Relationships between $\delta^{13}C$, $\delta^{18}O$ and grain yield in bread wheat genotypes under favourable irrigated and rain-fed conditions

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Abstract

In previous investigations, carbon isotope composition ($\delta^{13}C$) has been used in C_3 cereals to screen for genotypes with high transpiration efficiency and oxygen isotope composition ($\delta^{18}O$) has been shown to correlate with transpiration rate. We examined associations of $\delta^{13}C$ of the grain and flag leaf and $\delta^{18}O$ of the flag leaf with respect to grain yield in wheat cultivars in UK field conditions. Field experiments were carried out at University of Nottingham in 2009-10 and 2010-11 testing 17 wheat cultivars under fully irrigated and rain-fed conditions. Averaging across years grain yield was reduced by 1.69 t ha^{-1} (16.5%) in the rain-fed treatment ($P < 0.001$). There was a negative linear relationship between grain yield and grain $\delta^{13}C$ amongst cultivars, under both irrigated ($R^2 = 0.47$, $P < 0.01$) and rain-fed ($R^2 = 0.70$, $P < 0.001$) conditions. Grain $\delta^{13}C$ was negatively correlated with flag-leaf stomatal conductance ($r = -0.94$, $P < 0.01$) in a subset of six of the cultivars, indicating that higher transpiration efficiency was associated with lower stomatal conductance. The associations between grain yield and flag-leaf δ^{13}C and flag-leaf δ^{18}O amongst cultivars under irrigated and rain-fed conditions were not statistically significant. There was a positive linear relationship between flag-leaf δ^{18}O and grain δ^{13}C amongst cultivars under irrigation ($R^2 = 0.38$, $P < 0.01$), indicating a trade-off between transpiration and transpiration efficiency (TE). Genetic variation in grain yield under rain-fed conditions was also associated with delayed onset of flag-leaf senescence in our experiments ($R^2 = 0.35$, $P < 0.05$). The 17 wheat cultivars ranged in year of release (YoR) from 1964 to 2009 and grain yield increased linearly under irrigated conditions by 60.4 kg ha^{-1} yr^{-1} (0.72 % yr^{-1}) and under rain-fed conditions by 47.5 kg ha^{-1} yr^{-1} (0.66 % yr^{-1}) over the 45 year period and grain δ^{13}C composition decreased by 0.0255 and 0.0304 ‰ yr^{-1}, respectively, indicating genetic gains in wheat yield potential in the UK seem likely to have been achieved through a lower TE, higher water uptake and lesser limitation of stomatal conductance.

1. Introduction

Worldwide, drought limits agricultural productivity more than any other single factor. Wheat (*Triticum aestivum* L.) provides, on average, one-fifth of the total calorific input of the world's population. In the UK, winter wheat is the most widely grown arable crop and contributes c. 16 million tonnes per annum with an average productivity of c. 8.5 t ha^{-1} (DEFRA 2015). The significantly warmer and more extreme conditions now arising from climate change (IPCC 2014) mean that new cultivars with greater drought resistance must be developed to maintain food security. In the UK, water deficits can commonly limit wheat yields in some years, where, typically, the onset of drought is post-anthesis, and losses are c. 20-30% (Foulkes et al., 2002).

Plants discriminate against the heavier carbon isotope (^{13}C) during photosynthesis and the extent of this discrimination depends on the ratio of intercellular versus external CO_2 concentration (C_i/C_a) in photosynthetic organs (Farquhar et al., 1982). The carbon isotope composition ($\delta^{13}C$) is negatively related to Ci/Ca ($\delta^{13}C$) (Farquhar et al., 1982) , which, in turn, is negatively related to the transpiration efficiency ~~(TE)~~ at the stoma (CO_2 assimilation/ transpiration). Therefore, carbon isotope composition ($\delta^{13}C$; frequently expressed as discrimination from the source air, $\Delta^{13}C$) is positively associated with TE. When measured in dry matter, $\delta^{13}C$ provides information on the long-term transpiration efficiency of C_3 plants (Farquhar and Richards 1984). Conditions that induce stomatal closure, such as water deficit, restrict the CO_2 supply to carboxylation sites, which then increases the $\delta^{13}C$ (or decreases $\Delta^{13}C$) of plant matter (Farquhar et al., 1989). The carbon isotope signature has been used as a selection indicator for high TE in commercial wheat breeding for water-limited environments (Rebetzke et al., 2002; Condon et al., 2002; Condon et al., 2004). Selection for $\Delta^{13}C$ was used to develop the Australian spring-wheat cultivars Drysdale and Rees (Richards, 2006). Under severe drought in Australia, $\Delta^{13}C$ of grain was negatively correlated with aerial biomass and grain yield for wheat (Rebetzke et al., 2002). In other instances of more mild droughts with relatively plentiful water up to anthesis, the relationship between $\Delta^{13}C$ and grain yield of wheat has been positive, associated with higher stomatal conductance (gs) and increased water use (WU; the total water absorbed and further transpired by the plant) and biomass in Mediterranean conditions (Araus et al., 2001, 2003; Condon et al., 2004; Zhou et al., 2015) and in the UK (Aravinda-Kumar et al., 2011). Therefore, except for severe drought conditions, water use appears to be a more important adaptive trait than the water-use efficiency (WUE; ratio of aerial biomass to evapotranspiration) (Slafer and Araus, 2007; Blum, 2009). An important breeding objective is therefore to identify sources of high WU in which any trade-off with WUE is minimized. The $\delta^{13}C$ signature provides no definitive information on whether grain variation in $\delta^{13}C$ is being driven by variation in stomatal conductance (g_s) or photosynthetic capacity, although the major role of stomatal conductance has been implied in diverse studies (Araus et al., 2001, 2003; Condon et al., 2004; Aravinda-Kumar et al., 2011; Zhou et al., 2015).

The oxygen isotope signature ($\delta^{18}O$) of vegetative tissues can be used as an indirect measure of transpiration and WU. The oxygen isotope abundance of plant matter (usually expressed as a composition, $\delta^{18}O$ of the bulk matter) can be used to separate the independent effects of assimilation (*A*) and g_s on $\delta^{13}C$ since it is unaffected by photosynthesis (Farquhar et al. 2007). The oxygen isotope signature integrates the evaporative conditions throughout the crop cycle (Barbour et al., 2000) and has been proposed as a proxy method for measuring transpiration and water use in different crop species (Barbour et al., 2000; Sheshshayee et al., 2005; Cabrera-Bosquet et al., 2009a, 2011). $\delta^{18}O$ is affected by air humidity and temperature (Barbour et al., 2000; Helliker and Ehleringer, 2002a, 2002b), soil moisture (Saurer et al., 1997; Ferrio et al., 2007) and source water (Williams et al., 2005; Asbjornsen et al., 2008). Nevertheless, when comparing genotypes growing under the same water conditions, $\delta^{18}O$ may be used to assess the effect of treatments and genotypic variability on yield in wheat, e.g. in bread wheat (Barbour et al., 2000; Ferrio et al., 2007; Cabrera-Bosquet et al., 2009a; Zhou et al., 2015) and durum wheat (Cabrera-Bosquet et al., 2011; Araus et al., 2013). A negative relationship between $\delta^{18}O$ and grain yield was reported in field experiments under fully-irrigated conditions across a set of eight bread wheat cultivars (Barbour et al., 2000) and in nine bread wheat cultivars grown under high-yielding Mediterranean conditions in Spain (Zhou et al., 2015). However, Ferrio et al. (2007) failed to find a phenotypic correlation between $\delta^{18}O$ and yield across a set of 24 bread wheat genotypes growing under three different water regimes. Therefore, studies reporting the use of $\delta^{18}O$ in water-limited cereals grown under real field conditions are still scarce and the results are contradictory.

The combined measurement of $\delta^{13}C$ and $\delta^{18}O$ in plant tissues is of interest in breeding due to their relationship to photosynthetic and transpiration performance of the plant during the course of crop growth, i.e. measurement of $\delta^{13}C$ and $\delta^{18}O$ can be potentially be used to identify genotypes which minimize any trade-off between TE and transpiration. This may help plant breeders to select genotypes that are better suited to drought. A positive relationship between genetic variation in grain $\delta^{18}O$ and grain $\delta^{13}C$ was observed (implying a negative relationship between TE and transpiration) in durum wheat for 10 cultivars under irrigated and unirrigated conditions (Araus et al., 2013) and in bread wheat for nine cultivars under high-yielding conditions (Zhou et al., 2015). Previously studies reporting the effect of water limitation on both the carbon and oxygen signatures amongst genotypes were conducted in pots in maize (Cabrera-Bosquet, et al. 2009b), in the field in seedlings of tropical tree species grown in a tropical environment (Cernusak et al., 2009) and in the field in durum wheat (Araus et al., 2013). However, no previous studies have investigated the effects of water limitation on both the carbon and oxygen signatures amongst bread wheat genotypes. Our objectives were firstly to examine associations between $\delta^{13}C$, $\delta^{18}O$ and grain yield and associated physiological mechanisms amongst 17 bread wheat cultivars with year of release from 1964 to 2009 grown under favourable irrigated and rain-fed conditions and secondly to quantify changes in grain yield and the carbon and oxygen signatures with year of release over the 45 year period in field experiments at Nottingham, UK in two seasons.

2. Materials and Methods

2.1 Experimental design and plot management

Seventeen wheat cultivars (Table 1) were grown under irrigated and rain-fed conditions in field experiments in 2009-10 and 2010-11 on a sandy loam soil type (Dunnington Heath Series) at University of Nottingham farm, Leicestershire UK (52.834 N, -1.243 W). Cultivars ranged in year of release (taken as their year first included on the UK Recommended List) from 1964 to 2009, and were chosen because they were representative of the most widely grown cultivars over the 45-year period (Table 1). The cultivars were known to contrast for possession of major genes including the semi-dwarf *Rht-B1* and *Rht-D1* genes and the photoperiod sensitivity *Ppd-D1* gene (Table 1). Fourteen of the cultivars were semi-dwarfs, and three were tall cultivars (Maris Widgeon, Cadenza and Paragon); fourteen were winter wheat cultivars, and three were spring wheat cultivars (Cadenza, Paragon and Xi19); and all were photoperiod sensitive cultivars apart from Soissons which was photoperiod insensitive. Eight of the cultivars were bread-making cultivars (M. Widgeon, Hereward, Cadenza, Rialto, Soissons, Paragon, Xi19, Cordiale and Panorama) and nine were feed or biscuit-making cultivars. The experimental design was a split-plot randomised block with three replicates. Irrigation treatments were randomised on main-plots and cultivars on sub-plots. In the irrigated treatment, a trickle irrigation system was used to maintain soil moisture deficit (SMD), calculated using the ADAS Irriguide model (Bailey & Spackman 1996), to < 0.50 available water (AW) up to GS61 + 28 days and < 0·75 AW thereafter. The AW capacity to 1.2 m soil depth was 176 mm. No water was applied in the rain-fed treatment.

Sub-plot size was 6 x 1.65 m. Previous cropping was winter oats in both seasons. In each experiment, the field was ploughed and power harrowed and rolled after drilling. Seed rate was adjusted by genotype according to 1,000 grain weight to achieve a target seed rate of 320 seeds m^{-2}; rows were 0.13 m apart. In each season, 200 kg ha^{-1} nitrogen fertilizer as ammonium nitrate was applied in a three-split programme. P and K fertilizers were applied to ensure that these nutrients were not limiting. Plant growth regulator was applied at GS31 to reduce the risk of lodging. Sowing dates were 6 October 2009 and 14 October 2010. Herbicides, fungicides and pesticides were applied as required to minimise effects of weeds, diseases and pests.

Table 1 here

2.2 Crop measurements

In all plots in each of the 2009-10 and 2010-11 field experiments, flag-leaf samples at GS61 and grain dry matter samples at harvest were assessed for carbon δ^{13}C composition. Flag-leaf samples at GS61 were also assessed for oxygen δ^{18}O composition.

2.2.1 Anthesis measurements

Date of GS61 was measured by recording Zadoks' stages for each sub-plot every 3-4 days through the flowering window. Crop growth was assessed in all sub-plots at anthesis from a defined area of 0.64 m^2 by cutting the shoots at soil level. All cultivars were sampled on the date of reaching the stage at anthesis. Following sampling, a random 10% sub-sample (by fresh weight) of plant material was taken on which the following measurements were carried out. The number of fertile (those with an ear) and infertile shoots was counted. For the fertile shoots, green areas were measured for: (i) flag-leaf lamina, (ii) remaining leaf lamina, (iii) stem and attached leaf sheath, and (iv) ear, using a Li-Cor 3100 leaf area meter (LI-COR Inc., Lincoln, NE), and the components summed to give the green area index (GAI). Aboveground dry matter was measured on each component of the fertile shoots (flag-leaf lamina, remaining leaf lamina, stem and attached leaf sheath, ear) and for the infertile shoots after drying for 48 h at 80 °C.

2.2.2 Harvest analysis

Plant samples were taken from a 0.64 m^2 area and a 10% sub-sample (by fresh weight) of plant material was assessed as follows: plant material was separated into ears and straw. Ears were counted and threshed, and the chaff (rachis, rachilla, glume, palea, and lemma), grain and straw were weighed separately after drying for 48 h at 80 ^{o}C. Harvest index was calculated as the fraction of AGDM present as grain. A sub-plot area of at least 5 m^2 was machine-harvested at harvest and the grain weighed. The grain yield is expressed at 0% moisture content.

2.2.3 Carbon isotope and oxygen composition

Carbon isotope analysis

From each sub-plot dried flag leaves (from sample at anthesis) and grains (from sample at harvest) were milled separately for use in ^{13}C:^{12}C isotope ratio analysis. The samples were ground to a fine powder using a cyclotec 1093 sample machine. The milled samples (1 mg) were then weighed out in tin cups and analysed through an online system composed of an elemental analyser (EA), a TripleTrap and a mass spectrometer (Carlo Erba 2100, Milan, Italy) to determine carbon isotope composition (Aravinda Kumar et al., 2011). The EA interfaced with an isotope ratio mass spectrometer (IRMS; Thermo-Finnigan Deltaplus Advantage, Bremen, Germany) to analyse ^{13}C:^{12}C ratio (R) of plant material. Results were expressed as δ^{13}C composition values, using a secondary standard calibrated against Vienna Pee Dee Belemnite calcium carbonate (VPDB), and the analytical precision was ~0.1‰ (Eqn 1).

$$\delta^{13}\mathrm{C}\ (^{o}/_{oo}) = [\ (\mathrm{R}_{\mathrm{sample}}/\mathrm{R}_{\mathrm{standard}}) - 1] \times 1{,}000 \qquad \text{Eqn 1}$$

Oxygen isotope analyses

From each sub-plot dried flag leaves (from sample at anthesis) were milled for ^{18}O:^{16}O isotope ratio analysis. The milled samples (1 mg) were weighed out in silver cups and analysed through an online system composed of an EA, a TripleTrap and a mass spectrometer (Carlo Erba 2100, Milan, Italy) to determine oxygen isotope composition. The ^{18}O:^{16}O ratios (R) were determined by an on-line pyrolysis technique using a thermo-chemical elemental analyser (TC/EA Thermo Quest Finnigan, Bremen, Germany) coupled with an IRMS (Delta C Finnigan MAT, Bremen, Germany). Results were expressed as δ^{18}O values, using a secondary standard calibrated against the Vienna standard mean oceanic water (VSMOW) (Eqn 2).

$$\delta^{18}\mathrm{O}\ (^{o}/_{oo}) = [(R_{\mathrm{sample}}/R_{\mathrm{standard}}) - 1] \times 1{,}000 \qquad \text{Eqn 2}$$

2.2.4 Flag-leaf leaf photosynthetic rate and stomatal conductance

Gas-exchange readings on flag leaves were carried out in the rain-fed treatment in a subset of six cultivars (Avalon, Cordiale, Glasgow, Hobbit, Maris Widgeon and Paragon) on two dates around anthesis in each year (2 and 24 June 2010 and 31 May and 7 June 2011). Light-saturated photosynthetic rate (A_{max}) and stomatal conductance (g_s) of the flag leaf was measured using a Li-Cor LI-6400XT Portable Photosynthesis System (Licoln, NE, USA). In each sub-plot in two replicates, three readings were taken per sub-plot on randomly selected fertile shoots between 11:00 and 15:00. The instrument was calibrated for 50-60% relative humidity and the settings for the A_{max} readings were: photosynthetically active radiation (PAR) 2000 µmol m^{-2} s^{-1}, sample chamber CO_2 concentration 360 µl l^{-1} and flow rate 500 µ mol s^{-1}.

2.2.5 Flag-leaf senescence

Senescence kinetics of the flag leaf were assessed visually by recording the percentage green area senesced using a standard diagnostic key based on a scale of 0-10 (10 = flag leaf 100% senesced). Visual scoring of the plots was carried out twice weekly after anthesis (GS61) until complete leaf senescence. The same diagnostic key was used in the two seasons, and one operator assessed senescence scores throughout a given season. The visual senescence score was fitted against thermal time (base temperature $0^{o}C$) post-anthesis (GS61) using an equation with five parameters consisting of a monomolecular and logistic function and the onset and end of the rapid phase of post-anthesis senescence was determined as described by Gaju *et al*. (2009).

2.2.6 Plant height

Two to three days before harvest, plant height from the ground to the tip of the ear was recorded in three locations per subplot.

2.3 Soil water measurements

In 2010 in the rain-fed treatment in each replicate, volumetric soil water content to 1.0 m was measured using a PR2 Soil Moisture Profile Probe (Delta T Devices, Burwell, UK) inserted in one polycarbonate access tube per sub-plot on 25 April at around onset of stem extension. Readings were taken approximately every 14 days at 10, 20, 30, 40, 60 and 100 cm soil depth. In addition, in both years in the rain-fed treatment, volumetric soil water content was assessed from gravimetric analysis of soil cores (four cores per sub-plot, 2.5 cm diameter) to 100 cm soil depth during stem extension, on 27 April 2011 and 20 May 2012, and at harvest.

2.4 Statistical analysis

Treatment means were compared using least significance differences (LSD) calculated from standard errors of the difference of the means using appropriate degrees of freedom when ANOVA indicated significant differences using GenStat 16th edition statistical package for windows (VSN International, Hemel Hempstead UK). Relationships between traits were evaluated using a simple linear regression analysis for both the fully irrigated and rain fed treatments. A cross-season ANOVA was applied to analyse irrigation treatments and genotype effects across seasons and the interaction with season, assuming irrigation treatments and genotypes were fixed effects and replicates and seasons were random effects using Genstat version 16. Pearson's correlation coefficient and linear regressions were calculated using mean data for replicates using Genstat version 16. Bi-plot procedures to test associations between traits were carried out using the R software Version 3.0-2 (http://www.R-project.org/).

3. Results

3.1 Growing conditions

Rainfall was below the long-term mean (LTM) during stem elongation in April and May in 2010 (-45% LTM) and 2011 (-44% LTM) (Table 2). During grain filling in June and July rainfall was slightly above the LTM in 2010 (+17%), but below the LTM in 2011 (-34%). These rainfall patterns resulted in pre- and post-anthesis drought occurring in both years. In 2010 onset of drought (assuming a limiting soil moisture deficit (SMD) of 50% AW, 88 mm; Foulkes et al., 2001) occurred in the third week of May at around flag-leaf emergence (Fig. 1). Thereafter, SMD increased progressively to harvest, apart from a temporary decrease during early grain filling due to significant rainfall of 50.3 mm from 6-10 June at the start of anthesis. In 2011, although soil water content wasn't measured using the PR2 probe, the gravimetric estimate of soil water content on 20 May at around flag-leaf emergence and at harvest and the daily pattern of rainfall indicated onset of drought occurred, i.e. the limiting SMD of 88 mm was exceeded, at a similar stage to 2010 at around early booting with SMD thereafter progressively increasing through late stem extension and grain filling.

Temperatures during stem extension were significantly above the LTM in both years, particularly during early to mid-stem extension in April. In 2010, temperatures were warmer than average during grain filling in June and July. In 2011, temperatures were close to the LTM during the first half of grain filling in June, and slightly cooler than average during later grain filling in July. Radiation was generally brighter than the LTM during stem elongation in both years, particularly during early to mid-stem extension. Solar radiation was above average during the first half of grain filling in June in 2010 and close to the LTM during the later grain filling in July. In 2011, radiation was above average during the whole of grain filling.

Table 2 here

Figure 1 here

3.2 Plant height and flowering time

Averaging across years, drought in the rain-fed treatment reduced plant height from 82.7 to 79.9 cm ($P < 0.05$) and advanced anthesis date by 1 day (10 June vs 11 June).. Averaging across irrigation treatments, cultivars differed in plant height in the range 69.5 (Cordiale) to 109.5 cm (M. Widgeon) and in anthesis date from 3 (Soissons) to 15 (Xi19) June ($P < 0.001$). There was a trend for an irrigation x genotype interaction for plant height ($P = 0.07$) with several cultivars showing minimal change in response to drought (e.g. Beaver +0.2 cm, Cadenza +0.1 cm, Glasgow +0.2 cm and Oakley +1.4 cm) and other cultivars showing decreased plant height under drought (e.g. Paragon -5.2 cm, Panorama -6.5 cm and Rialto -7.9 cm).

3.3 Grain yield and above-ground biomass at harvest

In 2010, drought decreased grain yield from 10.40 t ha^{-1} in the irrigated treatment to 8.54 t ha^{-1} (-17.9%; $P < 0.05$; Table 3). Averaging across irrigation treatments, cultivars ranged from 7.60 (Maris Widgeon) to 10.48 (Istabraq) t ha^{-1} ($P < 0.001$). The decrease with restricted water availability ranged amongst cultivars from 1.10 (-11.7%, Soissons) to 2.35 (-24.6%, Xi19) t ha^{-1} but did not differ significantly. In 2011, drought decreased yield overall from 11.41 to 9.90 t ha^{-1} (-13.2%, $P < 0.05$). Averaging over irrigation treatments, cultivars ranged from 8.03 (Maris Widgeon) to 11.66 (Panorama) t ha^{-1}; and cultivars differed in response to drought with decreases in the range 0.96 (-9.1%, Hobbit) to 2.46 (-22.4%, Cadenza) t ha^{-1} ($P <0.05$). Averaging across years, drought reduced grain yield from 10.91 to 9.22 t ha^{-1} (15.5%, $P < 0.05$). Responses to drought differed amongst cultivars in the range -1.10 (-11.5%, Hobbit) to -2.34 (-22.8%; Xi19) t ha^{-1} ($P < 0.05$). The year x irrigation x genotype interaction was not significant.

Above-ground biomass was reduced from 19.70 to 16.51 t ha^{-1} in 2010 (-16.2%; $P < 0.05$) and from 22.21 to 19.05 t ha^{-1} (-14.2%, $P < 0.05$) in 2011 under drought (Table 3). Overall cultivars ranged from 17.08 (Cordiale) to 19.72 t ha^{-1} (Istabraq) in 2010 and 18.93 (Cordiale) to 22.33 t ha^{-1} (Paragon) in 2011 ($P < 0.001$). The cultivars responded differently to drought with decreases in the range 0.43 (M. Widgeon) to 4.12 t ha^{-1} (Xi19) in 2011 ($P < 0.01$), but not in 2010. The cross-year ANOVA indicated that the irrigation x genotype interaction was not statistically significant.

Table 3 here

3.4 Anthesis crop growth

Averaging across years, GAI was decreased under drought from 5.18 to 4.04 ($P < 0.001$; Table 4). Overall cultivars differed in the range 3.66 (Rialto) to 5.79 (M. Widegon) ($P < 0.001$). The cultivars responded differently to drought with decrease in GAI ranging from 2.3% (Oakley) to 42.6% (Savannah) ($P < 0.001$). Averaging across years, above-ground biomass at GS61 decreased slighlty from 13.4 t ha^{-1} under irrigation to 12.8 t ha^{-1} under drought ($P < 0.05$). Cultivars overall ranged from 11.5 (Beaver) to 14.8 t ha^{-1} (Rialto; $P < 0.001$), and responded differently to drought with decreases ranging from +2% (Soissons) to -18% (Rialto) ($P < 0.01$).

Table 4 here

3.5 $\delta^{13}C$ and $\delta^{18}O$ and relationships with grain yield

Averaging across years, grain δ^{13}C composition increased from -28.1‰ under irrigated to -26.6 ‰ under rain-fed conditions ($P < 0.001$, Table 5). Cultivars overall varied from -28.1 (Savannah) to -26.2‰ (M. Widgeon) (Table 5). The irrigation x cultivar interaction was not statistically significant. Results showed a negative linear relationship between grain δ^{13}C and grain yield amongst the 17 cultivars under both irrigated ($R^2 = 0.31$, $P = 0.02$) and rain-fed ($R^2 = 0.67$, $P < 0.001$) conditions in 2010 (Fig. 2). Similarly in 2011, a negative linear relationship was found under both irrigated ($R^2 = 0.53$, $P < 0.001$) and rain-fed ($R^2 = 0.58$, $P < 0.001$) conditions. Averaging across years, the negative linear relationship was again significant under both irrigated ($R^2 = 0.47$, $P < 0.01$) and unirrigated ($R^2 = 0.70$, $P < 0.001$) conditions. Omitting the tallest (non semi-dwarf) cultivar Maris Widgeon released in 1964, which had the lowest yield and the highest grain δ^{13}C under both irrigated and rain-fed conditions, the linear relationship between between grain δ^{13}C and grain yield amongst the remaining 16 cultivars was still significant under both irrigated ($R^2 = 0.25$, $P < 0.05$) and rain-fed ($R^2 = 0.46$, $P < 0.01$) conditions. Averaging across years, there was no linear relationship between anthesis date and grain δ^{13}C amongst the 17 genotypes under either irrigated or rain-fed conditions ($R^2 = 0.04$, P = 0.42 and $R^2 = 0.01$, P = 0.67. respectively). The linear relationship between grain yield and flag-leaf δ^{13}C was not statistically significant amongst the 17 cultivars under either irrigated or rain-fed conditions in individual years or averaging across years.

Flag-leaf δ^{18}O composition overall increased from 23.9‰ under irrigated to 24.3‰ under rain-fed conditions ($P < 0.05$; Table 5). Cultivars differed overall in the range 23.5 (Soissons) to 24.5‰ (Avalon) ($P < 0.05$). From the cross-year ANOVA there was an irrigation x cultivar interaction with Hereward, Maris Widgeon, Panorama and Xi19 showing slight decreases in δ^{18}O under drought in contrast to increases for other cultivars. The linear relationship between grain yield and flag-leaf δ^{18}O was not statistically significant amongst the 17 cultivars under either irrigated or rain-fed conditions in 2010, 2011 or averaging across years (Fig. 3).

Fig 2 near here

Fig. 3 near here

Table 5 here

3.6. Relationship between δ^{13}C and δ^{18}O

Flag-leaf $\delta^{18}O$ is not strongly influenced by photosynthesis rate, so measurement of grain $\delta^{13}C$ and flag-leaf $\delta^{18}O$ may allow stomatal and photosynthesis effects on $\delta^{13}C$ to be teased apart. Under drought the assocation between grain $\delta^{13}C$ and flag-leaf $\delta^{18}O$ amongst cultivars was not significant, but under irrigated conditions there was a positve linear association in 2010 ($R^2 = 0.19$, $P = 0.08$), 2011 ($R^2 = 0.39$, $P < 0.01$) and averaging across years ($R^2 = 0.38$, $P < 0.01$, Fig. 4). This indicated that higher transpiration effciency (higher $\delta^{13}C$) was associated with lower transpiration (higher $\delta^{18}O$). Omiting the old, tall cultivar Maris Widgeon averaing across years there was still a positve linear association between grain $\delta^{13}C$ and flag-leaf $\delta^{18}O$ under irrigated conditions ($R^2 = 0.28$; $P < 0.05$). Therefore, present results implied there was a trade-off between TE and transpiration. However, there were apparent departures from this overall positive relationship between $\delta^{13}C$ and $\delta^{18}O$ for indidvual cultivars. Thus, Maris Widgeon, Avalon and Soissons maintained high TE (high grain $\delta^{13}C$) relative to transpiration (flag-leaf $\delta^{18}O$) , as indicated by large positive standardized residuals above the regression line in Fig. 4), and the opposite was the case for Rialto and Savannah.

Fig. 4 here

3.7 Flag-leaf stomatal conductance and leaf photosynthetic rate

Gas-exchange measurements on flag leaves were carried out in the rain-fed treatment in a subset of six cultivars on two dates around anthesis in each year. There were significant differences amongst the cultivars for stomatal conductance on 2 June in the range 282-412 mmol m^{-2} s^{-1} and on 24 June in the range 315-470 mmol m^{-2} s^{-1} in 2010 and for the overall mean across the four readings in the two seasons in the range 243 (M. Widgeon) - 332 (Paragon) mmol m^{-2} s^{-1} ($P < 0.05$, Table 6). For flag-leaf photosynthetic rate, cultivars differed on 2 June 2010 in the range 26.4-35.3 μmol m^{-2} s^{-1} ($P < 0.05$) but not for other readings or for the overall mean across the four readings in the two years.

Averaging over assessments and years, there was a strong trend for a positive correlation amongst cultivars between stomatal conductance and grain yield ($P = 0.06$; Table 6). Stomatal conductance was negatively correlated amongst cultivars with grain $\delta^{13}C$ on 24 June 2010 ($P < 0.05$) and 31 May 2011 ($P < 0.05$), and with the overall mean across the four assessments ($P < 0.01$; Table 6). Flag-leaf photosynthesis rate was positively correlated with grain δ^{13}C amongst cultivars on 31 May 2011 ($P < 0.05$) and for the mean across all four assessments ($P = 0.05$). There were no statistically significant associations between flag-leaf photosynthesis rate and grain yield.

Table 6 here

3.8 Flag-leaf specific weight and senescence rate

There was a trend for flag-leaf specific weight (FLSW) to increase slighlty under drought from 62.7 to 66.5 g m^{-2} (P = 0.07). FLSW differed amongst cultivars in the range 56.6-70.9 g m^{-2} under irrigation and 53.6-81.9 g m^{-2} under drought ($P < 0.001$; Table 4). There was an irrigation x cultivar interaction ($P < 0.05$), with response of FLSW to drought ranging from +25.9% (Istabraq) to -12.3% (Soissons). Interestingly, under irrigated conditions, there was a negative linear association between FLSW under irrigation and the standardized residuals for the linear regression of grain δ^{13}C on flag-leaf δ^{18}O amongst cultivars (a more positive residual indicating higher TE (higher grain δ^{13}C) relative to tranpsiration (flag-leaf δ^{18}O) Fig. 5a).

The onset of the rapid phase of flag-leaf senenscence was advanced from 669 ^{o}Cd post-GS61 under irrigated conditions to 460 ^{o}Cd under drought. Cultivars overall ranged from 483 (Avalon) to 653 ^{o}Cd (Beaver; $P < 0.001$); the advancemet of onset of senescence under drought ranged from 107 ^{o}Cd (Zebedee) to 285 ^{o}Cd (Paragon) ($P < 0.001$). Averaging across years, there was a positive linear association between grain yield and the onset of senescence under drought ($R^2 = 0.35$, $P < 0.05$), but no signficant association under irrigated conditions (Fig. 5b). Associations between the end of post-anthesis flag-leaf senescence and grain yield amongst cultivars were not statistically signcant (data not shown).

Fig. 5 here

3.9 Princial component analysis(PCA) for yield, yield components and phsyiologcal traits

The relationships amongst grain yield, yield components and physiological traits are shown in the biplots in Fig. 6. PCA confirmed the associations between grain δ^{13}C and grain yield under both rain-fed and irrigated conditions and the absence of statistically significant associations between either flag-leaf δ^{13}C or flag-leaf δ^{18}O and grain yield. Variation in grain yield amongst genotypes was strongly positively associated with grains m^{-2} and negatively associated with grain weight in both irrigated and rain-fed conditions. Plant height was correlated positively with harvest biomass and negatively with grain yield in both irrigation treatments.

Fig. 6 here

3.10 Changes with year of release (YoR)

Grain yield increased linearly with year of release by 60.4 kg ha^{-1} yr^{-1} (0.72 % yr^{-1}) ($P < 0.001$) under irrigation and 47.5 kg ha^{-1} yr^{-1} (0.66 % yr^{-1}) under drought ($P < 0.001$; Table 7). Grain yield progress was associated with increases in above-ground DM under irrigation (54.6 ($P < 0.05$) and rain-fed conditions (30.8 kg ha^{-1} yr^{-1} (P = 0.07); and there were genetic gains in HI (%) of 0.180 % yr^{-1}($P < 0.01$) and 0.179 % yr^{-1} ($P < 0.01$), respectively. Plant height decreased over the 45-year period by 0.47 cm yr^{-1} under irrigated and 0.49 cm yr^{-1} under rain-fed conditions. Grain $\delta^{13}C$ decreased by 0.0255 and 0.0304 ‰ yr^{-1} under irrigated and rain-fed conditions, respectively. There were no statistically significant changes in flag-leaf $\delta^{13}C$, flag-leaf $\delta^{18}O$, onset of flag-leaf senescence or anthesis date with YoR under irrigated or rain-fed conditions.

Table 7 here

4. Discussion

The data collected in these experiments allowed consideration of the potential value of physiological traits including $\delta^{13}C$ and $\delta^{18}O$ as selection tools for drought resistance and the implications for wheat breeding.

4.1 Yield responses to drought

In the present study, the overall yield loss under drought of 1.86 (17.9%) and 1.51 t ha^{-1} (13.2%) in 2010 and 2011, respectively, was slightly smaller than typical losses under drought for winter wheat in the UK of *ca.* 20-30% (Innes et al., 1985; Foulkes et al., 2002, 2007). The high-yielding genotypes under irrigated conditions tended to lose more grain yield under drought, and there was a positive linear relationship between absolute grain yield loss under drought and yield in irrigated conditions ($R^2 = 0.44$, $P < 0.05$). From the physiological standpoint, it is not surprising that absolute reduction in yield for a given reduction in water resource is strongly influenced by yield potential (Fischer and Maurer, 1978; Foulkes et al., 2007; Aravinda-Kumar et al., 2011). In spite of the relatively mild drought stress, the range of yield reductions amongst cultivars was high as indicated by the significant irrigation x genotype interaction. Anthesis date of cultivars differed by up to 13 days under irrigation and 12 days under drought, in part, associated with presence/absence of the *Ppd-D1a* allele determining photoperiod insensitivity, with Soissons possessing the *Ppd-D1a* allele. However, there was no association between grain yield response to drought and anthesis date amongst cultivars. Therefore, there was no evidence that the wheat plants were able to escape drought by completing their life cycle before the onset of water deficit, as has been reported under more severe terminal droughts (Chaves *et al*., 2003). In these experiments, taller lines were associated with linear reductions in grain yield under both irrigated and rain-fed conditions as expected with decreased plant height favourng higher HI and grain number per unit area (Fischer, 1985); three of the 17 cultivars were tall non semi-dwarf cultivars (M. Widgeon, Cadenza and Paragon), and the remaining cultivars were semi-dwarfs. However, there was no association between plant height and grain yield response to drought.

The genetic gain in grain yield with YoR from 1964 to 2009 was 47.5 kg $ha^{-1}yr^{-1}$ (0.6% yr^{-1}), which is lower than that reported previously by Shearman et al. (2005) for 8 UK winter wheat cultivars released from 1972 to 2009 of 117 kg ha^{-1} yr^{-1} (1.2% yr^{-1}). The genetic gain in grain yield was associated with gains in both above-ground DM (30.9 kg ha^{-1} yr^{-1}) and HI (0.00176% yr^{-1}), which were again lower than changes in AGDM and HI reported by Shearman et al, (2005) of 104 kg ha^{-1} yr^{-1} and 0.0026% yr^{-1}, respectively. Clarke et al. (2013) for a set of 9 UK landmark winter wheat feed cultivars released from 1953 to 2007 reported a genetic gain in grain yield of 61 kg ha^{-1} yr^{-1} and for 11 UK landmark winter wheat bread-making cultivars released from 1964 to 2008 of 49 kg ha^{-1} yr^{-1}, similar to the rate of yield gain in the present study. Excluding the three spring wheats in the present study, the genetic gain in grain yield with YoR for 14 winter wheat cultivars did not change significantly compared to the 17 cultivars at 65.8 kg $ha^{-1}yr^{-1}$ under irrigated conditions and 50.0 kg ha^{-1} yr^{-1} under rain-fed conditions.

4.2 Relationships between isotope signatures and grain yield and biomass

There was a linear negative association between grain $\delta^{13}C$ and yield under drought, indicating lower TE was associated with higher grain yield. Similar correlations between the carbon isotope signature and grain yield under drought were reported previously in Mediterranean environments (Araus et al., 2001, 2003; Condon et al., 2004; Zhou et al., 2015) and the UK (Aravinda-Kumar et al., 2011). Although in the present study the association between grain $\delta^{13}C$ and yield was stronger under drought ($R^2 = 0.70$; $P < 0.01$), there was also an association under irrigation ($R^2 = 0.47$, $P < 0.01$). Moreover, grain $\delta^{13}C$ measured under irrigated and rain-fed conditions was linearly associated amongst genotypes ($R^2 = 0.77$; $P< 0.01$), suggesting grain $\delta^{13}C$ may be a constitutive trait under mild UK droughts. Therefore, selection for this trait under favourable conditions may be useful for indicating drought performance in breeding programmes. Grain $\delta^{13}C$ showed a linear decrease with year of release in both irrigated and rain-fed conditions indicating TE has decreased with plant breeding. Two of the 17 cultivars, Avalon and Istabraq, showed high positive departures (standardized residuals of 1.79 and 1.57, respectively) from the overall negative linear regression of grain yield on grain $\delta^{13}C$ (i.e. high grain yield relative to TE), demonstrating scope for selecting genotypes combining high yields with moderately high TE in UK adapted germplasm.

Grain $\delta^{13}C$ under drought was positively associated with flag-leaf stomatal conductance amongst the subset of six cultivars, indicating $\delta^{13}C$ and TE were determined in part by g_s; g_s was also positively associated with yield under drought. Therefore, the ability to access and transpire more water and maintain photosynthesis during grain filling appeared to be the main mechanism determining higher grain yield under drought in the present experiments. Similar findings were reported previously under Mediterranean-type droughts (Araus et al., 2001, 2003; Blum, 2009; Zhou et al., 2015) and UK droughts (Aravinda Kumar et al., 2011). Present results showed no association between flag-leaf $\delta^{13}C$ at anthesis and grain yield under rain-fed conditionsHowever, the difference between flag-leaf $\delta^{13}C$ and grain $\delta^{13}C$ showed a negative linear association with grain yield under rainfed conditions ($R^2 = 0.46$, $P< 0.01$). This again implies that increased stomatal conductance during grain filling amongst genotypes was critical in determining grain yield under drought. It is possible that higher stomatal conductance was associated with a more effective root system contributing to higher yield in the rain-fed environment.

In the present study, we found significant genetic variation in flag-leaf $\delta^{18}O$ in the range 23.6-24.4‰ in irrigated conditions and 23.4-24.8‰ in rain-fed conditions. Genetic variation in $\delta^{18}O$ has been previously reported for bread wheat (Barbour et al., 2000; Ferrio et al., 2007; Zhou et al. 2015), durum wheat (Araus et al., 2013) and maize (Cabrera-Bosquet et al., 2009b; Araus et al., 2010). Present findings indicated, however, no significant correlation between flag-leaf δ^{18}O and grain yield amongst the 17 cultivars under either rain-fed~~unirrigated~~ or irrigated conditions. A negative relationship of the oxygen isotope signature for grain with grain yield was reported amongst bread wheat cultivars under fully irrigated conditions (Barbour et al, 2000), bread wheat cultivars in high yield conditions in Spain (Zhou et al., 23015) and durum wheat varieties and landraces in Spain under rainfed and irrigated conditions (Araus et al., 2007). However, no phenotypic correlation was found across a set of 24 bread wheat genotypes (Ferrio et al., 2007) under three different water regimes. The reason for the lack of correlation between flag-leaf δ^{18}O and grain yield in the present experiments is not certain. The analytical precision of flag-leaf δ^{18}O is usually smaller than for grain δ^{13}C (Araus et al., 2007), and it is also possible that that spatial variation in soil N across the experimental site (Cernusak *et al*. 2007) or in air humidity and temperature (Barbour and Farquhar 2000) caused by different phasing of flag-leaf development pre-anthesis with respect to calendar time amongst the cultivars may have contributed to the lack of correlation.

The significant positive relationship between grain δ^{13}C and flag-leaf δ^{18}O under irrigated conditions in our study supported the contention that genetic variation in grain δ^{13}C was driven in part by changes in stomatal conductance, assuming that δ^{18}O is inversely associated to transpiration (Barbour et al., 2000). However, we found no relationship between grain δ^{13}C and flag-leaf δ^{18}O under drought conditions, where a relationship might have been expected since flag-leaf g_s was positively related with grain δ^{13}C. The lack of a correlation between flag-leaf δ^{18}O and grain δ^{13}C under drought may suggest that the application of δ^{18}O as an approach in estimating genetic variation g_s is not straightforward under drought, in that other factors may also have been altering the δ^{18}O signal as mentioned above.

Interestingly, there was a negative linear relationship amongst cultivars between FLSW and the standardized residual of the linear regression of grain δ^{13}C on flag leaf δ^{18}O, a higher residual indicating higher transpiration efficiency (higher δ^{13}C) relative to transpiration (δ^{18}O). The physiological mechanisms underpinning this relationship for lower FLSW to favour higher TE relative to transpiration cannot be certain. Aravinda-Kumar et al. (2011) reported a positive linear relationship between flag-leaf specific N and grain Δ^{13}C isotope discrimination amongst lines of a Beaver x Soissons winter wheat doubled-haploid population under drought in UK field experiments. Assuming genetic variation in flag-leaf specific N to be indicative of FLSW, this would imply lower FLSW was associated with higher TE in that study which would be generally consistent with the present findings. It can be speculated that lower FLSW was associated with lower flag-leaf g_s which, in turn, was associated with higher TE and that the main driver underpinning the trade-off between FLSW and TE relative to transpiration was a positive relationship between FLSW and g_s; however, g_s was not measured in the irrigated treatment in the present study. Further work is required to investigate the basis of this apparent association between FLSW and TE relative to transpiration under irrigated conditions.

4.3 Relationships between stay-green and grain yield and biomass

Greater yield production associated with longer green canopy area duration (stay-green) amongst genotypes has been reported under drought in wheat (Gorny and Garczynski, 2002; Verma et al., 2004; Foulkes et al., 2007; Christopher et al., 2008), sorghum (Borrell and Hammer, 2000) and maize (Campos et al., 2004). In the present study, averaging across years, there was a positive correlation amongst the cultivars between onset of flag-leaf senescence and grain yield under drought, but no association under irrigation. Higher grain yield associated with stay-green under post-anthesis abiotic stress is likely due to source limitation of grain yield (Christopher et al., 2008; Bogard et al., 2011), and greener canopies maintain the active photosynthetic rate better (Joshi et al., 2007). Conversely, present results in the irrigated treatment suggested that grain growth was limited by sink size rather than source size (Borras et al., 2004). The present positive association between onset of flag-leaf senescence and grain yield amongst genotypesis consistent with previous investigations under UK drought for winter wheat genotypes (Verma et al., 2004; Foulkes et al., 2007). In our study there was a large effect of drought on onset of flag-leaf senescence; drought advanced onset of senescence by 210 ^{o}Cd (approximately 14 days). However, the grain yield decrease was relatively modest at 1.69 t ha^{-1} (15.5%), suggesting that photosynthesis of non-laminar green organs (such as the ear, peduncle or sheaths) was still contributing to grain filling during the lamina senescence. Nevertheless, genetic variation in onset of flag-leaf senescence showed a moderately strong association with yield under the mild drought conditions (R^2 0.35, $P = 0.01$). The mechanisms underlying the genetic differences in leaf senescence cannot be certain from present measurements. Prolonged leaf senescence duration under low N availability in wheat was associated with lower N remobilization efficiency (Gaju et al., 2009) and greater post-anthesis N uptake (Bogard et al., 2011). Under drought, stay green was associated with deeper roots under drought during the grain-filling period for two

CIMMYT wheat lines SeriM82 and Hartog compared to check lines (Christopher et al., 2009).

In summary, it is suggested that screening for grain $\delta^{13}C$ will have value in breeding programmes aimed at improving yields in high yielding, rain-fed environments, but where drought can also be a problem, such as the UK. Droughts within the UK cannot be predicted with certainty even on soils of low available water due to unpredictability of rainfall. Traits for maintaining yield under drought must therefore carry no yield penalty in the absence of drought. Present results showed grain ^{13}C may be such a trait as it was strongly correlated with grain yield across post-green revolution cultivars under high yielding conditions and that the correlation was even higher under rain-fed conditions. Therefore, genetic gains in wheat yield potential in UK seem likely to have been achieved through a lower TE, higher water uptake and lesser limitation of stomatal conductance. It is possible these effects were associated with a more effective root system contributing to grain yield in the rain-fed environment given the association of stomatal conductance and flag-leaf senescence with grain yield.

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Figure legends

Figure 1. a) Daily rainfall (closed squares) and soil moisture deficit to 1.2 m soil depth estimated from capacitance probe (closed diamonds) and gravimetric analysis of soil cores (open circles) in 2010 and b) daily rainfall (closed squares) and soil moisture deficit to 1.2 m soil depth estimated from gravimetric analysis of soil cores (open circles) in 2011 under rain-fed conditions.

Figure 2. Linear regression of grain yield (100% DM) on grain δ^{13}C composition amongst 17 wheat cultivars under irrigated (black circles) and rain-fed conditions (open circles) at Sutton Bonington in a) 2010, b) 2011 and c) mean 2010-2011.

Figure 3. Grain yield versus flag leaf δ^{18}O composition for 17 wheat cultivars under irrigated and rain-fed conditions at Sutton Bonington in a) 2010, b) 2011 and c) mean 2010 and 2011.

Figure 4. Linear regression of flag-leaf δ^{18}O composition on grain δ^{13}C amongst 7 wheat cultivars under irrigated and rain-fed conditions at Sutton Bonington in a) 2010, b) 2011 and c) mean 2010 and 2011.

Figure 5. a) Linear regression of flag-leaf specific weight at anthesis (GS61) versus residual of linear regression of grain δ^{13}C on flag leaf δ^{18}O under irrigated conditions for 16 cultivars, and b) grain yield versus onset of rapid phase of flag-leaf post-anthesis (GS61) senescence for 17 cultivars under irrigated and rain-fed conditions. Values represent means of 2010 and 2011.

Figure 6. Biplot a) irrigated and b) rain-fed conditions. Grain yield (GYD), above ground biomass (ADM), harvest index (HIN), grains m^{-2} (GM2), thousand ground weight (TGW), flag leaf δ^{13}C (FL_C), grain δ^{13}C (GN_C), flag leaf δ^{18}O (FL_O), onset rapid phase of post-anthesis flag-leaf senescence (OSEN), plant height (PHT), anthesis date (ATD). Values represent means of 2010 and 2011.

Table 1. Reduced height *Rht-B1a*/*Rht-B1b* and *Rht-D1a*/*Rht-D1b*, photoperiod insensitivity *Ppd-D1a*/*Ppd-D1b* and spring/winter classes and year of release (YoR) for 17 wheat cultivars grown in experiments in 2009-10 and 2010-11. The *Rht-B1b* and *Rht-D1b* alleles confer semi-dwarf stature and the *Ppd-D1a* allele confers photoperiod insensitivity.

Cultivar	*Rht-D1/Rht-B1*	Spring/winter	YoR	*Ppd-D1*
Maris Widgeon	*Rht-B1a*/*Rht-D1a*	Winter	1964	Ppd-D1b
Hobbit	*Rht-B1a*/*Rht-D1b*	Winter	1977	Ppd-D1b
Avalon	*Rht-B1a*/*Rht-D1b*	Winter	1980	Ppd-D1b
Beaver	*Rht-B1a*/*Rht-D1b*	Winter	1990	Ppd-D1b
Hereward	*Rht-B1a*/*Rht-D1b*	Winter	1991	Ppd-D1b
Cadenza	*Rht-B1a*/*Rht-D1a*	Spring	1994	Ppd-D1b
Rialto	*Rht-B1a*/*Rht-D1b*	Winter	1995	Ppd-D1b
Soissons	*Rht-B1a*/*Rht-D1b*	Winter	1995	Ppd-D1a
Savannah	*Rht-B1a*/*Rht-D1b*	Winter	1998	Ppd-D1b
Paragon	*Rht-B1a*/*Rht-D1a*	Spring	1999	Ppd-D1b
Xi19	*Rht-B1a*/*Rht-D1b*	Winter	2002	Ppd-D1b
Cordiale	*Rht-B1a*/*Rht-D1b*	Winter	2004	Ppd-D1b
Istabraq	*Rht-B1a*/*Rht-D1b*	Winter	2004	Ppd-D1b
Glasgow	*Rht-B1a*/*Rht-D1b*	Winter	2005	Ppd-D1b
Zebedee	*Rht-B1a*/*Rht-D1b*	Winter	2007	Ppd-D1b
Oakley	*Rht-B1b*/*Rht-D1a*	Winter	2007	Ppd-D1b
Panorama	*Rht-B1a*/*Rht-D1b*	Winter	2009	Ppd-D1b

Table 2. Monthly means for daily mean temperature, solar radiation and rainfall in 2010 and 2011 at Sutton Bonington, UK and percentage of LTM (1989-2009) in parenthesis.

Month	Rainfall (mm)		Temperature (^{o}C)		Radiation (MJ m^{-2})	
	2010	2011	2010	2011	2010	2011
March	36.0 (67)	1.2 (2)	6.23 (97.4)	6.76 (106)	8.94 (123)	9.26 (128)
April	24.0 (55)	23.0 (53)	9.19 (113)	11.54 (142)	14.8 (133)	15.57 (139)
May	16.2 (35)	27.8 (61)	11.2 (99)	12.3 (110)	17.88 (118)	17.90 (118)
June	69.2 (152)	45.4 (100)	15.5 (109)	14.43 (102)	20.8 (126)	19.12 (116)
July	42.6 (86)	17.8 (36)	17.3 (105)	15.75 (95)	16.01 (102)	17.09 (109)

Table 3. Grain yield, harvest above-ground DM and year of release (YoR) for 17 cultivars in unirrigated and rain-fed conditions in 2009-10 and 2010-11.

		Combine grain yield t ha^{-1} 100% DM						Above ground DM t ha^{-1}					
Cultivar	YoR	2010		2011		2010-11		2010		2011		201-11	
		Irr	Unirr	Irr	Unirr	Irr	Unirr	Irr	Unirr	Irr	Unirr	Irr	Unirr
M. Widgeon	‘64	8.37	6.82	8.42	7.64	8.40	7.23	18.82	15.4	20.29	19.8	19.56	17.6
Hobbit	‘77	9.40	8.16	10.75	9.80	10.08	8.98	18.03	14.8	20.81	17.9	19.42	16.4
Avalon	‘80	10.00	7.90	10.75	9.38	10.37	8.64	18.86	16.0	21.34	18.2	20.10	17.1
Beaver	‘90	10.62	9.11	11.95	10.65	11.29	9.88	19.57	16.8	22.77	18.7	21.17	17.8
Hereward	‘91	9.53	7.74	10.46	9.01	9.99	8.38	18.23	15.4	22.08	18.0	20.15	16.7
Cadenza	‘94	10.92	8.72	12.22	9.76	11.57	9.24	20.78	17.5	22.88	18.9	21.83	18.2
Rialto	‘95	10.02	8.04	11.18	9.51	10.60	8.78	19.22	15.3	22.91	18.8	21.07	17.0
Soissons	‘95	9.92	8.82	10.84	9.53	10.38	9.17	18.75	16.6	19.96	18.2	19.35	17.4
Savannah	‘98	11.21	9.01	12.08	10.70	11.64	9.85	21.68	16.5	22.29	19.3	21.98	17.9
Paragon	‘99	9.73	7.96	10.40	9.59	10.07	8.77	19.65	17.3	23.38	21.2	21.51	19.3
Xi19	‘02	10.72	8.37	12.22	9.87	11.47	9.12	19.70	16.9	23.62	19.5	21.66	18.2
Cordiale	‘04	10.53	8.48	11.40	9.61	10.97	9.04	18.43	15.7	20.57	17.2	19.50	16.5
Istabraq	‘04	11.35	9.61	12.17	10.49	11.76	10.0	21.54	17.8	24.58	19.7	23.06	18.8
Glasgow	‘05	11.26	9.21	12.18	10.70	11.72	9.95	19.80	16.4	22.17	19.3	20.99	17.9
Zebedee	‘07	10.65	9.09	12.21	10.74	11.43	9.92	20.38	16.6	22.06	19.3	21.22	18.0
Oakley	‘07	11.17	9.03	12.01	10.60	11.59	9.81	19.86	17.0	21.41	19.0	20.64	18.0
Panorama	‘09	11.47	9.18	12.68	10.66	12.07	9.92	21.53	17.8	24.46	20.1	22.99	19.0
Mean		10.40	8.54	11.41	9.90	10.91	9.22	19.70	16.5	22.21	19.0	20.95	17.7
SED (df)													
Irr (2†*, 4*‡*)*		*0.279**		*0.209**		*0.174****		*0.42**		*0.39**		*0.288****	
Gen (63,126)		*0.338****		*0.209****		*0.199****		*0.75****		*0.52****		*0.457****	
*Irr*Gen (63,126)*		*0.47* ^{ns}		*0.296***		*0.324**		*1.07* ^{ns}		*0.73***		*0.690* ^{ns}	
*Yr*Irr*Gen (126)*						*0.519* ^{ns}						*1.129* ^{ns}	

* Significance at the 5% (P = 0.05) level; ** Significance at the 1% (P = 0.01) level; *** Significance at the 0.1% (P = 0.001) level.

† SED for individual year.

‡SED for cross-year mean.

Table 4. Plant height, anthesis date (GS61, AD), green area index (GAI), above-ground dry matter (AGDM), flag-leaf specific dry weight (FLSW) at GS61, and onset of rapid phase of post-anthesis (GS61) flag-leaf senescence (ONSEN) and year of release (YoR) for 17 wheat cultivars in irrigated and rani-fed conditions . Values represent means of 2010 and 2011.

Cultivar	YoR	Plant height (cm)		AD (days after 31 May)		GAI		AGDM (t ha^{-1})		ONSEN (^{o}Cd)		FLSW (g m^{-2})	
		Irr	Unirr	Irr	Unirr	Irr	Unirr	Irr	Unirr	Irr	Unirr	Irr	Unirr
M. Widgeon	‘64	110.7	108.3	11.5	10.0	6.30	5.28	14.2	13.1	669.5	436.7	58.0	53.6
Hobbit	‘77	78.3	73.9	12.0	10.0	5.48	4.78	12.2	12.1	684.2	520.5	60.9	60.8
Avalon	‘80	89.2	89.1	11.0	9.5	5.19	4.50	11.8	11.9	586.5	378.7	58.4	62.6
Beaver	‘90	79.1	79.3	11.5	10.0	5.58	4.18	11.8	11.2	741.5	564	63.8	68.9
Hereward	‘91	80.8	77.1	13.5	11.5	5.68	4.20	13.5	13.6	697.5	437.0	59.4	61.7
Cadenza	‘94	81.9	82.0	11.5	10.5	4.66	3.10	12.6	11.8	683.5	409	59.9	58.6
Rialto	‘95	81.2	73.3	13.0	11.5	4.23	3.08	16.3	13.3	595.5	411.7	70.6	80.8
Soissons	‘95	77.4	76.2	3.0	2.0	4.52	4.15	12.1	12.3	618.5	434.2	64.2	56.3
Savannah	‘98	80.2	77.0	14.0	12.0	5.21	2.99	15.0	12.9	711.5	503.8	66.3	74.6
Paragon	‘99	96.6	91.4	11.5	10.0	6.46	5.02	13.8	12.8	684.8	400.3	56.6	54.7
Xi19	‘02	84.3	80.3	16.0	13.5	4.50	3.49	14.9	14.6	669.5	424.7	70.9	81.9
Cordiale	‘04	71.3	67.7	7.5	7.0	5.37	3.96	12.5	11.6	623.5	447.3	59.7	67.1
Istabraq	‘04	86.8	82.0	12.0	11.0	4.62	3.24	15.6	13.8	727.5	496.3	69.3	87.2
Glasgow	‘05	72.4	72.6	9.0	8.0	4.88	4.33	12.5	12.1	669.5	459.8	61.6	65.2
Zebedee	‘07	77.7	74.6	12.5	11.5	5.42	3.74	13.0	12.9	618.5	511.8	59.5	70.4
Oakley	‘07	74.9	76.3	12.5	11.5	4.72	4.61	13.3	13.1	711.5	476.5	64.1	59.6
Panorama	‘09	83.7	77.2	12.0	9.6	-	-	-	-	684.2	505.5	-	-
Mean		82.7	79.9	11.41	9.95	5.18	4.04	13.4	12.8	669.2	459.9	62.7	66.5
SED (df)													
Irr (4)		1.45*				0.107***		0.14**		15.94 ***		1.62 $^{0.07}$	
Gen (128)		1.19***				0.264***		0.50***		18.28 ***		3.61 ***	
Irr*Gen		2.26 $^{0.07}$				0.377*		0.70***		29.72***		5.20*	

* Significance at the 5% (P = 0.05) level.

** Significance at the 1% (P = 0.01) level.

*** Significance at the 0.1% (P = 0.001) level.

Table 5. Carbon isotope composition (δ^{13}C) in flag-leaf at GS61 and grain at harvest, oxygen isotope composition (δ^{18}O) in the flag leaf at GS61 and year of release (YoR) for 17 cultivars. Values represent means in 2009-10 and 2010-11.

Cultivar	YoR	Flag leaf (δ^{13}C)		Grain (δ^{13}C)		Flag leaf (δ^{18}O)	
		Irrigated	Unirrigated	Irrigated	Unirrigated	Irrigated	Unirrigated
M. Widgeon	‘64	-29.2	-28.6	-27.1	-25.3	24.4	24.2
Hobbit	‘77	-29.3	-28.7	-27.7	-26.6	23.9	24.3
Avalon	‘80	-29.1	-28.7	-27.2	-25.7	24.3	24.7
Beaver	‘90	-29.8	-29.1	-28.3	-27.0	23.8	24.3
Hereward	‘91	-29.3	-28.5	-27.9	-26.3	24.3	24.2
Cadenza	‘94	-29.7	-29.1	-28.4	-27.0	23.7	24.4
Rialto	‘95	-29.7	-29.0	-28.4	-26.7	24.3	24.5
Soissons	‘95	-30.1	-29.7	-28.1	-26.7	23.4	23.6
Savannah	‘98	-29.9	-29.1	-28.9	-27.3	23.6	24.6
Paragon	‘99	-29.7	-29.1	-28.2	-26.7	23.6	24.1
Xi19	‘02	-29.5	-29.1	-28.5	-26.8	23.6	23.6
Cordiale	‘04	-29.4	-28.8	-28.0	-26.7	24.2	24.6
Istabraq	‘04	-29.2	-28.6	-28.1	-26.8	24.2	24.6
Glasgow	‘05	-29.4	-29.0	-28.2	-26.8	24.1	24.2
Zebedee	‘07	-29.1	-28.6	-27.9	-27.0	23.9	24.8
Oakley	‘07	-29.7	-29.2	-28.6	-26.9	23.6	24.2
Panorama	‘09	-29.3	-29.1	-28.1	-26.8	24.2	23.4
Mean		-29.5	-28.9	-28.1	-26.6	23.9	24.3
SED (df)							
Irr (4)		0.068***		0.143***		0.095*	
Gen (126)		0.101***		0.155***		0.218***	
Irr*Gen (126)		0.187		0.259		0.313*	

* Significance at the 5% (P = 0.05) level.

** Significance at the 1% (P = 0.01) level.

*** Significance at the 0.1% (P = 0.001) level.

Table 6. Flag-leaf stomatal conductance and light-saturated photosynthetic rate (A_{max}) for six wheat cultivars and phenotypic correlations with grain yield and grain $\delta^{13}C$ in the unirrigated treatment.

		Stomatal conductance (mmol m^{-2} s^{-1})					A_{max} (µmol m^{-2} s^{-1})				
		2010		2011		Mean	2010		2011		Mean
Cultivar	YoR	2 Jun	24 Jun	31 May	7 Jun		2 Jun	24 Jun	31 May	7Jun	
Maris Widgeon	‘64	282	315	189	166	243	28.3	26.7	20.6	19.2	24.2
Hobbit	‘77	303	470	261	147	296	26.4	32.2	24.5	20.9	26.1
Avalon	‘80	369	343	199	167	281	34.1	28.4	20.8	18.2	26.4
Paragon	‘99	412	454	257	162	332	35.3	28.0	24.5	18.8	27.6
Cordiale	‘04	306	405	267	210	298	30.9	26.3	25.2	21.7	26.6
Glasgow	‘05	403	426	215	176	316	33.8	27.1	22.1	18.9	26.4
Mean		346	402	231	171	294	31.5	28.1	23.0	19.6	26.2
SED		36.1*	33.5*	39.0	18.6	17.5*	2.22*	3.52	1.73	1.79	1.22
Df		5	5	5	5	25	5	5	5	5	25
Corr. (r) vs GY		0.52	0.61	0.45	0.11	$0.79^{0.06}$	0.36	0.02	0.44	0.09	0.67
Corr. (r) vs $\delta^{13}C$		-0.54	-0.90*	-0.84*	-0.36	-0.94**	-0.26	-0.32	-0.86*	-0.52	-0.83*

* Significance at the 5% (P = 0.05) level.

** Significance at the 1% (P = 0.01) level.

*** Significance at the 0.1% (P = 0.001) level.

Table 7. Fitted parameter estimates for linear changes in crop traits with year of release in irrigated and unirrigated conditions for 17 UK wheat cultivars released from 1964 and 2009. Linear function ($y = a + bx$) was fitted to 2-yr cultivar means (2010 and 2011).

	Irrigated		Unirrigated	
	y (as in 1964)	b ± SE	y (as in 1964)	b ± SE
Grain yield, t ha^{-1}	8.90	0.0604± 0.011 ***	7.73	0.0475± 0.0099 ***
AGDM, t ha^{-1}	19.24	0.0546± 0.0201 *	16.08	0.0308±0.0156 P = 0.07
Harvest index %	46.7	0.1798± 0.0538 **	46.3	0.1780± 0.0683 **
Plant height, cm	97.3	-0.468± 0.160 *	95.2	-0.492± 0.151 **
Grain δ13C, ‰	-27.31	-0.0255± 0.00712**	-25.68	-0.0304± 0.0067 ***
Flag leaf $\delta^{13}C$, ‰	-	-0.00431± 0.00617 ns	-	-0.00804± 0.00607 ns
Flag leaf $\delta^{18}O$, ‰	-	-0.00797± 0.00650 ns	-	-0.00444± 0.00835 ns
ONSEN, ^{o}Cd	-	0.375± 0.959 ns	-	0.755± 0.105 ns
Anthesis (GS61) days	-	0.0019 ± 0.053 ns	-	0.0010 ± 0.060 ns

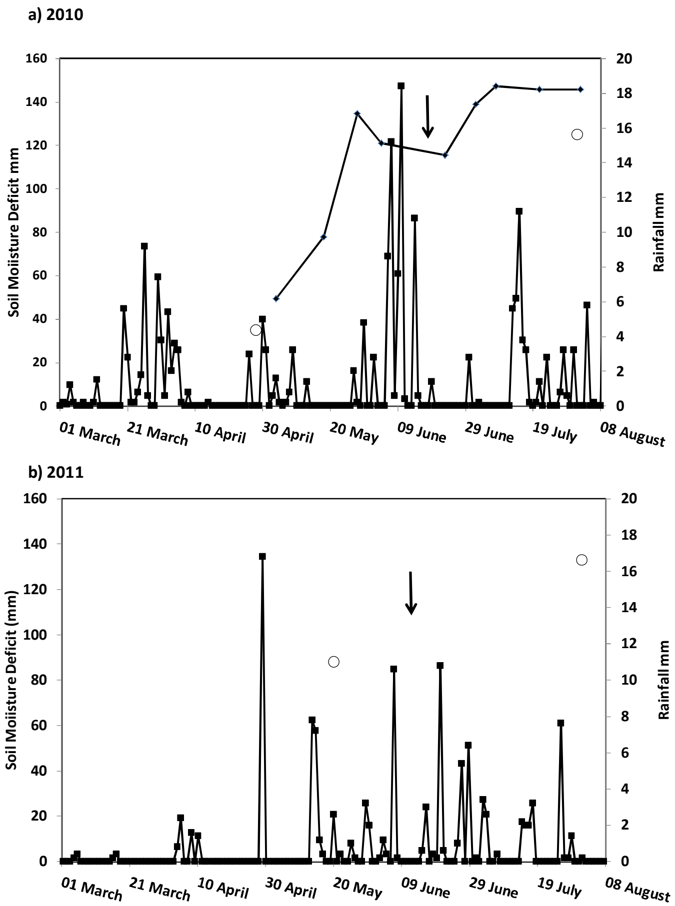

Figure 1. a) Daily rainfall (closed squares) and soil moisture deficit to 1.2 m soil depth estimated from capacitance probe (closed diamonds) and gravimetric analysis of soil cores (open circles) in 2010 and b) daily rainfall (closed squares) and soil moisture deficit to 1.2 m soil depth estimated from gravimetric analysis of soil cores (open circles) in 2011 in rain-fed conditions.

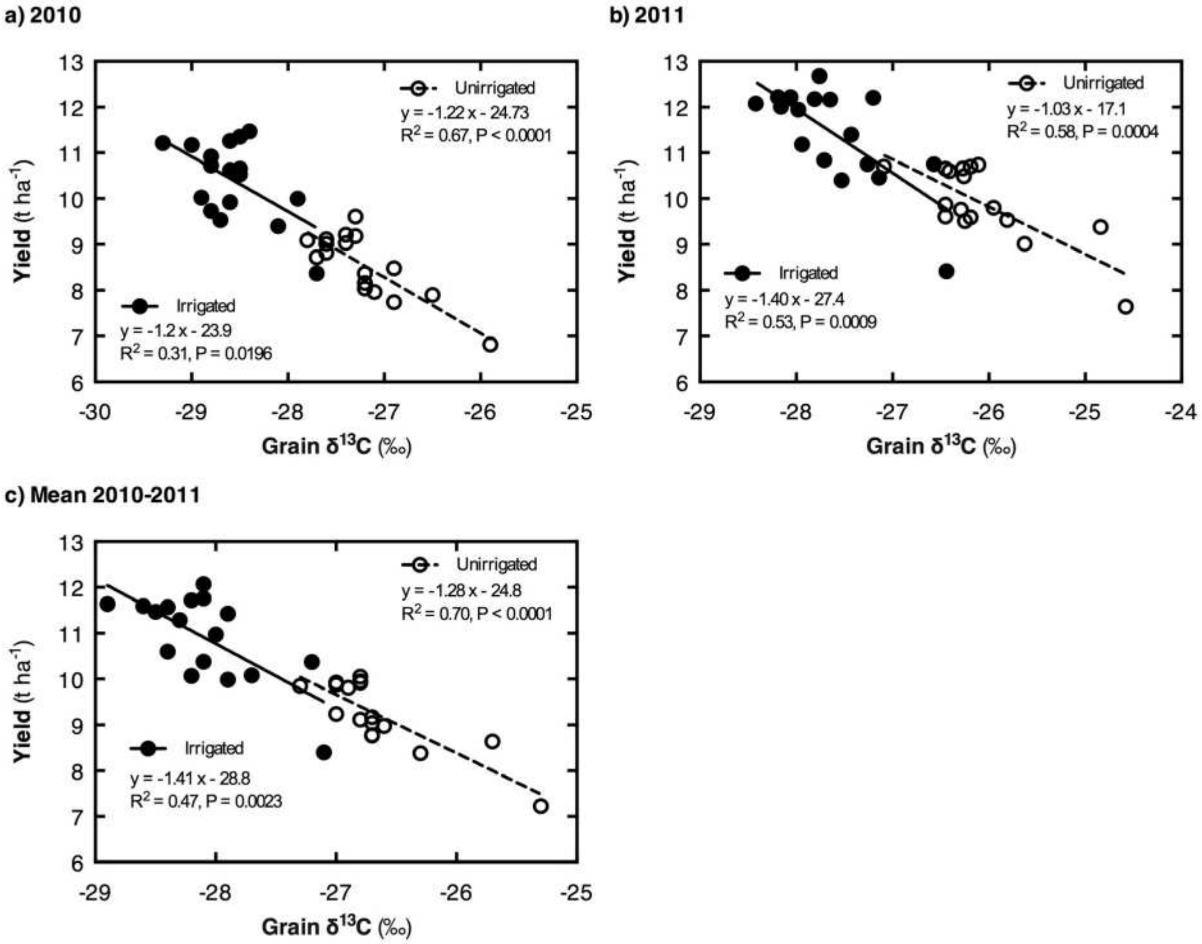

Figure 2. Linear regression of grain yield (100% DM) on grain δ^{13}C composition amongst 17 wheat cultivars under irrigated (black circles) and rain-fed conditions (open circles) at Sutton Bonington in a) 2010, b) 2011 and c) mean 2010-2011.

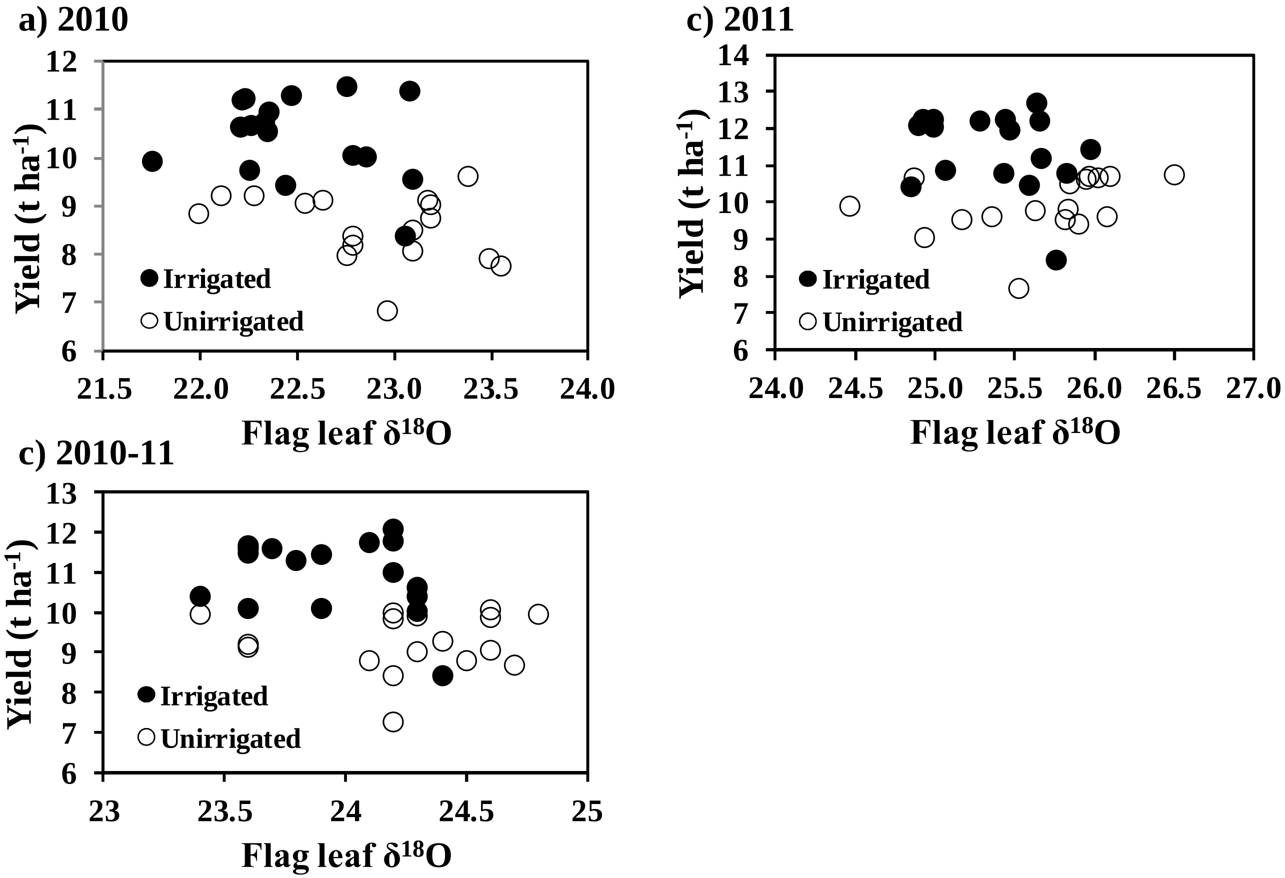

Figure 3. Grain yield versus flag leaf δ^{18}O composition for 17 wheat cultivars under irrigated and rain-fed conditions at Sutton Bonington in a) 2010, b) 2011 and c) mean 2010 and 2011.

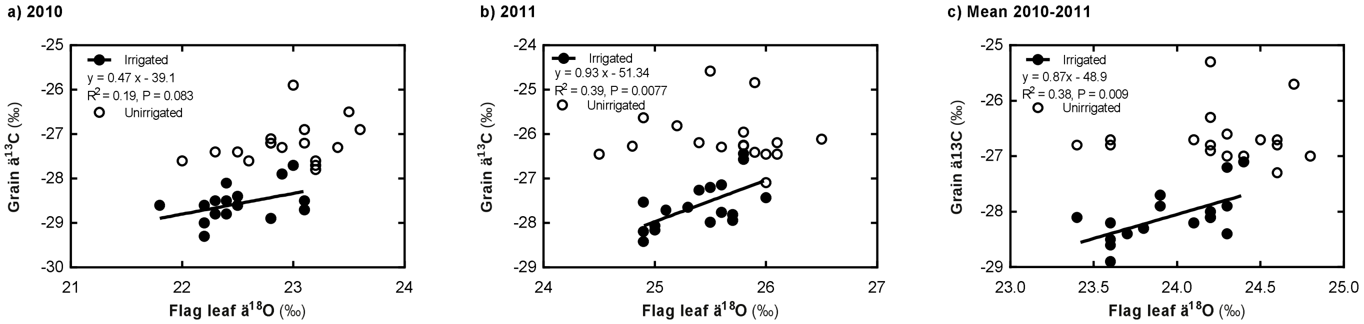

Figure 4. Linear regression of grain δ^{13}C composition on flag leaf δ^{18}O composition amongst 17 wheat cultivars under irrigated and rain-fed conditions at Sutton Bonington in a) 2010, b) 2011 and c) mean 2010 and 2011.

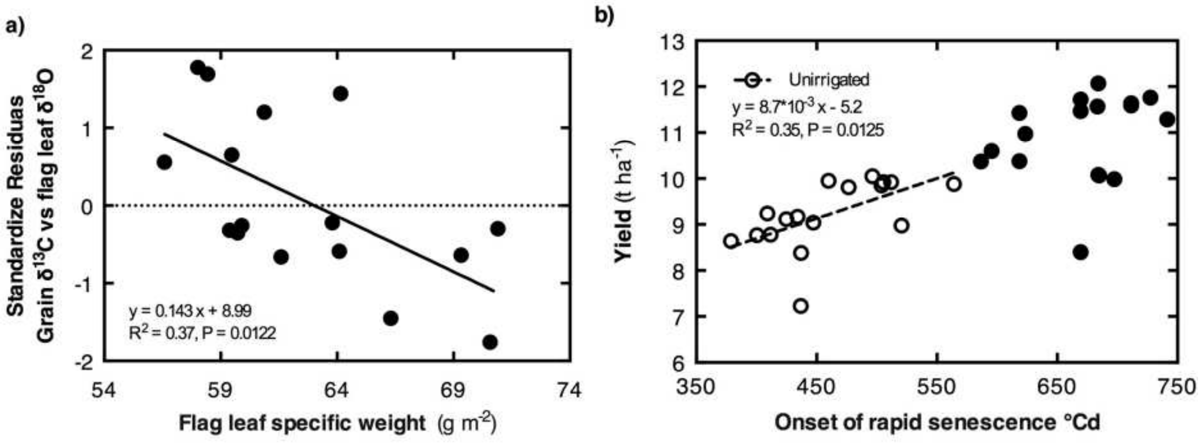

Figure 5. a) Linear regression of flag-leaf specific weight at anthesis (GS61) versus residual of linear regression of grain δ^{13}C on flag leaf δ^{18}O under irrigated conditions for 16 cultivars, and b) grain yield versus onset of rapid phase of flag-leaf post-anthesis (GS61) senescence for 17 cultivars under irrigated and unirrigated conditions. Values represent means of 2010 and 2011.

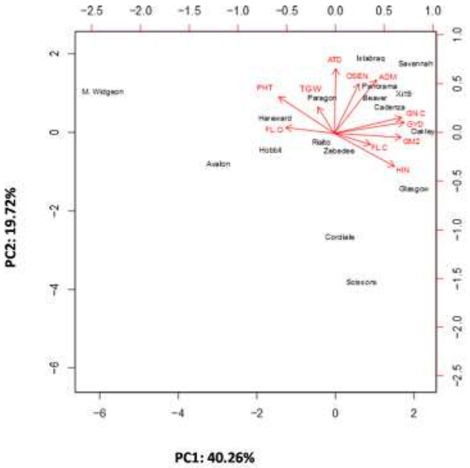

Figure 6a) Irrigated conditions

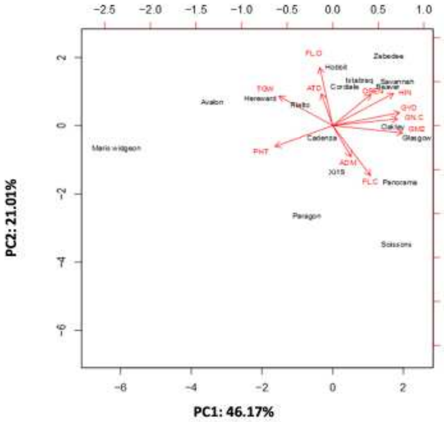

Figure 6b) Rain-fed conditions

Figure 6. Biplot a) irrigated and b) rain-fed. Grain yield (GYD), above ground dry matter (ADM), harvest index (HIN), grains m^{-2} (GM2), thousand ground weight (TGW), flag leaf δ^{13}C (FL_C), grain δ^{13}C (GN_C), flag leaf δ^{18}O (FL_O), onset of senescence (OSEN), plant height (PHT), anthesis date (ATD). Values represent means of 2010 and 2011.