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ARTICLE

Accumulation of water stress-responsive class-III type of boiling stable peroxidases (BsPOD) in different cultivars of wheat (*Triticum aestivum*)

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ABSTRACT Drought is one of the most important abiotic stress that affects plant growth and productivity. Class-III Peroxidases (PODs) are known to maintain oxidative stress induced-ROS at sub-lethal levels in plants under abiotic stress conditions, but, studies documenting how drought regulates boiling stable class-III PODs are still a matter of conjecture. In this study, changes in total protein content (TPC), water content (WC), H_2O_2, malondialdehyde (MDA) and ROS scavenging class-III boiling stable POD were studied in the shoots of seven cultivars of wheat at different stages of drought treatment (3 days: D3, 5 days: D5 and 7 days: D7) followed by recovery from stress (post stress: PS). Simultaneous analysis of H_2O_2, MDA contents and activities of ROS-scavenging class-III boiling stable POD (BsPOD) enzymes gave an integrative view of physiological state and detoxifying potential under conditions of sensitivity and tolerance. Drought stress increased TPC and decreased WC in all the cultivars of wheat. H_2O_2 content decreased considerably under various stage of drought stress in a genotype dependent manner. As a result, amount of MDA, a product of lipid peroxidation, was also less in all the cultivars at all stress durations. During initial stress conditions (D3), a marked increase in BsPOD activity was observed in cultivars PBW343, PBW550, PBW175, DBW17 and HD2967. An especially high increase (55-fold) was noticed in PBW175 accompanied by enhanced expression of boiling stable POD isoenzyme(s) suggesting that this cultivar has more efficient mechanisms to scavenge ROS species. In addition, this cultivar has also maintained higher BsPOD activity when the stress duration increased from D3 to D7. The other cultivars having lowest enzyme activities are mentioned as cultivars sensitive to drought stress. Compared to stress, significantly higher soluble protein content accompanied by BsPOD activity was observed after the exposure to recovery conditions in the majority of cultivars. Based on these results, the possible role of BsPOD activity and isoenzyme(s) to perform biological antioxidative reactions to combat drought-induced oxidative stress was discussed.
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KEY WORDS

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In nature, plants frequently encounter environmental conditions that may include stresses such as biotic and abiotic conditions, which drastically affects crop both in terms of quality and quantity (Chaves and Oliveira 2004; Kotak et al. 2007). Drought is one of the environmental stresses, which is the most significant factor restricting plant growth and crop productivity in the majority of agricultural fields of the world (Tas and Tas 2007). Compelling evidences have indicated that drought is associated with oxidative stress in plant cells and can cause the generation of reactive oxygen species (ROS) such as H_2O_2 (hydrogen peroxide), $O_2^{\cdot-}$ (superoxide) and OH (hydroxyl) radicals in a tissue dependent manner (Gao et al. 2008). Among them, H_2O_2 seems best suited to play the role of a signaling molecule due to its higher stability and longer half-life (Hung et al. 2005). If H_2O_2 serves as a stress signal, the fluctuation of the H_2O_2 level in plants should spatially and temporally reflect changes in the environment. Under normal conditions, ROS are inevitable generating from the essential aerobic metabolism including chloroplastic and mitochondrial processes, per-oxidation of membrane lipids and plasma membrane linked electron transport systems (Bi et al. 2009). Besides dangerous cytotoxic molecules, ROS have been shown to act as second messengers involved in the various stress signal transduction pathways, which control and regulate such biological processes as programmed cell death and hormone signaling (Graper and Dolan 2006). However, excessive ROS synthesis can cause an extensive peroxidation and de-esterification of membrane lipids as well as damages plants by oxidizing photosynthetic pigments, membrane lipids, proteins and nucleic acids, if the plant is not efficient in scavenging these molecules (Mittler 2002; Baruah et al. 2009). Besides this, increase and accumulation of ROS could cause lipid peroxidation leading to cell death. Malondialdehyde (MDA), which is the most abundant aldehydic lipid breakdown product, is considered as a suitable

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marker for membrane lipid peroxidation (Goel and Sheoran 2003). A decrease in membrane stability reflects the extent of lipid peroxidation caused by ROS. Oxidative stress arises due to an imbalance between generation and elimination of ROS, which can lead to changes in membrane permeability and membrane leakage. All these observations suggest the dual role of ROS in plant biology as dangerous molecules and key regulators of growth and defence pathways. Plants have ability to sense ROS and re-programme their gene expression in response to changing conditions of their environment. Microarray studies involving mutants and antisense lines that lack ROS-scavenging enzymes revealed that ROS act as signaling molecules involved in the expression of a large number of genes and biological processes (Miller et al. 2008). In order to combat ROS, plants have the detoxification mechanism that includes both enzymatic and non-enzymatic antioxidant components (Scandalios 2005). Enzymatic antioxidants systems involve the activity of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and dehydroascorbate reductase (DHAR) (Gill and Tuteja 2010). Non-enzymatic components includes ascorbate (AsA), glutathione (GSH), flavonoids, phenolic compounds, alkaloids, tocopherol and carotenoids, which act as antioxidant buffers (Mittler 2002; Foyer and Noctor 2005; Gratao et al. 2005). Out of the antioxidative enzymes involved in mitigating the effect of oxidative stress, class-III peroxidases (PODs) are the major antioxidative enzymes grouped in a super-family utilizing guaiacol as electron donor. They are located in the cytosol, cell wall and involved in the decomposition of H_2O_2 through the oxidation of phenolic compounds. Generally, peroxidases (PODs/PRXs) are divided in three different classes. Class-I mainly includes ascorbate peroxidases and cytochrome C peroxidases (CcPs); class-II contains fungal manganese and lignin peroxidases, while class-III involves all the secretary plant peroxidases (PODs) (for review, see Cosio and Dunand 2009). Several key roles have been attributed to plant class-III PODs in response to biotic and abiotic stresses, such as the modification of cell wall via suberin polymerization, cross-linking of structural non-enzymatic proteins, and cleaving cell wall polysaccharides; they may affect leaf expansion, fruit growth, germination and nodulation (Cosio and Dunand 2009). They may have diverse roles possibly due to the large number of their isoforms (isoenzymes). The quantitative and qualitative changes in the expression of antioxidant enzymes are often related to the levels of resistance to water stress. Some studies indicated that activities of antioxidant enzymes are correlated with plant tolerance to abiotic stress (Ozkur et al. 2009; Wang et al. 2009).

Many water-stress inducible proteins (e.g. HSPs and LEAs) are highly hydrophilic and remain soluble even after boiling. Thereafter, they have been termed as "boiling soluble proteins" (BSPs) (Jacobsen and Shaw1989). Many proteins, which were detected in total protein extracts, are lost after boiling the extracts (Pelah et al.1995). Earlier research also indicated that hydrophilins represent less than 0.2% of the total protein of a given genome, however, it represents the most significant part of the proteome in regulating tolerance to abiotic stresses in plants (for review, see Battaglia et al. 2008). Hence, to better elucidate the role of these boiling soluble proteins (BSPs), it is a prerequisite to examine their expression not only under water stress, but also after boiling of extracts. Moreover, the role of class-III type boiling stable peroxidases (BsPODs) under drought treatment is not well documented. Therefore, in the present study, we have analyzed the effect of drought on the biochemical activity and isoenzyme expression profile of BsPODs along with H_2O_2 and MDA contents in the shoots of different wheat cultivars. Analysis of isoforms of BsPOD coupled with biochemical analysis will provide new insights into drought-induced oxidative stress. Wheat is one of the most important crops in arid and semi arid areas worldwide and it is sensitive to drought and temperature stress. In order to facilitate the detection of BSPs, heat stable (HS) fractions that resist coagulation upon heating at 100 °C were focused.

Material and Methods

Seed germination and growth conditions

Seeds of commercially relevant lines of seven wheat (*Triticum aestivum* L.) cultivars (PBW343, PBW621, PBW527, PBW550, PBW175, DBW17 and HD2967) (Table 1) were selected for experimental purposes. All the cultivars were obtained from PAU Ludhiana, Punjab, India. They were selected as all are locally grown and a comparison of their responses could give a better understanding of the susceptibility/tolerance to the different varieties to drought. Seeds were surface sterilized with 1% (w/v) mercuric chloride followed by 70% (v/v) ethanol (Sharma et al. 2006). Seeds were thoroughly rinsed with deionized water and imbibed for 6 h. After imbibitions, seeds were placed in petri plates containing sterile filter sheets, moistened with water. Then, the seeds were incubated at 25±1 °C in a seed germinator in darkness and allowed to grow for 6 days. To impart water stress, watering of plants was completely withheld for the test period when plants were 6 days old. Sampling was done on the 3rd (D3), 5th (D5) and 7th (D7) day to study response of plants to the length of drought. In all experiments, samplings were also done at zero day of drought, which was designated as control. To study the post-stress (PS) effect, after the 7th day of drought stress, plants were re-watered. Water content (WC) was measured after imposing stress treatments. Fresh weights (FW) were determined within 2 h after collection. Dry weights (DW) were obtained after oven drying the samples for 72 h at 70 °C. WC was calculated from the following equation:

WC = FW-DW/FW.

Extraction of Boiling Stable Proteins

Boiling stable proteins were extracted as described previously (Sharma et al. 2006; Sharma et al. 2012). Briefly, tissues were homogenized with chilled mortar and pestle in extraction buffer (50 mM Tris buffer, pH 7.0). Crude extracts were centrifuged at 10 000 g for 10 min. The total extract was boiled for 15 min in order to get boiling stable protein fractions. The total soluble protein content in the supernatant was determined according to Lowry et al. (1951) using BSA as a standard.

BsPOD activity analysis

The BsPOD activity was measured according to the method described in Sharma et al. (2013). The mixture contained 50 mM TRIS (pH 7.0), 10 mM guaiacol and 5 mM H_2O_2. To this mixture An aliquot of 120 µg of protein was added to this mixture adjusting the total volume to 1 ml. The increase in absorbance was measured at 470 nm at intervals of 30 s. The activity was calculated as per min per milligram of tetraguaiacol production by using the extinction coefficient for tetraguaiacol of 26/mM/cm.

Isoenzyme analysis of BsPODs

Boling stable proteins were extracted as described above. For in-gel activity analysis, the proteins (240 µg) were separated by a non-denaturing 12% polyacrylamide gel electrophoresis as described by Sambrook et al. (1989). When electrophoresis was complete, the gel was washed three times in 50 mM sodium acetate buffer (pH 5.0). Peroxidase activity was visualized by incubating the gel in 50 ml of a solution containing 50 mM sodium acetate buffer (pH 5.0), 330 µl of guaiacol (9 M) and 1.5 ml of 6.6% H_2O_2. The gel was incubated at room temperature in dark until reddish-brown bands appeared. The gel was washed in distilled water and used for further analysis. Relative abundance of isoenzymes was quantified by Ultraquant software 13.3.26 of gel documentation system (Omega Lum USA)

Estimation of hydrogen peroxide content

The H_2O_2 content was measured by the method described by Mamik and Sharma (2014). The reaction mixture contained 0.5 ml of Tris-HCl buffer pH 7.0, 0.5 ml of 0.1% trichloroacetic acid (TCA), 120 µg of protein, 2 ml of 1 M KI. After 1 h of reaction in dark, the absorbance was measured at 390 nm. The amount of hydrogen peroxide (µM/gFW) was calculated using a standard curve prepared with known concentrations of H_2O_2.

Statistical analysis

The plants were distributed over a completely randomized design, with 175 treatment combinations, forming a 7×5×5 factorial (7 genotypes, 5 watering regimes and 5 samplings) at vegetative and reproductive phases. Statview ANOVA program was used for statistical analysis of the data. Values were compared using one-way analysis of variance and student's *t*-test for differences between pairs of data if the ANOVA ($LSD_{0.05}$) revealed significance. Means were tested by LSD at P 0.05 level ($LSD_{0.05}$).

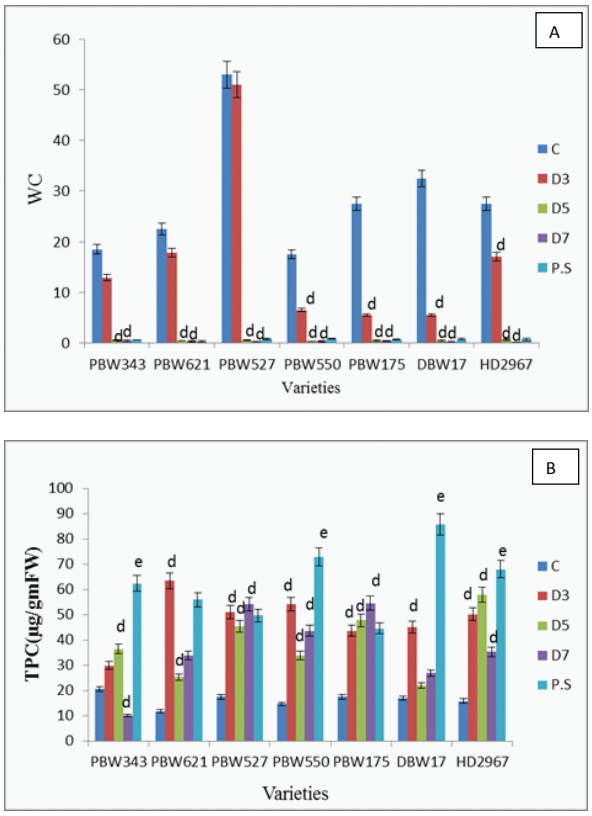

Figure 1. Effect of drought stress and recovery on WC (A), and TPC (B) in the shoots of different cultivars of *Triticum aestivum*. Data shown are averages ± SD (n=3). [d] represents significant difference with respect to control p≤0.5. [e] represents significant difference of PS relative to D7 p≤0.5.

Results and Discussion

Drought stress triggers the change of a wide variety of responses ranging from physiological to metabolic processes in plants. In the present study, effect of drought stress on BsPOD enzymes and other parameters were studied in seven different cultivars of wheat by imposing drought stress for 3 days (D3), 5 days (D5) and 7 days (D7) and followed by recovery from drought stress (PS).

Changes in the physiological parameters

As shown in Figure 1A, compared to the control (zero day), water content (WC) in all wheat cultivars showed a progres-

sive decrease as the intensity of stress increased from D3 to D7. However, only in PBW527, no substantial change in WC at D3 was observed. Most research has shown decreased WC in response to drought stress (Chakraborty and Pradhan 2012; Sanchez-Blanco et al. 2002; Chakraborty et al. 2002). Moreover, compared to D7, a notable increase (about 2- to 7-folds) in WC was observed following post stress recovery period (PS) in all cultivars, except PBW343 and PBW621.

The changes of total protein content (TPC) in the leaves of wheat cultivars treated with drought stress are shown in Figure 1B. Significantly increased TPCs were detected during the drought stress. Relative to the control, TPC increased significantly at all stages of drought in PBW621, PBW527, PBW550, PBW175 and HD2967. At the same time, in PBW343, a substantial decrease in TPC was observed at D7. Earlier studies reported that drought stress affects gene expression and protein synthesis (Lobato et al. 2008). Lee and Lee (2000) described elevated soluble protein content following water stress. As indicated by earlier studies, protein degradation under drought stress might be due to increased activity of proteases and catabolic enzymes or to fragmentation of proteins because of the toxic effects of ROS. These processes may result in reduced protein contents. Compared to D7, TPC increased considerably in cvs PBW343, PBW621, PBW550, DBW17 and HD2967 during the PS period.

Changes in MDA content

The drought-induced changes were further examined by measuring the accumulation of thiobarbituric acid (TBA)-reactive compounds, such as malondialdehyde (MDA). These compounds are by products of lipid peroxidation, a process resulting in the generation of ROS (Dhindsa et al. 1981). Lipid peroxidation in the cell membranes is said to be one of the most challenging and detrimental effect of water stress in the membranes of all cells exposed to various degree of stress (Thankamani et al. 2003). The degree of lipid peroxidation was measured in terms of MDA content which is one of the determinants indicating the severity of stress experienced by any plant. Compared to the control, significantly higher concentrations of TBA-reactive compounds were observed in drought stressed leaves of HD2967 and DBW17 at all stages (D3, D5, D7) of drought (Fig. 2A). In PBW621 and PBW527, the maximum MDA level was observed at D7. In PBW343, a linear increase of the MDA values were observed as stress duration raised from D3 to D5. Jiang and Huang (2001) reported increased MDA accumulation and reduced WC and photosynthetic pigment content under prolonged drought. Terzi and Kadioglu (2006) reported similar transient changes in MDA contents under drought stress. Earlier, Tatar and Gevrek (2008) also reported that MDA content increases in wheat with the increasing degree of stress. Tian et al (2012) also reported increased MDA content in the leaves and petals of Marigold plant in response to drought stress. Notably, in

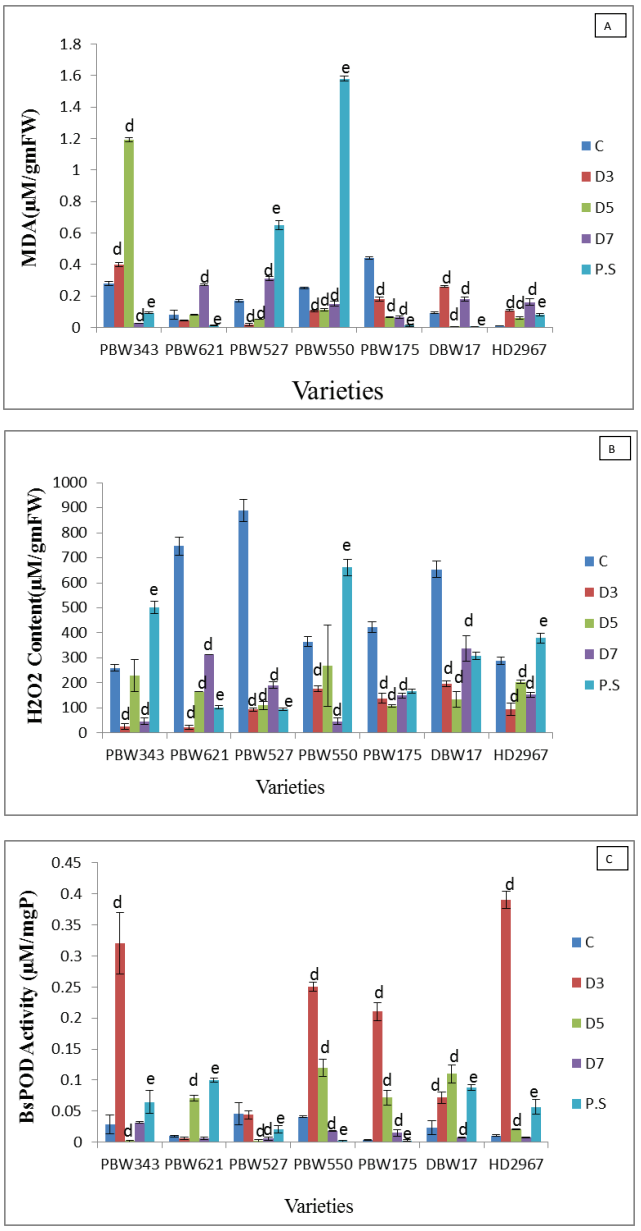

Figure 2. Effect of drought on MDA (A), and H_2O_2 (B) content and specific activity of BsPOD (C) in the shoots of different cultivars of *Triticum aestivum*. Data shown are averages ± SD (n=3). [d] represents significant difference relative to the control p≤0.5. [e] represents significant difference of PS relative to D7 p≤0.5.

PBW550 and PBW175, a substantial decrease in MDA levels were observed at D3, D5 or D7, suggesting that drought-induced BsPODs provided sufficient protection as seen in Figure 3A. The lower values of MDA in PBW175 and PBW550 may indicate that these cvs may have an efficient free radical quenching system, which can maintain higher membrane stability and lower peroxidation under drought treatment. These speculations are in agreement with previous studies

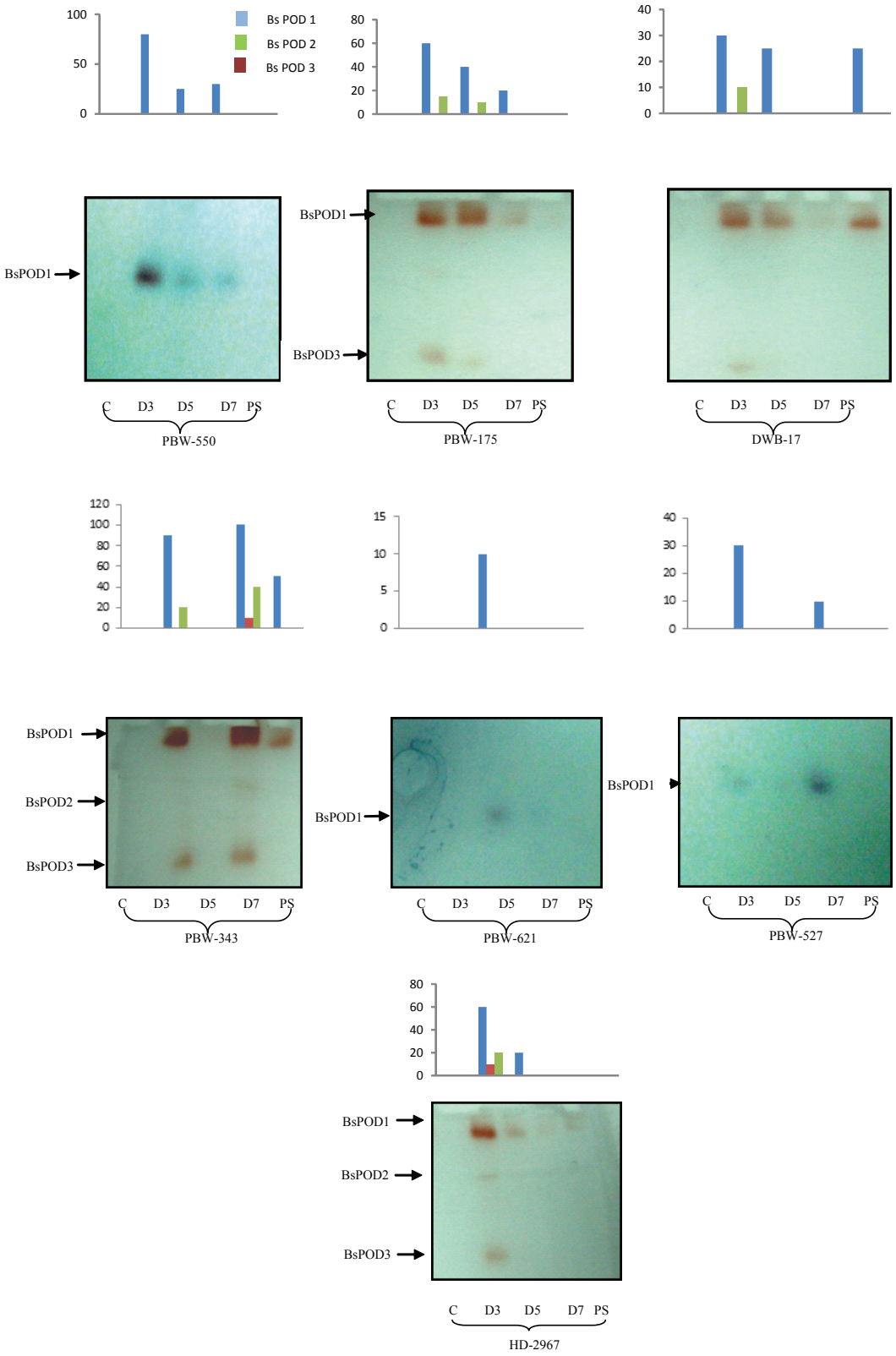

Figure 3. Drought-induced changes in BsPOD isoenzymes in shoots of different cultivars of *Triticum aestivum*. Bar graphs as shown in the top of panels indicate relative band intensities, which were determined using Ultraquant software of Gel Visualization, Documentation and Analysis system (Omega-Lum, USA).

of Sairam et al. (2000), Terzi et al. (2010) and Zhang et al. (2011) who found that low MDA levels were associated with drought stress tolerance in artichoke plans, common beans, and wheat, respectively. In PBW343, DBW17 and HD2967, MDA content increased in the early period of drought but it decreased during the further periods. During PS, MDA content was observed to be decreased in PBW621, PBW175, DBW17 and HD2967 while it increased substantially in PBW343 and PBW550. Earlier Simova-Stoilova et al. (2008) reported similar results in wheat varieties, which showed an increased MDA content on release of water stress.

Changes in H_2O_2

While ROS have the potential to cause oxidative damage to cells during environmental stresses, recent studies have shown that ROS play a key role in plants as signal transduction molecules involved in the mediation of responses to pathogen infection, environmental stresses, programmed cell death and different developmental stimuli (Mittler 2002). Among ROS, H_2O_2 seems best suited to play the role of signaling molecule due to its higher stability and longer half-life (Hung et al. 2005). If H_2O_2 serves as a stress signal, the fluctuation of H_2O_2 level in plants should spatially and temporally reflect changes in the environment. H_2O_2 content significantly decreased at D3 in PBW343, PBW550, PBW175, DBW17 and HD2967 (Fig. 2B) which might be a consequence of the increased BsPOD activities in these cultivars indicating that BsPOD activity have been involved in the detoxification of H_2O_2. Earlier Simova-Stoilova et al. (2008) reported similar results in four wheat varieties, which when subjected to drought stress showed a progressive decrease in H_2O_2 content due to the increased POD activities. Lee and Lee (2000) also documented similar findings upon cold stress in cucumber. In the present study, lower level of H_2O_2 coupled with lower values of MDA in these cvs indicate that these cultivars have an efficient free radical quenching system that offers protection against oxidative stress. Although H_2O_2 content decreased significantly at D3 day in PBW621 and PBW527, BsPOD activity also decreased, which might indicate that other antioxidant enzymes (such as APX, GR or CAT) might be involved in the detoxification of H_2O_2 in these cultivars. Earlier studies also documented that, besides antioxidant enzymes, non-enzymatic metabolites such as ascorbate and glutathione are involved in ROS scavenging. Furthermore, they, together with alpha-tocopherol, have been reported as to be involved in suppression of peroxidation of membrane lipids by reducing the MDA content and thus protecting the integrity of the bio-membranes (Gill and Tuteja 2010).Our results were in agreement with the studies of Bandurska et al. (1997), in which roots of two barley genotypes subjected to drought stress showed significantly decreased H_2O_2 content without increased peroxidase activity indicating that other mechanisms may also be involved in the detoxification of H_2O_2. During the post stress period, however, H_2O_2 levels increased significantly in PBW343 and PBW550 compared to those at drought stress. In PBW621 and PBW527, H_2O_2 content decreased considerably relative to that at D7.

Changes in BsPOD activity

The metabolism of ROS, such as that of H_2O_2, depends on various functionally interrelated antioxidant enzymes, such as PODs. Although abiotic stresses have been shown to induce one or more antioxidant enzymes, there has been little information on the role of POD at boiling level under various drought stress stages and recovery. To control the steady-state ROS levels, PODs are important enzymes of the antioxidant system converting H_2O_2 to water (Cosio and Dunand 2009; Miller et al. 2008).The changes in BsPOD activities in shoots of different wheat cultivars are shown in Fig. 2C. Our results showed that the seven cultivars responded to drought stress differently in terms of activities of antioxidant enzyme. Compared to the control, imposition of drought stress treatment significantly induced the BsPOD activity at D3 in PBW343 (11-fold), PBW550 (6-fold), PBW175 (52-fold), DBW17 (3-fold) and HD2967 (35-fold). The increased antioxidant enzyme activity correlated with decreased H_2O_2 content in drought stressed shoots. On the other hand, PBW621 showed a sharp decline in BsPOD activity after drought treatment and no change was observed in PBW527 at D3 stage, indicating a genotype specific regulation of BsPODs. As the duration of drought stress increased to severe stress (at D5), the BsPOD activity increased considerably in PBW621 and DBW17. In PBW343, PBW527 and HD2967, a significant decrease in the BsPOD activity was observed at D5. It should be emphasis that, as the duration of stress further increased to D7, only PBW175 maintained higher BsPOD activity (3.7-fold) compared to the control. This indicates that BsPOD activity is involved in maintaining the level of ROS and the stability of bio-membranes by controlling lipid peroxidation. As adaptive enzymes of the antioxidant system, PODs are known to play an important role in protecting membrane lipids from peroxidation and reducing cell damage being caused by oxidative stress in plants (Abedi and Pakniyat 2010; Lee and Lee 2000). All other cultivars showed a substantial decrease in BsPOD activity, which may reflect the low ROS scavenging capacity and increased damage in these cultivars. Tian et al (2012) also reported that POD activity declined significantly with increasing drought stress. Therefore, from the above described observation, it can be postulated that, out of the 7 varieties, antioxidant mechanisms in terms of BsPOD activity is enhanced in PBW175, which can be considered potentially tolerant and designated as best variety. Higher POD activity has been correlated with the relative drought tolerance of crop plants (Abedi and Pakniyat 2010; Mafakheri et al. 2011; Chakraborty and Pradhan 2012)

On the removal of stress after D7, a notable increase in Bs-

POD activity was observed in PBW343, PBW621, PBW527, DBW17 and HD2967 emphasizing the importance of cellular mechanisms that protect protein integrity and enable damage repair upon stress relief. Earlier studies on oxidative stress have also shown that the level of some antioxidants in relation with GR and APX may be higher during the recovery period than during water stress, as observed for example in cotton (Ratnayaka et al. 2003) or in pea (Mittler and Zilinskas 1994). This might indicate that either stress had induced an antioxidant response that 'hardens' the plants for future stressful conditions or/and that antioxidant protection in pivotal under recovery period. On the other hand, in PBW175 and PBW550, the BsPOD activity decreased significantly. The oxidative damage to cellular components is limited under normal conditions due to the efficient control of ROS through well coordinated and rapidly responsive antioxidant system consisting of several enzymes. Earlier, Mafakheri et al. (2011) also reported decreased activity of peroxidase during recovery in chickpea after drought stress conditions.

Changes in BsPOD isoenzyme

Patterns of BsPOD isoenzymes are shown is Fig. 3. After native-PAGE analysis, three BsPOD isoenzymes were detected in a genotype dependent manner. Earlier, Abedi and Pakniyat (2010) and Gratao et al. (2005) reported that number of isoenzymes of POD varies from plant to plant. Utilization of multiple isoforms of antioxidant enzymes is one of the major control mechanisms of cellular ROS detoxification. Imposition of drought treatment drastically provoked the expression of BsPOD1 isoenzyme at D3 in almost all cultivars except in PBW621. At D5, BsPOD1 isoenzyme was also induced in PBW621, PBW550, PBW175 and DBW17. Accumulation of BsPOD1 decreased in PBW343 and PBW527, which may be related to the low ROS scavenging capacity of these cultivars to remove ROS under drought treatment. Upon removal of water stress (PS), the BsPOD1 isoform was induced in DBW17, suggesting that the BsPOD1 isoform have participated in the repair of the cellular damage. Moreover, induction of new isoform(s) was also observed in the drought stressed shoots. Two minor differential boiling stable POD isoforms (designated as BsPOD2 and BsPOD3) were observed in shoots under drought treatment in PBW343, HD2967 and PBW175. Utilization of multiple isoforms in shoots may be one of the primary control mechanisms in plants to detoxify ROS. BsPOD2 isoform was induced in PBW343 at D3 and in HD2967 at D7. BsPOD3 was accumulated in PBW343, PBW175 and HD2967 at D3. During post stress period, differential BsPOD2 and BsPOD3 isoforms were not detected. In the different cultivars, along with various stages of drought stress, expression profile of isoenzymes well correlated with the changes observed in the biochemical activity. In PBW550, PBW175 and DBW17 a good correlation was observed between BsPOD1 and BsPOD activity at all the stages of drought stress. Increased band intensity and appearance of new isoenzyme bands may be an indication of an increase in the BsPOD activity under drought stress. Terzi and Kadioglu (2006) and Abedi and Pakniyat (2010) have also suggested that increased POD activity under stress conditions appears to be due to changes in the isoforms populations. Temporal and spatial drastic changes in expression of antioxidant isoforms have been reported previously (Baek et al. 2000; Kim et al. 2005).

To conclude, our results suggest that increased BsPOD activities due to temporal regulation or induction of new isoforms during the stress regimes were thereby dependent on plant genotype. Different wheat cultivars responded differently in response to different stages of drought stress. These results can be used as markers while breeding crops for drought stress tolerance in arid regions. In addition, PBW175 with high BsPOD activities, which may represent a higher capacity to protect against drought-induced oxidative damage, could be introduced to farmers as drought tolerant cultivar for arid and semi-arid environments. Out of the seven tested varieties, PBW175 showed much more pronounced antioxidant mechanisms and hence seemed to be protected from the negative effects of water stress even at the longer duration and increased severity.

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