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*Short Communication*

# Osmotic stress induced root growth and regulation of root development related genes in progenitors of wheat

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**Abstract**

**Maintenance of root growth during water deficit can significantly contribute to yield stability. In this study, total nine accessions, three each of *Triticum monococcum, Aegilops speltoides* and *Aegilops tauschii* were analysed for root traits under two levels of osmotic stress at seedling stage. *T. monococcum* accession (A2) showed highest increase in total root growth while 17% and 34% increase in primary root length was recorded in *T. monococcum* (A2) and *Ae. speltoides* (B3) under osmotic stress. Expression of *BREVIS RADIX* (*BRX*) and *NAM/ATAF/CUC 1*(*NAC1)* was analysed in three diploid accessions with contrasting root phenotype. *BREVIS RADIX*, a transcription factor modulating root length was up regulated in *T. monococcum* A2 accession while *NAC1* expression was up regulated in three of the accessions under osmotic stress. The accessions and genes analysed in the study can be good resource to explore the molecular-genetic mechanism of root growth under stress.**

**Key words:** *BREVIS RADIX,* diploid species, drought tolerance, seminal root

Roots are vital for water and nutrient acquisition from soil and contribute to both drought avoidance and heat tolerance in plants (Wasson et al. 2012; Li et al. 2019). Identification and optimization of root traits and their molecular genetic analysis is important for sustaining yield under abiotic stress conditions. In current scenario, where the focus of wheat breeding is on restricted or limited irrigation, stress induced root growth can be one of the useful adaptive root traits. Root growth under osmotic or drought stress has been analyzed in *Arabidopsis*, soybean, maize and wheat (*Triticum aestivum*) (van der Weele et al. 2000; Spollen et al. 2008, Yamaguchi and Sharp 2010; Song et al. 2016; Dalal et al. 2018). Diploid species of wheat have been used as sources of adaptive genes for various biotic and abiotic stresses (Hussien et al. 1997; Reynolds et al. 2007). However, these have not been sufficiently explored for root traits under abiotic stresses. Hence, in this study, diploid wheat species with AA, BB and DD genomes were analysed for root traits under osmotic stress conditions. Expression analysis of two genes viz. *BREVIS RADIX (BRX)* and, *NAM/ATAF/CUC 1* (*NAC1)*, involved in root development in *Arabidopsis* was also carried out to understand their regulation under osmotic stress.

Three accessions each of *T. monococcum, Aegilops speltoides* and *A. tauschii* were analysed in this study (Table 1). Based on studies on primary root elongation in rice and *Arabidopsis* (Xu et al. 2013), in present study two stress levels, 5% PEG and 10% PEG were selected. After 48 h of germination, uniformly germinated seeds of nine accessions were planted in sand filled pots saturated with water (Non-stress/ control), 5 % PEG 6000 (–0.50 bars) solution, and 10 % PEG6000 (–1.48 bars) solution, respectively. The pots were kept in culture room at 25±2$^{o}$ C with 16h/ 8h light/dark cycle. After 10 days of stress, the seedlings were carefully uprooted and washed with water. Primary root length and seminal root number were recorded manually while total root length, surface area and diameter were measured using

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**Table 1.** List of diploid accessions of wheat used in the present study

| S.No. | Name of species & accession no. | Abbreviation used in this study |
|---|---|---|
| 1 | *T. monococcum,* No 81 | A1 |
| 2 | *T. monococcum*, No 67 | A2 |
| 3 | *T. monococcum,* WLT 2804 | A3 |
| 4 | *Ae. speltoides* spp. *ligustica*, WLT-2003 | B1 |
| 5 | *Ae. speltoides* spp. *ligustica*, No. 84 | B2 |
| 6 | *Ae. speltoides,* No. 86 | B3 |
| 7 | *Ae. squarrosa* (*Ae. tauschii*), acc. 9810 | D1 |
| 8 | *Ae. squarrosa* (*Ae. tauschii*) acc. 3491 | D2 |
| 9 | *Ae. squarrosa* (*Ae. tauschii*) acc. 9788 | D3 |

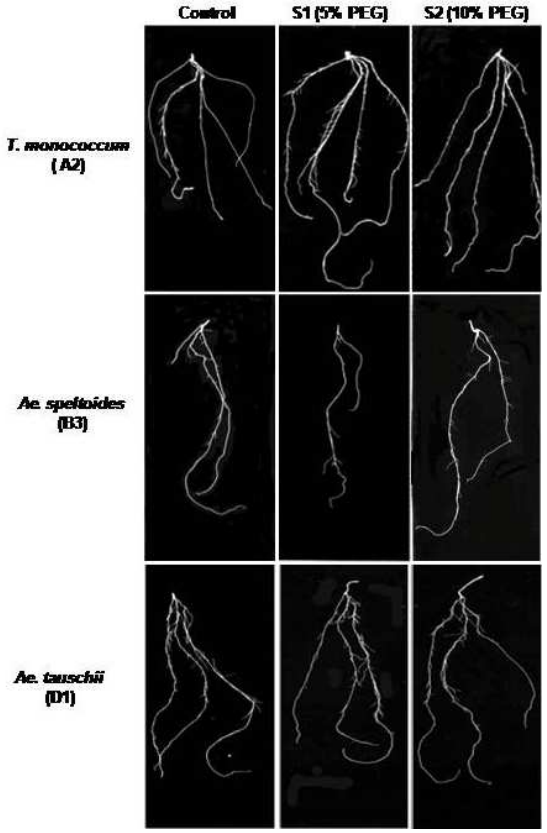
acc = Accession

WinRhizo2013a software (Regent Instruments Canada Inc.). The data were recorded for 5 plants in each treatment. For gene expression analysis, root samples were collected from control and stressed seedlings, frozen in liquid nitrogen and stored at -80 °C till further use.

Protein sequences of *Arabidopsis* genes *viz*., *BREVIS RADIX (BRX)* (*AtBRX;* At1G31880) and *NAM/ATAF/CUC 1* (*NAC1)* (*AtNAC1*; At1g56010) were used as query to BLAST search the wheat genome (http://plants.ensembl.org/Triticum_aestivum/Info/Index) and homologous sequences TraesCS6D02G245400 and TraesCS7A02G464100 were identified for BRX and NAC1, respectively. Gene specific primers were designed using primer 3 software (http://bioinfo.ut.ee/primer3-0.4.0/).

RNA was extracted using RNeasy® plant mini kit (QIAGEN) followed by on-column DNaseI (Sigma) treatment to obtain DNA-free RNA. First strand cDNA was synthesized from 2 µg of total RNA using Superscript–III reverse transcriptase (Invitrogen, USA). Since the primers were designed based on homologs of *BRX* and *NAC1* from *T. aestivum*, the full length coding sequences of *BRX* and *NAC1* in three genomes *viz*., A2, B3 and D1 were amplified, cloned and confirmed by sequencing (Acc. No MN640606, MN612105, MN612106, MN612107, MN612108). Three biological replicates were pooled and used for quantitative RT-PCR (qRT-PCR). The cDNA was diluted to 1:10 times and 1.0 µL of diluted cDNA was used as a template. The reaction mixture (10 µL) included cDNA, SYBRPremix (KAPA SYBR FAST qPCR kit) and gene specific forward and reverse primers (BRX-RTF: 5′-ACATCACCATCCGCGAG-3′, BRX-RTR: 5′-CTGCTACAGATACTGCGTGTG-3′; NAC-RTF: 5′-CATTGCCTTTGACCATCCTG-3′ and NAC-RTR: 5′-ATTGGAGAAGCAGGGCAC-3′). Expression data was normalized based on expression of endogenous control gene ADP-ribosylation factor (TaADPR-F: 5′-GCTCTCCAACAACATTGCCAAC-3′ and TaADPR-R: 5′-GCTTCTGCCTGTCACATACGC-3′) (Paolacci et al. 2009). Relative fold change was calculated using relative $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). The bars represent the mean expression with ± standard error of the expression of the three technical replicates. The root data and expression analysis data were analysed using two way and one way ANOVA, respectively.

There were significant phenotypic differences in root length, seminal root number and lateral roots among the diploid accessions Fig. 1. Under control




**Supplementary Fig. S1. Representative pictures depicting phenotypic variation in roots of diploid species. The seedlings were subjected to two levels of osmotic stress *viz*., 5 % PEG (S1, -0.50 bars), and 10 % PEG (S2, -1.48 bars) after 48 h of germination. Seedlings treated with water served as control (C)**

conditions, total root length of *T. monococcum* accessions A1 (184±11.2 cm) and A3 (140±5.4 cm) was significantly higher as compared to *T. monococcum* acc. A2 (64.1±2.6cm), as well as accessions of *Ae. speltoides* (mean 30.19±2.05 cm) and *Ae. tauschii* (mean 63.17±4.84 cm) (Fig. 2a). Under osmotic stress, the diploid accessions A2, A3 and D3 showed increase in total root length at both the stress levels. Accession A2 showed highest and significant increase of 51% and 40% in total root length under S1 and S2 stress (Fig. 2a). Under control conditions, primary root length was significantly higher in accession A1, followed by that of A3 and D1. Accession B3 showed increase of 20% (S1) and 34% (S2) while A2 showed 14% and 17% increase in primary root length at S1 and S2 stress respectively. Osmotic stress did not affect the primary root length of A3, B2, D2, and D3. D1 showed decrease of about 15% in primary root length under both the stresses (Fig. 2b). Lateral root length was highest in A1 accession under control conditions followed by A3, and *D1/D2/D3* accessions while *Ae. speltoides* accessions had the shortest lateral root length (Fig. 1c). Accessions A1, A2, A3, B2 and D3 showed significant increase in lateral root length under both S1 and S2 stress. Increase in total root length especially in case of A2, might be due to increase in primary as well as lateral root length under stress conditions (Fig. 2c). Osmotic stress increased the average root diameter of *T. monococcum* accession A2 and A3 at both the stresses, while A1 showed increase in root diameter at S1 stress. However, root diameter of *Ae. speltoides* and *Ae. tauschii* accessions (B1, B3, D1 and D3) was largely unaffected except B2 and D2 which showed decrease and increase in root diameter, respectively. Surface area of the roots was higher in three of the *T. monococcum* accessions at both S1 and S2 stresses. Root surface area was largely unaffected by osmotic stress in other accessions except B2 which showed decrease under stress. Seminal root number (SRN) per plant in *T. monococcum* accessions was significantly higher (3.8-5.9 per plant) than that of *Ae. speltoides* and *Ae. tauschii* which showed a mean of 2.9 and 2.8 seminal roots per plant, respectively, under control conditions (Fig. 2d). Accessions A3, B1, B2 and D1 showed significant reduction in seminal root number at both S1 and S2 stress while in other accessions, seminal root number reduced at S1 (A1 and B3) or S2 stress (A2 and D3) (Fig. 2d). Inter and intra-species variations in SRN have been recorded in

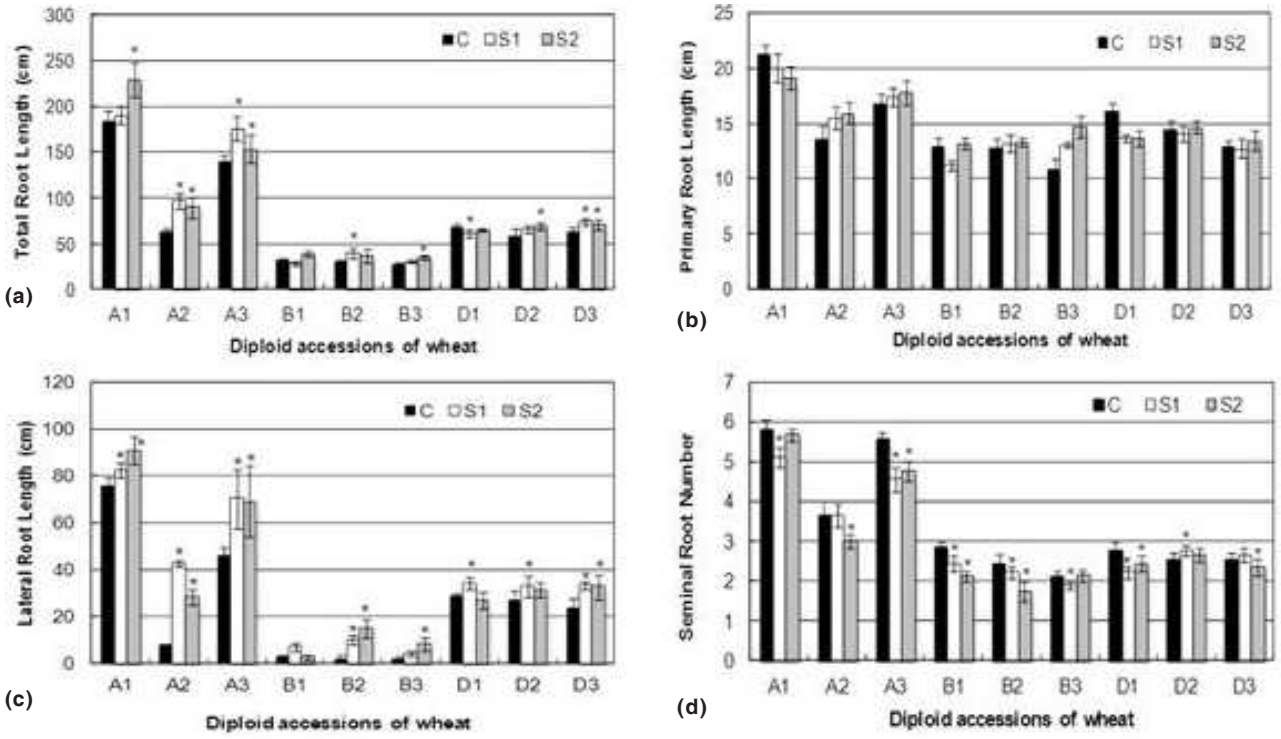



**Fig. 2. Root traits in diploid species of wheat under osmotic stress. Three accessions each from *T. monococcum* (A1, A2 and A3), *Ae. speltoides* (B1, B2, and B3) and *Ae. tauschii* (D1, D2 and D3) were subjected to two levels of osmotic stress. Asterisks indicate significant difference between control and stress (p<0.01)**

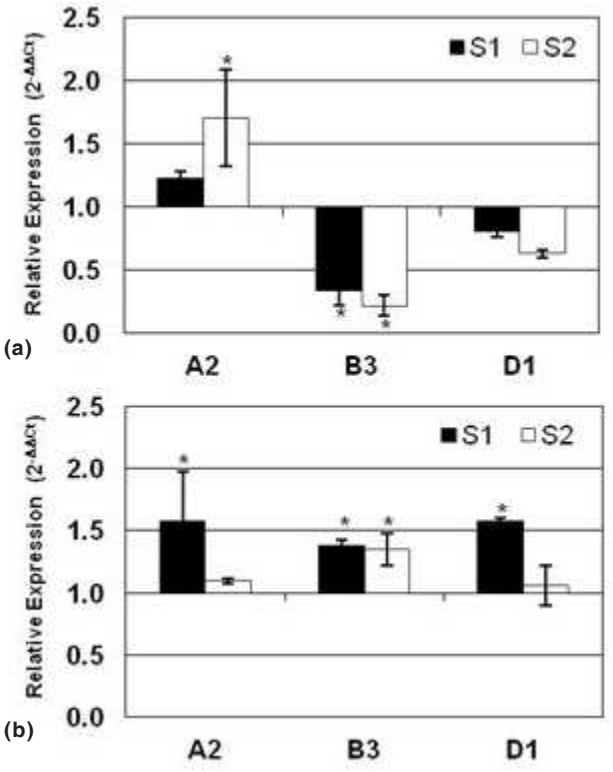



**Fig. 3. Relative expression of genes in root tissue of diploid species under osmotic stress conditions. a) *BRX*; and b) *NAC1* in *T. monococcum* accession A2, *Ae. speltoides* accession B3, and *Ae. tauschii* accession D1. Asterisks indicate significant difference between control and stress (p<0.05)**

diploid species (Golan et al. 2018). Inspite of the variation it appears that monococcum inherently has the higher SRN number followed by *Ae*. *tauschii* and *Ae. speltoides.*

As there were significant differences in root length and lateral roots among diploid accessions, expression of *BRX* (Mouchel et al. 2004) and *NAC1* (Xie et al. 2000) which are involved in root length and lateral root development, respectively was analysed under osmotic stress. Based on the phenotypic data A2, B3 and D1 representing A, B and D genomes respectively, were selected for qRT-PCR analysis. *BRX* expression level in *T. monococcum* A2 was significantly up regulated (~1.7 fold) at S2 stress (Fig. 3a) while it was down regulated in *Ae. Speltoides*, B3 and *Ae. Tauschii,* D1 under both the levels of osmotic stress (Fig. 3a).

*BRX* is a transcription factor associated and its expression is correlated with longer primary root length in *Arabidopsis* (Mouchel et al. 2004). A significant increase in *NAC1* transcript level was recorded in A2 and D1 at S1 stress while its expression decreased in S2 stress (Fig. 2b). *NAC1* is one of the main regulators of auxin induced lateral root development (Xie et al. 2000). The level of *NAC1* expression was found to relate with lateral root length in A2, B3 and D1 accessions under both S1 and S2 stress.

Thus *T. monococcum* accessions used in our study in general, showed better root traits in terms of primary root length and inductive root length, higher seminal root number and longer lateral roots under control and stress. The accessions and genes analysed in the study can be a good resource to explore the molecular-genetic mechanism of root growth under stress.

## Authors' contribution

Conceptualization of research (MD); Designing of the experiments (MD); Contribution of experimental materials (V); Execution of lab experiments and data collection (PA, ST); Analysis of data and interpretation (MD); Preparation of the manuscript (MD, V).

## Declaration

The authors declare no conflict of interest.

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