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Research article

Physiological and biochemical responses of two spring wheat genotypes to non-hydraulic root-to-shoot signalling of partial and full root-zone drought stress

Asfa Batool[a], Nudrat Aisha Akram[b], Zheng-Guo Cheng[a], Guang-Chao Lv[a], Muhammad Ashraf[a,c], Muhammad Afzal[c], Jun-Lan Xiong[a], Jian-Yong Wang[a], You-Cai Xiong[a,*,1]

[a] State Key Laboratory of Grassland Agro-Ecosystems, Institute of Arid Agroecology, School of Life Sciences, Lanzhou University, Lanzhou, 730000, China
[b] Department of Botany, GC University, Faisalabad, 38040, Pakistan
[c] Faculty of Agriculture, The University of Sargodha, Sargodha, 40100, Pakistan

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ABSTRACT

Non-hydraulic root-sourced signal (nHRS) is so far affirmed to be a unique positive early-warning response to drying soil, however its physiological and agronomic implications are still unclear. We designed two contrast methods to induce nHRS in two wheat (*Triticum aestivum* L.) genotypes released in different decades under pot-culture conditions. Partial root-zone stress (PS) was performed using the method of split-root alternative water supply (one half wetting and the other drying) to induce the continuous operation of nHRS, and full root-zone stress (FS) was subjected to whole root system to periodic operation of nHRS. nHRS-mediated signalling increased abscisic acid (ABA) production and triggered ROS (reactive oxygen species) generation, which, thereby, led to up-regulation of antioxidant defense system. Cytokinin synthesis reduced during drought stress while proline and malodialdehyde (MDA) content were increased. Regardless of drought treatment methods and wheat genotype, a significant decrease in grain yield, root biomass and above-ground biomass ($p < 0.05$) was observed, without significant changes in root-to-shoot ratio. Harvest index was increased, proposing that more energy was allocated to reproductive organs during the action of nHRS. Moreover, higher water use efficiency was witnessed in PS. The data suggest that nHRS triggered ABA accumulation, thereby closing stomata, and reducing water use and also decreases the production of ROS and improves the antioxidant defence enzymes, thus enhancing drought tolerance. This survey of different-decade genotypes suggests that advances in grain yield and drought tolerance would be made by targeted selection for a wheat genetic resource.

1. Introduction

Soil drought is one of the most adverse abiotic stresses affecting crop production globally (Araus et al., 2008; Hughes et al., 2017), and about 45% of crop production land is frequently subjected to continuous or terminal drought stresses worldwide (Ashraf and Foolad, 2007). Plants can respond to drought stress by developing physiological and biochemical mechanisms to maintain individual growth and grain production (Chaves et al., 2003). Stomatal behavior is the most sensitive physiological indicator by plants for adaptation to soil drying (Croker et al., 1998; Vialet-Chabrand et al., 2017). Stomatal conductance can be lowered in plants grown in soil drying conditions, remaining no significant changes in shoot water status (Yan et al., 2016). This phenomenon is generally defined as non-hydraulic root-sourced signalling (nHRS) (Blackman and Davies, 1985; Xiong et al., 2006b).

Non-hydraulic root-to-shoot signalling enables plants to sense soil drying (Batool et al., 2018) and then respond to it (Davies and Zhang, 1991; Xiong et al., 2006a) and is often considered to be essential in regulating shoot growth and water use. ABA is widely recognized as a main plant hormone to modulate the physiological response and adaptation to drought stress (Dar et al., 2017; Mehrotra et al., 2014). ABA biosynthesis generally occurs in vascular tissues, whereby ABA transportation is carried out from roots to the leaves (Boursiac et al.,

Abbreviations: ABA, Abscisic aid; CAT, Catalase; CK, Cytokinin; LRWC, Leaf relative water content; nHRS, Non-hydraulic root signals; POD, Peroxidase; ROS, Reactive oxygen species; SOD, Superoxide dismutase; ZR, Zeatin

* Corresponding author.
E-mail address: xiongyc@lzu.edu.cn (Y.-C. Xiong).
[1] Current address: School of Life Sciences, Lanzhou University, Lanzhou, 730000, China.

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2013; Holbrook et al., 2002). Stomatal closure occurs in leaves due to the accumulation of leaf ABA either originated from the roots (Dodd, 2005), illustrating that the root-generated signal is able to influence water use in leaves, or in the leaves (McAdam et al., 2016) which is later recirculated in the roots and xylem. Previous study suggested that ABA concentration in xylem sap was positively associated with stomatal conductance, which is involved in drought tolerance improvement (Du et al., 2012; Schachtman and Goodger, 2008). On the other hand, cytokinin (CK), a phytohormone which is well known for its involvement in plant growth and development, is also involved in root-to-shoot communication processes (Zwack and Rashotte, 2015), via interacting with other plant hormones such as gibberellin, auxin, and abscisic acid, etc. (El-Showk et al., 2013). Actually, CK and ABA work antagonistically in the plants grown in drying soil (Huang et al., 2018), which mechanically mediates drought stress responses (Li et al., 2015) Generally, leaf CK content decreases in plants under drought stress, induces stomatal closure by interacting with ABA and functions as one of the root sourced signals (Nishiyama et al., 2011). Moreover, CK maintains plant metabolism and growth, and inhibits the drought-induced oxidative damage in plant tissues and organs (Xu et al., 2016).

Drought stress causes inhibition of the respiration and photosynthesis processes which have been linked to reduction in chlorophyll content, cell membrane stability, and antioxidant enzyme activity (Fan et al., 2009; Henson et al., 1989). It also produces an imbalance in the electron-transfer chain and enhances the reactive oxygen species (ROS) production, including hydrogen peroxide (H_2O_2) and superoxide radical (O_2^-), which can cause damage in membrane lipids peroxidation, resulting in the loss of membrane permeability and modification in functionality (Apel and Hirt, 2004; Gill and Tuteja, 2010). Plants have developed an enzymatic antioxidant system to scavenge the ROS. This enzymatic system constitutes a superoxide dismutase (SOD) as the first line of defense against reactive oxygen species (ROS) by dismutating the superoxide radical to hydrogen peroxide. Later, H_2O_2 is broken down by catalases (CAT) and peroxidases that include guaiacol peroxidase (POD) and ascorbate peroxidase (APX) (Blokhina et al., 2003). This process is frequently viewed as antioxidant defence against oxidative damage (Antoni et al., 2013; Jiang and Zhang, 2002). It is reported in maize leaves that drought-induced ABA could trigger the antioxidant defence system (Jiang and Zhang, 2002).

Dryland wheat is grown in the erratic soil water environment, and its yield formation is mostly regulated by nHRS under soil drying (Fan et al., 2008, 2009; Xiong et al., 2006a). However, it is still unclear how regulation of physiological and biochemical characteristics occurs in response to non-hydraulic root-to-shoot signalling in different wheat genotypes. Also, variation in drought treatment methods frequently lead to different even opposite results in the researches of nHRS, particularly including partial and full root-zone drought stresses. Likewise, the wheat genotypes with different releasing time may respond to root-sourced signal differently. The main goals of this study were to: 1) examine the antagonistic crosstalk between the ABA and cytokinin signalling to fine tune the stomatal closure in two spring wheat genotypes, 2) characterize the eco-physiological and biochemical responses of plants exposed to partial and full root-zone drought stress, and 3) determine whether the root-to-shoot signalling improve the drought tolerance in terms of yield responses in different wheat genotypes.

2. Materials and methods

2.1. Plant materials and growth conditions

Two relatively independent but closely related pot-culture trials were conducted from March to August 2013 at the Yuzhong Experimental Station of Lanzhou University, Yuzhong County, Gansu Province (35°51′N, 104°07′ E, altitude 1620 m), Northwest China. The two spring wheat (*Triticum aestivum* L.) genotypes, Heshangtou (HST) with indehiscent awnless ears and naked grain and Longchun 8275 (L8275) with indehiscent awned ears and naked grain (Wang et al., 2017), were land races which had been extensively planted in semiarid Northwest China, are used in this study. HST was released before 1900, and L8275 was released in the 1993, were selected for their similar phenological development, but different yields under drought in previous experiments (Wang et al., 2017; Xiong et al., 2006a). The varieties were grown in a rainout shelter (50 m long × 24 cm wide × 5.7 m high) that can be opened and closed according to the weather forecast.

Seeds were prepared and vernalized at 4 °C for 24 h and kept on moistened filter paper by distilled water under dark for germination in an incubation cabinet at 25 °C. Each pot contained 11 kg soil mix of sieved loess soil-based substrate (loess soil: vermiculite (v/v) = 4 : 1). Before planting the seeds, 1.25 g N, 0.36 g P and 0.44 g K per pot was applied to avoid nutrition deficiency. Eighteen seeds per pot were sown in 36 plastic pots (28 cm diameter × 30 cm high). After germination, the seedlings were thinned to maintain 12 plants per pot for both experiments. After seeds emergence, all plants were watered daily to maintain soil water content within 80% FC before water stress initiation. Soil water content (SWC) at field capacity was determined by watering to excess and then allowed the pots to drain until 2 days before weighing. Harvest was taken at the flowering stage of both wheat cultivars according to their developmental period since imposing the water stress at jointing stage. To measure different biochemical and physiological attributes, fully expanded leaves were collected, three pots per treatment per wheat variety and immediately frozen in liquid nitrogen. Leaf RWC and gas exchange characteristics were measured between 9:00 a.m. and 11:00 a.m.

2.2. Trial 1

Split-root trial was conducted to reveal the signalling crosstalk of major root-sourced chemical signals and their association with other drought-stressed signals in two spring wheat varieties. Water treatments were exposed at the jointing stage, including 1) a control group with 80% FC maintenance throughout growing period (CK); 2) a split-root treatment group (PS group) with half wet and another half drying in two parts of root system; and 3) an intact root system drying group (FS group) with 55% FC to induce nHRS. For the split-root treatment (PS Group), a divider was placed in the middle of each pot to ensure no substance exchange between the two parts (Fig. S1), and an equal volume of the soil mix was filled into both halves of each pot. The pot was full irrigated before sown with 18 seeds along the boundary above the divider. Once the seed germination and emergence was completed, the top soil mix was gently collected, so that the roots of each of the 12 selected seedlings could be redirected to have ½ the roots in one side and the other ½ of the roots in the other side of the divider. The collected soil mix was then put back in the original place. Before imposing the drought stress both parts of the pot were maintained at the normal FC. In this study, SWC for the full root-zone stress (FS) was controlled gravimetrically by weighing and watering the pots in the late afternoon, daily, throughout the growing period to maturity. The SWC for the split-root treatment (PS group) was implemented as follows: when the pots SWC dropped from 65% FC to about 45% FC, ½ of the pot was irrigated to reach 65% FC, and the other ½ maintained at the 45% FC. Therefore, the whole pot, on a weight basis contained an average of 55% FC. At 2-day intervals, the pot was re-weighed, the drier half of the pot was re-watered to approximately 65% FC, and the wetter part maintained at about 45% FC. The SWC of the whole pot was once again at approximately 55% FC. This procedure was repeated until maturity. In all treatments, root samples were taken and washed for biomass determination. In the split-root treatment group, the roots were separately sampled and determined in each half of the pot. Water treatments started at jointing stage according to the developmental stage of each genotype. For each wheat genotype, considering their respective developmental stage by combining treatments, 12 pots were used to get the harvest with each variety replicated three times.

After imposing water treatments, SWC was measured daily, while leaf relative water content (RWC) and gas exchange characteristics including stomatal conductance (*gs*), transpiration rate (*E*), and photosynthetic rate (*Pn*) were measured at the flowering stage of each genotype for each pot. Each measurement had three and six replicates respectively, by selecting the upper fully expanded leaf (the 2nd leaf from the top for LRW, while 1st leaf from the top for gas exchange characteristics). Gas exchange characteristics for each replicate was the mean of five readings of each leaf, measured between 9:00 a.m. and 10:00 a.m. by using a LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA) following the recommended measuring precautions (Turner, 1988). Two thirds of the leaf discs (5 mm in diameter) were used to measure fresh weight (FW) by sampling and weighing immediately. The discs kept in the tubes having fresh distilled water for 8 h under 10 μmol m^{-2} s^{-1} PFD, then blotted dry using filter paper, and weighed to the saturated weight (SW). Dry weight (DW) was measured after drying at 80 °C in a forced-draught oven for 24 h. Leaf RWC was calculated as RWC = ((FW-DW)/(SW – DW)) × 100, where SW is saturated weight, DW is dry weight, and FW is the fresh weight.

2.3. Trial 2

In order to determine the role of different biochemical compounds on yield and yield components of two spring wheat varieties under drought, three water-stressed treatments were subjected from jointing stage of each variety. Water deficit was imposed by withholding water supply until the soil water content (SWC) reached up to the predetermined level: (i) 6 pots were maintained about 80% FC by sufficiently watering daily in the evening before sunset; (ii) 6 pots were used for split-root trial by imposing the divider, similar to the procedure used in trial 1. Each pot was allowed to dry until the SWC reached up to 45% FC in the half part of pot and watered in another part until 65% SWC. The cyclic operation was maintained and let pots to dry again until 45% FC and rewatered from another side until 65% FC; and (iii) 6 pots were used for fully wetting and drying in whole root system, without the divider used in pots. Each pot was watered till 65% FC and allowed to gradually dry till 45% FC. After that, the same operation was implemented periodically. Each treatment per cultivar was conducted with three replications till maturity stage.

At the physiological maturity (~110 DAS), whole plants were harvested as defined that completely disappearance of the glumes green color. Plant roots were washed free of soil by using screens (0.4 mm). Plant height, fertile spikelet number, yield and yield components per plant were recorded, and then divided into shoots (including leaves and husks), grain and roots, dried for 2 days at 80 °C and then weighed. Data for water use were collected by recording the daily water added during the plant's entire life. The following variables were determined: (i) HI (harvest Index) = grain weight/aboveground weight, (ii) root to shoot ratio = root weight/shoot weight, and (iii) WUE_G (water use efficiency for grain) = grain weight/total water used from sowing to harvest (Wang et al., 2017).

2.4. Determination of reactive oxygen species

Production of O_2^- was measured following the method described by Elstner and Heupel (1976) by observing nitrite formation from hydroxylamine in the presence of O_2^-. Data for H_2O_2 was measured by observing the titanium-peroxide complex absorbance at 415 nm as described by Brennan and Frenkel (1977). Absorbance values were calibrated by using the standard curve of known H_2O_2 concentrations.

2.5. Enzyme assays

Frozen leaf material (0.5g) was crushed to make fine powder by using mortar and pestle with liquid nitrogen. Soluble proteins extraction was done by homogenizing with 10 mL of 50 mM potassium phosphate buffer (pH 7.0) having 1% polyvinylpyrrolidone (PVP) and 1 mM EDTA, and addition of 1 mM ascorbate acid to perform an APX assay. This homogenous mixture was centrifuged at 4 °C and 12000*g* for 1200 s and the supernatant was used for the following antioxidant enzyme essays:

Catalase (CAT, EC 1.11.1.6) activity determined by H_2O_2 disappearance (extinction coefficient 39.4 mM^{-1} cm^{-1}) for 180 s at 240 nm as designated by Aebi (1984). 3 mL reaction mixture included 50 mM potassium phosphate buffer (pH 7.0), 10 mM H_2O_2, and 200 µL of enzyme extract. After adding the enzyme extract the reaction was started.

Total superoxide dismutase (SOD, EC 1.15.1.1) activity was measured by observing its ability to stop the photochemical reduction of NBT (nitro blue tetrazolium) (Giannopolitis and Ries, 1977). 3 mL reaction mixture contained 13 mM methionine, 50 mM potassium phosphate buffer (pH 7.8), 2 µM riboflavin, 75 µM NBT, 0.1 mM EDTA and 100 µL enzyme extract. Reaction mixtures were illumined for 900 s at a PPFD of 100 µmol m^{-2} s^{-1} provided by a cool-white fluorescent lamp. Absorbance was determined at 560 nm and the enzyme amount causing 50% inhibition of NBT reduction was described as one unit of SOD activity.

Peroxidase (POD, EC 1.11.1.7) activity was determined by following the method of Chance and Maehly (1955). Three ml of reaction solution contained 3 ml 20 m Mguaiacol, 10 µl extraction solution (50 mM potassium phosphate buffer, pH 7.0) and 10 µl 30% H_2O_2, and the enzyme activity was observed at 470 nm for 180 s.

2.6. Determinations of lipid peroxidation and free proline

The level of lipid peroxidation in leaves was determined by measurement of MDA (malondialdehyde) amount as described by Zhao et al. (1994). MDA content was calculated by its absorbance and mentioned as nmol MDA g^{-1} DW (Zhao et al., 1994). Free proline was determined by following the method of Bates et al. (1973).

2.7. ABA and ZR extraction, purification and quantification

ABA and ZR extraction and purification methods were modified and followed from those already described (Bollmark et al., 1988). Leaf segments were ground using silica in liquid nitrogen with a mortar and pestle, then extracted with ice-cold 80% methanol (v/v) contained 1 mM butylated hydroxytoluene for avoiding oxidation, subsequently moved to a centrifuge tube and kept overnight at 4 °C. The extract solution was then centrifuged for 900 s at 4 °C and 10 000*g*, then the supernatant was removed by a pipette into a centrifuge tube to centrifuge again at 4 °C and 10 000*g* for 900 s, the remnant was further suspended for 1 h at 4 °C into the same ice-cold extracting solution, then the supernatant was again by a pipette in the same centrifuge tube. This combined supernatant was then passed from Chromosep C18 columns (C18 Sep-Park Cartridge, Waters, Milford, MA, USA), prewashed using 5 mL 80% and 10 mL 100% methanol. The collected efflux was dried by evaporation using nitrogen. To determine the level of ABA and ZR, residues were dissolved in 10 mM phosphate-buffered saline (pH 7.5) containing 0.1% (w/v) gelatin and 0.1% (v/v) Tween 20. ABA and ZR measurement was done immunologically by using the ELISA (enzyme-linked immunosorbent assay) technique.

The immunoglobulin IgG-HRP (IgG-horseradish peroxidase) and the mouse monoclonal antigens and antibodies of ABA and ZR used in the ELISA technique were manufactured at the Phytohormones Research Institute of China Agricultural University. Microtitration plates (Nunc, Roskilde, Denmark) were covered and coated with synthetic ABA-ovalbumin conjugated using 50 mM $NaHCO_3$ buffer (pH 9.6), then stored overnight at 37 °C. In order to block nonspecific building, ovalbumin solution (10 mg mL^{-1}) was added into each well. Incubation for 1800 s at 37 °C, the standard ABA, and antibodies against ABA were added and then incubated for further 2700 s at 37 °C.

Then in each well IgG-HRP was added and incubated for 1 h at 37 °C. OPD (orthophenylenediamino) as colour-appearing solution was added after the incubation in each of the plates' well. The plates were stored in the dark at 37 °C for substrate reaction. After 15 min, progress of the reaction was inhibited by adding 50 μL 2 mM H_2SO_4. Absorbance from each well was observed at 490 nm with the ELISA Recorder (Model DG-3022 A; Huadong Electron Tube Factory, Shanghai, China). ABA quantification by the ELISA technique has already been described (Yang et al., 2001). ZR quantification followed by the method of ABA though antigens were used according to their respective antibodies. In the current study, ABA and ZR percentage recovery was monitored and calculated by addition of known standard ABA and ZR quantities to a split extract. Monoclonal antibody specificity was confirmed and the possibility of other nonspecific inhibitors was excluded in earlier studies (Yang et al., 2001).

2.8. Statistical analyses

Presentation of data was the means of three replicate samples in trial 1 while 15 replicate samples (3 pots * 5 plants for each pot) was analyzed in trial 2. All the data were examined by two-way ANOVA (treatments and genotypes). SPSS (SPSS 19.0 version, Chicago, IL) for Window was used to conduct all the data analyses and the means were compared by Duncan's multiple range tests at $P = 0.05$. Origin 9.0 (Microcal Software Inc) was used to draw the figures and perform the correlation analysis.

3. Results

3.1. Inducement of non-hydraulic root-sourced signal (nHRS) and the changes in leaf ABA and ZR concentrations in wheat genotypes

According to the definition of nHRS there is a significant reduction in stomatal conductance level without detectable change in leaf water status in the plant exposed to drying soil. In this study, we used leaf relative water content (RWC) to indicate the water status in leaves. There was no significant difference ($P < 0.05$) observed in the LRWC of two wheat varieties among three treatments WW, FS and PS (Fig. 1D). The results indicated that both partial and full root-zone water treatments induced the ABA signalling which maintained the leaf water status in both HST and L8275 wheat genotypes, by closing the stomata and enhancing the drought tolerance (Fig. 1). We also investigated the biosynthesis level of two major plant hormones ABA and ZR during the operation of nHRS.

ABA concentration varied among the three water stress treatments in both wheat varieties, but in general the trend was significantly enhanced. Leaf ABA concentration was reached up to a massively greater level in leaves of all plants especially in PS ($P < 0.05$) than its FS and WW controls of both varieties. Specifically, leaf ABA concentration increased by 34% and 79% than FS and WW in HST, while increased respectively by 29% and 62% in L8275 (Fig. 1A). These results indicate that under nHRS by splitting the root, there was enhanced drought-induced ABA concentration in both wheat varieties HST and L8275. ZR concentration markedly reduced under drought though there was no significant difference ($P < 0.05$) of each PS compared to its FS controls of both wheat genotypes whereas it was significantly reduced than its WW controls (Fig. 1B). The enhancement in ABA concentration was associated with a reduction in ZR (zeatin) concentration and this trend is observed in both wheat varieties (Fig. 1A and B). There was strong negative association found between ABA and ZR in HST ($R^2 = 0.74$) and L8275 ($R^2 = 0.74$), respectively (Fig. 4B).

Leaf stomatal conductance was significantly reduced in PS than FS and WW under the operation of nHRS (Fig. 1C). Crucially, ABA concentration was significantly increased under PS treatment of HST and L8275, inducing a reduction in stomatal conductance. This confirms that ABA was acting as nHRS chemical material. There was a strong negative association reported between ABA concentration and leaf stomatal conductance of two wheat genotypes, HST, $R^2 = 0.92$; and L8275, $R^2 = 0.83$, which shows that increased ABA concentration induced a reduction in stomatal conductance for all plants (Fig. 4A). ABA in the leaf continued to accumulate in PS systems as the partial root-zone water treatment produced more non-hydraulic root to shoot signalling since the treatment was imposed which led to a marked reduction in stomatal conductance (Fig. 1C). Overall, partial root-zone system induced ABA accumulation that reduced stomatal conductance.

3.2. Anti-oxidant, reactive oxygen species (ROS), proline and malondialdehyde (MDA) production under the regulation of nHRS

Drought induced accumulation of antioxidant enzyme activities such as CAT (catalase), POD (peroxidase), and SOD (superoxide dismutase) are shown in Fig. 3. Under soil drying, activities of CAT (Fig. 3A), POD (Fig. 3B), and SOD (Fig. 3C) increased significantly more in PS and FS than WW in both wheat varieties HST and L8275, respectively. Moreover, the enzymatic activities were highest in PS than FS and WW controls. Overall the drought induced accumulation of antioxidant enzymes increased under nHRS and tended to be higher in L8275 than HST (Fig. 3). Under nHRS, leaves O_2^- and H_2O_2 contents were significantly increased in FS and PS than WW in both wheat varieties (Fig. 2A and B), however O_2^- and H_2O_2 contents in PS were lower than its FS controls which showed that ABA might crosstalk with other signals by decreasing the reactive oxygen species production and enhancing the drought tolerance (Fig. 2A and B).

In response to drought stress proline content enhanced significantly than that of FS and WW controls and was much higher in PS of HST and L8275, respectively (Fig. 2C). Lipid membrane peroxidase, as measured in the form of MDA content was significantly lower under well-watered conditions (Fig. 2D). Split root systems had significantly reduced the MDA content in both wheat varieties, thereby declining lipid membrane oxidative damage.

3.3. Photosynthetic rate, transpiration rate and PCA analysis under the operation of nHRS

There were significant ($P < 0.05$) changes observed in gas exchange characteristics including photosynthesis and transpiration rates among three water treatments of both wheat varieties. Photosynthetic and transpiration rates in leaves were higher in HST than L8275 of WW control, however between PS and FS, it was highest in L8275 than HST (Table S1). PCA Analysis on biochemical substances indicated the crosstalk lies among all signals in two spring wheat varieties HST and L8275 (Fig. 5). The WW treatment of both genotypes lay in the same area of scale whereas two nHRS treatments were closely placed and lay opposite of WW. Moreover, ZR showed a negative association with all other biochemical signals, while ABA exhibited a strong positive crosstalk with antioxidants, proline, MDA and ROS, respectively. We also explained the mechanism of root to shoot signalling under the operation of nHRS by flow chart (Fig. 6).

3.4. Individual growth, grain yield and water use under the operation of nHRS

In order to compare the physiological and agronomic performance under the regulation of nHRS induced by partial and full root-zone drought stresses, we determined plant growth, grain filling and water consumption at the maturity stage. In two wheat varieties, consistent with reportedly higher yield stability, the grain yield of the HST was higher than the L8275 under three drought stress treatments (Table 1). The total water consumption across the whole growing period was recorded and analyzed in all the treatment groups/wheat genotypes. Root-zone water treatments led to substantial reduction in the total water consumption amount. For example, total water consumption was

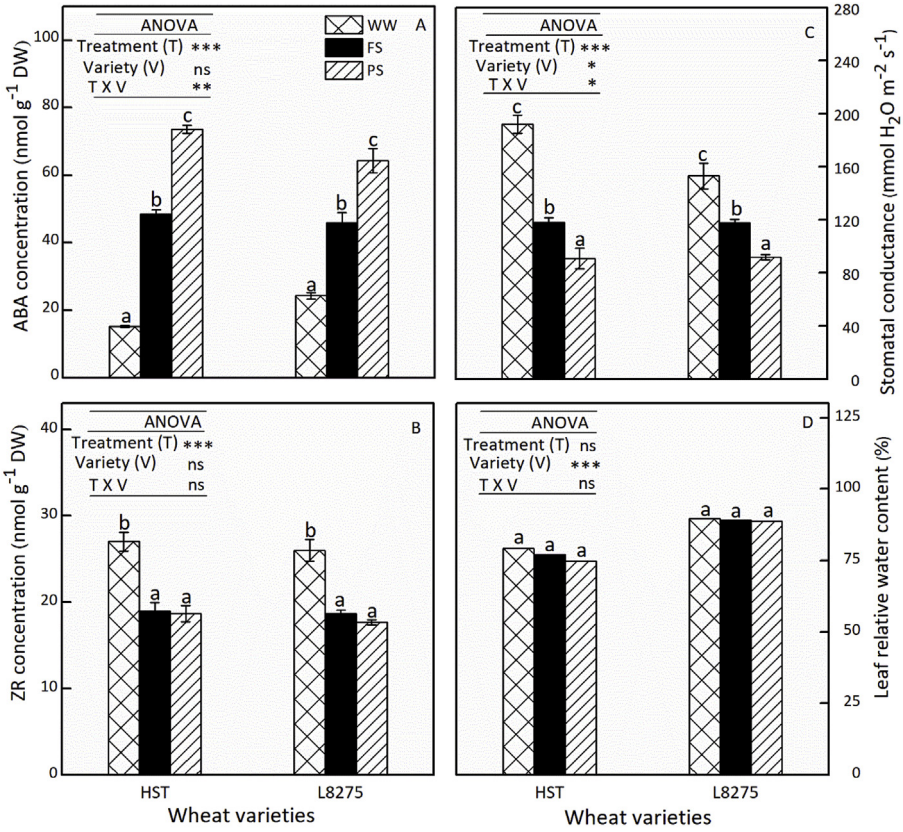

Fig. 1. Changes in the concentration of leaf ABA (A), ZR (B), stomatal conductance (C) and relative water content (RWC, %) (D) at the flowering stage of wheat genotypes, HST and L8275. Values are means ± 1 s.e. (n = 3, P = 0.05). Trial 1.

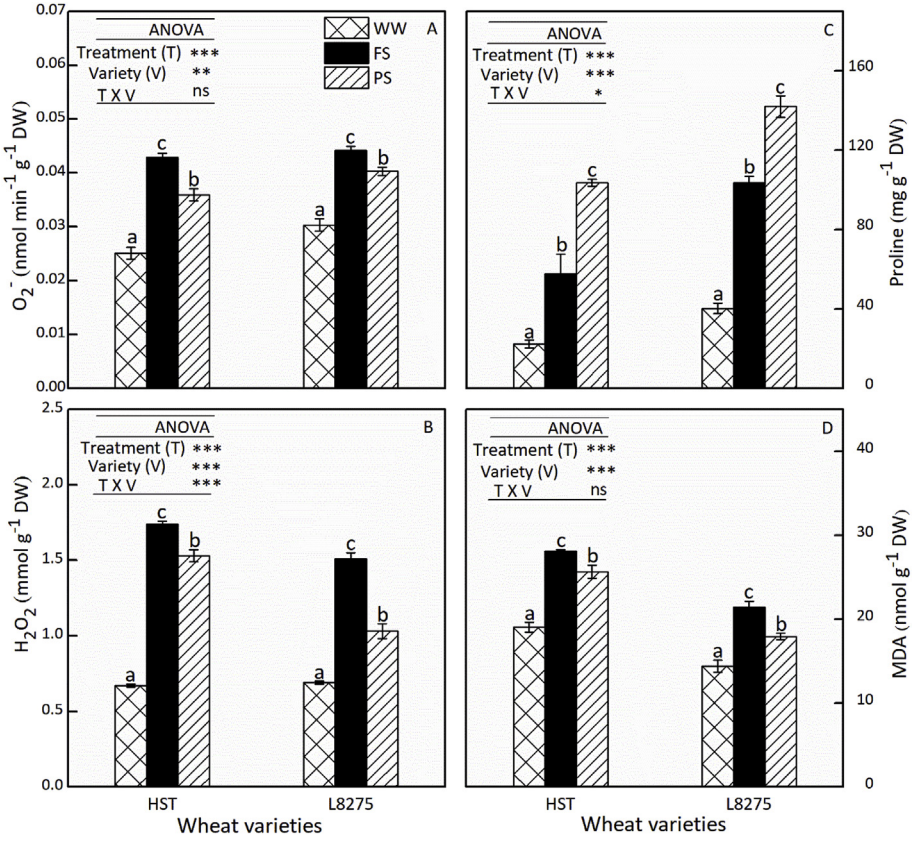

Fig. 2. Changes in production rate of reactive oxygen species (O_2^-) (A), hydrogen peroxide concentration (H_2O_2) (B), and osmoprotectants, such as proline (C) and malondialdehyde (MDA) concentration (D) in the leaves at the flowering stage of two wheat genotypes, HST and L8275. Values are means ± 1 s.e. (n = 3, P = 0.05). Trial 1.

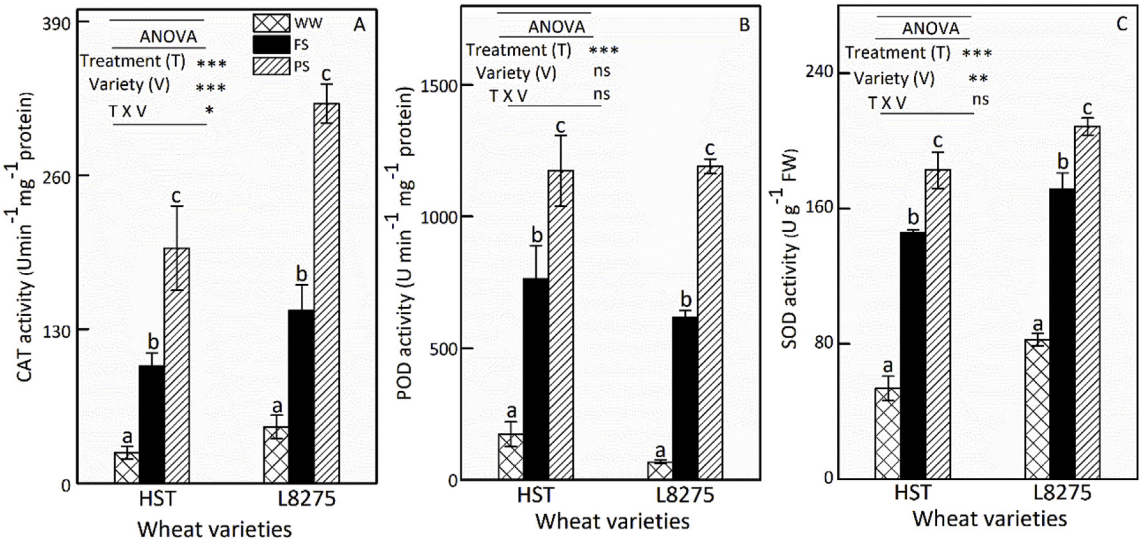

Fig. 3. Changes in the activity of the antioxidant enzymes: (A) catalase (CAT), (B) peroxidase (POD), and (C) superoxide dismutase (SOD) in the leaves at the flowering stage of two wheat genotypes, HST and L8275. Values are means ± 1 s.e. (n = 3, P = 0.05). Trial 1.

2.34 L per plant in sufficient water supply groups, whereas it was reduced to only 1.45 and 1.06 L per plant in FS and PS in HST, respectively (Table 1). It was significantly greater in FS than PS across wheat varieties, a pronounced trend. Full root-zone drought stress treatment generally brought about greater water consumption (Table 1).

Importantly, grain yield followed a similar trend as total water consumption in both wheat genotypes, and HST generally had greater grain yield than L8275. In sufficient water supply groups, grain yield was 4.29 g/plant in HST and 3.80 g/plant in L8275, respectively. In contrast, it was lowered to only 3.24 g/plant in HST and 2.47 g/plant in L8275, respectively, in the FS group. What's more, it was only 2.83 g/plant in HST and 2.36 g/plant in L8275 in the PS group, a general lower level. This phenomenon might be closely associated with their respective total water consumption. At the same time, the difference in above-ground biomass was consistent with that of grain yield. Sufficient water supply generally had the greatest biomass accumulation of above-ground parts, the FS treatment was in the middle and the PS treatment was the least across wheat genotypes (Table 1). Additionally, root biomass was totally reduced under drought stress and there was no significant difference between FS and PS among both varieties (Table 1). TKW (1000-kernel weight) was considerably higher in both

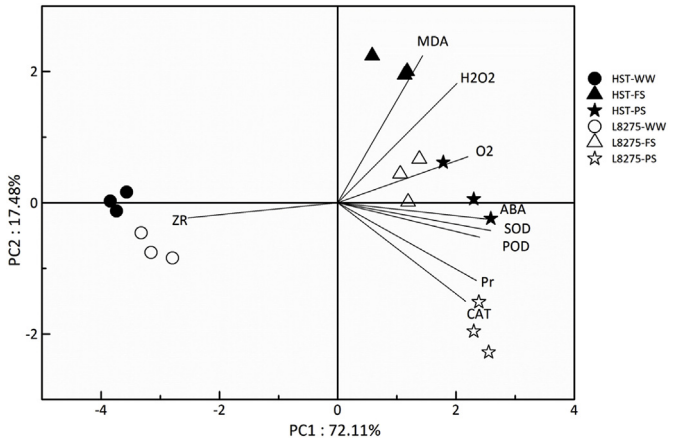

Fig. 5. PCA analysis on biochemical parameters among three drought treatments at the flowering stage of two wheat genotypes HST and L8275 (hexaploid), (n = 3). Trial 1. ZR, Cytokinin; ABA, Abscisic acid; SOD, superoxide dismutase; CAT, catalase; POD, peroxidase; H_2O_2, hydrogen peroxide; O_2^-, oxidase; Pr, proline; and MDA, malondialdehyde.

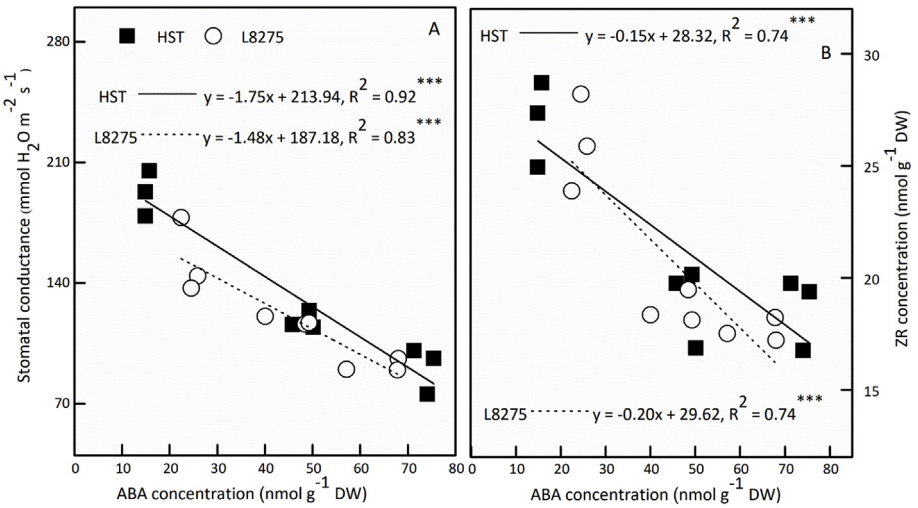

Fig. 4. The relationship between leaf ABA concentration and stomatal conductance (A), and between leaf ABA and ZR concentration (B) in two wheat genotypes HST and L8275 subjected to three water stress treatments. The fitted linear regressions are given: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Trial 1.

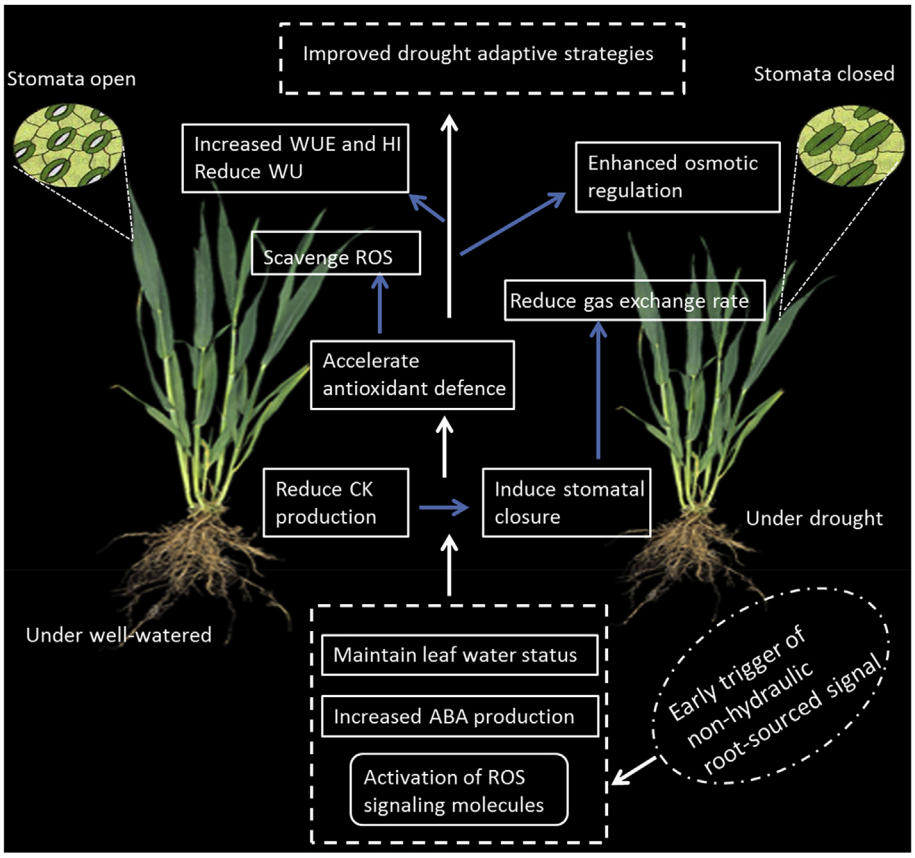

Fig. 6. A possible flow chart illustrating the mechanism of root-sourced signalling and its interaction with other stress signal to improve the drought adaptive strategies in wheat. Under drought early triggering of root-sourced signals activates the production of ROS (reactive oxygen species) which triggers the ABA synthesis in the leaf. Enhanced ABA (abscisic acid) productions reduced cytokinin (CK) concentration and maintain leaf water status, by inducing stomatal closure which led to reduction in gas exchange. In the meanwhile antioxidant defence mechanism accelerated and scavenges the ROS products. Osmotic regulation were enhanced to prevent plants tissues and cell from oxidative and membrane damage. Consequently, plant enhanced water use efficiency (WUE) and harvest index (HI), and reduce the water use (WU) to cope with drought stress.

FS and PS treatments of both wheat varieties. Drought stress reduced the grain number, spikelet number, and tiller number per plant in both wheat varieties, but overall it was highest in HST (Table 1). It was noted that water use efficiency (WUE) varied from wheat varieties and drought treatments in this study. The WUE_G has been mostly used in assessing the level of water use in higher plants in previous studies, and it was viewed as a typical parameter of water use. The data indicated that it was significantly increased in two wheat genotypes as a result of nHRS regulation. WUE_G was significantly greater in PS than FS in HST and L8275 and surprisingly it reached the highest value in early released genotype HST (Table 1). This tendency might be mostly related to the large root system of this genotype.

4. Discussion

The present study was conducted to insight the regulation of physiological, biochemical and growth responses in two wheat genotypes, HST and L8275, during the process of non-hydraulic root-to-shoot signalling of two contrasting soil drying methods. About one-half of the root system of PS wheat plants was watered between 65 and 45% FC throughout the drying cycle, and the plant leaf water status (measured as LRWC) was similar to that of both controls including intact root system drying groups (FS) and adequately watered control groups (WW). No difference in leaf relative water content across the treatments suggests that the significant decline in stomatal conductance of the half dried plants was likely due to the result of chemical root signals (Schachtman and Goodger, 2008). Leaf water potential and leaf relative water content are considered as a reliable indicator for leaf water status (Batool et al., 2018; Xiong et al., 2006a).

We witnessed the nHRS production in both drying treatments as compared to sufficient water supply. Much stronger evidence for nHRS signals was the higher concentration of ABA in PS relative to FS plants. Overall, the ABA concentration was higher in HST than L8275 to soil drying across all treatments (Fig. 1A). It could be possible that enhanced ABA concentration observed in PS wheat plants relative to WW would have been due to long term non-hydraulic signals. Therefore, having only WW controls for comparison with PS might have two disadvantages: (1) analytical instruments could have some small fluctuations, and (2) measurements of ABA and other signalling molecules might have temporary differences between PS and WW plants (Croker et al., 1998). FS treatment purpose was to compare the water supply of PS plants by half root systems relative to the same water supply in intact root systems and also with WW plants. Intact root (FS) group and split root half dried (PS) group plants had similar amounts of water in a cycle, so decreases in stomatal conductance and increase in ABA concentration of PS plants relative to FS could have been due to the direct nHRS crosstalk. Further, drying the intact root system to induce nHRS caused reduction in stomatal conductance in both wheat genotypes than control (80% FC). Although nHRS of soil drying is now clearly documented in various wheat species (Fan et al., 2009; Xiong et al., 2006b), its importance in early and recently released spring wheat genotypes for their similar phenological development, but different drought adaptive responses has been questioned.

To study the biochemical characteristics in both wheat genotypes during soil drying different attributes were studied. High MDA level is used to measure the membrane lipid peroxidation and thus increased soil drying on the stability of cell membranes. In PS and FS, MDA level was increased in two wheat genotypes at the flowering stage than WW, however MDA content was lower in PS plants than its FS controls (Fig. 2D). This observation is consistent with the study by Du et al. (2013) who conducted a pot experiment to study the two wheat cultivars and found increased MDA levels to soil water deficit. The half dried split root system significantly reduced the lipid peroxidation in terms of MDA contents in both accessions (Fig. 2D). It is possible that under non-hydraulic signalling, high ABA concentration prevents the lipid peroxidation of plants leading to the reduction in ROS accumulation, and it is

Table 1
Effects of water stress on growth parameters and yield and yield components of two wheat genotypes subjected to three drought treatments (WW, FS and PS). Trial 2.

Treatment	Variety	Plant height (cm)	Tiller Number	Spikelet number	Grain number	Aboveground Biomass (g/plant)	Grain yield (g/plant)	1000-Kernel Weight (g/plant)	Harvest Index	Root-shoot ratio	Root size (g/plant)	Total amount of water consumption/ plant	WUE_G
WW	HST	91.7 ± 2.6b	4.3 ± 0.4b	57.7 ± 1.6b	111.4 ± 5.5b	10.9 ± 0.4b	4.29 ± 0.3b	35.6 ± 0.3a	0.40 ± 0.02a	0.07 ± 0.01a	0.76 ± 0.04b	2.34 ± 0.03c	1.83 ± 0.1a
FS		80.2 ± 1.0a	3.3 ± 0.3a	35.1 ± 0.4a	72.7 ± 0.8a	6.03 ± 0.2a	3.24 ± 0.1a	39.2 ± 1.1b	0.54 ± 0.01b	0.07 ± 0.01a	0.45 ± 0.03a	1.45 ± 0.01b	2.24 ± 0.0b
PS		78.3 ± 0.9a	3.1 ± 0.2a	34.7 ± 0.6a	67.5 ± 0.7a	4.88 ± 0.1a	2.83 ± 0.1a	41.2 ± 0.3b	0.58 ± 0.01b	0.07 ± 0.01a	0.36 ± 0.03a	1.06 ± 0.01a	2.68 ± 0.0c
WW	L8275	86.1 ± 1.1b	2.3 ± 0.1b	41.6 ± 2.3b	96.5 ± 3.3b	9.73 ± 0.3b	3.80 ± 0.2b	36.9 ± 0.6a	0.39 ± 0.01a	0.05 ± 0.01a	0.53 ± 0.01b	2.15 ± 0.03c	1.76 ± 0.1a
FS		68.4 ± 1.0a	1.9 ± 0.1a	25.5 ± 1.3a	42.7 ± 1.8a	5.22 ± 0.1a	2.47 ± 0.1a	41.4 ± 0.8b	0.47 ± 0.01b	0.08 ± 0.01a	0.40 ± 0.02 ab	1.26 ± 0.03b	1.96 ± 0.0b
PS		66.2 ± 0.8a	1.8 ± 0.1a	23.9 ± 0.7a	41.7 ± 1.8a	4.64 ± 0.1a	2.36 ± 0.1a	42.4 ± 0.3b	0.51 ± 0.02b	0.07 ± 0.01a	0.31 ± 0.05a	1.03 ± 0.02a	2.30 ± 0.1c
Treatment (T)		*** (< 0.001)	** (0.004)	*** (< 0.001)	*** (< 0.001)	*** (< 0.001)	*** (< 0.001)	*** (< 0.001)	*** (< 0.001)	NS (0.110)	*** (< 0.001)	*** (< 0.001)	*** (< 0.001)
Variety (V)		*** (< 0.001)	*** (< 0.001)	*** (< 0.001)	*** (< 0.001)	** (0.003)	*** (< 0.001)	* (0.011)	*** (< 0.001)	NS (0.165)	** (0.001)	*** (< 0.001)	** (0.001)
T × V		NS (0.076)	NS (0.391)	NS (0.404)	NS (0.467)	NS (0.302)	NS (0.597)	NS (0.692)	NS (0.058)	NS (0.335)	* (0.024)	** (0.004)	NS (0.108)

Values are mean ± 1 s.e. (n = 15). Means within column having same letter are statistically similar at $P < 0.05$ according to Duncan's multiple range tests. *, **, *** indicate significant at 0.05, 0.01, and 0.001, respectively. Trial 2.

confirmed in the present study that there was an accumulation of ABA and a reduction of ROS in the PS plants (Fig. 2).

Proline is an important osmotic adjustment element which act as a metabolic signal (Fu et al., 2018) and its accumulation was significantly higher in PS than FS and WW controls (Fig. 2C). Excess of proline levels displays its ability for ROS scavenging and enhancing the activities of different enzymes (Alves et al., 2011). All of this is most likely due to the ABA induced accumulation of proline that is also reported in many previous studies (Man et al., 2011; Pal et al., 2018). Increase in an activity of antioxidant enzymes in PS individuals was likely to be responsible for the drought-induced ROS scavenging (Fig. 3). In addition, nHRS triggered ROS production and enhanced the antioxidant defence system in FS plants (Fig. 3). These results confirm the key role of ABA as nHRS material and its signalling crosstalk for controlling the balance between ROS and antioxidant enzyme activity and oxidative damage (Wilkinson and Davies, 2002; Zhou et al., 2014). Moreover, HST had a greater drought tolerance than L8275, possibly allied with the lower level of reactive oxygen species (Fig. 2A). However, the lipid membrane peroxide of the leaves was higher in HST than L8275 (Fig. 2D) and there was no difference for the negative correlation between the two varieties in the level of ZR to ABA (Fig. 4B). Higher ABA levels in HST would have been resulted into a slower rate of water loss in HST than L8275. This suggests that better yield under nHRS (higher desiccation tolerance) in HST than L8275 is the result of better adaptation to drought in terms of water loss, as well as an increase in the efficiency of water use (Tables S1 and 1).

Drought stress reduced the cytokinin concentration in the leaves of both wheat varieties (Fig. 1B). This illustrates that CK works antagonistically to ABA and its concentration reduced under drought leading to the stomatal closure (Dong et al., 2008). The strong negative relation between ABA and CK also confirms the interaction of two phytohormones. However, PS did not significantly reduce the CK concentration in leaves than its FS control (Fig. 1B). It can be explained in two possible ways: one is that ABA-induced signalling pathways for stomatal closure was more effective during root-to-shoot signalling (Blackman and Davies, 1985), which crosstalks with underlying mechanisms of other stress signals; and the other is that there are many further stress signalling molecules/hormones (auxin, ethylene, jasmonic acid and brassinosteroids) and ABA-independent pathways that are involved in the drought tolerance of plants (De Ollas and Dodd, 2016).

The sustained decline of stomatal conductance since the drought stress imposed indicates that signal continuously moved from roots-to-shoots under soil drying. Split root system when half of the roots are in drying soil leading to the production of non-hydraulic signals and root signals might be declined when the root growth in wet pot increased than in dry pot (Croker et al., 1998). In an intact root system and also in a split root system the roots in drying soil were rehydrated by subjecting the water in alternative cycle. In many split root system studies it has been observed that drought stressed plants have higher ABA levels and greater decline in stomatal conductance than that of intact systems and this higher ABA concentration by crosstalk with other plant signals improves the morpho-physiological adaptation and drought tolerance (Dodd et al., 2008; Holbrook et al., 2002; Martin-Vertedor and Dodd, 2011; Puertolas et al., 2015; Saradadevia et al., 2014).

We found that the early-released wheat genotype, HST had higher yields in both PS and FS than the recently-released cultivar L8275, however yields of PS and FS individuals were significantly lower than WW (Table 1). With adequate water supply, HST produced more root and aboveground biomass than L8275 which is also reported in previous studies on different wheat species (Wang et al., 2017). Grain yield was reduced by soil drying while there was no significant difference found between PS and its control FS (Table 1). In the present study, the decline in grain yield under water deficit is similar with the previous investigation (Saradadevia et al., 2014). The decline in shoot traits, such as plant height, tiller number, spikelet number per plant and

aboveground biomass, induced by drying soil was not significantly different between PS and FS of HST and L8275, which specifies that partial root-zone approach is enough to increase the grain yield especially in the genotypes with a large root system. Moreover, the water use efficiency for grain yield had also increased significantly in PS, particularly in HST, which shows that HST is more drought tolerant than L8275 under a half dried root system. One possible explanation for these results is that the larger root system of HST was significantly involved when only half roots of plants were working throughout their life span. Reduction in shoot biomass under nHRS in the FS treatment may have contributed to some extent to the decrease in water use (transpiration rate) by the two varieties, and the decline in water use was more than the decline in aboveground biomass, suggesting that decrease in stomatal conductance was also responsible for the decreased transpiration. Some other studies also showed an increase in WUE under nHRS (Du et al., 2012; Fan et al., 2008).

5. Conclusions

This study investigated partial and full root-zone drought stresses in two wheat (*Triticum aestivum* L.) genotypes HST and L8275 released in different decades under pot-culture conditions. Both wheat varieties under the operation of nHRS improved water use efficiency and harvest index and enhanced drought tolerance though the performance varied in early and recently released wheat genotypes. It suggested that the partial and full root zone system approaches achieved similar effectiveness on significantly improving drought adaptation regardless of wheat genotype. The stress induced hormone ABA and other biochemical substances were accordingly elevated in wheat plants. More importantly, partial root zone practice under drying conditions, a method to investigate the nHRS signals, provided insights into the mechanistic understanding of the tolerance of wheat crop plants to drought stress. Our findings indicate that the mechanism or nHRS signal concept of drought tolerance is applicable to a wider genetic background of wheat species.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Credit authorship contribution statement

Asfa Batool: Formal analysis, Writing – original draft. **Nudrat Aisha Akram**: Formal analysis, Writing – original draft. **Zheng-Guo Cheng**: Formal analysis, Writing – original draft. **Guang-Chao Lv**: Formal analysis. **Muhammad Ashraf**: Writing – review & editing. **Muhammad Afzal**: Formal analysis. **Jun-Lan Xiong**: Formal analysis. **Jian-Yong Wang**: Formal analysis. **You-Cai Xiong**: Writing – review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.03.001.

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