Effect of Drought at the Post-anthesis Stage on Remobilization of Carbon Reserves and Some Physiological Changes in the Flag Leaf of Two Wheat Cultivars Differing in Drought Resistance

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Abstract

Remobilization and transfer of the stored food in vegetative tissues to the grains in monocarpic plants require the initiation of whole plant senescence. However, mechanisms by which plant senescence promotes remobilization of assimilates are rather obscure. This study examined the relationship between the senescence induced by water deficits and C remobilization during grain filling. Two wheat cultivars (*Triticum aestivum* L.), Marvdasht and Zagros (sensitive and tolerant to terminal season drought, respectively) grown at a day: night temperature of 22:15°C from anthesis were held as well watered controls (Field Capacity), or subject to water deficit (50% FC) imposed either from anthesis to 14 days later (WS1) or from 14 days after anthesis to maturity (WS2). Relative water content, photosynthetic activity, chlorophyll content, soluble proteins of flag leaves, level of hexose sugars, sucrose and fructans in the peduncle (enclosed by the flag leaf sheath) and the penultimate internode and grain yield assessed. Results showed that water deficits enhanced the senescence by accelerating loss of leaf chlorophyll and soluble proteins and the loss was more under WS2 than WS1. The net CO_2 assimilation rate (P_N) in flag leaves during water deficit display a strict correlation with the drought sensitivity of the genotypes and showed an early reduction in Marvdasht. Water stress, both at WS1 and WS2, facilitated the reduction in concentration of total soluble sugars and fructans in the internodes but increased the sucrose level there, promoted the re-allocation of pre-stored C from the peduncle and penultimate to grains. There was an increase in internodes fructose and a fall in fructan level that preceded the loss of dry matter associated with water stress. WS2 resulted in more deleterious effect on grain yield than WS1 in both cultivars and led to a smaller kernels and lesser aerial biomass at maturity. The loss was more in Marvdasht than Zagros. Our results suggest that the senescence and remobilization promoted by water deficits during grain filling are coupled processes in wheat, and mass of soluble sugars in the stems is premier than sugar remobilization efficiency. Varietal differences in the extent of such trait existed. It would be advantageous to select genotypes with greater capacity to do this under water deficit conditions.

Keywords: Chlorophyll, Flag leaves, Grain filling, Grain yield, Hexose sugar, Internodes, Photosynthesis, Soluble proteins, Wheat (*Triticum aestivum* L.)

1. Introduction

The sensitivity of crop plants such as wheat (*Triticum aestivum* L.) to soil drought is particularly acute during the grain-filling period because the reproductive phase is extremely sensitive to plant water status. Extensive studies have demonstrated that post-anthesis water deficits result in early senescence and more mobilization of pre-anthesis stored assimilates to grains in cereals (Kobata *et al*., 1992; Palta *et al*., 1994; Yang *et al*. 2001, 2003). Growth of kernels is reduced depending upon the degree of water stress and on the rate of stress development, thereby limiting final grain yield (Kobata *et al*., 1992; Nicholas and Turner, 1992). The reduction was found to be more severe when the stress occurred suddenly rather than gradually (Stone and Nicholas., 1995b), and at early stages of grain filling rather than at later stages (Stone and Nicholas., 1995a).

The grain filling of wheat (*Triticum aestivum* L.) depends on carbon from two sources: current assimilation and remobilization of reserves stored in the stem either pre- or post-anthesis (Pheloung and Siddique 1991; Wardlaw

and Willenbrink 1994). The primary signs of leaf senescence are the breakdown of chlorophyll (Chl) and the decline of photosynthetic activity (Yang *et al*., 2001; Gregersen and Holm, 2007). It is generally accepted that genotypes that are able to sustain photosynthesis in the flag leaf for a longer time tend to yield more.

Under drought, there is a rapid decline in photosynthesis after anthesis, due to decrease in leaf stomatal conductance and net CO2 assimilation, limiting the contribution of current assimilates to the grain. Most of the drought-mediated reduction in CO_2 assimilation was attributed to stomatal closure, a part of it was attributed to the direct effect of water stress on the inhibition of CO_2 fixation (Sharkey and Seemann 1989). The relative magnitude of stomatal and non-stomatal factors in limiting photosynthesis depends on severity of the stress (*e.g*. Kicheva *et al*., 1994).

The contribution of stored carbohydrates may, thus, become the predominant source of transported materials (Bidinger *et al*., 1977; Blum *et al*., 1994). The main storage forms of non-structural carbohydrates (NSCs) in the stem of wheat are fructans and sucrose (Wardlaw and Willenbrink, 1994; Yukawa *et al*., 1995). This storage peaks well into the period of grain filling under adequate moisture conditions and declines during the later stages of kernel development as a result of supporting a high proportion of the concurrent kernel development (Wardlaw and Willenbrink, 1994). In fact, a high correlation was found between storage of non-structural carbohydrates of wheat stems and yield among several wheat cultivars under drought conditions (Gavuzzi *et al*., 1997). Fructan accumulated in the internodes during extension, although most of the fructan in an internode accumulated after it was fully extended. When WSC were mobilized from the stem, the mass of glucose, sucrose and fructan decreased but the mass of fructose first increased then decreased, indicating that fructan was hydrolysed at a faster rate than its product (Bonnett & Incoll, 1992b). In wheat, the peduncle and the penultimate internode contained the most storage (Wardlaw & Willenbrink, 1994), with variations in storage and remobilization under different experimental conditions being larger in the penultimate than in the fourth stem internode (Bonnett & Incoll, 1992a)

Ugalde and Jenner (1990) and Willenbrink *et al*. (1998) demonstrated a decrease in fructan content in wheat peduncle during grain filling, which was more pronounced under source-limiting conditions, but increased under sink-limiting conditions. Under conditions of water deficit, a decrease in stem fructans and an increase in fructose was found, associated with a rise in fructan exohydrolase and acid invertase (Wardlaw and Willenbrink, 2000).

The objective of this study was to determine if early senescence induced by water deficit during the grain filling could enhance carbon remobilization and if such enhancement could improve grain filling in, two wheat cultivars different in drought resistance. An additional objective of the present study was to compare some physiologic traits that are related to water stress, to finding direct correlations between these parameters and grain yield to facilitate the screening and selection of cultivars for drought tolerance.

2. Material and Method

2.1 Plant materials

The experiment was carried out on the Agricultural Biotechnology Research Institute of Iran (48°20 N; 31°41E; 20 m above sea level), in the growing season of 2009-2010. Two contrasting cultivars of *Triticum aestivum* L. Marvdasht (drought sensitive cultivar with high yielding potential under favorable condition) and Zagros (drought tolerant local cultivar) were used. Seeds were sown in porcelain pots (25 cm in height and 35 cm in diameter) filled with 4.1 kg of clay-sand-manure 1:1:1(v/v), cultivation was performed in a greenhouse with 16 h supplemental light (300 $\mu molm^{-2}s^{-1}$of photosynthetically active radiation 22 °C) and 8 h darkness (15°C), and at 55-60 air humidity. Five uniform plants in each pot were retained after seedling establishment and adequately irrigated with tap water. At three true leaves pots were placed in a field for forty days for vernalization. The experiment was 2 x 3 (two cultivars and three water regimes) factorial design with six treatment. Each of the treatment had three replication with three subsamples in a complete randomized block design.The imposition of water stress commenced at heading (stage 59, Zadoks scale, Zadoks *et al*.,1974) to 14 days later (WS1) and from 14 days after anthesis to physiological maturity (WS2, stage 92, Zadoks scale), to reach 50% FC at both treatments, water was withheld from the plants and the pots weighed daily until the desired stress level was reached (50% FC). Sufficient water was then added to maintain this value on a daily basis. In the control treatment (WW), the soil water status was maintained at FC (soil water potential, ψsoil, at -0.01 to -0.02 MPa) by weighing the pots daily and adding sufficient water to bring the soil moisture to its original value.

2.2 Sampling

Plants were harvested at 7, 14, 21, 28 and 35 days after-anthesis (DAA), both the length of peduncle enclosed by the flag leaf sheath and the penultimate internode were subdivided for soluble carbohydrate analyses. Samples

selected for soluble carbohydrate analysis were initially frozen in dry ice and stored at -20°C. These samples were later freeze dried to determine dry weight before the sugar extraction. At maturity, ears were harvested to determine the kernel weight, the number of kernels per spike, and the thousand-kernel weight. Each measurement was done on plants from three different pots.

2.3 Protein content determination

Leaf samples were ground in liquid nitrogen and the powder was dissolved in 1 ml of 50 mM HEPES-NaOH buffer pH 7.6 containing 3 mM DTT. After centrifugation for 10 min at 13000 g, the protein concentration was measured using the method Sedmak and Grossberg (1977), using BSA as standard protein. This allowed all enzymatic activities to be expressed relative to the soluble protein concentration.

2.4 Water soluble carbohydrate (WSC) analyses

Individual stem parts were chopped into short lengths and extracted directly into 10 ml of boiling water for 1 h . The supernatant from this extraction was collected and the residue washed with 5 ml of boiling water and finally rinsed in cold water (Bonnett and Incoll, 1993). All extracts and washings were combined and made up to 5 ml for analysis by high performance liquid chromatography (HPLC). There was no indication that this extraction procedure resulted in the hydrolysis of either sucrose or fructans. For HPLC analyses water extracts were first deionized by shaking with Amberlite MB-1 (mixed bed) resin. The resin was modified by drying at 40°C before use, as this avoided changes in sugar concentration and differential absorption of individual sugars. Using an HPLC system (Waters, Milford, MA, USA), soluble carbohydrates in the neutral fraction were separated on a 300×7.8 mm (Aminex) HPX 42C column (Bio Rad) at 80°C, with water as

eluent and a flow rate of 0.5 ml min^{-1}. Peaks were identified with a Waters differential refractometer (model R401). Standards used for identification by co-chromatography and quantification (Wardlaw and Willenbrink, 1994) were glucose, fructose, sucrose, raffinose stachyose and neo-sugar P (degree of polymerization) (DP≥3) fructans, (Wardlaw and Willenbrink, 2000).

2.5 Physiological Measurements

Relative water content (RWC) was measured in the blades of the flag leaf. Leaf blade segments were weighed (w_i), floated on distilled water at 4^0C overnight, weighed again (w_f), and dried at 80^0C for 48 h, after which, dry mass was determined (w_d). Relative water content was calculated as: $RWC = (w_i - w_d)\ (w_f - w_d)^{-1} \times 100$, (Boyer 1969).

The net photosynthetic rate (P_N), stomatal conductance (g_s) were measured with a portable photosynthesis system *LI-6400* (*LI-COR*, Lincoln, USA) on the flag leaves on 7, 14, 21, 28 and 35 DAA. Photosynthetically active radiation (PAR) of 1 800 μmol m^{-2} s^{-1} was provided at each measurement by the *6400-02* Light Source.

The fully expanded flag leaves on the above dates were homogenized in ice cold 100% (v/v %) acetone (1.5 mL for 250-mg sample)and extracted for 24 h. Samples were centrifuged at 5,000g for 15 min at 4^0C. The pellet was extracted again with 80% (v/v %) acetone (1.5 mL for 250-mg sample) for 24 h. After centrifugation (5,000g, 15 min, 4^0C), the supernatants were collected. The pigment composition was measured with a double-beam spectrophotometer using the method of Lichtenthaler and Wellburn (1983). This method involves measurement of the light absorbed in the plant extract at 646.8, and 663.2 nm. Six leaves were used for each treatment.

The results were analyzed for variance using the SAS statistical analysis package (version 6.12; SAS Institute, Cary, NC, USA). Data from each sampling date were analyzed separately. Means were tested by least significant difference at $P_{0.05}$ level (LSD 0.05).

3. Results

3.1 Plant water status and pigment content

Withholding irrigation resulted in reduction in RWC in both drought-sensitive and -tolerant genotypes; the reduction was more pronounced in the drought-sensitive cultivars

Marvdasht. At the end of WS1 (14 DAA), RWC in Marvdasht was lower than Zagros (55 compare to 77%), then increased in both cultivars after rewatering, and decreased again thereafter, but with a sharp slop in Marvdasht (Fig.1A). At the onset of inserting WS2, RWC dropped markedly at 21DAA in both cultivars, however the loss was more in Marvdasht (20 compare to 50%), then declined gradually and reach to zero at the end of experiment in both cultivars (Fig.1A-B). The RWC of flag leaf in both cultivars maintained higher under WW than WS treatments.

In the WW and WS plants, a relevant differences were observed in (Chl) of the leaves throughout the experiment (Fig.2A-B). Loss of chlorophyll (Chl) is an index of progress in leaf senescence. Chl a and b contents decrease

steadily in response to water stress in both withholding treatments (WS1 and WS2), and a significant changes were found in the Chl a and b contents at 21 DAA between treatments (Fig.2A-D). The lower Chl contents were measured in stress-watered flag leaves of the drought-sensitive Marvdasht. The senescence process started earlier in WS2 plants than WS1 in both cultivars (Fig.2 A-D).

3.2 Photosynthetic performance during grain filling

The P_N of both cultivars under well-watered condition was significantly higher than under water stress and the difference became more pronounced during the late stage of grain growth (Fig. 3A-B). The P_N of flag leaf in both cultivars under WW treatment exhibited a more moderate decline with a similar changing pattern in both cultivar, however Marvdasht had lower values in P_N nearly 5 contrast to 10 μmol m^{-2} s^{-1} CO_2 at the end of experiment. WS1 reduced P_N by 28% in Marvdasht and by 20% in Zagros at 14 DAA compared with those of control, while these values under WS2 were 74 and 31% in Marvdasht and Zagros in their respective to control treatment respectively at 21DAA.

Similar to P_N, values of g_s in well-watered treatment were significantly higher than under water stress (Fig.4A-B). Stomatal conductance under both stress regimes was significantly lower than the respective controls at all stages, and the differences kept remain with development. The effect of early stress (WS1) on g_s was evident throughout the experiment, while a rapid reduction in g_s of late stress (WS2) flag leaf corresponded to the time that treatment imposed. Irrespective of treatment, Zagros exhibited higher *g*s than Marvdasht after anthesis. The difference, however, was less pronounced under water stress.

3.3 Protein Contents

Amounts of soluble proteins reduced with time in all treatments (Fig.5A-B), although considerable differences were detected between treatments, as substantial reduction occurred in both cultivars under water stress compare to control treatment. Irrespective of treatment, Zagros revealed higher soluble proteins content than Marvdasht throughout all stages sampling. Reduction in soluble proteins under WS2 was more remarkable than WS1 from day 14 onwards in Marvdasht, since this difference was not evident until 28 DAA in Zagros (Fig. 5B)

3.4 Water soluble carbohydrates in the stem

Figs 6 and 7 show the changes with time of WSC content of the peduncle and penultimate internode under all treatments. The peak values for total WSC in the peduncle and penultimate internodes, irrespective treatments gained at 28 DAA in Zagros, while this event observed earlier in Marvdasht. The water stress, at either WS1 or WS2 reduced WSC, but the reduction was much more by WS1. The changes in WSC content under WS2 were greater in the penultimate internode than in the peduncle in both cultivars, but the patterns were similar.

The difference between the internodes is to be expected as the peduncle does not accumulate nonstructural (storage) carbohydrate until after anthesis when development (elongation) is complete. Up to 21 DAA in each treatment there was little change in total WSC of penultimate in both cultivars, however in this period there was a significant increase in fructans, an almost matching small drop in fructose, glucose and sucrose of both cultivars (Fig.6 C-E). From 21 to 28 DAA, WSC increased slightly and then substantially decreased under all treatment at the end of experiment in Zagros, the same pattern of reduction for WSC concentration was observed in Marvdasht, however a reduced WSC occurred 1 week sooner than Zagros. Results from final sample stage showed that the remobilization efficiency enhanced by water deficit. Total carbon remobilized reserve from peduncle and penultimate under WS1 by 70.4 and 81.4 and under WS2 by 105.9 and 150.4 for Marvdasht, and 112.1, 128.9 under WS1 and 129.9, 148.4 under WS2 for Zagros, respectively (Table 1).

A very similar changing pattern was observed for fructan concentration in the penultimate and peduncle in both cultivars from 21 to 35 DAA (Fig.6-9 B), suggesting that changes in WSC result mainly from changes in fructans. Glocose, fructose and sucrose concentration greatly declined under water stress compare to well-watered treatment in both cultivars.

The similar pattern almost for WSC changing was observed in peduncle of both cultivars but the fluctuation of other soluble sugars was different by the time, as fructans after slightly decrease, increased markedly in all treatments after 21DAA except under WS1 in Marvdasht, the glucose decreased moderately with a same changing pattern for all treatment in Zagros, but the effect of both stress was not evident until 21 DAA. In Marvdasht the initial concentration of glucose was much more contrast to Zagros under WW treatment (21 compare to 12 μg g^{-1}dw), however sharply declined closely to same value of Zagros cultivar at the end of this period (Fig. 8C).

From day 21 onwards a similar changing pattern for all soluble sugars like penultimate was observed in peduncle of both cultivars (Fig.7 & 9 B-E).

From 28 to 35 DAA there was a fall in WSCs of both internodes, of which a considerable part could be accounted for by the mobilization. The amount of WSC remobilization under water stress enhanced in both cultivar compare to WW treatment. Table 1 shows the disappearance of pre C stored in both internodes that was much more in Zagros than Marvdasht and under WS2 than WS1 (Table 1).

3.5 Kernel Weight and Grain Yield

Kernel weight was reduced by water deficits when compared with respective well-watered treatment in both cultivars (Table 2). However, reduction in kernel weight under WS2 was more remarkable than WS1 treatment. In comparison, the reduction in Marvdasht was higher than Zagros under both stress regimes.

The values reduction were 50 and 2.4% under WS1 in Marvdasht and Zagros cv, compare to 62 and 24% under WS2 in the same cultivars, respectively (Table 2). A similar result was obtained for grain yield (Table 2), possibly because only the kernel weight, rather than the spike number or kernel number per spike, was influenced by water deficits during grain filling.

Similar changing also was found for biomass and HI, the result showed that grain production of both cultivars affected by its biomass accumulation during its vegetative growth period. A positive relationship existed between HI and amount remobilization during grain filling. WS1 treatment increased mobilization of assimilate stored in vegetative tissue to grains, resulting in greater yield and HI than WS2 (Table 1-2).

4. Discussion

RWC in the leaves of the tolerant (Zagros) and sensitive (Marvdasht) genotypes decreased significantly in response to water deficit, but Marvdasht reached lower RWC values much earlier after water withdrawal, indicating that this cultivar respond to soil drought with a faster decrease in RWC than do tolerant genotype, however WS2 cause to more reduction than WS1 in both cultivars (Fig. 1A-B). A rapid development of water stress resulted in a significant reduction in the rate of flag leaf photosynthesis soon after water stress commencement causing premature senescence of the flag leaf Rawson *et al*., (1983). The significant changes in P_N during drought stress can be explained by earlier senescence, as drought may promote whole-plant senescence in monocarpic plants (Yang and Zhang, 2006). Earlier senescence during water deficit in sensitive and tolerant varieties was indicated by the earlier decline in pigment content (Fig.2 A-D). Marked differences between varieties observed for soluble proteins under all treatments (Fig. 5-A-B). As for Chl content, soluble proteins of flag leaves declined during grain filling, and water stress accelerated the decline, however the loss was more in Marvdasht and the differences achieved a maximum by day 28 from anthesis for each treatment. Our observation also showed that P_N of the flag leaves declined with age in both cultivars under well-watered treatment, but water stress enhanced such a decline with a more extent under WS2 than WS1, although Marvdasht showed earlier reduction under both stress treatments than Zagros cv (Fig. 3 A-B).Under this condition, *g*s was more affected than P_N (Fig. 4A-B), and Marvdasht showed a lower *g*s than Zagros under water stress. A similar result was reported by El Hafid *et al*., (1998a) that drought susceptible genotypes exhibited lower *g*s than tolerant genotypes upon exposure to the stress.

The decrease in P_N in water-stressed plants could be explained by the stomatal closure, which reduced CO_2 diffusion and thus *c*i. These results consistent with the recent view that an early decrease in photosynthesis under drought is due to increased stomatal resistance (Kicheva *et al*., 1994; El Hafid *et al*., 1998b).

Therefore, the photosynthates produced by the flag leaf during grain filling limit the growth of grain. A benefit from such a water deficit is that it can enhance remobilization of carbon reserves from vegetative tissues during grain-filling (Table 1). The contributions become greater when plants are grown under drought stress than under WW treatment.

The fast remobilization under both water stress regime coincide with the fast plant senescence induced by water deficit (Fig. 2A-D), and slightly more under in cases (WS1 versus WS2, penultimate versus peduncle and Zagros versus Marvdasht). However the WSC concentration substantially decreased by WS1 compare to their respective other treatments. The WW treatment left more WSC unused in both internodes cultivars than WS treatments (Fig.6 -9A). Compare with Zagros cv, Marvdasht cv. Had a lower remobilization under WW treatment, coinciding with its higher P_N during grain-filling (Fig. 3A-B), however Zagros cultivar maintained fairly higher P_N during post-anthesis periods under water stress. Marked differences among varieties in the relative contribution of pre- and post anthesis assimilates to grain yield were also reported by Przulj and Momcilovic (2001).

We observed in this study, however, that the change in total WSC coincided with that in fructan concentration in internodes (Fig. 6-9 A-B), which confirms early reports (Wardlaw and Willenbrink 1994; Yukawa *et al*., 1995) that fructans are the main storage form of NSC in wheat stems. It is notable that, with the decrease in fructan

contents, glucose and fructose concentrations were also reduced in WS plant internodes (Fig. 6-9 B-D). A probable explanation is that the fructose released from fructan hydrolysis, along with glucose, is metabolized to form sucrose, the sugar normally transported in the phloem of wheat (Fisher and Gifford, 1986).

The continuous growth of grains in the absence of current photosynthate would deplete the reserves and grain growth would cease (Westgate and Boyer, 1985). A reduction in grain yield due to a premature demise of sucrose activity (Westgate *et al*., 1989) and a reduction in post anthesis photosynthesis and the amount of remobilizable assimilate (Kobata *et al*., 1992) has been suggested to be the main account under water stress conditions. In accord with earlier findings (Evers, 1970) the duration of the cell production phase in wheat ranges from 12 to 19 DAA on the conditions and cultivar. Therefore it seems that the reduced grain weight under water stress conditions observed in the present study compared to their respective WW treatment, was via the effects of stress on cell division processes. Although, the WS2 treatment substantially decreases more kernel weight than WS1 treatment. This reduction was probably due to reduce endosperm cell number that led to reduced sink strength. This, in turn, could confer a critical survival advantage for few versus many seeds in terminal drought environments by reducing sink numbers at a key point in development and thus secure a sufficient sucrose supply for maturation of a few remaining seeds as observed under WS2 conditions.

In our experiment, obvious differences between treatments with a similar pattern for grain weight in both genotypes were found (Table 2). However the loss was more in Marvdasht than Zagros under water deficit. A similar result was obtained for grain yield under water stress treatments (Table 2). It is noteworthy that the grain number per spike, was more influenced by WS1 than WS2, but yield gain under WS2 condition, was not enough to fully compensate for the lower kernel weight (Table 2).

5. Conclusions

As the sink size was seriously affected by the WS in this experiment, we speculate that increased carbon remobilization from the stems to grains and accelerate grain-filling rate may be mainly attributed to an enhanced sink activity in Zagros under the WS1.The contribution of pre-anthesis assimilates to grain may be crucial for maintaining yield when adverse climatic conditions reduce photosynthesis and water uptake. Thus, the high contribution of remobilization to grain yield observed in Zagros may be responsible of its known yield stability over early water withdrawal, however these reserves deposited was not enough to compensate for the lower current assimilation under both water deficit treatments. Conversely, the low contribution of translocates could impair the var. Marvdasht to maintain high yield when water stress occur around anthesis, as it was suggested by Frederick and Bauer, (1999) for modern high yielding wheat varieties).

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Table 1. Maximum and minimum of total soluble sugar content of peduncle and penultimate (at flowering and maturity respectively), remobilization amount and remobilization efficiency in Zagros and Marvdasht genotypes under different water treatment, well watered (WW), withholding water from anthesis till 14 days later (WS1) and withholding water from 14 days after anthesis till maturity (WS2).

	Treatments	Maximum concentration of soluble sugars		Minimum concentration of soluble sugars		Remobilization amount		Remobilization efficiency	
Genotype		Peduncle	Penultimate	Peduncle	Penultimate	Peduncle	Penultimate	Peduncle	Penultimate
		mg Glucose g^{-1}						Remobilization percent	
Mardasht	WW	227±1.2	145±1.6	56±0.5	64±2.6	171±2.2	80±2.4	75.16	55.46
	WS1	146±3.2	183±2.4	40±2.4	32±1.3	105±4.1	150±3.3	72.33	82.11
	WS2	88±2.2	97±3.1	18±1.1	15±0.9	70±3.5	81±1.9	79.47	84.18
Zagrose	WW	169±2.5	187±3.3	59±0.8	68±2.6	110±4.5	118±4.1	65.1	63.28
	WS1	150±2.7	165±3.9	20±3.7	17±1.4	129±3.2	148±2.8	86.27	89.49
	WS2	129±2.1	141±2.6	17±2.8	12±0.7	112±2.5	128±2.3	86.26	91.2

± denotes standard error

Table 2. Effect of different water treatment, well watered (WW), withholding water from anthesis till 14 days later (WS1) and withholding water from 14 days after anthesis till maturity (WS2) on the final number of kernel per spike, kernel weight per spike, the thousand-kernel weight, aerial biomass of plant and harvest index in two wheat cultivars

Cultivars	Water-deficit treatment	No. of grains per ear	grain yield per ear (g)	1000 grain dry mass (g)	Aerial biomass (g plant^{-1})	Harvest Index (HI)
Marvdasht	WW	50.23 a	2.1 a	42.02 a	3.71 a	56 a
	WS1	41.97 c	0.902 d	21.1 c	2.17 d	41 d
	WS2	47.6 b	0.7 e	16.14 d	2.03 e	34 e
Zagros	WW	32.83 d	1.62 b	41.1 a	3.02 b	53 a
	WS1	28.43 e	1.22 c	40.3 a	2.42 c	50 b
	WS2	30.27 e	0.99 d	31.96 b	2.21 d	45 c
LSD (0.05)		2.526	0.115	0.102	0.005	2.546

Letters indicate statistical significance at $p_{0.05}$ within the same cultivar.

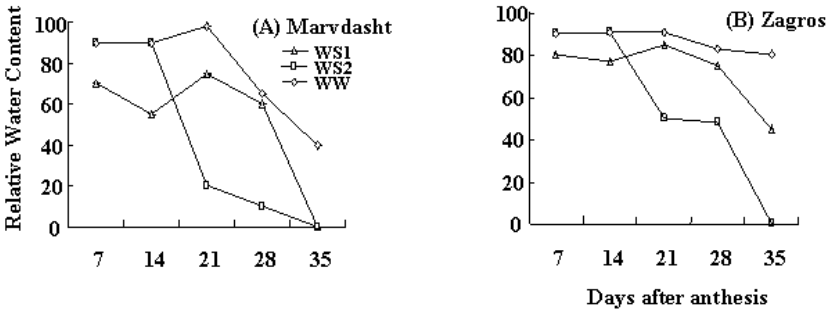

Figure 1. Changes in relative water content (RWC) under different water treatment, well watered (WW), withholding water from anthesis till 14 days later (WS1) and withholding water from 14 days after anthesis to maturity (WS2) flag leaves during grain filling in Marvdasht (A) and Zagros (B) wheat cultivars (*Triticum aestivum*). Data are means ± SE of three independent samples. SE bars are not shown where they are smaller than symbles

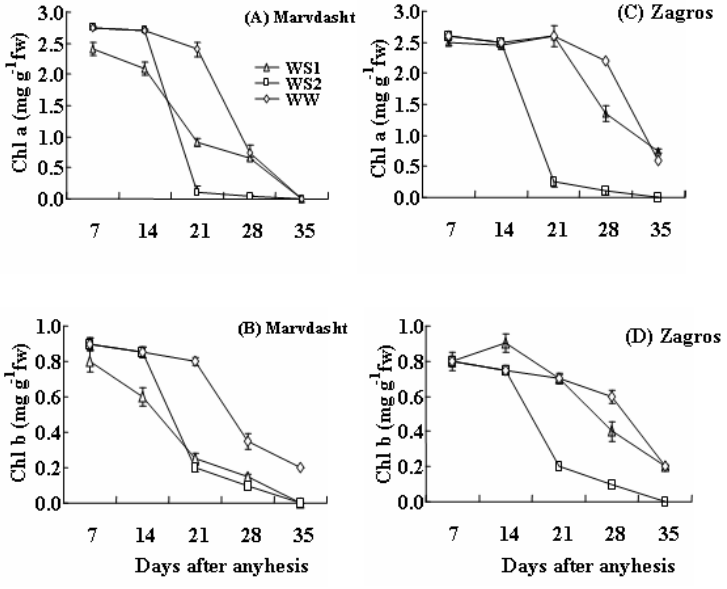

Figure 2. Changes in chlorophyll a & b content in well watered (WW), withholding water from anthesis till 14 days later (WS1) and withholding water from 14 days after anthesis to maturity (WS2) flag leaves during grain filling in Marvdasht (A) & (B) and Zagros (C) & (D) wheat cultivars (*Triticum aestivum*). Data are means ± SE of three independent samples. SE bars are not shown where they are smaller than symbles

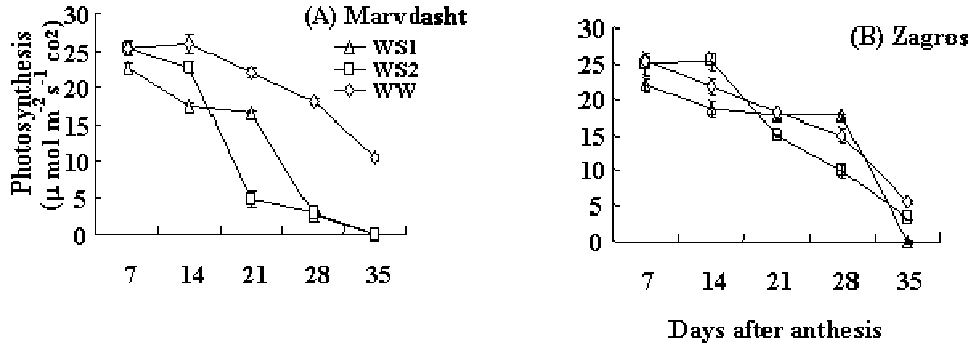

Figure 3. Changes in net photosynthetic rate in well watered (WW), withholding water from anthesis till 14 days later (WS1) and withholding water from 14 days after anthesis to maturity (WS2) flag leaves during grain filling in Marvdasht (A) and Zagros (B) wheat cultivars (*Triticum aestivum*). Data are means ± SE of three independent samples. SE bars are not shown where they are smaller than symbles

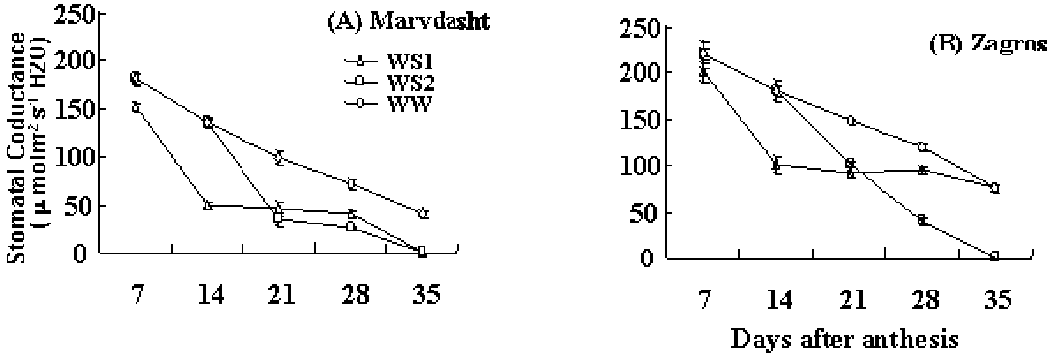

Figure 4. Changes in stomatal conductance (g_S) in well watered (WW), withholding water from anthesis till 14 days later (WS1) and withholding water from 14 days after anthesis to maturity (WS2) flag leaves during grain filling in Marvdasht (A) and Zagros (B) wheat cultivars (*Triticum aestivum*). Data are means ± SE of three independent samples. SE bars are not shown where they are smaller than symbles

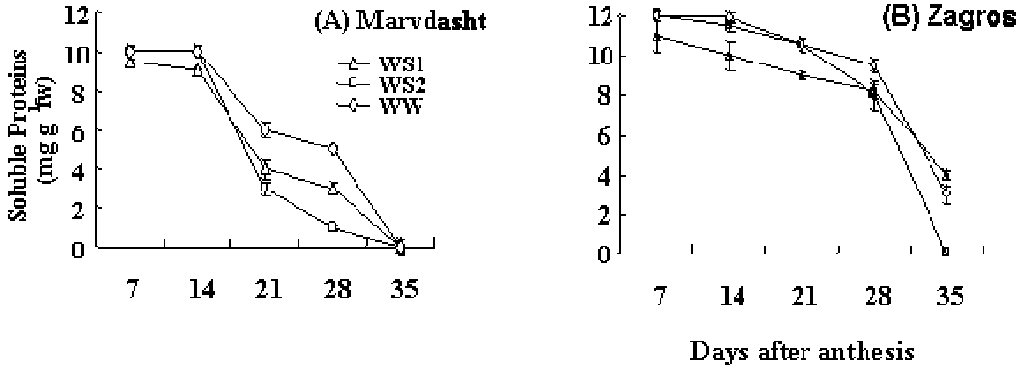

Figure 5. Changes in total soluble proteins in well watered (WW), withholding water from anthesis till 14 days later (WS1) and withholding water from 14 days after anthesis to maturity (WS2) flag leaves during grain filling in Marvdasht (A) and Zagros (B) wheat cultivars (*Triticum aestivum*). Data are means ± SE of three independent samples. SE bars are not shown where they are smaller than symbles.

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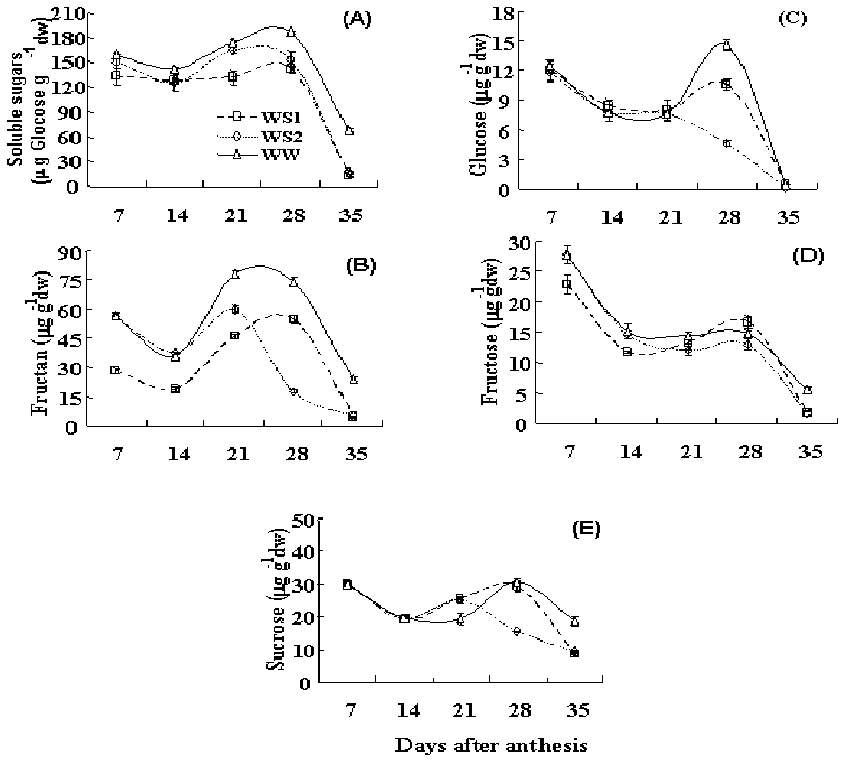

Figure 6. Changes in concentrations of total soluble sugars (a), fructans (b), glucose (c), fructose (d), and sucrose (e) content of the penultimate in Zagros wheat cultivar under different water treatment, well watered (WW), withholding water from anthesis to 14 days later (WS1) and withholding water from 14 days after anthesis to maturity (WS2). Vertical bars represent ± SE of the mean (n=3) where these exceed the size of the symbol

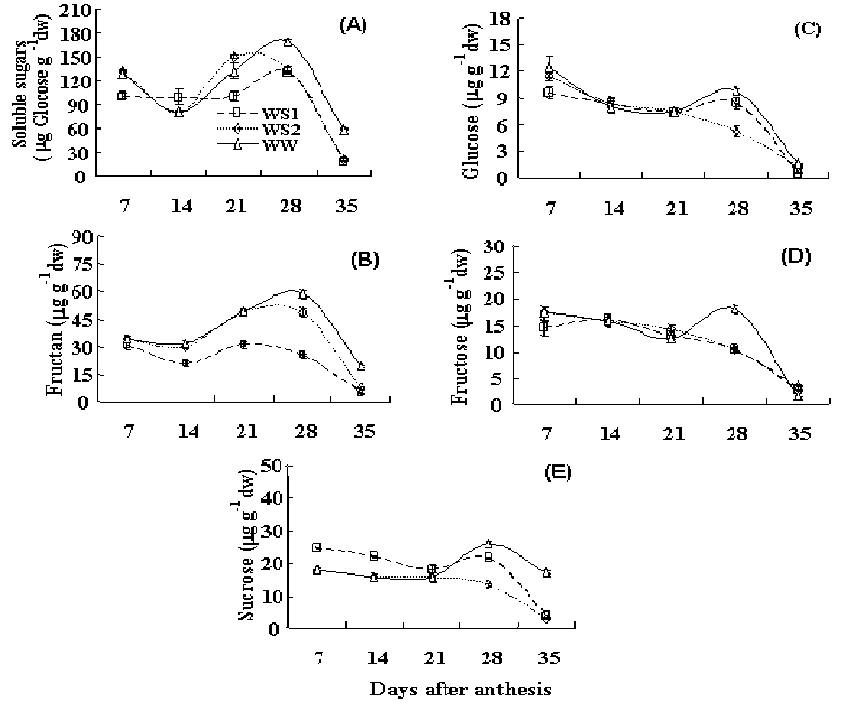

Figure 7. Changes in concentrations of total soluble sugars (a), fructans (b), glucose (c), fructose (d), and sucrose (e) content of the peduncle in Zagros wheat cultivar under different water treatment, well watered (WW), withholding water from anthesis to 14 days later (WS1) and withholding water from 14 days after anthesis to maturity (WS2). Vertical bars represent ± SE of the mean (n=3) where these exceed the size of the symbol

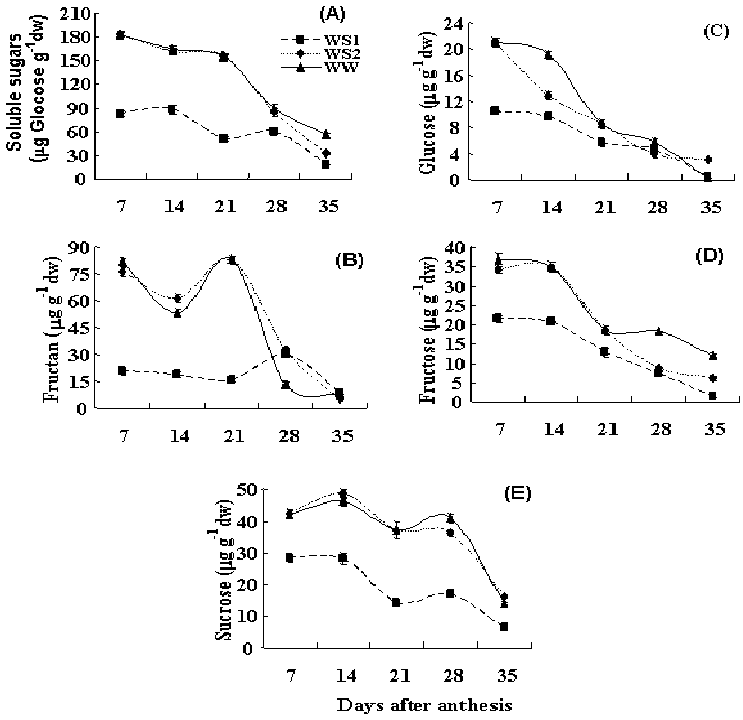

Figure 8. Changes in concentrations of total soluble sugars (a), fructans (b), glucose (c), fructose (d), and sucrose (e) content of the penultimate in Marvdasht wheat cultivar under different water treatment, well watered (WW), withholding water from anthesis to 14 days later (WS1) and withholding water from 14 days after anthesis to maturity (WS2). Vertical bars represent ± SE of the mean (n=3) where these exceed the size of the symbol

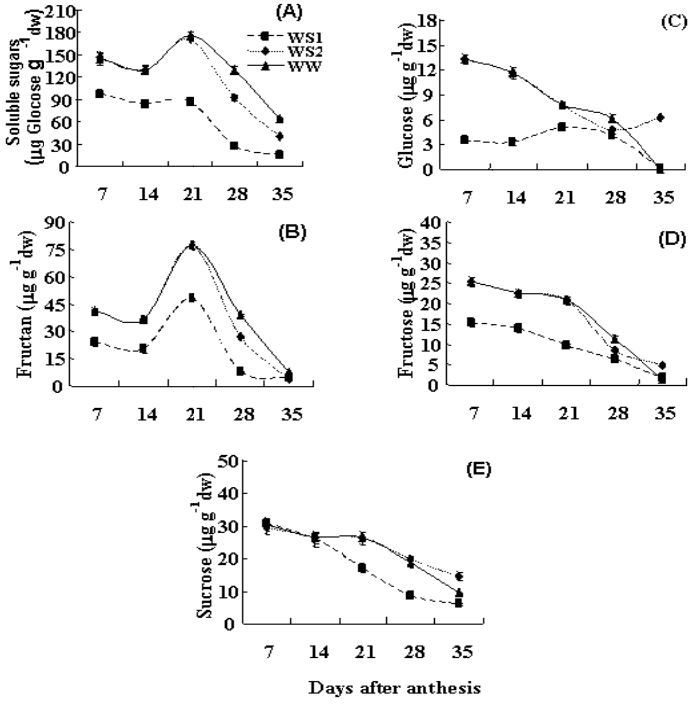

Figure 9. Changes in concentrations of total soluble sugars (a), fructans (b), glucose (c), fructose (d), and sucrose (e) content of the peduncle in Marvdasht wheat cultivar under different water treatment, well watered (WW), withholding water from anthesis to 14 days later (WS1) and withholding water from 14 days after anthesis to maturity (WS2). Vertical bars represent ± SE of the mean (n=3) where these exceed the size of the symbol