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Stem Water Soluble Carbohydrate Remobilization in Wheat under Heat Stress during the Grain Filling

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Abstract

The environmental conditions in regions with Mediterranean climate are favorable for wheat (*Triticum aestivum* L.) growth until the anthesis but afterwards exposed to terminal heat stress. Relative contribution of stem reserves during grain filling is assumed to increase compared with current photosynthesis. This study evaluated the ability of different wheat genotypes for accumulation and remobilization of stem water soluble carbohydrate (WSC) under the heat stress conditions during the grain filling. Ten spring bread wheat genotypes were grown under controlled conditions of phytotron with two temperature treatments i.e. normal and heat stress. There was significant variation among the genotypes for WSC concentration and remobilization. WSC remobilization increased under heat stress (on average 60%). Strong association between maximum WSC concentration in main stem and WSC remobilization was found. Under heat stress, the number of grains per spike correlated with WSC remobilization (r= 0.45) and correlation of percentage reduction of grain yield with WSC remobilization efficiency was negative (r = –0.47). However, there was no significant relation between grain yield and stem reserves remobilization under heat stress during grain filling. It seems that the tolerance to the heat stress would be provided either through maintenance of current photosynthesis or by high remobilization of stem reserves.

Keywords: Stem reserves; Remobilization; Heat stress; Grain filling; Wheat

Introduction

High temperature constrains yield of wheat (*Triticum aestivum* L.) during the grain filling period in many regions, especially in the Mediterranean climate. The needed assimilates for grain growth, are provided from current photosynthesis and remobilization of reserve carbohydrates from stem to grains (Schnyder, 1993; Blum *et al*., 1994; Nawaz *et al.*, 2013). Due to accelerated leaf senescence and damage to the photosynthetic apparatus (Al-Khatib and Paulsen, 1990), heat stress during grain filling leads to change in the relative contributions of stem during grain filling process. Therefore grain filling would be more dependent on stem reserves (Schnyder, 1993; Blum, 1998; Yang *et al*., 2002).

Stem reserve carbohydrates are commonly considered as total non-structural carbohydrates (TNC) or water soluble carbohydrates (WSC) and distinguished from the structural carbohydrates present in cell walls (Ruuska *et al*., 2006). Stem reserve carbohydrates principally consist of fructan, sucrose, glucose, fructose and starch, although fructan is the main reserve (Wardlaw and Willenbrink, 1994). Carbohydrate storage ability in stem and remobilization efficiency of reserves for grain development are effective components contributing to grain yield (Ehdaie *et al*., 2006a, b). Ability of carbohydrate storage in stem is determined by stem specific weight and stem length (Blum, 1998). The amount of accumulated WSC in stem depends on environmental conditions in pre- and post- anthesis until linear growth stage of grain (Blum, 1998; Takahashi *et al*., 2001). Stem reserve remobilization efficiency is also affected by sink strength (grain weight and number of grain per spike) and environment (Blum, 1998; Ehdaie *et al*., 2006a).

In order to estimate the apparent contribution of stem reserves in grain yield, different methods have been applied, such as labeling methods (Schnyder, 1992), inhibiting current photosynthesis during grain filling by leaf removal (Fokar *et al*., 1998), chemical desiccation (Blum *et al.*, 1994) or applying darkness (Yang *et al*., 2002). In addition, the difference between weight or WSC content of stem at anthesis and maturity has been used for estimation of apparent stem reserve contribution in grain yield (Blum *et al*., 1994; Cruz-Aguado *et al*., 2000; Ehdaie *et al*., 2006a, b).

Anyway, in different reports, apparent contribution of stem reserves in grain yield had wide variation depending on genotypes, environmental conditions and experimental techniques. The contribution estimated to be anywhere between 6–100% (Blum, 1998).

It seems that ability in stem reserve accumulation and remobilization can be considered as an appropriate trait in stress condition during grain filling to develop wheat more

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adapted to harsh environments as well. This study was conducted to evaluate the ability of different wheat genotypes for accumulation and remobilization of stem reserve carbohydrates under heat stress conditions during grain filling and their contribution to grain yield.

Materials and Methods

Plant Material

Experiment was conducted on ten spring bread wheat genotypes with similar phenology. Chamran (Attila) and Atrak (Kauz"s") are recognized as international heat stress tolerance genotypes and were entries in the International Maize and Wheat Improvement Centre (CIMMYT). Dez (Kauz*2/Opata//Kauz), Falat (Seri82) and Darab2 (Maya"s"/Nac) genotypes, also were provided in the CIMMYT. Original source of Aflak (Debeira) genotype is Sudan. Kavir and Pishtaz are local cultivars suitable for high temperature and temperate regions of Iran, respectively. In addition, S-83-3 and S-78-11 were advanced lines in high-temperature areas of Iran.

Growth Conditions and Experimental Factors

All genotypes were sown on November 18th, 2010 in pots (10 cm diameter and 50 cm deep) filled with mixture of silty loam soil (having 1.5% organic material) and sand using 3:1 ratio. Before planting, each pot was fertilized with 18.4 mg N as urea and 60 mg P using triple super phosphate. Five seeds were sown in each pot and after three weeks, three plants were maintained per pot. All pots were placed on Shahid Chamran university experimental farm (Ahvaz, Iran) from the sowing date until 10 days after anthesis. Temperature status at this period is shown in Table 1. Nitrogen was fertilized in splits, at the end of tillering and booting with 18.4 mg N per pot. All pots were irrigated after every two days or when needed to avoid any water stress.

All pots of each genotype 10 days after anthesis, were divided in two groups randomly, and then shifted to two phytotrons with different temperature treatments. Photoperiod in phytotrons was set on 16 h, and the photosynthetic photon flux density was 500 µ mol m^{-2} s^{-1} at 10 cm above the plants spike. Relative humidity range was 50–70%. Under normal conditions, daytime temperature was programmed at 25ºC for 4 h, 21℃ for 5 h before and after it, and 16℃ at night time. Under heat stress, daytime temperature was adjusted to 37℃ for 4 h, 31℃ for 5 h before and after it, and 25℃ for night temperature.

Measurements

In all pots, main stems were tagged as spikes emerged from the flag leaf sheaths. Randomly, three main stems were harvested from the surface of soil, 10 days after the anthesis and four-day intervals after the first harvesting until maturity. After each harvest, leaf blades and sheaths were removed and stems were dried in an oven at 75℃ for 48 h. Then, the weight of stems was measured and so the maximum dry stem weight during the sampling was determined. When a genotype reached to the maximum stem weight, its WSC concentration considered as the maximum WSC concentration and measured (Ehdaie *et al*., 2006b). The harvested samples at maximum stem weight and maturity were chopped into small pieces and grounded in a UDY cyclone mill to pass through a 1 mm sieve. To measure water soluble carbohydrate concentrations of the stem, 100 mg of dry material extracted with 15 mL (v/v) 80% ethanol and assayed by phenol-sulphuric acid method (Dubois *et al*., 1956). The difference between maximum stem WSC concentration and WSC concentration at maturity was used to estimate the mobilized WSC per unit dry matter. Stem WSC remobilization efficiency calculated by a ratio of stem mobilized WSC to the maximum stem WSC concentration.

The chlorophyll contents of flag leaf were assessed at four-day intervals after anthesis by using chlorophyll meter (Minolta Chlorophyll Meter SPAD-502) under heat treatment.

For main spike grain yield, the number of grains and grain weight, five main spikes at maturity were harvested.

Statistical Analysis

The experiment was arranged in a completely randomized block design with three replicates in each of phytotron. The combined analysis of variance was performed by GLM procedures across two phytotrons and genotypes for each measured trait. Also, means comparisons was performed by Fisher's protected least significant difference at 5% probability level. Associations between traits were examined by correlation analysis where appropriate.

Results

Water Soluble Carbohydrate Concentration

No significant difference was observed for the maximum stem weight and WSC concentration under heat stress and normal conditions. However, significant variation ($p<0.001$) was found among the genotypes for WSC concentration, and genotype × temperature treatment interaction was also non-significant. Among the genotypes, Atrak, Falat and S-83-3 had the highest and Aflak and Chamran had the lowest WSC concentration in the main stem (Table 2).

Water Soluble Carbohydrate Remobilization and its Efficiency

Heat stress increased WSC remobilization and its efficiency significantly (Table 3). Highly significant variation was observed among the genotypes for both traits. Genotype's response in both temperature treatments varied and genotype × temperature treatment interaction

Table 1: Mean of maximum and minimum temperatures and relative humidity for wheat growth duration in Ahvaz, Iran (2010-2011)

Month	Mean of air temperature (℃)		Mean of Relative humidity (%)
	maximum	minimum	
November	28.62	12.35	34.02
December	22.10	8.27	39.95
January	18.02	8.00	51.95
February	19.46	9.10	44.61
March	25.39	12.18	39.05

Table 2: Maximum water soluble carbohydrate (WSC) concentration of main stem for wheat genotype and temperature treatment

Genotype and Temperature treatment	Max. WSC concentration (mg g^{-1})
Genotype	
Chamran	183.80 e
Falat	278.60 ab
Aflak	186.60 e
Atrak	287.10 a
Dez	261.20 c
Kavir	272.80 abc
S-78-11	221.30 d
Darab2	270.60 bc
Pishtaz	231.00 d
S-83-3	277.30 ab
Temperature treatment	
Normal	249.90 a
Heat stress	244.10 a

Means, followed by similar letter are not significantly different ($p < 0.05$) according to LSD test

Table 3: Water soluble carbohydrate (WSC) remobilization and its efficiency of main stem for wheat genotype and temperature treatment

Genotype	WSC remobilization (mg g^{-1})		Remobilization efficiency (%)	
	Normal	Heat stress	Normal	Heat stress
Chamran	58.40 d	122.40 fg	31.22 de	67.60 bc
Falat	147.70 a	211.80 ab	52.57 a	76.64 a
Aflak	69.50 d	119.10 fg	37.16 bcd	63.95 c
Atrak	159.40 a	221.00 a	55.57 a	76.94 a
Dez	99.00 bc	168.60 cd	37.82 bc	64.39 c
Kavir	102.60 b	136.80 ef	36.83 bcd	51.24 d
S-78-11	77.30 cd	103.60 g	32.96 cde	49.41 d
Darab2	106.70 b	190.20 bc	38.54 bc	71.32 ab
Pishtaz	70.00 d	147.30 de	30.00 e	63.48 c
S-83-3	114.90 b	192.30 b	41.10 b	69.82 bc
Mean	100.60	161.30	39.38	65.28
Analysis of variance				
Temperature treatment (T)	***		***	
Genotype (G)	***		***	
Interaction T×G	*		**	

*, ** and ***: Significant at 5%, 1% and 0.1% probability levels, respectively; Means, followed by similar letter are not significantly different ($p < 0.05$) according to LSD test

for WSC remobilization and efficiency was significant. Anyway, in both temperature treatments, Atrak and Falat had the highest WSC remobilization and efficiency per unit dry matter. Under the heat stress, remobilization of WSC and its efficiency in S-78-11 was the minimum. Although, Chamran and Pishtaz had low remobilization under normal condition, while remobilization and its efficiency almost was two times under the heat stress condition (Table 3).

Also, in both temperature treatments, remobilized WSC per unit dry matter (mg g^{-1}) had high association with maximum WSC concentration. Genotypes with more WSC concentration had high WSC remobilization (Fig. 1).

Flag Leaf Chlorophyll Content

Genotypes showed variable response for chlorophyll content (Fig. 2). S-78-11, S-83-3, Dez and Atrak had the highest and Chamran the lowest rate of chlorophyll reduction per day. The decrease in chlorophyll was gradual for Pishtaz and Darab2, but the onset of chlorophyll loss in these genotypes started once heat stress imposed. Thus, 14 days after the anthesis their chlorophyll content was less than it in other genotypes.

Grain Yield and its Components

In both temperature treatments, significant variation was observed among genotypes for main spike grain yield and its components. Heat stress reduced yield of main spike, single grain weight and number of grains per main spike significantly on average by 31.61%, 28.58% and 5.26%, respectively (Table 4). The reduction in grain yield and yield components varied significantly among genotypes. S-78-11, Pishtaz and Kavir had the maximum reduction of grain yield while minimum reduction was in Atrak, Aflak and Dez genotypes. Reduction in single grain weight was low in Aflak followed by S-83-3 and was high in Falat. Under the heat stress treatment, no reduction in the number of grain per main spike in Atrak and Falat was observed and S-83-3 had the highest reduction (Table 4).

Discussion

WSC remobilization and its efficiency increases under heat stress during the grain filling (Blum *et al*., 1994; Fokar *et al*., 1998; Cruz-Aguado *et al*., 2000; Plaut *et al*., 2004; Tahir and Nakata, 2005). We also report WSC increase on average by 60 and 66% under heat stress, respectively. Highest remobilization of WSC under heat stress shows the decline in assimilate sources from current photosynthesis that leads to plants become more dependent on stem storage.

For instance, Blum *et al*. (1994) and Fokar *et al*. (1998) reported that stem reserve remobilization was a constitutive trait. But results of present study varied for different genotypes. Genotypes such as Falat and Atrak in both temperature treatments had the greatest ability for accumulation and remobilization of stem storage, constitutively. In contrast, Chamran and Pishtaz had the lowest remobilization of stem storage under normal condition. Nonetheless, remobilization and its efficiency

Table 4: Main spike grain yield and components in wheat genotypes grown under normal and heat stress and reduction (percent) yield and yield component under heat stress.

Genotype	Grain yield per spike (g)			Grain number per spike			Single grain weight (mg)		
	N†	H‡	R§(%)	N	H	R(%)	N	H	R(%)
Chamran	1.52 d	1.06 de	30.29 d	43.30 e	41.00 c	5.31 abc	35.07 de	26.12 bc	25.48 cd
Falat	1.65 bc	1.12 bcd	32.11 cd	46.00 cd	46.00 ab	0.03 c	36.88 bc	22.81 e	38.16 a
Aflak	1.52 d	1.12 bcd	25.90 e	43.67 de	40.33 c	7.63 ab	34.95 e	28.00 a	19.91 f
Atrak	1.54 d	1.15 abc	25.59 e	48.00 bc	48.00 a	0.00 c	32.14 f	23.10 e	28.13 c
Dez	1.60 cd	1.18 ab	25.95 e	50.67 a	48.33 a	4.59 abc	31.73 f	24.31 d	23.43 de
Kavir	1.69 ab	1.07 de	36.95 ab	44.67 de	41.33 c	7.46 ab	37.83 ab	25.90 c	31.54 b
S-78-11	1.76 a	1.09 cd	38.36 a	48.33 abc	44.67 b	7.49 ab	36.48 c	24.44 d	33.00 b
Darab 2	1.53 d	1.00 e	34.46 bc	42.33 e	40.67 c	3.95 bc	36.12 cd	24.70 d	31.62 b
Pishtaz	1.69 ab	1.07 de	36.90 ab	43.00 e	40.33 c	6.20 ab	38.83 a	26.07 bc	32.87 b
S-83-3	1.74 a	1.22 a	29.64 d	49.67 ab	44.67 b	9.96 a	34.65 e	27.12 ab	21.71 ef
Mean	1.62	1.11	31.61	45.97	43.53	5.26	35.47	25.26	28.58
Analysis of variance									
T¶	***			***			***		
G#	***			***			***		
T×G	***			ns			***		

†Normal condition, ‡Heat stress condition, §Reduction (percent) because of heat stress, ¶Temperature treatment, # Genotype; ns and ***: Non-significant and Significant at 0.1% probability levels, respectively; Means, followed by similar letter are not significantly different ($p < 0.05$) according to LSD test

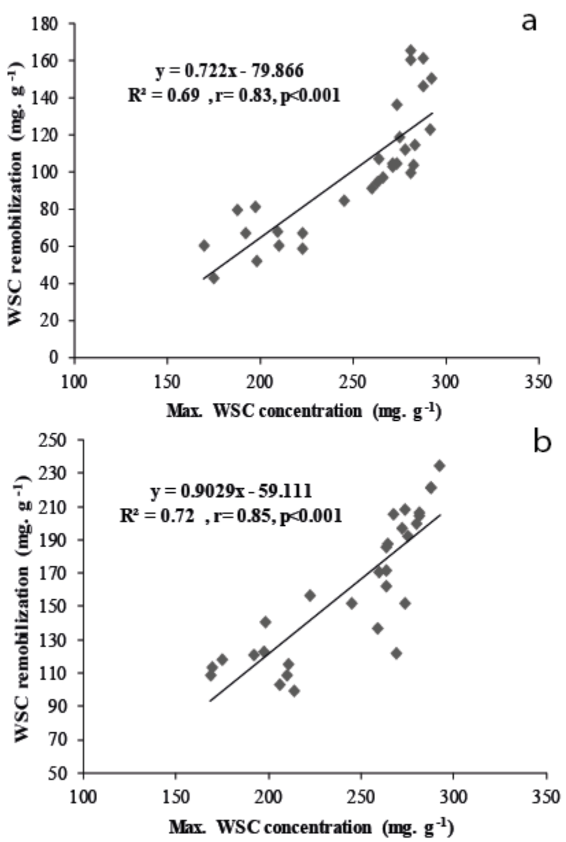

Fig. 1: Relationship between WSC concentration and WSC remobilization per unit dry matter of wheat genotypes under (a) normal and (b) heat stress temperature treatments

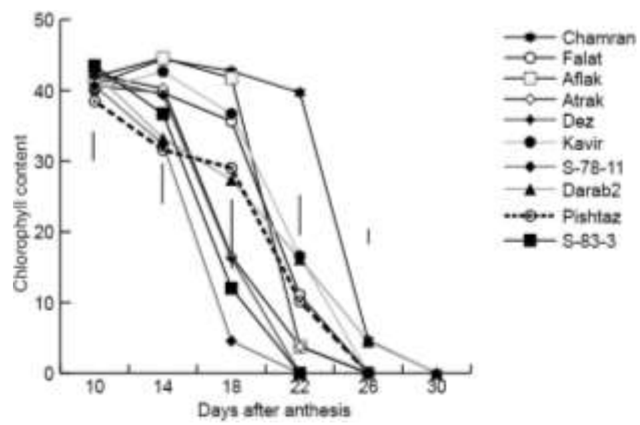

Fig. 2: Mean Changes in chlorophyll content of flag leaf wheat genotypes from 10 until 30 days after anthesis under heat stress; Each point is a mean of three observations; Vertical bars represent LSD ($p < 0.05$)

were almost doubled under the heat stress conditions. WSC remobilization under different temperature treatments are varied with genotypes (Tahir and Nakata, 2005).

In this study, the variation among genotypes for WSC concentration was significant, and genotype × temperature treatment interaction was non-significant. On the other hand, there was strong association between WSC concentration and WSC remobilization (Fig. 1). These results indicated that WSC concentration could be considered as a suitable trait for breeders in order to select proper genotypes with the maximum storage and remobilization of stem reserves. Heretofore, large stem WSC concentration had been suggested as a selection criterion for usage in breeding (Blum, 1998; Ehdaie *et al*., 2006b; Ruuska *et al*., 2006).

In both temperature treatments, the number of grains per spike correlated with the maximum WSC concentration in main stem ($n = 60$, $r = 0.43$, $p < 0.01$). Atrak and Falat with the maximum WSC concentration had maximum grain number per spike (Table 2, 4). This shows that high stem WSC concentration does not reduce the size of the sink

(Blum *et al.,* 1994). In the Mediterranean climate, conventional sowing date for wheat is from third half of the fall to early winter and the pre-anthesis environmental condition is suitable for the plant growth. Therefore, the high capacity for reserve storage of stem did not lead to reduction in the number of grains per spike.

In this study, association between the number of grains per main spike and WSC remobilization under heat stress treatment ($n = 30$, $r = 0.45$, $p < 0.01$) shows the effect of sink strength on increased WSC remobilization (Blum, 1998). Also, there was negative correlation between grain yield reduction under heat stress with WSC remobilization efficiency ($n = 30$, $r = –0.47$, $p < 0.01$). However, there was no significant relation between grain yield and WSC remobilization under heat stress during grain filling. Atrak, with the lowest grain yield reduction under the heat stress, had the highest WSC concentration and the greatest WSC remobilization and efficiency. Despite the high rate of chlorophyll loss per day, Atrak used stem storage as a carbon reserve for grain growth, consequently it led to the minimum reduction in the main stem grain yield. Among genotypes, S-78-11 with the greatest grain yield reduction under heat stress had the lowest WSC remobilization and high rate of chlorophyll loss per day. These results showed high sensitivity of this genotype and its inability in remobilization and using the stem reserves. Moreover, Dez and S-83-3 genotypes by using the stem reserves and Chamran and Aflak genotypes by preserving green leaf and current photosynthesis under heat stress had slight reduction in grain yield. Yang *et al*. (2002) reported high variation among genotypes for relative contribution of photosynthesis and stem reserves in wheat grain yield under the heat stress condition. Some genotypes tolerated the heat stress by preserving the stable photosynthesis and some others via the stem reserves.

It seems maintenance of photosynthesis or high WSC remobilization makes genotypes less sensitive to high temperature. However, the activities of key enzymes in endosperm starch biosynthesis are inhibited at temperature above 34℃ (Keeling *et al*., 1993), the starch accumulation will be reduced even if the assimilate source would be available. As Yang *et al*. (2002) stated that combination of a steady source of assimilates with a stable system for their use might be means for improving grain yield under heat stress.

In conclusion, either stable photosynthesis or high remobilization of stem reserves could improve heat tolerance of wheat. On the other hand, WSC concentration could be considered as a suitable trait for breeders in order to select proper genotypes with the most stem reserves remobilization ability under terminal heat stress conditions.

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