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Variation in grain weight among Iranian wheat cultivars: the importance of stem carbohydrate reserves in determining final grain weight under source limited conditions

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Abstract

Wheat (*Triticum aestivum* L.) plants accumulate fructan and sucrose in their stems during vegetative growth, which are remobilized during grain filling. The aims of the study were to evaluate the grain weight susceptibility of different wheat cultivars to decreased source leaf capacity, and to assess the contribution of stem carbohydrate reserves to the variation of grain weight in source-limited plants. Eighty-one wheat cultivars (mainly *T. aestivum* and few *T. durum*) were grown in the field in two consecutive years. Plants were fully defoliated or partially defoliated, leaving only the flag leaf attached, at anthesis or 14 days after anthesis (14 DAA) and final grain weight compared with non-defoliated control plants. The effect of defoliation tended to be smaller in modern varieties compared to older varieties, and in cultivars bred for subtropical regions compared to those bred for cold climates. Two contrasting varieties were grown under controlled environment conditions for physiological analysis: cv. Hamon, which showed no response to defoliation and cv. Maron, which strongly affected by defoliation. The non-responsive cultivar Hamon accumulated 4-5 times more water soluble carbohydrate reserves were remobilized during grain filling. These results suggest that cv. Hamon has sufficient stem carbohydrate reserves to support normal grain filling even if leaf area has been severely reduced. In contrast, the much smaller stem reserves in cv. Maron can only partially compensate for loss of photosynthetic capacity, leading to a marked reduction in final grain weight after defoliation. It is concluded that differences in stem carbohydrate reserves are likely to be a major factor underlying the variation in the effect of defoliation on final grain weight among wheat cultivars grown in the field.

Keywords: defoliation; flag leaf; peduncle; penultimate; remobilization; *Triticum aestivum* L; water soluble carbohydrates. **Abbreviations:** DAA- days after anthesis; WSCs- water soluble carbohydrates.

Introduction

To satisfy the food demands of a rapidly growing world population, which is projected to be over 9 billion people within the next 40 years, production of wheat (Triticum aestivum L.), as a staple food and one of the main sources of calories for human, needs to be increased by 70-100 % (Shewry, 2009; Godfray et al., 2010). Therefore, improvements in grain weight at maturity are a high priority for wheat breeders. Although it is well known that grain number per unit area is the most important yield component (Fischer, 2008), grain weight is also an important contributor to grain yield since for any given grain number there is a wide range in achievable yield due to variation in mean grain dry weight (Borras et al., 2004). Also, the potential grain number is determined prior to anthesis and any decrease in photosynthesis thereafter will mainly affect grain weight. Therefore, understanding the causes of grain weight determination could be critical to plant breeders and agronomists aimed to increase yield or yield stability, particularly in the Mediterranean region where characterized by terminal stresses. The first step towards increasing yield potential is to assess whether the growth of developing wheat grains, which are non-photosynthetic sink organs, is limited

by the availability of substrates (source limited), or by the capacity of the developing grains to import and utilize the available supplies (sink limited) (Schnyder, 1993). Manipulation of source: sink ratio through artificial defoliation of the plants is a classical approach used to address this question (Ahmadi et al., 2009). However, the results of such experiments show considerable variation from one region to another and between different cultivars. For instance, the plants are less likely to be source limited if the experiment is conducted in an area with high light intensity during anthesis and grain filling period than in an area with lower solar radiation (Calderini et al., 2006). Even within a given region, there can be large variations between cultivars in their response to source-sink manipulations. Kruk et al. (1997) investigated the effect of defoliation treatments on final grain weight in seven wheat cultivars chosen as representative of those released over last 70 years in Argentina, and reported that new high-yielding cultivars are more source limited than older low-yielding ones. However, other researchers have reported that there is still sink limitation even in modern cultivars since grain filling mostly occurs under conditions with saturated assimilate availability

(Borras et al., 2004; Cartelle et al., 2006; Richard, 2008). Grain filling in wheat depends on two major sources of carbon, namely, current photosynthesis in leaves and mobilization of stored water-soluble carbohydrates (WSCs) from the stem internodes into the growing grains (Ehdaie et al., 2006). Stem WSCs in wheat consist mainly of fructans, followed by sucrose and usually lesser amounts of glucose and fructose (Virgona and Barlow, 1991). In contrast to other cereals, such as maize and rice, wheat stores very little starch in the stems. The contribution of remobilized stem WSC reserves to grain filling becomes more important when current photosynthesis is reduced by partial defoliation (Borras et al., 2004). However, it has also been observed that there is a compensatory increase in photosynthetic rates in any remaining leaves and in other chlorophyll containing organs, especially the spike structure (Guitman et al., 1991; Maydup et al., 2010). Wheat is the main cereal crop in Iran and in many other regions with a Mediterranean climate, which have mild wet winters (when the wheat is sown) and hot dry summers. Despite the high irradiance, photosynthesis during the critical grain filling stage is often limited by water availability as conditions become hotter and drier, which could lead to a substantial reduction in grain yield. Since vast amount of absorbed water by roots is transpired through the leaves, plant water demand may be reduced by leaf area reduction. However, flag leaf is the most important photoassimilate supplier for growing grains (Zhang et al., 2006) and it is interesting to evaluate changes in grain weight of different wheat cultivars when just the flag leaf is attached on the stem. Furthermore, to the best of our knowledge, there have been no large scale studies of the effects of source limitation on final grain weight on wheat growing under Mediterranean conditions. Therefore, we carried out replicated field trials on 77 cultivars of bread wheat (T. aestivum L.) and 4 cultivars of durum wheat (T. durum L.), including both modern and older (up to 70 years) cultivars, and varieties recommended for cultivation in different climate zones. In order to fully differentiate this broad range of cultivars, the two severe defoliation treatments (i.e. partial defoliation, leaving only the flag leaf, or full defoliation) were applied on all the cultivars at either anthesis, initiation of grain filling, or 14 days after anthesis (14DAA) (Supplementary fig. 1). Then, plants final grain weight was compared with non-defoliated control plants

Based on the results of the field experiments, the cultivar showing the strongest response to defoliation (cv. Maron) and a non-responsive cultivar (cv. Hamon) were selected for further physiological analysis. These two cultivars were grown under controlled environmental conditions and the carbohydrate content of different parts of the plant were determined to assess the role of stem reserves in compensating reduced leaf area during grain filling.

Results and Discussion

Grain weight response of the cultivars to defoliation treatments

Significant variation was observed between wheat cultivars in the degree to which final grain weight was decreased in response to the defoliation treatments, with <1% loss in grain weight in the most resistant cultivars up to 34 % loss in grain weight in the most susceptible cultivars (Supplementary Tables 4, 5 and 6). Analysis of variance showed that the cultivars were one of the sources of variance (Table 1). In contrast, the year of the experiment was a much less important factor, and this is reflected in the high correlation

in grain weight observed for each cultivar/treatment in the two successive growing seasons (2007-2008 and 2008-2009) (Supplementary Fig. 2). Together, these results indicate a strong genetic component underlying the different sensitivity of the cultivars to restriction of source capacity. In the most susceptible cultivars, such as Maron and Zarrin, grain weight was decreased by even the least severe defoliation treatment (i.e. partial defoliation at 14 DAA). In contrast, the grain weight of some cultivars such as Hamon and Marvdasht was resistant to even severe reduction in source capacity (i.e. full defoliation at anthesis). These results are presented as supplementary Tables 1, 2 and 3. It has been estimated that less than 1 % of total water absorbed by plant roots is used directly by photosynthesis and that the vast majority of water taken up (>90%) is lost through leaf transpiration (Morison et al., 2010). Based on such estimates, it has been suggested that reducing leaf transpiration by timely defoliation could be a useful strategy to maximize wheat grain weight in the regions where the plants encounter terminal drought. Such modification of the crop canopy should help to maintain the availability of more water in the soil profile during the grain filling period and reduce the probability of crop failure (Zhu et al., 2004). Direct genetic modification of leaf area has been also suggested for yield improvement when water is limited (Richards, 1983). We suggest that the cultivars found in the current study to maintain high grain yield even after full defoliation could provide an excellent gene pool for breeding improved varieties with lower total leaf area to reduce water loss via transpiration, and thereby increase the chance that water will still be available to the plants during the grain filling stage. Such a strategy would be most effective in regions where water loss from the soil surface is not excessive. The average effects of the four defoliation treatments on grain weight are shown in Fig. 1. As might be expected, full defoliation had a greater effect on grain weight than partial defoliation, presumably reflecting the continued provision of carbohydrates from the remaining flag leaf in the latter. For both full and partial, defoliation at anthesis led to more severe loss of grain weight than the corresponding treatments performed at 14 DAA. The number of endosperm storage cell within the grain and grain number per spike are two of the major physical factors affecting sink strength, and these parameters are largely determined by photoassimilate availability at the anthesis stage (Borras et al., 2004). Thus, even a slight reduction in photoassimilate supply at anthesis can have a major impact on the eventual grain yield. Our observations that defoliation treatments at anthesis lead to greater losses in final grain weight than defoliation at 14 DAA are consistent with anthesis being a critical developmental stage for determining wheat yields.

The effect of defoliation on grain weight in old versus modern cultivars

When the percentage grain weight loss due to defoliation at anthesis was plotted against the release date of the cultivar (Fig. 2A), it has been found that there was a downward trend in the susceptibility of the cultivars to defoliation over time. A possible explanation for the downward trend is that grain number per spike/spikelet has been increased over time due to selection in breeding programmes. Most of this morphological change is related to the presence of innate small grains in florets 3 and 4 within the spikelets of the modern cultivars (Acreche and Slafer, 2006). Decreased photoassimilate supply at or shortly after anthesis probably leads to abortion of these small grains, diverting the available resources to florets 1 and 2 allowing them to develop into

Table 1. Analysis of variance of the factors affecting final grain weight in the two years field experiments.

Source of variation	DF	Mean Square
Year	1	2660.41 ^{ns}
Defoliation	4	1688.60^{**}
Cultivar	80	542.36**
Year×defoliation	4	5.97 ^{ns}
Year×cultivar	80	59.95**
Defoliation×cultivar	320	4.73**
Year×defoliation×cultivar	320	3.38 ^{ns}
CV= 8.17		
R-Square=0.86		

*, ** and ns indicate significant at $\overline{5\%}$, 1% and non significant, respectively. DF stands for degree of freedom. The experiments were carried out at the agriculture research farm of University of Tehran, Karaj, Iran during 2007-2008 and 2008-2009 growing seasons. Analysis of variance was performed using SAS 9.1 program (SAS Institute, Cary, NC, USA).

Table 2. Effect of full defoliation and partial defoliation treatments on final grain weight for wheat cultivars recommended for cultivation in different climatic zones.

	Grain weight redu	Grain weight reduction (%) in comparison with non-defoliated plant				
Region	FD (anthesis)	PD (anthesis)	FD (14DAA)	PD (14DAA)		
Subtropical	12.3 ± 3.0^{a}	6.9 ± 1.7^{a}	10.1 ± 1.7^{a}	4.7 ± 1.1^{a}		
Hot and dry	12.7 ± 4.3^{a}	7.3 ± 3.6^{a}	9.6 ± 2.4^{a}	4.9 ± 1.6^{a}		
Temperate	13.2 ± 4.1^{a}	7.2 ± 3.1^{a}	10.2 ± 3.6^{a}	4.9 ± 2.3^{a}		
Cold	13.5 ± 5.1^{a}	7.8 ± 3.5^{a}	10.0 ± 3.8^{a}	4.6 ± 1.8^{a}		

The data are the average of the two years. Differences between the two years were not significant. Each value is the mean of three replicates \pm SD. Numbers with the same letter within each column are not significant (P < 0.05). FD, full defoliation; PD, partial defoliation; DAA, days after anthesis; SD, standard deviation.

grains. The older cultivars, which generally lack these distal grains, are likely to have less flexibility in their allocation of the available photoassimilates. If the latter are limited then the plant might set more seeds than it has the capacity to support through to maturity, leading to lower overall grain yield. However, it must be emphasized that although the modern varieties on average showed a smaller effect of defoliation on grain weight than the older varieties, there is considerable variation between modern varieties, and some perform no better or even worse than the older ones. Grain weight of these modern varieties decreased up to 28% in response to full defoliation treatment at anthesis showing that grain weight of these cultivars are probably source limited. When the effects of defoliation at 14 DAA were plotted against cultivar release date (Fig. 2b) no obvious trend over time was observed. This suggests that if the plants have an adequate carbon supply around the time of anthesis then there is little difference between old and modern cultivars in the number of grains set, and if photoassimilate supply becomes limiting during the later stages of grain filling, the individual grain weight decreases in a similar way in both modern and old cultivars.

Defoliation responses in cultivars bred for different climatic regions

The cultivars were categorized into four groups based on their recommended cultivation regions: subtropical, hot and dry, temperate and cold. The average reduction in grain weight after defoliation at anthesis tended to be lower in cultivars selected for subtropical regions than in those from cold regions, but the differences were small and not statistically significant (Table 2). Differences in the response to defoliation at 14 DAA were even smaller and not statistically significant.



Fig 1. Final grain weight (gr) responses to full defoliation (FD) and partial defoliation (PD) treatments at anthesis or 14 DAA. Data show the averages for all 81 wheat cultivars over the two years of the replicate field experiments. Bars labeled with the same letters are not significantly different (P < 0.05) by ANOVA; error bars represent standard deviation.



Fig 2. Influence of cultivar release date on the grain weight response to full (FD) or partial (PD) defoliation at anthesis (A) or 14 DAA (B). The correlation was not significant

Accumulation and remobilization of water soluble carbohydrate reserves

In wheat, stem internodes and flag leaf sheath are the main parts in which surplus photoassimilate are extensively accumulated in the form of WSC during the vegetative growth phase (Takahashi et al., 2001). Amount of WSC reaches to its maximum level around 14 DAA. As accumulation of WSC in the different parts starts at different times but ceases at about the same time, the length of accumulation period varies among these parts (Schnyder, 1993). The carbohydrates can be remobilized from these parts to the growing grains if photoassimilate production is restricted for example by drought or defoliation. Carbohydrate is stored in the wheat stem mainly in the form of fructans, which are water soluble polymers of fructose synthesized from sucrose by fructosyltransferases (Lunn, 2008). The stems also accumulate soluble sugars, especially sucrose, glucose and fructose, but usually very little starch. Previous studies have shown that the stem reserves of water soluble carbohydrates (WSC) in wheat can be an important factor in determining grain yield, especially under terminal drought conditions (Van Herwaarden et al., 1998; Xue et al., 2008). We decided to compare the WSC content of two cultivars that showed strongly divergent responses to defoliation in our study of wheat cultivars grown under Mediterranean conditions: cv. Hamon, which showed no significant effect of defoliation on grain weight, and cv. Maron, which showed the severe loss in grain weight after defoliation. Plants were grown under controlled environmental conditions with high irradiance and temperature, similar to the conditions in the field trials. Nondefoliated plants were harvested at 14 DAA and maturity, and compared with plants that were partially defoliated at 14 DAA and harvested at maturity. WSC and starch were measured in lower stem internodes, penultimate stem internode, peduncle and flag leaf sheath in which surplus photoassimilates are accumulated.

Total water soluble carbohydrates

At 14 DAA, the stems of the non-responsive cv. Hamon contained substantially more WSCs than those of the defoliation-responsive cv. Maron (Fig. 3a). In lower stem internodes, the penultimate stem internode and peduncle, total WSCs levels were 4.1, 4.1 and 2 fold higher in cv. Hamon than cv. Maron. The physiological basis for variations in the accumulation of stem WSCs reserves in wheat is not understood. Potential contributing factors could include differences in: (i) photosynthetic rates, (ii) partitioning between storage and growth, (iii) fructosyltransferase activity in the stems and (iv) the storage capacity of the stem tissues. In the present study, lower stem internodes and peduncle were the main sites of WSC accumulation in cv. Hamon and cv. Maron, respectively. Previous studies have found that the main site of fructan accumulation can be influenced by growth conditions. The mature leaf sheath can accumulate substantial amounts of fructan, especially if growth is restricted for some reason but high rates of photosynthesis are maintained (Wardlaw et al., 1994; Schnyder, 1993), whereas in another study a major part of the fructan reserves were found in the penultimate internode (Joudi et al., 2012). In defoliated and nondefoliated plants at maturity, very little WSC remained in either cultivar, indicating that almost all of the WSCs had been remobilized during grain filling (Fig. 3a). Wheat cultivars grown in different environments showed fairly

consistent rankings in their total stem WSC reserves, indicating a high heritability for this trait (Ruuska et al., 2006). Therefore, high WSC content is considered to be a potentially useful selection criterion in breeding programs (Shearman et al., 2005; Ehdaie et al., 2006).

Sucrose, glucose and fructose

There were marked differences in the amounts of soluble sugars accumulated in the stems of the two cultivars at 14 DAA. All parts of the stem, as well as the flag leaf sheath, of cv. Hamon contained more glucose and fructose than the corresponding parts of Maron (Figs 3b and 3c). In all parts of the stem there was more glucose than fructose. In contrast to hexose sugars and total WSC, sucrose levels in stems of cv. Hamon were lower than in cv. Maron (Fig. 3d). It has previously been reported that some varieties of wheat accumulate higher levels of sucrose than others (Takahashi et al., 2001). As the total amount of WSC accumulated by cv. Hamon was greater than in cv. Maron (Fig. 3a), it seems unlikely that the higher level of sucrose in cv. Maron can be ascribed to higher rates of photosynthesis or sucrose import. A more likely explanation is that there are significant differences the activities of invertase and fructosyltransferases in the stems of the two cultivars. Any differences between the two cultivars in the rates of respiration of glucose and fructose could also contribute to the observed differences in hexose sugar levels. In both cultivars, the glucose and fructose contents of the stems of non-defoliated plants at maturity was very low (Figs 3b and 3c), suggesting that these sugars had been metabolized and exported to the developing grain, presumably as sucrose. In the partially defoliated plants, the hexose sugars were generally depleted to a greater extent than in the nondefoliated plants at maturity, consistent with sink demand from the developing grains being more dependent on the stem reserves to supply carbon due to the restricted supply from the remaining flag leaf. In general, the patterns of hexose accumulation and remobilization in the two cultivars closely resembled those of the stem total WSC reserves (Fig. 3a). In contrast, sucrose levels not only showed the opposite trend to the other WSC, being higher at 14 DAA in cv. Maron than cv. Hamon, but also responded differently to the partial defoliation treatment. In non-defoliated plants, almost all of the sucrose had disappeared from the stems at maturity, but in the partially defoliated plants a substantial amount of sucrose remained in the lower stem internodes of both cultivars, and in the penultimate internode, peduncle and flag leaf sheath of cv. Hamon (Fig. 3d). This might suggest that the defoliation treatment in some way impaired the capacity of the plants to remobilize all of their stem reserves or affected the sink strength of the developing grains and thus their ability to import sucrose. However, the decrease in total WSC content between 14 DAA and maturity in the partially defoliated plants was not significantly different to that in the nondefoliated control plants, except in the flag leaf sheath, where the fall was greater in the former than in the controls (Fig. 3a). As sucrose is the major transport sugar in wheat, these observations argue strongly against defoliation having an adverse effect on the capacity to export sucrose from the stems or import it into the grain. It is conceivable that the sucrose reserves in these plants are distributed in different cell types or intracellular compartments within the stem, and that a proportion of the sucrose is not readily available for export to the grain.



Fig 3. Total water soluble carbohydrates (a), glucose (b), fructose (c) and sucrose (d) accumulation and remobilization in stems and flag leaf sheathes of wheat cultivars Hamon and Maron grown in a controlled high-light environment (\Box , non-defoliated plants harvested at 14 DAA; \blacksquare , non-defoliated plants harvested at maturity; \blacksquare , plants partially defoliated at 14 DAA and harvested at maturity). Each bar is average of three replications. Bars related to corresponding parts of the two cultivars labeled with the same letter are not significantly different (P < 0.05) by t-test; error bars represent standard deviation.

Starch

At 14 DAA, the stems and flag leaf sheaths of both cultivars contained a small amount of starch, but this represented only 1-2% of the total non-structural carbohydrate reserves (Supplementary fig. 3). There were no significant differences in starch content between the two cultivars, except for a slightly higher level in the penultimate stem internode of cv. Hamon. In general, the starch content of the stems was lower at maturity than at 14 DAA in the non-defoliated plants, and decreased to a similar extent in the partially defoliated plants. Scofield et al. (2009) reported that the region of the peduncle enclosed by the flag-leaf sheath, but not the exposed part, contains starch. They also reported that significant but small amounts of starch are accumulated and then apparently remobilized from wheat stem storage parenchyma cells around the time of anthesis, before the major accumulation of WSC. This suggests that stem starch reserves may be utilized in the early development of the reproductive structures, or in peduncle growth processes, or as temporary carbon storage prior to the accumulation of fructan.

Materials and methods

Field experiments

Plant materials and growing conditions

Iranian bread wheat (Triticum aestivum L.; 75 cultivars) and durum wheat (Triticum durum L.; 4 cultivars) released within the last 70 years, plus two foreign bread wheat cultivars (bread wheat) were chosen for the field trials (Supplementary Table 1). Field experiments were carried out at the agriculture research farm of the University of Tehran, Karaj (35°49'N, 51°0'E and 1312 m altitude) during the 2007-2008 and 2008-2009 growing seasons. The experimental design of each year was lattice square with three replications. The region has a continental semi-arid climate with annual precipitation of 345 mm. Average photosynthetic photon flux density at canopy level during the growing seasons was 820 μ mol m⁻²s⁻¹. Seeds were sown on 1 Nov 2007 and 15 Nov 2008 at a density of 300 seeds m⁻². Soil type was a clay loam with pH of 7.8 and electrical conductivity of 0.44 dSm⁻¹. Plots were 4 m long with 4 rows spaced 0.2 m apart in a north-south direction. Fertilizers were applied at 200 kg ha⁻¹ diammonium phosphate [(NH₄)₂HPO₄) and 100 kg ha⁻¹ urea (CO (NH₂)₂] before planting, and 50 kg ha⁻¹ urea top-dressed at the jointing stage (Zadoks 31) (Zadoks et al., 1974). Diseases, insects and weeds were controlled by spraying recommended pesticides as required.

Defoliation treatments

At the flowering stage, 50 similar main stems with uniform height and spike size were tagged from interior rows within each plot. Full defoliation (removal of all leaves) or partial defoliation (removal of all leaves) or partial defoliation (removal of all leaves except the flag leaf) treatments were applied to 10 tagged stems per treatment at anthesis, and to another two sets of 10 tagged stems at 14 days after anthesis (14 DAA). The remaining 10 tagged stems were left intact (not defoliated) as controls (Supplementary fig. 1). At maturity, all the stems were separately harvested, the spikes were threshed and the grains were weighted.

Experiments at controlled environment

Plant materials and growing conditions

Bread wheat cultivars Hamon and Maron were grown in 20cm plastic pots containing a mixture of soil, perlite, sand, and peat moss (50:25:15:10 by volume) and osmocote slow release fertilizer in a high-light phytotron at the Max Planck Institute of Molecular Plant Physiology, Golm, Germany. The plants were illuminated with mercury vapor lamps providing a photosynthetic photon flux density of 700 µmol $m^{-2}s^{-1}$ at the leaf surface, with a 16 h photoperiod. The day and night temperatures were 25°C and 22°C, respectively and a constant relative humidity of 65% was maintained.

Defoliation treatments

At flowering, nine similar stems of each cultivar were tagged. Three of these tagged stems were randomly harvested at 14 DAA for carbohydrate analysis. Another three tagged stems were partially defoliated at 14 DAA and harvested at maturity. The other three tagged stems were left intact as a control and harvested at maturity. Each stem was rapidly separated into flag leaf sheath, peduncle, penultimate internode and lower internodes and frozen in liquid nitrogen. The frozen plant material was ground to a fine powder using a cryogenic grinding robot and aliquots of the tissue powder were weighed and stored at -80°C until analysis.

Water soluble carbohydrate determination

Total water soluble carbohydrate were extracted from 20 mg of tissue powder and determined using the anthrone method as described in van Herwaarden et al. (1998) with some modifications. Preliminary experiments were carried out with known amounts of hexose, sucrose or fructans to establish the optimal conditions for color development with the anthrone reagent. Glucose, fructose and sucrose were individually determined enzymatically, using an NADPH-linked spectrophotometric assay (Stitt et al., 1989), carried out in 96-well microplates. The assay (200 µl final volume) contained 50 µL of the aqueous-ethanolic extract and 150 µL of a reaction mixture containing: 70 mM Hepes-KOH, pH 7.0, 2 mM MgCl2, 2 mM ATP, 1 mM NADP and 0.25 U glucose-6-phosphate dehydrogenise (from yeast). Glucose, fructose and sucrose were determined by the successive addition of 9 U hexokinase, 0.6 U phosphoglucose isomerase and 100 U invertase, respectively. The increase in absorbance at 340 nm was recorded by an ELX-800-UV microplate reader (Bio-Tek) and the concentration of sugars in the extract calculated using an extinction coefficient of 6200 M⁻¹ cm⁻¹.

Starch

The pellet remaining after aqueous ethanolic extraction was resuspended in 400 μ L 0.1 M NaOH, vortex mixed and heated at 90 °C for 30 min to gelatinize the starch. After cooling to room temperature, 80 μ L of HCl/acetate buffer were added to neutralize the sample. Starch was determined as described in Hendriks et al. (2003). Duplicate 40 μ L aliquots of the resulting suspension were added to separate starch degrading reaction mixtures (110 μ l) containing amyloglucosidase and α -amylase in 96-well microplates and incubated at 37 °C for 16 h. The suspension was centrifuged (3400 rpm for 5 min) and 50 μ L of supernatant was taken for determination of glucose as described above.

Statistical analysis

All data were subjected to analysis of variance using SAS 9.1 program (SAS Institute, Cary, NC, USA). In figure 1, means were tested by least significant differences at $P_{0.05}$ level (LSD_{0.05}). In figure 2, linear regression was used to evaluate the relationships of cultivar release date and grain weight reduction percentage due to defoliation treatments. In figure 3, the means of carbohydrate in corresponding parts of the two cultivars were tested by Student's t-tests at $P_{0.05}$ level.

Conclusions

There is significant variation among wheat cultivars in the extent to which final grain weight is decreased by full or partial defoliation. Such variation could reflect the agronomic performance of different cultivars in wheat growing regions with a Mediterranean climate. It is concluded that differences in the amounts of stem carbohydrate reserves accumulated by different wheat cultivars are likely to be a major factor underlying the variation in the effect of defoliation on final grain weight among cultivars. Selection of genotypes with high stem WSC content could therefore be a useful strategy for breeding varieties that can maintain high grain yield even under terminal drought conditions, and so are better adapted for cultivation in regions with a Mediterranean climate.

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