Electronic Journal of Plant Breeding, 5(4): 641-650 (Sep 2014)
ISSN 0975-928X

Research Article

High temperature, drought and their interaction induced protein alterations in sensitive and tolerant wheat varieties

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(Received: 01 July 2014; Accepted: 17 Aug 2014)

Abstract
Two contrasting wheat (*Triticum aestivum* L.) cultivars WH730 (high temperature tolerant) and UP2565 (high temperature sensitive) were tested for differential response to combined and individually applied high temperature (HT) and drought (D) stress at seedling stage for peptide profile. Initial profile of the stress induced peptides was outlined via SDS electrophoresis of leaf extracts. Electrophoretic pattern of proteins revealed expression of new bands as well as disappearance of certain others in HT, D and interactive HT+D stress treated and revived samples in both wheat varieties relative to untreated control samples. Some of the bands that appeared in stress treated seedlings were also present after revival indicating their protective role, while some new peptides synthesized after stress but disappeared after revival period may be designated true stress proteins. However, all the plants from heat, drought and their interactive stress treatments continued to grow during recovery period. This suggests that these proteins and other newly synthesized proteins may have protective effects at high temperature (40°C) and water scarcity and provide plants for healthy growth during the recovery period. Furthermore, elucidating the functions of proteins expressed by genes in stress tolerant and susceptible plants may provide important information for designing new strategies for crop improvement.

Abbreviations: HSP- heat shock proteins, HT- high temperature, D- drought, SDS-PAGE- SDS polyacrylamide gel electrophoresis, M_W- molecular weight, LT- lethal temperature, R_f value- relative mobility, kDa- kilodalton, PVP- polyvinylpyrrolidone.

Key words: Wheat,.high temperature, drought, peptides, abiotic stress tolerance

Introduction

The world population grows exponentially; hence there is a need to increase both the wheat productivity and to expand production areas into warmer and dry climates. Both of these goals require significant breeding efforts to improve temperature and water stress tolerance of wheat and other cereals. In most of the studies on heat shock induced HSPs (Heat shock proteins) in plants, plant cells, tissues, organs and in particular whole plants, have been exposed to only one environmental stress factor i.e., high temperature. This experimental approach however may not reflect the conditions that plants may experience in the field. Under field conditions plants are often simultaneously exposed to soil drying and high temperature stress. These two stress factors could create water deficit in plant tissues, which, in turn, may affect the synthesis of HSPs. The accumulation and expression of small heat shock proteins during the exposure of plants to drought and heat stress suggest a general protective role in desiccation tolerance (Lopez *et al*., 2003; Efeoglu and Terzioglu, 2007; Demirevska *et al.*, 2008; Jangpromma *et al.*, 2010). Production of plants tolerant to high temperature and drought stress is of immense significance in the light of global warming and climate change. Plant cells respond to these stresses by re-programming their genetic machinery for survival and reproduction. High temperature tolerance in transgenic plants has largely been achieved either by over-expressing heat shock protein genes or by altering levels of heat shock factors that regulate expression of heat shock and non-heat shock genes (Grover *et al*., 2013). HSPs tend to associate with a wide range of "client" proteins, allowing the HSPs to perform a dizzying array of jobs. These can include helping newly formed amino acid chains to fold into their proper protein shapes, dismantling them after they have been damaged, escorting proteins to their intended mates and keeping them away from interlopers (Asha and Bhagyalakshmi, 2011). Based on the reports that HSP accumulate in field grown, heat and drought stressed plants (Lindquist,1986; Parsell and Lindquist,1994; Grigorova *et al.*, 2011), determining the role of heat shock genes in heritable thermal tolerance and the use of HSP as a selection criterion in improving plant germplasm for stressed environments is of great potential.

In past decade causal involvement of several HSPs in acquired thermo-tolerance of plants has been demonstrated (Stone, 2001; Maestri *et al.*, 2002; Laino *et al.*, 2010). Both germination and early

development in higher plants seems to be much affected by temperature fluctuations as well as water limitation and this causes harmful effects to the yield and productivity of plants by affecting all the physiological, biochemical and molecular processes in the plant cells (Xoconostle-Cazares *et al.*, 2010; Essemine *et al.*, 2010; Kaur *et al.*, 2008, Kaur and Behl, 2010). In view of the insufficient information on combined drought and heat stress effect on wheat and about the role of the above-described proteins in stress response, a specific study of the expression of these stress responsive peptides was initiated under high temperature (HT), drought (D) alone and combined high temperature and drought (HT+D) in tolerant and susceptible wheat cultivars.

Material and methods

*Plant materials and growing conditions***:** Grains of two contrasting varieties of wheat viz. WH730 (high temperature tolerant) and UP2565 (high temperature sensitive) were grown in plastic trays containing 2 kg sand in control conditions (day/night temperatures of 25/21°C, 200 µmol m^{-2} s^{-1} photosynthetic active radiation, 16h day/8h night, 50–60% humidity, 12% gravimetric soil moisture) in growth chamber. Soil moisture was controlled daily by gravimetric measurements of the trays to maintain relative maximal soil moisture capacity. Seven day old seedlings (fully developed first leaf and expanding second one) were subjected to individual high temperature (HT), drought (D) and combination of both the stresses (HT+D). For high temperature stress, seedlings were subjected to gradual temperature pretreatment (with rise of 5^0C h^{-1}) to achieve 35±1^{0}C. These acclimatized seedlings were exposed to lethal temperature (LT_{50} 40^{0}C) on the basis of wilting of the primary leaf for 2 h. For drought stress, the water supply was stopped on 4th day of sowing so as to attain soil moisture 3% (gravimetric). The control plants were watered optimally during the whole period. For combination of both high temperature and drought stresses, water supply was withheld as well as seedlings were exposed to high temperature. Three trays in each treatment and two replicates of leaf samples from each tray were used to study the electrophoretic pattern of seedlings. Peptide profile of the stressed seedlings was observed after stress and overnight revival period under control conditions of optimal temperature and watering.

SDS-PAGE: Samples for SDS-PAGE were prepared by crushing 300 mg of fresh wheat seedlings (frozen in liquid nitrogen prior to extraction) in 3 ml chilled Tris-HCl buffer (50 mM, pH 7.5) containing 50 mg insoluble polyvinylpyrrolidone (PVP). These were then centrifuged at 10,000 x g at 4^{0}C for 15 min. The pellets were discarded and protein in the supernatant was quantified according to Bradford (1976) with bovine serum albumin as a standard. The protein extract was transferred to an equal volume of 2 x sample buffer (Laemmli 2 x buffer), heated at 100^{0}C for 3 min, cooled and used for SDS-PAGE. An aliquot containing 20 µg of sample protein was used. Molecular weight markers (Genei) of medium range containing 6 proteins (97.4, 66.0, 43.0, 29.0, 20.1 and 14.3 kDa) were used. Leaf soluble proteins were separated by 10 % SDS-PAGE, using a dual mini vertical slab gel (8 x 7 cm) electrophoresis unit (Tarsons), according to Laemmli (1970). The three replicates of SDS-PAGE electrophoreses have produced similar profiles. One of them was selected to visualize the obtained protein pattern. After gel staining with Coomassie brilliant blue (0.1% CBBG-250), background destaining (20% methanol + 10% acetic acid), the relative mobilities (R_f values) were calculated for each of the marker protein and the resolved proteins. R_f value of marker proteins were plotted against log of molecular weights of the marker. Molecular weights of different proteins were estimated by matching their R_f values with appropriate point on the standard curve.

Results and discussion

Electrophoretic pattern of proteins resolved in 10% SDS-PAGE in two wheat varieties (WH730 and UP2565) is presented in Figure 1 and 2. The comparative details regarding alteration in number and molecular weight (kDa) of peptides resolved in stress treated as well as revived seedlings of var. UP2565 and var. WH730 are presented in Tables 1-3.

*SDS PAGE seedlings, stress:*Results presented in Fig. 1A and Table 1 show banding pattern in var. UP2565 after HT, D and combined HT+D stress treatments. The formation of new polypeptides and disappearance of existing proteins was evident as alteration in band number in stress treated plants. In UP2565 control, total of eight bands were observed. After HT treatment, out of total nine bands observed, five bands with M_W 100, 89, 79, 40.8 and 24 kDa were newly synthesized while four bands with M_W 56, 42.7, 35.5 and 19 kDa disappeared. After drought, peptides resolved in ten bands. Out of these six bands with M_W 100, 89, 74, 52.5, 33 and 21 kDa were newly synthesized and four bands with M_W 50, 35.5, 22.4 and 19 kDa disappeared. Five new bands were formed due to combined HT+D stresses, having M_W 95.5, 83, 74, 31.6 and 21 kDa, thus, making total of eleven bands that were seen in treated seedlings. In WH730 control, total nine bands were observed (Fig. 1B and Table 1). After HT treatment, six bands (M_W 95.5, 79.4, 56, 34.5, 21 and 16.6 kDa) were seen for the first time while 5 bands (M_W 83, 33, 28, 22.4 and 18 kDa) disappeared. Out of the

total ten bands that resolved in drought treated plants, 8 bands were newly synthesized. Compared to UP2565, where number of bands formed after interactive HT+D stresses were 11, total 15 bands were seen in WH730. Out of total twelve newly synthesized bands, six bands (M_W 53.7, 52.5, 24, 16.6, 14 and 13 kDa) were seen in combined stress while remaining were seen either due to HT stress (M_W 35.5, 56, 21 kDa) or during drought (M_W 76, 30, 20). Stress treatments resulted in synthesis of some stress specific proteins in both the varieties and the number as well as intensity of these proteins determines the stress tolerance potential of the concerned variety. More bands were resolved in WH730 than UP2565 indicating better thermo-tolerance of this variety.

SDS-PAGE seedlings, revival:The banding pattern in two wheat varieties (UP2565 and WH730) indicated that relative to stress (fig. 1; Table 1) the number of bands formed were lesser in both varieties after revival (fig. 2; Table 2). After HT termination, only one band having M_W 40 kDa was common in both stress and revived seedlings of UP2565 indicating its role during recovery phenomenon also four bands (M_W 100, 89, 79 and 24 kDa) appeared after stress termination but disappeared during revival indicating their role as the stress proteins (Table 3). Similar way results can be interpreted for D and HT+D from Table 3 which shows some bands were same in both stressed and revived seedling indicating their role in both stress and recovery phenomenon while some peptides appeared after stress but disappeared after revival indicating their role as true stress proteins. Total 12 bands resolved in interactive HT+D stresses revived seedlings in comparison to 15 bands formed after interactive stress termination. Only one new band of M_W 16.6 kDa and M_W 46 kDa persisted even after revival from HT and D stress while 3 new bands (M_W 56.0, 52.5 and 24 kDa) were persistent in combined HT+D (Table 3). Some of the newly synthesized proteins (M_W 40 kDa (HT), M_W 100, 89, 52.5 kDa (D) and M_W 31.6, 21.0 kDa (HT+D) in UP2565 and M_W 16.6 kDa (HT), M_W 46.0 kDa (D) and M_W 56.0, 52.5, 24.0 kDa (HT+D) in WH730 were observed after both stress termination and revival indicating their protective role during both phenomenon (Table 3). Disappearance of protein band may be interpreted as the turning off of protein synthesis machinery in response to stress treatment (Vierling, 1991 and Bora *et al*., 1999). It is more likely however, that disappearance of proteins as a result of stress is due to their denaturation, depressed protein synthesis and their increased degradation in plants. Combination of drought and heat stress provokes cessation of conventional protein synthesis, accompanied by increased translation of heat shock proteins (HSPs) and other stress related proteins. It is believed that this diversification of these proteins reflects an adaptation to tolerate the heat stress (Ahuja *et al.*, 2010; Al-Whaibi, 2011; Amudha and Balasubramani, 2011). Such results have been reported by numerous workers (Vierling, 1991; Waters *et al*., 1996; Maestri *et al*., 2002; Miroshnichenko *et al*., 2005). Sun *et al*. (2002) emphasized the importance of proteins which were up-regulated in response to stress in relation to stress tolerance.

Evaluation of electrophoresis gels in this study revealed several proteins to be differentially expressed as a result of individual and combined HT and D stress response. The thermotolerant variety WH730 exhibited the stronger proteases induction under combined stress and during the recovery period. Table 3 showed more specific changes in the abundance of some individual proteins in wheat leaves under treatment and revival conditions. Immediately after exposure to high temperature and drought, changes occur at molecular level altering the expression of genes and accumulation of transcripts, thereby acting as a stress tolerance strategy. According to Vierling (1991), temperature of 32-33°C is super optimal for normal wheat growth and development. In the present work, heat stress was imposed on plants for 2 h at 40°C in individual heat stress experiments and in a combined drought/heat stress. The more number of protective peptides and true stress peptides were observed in plants submitted to combined stress (HT+D). The responses of the two cultivars were quite similar although tolerant genotype WH730 had accumulated more peptides compared to susceptible one (UP2565) that were present after stress but disappeared after revival indicating their nature as true stress proteins indicating better thermo-tolerance (Table 3). The identified basic functions of stress induced peptides mentioned in the introduction present a reasonable explanation of the observed results. As obvious from the SDS-PAGE (Fig. 1), peptides with low Mw reached the highest contents in HT, D and HT+D samples, which supports their chaperoning activity (Mansfield *et al.*, 1987, Waters *et al.*, 1996, Smykal *et al.*, 2000). Our results regarding changes in protein profiles in response to high temperature (HT), drought (D) and dual stress (HT+D) vis-à-vis adaptation at seedling stage are in agreement with effect of these stresses on yield potential of these wheat varieties reported earlier by Chakraborty *et al.*, 2008; Kaur and Behl, 2010; Farooq *et al.*, 2011. This comparative study on drought and heat stress (applied separately and in combination) confirmed their influence on protein alterations in correspondence with previous results (Jiang *et al.*, 2002; Mittler, 2006; Demirevska *et al.*, 2008). Furthermore, elucidating the functions of proteins expressed by genes in stress tolerant

and susceptible plants will not only advance our understanding of plant adaptation and tolerance to environmental stresses, but also may provide important information for designing new strategies for crop improvement. In conclusion, the highest peptide expression was established under the combined drought and heat stress in wheat plants. The results differed strongly under individually applied heat shock or drought and combined stress. Therefore, a simple extrapolation of the results obtained after application of one of the stresses (heat or drought) separately will not produce a reliable basis to predict the effects of their combination. Such opinion was expressed earlier concerning *Arabidopsis* and tobacco by Ron Mittler (2006), who claimed that simultaneous exposure to different abiotic stresses would result in co-activation of the various stress response pathways with synergistic or antagonistic effect and that their combination should be regarded as a new state of abiotic stress in plants.

References

Ahuja, I., de Vos, C.H., Bones, A.M. and Hall, R.D. 2010. Plant molecular stress responses face climate change. *Trends Plant Sci.*, **15**(12): 664-674.

Al-Whaibi, M.H. 2011. Plant heat-shock proteins: A mini review. J. King Saud University – Sci., 23: 139–150.

Amudha, J. and Balasubramani, G. 2011. Recent molecular advances to combat abiotic stress tolerance in crop plants. *Biotech. Mol. Biol. Rev.*, **6**(2): 31-58.

Asha. S. and Bhagyalakshmi, K. 2011. Heat Shock Proteins. *J. Pharmacy Res.*, **4**(10): 3829-3832.

Bora, K.K., Roy, P.K. and Bohra, S.P. 1999. Heat shock proteins in plants. In : Advances in plant physiology, Vol. 2, A.J. Hemantranjan, (Ed.), Scientific Publishers, Jodhpur, India, pp. 63-86.

Bradford, M.M. 1976. A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein dye-binding. *Anal. Biochem.*, **72** : 248-254.

Chakraborty, D., Sharma, A., Rai, H.K., Jolly, M., Chander, S., Kumar, R., Bhadraray, S., Barman, D. Mittal, R.K. Lal, M. and Sehgal, M. 2008. Effect of increasing temperature on the yield of some winter crops in northwest India. *Curr. Sci.*, **94**(1) : 82-89.

Demirevska, K., Stoilova, L.S., Vassileva, V. Vaseva, I., Grigorova, B. and Feller, U. 2008. Drought induced leaf protein alterations in sensitive and tolerant wheat varieties. *Gen. Appl. Plant Physiol.*, **34**(1-2): 79-102.

Efeoglu, B. and Terzioglu, S. 2007. Varying patterns of protein synthesis in bread wheat during heat shock. *Acta Biologica Hungarica.*, **58**(1): 93-104.

Essemine, J., Ammar, S. and Bouzid, S. 2010. Impact of heat stress on germination and growth in higher plants: Physiological, Biochemical and Molecular repercussions and mechanisms of defence. *J. Biol. Sci.*, **10**(6): 565-572.

Farooq, M., Bramley, H., Palta, J.A. and Siddique, K.H.M. 2011. Heat Stress in wheat during reproductive and grain-filling phases. Critical Rev. *Plant Sci.*, **30**(6): 491-507.

Grigorova, B., Vasevai, I., Demirevska, K., Feller, U. 2011. Combined drought and heat stress in wheat: changes in some heat shock proteins. *Biol. Plant.*, **55**(1): 105-111.

Grover, A., Mittal, D., Negi, M. and Lavania, D. 2013. Generating high temperature tolerant transgenic plants: Achievements and challenges. *Plant Sci.*, **205-206**: 3 8-47.

Jangpromma, N., Kitthaisong, S., Lomthaisong, K., Daduang, S., Jaisal, P. and Thammasirirak, A. 2010. A proteomics analysis of drought stress responsive proteins as biomarker for drought tolerant sugarcane cultivars. *American J. Biochem. Biotech.*, **6**(2): 89-102.

Jiang, Y. and Huang, B. 2002. Protein alterations in tall fescue in response to drought stress and abscisic acid. *Crop Sci.*, **42**: 202-207.

Kaur, V., Behl, R.K. 2010. Grain yield in wheat as affected by short periods of high temperature, drought and their interaction during pre- and post-anthesis stages. *Cereal Res. Comm.*, **38**: 514-520.

Kaur, V., Behl, R.K., Shinano, T., Osaki, M. 2008. Interacting effects of high temperature and drought stresses in wheat genotypes under semiarid tropics - an appraisal. *Tropics*, **17**: 225-235.

Laemmli, J. 1970. Cleavage of structural proteins during the assembly of head of bacteriophage T_4. *Nature*, **227**: 680-685.

Laino, P., Shelton, D., Christine, F., Leonardis, A.M., Mastrangelo, A.M., Svensson, B., Lafiandra, D. and Masci, S. 2010. Comparative proteome analysis of metabolic proteins from seeds of durum wheat (cv. Svevo) subjected to heat stress. *Proteomics*, **10**(12): 2359-2368.

Lindquist, S. 1986. The heat shock response. *Annu. Rev. Biochem.*, **55**: 1151-1191.

Lopez, C.G., Banowetz, G.M., Peterson, C.J. and Kronstad, W.E. 2003. Dehydrin expression and drought tolerance in seven wheat cultivars. *Crop Sci.*, **43**: 577-582.

Maestri, E., Klueva, N., Perrotta, C., Gulli, M., Nguyen, H.T. and Marmiroli, N. 2002. Molecular genetics of heat tolerance and heat shock proteins in cereals. *Plant Mol. Biol.*, **48**: 667-681.

Mansfield, M. and Key, J. 1987. Synthesis of the low molecular weight heat shock proteins in plants. *Plant Physiol.*, **84**: 1007-1017.

Miroschnichenko, S., Tripp, J., Nieden, U., Neumann, D., Conrad, U. and Manteuffel, R. 2005. Immunomodulation of functions of small heat shock stress proteins prevents their assembly in cell death at sub-lethal temperatures. *Plant J.*, **41**: 269-281.

Mittler, R. 2006. Abiotic stress, the field environment and stress combination. *Trends Plant Sci.*, **11**: 15-19.

Parsell, D. A. and Lindquist, S. 1994. Heat shock proteins and stress tolerance. In: The biology

of heat shock proteins and molecular chaperones. R.I. Moromoto, A. Tissieres and C. Georgopoulous (Eds.) Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. pp. 457-494.

Smykal, P., Masin, J., Hardy, I., Konopasek, I. and Zarsky, V. 2000. Chaperone activity of tobacco HSP18, a small heat-shock protein, is inhibited by ATP. *Plant J.*, **23**: 703-713.

Stone, P. 2001. The effects of heat stress on cereal yield and quality. In: Crop responses and adaptations to temperature stress. A.S. Basra (Ed.), Food Products Press, Binghamton, NY. pp. 243-291.

Sun, W., Montagu, V.M. and Verbruggen, N. 2002. Small heat shock proteins and stress tolerance in plants. *Biochem. Biophys. Acta.*, **1577:** 1-9.

Vierling, E. 1991. The role of heat shock proteins in plants. Annual Rev. *Plant Physiol. Mol. Biol.*, **42**: 579-620.

Waters, E.R., Lee, G.J. and Vierling, E. 1996. Evolution, structure and functions of the small heat shock proteins in plants. *J. Expt. Bot.*, **47**: 325-338.

Xoconostle-Cazares, B., Ramirez-Ortega, F.A., Flores-Elenes, L. and Ruiz-Medrano, R. 2010. Drought Tolerance in Crop Plants. *American J. Plant Physiol.*, **5**(5): 241-256.

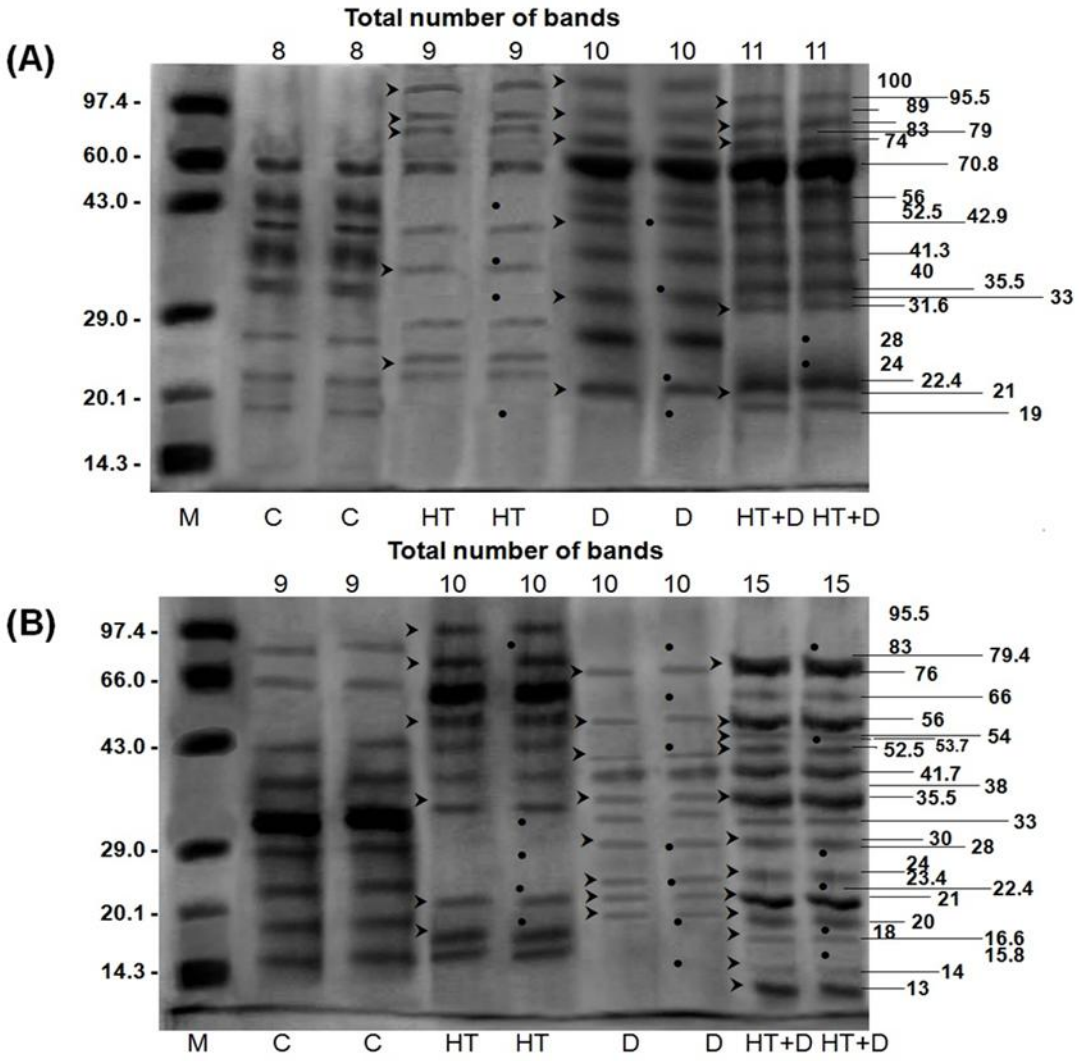

Figure 1. Protein profile through 10% SDS-PAGE showing the effect of individually applied high temperature (HT), drought (D) and combined (HT+D) stress on seedlings of wheat (A) var. UP2565 (B) var. WH730.

M – marker; C - control ; HT – high temperature; D – drought; HT+D – high temperature + drought

➢ newly synthesized band; • Disappeared band

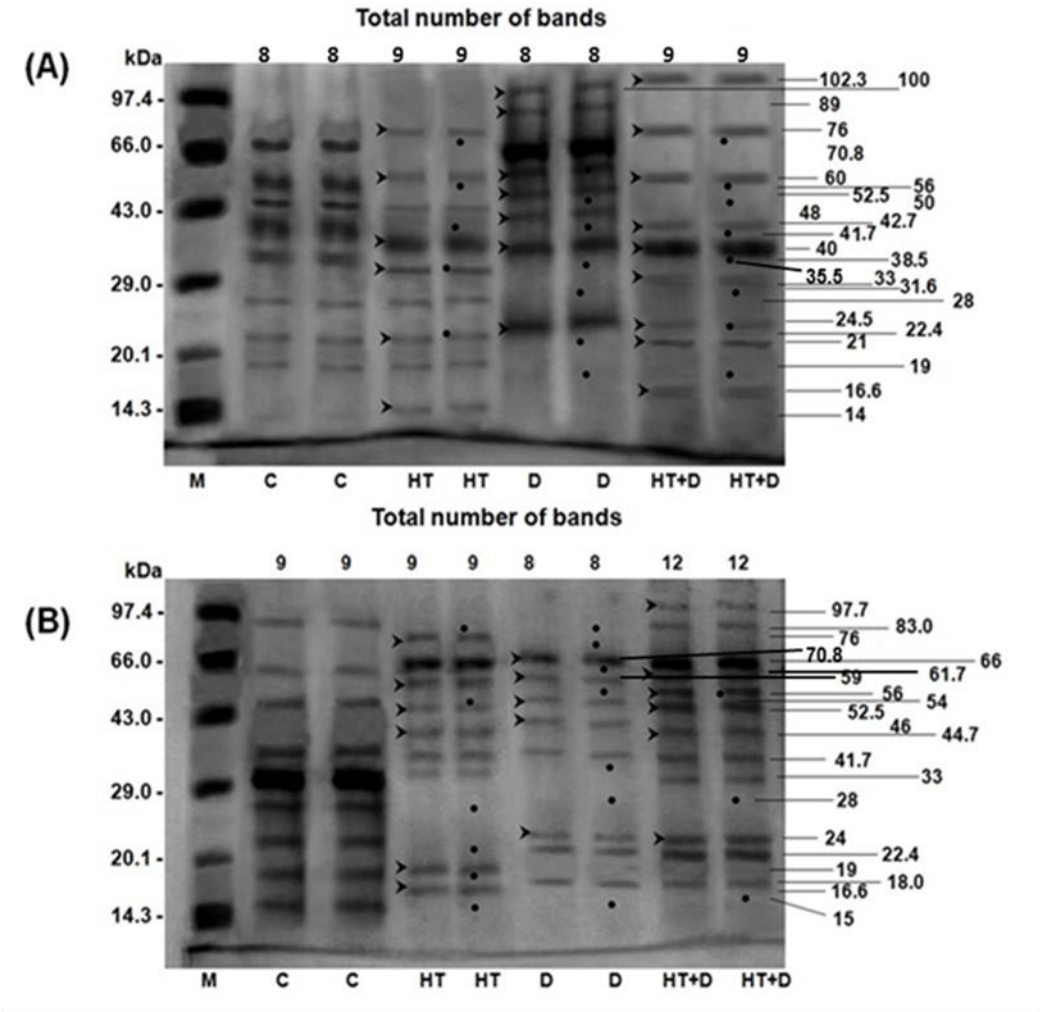

Figure 2. Protein profile through 10% SDS-PAGE in leaf (seedlings) of wheat (A) var. UP2565 (B) var. WH730 after revival from individual high temperature (HT), drought (D) and their combination (HT+D). M – marker; C - control ; HT – high temperature; D – drought; HT+D – high temperature + drought
➢ newly synthesized band; • Disappeared band

Table 1. Alteration in number and molecular weight (kDa) of peptides resolved under high temperature (HT), drought (D), applied individually and combined (HT+D) in susceptible (UP2565) and tolerant (WH730) wheat cultivars in comparison to control (C).

Peptides resolved→ / Treatments ↓		Total number of peptides resolved	Newly Synthesized peptides	Disappeared peptides	Common appearance in control and stress
UP2565	C	8 (70.8, 56, 50, 42.7, 35.5, 28.0, 22.4, 19)	-	-	-
	HT	9 (100, 89, 79, 70.8, 50, 40.8, 28, 24, 22.4)	5 (100, 89, 79, 40.8, 24)	4 (56, 42.7, 35.5, 19)	4 (70.8, 50, 28, 22.4)
	D	10 (100, 89, 74, 70.8, 56, 52.5, 42.7, 33, 28, 21)	6 (100, 89, 74, 52.5, 33, 21)	4 (50, 35.5, 22.4, 19)	4 (70.8, 56, 42.7, 28.0)
	HT+D	11 (95.5, 83, 74, 70.8, 56, 50, 42.7, 35.5, 31.6, 21, 19)	5 (95.5, 83, 74, 31.6, 21)	2 (28, 22.4)	6 (70.8, 56, 50, 42.7, 35.5, 19)
WH730	C	9 (83, 66, 54, 41.7, 33, 28, 22.4, 18, 15.8)	-	-	-
	HT	10 (95.5, 79.4, 66, 56, 54, 41.7, 34.5, 21, 16.6, 15.8)	6 (95.5, 79.4, 56, 34.5, 21, 16.6)	5 (83, 33, 28, 22.4, 18)	4 (66, 54, 41.7, 15.8)
	D	10 (76, 56, 52.5, 35.5, 30, 20, 41.7, 33, 23.4, 21)	8 (76, 56, 52.5, 35.5, 30 23.4, 21)	7 (83, 66, 54, 28, 22.4, 18 ,15.8)	2 (41.7, 33)
	HT+D	15 (76, 56, 53.7, 52.5, 41.7, 35.5, 33, 30, 24, 21, 20, 16.6, 14, 13)	12 (76, 56, 53.7, 52.5, 35.5, 30, 24, 21 ,20, 16.6, 14, 13)	6 (83, 54, 28, 22.4, 18, 15.8)	3 (66, 41.7, 33)

(Values outside parenthesis show number and values inside parenthesis show molecular weight (kDa) of the peptides resolved by 10% SDS-PAGE)

Table 2. Alteration in number and molecular weight (kDa) of peptides resolved after revival from high temperature (HT), drought (D), applied individually and combined (HT+D) in susceptible (UP2565) and tolerant (WH730) wheat cultivars in comparison to control (C).

Peptides resolved→ / Treatments ↓		Total number of peptides resolved	Newly Synthesized peptides	Disappeared peptides	Common appearance in control and revival
UP2565	C	8 (70.8, 56, 50, 42.7, 35.5, 28, 22.4, 19)	-	-	-
	HT	9 (76, 60, 50, 40, 33, 28, 22.4, 21, 14)	6 (76, 60, 40, 33, 21, 14)	5 (70.8, 56, 42.7, 35.5, 19)	3 (50, 28, 22.4)
	D	8 (100, 89, 70.8, 60, 52.5, 48, 38, 24.5)	7 (100, 89, 60, 52.5, 48, 38, 24.5)	7 (56, 50, 42.7, 35.5, 28, 22.4, 19)	1 (70.8)
	HT+D	9 (102.3, 76, 60, 41.7, 40, 31.6, 24.5, 21, 16.6)	9 (102.3, 76, 60, 41.7, 40, 31.6, 24.5, 21, 16.6)	8 (70.8, 56, 50, 42.7, 35.5, 28, 22.4, 19)	-
WH730	C	9 (83, 66, 54, 41.7, 33, 28, 22.4, 18, 15)	-	-	-
	HT	9 (76, 66, 59, 52.5, 44.7, 41.7, 33, 19, 16.6)	6 (76, 59, 52.5, 44.7, 19, 16.6)	6 (83, 54, 28, 22.4, 18, 15)	3 (66, 41.7, 33)
	D	8 (70.8, 59, 52.5, 46, 41.7, 24, 22.4, 18)	5 (70.8, 59, 52.5, 46, 24)	6 (83, 66, 54, 33, 28, 15)	3 (41.7, 22.4, 18)
	HT+D	12 (97.7, 83, 66, 61.7, 56, 52.5, 44.7, 41.7, 33, 24, 22.4, 18)	6 (97.7, 61.7, 56, 52.5, 44.7, 24)	3 (54, 28, 15)	6 (83, 66, 41.7, 33, 22.4, 18)

(Values outside parenthesis show number and values inside parenthesis show molecular weight (kDa) of the peptides resolved by 10% SDS-PAGE)

Table 3. Peptides resolved under high temperature (HT), drought (D), applied individually and combined (HT+D) in susceptible (UP2565) and tolerant (WH730) wheat cultivars in comparison to revival suggesting differential roles of stress induced peptides.

Peptides resolved→ Treatments ↓	UP2565		WH730	
	Newly synthesized peptides present after stress as well as revival (Protective phenomenon)	Peptides present after stress but disappeared after revival (True stress proteins)	Newly synthesized peptides present after stress as well as revival (Protective phenomenon)	Peptides present after stress but disappeared after revival (True stress proteins)
HT	1 (40)	4 (100, 89, 79, 24)	1 (16.6)	5 (95.5, 79.4, 56, 35.5, 21)
D	3 (100, 89, 52.5)	3 (74, 33, 21)	1 (46)	7 (76, 56, 38, 30, 23.4, 21, 20)
HT+D	2 (31.6, 21)	3 (95.5, 83, 74)	3 (56, 52.5, 24)	9 (76, 53.7, 35.5, 30, 21, 20, 16.6, 14, 13)

(Values outside parenthesis show number and values inside parenthesis show molecular weight (kDa) of the peptides resolved by 10% SDS-PAGE)