

Study on effect of high temperature stress in wheat genotypes using SDS protein profile

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Abstract

This study was carried out in order to investigate the effects of heat stress on SDS-Protein profile in wheat (*Triticum aestivum* L.). Ten wheat genotypes differing in thermotolerance were first grown in natural day light condition for 10 days and then seedlings were shifted to BOD at a temperature $25 \pm 1^\circ\text{C}$ for 24 h, and subsequently gradually exposed to 30°C for 1h, 35°C for 1h, 40°C for 2h and at 46°C for 3h. Genetic variability assayed by cell viability test revealed that wheat genotypes NIAW-34 and AKAW-4627 lowest TTC reduction and higher cell viability were considered as thermotolerant genotypes. The study revealed that SDS-PAGE protein profile exhibited new set of proteins when exposed to sub lethal to lethal heat stress (40°C for 2h to 46°C for 3h). Small molecular weight proteins appeared with different intensity during temperature induction treatments. Protein with 60 kDa size and 14 kDa size are observed constantly in all thermotolerant wheat cultivars during high temperature stress. Proteins with 7 and 11.2 kDa size are also observed at 46°C for 3h heat stress in thermotolerant NIAW-34 and AKAW-4627 and in thermo-susceptible wheat NIAW-917. It is also observed that normal wheat seedlings when shifted to temperature above five or more optimum growth temperature (25°C) synthesis of most normal proteins was repressed and translation of new protein was induced.

Keywords: Wheat, SDS-PAGE profile, temperature stress

1. Introduction

In India, wheat sowing time varies from October to December with temperature range of 10 to 32°C . In life cycle of wheat all stages of development are sensitive to temperature. It is the main factor controlling the rate of crop development. It is reported that 12 to 22°C is suitable temperature for proper growth in wheat (Farooq *et al.*, 2011) however, the optimum temperature for best wheat production is 25°C and the optimum threshold limit is 30°C (Hasanuzzaman, 2013). High temperature stress affects essentially every aspect of plant growth and development by influencing the entire metabolism. Plants overcome high temperature stress by adopting several physiological and biochemical mechanism such as excess heat dissipation through evaporative cooling, maintaining membrane integrity and synthesis of heat shock proteins. Several other studies have provided evidence that the genetic variability in plant that will survive when exposed to a temperature that would be lethal to a non-acclimated plant is mainly due to differential expression of stress-

responsive genes and have reported correlations between the acquisition of thermotolerance and the synthesis and accumulation of HSPs (Ristic *et al.*, 2008).

Improving tolerance to heat stress is the major challenge in wheat improvement programme. A simple and realistic test to identify high temperature tolerant cultivars however, has been a major limitation in this programme. Number of workers employed different techniques to assess the stress effects are the extent of membrane leakage (Blum *et al.*, 2001), cell viability (Babele and Kandya, 1986), chlorophyll stability (Haldimann *et al.*, 1996), quantification of malondialdehyde (MDA) (Jiang and Huang, 2001) and activities of free radical scavenging enzymes (Almeselmani *et al.*, 2009). In addition to this, heat stress tolerance is also quantified by mitochondrial electron transport activity, which is determined through reduction of TTC (2, 3, 5-triphenyl tetrazolium chloride). The number of workers used TTC reduction assay to differentiate the acquired thermotolerance of different genotypes (Krishnan *et al.*, 1989; Yildiz and Terzi, 2008;

Dhanda and Munjal, 2009). In this report we used this method to evaluate the ten wheat cultivars for their acquired thermotolerance using temperature induction response technique.

Plant synthesizes HSPs proportionally with severity of heat until maximum level. HSPs synthesis is completely induced for surviving with maximum activation of other protection mechanism at near deadly temperatures. However plants probably synthesize middle level HSPs at mild heat stress conditions at first, but if heat stress continues they synthesize more HSPs (Efeoglu, 2009). In general, threshold temperature for HSPs induction is correlated with the typical temperatures at which species live. When plants are exposed to high temperature, they synthesize both high molecular mass HSPs (from 60 to 110 kDa) and small HSPs (from 15 to 45 kDa) (Timperio *et al.*, 2008; Hasan and Barthakur, 2014; Pandey *et al.*, 2015). Heat shock proteins are known to be induced not only in response to short-term stress, but their production is a necessary step in plant heat acclimation. The heat shock proteins which constitutively express and protect intracellular proteins from denaturation and preserve their stability and function through protein folding; thus it acts as chaperones (Baniwal *et al.*, 2004). Most HSPs are thought to be intimately associated with reactive oxygen species (ROS) and are thought to be controlled by the ROS gene network of plants, playing a key role in mediating important signal transduction events (Mittler *et al.*, 2002).

In wheat, the appearance of high molecular weight and low molecular weight HSPs during high temperature and under cold response has been reported by Krishnan *et al.* (1989); Joshi *et al.* (1997); Skylas *et al.* (2002); Sharma Natu *et al.* (2007); Chauhan *et al.* (2009); Khalil *et al.* (2009). Some cultivars of wheat have versatile characters to survive above threshold level temperatures. The genotypes selected for this study have been released for cultivation in Maharashtra state except NIAW-1161 which was tested at national level in short duration cum late heat tolerance nursery at ARS, Niphad. Other genotypes (GW-322, NIAW-301, NIAW-917, MACS-2496 and HD-2189) were recommended for timely sown irrigated condition and NIAW-34, RAJ-4083, HD-2932 and AKAW-4627 were recommended for late sown irrigated condition. As Peninsular Zone is prone to late heat damage hence, to know the magnitude of tolerance, the study was undertaken to assays acquired thermotolerance and to investigate the SDS-PAGE protein profile with a view to generate information which will be used for further field and molecular studies aim with as for developing wheat thermotolerant genotypes.

2. Materials and methods

2.1 Plant material: Seeds of uniform size of 10 wheat genotypes viz., NIAW-34, RAJ-4083, NIAW-1161, HD-2932, AKAW-4627, GW-322, NIAW-301, NIAW-917, MACS-2496, HD-2189 were procured from ARS Niphad Maharashtra. The genotypes selected for this study have been released for cultivation in Maharashtra state except NIAW-1161 which was tested at national level in short duration cum late heat tolerance nursery at ARS, Niphad. Out of these genotypes five recommended for late sowing irrigated conditions were categorized as a thermotolerant viz., NIAW-34, RAJ-4083, NIAW-1161, HD-2932, AKAW-4627 and five genotypes recommended for normal sowing irrigated conditions were categorized as a thermo sensitive viz., GW-322, NIAW-301, NIAW-917, MACS-2496, HD-2189.

2.2 Growth condition for assaying acquired thermotolerance and SDS-PAGE: For assaying acquired thermotolerance in between the genotypes at germination stage, the seeds were surface sterilized by stirring them with 0.2% (w/v) mercuric chloride solution for 5 minutes and then washed with distilled water thrice. After imbibitions for 6 h, seeds were placed in petri plates containing sterile filter sheets moistened with water. The plates were incubated at $25 \pm 1^\circ\text{C}$ in a seed germinator for 41 h in darkness and allowed to grow. The germinated seedlings of wheat cultivars were transferred to incubator for temperature induction response. The plant material subjected to a gradual temperature induction treatment of 30°C for 1 h; 35°C for 1h; 40°C for 2h followed by challenge temperature of 46°C for 3h. Before subjecting to lethal temperature (46°C for 3h) seedlings were allowed to recover for 68 h at $25 \pm 1^\circ\text{C}$ as described by Sharma Natu *et al.* (2007). In non-induced (direct exposure), temperature was increased by 5°C min^{-1} up to 46°C for 3h. The seedlings from all treatments and control were harvested and pooled for acquired thermotolerance by cell viability assay.

In second experiment surface sterilized and imbibed seeds were sown in plastic protray containing soil medium and allowed to grow for 10 days in natural day light conditions. Seedlings were equilibrated with water as when required. After ten days, these seedlings were transferred in BOD incubator having 15/9h light/dark cycle with 1500-lux light intensity at $25 \pm 1^\circ\text{C}$ temperature for 24 h and subsequently exposed gradually to temperature stress at 30°C for 1h; 35°C for 1h; 40°C for 2h and 46°C for 3h (challenge temperature) as per the method described by Sharma-Natu *et al.* (2007) with slight modification.

2.3 Cell viability assay: The ability of seedlings to acquire thermotolerance was assessed by examining the extent of 2, 3,5- triphenyl tetrazolium chloride reduction (cell

viability assay) by induced wheat seedlings as per the method described by Krishnan *et al.* (1989) and Sharma-Natu *et al.* (2007) with slight modification in temperature. Uniformly germinated 10 seedlings of wheat genotypes were exposed to gradual temperature induction treatments from 30°C for 1 h; 35°C for 1 h; 40°C for 2 h and then subjected to challenge temperature stress (46°C for 3 h). At the end of stress, the roots of seedlings (0.1g) were excised and transferred to 5 mL of 0.1% TTC prepared in 50 mM sodium phosphate buffer, (pH 7.4) and incubated for 18-20 h in dark. After incubation the root segments were removed from TTC solution, rinsed with distilled water and placed in a beaker containing 5 mL of methoxyethanol. The samples were boiled to total dryness and resuspended in 5 mL of methoxyethanol. The colour recovered in 2-methoxyethanol was measured at 530 nm. Same quantity of seedlings maintained at 25±1°C served as absolute control. The percent TTC reduction of absolute control was taken as a measure to quantify the acquired thermotolerance from induced and non induced contrasting wheat cultivars. Cell viability assays during germination stage was statistically analyzed by completely randomized block design with three replications.

2.4 Extraction of protein: High temperature exposed seedlings leaf material of wheat genotypes were weighed separately. The leaf samples were homogenized in protein extraction buffer (PH 8.0) containing 0.1M Tris-HCl, 0.01M MgCl₂, 18% Sucrose, 40mM 2-β-mercaptoethanol in the proportion of 1:5. The extract was then centrifuged at 10,000 rpm at 4°C for 10 min. The supernatant was collected in a fresh micro centrifuge tube. The volume of supernatant was noted. An equal proportion of 10 % TCA was mixed in to the tube. These tubes were kept in ice for 30 minutes. Mixture was centrifuge at 10,000 rpm for 10 minute. Supernatant was discarded and the pellets were dissolved in 50 ul of 0.2 NaOH. The pellets were stored at - 20°C for further analysis as suggested by De Britto *et al.* (2011). Protein concentration per unit volume in each sample was adjusted by using the sample buffer and resolved for SDS-PAGE.

2.5 SDS-PAGE protein profile:

Soluble protein from leaf samples of each treatments determined by Lowry (1951) method and the samples of each corresponding to 25 µg protein were mixed with sample buffer to function it as the tracking dye and loaded carefully by syringe in separate wells. The gel was prepared by using 12% concentration of separating gel and 4.5% concentration of stacking gel. It was then connected to power supply unit. Initially a current 1.5 mA/well was applied till the sample migrated into the spacer gel and then adjusted to 2mA/well. The electrophoresis apparatus except power supply was

kept in a refrigerator at a constant temperature of 4°C. The electrophoresis was carried out until the bromophenol blue (tracking dye) migrated almost to the end of the gel. The heat shock proteins were resolved on 12 % SDS-PAGE. The protein bands were resolved using ATTO, AE-8450 model of Japan make electrophoresis unit as per the method described by Laemmli (1970). The molecular weight of resolved protein bands were calculated from the relative mobility (Rm) of bands as follows:

$$R_m = \frac{\text{Distance migrated by protein band (cm)}}{\text{Distance migrated by tracking dye from top of the separation gel (cm)}}$$

3. Results and discussion

3.1 Cell viability assay: Cell viability assay in terms of percent TTC reduction of ten wheat cultivars was carried out at control 25°C and at lethal temperature stress (46°C for 3h) in root of acclimated 25°C→30°C for 1h→35°C for 1h→40°C for 2 h and non acclimated wheat seedlings by measuring O.D at 530 nm (Table 1). Overall mean values of absorbance decreased from 1.295 to 0.431 in non acclimated seedlings and from 1.295 to 0.725 in acclimated seedlings after exposure to lethal temperature stress (46°C for 3 h). The mean values showed that non acclimated wheat recorded highest percent TTC reduction (66.72%), whereas it was lowest (43.15%) in acclimated wheat seedlings at different high temperature. Thermotolerant wheat cultivars recorded lowest percent TTC reduction both at non acclimated(52.39%) and acclimated(33.25%) condition as compared to thermosusceptible seedling where it was 81.04% in non acclimated and 52.65% in acclimated seedlings. Two thermo tolerant wheat cultivar NIAW-34 and AKAW-4627 recorded higher cell viability as evident from lowest percent reduction in TTC 47.28% and 50.52% at non acclimated condition and 25.78% and 29.63% under acclimated condition. Among thermo sensitive wheat NIAW-917 recorded lower TTC reduction both at non acclimated (64.02%) and (40.92%) at acclimated treatment. NIAW-917 recorded TTC reduction of 40.92%, which was less than RAJ-4083 and HD-2932 a thermotolerant cultivars. HD-2189 recorded highest reduction in cell viability both at acclimated and non acclimated condition i.e. 63.88 and 88.01% respectively. The mean values of ten wheat cultivars depicted in Table 1, indicated that less percent TTC reduction in acclimated condition (43.15%) than the non acclimated condition (66.72%). From the mean values of thermotolerant (33.25%) and thermo - susceptible (52.65%) of wheat seedlings it may be concluded that thermotolerant group has higher acquired thermotolerance than the thermo susceptible group.

Table 1. Percent TTC reduction in acclimated and non acclimated seedlings after exposed to high temperature stress at 46°C for 3 h.

Name of cultivars	OD at control (25°C)	Acclimation		Non-acclimation		Rank
		O.D	%TTC	O.D	%TTC	
Thermotolerant wheat cultivars						
RAJ-4083	1.394	0.818	41.32	0.637	54.30	4
NIAW-34	1.288	0.956	25.78	0.679	47.28	1
NIAW-1161	1.310	0.759	42.06	0.563	57.02	5
HD-2932	1.487	0.844	43.26	0.540	63.68	6
AKAW-4627	1.350	0.950	29.63	0.668	50.52	2
Mean	1.296	0.865	33.25	0.617	52.39	
Thermosusceptible wheat cultivars						
GW-322	1.310	0.513	60.84	0.157	88.02	9
NIAW-301	1.084	0.638	51.13	0.229	78.87	8
NIAW-917	1.312	0.774	40.92	0.472	64.02	3
MACS-2496	1.138	0.568	50.18	0.216	81.02	7
HD-2189	1.276	0.461	63.88	0.153	88.01	10
Mean	1.294	0.656	52.65	0.245	81.04	
Grand Mean	1.295	0.725	43.15	0.431	66.72	
SE±	0.042	0.071		0.017		
CD at 5 %	0.125	0.227		0.049		
CV	5.672	13.979		3.964		

TTC reduction by seedlings during acclimated condition is an indicator of mitochondrial functioning which in turn reflects the cell viability (Kalina and Palmer, 1968). The decrease in cell viability resulting from high temperature treatments may be attributed to uncoupling of the electron transport chain through disruption of inner mitochondrial

membrane and or inactivation of enzymes of the respiratory pathway. The results of this study are in agreement with the earlier reports in wheat by Porter *et al.* (1994), Sharma-Natu *et al.* (2007) and Yildiz and Terzi (2008), which showed a positive correlation between cell viability and acquisition of thermo - tolerance. It, thus, appears that in thermotolerant cultivars cell membrane may remains functional during heat stress and may assist plant to adapt to heat stress condition (Dhanda and Munjal, 2009). It has been reported that heat stress applied ($33\pm 1^{\circ}\text{C}$) at germination stage resulted in greater reduction in TTC cell viability of thermo - sensitive wheat cultivar PBW-550 than heat tolerant C306 suggesting relative susceptibility of mitochondrial respiratory system in thermo - sensitive genotype during heat stress (Kalina and Palmer, 1968; Bavita *et al.*, 2012). Similar observations were recorded in respect of cell viability in other crops like *Phragmites communis* (Ding *et al.*, 2010) and cotton (Ehab Abou Kheir *et al.*, 2012).

Based on cell viability wheat cultivars AKAW-4627 and NIAW-34 exhibited significant higher ability to acquired thermotolerance and were found to be heat tolerant for

temperature stress. Wheat cultivar RAJ-4083, NIAW-1161, HD-2932 and to some extent NIAW-917 were found to be moderately thermotolerant and cultivars GW- 322, NIAW-301, MACS-2496 and HD-2189 were found to be susceptible to temperature stress.

3.2 SDS-protein profile at 25°C (Control): The protein bands appeared at control (25°C) treatment differed in their molecular mass and their intensity in thermotolerant and thermosusceptible wheat cultivars (Fig-1). Total 17 different molecular mass protein bands were observed in each variety of wheat and visualized with different intensities. Out of a total 17 protein bands, 3 bands were of high molecular mass *viz.*, 130, 110 and 100 kDa size, 6 bands were medium molecular mass (70, 67, 60, 56, 47 and 35 kDa) size and 8 small molecular mass proteins band (30, 29, 27, 25, 23, 18.9, 17 and 14kDa size) appeared at control (25°C) treatment. The 130 and 56 kDa proteins were found intense in all ten wheat genotypes. Small molecular weight 14kDa size protein band appeared as with high intense band in all thermotolerant wheat cultivars however this band was a moderate intense and low intense in thermosusceptible wheat cultivars

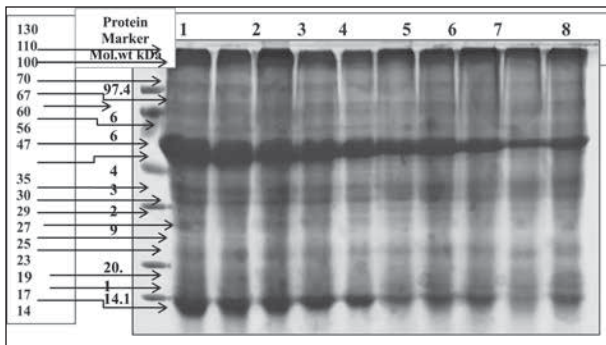


Fig. 1 SDS-PAGE protein profile at 25°C (Control). Lane 1. NIAW-34, 2. AKAW-4627, 3. NIAW-1161, 4. HD-2932, 5. RAJ-4083, 6. GW-322, 7. NIAW-301, 8. NIAW-917, 9. HD-2189, 10. MACS-2496

3.3 SDS-protein profile at 35°C for 1 h: After acclimation at 30°C for 1 h temperature stress treatment seedlings were subjected to 35°C for 1 h temperature stress treatment (Fig 2). A total of 24 protein bands appeared in wheat seedlings when shifted to 10°C higher temperature than control (25°C) temperature. Out of 24 bands, 8 bands appeared with high molecular weight, 5 bands of medium molecular weight protein were visualized and 11 low molecular/small molecular weight proteins bands were observed. The four protein bands of 58, 39, 25 and 21 were disappeared from all sensitive wheat cultivars whereas small HSP 16kDa size protein bands only appeared in NIAW-917 a thermo-susceptible wheat cultivar at 35°C 1 h temperature stress. Eight new protein bands of different sizes viz., 82, 58, 56, 37, 30.7, 28, 22 and 19.4 kDa appeared in all thermotolerant and susceptible wheat cultivars when exposed to 35°C 1 h heat stress. Protein band of 60 kDa was constitutively present as high intense in all thermo-tolerant and not in thermosusceptible genotypes. Protein bands with 58, 39, 25 kDa size appeared as intense to moderate intense in all

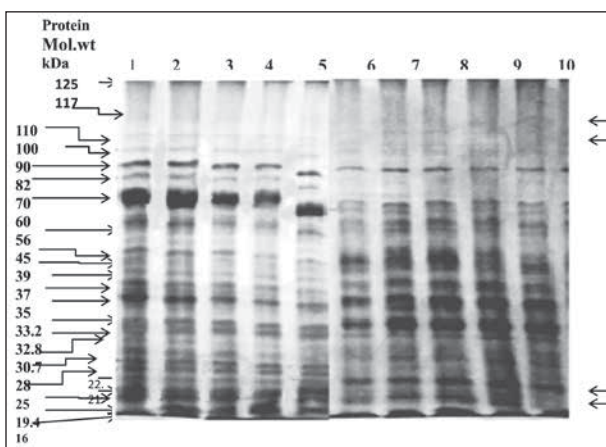


Fig. 2 SDS-PAGE protein profile at high temperature stress 35°C for 1h. Lane 1. NIAW-34, 2. AKAW-4627, 3. NIAW-1161, 4. HD-2932, 5. RAJ-4083, 6. GW-322, 7. NIAW-301, 8. NIAW-917, 9. HD-2189, 10. MACS-2496

the thermotolerant wheat but not found in the susceptible wheat cultivars. A 27 kDa protein band disappeared in all thermosusceptible wheat cultivars. A protein band of 28 kDa size was not found in HD-2932 but appeared as intense in all other thermotolerant and as a low intense in all thermosusceptible wheat cultivars. Whereas, intense protein band of 16kDa size appeared in all thermotolerant and thermo-susceptible wheat cultivar except GW-322 and NIAW-301.

3.4 SDS-protein profile at 40°C for 2 h: Prior acclimated wheat seedlings (30°C for 1 h → 35°C for 1 h HS) when exposed to high temperature treatment for 2 h at 40°C, some new protein bands of 125, 75, 40 kDa sizes appeared in NIAW-34 a thermotolerant wheat cultivars; a 40 kDa in AKAW-4627 whereas low intense band of 12kDa size appeared both in thermotolerant and thermosusceptible wheat cultivars (Fig 4). Medium range protein band with 40 kDa, 37kDa and seven small molecular mass proteins bands of 27, 25, 21.5, 20.1, 15.6, 14 and 12 kDa sizes were visualized with various intensities. It was observed that about 50 % protein bands were not observed at 40°C for 2 h. The protein band of 75, 90, 100 and 125 sizes found at 35°C disappeared at 40°C for 2h in all thermosusceptible wheat cultivars and three thermotolerant cultivars viz., RAJ-4083, NIAW-1161 and HD-2932. However, they were present in NIAW-34 and AKAW-4627 wheat cultivars. Temperature stress at 40°C 2 h resulted in appearance of low molecular protein bands (sHSP) in all thermo-tolerant and thermosusceptible wheat cultivars as moderate to low intense.

3.5 SDS-protein profile at 46°C for 3h: Seedlings acclimated at 40°C for 2h temperature stress treatment were shifted to lethal heat stress at 46°C for 3h. The Fig-4 showed that as temperature and time of exposure increased, protein profile was significantly changed. Total 12 new protein

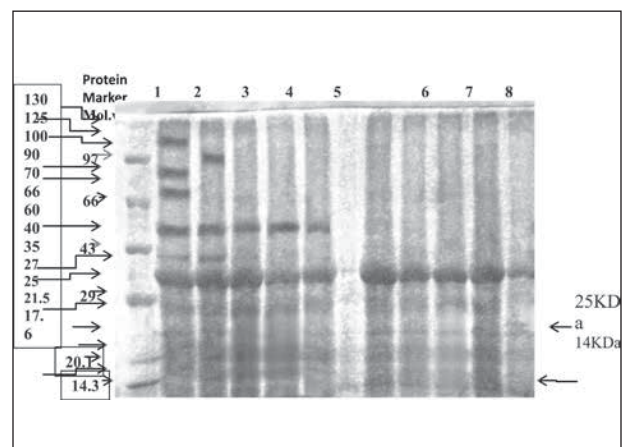


Fig. 3 SDS-PAGE protein profile at high temperature stress 40°C for 2h. Lane 1. NIAW-34, 2. AKAW-4627, 3. NIAW-1161, 4. HD-2932, 5. RAJ-4083, 6. GW-322, 7. NIAW-301, 8. NIAW-917, 9. HD-2189, 10. MACS-2496

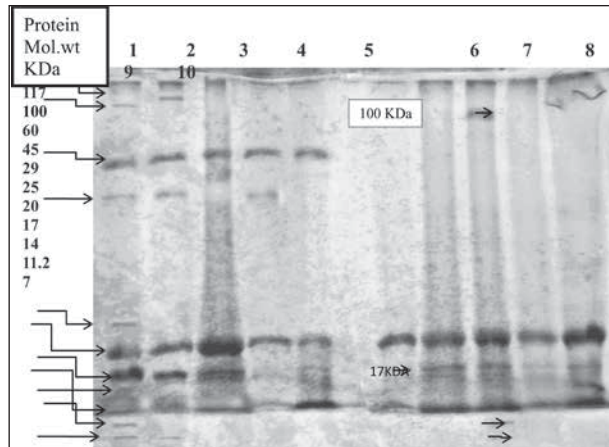


Fig. 4 SDS-PAGE protein profile at high temperature stress 46°C for 3h. Lane 1. NIAW-34, 2. AKAW-4627, 3. NIAW-1161, 4. HD-2932, 5. Raj-4083, 6. GW-322, 7. NIAW-301, 8. NIAW-917, 9. HD-2189, 10. MACS-2496

bands were synthesized at 46°C for 3h temperature stress. The protein bands of HSP100, HSP60 and HSP 14kDa protein bands disappeared at 46°C for 3h temperature stress. Out of 12 bands, 3 bands of high molecular weight (120, 117, 100 and 60 kDa), 1 band of medium molecular weight (45 kDa) and seven hsp bands of low molecular weight (7, 11.2, 14, 17, 20, 25 and 29 kDa) were observed at 46°C for 3h. A new 120 kDa protein bands visualized as intense in all wheat genotypes except GW-322 and found as moderate intense. A protein band of 117 kDa was observed only in thermotolerant AKAW-4627 cultivar. A protein band of 100 kDa was found only in NIAW-34 and NIAW-917. High intense band of HSP-60 (60 kDa) protein appeared in all thermotolerant cultivars and remained absent in thermosusceptible wheat cultivar. A new low range protein band of 45 kDa was found in NIAW-34, AKAW-4627 and NIAW-1161 whereas, it was absent in thermotolerant RAJ-4083, HD-2932 and in all thermosusceptible wheat cultivars.

A common protein band of 25 kDa which expressed previously at 30°C 1 h 35°C 1 h and 40°C 2 h also appeared as high intense band at lethal temperature (46°C 3h). A new protein band of 28.7 kDa found only in thermotolerant NIAW-34. Small molecular weight protein band of 20 kDa observed as high intense in thermotolerant NIAW-34, AKAW-4627 and as intense band in RAJ-4083, NIAW-301, NIAW-917, MACS-2496 and HD-2189 wheat cultivars at 46°C 3h. This band was observed as low intense in thermotolerant HD-2932 and absent in GW-322 wheat cultivar. A protein band of 17 kDa size appeared as moderate to low intense in thermotolerant NIAW-34, AKAW-4627 and four thermosusceptible NIAW-301, NIAW-917, MACS-2496 and HD-2189 wheat cultivars. New small mass weight protein bands HSP7 and HSP11.2 kDa size as moderate intense were found in NIAW-34,

AKAW-4627 at lethal temperature 46°C for 3h, whereas, it appeared as very low intense in a thermosusceptible NIAW-917 wheat cultivar.

As temperature and duration of exposure to temperature increased some quantitative and qualitative changes were observed in protein profile of wheat cultivars. In this study, we observed that the wheat genotypes synthesized new proteins (heat shock protein) when exposed to sub lethal (40°C for 2 h) and at lethal heat stress (46°C for 3h) which are differed significantly in their molecular mass. Protein band at 60 kDa size and 14 kDa sizes were expressed constantly in all thermotolerant wheat cultivars during high temperature stress. Protein bands with 7 and 11.2 kDa size expressed during severe heat stress in thermotolerant NIAW-34, AKAW-4627 and in two thermosusceptible NIAW-917, MACS-2496 wheat cultivars. A positive correlation of heat shock proteins with different intensity of temperature stress from 25 to 46°C has been recorded in wheat by Zivey (1987) and in tomato by Young *et al.* (2006).

Similar study carried in wheat by Krishnan *et al.* (1989) at seedling stage revealed that after exposure of wheat seedlings to heat shock 34 and 37°C several high molecular weight HSPs (83 to 94 kDa) and low molecular weight HSPs (16 to 42 kDa) appeared in Mustang and Sturdy varieties of wheat with distinct levels of acquired thermotolerance. Another study in wheat by Joshi *et al.* (1997) reported that HSP 16.9 a member of multigene family expressed in wheat is known to induce acquired thermotolerance. Recently genome-wide identification and characterization of 27 newly TaHSP20 candidate genes in wheat and 13 HvHSP20 in barley has been reported by Pandey *et al.* (2015) describing structures, phylogenetic relationships, conserved protein motifs, and expression patterns. This study also illustrates for the first time 3D model prediction of full-length wheat HSP20 (*TaHSP20*) protein and ACD region which revealed a widespread distribution of the sHSP family genes at various developmental stages of wheat and barley under 35°C and 42°C heat stress conditions. A growth stage specific expression profile study carried out under rainfed field condition and under high temperature stress condition in seedling stage revealed important role of Hsp 70 gene and their use as biomarkers for identifying phenophasic and developmental stage specific vulnerability for early and terminal stress tolerance trait (Hasan and Barthakur, 2014).

Mild heat shock is known to induce acquired thermotolerance in plants that is associated with concomitant production of heat shock proteins that belongs to different multigene families. Sharma-Natu *et al.* (2007) reported in wheat that high temperature stress from 30 to

45°C at different time interval enhanced the expression of HMW hsp70, hsp90 and hp104 as well a LMW hsp 2 in tolerant cultivar UP-2338 and HD-2285 as compared to thermo- susceptible cultivar HD2428 and HD2329. This results also suggested that low molecular weight HSPs can be used as a biological marker for identifying high temperature tolerant cultivar under serve heat stress conditions. The differences in SDS-PAGE protein profile after gradually exposed to different temperature are in agreement with the earlier results reported in wheat by Krishnan *et al.* (1989), Skylas *et al.* (2002), Sharma-Natu *et al.* (2007), Chauhan *et al.* (2009), Khalil *et al.* (2009) and Kumar *et al.* (2012).

In conclusion, our results of screening experiment conducted for assaying thermotolerance suggested that the TIR technique is a useful technique to identify genetic variability in high temperature tolerance in wheat within a short time period and suitable for screening a large number of genotypes and needs further confirmation at molecular levels. This study also demonstrates that TTC reduction test is a simple and realistic method for screening wheat seedlings for heat tolerance. Using this technique it was demonstrated that there is sufficient genetic variability present among wheat cultivars for high temperature tolerance. SDS-PAGE profile study revealed that normal wheat seedlings when shifted to temperature above five or more optimum growth temperature (25°C) synthesis of most normal proteins was repressed and translation of new protein was induced. Differential SDS-PAGE protein profile exhibited new set of proteins (heat shock protein) during sub lethal to severe heat stress (40°C for 2h to 46°C for 3h). Small molecular weight proteins appeared with different intensity during temperature induction treatments. Protein with 60kDa size and 14kDa size are observed constantly in all thermotolerant wheat cultivars during high temperature stress. Proteins with 7 and 11.2 kDa size are also observed at 46°C for 3h heat stress in thermotolerant NIAW-34, AKAW-4627 and also in thermosusceptible wheat NIAW-917 might help these genotypes to exhibit acquired thermotolerance that can be used as a marker for screening high temperature tolerant wheat genotypes. The information generated from this experiment will be useful for further study and needs to be evaluated at molecular levels.

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