Short communication

# Expression analysis of some boiling stable proteins (Hydrophilins) under combined effect of drought stress and heat shock in drought tolerant and susceptible cultivars of *Triticum aestivum*

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**Abstract:** The combined effect of drought stress and heat shock on the induction of boiling stable proteins viz: WGA, SOD, HSP90,Aquaporin, CyPs, APase and LEA proteins was studied in 3-days old seedlings of drought tolerant and drought susceptible cultivars of wheat. Boiling stable protein profile was outlined via SDS electrophoresis of tissue extracts. The results obtained were confirmed by Immunoblot analysis with anti-WGA, anti-SOD, anti-HSP90, anti-APase, anti-Aqua, anti-Cyp and anti-LEA antibodies. Western blot analysis revealed the induction of boiling stable proteins (SOD, HSP90, Aquaporin, CyPs) during combined drought and heat stress (DH) conditions as compared to separately applied heat (H) and drought treatments (D) in drought tolerant cultivars of wheat, indicating their role in water stress adaptation under simultaneous applied abiotic stress conditions. Alternation in boiling stable protein expression was more pronounced in seeds as compared to shoots of both the cultivars. Based upon these observations the possible role of hydrophilins in water stress tolerance is discussed.
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## Introduction

Throughout their life cycle, plants are subjected to many adverse environmental conditions such as drought, heat, cold and flooding etc. that dramatically affect plant survival and limit productivity (Serrano et al. 1999). Some environmental factors (such as temperature) can become stressful in a few minutes, others may take days to weeks (soil water) or even months (mineral nutrients) to become stressful. Most cultivated crop plants are highly sensitive and either die or display reduced productivity after they are exposed to long periods of abiotic stresses. All these stresses are often interconnected and may induce similar cellular damage (Ingram and Bartels, 1996). Under field conditions, plants are often simultaneously exposed to soil drying and high temperature stresses. These two stress factors could create water deficit in plant tissues, which in turn may affect the synthesis of stress-induced proteins. In most of the studies on stress-associated proteins; plants have been exposed to only one environmental stress factor viz: high temperature. Although abiotic stress response has been studied considerably in recent years (Chaves et al. 2003), however, analyzing the effect of single stress on plants can be very different from conditions encountered by plants in the field in which a number of different stresses may occur simultaneously. These can alter plant metabolism in a novel manner that may be different from that caused by each of the different stresses applied individually. It may require a new type of response that would not have been induced by each of the individual stresses. Plants inherently posses various molecular-biochemical mechanisms that are involved in stress tolerance (Ingram and Bartels, 1996). Some of these stress-responsive genes encode regulatory proteins, soluble proteins, appearance of new isozymes; whereas others protect cells by causing the accumulation of metabolic proteins and cellular protectants including sugars (Ingram and Bartels, 1996). These stress-induced responses enable the plant to adapt its physiology and survive. Stress induced proteins play a definite role in protecting plants from possible damage by these conditions. Concomitant to induced stress tolerance, protein metabolism of the cells undergoes changes in terms of acquiring specific stress proteins, which are either not detected or present in low amounts in the un-induced cells (Ingram and Bartels 1996). A growing body of evidence suggest that stress response involves synthesis of one set of proteins and degradation of the other (Serrano and others. 1999). Therefore, stress responsive changes in gene expression in general and protein profiles in particular have been targeted for intensive investigation. One of these mechanisms that may confer stress tolerance is the activation of a large set of genes, which leads to the accumulation of specific cellular proteins. Heat shock proteins (HSPs), dehydrins and late embryogenesis proteins

(LEAs), are the major groups of stress-induced proteins which believed to contribute to the protection of cellular structures and metabolites during water stress (Chaves and others. 2003). In addition, these proteins accumulate to high levels during natural growth of seed development and maturation when a loss of water from the cell occurs (Rurek , 2010). These proteins also seem to respond similarly to the application of ABA (Chaves and others. 2003). Some drought stress-induced proteins (e.g. dehydrins, LEAs) are highly hydrophilic and remain soluble even after boiling (Close et al. 1989), a characteristic that has been termed "boiling stability" (Jacobsen and Shaw 1989). Even some of the proteins detected in total protein extracts, under drought stress, are lost in boiling treated extracts (Pelah and others. 1995). Earlier research also indicated that hydrophilins represent less than 0.2% of the total protein of a given genome (for review see Battaglia et al. 2008). Bioinformatic analyses of hydrophilins from several kingdoms including plant, bacteria and fungi have revealed the conservation of glysine-rich regions in these proteins, thus, suggesting an evolutionary role for these cellular boiling stable proteins during water-deficits (Arroyo and others. 2000). Accordingly, data suggest that hydrophilins have evolved independently in different protein families and in different organisms, but with the similar goal of protecting specific functions under partial dehydration. It is noteworthy that all hydrophilins from different phyla show higher expression under water limiting conditions, imposed by environment. This is not only the case for LEA and non-LEA like hydrophilins from plants, but also for hydrophilins expressed in bacterial and fungal spores. Although the functional role of hydrophilins remains speculative, there is evidence supporting their participation in accilimation and/or in the adaptive response to abiotic stresses. Overexpression analysis of some hydrophilins (LEAs) revealed enhanced salt, cold and drought tolerance in plants, indicating their role in water limiting conditions (Battaglia and others. 2008). It was reported earlier that the large number of more hydrophilic residues like Gly probably confers a very flexible backbone and this is likely responsible for the boiling stability of these proteins. It facilitates the formation of intramolecular hydrogen bonding and thus gives the protein a random coil conformation. This property allows the protein to stretch, bend and expand in all directions, a property that could be useful to protect cellular structures against water stress.

At present hundreds of genes induced under water stress have been identified which may allow plants to adapt to water limiting conditions. Because plant responses to environmental stresses are complex and multigenic, the functions of many of the induced genes and their related products are still a matter of conjuncture (Bray, 2002). Therefore, to better understand the role of these proteins in water stress tolerance, it is a prerequisite to examine their expression not only under water stress, but also after boiling of extracts. Thereafter, the sequencing of the relevant hydrophilic proteins and cloning of the corresponding genes will generate probes for early selection of drought resistant genotypes. Therefore, to assess the role of these proteins in water stress adaptation it is imperative that variability in boiling stable proteins (BSPs) should be studied in stress tolerant and susceptible cultivars of a crops. In the light of these observations, the proposed study was undertaken to investigate the effect of combined drought and heat stresses on the expression of some boiling stable proteins like: SOD, WGA, HSP90, Aquaporins, CyPs, APases, and LEA in the drought tolerant and susceptible cultivars of wheat so as to gain an insight into the physiological role of these proteins in water stress adaptation and the possible implication as a marker for drought stress tolerance. Wheat is one of the most important crops in arid and semi arid areas worldwide and is sensitive to drought and temperature stress. In view of this, we have chosen wheat as an important tropical crop for the present investigation. To facilitate the detection of BSPs, we focused on heat stable (HS) fractions that resists coagulation upon heating at 100°C. By this method, the soluble protein extract containing hydrophilic proteins could enriched with BSPs and devoid of storage proteins.

## Materials and Methods

*Seed germination and growth conditions*

The seeds of *Triticum aestivum* L. cvs. PBW 527 (drought tolerant) and PBW 343 (drought sensitive) were procured from PAU Ludiahana, Punjab, India. Seeds were thoroughly rinsed with deionized water and imbibed for 6 h. After imbibition, seeds were placed in Petri plates containing sterile filter sheets, moistened with water. The plates were incubated at 25 ±1°C in a seed germinator in darkness and allowed to grow for 3 days (Sharma and others. 2012). Stress treatments were performed on 3 M Whatman filter paper. For combined drought stress and heat shock treatment, 3-day old seedlings were exposed to 3-day drought stress followed by 4-h heat shock (42°C). Individual drought stress was imposed to 3-day old seedlings for 3 days. Heat treatment was imposed to 6-day old seedlings for 4-h at 42°C. The tissues (seeds/shoots) from all treatments were harvested and pooled for further analysis.

*Extraction of proteins*

Tissues (seeds/shoots) were homogenized with chilled mortar and pestle in extraction buffer (50 mM Tris-HCl, pH 7.0). Crude extracts were centrifuged at 10,000 g for 10 min, and total protein content in the supernatant was determined by the Bradford method busing BSA as a standard (Bradford, 1976). Protein samples were resolved on SDS-PAGE on 15% (w/v) polyacrylamide gel and visualized by Coomassie brilliant blue as described in Sambrook and others. (1989).

*Western blot analysis*

Western blot analysis was carried out with antibodies against WGA (wheat germ aggulitinin), SOD (superoxide dismutase), Aquaporin, HSP90 (heat shock protein 90 kDa), APase (Acid phosphatase) and CyP (Cyclophilin). After electrophoresis, proteins were electroblotted to a nitrocellulose membrane (Hybond C extra, GE Healthcare). Protein blots were reacted with anti-WGA (1:500 dilution), anti-SOD (1:2000 dilution), anti-Aqua (1:3000 dilution), anti-APase (1:2000 dilution), anti-Cyp (1:1000 dilution) and anti-LEA (1:500 dilution) and developed using an alkaline phosphatase-conjugated secondary antibody (1: 500 dilution) and 5-bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt/*p*-nitroblue tetrazolium chloride reagent systems (Sambrook and others. 1989).

**Results and discussion**

In the present study, effect of combined drought stress and heat shock (DH) was studied on the expression of some boiling stable proteins viz: WGA, SOD, HSP90, Aquaporin, APase, CyP and LEA in drought tolerant (PBW 527) and drought susceptible (PBW 343) cultivars of wheat. Our results strongly suggest that the effect of this combination (DH) on plants is very different from that of drought and heat shock applied individually. Earlier, Mittler and others. (2006) also claimed that simultaneous exposure to different stresses would result in co-activation of various stress response pathways with synergetic or antagonistic effects and that their combination should be regarded as new state of abiotic stress in plants. To examine the role of boiling stable hydrophilins, the BSPs expression was examined in the samples collected, run on a 12% SDS-PAGE followed by immuno-blotting and analyzed. Figure 1 shows the boiling stable protein (BSP) profile of seeds and shoots of drought tolerant cultivar (PBW 527) and drought sensitive cultivar (PBW 343) under drought (D), Heat (H) and combined heat and drought (DH) conditions. As can be seen in both the cultivars, seeds exhibited a more number of protein bands (high mol wt and low mol wt), however, in shoots very few barely detectable BSPs (high mol wt) were observed. Such observation is quite expected, since it is well known that an intense proteins synthesis take place in the reproductive plant structures (Duck et al. 1989). In seeds several groups have proposed that proteins were critical for protection of cellular components during seed development. Although both lines almost had almost similar pattern of synthesis of low mol wt and high mol wt proteins, some quantitative differences in the synthesis of proteins between the two lines might exist, which were further analyzed by immunoblot analysis.

**Western blot analysis of BsWGA49 under drought (D) heat (H) and combined DH conditions**

Wheat germ agglutinin (WGA) is the best characterized lectin, which supposed to be maximally synthesized in the developing embryos (Triplett and Quatrano, 1982). Enhanced accumulation of WGA in response to abiotic stresses has been reported earlier (Plant and others. 1991), however, its physiological function as boiling stable proteins is still a matter of conjuncture. Anti-WGA immuno-blotting of seed samples of drought tolerant cv. PBW-527, detected a strong cross-reacting protein band at about 49-kDa (BsWGA49) under heat (H) stress only, indicating its role during higher temperature. However, no expression of BsWGA49 was observed under D and DH conditions (Fig 1A). The absence of WGA expression in response to D and DH conditions indicates that the regulation of these proteins differ from that of number of other high-temp induced proteins. It is also plausible that at high temp, BsWGA49 may be involved in seedling development. Earlier studied also documented the role of wheat germin during seed germination (Hurkman and others. 1991). Jaikaran and others., 1990, also speculated that wheat germin could have a role in cell wall expansion during seed germination in cereals.

**Immuno-blot analysis of BsSOD under drought (D) heat (H) and combined DH conditions**

It was reported earlier that water deficit stress often results in oxidative stress. It arises from the production of free radicals or ROS (reactive oxygen species) which damage proteins by amino acid modifications, fragmentations of aggregation of cross linked reaction products, or increase susceptibility to proteolysis (Bowler, 1992). Plants contain a number of enzymes like SOD, catalase, peroxidase, GST that catalysis the cascade of ROS and convert them into less reactive products (Gazanchian and others. 2007). Superoxide dismutase (SOD, EC 1.15.1.1) is ubiquitous, being widely

distributed among $O2^-$ consuming organisms and is the first line of defense against oxidative stress. Anti-SOD Immuno-blotting of seed samples of both the cultivars, detected BsSOD30 band under combined DH conditions. However, in cv. PBW 527, the relative expression of BsSOD30 was remarkably higher, as compared to sensitive cv. PBW 343 under combined DH conditions (Fig 1B). Consequently higher expression of BsSOD30 may represent a kind of anticipating mechanism for protection of developing seeds against stress-provoking factors. Applied alone, drought stress (D) did not provoke any changes in BsSOD30 levels. However, under heat (H) stress alone, we also detected another differential protein band of 35 kDa (indicated by arrow), having antigenic similarity with SOD, in cv. PBW 527, indicating its important role in response to heat stress. Earlier, abundance of heat stable SOD was shown to be up regulated in chenopodium (Khanna Chopra and Sabarinath, 2004), However, in shoots, no detectable cross-reacting protein bands were observed under all the conditions, suggesting tissue specific induction of BSPs. Differences in the expression of specific gene products between stress-sensitive and stress tolerant cultivars indicate that tolerance is conferred by genetically encoded mechanisms (Bray, 1993) so, it is reasonable to expect the inter- and intra-specific differences in the pattern of protein synthesis between plants which differ in their stress resistance.

**Immuno-blot analysis of BsHSP90 under drought (D) heat (H) and combined DH conditions**

HSPs represent a large protein family that includes several subfamilies (HSP 90, HSP 70 and HSP60). HSPs are found in all organisms exposed to high temperature stress and many posses molecular chaperone activities, which involve in the proper folding of native polypeptides and in helping damaged proteins to regain their biological active structures (for review see Waters and others. 1996). Accumulating evidence showed that plants HSPs are not only expressed in response to heat shock, but also upon water, salt and oxidative stress and at low temperature (Waters and others. 1996). The antiHsp90 monoclonal antibody that we used for immune-blot detection, recognized protein band of Mr 66 kDA in seeds as well as shoots of both the cultivars, but in different amounts (Fig 1C). During DH conditions, in cv. PBW 527, the BsHSP66 level was substantially higher as compared to drought sensitive cultivar PBW 343, confirmed its important role for survival under combined stress conditions. Common responses to different stress conditions in both the tissues may indicate similar functions of stress-responsive gene products for plants under stress conditions involving water deficit.

**Immuno-blot analysis of BsApase under drought (D) heat (H) and combined DH conditions**

Acid phosphatases (APases) are widely found in plants having intracellular and extracellular activities. APases are believed to be important for Pi scavenging and remobilization in plants, but role of boiling stable APases has not been critically evaluated under abiotic stresses. Acid Phosphatases (APases; EC 3.1.3.2) largely catalyze the hydrolysis of Pi from small molecules, which are believed to be important for many physiological processes, including regulation of soluble phosphorous (Pi) (Yan et al., 2001). As shown in Fig. 1 D, western blot analysis detected a strong protein band (BsAPase67) being constitutively expressed in both the tissues of cv. PBW 527 and cv. PBW 343. Contrary to cv. PBW 343, the relative concentration of BsAPase67 was rather higher in cv. PBW 527 under heat stress (H) in seeds and droughted (D) shoot samples. From these observations it was suggested BsAPase14 may be playing a significant role in the maintenance of orthophosphate (Pi) levels in the germinated tissues under stress conditions. Earlier studies also reported that APases were implicated in providing Pi during seed germination from stored phytate (for review see Vance and others. 2003). It may also be possible that under conditions of drought, delivery of phosphate (Pi) is impaired, thus, resulting in the activation of the cellular phosphatases that release soluble phosphate from its insoluble compounds thereby modulate osmotic adjustment by free phosphate uptake mechanism. Olmos and Hellin (1997) also observed that acid phosphatases are known to act under salt stress by maintaining a certain level of inorganic phosphate which can be co-transported with $H^+$ along a gradient of proton motive force.

**Immuno-blot analysis of BsAuap under drought (D) heat (H ) and combined DH conditions**

Aquaporins (Auap's/AQPs) belong to the major intrinsic proteins (MIP family), members which are found in almost all living organisms, are believed to increase water permeability in roots and also maintain the physiology and development of leaves (for review see Heinen and others. 2009). Regulation of their expression and activity has been reported to be modulated by dehydration and ABA. Evidences are accumulating that AQPs play an important role in plant hydraulic relations at the cell, tissue, organ and whole plant level. They facilitate the rapid, passive exchange of water across cell membranes and are responsible for up to 95% of water permeability of plasma membranes (Heinen

and others. 2009). The mechanism by which AQPs synthesis is enhanced and its in vitro remain poorly understood. In this study, the antiAqua5 antibody that we used for immuno-blot detection recognized five distinctive, tissue specific bands. These bands with molecular mases of 60kDa, 48kDa, 34kDa,29kDa and 28kDa were herein designated as BsAqua1, BsAqua 2, BsAqua 3, BsAqua 4, and BsAqua 5, respectively (Fig 1E). Although it is reasonable to suppose that these bands represent distinct AQPs isoforms, the possibility that at least some of them are degradation products of those with higher molecular masses can not be ruled out. As compared to drought susceptible cv. PBW 343, the expression of BsAqua 1 was considerably higher in cv. PBW 527 during combined DH conditions. Interestingly, no other isoform except BsAqua 1 was detected in shoots under all conditions in both the cultivars. The BsAqua 2 was the only AQP that was exclusively present in the seed sample of cv. PBW 527 under H stress. Our study clearly indicated that the relative concentration of the examined AQPs was disproportionately distributed over distinct tissues in both the cultivars. The unequal distribution of AQPs bands in the shoots versus seed tissues under physiological conditions in both studied populations suggest that different species of this protein may serve diverse cellular roles with in different tissues. Different responses of AQPs (up/does-regulated/ no change) to abiotic stresses suggest that AQP isoforms can be divided to different groups which can contribute differently to water transport and regulation, with some being stress responsive. Further, differential responses of AQPs to water stress were found in drought tolerant and susceptible cultivars, indicating that AQPs present in the same species, but in different cultivars can respond differently to water stress depending upon their tolerance to water deficit. High expression of AQPs in seeds may be indicating that cells grow through irrereverseble expansion of cells, a process that requires the continues uptake of water. So it is tempting to believe that they are involved in the growth process under water stress conditions. This suggestion has been strengthen by demonstrations that introduction of aquaporin gene (OsPIP1) in drought-sensitive cultivar of rice resulted in higher leaf water potential and transpiration rare, indicating the role of OsPIP1 in drought resistance (Heinen et al. 2009). Earlier studies also indicated that overexpression of Arabidopsis homolog of MtPM25 in germinating seeds led to improved growth under high NaCl, KCL and sorbitol conditions (Borrell et al. 2002). Liu and Zheng (2005) also reported similar findings by overexpressing PM2 in *E coli*. Recently, Zhu et al. (2007) has reported heat protection in *Arabidopsis thaliana* by overexpressing Aspen sp1.

**Immuno-blot analysis of BsCyP under drought (D) heat (H) and combined DH conditions**

Cyclophilins are involved in Protein folding *in vivo* and by virtue of their stress-inducibility the different genes have been proposed to play a role in stress adaptation of plants (reviewed in Chou and Gasser, 1997). However, a direct relationship between stress tolerance and expression of BSPs has not been reported as yet. Immuno-blot analysis of seed samples revealed the presence of BsCyp53 in both the cultivars under DH conditions (Fig 1 F), however, the BsCyp53 expression was substantially higher in cv. PBW 527. Under D conditions, scarcely visible bands of BsCyp53 were detected with no substantial quantitative differences in both the cultivars. Applied alone, heat stress (H) did not provoke any BsCyp53 expression in both the cultivars. So, taken together, it can be suggested that by virtue of its hydrophilicity, BsCyp53 belongs to the broad family of boiling-soluble proteins, including those associated with cellular dehydration, either as a result of environmental stress (dehydrins), or during normal seed desiccation (HSPs)(Dure and others. 1989). From these observations it is also suggested that like other stress regulated proteins (HSPs/dehydrins proteins), BsCyp53 may be playing a significant role in water stress tolerance in drought tolerant cv. PBW 527, but not in drought sensitive cv. PBW 343. The specific induction of this protein during a combination of drought and heat shock may suggest that this combination is accompanied by the activation of a unique protein, which is not activated when each of these stresses was applied individually. Thus it may be possible to enhance the tolerance of plants to multiple stresses by manipulating the expression of cyclophilins. It is plausible that BsCyp53 may assist in import, folding and assembly of storage proteins in ER and may be essential for post translational processing of storage proteins. BsCyp53 may be helping other stress induced proteins to maturation besides regulating the expression of other genes imparting stress tolerance. Due to their hydrophilic nature, BsCyP53 may also function specifically in the protection of membranes and proteins against desiccation damage, possibly by binding water tightly or providing hydrophilic interactions in the absence of free water and by preventing the crystallization of cellular components through their ability to act as stabilizing “solvents’ (Close and others. 1989). Another possible role of BsCyp53 may be is to bind with the accumulated ions (ion sequestering) under water stress and to control solute concentration in the cytoplasm. Earlier studies

indicated that at high moisture contents, some BSPs like LEA proteins act as compatible solutes that preferentially exclude chaotropic agents (such as salts) from the surface of macromolecule (Liu and Zheng 2005). Likewise when hydration shell is removed they might exert their protective effects in the dry state by replacing water molecule by hydrogen bonding and/or forming a glass which stabilizes the system in the dried state (Wolkers and others. 2001). The high concentration of BsCyp53 induced by combined DH conditions prompted their consideration as essential factors of the adaption process to this type of environmental insult.

**Immuno-blot analysis of BsGST under drought (D) heat (H) and combined DH conditions**

Plant glutathione *S*-transferases (GSTs, EC 2.5.1.18) are a family of multifunctional enzymes involved in the intracellular detoxification of xenobiotics and toxic compounds produced endogenously (Edwards et al. 2000). Most of the enzymes are stress-inducible and play a role in the protection of plants from adverse effects of stresses (Marrs and Walbot, 1997). The GSTs have been associated with both normal cellular metabolism as well as in the detoxification of xenobiotics, limiting oxidative damage and other stress responses in plants. In the present study, Anti-GST immuno-blotting resulted only in scarcely visible trace reactions in seeds of both the cultivars under all conditions. In cv. PBW 527, the BsGST55 expression was substantially higher under H treatment. In contrast, no band was recognized by the anti-GST antibodies in seed samples of cv. PBW 343, suggesting specific role of GST proteins in drought tolerant cultivar. Earlier studies also reported enhanced GST expression by 2.12 folds upon drought stress (Gazanchian and others. 2007). Further, no protein band antigenicaly similar to GST was detected in shoots samples of both cvs. PBW 527 and PBW 343 under all the abiotic stress conditions, again indicating tissue specific expression of proteins.

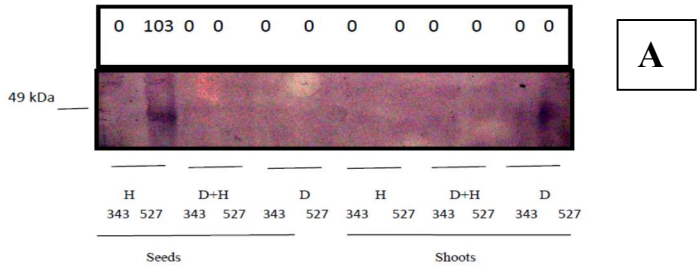
**Immuno-blot analysis of BsLEA under drought (D) heat (H ) and combined DH conditions**

Besides, other hydrophilins (HSPs, CyPs and GSTs), LEA proteins are proposed to protect membranes and protein structures against drought induced damage. LEA proteins accumulate in seeds during the later stages of embryogenesis (Close and others. 1989) and some also accumulate in vegetative tissues in response to osmotic stress. They are supposed to act as solubilizing agents with chaperonic activities, maintaining cellular structural organization and prevent ion crystallization during desiccation. But surprisingly, in our study no specific band was detected in the both the cultivars under all conditions. Earlier also in cereals, it was reported that the expression of high molecular weight heat stable polypeptides was relatively high but did not cross react with antibodies against LEA or RAB (responsive to ABA) (Knight and others. 1995).

**Conclusion**

To conclude, enhanced expression of BsSOD, BsHSP, BSAQPs and BsCyPs particularly during DH conditions suggested that a combination of drought and heat shock affects plants differently from individual stress. These hydrophilins may be necessary to maintain protein function during this specific type of abiotic streses (Reyes and others. 2005). It is been possible that some hydrophilins particularly BsCyP may target molecular chaperones, and in combination, they could contribute to protect proteins under conditions. Other studies have shown that certain hydrophilins afford protection to other proteins like LDH against freeze-induced inactivation in vitro (Houde and others. 1995). A LTP1 protein, in addition to be responsive to abiotic stresses, has been suggested to be involved in transport of cutin monomers and flowering (Lindorff-Larsen and Winther 2001). Similarities in the conditions under which BsSOD, BsHSP, BsAQPs and BsCyPs proteins expressed, together with their hydrophilic character, may underlie a common function for at least a subset of these proteins, possibly in ameliorating the injurious effects of cellular dehydration. It has been reported earlier in tobacco and maize that several HSPs or transcriptional factors such as a pathogenesis related factor (WRKY) and ethylene responsive transcriptional co-activator (ERCTCA), are induced or accumulate during drought stress and heat shock treatments which supports the presence of key regulators involved in this response (Jacobsen and Shaw, 1989). Further, differences in the expression of specific gene products between stress-sensitive and stress tolerant plants indicate that tolerance is conferred by genetically encoded mechanisms (Bray, 1993). It is reasonable to expect that inter- and intraspecific differences in the pattern of protein synthesis between plants which differ in their stress resistance, however, this has also been some controversial issue.

A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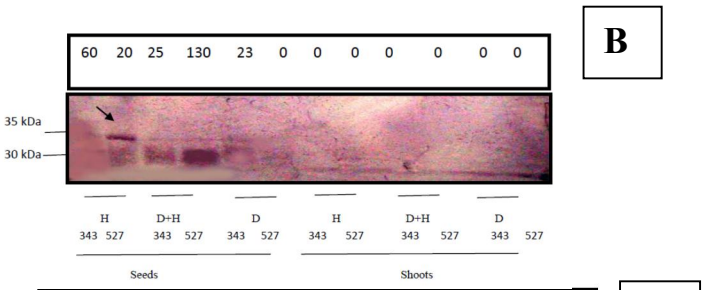
Shoots

A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Seeds
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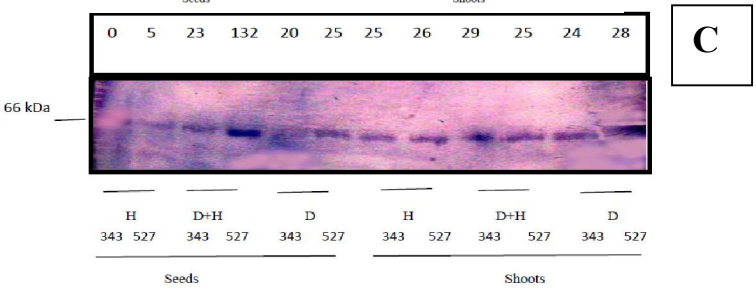
B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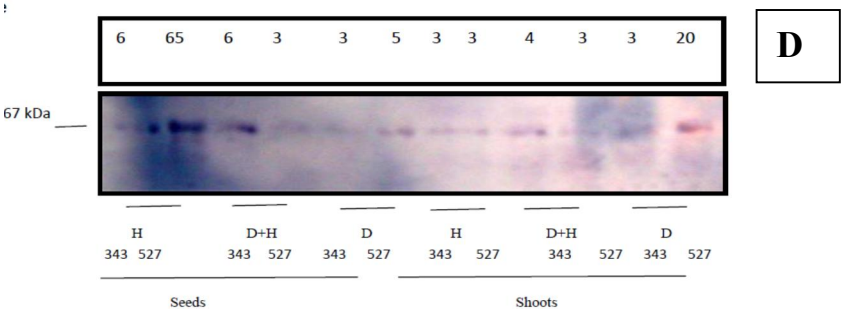
Seeds
Shoots

C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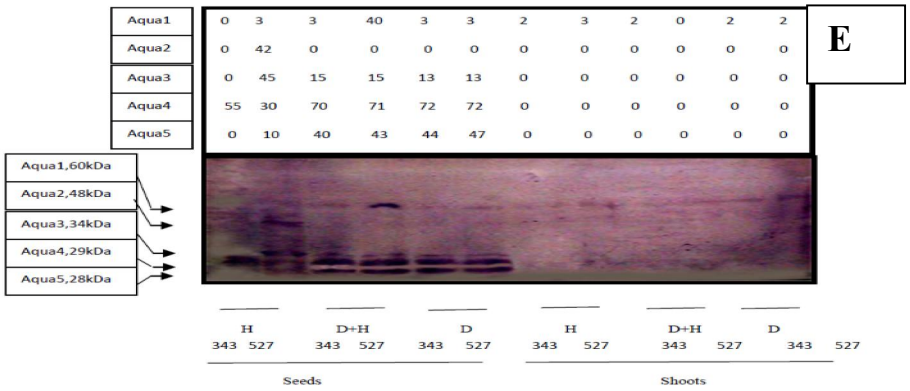
D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Aqua5 0 10 40 43 44 47 0 0 0 0 0 0
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Aqua2,48kDa
Aqua3,34kDa
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Seeds
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E

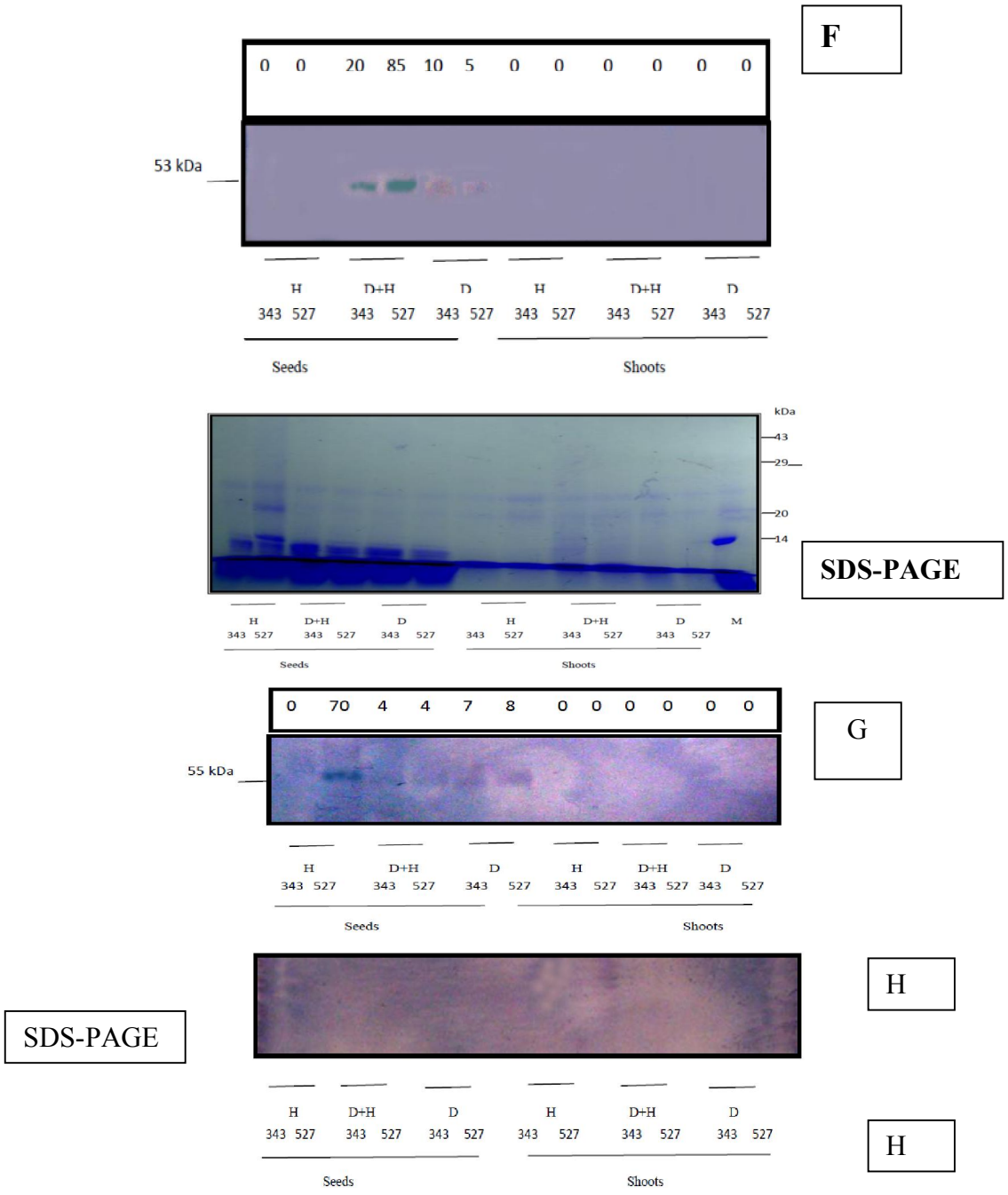


Figure 1: An SDS-PAGE profile of boiling stable proteins of seeds and shoots of drought tolerant (PBW 527) and drought sensitive (PBW 343) cultivars *Triticum aestivum* after stress treatments. Each lane was loaded with 60 µg of boiling stable proteins. D: drought, H: heat, DH: combined drought and heat, M: molecular weight marker.
Immunoblot analysis of BsWGA(A), BsSOD(B), BsHSP90(C), BsAPase(D), BsCyP(E), BsAqua(F), BsGST(G) and BsLEA(H) in seeds and shoots of drought tolerant (PBW 527) and drought sensitive (PBW 343) cultivars *Triticum aestivum* after stress treatments. Numerical values as shown in the top of Panels, indicates relative band intensities, which were determined using Gel Visualization, Documentation and Analysis system (Bio-Rad, USA). Numerical comparisons are only valid within panels and cannot be made between panels. Each lane loaded with 60µg of boiling stable proteins was resolved on 12% SDS-PAGE and transferred to nitrocellulose membrane and probed with different antiserums.

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