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RESEARCH ARTICLE

Molecular and biochemical mechanisms associated with differential responses to drought tolerance in wheat (*Triticum aestivum* L.)

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Drought stress is a common abiotic stress in wheat. In this study, PEG-induced drought stress caused significant decline in morpho-physiological characteristics in Bijoy but not in BG-25, suggesting that drought tolerance mechanisms exist in BG-25. Semi-quantitative RT-PCR (reverse transcriptase) revealed the upregulation of *TaCRT1* (calreticulin $Ca2^{+}$-binding protein) and *DREB1A* (dehydration responsive transcription factor) transcripts in drought-stressed roots of BG-25 and Bijoy, albeit to a lesser extent. These imply that increased *TaCRT1* expression may be associated with the survival of the wheat plants under drought conditions. In addition, *DREB1A* suggests its involvement in gene regulation associated with drought tolerance. Higher antioxidant enzyme capacity (catalase, peroxidase and glutathione reductase) along with less MDA content in roots of BG-25 suggests that wheat tolerance to drought stress could be associated with higher oxidative scavenging ability. Finally, elevated S-metabolites (glutathione, methionine and cysteine) and proline in BG-25 indicates that strong antioxidant defense play a vital role in drought tolerance in wheat.

Keywords: Drought tolerance; *TaCRT1*; *DREB1A*; glutathione; proline

Introduction

Wheat (*Triticum aestivum* L.) is the most important cereal crop after rice to meet the food requirements of the world. However, drought is one of the most common environmental stresses that affect growth and development of wheat leading to reduced yields in many countries. Drought tolerance is a quantitative trait, with complex phenotype and genetic control (McWilliam 1989; Budak et al. 2013) and is the ability of plant to sustain itself in limited water supply (Ashley 1993). Two essential building blocks of any crop improvement program are genetic variation any sources of germplasm containing enhanced expression of desired traits. Though genotypic variations do exist in wheat, studying the mechanisms underlying drought tolerance in still a challenge.

The mechanisms of drought tolerance are complex and involve diverse and multiple molecular and physiological mechanisms (Shinozaki & Yamaguchi-Shinozaki 2007; Farooq et al. 2009). Calcium (Ca^{2+}) is perhaps the best-known second messenger but is also required for proper cell wall structure and membrane integrity (Maathuis 2009). Calreticulin (CRT) is a ubiquitously expressed, high capacity Ca^{2+}-binding protein that is involved in plant development regulations and in stress responses (An et al. 2011). CRT plays a crucial role in many cellular processes including $Ca(^{2+})$ storage and release, protein synthesis, and molecular chaperone activity (Jia et al. 2008). In a previous study, upregulation of *TaCRT* transcript was observed in wheat seedlings in response to PEG-induced drought stress (Jia et al. 2008). Furthermore, compared to the wild-type and GFP-expressing plants, *TaCRT*-overexpressing tobacco plants grew better and exhibited less wilt under the drought stress.

Transcription factors are key regulators of the changes in gene expression and environmental stress responses. The transcription factor *DREB1A* (dehydration responsive binding protein), identified from the model plant *Arabidopsis thaliana*, has been reported to enhance stress tolerance against drought stress (Ravikumar et al. 2014). In several transgenic studies, Arabidopsis *DREB1A* has been used to improve abiotic stress tolerance in japonica rice (Oh et al. 2005; Datta et al. 2012; Ravikumar et al. 2014). Datta et al. (2012) further reported that the expression of *DREB1A* was associated with an increased accumulation of the osmotic substance proline, maintenance of chlorophyll, increased relative water content and decreased ion leakage under drought stress.

Pellegrineschi et al. (2004) reported that transgenic wheat expressing the Arabidopsis *DREB1A* gene showed substantial tolerance to osmotic stress. However, evidences of *DREB1A* expression associated with drought tolerance are still vague in natural wheat genotypes.

Plants have developed a series of antioxidant enzymes and redox metabolites to counteract antioxidant system, thereby protecting cells from oxidative damage (Sairam & Tyagi 2004; Devi et al. 2011). These include catalase (CAT), peroxidase (POD), superoxidase dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and redox metabolites such as ascorbic acid, proline and glutahione. In a previous study on wheat, drought induced the upregulation

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of CAT, POD, APX and GR in drought tolerant wheat (Devi et al. 2011). Similarly, drought tolerant wheat cultivar Sids showed antioxidant enzymes (CAT, GPX, APX and SOD) than drought sensitive Gmiza under water stress (Hassan et al. 2015). The plants also accumulate some kind of organic and inorganic solutes in the cytosol to raise osmotic pressure and thereby maintain both turgor and the driving gradient for water uptake (Rhodes & Samaras 1994). Among these solutes, proline is the most widely studied (Delauney & Verma 1993).

Despite the large wheat germplasm in Bangladesh, attempts to improve drought tolerance trait through conventional breeding have met with little success. Therefore, the present work is aimed at studying the molecular and biochemical mechanisms associated with drought tolerance in contrasting wheat genotypes.

Materials and methods

Plant materials and growth conditions

Seeds of two wheat cultivars (BG 25 and Bijoy) were obtained from Wheat Research Center, Bangladesh Agricultural Research Institute, Rajshahi, Bangladesh. After being sterilized with 75% ethanol and washed with sterilized water, seeds were placed in moist filter paper for germination in the dark at room temperature. Afterwards, only healthy and uniform seedlings were transplanted to solution culture. A basal nutrient solution (Hoagland & Arnon 1950) was used with the following nutrient concentrations (µM): KNO_3 (16000), $Ca(NO_3)_2.4H_2O$ (6000), $NH_4H_2PO_4$ (4000), $MgSO_4.7H_2O$ (2000), KCl (50), H_3BO_3 (25), Fe-EDTA (25), $MnSO_4.\ 4H_2O$ (2), $ZnSO_4$ (2), $Na_2MoO_4.2H_2O$ (0.5) and $CuSO_4.5H_2O$ (0.5). Plants were grown in 2 l of aerated solution and the environment was strictly maintained under 10 h light and 14 h dark (550–560 µmol s^{-1} per µA). The pH was pH adjusted to 6.3 by using NaOH or HCl. For drought treatment, 15% polyethylene glycol (PEG-6000) was added to the culture solution (Liu et al. 2013). Non-stressed control plants were grown concurrently and harvested at the same time. Nutrient solution was changed in every day's interval for both control and treatment.

Measurement of morphological characters

Shoot height, root length, shoot dry weight and root dry weight were measured on 2-week old plants grown on solution culture. Total roots developed by each plant sample were washed in distilled water to remove nutrient and then quickly blotted in tissue paper. Shoots and roots were then dried in oven at 70°C for 2 days before taking the dry weight.

Determination of chlorophyll concentrations

A chlorophyll concentration of young leaves (2-week old after treatment was imposed) was determined spectrophotometrically as previously described with some modifications (Lichtenthaler & Wellburn 1985) on hydroponically grown plants. Firstly, 0.1 g leaf was weighted and placed in 95% acetone in a 5 mL falcon tube. The leaf sample was then grinded using mortar–pestle. The homogenate was filtered through whatman filter and was centrifuged at 2500 rpm for 10 min. The supernatant was separated and the absorbances were read at 662 nm (chlorophyll a) and 646 nm (chlorophyll b) on a spectrophotometer (UV-1650PC, Shimadzu). The amount of these pigments was calculated based on formula (Lichtenthaler & Wellburn 1985).

RNA isolation and semi-quantitative RT-PCR

Expression of genes (*Actin, TaCRT1 and DREB1A*) was studied by semi-quantitative RT-PCR (reverse transcription PCR) in roots of 2-week old plants grown under PEG– and PEG+ hydroponic conditions. Around 100mg of root tissues was ground to a fine powder in liquid nitrogen using a mortar and pestle. Total RNA was extracted according to the protocols given by SV Total RNA Isolation System (cat. no. Z3100) manufactured by Promega Corporation, United States. The amount and purity of RNA in each sample was checked by Micro Nanodrop and RNA integrity was checked by denaturing agarose gel electrophoresis. RNA was used for first-strand cDNA synthesis using GoScript™ Reverse Transcription System (Cat no. A5001) supplied by Promega Corporation, USA. The primers were used to perform BLASTN searches to confirm that they would specifically amplify the gene of interest. The first-strand cDNA was then amplified using gene specific primers (*Actin*-fw: GAATCCATGAGACCACCTAC, *Actin*-rev: AATCCAGACACTGTACTTCC; *TaCRT1*-fw: GAAGCCCCCCAAATCTT, *TaCRT1*-rev: CCTCACACGAGACAAGAAACAC; *DREB1A*-fw: AAGAAAACAGGCGACAAGAT, *DREB1A*-rev: ACGAAGCACAAAAAACTAGC) in a MultiGene™ OptiMax Thermal Cycler, Labnet International Inc. The PCR program used was as follows: 4 min at 95°C, 35 cycles of 30 s at 95°C, 45 s at 55°C, 1 min at 72°C and 10 min at 72°C.

Enzymatic analysis

Enzyme extraction for CAT, POD and SOD was performed in roots as previously described with slight modifications (Goud & Kachole 2012) on 2-week old plants. Briefly, 100 mg of root sample ground in 5 ml of 100mM phosphate buffer (KH_2PO_4). The homogenate was centrifuged for 10 min and the supernatant was used for the enzyme assay.

For CAT analysis, the reaction mixture in a total volume of 2 ml contained 100 mM potassium phosphate buffer (pH 7.0) mixed with 400 µl of 6% H_2O_2 and 100 µl root extract. Root extract was the last component to be added and the decrease in absorbance was recorded at 240 nm (extinction coefficient of 0.036 mM^{-1} cm^{-1}) using a UV spectrophotometer at 30 s intervals up to 1 min. The specific activity of enzyme is expressed as

μmol of H_2O_2 oxidized min^{-1} (mg protein)$^{-1}$. For POD, the reaction mixture in a total volume of 2 ml contained 100 mM potassium phosphate buffer (pH 6.5) mixed with 1 ml of 0.05 M pyrogallol solution, 400 μl of 200 mM H_2O_2 and 100 μl root extract. The change in absorbance was recorded at 430 nm (extinction coefficient 12 mM^{-1} cm^{-1}) in a spectrophotometer from 30 s up to 1.5 min. The specific activity of enzyme is expressed as μmol pyrogallol oxidized min^{-1}(mg protein)$^{-1}$. For SOD analysis, the assay mixture in a total volume of 1.5 ml contained 50 mM sodium carbonate/bicarbonate buffer (pH 9.8), 0.1 mM EDTA, 0.6 mM epinephrine and enzyme (Sun & Zigman 1978). Epinephrine was the last component to be added. The adrenochrome formation in the next 4 min was recorded at 475 nm in a UV–Vis spectrophotometer. One unit of SOD activity is expressed as the amount of enzyme required to cause 50% inhibition of epinephrine oxidation under the experimental conditions. For GR analysis, 100 μl of enzyme extract was added to 1 ml of 0.2 M phosphate buffer (pH 7.0) containing 1 mM EDTA, 0.75 ml distilled water, 0.1 ml of 20 mM oxidized glutathione (GSSG) and 0.1 ml of 2 mM NADPH. Oxidation of NADPH by GR was monitored at 340 nm and the rate (nmol min^{-1}) was calculated using the extinction coefficient of 6.12 mM^{-1} cm^{-1} (Halliwell & Foyer 1978).

Lipid peroxidation activity was analyzed as previous described (Kosugi & Kikugawa 1985). Malondialdehyde (MDA) is one final decomposition product of lipid peroxidation and an indicator lipid peroxidation status. Thiobarbituric acid reactive substances representing the lipid peroxidation product were extracted by homogenization of 0.2 g leaf in 5 ml of a solution containing 20% trichloroacetic acid and 0.5% 2-thiobarbituric acid. The mixture was heated at 95°C for 30 min and the reaction was arrested by quickly transferring the mixture to an ice bath. The cooled mixture was centrifuged at 5000 g for 10 min at 25°C and the absorbance of the supernatant at 532 and 600 nm was recorded. After subtracting the nonspecific turbidity at 600 nm, the MDA concentration was determined by its molar extinction coefficient 155 mmol/l cm (Kosugi & Kikugawa 1985).

Analysis of plant metabolites by HPLC (high-performance liquid chromatography)

HPLC analysis was performed as previously described with some modifications (Kabir et al. 2015). Briefly, harvested roots were grinded in mortar pestle using methanol and were centrifuged at 1500 g for 10 min before storing the supernatant at −20°C. Amino acids in roots were then analyzed by HPLC (Binary Gradient HPLC System, Waters Corporation, Milford, Massachusetts, USA) using Empower2™ software. This comprised a Waters 515 HPLC pump and Waters In-line degasser AF. Compound separations were achieved with a C18 reverse phase-HPLC column (particle size: 5 μm, pore size: 300 A, pH Range: 1.5–10, Dimension: 250 mm × 10 mm). Buffer A (water and 0.1% TFA) and buffer B (80% acetonitrile and 0.1% TFA) were used as mobile phase at the gradient of: 1–24 min 100% A, 25–34 min 100% B, 35–40 min 100% A.

Standards for each amino acid were purchased from Sigma-Aldrich, Co., St. Louis and Carl Roth. All standard stock solutions (0.5 mM) were prepared in LC-MS grade water. In addition, samples were diluted 100 times in LC-MS grade water before injection. Both standards and samples were filtered using 0.22 μm Minisart Syringe Filters (Sartorius Stedim Biotech, Germany) before injection. Metabolites were detected with a Waters 2489 dual absorbance detector (Waters Corporation, Milford, Massachusetts, USA) at 280 and 360 nm. Peak identifications were achieved by comparing retention times and mass spectra of sample peaks with those of authentic standards.

Statistical analysis

All experiments were performed having at least three replications for each sample. Statistical analyses (*t*-test) were performed using Genstat software (14th edition) and graphical presentation was prepared using GraphPad Prism 6. Significance was set at $P \leq .05$.

Results

Morphological features

No visible differences were observed between control and PEG treated BG-25 grown in hydroponic culture for 2-week (Figure 1). However, Bijoy plants showed stunted growth and anemic looking due to PEG treatment. PEG-induced drought stress did not significantly influence the shoot height, shoot dry weight, root length and root dry weight in BG-25 plants (Table 1). In contrast, a significant decrease was observed under drought stress in Bijoy plants in the aforesaid morphological characteristics (Table 1). Chlorophyll contents in leaves were also determined 2 weeks after the imposition of PEG-induced drought stress conditions. Total chlorophyll concentrations (a and b) were not significantly affected in BG-25 under PEG stress compared with control plants (Figure 2). In contrast, total chlorophyll concentrations (a and b) significantly decreased due to PEG treatment in Bijoy compared to controls.

Expression of genes associated with drought tolerance

The expression pattern of *TaCRT1 and DREB1A* transcripts was studied by semi-quantitative reverse transcriptase PCR, using samples from roots. *TaCRT* showed higher expression in PEG treated plants than control plants in both BG-25 and Bijoy. However, expression was higher in BG-25 than Bijoy plants (Figure 3). *DREB1A* showed constitutive expression in both genotypes under control and PEG treated drought stress. Comparatively, *DREB1A* gene was upregulated due to PEG treatment compared to control plants; however, the expression was predominant in BG-25 than Bijoy (Figure 3).

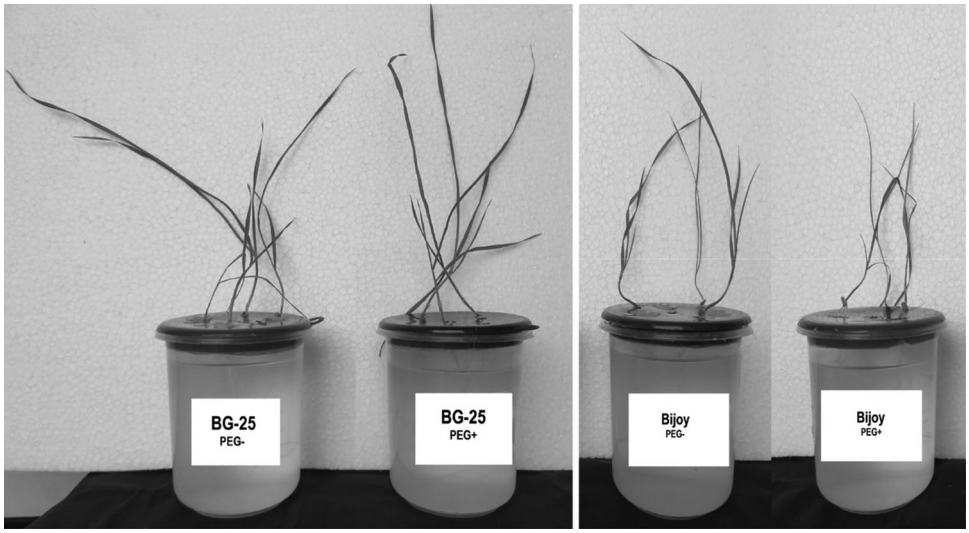

Figure 1. Morphological view of BG-25 and Bijoy grown under PEG– and PEG+ hydroponic conditions.

Table 1. Morphological features of BG-25 and Bijoy grown in the absence and presence of PEG under hydroponic conditions.

	BG-25		Bijoy	
Features	PEG–	PEG+	PEG–	PEG+
Shoot height (cm)	22.3 ± 0.56^a	21.15 ± 0.77^a	22.4 ± 2.15^a	16.4 ± 1.35^b
Shoot dry weight (g)	0.16 ± 0.03^a	0.16 ± 0.004^a	0.12 ± 0.05^a	0.062 ± 0.015^b
Root length (cm)	7.7 ± 0.70^a	6.4 ± 0.28^a	12.13 ± 0.93^a	7.9 ± 0.26^b
Root dry weight (g)	0.016 ± 0.003^a	0.01 ± 0.006^a	0.013 ± 0.003^a	0.0065 ± 0.002^b

Note: Different letters indicate significant differences between means ± SD of treatments ($n = 3$). Data were taken on 2-week old plants.

Activities of antioxidant enzymes

Activity of CAT, POD and GR significantly increased due to PEG treatment in roots of BG-25 compared to control plants (Table 2). In contrast, activity of these enzymes was not significantly changed due to PEG-induced drought stress in Bijoy though a slight non-significant increase was observed in CAT and GR activity. Further, SOD activity was not significantly differed under PEG treatment in neither of the genotypes compared with control plants. Interestingly, MDA content showed significant decrease and increase in BG-25 and Bijoy, respectively, when treated with PEG compared with the plants grown without PEG treatment (Table 2).

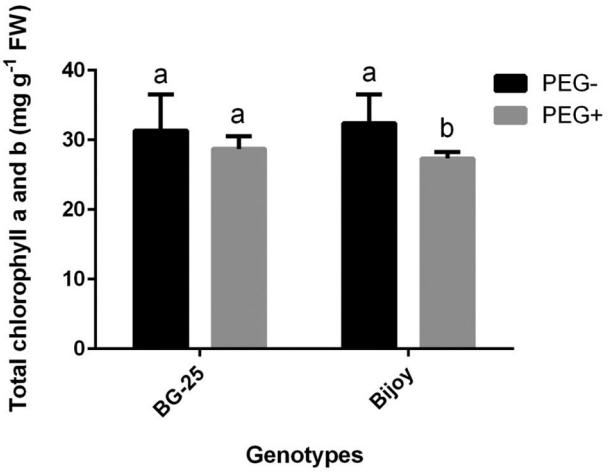

Figure 2. Chlorophyll concentrations (a and b) in leaves of BG-25 and Nijoy grown in PEG– and PEG+ hydroponic culture. Values are the means of three independent replications with standard deviations. Different letters indicate significant differences between means±SD of treatments ($n = 3$). Leaves were harvested from 2-week old plants.

HPLC analysis of amino acids

HPLC technique was employed to study the changes of few key amino acids in roots of BG-25 and Bijoy grown in control and PEG treated hydroponic conditions. Among the S-containing amino acids, glutathione (1.48-

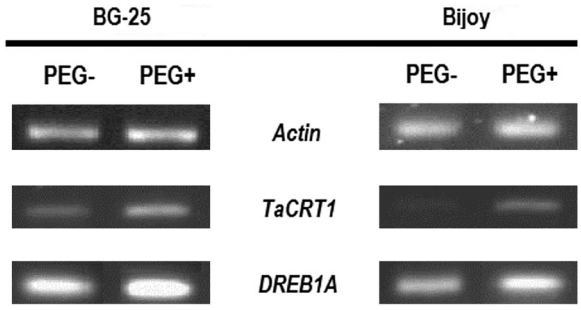

Figure 3. Expression analysis of *Actin* (control), *TaCRT1* and *DREB1A* transcripts in roots of BG-25 and Bijoy grown under PEG– and PEG+ hydroponic conditions. Roots were harvested from 2-week old plants.

Table 2. Activities of different antioxidant enzymes in roots of BG-25 and Bijoy grown in the absence and presence of PEG under hydroponic conditions.

	BG-25			Bijoy		
Enzyme activities	PEG–	PEG+	Fold (ratio)	PEG–	PEG+	Fold (ratio)
CAT min^{-1} [(mg protein)$^{-1}$]	1.52 ± 0.093[a]	2.17 ± 0.044[b]	1.43-fold increase	0.89 ± 0.140[a]	1.12 ± 0.204[a]	1.26-fold increase
POD min^{-1} [(mg protein)$^{-1}$]	0.22 ± 0.040[a]	2.16 ± 0.250[b]	9.48-fold increase	0.20 ± 0.040[a]	0.16 ± 0.017[a]	1.23-fold decrease
GR [nmol.NADH.min^{-1} mg protein^{-1}]	0.033 ± 0.004[a]	0.058 ± 0.008[b]	1.76-fold increase	0.072 ± 0.016[a]	0.122 ± 0.031[a]	1.68-fold increase
SOD [U.mg^{-1} protein]	4.38 ± 0.462[a]	5.31 ± 0.088[a]	1.21-fold increase	8.64 ± 0.370[a]	7.93 ± 0.972[a]	108-fold decrease
MDA content (nmol g^{-1} FW)	1.87 ± 0.045[a]	0.79 ± 0.124[b]	2.36-fold decrease	1.70 ± 0.074[a]	2.08 ± 0.130[a]	1.22-fold increase

Note: Different letters indicate significant differences between means ± SD of treatments ($n = 3$). Data were taken on 2-week old plants.

fold), cysteine (1.21-fold) and proline (1.22-fold) significantly increased due to PEG treatment in roots of BG-25 compared to controls plants (Table 3). However, PEG treatment caused non-significant increase and significant decrease in glutathione and methionine, respectively, in Bijoy compared to the plants grown without PEG treatment. Among the N-metabolites, glycine showed no significant difference between control and PEG treatment. Interestingly, PEG-induced drought stress caused a significant increase and decrease in BG-25 and Bijoy, respectively, compared to non-treated control plants (Table 3). In addition, leucine was only detected under PEG treatment in BG-25; whereas this amino acid did not significantly change between control and treatment in Bijoy.

Discussion

Drought tolerance is a trait of great importance in wheat given the prevalence of drought conditions in South Asia and other parts of the world. To gain insights into the determinants underlying drought tolerance, the present study deals with the molecular and biochemical analyses underlying differential tolerance in wheat in response to drought.

Morpho-physiological variations in contrasting genotypes

Genetic variation is an essential prerequisite for any crop improvement program. Based on morphological features and leaf chlorophyll concentration, it does suggest that genotypic variations exist in BG-25 and Bijoy in response to drought stress (Figure 2, Table 1). Drought stress was not able to affect growth and chlorophyll synthesis in BG-25 and thus, it appears that BG-25 is able to tolerate drought stress and continue normal growth and development. On the contrary, Bijoy is not efficient to operate mechanisms conferring drought tolerance as evident from their significant reduction in morpho-physiological features. Drought causes losses in tissue water content which reduce turgor pressure in cell, thereby inhibiting enlargement and division of cell causing of reduce of plant growth and dry mass accumulation (Delfine et al. 2002). The decrease in chlorophyll concentration under drought stress could be considered as a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation (Farooq et al. 2009). The results reported in our study are similar to earlier studies of Dhanda et al. (2004) in wheat; and Radhouane (2007) in tomato. Taken together, our results suggest that cultivar Bijoy is the drought sensitive cultivar while BG-25 is drought tolerant as it showed a better degree of survival on external drought stress.

Expression of genes associated with drought tolerance

Plants exhibiting drought-related genes are to be used in the improvement of modern crop varieties. In this current study, *TaCRT1* showed upregulation in both genotypes when plants were treated with PEG in hydroponic culture. However, expression was higher in roots of

Table 3. HPLC analysis of amino acids (µg/mg) in roots of BG-25 and Bijoy grown under PEG– and PEG+ hydroponic conditions.

	BG-25			Bijoy		
Metabolites	PEG–	PEG+	Fold (ratio)	PEG–	PEG+	Fold (ratio)
Glutathione	5.91 ± 0.53[a]	8.80 ± 1.10[b]	1.48-fold increase	2.19 ± 0.14[a]	2.33 ± 0.07[a]	1.06-fold increase
Cysteine	2.77 ± 0.25[a]	3.36 ± 0.42[b]	1.21-fold increase	ND	ND	NA
Methionine	5.95 ± 0.53[a]	7.28 ± 0.91[b]	1.22-fold increase	6.19 ± 0.41[a]	1.16 ± 0.03[b]	5.31-fold decrease
Glycine	4.21 ± 0.38[a]	4.43 ± 0.55[a]	1.05-fold increase	ND	ND	NA
Proline	5.38 ± 0.48[a]	6.74 ± 0.84[b]	1.25-fold increase	9.68 ± 0.65[a]	2.95 ± 0.09[b]	3.27-fold decrease
Leucine	ND	6.27 ± 0.78[b]	NA	1.91 ± 0.12[a]	1.33 ± 0.04[a]	1.43-fold decrease

Note: Different letters indicate significant differences between means ± SD of treatments (n = 3). Roots were harvested from 2-week old plants.
ND, not detected; NA, not applicable.

drought tolerant BG-25 than the sensitive Bijoy. *TaCRT1* gene, responsible for calreticulin proteins are highly abundant and structurally conserved soluble proteins in endoplasmic reticulum (Michalak et al. 2009) and it contains N, P and C conserved domains (Chen 1998). Calreticulin proteins from higher plants were divided into group I, group II and group III. Different expression patterns of plant calreticulin genes are found when under diverse stress treatments or in defense responses. Some members are upregulated by stress factors, such as Arabidopsis *AtCRT3* by salt stress, wheat *TaCRT* by drought, rice *OsCRO1* by low temperature, and papaya *CpCRT* by papaya ringspot virus infection (An et al. 2011). *CRT1* or a $Ca2^{+}$-binding peptide (CBP) consisting of only the CRT domain can not only increase Ca^{2+} stores, but also enhance the survival of plants (Persson et al. 2001). In a recent report, a solution of Ca^{2+} was reported to be beneficial when sprayed directly onto the leaves of drought-stressed tea plants (Upadhyaya et al. 2011). Being consistent with these reports, our study suggest that enhanced expression *TaCRT1* in drought tolerant BG-25 may be involved with increased Ca^{2+} and thus, contribute to its tolerance in response to PEG-induced drought stress.

Transcription factors have been shown to produce multiple phenotypic alterations, many of which are involved in stress responses. *DREB1A*, a transcription factor that recognizes dehydration response elements, has strongly induced due to PEG treatment in roots of both BG-25 and Bijoy. Furthermore, relatively higher expression in BG-25 implies that *DREB1A* play a crucial role in promoting the expression of drought-tolerance genes in wheat. Recently, *AtDREB1A*, identified from the model plant *Arabidopsis thaliana*, has been reported to enhance stress tolerance in rice against drought stress (Ravikumar et al. 2014). They further demonstrated that of *AtDREB1A* was induced by drought stress in transgenic rice lines, which were highly tolerant to severe water deficit stress in both the vegetative and reproductive stages without affecting their morphological or agronomic traits. Furthermore, drought stress differentially regulated the expression level of *DREB* gene in wheat cultivars (Hassan et al. 2015). The increased *DREB* expression by drought was reported in soybean (Chen et al. 2006).

Role of antioxidant system in drought tolerance

The results of antioxidant enzymes showed variations between tolerant and sensitive genotypes. CAT, POD and GR enzyme showed higher significant activity in roots of BG-25 when plants were stressed with PEG. Contrarily, there was no significant different in these enzymatic activities in response to drought stress in the sensitive cultivar Bijoy. Rivero et al. (2007) also reported that CAT remained more active for a greater duration of drought stress. APX and POD might be responsible for the fine modulation of reactive oxygen species (ROS) for signaling, whereas CAT might be responsible for or the removal of excess ROS during stress (Mittler 2002). GR is involved in the regulation of ROS and loss of their activities resulted in building up of ROS to high levels that resulted in CAT induction (Hassan et al. 2015). GR activates in glutathione-ascorbate cycle and coverts GSSG to reduced glutathione (Vega et al. 2003). MDA is regarded as a marker for evaluation of lipid peroxidation, which is linked to the activity of antioxidant enzymes for example, with the increase of CAT, SOD, APX, GR, etc, oxidative stress tolerance is enhanced and MDA is decreased (Esfandiari et al. 2007). Being consistent with this theory, MDA content significantly decreased in BG-25, while Bijoy showed a non-significant increase due to drought stress. The association between the levels/activities of antioxidant enzymes and plant drought tolerance has been previously observed in wheat (Hassan et al. 2015) and maize (Benesova et al. 2012). From our investigations, it appears that there was an association between the higher antioxidant capacity and higher tolerance to drought stress in this tolerant wheat cultivar BG-25. In addition, all these antioxidant enzymes were limiting factors that led to the sensitivity of Bijoy to drought stress.

Changes of root metabolites due to drought stress

In this study, drought stress caused significant changes in some key metabolites in roots of BG-25 and Bijoy. Among S-metabolites, we observed marked increases in glutathione, cysteine and methionine only in BG-25 roots due to drought stress (Table 3). In contrast, methionine was significantly decreased and cysteine was not detected in Bijoy roots. Elevated level of these three S-containing amino acids in BG-25 suggests that drought tolerance is associated with the accumulation of these metabolites in roots. One likely role for glutathione is to work as an antioxidant compound to protect cells from PEG-induced oxidative injury in wheat. Proline, which is a common compatible osmoprotectant, accumulates in response to drought stress and reduces cell injury (Ravikumar et al. 2014; Hassan et al. 2015). During the drought stress treatment, the proline level was only significantly increased drought tolerant cultivar BG-25 compared to the control plants. Thus, it appears that osmotic adjustment by the enhanced accumulation of proline may contribute immensely to drought tolerance in this cultivar.

Conclusion

We investigated the contribution of a few molecular and biochemical determinants, leading to drought tolerance in two naturally occurring wheat cultivars, BG-25 and Bijoy. The two cultivars exhibited marked differences in physiological growth and chlorophyll concentrations under drought stress with BG-25 plants noticeably more tolerant to drought stress than Bijoy. Expression of two genes (*TaCRT1* and *DREB1A*) greatly induced in roots of BG-25, suggesting the roles in drought tolerance in this genotype. In addition, higher antioxidant capacities in roots of BG-25 further implicate that this genotypes is efficient scavenging ROS generated by drought stress.

Disclosure statement

No potential conflict of interest was reported by the authors.

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