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

3

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19 **Abstract**

20 In previous investigations, carbon isotope composition ( $\delta^{13}\text{C}$ ) has been used in  $\text{C}_3$  cereals to  
21 screen for genotypes with high transpiration efficiency and oxygen isotope composition  
22 ( $\delta^{18}\text{O}$ ) has been shown to correlate with transpiration rate. We examined associations of  $\delta^{13}\text{C}$   
23 of the grain and flag leaf and  $\delta^{18}\text{O}$  of the flag leaf with respect to grain yield in wheat  
24 cultivars in UK field conditions. Field experiments were carried out at University of  
25 Nottingham in 2009-10 and 2010-11 testing 17 wheat cultivars under fully irrigated and rain-  
26 fed conditions. Averaging across years grain yield was reduced by  $1.69 \text{ t ha}^{-1}$  (16.5%) in the  
27 rain-fed treatment ( $P < 0.001$ ). There was a negative linear relationship between grain yield  
28 and grain  $\delta^{13}\text{C}$  amongst cultivars, under both irrigated ( $R^2 = 0.47$ ,  $P < 0.01$ ) and rain-fed ( $R^2$   
29  $= 0.70$ ,  $P < 0.001$ ) conditions. Grain  $\delta^{13}\text{C}$  was negatively correlated with flag-leaf stomatal

30 conductance ( $r = -0.94$ ,  $P < 0.01$ ) in a subset of six of the cultivars, indicating that higher  
31 transpiration efficiency was associated with lower stomatal conductance. The associations  
32 between grain yield and flag-leaf  $\delta^{13}\text{C}$  and flag-leaf  $\delta^{18}\text{O}$  amongst cultivars under irrigated  
33 and rain-fed conditions were not statistically significant. There was a positive linear  
34 relationship between flag-leaf  $\delta^{18}\text{O}$  and grain  $\delta^{13}\text{C}$  amongst cultivars under irrigation ( $R^2 =$   
35  $0.38$ ,  $P < 0.01$ ), indicating a trade-off between transpiration and transpiration efficiency (TE).  
36 Genetic variation in grain yield under rain-fed conditions was also associated with delayed  
37 onset of flag-leaf senescence in our experiments ( $R^2 = 0.35$ ,  $P < 0.05$ ). The 17 wheat cultivars  
38 ranged in year of release (YoR) from 1964 to 2009 and grain yield increased linearly under  
39 irrigated conditions by  $60.4 \text{ kg ha}^{-1} \text{ yr}^{-1}$  ( $0.72 \% \text{ yr}^{-1}$ ) and under rain-fed conditions by  $47.5$   
40  $\text{kg ha}^{-1} \text{ yr}^{-1}$  ( $0.66 \% \text{ yr}^{-1}$ ) over the 45 year period and grain  $\delta^{13}\text{C}$  composition decreased by  
41  $0.0255$  and  $0.0304 \text{ ‰ yr}^{-1}$ , respectively, indicating genetic gains in wheat yield potential in  
42 the UK seem likely to have been achieved through a lower TE, higher water uptake and lesser  
43 limitation of stomatal conductance.

## 44 1. Introduction

45 Worldwide, drought limits agricultural productivity more than any other single factor.  
46 Wheat (*Triticum aestivum* L.) provides, on average, one-fifth of the total calorific input of the  
47 world's population. In the UK, winter wheat is the most widely grown arable crop and  
48 contributes c. 16 million tonnes per annum with an average productivity of c. 8.5 t ha<sup>-1</sup>  
49 (DEFRA 2015). The significantly warmer and more extreme conditions now arising from  
50 climate change (IPCC 2014) mean that new cultivars with greater drought resistance must be  
51 developed to maintain food security. In the UK, water deficits can commonly limit wheat  
52 yields in some years, where, typically, the onset of drought is post-anthesis, and losses are c.  
53 20-30% (Foulkes et al., 2002).

54 Plants discriminate against the heavier carbon isotope (<sup>13</sup>C) during photosynthesis and  
55 the extent of this discrimination depends on the ratio of intercellular versus external CO<sub>2</sub>  
56 concentration (C<sub>i</sub>/C<sub>a</sub>) in photosynthetic organs (Farquhar et al., 1982). The carbon isotope  
57 composition (δ<sup>13</sup>C) is negatively related to C<sub>i</sub>/C<sub>a</sub> (δ<sup>13</sup>C) (Farquhar et al., 1982), which, in  
58 | turn, is negatively related to the transpiration efficiency (~~TE~~) at the stoma (CO<sub>2</sub> assimilation/  
59 transpiration). Therefore, carbon isotope composition (δ<sup>13</sup>C; frequently expressed as  
60 discrimination from the source air, Δ<sup>13</sup>C) is positively associated with TE. When measured in  
61 dry matter, δ<sup>13</sup>C provides information on the long-term transpiration efficiency of C<sub>3</sub> plants  
62 (Farquhar and Richards 1984). Conditions that induce stomatal closure, such as water deficit,  
63 restrict the CO<sub>2</sub> supply to carboxylation sites, which then increases the δ<sup>13</sup>C (or decreases  
64 Δ<sup>13</sup>C) of plant matter (Farquhar et al., 1989). The carbon isotope signature has been used as a  
65 selection indicator for high TE in commercial wheat breeding for water-limited environments  
66 (Rebetzke et al., 2002; Condon et al., 2002; Condon et al., 2004). Selection for Δ<sup>13</sup>C was used  
67 to develop the Australian spring-wheat cultivars Drysdale and Rees (Richards, 2006). Under  
68 severe drought in Australia, Δ<sup>13</sup>C of grain was negatively correlated with aerial biomass and  
69 grain yield for wheat (Rebetzke et al., 2002). In other instances of more mild droughts with  
70 relatively plentiful water up to anthesis, the relationship between Δ<sup>13</sup>C and grain yield of  
71 wheat has been positive, associated with higher stomatal conductance (g<sub>s</sub>) and increased  
72 water use (WU; the total water absorbed and further transpired by the plant) and biomass in  
73 Mediterranean conditions (Araus et al., 2001, 2003; Condon et al., 2004; Zhou et al., 2015)  
74 and in the UK (Aravinda-Kumar et al., 2011). Therefore, except for severe drought  
75 conditions, water use appears to be a more important adaptive trait than the water-use  
76 efficiency (WUE; ratio of aerial biomass to evapotranspiration) (Slafer and Araus, 2007;  
77 Blum, 2009). An important breeding objective is therefore to identify sources of high WU in

78 which any trade-off with WUE is minimized. The  $\delta^{13}\text{C}$  signature provides no definitive  
79 information on whether grain variation in  $\delta^{13}\text{C}$  is being driven by variation in stomatal  
80 conductance ( $g_s$ ) or photosynthetic capacity, although the major role of stomatal conductance  
81 has been implied in diverse studies (Araus et al., 2001, 2003; Condon et al., 2004; Aravinda-  
82 Kumar et al., 2011; Zhou et al., 2015).

83 The oxygen isotope signature ( $\delta^{18}\text{O}$ ) of vegetative tissues can be used as an indirect  
84 measure of transpiration and WU. The oxygen isotope abundance of plant matter (usually  
85 expressed as a composition,  $\delta^{18}\text{O}$  of the bulk matter) can be used to separate the independent  
86 effects of assimilation ( $A$ ) and  $g_s$  on  $\delta^{13}\text{C}$  since it is unaffected by photosynthesis (Farquhar et  
87 al. 2007). The oxygen isotope signature integrates the evaporative conditions throughout the  
88 crop cycle (Barbour et al., 2000) and has been proposed as a proxy method for measuring  
89 transpiration and water use in different crop species (Barbour et al., 2000; Sheshshayee et al.,  
90 2005; Cabrera-Bosquet et al., 2009a, 2011).  $\delta^{18}\text{O}$  is affected by air humidity and temperature  
91 (Barbour et al., 2000; Helliker and Ehleringer, 2002a, 2002b), soil moisture (Saurer et al.,  
92 1997; Ferrio et al., 2007) and source water (Williams et al., 2005; Asbjornsen et al., 2008).  
93 Nevertheless, when comparing genotypes growing under the same water conditions,  $\delta^{18}\text{O}$   
94 may be used to assess the effect of treatments and genotypic variability on yield in wheat, e.g.  
95 in bread wheat (Barbour et al., 2000; Ferrio et al., 2007; Cabrera-Bosquet et al., 2009a; Zhou  
96 et al., 2015) and durum wheat (Cabrera-Bosquet et al., 2011; Araus et al., 2013). A negative  
97 relationship between  $\delta^{18}\text{O}$  and grain yield was reported in field experiments under fully-  
98 irrigated conditions across a set of eight bread wheat cultivars (Barbour et al., 2000) and in  
99 nine bread wheat cultivars grown under high-yielding Mediterranean conditions in Spain  
100 (Zhou et al., 2015). However, Ferrio et al. (2007) failed to find a phenotypic correlation  
101 between  $\delta^{18}\text{O}$  and yield across a set of 24 bread wheat genotypes growing under three  
102 different water regimes. Therefore, studies reporting the use of  $\delta^{18}\text{O}$  in water-limited cereals  
103 grown under real field conditions are still scarce and the results are contradictory.

104 The combined measurement of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  in plant tissues is of interest in breeding  
105 due to their relationship to photosynthetic and transpiration performance of the plant during  
106 the course of crop growth, i.e. measurement of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  can be potentially be used to  
107 identify genotypes which minimize any trade-off between TE and transpiration. This may  
108 help plant breeders to select genotypes that are better suited to drought. A positive  
109 relationship between genetic variation in grain  $\delta^{18}\text{O}$  and grain  $\delta^{13}\text{C}$  was observed (implying a  
110 negative relationship between TE and transpiration) in durum wheat for 10 cultivars under  
111 irrigated and unirrigated conditions (Araus et al., 2013) and in bread wheat for nine cultivars

112 under high-yielding conditions (Zhou et al., 2015). Previously studies reporting the effect of  
113 water limitation on both the carbon and oxygen signatures amongst genotypes were  
114 conducted in pots in maize (Cabrera-Bosquet, et al. 2009b), in the field in seedlings of  
115 tropical tree species grown in a tropical environment (Cernusak et al., 2009) and in the field  
116 in durum wheat (Araus et al., 2013). However, no previous studies have investigated the  
117 effects of water limitation on both the carbon and oxygen signatures amongst bread wheat  
118 genotypes. Our objectives were firstly to examine associations between  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$  and grain  
119 yield and associated physiological mechanisms amongst 17 bread wheat cultivars with year  
120 of release from 1964 to 2009 grown under favourable irrigated and rain-fed conditions and  
121 secondly to quantify changes in grain yield and the carbon and oxygen signatures with year  
122 of release over the 45 year period in field experiments at Nottingham, UK in two seasons.

123

## 124 **2. Materials and Methods**

125

### 126 *2.1 Experimental design and plot management*

127 Seventeen wheat cultivars (Table 1) were grown under irrigated and rain-fed  
128 conditions in field experiments in 2009-10 and 2010-11 on a sandy loam soil type  
129 (Dunnington Heath Series) at University of Nottingham farm, Leicestershire UK (52.834 N, -  
130 1.243 W). Cultivars ranged in year of release (taken as their year first included on the UK  
131 Recommended List) from 1964 to 2009, and were chosen because they were representative of  
132 the most widely grown cultivars over the 45-year period (Table 1). The cultivars were known  
133 to contrast for possession of major genes including the semi-dwarf *Rht-B1* and *Rht-D1* genes  
134 and the photoperiod sensitivity *Ppd-D1* gene (Table 1). Fourteen of the cultivars were semi-  
135 dwarfs, and three were tall cultivars (Maris Widgeon, Cadenza and Paragon); fourteen were  
136 winter wheat cultivars, and three were spring wheat cultivars (Cadenza, Paragon and Xi19);  
137 and all were photoperiod sensitive cultivars apart from Soissons which was photoperiod  
138 insensitive. Eight of the cultivars were bread-making cultivars (M. Widgeon, Hereward,  
139 Cadenza, Rialto, Soissons, Paragon, Xi19, Cordiale and Panorama) and nine were feed or  
140 biscuit-making cultivars. The experimental design was a split-plot randomised block with  
141 three replicates. Irrigation treatments were randomised on main-plots and cultivars on sub-  
142 plots. In the irrigated treatment, a trickle irrigation system was used to maintain soil moisture  
143 deficit (SMD), calculated using the ADAS Irriguide model (Bailey & Spackman 1996), to <  
144 0.50 available water (AW) up to GS61 + 28 days and < 0.75 AW thereafter. The AW  
145 capacity to 1.2 m soil depth was 176 mm. No water was applied in the [rain-fed](#) treatment.

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

154

155 **Table 1 here**

156

## 157 *2.2 Crop measurements*

158

159 In all plots in each of the 2009-10 and 2010-11 field experiments, flag-leaf samples at  
160 GS61 and grain dry matter samples at harvest were assessed for carbon  $\delta^{13}\text{C}$  composition.

161 Flag-leaf samples at GS61 were also assessed for oxygen  $\delta^{18}\text{O}$  composition.

162

### 163 *2.2.1 Anthesis measurements*

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

176

### 177 *2.2.2 Harvest analysis*

178 Plant samples were taken from a 0.64 m<sup>2</sup> area and a 10% sub-sample (by fresh  
179 weight) of plant material was assessed as follows: plant material was separated into ears and

180 straw. Ears were counted and threshed, and the chaff (rachis, rachilla, glume, palea, and  
181 lemma), grain and straw were weighed separately after drying for 48 h at 80 °C. Harvest  
182 index was calculated as the fraction of AGDM present as grain. A sub-plot area of at least 5  
183 m<sup>2</sup> was machine-harvested at harvest and the grain weighed. The grain yield is expressed at  
184 0% moisture content.

185

### 186 2.2.3 Carbon isotope and oxygen composition

#### 187 *Carbon isotope analysis*

188 From each sub-plot dried flag leaves (from sample at anthesis) and grains (from  
189 sample at harvest) were milled separately for use in <sup>13</sup>C:<sup>12</sup>C isotope ratio analysis. The  
190 samples were ground to a fine powder using a cyclotec 1093 sample machine. The milled  
191 samples (1 mg) were then weighed out in tin cups and analysed through an online system  
192 composed of an elemental analyser (EA), a TripleTrap and a mass spectrometer (Carlo Erba  
193 2100, Milan, Italy) to determine carbon isotope composition (Aravinda Kumar et al., 2011).  
194 The EA interfaced with an isotope ratio mass spectrometer (IRMS; Thermo-Finnigan  
195 Deltaplus Advantage, Bremen, Germany) to analyse <sup>13</sup>C:<sup>12</sup>C ratio (R) of plant material.  
196 Results were expressed as δ<sup>13</sup>C composition values, using a secondary standard calibrated  
197 against Vienna Pee Dee Belemnite calcium carbonate (VPDB), and the analytical precision  
198 was ~0.1‰ (Eqn 1).

199

$$200 \delta^{13}\text{C} (\text{‰}) = [ (R_{\text{sample}}/R_{\text{standard}}) - 1 ] \times 1,000 \quad \text{Eqn 1}$$

201

#### 202 *Oxygen isotope analyses*

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

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$$213 \delta^{18}\text{O} (\text{‰}) = [ (R_{\text{sample}}/R_{\text{standard}}) - 1 ] \times 1,000 \quad \text{Eqn 2}$$



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#### 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  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , sample chamber  $\text{CO}_2$  concentration 360  $\mu\text{l l}^{-1}$  and flow rate 500  $\mu\text{mol s}^{-1}$ .

#### 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°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

248 | cm soil depth. In addition, in both years in the rain-fed treatment, volumetric soil water  
249 | content was assessed from gravimetric analysis of soil cores (four cores per sub-plot, 2.5 cm  
250 | diameter) to 100 cm soil depth during stem extension, on 27 April 2011 and 20 May 2012,  
251 | and at harvest.

252

## 253 | *2.4 Statistical analysis*

254

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

267

## 268 | **3. Results**

### 269 | *3.1 Growing conditions*

270

271 | Rainfall was below the long-term mean (LTM) during stem elongation in April and  
272 | May in 2010 (-45% LTM) and 2011 (-44% LTM) (Table 2). During grain filling in June and  
273 | July rainfall was slightly above the LTM in 2010 (+17%), but below the LTM in 2011 (-  
274 | 34%). These rainfall patterns resulted in pre- and post-anthesis drought occurring in both  
275 | years. In 2010 onset of drought (assuming a limiting soil moisture deficit (SMD) of 50%  
276 | AW, 88 mm; Foulkes et al., 2001) occurred in the third week of May at around flag-leaf  
277 | emergence (Fig. 1). Thereafter, SMD increased progressively to harvest, apart from a  
278 | temporary decrease during early grain filling due to significant rainfall of 50.3 mm from 6-10  
279 | June at the start of anthesis. In 2011, although soil water content wasn't measured using the  
280 | PR2 probe, the gravimetric estimate of soil water content on 20 May at around flag-leaf  
281 | emergence and at harvest and the daily pattern of rainfall indicated onset of drought occurred,



314 restricted water availability ranged amongst cultivars from 1.10 (-11.7%, Soissons) to 2.35 (-  
315 24.6%, Xi19) t ha<sup>-1</sup> but did not differ significantly. In 2011, drought decreased yield overall  
316 from 11.41 to 9.90 t ha<sup>-1</sup> (-13.2%,  $P < 0.05$ ). Averaging over irrigation treatments, cultivars  
317 ranged from 8.03 (Maris Widgeon) to 11.66 (Panorama) t ha<sup>-1</sup>; and cultivars differed in  
318 response to drought with decreases in the range 0.96 (-9.1%, Hobbit) to 2.46 (-22.4%,  
319 Cadenza) t ha<sup>-1</sup> ( $P < 0.05$ ). Averaging across years, drought reduced grain yield from 10.91 to  
320 9.22 t ha<sup>-1</sup> (15.5%,  $P < 0.05$ ). Responses to drought differed amongst cultivars in the range -  
321 1.10 (-11.5%, Hobbit) to -2.34 (-22.8%; Xi19) t ha<sup>-1</sup> ( $P < 0.05$ ). The year x irrigation x  
322 genotype interaction was not significant.

323 Above-ground biomass was reduced from 19.70 to 16.51 t ha<sup>-1</sup> in 2010 (-16.2%;  $P <$   
324 0.05) and from 22.21 to 19.05 t ha<sup>-1</sup> (-14.2%,  $P < 0.05$ ) in 2011 under drought (Table 3).  
325 Overall cultivars ranged from 17.08 (Cordiale) to 19.72 t ha<sup>-1</sup> (Istabraq) in 2010 and 18.93  
326 (Cordiale) to 22.33 t ha<sup>-1</sup> (Paragon) in 2011 ( $P < 0.001$ ). The cultivars responded differently  
327 to drought with decreases in the range 0.43 (M. Widgeon) to 4.12 t ha<sup>-1</sup> (Xi19) in 2011 ( $P <$   
328 0.01), but not in 2010. The cross-year ANOVA indicated that the irrigation x genotype  
329 interaction was not statistically significant.

330

331 **Table 3 here**

332

### 333 *3.4 Anthesis crop growth*

334

335 Averaging across years, GAI was decreased under drought from 5.18 to 4.04 ( $P <$   
336 0.001; Table 4). Overall cultivars differed in the range 3.66 (Rialto) to 5.79 (M. Widegon) ( $P$   
337  $< 0.001$ ). The cultivars responded differently to drought with decrease in GAI ranging from  
338 2.3% (Oakley) to 42.6% (Savannah) ( $P < 0.001$ ). Averaging across years, above-ground  
339 biomass at GS61 decreased slightly from 13.4 t ha<sup>-1</sup> under irrigation to 12.8 t ha<sup>-1</sup> under  
340 drought ( $P < 0.05$ ). Cultivars overall ranged from 11.5 (Beaver) to 14.8 t ha<sup>-1</sup> (Rialto;  $P <$   
341 0.001), and responded differently to drought with decreases ranging from +2% (Soissons) to -  
342 18% (Rialto) ( $P < 0.01$ ).

343

344 **Table 4 here**

345

### 346 *3.5 $\delta^{13}C$ and $\delta^{18}O$ and relationships with grain yield*

347

348 Averaging across years, grain  $\delta^{13}\text{C}$  composition increased from -28.1‰ under  
349 irrigated to -26.6‰ under rain-fed conditions ( $P < 0.001$ , Table 5). Cultivars overall varied  
350 from -28.1 (Savannah) to -26.2‰ (M. Widgeon) (Table 5). The irrigation x cultivar  
351 interaction was not statistically significant. Results showed a negative linear relationship  
352 between grain  $\delta^{13}\text{C}$  and grain yield amongst the 17 cultivars under both irrigated ( $R^2 = 0.31$ ,  
353  $P = 0.02$ ) and rain-fed ( $R^2 = 0.67$ ,  $P < 0.001$ ) conditions in 2010 (Fig. 2). Similarly in 2011, a  
354 negative linear relationship was found under both irrigated ( $R^2 = 0.53$ ,  $P < 0.001$ ) and rain-  
355 fed ( $R^2 = 0.58$ ,  $P < 0.001$ ) conditions. Averaging across years, the negative linear relationship  
356 was again significant under both irrigated ( $R^2 = 0.47$ ,  $P < 0.01$ ) and unirrigated ( $R^2 = 0.70$ ,  $P$   
357  $< 0.001$ ) conditions. Omitting the tallest (non semi-dwarf) cultivar Maris Widgeon released  
358 in 1964, which had the lowest yield and the highest grain  $\delta^{13}\text{C}$  under both irrigated and rain-  
359 fed conditions, the linear relationship between between grain  $\delta^{13}\text{C}$  and grain yield amongst  
360 the remaining 16 cultivars was still significant under both irrigated ( $R^2 = 0.25$ ,  $P < 0.05$ ) and  
361 rain-fed ( $R^2 = 0.46$ ,  $P < 0.01$ ) conditions. Averaging across years, there was no linear  
362 relationship between anthesis date and grain  $\delta^{13}\text{C}$  amongst the 17 genotypes under either  
363 irrigated or rain-fed conditions ( $R^2 = 0.04$ ,  $P = 0.42$  and  $R^2 = 0.01$ ,  $P = 0.67$ . respectively).  
364 The linear relationship between grain yield and flag-leaf  $\delta^{13}\text{C}$  was not statistically significant  
365 amongst the 17 cultivars under either irrigated or rain-fed conditions in individual years or  
366 averaging across years.

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

375

376 **Fig 2 near here**

377 **Fig. 3 near here**

378 **Table 5 here**

379

380 *3.6. Relationship between  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$*

381

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

397

398

**Fig. 4 here**

399

### 400 *3.7 Flag-leaf stomatal conductance and leaf photosynthetic rate*

401

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

410

411 Averaging over assessments and years, there was a strong trend for a positive  
412 correlation amongst cultivars between stomatal conductance and grain yield ( $P = 0.06$ ; Table  
413 6). Stomatal conductance was negatively correlated amongst cultivars with grain  $\delta^{13}\text{C}$  on 24  
414 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

415 grain  $\delta^{13}\text{C}$  amongst cultivars on 31 May 2011 ( $P < 0.05$ ) and for the mean across all four  
416 assessments ( $P = 0.05$ ). There were no statistically significant associations between flag-leaf  
417 photosynthesis rate and grain yield.

418

419

**Table 6 here**

420

421 *3.8 Flag-leaf specific weight and senescence rate*

422

423 There was a trend for flag-leaf specific weight (FLSW) to increase slightly under  
424 drought from 62.7 to 66.5 g m<sup>-2</sup> ( $P = 0.07$ ). FLSW differed amongst cultivars in the range  
425 56.6-70.9 g m<sup>-2</sup> under irrigation and 53.6-81.9 g m<sup>-2</sup> under drought ( $P < 0.001$ ; Table 4).  
426 There was an irrigation x cultivar interaction ( $P < 0.05$ ), with response of FLSW to drought  
427 ranging from +25.9% (Istabraq) to -12.3% (Soissons). Interestingly, under irrigated  
428 conditions, there was a negative linear association between FLSW under irrigation and the  
429 standardized residuals for the linear regression of grain  $\delta^{13}\text{C}$  on flag-leaf  $\delta^{18}\text{O}$  amongst  
430 cultivars (a more positive residual indicating higher TE (higher grain  $\delta^{13}\text{C}$ ) relative to  
431 transpiration (flag-leaf  $\delta^{18}\text{O}$ ) Fig. 5a).

432

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439

The onset of the rapid phase of flag-leaf senescence was advanced from 669 °Cd  
post-GS61 under irrigated conditions to 460 °Cd under drought. Cultivars overall ranged  
from 483 (Avalon) to 653 °Cd (Beaver;  $P < 0.001$ ); the advancement of onset of senescence  
under drought ranged from 107 °Cd (Zebedee) to 285 °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 significant association under irrigated  
conditions (Fig. 5b). Associations between the end of post-anthesis flag-leaf senescence and  
grain yield amongst cultivars were not statistically significant (data not shown).

440

441

**Fig. 5 here**

442

443 *3.9 Principal component analysis(PCA) for yield, yield components and physiological traits*

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445

446

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448

The relationships amongst grain yield, yield components and physiological traits are  
shown in the biplots in Fig. 6. PCA confirmed the associations between grain  $\delta^{13}\text{C}$  and grain  
yield under both rain-fed and irrigated conditions and the absence of statistically significant  
associations between either flag-leaf  $\delta^{13}\text{C}$  or flag-leaf  $\delta^{18}\text{O}$  and grain yield. Variation in grain

449 yield amongst genotypes was strongly positively associated with grains m<sup>-2</sup> and negatively  
450 associated with grain weight in both irrigated and rain-fed conditions. Plant height was  
451 correlated positively with harvest biomass and negatively with grain yield in both irrigation  
452 treatments.

453

454

**Fig. 6 here**

455

456 *3.10 Changes with year of release (YoR)*

457

458 Grain yield increased linearly with year of release by 60.4 kg ha<sup>-1</sup> yr<sup>-1</sup> (0.72 % yr<sup>-1</sup>)  
459 ( $P < 0.001$ ) under irrigation and 47.5 kg ha<sup>-1</sup> yr<sup>-1</sup> (0.66 % yr<sup>-1</sup>) under drought ( $P < 0.001$ ; Table  
460 7). Grain yield progress was associated with increases in above-ground DM under irrigation  
461 (54.6 ( $P < 0.05$ )) and rain-fed conditions (30.8 kg ha<sup>-1</sup> yr<sup>-1</sup> ( $P = 0.07$ )); and there were genetic  
462 gains in HI (%) of 0.180 % yr<sup>-1</sup> ( $P < 0.01$ ) and 0.179 % yr<sup>-1</sup> ( $P < 0.01$ ), respectively. Plant  
463 height decreased over the 45-year period by 0.47 cm yr<sup>-1</sup> under irrigated and 0.49 cm yr<sup>-1</sup>  
464 under rain-fed conditions. Grain  $\delta^{13}\text{C}$  decreased by 0.0255 and 0.0304 ‰ yr<sup>-1</sup> under irrigated  
465 and rain-fed conditions, respectively. There were no statistically significant changes in flag-  
466 leaf  $\delta^{13}\text{C}$ , flag-leaf  $\delta^{18}\text{O}$ , onset of flag-leaf senescence or anthesis date with YoR under  
467 irrigated or rain-fed conditions.

468

469

**Table 7 here**

#### 470 **4. Discussion**

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

474

##### 475 *4.1 Yield responses to drought*

476

477 In the present study, the overall yield loss under drought of 1.86 (17.9%) and 1.51 t  
478 ha<sup>-1</sup> (13.2%) in 2010 and 2011, respectively, was slightly smaller than typical losses under  
479 drought for winter wheat in the UK of *ca.* 20-30% (Innes et al., 1985; Foulkes et al., 2002,  
480 2007). The high-yielding genotypes under irrigated conditions tended to lose more grain yield  
481 under drought, and there was a positive linear relationship between absolute grain yield loss  
482 under drought and yield in irrigated conditions ( $R^2 = 0.44$ ,  $P < 0.05$ ). From the physiological



483 standpoint, it is not surprising that absolute reduction in yield for a given reduction in water  
484 resource is strongly influenced by yield potential (Fischer and Maurer, 1978; Foulkes et al.,  
485 2007; Aravinda-Kumar et al., 2011). In spite of the relatively mild drought stress, the range  
486 of yield reductions amongst cultivars was high as indicated by the significant irrigation x  
487 genotype interaction. Anthesis date of cultivars differed by up to 13 days under irrigation and  
488 12 days under drought, in part, associated with presence/absence of the *Ppd-D1a* allele  
489 determining photoperiod insensitivity, with Soissons possessing the *Ppd-D1a* allele. However,  
490 there was no association between grain yield response to drought and anthesis date amongst  
491 cultivars. Therefore, there was no evidence that the wheat plants were able to escape drought  
492 by completing their life cycle before the onset of water deficit, as has been reported under  
493 more severe terminal droughts (Chaves *et al.*, 2003). In these experiments, taller lines were  
494 associated with linear reductions in grain yield under both irrigated and rain-fed conditions as  
495 expected with decreased plant height favouring higher HI and grain number per unit area  
496 (Fischer, 1985); three of the 17 cultivars were tall non semi-dwarf cultivars (M. Widgeon,  
497 Cadenza and Paragon), and the remaining cultivars were semi-dwarfs. However, there was no  
498 association between plant height and grain yield response to drought.

499 The genetic gain in grain yield with YoR from 1964 to 2009 was  $47.5 \text{ kg ha}^{-1}\text{yr}^{-1}$   
500 ( $0.6\% \text{ yr}^{-1}$ ), which is lower than that reported previously by Shearman et al. (2005) for 8 UK  
501 winter wheat cultivars released from 1972 to 2009 of  $117 \text{ kg ha}^{-1} \text{ yr}^{-1}$  ( $1.2\% \text{ yr}^{-1}$ ). The genetic  
502 gain in grain yield was associated with gains in both above-ground DM ( $30.9 \text{ kg ha}^{-1} \text{ yr}^{-1}$ ) and  
503 HI ( $0.00176\% \text{ yr}^{-1}$ ), which were again lower than changes in AGDM and HI reported by  
504 Shearman et al. (2005) of  $104 \text{ kg ha}^{-1} \text{ yr}^{-1}$  and  $0.0026\% \text{ yr}^{-1}$ , respectively. Clarke et al. (2013)  
505 for a set of 9 UK landmark winter wheat feed cultivars released from 1953 to 2007 reported a  
506 genetic gain in grain yield of  $61 \text{ kg ha}^{-1} \text{ yr}^{-1}$  and for 11 UK landmark winter wheat bread-  
507 making cultivars released from 1964 to 2008 of  $49 \text{ kg ha}^{-1} \text{ yr}^{-1}$ , similar to the rate of yield  
508 gain in the present study. Excluding the three spring wheats in the present study, the genetic  
509 gain in grain yield with YoR for 14 winter wheat cultivars did not change significantly  
510 compared to the 17 cultivars at  $65.8 \text{ kg ha}^{-1}\text{yr}^{-1}$  under irrigated conditions and  $50.0 \text{ kg ha}^{-1} \text{ yr}^{-1}$   
511 under rain-fed conditions.

512

#### 513 *4.2 Relationships between isotope signatures and grain yield and biomass*

514

515 There was a linear negative association between grain  $\delta^{13}\text{C}$  and yield under drought,  
516 indicating lower TE was associated with higher grain yield. Similar correlations between the

517 carbon isotope signature and grain yield under drought were reported previously in  
518 Mediterranean environments (Araus et al., 2001, 2003; Condon et al., 2004; Zhou et al.,  
519 2015) and the UK (Aravinda-Kumar et al., 2011). Although in the present study the  
520 association between grain  $\delta^{13}\text{C}$  and yield was stronger under drought ( $R^2 = 0.70$ ;  $P < 0.01$ ),  
521 there was also an association under irrigation ( $R^2 = 0.47$ ,  $P < 0.01$ ). Moreover, grain  $\delta^{13}\text{C}$   
522 measured under irrigated and rain-fed conditions was linearly associated amongst genotypes  
523 ( $R^2 = 0.77$ ;  $P < 0.01$ ), suggesting grain  $\delta^{13}\text{C}$  may be a constitutive trait under mild UK  
524 droughts. Therefore, selection for this trait under favourable conditions may be useful for  
525 indicating drought performance in breeding programmes. Grain  $\delta^{13}\text{C}$  showed a linear  
526 decrease with year of release in both irrigated and rain-fed conditions indicating TE has  
527 decreased with plant breeding. Two of the 17 cultivars, Avalon and Istabraq, showed high  
528 positive departures (standardized residuals of 1.79 and 1.57, respectively) from the overall  
529 negative linear regression of grain yield on grain  $\delta^{13}\text{C}$  (i.e. high grain yield relative to TE),  
530 demonstrating scope for selecting genotypes combining high yields with moderately high TE  
531 in UK adapted germplasm.

532 Grain  $\delta^{13}\text{C}$  under drought was positively associated with flag-leaf stomatal  
533 conductance amongst the subset of six cultivars, indicating  $\delta^{13}\text{C}$  and TE were determined in  
534 part by  $g_s$ ;  $g_s$  was also positively associated with yield under drought. Therefore, the ability to  
535 access and transpire more water and maintain photosynthesis during grain filling appeared to  
536 be the main mechanism determining higher grain yield under drought in the present  
537 experiments. Similar findings were reported previously under Mediterranean-type droughts  
538 (Araus et al., 2001, 2003; Blum, 2009; Zhou et al., 2015) and UK droughts (Aravinda Kumar  
539 et al., 2011). Present results showed no association between flag-leaf  $\delta^{13}\text{C}$  at anthesis and  
540 grain yield under rain-fed conditions. However, the difference between flag-leaf  $\delta^{13}\text{C}$  and  
541 grain  $\delta^{13}\text{C}$  showed a negative linear association with grain yield under rainfed conditions ( $R^2$   
542 = 0.46,  $P < 0.01$ ). This again implies that increased stomatal conductance during grain filling  
543 amongst genotypes was critical in determining grain yield under drought. It is possible that  
544 higher stomatal conductance was associated with a more effective root system contributing to  
545 higher yield in the rain-fed environment.

546 In the present study, we found significant genetic variation in flag-leaf  $\delta^{18}\text{O}$  in the  
547 range 23.6-24.4‰ in irrigated conditions and 23.4-24.8‰ in rain-fed conditions. Genetic  
548 variation in  $\delta^{18}\text{O}$  has been previously reported for bread wheat (Barbour et al., 2000; Ferrio et  
549 al., 2007; Zhou et al. 2015), durum wheat (Araus et al., 2013) and maize (Cabrera-Bosquet et

550 al., 2009b; Araus et al., 2010). Present findings indicated, however, no significant correlation  
551 between flag-leaf  $\delta^{18}\text{O}$  and grain yield amongst the 17 cultivars under either rain-  
552 fedunirrigated or irrigated conditions. A negative relationship of the oxygen isotope signature  
553 for grain with grain yield was reported amongst bread wheat cultivars under fully irrigated  
554 conditions (Barbour et al, 2000), bread wheat cultivars in high yield conditions in Spain  
555 (Zhou et al., 2015) and durum wheat varieties and landraces in Spain under rainfed and  
556 irrigated conditions (Araus et al., 2007). However, no phenotypic correlation was found  
557 across a set of 24 bread wheat genotypes (Ferrio et al., 2007) under three different water  
558 regimes. The reason for the lack of correlation between flag-leaf  $\delta^{18}\text{O}$  and grain yield in the  
559 present experiments is not certain. The analytical precision of flag-leaf  $\delta^{18}\text{O}$  is usually  
560 smaller than for grain  $\delta^{13}\text{C}$  (Araus et al., 2007), and it is also possible that that spatial  
561 variation in soil N across the experimental site (Cernusak *et al.* 2007) or in air humidity and  
562 temperature (Barbour and Farquhar 2000) caused by different phasing of flag-leaf  
563 development pre-anthesis with respect to calendar time amongst the cultivars may have  
564 contributed to the lack of correlation.

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

574 Interestingly, there was a negative linear relationship amongst cultivars between  
575 FLSW and the standardized residual of the linear regression of grain  $\delta^{13}\text{C}$  on flag leaf  $\delta^{18}\text{O}$ , a  
576 higher residual indicating higher transpiration efficiency (higher  $\delta^{13}\text{C}$ ) relative to  
577 transpiration ( $\delta^{18}\text{O}$ ). The physiological mechanisms underpinning this relationship for lower  
578 FLSW to favour higher TE relative to transpiration cannot be certain. Aravinda-Kumar et al.  
579 (2011) reported a positive linear relationship between flag-leaf specific N and grain  $\Delta^{13}\text{C}$   
580 isotope discrimination amongst lines of a Beaver x Soissons winter wheat doubled-haploid  
581 population under drought in UK field experiments. Assuming genetic variation in flag-leaf  
582 specific N to be indicative of FLSW, this would imply lower FLSW was associated with

583 higher TE in that study which would be generally consistent with the present findings. It can  
584 be speculated that lower FLSW was associated with lower flag-leaf  $g_s$  which, in turn, was  
585 associated with higher TE and that the main driver underpinning the trade-off between FLSW  
586 and TE relative to transpiration was a positive relationship between FLSW and  $g_s$ ; however,  
587  $g_s$  was not measured in the irrigated treatment in the present study. Further work is required  
588 to investigate the basis of this apparent association between FLSW and TE relative to  
589 transpiration under irrigated conditions.

590

#### 591 *4.3 Relationships between stay-green and grain yield and biomass*

592 Greater yield production associated with longer green canopy area duration (stay-green)  
593 amongst genotypes has been reported under drought in wheat (Gorny and Garczynski, 2002;  
594 Verma et al., 2004; Foulkes et al., 2007; Christopher et al., 2008), sorghum (Borrell and  
595 Hammer, 2000) and maize (Campos et al., 2004). In the present study, averaging across  
596 years, there was a positive correlation amongst the cultivars between onset of flag-leaf  
597 senescence and grain yield under drought, but no association under irrigation. Higher grain  
598 yield associated with stay-green under post-anthesis abiotic stress is likely due to source  
599 limitation of grain yield (Christopher et al., 2008; Bogard et al., 2011), and greener canopies  
600 maintain the active photosynthetic rate better (Joshi et al., 2007). Conversely, present results  
601 in the irrigated treatment suggested that grain growth was limited by sink size rather than  
602 source size (Borras et al., 2004). The present positive association between onset of flag-leaf  
603 senescence and grain yield amongst genotypes is consistent with previous investigations under  
604 UK drought for winter wheat genotypes (Verma et al., 2004; Foulkes et al., 2007). In our  
605 study there was a large effect of drought on onset of flag-leaf senescence; drought advanced  
606 onset of senescence by 210 °Cd (approximately 14 days). However, the grain yield decrease  
607 was relatively modest at 1.69 t ha<sup>-1</sup> (15.5%), suggesting that photosynthesis of non-laminar  
608 green organs (such as the ear, peduncle or sheaths) was still contributing to grain filling  
609 during the lamina senescence. Nevertheless, genetic variation in onset of flag-leaf senescence  
610 showed a moderately strong association with yield under the mild drought conditions ( $R^2$   
611 0.35,  $P = 0.01$ ). The mechanisms underlying the genetic differences in leaf senescence cannot  
612 be certain from present measurements. Prolonged leaf senescence duration under low N  
613 availability in wheat was associated with lower N remobilization efficiency (Gaju et al.,  
614 2009) and greater post-anthesis N uptake (Bogard et al., 2011). Under drought, stay green  
615 was associated with deeper roots under drought during the grain-filling period for two

616 CIMMYT wheat lines SeriM82 and Hartog compared to check lines (Christopher et al.,  
617 2009).

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

631

632

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637

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777 Figure legends

778

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

784

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

788

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

791

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

795

796 Figure 5. a) Linear regression of flag-leaf specific weight at anthesis (GS61) versus residual  
797 of linear regression of grain  $\delta^{13}\text{C}$  on flag leaf  $\delta^{18}\text{O}$  under irrigated conditions for 16 cultivars,  
798 and b) grain yield versus onset of rapid phase of flag-leaf post-anthesis (GS61) senescence  
799 for 17 cultivars under irrigated and rain-fed conditions. Values represent means of 2010 and  
800 2011.

801

802 Figure 6. Biplot a) irrigated and b) rain-fed conditions. Grain yield (GYD), above ground  
803 biomass (ADM), harvest index (HIN), grains  $\text{m}^{-2}$  (GM2), thousand ground weight (TGW),  
804 flag leaf  $\delta^{13}\text{C}$  (FL\_C), grain  $\delta^{13}\text{C}$  (GN\_C), flag leaf  $\delta^{18}\text{O}$  (FL\_O), onset rapid phase of post-  
805 anthesis flag-leaf senescence (OSEN), plant height (PHT), anthesis date (ATD). Values  
806 represent means of 2010 and 2011.

807

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      | <i>Rht-D1/Rht-B1</i>   | Spring/winter | YoR  | <i>Ppd-D1</i> |
|---------------|------------------------|---------------|------|---------------|
| Maris Widgeon | <i>Rht-B1a/Rht-D1a</i> | Winter        | 1964 | Ppd-D1b       |
| Hobbit        | <i>Rht-B1a/Rht-D1b</i> | Winter        | 1977 | Ppd-D1b       |
| Avalon        | <i>Rht-B1a/Rht-D1b</i> | Winter        | 1980 | Ppd-D1b       |
| Beaver        | <i>Rht-B1a/Rht-D1b</i> | Winter        | 1990 | Ppd-D1b       |
| Hereward      | <i>Rht-B1a/Rht-D1b</i> | Winter        | 1991 | Ppd-D1b       |
| Cadenza       | <i>Rht-B1a/Rht-D1a</i> | Spring        | 1994 | Ppd-D1b       |
| Rialto        | <i>Rht-B1a/Rht-D1b</i> | Winter        | 1995 | Ppd-D1b       |
| Soissons      | <i>Rht-B1a/Rht-D1b</i> | Winter        | 1995 | Ppd-D1a       |
| Savannah      | <i>Rht-B1a/Rht-D1b</i> | Winter        | 1998 | Ppd-D1b       |
| Paragon       | <i>Rht-B1a/Rht-D1a</i> | Spring        | 1999 | Ppd-D1b       |
| Xi19          | <i>Rht-B1a/Rht-D1b</i> | Winter        | 2002 | Ppd-D1b       |
| Cordiale      | <i>Rht-B1a/Rht-D1b</i> | Winter        | 2004 | Ppd-D1b       |
| Istabraq      | <i>Rht-B1a/Rht-D1b</i> | Winter        | 2004 | Ppd-D1b       |
| Glasgow       | <i>Rht-B1a/Rht-D1b</i> | Winter        | 2005 | Ppd-D1b       |
| Zebedee       | <i>Rht-B1a/Rht-D1b</i> | Winter        | 2007 | Ppd-D1b       |
| Oakley        | <i>Rht-B1b/Rht-D1a</i> | Winter        | 2007 | Ppd-D1b       |
| Panorama      | <i>Rht-B1a/Rht-D1b</i> | 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 (°C) |             | Radiation (MJ m <sup>-2</sup> ) |             |
|-------|---------------|------------|------------------|-------------|---------------------------------|-------------|
|       | 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.

| Cultivar         | YoR | Combine grain yield t ha <sup>-1</sup> 100% DM |       |          |       |                     |       | Above ground DM t ha <sup>-1</sup> |       |         |       |                     |       |
|------------------|-----|--|-------|----------|-------|---------------------|-------|------------------------------------|-------|---------|-------|---------------------|-------|
|                  |     | 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 <sup>ns</sup>                             |       | 0.296**  |       | 0.324*              |       | 1.07 <sup>ns</sup>                 |       | 0.73**  |       | 0.690 <sup>ns</sup> |       |
| Yr*Irr*Gen (126) |     |  |       |          |       | 0.519 <sup>ns</sup> |       |                                    |       |         |       | 1.129 <sup>ns</sup> |       |

\* 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 rain-fed conditions. Values represent means of 2010 and 2011.

| Cultivar   | YoR | Plant height (cm)    |       | AD (days after 31 May) |       | GAI      |       | AGDM (t ha <sup>-1</sup> ) |       | ONSEN (°Cd) |       | FLSW (g m <sup>-2</sup> ) |       |
|------------|-----|----------------------|-------|------------------------|-------|----------|-------|----------------------------|-------|-------------|-------|---------------------------|-------|
|            |     | 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 <sup>0.07</sup>      |       |
| Gen (128)  |     | 1.19***              |       |                        |       | 0.264*** |       | 0.50***                    |       | 18.28***    |       | 3.61***                   |       |
| Irr*Gen    |     | 2.26 <sup>0.07</sup> |       |                        |       | 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 ( $\delta^{13}\text{C}$ ) in flag-leaf at GS61 and grain at harvest, oxygen isotope composition ( $\delta^{18}\text{O}$ ) 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 ( $\delta^{13}\text{C}$ ) |             | Grain ( $\delta^{13}\text{C}$ ) |             | Flag leaf ( $\delta^{18}\text{O}$ ) |             |
|---------------|-----|-------------------------------------|-------------|---------------------------------|-------------|-------------------------------------|-------------|
|               |     | 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  $\delta^{13}\text{C}$  in the unirrigated treatment.

| Cultivar                           | YoR | Stomatal conductance ( $\text{mmol m}^{-2} \text{s}^{-1}$ ) |        |        |       |                      | $A_{\max}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) |        |        |       |        |
|------------------------------------|-----|---|--------|--------|-------|----------------------|---|--------|--------|-------|--------|
|                                    |     | 2010  |        | 2011   |       | Mean                 | 2010  |        | 2011   |       | Mean   |
|                                    |     | 2 Jun   | 24 Jun | 31 May | 7 Jun |                      | 2 Jun   | 24 Jun | 31 May | 7 Jun |        |
| 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 <sup>0.06</sup> | 0.36  | 0.02   | 0.44   | 0.09  | 0.67   |
| Corr. (r) vs $\delta^{13}\text{C}$ |     | -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 <sup>-1</sup> | 8.90           | 0.0604± 0.011 ***    | 7.73           | 0.0475± 0.0099 ***     |
| AGDM, t ha <sup>-1</sup>        | 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 δ <sup>13</sup> C, ‰      | -27.31         | -0.0255± 0.00712**   | -25.68         | -0.0304± 0.0067 ***    |
| Flag leaf δ <sup>13</sup> C, ‰  | -              | -0.00431± 0.00617 ns | -              | -0.00804± 0.00607 ns   |
| Flag leaf δ <sup>18</sup> O, ‰  | -              | -0.00797± 0.00650 ns | -              | -0.00444± 0.00835 ns   |
| ONSEN, °Cd                      | -              | 0.375± 0.959 ns      | -              | 0.755± 0.105 ns        |
| Anthesis (GS61) days            | -              | 0.0019 ± 0.053 ns    | -              | 0.0010 ± 0.060 ns      |

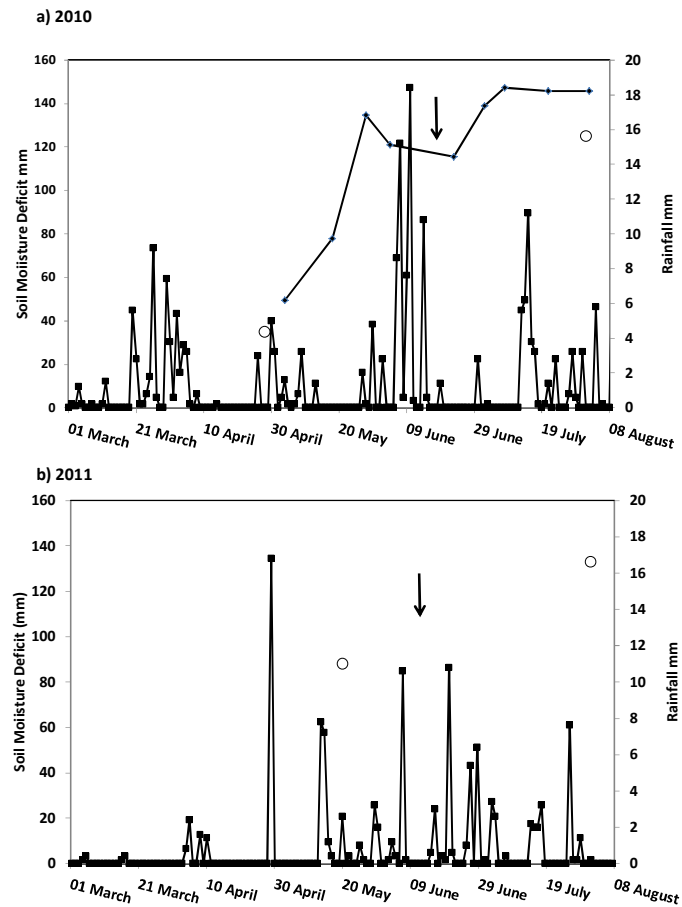
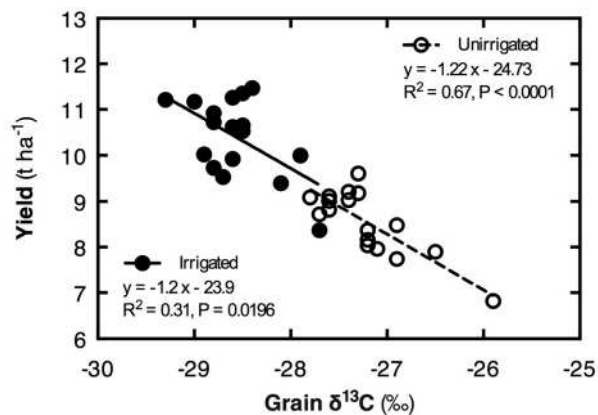
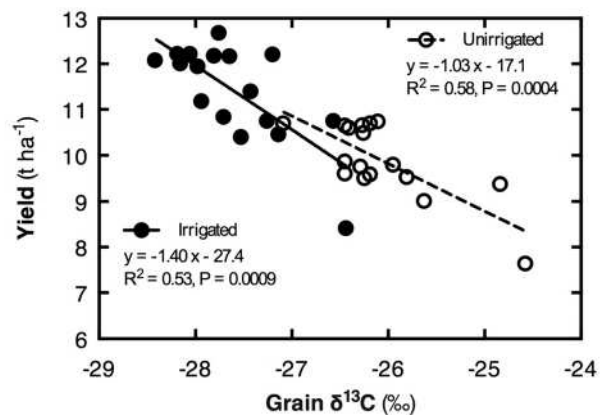


Figure 1. a) Daily rainfall (closed squares) and soil moisture deficit to 1.2 m soil depth estimated from capacitance probe (closed diamonds) and gravimetric analysis of soil cores (open circles) in 2010 and b) daily rainfall (closed squares) and soil moisture deficit to 1.2 m soil depth estimated from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

a) 2010



b) 2011



c) Mean 2010-2011

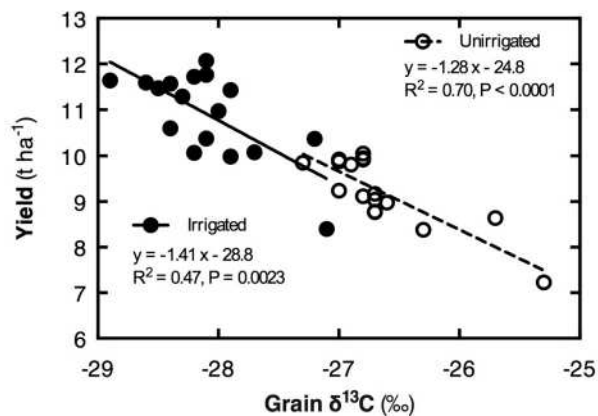


Figure 2. Linear regression of grain yield (100% DM) on grain δ<sup>13</sup>C composition amongst 17 wheat cultivars under irrigated (black circles) and rain-fed conditions (open circles) at Sutton Bonington in a) 2010, b) 2011 and c) mean 2010-2011.

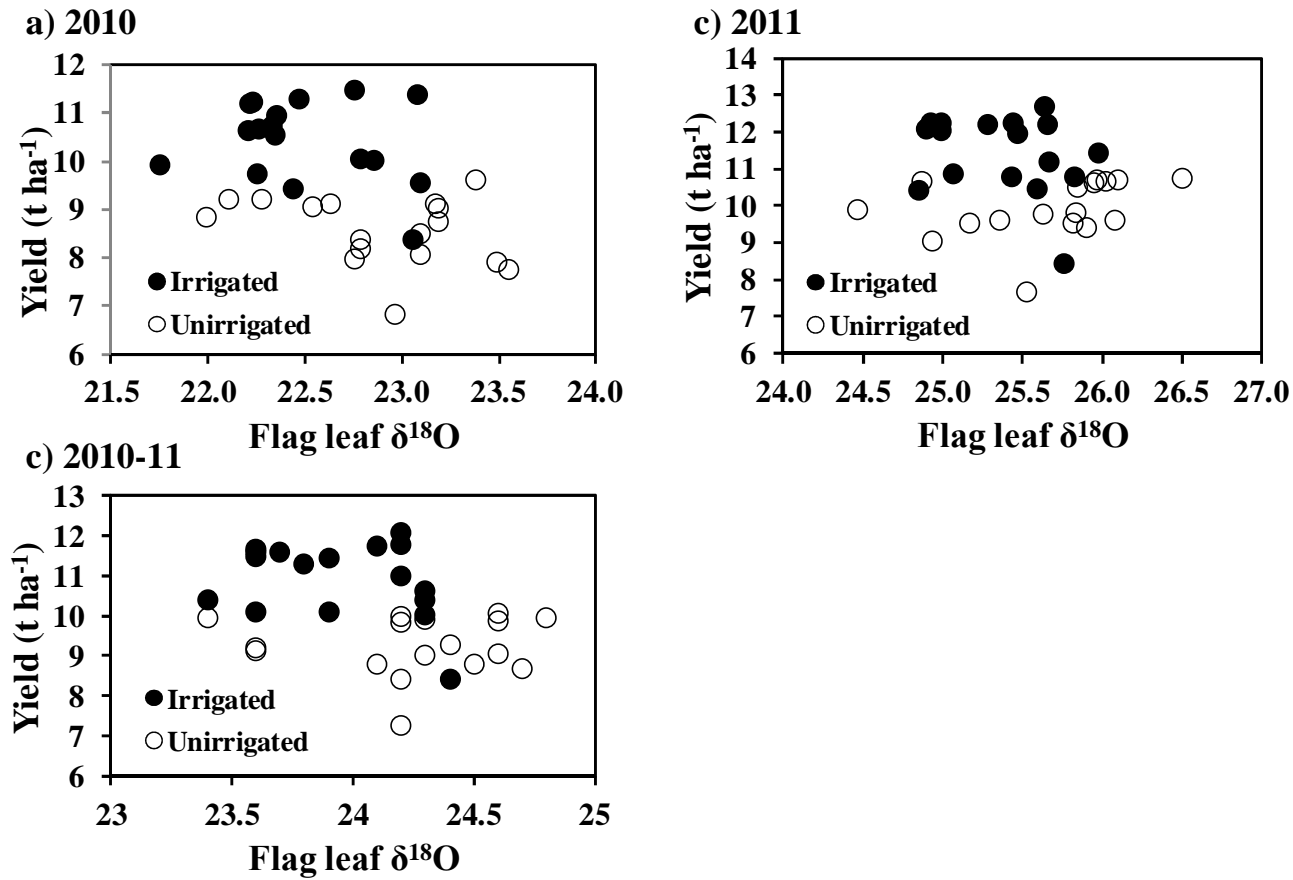


Figure 3. Grain yield versus flag leaf  $\delta^{18}\text{O}$  composition for 17 wheat cultivars under irrigated and rain-fed conditions at Sutton Bonington in a) 2010, b) 2011 and c) mean 2010 and 2011.

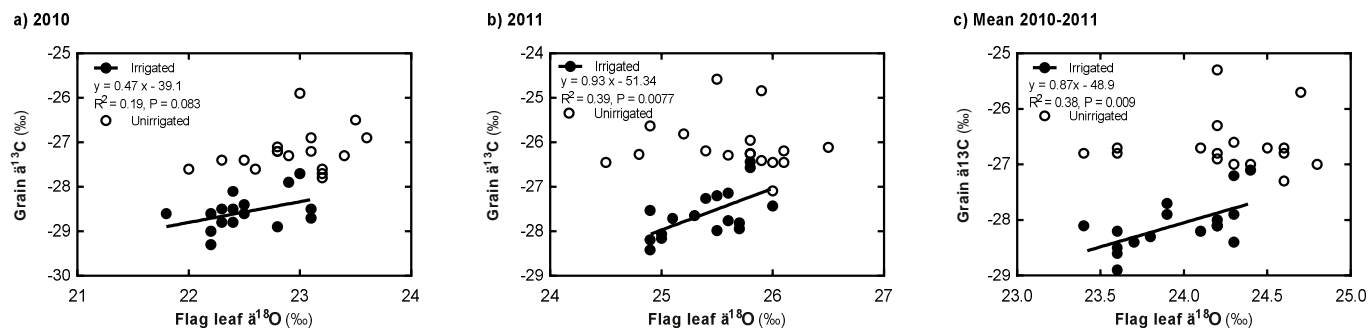


Figure 4. Linear regression of grain  $\delta^{13}\text{C}$  composition on flag leaf  $\delta^{18}\text{O}$  composition amongst 17 wheat cultivars under irrigated and rain-fed conditions at Sutton Bonington in a) 2010, b) 2011 and c) mean 2010 and 2011.

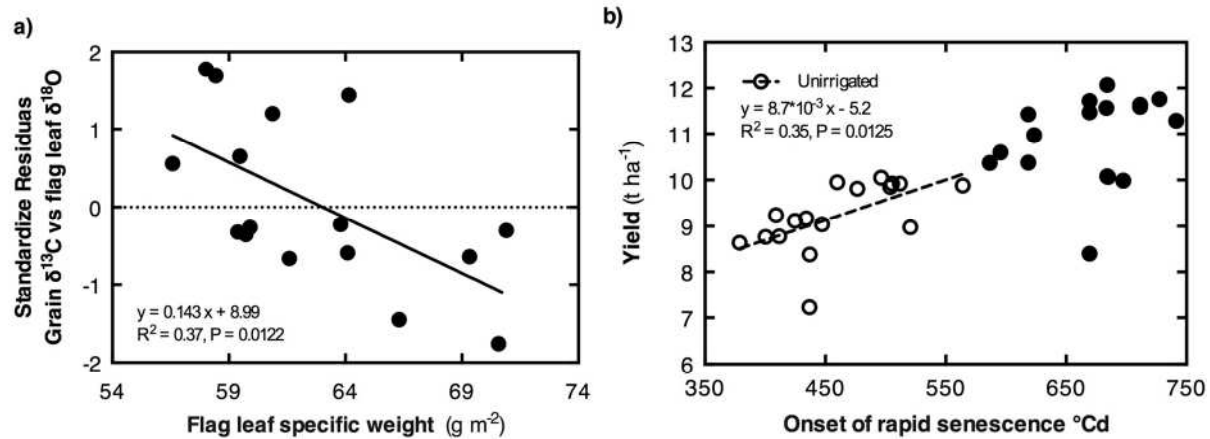


Figure 5. a) Linear regression of flag-leaf specific weight at anthesis (GS61) versus residual of linear regression of grain  $\delta^{13}\text{C}$  on flag leaf  $\delta^{18}\text{O}$  under irrigated conditions for 16 cultivars, and b) grain yield versus onset of rapid phase of flag-leaf post-anthesis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

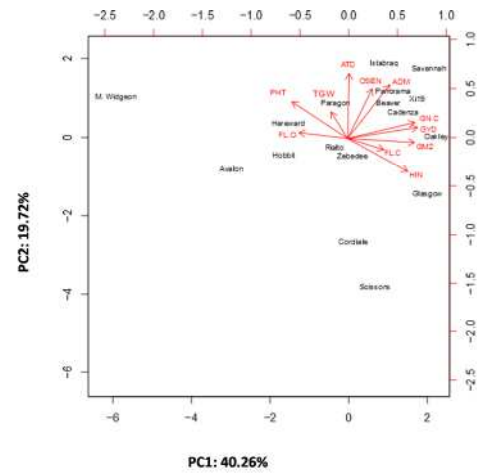


Figure 6a) Irrigated conditions

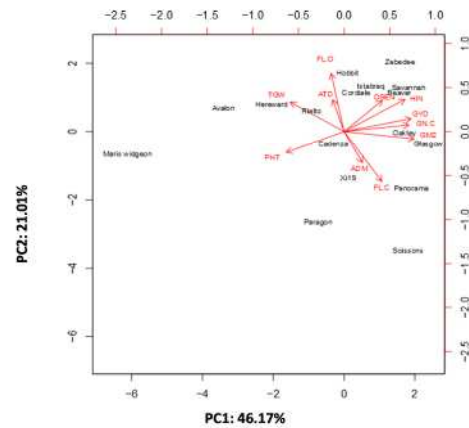


Figure 6b) Rain-fed conditions

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  $\delta^{13}C$  (FL\_C), grain  $\delta^{13}C$  (GN\_C), flag leaf  $\delta^{18}O$  (FL\_O), onset of senescence (OSEN), plant height (PHT), anthesis date (ATD). Values represent means of 2010 and 2011.