

Activities of fructan- metabolizing enzymes in barley stems subjected to terminal drought stress

Mona Pureisa^{1*}, Majid Nabipur², Musa Meskarbashi²

¹Department of Agriculture, Payame Noor University (PNU), Iran.

² Agronomy and plant breeding department, Shahid Chamran University, Ahvaz, Iran.

(Received: 28/05/2018- Accepted: 03/10/2018)

Abstract

Barley crop grown in semiarid areas may experience water deficit especially during grain filling that makes them more dependent on stem water-soluble carbohydrates (WSC). Fructans are the most important reserved carbohydrates. A pot experiment was undertaken at Shahid Chamran University in the duration of 2010-2011 growing seasons to investigate the accumulation and loss of WSC and the activity of fructan-metabolizing enzymes under water stress in four barley (*Hordeum vulgare* L.) cultivars namely Nimruz, Jonub, Nosrat and Torkaman. Cultivars were different in WSC-related traits such as WSC concentration (WSCc) at anthesis, maximum WSCc and rate of WSC accumulation in ten-day period after anthesis. Water withholding elevated WSC remobilisation efficiency. The activity of sucrose:sucrose 1-fructosyl transferase (1-SST; EC 2.4.1.99) harmonized the rate of WSCc increase in cultivars. The change in fructan 1-exohydrolase (1-FEH; EC 3.2.1.80) activity on the 15th day post-anthesis was along with the change in stem reserve remobilisation. Considering acid invertase (EC 3.2.1.26) activity, a significant difference was observed between cultivars on the 15th day post-anthesis under water-stressed conditions. Cultivars showed different manners in use of current photosynthesis and stem reserves that could be an important reason in different decreases in grain weight affected by water stress.

Keywords: Barley, drought, Fructan 1-exohydrolase, Penultimate internode, Sucrose:sucrose 1-fructosyl transferase, Water soluble carbohydrate

Introduction

In semiarid regions with a Mediterranean climate, grain-filling period of barley faces different kinds of biotic and abiotic stresses including water deficit, heat stress and foliar diseases. Current assimilation as a carbon source for grain filling is dependent on light absorptive green surfaces after anthesis. This source decreases normally due to natural senescence and different stresses. Simultaneously, demand of growing grain increases in addition to the demand of maintenance and respiration of live plant biomass. So stem reserves are one of the important carbon reserves for grain filling. Even under moderate growth conditions, current assimilate may be limited for grain filling (Blum, 1998). Stem reserves are mainly in the form of WSC (Kuhbauch and Thome, 1989; Przulj and Momcivic, 2001; Schnyder, 1993). At the time of maximum WSCc, fructans show 85% of WSC in wheat stem (Zhang *et al.*, 2009). High concentration of fructans reserve in vacuoles of parenchyma cells that surround the sieve tube (Sadras, 2009). Graminan types of fructans are predominant in growing tissue of temperate grasses

such as wheat and barley (Zhang *et al.*, 2009). SST catalyses the first step and likely the most important step in fructan synthesis (Blum, 1998). The activity of this enzyme seems related to the sucrose concentration that itself is affected by the activity of sucrose synthase in stem. The activities of enzymes related to the synthesis and remobilisation of stem WSC showed various reactions to time, soil moisture content and amount of nitrogen. SST activity in the stems of well-watered plants increased drastically from 6 to 15 days post-anthesis in normal nitrogen and from 6 to 27 days post-anthesis in high amount of nitrogen and then decreased and at the same time it showed high positive correlation with fructan concentration. Water deficit decreased SST activity in both levels of nitrogen. Fructan exohydrolase (FEH) enzyme catalyses the fructan hydrolysis leading to release fructose that should be converted to precursors needed for the re-synthesis of sucrose (Yang *et al.*, 2004). FEHs consist of 1-FEHs and 6-FEHs. Sucrose inhibits the activity of 1-FEH that has a role in fuctan cleaving. Therefore, when the amount of sucrose is high, the fructan cleaving doesn't

*Corresponding Author, Email: m_pureisa@pnu.ac.ir

occur. When the amount of sucrose can't provide the need of grain filling, fructans are degraded by 1-FEH to release more sucrose and fructose. Hence, 1-FEH is really important for maintaining the carbon flow needed for grain filling. Although 6-FEH isn't inhibited by sucrose, showing that it may doesn't have any roles in reserve mobilisation (Zhang *et al.*, 2009). In contrast to the decrease in the activity SSTs due to water stress, the increase in the activity of FEHs is closely associated with fructan remobilization from stem. (Yang *et al.*, 2004). During both mild stress and drastic stress conditions, the activity of FEHs and invertase was more than non-stress conditions, in the period of post-anthesis in a wheat variety (Wardlaw and Willenbrink, 1994). There is a big disagreement among researchers about the activity of invertase which hydrolyses irreversibly sucrose into glucose and fructose and its role in fructan metabolism. Although, some investigations in wheat (Prudhomme *et al.*, 1992; Simpson *et al.*, 1991) and barley (Simmen *et al.*, 1993) indicated that the invertase activity in stem decreased in the period of fructan accumulation and increased at starting time of remobilisation, two other researches in wheat (Gallagher *et al.*, 2004) and barley (Wagner *et al.*, 1986) didn't observe any changes in invertase activity. It has been shown that the activity of invertase was high at anthesis and decreased drastically during grain filling (Bancal and Triboni, 1993). Yang *et al.* (2004) showed that invertase activity didn't change significantly from anthesis till 27 days post-anthesis and after that it decreased drastically. When the stress began 9 days post-anthesis, invertase activity increased until 10 days after starting of stress and then decreased. In preliminary experiment a diverse set of tow- and six-rowed spring and facultative barley cultivars including Iranian and Japanese barley that were different in height were planted and four cultivars namely Nimruz, Jonub, Nosrat and Torkaman that showed extreme amount of WSC- related traits were selected. The current study was aimed to evaluate enzymatic mechanism of synthesis and degradation of fructans and the effect of drought conditions on enzyme activity. Also, the association between enzyme activity and the change trend of WSC during post-anthesis period was investigated.

Materials and methods

Plant material and treatments: A pot experiment was carried out as factorial experiment in randomized complete blocks design (RCBD) with three replicates at the faculty of agronomy, University of Shahid Chamran, Ahvaz, Iran, in the 2010-2011 growing season. Four spring barley cultivars (*Hordeum vulgare* L.) including Nimruz, Jonub, Nosrat and Torkaman were grown under two water treatments including water stress vs. fully irrigation. The pots with a surface area of 0.2 square meters were filled with farm soil that contained organic matter at 0.4%. 1 g N as urea, 1 g P as triple super phosphate and 2.2 g potassium as sulphate

potassium were incorporated into the soil in each pot (equivalent to 46, 46 and 92 kg ha⁻¹, respectively). N as urea was also top-dressed into each pot by the rate of 0.5 g (equivalent to 23 kg ha⁻¹) at the end of tillering. The pots were placed in the field. Seeds were planted in 3 cm intervals after treating with Vitavax. Seedlings were thinned to 54 plants per pot (equivalent to a density of 225 plants per m²). Each plot consisted of three pots. Soil moisture content for plants in fully irrigated conditions was maintained at field capacity until physiological maturity while water was withheld at on tenth day in water-stressed treatment. Water-stressed plants were irrigated when water potential reached -1.7 MPa based on a soil water potential curve drawn before the onset of the drought treatment. In each pot the plants which showed simultaneous anther exit were tagged.

Observation and measurements: In each pot, same-age plants were tagged as spikes emerged from the flag leaf sheaths and used for observation and measurements. The chlorophyll content of flag leaf was assessed from anthesis to maturity at 5-day intervals by using chlorophyll meter (Minolta Chlorophyll Meter SPAD-502). Sampling was done from anthesis to maturity at 5-day intervals from the same-age plants. The spikes were removed and the penultimate internodes were separated from stems and then frozen in liquid nitrogen after removing leaf sheets. Penultimate internodes were stored at -80°C for WSC and enzymatic measurements. Five main spikes at maturity were harvested for assessment of main spike grain yield, the number of grains and grain weight. WSC were extracted based on Sonnewald *et al.* (1992) and measured according to Dubois *et al.* (1956). Results are expressed as milligrams of WSC per gram of fresh weight for WSC concentration. The mobilized WSC (MWSC) in penultimate internode was estimated as the difference between post-anthesis maximum and minimum WSC concentration. Remobilization efficiency (Re) of WSC was estimated by the proportion (%) of post-anthesis maximum WSC concentration of penultimate internode that was mobilized.

Extraction and enzyme assays of invertase and 1-SST were modified from Savitch *et al.* (2000). The frozen penultimate internodes were pulverized with a mortar and pestle when they were flooded with liquid nitrogen. The powder was ground with extraction buffer containing 50 mM Na-acetate (pH 5.0), 20 mM EDTA, 5 mM MgCl₂, 3 mM dithiothreitol (DTT), 0.04% (w/v) BSA, 0.04% (w/v) polyvinylpyrrolidone (PVPP) and 20 mM β-mercaptoethanol. The homogenate was centrifuged for 15 mins. at 15,000 g at 4 °C. The pellet was resuspended in the extraction buffer and desalted by centrifugal filtration on Sephadex G-25 columns equilibrated with extraction buffer minus BSA, PVPP and β-mercaptoethanol. 80 µl of supernatant desalted by sephadex, was incubated at 95°C for 4 minutes showing zero time or non-enzymatic activity. Enzyme solution containing 80 µl of supernatant and 20 µl of 0.5 M sucrose was incubated at 30°C for 45 minutes and then

incubated at 95°C for 4 minutes to stop the reaction. Finally, the amount of sucrose and fructose were measured by HPLC (Aminex HPX-87P, flow speed of 0.6 ml per min, column temperature of 80°C, IR type of detector and deionized water as a mobile phase). The excess amount of glucose over fructose was used to indicate the 1-SST activity while the amount of produced fructose was considered as invertase activity. Protein content was determined according to Bradford (1976), using bovine serum albumin (BSA) as standard. Enzyme activity was reported as μmol glucose or fructose per mg protein per hour.

The activity of 1-FEH was measured based on Yang *et al.* (2014). Briefly, 150 mg of penultimate internodes were pulverized with a mortar and pestle by liquid nitrogen. The powder was ground with 300 μl of ice-cold 50 mM citrate-phosphate buffer (pH 5.5), containing 5 mM MgCl_2 , 5 mM DTT, 0.2% (w/v) PVPP, 0.1% (w/v) BSA. The homogenate was centrifuged at 12,000 g for 10 min. The enzyme activity was measured after desalting supernatant by Sephadex G-25 column equilibrated with citrate-phosphate buffer (pH 5.5). Inulin 10% (w/v) was added and incubated at 30°C for 45 min. The reaction was stopped by incubation of reaction mixture at 95°C for 4 min. The amount of fructose was measured by HPLC and the activity of 1-FEH was reported as μmol fructose per mg protein per hour. Data was analyzed using ANOVA with the Statistical analysis system package (SAS Institute Inc., Cary, USA). Means were compared using the LSD test (at 5% level of significance).

Results

Water-soluble carbohydrates: Significant differences were observed among cultivars in WSCc at anthesis. Jonub showed the highest and Nosrat showed the lowest concentrations (Fig. 1). In spite of lower WSCc at anthesis, Torkaman and Nimruz could be set in the same group with Jonub, based on maximum WSCc (Fig. 1). The ratio of preanthesis WSC to total reserved WSC was different among cultivars. Jonub had the highest ratio, while Torkaman had the lowest ratio.

Decreasing trend of WSCc after reaching the maximum started after 10 days post-anthesis under both normal and stressed conditions. Under water stress WSCc reached zero on 20th day post-anthesis in Jonub and Torkaman, although it didn't happen in Nimruz and Nosrat until maturity (data not shown). There was no significant difference among Nimruz, Jonub and Torkaman in mobilised WSC but Nosrat showed lower amount under both conditions. Besides the least amount of mobilised WSC, Nosrat showed the least amount of remobilisation efficiency and ranked second under well-watered condition. Water withholding elevated remobilisation efficiency by 10%. Nosrat was mostly affected by water-stressed conditions by increase of 47% in mobilised carbohydrates and increase of 44% in remobilisation efficiency. On the other hand, Nimruz and Jonub had high level of WSC remobilisation in both

conditions and these cultivars weren't highly affected by drought (Table 1).

Nimruz and Nosrat were selected for more investigation of enzyme activity related to carbohydrate synthesis because of its highest and lowest amount of WSC accumulation during 10 days post-anthesis. On the other hand, Nosrat was considered to analyze WSC due to its highest change in mobilised WSC and remobilisation efficiency.

Flag Leaf Chlorophyll loss: Water withholding increased the rate of chlorophyll loss by 66%. There was a significant difference among cultivars in the case of this increase. Nosrat and Nimruz showed the highest and the lowest rate of chlorophyll loss in both circumstances, respectively. On the other hand, Jonub and Nosrat showed the highest and the lowest increase respectively in this feature by entering drought (Fig. 2).

Enzyme activity: There was a significant difference between cultivars with respect to 1-SST activity on 5th day post-anthesis. Nimruz showed more enzyme activity than Nosrat. The activity of this enzyme showed a drastic decrease on 15th day post-anthesis in both cultivars and water regimes (Fig. 3a). The 1-FEH activity on fifth days post-anthesis was much less than that of 15th day post-anthesis. There weren't any significant differences between two cultivars on fifth day post-anthesis while a significant difference was observed between cultivars on 15 day post-anthesis. Enzyme activity was higher in Nimruz than Nosrat in both conditions. Moreover, there wasn't significant increase in enzyme activity in Nimruz while Nosrat showed obvious enhancement by entering drought conditions (Fig. 3b). There was no significant difference between two cultivars with respect to acid invertase activity on 5th day post-anthesis. Although there was no increase in activity on 15th day post-anthesis under normal conditions, we could observe the difference between two cultivars in water stress. Although, Nimruz showed increase in enzyme activity due to water stress. Nosrat didn't show any change (Fig. 3c).

Grain yield: Reduction by 17% was observed for yield of main spike by entering drought that was mainly due to the decrease of grain weight by 15%. Because grains per spike didn't change (Table 2).

Discussion

The start of WSC accumulation in different internodes is close to fulfillment of its length growth which is simultaneous with the rapid elongation of upper internodes (Bonnet and Incoll, 1993). Some factors affect the WSCc at anthesis and tenth day after anthesis, including the amount of sucrose inflow into stem affected by sink-source relationships and the sucrose allocation between reserving in parenchyma cells as water soluble carbohydrates and formation of structural tissues as celluloses and hemicelluloses. Since the accumulation of WSC in penultimate internode occurs in both before and after anthesis, the differences in these features can explain the different WSC concentrations.

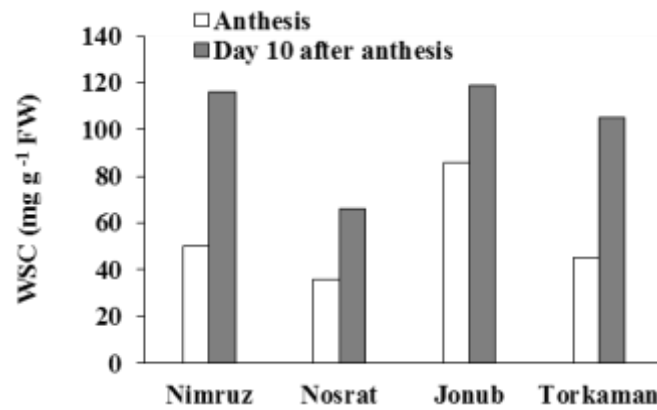


Fig. 1. Penultimate internode WSC concentration of barley cultivars at anthesis and 10 days post-anthesis. Values shown are means for three replicates. L.S.D._{0.05} = 10.08 and 20.34 for anthesis and 10 days post-anthesis, respectively.

Table 1. Penultimate content of mobilised WSC (MWSC) and remobilisation efficiency (RE) in barley cultivars under well-watered (WW) and water-stressed (WS) conditions

| Genotype | MWSC (mg g ⁻¹) | | | Re (%) | |
|----------------------|----------------------------|-----|------------------|------------------|-----------------|
| | WW | WS | Mean | WW | WS |
| Nimruz | 112 | 106 | 109 ^a | 91 ^b | 97 ^a |
| Nosrat | 44 | 65 | 54 ^b | 67 ^b | 97 ^a |
| Jonub | 116 | 118 | 117 ^a | 98 ^a | 98 ^a |
| Torkaman | 92 | 106 | 99 ^a | 95 ^{ab} | 93 ^a |
| Irrigation treatment | | | | | |
| WW | 90.83 ^a | | | 88 ^a | |
| WS | 98.35 ^a | | | 96 ^b | |

Means in each column, followed by similar letters are not significantly different at 5% probability level, using LSD test.

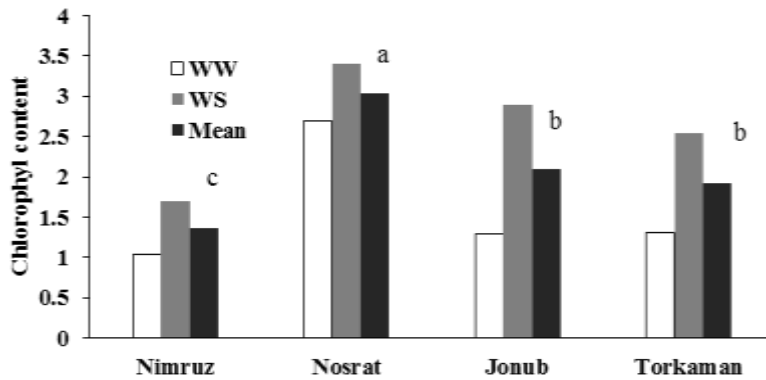


Fig. 2. Rate of chlorophyll loss of barley cultivars under well-watered (WW) and water-stressed (WS) conditions. Column not followed by the same letter present statistical difference as determined by least significant difference (LSD).

Pre-anthesis source is the photosynthesis of green leaves and the sinks include structural growing peduncle and tillers, the respiration of growing organs and WSC accumulation in penultimate internode. The relationship between these sources and sinks determines the amount of entering sucrose into parenchyma cells of penultimate internode. On the other hand, a genotypic variation was observed in sucrose partitioning (Xue *et al.*, 2008). The exported sucrose from photosynthetic organs can be either reserved in vacuole of parenchyma cells as WSC or spent on producing celluloses and hemicelluloses that lead to thickening of cell wall. These structural sucrose reserves can't be remobilised to

spike in the period of grain growth. Therefore, cultivar difference in penultimate internode WSCc at anthesis could be due to the different source power and physiological active sink power relationship in the period of time before anthesis. This point showed that in Jonub, perhaps its sources outweighing the sinks or allocating of sucrose to reserve were more than other genotypes.

The grain filling procedure has been partitioned into three phases: the lag phase, the effective grain filling period and the maturation drying phase (Zhang *et al.*, 2013). In the lag phase of grain filling the most powerful source forms because photosynthetic tissues

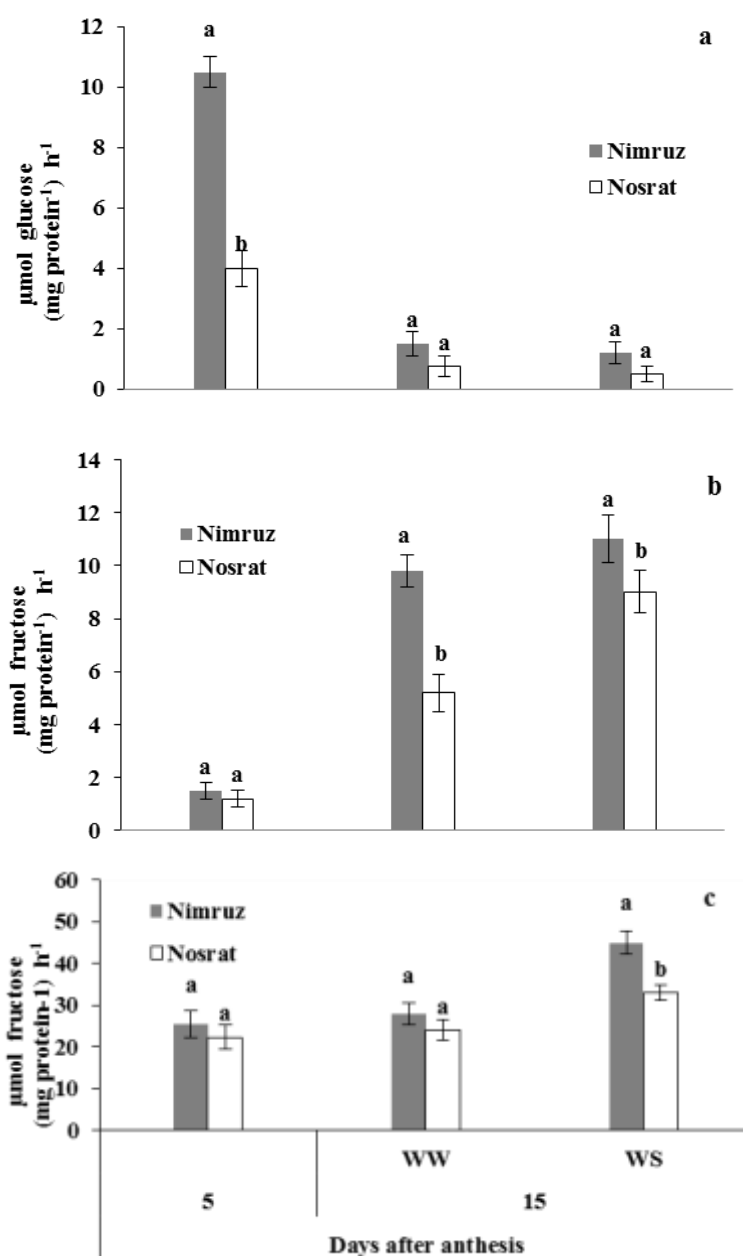


Fig. 3. Enzymatic activities of 1-SST (a); 1-FEH (b) and INV (c) in penultimate internode of barley cultivars under well-watered (WW) and water-stressed (WS) conditions. Column not followed by the same letter present statistical difference as determined by least significant difference (LSD). Vertical bars present \pm SE of the mean of three replicates

Table 2. Number of grains per main spike (NG), 1000-grain weight of main spike (GW) and grain yield of main spike (GY) in barley cultivars under well-watered (WW) and water-stressed (WS) conditions

| | NG (no.) | GW (gr) | GY (Kg/h) |
|----------------------|-----------------|------------------|-------------------|
| Genotype | | | |
| Nimruz | 22 ^b | 41 ^a | 815 ^b |
| Nosrat | 37 ^a | 35 ^{bc} | 1218 ^a |
| Jonub | 40 ^a | 33 ^c | 1191 ^a |
| Torkaman | 35 ^a | 37 ^b | 1173 ^a |
| Irrigation treatment | | | |
| WW | 34 ^a | 39 ^a | 1200 ^a |
| WS | 33 ^a | 34 ^b | 999 ^b |

Means in each column, followed by similar letters are not significantly different at 5% probability level, using LSD test.

including leaves, leaf sheets, green parts of spike and extrogen (the exposed part of the peduncle) produce

Table 3. Penultimate content of accumulated WSC (AWSC), WSC accumulation rate (WSCAR) and pre-anthesis WSC/total WSC in barley cultivars

| Genotype | AWSC (mg) | WSCAR (mg day ⁻¹) | Preanthesis WSC/total WSC (%) |
|----------|-----------------|----------------------------------|-------------------------------|
| Nimruz | 66 ^a | 7 ^a | 46 ^b |
| Nosrat | 30 ^b | 3 ^b | 54 ^b |
| Jonub | 33 ^b | 3 ^b | 74 ^a |
| Torkaman | 60 ^a | 6 ^a | 44 ^b |

Means in each column, followed by similar letters are not significantly different at 5% probability level, using LSD test

maximum photo-assimilates due to the maximum leaf area and the appearance of flag leaf. The amount of chlorophyll was at maximum level until 10 days after anthesis, then it started to decrease (data not shown). At this time the first sink, growing grain, as the most important sink of plant isn't powerful because of being in delay phase. Other active sinks in the mentioned period includes growing tissues, main spike respiration, producing structural tissues of tillers and WSC accumulation in stem. So under non-stressed conditions sources are much more powerful than sinks in this period of time which leads to more inflow of sucrose to vacuoles of parenchyma cells. Higher accumulation rate of WSC (mg per day) in Torkaman and Nimruz than Jonub (Table 3) can be due to more powerful sources of these cultivars than Jonub's. This feature compensated their low WSC at anthesis and resulted in setting of these two cultivars and Jonub in the same group considering maximum WSC.

Furthermore, the activity of fructan metabolizing enzymes determines the amount of fructan accumulation in the vacuoles of parenchyma cells. These enzymes include 1-SST that catalyses the production of smallest molecule of fructans (1-kestose) (Van den Ende *et al.*, 2004) and 1-FFT and 6-SFT that catalyze the lengthening of fructan chain by adding fructosyl units. The first and likely the most important step in fructan synthesis is catalysed by 1-SST (Wardlaw and Willenbrink, 1994). 1-SST enzyme is more important than other fructan producing enzymes and it is affected more by conditions (Wagner *et al.*, 1986). Sucrose goes to the place where fructans are synthesised in internodes that finish their growth. The start time of 1-SST activity is related to sucrose concentration and it is the time when sucrose reaches the maximum amount to make this enzyme induced. Positive relationship has been observed in wheat between sucrose concentration and 1-SST activity that shows sucrose not only is a substrate for fructan producing but also has a regulating effect on induction of 1-SST activity in wheat (Dubois *et al.*, 1990) and barley (Wagner *et al.*, 1986). The difference in increasing slope of WSC and total accumulated carbohydrates in this period (Table 3) was coordinated with 1-SST activity in Nimruz and Nosrat. The 1-SST activity of Nosrat was less than Nimruz on 5th day after anthesis (Fig. 3). That was due to less inflow of sucrose to vacuoles of parenchyma cells stemmed from the existence of stronger sink such as accumulation of structural carbohydrates in growing peduncle at that

time. The ratio of peduncle length to stem length in Nosrat (30%) was the highest among investigated cultivars (data not shown). Therefore, the structural growth of peduncle could be considered as a strong sink which can lower the sucrose inflow to penultimate internode for WSC reserving. Lower synthesis of fructans in penultimate internode could also be due to the lower 1-SST activity in equal inflow sucrose. Two reasons can be considered here. The first one may refer to the genotypically difference between cultivars in sensitivity of 1-SST to sucrose amount. It may mean Nosrat needs further amount of sucrose to induce 1-SST activity. The second reason is the difference in the location of sucrose and fructans. The former can exist in several locations including total of cell, apoplast and phloem while the latter can be seen only in vacuoles (Wagner *et al.*, 1986). Indeed, 1-SST is induced by the increase of cytoplasmic sucrose. 1-SST activity decreased significantly and stopped approximately on 15th day after anthesis in both Nimruz and Nosrat and also in both normal and water- stressed conditions (Fig. 3). This period is simultaneous with the decrease of WSCc. By entering linear phase of grain filling, growing grains become much stronger sinks than stem and make current assimilate get to grains for starch accumulation. As WSC reserving in internodes is weaker sink than growing grains and these two sinks don't compete on sucrose, entering sucrose flow to internodes and 1-SST activity stop. At the point of mobilised WSC from maximum time to physiological maturity, there were two different groups in genotypes. The increase of 47% in mobilised carbohydrates induced by drought in Nosrat (Table 1) was the most among cultivars. The amount of mobilised WSC in Nimruz and Nosrat under both well-watered and water-stressed conditions was in accordance with the difference in the activity of 1-FEH enzyme that hydrolyzed fructans. More activity of 1-FEH without significant difference in both circumstances led to more hydrolysis of fructan in Nimruz than Nosrat. On the other hand, an increase in 1-FEH activity induced by drought was consistent with the changes in fructan hydrolysis that was followed by more WSC remobilisation in Nosrat under drought conditions. In the second phase of grain filling when the entering sucrose decreases, FEH becomes active and causes decomposition of fructans to release more sucrose and fructose. Therefore, 1-FEH is important for maintaining carbon flow needed for grain filling. It has been shown

that the activity of 1-FEH was more under moderate and severe stress conditions than non-stressed conditions (Wardlaw and Willenbrik, 2000; Yang *et al.*, 2004) and this change accompanied by decrease of stem WSC and increase of their remobilisation from stem to grains (Yang *et al.*, 2004). During grain filling the gene expression in stem and especially in penultimate internode was more than in other organs (Van Riet *et al.*, 2008). Cultivars that showed higher gene expression related to 1-FEH enzyme under water-deficit conditions than normal conditions also had higher increase in remobilisation under water-stressed conditions than well-watered conditions. Dominate expression of 1-FEH can be an indicator of the high remobilisation efficiency of WSC (Zhang *et al.*, 2009). Drought can increase the WSC remobilisation by increasing the expression of 1-FEH. There is a positive relationship between abscisic acid (ABA) content and enzyme activity. Moreover, the activity of these enzymes increased significantly by external ABA. Use of fluridon (ABA synthesis inhibitor) had opposite effect as well (Yang *et al.*, 2004). The role of ABA in the regulation of FEH enzyme activity has been emphasized by Ruuska *et al.* (2008). ABA may have an important role in the increase of enzyme activity, but more investigation is needed for finding the start point of the way that leads to more production of ABA followed by processes including higher expression of 1-FEH during water stress. On the other hand, the amount of entering sucrose into fructan reserves locations can have opposite relationship with 1-FEH enzyme activity. It means that when the sucrose inflow decreases due to the decrease of source power, the activity of 1-FEH increases to produce sucrose for compensating the lack of sucrose for growing grain. 1-FEH activity can be regulated at two levels including the transcriptional level by abiotic stress and protein level by sucrose (Sonnewald, 1992). It means that maybe the increase of transcription of 1-FEH gene due to abiotic stress doesn't lead to increase of 1-FEH activity because of high level of sucrose that has protein level regulation. There is a complex regulation of fructan metabolic genes by abiotic stresses and overall sugar status of plant. Since the carbohydrates and hormonal signalling are closely related, hormonal balance most likely plays an important role as well (Valluru and Van den Ende, 2008). The role of ABA in induction of 1-FEH activity in wheat was also confirmed by other researchers (Ruuska *et al.*, 2008; Yang *et al.*, 2004).

Three enzymes are directly involved in sucrose metabolism. Sucrose phosphate synthase (SPS, EC 2.4.1.14) that has a main role in sucrose biosynthesis, sucrose synthase (SS, EC 2.4.1.13) that catalyses the reversible conversion of sucrose and UDP into fructose and UDP-glucose and invertase which hydrolyses irreversibly sucrose into glucose and fructose are included (Yang *et al.*, 2004). Sucrose is degraded by two enzymes, invertase and sucrose synthase. There is a range of invertase activity in higher plants that are

different in optimum pH, intracellular localization, solubility in buffer and the ability of catalyzing other glucosyl transition reactions. Sucrose synthase catalyses the sucrose synthesis. This reaction is reversible. It means that sucrose synthase can participate in sucrose production. Sucrose synthase exist in cytoplasm while invertase is located in vacuole, cell wall and cytoplasm. Their different places and patterns for sucrose degradation lead to different carbohydrate signals in cell and consequently in growth and development. Different trends of invertase activity from anthesis to maturity have been observed in experiments. The activity of acid invertase decreased during the period of fructan accumulation in stem while it increased simultaneously with the start of remobilization in ryegrass (*Lolium perenne* L.) (Prudhomme *et al.*, 1992) wheat (Simpson *et al.*, 1991) and in barley (Simmen *et al.*, 1993) Yet, some other researches on *Lolium temulentum* L. (Gallagher, *et al.*, 2004) and barley (Wagner *et al.*, 1986) didn't show any changes. Invertase might compete with fructosyl transferase enzymes (such as 1-SST) on sucrose. It means that this enzyme plays the role of fructan producing enzymes (Cairns and Ashton, 1993), although Simmen *et al.* (1993) showed that invertase didn't have any roles in fructan synthesis. Such an increase under water withholding was also observed in another investigation (Xue *et al.*, 2008) that led to more sucrose degradation. Persistent period of sucrose degradation and synthesis is a general characteristic of sucrose metabolism in lots of plant systems. Increase in the activity of both SPS and acidic invertase in stems might be related to the fast circulation of sucrose. Increase of invertase activity under stressed conditions is in accordance with sucrose degradation as the second part of carbohydrate reserves in Nimruz.

Cultivars showed two types of reactions to drought conditions in terms of mobilised WSC. Nosrat showed drastic increase of 47%. This feature was along with the least increase in chlorophyll loss leading to intermediate decrease of grain weight by entering drought stress (14%) (Fig. 2) that showed two sources of assimilates including stem reserves and current assimilates which were able to provide the needs of growing grains under drought conditions. The other cultivars didn't show any changes in mobilized WSC by entering drought. This characteristic was along with the high amount of increase in chlorophyll loss (94%) (Fig. 2) leading to the most decrease of grain weight (18%) by water stress in Torkaman. On the other hand, Jonub showed the least decrease in grain weight in spite of showing 120% increase in chlorophyll loss by drought (Fig. 2). This meant that stem reserves were mainly related to preanthesis period and they were able to provide the needs of growing grains even under stressed conditions. Finally, in Nimruz the intermediate increase in chlorophyll loss (Fig. 2) led to intermediate loss of grain weight. The amount of stem reserves, the remobilization and their changes induced by water withholding harmonized the activity of fructosyl transferase enzymes

and exohydrolase enzymes, but these changes were not necessarily in favour of maintaining grain weight similar to non-stressed conditions.

Conclusion

Cultivars were different in WSC concentration at anthesis and 10 days after anthesis. Drought conditions enhanced remobilisation efficiency. The activity of 1-SST harmonized the rate of WSCc increase in cultivars during the 10 days after anthesis. Water withholding enhanced the 1-FEH activity that catalysed the hydrolyzing of fructan. Considering invertase activity, a significant difference was observed between cultivars only on 5th day post-anthesis and water-stressed conditions. Meanwhile, only Nimruz showed increase in

invertase activity due to water stress which was related to degradation of sucrose as the second type of WSC and also the degradation and remobilisation of fructans. The decrease of 15% in grain weight led to decrease of 17% in grain yield of main stem. Cultivars showed different habits in use of current photoassimilates and reserved WSC in the second phase of grain filling that led to different changes in grain weight by entering drought conditions.

Acknowledgments

This work was supported by Shahid Chamran University of Ahvaz.

References

- Bancal, P. E. and Triboui, E. (1993) Temperature effect on fructan oligomer contents and fructan-related enzyme activities in stems of wheat (*Triticum aestivum* L.) during grain filling. *New Phytologist* 123: 247-253.
- Blum, A. (1998) Improving wheat grain filling under stress by stem reserve mobilization. *Euphytica* 100: 77-83.
- Bonnet, G. and Incoll, L. (1993) Effect on the stem of winter of barley of manipulating the source and sink during grain filling. 1. Change in composition of water-soluble carbohydrate of internodes. *Journal of Experimental Botany* 44: 83-91.
- Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram -quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248-254.
- Cairns, A. J. and Ashton, J. E. (1993) Species-dependent patterns of fructan synthesis by enzymes from excised leaves of oat, wheat, barley, and timothy. *New Phytologist* 124: 381-388.
- Dubois, M., Gilles, K., Hamilton, J., Rebers, P. and Smith, F. (1956) Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* 28: 350-356.
- Dubois, D., Winzeler, M. and Nosberger, J. (1990) Fructan accumulation and sucrose: sucrose fructosyltransferase activity in stems of spring wheat genotypes. *Crop Science* 30: 315-319.
- Gallagher, J. A., Cairns, A. J. and Pollock, C. J. (2004) Cloning and characterization of a putative fructosyltransferase and two putative invertase genes from the temperate grass *Lolium temulentum* L. *Journal of Experimental Botany* 55: 557-569.
- Huber, S. C. and Huber, J. L. (1996) Role and regulation of sucrose-phosphate synthase in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 47: 431-444.
- Kuhbauch, W. and Thome, U. (1989) Nonstructural carbohydrates of wheat stems as influenced by sink-source manipulations. *Journal of Plant Physiology* 134: 243-250.
- Prudhomme, M., Gonzalez, B., Billard, J. and Boucaud, J. (1992) Carbohydrate content, fructan and sucrose enzyme activities in roots, stubble and leaves of ryegrass (*lolium perenne* L.) as affected by source/sink modification after cutting. *Journal of Plant Physiology* 140: 282-291.
- Przulj, N. and Momcilovic, V. (2001) Genetic variation for dry matter and nitrogen accumulation and translocation in two-rowed spring barley I. Dry matter translocation. *European Journal of Agronomy* 15: 241-254.
- Ruuska, S., Rebetzke, G., van Herwaarden, A. and Richards, R. (2006) Genotypic variation in water-soluble carbohydrate accumulation in wheat. *Functional Plant Biology* 33: 799-809.
- Ruuska, S., David, L., Kennedy, G., Robert, T. and Furbank Jenkins, L. D. (2008) Effects of nitrogen nutrition on accumulation of stem carbohydrate reserves in reproductive stage wheat. *Plant Molecular Biology* 66: 15-32.
- Sadras, V. O. and Denison, R. F. (2009) Do plant parts compete for resources? An evolutionary viewpoint. *New Phytologist* 183: 565-574.
- Savitch, L. V., Harhey, T. N. P. and Huner, N. P. (2000) Sucrose metabolism in spring and winter wheat in response to high irradiance, cold stress and cold acclimation. *Physiologia Plantarum* 108: 270-278.
- Schnyder, H. (1993) The role of carbohydrate storage and redistribution in the source-sink relations of wheat and barley during grain filling. *New phytologist* 123: 233-245.
- Simmen, U., Obenland, D., Boller, T. and Wiemken, A. (1993) Fructan synthesis in excised barley leaves. *Plant Physiology* 101: 459-468.
- Simpson, B., Walkers, R. and Pollock, C. (1991) Fructan exohydrolase activity in leaves of *Lolium Temulentum* L. *New Phytologist* 89: 499-507.
- Sonnenwald, U. (1992) Expression of *E. coli* inorganic pyrophosphatase in transgenic plants alters photoassimilate partitioning. *The Plant Journal* 2: 571-581.

- Valluru, R. and Van den Ende, W. (2008) Plant fructans in stress environments: emerging concepts and future prospects. *Journal of Experimental Botany* 59: 2905-2916.
- Van den Ende, W., Barbara, D. C. and Andre, V. L. (2004) Plant fructan exohydrolases: a role in signaling and defense? *Trends in Plant Science* 9: 523-528.
- Van Riet, L., Altenbach, D., Vergauwen, R., Clerens, S., Kawakami, A., Yoshida, M., Van den Ende, W., Wiemken, A. and Van Laere, A. (2008) Purification, cloning and functional differences of a third fructan 1-exohydrolase (1-FEHw3) from wheat (*Triticum aestivum*). *Physiologia Plantarum* 133: 242-253.
- Wagner, W., Wiemken, A. and Matile, P. (1986) Regulation of fructan metabolism in leaves of barley (*Hordeum vulgare* L. cv Gerbel). *Plant Physiology* 81: 444-447.
- Wardlaw, I. F. and Willenbrink, J. (1994) Carbohydrate storage and mobilization by the culm of wheat between heading and grain maturity: The relation to sucrose synthase and sucrose-phosphate synthase. *Australian Journal of Plant Physiology* 21: 255-271.
- Wardlaw, I. and Willenbrik, J. (2000) Mobilization of fructan reserves and changes in enzyme activities in wheat stems correlate with water stress during kernel filling. *New Phytologist* 148: 413-422.
- Xue, G. P., McIntyre, C. L., Jenkins, C. L. D., Glassop, D., van Herwaarden, A. and Shorter, R. (2008) A molecular dissection of variation in carbohydrate metabolism related to water-soluble carbohydrate accumulation in stems of wheat (*Triticum aestivum* L.). *Plant Physiology* 146: 441-454.
- Yang, J., Zhang, J. and Wang, Z. (2004) Activities of fructan- and sucrose-metabolizing enzymes in wheat stems subjected to water stress during grain filling. *Planta* 22: 331-343.
- Zhang, J., Dell, B., Conocono, E., Waters, I., Setter, T. and Appels, R. (2009) Water deficits in wheat: fructan exohydrolase (1-FEH) mRNA expression and relationship to soluble carbohydrate concentrations in two varieties. *New Phytologist* 181: 843-850.
- Zhang, Z. h., Liu, Z., Cui, Z., Hu, Y., Wang, B. and Tang, J. (2013) Genetic analysis of grain filling rate using conditional qtl mapping in maize. *PLoS One* 8 1-10.