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Research Article

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Investigation of the expression pattern of some nitrogen transporter genes in root in two different cultivars of wheat

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ABSTRACT- In crop production, one important limiting factor is the availability of nitrogen (N) and N derivatives, as they are of much significance in plant metabolism and growth. Various nitrate transporter genes including GS2a, TaNRT2.1, TaNRT2.2, TaNRT1, ASN1, cysteine protease, GST1, and *TaAMT2.1* mediate the uptake, transport, and remobilization of N in the plants. However, their role is not quite understood with respect to the importance of grain-filling stages and the plant's overall yield. Herein, the expression levels of a number of genes and enzymes involved in N uptake and metabolism were measured over the course of anthesis and under N-treatet and N-deficit conditions in two genotypes of wheat, namely Morvarid and Gonbad. The samples were taken 10, 15, and 20 days after anthesis (DAA), which is a critical stage in grain filling, and the relation between gene expression, enzyme activity, and DAA was demonstrated. It was observed that N-related gene expression significantly increases under N-deficit conditions and over the course of anthesis, suggesting the significance of the N-related genes and enzymes in maintaining the metabolism and growth of the plants under N-deficit conditions. The results of this study also showed that the amount of nitrogen in the plant can increase the amount of seed protein, leaf chlorophyll content, the size and volume of cell protoplasm, and also affect the leaf surface and photosynthetic activity, so it is important to use modified cultivars with high expression of transgenic nitrogen genes.

INTRODUCTION

The availability of nitrogen is a particular factor in crop production. Therefore, the amount of N fertilizers used has increased significantly each year. Nitrate (NO_3^-) and ammonium (NH_4^+), as derivatives of N, are of significant importance to plant growth. Nitrogen absorption has different mechanisms in plants. In fact, plants uptake both nitrate and ammonium forms (Schroeder et al., 2013; Ho and Tsay, 2010). The transfer of NO_3^- or NH_4^+ to the epidermal cells of the root is considered the first step in the process of nitrogen absorption and consumption by plants (Forde. 2000). At least two specific transporters mediate NO_3^- transport, namely the high-affinity transport system (HATS) and the low-affinity transport system (LATS), encoded by the *NRT2* and *NRT1* gene families, respectively (Forde. 2000; Léran et al., 2014). Similarly, in the case of ammonium, there are low and high-affinity ammonium transport systems (AMTs) encoded by the *aminomethyltransferase* (*AMT*) gene family (Duan et al., 2016). In the next step, NO_3^- is reduced to nitrite, and nitrite to ammonium, by NO_3^- reductase (NR) and nitrite reductase (NiR) in the second step, respectively (Leydecker et al., 2000).

The distribution of nitrate- and N-containing metabolites in different tissues is influenced by plant growth following initial root-mediated uptake. In the case of cereal crops, seed filling after pollination as a critical step involves not only uptake from the soil but also the re-transport of previously acquired nitrogen (Buchner and Hawkesford 2014; Gregersen et al., 2008). For example, up to 90% of the nitrogen in wheat and barley undergoes translocation from vegetative tissues to the seed, Also, during senescence, nitrogen remobilization has an important effect on overall grain quality and thus yield (Buchner and Hawkesford 2014; Gregersen et al., 2008)

It has been shown that the novel varieties of wheat have great nutrient consumption efficiency through the remobilization of nitrogen from the old leaves to the newer ones, and into the grain (Buchner and Hawkesford 2014;

Gregersen et al., 2008). After distribution, biomolecules of nitrogen are converted into other organic molecules. For example, ammonium is catalyzed into organic compounds by glutamine synthetase (*GS2a*). Also, glutamine and aspartate are catalyzed by asparagine synthetase (*ASN1*) from glutamate and asparagine, respectively. (Good et al., 2004). *GS* is a key enzyme because it catalyzes the adenosine triphosphate (ATP)-dependent fixation of ammonium to the δ-carboxyl group of glutamate to form glutamine (Bernard and Habash 2009). Cysteine protease is another important enzyme involved in metabolite distribution in plants and functions in re-transporting nutrients and reorganizing plant metabolism (Grudkowska and Zagdanska 2004).

Many signals can be functional regulators of N transport systems. Among them, we can mention root NH_4^+, amino acids, and root NO_3^-. In fact, these biomolecules are both the main metabolites of nitrogen and modulate plant growth and metabolism (Crawford and Glass1998; Cai et al., 2007; Zhao et al., 2004). In addition, *NRT2* expression is significantly increased by N resupply after a period of NO3 deficiency and thus regulates N uptake in roots (Okamoto et al., 2003; Zhuo et al., 1999). On the other hand, amino compounds, especially Glutamic Acid (Gln), reduce the regulation of N uptake and decrease *NRT2* transcript abundance (Lejay et al., 1999; Vidmar et al., 2000). Therefore, the expression of *NRT2* genes is believed to be regulated by nitrate availability and other factors (Zhuo et al., 1999; Orsel et al., 2002; Orsel et al., 2006).

Although wheat is one of the most important crops in the world, the molecular aspects of nitrogen assimilation in wheat have not been well studied, which can be attributed to the large hexaploid genome of wheat. Likewise, the function and expression of only a few *AMT* and the *nintrate transporter* (*NRT*) genes have been studied in wheat (Duan et al., 2016). However, the expression of two putative *NRT* genes, *TaNRT2.1*, and *TaNRT2.3* has been shown to be root-specific in wheat, which can also be induced by nitrogen resupply after a period of nitrate deficiency (Zhao et al., 2004; Yin et al., 2007). Exogenous abscisic acid and glutamine have been shown to regulate the expression of these high-affinity *NRT* genes in wheat roots (Cai et al., 2007). On the other hand, it has been reported that ammonium transport in wheat is regulated by TaAMT1.1 and TaAMT1.2, as two *AMT* genes (Jahn et al., 2004; Sogaard et al., 2009).

Overall, there is limited information on the expression of such *AMT* genes in wheat roots (Duan et al., 2016). In this study, various genes and enzymes involved in nitrogen absorption and re-transport were analyzed with the aim of better understanding their performance under N deficiency conditions and during the pollination period in two wheat genotypes including Morvarid and Gonbad.

MATERIALS and METHODS

Plant materials and growth conditions

A factorial experiment was conducted in a randomized complete block design with three replicates in the educational farm of the Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources in the growing season 2018-2019. Experimental treatments included Morvarid (with high efficiency of nitrogen fertilizer metabolism) and Gonbad cultivars, and two different regimes of urea fertilizer (nitrogenous) including 150 kg/ha (50 kg at the planting and 100 kg at the stem stage) and control (without fertilizer treatment) as well as three sampling times including 10, 15 and 20 DAA. It should be noted that before planting, field soil sampling was performed to determine the absorbable solution nitrate. To prepare the sample, first, the upper part of the plant was cut from the soil. Afterwards, the sampling tube was placed on the crown and the tube was rammed to a depth of 30 cm. Thus, the plant's root and its relevant soil were brought out and washed with deionized water, transferred to the laboratory inside liquid nitrogen, and stored in a freezer at -80 ° C.

RNA extraction and reverse transcription

To extract *GS* enzyme, one g of frozen shoots with some sand was grounded into a pre-cooled mortar with a pestle and then homogenized in extraction buffer containing 50 mmol La Tris - HCl (pH 8.0), 0.5 mmol L EDTA, 2.0 mmol L: $MgSO_4$ $27H_20$ and 4.0 mmol L. DTT. The homogenates were centrifuged at 25000x*g* for 20 min. The supernatant was used to assay enzyme activity and soluble protein content.

Total RNA was isolated using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) from the root according to the manufacturer's instructions. The concentration and purity of total RNA were determined by measuring the absorbance at 260 and 280 nm using a Bio photometer (Eppendorf Company Germany). Equal amounts of 4 µg of total RNAs were reverse transcribed (RT) into first-strand cDNAs, each in a 20 µl reaction mixture using Moloney Murine Leukemia Virus Reverse Transcriptase (M–MLV, 1 U), following the manufacturer's (Fermentas) recommended protocol.

The quantitative polymerase chain reaction was performed using SYBR Green dye technology and Cyberbiopars kit (Gorgan University of Agricultural Sciences and Natural Resources, Iran) in an iQ5 device (Bio-Rad Company, USA). Spectrophotometry and electrophoresis (performed on 1.5% agarose gel) were used to determine the quantity and quality of RNA, respectively. A dilution of 1 to 100 RNA samples in distilled water and a 600 BT spectrophotometer were used in the spectrophotometric method. The amount of UV absorbed by a solution is directly proportional to the amount of RNA in the sample. Absorption (optical density, OD) is typically measured at 260 nm and this is the wavelength at which an absorption unit of 40 micrograms of RNA per microlitre occurs (https://www.hellovaia.com/textbooks/biology/essentials-of-genetics-10-edition/chapter-9/problem-20-how-is-the-absorption-of-ultraviolet-light-by-dna/). So, Equation (Eq.) 1 was used to measure the amount of RNA (mg per ml) of each sample as follows: Dilution factor * 40 * OD read at 260 nm = amount of RNA (µg per ml) Eq. 1

Real-time PCR (RT_PCR) analysis of genes

The quantitative real-time polymerase chain reaction (RT-PCR) was performed using specific primers. Using available information on the NCBI website, and a primer

software, the primers were designed. The Characteristics of primers are shown in Table 1.

To confirm the cDNA constructs and to evaluate the specific gene primers of target genes, the β-actin housekeeping gene primer pair (Table 1) was used as the internal control in RT-PCR according to the thermal cycling of RT-PCR as shown in Table 2 with a Primer annealing temperature at 60 ◦C. Relative gene expression was calculated using the Pfaffl formula (Permutation tests) (Pfaffl et al., 2002). The expression ratio of target genes and β-actin housekeeping gene the internal control was analyzed by the REST software. To check the specificity of the PCR reaction, melting curves were analyzed for each data point and graphs were drawn by Excel software.

RESULTS

Expression of glutamine synthetase (*GS2a*) gene

Ten days after anthesis (DAA), the expression of the *GS2a* gene in the Morvarid wheat genotype was higher by 1.66-fold under N-deficit conditions compared to the N-treated soil. The same results were observed in 15 and 20 DAA by 1.92- and 1.58-fold, respectively. However, the gene's expression level was the highest in 10 DAA and it gradually decreased, lowering to almost half the initial expression level by day 20, under both the N-deficit and N-treated conditions. For Gonbad wheat genotype, despite the fact that the expression of the gene was generally lower as compared to that of the Morvarid genotype, same differences in data were observed; that was, higher expression under N-deficit conditions (1.45-fold at 10 DAA, 1.66-fold at 15 DAA, and 1.25-fold at 20 DAA) with its peak which was observed in 10 DAA, as well as gradual decrease in expression level for both conditions (Fig. 1).

Expression of *TaNRT2.1* gene

In the Morvarid genotype, the expression of the *TaNRT2.1* gene was higher by 4.1-, 2.61-, and 1.75-fold under N-deficit conditions in 10, 15, and 20 DAA, respectively, compared to the N-treated conditions (Fig. 2). Although the expression level of this gene for the Morvarid genotype was shown to double over time (from 10 DAA to 20 DAA) under N-treated conditions, its expression was reduced by 0.85-fold in the same period of time under N-deficit conditions (Fig. 2). For the Gonbad genotype, however, the expression of this gene was lower by 0.86-fold in 10 DAA, higher by 1.17-fold in 15 DAA, and 1.63-fold higher in 20 DAA under N-deficit conditions compared to the N-treated conditions (Fig. 2). Also, the expression of *TaNRT2.1* gene was observed to increase (by 1.13-fold for the N-treated and 1.53-fold for the N-deficit conditions) from day 10 to day 15 after anthesis, and decrease (by 0.64-fold for the N-treated and 0.9-fold for the N-deficit conditions) from day 15 to day 20 after anthesis(Fig. 2). Interestingly, the expression of the *TaNRT2.1* gene in the Morvarid genotype was higher than the Gonbad genotype under N-deficit conditions by 3.15-, 1.7-, and 1.94-fold at 10 DAA, 15 DAA, and 20 DAA, respectively (Fig. 2). On the other hand, under N-treated conditions, the expression of this gene for the Gonbad genotype was higher by 1.5-fold at 10 DAA and by 1.3-fold at 15 DAA compared to the Morvarid genotype at the same sampling days, respectively, and yet it was lower than the Morvarid genotype by 0.55-fold at 20 DAA (Fig. 2).

Table 1. Characteristics of primers used to perform real-time quantitative polymerase chain reaction

Gene	Accession no.	Forward primer sequence (5′- 3′)	Reverse primer sequence (5′ - 3′)	Amplicon length (bP)
GS2a	DQ124212	GGAACCCGTCCCTACTAAC	GTCTCCCGCATCAATACC	340
TaNRT2.1	AF332214	GCCGCTTGTCTTCCACGCA	GTCCTTGGCCATGTCTCCCTTCT	181
TaNRT2.2	AF332214	TCCGGTTCCTCATTGGCTTC	TGGAAGACAAGCGGCATGAT	163
TaNRT1	AY587264	GGCAAGGACCCCAGTACTTC	GTGAATACCGGCACAAGGGA	184
ASN1	AY621539	GAGCATCTCCCAGCAACCATCATG	GGCAAGCAGGACAGGACACCATCAAC	111
Cysteine protease	AB109216	GCCGGAGAAGAGTACTGGAT	TACATGACGGGGTAGGCAC	140
GST1	AF184059	GGCTACACCCTACGCATCTC	AGACAGGATCGCAAGCACAA	244
TaAMT2.1	AY428038	AAGAAGAAGTGGGCGGTCAA	GAACACGCACTGGAAGTAGACG	273
Actin	AB181991	GTCTGGATCGGTGGCTCTAT	GCAGCAAGTCCCCTTTGTAA	152

Table 2. Real-time polymerase chain reaction thermal cycling

Number of cycles	Step	time	Temperature (◦C)
1	Initial Denaturation	3 Minutes	95
	Denaturation	10 Seconds	95
40	Primer Annealing	10 Seconds	60
	Extension	10 Seconds	72
1	Final Extension	5 Minutes	72
81	Melting	10 Seconds	95-55

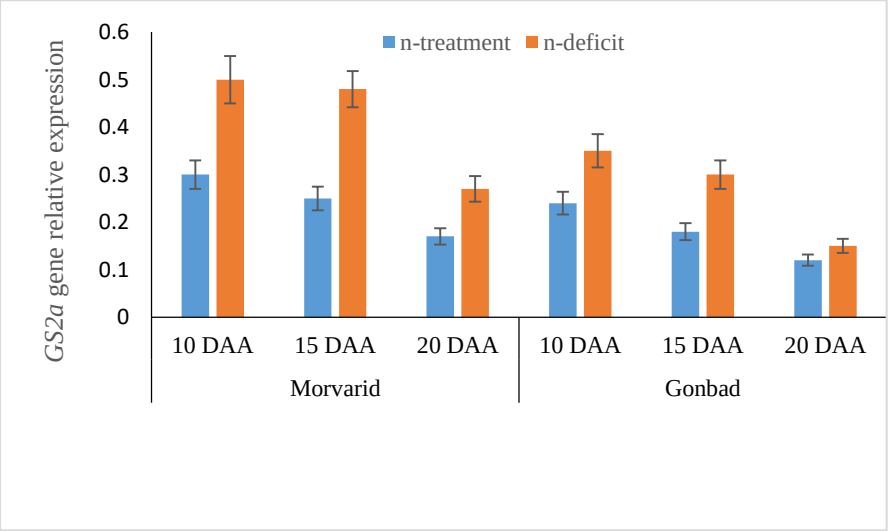

Fig. 1. The relative expression of the *GS2a* gene

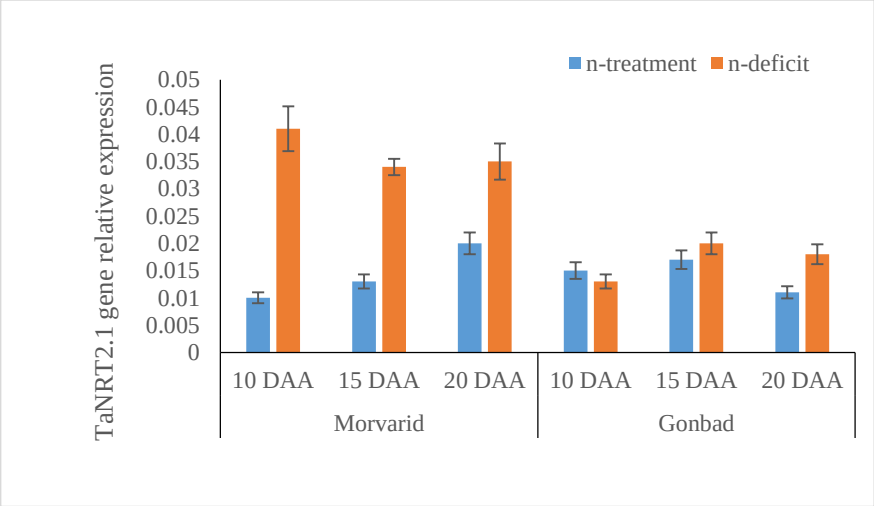

Fig. 2. The relative expression of the *TaNRT2.1* gene

Expression of *TaNRT2.2* gene

The expression of the *TaNRT2.2* gene decreased over time (from 10 DAA to 20 DAA) by 0.57-fold under N-treated conditions for the Morvarid genotype (Fig. 3). For the Gonbad genotype at the same conditions, however, there was a slight increase (1.13-fold) in expression from day 10 to 15 and a slight decrease (0.88-fold) from day 15 to 20 after anthesis (Fig. 3) The expression under N-deficit conditions, on one hand, first increased (1.1-fold) and then decreased (0.77-fold) for the Morvarid genotype from day 10 to 15 and 15 to 20 after anthesis, respectively (Fig. 3). On the other hand, the expression of this gene under N-deficit conditions was reduced by 0.57-fold over time (from day 10 to 20 after anthesis) for the Gonbad genotype (Fig. 3). Also, the expression of *TaNRT2.2* gene was higher under all conditions for the Morvarid genotype, compared to the Gonbad genotype (by 1.86-fold for N-treated and 2.3-fold for N-deficit conditions in 10 DAA, by 1.35-fold for N-treated and 2.8-fold for N-deficit conditions in 15 DAA, and by 1.06-fold for N-treated and 3.42-fold for N-deficit conditions in 20 DAA, Fig. 3).

Expression of *TaNRT1.1* gene

According to Fig. 4, the expression of the *TaNRT1.1* gene under N-treated conditions first increased by 1.28- and 1.45-fold from day 10 to 15 and then decreased by 0.83- and 0.75-fold from day 15 to 20 after anthesis for Morvarid and Gonbad genotypes, respectively. However, the expression of this gene increased by 1.36-fold over time (from 10 DAA to 20 DAA) under N-deficit conditions for the Morvarid genotype. By contrast, its expression decreased by 0.68-fold over time (from day 10 to 20 after anthesis) under the same conditions for the Gonbad genotype. In general, the expression of the *TaNRT1.1* gene was higher under N-deficit conditions compared to N-treated conditions for either genotype. Similarly, the expression was higher for the Morvarid genotype compared with the Gonbad genotype at each three measured stages and both conditions.

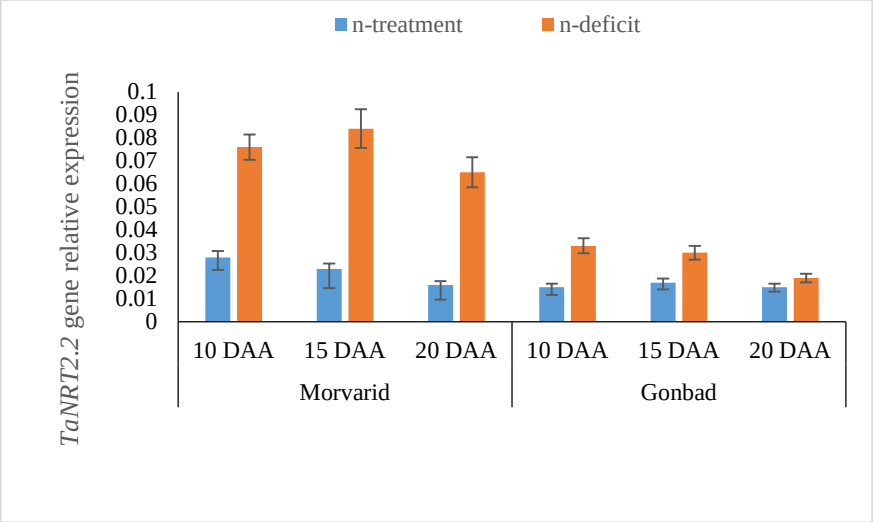

Fig. 3. The relative expression of the *TaNRT2.2* gene

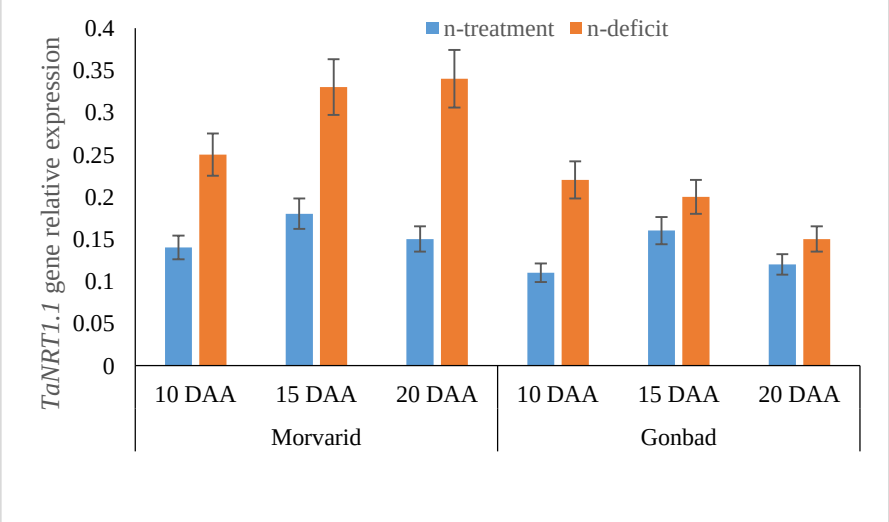

Fig. 4. The relative expression of the *TaNRT1.1* gene

Expression of asparagine synthetase (*ASN1*) Gene

The expression of the *ASN1* gene was measured under non-treated and N-treated conditions. Under the latter conditions, the expression of this gene in the Morvarid genotype did undergo a 2.14-fold increase from day 10 to 15 and a 0.89-fold decrease from day 15 to 20 after anthesis (Fig. 5). The same pattern was observed under N-limited conditions; that was, a 1.87-fold increase from 10 DAA to 15 DAA, and a 0.91-fold decrease from 15 DAA to 20 DAA (Fig. 5). However, the overall expression for the Morvarid genotype was higher under non-treated conditions compared to N-treated conditions at days 10, 15, and 20 after anthesis by 1.38-, 1.19-, and 1.23-fold, respectively (Fig. 5). Likewise, the expression of the *ASN1* gene in the Gonbad genotype first (from 10 DAA to 15 DAA) increased by 1.32- and 1.87-fold, and then (from 15 DAA to 20 DAA) decreased by 0.82- and 0.88-fold under non-treated and N-treated conditions, respectively (Fig. 5). Unlike that of the Morvarid genotype, the expression of the *ASN1* gene in the Gonbad genotype 10 days after anthesis under the non-treated condition was lower than the N-treated condition by 0.77-fold. However, on days 15 and 20 after anthesis and under the non-treated conditions the expression of the *ASN1* gene was higher by 1.09- and 1.17-fold compared to N-treated conditions, respectively. (Fig. 5).

Expression of *cysteine protease gene*

The expression of the *cysteine protease gene* in the Morvarid cultivar was the highest in 20 DAA under non-treated conditions and was twice the N-treated conditions at the same of sampling date (Fig. 6). In the Gonbad cultivar, again the highest *cysteine protease gene* expression was observed at 20 days after pollination under no consumption of N conditions, but this value was lower than that of the Morvarid cultivar at the same of sampling date (Fig. 6). The lowest relative value of gene expression of the *cysteine*

protease gene under N-treated conditions was 0.4 in Gonbad variety at 10 DAA (Fig. 6).

Expression of glutathione S-transferase (*GST-1*) gene

Under N-treated conditions, the expression of the *GST-1* gene in the Morvarid genotype increased by 1.26-fold from 10 DAA to 20 DAA (Fig. 7). The same results were observed for the Gonbad genotype (1.48-fold increase). By contrast, under non-treated conditions, the expression first (from 10 DAA to 15 DAA) increased by 1.45-fold for both genotypes and then (from 15 DAA to 20 DAA) decreased by 0.85- and 0.95-fold for the Morvarid and Gonbad genotypes, respectively (Fig. 7).

Expression of ammonium transporter 2.1 (*TaAMT2.1*) gene

For the Morvarid genotype, the expression of the *AMT2.1* gene in 20 DAA increased by 1.78-fold under N-treated conditions and decreased by 0.82-fold under N-deficit conditions (Fig. 8). For the Gonbad genotype, the expression of the *AMT2.1* gene gradually decreased (finally decreased by 0.71-fold from 10 DAA to 20 DAA) under N-deficit conditions, while under N-treated conditions, first (from 10 DAA to 15 DAA) increased by 1.45-fold and then (from 15 DAA to 20 DAA) decreased by 0.75-fold (Fig. 8).

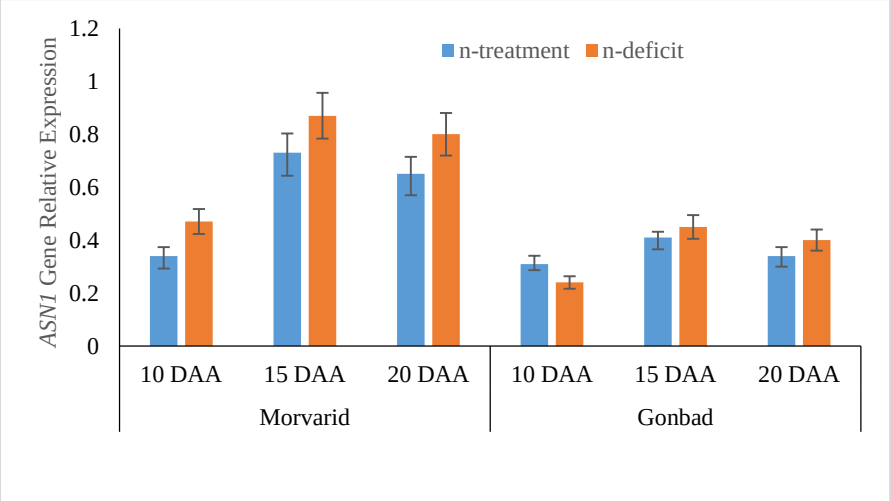

Fig. 5. The relative expression of the *ASN1* gene

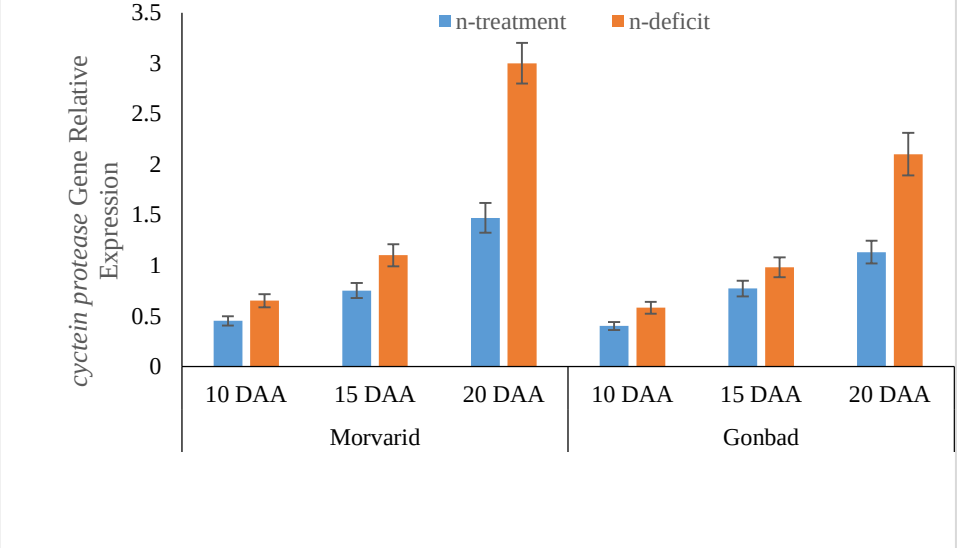

Fig. 6. The relative expression of the *Cysteine Protease* gene

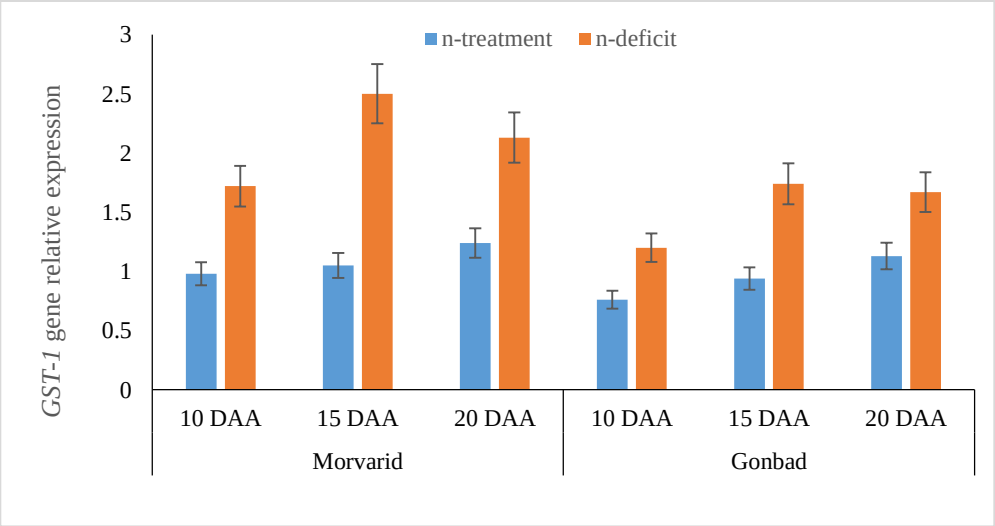

Fig. 7. The relative expression of *GST-1* gene

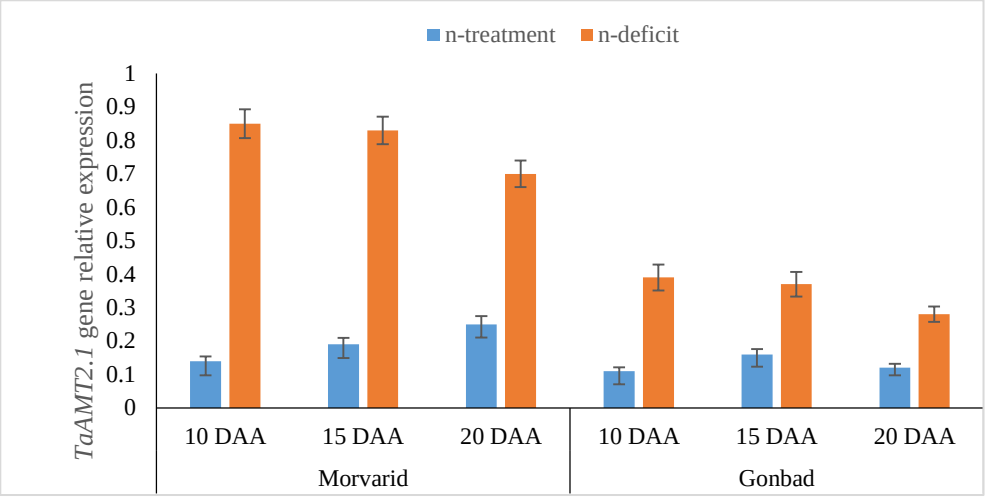

Fig. 8. The relative expression of the *TaAMT2.1* gen

Total nitrogen percent in grains

Total nitrogen percent in grains increased with increasing in time from 10 DAA to 20 DAA under both N-treated and N-deficit conditions for both Morvarid and Gonbad genotypes (Fig. 9). However, the total amount of N was higher in the N-treated group compared to the non-treated group for both Morvarid and Gonbad genotype (Fig. 9).

Glutamine synthetase (GS) enzyme activity

Under N-treated conditions, the activity of the *GS* enzyme first (from 10 DAA to 15 DAA) increased and then (from 15 DAA to 20 DAA) decreased for both Morvarid and Gonbad genotypes (Fig. 10). However, under N-deficit conditions, the activity of the enzyme gradually (from 10 DAA to 20 DAA) increased for Morvarid genotype (Fig. 10). Likewise, the activity of the enzyme increased for Gonbad genotype under N-deficit conditions from 10 DAA to 15 DAA. Nevertheless, it slightly decreased from 15 DAA to 20 DAA (Fig. 10).

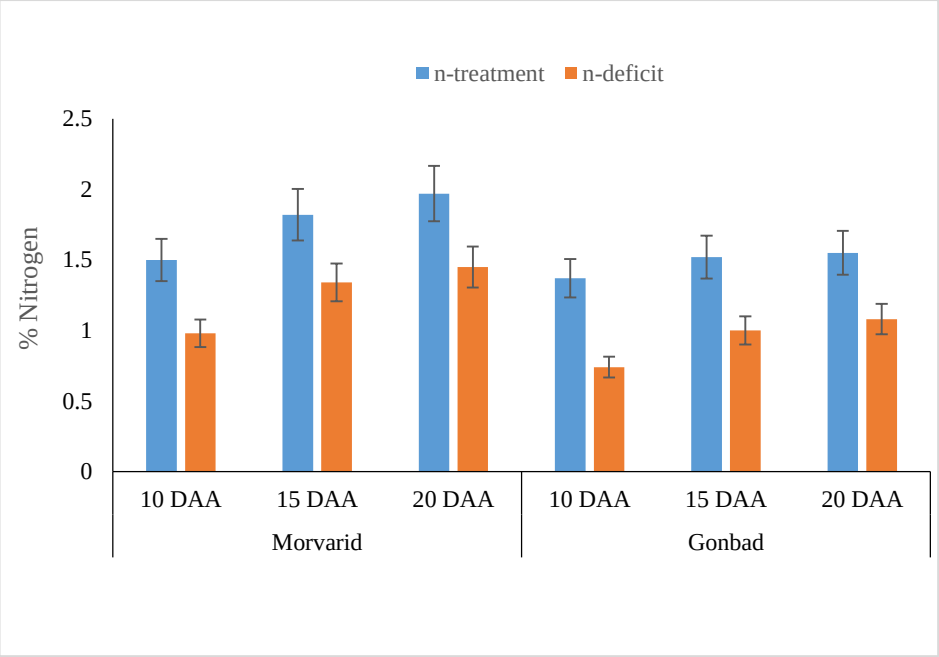

Fig. 9. Total nitrogen percent in grains

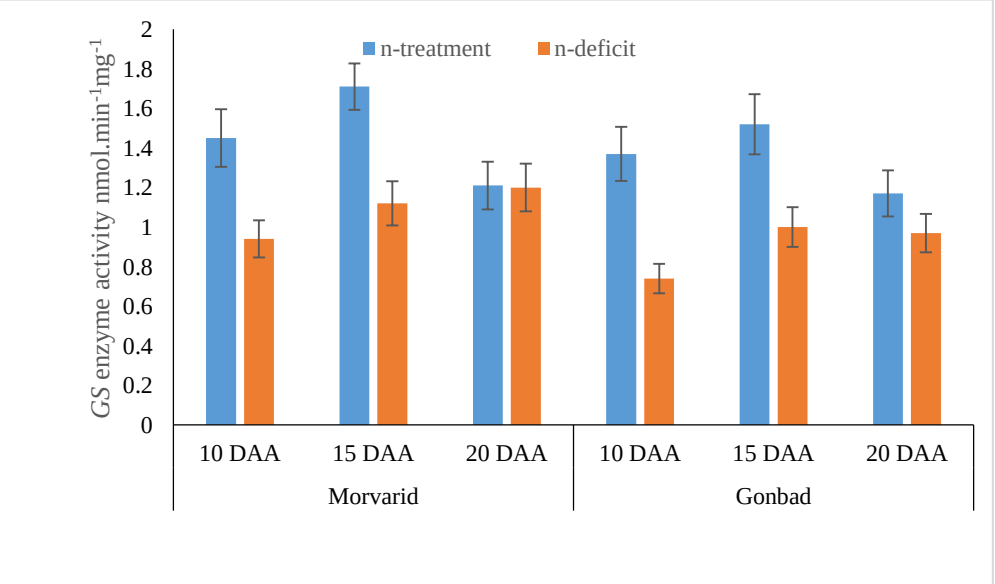

Fig. 10. The relative expression of the *glutamine synthetase* enzyme activit

DISCUSSION

It was shown in this study that the activity of nitrate transporter genes in two wheat genotypes, including pearl (Morvarid) and dome (Gonbad), increased in most of the studied genes with an increase in days after pollination under N deficiency conditions. The results suggest the significance of these genes in plant growth and overall yield because they are responsible for both N uptake and transport in the plant, as the role of N in plant growth and metabolism has been demonstrated in previous studies (Forde 2002; Walch-Liu et al., 2006; Zhang and Forde, 2000).

Seed filling after pollination is a critical stage in plant growth, which has been shown to involve nitrogen uptake and re-transportation (Buchner and Hawkesford 2014; Gregersen et al., 2008). The results of the current study suggested that more N uptake and remobilization take place in the plant, regardless of the N-treated conditions, in order to fill the grains as much as possible. Moreover, the results demonstrated the enhanced activity of N transporter genes. Such efforts to fill the grains with N could suggest the attempts of the plant to increase the overall yield. N and N-containing biomolecules are converted into organic molecules in plants. For instance, it has been shown that ammonium is catalyzed into organic compounds by the *GS2a* gene. Likewise, glutamine and aspartate are catalyzed from glutamate and asparagine, respectively, by the *ASN1* gene (Good et al., 2004).

It has been reported that the *GS* enzyme is closely related to grain N concentration, grain size, and yield components of crops (Gao et al., 2019). The distribution and role of *GS* enzyme in grains remain largely unknown. During a study, it was discovered that

T*riticum aestivum GS* (*TaGS*) isoenzymes are mainly located in the transport tissue of the embryo and seed and synergistically carry out NH_4^+ absorption in the seed (Wei et al., 2021). Additionally, it has been identified that *GS* enzyme activity is an adequate marker to recognize the nitrogen status in barley, maize, and wheat (Avila-Ospina et al., 2015). Also, *cysteine protease* plays key roles in nutrient remobilization, reorganization of plant metabolism, and in the process of nitrogen remobilization during the leaf aging and root remobilization and approximately makes the yields stable when nitrogen availability is low (Grudkowska and Zagdanska 2004; Martínez et al., 2008; James et al., 2019).

It has been shown that in *Arabidopsis*, the *NRT* transporter family especially the *NRT2* genes has an important role in the nitrate uptake from the soil in the environment (Chopin et al., 2007). Likewise, it has been reported that *NRT2* is expressed under conditions of nitrogen limitation and non-limitation in the environment (Lezhneva et al., 2014). Also, it has been shown that both *NRT1.1* and *NRT1.2* participate in nitrate absorption in the roots, and *NRT1.1* involves in auxin transport (Kiba et al., 2012). One research has also demonstrated that high expression of *AMT* genes can increase ammonium uptake in rice (Hoque et al., 2006). According to another survey (Liu et al., 2015), nitrate and ammonium transporters have an important effect on nitrogen absorption. It seems all *AMT*s and *NRT*s are involved in this process (Liu et al., 2015).

Asparagine synthetase (*ASN*) is another key enzyme of N metabolism in plants. The product of *ASN* is asparagine, which is involved in N transport and storage (Iqbal et al., 2022). In cotton plants, the majority of *ASN*s have up-regulation in both vegetative and reproductive organs (Iqbal et al., 2022). Therefore, considering the importance of the role of *glutamine synthetase*, *Asparagine synthetase*, and c*ysteine protease* in N metabolism in plants, their expression was analyzed in the current study.

In the current study, it was observed that the expression of *GS2a* was decreased over time (from 10 DAA to 20 DAA) under the N-treated conditions, while over-expressed under the N-deficit conditions. This could suggest that more effort is put by the plant for converting N-containing biomolecules into other essential organic compounds under the N-deficit conditions. On the other hand, the expression of *ASN1* first (from 10 DAA to 15 DAA) increased and then (from 15 DAA to 20 DAA) decreased, and the expression of the *cysteine protease* gene increased over time (from 10 DAA to 20 DAA). For both genes, the expression was higher under the N-deficit conditions, suggesting that more nutrient conversion and remobilization as well as reorganization of plant metabolism take place under the N-deficit conditions in order to increase the chances of survival and development. Very high *GS* activity in leaves indicates excessive green stay (Lea and Miflin 2003). *GS* is an enzyme required for nitrogen metabolism as it catalyzes the assimilation of all inorganic nitrogen incorporated into organic compounds such as proteins and nucleic acids (Lea and Miflin 2003). This response is associated with the formation of glutamate by glutamate synthase (glutamine oxoglutarate aminotransferase, GOGAT) as part of the GS/GOGAT cycle (Lea and Miflin 2003). As a multifunctional enzyme family, *Glutathione S-Transferases* (*GSTs*) have various roles such as the formation of non-toxic water-soluble substrates by the addition of glutathione to toxic molecules, anti-oxidative roles, and intracellular carrier functions (Roxas et al., 1997; Bartling et al., 1993). Analysis of the results of the *GST* genes indicated that their expression first increased and then decreased from 10 DAA to 15 DAA and 15 DAA to 20 DAA, respectively. However, the overall expression was higher under the N-deficit conditions compared to N-treated conditions, suggesting the effort of the plant to remobilize as much N as possible while countering oxidative stresses.

CONCLUSIONS

According to the results of this study, it can be suggested that the expression of certain genes influences N uptake and its remobilization to increase the response to N deficiency and consequently compensate for N shortage, as N is of significant importance for plant metabolism and development. These genes included *TaNRT* and *TaAMT* genes as well as *GS2a*, *ASN1*, *cysteine protease*, and *GST-1*. In the current research,, the relations between gene expression, total N percent in grains, the activity of *GS* enzyme, and days after anthesis were also demonstrated. It was concluded that higher expression of the genes eventually resulted in a higher N percentage in grains over the course of anthesis. That is, from day 10 to day 20 after anthesis, the overall N percentage for both tested genotypes increased under both the N-treated and N-deficit conditions, demonstrating the role of the afoementioned genes and enzymes in N uptake and transport in the plants.

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بررسی الگوی بیان برخی از ژن های ناقل نیتروژن در ریشــه در دو رقم مختلف گندم

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چکیده - در تولید محصولات زراعی، یکی از عوامل محدود کننده مهم در دسترس بودن نیتروژن (N) و مشتقات N است، زیرا آنها در متابولیسم و رشد گیاه اهمیت زیادی دارند. ژن¬های مختلف ناقل نیترات از جمله *GS2a*، *TaNRT2.1*، *TaNRT2.2*، *TaNRT1*، *ASN1*، *سیستئین پروتئاز*، *GST1* و *TaAMT2.1* جذب، انتقال و انتقال مجدد نیتروژن را در گیاهان واسطه گری می¬کنند. با این حال، نقش آنها با توجه به اهمیت مراحل پر شدن دانه و عملکرد کلی گیاه کاملاً درک نشده است. در این مطالعه، میزان بیان تعدادی از ژن‌ها و آنزیم‌های دارای نقش در جذب و متابولیسم نیتروژن در طول دوره گرده‌افشانی و در شرایط تیمار N و کمبود N در دو ژنوتیپ گندم بنام مروارید و گنبد اندازه‌گیری شد. نمونه‌ها ۱۰، ۱۵ و ۲۰ روز پس از گرده‌افشانی که مرحله‌ای حیاتی در پر شدن دانه است، گرفته شد و ارتباط بین بیان ژن ها، فعالیت آنزیم ها و روزهای پس از گرده‌افشانی نشان داده شد. مشاهده شد که بیان ژن مربوط به N به طور قابل توجهی در شرایط کمبود N و در طول دوره گرده‌افشانی افزایش می‌یابد، که نشان‌دهنده اهمیت ژن‌ها و آنزیم های مرتبط با N در حفظ متابولیسم و رشد گیاهان در شرایط کمبود N است. نتایج این مطالعه همچنین نشان داد که مقدار نیتروژن موجود در گیاه می تواند میزان پروتئین بذر، محتوای کلروفیل برگ، اندازه و حجم پروتوپلاسم سلولی را افزایش دهد و همچنین بر سطح برگ و فعالیت فتوسنتزی تأثیر بگذارد، بنابراین استفاده از ارقام اصلاح شده با قابلیت بیان بالای ژن های تراریخته نیتروژن مهم است.