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EXPRESSION OF NITROGEN TRANSPORTER GENES IN WHEAT LEAVES (*Triticum aestivum* L.) AND FIELD PERFORMANCES UNDER ORGANIC CONDITIONS

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ABSTRACT

This study aimed to investigate the expression patterns of nitrogen transporter genes in wheat (*Triticum aestivum* L.) grown in organic farming systems. Expression profiling of these genes will provide valuable insights into the molecular mechanisms involved in nitrogen uptake and transport in wheat plants. In addition, the agronomic responses of the wheat genotypes were evaluated under organic conditions. This will help in the creation of genotypes that support effective nitrogen use in organic farming systems. The study showed that the nitrate transporter gene family member TaNRT2.1 exhibited the highest expression level. The Alada cultivar performed well in terms of yield under field conditions and showed the highest expression levels of TaNRT2.1, NRT2.1, and TaGS1. The 6DZT-03-01 genotype had the highest expression levels of NPF6.3 and AMT2.1. Alada, YE-4, and YE-31 were identified as the most suitable genotypes for organic farming. Several genes, including TaNRT2.1, were more highly expressed in the high-yielding genotype, Alada, than in the other genotypes. This suggested that nitrate and ammonium transporters play an important role in determining how different genotypes of wheat take in nitrogen. Further research is needed to understand the complex mechanisms that contribute to genotypic variation in nitrogen uptake in wheat. To fully understand the genetic basis of nitrogen uptake in wheat and to develop ways to improve nutrient management in organic farming systems, the expression levels of other genes involved in nitrogen metabolism should also be examined.

Keywords: Bread wheat, Yield, Gene expression, Nitrogen transporter genes, qRT-PCR

INTRODUCTION

Wheat was one of the first crops to be cultivated and is widely produced and consumed worldwide (Silva *et al.,* 2020). Because of its high adaptability, it can be grown in various climates and regions, making it a crucial crop. Wheat holds a pivotal role in human nutrition. It is an essential food for more than 35% of the world's population, providing 20% of daily protein and food calories (FAOSTAT, 2022). In recent years, organic farming has substantially expanded in both industrialized and developing nations. Organic agriculture has been implemented in 187 countries, covering an area of 72.3 million hectares. This agricultural approach engages 3.1 million producers, who contribute to a worldwide organic market valued at $89.7 billion. Australia, Argentina, and Spain are among the countries with notable levels of organic farming, according to FAOSTAT's (2020) report.

Globally, organic cereals, primarily wheat and rice (*Oryza sativa* L.), are cultivated on 4.1 million hectares of land, with significant contributions from countries such as China (811 thousand hectares) and Kazakhstan (130 thousand hectares). Despite its relatively small footprint, organic cereal products account for 0.6% of the world's vast agricultural area, totaling 1.6 billion hectares. Specifically, organic wheat accounts for 36% of organic grain production (IFOAM, 2020). In Türkiye, wheat is predominantly cultivated as part of organic cereal farming, resulting in the production of approximately 870.2 thousand tons of organic wheat in 2020. Ensuring that plants receive the necessary nutrients is a crucial barrier in organic farming as it can result in decreased crop productivity. One contributing factor is the prolonged dissolution time of the nitrogen fertilizers used in organic farming. Nitrogen plays a pivotal role in various physiological processes, including carbon and amino acid metabolism, and protein synthesis.

Plants possess at least three nitrate transport systems: low-affinity transport system (LATS), high-affinity transport system (HATS), and dual-affinity transport system (DATS) (Li *et al.,* 2021). NPF family genes (nitrate transporter 1/peptide transporter family) have the potential to encode all three types of nitrate transporter proteins because their variable affinities are partly determined through post-translational regulation (Wen *et al.,* 2017). Furthermore, a reliable prediction of the affinity of NRTs based solely on the homology of their amino acid sequences is not feasible (Li *et al.,* 2021). There are two ways for plant cells to obtain ammonium ions from the rhizosphere: directly through ammonium transporter genes (AMTs), or indirectly through pathways that break down nitrogen and change nitrate (Patterson *et al.,* 2010). AMT genes, categorized into two subfamilies (AMT1 and AMT2), play distinct roles in ammonium uptake. AMT1 genes are predominantly expressed in the roots and are essential for efficient ammonium uptake from the soil (Mayer *et al.,* 2006). In contrast, AMT2 genes are expressed at lower levels and are distributed across various plant tissues, including the roots, shoots, and leaves (Yuan *et al.,* 2007).

The NPF and NRT2 gene families are important for obtaining nitrogen from the soil to plant roots. This means that they can be used to improve the ability of wheat cultivars to take in nitrogen (Nacry *et al.,* 2013). Nitrate is the primary form of nitrogen in plants grown in aerobic soils, such as Arabidopsis and barley, and it is taken up by the nitrate transporter gene families NRT1 (including NPF6.3) and NRT2, which are found in the root plasma membrane. (Plett *et al.,* 2010; Møller *et al.,* 2011). NRT1 genes mediate low-affinity nitrate transport, whereas NRT2 genes mediate high-affinity nitrate transport. In a recent study, Kumar *et al.* (2022) reported 46 NRT2 genes and 8 NAR2 (nitrate assimilation related protein) genes belonging to the nitrate-responsive family. These genes were found to respond to nitrate limitation in various plant tissues, such as roots, leaves, and stems. In addition, they observed a preference for homolog expression in the two wheat genotypes. The objective of this study was to examine the levels of gene expression related to nitrogen use efficiency and their relations with grain production in bread-wheat genotypes cultivated under organic farming conditions.

MATERIALS AND METHODS

Plant materials: The performance of twelve different bread wheat genotypes was evaluated in this study (Table 1) to determine their performance in organic farming. Grain yield, protein content, physiological and morphological traits, and expression levels of genes controlling nitrogen use efficiency were the main variables investigated.

Table 1. The bread wheat genotypes used in the research.

Genotype	Variety or Line	Maturing Time	Breeder Organization/Origin
Karacadağ-98	Variety	Early	GAP Agricultural Research and Training Center, Diyarbakır-Türkiye
6DZT-03-01	Line	Early	Dicle University, Faculty of Agriculture, Diyarbakır-Türkiye
DZ17-1	Line	Early	Dicle University, Faculty of Agriculture, Diyarbakır-Türkiye
TBT16-2	Line	Early	Dicle University, Faculty of Agriculture, Diyarbakır-Türkiye
YE-41	Landrace	Medium early	Dicle University, Faculty of Agriculture, Diyarbakır-Türkiye
Alada	Variety	Medium early	Maize Research Institute, Sakarya-Türkiye
Murat-1	Variety	Medium early	Trakya Agricultural Research Institute, Edirne-Türkiye
Tosunbey	Variety	Medium early	Field Crops Central Research Institute, Ankara-Türkiye
Aşure	Landrace	Late	Southeastern Anatolia Region Local Bread Wheat, Türkiye
Seri-2013	Variety	Late	Eastern Mediterranean Agricultural Research Institute, Adana-Türkiye
YE-4	Landrace	Late	Dicle University, Faculty of Agriculture, Diyarbakır-Türkiye
YE-31	Landrace	Late	Dicle University, Faculty of Agriculture, Diyarbakır-Türkiye

Research field and soil properties: Field experiment was conducted in the organic farming experimental area of Dicle University's Faculty of Agriculture in Diyarbakır, between 37° 53 'N and 40° 16' E. The research was conducted between 2019-2020 and 2020-2021. Soil pH in the experimental area ranged from 8.04 to 8.15, indicating alkaline and moderately calcareous soils with low organic matter content. Furthermore, the soil in the experimental field exhibited deficiencies in nitrogen and phosphorus, but was rich in potassium (Table 2).

Climatic conditions of the experimental field area: Table 3 shows the climate data of experimental area during study period (2019/2020 and 2020/2021) and the long-term (1991-2020). The total precipitation for the 2019-2020 period was higher than that for 2020-2021. The highest amounts of monthly precipitation were recorded in March and December for 2019-2020 and March and November for 2020-2021. The temperature values for both years of the experiment were above the long-term average, but the average temperatures in the first year of the study were higher than those in the second. Therefore, drought occurred during the second year of this study. During the second year of the study, there was a significant decrease in precipitation, particularly in April and May. Because of the drought that occurred during this period, approximately 20 mm of supplemental irrigation was applied to the experimental plots during the booting stage.

Experiment design and agronomic practices: The experiment was conducted according to a randomized complete block design, with four replicates. The sowing norm was determined by calculating 500 seeds per square meter, based on the thousand-grain weight of the seeds used. Each genotype was sown in six rows, and the plot area was 4.8 m^2 (4 m long x 1.2 m wide). Sowing was done on November 24 in both years (2019-2020 and 2020-2021) using a fully automatic trial sowing seeder. Certified organic fertilizer (Organoferm 6 kg ha^{-1}) with 50% organic matter content was applied at sowing, and liquid organic fertilizer with 40% organic matter content (Naturamix 300 ml/100 liters) was applied to the plots during the tillering stage (Table 4). Harvesting was manually performed in June.

Measurements: Yield, quality, agronomic, and physiological traits were investigated in the study. Heading time was measured as the number of days from sowing to the day when the first spike emerged from the flag leaf. Plant height was measured randomly from 10 plants per plot in centimeters from the soil surface to the awn tip. The normalized difference vegetation index (NDVI) was measured during the heading period using a Trimble GreenSeeker handheld sensor. The leaf area index (LAI) was measured as the area covered by the plants in the plot using the LAI-2000 (LI-COR, Lincoln, NE). The thousand kernel weights (TWK) were determined by randomly sampling 20 plants from each plot. The grain yield (GY) was measured by harvesting the whole area of each plot. For protein content determination, the grains from each plot were analyzed using a portable NIT (Near-Infrared Transmission) device named GrainSense (Özkan, 2021).

Table 2. Soil analysis results of the experiment area

Analysis Name	Results	
Saturation (%)	63.00	Clay Loam
Salinity (Saturation Sludge-dS/m)	0.92	No salt
% Salt (With Calculation) TS 8334	0.04	No salt
pH (Saturation Sludge)	8.11	Slightly Alkaline
Lime (Calcimetric-%)	11.24	Medium
Organic Matter (Walkley Black-%)	0.71	Low
Nitrogen (by Calculation-%)	0.04	Low
Phosphorus (Olsen Spectrometer-ppm)	4.00	Low
Potassium (A. Acetate-ICP-ppm)	314.45	High
Calcium (A. Acetate-ICP-ppm)	10717.89	Very high
Magnesium (A. Acetate-ICP-ppm)	471.78	Medium
Sodium (A. Acetate-ICP-ppm)	26.65	Low
Iron (DTPA-ICP-ppm)	9.29	Very high
Copper (DTPA-ICP-ppm)	1.61	Medium
Manganese (DTPA-ICP-ppm)	16.50	Medium
Zinc (DTPA-ICP-ppm)	0.08	Low

Table 3. Climatic values for the research year and long term (Meteorological Service, 2021).

Month	Precipitation (mm)			Temperature (^{0}C)			Humidity (%)	
	2019-2020	2020-2021	Long Years	2019-2020	2020-2021	Long Years	2019-2020	2020-2021
November	7.5	54.7	55.2	18.3	11.6	9.6	61.7	65.8
December	160.8	30.8	73.1	11.6	5.7	4.0	88.3	83.5
January	73.9	41.2	70.9	4.7	5.2	1.7	77.6	71.8
February	59.5	37.7	67.7	4.5	8.3	3.6	79.2	63.1
March	191.6	57.9	65.6	11.5	9.6	8.3	75.9	63.4
April	112	7.1	69.5	14.7	17.2	13.7	73.3	52.1
May	74.3	3.8	44.2	20.9	24.7	19.2	54.4	30.7
June	0.0	0.0	8.8	27.4	28.6	26.0	30.0	25.5
July	2.9	1	1.3	32.4	32.7	30.9	22.8	24.9
Total/Mean	**682.5**	**234.2**	**456.3**	**16.2**	**15.9**	**13.0**	**62.5**	**53.4**

Table 4. Content of organic fertilizers used in the research.

Content	Organoferm	Naturamix
Form	Granule (%)	Liquid (%)
Organic Matter	50	40
Organic Carbon	25	-
nitrogen (N)	7	5
Organic Nitrogen (N)	7	0.03
P_2O_5	4	-
Potassium Oxide (K2O)	4	6
Iron (Fe)	0.3	-
Humic Acid + Fulvic Acid	25	25
Ammonium Nitrogen (NH3)	-	1.2
Urea Nitrogen (N)	-	3.5
pH	-	7.5-9.5

Gene Expression

RNA extraction from samples and cDNA synthesis: The genotypes were sown on August 18, 2021, in individual pots with three replicates and one seed in each pot. The experiment was conducted according to a randomized plot experimental design. Table 2 shows the chemical and physical content of the soil used in the experiment. During sowing, 0.8 g of Organoferm organic fertilizer was added to each pot and diluted with water before applying at 50 ml per pot. Leaf samples were collected (September 16, 2021) from the plants and stored at -80 °C for RNA extraction. The gene expression analysis was conducted in the Molecular Biology

Laboratory of the Faculty of Agriculture at Dicle University as a part of this study.

Total RNA was extracted from leaf tissues using the TRIzol (TRI Reagent®, Sigma-Aldrich, Germany) method (Erayman *et al.,* 2015). Briefly, 1 mL of TRIzol reagent was added to 2 mL tubes and kept at +4 ºC. Next, 100 mg of each leaf sample was ground to a fine powder using liquid nitrogen in a mortar, and the powdered samples were transferred to tubes. The tubes were shaken until a homogeneous mixture was obtained, and incubated at room temperature for 10 min. Then, 0.2 mL of chloroform was added to the tubes for each mL of TRIzol, followed by vigorous shaking for 15 s and a 5-minute incubation at room temperature. The tubes were centrifuged at 15,000 rpm for 17 min at 4 °C and the upper liquid phase was transferred to new tubes. Next, 500 µL of isopropyl alcohol (half the amount of TRIzol) was added to the tubes and incubated for 10 min at room temperature. The tubes were then centrifuged at 12,000 rpm and 4 °C for 10 min, and the resulting supernatant was discarded. After precipitation, RNA at the bottom of the tube was washed with 1 ml of 75% ethanol. Next, RNA samples were centrifuged at 10,000 rpm for 5 min at 4 °C. The supernatant was carefully removed, leaving the RNA precipitate at the bottom to dry completely. To each tube, 30 µl of ddH_2O was added, and the samples were stored at -80 °C for later use. To determine the purity and concentration of the RNA samples extracted from the leaf tissues, a BioDrop spectrophotometer (BioDrop µLITE, Biodrop Inc.) was used.

The iScript cDNA Synthesis Kit (Bio-Rad, Catalog no: 1708891) was used for cDNA synthesis. All RNA samples were normalized to 1500 ng before cDNA synthesis was performed to ensure consistency. The cDNA synthesis protocol followed the method used by Özkan (2021). Specifically, 4 µl of 5x iScript Reaction Mix, 1 µl of iScript Reverse Transcriptase enzyme, and 1500 ng of RNA were mixed in a total volume of 20 µl using ddH_2O. PCR protocol was then applied to the prepared samples for 5 min at 25 °C, 20 min at 46 °C, 1 min at 95 °C, and then held at 4 °C indefinitely.

qRT-PCR analysis: Real-time PCR analysis was used to examine the gene expression levels linked to how well some bread wheat genotypes use nitrogen. The primer sequences used to amplify the genes associated with nitrogen utilization efficiency are listed in Table 5, and were specifically chosen to target the regions of interest.

Table 5. Primers used for qRT-PCR reactions.

Primer	Primer Sequences	References
Actin	**F:** CAGCAACTGGGATGATATGG **R:** ATTTCGCTTTCAGCAGTGGT	(Wang *et al.,* 2012)
TaNRT2.1	**F:** ACAAGCTGCTTGTGGTGCTGTA **R:** GTGATGAACAGTAAAATTCTTAGGTG	(Wang *et al.,* 2012)
NRT2.1	**F:** GTGGTGCCACACAACTCATC **R:** TTCTGGAGACTCGCAAGGTT	(Lupini *et al.,* 2021)
AMT2.1	**F:** AGCCGAACCTCTGCAATCTA **R:** TGACGACGCAGATAATGGAC	(Lupini *et al.,* 2021)
TaGS1	**F:** CAACCCTGATGTTGCCAAG **R:** GTAGGCGGCGATGTGCT	(Wang *et al.,* 2012)
NPF6.3	**F:** CACAGCGAATAGGGATTGGT **R:** CGCCTAGCAGGAAGTACTGG	(Lupini *et al.,* 2021)

The SYBR Green Supermix Kit (Bio-Rad, Catalog no: 1725120) was used to measure relative gene expression levels in leaf tissues of various wheat genotypes using RT-PCR analysis in this study. To minimize experimental errors, each sample was prepared in triplicates. Actin gene was used as an internal control to normalize the expression levels of the samples.

The qRT-PCR protocol was adapted from that of a previous study by Inal *et al.* (2014). Briefly, the protocol was prepared as a mixture of 0.1 µL of forward primer, 0.1 µL of reverse primer, 5.0 µL of iTaq Universal SYBR Green Supermix, and 4.8 µL of nuclease-free water, resulting in a total volume of 10 µL per reaction. This mixture was dispensed into a 72-well plate (10 µL per well) and 2 µL of cDNA from each sample was added to the wells. The plate was then loaded into a qRT-PCR device (Rotor-Gene Q, QIAGEN) for analysis.

Data Analysis*:* Gene expression levels were calculated according to the $2^{-\Delta\Delta Ct}$ algorithm (Livak and Schmittgen, 2001). The values obtained for the examined traits were analyzed using the JMP Pro 13 statistical package.

RESULTS AND DISCUSSION

Field experiments: The amount of rainfall varied between the seasons. In the first year of the study, there was a 49.6% increase in rainfall compared with the long-term average, whereas in the second year, there was a 48.7% decrease. Moreover, the total rainfall in the first year was 191.4% higher than that in the second year

(Table 3). In the second year of the study, a decrease in rainfall in April and May, coinciding with the booting and heading stages, resulted in poor genotype development. Compared to the first year, the genotypes had earlier heading times and shorter plant heights. NDVI and LAI values were significantly reduced because of poor development of the plant's vegetative organs. Because of the drought that occurred during and after the booting period at second year, supplementary irrigation was necessary to ensure healthy spike formation and grain filling. Supplementary irrigation during the booting stage had a positive effect on the yield.

Significant statistical differences in examined parameters were observed among the genotypes in this study. Significant differences were observed between years for all traits examined (Table 6-7). The means of the genotypes for each year were as follows: heading time 142.46 - 130.65 days; plant height 87.42 - 47.74 cm; NDVI 0.59 - 0.30; LAI 3.78 - 1.01; thousand kernel weight 41.35 - 29.59 g; grain yield 1493 - 1384 kg ha^{-1}; protein content 12.24% - 10.56%, respectively.

Based on the two-year mean, the earliest genotype was Karacadağ-98 (131.75 days) and the latest was YE-31 genotype (142.00 days) (Table 6). Özkan *et al.* (2021) reported a heading time range of 97.00-108.00 days, Kendal *et al.* (2012) 108.5 - 114.5 days, Karaman and Aktaş (2020) 153.5-166.8 days, Bayhan *et al.* (2019) 128.6 - 133.3 days.

The tall plant height of wheat plants in wheat farming offers several advantages. Long stems provide an opportunity for plants to capture more sunlight and produce more energy through photosynthesis. In addition, taller wheat plants have a more effective competitive edge against weeds, hindering their growth by shading them. Extensive root systems enable plants to draw water from deeper layers of the soil, enhancing their access to water sources, particularly under drought conditions. Plant height in cereals varies according to the genetics of the variety, sowing method and time, fertilization, weed control, soil structure, and climatic data (Doğan and Kendal, 2013). The tallest plant height was observed in YE-41 (92.33 cm) and YE-31 (88.48 cm), while the shortest plant height was recorded in 6DZT-03-01 (54.10 cm) (Table 6). A significant height difference of 38.2 cm between the shortest and tallest genotypes. In previous research, Yıldız (2023) reported a range of 61.03 - 83.97 cm, Kılıç *et al.* (2014) found a range of 70 - 100 cm, and Ulucan and Atak (2020) observed a range of 83.6 - 105 cm.

High NDVI values indicate healthy plant growth. The highest NDVI value was recorded for YE-31 (0.57), whereas the lowest value was recorded for the 6DZT-03-01 (0.35) lines (Table 6). Gamon *et al.* 1992 and Gamon *et al.* 1997 stated that the Normalized Difference Vegetation Index (NDVI) exhibits a decline of up to 0.3 during the grain filling stage, indicating plant stress and a diminished capacity to absorb Photosynthetically Active Radiation (PAR). Özkan *et al.* (2019) found a NDVI values range of 0.35 - 0.45, Bayhan *et al.* (2020) observed a range of 0.53 - 0.67, Albayrak *et al.* (2021) noted a range of 0.31 - 0.66 and Özkan *et al.* (2022) reported a range of 0.44 - 0.63.

Leaf Area Index (LAI) is a crucial parameter for assessing plant growth and drought severity. LAI, the total surface area of leaves per unit of ground area (m^2 m^{-2}), is a collective measure of the foliar portion of the vegetation canopy structure. The highest LAI value was in Alada (3.25), and the lowest value was in 6DZT-03-01 (1.35) (Table 6). Bayhan *et al.* (2019) reported that the LAI ranged from 0.67 to 1.13 at drought stress conditions. Instead of focusing on high or low LAI values, it is crucial to identify the optimal value for each genotype. Koç and Barutçular (2000) noted that the biological yield and grain yield reached their optimum values when the LAI was approximately 6.5 - 7.0, and yields decreased at higher LAI values.

The highest thousand-kernel weights were observed in YE-41 (39.36 g) and DZ17-1 (39.35 g), while Alada (31.77 g) and Murat-1 (32.11 g) had the lowest values (Table 7). The grain weight of the wheat varieties increased when the environmental conditions were favorable after heading. However, a significant decrease in precipitation during the spike period had a detrimental impact on the grain weight. This was due to the plants being unable to adequately fulfill their water requirements, as stated by Sakin *et al.* (2016). In a previous research, Koca *et al.* (2011) found a range of 22.1 - 42 g, and Aktaş and Eren (2014) observed a range of 31.3 - 38.2 g.

The grain yields were highest in YE-4 (2123 kg ha^{-1}), YE-31 (2112 kg ha^{-1}), and Alada (2091 kg ha^{-1}), whereas Tosunbey had the lowest grain yield (932 kg ha^{-1}). The mean values for the initial and subsequent years were 14.93 and 13.84 kg per hectare, respectively, as indicated in Table 7. Wheat yield was formulated as multiplicated of the spike number per m², grain number per spike, and grain weight (Gençtan and Balkan, 2006). In previous studies, Bahar and Bahar (2016) 1515 - 2673 kg ha^{-1}, Kodaş *et al.* (2015) 1900 - 3290 kg ha^{-1}, Yıldırım *et al.* (2021) 901 - 5096 kg ha^{-1}, Yorulmaz *et al.* (2022) 894 - 3757 kg ha^{-1}. Özkan and Akıncı (2021) found that the mean grain yield was 3443 kg ha^{-1} under organic conditions and 4499 kg ha^{-1} under conventional conditions in their study. For example, in this field experiment, the genotypes YE-31 and DZ17-1 showed the highest grain yield in different years. However, it is important to consider that grain yield can be influenced by various factors such as soil quality, weather conditions, and management practices, making it difficult to directly compare results from different studies and draw definitive conclusions about the superiority of specific genotype.

Protein content varies between 6 and 22 % depending on the variety, climate, environment, fertilization, and many other factors (Yorulmaz *et al.,* 2023). The highest protein content was in 6DZT-03-01 (12.75 %), and the lowest value was in Karacadağ-98 (10.27 %) (Table 7). According to Bahar and Bahar (2016), the protein content ranged from 13.00% to 14.70% under organic conditions. Özkan and Akıncı (2021) found that the mean protein content of the genotypes was 12.05% under organic conditions and 12.74% under conventional conditions. These findings suggest that there is considerable variation in protein content among different genotypes and across different years of the study. The results also indicate that both organic and conventional conditions can influence protein content, with slightly higher values observed under organic conditions. Albayrak *et al.* (2020) observed a protein content range of %12.15 - 16.24, Yıldırım *et al.* (2022) noted a range of %15.1 - 18.9 and Özkan *et al.* (2022) found a range of %14.2 - 17.64.

qRT-PCR analysis: Using leaf tissues from wheat genotypes grown under organic conditions, qRT-PCR analysis was performed to determine the expression levels of genes related to nitrogen use efficiency (Figure 1). The TaNRT2.1 gene exhibited the highest expression level, while the NRT2.1 gene demonstrated the lowest expression level among the genes (Figure 1).

Researchers have already identified and discussed the expression patterns of NPF genes, which are low-affinity nitrate transporter genes, in bread wheat (Buchner and Hawkesford, 2014; Wang *et al.,* 2020). Bajgain *et al.* (2018) identified 77 NRT genes, encompassing NRT1/NPF, NRT2, and NRT3 families, in wheat, which were orthologous to NRT genes in Arabidopsis, rice, barley, and maize. Plants require nitrate transporter genes for nitrogen uptake, nitrate transporters are categorized into high-and low-affinity systems, which belong to the NRT gene family in plants. Similarly, AMT gene family includes ammonium transporters. In this study, we examined three genes responsible for nitrate transport and two genes responsible for ammonium transport. We observed that the expression levels of nitrate transporter genes were higher than those of ammonium transporter genes. Additionally, we observed that Alada cultivar, which performed well in terms of grain yield in field conditions (Table 5), had the highest expression levels for TaNRT2.1, NRT2.1, and TaGS1 genes, while 6DZT-03-01 genotype had the highest expression levels for NPF6.3 and AMT2.1 genes (Figure 1). Notably, the uptake of nitrogen in plants is a complex process involving various genes and environmental factors.

Local wheat genotypes exhibited lower expression levels than the modern cultivars and lines. However, the old local genotype Aşure showed higher gene expression levels than other local genotypes. This finding aligns with the results of Lupini *et al.* (2021), who reported that local genotypes generally exhibited higher gene expression levels than modern cultivars. The combination of nitrogen and water stress has been reported to affect nitrogen use efficiency (NUE) and may be useful for identifying genotypes that are more resistant to water stress (Islam *et al.,* 2021; Lupini *et al.,* 2021). According to Buchner and Hawkesford (2014), the expression of nitrate transporter genes varies among different tissues. TaNRT2.1 and TaNRT2.3 genes in wheat are mostly root-specific (Zhao *et al.,* 2004; Yin *et al.,* 2007). Furthermore, the genes NPF6.1, NPF6.2, and NPF6.4 have higher levels of expression in roots and lower levels in shoots, although NPF6.3 is equally expressed in both tissues (Buchner and Hawkesford, 2014). High-affinity AMT genes facilitate ammonium uptake in ammonium-deficient soils (Garnett *et al.,* 2009). Some studies have indicated that the expression levels of NRT and AMT genes increase under drought stress conditions. In hydroponic studies on rice and Arabidopsis, Chen *et al.* (2012) and Hu *et al.* (2006) reported that the expression of certain NRT genes belonging to the NRT1 family was stimulated by drought stress. According to Liu *et al.* (2015), the TaNRT2.1 gene, a member of the nitrate transporter gene family, plays a crucial role in determining the genotypic variation in nitrogen uptake in wheat. Lupini *et al.* (2021) reported an increase in the expression of the NRT2.1 gene in all cultivars, ranging from 21.00% to 50.70%, during the milk development stage under nitrogen stress. However, gene expression decreased under both water and nitrogen stress conditions.

It is noteworthy that, although there are some differences between cultivars, nitrogen stress was found to increase the expression of AMT2.1, which belongs to the ammonium transporter gene family, in all genotypes (Lupini *et al.,* 2021). However, during both the stem elongation and milk development stages, water stress and low nitrogen treatment significantly decreased the expression of AMT2.1 in modern cultivars compared with the control treatment.

Taranto *et al.* (2020) outlined the impact of selection on the genetic diversity of Italian durum wheat. Analysis of diversity patterns resulted in the detection of major QTLs that could define the differences among ancient and modern varieties. It is important to note that both low- and high-affinity NRT genes play essential roles in mediating nitrate uptake by plants from the soil (Duan *et al.,* 2016). The concentration of nitrate in the soil can vary from 10 mM-100 mM due to changes in soil moisture (Xu *et al.,* 2012). Additionally, Duan *et al.* (2016) found that water stress significantly stimulates the expression of ammonium transporter genes during the vegetative period of wheat.

Table 6. Mean of the heading time, plant height, NDVI and LAI in wheat (*Triticum aestivum* L.).

Genotype	Heading Time (day)			Plant Height (cm)			Normalized Difference Vegetation Index (NDVI)			Leaf Area Index (LAI)		
	2019-2020	2020-2021	Mean	2019-2020	2020-2021	Mean	2019-2020	2020-2021	Mean	2019-2020	2020-2021	Mean
6DZT-03-01	140.00 ^{c}	127.25 ^{g}	133.63 $^{f-h}$	61.40 ^{e}	46.80 $^{b-d}$	54.10 ^{e}	0.43 ^{h}	0.28	0.35 ^{f}	1.70 ^{h}	1.00	1.35 ^{c}
Alada	141.00 ^{c}	130.75 ^{de}	135.88 ^{ef}	75.60 ^{d}	44.65 ^{cd}	60.13 $^{c-e}$	0.61 ^{e}	0.28	0.44 $^{b-e}$	5.40 ^{a}	1.10	3.25 ^{a}
Aşure	147.00 ^{b}	130.25 $^{d-f}$	138.63 ^{cd}	78.00 ^{cd}	45.77 $^{b-d}$	61.88 $^{b-e}$	0.63 ^{de}	0.29	0.46 $^{b-d}$	5.50 ^{a}	0.93	3.21 ^{a}
DZ17-1	138.00 ^{d}	128.50 ^{fg}	133.25 ^{gh}	76.60 ^{d}	49.45 ^{bc}	63.03 $^{b-e}$	0.54 ^{f}	0.29	0.41 $^{c-f}$	3.00 ^{d}	1.20	2.10 ^{bc}
Karacadağ-98	135.00 ^{e}	128.50 ^{fg}	131.75 ^{h}	81.00 ^{c}	51.89 ^{ab}	66.44 $^{b-d}$	0.54 ^{f}	0.27	0.40 $^{d-f}$	2.70 ^{e}	0.90	1.80 ^{c}
Murat-1	140.00 ^{c}	131.50 ^{cd}	135.75 ^{ef}	75.00 ^{d}	50.40 $^{a-c}$	62.70 $^{b-e}$	0.47 ^{g}	0.30	0.38 ^{ef}	2.10 ^{g}	1.23	1.66 ^{c}
Seri-2013	150.00 ^{a}	133.00 $^{a-c}$	141.50 ^{ab}	76.80 ^{d}	44.03 ^{cd}	60.42 $^{c-e}$	0.44 ^{gh}	0.31	0.37 ^{ef}	2.40 ^{f}	0.88	1.64 ^{c}
TBT16-2	140.75 ^{c}	128.75 $^{e-g}$	134.75 ^{fg}	75.60 ^{d}	41.65 ^{d}	58.62 ^{de}	0.67 ^{bc}	0.32	0.49 ^{b}	4.60 ^{c}	1.03	2.81 ^{ab}
Tosunbey	140.75 ^{c}	128.50 ^{fg}	134.63 ^{fg}	95.20 ^{b}	48.70 ^{bc}	71.95 ^{b}	0.65 ^{cd}	0.31	0.48 ^{bc}	5.30 ^{a}	0.93	3.11 ^{a}
YE-31	150.00 ^{a}	134.00 ^{ab}	142.00 ^{a}	127.60 ^{a}	49.35 ^{bc}	88.48 ^{a}	0.78 ^{a}	0.35	0.57 ^{a}	4.60 ^{c}	1.10	2.85 ^{ab}
YE-4	147.00 ^{b}	132.25 $^{b-d}$	139.63 ^{bc}	98.20 ^{b}	43.55 ^{cd}	70.88 ^{bc}	0.66 ^{cd}	0.31	0.48 ^{bc}	3.00 ^{d}	0.93	1.96 ^{c}
YE-41	140.00 ^{c}	134.50 ^{a}	137.25 ^{de}	128.00 ^{a}	56.65 ^{a}	92.33 ^{a}	0.70 ^{b}	0.32	0.51 ^{ab}	5.00 ^{b}	0.95	2.98 ^{a}
Mean	142.46 ^{A}	130.65 ^{B}	136.56	87.42 ^{A}	47.74 ^{B}	67.58	0.59 ^{A}	0.30 ^{B}	0.45	3.78 ^{A}	1.01 ^{B}	2.40
LSD	1.46**	2.02**	2.36**	4.09**	6.94**	10.83**	0.03**	-	0.07**	0.24**	-	0.78**

*Significant differences at $p < 0.05$; **Significant differences at $p < 0.01$

Table 7. Mean of thousand kernel weight, grain yield and protein content in wheat (*Triticum aestivum* L.).

Genotype	1000-Grain Weight (g)			Grain Yield (kg ha^{-1})			Protein Content (%)		
	2019-2020	2020-2021	Mean	2019-2020	2020-2021	Mean	2019-2020	2020-2021	Mean
6DZT-03-01	43.09 ^{c}	33.67 ^{a}	38.38 ^{ab}	1008 ^{fg}	1100 ^{cd}	1054 ^{ef}	12.88 ^{a}	12.62 ^{a}	12.75 ^{a}
Alada	40.00 ^{d}	23.54 ^{e}	31.77 ^{f}	2508 ^{c}	1681 ^{ab}	2091 ^{a}	12.88 ^{a}	8.85 ^{f}	10.87 ^{de}
Aşure	36.36 ^{ef}	29.10 ^{b}	32.73 ^{ef}	1295 ^{e}	1495 $^{b-d}$	1395 ^{cd}	12.26 $^{a-c}$	9.36 ^{ef}	10.81 ^{de}
DZ17-1	45.28 ^{a}	33.43 ^{a}	39.35 ^{a}	1002 ^{fg}	2037 ^{a}	1520 ^{bc}	12.76 ^{ab}	10.19 $^{c-e}$	11.47 $^{b-d}$
Karacadağ98	42.51 ^{c}	26.77 ^{cd}	34.64 $^{c-e}$	1081 ^{ef}	1185 $^{b-d}$	1133 $^{d-f}$	10.19 ^{d}	10.35 $^{c-e}$	10.27 ^{e}
Murat-1	37.66 ^{e}	26.55 ^{d}	32.11 ^{f}	797 ^{g}	1112 ^{cd}	955 ^{ef}	10.78 ^{cd}	11.36 $^{a-c}$	11.07 $^{c-e}$
Seri-2013	42.65 ^{c}	28.54 $^{b-d}$	35.60 ^{c}	980 ^{fg}	1072 ^{cd}	1026 ^{ef}	11.63 $^{a-d}$	10.09 $^{d-f}$	10.86 ^{de}
TBT16-2	40.78 ^{d}	29.98 ^{b}	35.38 ^{cd}	1834 ^{d}	1571 $^{a-c}$	1702 ^{b}	13.05 ^{a}	9.95 $^{d-f}$	11.50 $^{b-d}$
Tosunbey	35.79 ^{f}	30.08 ^{b}	32.93 $^{d-f}$	430 ^{h}	1433 $^{b-d}$	932 ^{f}	11.02 $^{b-d}$	10.44 $^{b-e}$	10.73 ^{de}
YE-31	43.78 ^{bc}	30.30 ^{b}	37.04 $^{a-c}$	3226 ^{a}	999 ^{d}	2112 ^{a}	13.01 ^{a}	11.20 $^{b-d}$	12.11 $^{a-c}$
YE-4	43.66 ^{bc}	28.98 ^{bc}	36.32 ^{bc}	2884 ^{b}	1363 $^{b-d}$	2123 ^{a}	13.46 ^{a}	10.67 $^{b-d}$	12.06 $^{a-c}$
YE-41	44.59 ^{ab}	34.13 ^{a}	39.36 ^{a}	884 ^{fg}	1558 $^{a-c}$	1221 ^{de}	12.92 ^{a}	11.67 ^{ab}	12.30 ^{ab}
Mean	41.35 ^{A}	29.59 ^{B}	35.47	1493 ^{A}	1384 ^{B}	1438	12.24 ^{A}	10.56 ^{B}	10.40
LSD	1.45**	2.29**	2.53**	2.81**	5.18*	2.85**	1.84**	1.25**	1.12**

*Significant differences at $p < 0.05$; **Significant differences at $p < 0.01$

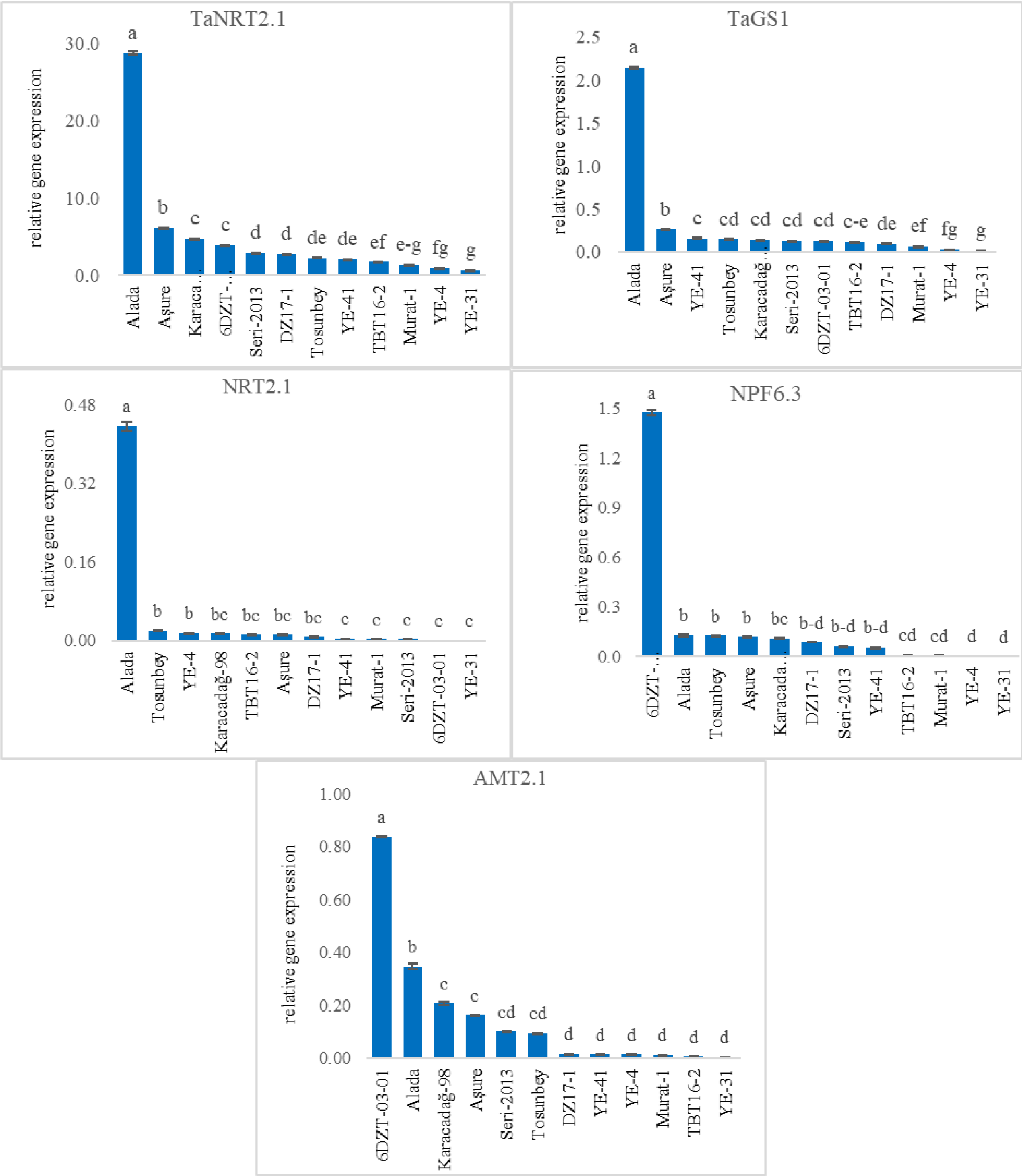

Figure 1. Gene expression levels of nitrogen transporter genes of wheat genotypes

Conclusions: In this study, qRT-PCR analysis was used to determine whether N transporter genes were present and to what extent they were expressed in 12 different wheat genotypes under organic conditions. The results showed that TaNRT2.1, a member of the nitrate transporter gene family, had the highest gene expression level. Furthermore, under organic farming conditions, the expression levels of nitrate transporter genes were higher than those of ammonium transporter genes. The Alada cultivar performed well under organic farming conditions and exhibited the highest expression levels of TaNRT2.1, NRT2.1, and TaGS1. On the other hand, the 6DZT-03-01 line showed the highest expression levels of NPF6.3 and AMT2.1 genes.

Based on the analysis of traits in field conditions, the Alada, YE-4, and YE-31 genotypes demonstrated superior performance compared to the other genotypes. Furthermore, the Alada variety exhibited outstanding performance, both in terms of gene expression and grain yield.

When the performances of the genotypes grown under rainfed conditions were evaluated as a whole, it was revealed that high grain yield and high-quality traits can be obtained under suitable variety and management conditions in Diyarbakır ecological conditions, which is one of the regions with the earliest harvest period in Türkiye.

These findings provide a foundation for future research aimed at understanding the molecular mechanisms underlying the functions of nitrate transporter genes in nitrogen use and optimizing productivity in wheat. Information on these gene families will pave the way for utilizing them to improve nitrate uptake, nitrogen transport, and nitrogen use efficiency in wheat.

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