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The effect of drought stress on the expression of genes *TaMOR*, *TaNAC69-1* and *TaExpb23* in different bread wheat (*Triticum aestivum* L.) cultivars

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Abstract

For evaluating the effects of *TaMOR*, *TaNAC69-1* and *TaExpb23* expression on root system architecture and drought tolerance of wheat, 10 bread wheat varieties were compared through germination test, pot experiment and field trial. In germination test, genotypes were evaluated under 0, -3 and -6 bar osmotic potentials. In the pot experiment genotypes were compared at 50%, 75% and 100% field capacity (FC) irrigation regime. In the field trial, genotypes were grown under well-watered and rain fed conditions. Based on the results of this experiment, it was concluded that water deficit had a significant effect on all traits except for seminal root numbers. Significant genotypic differences were observed for all traits indicating the presence of genetic variability among the genotypes. Correlation coefficients showed that *TaMOR* gene expression had a positive correlation with plant yield under 75% FC stress (r=0.733*). *TaNAC69-1* gene expression had positive correlations with plant yield and plant biomass under 50% FC (r=0.634* and r=0.711*). *TaNAC69-1* gene expression also showed a positive correlation with root numbers under 50% FC condition (r=0.622*). *TaNAC69-1* gene expression also showed a positive correlation with seminal root angle ($r=0.648^*$) under severe stress. *TaExpb23* expression showed positive correlation with plant yield and biomass under 75% FC (r=0.663* and r=0.723*). Based on the results of this experiment, it was concluded that under different stress levels, *TaMOR*, *TaNAC69-1* and *TaExpb23* gene expressions had significant correlations with traits. Due to significant G×E interactions, it was also concluded that the effect of expression of *TaMOR*, *TaNAC69-1* and *TaExpb23* genes on traits is different under distinct stress levels.

Key words: Gene expression, Root system architecture, Tolerance, Water deficit.

INTRODUCTION

Drought is a major abiotic stress and is a widespread limitation for cereals production especially in arid and semi-arid regions of the world. Rain-fed wheat production in many parts of the world is dependent on stored soil moisture (Wasson *et al*., 2012). When the in-season rainfall is unpredictable, stored water becomes a valuable source of water, since its uptake coincides with grain filling when plants are vulnerable to drought (Jia *et al*., 2019). Roots are the main organs to sense, respond and maintain crop performance under drought stress. Plants with deeper root systems extract water from deeper soil layers and it, in turn, helps the plants to avoid drought stress (Cooper *et al*., 2009).

Direct selection for root traits could enhance crops yield in drought stress conditions (Comas *et al*., 2013; Li *et al*, 2016). Indirect or trait-based selection complements empirical breeding approaches for yield. Indirect breeding for root traits is more difficult than for shoot traits (Wasson *et al*., 2012). To overcome this problem, fast laboratory-based selection methods and genomic approaches for root traits have been developed. Understanding the molecular mechanisms of root growth and development for future crop improvement is essential.

Root growth and development are inextricably linked to plant hormones (Overvoorde *et al*., 2010). Auxin, is responsible for root architecture and plant development at various stages (Lavenus *et al*., 2013). *TaMOR* is a transcription factor gene involved in auxin signaling in the initiation of both lateral and crown roots. Results of Li *et al*. (2016) showed that *TaMOR* is a promising candidate gene for root improvement and grain yield enhancement in crops. Studies of specific genes from *NAC* transcription factor family showed that some of them may play a role in drought tolerance and simultaneous control of root architecture (Janiak *et al.*, 2016). Lines of evidence on the regulation of plant growth, including root development via *NAC* transcription factors were found in the study of the *TaNAC69* gene in wheat, where the line overexpressing *TaNAC69* developed longer roots and greater root systems and shoot biomass in comparison to non-transgenic plants, when subjected to osmotic stress (Xue *et al*., 2011). *TaNAC69-1* expression in the roots down-regulates the expression of *TaSHY2* and *TaIAA7*, which are members of the auxin/IAA transcriptional repressor gene family. The expression of *TaSHY2* and *TaIAA7* in roots inhibits root growth (Chen *et al*., 2016). One of the gene families which are involved in the root system architecture of plants are expansins (Chen *et al*., 2016). Expansins are cell wall proteins inducing cell wall loosening (Han *et al*., 2015) and, increased expansin activity involved in maintaining the growth of primary roots at a low water potential (Wu *et al*., 2001). Current study aimed to evaluate the effects of *TaMOR*, *TaNAC69-1* and *TaExpb23* genes expression on root system architecture and their potential use as selection criteria for root characters and drought tolerance of bread wheat.

MATERIALS AND METHODS

Plant materials

Ten rain-fed and irrigated bread wheat varieties adapted for cold region (Table 1) were compared in germination test, pot experiment and field trial as follows:

Germination test

The seminal root characters including their number, length, dry weight and growth angle were measured following the blotter-paper germination protocol reported by Hakizimana *et al*. (2000). Seeds of above-mentioned cultivars were surface sterilized. A germination towel (Whatman filter paper, was cut to 15×20 cm) was moistened with distilled water (as control) or PEG 6000 solution with -3 and -6 bars osmotic potential and placed flat on a table. A guide line was drawn 15 cm from the bottom of the paper. Five seeds for each cultivar were placed on the line with the germ end down. A second wet germination towel was placed on top of the seeds. The towels were rolled without disturbing the seed position in the paper, and secured with a rubber band. The rolled towels were placed vertically in a glass graduated cylinder and then placed in a dark incubator at 20 ºC for 10 days. To avoid water losses, the top of cylinders was sealed with Parafilm. The experiment was conducted as factorial design based on randomized complete block design, with three replicates in which the first factor consisted of cultivar and second factor consisted of osmotic stress.

Table 1. List of wheat cultivars used in the experiment with their yield under stress and normal conditions.

No.	Cultivar/Genotype	Adaptability	Pedigree
1	Heidari	Irrigated	Ghk"s"/Bow"s"//90Zhong87/3/Shiroodi
2	Baran	Rainfed	Ptzniska/Ut1556-170//Unknown TCI97AP
3	Zareh	Irrigated	130L1.11/F35.70/Mo73/4Ymh
4	Saeen	Rainfed	Azar2 /Zhong85
5	C-88-4	Irrigated	---
6	Azar2cross	Rainfed	---
7	Oroum	Irrigated	Alvand //NS732/Her
8	C-90-11	Irrigated	Eudiele
9	Pishgam	Irrigated	90-zhong87/Barakat
10	Homa	Rainfed	Pure line selection from Sardari landrace

Pot experiment

The above mentioned cultivars compared in a pot experiment with 50, 75 and 100% of field capacity irrigation regimes in a completely randomized design with 3 replicates. Three seeds were grown in polyvinylchloride tubes,100 cm depth and 25 cm diameter, filled with 15 kg of soil composed of a mixture of garden soil, vermicompost and sand (1:1:1, v/v). Well-watered plants were irrigated on alternate days to keep them at FC during the whole growing period. Water stress was started at the stem elongation stage (Zadoks stage, 37) by withholding water until reaching the soil moisture to 50% and 75% of FC, using soil water depletion curve (equivalent to a water content of 20 and 13.5 v/v% measured by Extech MO750 soil Moisture Meter, USA). At these moisture levels, pot moistures were brought to FC by re-watering. Stress regime continued until maturity. Sampling from root tissue was done at flowering stage and expression of *TaMOR*, *TaNAC69-1* and *TaExpb23* genes was measured by quantitative real time PCR. Root length, root main branches, root dry weight, plant yield and biomass were measured at maturity.

Gene expression analysis

Total RNA was extracted from roots using RNX-Plus total RNA extraction kit (CinnaGen), RNA concentrations and quality were assessed by spectrophotometry (NanoDrop,Thrmo). With 1 microgram template RNA, cDNA synthesis was done using SinaClon cDNA synthesis Kit. Reverse transcriptase-negative and RNA template-negative controls were carried out in parallel for each of the analyzed samples. Quantitative real-time PCR was performed using Sina SYBR Blue HS-qPCR Mix, with 500 nanogram cDNA, using a Rotor-Gene 2000 fluorometric thermal cycler (Corbett Research; Australia). The negative control was real time PCR reaction without cDNA template and positive control was *wzy2*. Actin gene expression was used as an internal control. Differences between the Ct values of the target gene and Actin were calculated as $\Delta Ct=Ct$ target gene – Ct actin, and the relative expression levels of the target genes were determined by the expression of $e^{-\Delta\Delta Ct}$, where "e" is the amplification efficiency. Primers were designed using Primer 3.0 software (Koressaar *et al.,* 2018) with primer lengths of 19–20 bp (Table 2).

Field trial

In order to investigate the relationship between root characters and gene expression data with real performance of varieties on field, varieties were grown in field under well-watered and rain-fed conditions using split plot design based on randomized complete block design with 3 replicates. Thirty kg of N ha-1 and 60 kg of P2O5 ha-1 were applied to the soil prior to planting. Drought was considered as the main factor and cultivar as the sub factor. Only well-watered plots were irrigated. Irrigation started when tensiometer indicated a soil water suction of 50 KPa. At each irrigation, 40 mm of water was applied to the plots, totaling to 200 mm of water during the whole period. In rain-fed conditions, drought was imposed by withholding irrigation and using a rain exclusion shelter after anthesis. Each plot consisted of six 3 m long rows spaced 20 cm apart. After maturity, grain yield was determined from $1m^2$ harvested area.

Statistical analysis

ANOVA of germination test, pot experiment and field trial were conducted in randomized complete blocks, completely randomized and split-plot design. Kolmogorov-Smirnov test was used for test of normality. Turkey's test was used for means comparisons at 0.05 probability level. Multiple regression analysis, using stepwise method and Pearson correlation coefficients were used to investigate the relations between traits. The hierarchical cluster analysis using Ward's method was used for grouping of genotypes. Statistical

Table 2. Designed specific primers for the selected target genes and actin as the reference gene.

Gene (accession number)	Forward (5´ → 3´)	Tm (°C)	Reverse (5´ → 3´)	Tm (°C)	Size of amplicon (bp)
Actin (GQ339780.1)	CGTGTTGGATTCTGGTGATG	55	GAGCTTCTCCTTGATGTCC	54	157
Ta Mor (KU158416.1)	CAGCAACGTCTCCAAGCTC	57	CTCCCACGTAGCCCTTGAC	58	203
EXPB23 (AY260547.1)	TACCCGTTCTCCTCCATGAC	57	TGGCAACCGGGTAGTAGTTC	58	120
TaNAC69-1 (AY625682.1)	TCGTCTTCTACCAGGGGAGG	58	AGAACACCCTGCATAGCACC	58	183

analysis was carried out with the SAS 9.2 and SPSS 20 software.

RESULTS

The results of ANOVA on the germination test showed that osmotic stress, had significant effects on all traits except on seminal root numbers (Table 3). In the pot experiment, root branches, root length, root dry weight, plant biomass and plant yield were decreased under drought conditions, however *TaMOR* expression was increased. *TaNAC69-1* and *TaExpb23* expressions were firstly increased and then decreased with the progression of water deficit (Table 4). Up-regulation of *TaExpb23* gene under drought conditions is in accordance with the results of Wu *et al*. (2001). It has been observed that under severe water deficits, limited root growth may occur because of very low soil water availability and high soil impedance (Comas *et al*., 2013). Xue *et al*. (2011) also found that *TaNAC69* expression was predominantly induced in drought-treated roots. In a study on maize, Barker *et al*. (2005) reported that 27% of gene expression was up-or down-regulated when maize plants were stressed for 5 days in buckets. Seminal root dry weight decreased whereas seminal root length increased in response to osmotic stress. ABA concentration is known to be elevated in the roots of drought-stressed plants and elevation of ABA in roots during mild drought or dehydration stress stimulates primary root elongation (Xu *et al*., 2013). This is explained by the ectopic overexpression of transcription factors, which directly promotes the transcription of genes encoding cell wall loosening proteins. These proteins promote cell elongation in the root, leading to an extended root system (Xu *et al*., 2013). Seminal root angle increased in response to osmotic stress. Polar auxin transport by influx and efflux carriers determines auxin distribution in the root, which is important for bending and gravitropism of the roots. The magnitude of the difference in auxin concentration between the upper and lower sides of the root determines the amount of root bending. auxin transport is inhibited during drought stress due to the inhibition of *PIN1* expression, which might facilitate increased downward bending of the roots (Roychoudhry *et al*., 2013). Drought also caused a decrease in the grain yield of cultivars under field conditions (Table 5), considering that, due to non-significant main factor error in field trial, the split-plot designe was analyzed as the simple factorial. Significant genotypic differences were observed for all traits, indicating the presence of genetic variability among the cultivars under study (Tables 3 and 4). Genotype×stress interactions were significant in root branches, root dry weight, plant biomass, plant yield,

Table 3. Mean squares in analysis of variance of traits in the pot experiment.

Source of variation	df	*Ta Mor*	*Ta NAC*	*Ta EXP*	RL	RN	RDW	PB	PY
Stress	2	128.4**	15.3**	12.34**	112.5**	3587**	3.03**	310**	4.74**
Genotype	9	150.6**	18.3**	12.3**	37.1**	421.1**	0.657**	20.5**	0.28**
G×E	18	15.4^{ns}	9.11**	8.5^{ns}	43.7**	238**	0.326**	17.7**	0.24**
Coefficient of variation (%)		67	58	83	9.75	30.6	51	45.9	29.3

^{ns}, * and **: are no significant and significant at p<0.05 and 0.01 probability levels respectively.
RL: Root length, RN: Root numbers, RDW: Root dry weight, PB: Plant biomass, PY: Plant yield.

Table 4. Mean squares in analysis of variance of traits in the germination test and the field trial (grain yield).

Source of variation	df	GY	SRA	SRN	SRDW	SRL
Rep	2	2670*	62.6^{ns}	0.67^{ns}	727**	0.59^{ns}
Stress	2	1446759**	1229**	1.08^{ns}	1854**	433.8**
Genotype	9	4664**	803.6**	2.07**	613**	4.0*
G×E	18	7642**	199.0^{ns}	0.96^{ns}	154.6*	3.26*
Coefficient of variation (%)		4.8	29	20.9	30.7	18.6

^{ns}, * and **: are no significant and significant at p<0.05 and 0.01 probability levels respectively.
GY: Grain yield (field experiment), SRA: Seminal root angle, SRN: Seminal root numbers, SRDW: Seminal root dry weight, SRL: Seminal root length.

TaNAC69-1 gene expression (Pot experiment), seminal root dry weight, seminal root length (germination test) and grain yield (field experiment), indicating the different response of cultivars to different stress levels (Tables 3 and 4). Most root traits are controlled by multiple genes, each governing small effect and often with a degree of epistasis or interaction effects that can be changed by environmental conditions (Cooper *et al*., 2009).

Mean comparisons showed that in the pot experiment, under stress (75% and 50% FC) Baran, Azar2 cross, Saeen and Zareh illustrated higher *TaMOR* expressions. The expression of TaNAC69-1 was high in C-88-4 and Azar2 cross under 75% FC and in Azar2 cross under 50% FC, respectively (Table 5). *TaExpb23* gene expression was high in Baran, Azar2 cross and Pishgam under 75% FC and in Baran, Saeen and Azar2 cross under 50% FC. In terms of plant yield, under non-stress condition, Oroum, Pishgam and C-88-4 produced higher yields. Under 75% FC, Azar2cross, Saeen, Pishgam, Baran and Zareh were located in the first rank. Under 50% FC, Pishgam, C-88-4 and Azar2 cross had the highest plant yield. Under non-stress conditions, C-90-11, Pishgam, C-88-4, Baran and Zareh had the highest root length. Under 70% FC, all genotypes were located in the first group except for Oroum, C-90-11 and Pishgam. However, under 50% FC, Pishgam, Saeen, C-88-4, Azar2 cross and Baran showed the highest root length. The highest main root numbers belonged to Pishgam in non-stress conditions, however, under 75% FC, all genotypes were located in the first group except for C-88-4 and under 50% FC, Azar2 cross had the highest values. In non-stress conditions, the highest root dry weight belonged to Oroum, Baran, Heidari, Zareh and Pishgam. Under 75% FC treatment, Azar2cross, Pishgam, C-90-11, Oroum, Zareh and under 50% FC, Oroum, Pishgam and Azar2 cross had the highest root dry weight. In the germination test, under moderate osmotic stress (-3bar), 8 out of 10 genotypes and in severe osmotic stress (-6bar), 9 out of 10 genotypes had higher seminal root numbers (Table 5). In seminal root length under non-stress conditions, Saeen, Homa and Baran along with 3 other genotypes had the highest values. Under moderate osmotic stress, 7 out of 10 genotypes were located in superior group, while under severe stress all genotypes were grouped in the superior class, except for Baran and Oroum. In seminal root angle, under non-stress conditions Pishgam along with 8 latter genotypes had the steepest roots. Under osmotic stress (-3bar and – 6 bar), Azar2 cross, Oroum, Homa and Pishgam had steeper root angles. In the field experiment the irrigated genotypes (Heidari, Pishgam and Oroum) had the highest grain yield under non-stress conditions. Under drought stress, Baran, Azar2 cross and Heidari were superior (Table 5).

Correlations

In order to determine the applicability of the above-mentioned root characters and gene expression levels in the field as selection criteria, correlation of traits in all 3 experiments were obtained. *TaMOR* gene expression had positive correlation with plant yield in 75% FC (Table 6), additionally, its correlation with grain yield (drought condition) showed a tendency toward significance (r=0.595, p=0.069). Undoubtedly, with higher number of genotypes, such correlations could be significant. Under 75% FC treatment, correlation of *TaMOR* expression with root length also showed a tendency toward significance (r=0.570, p=0.086). Li *et al*. (2016) observed that overexpression of *TaMOR* gene from wheat results in more roots and higher grain yield in rice. *TaNAC69-1* gene expression had positive correlations with plant yield under 50% FC and with plant biomass 50% FC stress conditions. Chen *et al*. (2016) showed that *TaNAC69-1* transgenic wheat lines produced 32% and 35% more above-ground biomass and grains than wild-type plants, respectively. *TaNAC69-1* gene expression also showed significant correlation with root branches under 50% FC, which is in accordance with the results of Chen *et al*. (2016). Additionally, its correlation with root dry weight under 75% FC showed a tendency toward significance (r=0.599, p=0.067). This result is in accordance with the results of Xue *et al*. (2011). *TaNAC69-1* is likely to be involved in promoting root elongation in drying soil (Chen *et al*., 2016). Importantly, *TaNAC69-1* gene expression showed a positive correlation with seminal root angle. *TaExpb23* gene expression showed positive correlation with plant yield and biomass under 75% FC. Han *et al*. (2015) reported that *TaEXPB23* improves oxidative stress tolerance in transgenic tobacco plants. Among the traits, root length was significantly correlated with plant yield and biomass under 50% FC, which is in accordance with the results of other researchers (Wu *et al*., 2001; Comas *et al*., 2013). Correlation of root length under 75% FC, with grain yield (under stress) showed a tendency toward significance (r=0.604, p=0.064). Root numbers showed a positive correlation with plant yield under non-stress and 50% FC conditions and with plant biomass in all conditions. Root numbers also showed positive correlation with seminal root angle under severe stress and with grain yield under non-stress conditions. Root dry weight had positive correlations with seminal root angle under moderate and sever stresses,

Table 5. Mean comparisons of traits in the germination test under different osmotic stresses and the pot experiment under different drought stresses.

Cv.	SRN			SRL (cm)			SRDW (mg)			SRA (°)		
	0 bar	-3 bar	- 6bar	0 bar	-3 bar	- 6bar	0 bar	-3 bar	- 6bar	0 bar	-3 bar	- 6bar
1	3.89^{a}	4.0^{abc}	3.94^{ab}	2.78^{abc}	9.83^{ab}	8.67^{ab}	30.2^{de}	27.4^{cde}	13.3^{c}	40.00^{ab}	40.0^{abc}	45.0^{bc}
2	4.33^{a}	4.44^{ab}	4.00^{ab}	2.89^{abc}	8.22^{ab}	8.00^{b}	53.9^{a}	41.6^{abc}	21.8^{bc}	44.44^{ab}	30.0^{c}	41.67^{c}
3	4.83^{a}	4.89^{a}	4.44^{a}	2.75^{abc}	10.78^{a}	9.44^{ab}	50.3ab	50.70^{a}	24.4^{abc}	53.33^{ab}	37.8^{abc}	48.3^{bc}
4	4.11^{a}	4.2^{abc}	4.44^{a}	3.11^{a}	9.56^{ab}	8.83^{ab}	40.7^{bcd}	43.6^{ab}	33.3^{a}	45.00^{ab}	50.0^{abc}	59.4^{abc}
5	5.00^{a}	4.0^{abc}	4.89^{a}	2.22^{bc}	7.44^{b}	9.56^{ab}	47.9^{abc}	31.7^{bcd}	30.4^{ab}	40.00^{ab}	32.2^{bc}	59.4^{abc}
6	4.22^{a}	3.22^{bc}	3.33^{ab}	2.11^{c}	11.0^{a}	11.11^{a}	27.7^{ef}	35.7^{abc}	16.5^{c}	55.56^{an}	61.11^{a}	71.1^{ab}
7	3.98^{a}	3.4^{abc}	3.44^{ab}	2.24^{abc}	8.56^{ab}	8.00^{b}	25. 3^{ef}	17.8^{de}	18.5^{c}	40.56^{ab}	57.78^{ab}	71.1^{ab}
8	3.56^{a}	4.56^{ab}	4.89^{a}	2.19^{bc}	6.78^{b}	10.17^{ab}	19.6^{f}	28.2^{b-e}	19.9^{bc}	37.16^{b}	52.2^{abc}	46.1^{bc}
9	4.61^{a}	3.7^{abc}	4.00^{ab}	2.00^{c}	6.83^{b}	9.89^{ab}	31.9^{de}	36.2^{abc}	23.1^{abc}	72.78^{a}	48.9^{abc}	78.89^{a}
10	4.28^{a}	2.83^{c}	2.50^{b}	3.05^{ab}	9.06^{ab}	10.28^{ab}	38.5^{cd}	13.80^{e}	13.1^{c}	56.33^{ab}	53.9^{abc}	62.8^{abc}
mean	4.28^{a}	3.93^{a}	3.99^{a}	2.53^{b}	8.81^{a}	9.39^{a}	36.6^{a}	32.7^{a}	21.4^{b}	48.5^{b}	46.4^{b}	58.4^{a}

Cv.	RN			RL (cm)			RDW (gr)			PB (gr)		
	FC	75%FC	50%FC	FC	75%FC	50%FC	FC	75%FC	50%FC	FC	75%FC	50%FC
1	28.0^{def}	17.0^{ab}	2.3^{d}	30.0^{bc}	24.0^{ab}	18.7^{c}	1.22^{abc}	0.167^{c}	0.056^{d}	6.85^{cd}	0.77^{ab}	0.156^{c}
2	44.5^{bc}	18.3^{ab}	5.0^{d}	36.7^{ab}	31.3^{a}	27.3^{ab}	1.59^{ab}	0.48^{bc}	0.056^{d}	8.02^{c}	3.67^{ab}	0.205^{c}
3	37.0^{cd}	19.3^{ab}	6.7^{cd}	34.0^{abc}	23.3^{abc}	18.7^{c}	1.19^{abc}	0.63^{abc}	0.221^{cd}	7.86^{c}	1.56^{ab}	0.467^{c}
4	27.5^{def}	16.5^{ab}	15.0^{bc}	21.0^{de}	25.3^{abc}	32.0^{a}	0.86^{cd}	0.55^{bc}	0.215^{cd}	6.72^{cd}	1.40^{ab}	1.79^{bc}
5	36.0^{cde}	14.2^{b}	21.0^{b}	37.0^{ab}	28.7^{ab}	32.0^{a}	1.10^{bc}	0.27^{bc}	0.43^{bcd}	12.06^{b}	0.89^{ab}	2.52^{abc}
6	25.3^{ef}	24.5^{ab}	35.0^{a}	14.0^{e}	29.0^{ab}	28.0^{ab}	1.05^{bc}	1.19^{a}	0.82^{abc}	3.37^{e}	3.89^{a}	4.96^{a}
7	48.7^{b}	23.3^{ab}	12.0^{bcd}	26.0^{cd}	21.3^{bcd}	16.7^{cd}	1.74^{a}	0.66^{abc}	1.30^{a}	6.44^{cd}	1.35^{ab}	1.39^{bc}
8	21.3^{f}	15.5^{ab}	3.7^{d}	42.0^{a}	14.0^{d}	9.3^{d}	0.75^{cd}	0.853^{ab}	0.378^{cd}	4.62^{de}	0.53^{b}	0.173^{c}
9	60.3^{a}	25.5^{a}	22.0^{b}	40.0^{a}	20.0^{cd}	33.0^{a}	1.13^{abc}	0.875^{ab}	1.01^{ab}	15.4^{a}	2.46^{ab}	3.69^{ab}
10	17.5^{f}	20.0^{ab}	11.3^{bcd}	28.0^{cd}	26.6^{abc}	24.0^{bc}	0.29^{d}	0.495^{bc}	0.313^{cd}	3.25^{e}	3.05^{ab}	2.92^{abc}
mean	34.6^{a}	19.4^{b}	13.4^{c}	30.8^{a}	24.4^{b}	23.9^{b}	1.09^{a}	0.62^{b}	0.48^{b}	7.46^{a}	1.96^{b}	1.83^{b}

Cv.	*Ta Mor* (fold change)		*Ta NAC69* (fold change)		*Ta EXP23* (fold change)		GY (gr/m2)		PY (gr)		
	75%FC	50%FC	75%FC	50%FC	75%FC	50%FC	Non-stress	Drought stress	FC	75%FC	50%FC
1	4.3^{b}	0.5^{d}	1.43^{bc}	0.25^{b}	3.31^{cd}	0.50^{c}	702a	353^{abc}	0.99^{cd}	0.24^{bc}	0.047^{c}
2	12.3^{a}	19.3^{b}	0.76^{c}	0.76^{b}	8.07^{a}	11.3^{a}	644bc	380^{a}	0.57^{ef}	0.38^{abc}	0.140^{c}
3	3.98^{b}	25.9^{a}	3.20^{b}	1.81^{b}	3.3^{bcd}	0.23^{c}	582ef	330^{bcd}	0.76^{de}	0.27^{abc}	0.146^{c}
4	10.65^{a}	11.6^{c}	1.32^{bc}	0.86^{b}	3.9^{bcd}	7.09^{b}	599de	333^{bcd}	0.77^{de}	0.50^{ab}	0.303^{bc}
5	0.68^{b}	0.4^{d}	3.34^{ab}	1.30^{b}	0.81^{c}	0.81^{c}	640cd	319^{cde}	1.38^{b}	0.20^{c}	0.603^{a}
6	11.9^{a}	11.9^{c}	5.09^{a}	7.30^{a}	5.98^{ab}	6.70^{b}	608cde	365^{ab}	0.56ef	0.53^{a}	0.433^{ab}
7	4.98^{b}	0.5^{d}	1.90^{bc}	1.00^{b}	3.1^{bcd}	0.50^{c}	683ab	239^{f}	1.69a	0.15^{c}	0.085^{c}
8	1.00^{b}	1.0^{d}	2.19^{bc}	0.35^{b}	0.09^{d}	0.54^{c}	620cde	307de	1.04c	0.23^{bc}	0.054^{c}
9	1.16^{b}	1.16^{d}	1.98^{bc}	2.00^{b}	5.1^{abc}	0.51^{c}	690a	281^{e}	1.52b	0.40^{abc}	0.66^{a}
10	1.39^{b}	0.76^{d}	1.96^{bc}	1.78^{b}	1.8^{bcd}	2.10^{c}	553f	310de	0.48f	0.20^{c}	0.233^{bc}
mean	5.24^{b}	7.32^{a}	2.32^{a}	1.74^{b}	3.54^{a}	3.03^{ab}	6322.8^{a}	3217.1^{b}	0.98^{a}	0.31^{b}	0.27^{b}

Means showing the same letter in each column haven't significant differences in $p \leq 0.05$.

Cv.: Cultivar, SRN: Seminal root numbers, SRA: Seminal root angle, SRDW: Seminal root dry weight, SRL: Seminal root length, PY: Pot yield, PB: Pot biomass, RL: Root length, RN: Root numbers, RDW: Root dry weight, GY: Grain yield.

Table 6. Correlation coefficients among selected genes and root traits under normal and two water deficit conditions.

	Cond.	GY_N	GY_S	*Ta Mor*	*TaNAC*	*Ta EXP*	PY	PB	RL	RN	RDW	SRL	SRN	SRA	SRDW
	N	----	-----	1											
Ta Mor	MS	----	0.595	1											
	SS	----	0.263	1											
	N	----	----	----	1										
Ta NAC	MS	----	0.105	0.009	1										
	SS	----	-0.243	0.225	1										
	N	----	----	----	----	1									
Ta EXP	MS	----	0.467	0.777**	-0.101	1									
	SS	----	0.428	0.527	-0.051	1									
	N	0.707*	---	----	----	----	1								
PY	MS	----	0.528	0.733*	0.207	0.663*	1								
	SS	----	0.003	-0.175	0.634*	0.012	1								
	N	0.525	----	----	-----	-----	0.608*	1							
PB	MS	----	0.143	0.313	0.091	0.723*	0.542	1							
	SS	----	-0.075	-0.201	0.711*	0.084	0.773**	1							
	N	0.257	----	----	----	----	0.354	0.556	1						
RL	MS	----	0.604	0.570	0.141	0.533	0.333	0.308	1						
	SS	----	0.256	0.091	0.477	0.438	0.815**	0.612*	1						
	N	0.632*	----	----	----	----	0.636*	0.788**	0.351	1					
RN	MS	----	-0.317	0.154	0.273	0.496	0.284	0.752*	-0.03	1					
	SS	----	-0.036	-0.075	0.622*	0.165	0.788**	0.940**	0.629*	1					
	N	0.674*	----	----	----	----	0.448	0.331	0.056	0.704*	1				
RDW	MS	----	-0.207	0.110	0.599	0.099	0.441	0.419	-0.35	0.639*	1				
	SS	----	-0.77**	-0.374	0.409	-0.306	0.346	0.535	0.045	0.548	1				
	N	-0.449	----	----	----	----	-0.639*	-0.356	-0.201	-0.399	-0.255	1			
SRL	MS	----	0.445	0.519	0.387	0.294	0.335	0.022	0.427	0.174	0.094	1			
	SS	----	0.098	-0.112	0.510	-0.153	0.439	0.662*	0.083	0.573	0.130	1			
	N	-0.153	----	----	----	----	0.069	0.610*	0.171	0.395	0.087	-0.054	1		
SRN	MS	----	0.285	0.063	-0.221	-0.015	0.020	-0.419	-0.22	-0.544	-0.247	-0.077	1		
	SS	----	0.079	0.141	-0.352	-0.106	0.084	-0.435	-0.057	-0.206	-0.273	-0.154	1		
	N	-0.100	-----	----	----	----	-0.056	0.372	-0.030	0.381	-0.214	-0.179	0.426	1	
SRA	MS	----	-0.431	0.036	0.307	-0.125	0.172	0.194	-0.37	0.567	0.749*	0.154	-0.618*	1	
	SS	----	-0.553	-0.370	0.648*	-0.184	0.656*	0.838**	0.448	0.772**	0.846**	0.347	-0.412	1	
	N	-0.303	-----	----	----	----	-0.346	0.303	0.159	0.165	0.131	0.523	0.727*	0.053	1
SRDW	MS	----	0.506	0.438	0.119	0.452	0.607	0.128	0.191	-0.099	0.075	0.270	0.712*	-0.452	1
	SS	----	-0.053	0.268	0.101	0.189	0.429	-0.027	0.491	0.161	-0.102	-0.190	0.693*	0.017	1

* and **: are significant at $p<0.05$ and 0.01 probability levels respectively.

Cond.: Conditions, N: Normal, MS: Moderate stress, SS: Severe stress, PY: Plant yield, PB: Plant biomass, RL: Root length, RN: Root numbers, RDW: Root dry weight, SRL: Seminal root length, SRN: Seminal root numbers, SRA: Seminal root angle, SRDW: Seminal root dry weight, GY_N: Grain yield (nonstress), GY_S: Grain yield (stress).

indicating that plants with steeper root growth, may also produce more root biomass. Seminal root number showed a positive correlation with plant biomass under non-stress conditions and with seminal root dry weight in all conditions. It was negatively correlated with seminal root angle under drought stress conditions. It seems that under drought, lower root numbers and steeper root system can simultaneously be achieved.

Regression analysis

Plant yield in the pot experiment and grain yield in the field trial were considered as dependent variables in two distinct regression analyses. Considering field grain yield as the dependent variable, results showed that under non-stress conditions only root dry weight remained in the final model and explained the 96.5% of grain yield variance. Under moderate stress (75% FC or -3 bar) none of the traits entered the regression model. Under severe stress (50% FC or -6 bar), root dry weight and root numbers remained in the final model and explained 74.5% of variance (Table 7). When plant yield was considered as the dependent variable, root numbers, *TaMOR* expression rate and root length remained in the final model in all three stress conditions (Table 8). Capacity for deep root growth may improve root acquisition of water when ample water at depth is available (Comas *et al*., 2013). The results of Barker *et al*. (2005) showed that genes regulated in pot experiment may differ from those regulated in the field conditions. The compilation of these studies could lead to the identification of constitutive gene and trait expressions that are crucial across multiple environments for improving drought tolerance in the field.

Cluster analysis

Cluster analysis could separate genotypes only under stress conditions. Under moderate stress, genotypes were grouped in 3 clusters, discriminant analysis was used to confirm proper grouping (data not shown); there were significant differences among clusters in root numbers, *TaMOR* and *TaExpb23* expression rate and grain yield (Figure 1). The first cluster which consisted of Heidari, Baran and Azar2cross, had higher numbers of root number, higher *TaMOR* and *TaExpb23* expression rates and higher grain yields, respectively. Second cluster which consisted of C-90-11, Homa, C-88-4, Zareh and Saeen were located in the second rank in terms of grain yield. The third cluster consisted of Oroum and Pishgam had the lowest *TaMOR* expression rate and grain yield. Under severe stress, genotypes were grouped in 2 clusters; there were significant differences among clusters in root dry weight, *TaMOR* and *TaExpb23* expression rate and grain yield, respectively. The first cluster which consisted of Zareh, Saeen, Homa, C-88-4, Azar2 cross, Heidari and Baran, had higher *TaMOR* and *TaExpb23* expression rates and higher grain yields and lower root dry weights (Table 9). As shown in both stress levels, genotypes having the higher expression rates of *TaMOR* and *TaExpb23* had the highest grain yield. Divergent clusters and genotypes

Table 7. Multiple regression coefficients (grain yield in field experiment as dependent variable).

Stress condition	Trait	Unstandardized coefficients	Standardized coefficients	t	Sig.	R^2
Non-stress	RDW	808.6*±313	0.674	2.58	0.033	0.965
50%FC	RDW	-1205**±226.8	-1.07	5.31	0.001	0.745
	RN	25.64*±9.38	0.551	2.74	0.029	

* and **: are significant at p<0.05 and 0.01 probability levels respectively.
RDW: Root dry weight, RN: Root numbers.

Table 8. Multiple regression coefficients (plant grain yield in the pot experiment as a dependent variable).

Stress condition	Trait	Unstandardized coefficients	Standardized coefficients	t	Sig.	R^2
Non-stress	RN	0.02*±0.009	0.636	2.33	0.048	0.404
75%FC	*TaMor*	0.021*±0.007	0.733	3.051	0.016	0.538
50%FC	RL	0.046**±0.012	0.815	3.98	0.004	0.622

* and **: are significant at p<0.05 and 0.01 probability levels respectively.
RN: Root numbers, RL: Root length.

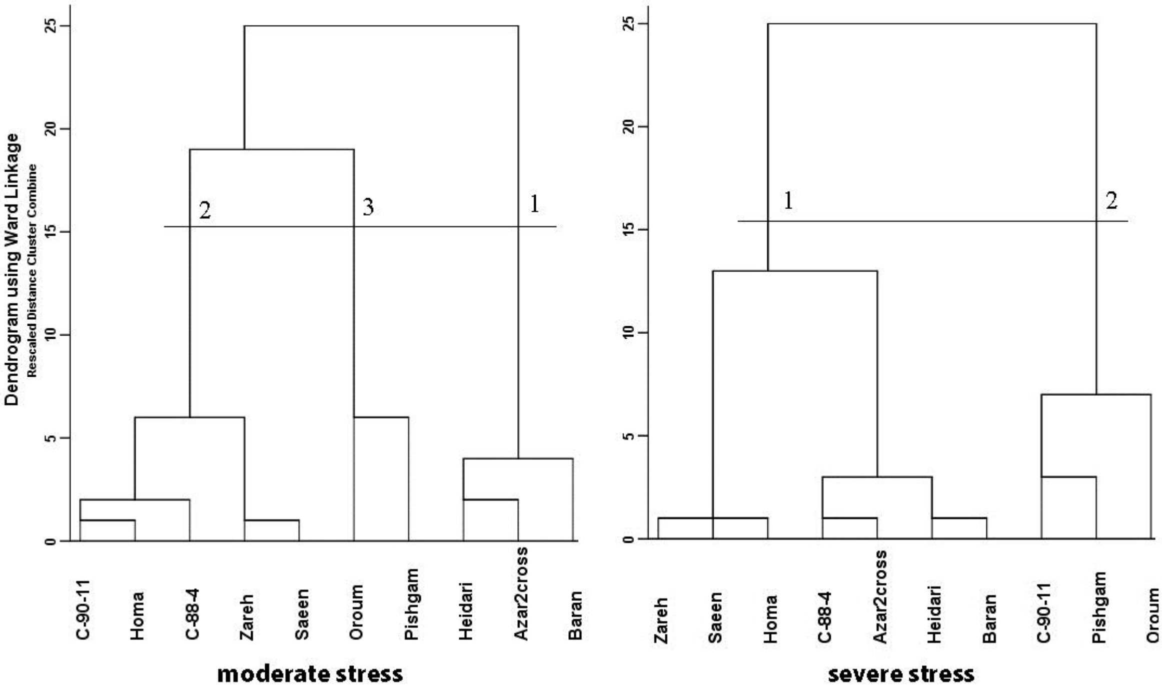

Figure 1. Cluster analysis using Ward's method for genotypes under moderate and severe stress.

can be further evaluated for their breeding values as parents and can be exploited in wheat breeding programs for superior root characters. For example, crossing between genotypes of clusters 1 and 2 under severe stress, can be resulted in progenies with higher root biomass, higher *TaMor* and *TaEXP* expression and higher grain yield.

DISCUSSION

Most of wheat plants grown in Iran are rain-fed and are sown at mid or late fall. In this region, most of rain falls in the fall and winter and wheat relies largely on the moisture stored in the soil. Thus, root growth, deep into the soil during the season may increase water capture. Therefore, a better understanding of root-related traits and how traits are related to crop plant productivity under different drought conditions is required. In this regard, the presence of genetic variability among the genotypes indicates the possibility of development of these traits through breeding. Correlation and regression analyses revealed that there are significant relations between root characters, gene expression and plant performance. Among the root traits, only those measured in the pot experiment, showed significant relations with grain yield (per plant or per unit area), suggesting that germination traits measured at early stages of growth may not necessarily be a surrogate for empirical selection of yield. Among the genes studied, only *taMOR* remained in the final regression model. *taMOR* expression had significant relation with both plant yield and grain yield, and it tend to have a positive correlation with root length, concluding its possible importance in the development of root system architecture. Tardieu *et al.* (2017) also suggested that TaMOR offers an opportunity to improve root architecture and increase yield in crops, which is in accordance with our findings. According to Itam *et al.* (2020) to improve the drought tolerance of wheat varieties, this transcription factor, among other favorable genes, can be transferred via crossing. *TaNAC69-1* gene expression had positive correlations with plant yield, root numbers and seminal root angle. Some members of the NAC family have been shown to be involved in promoting lateral root development (He *et al*., 2005), Xue *et al.* (2011) suggested that TaNAC69 is involved in regulating stress up-regulated genes and wheat adaptation to drought stress. As mentioned above *TaNAC69-1* expression in the roots down-regulates the expression of auxin/IAA transcriptional repressor genes and suppresses the inhibition of root growth (Chen *et al*., 2016), therefore, it can be regarded as a means for the regulation of root system architecture. *TaExpb23* expression also showed a positive correlation with plant yield and biomass and tend to have a positive correlation with grain yield but did not showe any correlation with root traits. So, it can be concluded that *TaEXPB23* improved plant productivity through other mechanisms such as decreasing oxidative damage (Han *et al.,* 2015). Several studies have provided evidence that expansins are associated with environmental stress tolerance in plants (Zhao *et al.,* 2011; Han *et al.,* 2015; Zhou *et al.,* 2015). It also was concluded that Azar2 cross was the only cultivar which had the higher expression rate for each of the three genes. This genotype had a higher plant yield, plant biomass, root length, number of root branches, root dry weight, seminal root length, seminal root angle and finally grain yield indicating the importance of the accumulation of favorable alleles in the productivity of plant. Jia *et al*. (2019) showed that barley genotypes with 15 beneficial alleles of root system depth loci showed a deeper rooting phenotype. In their study additive effect was also observed for root spreading angle so that lines carrying favorable alleles for root spreading angle, result in even steeper roots revealing that there is a potential for pyramiding these alleles in the breeding practice. Finally, the results of this study suggested that the expression pattern of genes involved in root system architecture can be used for the development of root traits and plant yield under drought stress. However, due to the use of polyvinylchloride tubes for the evaluation of root traits of mature plants in this study, root growth angle was evaluated only in early stages in a towel paper. Therefore, evaluation of root characters of mature plants using tools such as rhizotron can lead to more accurate results. Application of molecular markers associated with higher expression of these genes also was suggested.

Table 9. Mean of clusters derived from Cluster analysis under moderate and severe stresses.

Stress		PY	RL	RN	RDW	*TaMor*	*TaNac*	*TaEXP*	SRL	SRN	SRA	SRDW	GY
Moderate	Cluster1	0.381^{a}	14.1^{a}	19.9^{ab}	0.546^{a}	9.51^{a}	2.42^{a}	5.78^{a}	9.68^{a}	3.89^{a}	43.7^{a}	34.9^{a}	3659^{a}
	Cluster2	0.282^{a}	11.8^{a}	17.1^{b}	0.560^{a}	3.54^{b}	2.40^{a}	1.98^{b}	8.72^{a}	4.10^{a}	45.2^{a}	33.6^{a}	3199^{b}
	Cluster3	0.268^{a}	10.3^{a}	24.4^{a}	0.766^{a}	3.07^{b}	1.94^{a}	4.1^{ab}	7.69^{a}	3.56^{a}	53.3^{a}	27.0^{a}	2598^{c}
	F value	0.58^{ns}	1.59^{ns}	4.56*	0.30^{ns}	2.53*	0.09^{ns}	4.1*	1.07^{ns}	0.47^{ns}	0.27^{ns}	0.27^{ns}	26.4**
Severe	Cluster1	0.272	12.91	13.7	0.30	9.89	1.18	4.03	9.41	3.93	55.4	21.8	3586
	Cluster2	0.267	9.83	12.6	0.89	0.72	1.12	0.35	9.35	4.11	65.4	20.5	2756
	T value	0.035^{ns}	1.15^{ns}	0.162^{ns}	2.62*	2.34*	1.22^{ns}	2.23*	0.082^{ns}	0.32^{ns}	1.15^{ns}	0.26^{ns}	4.42*

Means showing the same letter in each column haven't significant differences in $p \leq 0.05$.

^{ns}, * and **: are no significant and significant at $p<0.05$ and 0.01 probability levels respectively.

PY: Plant yield, RL: Root length, RN: Root numbers, RDW: Root dry weight, SRL: Seminal root length, SRN: Seminal root numbers, SRA: Seminal root angle, SRDW: Seminal root dry weight, GY: Grain yield.

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