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The effect of post anthesis source limitation treatments on wheat cultivars under water deficit

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Abstract

This paper attempts to determine the effect of post-anthesis water deficiency at the early and late grain growth periods separately and also the roles of main ear (spike) and total leaves photosynthesis and stem remobilization in yield production of wheat. Two modern bread wheat cultivars that differed in water deficiency resistance and yield potential were grown in greenhouse. The changes in grain yield components and different soluble sugars (glucose, fructose, sucrose and fructan) caused greater reduction in grain yield (72.6%), biomass and grain weight and number in tolerant cultivar. The photosynthesis of spike and leaves and carbohydrate remobilization from stem made significant contributions to the growing grains about 43%, 25% and 32%, respectively. In both cultivars, penultimate internodes had more soluble sugar concentration than peduncle. Compared to the sensitive cultivar, peduncle and penultimate internode of tolerant cultivar had more glucose, fructose, sucrose, fructan and starch concentrations at grain growth stages. Moreover, the water deficiency and photosynthesis inhibition treatments caused more remobilization of soluble sugars in peduncle and penultimate internodes of resistant cultivar. We suggest that the role of spike photosynthesis is more important than carbohydrate remobilization and leaves photosynthesis in grain yield formation. In addition, grain yield of these cultivars under the conditions tested was more controlled by source than sink limitation.

Keywords: Wheat, spike, remobilization, sucrose, fructan.
Abbreviations: CD_cell division, GF_grain filling, GG_grain growth, KI_iodide potassium, WSC_water soluble sugar concentration.

Introduction

In arid and semi-arid areas, prevailing of water deficit during grain growth of wheat due to shortage of irrigation and high evaporative demand is a common abiotic stress that reduces grain yield (Ehdaie and Waines, 1989). The yield reduction via water deficit is mediated through reduced leaf growth (Gan and Amasino, 1997; Ephrath and Hesketh, 1991), chlorophyll concentration (Brevedan and Egli, 2003), soluble protein (Rudriguez et al., 2002), stomatal conductance (Liang et al., 2002) and consequential lower photosynthesis productivity (Chen et al., 1993; Brevedan and Egli, 2003). Grain growth of wheat has two stages include cell division and grain filling. The endosperm cells are developed during an approximately 14 day post-anthesis at cell division phase (Brocklehurst, 1977). When cell division ceased, a major transition point occurs at about 14 days of post-anthesis (at the end of endosperm cell division stage and the start of grain filling) in wheat grown in temperate climates with the deposition of starch and gluten proteins in these cells (Evers and Millar, 2002). The carbohydrates that are needed for grain growth are provided from two sources (1) during GF via leaves and spike (Abbad et al., 2004; Tambussi et al., 2007; Maydup et al., 2010) and (2) excess carbohydrates that are produced after and before anthesis, stored in the stem and remobilized to the grains during GF stage (Alvaro et al., 2008a; Ehdaie et al., 2008). Several studies have been reported that the contribution of stem remobilization in grain yield formation depends on genotypes and water regimes and ranges from 10% to 50% (Plaut et al., 2004; Ehdaie et al., 2006a, b and 2008). The contribution of leaves and spike photosynthesis and carbohydrate remobilization from stem affect the final grain weight. Traditionally, the flag leaf has been considered as the main photosynthetic organ in grain yield formation (Evans et al., 1972) but Ahmadi et al. (2009) and Aggarwal et al. (1990) reported that defoliation at anthesis had only small effects on grain yield of wheat, and they stated that the yield of cultivars used under those conditions was more controlled by sink than source strength. Recently, Maydup et al. (2010) have indicated that the defoliation significantly reduced the total grain weight per spike about 25% in two wheat cultivars. There is evidence that when a photosynthesis organ of plant is detached, the compensations in the remaining photosynthesis tissues or remobilization may occur and diminish the photo assimilate reduction (Chanishvili et al., 2005). Thus, the source limitation of grain yield in previous works (Ahmadi et al., 2009; Aggarwal et al., 1990) may be because of the fact that the photosynthetic role of spike was neglected. The contribution role of ear photosynthesis in grain yield formation in wheat and barley has been reported from 10% to 76%, respectively (Bisco et al., 1985; Daffus et al., 1975). A recent study by Maydup et al. (2010) showed that the ear photosynthesis makes a significant contribution to grain yield of wheat from 13% to 33% in control and 22% to 45% under water deficiency conditions. So far, there is no report about the source manipulation when defoliation and inhibition of ear photosynthesis are practiced at the beginning of GF stage of grain growth and simultaneously in irrigated and drought

stress conditions. Moreover, there has been little evidence about application of these treatments in semiarid region such as Iran, where the wheat grain growth takes place under high temperature and high radiation conditions. The objectives of this research were to determine the role of ear and leaf photosynthesis and photo-assimilate remobilization from stem to the growing grain at GF stage. In addition, it was also attempted to evaluate the roles of different soluble sugars (glucose, fructose, sucrose and fructan) and the insoluble sugar (starch) of peduncle and penultimate internode of two bread wheat cultivars in grain yield production under controlled and post anthesis water deficit treatments.

Results

Water deficiency and yield

In the control condition (Table 1), significant differences were observed in grain yield, biomass and grain spike^{-1}. In this situation, the above traits were significantly higher in the sensitive cultivar compared to the tolerant cultivar. Two levels of water deficiency at cell division (CD) and grain filling (GF) stage of grain growth (GG) significantly decreased the grain yield, biomass and grain spike^{-1} in both cultivars. Water deficiency applied at GF stage caused more reduction in all evaluated traits than CD stage. Under two levels of water deficiency more significant reduction of all traits was observed in sensitive cultivar (Table 1). For example, application of water deficiency at CD and GF stages diminished grain yield in the sensitive cultivar by about 57.4 and 61.9 percent and in the tolerant cultivar by about 14.1 and 30.1 percent, respectively (Table 1). In average, water deficiency at CD and GF caused 28.86 and 48.91 percent reduction in grain yield of two cultivars, respectively (Table 4).

Photosynthesis inhibition

Inhibition of spike and total plant photosynthesis with iodide potassium (KI) significantly decreased grain yield, biomass, 1000 grains weight, harvest index, grain spike^{-1} and specific weight of peduncle and penultimate internode of two cultivars (Table 2 and 3). Under inhibition of ear and whole plant photosynthesis treatments, the relative reduction of grain yield, biomass, harvest index, 1000 grain weight, grain spike^{-1} and specific weight of peduncle internode in the tolerant cultivar were higher compared to sensitive cultivar (Table 2). Inhibition of whole plant photosynthesis caused more reduction in grain yield (approximately 68.46 percent) than inhibition of ear photosynthesis (approximately 42.16 percent; Table 2 and 4).

Soluble and insoluble sugars

Concentrations of water soluble and insoluble sugars in penultimate internode were higher than peduncle internode (Table 5). In control treatment, the specific weight of peduncle and penultimate internodes of sensitive cultivar was higher than tolerant. There was no significant difference between peduncle and penultimate internodes length of two cultivars but the peduncles were, on average, longer than the penultimate internodes in this evaluation (Table 1). In control plants of tolerant cultivar (peduncle and penultimate internodes), starch, sucrose, glucose, fructose and fructan concentration were higher than the control plants of sensitive cultivar (Table 6). In this condition, there were no correlations between higher soluble sugars concentration in the stem and higher grain yield, since control plants of the sensitive cultivar with higher grain yield had lower soluble sugars in their stems compared to control plants of tolerant cultivar. The water deficiency at CD and GF stage significantly decreased concentration of water soluble and insoluble sugars in the stem of two cultivars. This reduction in the stem of tolerant cultivar was higher than sensitive cultivar (Table 6). More reduction of soluble sugars concentration was seen in the stem of both cultivars when water deficiency applied at GF stage. Between soluble sugars, fructose and sucrose had the highest and lowest concentrations, respectively, in the stem of two cultivars at maturity stage (Table 5). In comparison to the control treatment, inhibiting of spike and whole plant (leaves and spike) photosynthesis at the beginning of GF stage significantly decreased soluble and insoluble (starch) sugar concentrations in the stem of two cultivars (Table 6). The total inhibition of current photosynthesis at the beginning of GF had the highest effect on sugars concentration in the stems of two cultivars. Starch concentration in stem had less reduction in comparison to soluble sugars. In inhibition of current photosynthesis treatments, fructan and other sugars had lower decreasing rate of concentration in the stem of tolerant cultivar (Table 6). These treatments also significantly decreased the soluble and insoluble sugar concentrations in peduncle and penultimate internodes of two cultivars. The relative decrease of fructan and sucrose concentration in the peduncle, and glucose and fructose in penultimate internodes were higher. In these treatments and the control treatment (except fructose in all treatments and glucose and fructose in the control treatment), concentration of starch, glucose, sucrose and fructan decreased in stem at maturity in comparison to the beginning of the GF stage. In these treatments, the highest relative decrease rate was observed in sucrose and fructan concentration, respectively (Table 6).

Discussion

Higher grain yield and biomass of control plants of sensitive cultivar may be the result of higher potential of this cultivar in employing the environmental factors specially soil water to produce grain yield. Grain number and grain weight are two main components of yield in wheat (Duggan et al., 2002; Dreccer et al., 2009). Based on the results, higher grain number was the main reason for greater grain yield of control plants of sensitive cultivar in comparison to tolerant cultivar (Table 1). Fischer (2007) also reported that yield in modern bread wheat cultivar has been frequently correlated with grain number. The formation of yield potential after anthesis may be limited by sink (Fisher et al., 1977; Kruk et al., 1997; Ahmadi et al., 2009) or source activity (Fischer and Hillerislambers, 1978). Assimilate production after 15-20 days from anthesis exceeded the demand of growing kernels in cereal crops such as wheat and barley and there is evidences still about sink limitation in these crops for more grain yield production (Bingham et al., 2007; Borras et al., 2004; Slafer and Savin, 1994). It was found that post anthesis water deficit at two levels have no significant effect on the grain number but significantly decreased the grain weight of both cultivars. Grain number is established in the period between 20 and 30 days before anthesis and few days after anthesis (Abbate et al., 1995; Fischer 1985). This period coincides with tiller and floret mortality, along with the active growth of the stem (peduncle) and spike. Post anthesis water deficiency through reduction of grain weight

Table1. Estimation of grain yield and some related characteristics under two levels of water deficit treatment (at cell division and grain filling of grain growth) in two wheat cultivars. Mean values followed by the same letter (a-e) are not significantly different according to Duncan (P<0.05).

Water deficiency treatment	Grain Yield (g plant^{-1})		Biomass (g plant^{-1})		Harvest index (%)		1000 Grain weight (g)	
	Tolerant	Sensitive	Tolerant	Sensitive	Tolerant	Sensitive	Tolerant	Sensitive
Control	1.56 b	2.26 a	2.85 b	3.58 a	59.6 a	63.0 a	40.0 a	42.3 a
Cell division	1.34 c	0.99 d	2.65 c	2.38 de	50.6 b	41.7 c	39.0 a	21.1 c
Grain filling	1.09 d	0.86 e	2.43 d	2.23 e	44.6 c	38.8 d	31.9 b	16.1 d
Relative decrease (%)(1)	14.1	57.4	7.02	33.5	15.1	33.8	2.5	50.1
Relative decrease (%) (2)	30.1	61.9	14.7	37.7	25.2	38.4	20.3	61.9

Water deficiency treatment	Grain spike^{-1}		Specific weight of Peduncle(mg/cm)		Specific weight of Penultimate(mg/cm)		Length of peduncle (cm)		Length of penultimate (cm)	
	Tolerant	Sensitive	Tolerant	Sensitive	Tolerant	Sensitive	Tolerant	Sensitive	Tolerant	Sensitive
Control	36.9 d	56.4 a	21.5 a	21.6 a	27.5 b	30.5 a	27.1 a	27.9 a	13.4 a	13.1 a
Cell division (1)	31.9 e	47.1 c	14.8 b	19.9 a	24.4 c	23.9 c				
Grain filling (2)	34.0 e	53.5 b	20.3 a	21.9 a	20.6 d	25.7 c				
Relative decrease (%) (3)	13.6	16.5	31.1	7.70	11.4	21.8				
Relative decrease (%) (4)	7.8	5.14	5.39	0	25.4	15.8				

(1) and (2) from anthesis until 14 days after and from 14 days after anthesis until maturity.
(3) and (4): Percentage decrease down control when water deficiency was applied at cell division and grain filling stage, respectively.

Table 2. Changes in grain yield and some related characteristics under two photosynthesis inhibition treatments with desiccant application on ear and whole plant in two wheat cultivars. Mean values followed by the same letter (a-e) are not significantly different according to Duncan (P<0.05).

Photosynthesis inhibition treatments	Grain Yield (g plant^{-1})		Biomass (g plant^{-1})		Harvest index (%)		1000 Grain weight (g)	
	Tolerant	Sensitive	Tolerant	Sensitive	Tolerant	Sensitive	Tolerant	Sensitive
Non-treated (control)	1.33 a	1.37 a	2.64 a	2.73 a	49.9 a	47.8 a	38.8 a	35.7 a
Spraying only on ears	0.70 c	0.90 b	1.71 d	2.21 b	41.1 b	40.7 b	27.7 b	20.2 c
Spraying on whole plant	0.36 e	0.58 d	1.61 d	2.07 d	22.3 d	27.8 c	11.6 e	15.2 d
Relative decrease (%)(1)	47.4	34.4	35.3	19.1	17.8	14.9	28.4	43.6
Relative decrease (%)(2)	72.6	58.0	39.0	24.3	55.5	42.1	70.2	57.3

Photosynthesis inhibition treatments	Grain spike^{-1}		Specific weight of Peduncle (mg/cm)		Specific weight of Penultimate (mg/cm)	
	Tolerant	Sensitive	Tolerant	Sensitive	Tolerant	Sensitive
Non-treated (control)	34.3 d	52.4 a	18.9 b	21.1 a	23.6 b	27.3 a
Spraying only on ears	25.9 e	43.7 b	9.0 d	9.18 d	11.7 cd	12.9 c
Spraying on whole plant	31.6 d	38.1 c	9.20 d	10.5 d	11.7 cd	10.8 d
Relative decrease (%)(1)	52.7	50.1	56.6	52.4	16.5	24.5
Relative decrease (%)(2)	60.9	50.3	50.5	51.3	27.3	7.82

(1) and (2): Percentage decrease down control when Photosynthesis inhibition treatments were applied only on spikes and whole plant, respectively.

reduced the grain yield production. Decreasing of grain weight under post anthesis water deficit also has been elucidated by many reports as the main factor in determination of yield (Royo et al., 2000; Gooding et al., 2003). Water deficit may act through reduction in photo assimilate production and enzyme activity in growing grains to reduce the grain weight. More reduction of grain yield and grain weight of two cultivars showed that sink activity (GF stage) might be more important than sink size (CD stage) in determination of grain yield, when water deficiency is applied at GF stage. Water deficiency at CD stage normally reduces the grain yield and grain weight through in cell division reduction and reduction of sink size (Michihiro et al., 1994) and at GF stage through the reduction in enzyme activities that involve in starch biosynthesis (Ahmadi and Baker, 2001) and accumulation (Blum, 1998). At this level of water deficiency (40% field capacity) cell division (CD) process may be less sensitive to water deficit than enzyme activities that involves in GF. The sensitive cultivar had more sensitivity to water deficit at two levels. More differences were shown when water deficit applied at CD stage. In this situation grain yield and grain weight reduction of sensitive cultivar were 57.4% and 50.1% and in tolerant cultivar 14.1% and 2.5%, respectively. Therefore, more sensitivity is possible to happen in CD processes of sensitive cultivar such as enzyme activities, hormonal changes or sugar concentration. Our previous research on these two cultivars showed the activities of different isoforms of invertase

involved in CD of sensitive cultivar were more susceptible to water deficiency and caused more reductions in their activities under water deficit treatments (Unpublished data). The reduction of harvest index under application of water deficiency showed that water stress caused more reduction in grain yield than biomass production (Table 1).

Yang et al. (2003, 2004) showed that mild water deficiency increased the harvest index through increasing the carbohydrate remobilization from stems to the growing grains but severe water deficiency reduced the harvest index (Ehdaie and Waines, 1994; Araus et al., 2002). When KI inhibited current photosynthesis of ear and whole plant, more reduction in grain yield of tolerant cultivar (Table 2) showed that the main reason for the tolerance of this cultivar to post anthesis water deficit may be because of more stability in its current photosynthesis rate under water deficiency and also may be due to more carbohydrate remobilization from stem to the growing grain. According to different researches on wheat, the contribution of stem remobilization in grain yield formation depends on genotypes and water regimes and ranges from 10% to 50% (Plaut et al., 2004; Ehdaie et al., 2006a, b and 2008). But in sensitive cultivar when KI treatment applied at GF stage and water deficiency at CD, the role of remobilization in grain yield production was more than 50% because of additive effect of these treatments.

About the roles of current photosynthesis in grain yield production Ahmadi et al. (2009) and Aggarwal et al. (1990) reported that defoliation at anthesis had only small effects on grain yield of wheat and stated that the yield of cultivars used under those conditions was more controlled by sink than source strength. Recently, Maydup et al. (2010) have indicated that defoliation significantly reduced total grain weight per ear (about 25%) in wheat cultivars. Also, we found that inhibition of leaves photosynthesis at GF stage caused 25.26% reduction in grain yield. There is evidence that when a photosynthesis part of a plant is inhibited, the compensations in the remaining photosynthesis tissues such as spike or remobilization may occur and diminish the photo assimilates (Chanishvili et al., 2005). Thus, the lack of source limitation of grain yield in previous works may be the result of the fact that the photosynthetic role of spike was neglected (Ahmadi et al., 2009; Aggarwal et al., 1990). The inhibition of ear photosynthesis reduced grain yield of two cultivars. For wheat and barley, the contribution roles of spike photosynthesis in grain yield formation have been reported from 10% to 76% (Bisco et al., 1975). Maydup et al. (2010) recently have reported that blockage of ear photosynthesis with shading near anthesis stage makes a significant contribution to grain yield of wheat from 13% to 33% in control and 22% to 45% under water deficiency conditions. However, the findings of the current study do not support the previous research by Maydup et al. (2010) and showed that under control treatment, inhibition of ear photosynthesis at early GF caused more reduction in grain yield than water deficiency condition. In sensitive and tolerant cultivars, increasing of carbohydrate remobilization from stem to the growing grains in two levels of water deficiency did not prevent the yield loss. This finding suggests that the main reason for yield stability of tolerant cultivar under post anthesis water deficit may be the stability of its photosynthesis rate under this condition. Thus, longevity of the photosynthetic machinery could be the main reason in higher yield formation of this cultivar under water deficiency. The main storage of WSCs in wheat stems are fructans and sucrose (Kühbauch and Thome, 1989; Wardlaw and Willenbrink, 1994; Yukawa et al., 1995). Lower concentration of sucrose concentration in the stem of two cultivars at maturity showed that these sugars may be quickly remobilized to the grains and the enzymes involved in hydrolyzing of fructan and biosynthesis of sucrose from fructans may be the most determinant of sucrose remobilization to the growing grains around this time.

Penultimate internodes had more sugars concentration than peduncle and were more efficient in remobilization of soluble sugars to the grains in both cultivars. Wardlaw and Wilnbrink (2000) also reported that soluble sugar concentration in the penultimate internode was greater than peduncle, but the patterns were similar. The differences between soluble sugar concentrations of internodes are to be expected as the peduncle does not accumulate nonstructural carbohydrate until after anthesis, when the development is completed. In the stem, less decrease of starch concentration in comparison to soluble sugars showed that this insoluble sugar may not be efficient in carbohydrate remobilization to the growing grains. Kiniry (1993), reported that starch in wheat stems is not involved in remobilization to the growing grains under shading conditions. Therefore, the soluble sugars especially fructans are the main sugar involved in remobilization (Dubois et al., 1990). Under inhibition of photosynthesis of whole plant and ear at the beginning of GF, the role of remobilization in grain yield formation was similar.

Thus, two treatments approximately had the same effect on remobilization and spike photosynthesis and the main role in yield formation. When two levels of water deficit were applied, the inhibition of photosynthesis at the beginning of GF caused more reduction in grain yield. In addition, less contributory role of carbohydrates remobilization in grain yield formation of tolerant cultivar was observed. However, higher content and remobilization rates of WSCs in the stem of tolerant cultivar showed that there may be more respiratory losses of remobilized carbohydrates (Austin et al., 1977) or especially more movement of remobilized carbohydrates to alternative sinks such as young developing tillers (Wardlaw and Porter, 1967) in tolerant cultivar. The use of ^{14}C has provided evidence that the proportion of reserves used in respiratory processes is low, indicating that respiration relies largely on current assimilation rather than on mobilized reserves (Bell and Incoll, 1990; Cruz-Aguado et al., 2000). Therefore, more yield stability of this cultivar in addition to longer duration of photosynthesis at GF might be the result of having a greater number of fruitful tillers in comparison to the sensitive cultivar.

The decrease of photo-assimilate under inhibition of total and spike photosynthesis treatment, similar to the water deficiency, may have caused the production of signals that involved in inducing remobilization and greater reduction of fructan and sucrose concentration in stem (Yang et al., 2004; Wang et al., 2000). In addition to ABA that plays an important role in the regulation of both senescence and assimilate remobilization (Tadas et al., 1999; Yang et al., 2001, 2002, 2003), there may be other signals that are produced in response of current photosynthesis inhibition, at the beginning of carbohydrate remobilization.

The Fructan exohydrolase (FEH; EC 3.2.1.80) which catalyzes the hydrolysis of fructans, leads to release of more fructose and glucose in stems at grain filling and sucrose concentration at the end of grain filling (Simpson and Bonnett, 1993; Willenbrink et al., 1998). After that, the

sucrose phosphate synthase (SPS; EC 2.4.1.14) which is thought to play a major role in sucrose biosynthesis, re-synthesize the sucrose from mono-saccharides such as fructose (Huber and Huber, 1996) before phloem loading to remobilize. Perhaps the determinant factors in the amount of remobilization from stem not only are the non-structural sugar concentrations but also the enzyme activities that are involved in sucrose re-synthesis from fructose and glucose (produced mainly from fructans and starch, respectively). These may be the most important factors that co-operates in yield formation, especially under post anthesis water deficit. So, the evaluation of these enzyme activities in relation to remobilization after anthesis may be the most important factor in improving yield stability via WSCs remobilization.

Material and methods

Plant material and drought treatments

Two contrasting cultivars of bread wheat (*Triticum aestivum* L.), Zagrose (low yield potential) and Marvdasht (high yield potential) which are defined as tolerant and sensitive cultivars to post anthesis water deficiency, respectively, were selected for this experiments. Plastic pots (15 cm diameter and 16.5 cm height) were filled with 2.5 kg of a mixture of clay loam soil, sand, and farmyard manure in a ratio of 2:1:1, respectively. Fertilizers were applied at the rate of 100:50:50 kg/ha of N: P: K and kept under ambient conditions in the greenhouse. Ten seeds were planted per pot and one week after their emergence, the number of the seedlings was reduced to 5 per pot. Plants were subjected to three levels of water regimes (1) control conditions in which plants were regularly watered to allow appropriate growth, (2) and (3) water deficiency conditions in which water level of the pots was adjusted to about 40% of field capacity from anthesis until 14 days after (CD: cell division) and from 14 days after anthesis until maturity (GF: grain filling) (Brocklehurst, 1977; Evers and Millar, 2002). Each treatment was replicated 3 times.

Desiccant application

The desiccant treatment was applied according to method of Nicolas and Turner (1993). The pots were divided into three groups. A 0.4% (W/V) solution of potassium iodide (KI) was sprayed uniformly and precisely on the plants of first group (whole plant) 12 days after anthesis and on the second group was applied only on spikes of plants and third group was used a control group (without KI application) and only treated with distilled water.

Sampling

The samplings were conducted at two developmental stages (14 days after anthesis and at maturity). Ten plants from two pots in each treatment were harvested at maturity for determination of grain yield and its components (grain weight and grain number). Ten main tillers from other pots, randomly harvested at 14-d after anthesis (beginning of GF) and at maturity. The main tillers were harvested from the soil surface. Leaf blades were removed from main tillers and immediately dried in a forced-air drier at 80°C for 72 h. Then, each main tiller was divided into two segments, namely peduncle (first internode below the ear) and penultimate internode (the internode below the peduncle). The length and dried weight of each segment were measured, and its specific weight (linear density) was calculated as the ratio of its weight to its length.

Soluble and insoluble sugars determination

The peduncle and penultimate internodes in each treatment were chopped separately into powder. These powders were used for total soluble sugar, main soluble sugars (glucose, fructose, sucrose and fructan) and insoluble sugar (starch) determination. As described with Dubois et al. (1990), with some modifications, 100 mg of powder in each treatment was used for extraction of soluble sugars. Ethanol (80%) at 80°C for 60 minutes was used as the extraction buffer. Subsequently, samples were centrifuged at 14000 rpm for 15 min and supernatant was collected. This step was repeated three times. Supernatant was used for the determination of total soluble sugars (AOAC, 1995) and glucose, fructose, sucrose and fructan (Dubois et al., 1990) by using a HPLC (Preparative HPLC system smartline model, Knauer Company, Germany; Eurokat H column at room temperature; RI detector; flow rate 1ml/min; solvent sulfuric acid 0.02N). The remaining shoot dry material was washed twice in ethanol and water and dried under the bench for 30 minutes. Determination of starch was carried out as described by AOAC (1995).

Statistical analysis

The Analysis of variance using ANOVA software was performed for each parameter measured or calculated. The means were compared using the Duncan's Multiple Range (DMR) test at level of 0.05 probability (Steel et al., 1997).

Conclusions

In summary, the results suggested that the grain yield of wheat cultivars under water deficiency and also photosynthesis inhibition of sources at the beginning of GF is more controlled by source than sink limitation. With respect to the future requirements for the production of wheat cultivars with higher grain yield, among the different sources of assimilates, spike (ear) photosynthesis had the main role in GF and should be more considered in future breeding programs. Moreover, as post-anthesis water deficit at GF stage of wheat occurres almost every year in arid and semi-arid regions, another main strategy for higher grain yield stability must be the selection of genotypes with greater amounts of carbohydrates storage in stem with higher remobilization rate to the growing grains before anthesis stage. The over-expanded leaf areas and delayed senescence will result in more water consuming by plant that could be used for other purposes.

Table 3. Mean comparisons of cultivar × water deficiency × photosynthesis inhibition treatment interaction on grain yield and yield reduction comparison with control treatment. Mean values followed by the same letter (a-e) are not significantly different according to Duncan (P<0.05).

Water deficiency treatment	Cultivar	Photosynthesis inhibition treatment	Grain Yield (g plant^{-1})	Decrease down to control (%)
At grain filling Stage (1)	Sensitive	T_0*	0.86 f	
		T_1	0.66 gh	23.5
		T_2	0.41 jk	52.8
	Tolerant	T_0	1.09 d	
		T_1	0.56 i	48.8
		T_2	0.25 l	76.7
At cell division Stage (2)	Sensitive	T_0	0.99 e	
		T_1	0.74 g	25.8
		T_2	0.60 hi	39.6
	Tolerant	T_0	1.34 c	
		T_1	0.68 gh	49.5
		T_2	0.36 k	73.1
Control	Sensitive	T_0	2.26 a	
		T_1	1.30 c	42.4
		T_2	0.71 g	68.4
	Tolerant	T_0	1.56 b	
		T_1	0.86 f	44.7
		T_2	0.48 j	69.2

*T_0: Non-treated (control), T_1: Spraying only on ears and T_2: Spraying on whole plant. (1) and (2) from 14 days after anthesis until maturity and from anthesis until 14 days after.

Table 4. Changes in grain yield under different treatments of photosynthesis inhibition with KI (Iodide potassium) and water deficiency in two wheat cultivars and yield. Mean values followed by the same letter (a-e) are not significantly different according to Duncan (P<0.05).

Photosynthesis inhibition treatment	Grain Yield (g plant^{-1})	Decrease down control (%)
Non-treated (control)	1.90 a	-
Spraying only on ears	1.08 b	- 42.16
Spraying on whole plant	0.60 c	- 68.46
Control	1.91 a	-
Water deficit at cell division	1.17 b	- 28.86
Water deficit at grain filling	0.98 c	- 48.91

Table 5. Different soluble and insoluble sugars concentration in stem of two wheat cultivars at two dates of sampling after anthesis and in different segments of stem. Mean values followed by the same letter (a-e) are not significantly different according to Duncan (P<0.05).

Cultivar	Date of sampling	Soluble sugars	Starch	Sucrose	Glucose	Fructose	Fructan
		mM/g dw					
Tolerant	14 days after anthesis	162 b	885	60 a	17.8 b	39.4 c	133 b
	Maturity	44 c	624	2.6 d	18.2 b	67.6 b	57.2 c
Relative Decrease (%) (1)		-72.9	-29.5	-95.6	2.6	71.6	-57.2
Sensitive	14 days after anthesis	192 a	881	54.5	18.8 b	40.1 c	185 a
	Maturity	47 c	679	4.7	29.8 a	84.6 a	55.6 c
Relative decrease (%)(1)		-75.6	-22.9	-91.4	34.5	110	-70
Cultivar	Segment of stem	Soluble sugars	Starch	Sucrose	Glucose	Fructose	Fructan
		mM/g dw					
Tolerant	Peduncle	36.1 b	634 c	2.59 c	23.4 b	79.4 b	45.2 b
	Penultimate	57.4 a	724 a	6.76 a	36.2 a	89.6 a	65.9 a
Sensitive	Peduncle	28.9 c	547 d	1.80 d	16.6 d	67.5 c	47.1 b
	Penultimate	58.9 a	701 b	3.43 b	19.9 c	65.7 c	67.3 a

(1) Percentage decrease concentration of different sugars in stem down to 14 days after anthesis.

Table 6. Estimations of soluble and insoluble sugars concentration in the stems of two wheat cultivars under different water deficiency and photosynthesis inhibition treatments. Mean values followed by the same letter (a-e) are not significantly different according to Duncan (P<0.05).

Cultivar	Water deficiency treatment	Soluble sugars	Starch	Sucrose	Glucose	Fructose	Fructan
		mM/g dw					
Tolerant	Control	74.7 a	724 a	10.4 a	46.0 a	124.2 a	107.8 a
	Cell division (1)	43.3 c	665 b	2.78 c	27.6 b	76.1 c	42.1 d
	Grain filling (2)	22.2 f	647 bc	0.83 e	15.8 d	53.4 e	16.8 e
Relative Decrease (%)(3)		-42.0	-8.3	-73.2	-39.9	-38.7	-60.9
Relative Decrease (%)(4)		-70.3	-10.6	-91.9	-65.6	-57.0	-84.4
Sensitive	Control	61.5 b	659 bc	3.65 b	25.3 c	82.5 b	72.6 b
	Cell division	38.9 d	635 c	2.67 c	16.3 d	66.5 d	54.6 c
	Grain filling	31.3 e	577 d	1.51 d	13.2 e	53.8 e	44.4 d
Relative Decrease (%)(3)		-36.7	-3.5	-26.6	-35.7	-19.2	-24.7
Relative Decrease (%)(4)		-49.0	-12.4	-58.4	-47.9	-34.7	-38.7
Cultivar	Photosynthesis inhibition treatments	Soluble sugars	Starch	Sucrose	Glucose	Fructose	Fructan
		mM/g dw					
Tolerant	Non-treated (control)	81.2 a	816 a	8.13 a	66.9 a	107 a	80.8 b
	Spraying only on ears	42.9 c	682 b	4.68 c	14.4 c	80.1 b	45.6 c
	Spraying on whole plant	16.2 f	540 b	1.20 e	8.03 e	66.0 c	40.3 d
Relative Decrease (%)(5)		-47.2	-16.5	-42.4	-78.4	-25.6	-43.6
Relative Decrease (%)(6)		80.1	-33.8	-85.2	-88.0	-38.6	-50.5
Sensitive	Non-treated (control)	71.4 b	744 b	5.14 b	37.6 b	81.7 b	89.2 a
	Spraying only on ears	35.8 d	660 c	1.80 d	10.4 d	67.7 c	44.3 c
	Spraying on whole plant	24.7 e	467 e	0.90 f	6.67 e	53.4 d	38.1 d
Relative Decrease (%)(5)		-49.8	-11.3	-65.0	-72.2	-17.2	-50.3
Relative Decrease (%)(6)		-65.4	-37.2	-82.5	-82.3	-34.6	-57.4

(1) and (2) from anthesis until 14 days after and from 14 days after anthesis until maturity.
(3) and (4): Percentage decrease down control when water deficiency was applied at cell division and grain filling stage respectively.
(5) and (6): Percentage decrease down control when Photosynthesis inhibition treatments were applied only on ears and whole plant respectively.

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