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Expression profiling of two sucrose transporter genes during post-anthesis in wheat

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Abstract

Sucrose transporters (SUTs) are critical for distribution and storage of sucrose between the source and sink tissues and considered as an important key for an efficient growth of common wheat (*Triticum aestivum* L.) during post-anthesis as well as during senescence stage. Therefore, a complete study including molecular and biochemical experiments was carried out to assess the *TaSUT1* and *TaSUT2* genes expression, reactive oxygen species: superoxide radical ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), production and antioxidant enzymes: superoxide dismutase (SOD) and catalase (CAT), activity as well as non-enzymatic antioxidants: ascorbate, α-tocopherol and carotenoid, content in flag leaf and other leaves of promising line N81-18 of wheat during post-anthesis. The *TaSUT1* and *TaSUT2* genes expression was identified in both flag leaf and other leaves, as the flag leaf had the highest genes expression. The activity of SOD and CAT significantly increased in both flag leaf and other leaves, which caused a decrement of $O_2^{\bullet-}$ and H_2O_2 content, respectively. Moreover, the ascorbate, α-tocopherol and carotenoid content significantly increased as a cellular defence mechanism to minimize the damage due to oxidative stress caused during post-anthesis and leaf senescence. Overall, it can be concluded that flag leaf had the highest *TaSUT1* and *TaSUT2* genes expression as well as non-enzymatic antioxidants that could be considered as the main key for future genetic studies and practical breeding programs.

Key words: antioxidant, ascorbate content, α-tocopherol, catalase, hydrogen peroxide, superoxide radical.

Introduction

Common wheat (*Triticum aestivum* L.), as a strategic crop, is the main source of staple foods providing 70–90% of all calories and 66–90% of the protein consumed. According to the cultivated area and total production, wheat is considered as the major cereal crop in the world, including Iran. Considering that the global demand for wheat has grown, its global production will need to increase by 1.6–2.6% annually until 2020, in order to eliminate the wide gap between productivity and consumption (Salim, 2016; Houshyar, Grundmann, 2017). Therefore, to achieve this goal, research should be focused on physiological, biochemical and metabolic processes occurring in wheat during the growing season.

Recently the most promising methods for increasing crop yield have appeared to be those based on the genetic engineering of developmental or bioenergetic processes, such as photosynthesis. Photosynthesis is mutually connected and restricted by the light and dark reactions, which are used by photoautotrophic organisms to assimilate atmospheric carbon dioxide (CO_2) into organic molecules and produce sugars (Pribil, Leister, 2017). Sucrose, a highly soluble disaccharide, is one of the main end products in photosynthetically active leaves that provide a mobile energy source for the distribution of carbohydrates within all parts of higher plants (Levine, 2011). Moreover, sucrose plays an important role in plant growth and development, crop yield and quality as well as responses to abiotic stresses. In plants, it acts not only as an essential source of carbon but also as signalling molecules in response to the integration of information from environmental signals as well as developmental and metabolic cues (Lastdrager et al., 2014). Various metabolic reactions and regulations directly link soluble sugars, such as sucrose, with the production rates of reactive oxygen species (ROS), such as mitochondrial respiration or photosynthesis regulation, and, conversely, with antioxidative processes, such as the oxidative pentose-phosphate pathway, secondary metabolites and carotenoid biosynthesis. In reverse, sucrose can also feed nicotinamide adenine dinucleotide phosphate (NADPH)-producing metabolic pathways, such as the oxidative pentose-phosphate (OPP) pathway, which can contribute to ROS scavenging (Couee et al., 2006; Van den Ende, Valluru, 2008).

The procedure of sucrose entrance into the phloem and its delivery into the sink cells involves

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membrane transporter proteins well known as sucrose transporters (SUTs). SUTs are proteins with 12 transmembrane domains that form a pore in the membrane to allow the passage of sucrose. SUTs function as sucrose / proton symporters that use the energy stored in the proton gradient across the membrane generated by H^+-ATPases to drive sucrose movement (Braun et al., 2014). SUTs also have the main role in plant resistance under abnormal conditions by carbohydrate distribution; facilitate the production of secondary metabolites and ROS scavenging (Cai et al., 2017; Rezaul et al., 2019). Genes encoding plant SUTs have been isolated from different plants, such as rice (Eom et al., 2011), wheat (Deol et al., 2013), sorghum (Milne et al., 2013) and *Saccharum* species (Zhang et al., 2016), and are known to form small gene families. SUTs are divergent in function and deferentially expressed in different tissue types or at different plant developmental stages (Aoki et al., 2004).

Sucrose metabolism and transport are very critical for growth and senescence. Flag leaf photosynthesis is an important factor to produce photoassimilates for grain filling and the initiation of grain filling coincides with the onset of senescence: therefore, photosynthesis of the flag leaf is the most important reason for the increment of grain yield, and the start and rate of senescence are important factors for assessment of grain yield (Zhang et al., 2006; Wang et al., 2015).

Leaf senescence is the main physiological process, which is considered as the final step of leaf development. Senescence enhances the production of ROS and subsequently, ROS negatively affect grain filling, remobilization of nutrients, degradation of pigments content and lipid peroxidation. However, a scavenging system including enzymatic and non-enzymatic antioxidants can reduce cellular damage caused by ROS (Guo et al., 2015).

Sucrose plays an important role in the development of wheat grains; identification and characterization of new SUTs in wheat provide insights into their role in determining seed yield. On the other hand, few studies have been monitoring SUTs expression during post-anthesis of wheat with regards to leaf senescence process. Therefore, in the present study, the *TaSUT1* and *TaSUT2* genes expression, ROS production and antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) activities, and also non-enzymatic antioxidants (ascorbate, α-tocopherol and carotenoid) content during post-anthesis in flag leaf and other leaves of promising line N81-18 of wheat was compared.

Materials and methods

Plant growth conditions. The present study was done at the Gorgan University of Agriculture and Natural Sciences, Iran, in 2016. Seeds of the promising line N81-18 of common wheat (*Triticum aestivum* L.) were obtained from the Rice Research Institute of Rasht, Iran. It should be noted that promising line N81-18 is one the most popular and cultivated wheat lines in Iran. Seeds were surface sterilized with 2% (w:v) sodium hypochlorite for 10 min and rinsed four times thoroughly with distilled water. Mature dry seeds were imbibed on a moist sterile Whatman #1 filter paper in a Petri plate (15 seeds per plate) in darkness for three days. Then, germinated seedlings were planted in 4 L (20.0 × 15.5 × 16.5 cm) pot (1 seedling per pot) filled with the LA4 mix (containing sphagnum peat moss, sand, starter nutrient with gypsum and dolomitic limestone; sun-grow). The seedlings were grown at 16–22/14–18°C day/night temperature under a 16/8 h photoperiod with cool white fluorescent light (175 µmol m^{-2} s^{-1}). The seedlings were irrigated every other day and fertilized with 20:20:20 (N:P:K) mixture once a week (Deol et al., 2013). Sampling was done individually from flag leaf and other leaves at 2, 7, 12 and 17 days after anthesis (DAA). Three biological replications were taken for each evaluated time. Samples were frozen in liquid nitrogen and then stored at −80°C until further traits measurement.

Analysis of genes expression. The overexpression of wheat sucrose transporter (*TaSUT1* and *TaSUT2*) genes was evaluated using the Deol et al. (2013) method with slight modifications. Briefly, flag leaf and other leaves (100 mg fresh weight (FW) per sample) of the promising line N81-18 of wheat individually were pooled and ground into fine powder in liquid nitrogen using sterile mortar and pestle. Total RNA was extracted using Biozol kit (CinnaGen, Iran) as recommended by the manufacturer. Extraction of total RNA from developing leaves was performed as described by Li and Trick (2005). The RNA samples were then subjected to cDNA synthesis using the DNase I Kit (Thermo Fisher Scientific, Germany) following the manufacturer's protocol. The cDNA samples were amplified using specific primers designed.

Table shows properties and sequences of primers for genes used in the experiment, wheat β-actin (*Taβ-actin*) used as reference gene. The quantity of mRNA corresponding to each gene was measured by SYBR Green method using Sybr Biopars Kit (GUASNR, Iran) by a quantitative real-time polymerase chain reaction (q-PCR) device (Corbett Research, Australia). Each sample was evaluated in three technical and three biological replications.

Table. Features of primers applied in q-PCR experiment

Genes		Primers sequence amplicon	Length bp
TaSUT1	FP	TGGATTCTGGCTCCTTGAC	150
	RP	GCCATCCAAGAACAGAAGATT	
TaSUT2	FP	TACGGAGTCCTGCTCTGTCA	150
	RP	CTCGTCGCTTCCGAAAGTA	
Taβ-actin	FP	GCTGTTCCAGCCATCTCATGT	156
	RP	GCTGTTCCAGCCATCTCATGT	

FP – forward primer, RP – reverse primer

Measurement of reactive oxygen species. Superoxide radical ($O_2^{\bullet -}$) was measured as described by Elstner and Heupel (1976). This assay is based on the formation of nitrite (NO_2^-) from hydroxylamine in the

presence of $O_2^{\bullet-}$. The absorbance of the coloured aqueous phase was recorded at 530 nm by atomic absorption spectrometer Uvikon 930 (Kontron Instruments, UK). A standard curve prepared with sodium nitrite ($NaNO_2^-$) was used to calculate the production rate of $O_2^{\bullet-}$. Hydrogen peroxide (H_2O_2) content was measured by following titanium-peroxide complex method according to the Brennan and Frenkel (1977). The absorbance values via oltrasfer column (OD = 5 μm) at 415 nm were calibrated to a standard graph generated with known concentrations of H_2O_2.

Measurement of antioxidant enzymes activity. The activity of superoxide dismutase (SOD; EC 1.15.1.1) was determined based on the method of Giannopolitis and Ries (1977) with some modifications. Samples (1.0 g) were homogenized with pre-chilled mortar and pestle, in 5 ml of 0.1 M phosphate buffer (pH 7.0). The extract was centrifuged in cold centrifuge at 15.000× g for 20 min at 48°C and the supernatant was used as enzymes source. The reaction solution contained 50 mM phosphate buffer (pH 7.8), 2 μM riboflavin (7,8-dimethyl-10-ribitylisoalloxazine), 13 mM methionine (2-amino-4-(methyl-thio)-butyric acid), 0.1 mM EDTA (ethylenediaminetetraacetic acid), 75 μM nitro blue tetrazolium (NBT, 2,2′-di-p-nitrophenyl-5,5′-diphenyl-(3,3′-dimethoxy-4,4′-diphenylene) ditetrazolium chloride) and 100 mL of extracted solution. The mixture was held in a test tube and placed under fluorescent lights (100 μmol photons s^{-1} m^{-2}) for 15 min. The photo-reduction of NBT (formation of purple formazone) was measured by spectrophotometer Uvikon 930 (Kontron Instruments) at 560 nm. One unit of SOD activity is defined as the amount of enzyme, which is required to cause 50% inhibition in the reduction of NBT. Finally, SOD activity was expressed as U mg^{-1} of protein.

Catalase (CAT; EC 1.11.1.6) activity was determined in terms of decrease in absorbance by spectrophotometer Uvikon 930 (Kontron Instruments) due to decomposition of H_2O_2 at 240 nm using an extinction coefficient of 39.4 mM^{-1} cm^{-1} (Aebi, 1984). Three ml reaction mixture containing 1.5 ml of 50 mM potassium phosphate (KH_2PO_4) buffer (pH 7.8), 0.5 ml of 75 mM H_2O_2, 200 μl enzyme extraction and distilled water to make up the volume to 3 ml. CAT activity was expressed as μM min^{-1} mg^{-1} of protein min^{-1}.

Measurement of non-enzymatic antioxidants. The ascorbate content was determined according to the method of Arakawa et al. (1981). In brief, 500 mg of samples were macerated in liquid nitrogen, added to 20% PVPP (m:m) and homogenized in 10 mL of trichloroacetic acid (TCA) 5% (m:v). The homogenate was then centrifuged at 14.000× g for 10 min at 4°C. Aliquots (40 μL) of the supernatant were added to the reaction medium, which was composed of TCA 5% (m:v), ethanol 99.8% (v:v), phosphoric acid (H_3PO_4) 0.4% in ethanol (v:v), bathophenanthroline 0.5% in ethanol (m:v) and iron(III) chloride ($FeCl_3$) 0.03% in ethanol (m:v). The mix was homogenized thoroughly and incubated at 30°C for 90 min. Finally, the absorbance of the extracted mixture was measured by spectrophotometer Uvikon 930 (Kontron Instruments) at 534 nm. A standard curve in the range 0–25 nmol ascorbate was used to calculate ascorbate, and the ascorbate content was expressed as μm g^{-1} FW. α-Tocopherol was determined using high-performance liquid chromatography (HPLC) Merck-Hitachi L-7100 (Germany) as described by Munné-Bosch et al. (1999). Samples (1 g) were frozen in liquid nitrogen followed by grinding with 5 mL methanol containing ascorbate (1%) and 4 mL hexane. Aqueous part of the samples was centrifuged for 10 min at 1500 g. The supernatant of centrifuged samples was filtered through disposable 0.45-mm syringe filter. Then 2 mL of the filtered sample was injected into the HPLC. UV detection was carried out at 295 nm and fluorescence detection was performed at an excitation wavelength of 295 nm and emission at 340 nm. α-Tocopherol (98.4% purity) was used for calibration. Finally, α-tocopherol content was expressed as μg g^{-1} dry weight (DW). The carotenoid content was determined by spectrophotometer Uvikon 930 (Kontron Instruments), according to the Lichtenthaler (1987). Extraction was done in methanol and the extract was filtered. The absorbance (A) of the extracted mixture was recorded at 480, 663 and 645 nm, and the content of carotenoid was calculated by the formula:

$$A_{480} - (1.144 \times A_{663} - 0.638 \times A_{645}).$$

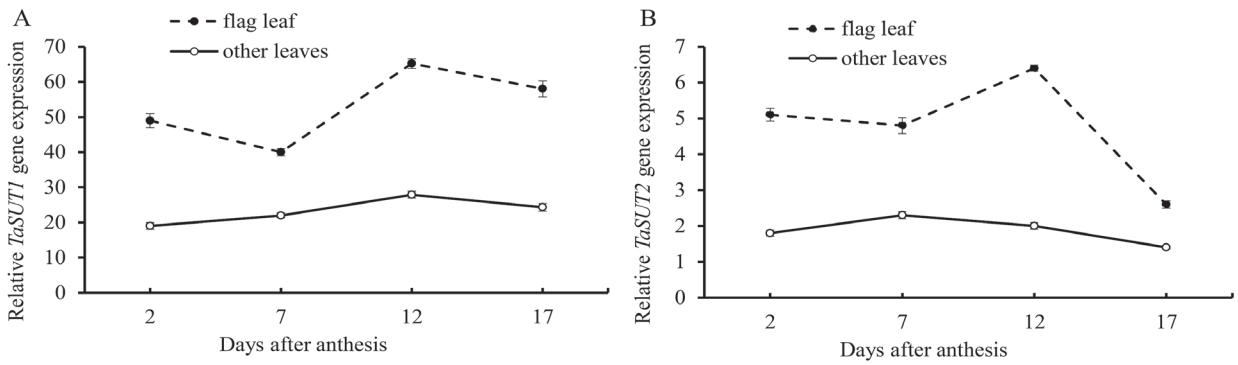
Carotenoid content was expressed as μg g^{-1} DW.

Statistical analysis. The experiment was carried out according to a split plot design based on a completely randomized block design with three replications. Enzymatic and non-enzymatic antioxidants activities as well as reactive oxygen species (ROS) content were analysed by software *SAS*, version 9.1 (SAS Institute Inc., USA). The ratio between the target gene and reference gene was analysed by the software *REST*, version 2.0.13 (Qiagen, USA).

Results and discussion

TaSUT1 and TaSUT2 genes expression. The results showed that *TaSUT1* and *TaSUT2* genes expression was significantly affected by the simple and the interaction effects of sampling type and sampling time. *TaSUT1* gene expression in flag leaf significantly decreased at 7 days after anthesis (DAA), then significantly increased and decreased again at 17 DAA, while in other leaves *TaSUT1* gene expression gradually increased until 12 DAA and then slightly decreased (Fig. 1A). In the flag leaf, *TaSUT2* gene expression slightly decreased at 7 DAA, then significantly increased until 12 DAA and finally significantly decreased at 17 DAA, but in other leaves, *TaSUT2* gene expression slightly increased at 7 DAA and then gradually decreased until 17 DAA (Fig. 1B).

The differential expression patterns of the *TaSUT1* and *TaSUT2* genes in this work suggest that the *SUT* gene family has many roles in both source and sink tissues, and at different developmental stages and different plant tissues. These results are in agreement with Aoki et al. (2003). Expression patterns of *SUT* genes in developing rice caryopses suggested that the physiological role(s) of *SUT1* may differ from those of the other *SUT2* gene when caryopses differentiate, elongate and fill (Aoki et al., 2003). Moreover, it was reported that *SUT1* and *SUT2* genes expression patterns were different, while *SUT1* is mainly expressed in sucrose exporting source leaves, and *SUT2* is expressed predominantly in sink organs, such as sink leaves, stems and fruits (Barker et al., 2000).



Note. When larger than symbols, vertical bars represent ± SE of the mean for n = 3.

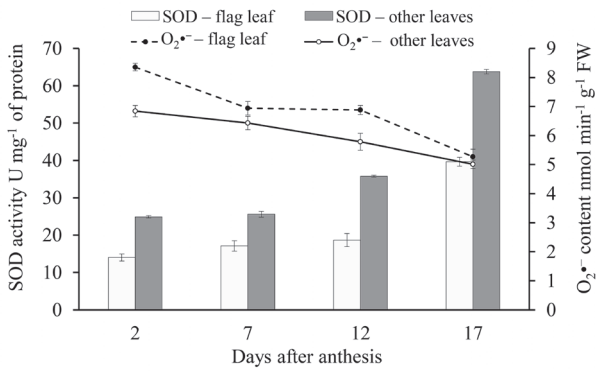
Figure 1. Changes of *TaSUT1* (A) and *TaSUT2* (B) genes relative expression in flag leaf and other leaves of wheat during 17 days after anthesis

SUTs are essential proteins for the distribution of assimilate from source to sink in higher plants. The orthologous transporter of wheat *TaSUT1* is assumed to be directly involved in sugar transfer across the scutellar epithelium and to have a transport function in enucleate sieve elements (Aoki et al., 2004). Furthermore, Deol et al. (2013) concluded that *TaSUT2* gene is involved in the exchange of sucrose between the vacuolar and cytoplasmic cell compartments in both sink and source tissues.

In agreement with our results, the expression of *TaSUT1* (Aoki et al., 2002; 2004; 2006) and *TaSUT2* (Deol et al., 2013) genes has been identified and characterized in wheat. It was reported that the transcriptional regulation of SUTs genes depends on tissue types, various developmental activities, environmental factors, such as light and different stresses, and endogenous signals, such as sugars and hormones (Fukuzawa et al., 2012). It was noted that in wheat the expression of *TaSUT* gene in flag leaf blades, leaf sheaths and internodes was the highest before heading and decreased immediately after flowering when an increased level of its transcript was evident in the developing seeds (Aoki et al., 2004). The decrement in the expression of *TaSUT* genes in the flag leaf blade during the later stages of seed filling can be associated with the senescence of leaves and decreased accumulation of storage sucrose in the vacuole (Schnyder, 1993; Kong et al., 2010; Deol et al., 2013).

On the other hand, the wheat tonoplast-localised *TaSUT2* gene is greatly expressed in the flag leaf blade before anthesis and during early grain filling, suggesting intracellular partitioning in leaves to allocate assimilates from leaves to grains. *TaSUT1* and *TaSUT2* genes are functionally and temporally co-ordinated during seed development (Deol et al., 2013), whereas *TaSUT2* might control cytosolic sucrose homeostasis in leaves, and *TaSUT1* secures sucrose loading in the endosperm. Tonoplast *HvSUT2* in barley and *OsSUT2* in rice (Sun et al., 2008) are greatly expressed in source leaves, consistent with the fact that cereal leaves transiently accumulate sucrose rather than starch in vacuoles (Martinoia et al., 2007).

Antioxidant enzymes activity and reactive oxygen species (ROS) content. As shown in Figure 2, the simple and the interaction effects of sampling type and sampling time significantly influenced the SOD activity as well as the $O_2^{\bullet-}$. In both flag leaf and other leaves SOD activity significantly increased during 17 DAA, as this increment was higher in other leaves (from 3.2 to 8.2 U mg^{-1} of protein) as compared with flag leaf (from 1.8 to 5.1 U mg^{-1} of protein). The $O_2^{\bullet-}$ content significantly reduced in both flag leaf (from 65.3 to 41.2 nmol min^{-1} g^{-1} FW) and other leaves (from 53.2 to 39.1 nmol min^{-1} g^{-1} FW) in response to the SOD activity. Moreover, 17 DAA no significant difference was observed between $O_2^{\bullet-}$ content in both flag leaf (41.2 nmol min^{-1} g^{-1} FW) and other leaves (39.1 nmol min^{-1} g^{-1} FW).

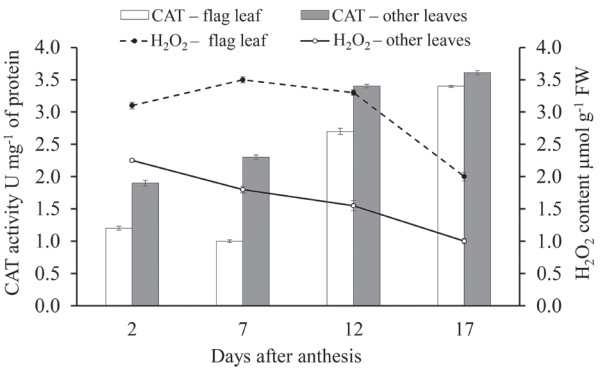


Explanation under Figure 1

Figure 2. Changes of superoxide dismutase (SOD) enzyme activity and superoxide radical ($O_2^{\bullet-}$) content in flag leaf and other leaves of wheat during 17 days after anthesis

It was found that the simple and the interaction effects of sampling type and sampling time significantly affected the CAT activity and H_2O_2 content. The CAT activity in flag leaf slightly diminished from 1.2 to 1.0 U mg^{-1} of protein at 7 DAA and then significantly enhanced to 3.4 U mg^{-1} of protein until 17 DAA, while CAT activity in other leaves significantly increased from 1.9 to 3.6 U mg^{-1} of protein during post-anthesis (Fig. 3). According to Figure 3, the H_2O_2 content of flag leaf significantly increased from 3.1 to 3.5 µmol g^{-1} FW at 7 DAA and then significantly decreased to 2.0 µmol g^{-1} FW until 17 DAA, while in other leaves H_2O_2 content significantly decreased from 2.3 to 1.0 µmol g^{-1} FW during post-anthesis.

Our results are in agreement with the findings of Rogers and Munné-Bosch (2016). The final stage of leaf development is inevitably senescence with a genetically programmed sequence of biochemical and physiological



Explanation under Figure 1

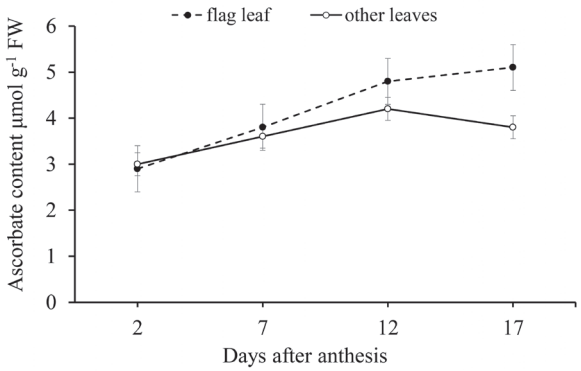
Figure 3. Changes of catalase (CAT) enzyme activity and hydrogen peroxide (H_2O_2) content in flag leaf and other leaves of wheat during 17 days after anthesis

changes that occur during the plant life. As green tissues progress toward senescence, the photosynthetic rate would decrease due to the ultrastructural alterations of chloroplasts and the ROS (such as $O_2^{\bullet-}$ and H_2O_2) levels increase, which can then lead to oxidative damage to the photosynthetic apparatus and cellular macromolecules (Kong et al., 2015).

In plants, $O_2^{\bullet-}$ which is continually being created through endogenous processes and exogenous sources can through various pathways or chain reactions cause the production of numerous other reactive species. It can directly produce hydroxy radical or indirectly from H_2O_2. $O_2^{\bullet-}$ is generated in different cell compartments, including chloroplasts, peroxisomes, apoplast, the mitochondrial electron transport chain and the plasma membrane. Furthermore, $O_2^{\bullet-}$ is a moderately reactive, short-lived ROS with a half-life of approximately 2–4 µs; therefore, it cannot cross biomembranes and is easily dismutated to H_2O_2. It should be noted that a signalling role of $O_2^{\bullet-}$ during senescence and abiotic stresses seems unconvincing (Jajic et al., 2015). H_2O_2 is a non-radical ROS produced in a two-electron reduction of molecular oxygen. Several sites have been recognized as H_2O_2 sources, including organelles (mitochondria, peroxisomes and chloroplasts), the apoplastic and the plasma membrane and also cell-wall associated enzymes (Chakraborty, Pradhan, 2012).

The oxidative damage to cellular elements is restricted during normal growing conditions due to the effective processing of ROS. Plants possess very efficient antioxidant defence systems, which work in concert to regulate unrestrained oxidation processes and reduce the $O_2^{\bullet-}$ and H_2O_2 production, thereby preventing membrane peroxidation (Ahmad, 2014). SOD and CAT are two key enzymes in the active-oxygen scavenging system. SODs are the first step of cellular defence to scavenge ROSs; they transform $O_2^{\bullet-}$ and water (H_2O) to H_2O_2 and molecular oxygen (O_2). Then the produced H_2O_2 is quickly converted to H_2O and ½ O_2 by the activity of CAT (Gupta, Huang, 2014). The increment of SOD and CAT activities that was observed in our study might be due to the plant response to senescence processes and excessive ROS production. Subsequently, the cellular $O_2^{\bullet-}$ and H_2O_2 content significantly decreased as a result of the increment of antioxidant enzymes activity.

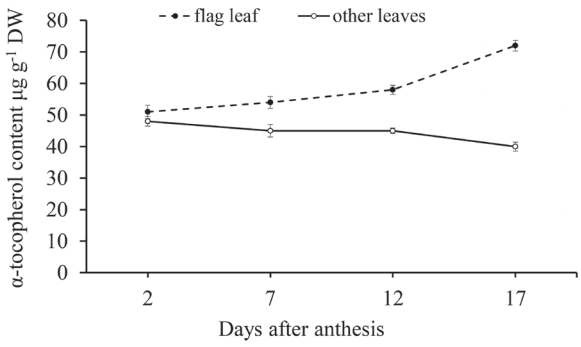
Non-enzymatic antioxidants. As shown in Figure 4, ascorbate content was significantly influenced by the simple and the interaction effects of sampling type and sampling time. In flag leaf, the ascorbate content significantly increased from 2.9 to 5.1 µmol g^{-1} FW during post-anthesis, while in other leaves it significantly increased until 12 DAA (from 3.0 to 4.2 µmol g^{-1} FW) and then slightly decreased (to 3.8 µmol g^{-1} FW) at 17 DAA.



Explanation under Figure 1

Figure 4. Changes of ascorbate content in flag leaf and other leaves of wheat during 17 days after anthesis

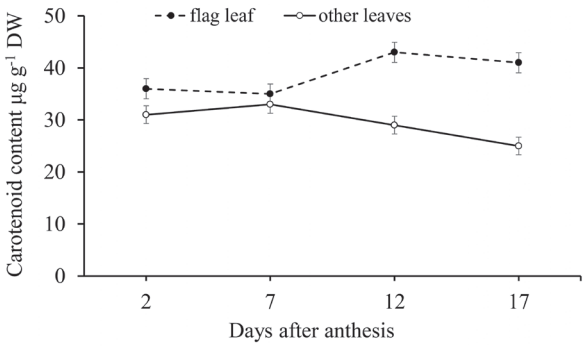
Our results revealed that the simple and the interaction effects of sampling type and sampling time significantly affected the α-tocopherol content. Moreover, the α-tocopherol content significantly increased from 51.3 to 72.6 µg g^{-1} DW in flag leaf of wheat during post-anthesis, whereas other leaves showed slight decrement of α-tocopherol content from 48.1 to 40.7 µg g^{-1} DW during 17 DAA (Fig. 5).



Explanation under Figure 1

Figure 5. Changes of α-tocopherol content in flag leaf and other leaves of wheat during 17 days after anthesis

As is seen from Figure 6, the carotenoid content of flag leaf and other leaves in wheat was significantly affected by the simple and the interaction effects of sampling type and sampling time. In flag leaf, carotenoid content slightly decreased from 36.2 to 35.1 µg g^{-1} DW at 7 DAA and then until 12 DAA significantly increased to 43.7 µg g^{-1} DW and finally decreased to 41.4 µg g^{-1} DW at 17 DAA. But carotenoid content in other leaves slightly increased from 31.6 to 33.7 µg g^{-1} DW and then significantly decreased to 25.6 µg g^{-1} DW until 17 DAA.



Explanation under Figure 1

Figure 6. Changes of carotenoid content in flag leaf and other leaves of wheat during 17 days after anthesis

These results are in agreement with Farouk (2011) and Lou et al. (2018). To diminish the damage due to oxidative stress, plants have evolved complex enzymatic and non-enzymatic antioxidant systems, such as low-molecular-mass antioxidants (ascorbate, α-tocopherol, carotenoids, etc.). It is well evident that plant cells are strongly redox-buffered due to very large quantities of the water-soluble antioxidants. The physiologically effective model of ascorbic acid (vitamin C) is the resonance stabilized anionic form (formed due to deprotonation of the hydroxy group at C_3), which is known as ascorbate (Akram et al., 2017). It is one of the most influential antioxidant molecules due to the regenerative nature of ascorbate, which plays a vital role in suppressing intermediate / excited reactive forms of molecular oxygen either directly or through enzymatic catalysis (Zhang, 2013).

α-Tocopherol is a lipophilic membrane located compound present in chloroplasts. α-Tocopherol is believed to protect chloroplast membranes from photo-oxidation and to help provide an optimal environment for the photosynthetic machinery. The most prominent function of α-tocopherol is protection of polyunsaturated fatty acids from lipid peroxidation (Farouk, 2011). Additionally, α-tocopherol is another non-enzymatic antioxidant, which can alleviate the harmful effect of ROS. The antioxidant activity of α-tocopherol is mainly due to its ability to donate its phenolic hydrogens to lipid free radicals, involved in both electron transport of photosystem II (PSII) and antioxidizing system of chloroplasts and act as membrane stabilizers and multifaceted antioxidant that scavenge ROS (Orabi, Abdelhamid, 2016).

Carotenoids are lipophilic organic compounds present in the plastids of both photosynthetic and non-photosynthetic plant tissues. Carotenoids play a multitude of functions in plant metabolism including the role in oxidative stress tolerance. In chloroplasts, carotenoids function as accessory pigments in light harvesting; however, perhaps a more important role is their capacity to detoxify different types of oxygen free radicals (Karuppanapandian et al., 2011).

Similarly in our study, it was found that during post-anthesis and along with the progress of senescence, the non-enzymatic antioxidants content, such as ascorbate, α-tocopherol and carotenoid, significantly increased for scavenging of ROS.

Conclusion

This study identified the *TaSUT1* and *TaSUT2* genes expression in both flag leaf and other leaves of the promising line N81-18 of wheat during post-anthesis, as the highest relative *TaSUT1* and *TaSUT2* genes expression was obtained in the flag leaf. During post-anthesis and along with leaf senescence, superoxide dismutase (SOD) and catalase (CAT) activities significantly increased in order to scavenge the superoxide radical ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) content. Additionally, the highest antioxidant enzymes activity was found in other leaves as compared with flag leaf. The content of ascorbate, α-tocopherol and carotenoid as non-enzymatic antioxidants significantly increased during post-anthesis, as the flag leaf showed the highest non-enzymatic antioxidants content. The increment of total antioxidant capacity would lead to minimizing the damage of oxidative stress during post-anthesis and along with leaf senescence.

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Dviejų sacharozės pernešėjų genų raiška paprastojo kviečio grūdų vystymosi ir brendimo tarpsniais

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Santrauka

Sacharozės pernešėjai yra labai svarbūs sacharozės paskirstymui ir kaupimui tarp gaminančių bei vartojančių audinių ir yra laikomi itin reikšmingais paprastojo kviečio (*Triticum aestivum* L.) grūdų vystymosi, brendimo ir senėjimo tarpsniais. Siekiant įvertinti genų *TaSUT1* ir *TaSUT2* ekspresiją reaktyvios rūšies deguonies (superoksido radikalo ($O_2^{\bullet-}$) ir vandenilio peroksido (H_2O_2)) gamybai ir antioksidantinių fermentų (superoksido dismutazės (SOD) bei katalazės (CAT)) aktyvumui, taip pat nefermentinių antioksidantų (askorbato, α-tokoferolio bei karotinoidų) kiekį perspektyvios kviečių linijos N81-18 viršūniniame ir kituose lapuose grūdų vystymosi, brendimo ir senėjimo tarpsniais, buvo atliktas išsamus tyrimas, apimantis molekulines ir biochemines analizes. Genų *TaSUT1* ir *TaSUT2* ekspresija buvo nustatyta viršūniniame ir kituose lapuose, tačiau viršūninis lapas pasižymėjo didžiausia šių genų ekspresija. SOD ir CAT aktyvumas reikšmingai padidėjo ir viršūniniuose, ir kituose lapuose, dėl to atitinkamai sumažėjo $O_2^{\bullet-}$ ir H_2O_2 kiekis. Be to, askorbato, α-tokoferolio ir karotenoidų kiekis reikšmingai padidėjo kaip ląstelės gynybos mechanizmas siekiant sumažinti oksidacinio streso žalą, kuri pasireiškia grūdų vystymosi, brandos bei senėjimo tarpsniais. Galima daryti išvadą, kad viršūninis lapas pasižymėjo didžiausia genų *TaSUT1* bei *TaSUT2* ekspresija ir didžiausiu kiekiu nefermentinių antioksidantų, kurie ateityje galėtų būti laikomi pagrindiniu genetinių tyrimų ir praktinių selekcinių programų raktu.

Reikšminiai žodžiai: antioksidantas, askorbato kiekis, α-tokoferolis, katalazė, superoksido radikalas, vandenilio peroksidas.