



Transcript Profiling of Genes Encoding Fructan and Sucrose Metabolism in Wheat Under Terminal Drought Stress

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Abstract

Maintaining photosynthetic performance and remobilization of assimilates stored in vegetative tissues are strategies of superior wheat genotypes under drought stress conditions. To better understand the response of vegetative tissues to drought stress at the grain filling period, transcript profiling of genes encoding fructan and sucrose metabolism were studied in the stem (penultimate internode) and root of two drought-tolerant genotypes. Based on a preliminary screening, the cultivars T-65-7-1 (the mutant line) and Tabasi (wild type) were selected for further study with respect to the parameters associated with photosynthesis and stem remobilization under rain-fed conditions. The expression of photosynthetic genes, chlorophyll content and relative water content were sharply reduced in the T-65-7-1 compared to Tabasi, as a result of drought-induced leaf senescence. Under drought stress, fructan remobilization in the stem and root of T-65-7-1 was significantly higher than Tabasi, which was due to the over-expressed genes involved in the synthesis and hydrolysis of fructan, as well as the synthesis, hydrolysis and transport of sucrose. The stem and root tissues depicted similar assimilate remobilization behaviours under drought stress. The grain yield reduction was less in T-65-7-1 than Tabasi under drought stress during the grain filling period, therefore, the remobilization of assimilates to the grains was a more effective strategy than maintenance of photosynthesis under drought stress conditions during the grain filling period. This research provides valuable molecular indicators for selecting drought-tolerant wheat genotypes with high fructan content and increased remobilization in wheat breeding programs.

Keyword Remobilization · Gene expression · Photosynthesis · Drought stress

Introduction

The majority of the world's wheat (*Triticum aestivum* L.) is produced under rain-fed conditions, especially in the Mediterranean climate, where the crop faces drought stress during the grain filling period (Blum 1998). Current photosynthesis of leaves (mainly flag leaf) and the remobilization of stored materials in vegetative tissues (that is, stem, leaves and roots) are the two main sources of carbon

supply for the filling of grains (Wardlaw and Willenbrink 2000; Zhang et al. 2016). Plant varieties employ different strategies when facing drought, either via maintaining photosynthetic performance by using photoassimilates or through the remobilization of assimilates stored in vegetative tissues to grains (Yang and Zhang 2006; Bazargani et al. 2011; Farooq et al. 2014). Drought stress can induce early senescence and increase the remobilization of assimilates to grains (Yang and Zhang 2006; Bazargani et al. 2012; Distelfeld et al. 2014). The most important event that occurs during leaf senescence is degradation of the photosynthetic apparatus. Despite the decrease of photosynthesis in plants under drought stress, a large amount of water-soluble carbohydrates (WSCs), including glucose, fructose, sucrose and fructan, accumulates in vegetative tissues (Yang et al. 2004; Joudi et al. 2012). WSCs may temporarily accumulate in the stem, leaf sheath and roots of wheat from stem elongation to the early stages of grain filling and might be utilized for grain filling (Gebbing 2003; Xue et al. 2013). The WSCs of wheat in vegetative tissue are mostly comprised of

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fructan (Chalmers et al. 2005; Van den Ende and El-Esawe 2014). Wheat fructans are made in vacuoles and are mainly graminan-types (Verspreet et al. 2013). Fructans are derived from sucrose, linear or branched polysaccharides based on fructose with β -(2–1) and β -(2–6) linkages (Verspreet et al. 2013).

The biosynthesis of fructan is done by the action of sucrose:sucrose 1-fructosyltransferase (1-SST), sucrose:fructan 6-fructosyltransferase (6-SFT) and fructan:fructan 1-fructosyltransferase (1-FFT) (Van den Ende et al. 2003; Xue et al. 2008a, b). First, 1-SST produces trisaccharide 1-kestotriose, and then 1-kestotriose acts as a substrate for 6-SFT and produces the branched trisaccharide bifurcose (1 and 6 kestotetraose). The enzyme 6-SFT also produces 6-kestotriose from sucrose. 1-Kestotriose also acts as a substrate of the enzyme 1-FFT and leads the production of 1,1-kestotetraose (Ritsema and Smeekens 2003; Valluru and Van den Ende 2008; Xue et al. 2013). Furthermore, fructan is hydrolyzed by fructan 1-exohydrolases (1-FEHs) and fructan 6-exohydrolases (6-FEHs) and is transformed into sucrose and fructose (Van den Ende et al. 2003; Zhang et al. 2009).

Sucrose is synthesized from fructose-6-phosphate and uridine diphosphate glucose (UDP-Glc) in plant tissues by sequential actions of sucrose phosphate synthase (SPS) and sucrose 6-phosphate phosphohydrolase (SPP). In addition, sucrose can be synthesized from the combination of UDP-Glc and fructose by the action of SuS (Xue et al. 2008a, b). The hydrolysis of sucrose to α -D-glucose and fructose is facilitated by different invertase enzymes, of which vacuolar invertase (INV) plays the most important role (Königshofer and Löppert 2015). Sucrose is the most important sugar in the phloem, and is transiently stored in the vacuole (Lemoine et al. 2013). Sucrose transport (SUT) from vacuoles to the cytosol is done by SUT2, which is located in the tonoplast (Deol et al. 2013). Moreover, during phloem loading, the transport of sucrose across the plasma membrane occurs through SUT1 (Aoki et al. 2004).

Studies have been performed on the expression of some genes involved in the biosynthesis, hydrolysis and transport of important carbohydrates in wheat during the grain filling period under terminal drought stress (Xue et al. 2008a, b, 2013; Zhang et al. 2009; Khoshro et al. 2014). However, the gene expression patterns have been studied in the leaf and stem, but there have been no studies related to the genes involved in carbohydrate metabolism and remobilization under drought stress in the root system of wheat. In this study, two drought-tolerant genotypes with contrasting behaviours in current flag leaf photosynthesis and remobilization of stored assimilates under rain-fed conditions were selected for further study based on a preliminary screening. To better understand the response of vegetative tissues to drought stress at the grain filling period, the transcription

profiles of genes encoding fructan and sucrose metabolism were studied by quantitative real-time PCR (qRT-PCR) in the stem, penultimate internode (pn) and root of the two selected genotypes with the aim of (1) comparing the behaviours of stem and root tissues in the remobilization of assimilates to the grain and (2) determining a suitable strategy for the development of wheat genotypes under drought stress during grain filling.

Materials and Methods

Two experiments were carried out at the research farm and greenhouse of Gorgan University of Agricultural Sciences and Natural Resources (36°54'N, 54°24'E and 13 m) from 2012 to 2015.

Field Experiment

The Tabasi mutated lines were produced by the Institute of Agricultural, Medical and Industrial Research, Tehran, Iran. These mutant lines were the result of a mutation breeding program by Co-60 gamma radiation, 300 Gray at 55 rad min⁻¹ (Daei et al. 2009). Tabasi is a spring cultivar with an acceptable level of drought tolerance (Korde-naeej et al. 2008). Fifteen mutant lines (M6) of bread wheat along with their wild type (Tabasi cultivar) were grown under rain-fed conditions for 2 years (2012–2014) in the field (listed in Supplemental Table 1). In this area, the grain filling period occurs during June and July, and the average daily temperatures and precipitation in June 2013/2014 were 26.4/26.6 °C and 12.2/7.8 mm, respectively. In July 2013/2014, the average daily temperature was 27.5/28.1 °C and there was no precipitation. The experiment was conducted in a randomized complete block design (RCBD), with three replications. There were five rows in each plot, the distance between rows was 20 cm, and the length of each row was 2 m. The measurements were randomly taken from the tagged main stems with the same length in four stages (each experimental unit consisting of eight plants) after anthesis at intervals of 10 days (at 10, 20, 20 and 40 days after anthesis) from the stem and the flag leaf. To determine the dry weight, stem samples were first dried in an oven at 80 °C for 48 h and then weighed. Remobilization based on specific weight was calculated as the difference between the maximum and minimum of specific weight of stems after anthesis (Ehdaie et al. 2006a, b). The specific weight was calculated by dividing the stem weights by their lengths. The remobilization efficiency was calculated as the ratio of remobilization to the maximum specific weight (Ehdaie et al. 2006a, b). Furthermore, to determine the photosynthetic capacity of the flag leaf, stomatal conductance was measured using a porometer (Delta T AP4, UK), and the

chlorophyll content was obtained using a chlorophyll meter (SPAD CCM-200, USA), reporting the average of the four stages. Genotypes were plotted against stomatal conductance and chlorophyll content of the flag leaf, remobilization and remobilization efficiency of the main stem. Consequently, the T-65-7-1 mutant line (with a high remobilization and a low photosynthetic capacity) and the Tabasi wild type (with a low remobilization and a high photosynthetic capacity) were selected for further study.

Greenhouse Experiment

The two selected genotypes (Tabasi, the wild type, and T-65-7-1, the mutant line) were grown in a greenhouse in a completely randomized design (2×2 factorial experiment) with five replications during the growing season of 2014–2015. Each pot was filled with 10 kg of soil containing a combination of clay, loamy sand and animal fertilizer at a 1:2:1 ratio. The diameter and depth of the pots were 28 and 40 cm, respectively, and there were 10 plants in each experimental unit (pot). The plants were grown in the controlled greenhouse environment with 16 h of light (28 °C) and 8 h of dark (18 °C). The relative humidity was 55–60% and the photon flux density was approximately $250 \mu\text{mol m}^{-2} \text{s}^{-1}$. The plants were grown in the same conditions under well-watered conditions until stress was imposed. Plants were irrigated three times with 1/2 Hoagland solution (Supplemental Table 2) until flag leaf emergence (Zadoks 37).

Imposing Stress and Sampling

Imposing stress by terminating irrigation was initiated at the Zadoks 60 (full heading emergence) stage (Zadoks et al. 1974). The main stems of the plants in each pot were tagged as the first spikes emerged from the flag leaf sheaths. The soil moisture of plots under well-watered conditions was kept at 90% field capacity (FC) through regular irrigation, whereas under drought stress conditions, the soil moisture was held at 40% of the field capacity using the procedure explained by Bazargani et al. (2011) through regular weighing. Random sampling of flag leaf, pn internode (from the main stem) and roots occurred at five stages (S1–S5) from anthesis to ripening at 7-day intervals (0, 7, 14, 21, and 28 days), with each plot consisting of five plants. According to previous studies, among the stem internodes, the pn internode has the highest level of stored carbohydrates and the highest remobilization efficiency; therefore, in this study, the pn internodes (with leaf sheath) were used (Blum 1998; Wardlaw and Willenbrink 2000; Scofield et al. 2007; Sharbatkhari et al. 2016). Roots with soil sticking to them were rapidly put on a wire mesh and washed with water until the soil was completely eliminated (Ehdaie et al. 2012). Tissue

samples needed for gene expression evaluation were kept in -80 °C after being frozen in liquid nitrogen.

Fructan Remobilization and Remobilization Efficiency of the pn Internode

Fructan remobilization of the pn internode was calculated as the difference between the maximum and minimum fructan concentrations after anthesis. Remobilization efficiency was the ratio of remobilization to the maximum fructan concentration (Ehdaie et al. 2006).

Chlorophyll Content and Relative Water Content (RWC) of the Flag Leaf

The flag leaf chlorophyll content was measured using the spectrophotometric method and by employing pure acetone (Lichtenthaler 1987). The leaf RWC was measured using the method described by Dhanda and Sethi (1998).

Water Soluble Carbohydrate (WSC), Sucrose and Fructan Contents

The total WSCs of stems (pn) and roots was extracted using water at 70 °C and measuring the concentration of WSCs with the phenol–sulphuric acid approach (Dubois et al. 1990). Glucose, fructose and sucrose were measured in accordance with the method described by Hajirezaei et al. (2000). Glucose and fructose were measured by determining the D-glucose and D-fructose contents due to the activity of hexokinase, phosphoglucose isomerase and glucose 6-phosphate dehydrogenase. The NADPH content formed in this reaction is stoichiometric with the content of D-glucose D-fructose. It is the NADPH which is determined by the increase in absorbance at wavelengths of 340 nm. Sucrose is hydrolyzed to D-fructose and D-glucose using β -fructosidas. By subtracting the amounts of D-fructose and D-glucose obtained from the previous reaction, the amount of sucrose was calculated. Fructans and sucrose were hydrolyzed to glucose and fructose by perchloric acid (HClO_4). Increasing the concentration of fructose indicated the concentration of sucrose and fructans, and consequently the concentration of fructans was obtained by subtracting the amount of the primary proportion of sucrose and fructose in the sample.

Quantitative Real-Time PCR Analysis

To evaluate the expression of genes, total RNA was extracted from 100 mg of the samples prepared using the P-Biozol kit (Bio Flux-Bioer, Tokyo, Japan). After DNaseI treatment, the quantity and quality of extracted RNA were determined by spectrophotometry and agarose gel electrophoresis. The cDNA strand was synthesized with an RT reagent kit

(Fermentas company, Vilnius, Lithuania) according to the manufacturer's protocol. Quantitative real-time PCR (qRT-PCR) was done using SYBR Green I in a 20 µl reaction volume that contained 1× of SYBR Bio Pars (GUASNR). Reactions were carried out in an iCycler iQ5 thermocycler (Bio Rad Company). Specific primers used to assess the transcript profiles of fructan (*1-SST*, *6-SFT*, *1-FFT*, *1-FEHw3*, *6-FEH*), sucrose (*SPS*, *SPP*, *SuS*, *INV*, *SUT1*, *SUT2*) and photosynthetic genes involved in the Calvin cycle (*RBCL*: Ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit, *RBCS*: Ribulose 1,5-bisphosphate carboxylase/oxygenase small subunit, *RCA*: Ribulose bisphosphate carboxylase activase) are shown in Table 1. Some of these primers were used in other studies (Table 1), and others were designed using the software AllelID7 (PREMIER Biosoft, Palo Alto, CA, USA). Genes involved in the metabolism of sucrose and

fructan were studied in the stem (pn internode along with leaf sheath) and root, and photosynthesis genes were studied only in the flag leaf. The housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as an internal control (Gonçalves et al. 2005; Zhang et al. 2009). Gene expression was assessed by $2^{-\Delta\Delta CT}$ (Pfaffl 2001) in relation to the control plants at the same stage with three biological replicates (each replicate consisted of four pooled plants).

Statistical Analysis

Gene expression data were analyzed using GenEx 6 (MultiDAnalyses, Goteborg, Sweden). The analysis of morphophysiological data was conducted using SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA) with proc GLM, and the

Table 1 Specific primer pairs used in the study of transcript profiling of fructan and sucrose metabolism in wheat

Primer name	Sequence (5' → 3')	GenBank accession no.	Tm (°C)	Product length (bp)	References
<i>1-SST</i>	F: GCGACTCTGCCTATCACTTC R: CATAGCCCTGTCATCAACAC	AB029888.1	60	88	
<i>6-SFT</i>	F: CGATCACTCGTATGTTCATG R: CACGGATAGATGTTTCTGTTC	AB029887.1	60	118	
<i>1-FFT2</i>	F: GGTCCTGGAGCTTCCTTTTAT R: CCGGAACCAAAAACATAAATCAA	AB088410.1	60	93	Xue et al. (2008a)
<i>1-FEHw3</i>	F: CCGCGTTAGTGCGGGACA R: GCCTGATTTTGATCTATGTCAC	FJ184990.1	59	121	Zhang et al. (2008)
<i>6-FEH</i>	F: GAGCACACGGCTATCTTCTTCA R: CCACTACCGAATGGTCAATCAA	AM075205.1	60	193	Khoshro et al. (2014)
<i>SPS</i>	F: AAGCGGCGATACAGATTATGAA R: GATACGACATCCTCTAGCGAATA	HG670306.1	60	129	
<i>SPP1</i>	F: AAGAAATCGTAGCGAAAGCATGA R: CGTAGACATCATCCAGCCCAGTA	AF321556.1	60	98	Xue et al. (2008a)
<i>SUS1</i>	F: GGACAGGAACAAGCCAATCATC R: TCTTGCCGTACATCTCAACCAA	AJ001117.1	60	86	Xue et al. (2008a)
<i>INV</i>	F: GACGACTCCTACAACCAGAT R: CTCCATCTCCATCCATCCAT	AF069309.1	60	173	
<i>SUT1</i>	F: CGCATAGGCGTAACTAGGG R: CACATACAGACACAACACATACA	AF408842.1	60	91	
<i>SUT2</i>	F: TACGGAGTCTGCTCTGTCA R: CTCGTCGCTTCCGAAAGTA	KJ812204.1	60	130	Deol et al. (2013)
<i>RBCL</i>	F: GGTGGAGGAACTTTAGGACAT R: TCGCCTTCCATACTTCAACA	AY328025.1	60	187	
<i>RBCS</i>	F: ACTGGACAATGTGGAAGC R: ACTCCTTCTTGACCTCCTC	AB042069.1	60	84	
<i>RCA</i>	F: TACGACATCTCCGATGACCA R: CTCGTAGGAGCTCAGGATGG	KT835665.1	60	114	Kumar et al. (2016)
<i>GAPDH</i>	F: TCACCACCGACTACATGACC R: ACAGCAACCTCCTTCTCACC	KR029492.1	60	121	

F forward primer, R reverse primer

comparison of means was performed using LSD at a 5% probability level.

Results

Current Photosynthesis and Remobilization of Assimilates

Based on the results of the field experiment, the genotypes differed in their parameters associated with photosynthesis (Supplemental Table 1) and stem remobilization under rain-fed conditions. For instance, the flag leaf SPAD value in genotypes varied between 26.95 and 42.88. The flag leaf stomatal conductance of genotypes ranged from 25.87 to 63.34 $\text{m mol H}_2\text{O m}^{-2} \text{s}^{-1}$ (Fig. 1a). Furthermore, the SPAD value and the stomatal conductance of the flag leaf as photosynthetic parameters had a strong relationship ($r=0.87^{**}$) (Fig. 1a). The stem remobilization of genotypes varied from 3.66 to 6.92 mg cm^{-1} , and the remobilization efficiency varied between 19.61 and 34.13% (Fig. 1b). The correlation between remobilization and remobilization efficiency was also strong ($r=0.80^{**}$) (Fig. 1b). The behaviours of Tabasi and T-65-7-1 were completely opposite with respect to these parameters. Tabasi depicted a high current photosynthesis and a low remobilization efficiency whereas T-65-7-1 showed lower current photosynthesis and higher remobilization efficiency (Fig. 1). However, the two genotypes did not differ in terms of flowering time and fertile tillers (data not shown). Based on the results of the field experiment, the

T-65-7-1 mutant line and the Tabasi wild type were selected for further study in the greenhouse environment.

Grain Yield, Flag Leaf Chlorophyll Content, and RWC in T-65-7-1 and Tabasi

Under both moisture conditions, the grain yield of T-65-7-1 was significantly higher than Tabasi (Fig. 2a). Drought stress reduced grain yield per plant in the both genotypes, and this yield reduction was lower in T-65-7-1 (17.43%) than Tabasi (25.34%) (Fig. 2a). Two genotypes under well-watered conditions had no significant differences in chlorophyll content during the grain filling period excluding S5 (Fig. 2b). Drought stress conditions significantly reduced chlorophyll content in T-65-7-1 at all stages, but the decline in Tabasi was not significant at S1, S2 and S3 (Fig. 2b). Similarly, under well-watered conditions, these two genotypes were not significantly different in terms of RWC (Fig. 2c). Flag leaf RWC decreased in T-65-7-1 at all stages under drought stress conditions (Fig. 2c). Flag leaf RWC of Tabasi was significantly higher than T-65-7-1 during the grain filling period under drought stress conditions (Fig. 2c).

Fructan and Sucrose Contents of the Stem (pn Internode) During the Grain Filling Period

Generally, under well-watered conditions, the trend of fructan changes in the stem (pn) was similar for both genotypes, in which after anthesis and during grain filling, the stem (pn) fructan started to increase and peaked at S3, and then, after decreasing, reached a minimum at

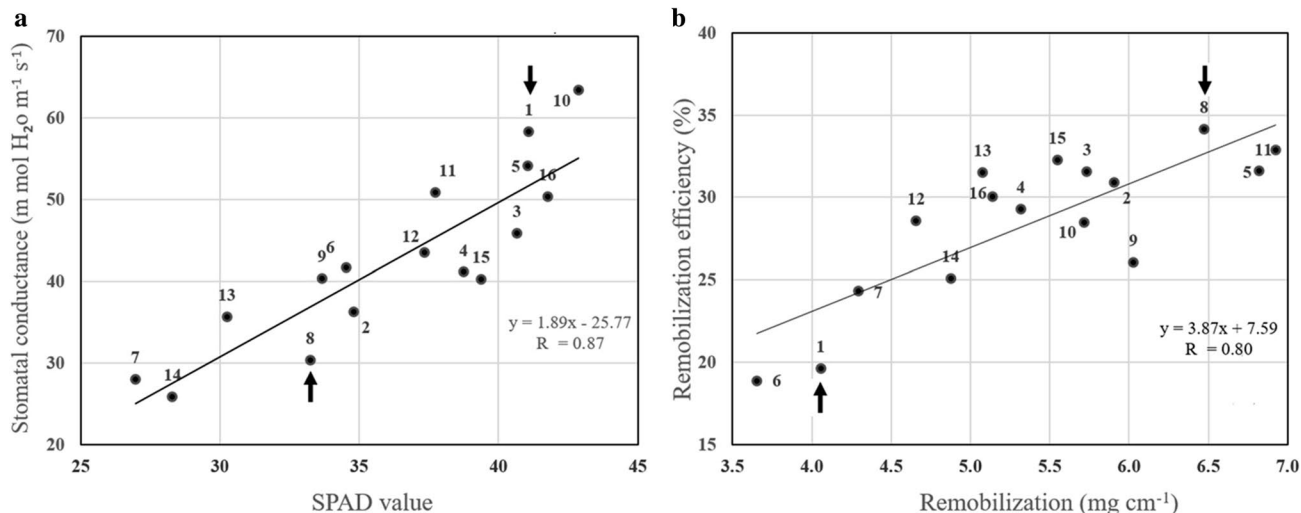


Fig. 1 Scatter plots: **a** stomatal conductance and SPAD value, **b** remobilization and remobilization efficiency in the main stem of 16 wheat genotypes over two growing seasons (2012–2014) under natural rain-fed conditions. Each point represents a mean computed from

192 (a) and 48 (b) independent main stems. Arrows show genotypes, 1 (Tabasi; wild type) and 8 (T-65-7-1; mutant line). Remobilization = maximum specific weight – specific minimum weight. Remobilization efficiency = (remobilization/maximum specific weight) \times 100

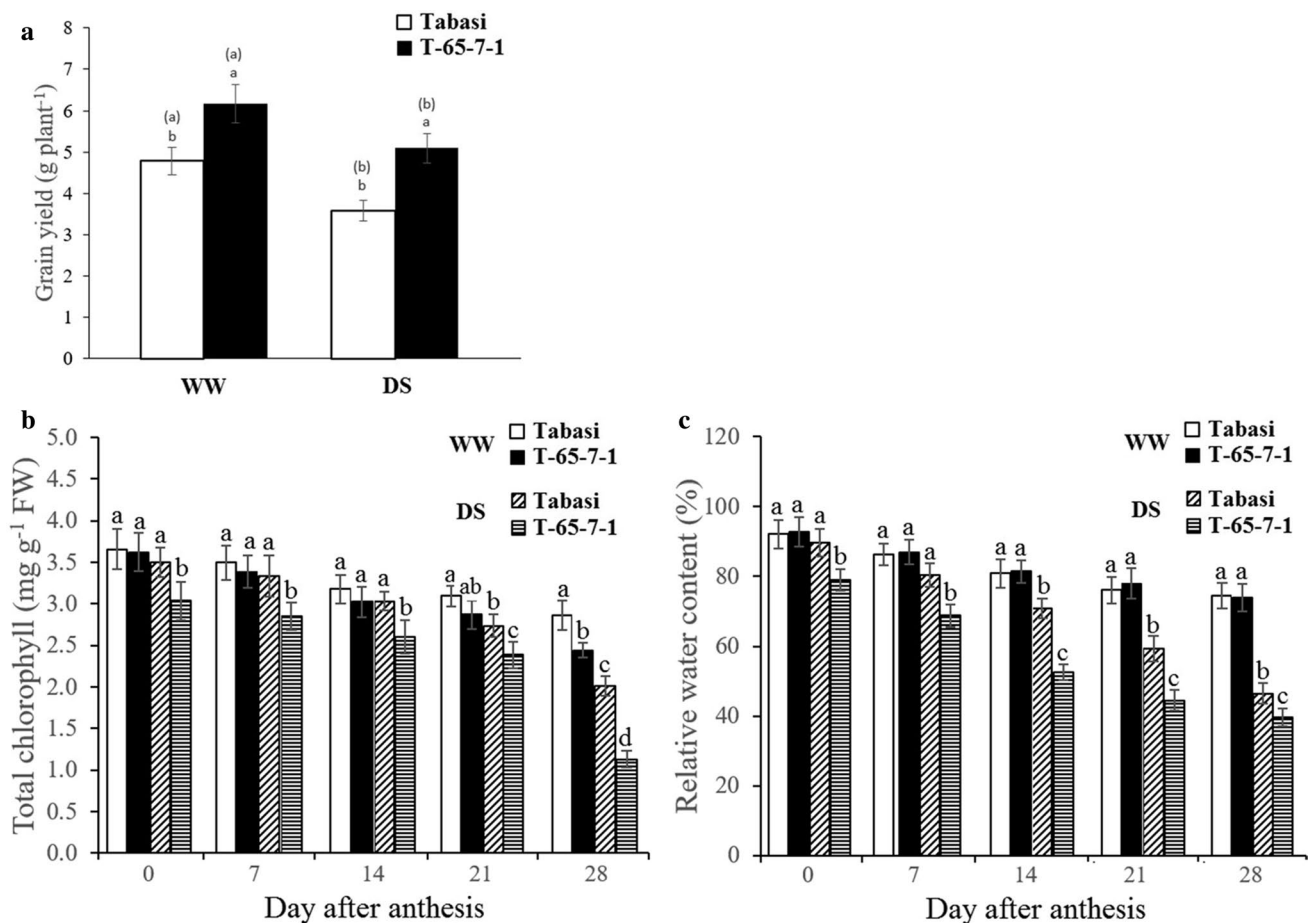


Fig. 2 Grain yield (a), chlorophyll content (b) and relative water content (c) in Tabasi and T-65-7-1 in well-watered (WW) and drought stress (DS) conditions in the controlled greenhouse environment. The vertical lines represent \pm SE of the mean of three replicates. Stages represent days after anthesis 0 (S1), 7 (S2), 14 (S3), 21 (S4) and 28

(S5), respectively. Means followed by same letters are not significantly different ($P < 0.05$). Same letters in parentheses indicate non-significant difference between each genotype under WW compared to DS ($P < 0.05$)

S5 (Fig. 3). Under both moisture conditions, the peak of fructan concentration in T-65-7-1 was greater than Tabasi. The fructan concentration under drought stress conditions reached the peak earlier at S2 in T-65-7-1. Additionally, the fructan concentration in T-65-7-1 decreased more sharply after attaining the peak under drought stress conditions (Fig. 3a). Under well-watered conditions, the differences in fructan remobilization and remobilization efficiency of stem tissues (pn) in the two genotypes were not significant, but under drought stress conditions, remobilization was significantly greater in T-65-7-1 than Tabasi (Fig. 5a).

The sucrose concentration of the stem (pn) reached the peak earlier in T-65-7-1. After anthesis, T-65-7-1 (at S3) and Tabasi (at S4) peaked and then reached a minimum at S5 (Fig. 3b). The peak of sucrose concentration in both conditions was greater in T-65-7-1 than Tabasi. Drought increased the concentration of sucrose at the S2 and S3 stages in both

genotypes. Compared to Tabasi, the sucrose concentration of T-65-7-1 decreased more intensively after peaking (Fig. 3b).

Fructan and Sucrose Contents in the Root During the Grain Filling Period

Under both moisture conditions, trends in fructan changes of roots were similar for both genotypes. The peak of fructan concentration was observed at S2 (Fig. 4a). Drought stress conditions caused a significant increase in the fructan concentration of T-65-7-1, so that the peak of fructan concentration in roots of T-65-7-1 was significantly greater than Tabasi (Fig. 4a). Drought stress conditions caused a significant increase in sucrose concentrations in T-65-7-1 at S2 and S3. Compared to Tabasi, T-65-7-1 sucrose concentrations decreased more sharply after peaking under drought stress conditions (Fig. 4b). Additionally, similar to the stem (pn) under well-watered conditions, the two genotypes did not significantly differ in fructan

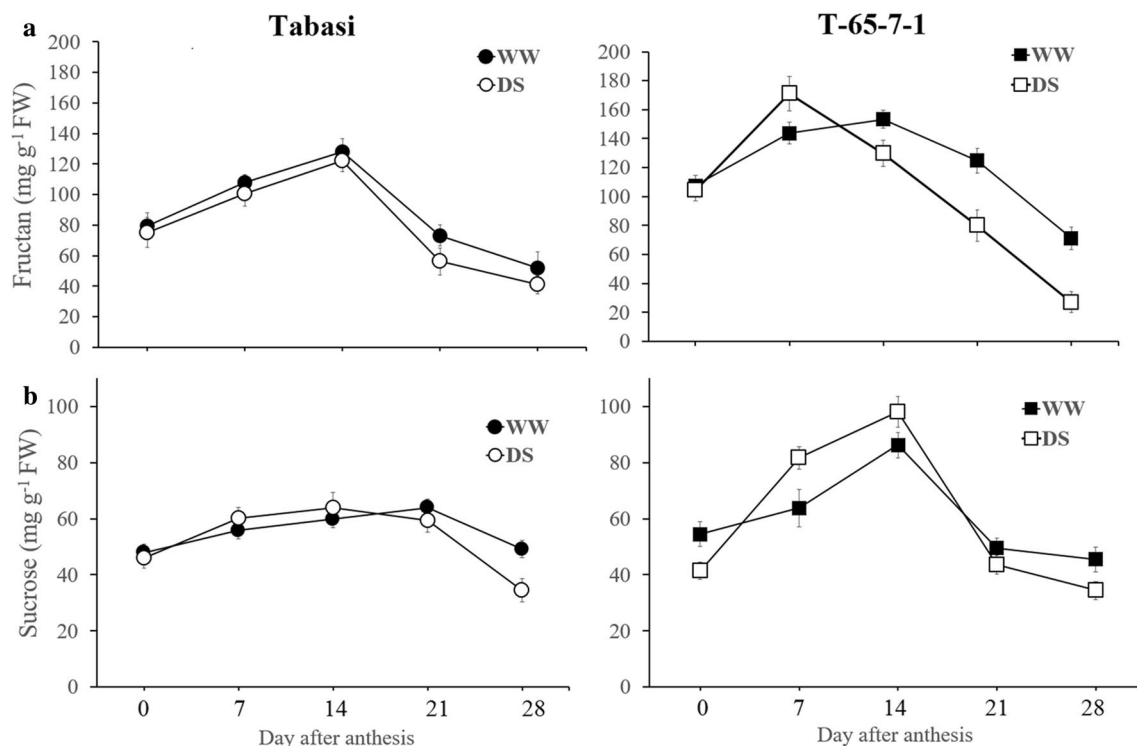


Fig. 3 Fructan (a) and sucrose (b) contents of the stem (pn) in Tabasi and T-65-7-1 in well-watered (WW) and drought stress (DS) conditions. Stages represent days after anthesis 0 (S1), 7 (S2), 14 (S3), 21

(S4) and 28 (S5), respectively. The vertical lines represent \pm SE of the mean of three replicates

remobilization and remobilization efficiency of the root, but under drought stress conditions, remobilization was significantly higher in T-65-7-1 than Tabasi (Fig. 5b).

Expression of Photosynthetic Genes Under Drought Stress in the Flag Leaf

The expression of photosynthetic genes showed that the photosynthetic apparatus of T-65-7-1 was affected more quickly than Tabasi under drought stress (Fig. 6). For T-65-7-1, drought stress caused a significant reduction in the expression of photosynthetic genes after S2, while Tabasi exhibited a significant reduction at S4 and S5 (Fig. 6). The reduction in expression of *RBCL*, *RBCS* and *RCA* at S2 to S5 was significantly higher in T-65-7-1 than Tabasi (except at S2 for *RBCL*). As the plants reached the final stages of the grain filling period, the differences in expression of photosynthetic genes became greater between T-65-7-1 and Tabasi (Fig. 6).

Expression of Genes Involved in the Metabolism of Sucrose and Fructan Under Drought Stress in the Stem (pn Internode) and Root

The expression of key genes involved in fructan biosynthesis (*1-SST*, *6-SFT* and *1-FFT*) in the stem (pn) and root

showed that drought stress conditions caused a significant increase in the *1-SST* and *6-SFT* genes (especially at S2, S3 and S4), whereas the expression of the *1-FFT* gene was not significantly changed due to drought stress (Fig. 7). The expression of *1-SST* and *6-SFT* in the stem (pn) and root of T-65-7-1 peaked earlier (S2), whereas the peak of expression was observed at S3 in Tabasi. In general, the expression of genes involved in fructan biosynthesis under drought stress conditions in the stem (pn) and root of T-65-7-1 was equal to or greater than Tabasi at all of the stages (Fig. 7). Under drought stress conditions, expression of *1-FEHw3* in T-65-7-1 was significantly greater than Tabasi in both tissues at S3, S4 and S5 (Fig. 8). Also expression of *6-FEH* in the stem (pn) of T-65-7-1 was significantly higher than Tabasi at S1, S3, S4 and S5, and in the root at S3, S4 and S5 (Fig. 8).

Under drought stress conditions, the gene expression changes of *SPS* and *SPP* were similar in both stem (pn) and root tissues, peaking at S3 and then declining. *SPS* gene expression was significantly higher than Tabasi in the stem (pn) of T-65-7-1 at stages S3 to S5, and in the root at all stages excluding S5 (Fig. 9). Under drought stress conditions, *SPP* gene expression in the stem (pn) and root was significantly higher in T-65-7-1 than Tabasi (Fig. 9) at all stages excluding S5 for the root. Significant changes in *SuS* gene expression were not observed in the stem (pn)

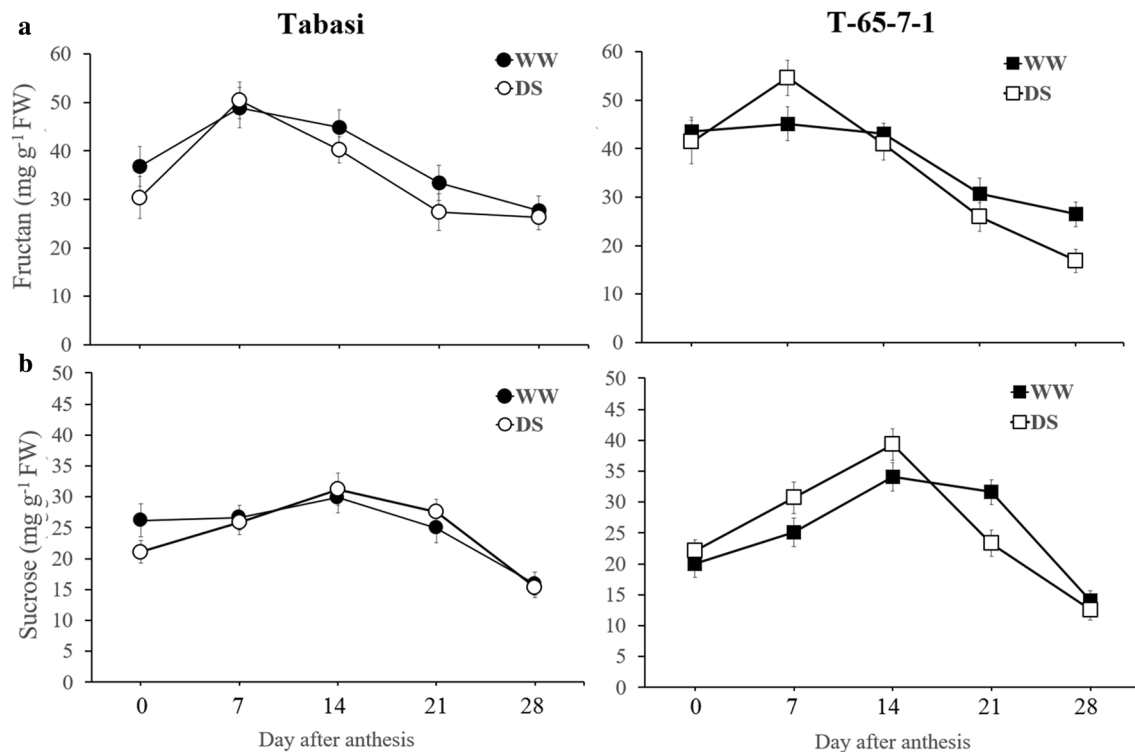


Fig. 4 Fructan (a) and sucrose (b) contents of the root in Tabasi and T-65-7-1 in well-watered (WW) and drought stress (DS) conditions. Stages represent days after anthesis 0 (S1), 7 (S2), 14 (S3), 21 (S4)

and 28 (S5), respectively. The vertical lines represent \pm SE of the mean of three replicates

or root of both genotypes under drought stress conditions (Fig. 9). Expression of *INV* increased under drought stress conditions at all of the stages in the stem (pn) and root of T-65-7-1. In T-65-7-1, *INV* gene expression was higher than Tabasi in both tissues since the expression of Tabasi remained almost constant during the grain filling period (Fig. 9).

The expression patterns of sucrose transporter genes (*SUT1* and *SUT2*) were almost identical in both genotypes (Fig. 10). After anthesis in T-65-7-1, the sucrose transporter gene expression increased as a result of drought stress, peaked at S3, and then decreased (Fig. 10). Changes in *SUT1* gene expression in the stem (pn) and root of the wild type under drought stress conditions were not significant at any of the stages but were significantly higher in the stem (pn) (S3 and S4) and root (S2, S3 and S4) of T-65-7-1 and were higher than Tabasi (Fig. 10). Under drought stress conditions, *SUT2* gene expression in the stem (pn) and root of Tabasi increased significantly at only one stage (stem S2, root S3). However, *SUT2* increased significantly in T-65-7-1 at three stages (S2, S3, S4). In the stem (pn), *SUT2* increased in two stages (S3 and S4), and in the root, *SUT2* increased in three stages (S2, S3 and S4), and this increase was significantly greater than Tabasi (Fig. 10).

Correlation of the Expression of Genes Involved in Carbohydrate Metabolism with Contents of Fructan, Sucrose and WSCs

There was a positive and significant correlation between the expression of genes involved in fructan biosynthesis (*I-FFT*, *6-SFT*, *1-SST*), and the fructan and WSC contents in both tissues (Table 2). A positive and significant correlation between the expression of *SPS* and *SPP* and sucrose was observed in the stem (pn) and root (Table 2). In both tissues, there was a positive and significant correlation between sucrose transporter gene expression (*SUT1* and *SUT2*) and sucrose (Table 2). In contrast, a negative significant correlation was observed for *1-FEHw3* and *6-FEH* with fructan and WSC contents in the stem (pn) and root. Moreover, the correlation between *INV* gene expression and fructan, sucrose and WSC contents was negative in both tissues (Table 2).

Correlation of the Expression of Genes Involved in Carbohydrate Metabolism Between Stem (pn Internode) and Root

Among all of the evaluated genes involved in the metabolism of fructan and sucrose, the correlation between stem (pn) and root was not significant for only *I-FFT*. In the

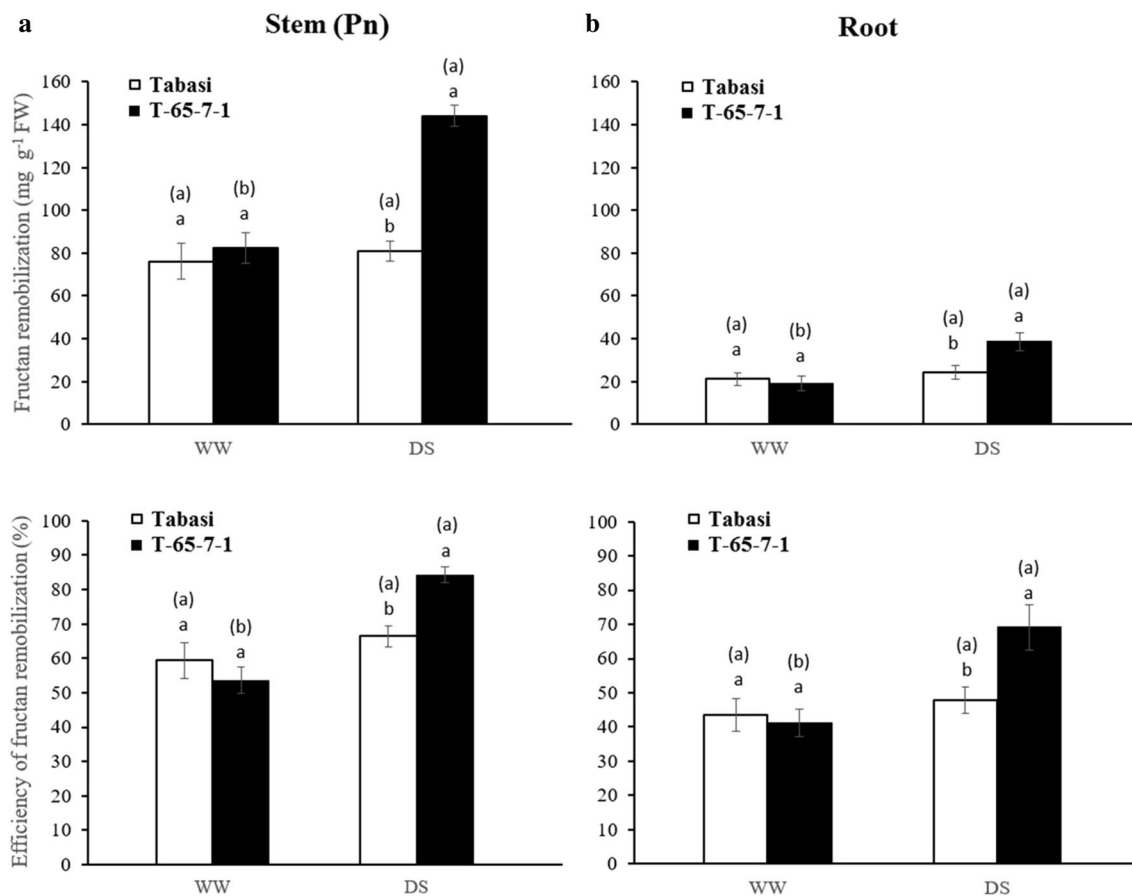


Fig. 5 Fructan remobilization and remobilization efficiency, **a** in the stem (pn) and **b** root in Tabasi and T-65-7-1 in well-watered (WW) and drought stress (DS) conditions. The vertical lines represent \pm SE of the mean of three replicates. Means followed by same letters are

not significantly different ($P < 0.05$). Same letters in parentheses indicate non-significant difference between each genotype under WW compared to DS ($P < 0.05$)

expression of the other genes in the stem (pn) and root, correlations were positive and significant (Supplemental Fig. 1).

Discussion

In plants that are not drought-escaping, some genotypes mostly react to drought stress by resisting the reduction of photosynthesis and some by remobilizing assimilates stored in vegetative tissues (Farooq et al. 2014). In the screening of bread wheat mutant lines and their wild type, a huge difference was observed with respect to the parameters associated with photosynthesis and stem remobilization under rain-fed conditions (Fig. 1). In other studies, genetic variation is frequently mentioned for remobilization and remobilization efficiency (Ehdaie et al. 2006a, b; Ruuska et al. 2006; Xue et al. 2009; Gupta et al. 2011; Joudi et al. 2012).

The results showed that under both moisture conditions, grain yield per plant in T-65-7-1 was significantly greater than Tabasi. Drought stress conditions caused lower

reductions in the yield of T-65-7-1 (17.43%) compared to Tabasi (25.34%) (Fig. 2a). It seems that the remobilization of assimilates to grain during the grain filling period is a more effective strategy than maintenance of photosynthesis under drought stress conditions because drought stress is imposed during the grain filling period. Jagadish et al. (2015) reported that both staying green and senescence are physiological reactions of plants against abiotic stresses. When the plant is faced with stress before anthesis or fertility, the ability to stay green causes more fertility and, as a result, increases the grain number whereas under stress after anthesis and during grain filling, senescence leads to increased remobilization of stored material to grains and changes in grain weight (Jagadish et al. 2015).

In this study, based on the results of the expression of photosynthetic genes, flag leaf chlorophyll and RWC, it seems that Tabasi maintains and continues photosynthesis and uses the current assimilates during grain filling under drought stress (Figs. 2b, c, 6). The obvious signs of leaf senescence are the controlled changes of physiological

Fig. 6 Relative expression of photosynthetic genes **a** *RBCS*, **b** *RBCL* and **c** *RCA* in the flag leaf of Tabasi and T-65-7-1, under drought stress during the grain filling period. Stages represent days after anthesis 0 (S1), 7 (S2), 14 (S3), 21 (S4) and 28 (S5), respectively. The vertical lines represent \pm SE of the mean of three replicates

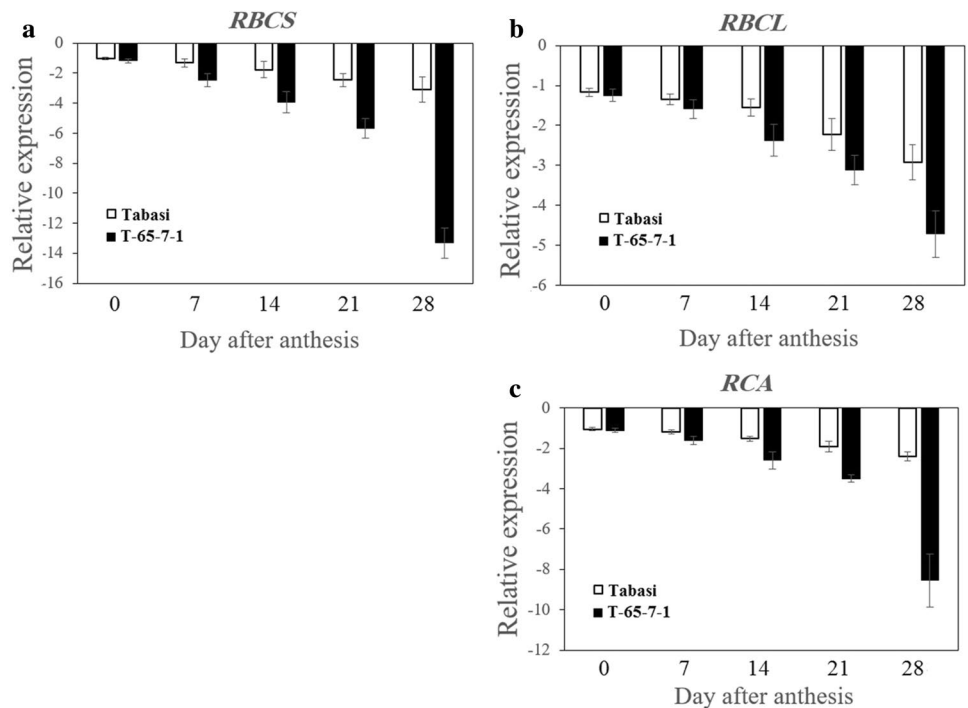
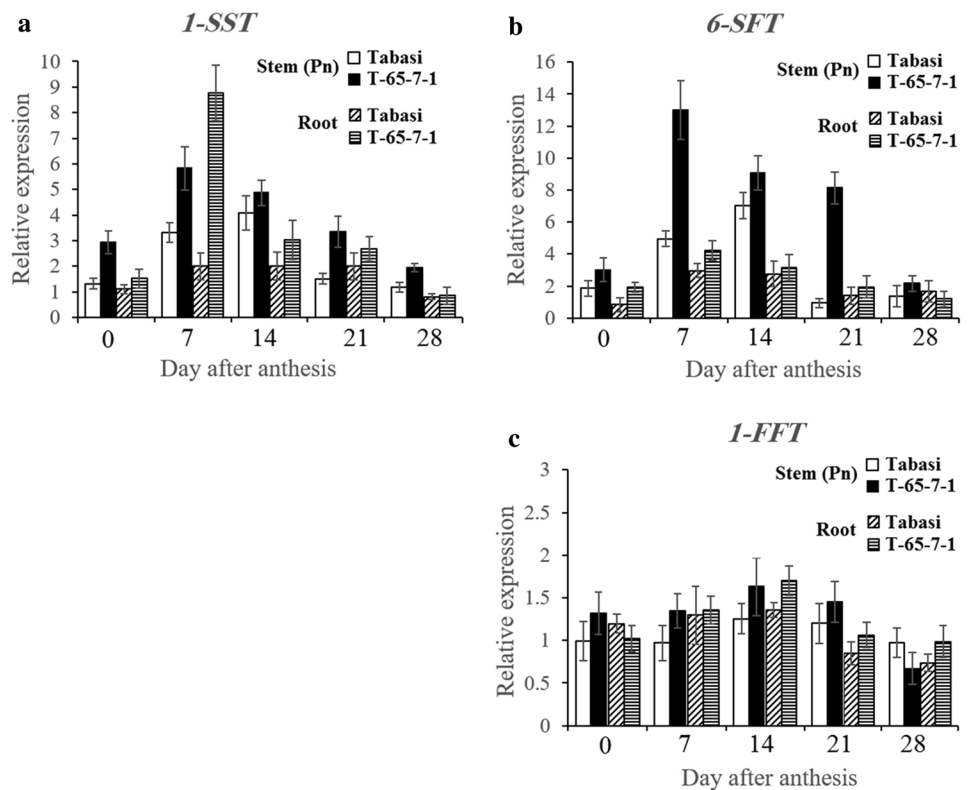


Fig. 7 Relative expression of fructan biosynthesis genes **a** *1-SST*, **b** *6-SFT* and **c** *1-FFT* in the stem (pn) and root of Tabasi and T-65-7-1, under drought stress during the grain filling period. Stages represent days after anthesis 0 (S1), 7 (S2), 14 (S3), 21 (S4) and 28 (S5), respectively. The vertical lines represent \pm SE of the mean of three replicates



reactions including photosynthesis decline, chloroplast degradation, a significant reduction in chlorophyll content and RWC (Chandlee 2001; Hörtensteiner and Feller 2002). The expression of photosynthetic genes (*RBCL*, *RBCS* and

RCA) during the grain filling period under drought stress conditions showed that the photosynthetic system of T-65-7-1 was affected more quickly and severely by drought than Tabasi (Fig. 6). Rubisco is degraded during senescence, and

Fig. 8 Relative expression of fructan hydrolysis genes **a** *1-FEHw3* and **b** *6-FEH* in the stem (pn) and root of Tabasi and T-65-7-1, under drought stress during the grain filling period. Stages represent days after anthesis 0 (S1), 7 (S2), 14 (S3), 21 (S4) and 28 (S5), respectively. The vertical lines represent \pm SE of the mean of three replicates

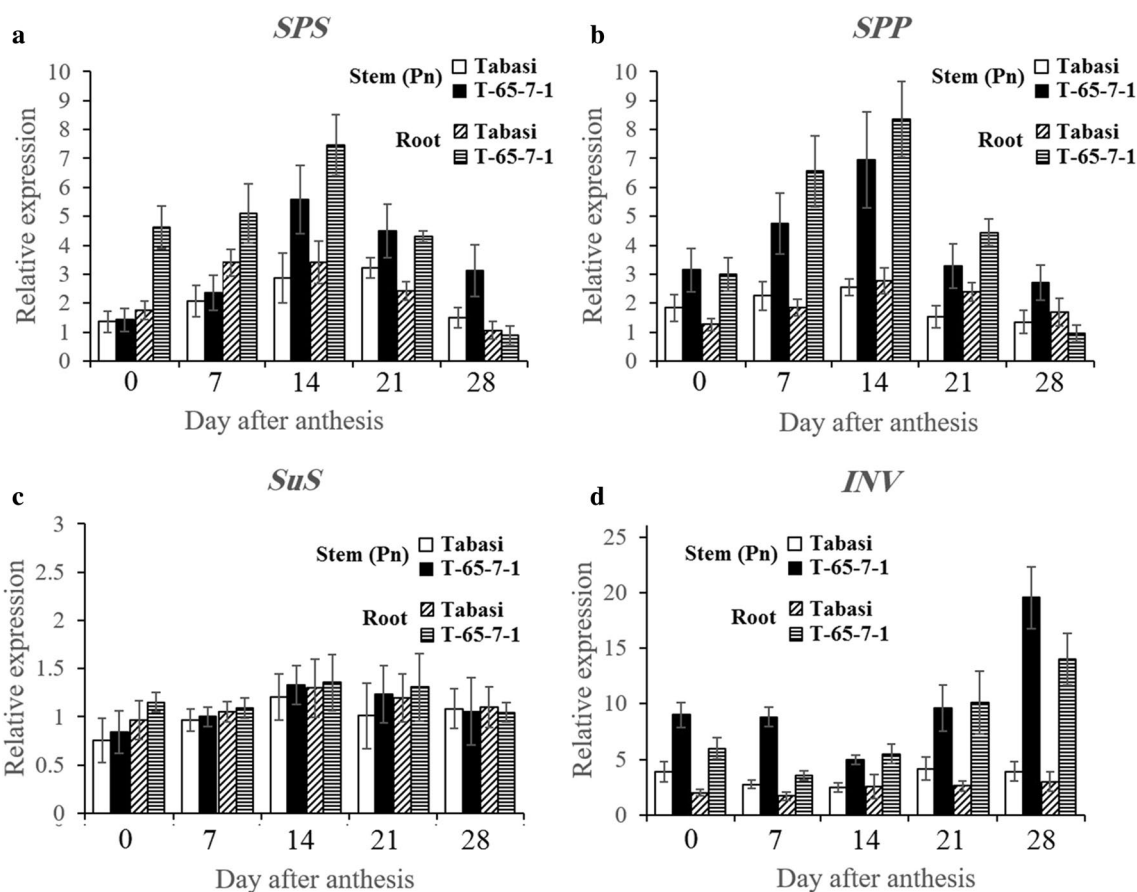
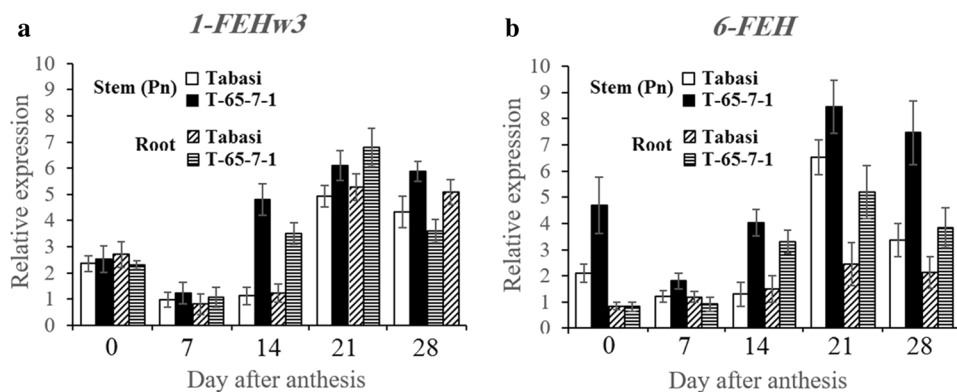


Fig. 9 Relative expression of sucrose biosynthesis/hydrolysis genes **a** *SPS*, **b** *SPP*, **c** *SuS* and **d** *INV* in the stem (pn) and root of Tabasi and T-65-7-1, under drought stress during the grain filling period. Stages

represent days after anthesis 0 (S1), 7 (S2), 14 (S3), 21 (S4) and 28 (S5), respectively. The vertical lines represent \pm SE of the mean of three replicates

its degradation products are re-utilized as a source for developing tissues (Suzuki et al. 2001). Senescence increases the remobilization of assimilates stored in vegetative tissue for grain filling in monocarpic plants such as wheat (Yang and Zhang 2006). The stem and root are capable of transporting the stored assimilates (Lawlor and Paul 2014). Our results showed that, under well-watered conditions, fructan

remobilization and remobilization efficiency of the stem and root were not significantly different between the two cultivars, whereas under drought stress conditions, these were significantly higher in T-65-7-1 than Tabasi (Fig. 5), which is probably due to early senescence. Bazargani et al. (2011) studied the proteome of two wheat landraces (contrasting in stem remobilization) and reported that the expression of

Fig. 10 Relative expression of sucrose transporter genes **a** *SUT1*, **b** *SUT2* in the stem (pn) and root of Tabasi and T-65-7-1 under drought stress during the grain filling period. Stages represent days after anthesis 0 (S1), 7 (S2), 14 (S3), 21 (S4) and 28 (S5), respectively. The vertical lines represent \pm SE of the mean of three replicates

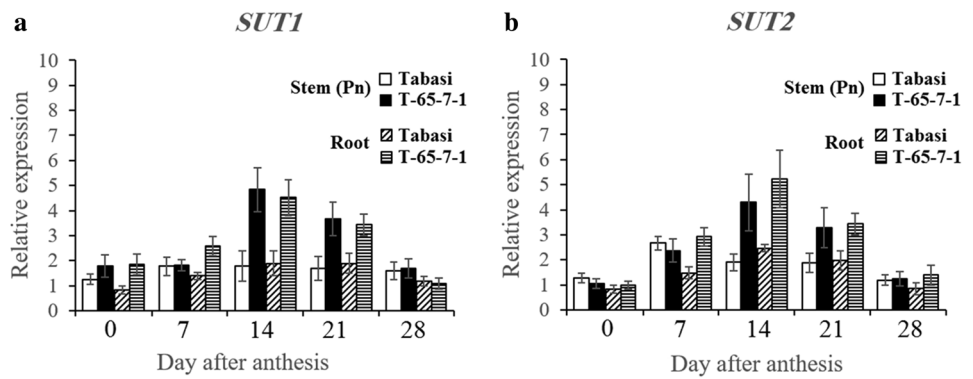


Table 2 Pearson correlation coefficients between relative gene expression involved in carbohydrate metabolism and fructan, sucrose and WSCs contents in the stem (pn) and root of wheat genotypes during grain filling period in five stages (S1–S5) under terminal drought stress

	<i>1-SST</i>	<i>6-SFT</i>	<i>1-FFT2</i>	<i>1-FEHw3</i>	<i>6-FEH</i>	<i>SPS</i>	<i>SPP1</i>	<i>SUS1</i>	<i>INV</i>	<i>SUT1</i>	<i>SUT2</i>
Stem (pn)											
Fructan	0.91**	0.85**	0.68*	-0.63*	-0.58*	0.15 ^{ns}	0.66*	0.15 ^{ns}	-0.30 ^{ns}	0.29 ^{ns}	0.48 ^{ns}
Sucrose	0.78**	0.72*	0.67*	-0.28 ^{ns}	-0.35 ^{ns}	0.58*	0.78**	0.43 ^{ns}	-0.34 ^{ns}	0.58*	0.74*
WSCs	0.73*	0.61*	0.60*	-0.63*	-0.66*	0.10 ^{ns}	0.53 ^{ns}	0.05 ^{ns}	-0.52 ^{ns}	0.24 ^{ns}	0.45 ^{ns}
Root											
Fructan	0.67*	0.86**	0.64*	-0.81**	-0.60*	0.65*	0.52 ^{ns}	0.06 ^{ns}	-0.54 ^{ns}	0.28 ^{ns}	0.27 ^{ns}
Sucrose	0.50 ^{ns}	0.70*	0.80**	-0.32 ^{ns}	-0.26 ^{ns}	0.84**	0.79**	0.57 ^{ns}	-0.41 ^{ns}	0.73*	0.78**
WSCs	0.59*	0.69*	0.84**	-0.60*	-0.62*	0.72*	0.55 ^{ns}	0.27 ^{ns}	-0.54 ^{ns}	0.39 ^{ns}	0.43 ^{ns}

*Significant at $P < 0.05$; **significant at $P < 0.01$, ^{ns} non-significant, $n = 10$

several related senescence proteins, and consequently the degradation of photosynthetic proteins, increased under drought stress conditions in the genotype with higher remobilization.

Several studies have been conducted on the remobilization from stem to grain under drought stress conditions in wheat (Wardlaw and Willenbrink 2000; Yang et al. 2000, 2004; Ehdaie et al. 2006a, b, 2008; Xue et al. 2009; Gupta et al. 2011; Zhang et al. 2015). There are few reports on the importance of remobilization of root assimilates during the grain filling period under drought stress conditions (Lopes and Reynolds 2010; Zhang et al. 2016), although there have been some studies of the role of root system architecture (Manschadi et al. 2006; Palta et al. 2011; Nakhforoosh et al. 2014). Drought stress conditions cause root development in deeper soil layers for moisture absorption (Wasson et al. 2012). In wheat breeding programs, varieties with deeper root systems were selected to create a drought-tolerant variety. However, in rain-fed Mediterranean regions, the evaporation rate is high due to the limited (or non-existent) rainfall and high temperatures at the end of the growing season. Therefore, any rainfall that occurs will only affect the uppermost soil layer and is not enough to help develop deeper roots (Zhang et al. 2016). Under these conditions, the importance of the remobilization of assimilates from root to grain

is vital. Roots, as a partial fructan pool, can be involved in assimilate remobilization (Zhang et al. 2016). In this study, the amount of root fructan remobilization was one-fourth of the pn stem internode (Fig. 5). Zhang et al. (2016) reported that the WSCs and root fructan were approximately one-third of the stem. Further, compared to Tabasi, the peak of fructan and sucrose contents in T-65-7-1 was higher in vegetative tissues (Figs. 3, 4).

Fructan comprises most of the WSCs in wheat vegetative tissues, which contain both β -(2–1)-linked and β -(2–6)-linked (fructose Van den Ende et al. 2003; Chalmers et al. 2005; Van den Ende and El-Esawe 2014). The β -(2–6)-linked fructans are formed by the sequential action of *1-SST* and *6-SFT*, while β -(2–1)-linked fructans are formed by the action of *1-FFT*; the latter reaction is least important in fructan synthesis (Verspreet et al. 2013). The present results showed that the expression of *1-SST* and *6-SFT* in the stem (pn) and root significantly increased under drought stress (especially at S2, S3, and 4S), whereas *1-FFT* gene expression was not affected by drought stress (Fig. 7). The reason that *1-FFT* is not affected may be because of a less important role in the synthesis of fructan. These results suggested that β -(2–6) linkages, the predominant form of fructan linkages, were affected by the drought. In this study, under drought stress conditions, the expression of *1-SST* and *6-SFT* in the

stem (pn) and root of T-65-7-1 were equal to or greater than Tabasi at all stages (Fig. 7). A significant positive correlation was observed between the expression of genes involved in fructan biosynthesis and contents of fructan and WSCs in both the stem (pn) and the root (Table 2). These results clearly indicate that the contents of fructan and WSCs in the root and stem are a function of the activity of enzymes encoded by *1-SST* and *6-SFT*. Moreover, the genetic variation in fructan accumulation among genotypes is caused by *1-SST* and *6-SFT* (Xue et al. 2008).

The hydrolysis of fructan and its conversion to sucrose and fructose are conducted by *1-FEHs* and *6-FEHs*, which preferentially cleave the β -(2–1)- and β -(2–6)-links, respectively (Van den Ende et al. 2003; Van Riet et al. 2006). There are three forms of *1-FEH*: *1-FEHw1* (*1-FEH-6A*), *1-FEHw2* (*1-FEH-6D*) and *1-FEHw3* (*1-FEH-6B*). However, *1-FEHw3* is the key gene in the remobilization of fructan (Zhang et al. 2009). The expression of *1-FEHw3* and *6-FEH* in the stem (pn) and root of T-65-7-1 were significantly higher at the final stages (S3, S4 and S5) than Tabasi (Fig. 8). Khoshro et al. (2014) studied the effect of terminal draught stress on two wheat cultivars and reported that the *1-FEHw3* and *6-FEH* is up-regulated during remobilization of assimilates. In this study, negative and significant correlations were observed between the *FEHs* genes (*1-FEHw3* and *6-FEH*) and fructan and WSC contents in the stem (pn) and root (Table 2), which represents a further reduction of fructan content under drought stress conditions at the final stages of grain filling, especially in T-65-7-1. The negative and significant correlations between the *FEHs* genes expression and WSCs under terminal drought stress has been reported by the other researchers (Zhang et al. 2009; Khoshro et al. 2014). When the demand for grain filling is high, sucrose is limited, and fructan is converted to sucrose and fructose by *FEHs* (Zhang et al. 2009). It seems that the higher sink strength (grain yield) of T-65-7-1 limits sucrose and increases *FEHs* genes expression to supply sucrose (Figs. 2a, 8).

After fructan hydrolysis, fructose is used as the substrate for resynthesis of sucrose (Joudi et al. 2012). In plant tissues, sucrose is synthesized by the activity of the enzymes *SPS*, *SPP*, and *SuS* (Xue et al. 2008a, b). At most of the stages, *SPS* and *SPP* gene expression were significantly higher in the stem (pn) and the root of T-65-7-1 than Tabasi (Fig. 9a, b). However, no significant change was observed in the *SuS* gene expression in the stem (pn) and root of both genotypes under drought stress (Fig. 9c). These results reflect the higher synthesis of sucrose in the stem (pn) and root of T-65-7-1 compared to Tabasi, especially at intermediate stages (S2, S3, and S4). A significant positive correlation between the expression of *SPS* and *SPP* and sucrose in the stem (pn) and root indicates the role of these genes in determining the sucrose content of these tissues (Table 2).

Hydrolysis of sucrose and its conversion into α -D-glucose and fructose in the vacuole is done by vacuolar invertase (*INV*) (Ruan 2014). The results showed that *INV* expression for T-65-7-1 under drought stress conditions was higher than Tabasi in both shoot and root tissues for all stages (Fig. 9d). Therefore, more hexose sugars are produced under drought stress conditions in T-65-7-1, and the required substrate for producing sucrose and fructan are provided. The effect of the increase in *INV* gene expression in the varieties with high remobilization under drought and salt stress has been reported by the other researchers (Khoshro et al. 2014; Sharbatkhari et al. 2016). In this study, a negative correlation was observed between *INV* gene expression and fructan and WSC contents in the stem (pn) and root, though this was not statistically significant (Table 2). This was not unexpected because the *INVs* are mostly regulated at the post-translational level by invertase inhibitors (Tauzin et al. 2014).

The metabolism and transport of sucrose are very important for growth and senescence (Wang et al. 2016). The transportation of sugars from source to sink is one of the main necessities of plant growth (Lemoine et al. 2013). Transport of sucrose from the vacuole to the cytosol and the cytosol to the apoplast are due to the action of *SUT2* and *SUT1*, respectively (Aoki et al. 2004; Deol et al. 2013). The expression of sucrose transporter genes (*SUT1* and *SUT2*) was increased in T-65-7-1 at most of the evaluated stages, while Tabasi was not affected by drought and had a constant trend at most stages (Fig. 10). The over-expression of sucrose transporter genes (*SUT1* and *SUT2*) in T-65-7-1 correlates an increase in sucrose transport in the remobilization from vegetative tissues to grains. In both tissues, a positive significant correlation was observed between the expression of sucrose transporter genes (*SUT1* and *SUT2*) and sucrose (Table 2), which represents the fast transport of sucrose from vegetative tissues to the sink during terminal drought, especially in T-65-7-1.

Our results showed that both stem (pn) and root tissues display similar behaviours in the remobilization of stored assimilates to the grain during grain filling under drought stress conditions. In most of the examined genes in the fructan and sucrose metabolism pathway, a positive and significant correlation was observed between gene expression in the stem (pn) and root (Supplemental Fig. 1). Drought-induced leaf senescence increases the remobilization of assimilates from vegetative tissues to the grains (Yang and Zhang 2006; Distelfeld et al. 2014). It seems that the wheat stem (pn) and root exhibit similar behaviours for responding to senescence.

The majority of the investigated genes in the current study, including *1-SST*, *6-SFT*, *1-FEHw3*, *6-FEH*, *SPS*, *SPP*, *INV*, *SUT1* and *SUT2*, provide valuable molecular indicators for achieving wheat genotypes with high fructan content and more remobilization under terminal drought stress and will

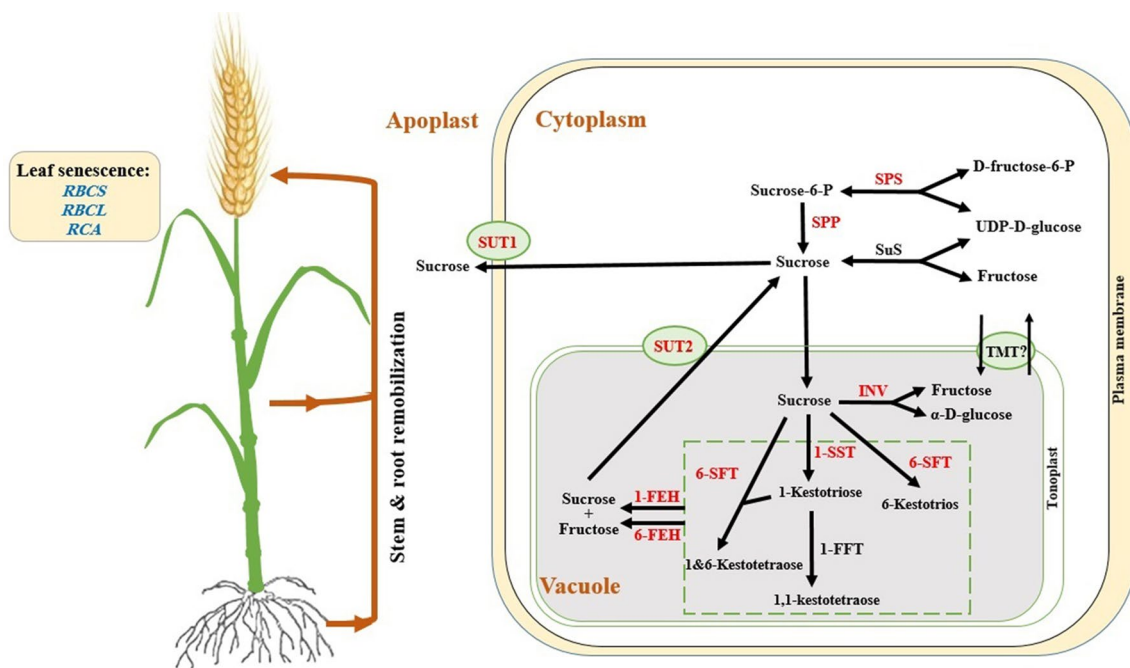


Fig. 11 The effect of flag leaf senescence on the increase of remobilization of WSCs from the stem (pn) and root to grain, and changes in the expression of genes involved in fructan and sucrose metabolism in the stem (pn) and root of T-65-7-1 compared to Tabasi. Overall expression changes of the genes are indicated by colour, with red for drought up-regulated genes, blue for drought down-regulated genes

aid in the development of new drought-tolerant genotypes in wheat breeding programmes.

Conclusions

Our results showed that senescence was induced in T-65-7-1 but not in Tabasi as a result of drought. Additionally, the expression of photosynthetic genes, chlorophyll content, and RWC of the flag leaf were sharply reduced in T-65-7-1. These changes may have contributed to the damage of the photosynthetic apparatus under drought stress conditions. Higher fructan remobilization through vegetative tissues to the grains in T-65-7-1 compared to Tabasi under drought stress was caused by the regulation of genes involved in the synthesis and hydrolysis of fructan (*1-SST*, *6-SFT*, *1-FEHw3*, *6-FEH*) as well as the synthesis, hydrolysis, and transport of sucrose (*SPS*, *SPP*, *INV*, *SUT1*, *SUT2*) (Fig. 11). WSCs stored in the root as a partial pool participated in the remobilization of assimilates during the grain filling period, while root fructan remobilization was one-fourth of the stem. Stem (pn) and root tissues exhibited similar behaviour in the remobilization of assimilates to grain during grain filling under drought stress. Our results showed that the remobilization of assimilates to the grains during

and black for genes with no significant change in T-65-7-1 compared to Tabasi. Sucrose transport from the vacuole to the cytosol and the cytosol to the apoplast occurs by *SUT2* and *SUT1*, respectively. The tonoplast monosaccharide transporters (TMTs) reversibly transfer glucose and fructose between the vacuole and cytosol, which is still poorly understood in wheat. (Color figure online)

the grain filling period was a more effective strategy than maintenance of photosynthesis under terminal drought stress conditions, so that the grain yield reduction in T-65-7-1 was lower than Tabasi. This research provides molecular indicators for selecting drought-tolerant wheat genotypes with high fructan content and greater remobilization under drought stress during the grain filling period in wheat breeding programmes.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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