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Role of Boiling Soluble Protein Disulphide Isomerase (BsPDI) under Drought Stress in Divergent Cultivars of Wheat

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Background: To protect them from water stress induced-ROS- mediated protein unfolding and aggregation, plants are equipped with a wide range of antioxidant redox molecular chaperonic proteins like Protein disulphide Isomerase (PDI) (E.C.5.3.4.1). These are a diverse group of proteins that *in vivo* bind to misfolded or unfolded proteins and play an important role to form specific three dimensional conformation of the functional proteins. In addition, stress conditions induce altered and intensified PDI expression in plant cell, thereby highlighting the role of these proteins under abiotic stress conditions.

The context and purpose of the study; The main objective of the study was to determine drought stress- induced changes in the modulation of the boiling soluble protein disulphide isomerase (BsPDI) in response to drought at two different developmental stages {38 Days Post Anthesis (DPA) and 52 DPA} in *Triticum aestivum.*

Results, the main findings; A temporal regulation of BsPDI accumulation in a cultivar dependent manner was observed under control and drought stress. SDS-PAGE and Western blot analysis revealed strong induction of BsPDI17 under drought conditions only in the tolerant cv. PBW 527 at 38 DPA. Contrary to this, unchanged BsPDI17 accumulation was detected in the sensitive cv. PBW 343 at 38 DPA under drought. However, at 52 DPA, there was a marked decline in BsPDI17 accumulation in the sensitive cv. PBW 621 under stress conditions.

Conclusions, brief summary and potential implications: Based upon our results, significance of BsPDI in the wheat cultivars differing in drought resistance during stress conditions is discussed.

Key words: Boiling soluble protein disulphide isomerase, chaperones, Days post Anthesis, drought stress, Triticum aestivum.

Drought stress occurring during grain filling (reproductive) stage of majority of cereal crops including wheat has become an important restraining factor for food security worldwide (Barnabas *et al.* 2008). With increasing burden on water supply, a major swing is now underway to improve the level of abiotic stress tolerance to meet the food demands (Peng *et al.* 2009). However, plant tolerance mechanism is a polygenic, complex and dynamic process aimed at an establishment of novel homeostasis through various morphological, biochemical and physiological responses at the cellular and whole organism level. Many are apparently adaptive, and most responses include a multitude of biochemical pathways associated with signal perception, transduction and regulation of gene expression in a spatial and temporal manner. A myriad number of genes, gene products and pathways associated with drought response have been recognized by a variety of experimental approaches (Gowda *et al.* 2004), yet the function of substantial number of the genes with altered expression under drought is in its infancy. Concerted efforts in identifying novel genes and studying their expression patterns in response to various abiotic stress conditions at different developmental stages of grains are required.

Plant cells produce various kinds of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anions and hydroxyl radicals under normal and stress conditions__that can damage cellular components, mediate protein unfolding and aggregation or act as important signal transduction molecules to trigger the cellular defense signalling cascades (Schwarzlander and Finkemeier 2013). Excess of unfolded or misfolded protein aggregates in the ER ensues ER stress which is alleviated through Unfolded Protein response (UPR) (Oslowski and Urano 2011). Protein disulphide isomerase (PDI) (E.C.5.3.4.1) is a component of the UPR along with other chaperones such as peptidyl prolyl cis transisomerase (PPI), BiPs, (also called Calnexin, CNX) and Calreticulin, CRT. PDI is a dithiol disulphide oxidoreductase catalysing dithiol/disulphide interchange reactions depending upon their polypeptide substrates. Typical plant PDI consists of four domains and an ER resident signal peptide Lys-Asp-Glu-Leu (KDEL) at the C terminus. The 2 domains (a and a') have high sequence similarity to thioredoxin containing the active site -Cys-Gly-His-Cys-. The 2 remaining domains (b and b') are responsible for providing the principal substrate binding site (Kim *et al.* 2012). These proteins were primarily studied in yeast and mammals but later homologs of these ER molecular chaperones have also been discovered in higher plants (Vitale and Boston 2008). Intriguingly, the conservation of proteins among different species and kingdom highlights the existence of common protein folding pathway. The molecular chaperones along with ascorbate, glutathione, thioredoxin determine the half-life of various ROS molecules and the redox potential of the plant cells (Foyer and Noctor 2013). These UPR proteins switch their protein function and structure from low to high molecular weight complexes in a redox dependent manner in response to various environmental cues (Chi *et al.* 2013).

Despite recent data on the complexity and diversity of the PDI gene family in plants, there are still a number of unanswered questions concerning the physiological functions of PDI at boiling level under abiotic stress conditions including drought. Some of the drought responsive proteins are boiling soluble having the ability to remain in solution after the boiling treatment and being termed as "boiling stable proteins" (Jacobsen and Shaw 1989). These proteins are exemplified by random coil conformation and hydrophilicity property empowering them to trap water inside the cells so as to attenuate the damage due to drought stress. Many of the proteins detected in total protein extracts, under drought stress, are lost in the boiling-soluble fractions (Pelah *et al.*1995). Therefore, to better understand the biochemical role of PDI in drought-stress tolerance, it is a prerequisite to examine their accumulation not only under water stress but also after boiling. The response of the plants to water stress depends upon several factors such as developmental stage, severity and duration of stress and cultivar genetics (Beltrano and Marta 2008). Therefore, understanding the biochemical and physiological basis of water stress tolerance in different wheat genotypes at different stages of

development is a prerequisite to select plants for improving crop water stress tolerance. Accordingly, this study was conducted in the seeds of drought-tolerant as well as drought-sensitive cultivars of wheat in order to determine the likely alterations in the accumulation of boiling soluble protein disulphide isomerase (BsPDI) in conferring drought resistance in different wheat cultivars. These proteins could be chosen as putative key elements for understanding molecular mechanisms involved in proper protein synthesis/ assembly under drought. Maintaining crop yields under adverse environmental conditions is probably the major challenge facing modern agriculture where PDI can play important role.

MATERIALS AND METHODS

Growth and stress conditions

Seeds of *Triticum aestivum* L. cvs. PBW 175, PBW 527 (drought-tolerant) and PBW 621, PBW 343 (drought-sensitive) divergent in degree of drought resistance (Sharma *et al.* 2014) were obtained from PAU Ludhiana, Punjab, India. The seeds were surface-sterilised with 1% (w/v) mercuric chloride and 70% ethanol. Plants were raised up in 5-dm^3 pots and kept in a net house under natural conditions. Seeds at two reproductive phase (38 and 52 days post-anthesis (DPA) were water-stressed by withholding water supply for consecutive 5 days, whereas the control plants were watered daily. The samples were harvested in triplicate, pooled and used for further analysis. Water content (WC) was calculated as: (FW – DW) / DW, where FW is fresh weight and DW is dry weight. Fresh seed tissues were weighed to obtain FW; for DW, seeds were dried at 72 º C for 72 h until constant weight was achieved.

Extraction of proteins soluble after boiling and Western blot analysis

The Boiling Soluble Proteins (BSPs) were extracted using 50 mM Tris-Cl buffer (pH 7.0) as described previously (Rakhra *et al.* 2015). Western blot analysis was carried out according to Rakhra *et al.* 2015 with minor modifications using antibody against Protein disulphide isomerase (Anti-Protein Disulphide isomerase Rabbit ,pAb, Calibiochem®) at a dilution of 1:1000.

Statistical analyses

The plants were distributed over a completely randomised design, with 16 treatment combinations, forming a 4 X 2 X 2 factorial (four genotypes, two watering regimes and two samplings) at reproductive phases. All results are presented as mean of three replicates ± S.E. (standard error). Each replicate contains a set of three seeds. Data was subjected to Student's t-test for differences between pairs of data. Means that showed significant difference at $P \leq 0.05$ were considered.

RESULTS AND DISCUSSION

In the present study, effect of drought stress on BsPDI at two developmental stages of caryopses in drought-tolerant and drought-susceptible wheat cultivars were investigated.

Drought-induced changes in the Water Content (WC) of seeds

Imposition of drought stress resulted in either decrease or no change in Water content (WC) at two different growth stages. At 38 DPA, WC recorded a substantial depreciation in all the cultivars under drought stress. However, at 52 DPA, WC decreased considerably under stress in all the cultivars except cv. PBW 343, in which it remained unchanged (Fig.1).

Drought-induced changes in the expression of BSPs in seeds

Fig. 2 shows the protein profile in seeds of drought-tolerant cvs. PBW 175, PBW 527 and drought-susceptible cvs. PBW 621, PBW 343 at 38 and 52 DPA. At 38 DPA, after boiling treatment of 15 min, many protein bands disappeared while some medium- and low-molecular-weight (LMW) protein bands in the range 14–29 kDa were observed in seeds of all the cultivars under control and drought conditions (Fig.2a). This indicates boiling soluble nature of the existing peptides under control and drought in all the cultivars. It is remarkable here that in cv. PBW 527, accumulation of BSP with molecular weight ~17 kDa (marked by an arrow) was enhanced under drought at 38 DPA suggesting the role of this boiling soluble protein in conferring stress tolerance in tolerant cv. PBW 527. However, at 52 DPA, few barely detectable LMW protein

bands were seen under control and drought conditions in all the cultivars (Fig.2b). Quantitative differences in the synthesis of boiling soluble protein disulphide isomerase (BsPDI) among the four cultivars were further analysed by western blot analysis at 38 and 52 DPA.

Changes in BsPDI

PDI are ubiquitous thiol- disulphide oxidoreductase catalyzing dithiol oxidation, disulphide bond reduction and isomerisation of its substrate proteins until they fold into a native stable conformation (Gruber *et al.* 2007). PDI proteins participate either in a normal protein folding pathway or for rescuing proteins that have become misfolded under stress. Western blot analysis using anti-PDI antibody detected BsPDI17 at 38 DPA in the cvs. PBW 175 and 343, with expression remaining same under drought conditions (Fig.3a). However, in cv. PBW 621, BsPDI17 expression was enhanced slightly under drought condition at 38 DPA. Interestingly, in the tolerant cv. PBW 527, there was a strong induced expression of the BsPDI17 under stress condition which corresponds to a differential band (~ 17 kDa) marked by an arrow in SDS PAGE gel (Fig 2a). The induced BsPDI17 expression in the tolerant cv. PBW 527 at 38 DPA under stress indicate its role in improving protein folding and transport during stress. Being a part of the antioxidative defense system, PDI has an important function in the thioredoxin based redox pathway by (1) eliminating aberrant disulphides introduced by ROS mediated oxidative conditions (2) reduce disulphides to activate antioxidative defense proteins (Rigobello *et al.* 2001).

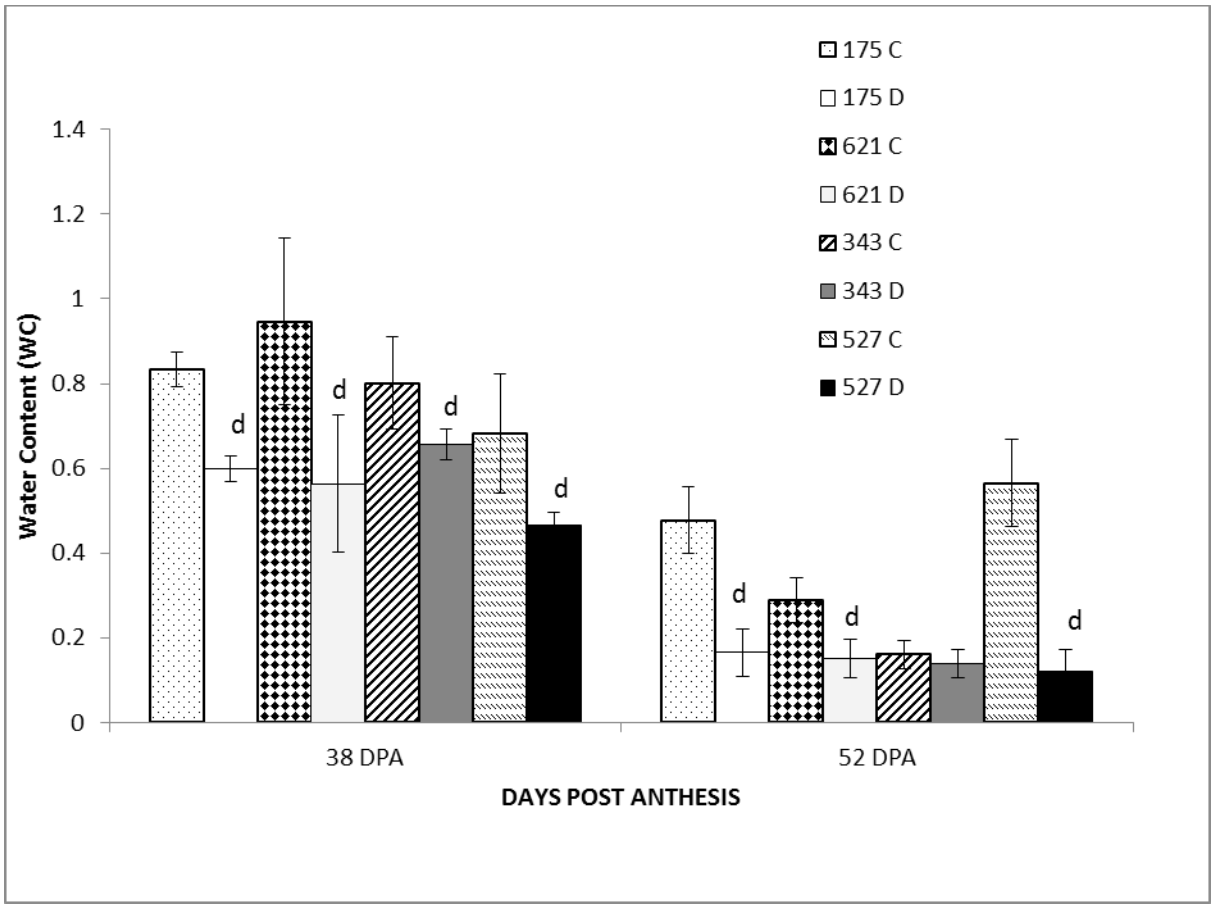

Figure 1. Effect of drought stress on water content (WC) in seeds of drought-tolerant (PBW 175 and PBW 527) and drought-sensitive (PBW 621 and PBW 343) cultivars of *Triticum aestivum* at two different developmental stages (38 and 52 DPA) under control (C) and drought (D) conditions. Data shown are Mean ± SE of three replicates. Each replicate contain a set of three caryopses. ^{d} indicates significant difference vs. control at $P \leq 0.05$. Vertical bars indicate standard error. When no bar is shown, the standard error is smaller than the symbol.

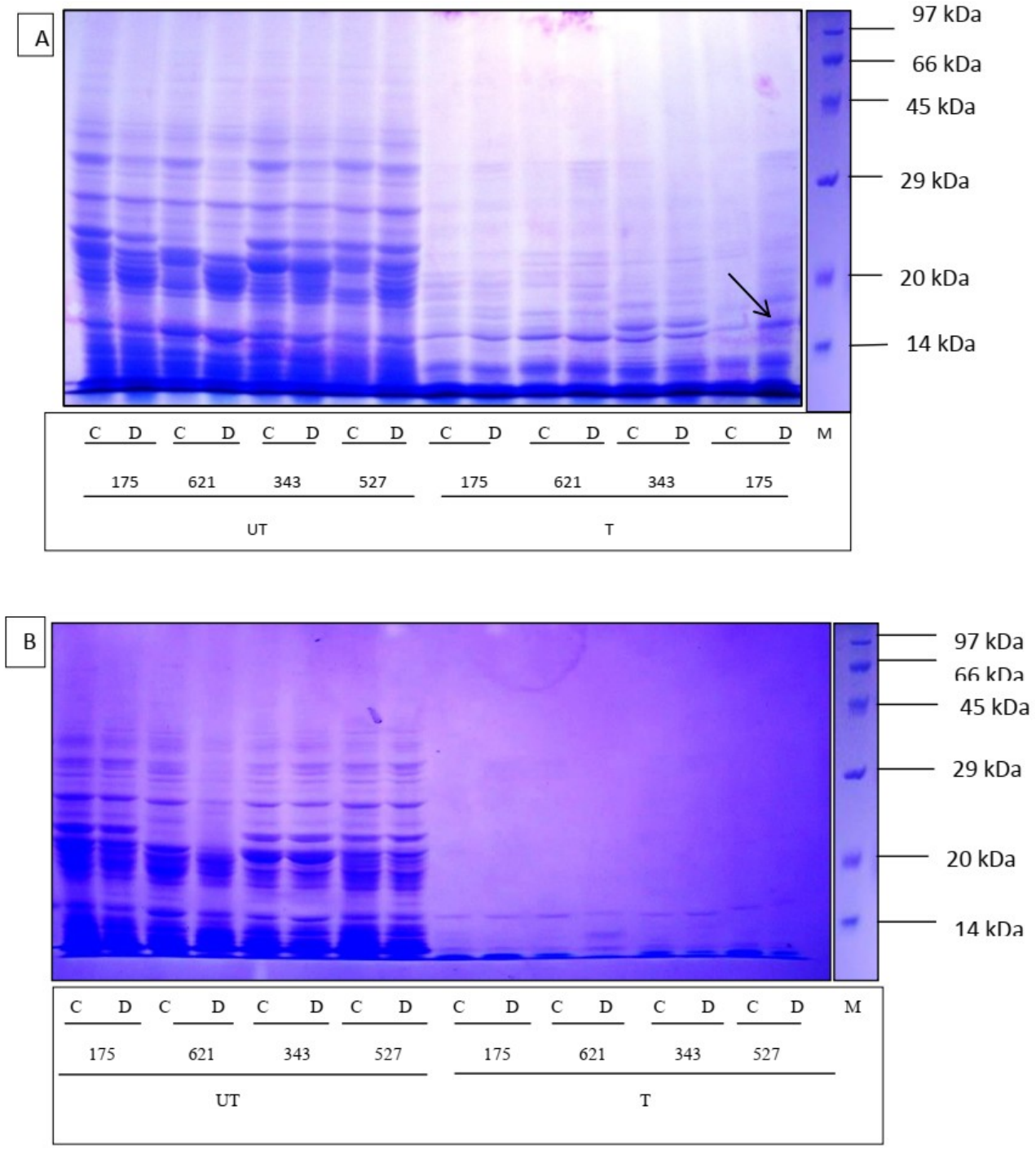

Figure 2. SDS PAGE profile of proteins in seeds of drought tolerant (PBW 175 and PBW 527) and drought sensitive (PBW621 and PBW343) cultivars of *Triticum aestivum* harvested at two different reproductive stages : 38 DPA (A) and 52 DPA (B) under control (C) and drought (D) conditions. Each lane loaded with 120 µg of protein sample. DPA: Days Post Anthesis. M: Molecular weight marker. UT- Untreated / Unboiled protein samples, T- Treated/Boiled protein samples.

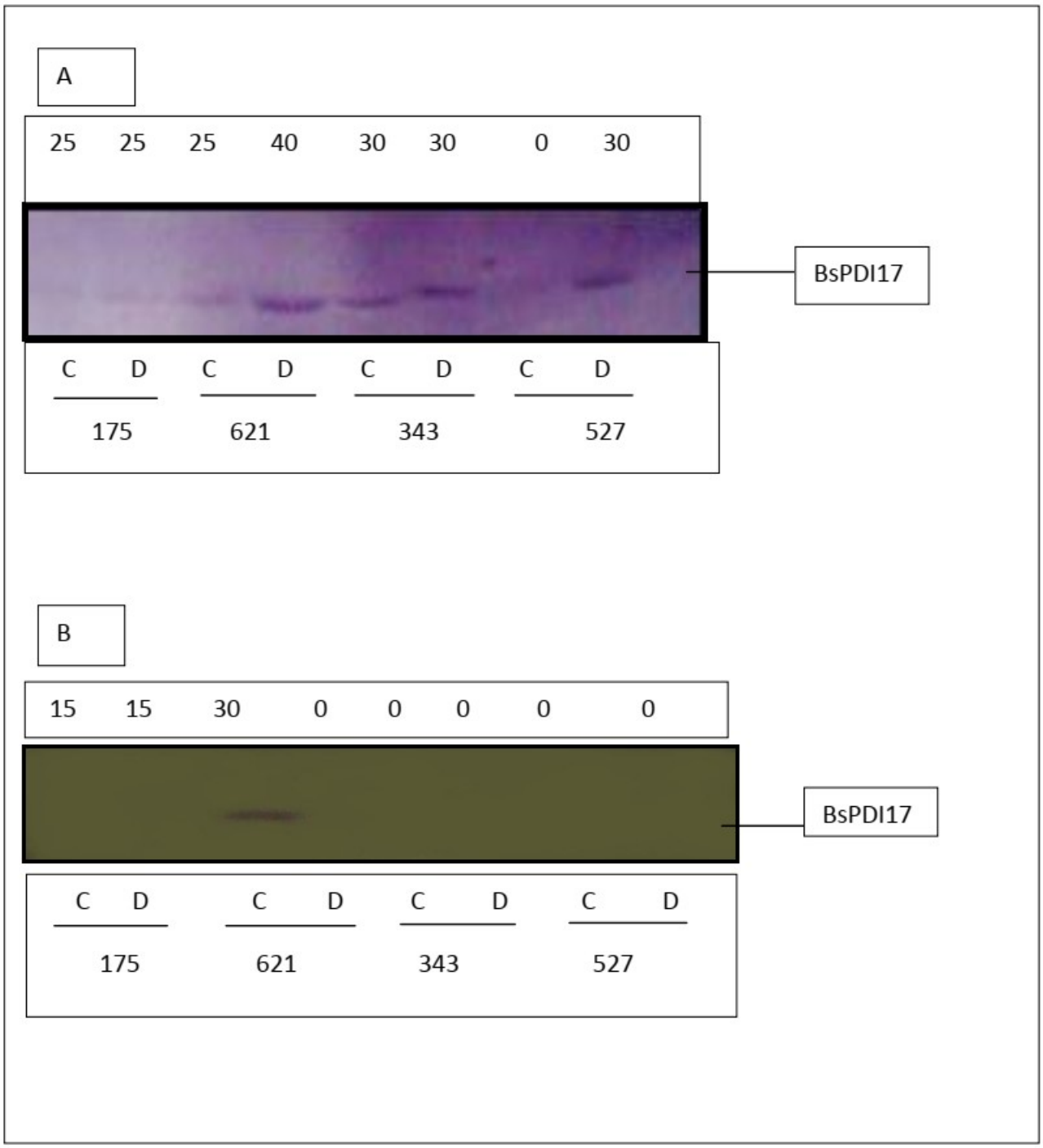

Figure 3. Immuno-blot analysis of boiling soluble protein disulphide isomerase (BsPDI) in seeds of drought tolerant (PBW 527, PBW 175) and drought sensitive (PBW 343, PBW 621) cultivars of *Triticum aestivum* at two different reproductive stages : 38 DPA (A) and 52 DPA (B) under control (C) and drought (D) conditions. Numerical values as shown in the top of panels indicates relative band intensities which were determined using Gel visualisation, Documentation and Analysis system (Bio- Rad,USA).

Consistent with our study, Zhu *et al.* 2014 studied the effect of PEG6000 on the PDI expression in Brachypodium and found that four genes (*BdPDIL1-1, BdPDIL1-2, BdPDIL7-2, BdPDIL2-1* were significantly upregulated. Also, high expression of BsPDI17 in the developing grains of wheat under control conditions in all the cultivars at 38 DPA seems congruent with its metabolic role of participating in the redox regulation of cytosolic AGPase (an enzyme involved in the starch synthesis) as has been postulated by d Aloisio *et al.*

2010. Besides this, BsPDI17 expression at 38 DPA is consistent with their proposed role for the correct synthesis and accumulation of seed storage proteins like prolamins which is related to the gluten quality. However, at 52 DPA, there was a remarkable decline in the accumulation of BsPDI17 under stressful conditions in the sensitive cv. PBW 621. Decline in BsPDI17 accumulation in the sensitive cv. PBW 621 indicates that drought damage may be related to the disruption of protein folding and stability leading to chaperonic dysfunction. In line to our findings, Xu *et al.* 2008 also reported that PDI was downregulated only in the roots of heat sensitive *Agrostis stolonifera*. No immunoreactive protein band of BsPDI17 was detected in the cvs. PBW 343, PBW 175 and PBW 527 under control and drought conditions at 52 DPA. Thus, based upon our observations, we can say that diversified expression exhibited by BsPDI can be explained by the need of the temporal regulation to accomplish the diverse roles of PDI in caryopses of wheat at different developmental stages.

CONCLUSION

The present study highlights the role of BsPDI in wheat under normal and stress conditions. Notable induced BsPDI17 expression in the tolerant cv. PBW 527 at 38 DPA and a marked decline of BsPDI17 in the sensitive cv. PBW 621 at 52 DPA is indicative of its enhanced role as a molecular chaperone under stress in the tolerant cv. PBW 527. Accumulation of the BsPDI in drought tolerant and susceptible cultivars of *Triticum aestivum,* as evident in our study, could reveal the potential of this protein as biochemical marker in breeding programmes for making drought tolerant high yielding crops and can suggest possible targets for the enhancement of drought tolerance in crops by genetic engineering. Further studies having more diverse wheat cultivars using proteomic and molecular approaches are required to elucidate the further role of BsPDI in water stress tolerance.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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