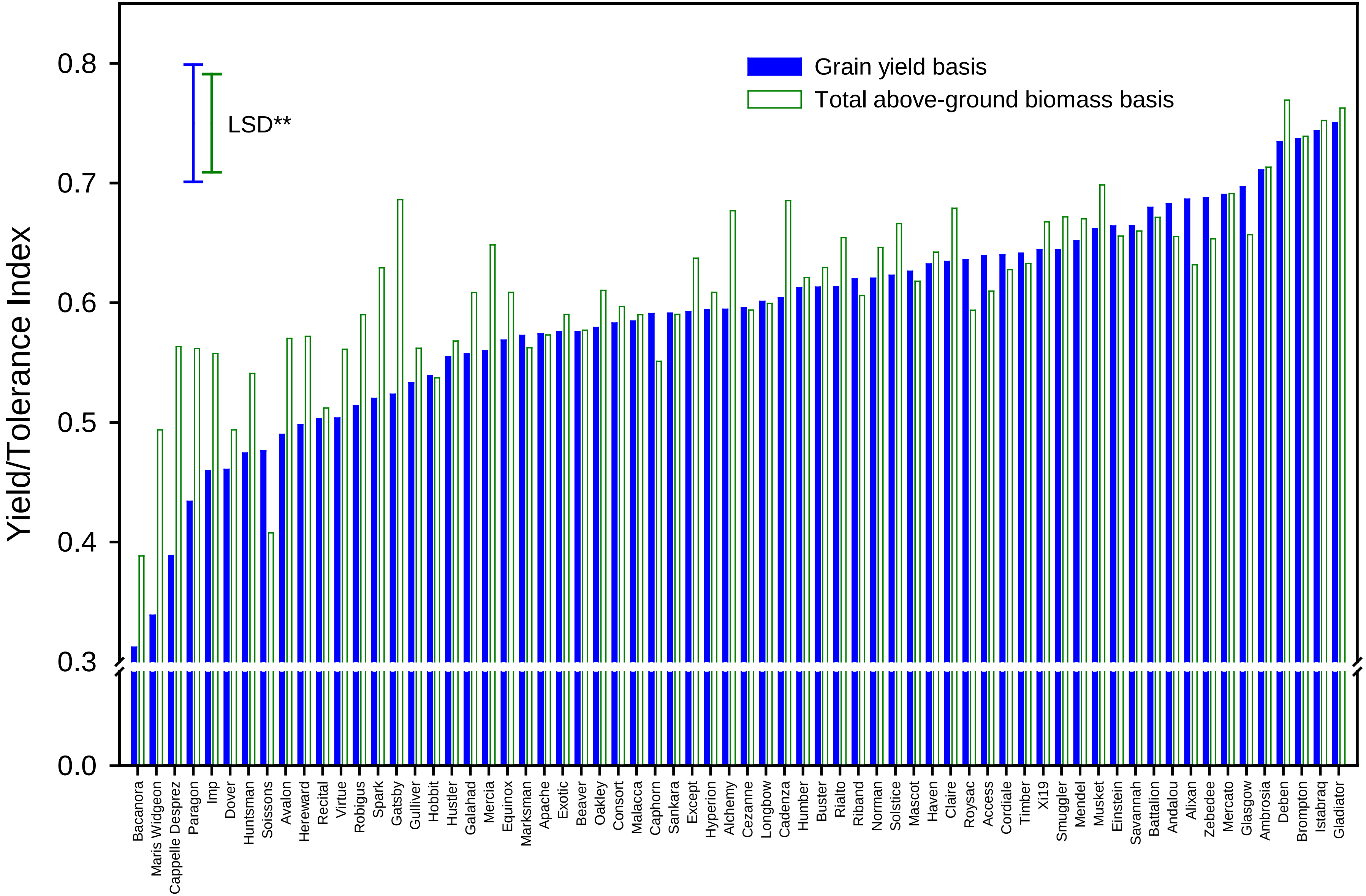


Fig. 14. Yield/tolerance index (YTI) expressed on the basis of grain yield or above-ground biomass for lines tested at Broom's Barn in 2009. In this experiment, the lines with large differences in YTI on the biomass and grain bases were usually associated with poor harvest index.

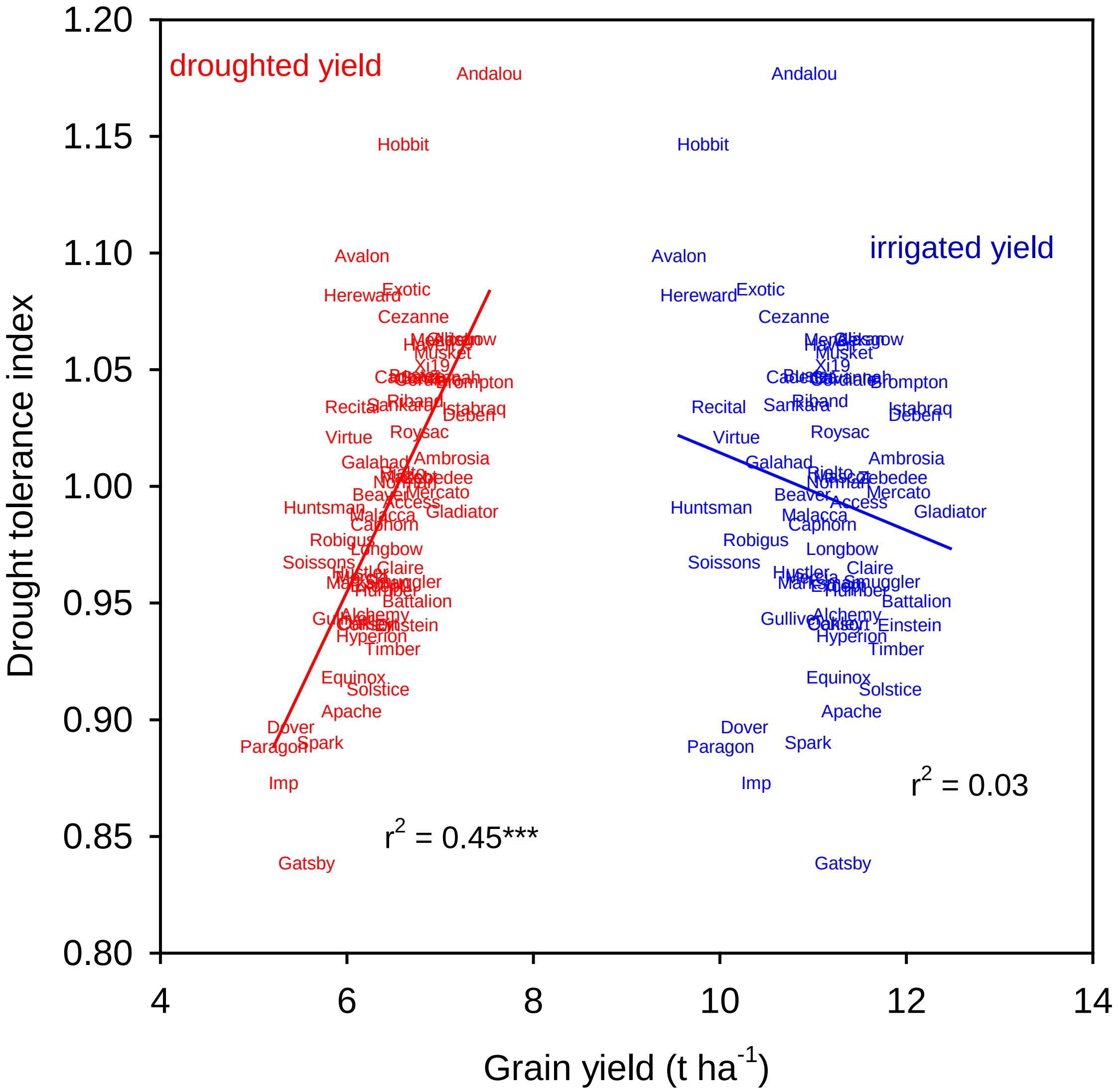


Fig. 15. Relationship between yields under irrigated and droughted conditions at Broom's Barn in 2009 and drought tolerance index (DTI). DTI was strongly influenced by yield under droughted conditions, with only a slight negative trade-off with yield potential (irrigated yield). Varieties that fall to the right side of the Figure and above the regression line fitted to irrigated yields have the best combination of drought tolerance and yield potential.

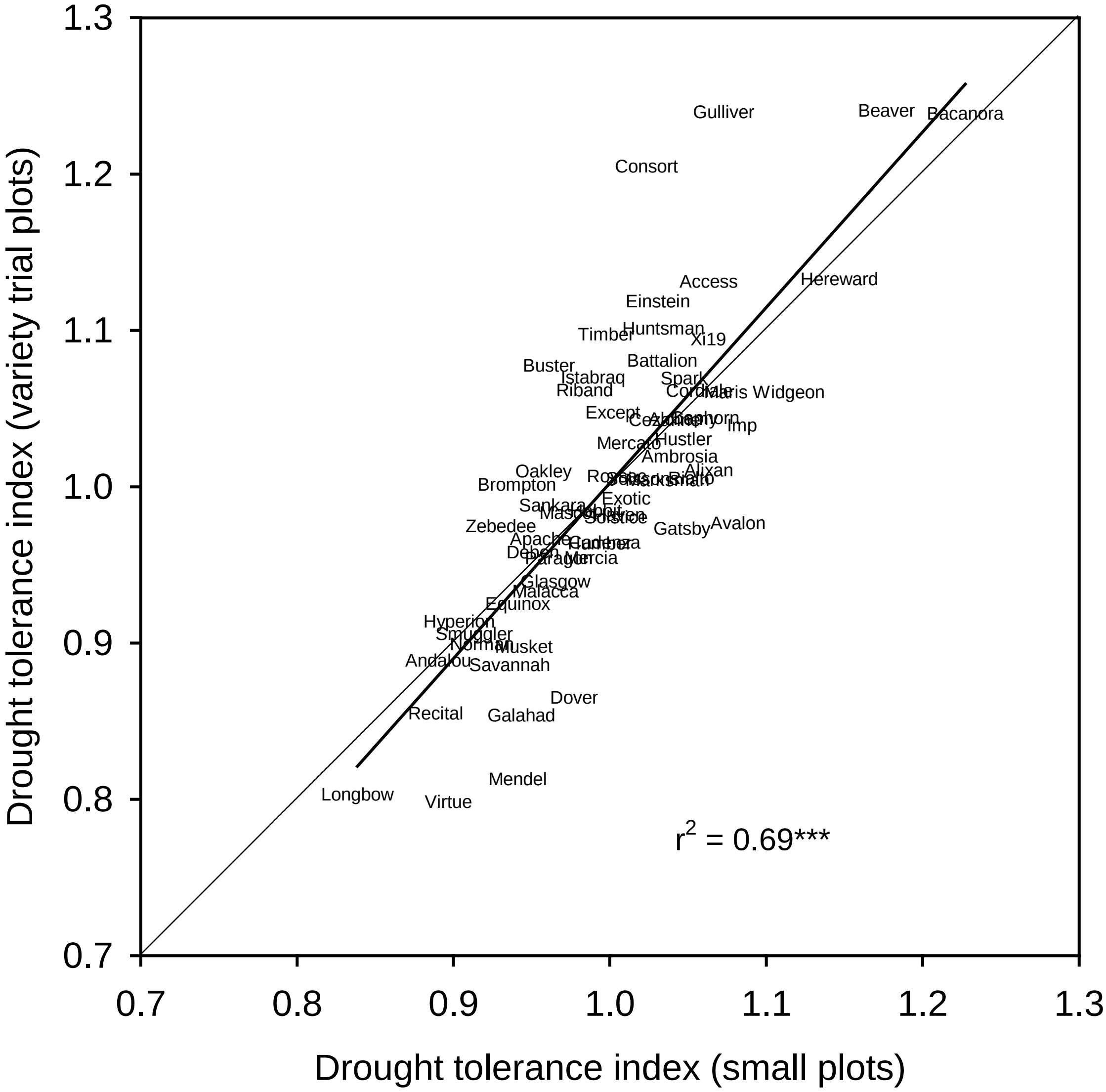


Fig. 16. Drought tolerance index (DTI) in 2009 calculated using different denominators of yield potential, based on irrigated yields obtained at Broom's Barn in small experimental plots, or using the yield of a variety averaged across all breeders' UK trials. Good agreement between the methods indicates that DTI based on the yield potential of irrigated experimental plots was sufficiently accurate. The 1:1 line is shown for reference.

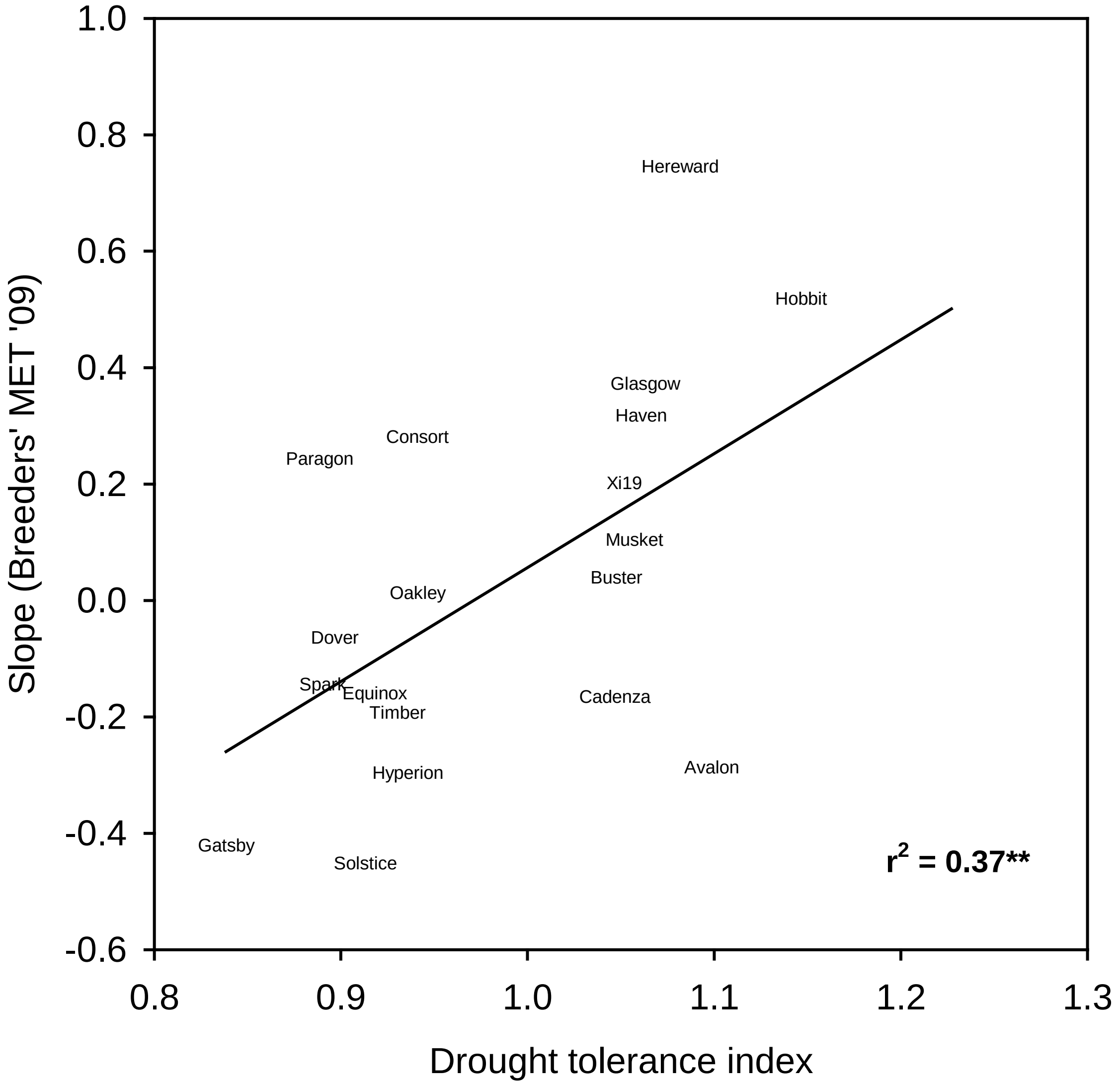


Fig. 17. The association between the top- and bottom-ranked varieties for DTI derived from experiments under managed drought conditions at Broom's Barn in 2009, and the slopes computed from yields across breeders' multi-environment trial (MET) sites in 2009.

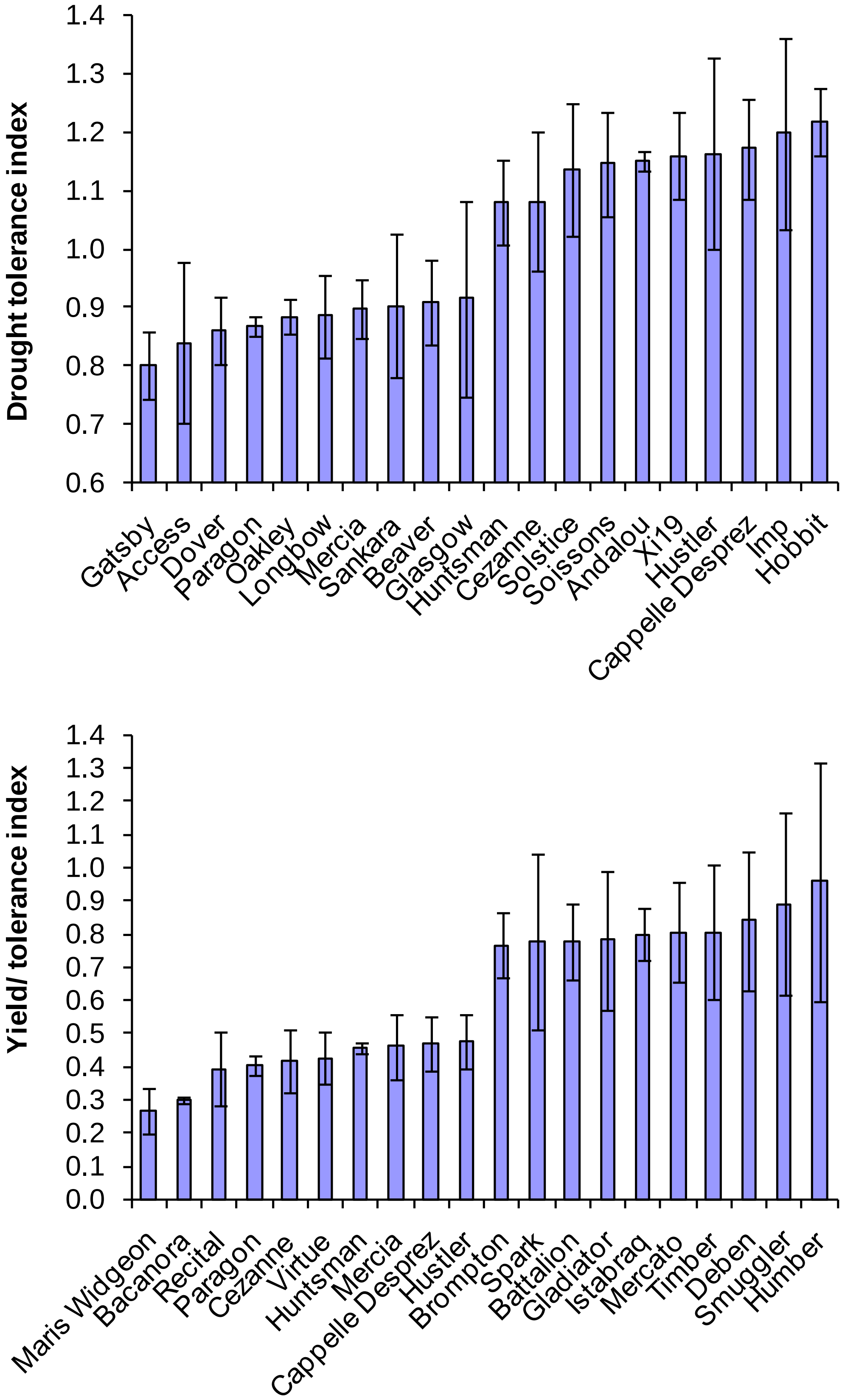


Fig. 18. Drought tolerance index (DTI) and YTI of varieties tested across three years at Broom's Barn. The 10 varieties with the smallest and largest mean scores are shown. The repeatability of the indices indicates that these variety subsets could reliably be used to further dissect mechanisms and traits related to drought tolerance. Bars are mean ± se (n = 3).

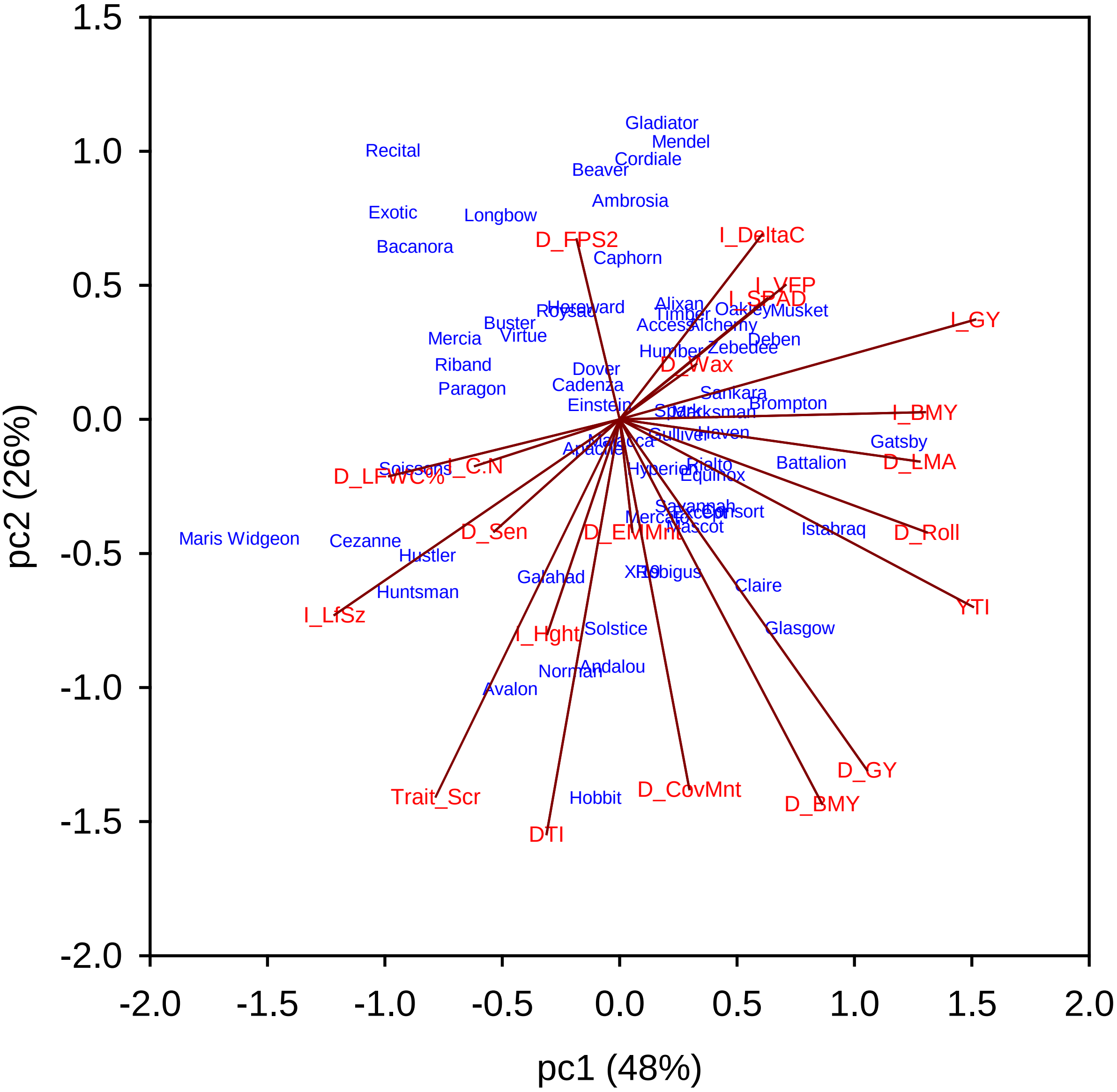


Fig. 19. A genotype x trait biplot based on yield and morpho-physiological traits measured in the 2008 experiment. The first two principal components are plotted, each accounting for a proportion of the variance in the original dataset, shown in parentheses. Genotypes (in blue) are plotted according to scores on each principal component, and traits (in red) are plotted on the basis of the eigenvectors (loadings) on each principal component. For a guide on full interpretation of GGT biplots, see Yan and Kang (2003) and Ober *et al*. (2005). Trait abbreviations: CovMnt, the proportion of green canopy cover in irrigated plots maintained under drought, measured at anthesis; BMY, biomass yield; GY, grain yield; Roll, leaf rolling score; LMA, leaf mass:area ratio; SPAD, flag leaf chlorophyll content; Wax, leaf wax score; VFP, viscous flow porometer reading (inversely related to transpiration rate); Delta C, carbon isotope discrimination ratio in flag leaves at anthesis; FPS2, photosynthetic efficiency; C:N, flag leaf C:N ratio; LFWC%, flag leaf water content at anthesis; Sen, canopy senescence score; LfSz, projected area of flag leaf. Height, stem height; EMMnt, proportion of ear dry mass at anthesis in irrigated plots maintained in droughted plots; TraitScr, standardised composite trait score (see Fig. 20). Trait prefix ‘D_’ denotes measurements made on droughted plots; ‘I_’ for irrigated plots.

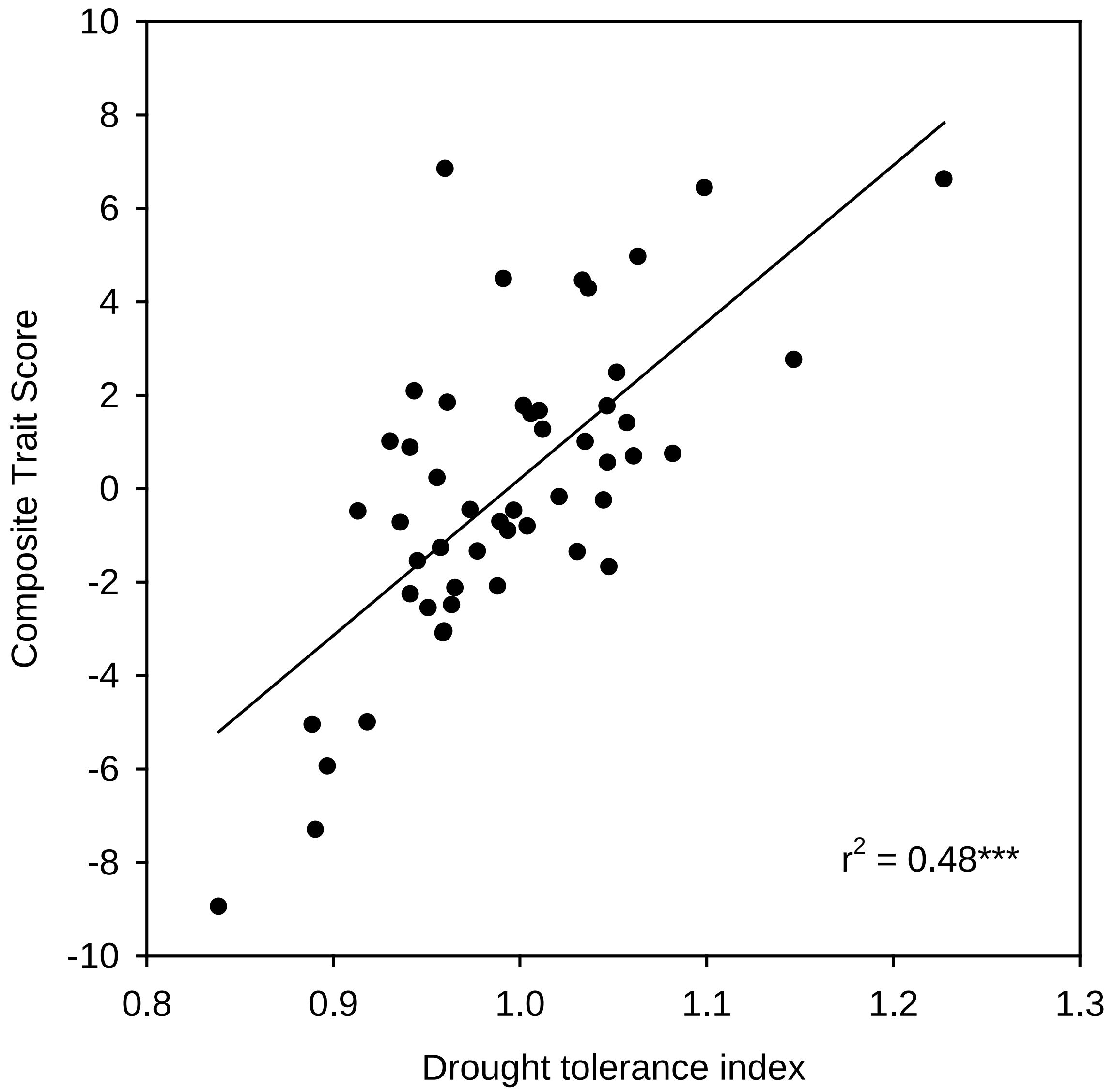


Fig. 20. The DTI from 2009 plotted against a composite trait score derived from summed and standardised values for: flag leaf Delta (irrigated)*; the proportion of irrigated green canopy maintained under drought at anthesis; stem dry matter at anthesis (irrigated), flag leaf senescence*; flag leaf mass:area (irrigated LMA); stem water-soluble carbohydrate concentration at anthesis (droughted). *The sign of traits with negative correlation with DTI was inverted before summing. The composite trait score, which explains 48% of the variation in DTI, compiles these traits into one value and provides a quantitative measure of a complex ideotype for dry conditions. The broad utility of the composite score will be proven if it describes well the DT of other populations in other environments.

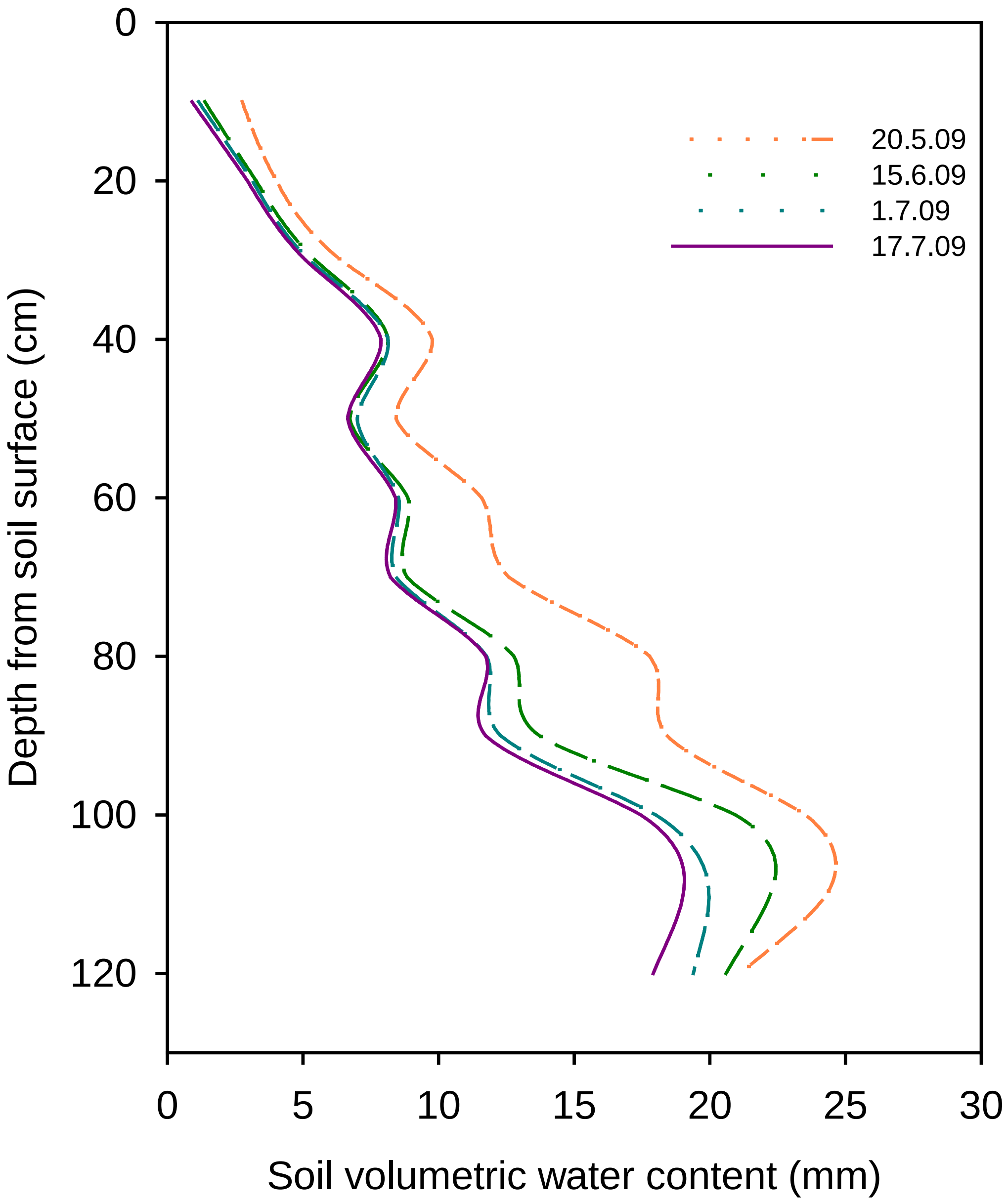


Fig. 21. Soil volumetric water content measured every 10 cm down the soil profile on four dates. Data are from an individual plot of Gladiator grown under droughted conditions in 2009. Lines are spline curves fitted to datapoints (SigmaPlot software).

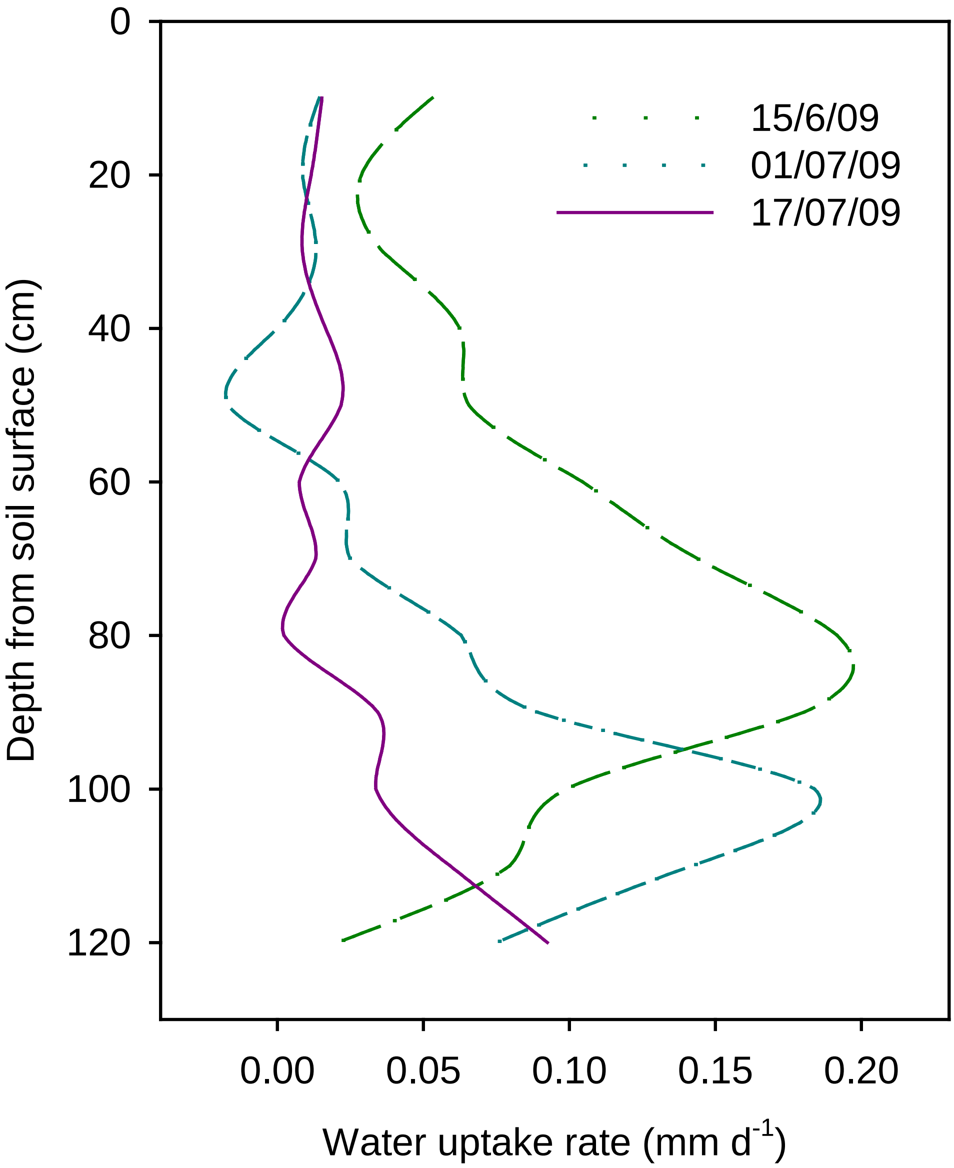


Fig. 22. Rates of soil water uptake computed from changes in soil moisture content between measurement dates. Data are from a single plot of Gladiator grown under droughted conditions in 2009 (see Fig. 21). The peak in water use should indicate the depth at which there is the greatest root activity, which moved from 80 cm on 15 June to 100 cm on 1 July. Water use in upper soil layers decreased as available soil water was exhausted.

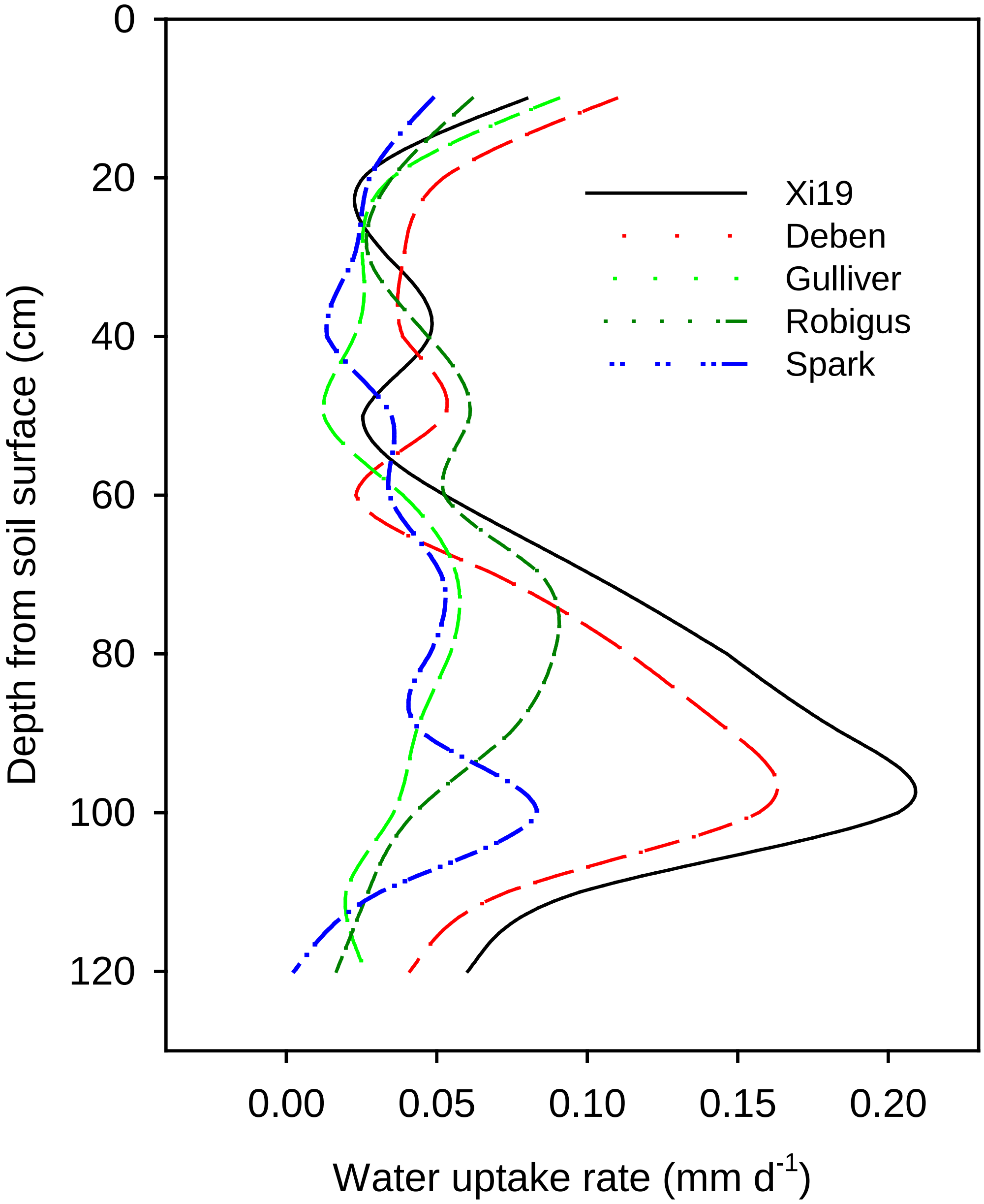


Fig. 23. Rates of soil water uptake computed from changes in soil moisture content between measurement dates in droughted plots in 2009 (see Fig. 22). Data are means (n = 4) for each variety. Xi19 and Deben showed greater rates of water uptake from deep soil layers than the other varieties. Xi19 and Deben also showed consistently greater DTI and YTI values (Fig. 18).

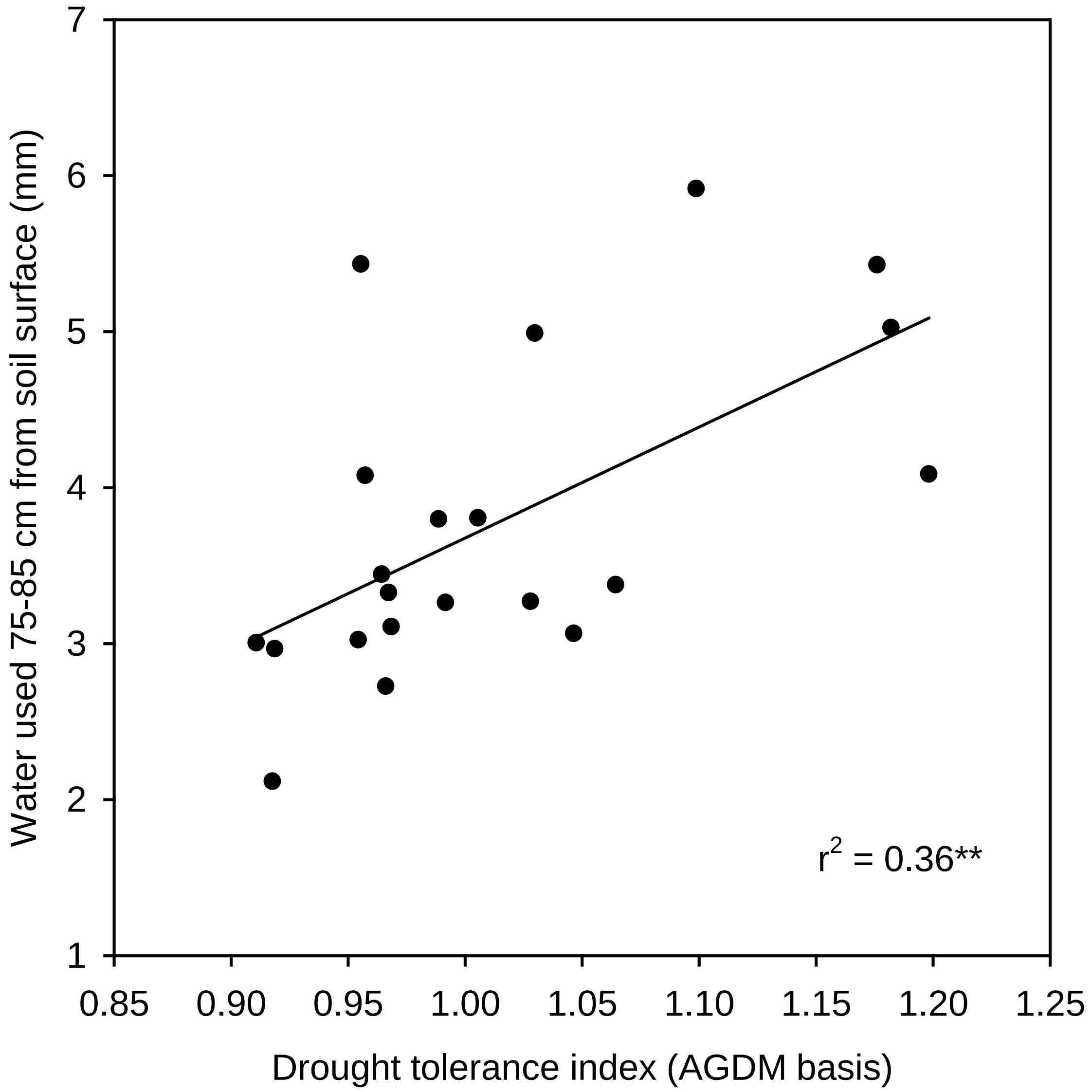


Fig. 24. Correlation between DTI computed on an above-ground dry matter (AGDM) basis and seasonal water use in droughted plots extracted from the soil layer centred at 80 cm from the soil surface. Each point represents the mean value for each variety (n = 4).

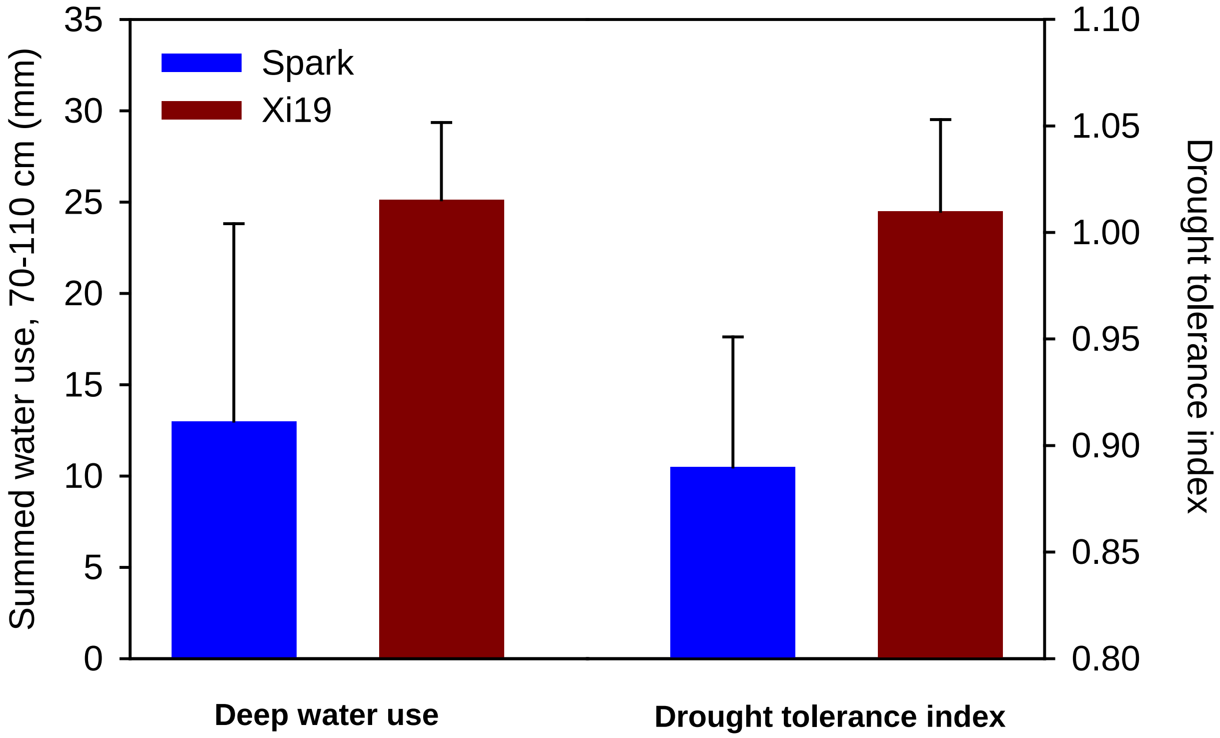


Fig. 25. Compared with Xi19, Spark showed less water use from deep soil layers summed over the season in droughted plots in 2009. Spark also had smaller droughted yields (5.7 t ha^{-1}) than Xi19 (6.9 t ha^{-1}) and smaller DTI. Bars are means ± se (n = 4).

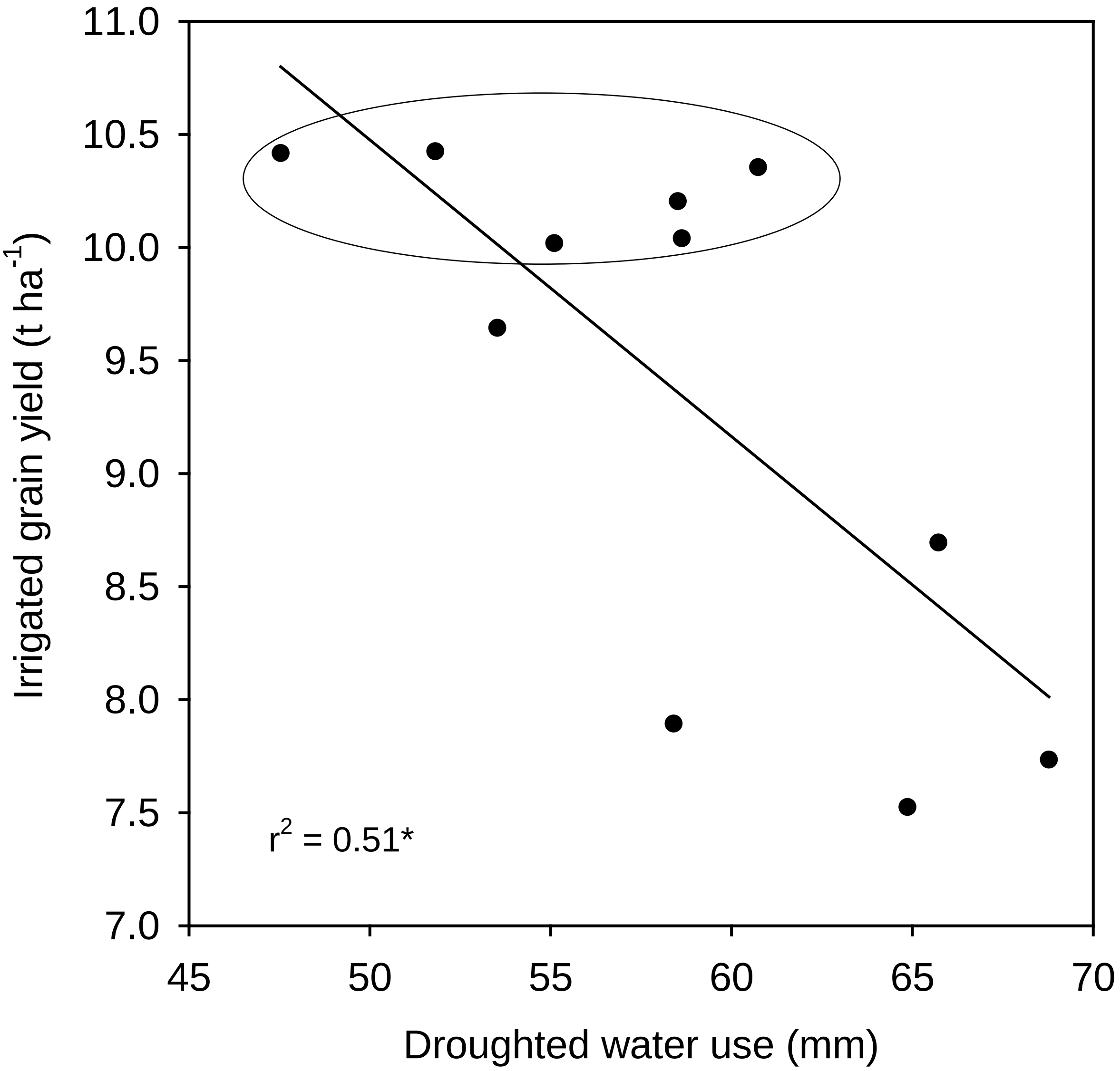


Fig. 26. The negative correlation between irrigated yield potential and water used summed over the period plots were covered. The varieties within the oval indicate that within a range of high yield, there was variation for water use; i.e., some varieties could mine more water from the soil without a penalty in yield. Data points are the mean values (n = 3) of genotypes measured under managed drought conditions in 2007.

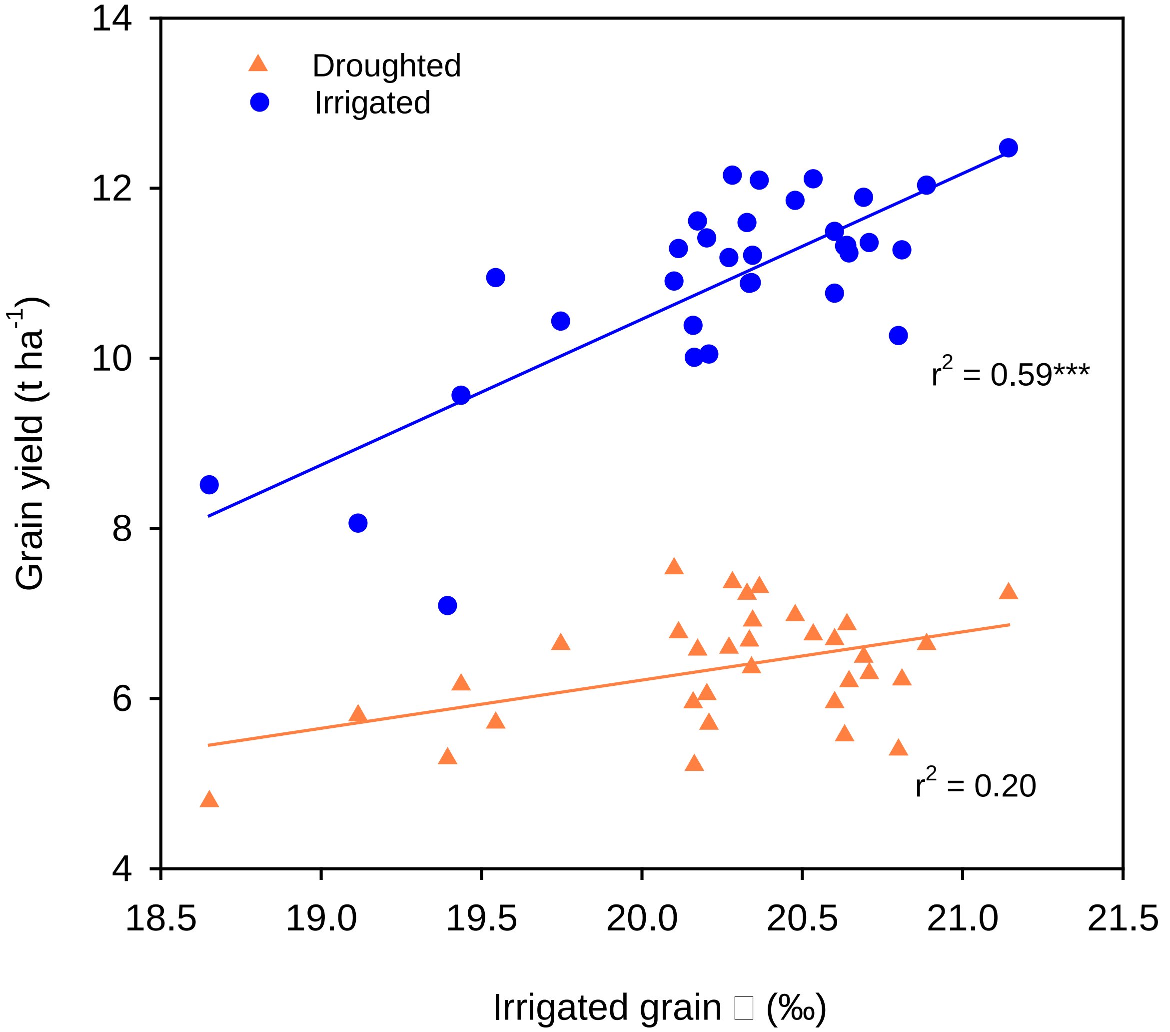


Fig. 27. The relationship between carbon isotope discrimination ratio (Delta, Δ) measured in grain harvested from irrigated plots, and grain yield under irrigated and droughted conditions.

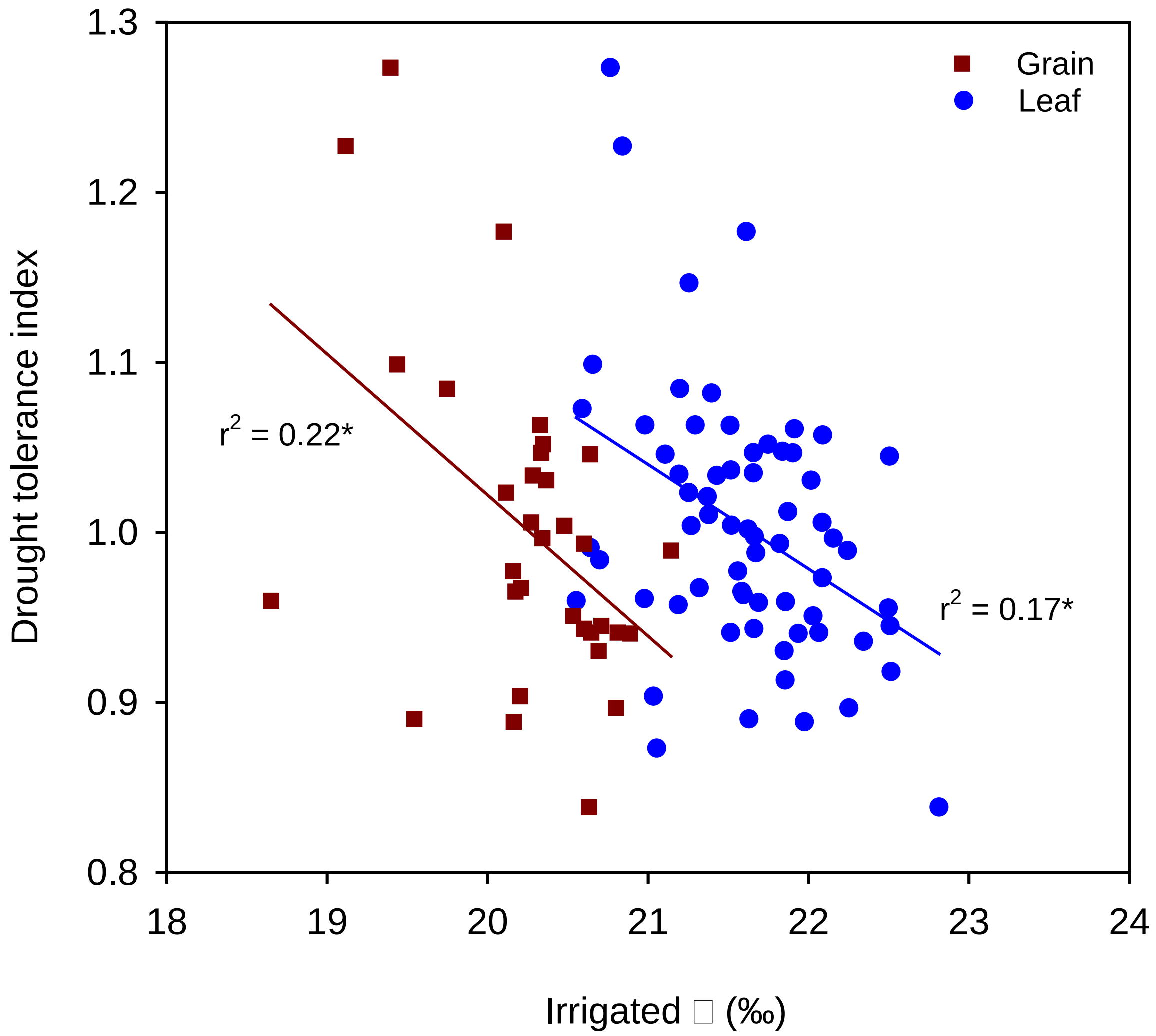


Fig. 28. The relationship between carbon isotope discrimination ratio (Delta, Δ) measured in flag leaves at anthesis and grain harvested from irrigated plots, and drought tolerance index.

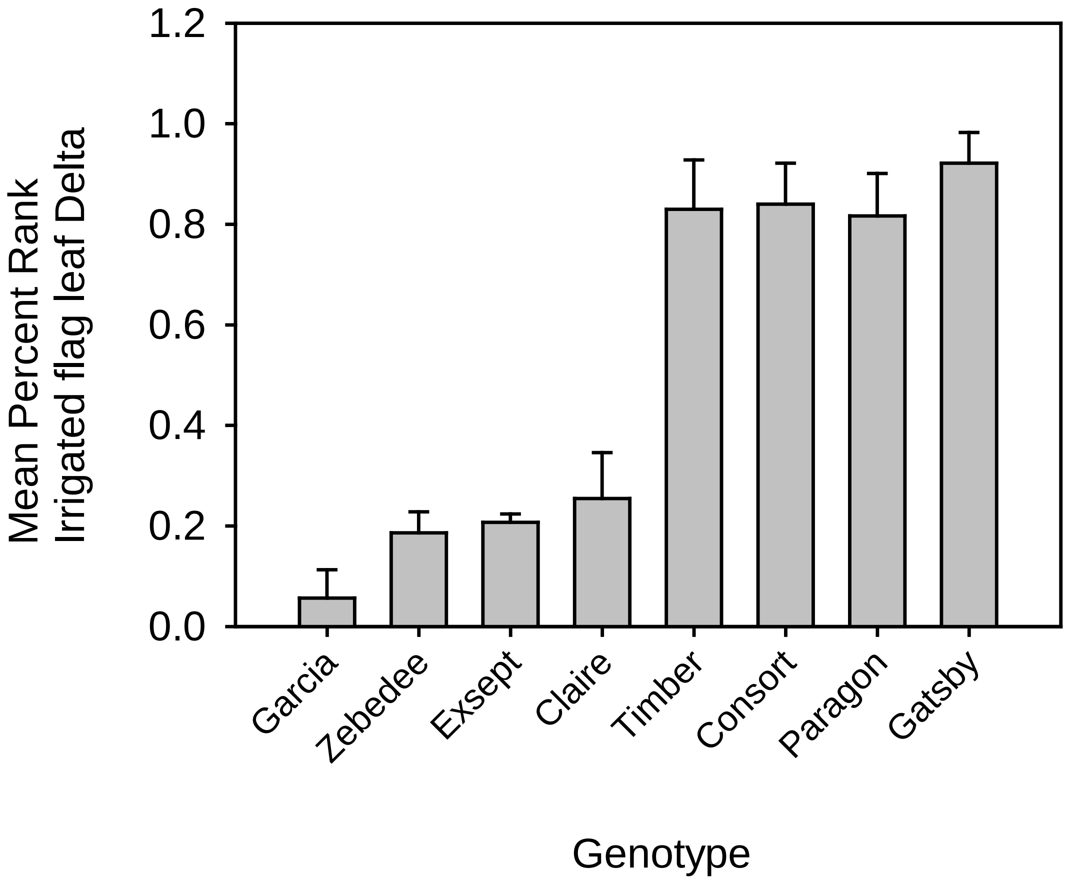


Fig. 29. Four varieties that showed consistently low values for carbon isotope discrimination ratio (Delta, Δ) measured in flag leaves at anthesis, and four that showed consistently high values. Bars are the mean of the percentage rank values calculated in each year, 2007-2009 ± se (n = 3).

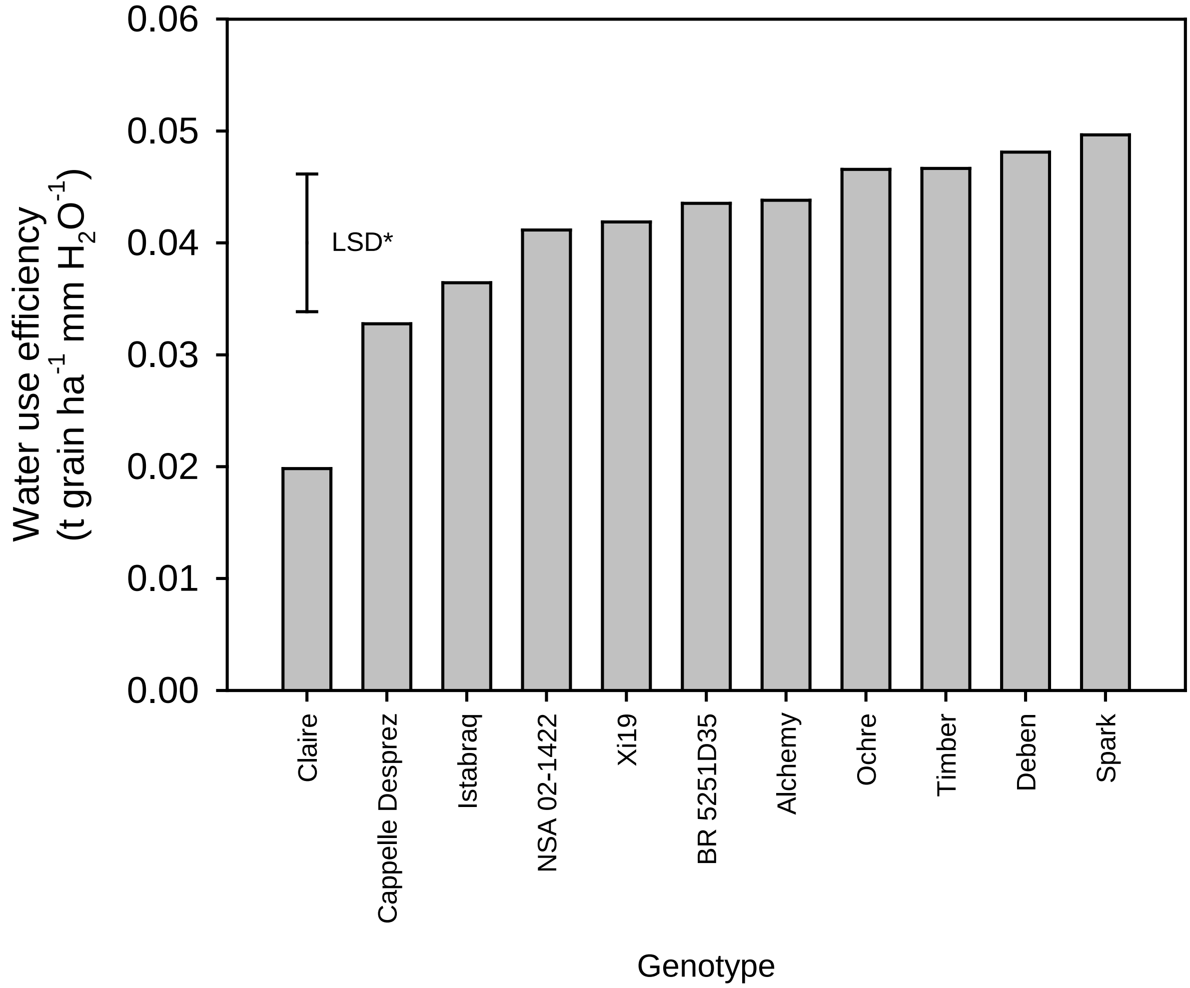


Fig. 30. Water use efficiency of replicated varieties measured under droughted conditions in 2007. The LSD (P = 0.05) for the genotype effect is shown.

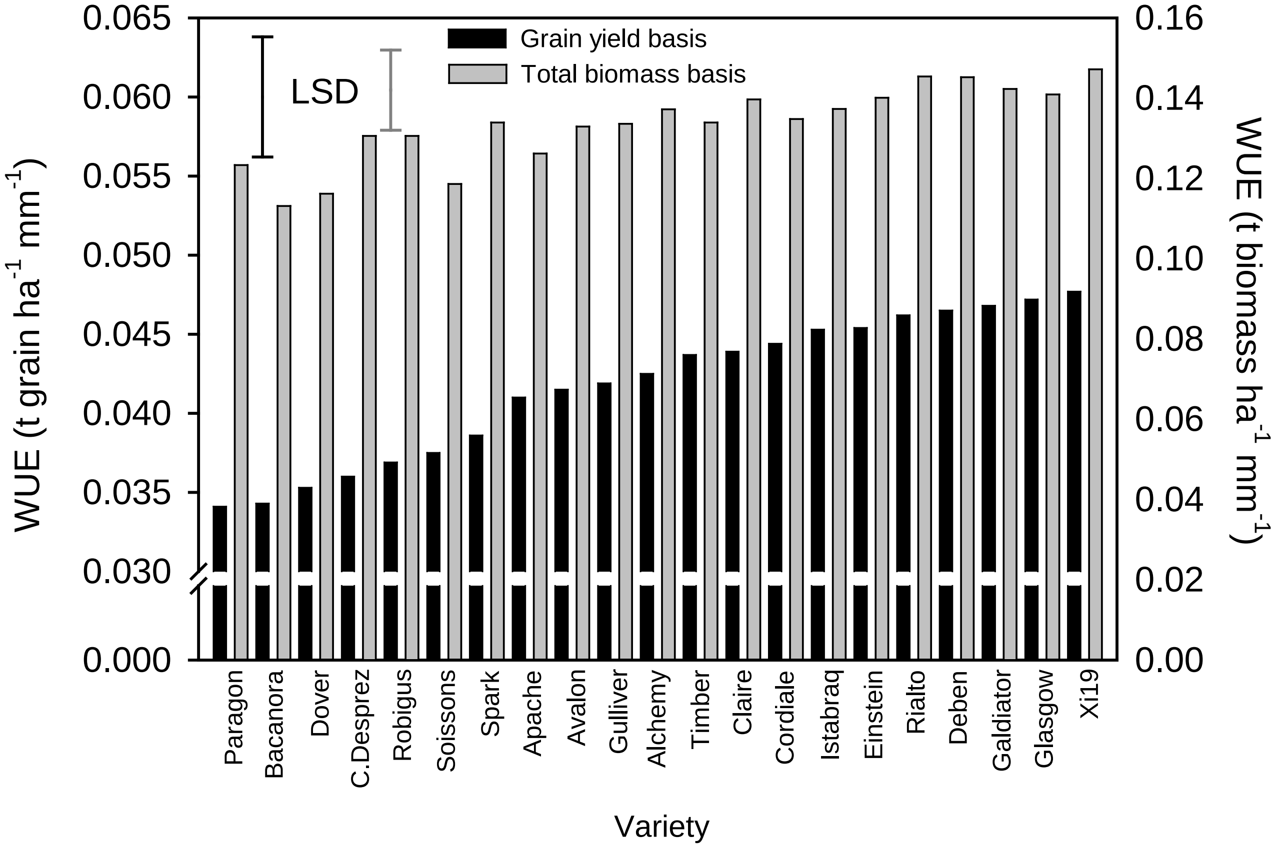


Fig. 31. Water use efficiency, computed on the basis of grain yield and above-ground biomass yield, for varieties measured under droughted conditions in 2009. The LSD bars (P = 0.05) for the genotype effect from ANOVA are shown.

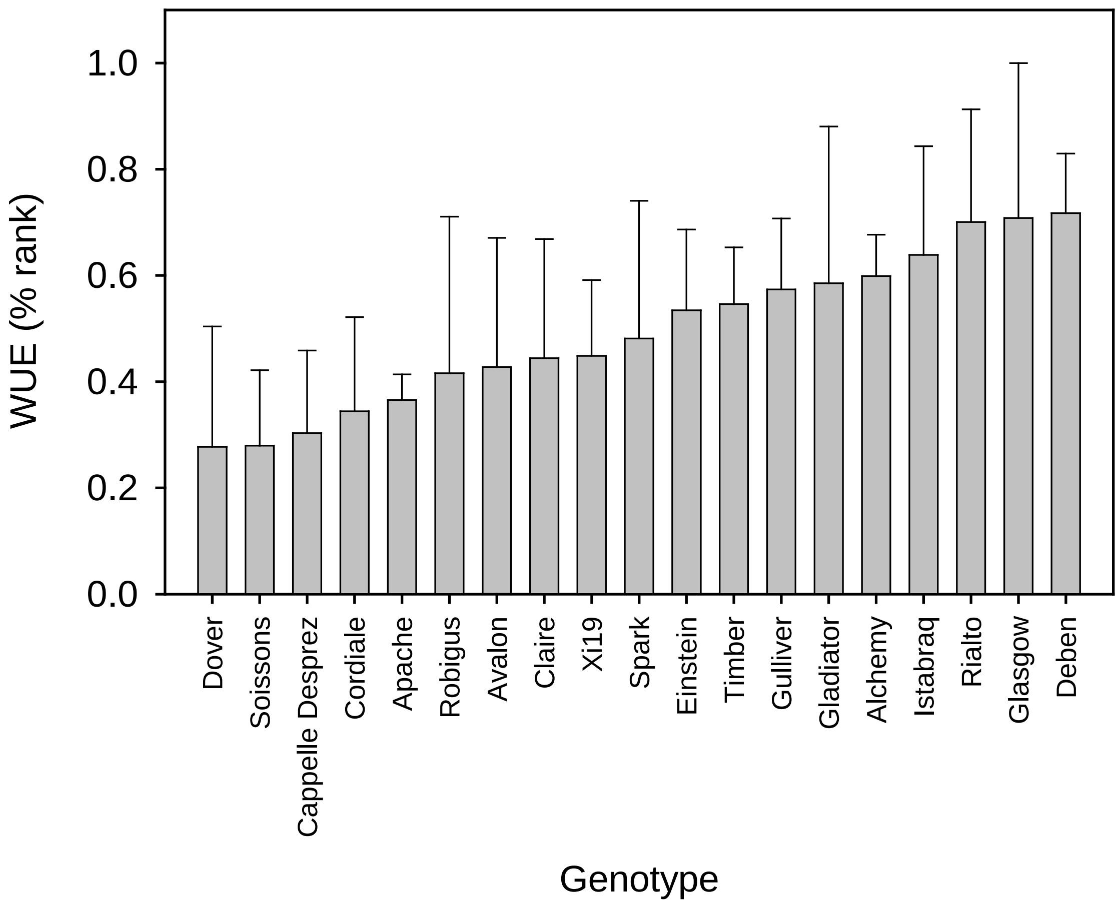


Fig. 32. The mean percentage rank of varieties for water use efficiency (WUE), computed on the basis of grain yield measured under droughted conditions from 2007-9. Bars are the mean ± se (n = 2-3).

Table 2. Cropping details for experiments at Broom's Barn from 2007 to 2009. The grain yield loss was computed as the proportion of irrigated grain yield lost under droughted conditions. In brackets is the inverse, the drought intensity index (DII).

Crop Year	Sowing date	Harvest Date	Date plots covered	Mean irrigated grain yield	Mean droughted grain yield	Yield loss (DII)
				$t\ ha^{-1}$	$t\ ha^{-1}$	%
2007	2.11.06	30.8.07 (droughted 29.8.07 (irrigated)	24.5.07	8.81	7.56	14 (0.86)
2008	19.10.07	8.8.08 (droughted 10.9.08 (irrigated)	20.5.08	13.95	7.74	45 (0.55)
2009	23.10.08	27.7.09 (droughted) 18.8.09 (irrigated)	2.6.09	10.97	6.43	41 (0.59)

Table 3. Summary ANOVA results for trials conducted at Broom's Barn 2007-2009. The number of lines tested (n) and the F probabilities are shown for the factors genotype (G), water regime (W) and the interaction term (G x W). The LSD (P = 0.05) are shown for the genotype and interaction terms. The trial precision based on residual and total sums of squares is shown as the R^2 value (Bowman and Watson, 1997). Yield components for the irrigated trial in 2007 are not reported because of grain sprouting due to rain-delayed harvest. Abbreviations: AGDM, above-ground dry matter; TGW, thousand grain weight; DTI, drought tolerance index; YTI, yield/tolerance index.

	Grain yield	AGDM	HI	Grain mass /ear	Grain No. /ear	TGW	Ear No. /m^2	DTI	YTI
2007	t ha^{-1}	t ha^{-1}	-	g	-	mg	-	-	-
F_{pr} genotype	<0.001	0.026	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
F_{pr} water regime	<0.001	0.128	<0.001	-	-	-	0.086	-	-
F_{pr} GxW	<0.001	0.831	<0.001	-	-	-	0.09	-	-
LSD (G)	1.6	4.6	0.09	0.8	12.4	5.7	54	0.3	0.27
LSD (GxW)	2.3	6.6	0.12	-	-	-	77	-	-
R^2	0.67								
n = 118									
2008									
F_{pr} genotype	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.202	0.044	<0.001
F_{pr} water regime	<0.001	<0.001	<0.001	0.003	0.004	0.064	<0.001	-	-
F_{pr} GxW	0.066	0.874	0.002	0.122	0.029	0.055	0.757	-	-
LSD (G)	2.0	3.0	0.04	0.5	7.4	5.6	218	0.4	0.22
LSD (GxW)	2.8	4.3	0.05	0.7	10.5	7.9	309	-	-
R^2	0.85								
n = 120									
2009									
F_{pr} genotype	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.044	<0.001
F_{pr} water regime	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-	-
F_{pr} GxW	0.01	0.246	0.395	0.233	0.564	0.031	0.227	-	-
LSD (G)	0.9	1.7	0.03	0.31	5.8	3.1	72	0.4	0.22
LSD (GxW)	1.3	2.4	0.05	0.43	8.3	4.4	102	-	-
R^2	0.92								
n = 66									

Table 4. Summary ANOVA results for trials conducted at Broom's Barn 2007-2009 comprising a subset of varieties selected for measurement of water use efficiency (WUE) on the basis of grain yield (GY) and above-ground dry matter (AGDM) under water-limited conditions. Seasonal water use was calculated based on adjusted reference evapotranspiration from 1 April until the date plots were covered to exclude rainfall, and thereafter crop water use was determined by measuring changes in soil moisture content (see text for detail). The F probabilities are shown for the genotype factor. The LSD (P = 0.05) and the number of lines tested (n) are shown.

	Grain yield	**AGDM**	**Water Use**	**WUE-GY**	**WUE-AGDM**
2007	t ha^{-1}	t ha^{-1}	mm	t $ha^{-1}mm^{-1}$	t $ha^{-1}mm^{-1}$
F_{pr} genotype	0.008	0.697	0.648	0.007	0.758
mean	8.4	18.2	206	0.04	0.08
LSD	2.5	8.6	22	0.01	0.04
n = 11					
2008					
F_{pr} genotype	0.716	0.716	0.458	0.811	0.701
mean	7.7	12.0	154	0.05	0.08
LSD	3.1	4.8	21	0.02	0.03
n = 21					
2009					
F_{pr} genotype	<0.001	0.005	0.561	0.001	0.121
mean	7.6	13.9	152	0.05	0.09
LSD	1.3	1.7	15.5	0.009	0.013
n = 21					

3.4. Discussion

3.4.1. Evaluation of multi-location variety trial data

Conclusions

It is well known that varieties yield differently depending on the year and test location. This fact, however, generally remains a nuisance to the breeder, variety trial manager and farmer. Currently, varieties are ranked by their relative yield averaged across a range of sites representing different regions and soil types. This mean relative yield is a crude but effective indicator of overall performance; however, there is a great deal more information for each variety that can be extracted from multi-location test data. We have shown that a drought stress index can be assigned to each location based on the actual moisture-holding capacity of the soil and the local weather conditions. Varieties then can be judged according to how well they perform relative to other varieties along a gradient of wet to dry locations. It is a simple approach, but provides information about the potential drought susceptibility of a variety that would otherwise be buried in the data. The analysis is a cost-effective and practical use of data already gathered as part of the Recommended List process.

In addition, these data can provide information on the relative yield stability of a variety. The same data that are collected for simple yield evaluations—with appropriate application of modern statistical tools—can be used to rank varieties according to how reliable the yield performance is across a range of conditions. This information would be useful to farmers wishing to decrease risk by planting some proportion of the crop with a stable variety instead of a riskier but potentially higher yielding variety.

In order for these types of evaluations to be conducted on a routine basis every year, several aspects are important to note. Firstly, variety trial locations must reflect the environmental conditions of the market area; in the UK this means that some sites should be drought-prone, and the crop should be permitted to develop stress. Often, variety trial managers discourage this because trial precision sometimes decreases due to unexplained error variances. However, with judicial choice of trial sites with uniform fields and good statistical design, these error variances can be decreased to within allowable limits. It is also vital to collect soil texture information over the entire soil profile (not just topsoil), including physical barriers and stone content. These samples can be done quickly and cheaply, and once entered into the database, can be referenced anytime a trial returns to that location. Finally, it is important to have complete and accurate daily rainfall data for each trial location. Weather stations even 20 km from test sites are not adequate as the hit-and-miss nature of thunderstorms can have a large influence on the calculated stress index.

3.4.2. Drought tolerance, WUE and morpho-physiological traits

There are many ways to define drought tolerance, which depends on the scale at which the problem is viewed (Passioura, 2007). For the cellular biologist, the activation of drought-responsive genes may be the main importance; at the other end of the scale, the geo-ecologist looks at weather patterns and the productivity of vegetation on a regional or global level. For the breeder or farmer, maximising yields and therefore profits across fields and seasons is of prime importance. Therefore, in this work, drought tolerance is defined on the basis of yield. The DTI provides information about the intrinsic ability of a variety to maintain its yield potential, relative to other varieties, regardless of the actual yield potential. The variety Bacanora, for instance, has a high DTI, but very low yield potential. As such, not a variety for use in the UK, but the fact that it is relatively insensitive to drought can provide useful information. For example, beneficial traits exhibited by Bacanora could be bred into an elite UK line with high yield potential, improving the drought tolerance of that line. Conversely, a variety with low DTI such as Gatsby had much smaller droughted yields compared with the yields obtained with irrigation; hence, it is relatively sensitive to droughts encountered in this study. This information could also provide clues about plant characteristics that cause plants to grow relatively less well when water is limiting. However, the absolute levels of droughted yields of Gatsby were greater than the droughted yields of some varieties with much greater DTI values. This is because the overall yield capacity of Gatsby was high. Therefore, a grower given the choice of Bacanora (high DTI), and Gatsby (low, DTI), would always choose, in this case, the ‘drought susceptible’ variety because even under poor conditions, Gatsby still yields better than Bacanora. Absolute yields, not indices or ratios pay the farmer’s bills.

Although there was a strong positive correlation between yields in irrigated and droughted conditions, the yield potential of a variety is not a foolproof indicator of its performance under sub-optimum conditions. Unfortunately, varieties with high yield potential often realise high yields only under the most favourable conditions, and there is large variability in their yields across a range of environments; i.e. yield stability is often low in high-yielding varieties. Although yield and quality are vital, yield stability is also important to farmers. We derived information about yield stability of varieties from the multi-location variety trial data, and this kind of evaluation could provide farmers with clues as to which high-yielding varieties also show the best yield stability.

Varieties that look good under ideal conditions, but then fall apart with water limitation could end up being marketplace disasters, costing breeders and growers. Breeders obviously have a keen interest in culling lines of this sort before reaching the RL stage. An important question is whether the private in-house and official variety trial processes include enough stress-susceptible (lighter land) sites in variety evaluation that such lines would be discovered. If not, traits or combinations of traits that help identify high-yielding but fragile lines would help avoid progression of such lines to the RL stage.

Improvements in ear fertility may be a potentially important way to increase the drought tolerance of UK wheat varieties. Dry conditions at the time of pollen development (corresponding approximately to stem extension and early boot stages) can lead to pollen sterility, causing decreased grain numbers at maturity (Bingham, 1966; Ji *et al*., 2010; Dolferus *et al*., 2011). Increased partitioning of assimilate to developing reproductive structures should increase the number of filled grains. The role of invertase is probably vital for maintaining the flux of sucrose into these tissues, but the dynamics of carbon partitioning amongst competing sinks in the growing plant is complex (Liu *et al*., 2005). More research is needed to identify specific targets amenable to selection for variety improvement.

The utility of morpho-physiological traits as indirect selection criteria to improve drought tolerance depends on several conditions being met: 1) the trait must show sufficient genetic correlation with the target trait such as yield or drought tolerance per se; 2) the trait must show good heritability; 3) molecular markers must be linked to loci controlling a significant proportion of the phenotypic variance for the trait, or 4) direct measurement of the trait must be done with minimal time and cost to enable high-throughput screening. The challenge is to combine efforts of physiologists, agronomists, modellers, molecular geneticists, bioinformaticians and plant breeders to enable the incorporation of new knowledge, such as the results described here, into a practical and cost-effective breeding programme.

3.4.3. Genotyping

Marker data for the major genes listed above were used to segregate the population of lines into subgroups according to, for example, the presence or absence of different dwarfing genes. This allowed comparison of phenotypes within and between subgroups.

The 94 lines were genotyped with 2499 DArT markers with an overall call rate of 96.9%. Only one of the markers from the literature was deemed unscorable, and two others were poor markers that were difficult to score. In collaboration with Dr. Jackie Barker at Rothamsted, the DArT data were used to develop a preliminary dendogram that shows the relatedness of the varieties. Near-neighbours on the dendogram that share known pedigrees corroborate the analysis. These genotypic data, in conjunction with the phenotypic data collected here, feasibly could be used in an association study, as done previously in durum wheat (Maccaferri *et al*. 2010). In anticipation of this, a collaboration agreement with Triticarte Pty, Ltd has been established. However, association or other mapping exercises were not an objective of the current study; the data will be available for further analysis in future work.

3.4.4. Making best use of water resources through breeding

Recent developments in drought research

Over the last 30 years, plant breeders have improved crop yields for dry conditions, agricultural scientists have improved management techniques for saving water, and plant scientists have furthered understanding of plant responses to water (Parry *et al*., 2005). Recent work in durum wheat shows significant genetic variability and high heritability (additive and dominant control) for WUE (Solomon and Labuschagne, 2004). In bread wheat, an important advance was the release of three new Australian wheat varieties with improved WUE that were bred based on selections for low $^{13}C/^{12}C$ isotope discrimination ratio (Delta) (Condon *et al*., 2004). Currently, there is substantial international effort to address these problems at all levels. Despite the scale of the endeavour, gaps remain in the basic understanding of processes that control WUE and yield, and breeding progress is slow. In addition, many of these research programmes are focused on low-yielding environments in arid and semi-arid areas of the world, characterised by distinct wet and dry seasons and high temperatures. Such programmes are not easily applied to the cropping situation in the UK where rainfall is unpredictable and variable. An important requirement of new UK varieties with enhanced drought tolerance and WUE is that they must not carry a significant yield penalty if they are to be commercially successful.

In arid regions, improvements in drought tolerance have been made largely through conventional empirical breeding; that is, selecting varieties that yield well in the target dry environments. However, even though there is genetic variability for WUE and drought tolerance in wheat (data presented here and by Foulkes *et al.,* 2001), breeders find it difficult and costly to breed effectively for these traits. Genetic gain is low because yield shows low heritability due to genotype x environment interactions (and drought tolerance is defined on the basis of yield). Marker-assisted selection (MAS) is an attractive option, but this requires the identification of key loci for yield performance under drought. For example, MAS is leading to improvements in drought tolerance of upland rice (Steele *et al*., 2006) and pearl millet (Serraj *et al*., 2005). However, in other species including wheat, quantitative trait loci (QTLs) for drought tolerance *per se* have so far shown limited utility (Blum, 2005; Richards *et al*., 2010). Reasons for the failure of these QTLs to quickly lead to practical markers include: poor quality or insufficient phenotypic data; too many QTLs each with small effect; large QTL by environment interactions; QTLs that are population-specific; use of parental lines too far removed from actual breeding stocks. In barley, many QTLs for yield under drought conditions simply coincided with loci controlling phenology rather than traits conditioning physiological mechanisms of drought tolerance (Forster *et al*., 2004). For example, in UK winter wheat, advantages of drought escape through earliness may be offset by lower yield potential or smaller root system size (Foulkes *et al*., 2004).

Transgenic crops are an alternative approach to improvement in drought tolerance. In the private sector, these products are still in development phases. For instance, both Monsanto and Dupont/Pioneer have been field-testing maize lines carrying gene events that appear to improve drought tolerance (Habben, 2005; Nelson *et al*., 2007); a Canadian company has put transgenic rape in field trials (Wang *et al*., 2005); and CIMMYT has tested wheat lines over-expressing the drought-related transcription factor DREB1A (Pelligrineschi *et al*., 2004). Despite the number of claims that findings in these areas will be useful to breeders, breeders are still waiting for the promises and investments of the genomic era to bear fruit (Blum, 2005). Reasons for these failures include poor communication between biotechnologists, physiologists and breeders, little testing of ideas at the field level (Edmeades *et al*., 2004), and significant penalties in yield potential associated with the constitutive or over-expression of certain genes (Nelson *et al*., 2007). It is now widely recognised that tools for genotyping have outpaced the ability to provide high quality phenotypic information of genetic stocks, particularly under field conditions.

Another approach to improve genetic gain for yield under water-limited conditions is to base selections on secondary traits that are associated with drought tolerance and WUE (Reynolds *et al*., 2005). Many of these traits have been based on studies of the physiological and morphological characters that contribute to yield and WUE, but successful implementation in practice requires a multi-disciplinary team interacting with breeders (Lafitte *et al*., 2006; Richards, 2006). For the 'indirect trait' approach to be successful, the trait must have high genetic correlation with yield, high heritability, and selection methods must be applicable on a large scale (i.e. quick and inexpensive).

In maize, after a period of intense investment by CIMMYT and Pioneer in ‘drought' QTLs, attention has turned back to physiology-based development of markers using large-scale managed drought conditions for phenotypic evaluation (Bruce *et al*., 2002). One reason for this shift in research emphasis is that component traits are often controlled by a smaller number of major QTLs compared with yield QTLs. Such secondary traits often show high heritability; examples include osmotic adjustment (Moinuddin *et al*., 2005), transpiration efficiency (Malik *et al*., 1999; Solomon and Labuschagne, 2004), relative leaf water content (Schonfeld *et al*., 1988), leaf conductance (Rebetzke *et al*., 2003) and leaf rolling (Price *et al*., 2002). Also, carbon isotope discrimination ratio (Delta, Δ), an indirect measure of WUE, is controlled by one major QTL linked to the *ERECTA* gene in the model species *Arabidopsis* (Masle *et al*., 2005). Delta has been used as the selection criterion for the development of three new Australian wheat varieties with significant improvement in WUE (Condon *et al*., 2004).

Candidate genes and traits for UK conditions

Work done specifically on UK wheat with regard to drought tolerance and WUE has provided an important foundation of data. For instance, the relative importance of earliness, stem reserves, flag leaf persistence and dwarfing genes has been studied (Austin, 1987; Foulkes *et al*., 2002; Foulkes *et al*., 2007; Foulkes *et al*., 2004; Innes and Quarrie, 1987), and putative QTLs for drought-related characters have been found (Quarrie *et al*., 2003; Verma *et al*., 2004), which could aid introgression of favourable traits into elite germplasm. However, there may be varieties or breeding lines that already have good drought tolerance and WUE. It is important to breeders to exploit first the range of drought tolerance available within the UK pool because of the considerable time and resources needed to introgress exotic germplasm into elite UK material. Secondly, it is also important to explore sources of drought tolerance that are genetically quite distinct from the UK pool (Rattey *et al*., 2011; Reynolds *et al*., 2007). These materials may harbour alleles conferring drought tolerance that are qualitatively and quantitatively different from the alleles present in largely European germplasm. Some of the traits in foreign material may be appropriate to UK conditions, while others may not be.

One of the obstacles to improved drought tolerance and WUE is the negative association (trade-offs) between certain stress-adaptive traits and productivity in ideal environments (Chapin *et al.*, 1993). Many studies (predominantly using transgenic *Arabidopsis* or tobacco) that demonstrate ‘drought tolerance' in the laboratory have little relevance to agricultural conditions because growth is affected under unstressed conditions (Vinocur and Altman, 2005). The genes modified in some of these studies merely affect stomatal control, the rate of gas exchange, and hence growth (Passioura, 2006). However, some beneficial traits, such as stem reserves, are correlated with yield under all conditions (Foulkes *et al*., 2007; Shearman *et al*., 2005). In environments characterised by predictable dry periods, growers are willing to sacrifice some yield potential in order to achieve better yield under limiting conditions. In variable environments, the trade-offs have to be balanced more delicately, which requires knowledge of the water-holding capacity of the soil and the relative drought-susceptibility of current varieties.

Other genes that have been proposed to improve drought tolerance include:
1) proteins (e.g. dehydrins) that help protect cellular structures when plant cells become dehydrated (Lopez *et al*., 2003);
2) transcription factors (e.g. the DREB/CBF family) or signal transduction factors (Creelman, 2005; Pelligrineschi *et al*., 2004);
3) proteins that maintain redox balance and/or handle damaging reactive oxygen species that accumulate in stressed plants (Foyer and Noctor, 2005).
4) genes that control osmotic adjustment (Moinuddin *et al*., 2005) or plant morphology, such as root length and thickness, stomatal density, epicuticular wax deposition, etc. Clearly, deeper, more effective root systems are important to avoid or delay the effects of drought. Improved rice cultivars have been developed by introgressing alleles conditioning root depth and penetration ability via markers linked to root trait QTLs. For UK winter wheat, it remains unclear how much, if at all, rooting depth limits WUE, and whether or not dwarfing genes affect root system size.
5) proteins that sense and regulate assimilate flux between source and sink organs. These could be key to determining aspects such as floret abortion, grain size and number, and mobilization of stem reserves. These processes are also the least understood. Genes that respond to abscisic acid (ABA) and sucrose are probably key players; apoplastic acid invertase is one likely target (Boyer and Westgate, 2004; Koch, 2004; Liu *et al*., 2005; Wardlaw and Willenbrink, 2000). Can alteration in the expression of one gene make a difference? The *ERECTA* gene in *Arabidopsis,* which controls differences in WUE, is a developmental gene that affects leaf thickness, stomatal density, and ultimately, the conductance to CO_2 within the leaf. (Masle *et al*., 2005). This study shows that manipulation of a single gene may have profound effects on WUE without penalising growth.

3.4.5. Conclusions

Improvements in wheat yields under water-limited conditions are a global challenge and pressing need. Despite decades of world-wide research effort, drought tolerance remains a difficult and elusive target for breeders. Continued progress in yield potential generally benefits productivity in optimal and stressed environments, as shown by positive correlation between irrigated and droughted yields. Therefore, in the absence of varieties bred specifically for drought-prone environments, farmers generally would simply choose a high-yielding variety. However, yield instability of high-yielding varieties sometimes can be large, leading to loss of profits for growers and market failures for seed companies. In arid areas, development of varieties that yield well when water is limiting is done empirically by direct selection for yield under these conditions. However, this is not practical in the UK or other regions that have periods of dry weather that are unpredictable in timing and severity. There has been considerable interest in secondary traits associated with drought tolerance and WUE as criteria for indirect selection for these targets. Selection can be via phenotypic expression screens or via molecular markers linked to loci conditioning specific physiological or morphological traits. Before breeding companies will invest in a marker-assisted breeding programme using secondary traits, they need sufficient evidence that selection for those traits will bring about the desired improvements without deleterious side effects or a conspicuous drag on yield. Currently, very few of the traits, markers or candidate genes suggested in the literature have provided any practical aid to the breeder for improving drought tolerance, though some of the success cases are notable.

Findings from this project reveal that the ideal drought-tolerant UK wheat phenotype (ideotype) is comprised of several characters, each of which individually makes a partial contribution to success under droughted conditions. The challenge is to quantify this ideotype to enable phenotypic selection on a large scale in breeding nurseries, or to pyramid a sufficient number of markers.

Results also showed that one ideotype is not sufficient for the UK environment: varieties that are most successful during a water deficit prior to flowering, but with recovery of stress during grain filling, are different than those that yield relatively better when deficits develop only after flowering. It is impossible for breeders to know which kind of drought predominates, so focus on traits such as maintenance of green canopy, which is important regardless of the timing of deficits, could be more fruitful than attempting to match sensitive developmental phases with potentially stress-free periods. Recent work also highlights the growing importance of heat stress (Semenov, 2010), which was not explicitly considered in this project. Tolerance to heat, or heat combined with water deficit, also needs to be considered for future UK varieties.

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3.7. Abbreviations

DELTA	carbon isotope discrimination ratio
DSI	drought stress index
DTI	drought tolerance index
ET	evapotranspiration
GGT	genotype and genotype x trait
GS	growth stage
LMA	leaf mass : area ratio
LSD	least significant difference
MDA	malonyldialdehyde
NDVI	normalised difference vegetation index
NRR	normalised reflectance ratio
QTL	quantitative trait loci
RL	Recommended List

SMD	soil moisture deficit
TBARS	thiobarbituric acid reactive substances
TSI	transpiration-based stress index
WGIN	wheat genetics improvement network
WSC	water-soluble carbohydrates
WUE	water use efficiency
YTI	yield/tolerance index
*,**,***	Probability at $P < 0.05$, 0.01, 0.001, respectively

APPENDICES

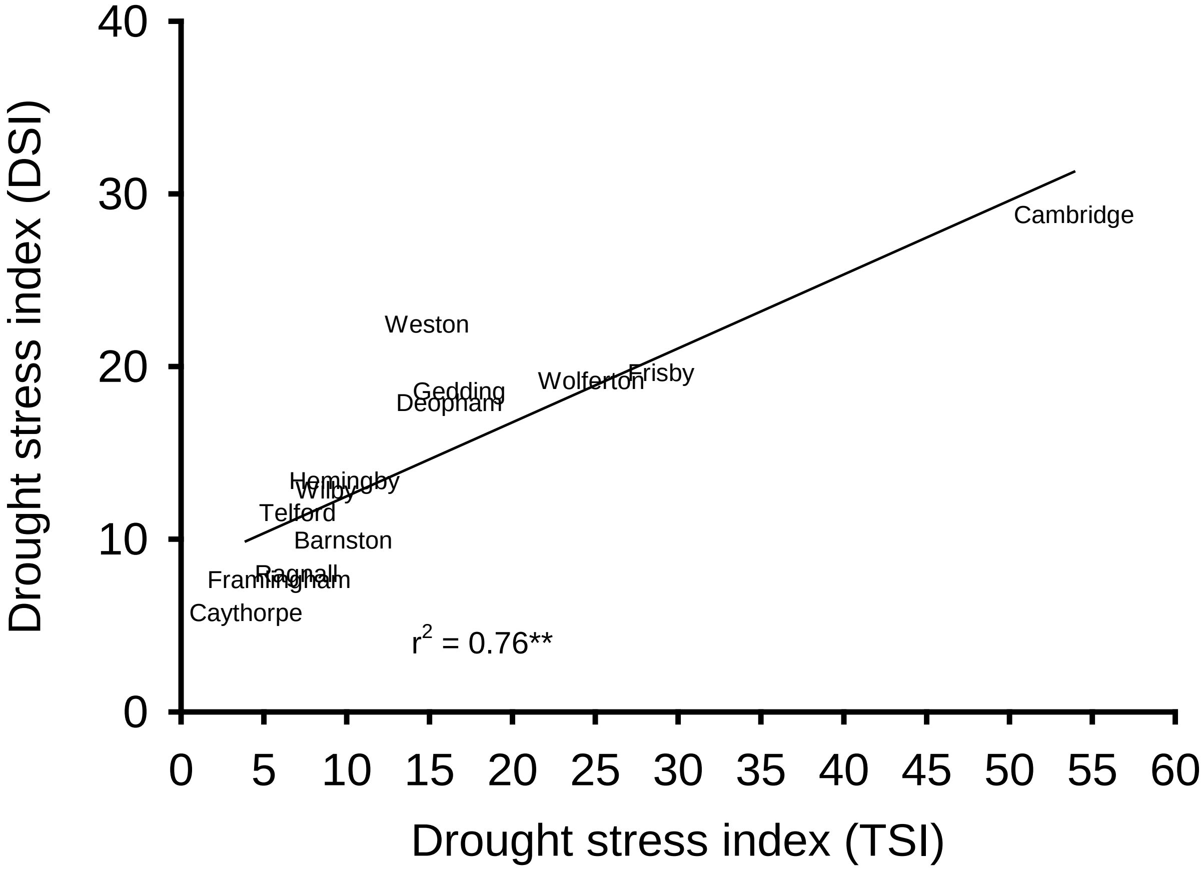
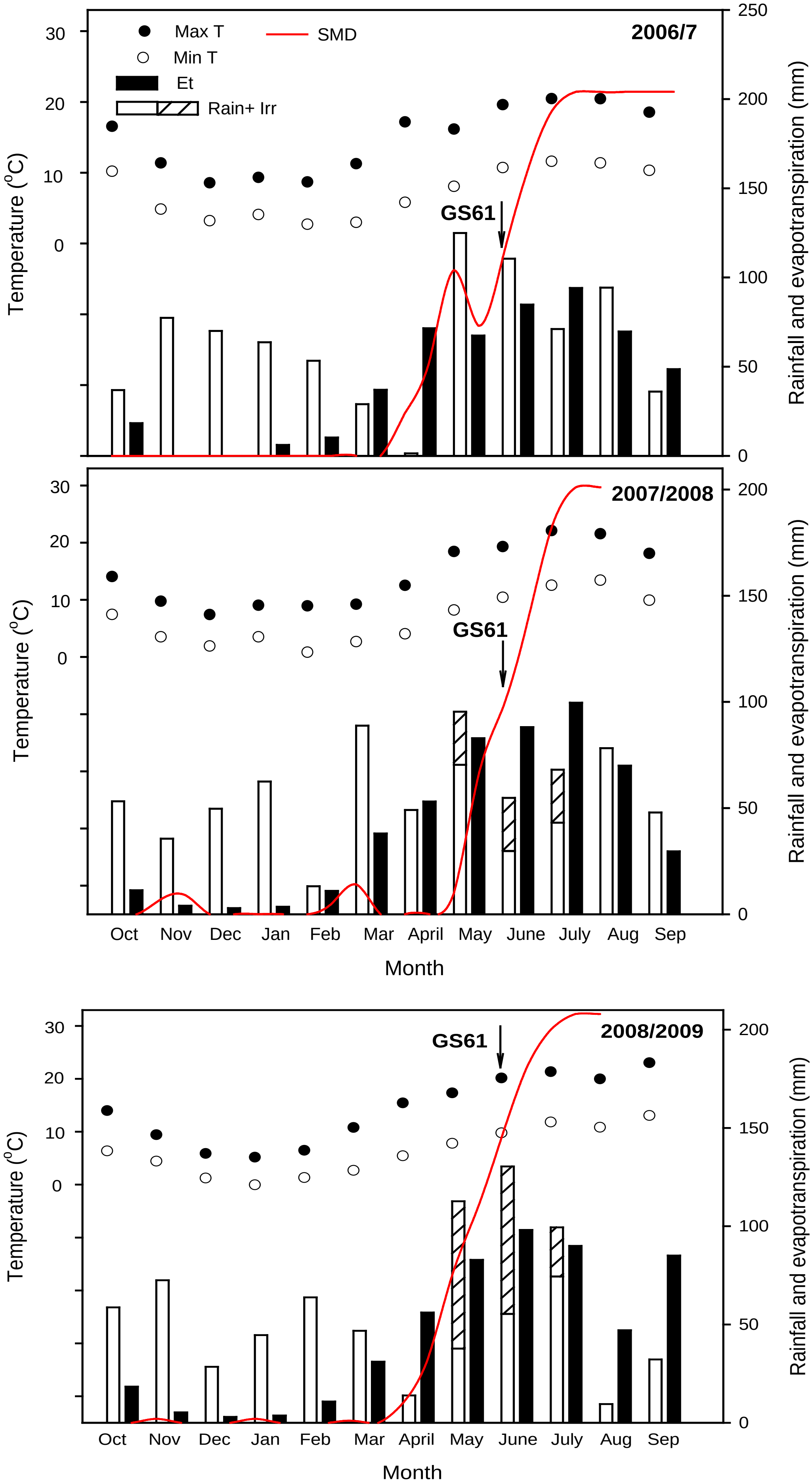


Fig. 1. Relationship between the drought stress index based on simulated grain yield (DSI) and crop transpiration rates (TSI) for RL trial sites in 2006. Both indices would serve equally well as an environmental descriptor.

Fig. 2. Following page. Environmental conditions for the field experiments at Broom’s Barn from 2007-2009. Temperatures, rainfall (open bars) and evapotranspiration rates (solid bars) were measured at a weather station within 500 m of the experiment. The solid line indicates the daily accumulated soil moisture deficit (SMD = ET - rainfall) in the droughted treatment using the polytunnel rainout shelter. The arrow indicates the date of anthesis for the variety Avalon. Irrigation (hatched bar) was applied to the irrigated portion of the experiment outside and adjacent to the rain shelter.



Max T
Min T
Et
Rain+ Irr
SMD
2006/7
2007/2008
2008/2009
GS61
Temperature (°C)
Rainfall and evapotranspiration (mm)
Oct
Nov
Dec
Jan
Feb
Mar
April
May
June
July
Aug
Sep
Month

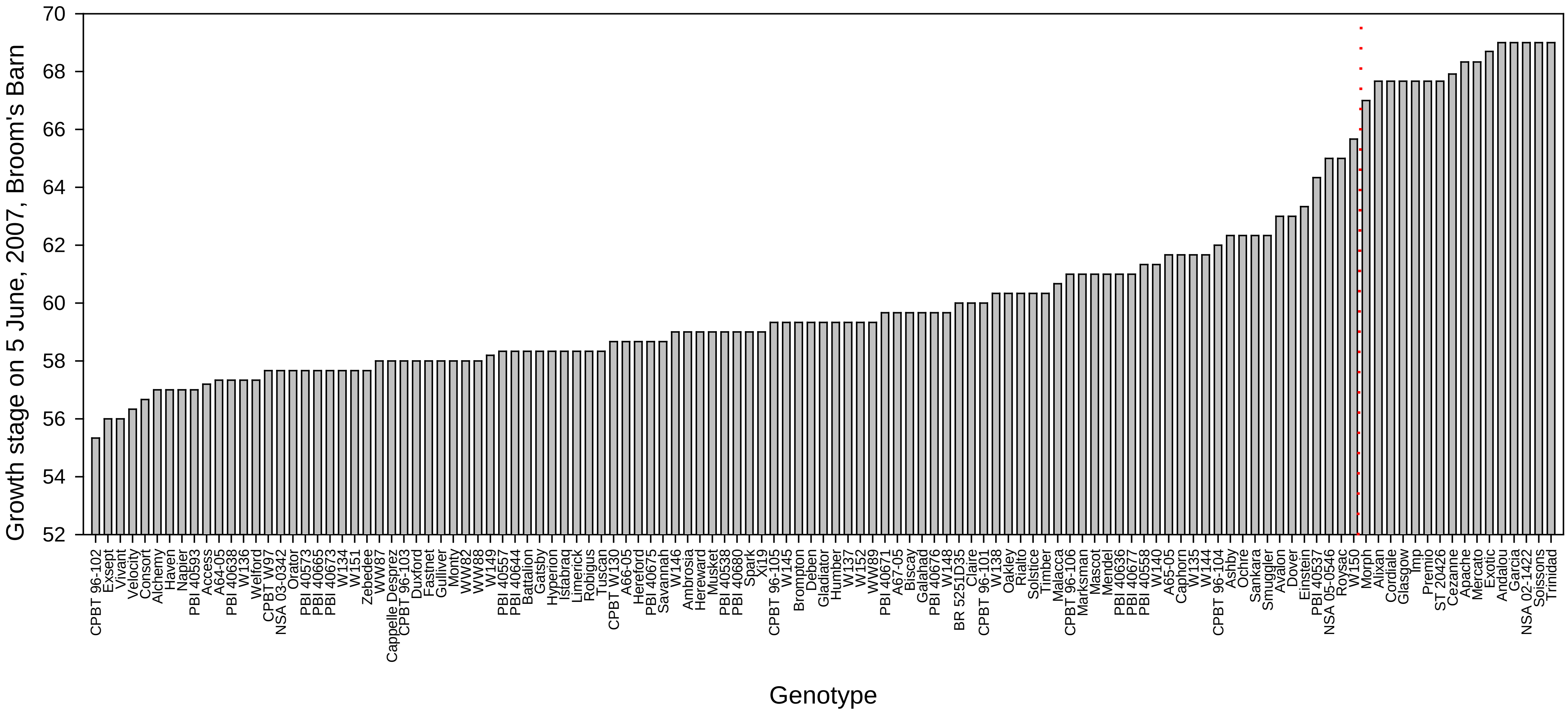


Fig. 3. Growth stages assessment of genotypes grown at Broom's Barn in 2007. The cut-off between 'earlier-flowering' lines and 'later-flowering' lines is shown by the dotted red line. In subsets of lines grown and assessed in breeders' trials, there was good association between different measures of phenology in different experiments.

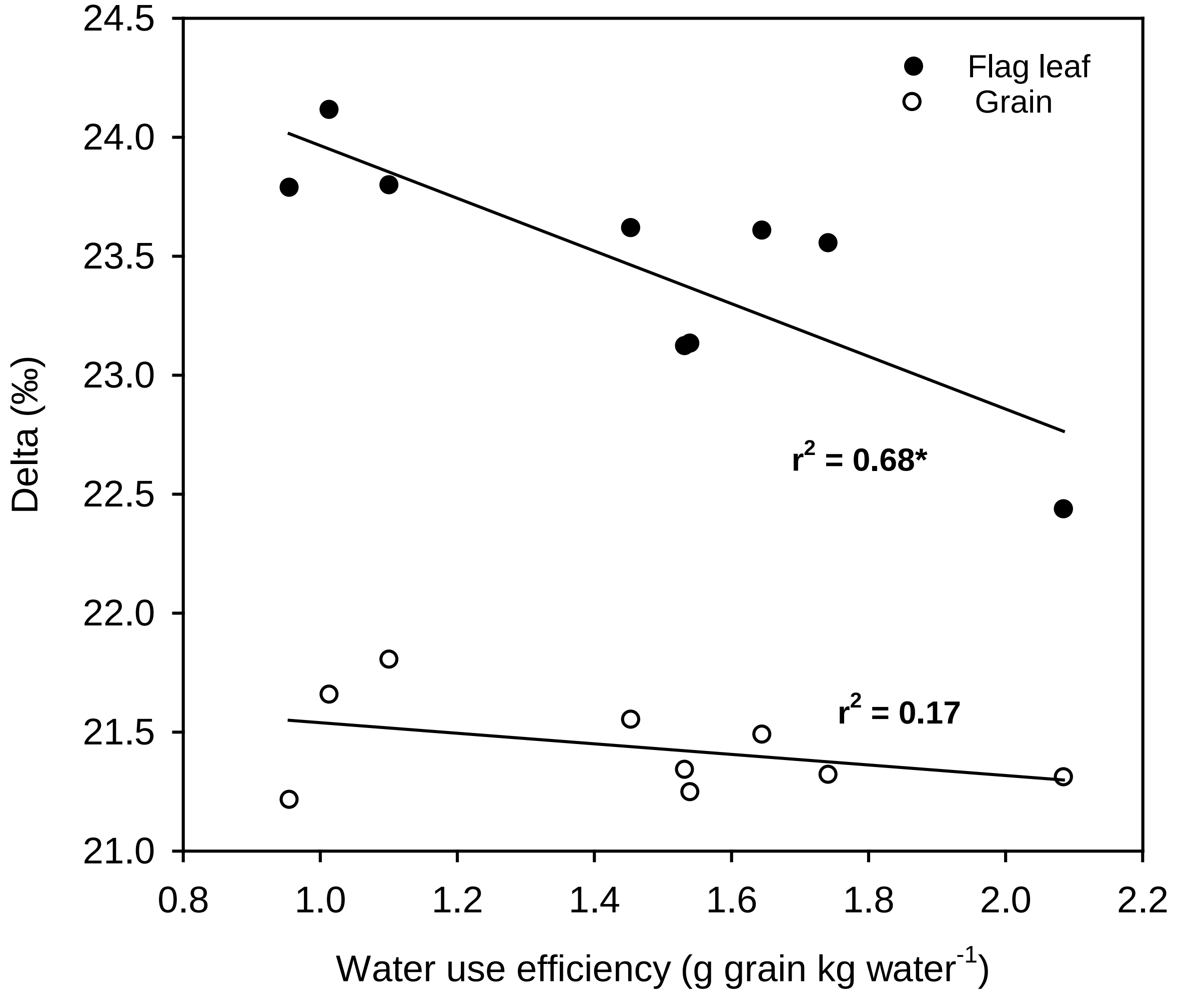


Fig. 4. Water use efficiency measured in large weighing lysimeters in 2009, vs. carbon isotope discrimination ratio (Delta) measured in flag leaves at anthesis or in mature grain. Each symbol represents the values for a single lysimeter. Three replicate lysimeters of three varieties are represented.

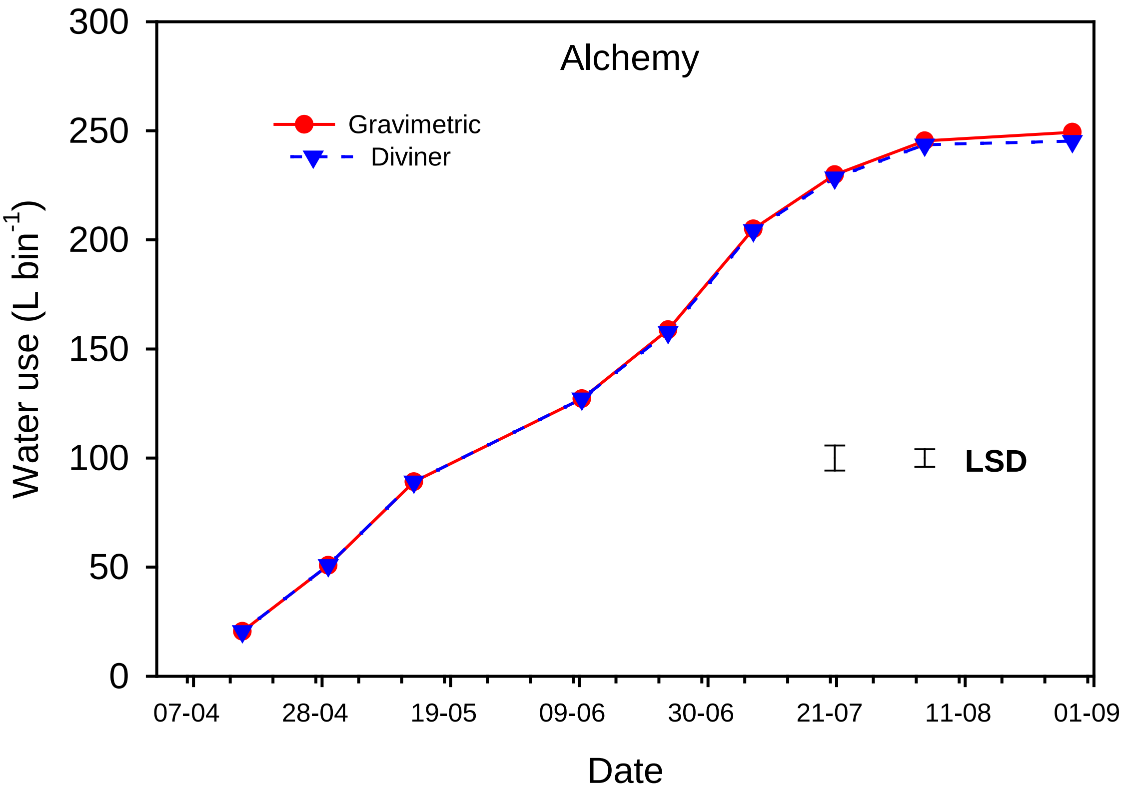


Fig. 5. Comparison of seasonal crop water use of plants in large weighing lysimeters determined gravimetrically (circles) or by measuring changes in soil moisture content with a capacitance-type soil moisture probe (Diviner, triangles). Data are from 2009 for an individual bin planted with Alchemy. Similar good agreement was observed in the other lysimeters.

Table 1. RL sites used in 2006 for variety evaluation. The simulated yields using Sirius under actual or stress-free conditions are shown for the calibration variety Mercia. The drought stress index (DSI) and the transpiration stress index (TSI) were calculated for each site using actual soil samples and weather from the nearest MET Office weather station to the site.

Unique Site ID	Nearest Settlement	Actual Mean site Yield	Simulated Yield	Simulated Potential Yield	DSI	TSI
		t/ha	t/ha	t/ha	%	%
06WW121	Barnston	11.8	8.0	8.9	9.8	9.9
06WW115	Cambridge	9.0	4.2	9.1	53.9	28.8
06WW112	Caythorpe	7.5	8.4	8.8	3.9	5.8
06WW118	Deopham	9.2	7.8	9.3	16.2	17.9
06WW120	Framlingham/Bedfield	11.9	8.7	9.2	5.9	7.7
06WW124	Frisby on the Wreake	10.5	6.7	9.4	29.0	19.6
06WW116	Gedding	10.7	8.3	9.9	16.8	18.6
06WW111	Hemingby	9.6	8.1	9.0	9.9	13.4
06WW109	Ragnall	9.3	7.8	8.4	7.0	8.0
06WW123	Telford	10.2	9.7	10.5	7.0	11.5
06WW119	Weston	11.9	9.1	10.6	14.9	22.5
06WW113	Wilby/Quidenham	6.9	8.3	9.1	8.7	12.9
06WW114	Wolferton	9.8	7.5	9.9	24.8	19.2

Table 2. RL sites used in 2007 for variety evaluation. The simulated yields using Sirius under actual or stress-free conditions are shown for the calibration variety Mercia. The drought stress index (DSI) and the transpiration stress index (TSI) were calculated for each site using actual soil samples and weather from the nearest MET Office weather station to the site.

Unique Site ID	County	Nearest Settlement	Actual Mean site Yield	Simulated Yield	Simulated Potential Yield	DSI	TSI
			t/ha	t/ha	t/ha	%	%
07WW107	East Yorkshire	Ottringham	8.9	8.0	7.9	0.00	0
07WW109	Lincolnshire	Ulceby	9.1	8.0	8.0	0.00	0
07WW110	North Yorkshire	Rudston	7.9	9.9	9.8	0.00	0
07WW112	Lincolnshire	North Rauceby	8.8	10.3	11.2	8.25	10.45
07WW113	Norfolk	Kenninghall	9.3	9.7	9.7	0.06	0.09
07WW114	Norfolk	Wolferton	9.1	9.5	9.5	0.00	0
07WW115	Cambridgeshire	Histon	10.0	8.8	8.8	0.07	0
07WW119	Lincolnshire	Weston	9.4	8.8	8.8	0.00	0
07WW120	Suffolk	Bedfield	10.1	10.3	10.3	0.00	0
07WW121	Essex	Barnston	10.9	9.6	9.6	0.00	0
07WW122	Kent	Ivychurch	10.7	10.1	10.1	0.00	0
07WW123	Shropshire	Telford	10.3	10.9	10.8	0.00	0
07WW124	Leicestershire	Frisby on the Wreake	8.9	8.9	8.9	0.00	0

Table 3. RL sites used in 2008 for variety evaluation. The simulated yields using Sirius under actual or stress-free conditions are shown for the calibration variety Mercia. The drought stress index (DSI) and the transpiration stress index (TSI) were calculated for each site using actual soil samples and weather from the nearest MET Office weather station to the site.

Unique Site ID	County	Nearest Settlement	Actual Mean site Yield	Simulated Yield	Simulated Potential Yield	DSI	TSI
			t/ha	t/ha	t/ha	%	%
08WW106	North Yorkshire	Bugthorpe	10.0	8.5	8.5	0.00	0
08WW107	East Yorkshire	Ottringham	11.5	9.1	9.1	0.00	0
08WW108	Durham	Middridge/Redworth	9.9	10.3	10.3	0.03	0.14
08WW110	North Yorkshire	Rudston	10.0	10.3	10.3	0.16	0.51
08WW111	Lincolnshire	Baumber	12.1	9.5	9.7	1.90	3.98
08WW112	Lincolnshire	Welbourn/Caythorpe	12.3	9.6	9.8	2.93	4.93
08WW114	Norfolk	Wolferton	13.0	10.7	10.7	0.36	1.51
08WW116	Suffolk	Woolpit	8.8	9.8	9.9	1.20	3.33
08WW119	Lincolnshire	Weston Hills	11.2	9.1	9.1	0.18	1.6
08WW120	Suffolk	Bedfield/Framlingham	12.7	11.0	11.0	0.11	0.67
08WW122	Kent	Newchurch	12.3	10.6	10.6	0.00	0
08WW124	Leicestershire	Frisby on the Wreake	12.4	10.6	10.6	0.00	0

Table 4. RL sites used in 2009 for variety evaluation. The simulated yields using Sirius under actual or stress-free conditions are shown for the calibration variety Mercia. The drought stress index (DSI) and the transpiration stress index (TSI) were calculated for each site using actual soil samples and weather from the nearest MET Office weather station to the site.

Unique Site ID	County	Nearest Settlement	Actual Mean site Yield	Simulated Yield	Simulated Potential Yield	DSI	TSI
			t/ha	t/ha	t/ha	%	%
09WW108	N. Yorkshire	Croft on Tees	11.4	10.4	10.6	2.01	2.73
09WW109	N. Lincolnshire	Ulceby	9.6	10.6	11.0	3.49	3.51
09WW110	E. Yorkshire	Rudston	9.8	10.3	10.4	0.90	1.72
09WW111	Lincolnshire	Horncastle	7.1	8.6	10.3	16.97	14.4
09WW112	Lincolnshire	Welbourn	13.0	8.6	10.3	16.07	14.57
09WW114	Norfolk	Wolferton	13.0	10.0	10.4	3.75	3.45
09WW115	Cambridgeshire	Cambridge	8.8	9.0	10.3	12.87	13.92
09WW116	Suffolk	Woolpit	9.1	9.4	11.3	16.53	23.73
09WW119	Lincolnshire	Weston Hills	13.7	8.1	11.0	26.35	29.11
09WW120	Suffolk	Bedfield	13.0	9.4	10.3	9.50	9.98
09WW121	Essex	Youngs End	8.9	8.6	10.2	15.63	15.2
09WW122	Kent	Newchurch	13.2	9.6	10.3	6.28	8.58

Table 5. Mean site yields for each of the breeders' trial locations 2007-2009. *Shelford was used instead of Grantchester in 2008. †Trumpington was used instead of Langton in 2007. In 2007 Thriplow was discarded. The LINK-ERYCC site at Terrington was not started until 2008.

Crop Year	Arras	Grantchester*	Clopton	Framlingham	Langton†	Maldon	Riseholme	Thriplow	Terrington
	t ha^{-1}								
2007	8.5	8.3	9.9	8.8	8.8	13.6	10.2	-	-
2008	10.8	10.5	14.5	11.3	12.3	12.1	13.5	11.7	10.7
2009	8.5	10.8	9.9	12.8	10.1	10.9	10.3	9.6	8.6

Table 6. Drought stress indices for breeders' sites in 2009 based on modelled crop transpiration ratios (TSI) and simulated yield ratios (DSI) using the Sirius wheat model and Mercia as a calibration variety. The simulated yield was based on actual weather; the potential yield was based on actual weather with water deficit removed. Drought stress indices were not calculated for 2007 and 2008 on breeders' trials because very little stress occurred in those years.

Site	Region	Yield	Potential Yield	TSI	DSI
		t/ha	t/ha	%	%
Framlingham	Suffolk	10.0	10.7	9.1	6.6
Thriplow	Cambridgeshire	9.3	10.7	13.6	12.9
Arras	France	10.6	11.6	14.8	8.7
Clopton	Suffolk	8.8	10.7	15.5	17.6
Maldon	Essex	8.6	10.8	16.5	20.5
Riseholme	Lincolnshire	9.9	10.8	9.2	8.1
Langton	North Yorkshire	10.9	11.2	5.7	2.7
Grantchester	Cambridgeshire	10.0	10.7	8.9	6.5
Terrington	North Yorkshire	10.6	10.7	2.8	1.7

Table 7. Finlay-Wilkinson regressions of relative grain yields of varieties vs. DSI using 13 selected RL sites in 2006 (see Table 1, Appendix). The y-intercept (A) indicates the stress-free yield potential of the variety, while the slope (b) indicates the relative sensitivity to available water supply: negative slopes reflect drought sensitivity while positive slopes indicate that varietal performance improves (compared to the trial mean) as conditions become drier. The only variety showing a significant slope ($R^2 \geq 0.33$, 10 df) was Sahara.

Variety	**Intercept**	**Slope**	**R2**
Alchemy	97.5	0.02	0.00
Ambrosia	102.7	0.27	0.17
Battalion	95.7	0.49	0.24
Benedict	105.3	-0.09	0.01
Brompton	105.9	-0.20	0.21
Claire	96.9	-0.05	0.01
Consort	97.7	-0.28	0.18
Contender	98.9	0.03	0.00
Cordiale	100.4	0.08	0.03
Deben	102.1	-0.06	0.04
Dover	96.1	0.00	0.00
Einstein	99.5	0.18	0.07
Gatsby	96.2	-0.11	0.03
Gladiator	102.1	0.18	0.15
Glasgow	105.9	-0.04	0.00
Gulliver	100.6	-0.03	0.00
Hereward	95.3	-0.26	0.14
Humber	103.1	0.24	0.20
Hyperion	100.2	-0.03	0.00
Istabraq	100.7	0.11	0.06
Kipling	101.7	0.00	0.00
Malacca	91.8	-0.20	0.13
Mascot	99.1	-0.13	0.07
Nijinsky	94.1	0.10	0.06
Oakley	102.9	-0.23	0.18
Robigus	96.7	-0.10	0.02
Sahara	95.2	0.36	0.44
Soissons	100.1	-0.10	0.37
Solstice	99.3	0.01	0.00
Timber	99.1	0.24	0.08
Welford	100.3	-0.16	0.14
Xi19	105.3	-0.24	0.24
Zebedee	107.2	-0.09	0.02

Table 8. Finlay-Wilkinson regressions of relative grain yields of varieties vs. DSI using selected RL sites in 2009 (see Table 4, Appendix). The y-intercept (A) indicates the stress-free yield potential of the variety, while the slope (b) indicates the relative sensitivity to available water supply: negative slopes reflect drought sensitivity while positive slopes indicate that varietal performance improves (compared to the trial mean) as conditions become drier. The only variety showing a significant slope ($R^2 \geq 0.33$, 10 df) was Scout.

Variety	**Intercept**	**Slope**	**R^2**	**n**
Alchemy	100.7	-0.06	0.02	12
Battalion	97.9	0.15	0.22	12
Beluga	109.5	-0.45	0.67	4
Cassius	103.2	-0.03	0.00	12
Claire	97.7	0.28	0.25	4
Conqueror	108.8	-0.42	0.28	12
Cordiale	95.9	0.06	0.01	12
Duxford	104.7	-0.12	0.06	12
Edmunds	102.8	-0.01	0.00	12
Einstein	96.1	0.03	0.00	12
Gallant	91.2	0.30	0.23	12
Gladiator	96.7	0.15	0.13	12
Glasgow	101.5	-0.01	0.00	12
Grafton	102.0	-0.18	0.17	12
Humber	99.3	0.03	0.00	12
Invicta	103.8	-0.06	0.02	12
JB-Diego	100.6	0.18	0.18	12
Ketchum	97.5	0.36	0.28	12
Kingdom	94.2	0.17	0.10	12
KWS_Curlew	98.2	0.11	0.05	12
KWS_Horizon	98.8	-0.22	0.17	12
KWS_Quartz	98.7	-0.08	0.13	12
KWS_Sterling	102.3	0.03	0.00	12
Marksman	96.1	0.14	0.08	12
Oakley	110.0	-0.27	0.26	12
Panorama	99.4	0.14	0.16	12
Qplus	97.6	-0.07	0.02	12
Robigus	103.4	-0.18	0.08	12
Scout	101.1	-0.26	0.39	12
Solstice	93.1	0.12	0.08	12
Timber	97.3	0.12	0.06	12
Viscount	102.8	-0.04	0.02	12
Warrior	101.0	0.07	0.02	12

Table 9. Finlay-Wilkinson regressions of relative grain yields of varieties vs. DSI using nine breeders' sites in 2009 (see Table 6, Appendix). Six varieties in boldface showed statistically significant slopes ($R^2 \geq 0.44$, 7 df).

Variety	R^2	Slope	Intercept
ACCESS	0.02	-0.08	110
ALCHEMY	0.03	0.11	107
ALIXAN	0.02	-0.16	101
AMBROSIA	0.00	-0.04	107
ANDALOU	0.01	0.05	97
APACHE	0.15	-0.28	102
AVALON	0.18	-0.29	95
BACANORA	0.02	-0.14	69
BATTALION	0.38	-0.44	110
BEAVER	0.10	0.26	99
BROMPTON	0.31	-0.41	115
BUSTER	0.00	0.04	100
CADENZA	0.01	-0.17	100
CAPHORN	0.01	-0.10	96
CAPPELLEDESPREZ	0.07	0.42	74
CEZANNE	0.40	-0.47	99
CLAIRE	0.10	0.18	104
CONSORT	0.10	0.28	98
CORDIALE	0.07	-0.19	103
DEBEN	0.24	0.55	101
DOVER	0.04	-0.06	100
EINSTEIN	0.02	0.13	103
EQUINOX	0.04	-0.16	105
EXOTIC	**0.47**	-0.53	104
EXSEPT	0.28	0.50	96
GALAHAD	0.06	0.16	94
GATSBY	0.11	-0.42	108
GLADIATOR	**0.67**	0.40	101
GLASGOW	0.21	0.37	102
GULLIVER	0.00	-0.01	104
HAVEN	0.27	0.32	101
HEREWARD	0.08	0.75	84
HOBBIT	**0.56**	0.52	97
HUMBER	0.07	0.24	103
HUNTSMAN	0.00	0.08	88
HUSTLER	0.02	0.16	99
HYPERION	0.11	-0.30	108
ISTABRAQ	0.02	0.06	108
LONGBOW	0.05	-0.10	105
MALACCA	0.01	-0.05	98
MARISWIDGEON	0.06	0.30	71
MARKSMAN-06	0.15	-0.29	108
MASCOT	0.02	-0.07	100
MENDEL	0.00	-0.02	99
MERCATO	0.06	0.16	101
MERCIA	0.17	-0.21	94
MUSKETEER	0.02	0.10	101
NORMAN	**0.53**	0.41	100
OAKLEY	0.00	0.01	110
PARAGON	0.10	0.24	91
RECITAL	0.30	-0.64	98
RIALTO	0.36	-0.60	105
RIBAND	0.01	0.07	104
ROBIGUS	0.16	0.39	103
ROYSAC	0.15	-0.19	97
SANKARA	0.10	-0.25	103
SAVANNAH	0.28	-0.34	110
SOISSONS	**0.45**	-0.59	97
SOLSTICE	0.28	-0.45	106
SPARK	0.01	-0.14	101
TIMBER	0.04	-0.19	106
VIRTUE	**0.56**	0.38	95
XI19	0.04	0.20	104
ZEBEDEE	0.33	0.50	106

Table 10. Response of varieties to available soil moisture in a combined dataset of RL (13 sites) and breeder trials (8 sites) in 2009. Only a subset of varieties had representation in both data sets. None of the slopes were statistically significant.

Variety	R^2	Slope	Intercept	Number of Trials
Alchemy	0.02	-0.11	105	21
Battalion	0.02	-0.09	103	21
Claire	0.14	0.28	102	13
Cordiale	0.00	-0.04	99	21
Einstein	0.00	-0.04	100	21
Gladiator	0.04	0.12	100	21
Glasgow	0.02	0.07	103	21
Humber	0.00	0.01	102	21
Marksman	0.01	-0.08	102	21
Oakley	0.05	-0.15	110	21
Robigus	0.00	-0.03	104	21
Solstice	0.02	-0.10	98	21
Timber	0.01	-0.07	102	21

Photo 1. Images of the polytunnel rainout shelters at Broom's Barn used to impose managed drought conditions. Irrigated plots were in blocks adjacent to the droughted area. Note the striking visual differences between genotypes, reflecting the genetic diversity of the materials.

Photo 2. Illustration of genotypic contrasts in leaf rolling behaviour in droughted plots at Brrom's Barn. Photos were taken between 12:00 and 12:30 on 10-6-08. Values represent the visual score for leaf rolling assigned to each plot.

Photo 2. Illustration of genotypic contrasts in floret sterility. Samples were taken from droughted plots at Broom's Barn on 22-6-09.

Photo 2. Illustration of genotypic contrasts in leaf wax. Samples were taken from droughted plots at Broom's Barn on 19-6-07. Values represent the mean visual score value assigned to plots of each genotype.

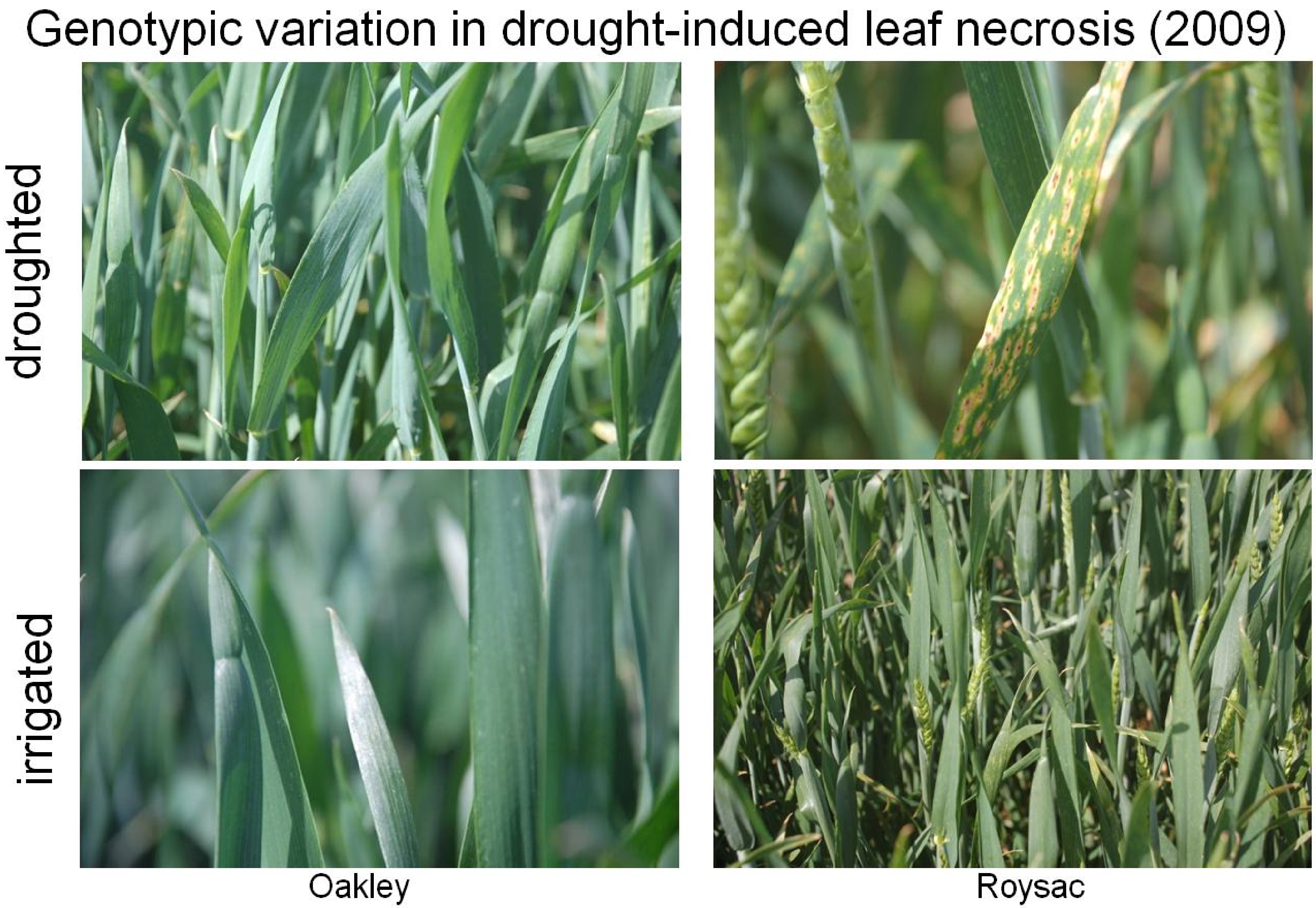


Photo 2. Illustration of genotypic contrasts in drought-induced leaf necrosis. Photos were taken on 18-5-09.