The Wheat Genetic Improvement Network (WGIN) – Improving the Environmental Footprint of Farming through Crop Genetics and Targeted Trait Analysis

WGIN Sub-contract: Quantifying relationships between carbon and oxygen isotope screens and drought performance in UK wheat

DEFRA WGIN Sub-Contractor Final Report on a 2 year project

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Executive Summary

In previous work, carbon isotope discrimination ($\Delta^{13}C$) has been used in C3 cereals to screen for genotypes with high transpiration efficiency and ^{18}O isotope enrichment has been shown to correlate with transpiration rate. We explored the use of $\Delta^{13}C$ and $\Delta^{18}0$ isotope screens of the grain and flag leaf in wheat as new tools for phenotyping with respect to drought performance. Field experiments were carried out at University of Nottingham in 2009-10 and 2010-11 testing 17 cultivars under fully irrigated and unirrigated conditions. The main findings were:

- There was a positive linear relationship in both years between grain yield and grain $\Delta^{13}C$ amongst cultivars, under both non-drought (R^2 = 0.70. P< 0.01) and drought (R^2 = 0.41, P< 0.05) conditions. This demonstrates the usefulness of this technique as a phenotyping screen for grain yield under non-drought and drought conditions in the UK.
- The associations between flag-leaf $\Delta^{13}C$ (R^2 = 0.11, ns) and flag-leaf $\Delta^{18}O$ (R^2 = 0.01, ns) and grain yield amongst cultivars under drought were not statistically signficant. These screens may not be applicable as direct screens of grain yield performance under drought conditions in the UK.
- There was a signficant negative linear relationship between flag-leaf $\Delta^{18}O$ and grain $\Delta^{13}C$ amongst cultivars (R^2 = 0.45, *P*< 0.01) under irrigation, and the combination of these two traits was found to be useful in identifying genotypes combining high water use with high water-use effciency under irrigated conditions.

  In both years, grain yield was not found to be significantly associated with mineral ash content of the flag leaf either under irrigated or unirrigated conditions.

The grain $\Delta^{13}C$ isotope screen was shown to have scope for use as an indicator of crop water-use efficiency and grain yield under drought and non-drought conditions. Whereas the relationship between flag-leaf $\Delta^{18}O$ and grain $\Delta^{13}C$ was able to identify genotypes combining high water use with high water-use effciency under irrigated conditions on the Nottingham Experimental Farm. Further experiments are required to test the stability of these correlations with performance across a greater range of germplasm, sites and seasons.

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1. Background and Objectives

1.1 Background

Carbon isotope discrimination ($\Delta^{13}C$) has been used in wheat to screen for genotypes with high transpiration efficiency (Rebetske et al., 2002) and this has led to the release of new wheat varieties (e.g. Rees and Drysdale) in Australia better suited to drier conditions during grain filling. More recently another isotope-based screen using ^{18}O enrichment has been shown in maize (Barbour, 2007) and wheat to correlate with yield potential and drought resistance (Cabrera-Bosquet et al. 2009a). The $\Delta^{18}O$ signature of vegetative tissues is known to reflect variation in evaporative enrichment in leaves due to transpiration, and has been shown to be negatively correlated with transpiration rate (Barbour et al., 2000). Therefore, measurement of the $\Delta^{18}O$ signature might provide a powerful tool for plant breeders to track genotypic differences in drought resistance. In addition, the total leaf ash content of plant tissues has been suggested as a useful tool to predict yield performance under drought (Araus et al., 2002). The mechanism of mineral accumulation in plant tissues appears to be explained through the passive transport of minerals via xylem driven by transpiration. Thus, ash content measured in plant tissues may provide an indicator of transpirative gas-exchange activity and therefore of the total water transpired. This analysis, which is less expensive than analysis of $\Delta^{13}C$ or $\Delta^{18}0$ of plant tissues, could therefore have potential application with future prebreeding research activities as well as in breeders' trials. With a view to phenotyping large association genetics panels in future projects where costs may become prohibitive, relationships between ash content and genotypic differences in grain yield were also tested in the present WGIN2 drought trials.

The $\Delta^{18}0$ value was expected to provide added value to the WGIN drought trials (in addition to the existing $\Delta^{13}C$ value) because, although much attention has focused on improving water-use efficiency (above-ground biomass per unit crop evapotranspiration; WUE) when breeding for drought adaptation, it seems that, except for very severe drought conditions, water use (WU, i.e. the total water absorbed and further transpired by the plant) is a more important adaptive trait than WUE (Slafer & Araus, 2007). This is related to the genotypic capacity to use available water and therefore to sustain transpiration under unfavourable conditions. The $\Delta^{18}0$ of vegetative tissues can be used as an indirect measure of transpiration and WU.

1.2 Objectives

In this sub-contract we explore the use of $\Delta^{18}0$ and $\Delta^{13}C$ of the flag leaf and total mineral ash content of flag-leaf in wheat as new tools for phenotyping wheat with respect to water inputs. We study a panel of 17 wheat genotypes which were grown under irrigated and unirrigated conditions in the DEFRA WGIN Drought Tolerance sub-project (Activity 9) in experiments carried at University of Nottingham in 2009-10 and 2010-11. We compare genetic variability in $\Delta^{18}0$ (flag leaf), $\Delta^{13}C$ (flag leaf) and total mineral ash content (flag leaf) with that for $\Delta^{13}C$ (grain) which was also measured as a part of the core WGIN UoN funding and respective correlations with grain yield under drought.

2. Materials and methods

2.1 Experimental design and plot management

Sixteen winter wheat cultivars and one spring wheat cultivar Paragon were used to identify physiological isotope screens indicative of water-use efficiency, water use and drought tolerance. The varieties were grown under irrigated and unirrigated 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). The experimental design was a split plot block (randomised block) with three replicates. Irrigation treatments were randomised on main plots and cultivars on sub plots. Sub plot size was 6 x 1.65 m. Previous cropping was winter oats. 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 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 in the first half of October in both years. Herbicides, fungicides and pesticides were applied as required to minimise effects of weeds, diseases and pests.

Irrigation Treatment: 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 1·2m soil depth was 176 mm. No water was applied in the unirrigated treatment.

Wheat Genotypes tested (17):

1. Avalon
2. Beaver
3. Cadenza
4. Cordiale
5. Glasgow
6. Hereward
7. Hobbit
8. Istabraq
9. M. Widgeon
10. Oakley
11. Panorama
12. Paragon
13. Rialto
14. Savannah
15. Soissons
16. Xi19
17. Zebedee

2.2 Experimental measurements

In all plots in each of 2009/10 and 2011/12, flag leaf samples at GS61 and grain dry matter samples at harvest were assessed for carbon 12/13 isotope discrimination (grain samples through core funding). Flag leaf samples at GS61 were also assessed for oxygen 16/18 isotope enrichment in both seasons and for mineral ash content in 2009-10.

Date of GS61 was measured by recording Zadoks' stages for each sub-plot every 3-4 days through the flowering window. Plant height was recorded in all 108 sub-plots from GS75 to harvest. Growth analysis samples were taken from a 0.64 m^2 area and 10% sub-sample of plant material processed and dry weighted. Flag leaves from the 10% sub-sample were used for the isotope analysis. A sub-plot area of at least 5 m^2 was machine-harvested at harvest and grain weighed and grain yield expressed at 15% mc.

Carbon 12/13 isotope ($\Delta^{13}C$) and Oxygen isotope ($\Delta^{18}O$) discrimination analysis

From each sub-plot dried flag leaves and grain were milled separately for use in carbon12/13 and oxygen 16/18 isotope analysis. The samples were dried for 48 hours at 80 °C, then ground to a fine powder using a cyclotec 1093 sample machine. The milled samples were then submitted to the isoptope laboratory at The James

Hutton Institute and analysed through an online system composed of an elemental analyser, a TripleTrap and a mass spectrometer to determine carbon isotope composition (Aravinda Kumar et al., 2011).

Mineral ash analysis

From each plot, milled flag leaf samples were submitted to the chemical analysis laboratory for mineral ash determination by combustion analysis (Cabrera-Bosquet et al, 2009b).

Crop water uptake

Soil water uptake was measured directly for eight cultivars (Cordiale, Hobbit, Glasgow, M. Widgeon, Xi19, Rialto, Savannah, Soissons) in the unirrigated plots in both years by gravimetric analysis of four soil cores per plot to 120 cm. In addition, soil water uptake was measured for Paragon in 2010-11. Soil cores were taken in mid April at around GS31 and in August after harvest and the water uptake calculated as the difference between these two estimates of soil water content plus rainfall in the intervening period.

Statistical analysis

Data were entered into Excel spreadsheets and analysed using GenStat 16th edition statistical package for windows (VSN International, Hemel Hempstead UK). 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. Relationships between traits were evaluated using a simple linear regression analysis for both the fully irrigated and unirrigated treatments.

3. Results for WGIN Drought Tolerance trial 2009-10

3.1 Growing conditions

In both 2010 and 2011, rainfall was significantly below the long-term mean (LTM) in March, April, May and July. In June, rainfall was above the LTM in 2010, and the same as the LTM in 2011. The results of these rainfall patterns was that post-anthesis drought occurred in both years, with the severity of drought slightly greater in 2010 than 2011.

Table 1. Monthly rainfall at University of Nottingham Farm in 2010 and 2011 and percentage of long-term mean (LTM)

Month	Rainfall (mm) (% LTM)	
	2010	2011
January	33.0 (62)	33.2 (62)
February	41.6 (95)	44.6 (101)
March	36 (67)	1.2 (2)
April	24 (55)	23 (53)
May	16.2 (35)	27.8 (61)
June	69.2 (152)	45.4 (100)
July	42.6 (86)	17.8 (36)
Total	262.6 (78)	193.0 (57)

3.2 Crop measurements

From the core WGIN DEFRA funding, grain $\Delta^{13}C$ results showed a highly significant positive linear relationship with grain yield amongst the 17 cultivars under both irrigated (R^2 = 0.58, P< 0.001) and unirrigated (R^2 = 0.44. P< 0.01) conditions in 2009-10 (Fig.1). Similarly in 2010-11, a positive linear relationship was found between grain $\Delta^{13}C$ and grain yield under both irrigated (R^2 = 0.69. P< 0.01) and unirrigated (R^2 = 0.30, P< 0.05) conditions. Averaging across years, the linear relationship was significant under both irrigated (R^2 = 0.70. P< 0.01) and unirrigated (R^2 = 0.41, P< 0.05) conditions. The corresponding relationships between the grain yield and flag-leaf $\Delta^{13}C$ were not statistically significantly under irrigation or unirrigated conditions (Fig. 2). However, fitting a linear relationship across the 34 irrigation/cultivar treatment combinations, there was a significant linear regression in 2009-10 (R^2 = 0.30, P< 0.01), 2010-11 (R^2 = 0.37, P< 0.001) and averaging over years (R^2 = 0.38, P< 0.001).

Both grain $\Delta^{13}C$ and flag-leaf $\Delta^{13}C$ results indicated that WUE was negatively associated with grain yield (i.e. suggesting water use was positively correlated with grain yield).

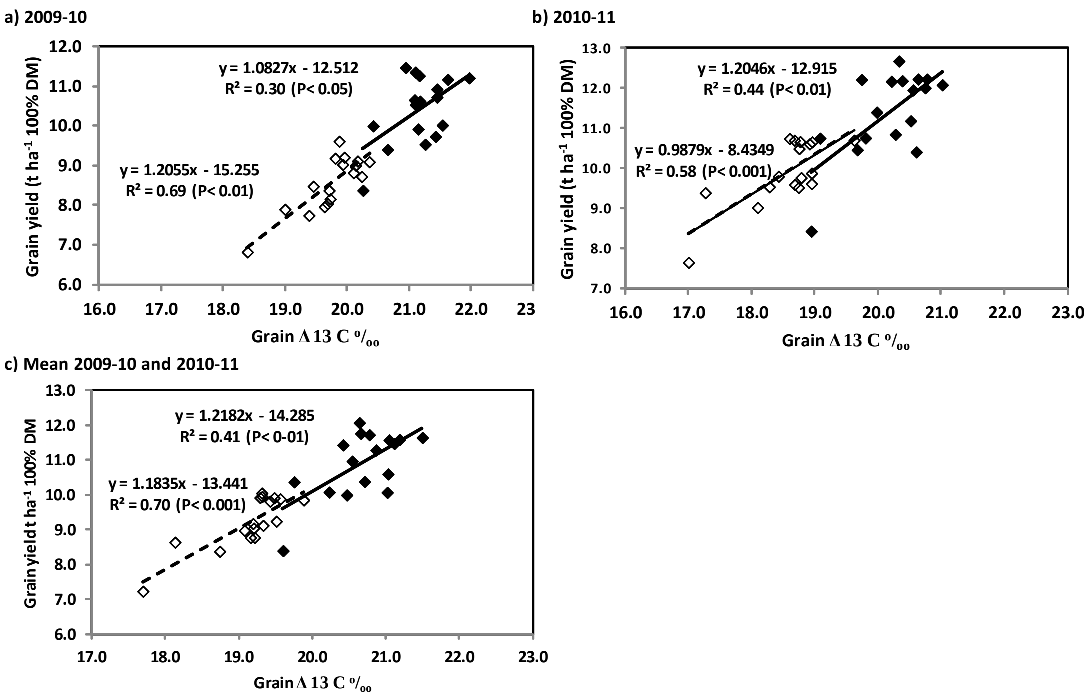

Fig. 1 Linear regression of grain yield on grain $\Delta^{13}C$ discrimination amongst 17 wheat cultivars under irrigated (black diamond) and unirrigated conditions (open diamonds) at Sutton Bonington in a) 2009-10, b) 2010-11 and c) mean 2009-10 and 2010-11.

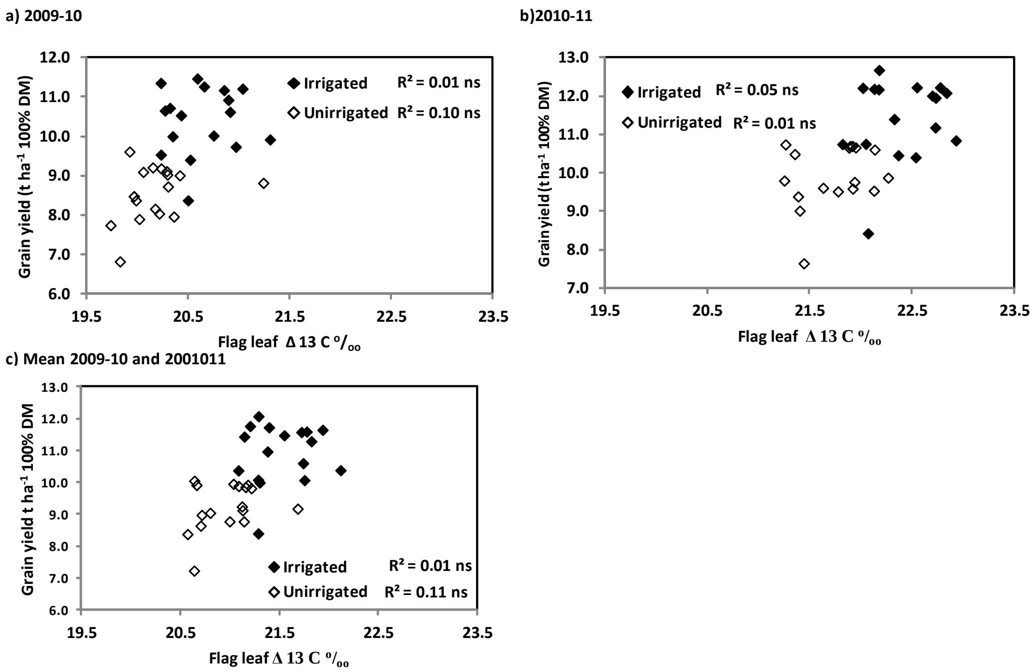

Fig. 2 Linear regression coefficients for grain yield versus flag leaf Δ^{13}C discrimination for 17 wheat cultivars under irrigated (black diamonds) and unirrigated conditions (open diamonds) at Sutton Bonington in a) 2009-10, b) 2010-11 and c) mean 2009-10 and 2010-11.

The linear relationship between grain yield and flag-leaf Δ^{18}O was not statistically significant amongst the 17 cultivars under either irrigated or unirrigated conditions in 2009-10, 2010-11 or averaging across years. Neither was there a significant linear relationship across irrigated and unirrigated treatment combinations (Fig. 3). Grain yield was not significantly associated with mineral ash content of the flag leaf either under irrigated or unirrigated conditions amongst the 17 cultivars in 2009-10, or combining data across the two irrigation treatments (Fig. 4).

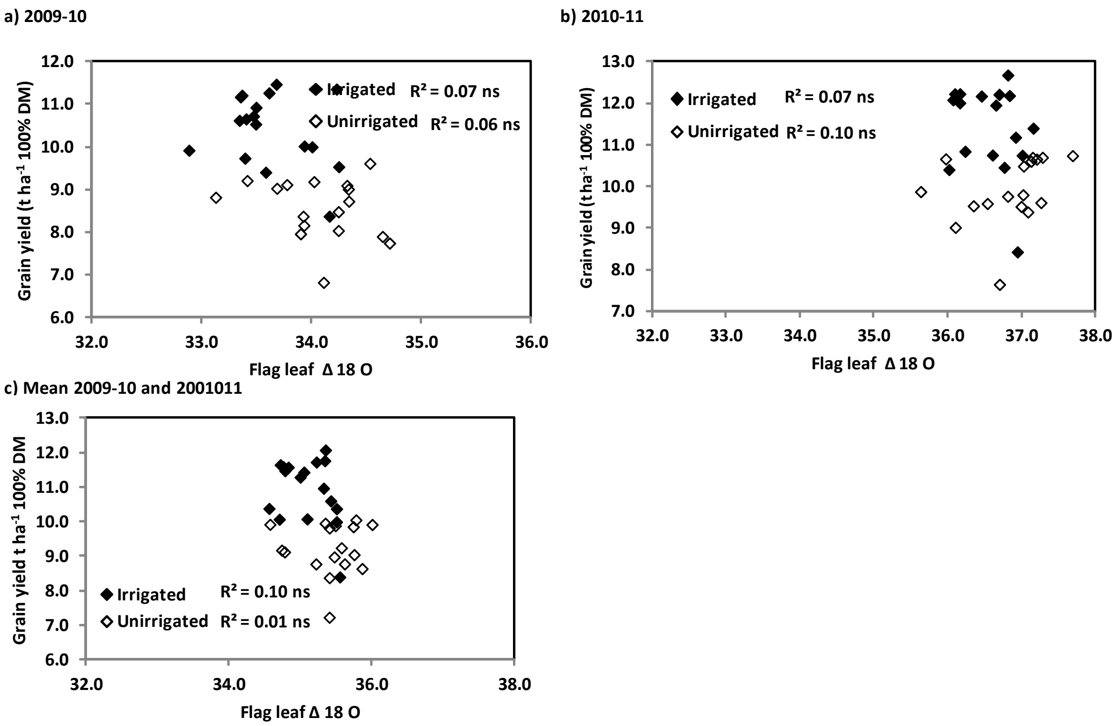

Fig. 3 Linear regression coefficients for grain yield versus flag leaf $\Delta^{18}O$ enrichment for 17 wheat cultivars under irrigated and unirrigated conditions at Sutton Bonington in a) 2009-10, b) 2010-11 and c) mean 2009-10 and 2010-11.

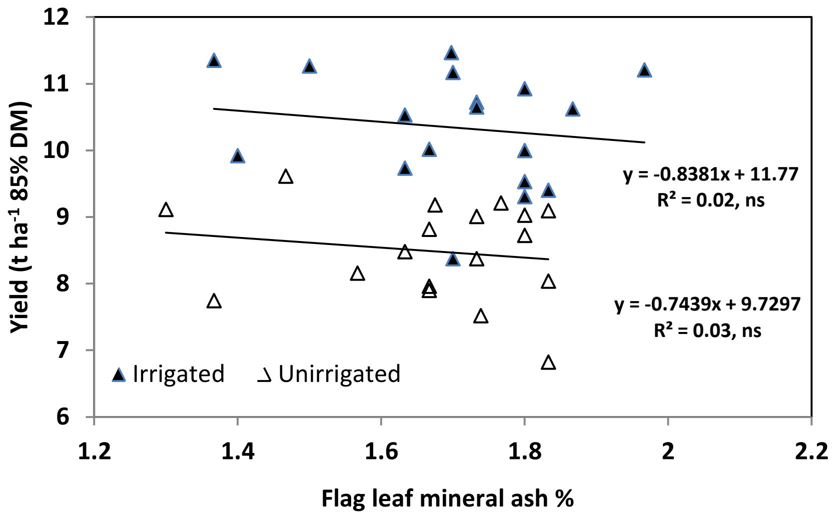

Fig. 4. Linear regression of grain yield on total mineral ash content of flag leaf (%) amongst 17 wheat cultivars under irrigated and unirrigated conditions at Sutton Bonington 2009-10.

Flag-leaf $\Delta^{18}O$ is not strongly influenced by photosynthesis rate, so measurement of grain $\Delta^{13}C$ and flag-leaf $\Delta^{18}O$ allows stomatal and photosynthesis effects on $\Delta^{13}C$ to be teased apart. Fig. 5 shows that under drought the assocation between grain $\Delta^{13}C$ and flag-leaf $\Delta^{18}O$ was not significant, but under irrigated conditions it was in each year and averaging across years ($P< 0.05$), i.e. higher water-use effciency was associated with lower water use (hence yield). The relationhship between grain $\Delta^{13}C$ and flag-leaf $\Delta^{18}O$ was also signficant for values averaged across irrigation treatments; with a negative linear relationship amongst the 17 cultivars in 2009-10 and also for the mean of 2009-10 and 2010-11 ($R^2 = 0.18$, $P = 0.09$; $R^2 = 0.20$, $P = 0.06$, respectively). Furthermore, there was a negative linear relationship between grain $\Delta^{13}C$ and flag-leaf $\Delta^{18}O$ across the 34 irrigation/cultivar treatment combinations in 2009-10 and averaging over years ($R^2 = 0.34$, $P< 0.05$; $R^2 = 0.25$, $P< 0.05$, respectively). Therefore, present results indicated there was a trade-off between water-use efficiency and water use. However, there were some departures from this overall negative relationship for indidvual cultivars. Thus, under irrigation, Soissons maintained high WUE relative to its water use (as indcated by its position below the regression line in Fig. 5), and the opposite was the case for Panorama.

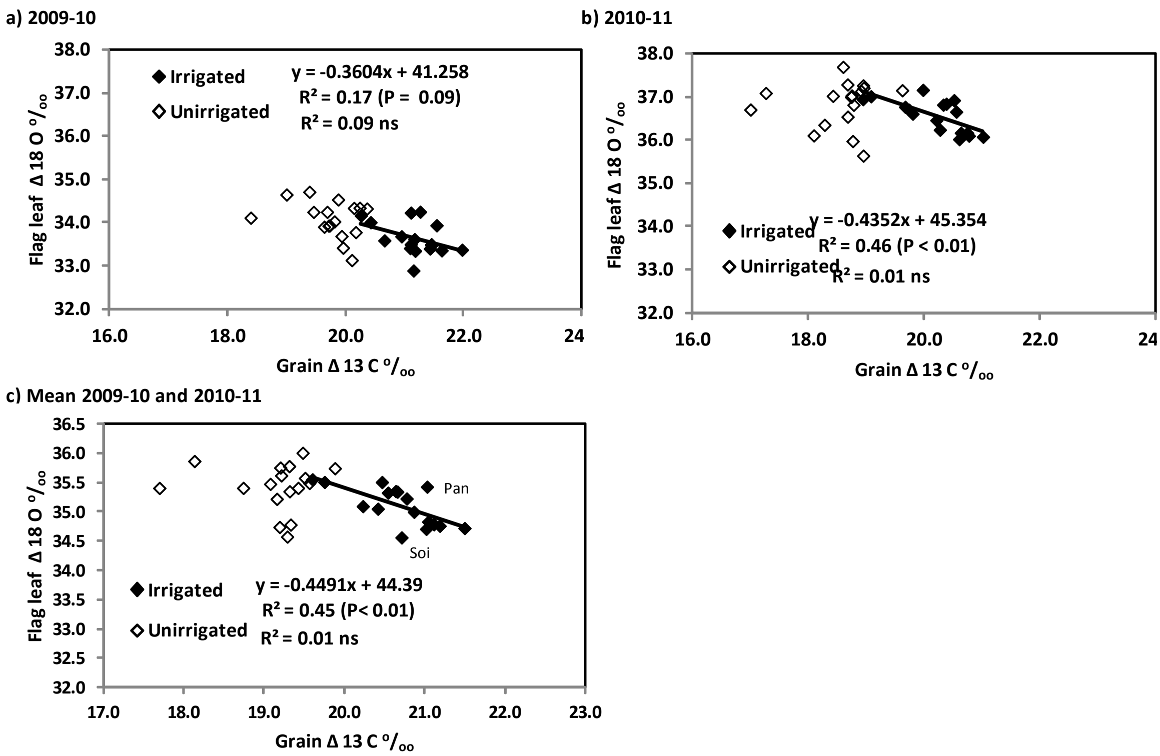

Fig. 5 Linear regression of flag-leaf $\Delta^{18}O$ enrichment on grain $\Delta^{13}C$ discrimination amongst 17 wheat cultivars under irrigated and unirrigated conditions at Sutton Bonington in a) 2009-10, b) 2010-11 and c) mean 2009-10 and 2010-11.

There was a negative linear relationship between flag-leaf $\Delta^{18}O$ and crop water uptake from 21 April to harvest measured by gravimetric analysis of soil cores in 2009-10 (Fig. 6, P< 0.05). However, the corresponding relationship was not statistically signficant in 2010-11 although there again was a tendency for a negative realtionship.

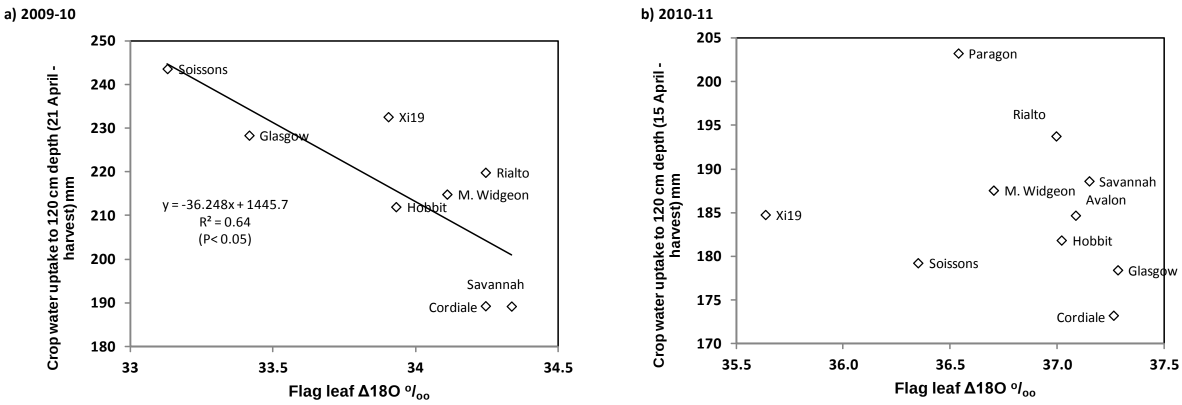

Fig. 6 Linear regression of crop water use on flag-leaf Δ^{18}O amongst 8 wheat cultivars in 2009-10 and 9 cultivars in 2010-11 under unirrigated conditions at Sutton Bonington.

4. Conclusions

In the WGIN project, the present trait analysis directly relates to the "Targeted Traits" section of "Resource Development", specifically "improvement of water use efficiency and drought tolerance traits". The potential value of Δ^{18}0 and mineral ash content as new phenotypic screens in wheat for adaptation and yield potential under varying levels of water availability has been evaluated in two years. These results indicated the mineral ash content screen may not be a useful technique for indicating drought performance under UK conditions. The present results indicated there was a negative linear relationship between flag-leaf Δ^{18}O and crop water use in one year out of two and tendency for a negative relation in the second year. This screen may have scope for use as an indicator of season long crop water use under drought, but further experiments across a greater range of sites and seasons are required to test further the stability of the correlation with crop water use across environments. The flag-leaf Δ^{13}C and flag-leaf Δ^{18}O associations with grain yield were not as strong as that of grain Δ^{13}C with grain yield. These flag-leaf isotope screens may not be applicable as direct screens of grain yield under drought. However, the present results demonstrate that the combination of flag-leaf $\Delta^{18}O$ and grain $\Delta^{13}C$ is potentially useful to plant breeders in that it provides a mechanism to identify those genotypes combining high WUE with high water use, and hence new germplasm for incorporation into breeding programmes for drought tolerance. Generally the isotope screens seemed to be operating in a constitutive manner in these experiments, with genotype rankings generally similar with and without drought. Although R^2 values were generally higher under drought conditions, the linear relationship between grain yield and grain $\Delta^{13}C$ observed under drought was also observed under irrigated conditions. This is encouraging since it indicates grain $\Delta^{13}C$ may be selected for in non-drought seasons and that high expression of grain $\Delta^{13}C$ is not detrimental for grain yield in the absence of drought. It is encouraging that cultivars with high WUE and high WU under drought were identified in the present study, e.g. Soissons. The flag-leaf $\Delta^{13}C$, flag-leaf $\Delta^{18}O$ and flag-leaf mineral ash content screens have not previously been assessed on UK winter wheat germplasm.

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