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Evaluation of Fructan metabolism enzymes in contrasting barley (*Hordeum vulgare*) genotypes under drought stress

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Abstract: In terminal drought conditions, the limitations on current photosynthesis can improve the influence of cereal stem carbohydrate reserves on preserving grain yield. This study investigated how the remobilization of stem fructans affects barley yield under drought conditions. The present study performed comparative physiological and gene expression analyses using flag leaf, penultimate, and peduncle tissues from four barley genotypes (G1, G2, G3, and G4). We examined the expression levels of fructan metabolism genes in drought-stressed leaf, stem penultimate, and peduncle tissues during the grain-filling stage, comparing them to those under normal conditions. Notably, genetic variability among the cultivars influences the expression of drought-responsive genes associated with tolerance. Specifically, genotypes 1 and 3 showed an up-regulation of fructan metabolism genes in response to drought, while genotypes 2 and 4 exhibited a down-regulation. Our findings indicated that increased fructan accumulation and its subsequent remobilization significantly contribute to the yield stability of G1 and G3 under drought stress conditions. Barley genotypes that demonstrate a strong capacity for fructan production and remobilization in response to terminal drought stress could serve as valuable resources for breeding programs aimed at improving drought tolerance through the selection of these traits.

Keywords: Barley, drought tolerance, fructan metabolism, gene expression, seed filling.

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Introduction

Barley, *Hordeum vulgare*, is the fourth most significant grain crop globally, following by wheat, maize, and rice (FAO, 2020). Barley crops grown in semi-arid regions may become dehydrated during growth, especially during anthesis and seed filling, making them more dependent on water-soluble carbohydrates (WSCs). WSCs include fructan, glocuse, and sucrose, with fructans being the most important stored carbohydrates (Goggin and Setter, 2004; Hübner et al., 2015). Drought stress triggers a range of biochemical and physiological responses in plants, ultimately resulting in decreased crop production (Nosratiazar et al., 2024). Drought is a critical environmental factor that limits plant growth and productivity, particularly during the flowering and seed-filling stages of barley. It is the second leading cause of grain yield loss, following plant pathogens (Liliane and Charles, 2020). In general, seed filling depends on the supply of carbon from two sources: post-anthesis carbohydrates that are mobilized directly to the seed (current photosynthesis) and remobilization of storage material from the stem to grain (Siah et al., 2003; Esmaeilpour-Jahromi et al., 2012). In drought conditions, the role of stem reserves and their remobilization becomes increasingly vital due to restricted photosynthesis, significantly impacting the final yield (Sehgal et al., 2018). The carbohydrate reserves in barley stem tissues serve as a key carbon source during the initial grain-filling stages. Water scarcity can lead to a reduction in yield of up to 50-60%, primarily due to decreased seed weight and fewer spikes. Genotypes that are sensitive experience reductions in yield-related traits, including pollen viability, panicle number, and seed weight when subjected to stress conditions (Barnabás et al., 2008). When faced with drought, the remobilization of these stem reserves can aid in grain filling. Under terminal drought conditions, the remobilization of carbohydrates from the stem to the seeds can enhance grain yield by approximately 40% (Liu et al., 2020). To elucidate the molecular basis of genotypic variation in the concentration of WSC, a microarray analysis was conducted on the stem. Different barley genotypes exhibit varying capacities for WSC accumulation in the stem internode (Badigannavar et al., 2018). Physiological, morphological, and molecular traits can be surveyed to better understand the molecular mechanism of stem reserve remobilization in response to drought conditions. Drought stress can impede grain photosynthesis and assimilation, leading to reduced grain yield by diminishing stomatal conductance and fostering protein degradation, and causing significant declines in chlorophyll levels (Farooq et al., 2017). Chlorophyll is a pigment that plays a key role in photosynthesis by capturing light energy (Adl et al., 2023). Concurrently developing varieties with multiple stress resistance may not be possible using conventional breeding (Bita and Gerats, 2013; Saidi and Hajibarat, 2020).

Fructans constitute a significant component of carbohydrate storage in cereal plants and exhibit a notable increase under stress conditions within their vegetative structures. The biosynthesis of fructans occurs through fructosyl transferases. Key enzymes that facilitate fructan biosynthesis include sucrose:sucrose 1-fructosyltransferase (*1-SST*) and sucrose:fructan 6-fructosyltransferase (*6-SFT*) (Nagaraj et al., 2004). Studies have indicated that osmotic stress leads to an upregulation of the *1-SST* and *6-SFT* gene expressions in wheat stems (Xue et al., 2008). Fructans are primarily stored in stem internodes until around the mid-grain filling stage (Schnyder, 1993). The significance of utilizing these stem reserves during drought stress is highlighted when photosynthetic efficiency declines during the grain filling phase (Blum, 1998). As the demand for grain filling intensifies and sucrose becomes scarce, fructans are broken down to release additional fructose and sucrose. The mobilization of stored carbohydrates necessitates the hydrolysis of fructans, a process facilitated by fructan exohydrolases (*FEHs*), predominantly the fructan-1-exohydrolases (*1-FEHs*). Both *FEHs* and fructosyl transferases are likely regulated at the transcriptional level (Van Laere and Van Den Ende, 2002; Zhang et al., 2020). Research by Zhang et al. (2020) revealed that the expression of the *1-FEH* w3 gene increased in wheat stems under terminal drought, approximately 20-25 days after anthesis. One investigation demonstrated that the *1-FEH* w3 gene plays a crucial role in the remobilization of stem fructans and serves as a valuable marker for selecting wheat with efficient fructan remobilization (Zhang et al., 2015). Since fructans cannot be directly transferred from vacuoles, they must be degraded and moved as sucrose. As sucrose is transported from source to sink via the phloem, sucrose transporters (SUTs) are essential for facilitating its active transport across plasma membranes (Lalonde et al., 2003). SUT proteins are divided into two subgroups: *SUT1* and *SUT2* (Endler et al., 2006). However, few studies have explored the activity or expression patterns of these transporters in different wheat and barley internodes under terminal drought conditions. The expression of *SUT1* peaks 16-20 days after anthesis (DAA), underscoring its vital role in regulating carbon distribution during the grain filling process in cereals (Aoki et al., 2002). In this study, the accumulation and remobilization rates of fructans in the penultimate internode were analyzed in different genotypes subjected to terminal drought stress. Additionally, the research included transcriptional profiling of genes that encode vital enzymes involved in fructan metabolism, resulting in the identification of correlations between transcript levels and grain yield. This study aims to compare different barley genotypes in terms of their fructan metabolism enzymes during drought stress at the grain filling stage.

Materials and Methods

Study sites and planting materials

Four different barley genotypes were subjected to field trials at the Varamin Agricultural Research Station, Ministry of Jehad Agriculture, focusing on both normal and water-deficient conditions. Water scarcity poses a significant challenge in these regions. The plant material was supplied from seed collection of Seed and Plant Improvement Institute ([SPII), ministry of Jehad Agriculture in Karaj. The experiment included two treatments: a well-watered control and a water-stressed treatment. A randomized complete block design (RCBD) was implemented for the study, with three replications conducted across three sites. Each genotype was sown in a single-row plot measuring 5 meters in length, with a width of 25 cm and a row spacing of 75 cm. Irrigation was applied once after seeding in the fall and three times during spring for tillering, stemming, and flowering. A water shortage facilitated the achievement of 50% flowering. Irrigation was discontinued at the 50% spike stage, which is when half of the plants in the plots had begun to flower. Each plot featured six planting lines spaced 20 cm apart on two 6-meter stacks. The total area for each plot was 7.2 square meters; after excluding 0.5 meters from both ends to account for edge effects, the area designated for harvesting became 6 square meters. All plots were planted around the same time, specifically in early November. Fertilization included 80 kg of nitrogen per hectare at planting and an additional 100 kg per hectare as top dressing on April 1st. Phosphorus and potassium fertilizers were applied at 200 kg and 50 kg per hectare, respectively, at the time of planting. The seeding rate was set at 350 seeds per square meter, with the required seed weight per plot calculated based on the weight of a thousand seeds of each cultivar. To control broadleaf weeds, Granstar herbicide was employed, while Puma Extra herbicide was used to target narrow-leaved weeds. List of barley genotypes used in the study is given in Table 1.

Physiological responses to water deficit

Three types of tissues (peduncle, penultimate leaf, and flag leaf) were examined to assess the physiological responses of four barley genotypes. To study these traits, both normal and water-stressed barley tissues were collected 28 days after anthesis at the seed filling stage. Fresh samples were rinsed with distilled water in the laboratory and then left to air dry at room temperature (18 °C) for 6 hours before analyzing chlorophyll a (Chl-a), chlorophyll b (Chl-b), total chlorophyll, and carotenoid levels. A precise weight of 0.5 g of fresh plant material was homogenized in a tissue homogenizer with 10 ml of acetone as the extraction solvent. The homogenate was then centrifuged at 12,000 rpm and 4 °C for 15 minutes. One milliliter of the resulting supernatant was combined with 4 ml of acetone. The resulting solution was analyzed for Chl-a, Chl-b, total chlorophyll, and carotenoids using spectrophotometry according to the following formulas:

$\text{Chl-a} = 12.25A_{663.2} - 279A_{646.8}$

$\text{Chl-b} = 21.5A_{646.8} - 5.1A_{663.2}$

$C\ x{+}c = (1000A_{470} - 1.82Ca - 85.02Cb) / 198$

$\text{Total chlorophyll (mg/g)} = [20.2\ (A_{645}) + 8.02\ (A_{663})]$

Table 1. Pedigree and yield of barley genotypes used in this study.

Genotype	Pedigree	Yp	Ys
G1	Rojo/3/LB.IRAN/Una8271//Gloria"S"/Com"S"/4/Nik	4.933	5.133
G2	Triton/Yazd-5//Nik/3/Rhn03	5.383	5.181
G3	Rojo/3/LB.IRAN/Una8271//Gloria"S"/Com"S"/4/ Rojo/3/LB.IRAN/Una8271//Gloria"S"…	4.947	4.719
G4	Triton/Yazd-5//Nik/3/Rhn03	4.008	3.778

Yp: Yield under normal conditions; Ys: Yield under drought stress.

Table 2. Genes ID and primer sequences of fructan metabolism-related genes in barley were used in this study.

Gene ID	Sequence of primer
1-SST	F: TATGGACTCGTCGTACAACC
1-SST	R: TGTGTCTAGGAGAAAGGGAG
SUT1	F: TATTCCTGCTGCCCAAGATC
SUT1	R: GAGAGCCACCTAGTTACGAC
1-FEH	F: TGTGGAGAAGGGTTGGAGTG
1-FEH	R: GATGGGAAGGGCTTACTAAG
6-SFT	F: ACAACCAGCTCTCCAATGAG
6-SFT	R: GTATGAATTGCCCTTCCCTG
SUT2	F: TGCAGACAAGGAAGGAAGGC
SUT2	R: GCCCTCCTACTTTTGCCC
HvActin	F: GGTCCATCCTAGCCTCACTC
HvActin	R: GATAACAGCAGTGGAGCGCT

A = Absorbance, Ch-a = Chlorophyll a, Ch-b = Chlorophyll b, C x+c = Carotenoids.

RNA extraction and expression profiles fructan metabolism genes

Samples from the penultimate, leaf, and peduncle were collected separately to isolate RNA from barley plants subjected to both normal and water deficit conditions during the grain filling stage. Total RNA was extracted from the drought-stressed and controlled penultimate and peduncle internodes utilizing the RNX-Plus kit (Sinaclone, Iran) under the manufacturer's guidelines. The RNA's purity and concentration were assessed using a Nano Drop spectrophotometer, and its integrity was verified via 1% agarose gel electrophoresis. Subsequently, cDNA synthesis was carried out following the instructions of a cDNA synthesis kit (SinaClon, Iran). Each gene was analyzed in triplicate, using the actin gene as a reference. All primers for gene expression analysis were designed using the Oligo program (Table 2). Gene expression assessments were performed with a real-time PCR instrument (ABI real-time, Agilent**)** employing SYBR green, as per the manufacturer's recommendations. After normalization, the relative expression levels of the genes were calculated using the $2^{-\Delta\Delta CT}$ method (Livak et al., 2013), with *HvActin* as the reference for determining Ct values for the fructan metabolism genes. Quantitative real-time PCR (RT-qPCR) was conducted to elucidate the expression profiles of *SUT1*, *SUT2*, *6-SFT*, *1-SST*, and *1-FEH* genes across three tissue types under both normal and water deficit conditions, with additional investigation into their expression levels during the seed filling stage.

The heatmap was created using TBtools to illustrate the differential expression of genes and the relationship between physiological traits and gene expression. Statistical analyses were conducted with SPSS version 20.0. Significant differences between means were assessed with Duncan's test at a significance level of $P < 0.05$.

Results

Leaf fructan metabolism gene expression under drought

The expression of the *SUT1* gene in three different tissues (peduncle, penultimate and leaf) showed a significant increase in genotype 1 in the stem penultimate (Figure 1). The expression of *SUT2* gene in all tissues revealed that the penultimate had a higher expression as compared to the other tissues. The expression of the *6-SFT* gene in leaves was higher than the other tissues in response to drought stress. The expression of *1-SST* gene in penultimate and peduncle showed higher expression as compared to leaf tissue. In genotype 2, the expression of *SUT1* gene was increased in the peduncle tissue. The expression of *SUT2* gene showed increased expression in the leaf tissues. The increased expression of *the 6-SFT* gene was observed in the penultimate tissue of the stem. The expression of *1-SST* and *1-FEH* genes increased in three tissues. The expression of *SUT1* gene in three different tissues (peduncle, penultimate, and leaf) showed that this gene was increased in penultimate and leaf of genotype 3. The expression of *SUT2* gene in penultimate and peduncle tissues showed a higher expression as compared to the leaf tissue. The expression of *6-SFT* and *1-SST* genes in penultimate and leaves in response to drought stress showed a greater increase as compared to the peduncle tissue. The *1-FEH* gene showed increased expression in the leaf tissue. In genotype 4, significant differences in expression was not observed for *SUT1* and *SUT2* genes in leaf, peduncle, and penultimate tissues.

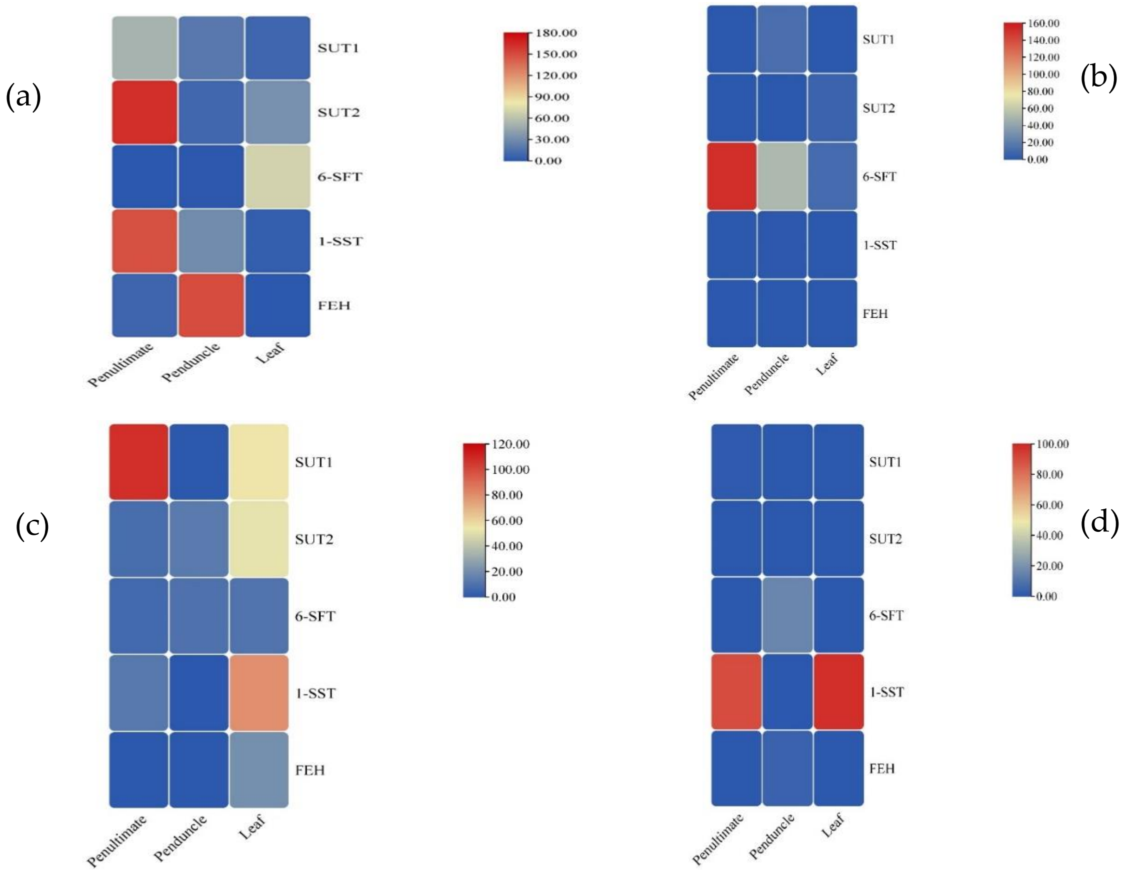

Figure 1. Fructan biosynthesis enzymes in genotype 1 (a), 2 (b), 3(c), and 4 (d) in three tissues under water deficit conditions.

The expression of *6-SFT* gene was significantly increased in the peduncle tissue. An increased expression of *1-SST* gene was observed in the penultimate tissue of stem and leaf. *1-FEH* gene expression increased in peduncle tissue.

Correlations between measured traits and gene expression levels under normal and water deficit conditions

Correlations Between Traits Under Normal Conditions

The analysis of the correlation between fructan metabolism genes and physiological traits under normal conditions revealed that chlorophyll a in the penultimate leaf had a positive and significant correlation with both chlorophyll b in the penultimate leaf and the total chlorophyll content of the penultimate leaves. Additionally, chlorophyll b exhibited a positive and significant correlation with the total chlorophyll content of the penultimate leaves, as well as with chlorophyll b present in the peduncle and leaf, and also with the *6-SFT* gene expressed in the peduncle. Conversely, chlorophyll b displayed both positive and negative correlations with the *SUT1* and the *1-SST* genes in the peduncle. Furthermore, the total chlorophyll content in the penultimate leaves showed a positive and significant correlation with both the total chlorophyll and chlorophyll a content in the leaves; however, it demonstrated a negative and significant correlation with the *SUT1* gene in the peduncle. Chlorophyll a in the peduncle exhibited a positive and significant correlation with the total chlorophyll content of the peduncle and the *6-SFT* gene in the penultimate leaf. Similarly, chlorophyll b in the peduncle showed a positive and significant correlation with the total chlorophyll content of the peduncle as well as with the *6-SFT* and *1-SST* genes in the peduncle and the leaves, specifically in relation to *1-FEH*.

Finally, the total chlorophyll content of the peduncle had a positive and significant correlation with the *6-SFT* gene in the peduncle. The chlorophyll a content in the leaves indicated a positive and significant correlation with the total chlorophyll content of the leaves and yield, while also exhibiting a negative correlation with the *SUT1* and *SUT2* genes in the peduncle, along with the *SUT1* and *SUT2* genes in the penultimate tissues (Figure 2a). Chlorophyll b exhibited a positive and significant correlation with both leaf chlorophyll content and peduncle *6-SFT*. Conversely, chlorophyll b demonstrated a negative correlation with peduncle *SUT1* and peduncle *1-SST*. Penultimate *SUT1* revealed a positive and significant correlation with both *SUT1* and *SUT2*. Likewise, penultimate *SUT2* displayed a positive and significant correlation with peduncle *SUT2*, leaf *SUT1*, and leaf *6-SFT*. Furthermore, penultimate *1-SST* was positively and significantly correlated with both penultimate *FEH* and leaf *1-SST*. Penultimate *1-FEH* illustrated a positive and significant correlation with peduncle *1-FEH*. Peduncle *SUT1* was positively correlated with peduncle *1-SST*, while showing a negative correlation with peduncle *6-SFT*. Peduncle *SUT2* demonstrated a positive and significant correlation with leaf *SUT1*, *SUT2*, and *6-SFT*. Leaf *SUT1* showed a positive and significant correlation with leaf *SUT2* and *6-SFT*. Additionally, a positive and significant correlation was found between leaf *SUT2* and leaf *6-SFT*. The expression of the *1-SST* gene in leaves was positively and significantly correlated with leaf *1-FEH* (Figure 2a).

Correlations between traits at grain filling stage under water deficit

Correlations among various traits during the grain filling stage under water deficit conditions were analyzed. In particular, the relationship between physiological traits, yield, and the expression of fructan metabolism genes in response to stress demonstrated that penultimate chlorophyll exhibited a positive and significant correlation with total penultimate chlorophyll, peduncle chlorophyll a, chlorophyll a, total leaf chlorophyll, and yield. Conversely, chlorophyll a showed a negative and significant correlation with the *SUT2* gene in the peduncle, as well as with *SUT2* and *6-SFT* genes in the leaf. Additionally, penultimate chlorophyll b exhibited a positive and significant correlation with total penultimate chlorophyll, chlorophyll a and b, the total chlorophyll content in the peduncle, and the total chlorophyll content in the leaf. However, penultimate chlorophyll b also demonstrated a negative and significant correlation with *SUT1* in the peduncle. The total chlorophyll content in the penultimate stage exhibited a positive and significant correlation with chlorophyll a and the total chlorophyll content in both the peduncle and leaf, including chlorophyll a and b.

Furthermore, chlorophyll a in the peduncle showed a positive and significant correlation with chlorophyll b and the total chlorophyll content in the peduncle. On the negative front, the chlorophyll of the peduncle showed a significant negative correlation with *SUT1* in the peduncle. Chlorophyll b in the peduncle was positively and significantly correlated with both *SUT1* and *1-SST* genes. Notably, the penultimate *SUT1* gene displayed a positive and significant correlation with *SUT2* in the peduncle.

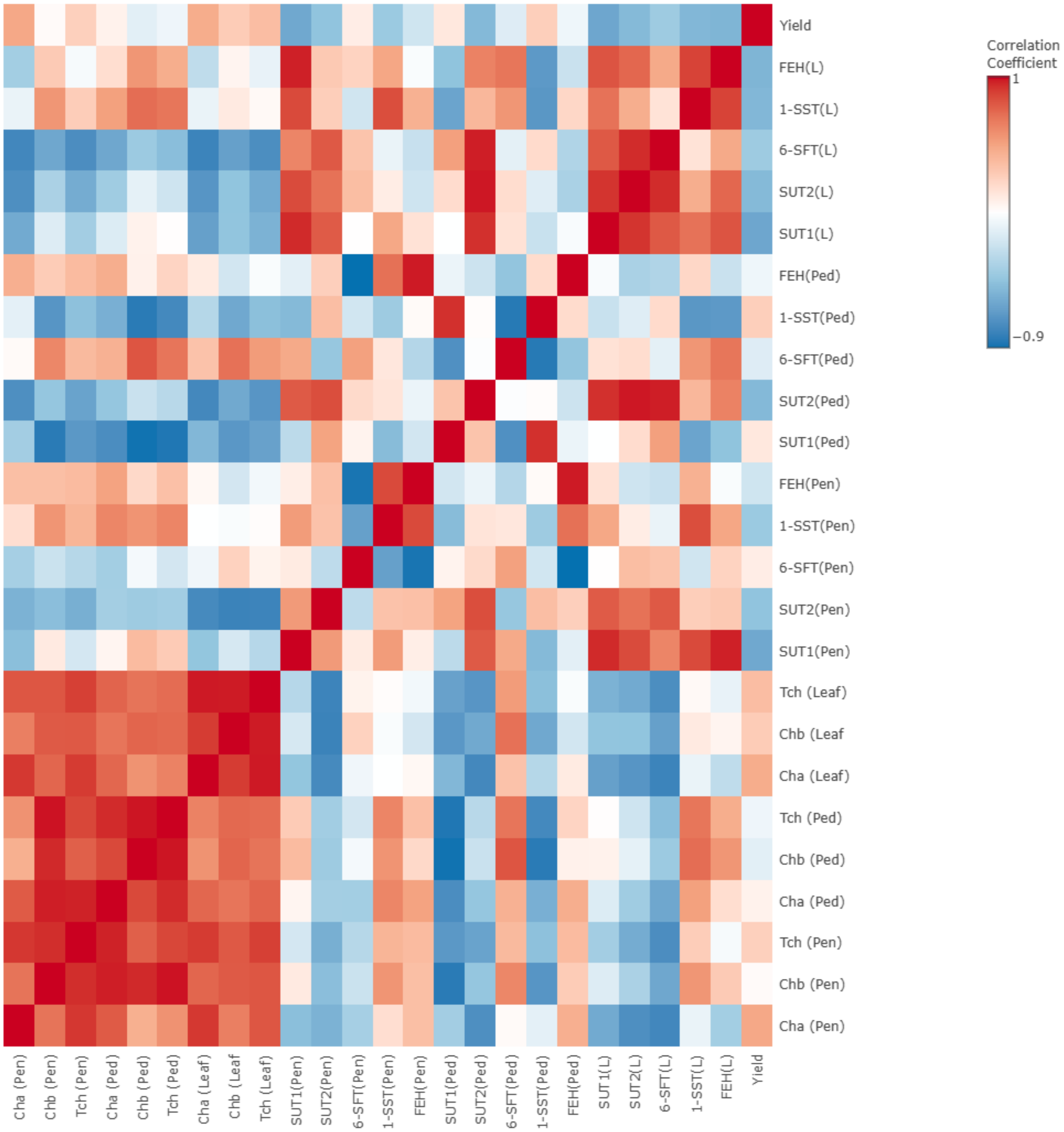

Figure 2. The correlation coefficients of physiological traits, yield, and gene expression under normal conditions.

The *SUT2* in the peduncle also revealed a positive and significant correlation with leaf *SUT1*, *SUT2* as well as leaf *1-SST* and *1-FEH*. A significant positive correlation was found between penultimate *SUT2* and peduncle *SUT2*. The penultimate *1-SST* gene offered a positive and significant correlation with both penultimate *1-FEH* and leaves *1-SST*. Furthermore, penultimate *1-FEH* exhibited a positive and significant correlation with peduncle *1-FEH*. A positive and significant correlation was observed between peduncle *1-SST* and peduncle-*1-SST*, while peduncle *SUT1* was negatively and significantly correlated with peduncle-*6-SFT*. Additionally, peduncle *SUT2* displayed a positive and significant correlation with leaf *SUT2*, *SUT1*, and *6-SFT*. In the leaves, *SUT1* was positively and significantly correlated with both leaf *SUT2* and *6-SFT*. Significant positive correlations were also noted between leaf *SUT2* and leaf *6-SFT*, as well as between leaf *1-SST* and leaf *1-FEH* (Figure 3).

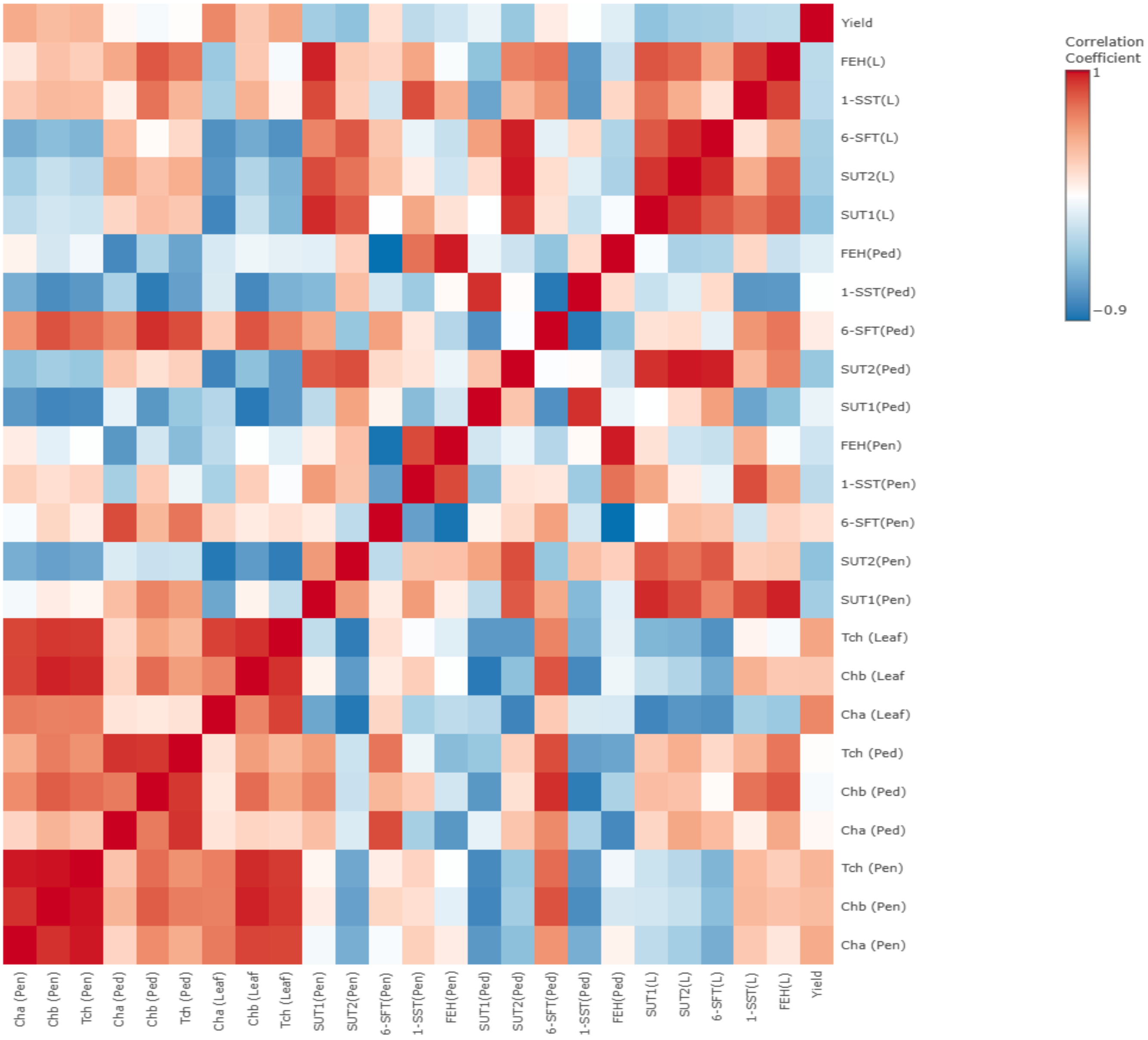

Figure 3. The correlation coefficients of physiological traits, yield, and gene expression, under drought stresses.

Discussion

Drought during the grain-filling phase significantly affects grain development and overall productivity. Breeders aim to select cultivars that possess a high capacity for nitrogen and carbon remobilization, along with genotypes that exhibit strong photosynthetic abilities (Rauf et al., 2016). Additionally, researchers must utilized the genetic foundations of yield potential (grain yield), physiological efficiency (chlorophyll content), and drought tolerance mechanisms to create cultivars capable of withstanding various stresses (Akbari et al., 2024; Hajibarat et al., 2024). Effective breeding strategies, incorporating molecular markers, should be employed to select genotypes that exhibit drought tolerance. In conditions of drought stress, stem storage serves as a crucial source for grain filling. However, the molecular processes related to the remobilization of reserves from stressed stems remain inadequately studied. Current knowledge indicates that under stress, plants draw upon water-soluble carbohydrates (WSC) from remobilized stem reserves, as well as carbon and nitrogen derived from the breakdown of leaf proteins, to support grain filling (Joudi, 2017). This process of sucrose mobilization from the stem to the wheat grain is particularly crucial for maximizing yield in conditions of terminal drought (Joudi, 2017). *1-SST* catalyzes the most important enzyme for fructan synthesis. The activity of this enzyme is related to the concentration of sucrose, which *1-SST* is affected by the activity of sucrose synthase in the stem. Fructans demonstrate a dual functionality under drought stress in barley: they contribute to osmotic adjustment, enhancing drought tolerance, and simultaneously serve as the primary polysaccharide storage in the stem, providing resources for grain filling. Previous stydy has indicated that the penultimate internode of both barley and wheat stems is the most efficient region for assimilate remobilization, with fructan and sucrose being the primary compounds involved (Zhang et al., 2015). This study further revealed that in stressed plants, genotypes 1 and 3 exhibit higher expression levels of genes crucial for fructan metabolism as compared to genotypes 2 and 4 within their internodes.

Based on the results, the higher expression of fructan mechanism enzyme under drought stress implied that genotypes 1 and 3 probably utilized the osmolytes like fructan for the osmotic adjustment and the stem reserve remobilization as compared to genotypes 2 and 4. These findings indicate that fructans may serve a dual function during drought stress, contributing both to osmotic adjustment and providing protective benefits, while also acting as the primary storage of polysaccharides in the barley stem for utilization during grain filling.The activity of *1-SST* enzymes related to the synthesis and remobilization of stems WSC showed that this enzyme has varing responces to the time and amount of soil moisture. *1-SST* activity in plant stems with normal irrigation decreased from 6 to 15 days after anthesis and then showed a high positive correlation with fructan concentration. Our findings align with previous studies that demonstrated a positive correlation between the expression levels of *1-SST* and *6-SFT* and stem WSC/fructan content. These earlier reports suggested a link between genetic variations in fructan accumulation and the expression levels of *1-SST* and *6-SFT* (Chen et al., 2024). Our findings showd that a significantly positive correlation was obtained between grain yield and chlorophyll content in both the leaves and the penultimate of stems under drought stress conditions. The activity of enzymes involved in the fructose mobilization pathway was investigated in several barley cultivars (Nosrat, Jonob, Nimrozo and Turkmen) under drought stress (Pureisa et al., 2019). The cultivars differed for WSC-related traits, reaching its maximum amount at 10 days after anthesis. The results revealed that sucrose activity: sucrose 1-fructosyltransferase (*1-SST*) increased WSCc in these cultivars. The change in fructan-1-exohydrolase (*1-FEH*) activity on the 15th day after anthesis was accompanied by a change in stem reserve remobilization. Cultivars with continued photosynthesis and stem reserve remobilization efficiency have higher yield stability under drought stress conditions. In this study, it was observed that the expression levels of key genes associated with fructan metabolism were higher in the internodes of tolerant genotypes than those of susceptible genotypes, regardless of whether the plants were well-watered or under stress. Drought stress notably enhanced the abundance of these transcripts in tolerant genotypes, whereas no such increase was seen in the susceptible genotypes. It was found that wheat cultivars exhibiting drought tolerance showed a rise in fructan exohydrolase (*1-FEH*) activity, which contributed to greater resistance to drought by facilitating the remobilization of larger quantities of fructan from the stem (Yang et al., 2020). Genotypes 1 and 3 displayed increased expression levels of the *1-FEH* gene, whereas genotypes 2 and 4 showed no significant variations in *1-FEH* gene expression. Additionally, the remobilization of fructan in drought-resistant barley cultivars began earlier compared to that in susceptible varieties (Khodaeiaminjan and Bergougnoux, 2021).

Conclusion

Terminal drought stress significantly reduces grain yield, but tolerant barley genotypes exhibit resilience due to their ability to store and mobilize fructans, a type of carbohydrate. Under drought stress, tolerant genotypes increase genes related to fructan metabolism, ensuring a consistent supply of carbohydrates from stored resources, and mitigating yield loss compared to susceptible genotypes. Genes associated with the synthesis and remobilization of fructans are significantly expressed in the penultimate internode of drought-tolerant barley, and their expression levels rapidly increase when exposed to drought conditions. This reaction leads to a greater accumulation of water-soluble carbohydrates (WSC) and speeds up their remobilization, which serves as an essential energy source for the plant. There was a positive significant correlation between grain yield and chlorophyll content, suggesting relationships among yield and physiological traits in response to drought stress. A strong relationship has been observed between the expression of genes related to fructan biosynthesis (*1-SST* and *6-SFT*) and the levels of fructan content, as well as between the expression of genes related to fructan remobilization (*1-FEH* and *SUT1*) in stress-tolerant cultivars during grain filling under drought conditions. These results indicated that the increased accumulation and subsequent remobilization of fructans are crucial for sustaining yield stability in drought-tolerant barley genotypes facing limited photosynthesis. There was a positive correlation between chlorophyll content (a/b, total) of penultimate with grain yield, indicating the key role of penultimate tissues under termnial drought stress conditions. The differences in fructan accumulation and remobilization mechanisms among various barley cultivars provide opportunities for breeding programs to enhance these traits, ultimately leading to the development of drought-resistant varieties.

Supplementary Materials

No supplementary material is available for this article.

Author Contributions

Conceptualization, Z.H. and A.S.; methodology, A.S.; software, Z.H.; validation, Z.H. and A.S.; formal analysis, A.S.; investigation, Z.H.; resources, Z.H.; data curation, A.S.; writing-original draft preparation, Z.H.; writing-review and editing, A.S.; visualization, Z.H.; supervision, A.S.; project administration, Z.H.; funding acquisition, A.S. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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مقاله پژوهشی

ارزیابی آنزیم‌های متابولیسم فروکتان در ژنوتیپ‌های متضاد جو (*Hordeum vulgare*) تحت تنش خشکی

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چکیده: حفظ عملکرد دانه در شرایط تنش خشکی انتهایی، با کمک انتقال مجدد ذخایر کربوهیدرات ساقه غلات ممکن می‌شود. این مطالعه به بررسی چگونگی انتقال مجدد فروکتان‌های ساقه بر عملکرد جو در شرایط خشکی پرداخته است. در مطالعه حاضر، تجزیه و تحلیل مقایسه‌ای فیزیولوژیکی و بیان ژن با استفاده از بافت برگ پرچم، پنالتیمیت و پدانکل در چهار ژنوتیپ جو (G1، G2، G3، G4) انجام شد. سطوح بیان ژن‌های متابولیسم فروکتان در بافت‌های برگ، پنالتیمیت و پدانکل ساقه در مرحله پر شدن دانه و در دو شرایط تنش خشکی و نرمال مقایسه شدند. شایان توجه است که تنوع ژنتیکی میان ارقام بر بیان ژن‌های مرتبط با پاسخ به تحمل خشکی، اثرگذار است.به طور خاص، ژنوتیپ‌های ۱ و ۳ در پاسخ به تنش خشکی، افزایش بیان ژن‌های متابولیسم فروکتان را نشان دادند، در حالی که ژنوتیپ‌های ۲ و ۴ کاهش بیان را بروز دادند. یافته‌های ما نشان داد که افزایش تجمع فروکتان و انتقال مجدد آن در طول دوره پرشدن دانه ممکن است به طور قابل‌توجهی به پایداری عملکرد ژنوتیپ‌های ۱ و ۳ در شرایط تنش خشکی کمک کند. ژنوتیپ‌های جو که توانایی بالایی در تولید فروکتان و بازتخصیص آن در واکنش به تنش خشکی انتهایی دارند، می‌توانند به‌عنوان منابعی ارزشمند در برنامه‌های به‌نژادی جو برای شرایط تنش خشکی مورد استفاده قرار گیرند.

کلمات کلیدی: جو، تحمل به خشکی، متابولیسم فروکتان، بیان ژن، پر کردن دانه.

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