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Changes in protein profile, ascorbic acid and chlorophyll stability index of wheat (*Triticum aestivum* L.) seedlings under heat stress and revival conditions

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Abstract

Screening of thirtysix genotypes for thermo-tolerance was done based on wilting of primary leaf and values of chlorophyll fluorescence. Four wheat genotypes, i.e., two tolerant (HW-2045 and WH-1021) and two susceptible (HS-277 and WH-147) were selected and polypeptide pattern, levels of ascorbic acid and chlorophyll stability index (CSI) were observed. The observations were recorded in the leaves of control, stressed and revived seedlings on 2nd and 4th day of revival. The least decrease in CSI under high temperature conditions was in tolerant genotype HW-2045 and highest decrease in susceptible genotype WH-147.The higher accumulation of ascorbic acid was observed under heat stress in all the genotypes but level was more in heat susceptible genotypes as compared to heat tolerant genotypes. SDS-PAGE of seedlings under stress conditions revealed the appearance of polypeptide bands of different molecular weight in tolerant and susceptible genotypes, and these polypeptides bands disappeared on revival of 2nd and 4th day. Based on genotypes screening and on polypeptide pattern, out of four genotypes, HW-2045 was found to be the most tolerant and WH-147 as the most susceptible genotype.

Keywords: Wheat, *Triticum aestivum*, heat stress, H_2O_2, protein profile

Introduction

Proteins are compounds of fundamental importance for all functions in the cell. It is well known that alteration of gene expression is always involved in preparing plants for an existence under stress. Protein variation is an essential part of plant response to environmental stress as well as for adaptation to environmental conditions.Exposure of plants to elevated temperature for short term (heat shock) results in a complex set of gene expression and selective translation of mRNA encoding heat shock proteins (HSPs), thereby, enhancing thermotolerance and improving cellular survival under subsequent heat stress (Gong *et al.*, 2001). In higher plants, these HSPs function as molecular chaperons that promote the degradation of misfolded proteins, aid in refolding of denatured proteins and prevent them from aggregation and resolubilize the proteins that have already aggregated (Boston *et al.*, 1996). A large number of studies reveal a positive correlation between induction of HSPs and acquisition of thermotolerance. However, the definite mechanism that imparts heat tolerance to plants still remains to be elucidated and there is pressing need to understand the precise physiological, biochemical and molecular mechanisms of heat tolerance in plants so that these attributes can be introduced in the species of interest through genetic engineering.

Improving tolerance to heat stress is a major challenge in many C3 crops given the threat of global warming. Heat stress leads to drastic change in the ascorbic acid, chlorophyll stability index and ultimately influences the sensors present in the membrane. Ascorbic acid is a biological oxidant. It protects the plants against oxidative damage resulting from aerobic metabolism due to the effect of wide range of biotic and abiotic stresses. Asocrbate protects the chloroplasts from photo-inhibition and suppresses the accumulation of photo-produced hydrogen peroxide in stroma. It also acts as the electron donor to ascorbate peroxidase and protects components of calvin cycle from inactivation (Hernandez *et al.*, 2004). The response mechanism of wheat to elevated temperature would help the development of wheat cultivars that perform better under heat stress. Therefore, present study was undertaken to analyze the changes in polypeptide pattern, levels of ascorbic acid and chlorophyll stability index (CSI) in wheat seedlings under heat stress and revival conditions.

Materials and Methods

Seeds of uniform size of 36 genotypes were selected and surface sterilized by stirring them with 0.2% (w/v) mercuric chloride solution for 5 minutes, and then, washed for 1 h under running tap water, and thereafter, the seeds were grown for 7 days in plastic trays containing sandy loam soil, which was equilibrated with distilled water. These soil filled trays were marked into 4 rows and 7 spots in each row. Five seeds were sown and the seedlings were raised by keeping the trays in B.O.D. incubator having 15/9 h light/dark cycle with 2500 lux light intensity at 25±1°C temperature. Seedlings of 7 days old were subjected gradually to temperature pretreatment (with rise of 5°C/h) to achieve 35±1°C, and thereafter, the acclimatized seedlings were exposed to 40 and 45°C for different duration to find out LT_{50}. Based on wilting of primary leaf and the values of chlorophyll fluroescence (Fv/Fm), screening was done against heat stress (Table 1).

Ascorbic Acid

For the extraction of metabolite, 1 g of seedlings each from control, stressed and revived plants were ground in 5 ml

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of chilled 0.8 N HClO4 and centrifuged at 10,000 rpm for 25 minutes. The clear supernatant was decanted carefully and used for the estimation of ascorbic acid. Ascorbic acid content was estimated by using the method of Roe (1964), which is based on the reduction of 2,6-dichlorophenol indophenol (2,6-DCPIP) dye by ascorbic acid. Seedlings were homogenized in 5 ml of 5% (w/v) meta-phosphoric acid in glacial acetic acid and the homogenate was centrifuged at 10,000 x g for 25 minutes. The supernatant, thus, obtained was used for the estimation of ascorbic acid content. An aliquot (0.5 ml) was titrated with 2,6-DCPIP reagent until a pink end point is reached. The quantity of ascorbic acid was calculated by comparing the amount of 2,6-DCPIP reagent used for unknown sample with that used with known quantities of ascorbic acid (5-40 mg).

Chlorophyll stability index

Chlorophyll stability index (CSI) was estimated using the method of Murthy and Majumder (1962). Chlorophyll estimation was done by incubating 50 mg of leaf material from middle portion of flag leaf in 10 ml of Dimethyl sulfoxide (DMSO) in a test tube for 3 h at 65°C temperature. Other set of same amount of leaf was exposed to high temperature treatment in water bath before putting 10 ml DMSO at 49°C temperature, and then, the absorbance of the solvent was recorded at 663 and 645 m for estimating chlorophyll content. Chlorophyll stability in terms of loss of chlorophyll (%) was calculated by considering control as 100%, and loss in chlorophyll at stress and revival was subtracted from control. The CSI was calculated using the formula given as under:

$$\text{CSI (\%)} = 1 - \frac{\text{Total chlorophyll of heated samples}}{\text{Total chlorophyll of non-heated samples}} \times 100$$

Electrophoretic protein banding pattern

For studying the polypeptide pattern, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of susceptible and tolerant genotypes was carried out by using the method of Laemmli (1970).

Calculation of Rf values: Rf values of each protein bands was calculated by the following relationship:

$$\text{Rf} = \frac{\text{Distance traveled by the protein band}}{\text{Distance traveled by the tracking dye}}$$

A calibration curve was drawn by plotting the relationship of log molecular weight and Rf value of standard proteins. Molecular weight of each band in the gel was calculated by using standard curve.

Results and Discussion

Table 1. Screening of the wheat genotypes based on chlorophyll fluorescence

S. No.	Genotype	Chlorophyll Fluroscence (Fv/Fm)	S. No.	Genotype	Chlorophyll Fluroscence (Fv/Fm)
1	WH-147	0.451±0.04	19	DBW-14	0.704±0.03
2	HS-277	0.480±0.03	20	PBW-579	0.706±0.11
3	WH-542	0.486±0.08	21	Cow-(w-1)	0.710±0.04
4	K -9107	0.495±0.07	22	HD-2781	0.718±0.12
5	DBW-22	0.496±0.08	23	Lok-54	0.720±0.03
6	PBW-574	0.496±0.09	24	NWS-(2-4)	0.720±0.08
7	Sonalika	0.520±0.03	25	PBW-373	0.720±0.09
8	Raj-4083	0.524±0.06	26	HS-240	0.721±0.02
9	HI-1544	0.537±0.06	27	K-0307(1)	0.721±0.04
10	WH-1022	0.568±0.05	28	HD-2824	0.721±0.07
11	NW-2036	0.569±0.07	29	HW-2004	0.722±.06
12	Lok-1	0.608±0.09	30	VL-616	0.723±0.02
13	NW-3069	0.663±0.05	31	Raj-4037	0.725±0.11
14	HD-2733	0.681±0.03	32	Raj-3765	0.727±0.09
15	HW-5021	0.690±0.09	33	Raj-4101	0.736±0.05
16	PBW-575	0.693±0.04	34	HD-2687	0.741±0.06
17	HS-375	0.698±0.07	35	WH-1021	0.742±0.04
18	HI-1539	0.701±0.08	36	HW-2045	0.762±0.06

The wheat genotypes showing higher value of Fv/Fm were selected as heat tolerant and those showing low value as heat susceptible genotype. Based on this experiment, the genotypes HW-2045 (0.762) and WH-1021 (0.742) were identified as thermotolerant, while the genotypes HS-277 (0.480) and WH-147 (0.451) as thermosusceptible.

Ascorbic Acid

The control seedlings of susceptible genotypes HS-277 and WH-147 had same ascorbic acid content, i.e., 20-21 mmoles g-1 fresh weight (Fig 1). Ascorbic acid content increased under stress conditions in leaves of all genotypes; susceptible genotypes showed higher increase (45-60%) enhancement over their respective control. Upon revival on 2nd and 4th day, the ascorbic acid content decreased in tolerant genotypes by 9-13% and in susceptible genotypes by 21-47% in comparison to the seedlings grown under stress conditions.The results of present investigation are in agreement with those of Ma *et al.* (2008) and Babu and Deveraj (2008) who reported elevated level of ascorbic acid in apple leaves and French bean seedlings, respectively in response to high temperature in comparison to control seedlings. Mehlhorn *et al.* (1996) reported an increase in ascorbic acid content under oxidative stress. It protects the plants against oxidative damage resulting from aerobic metabolism and from array of biotic and abiotic stresses (Smirnoff and Pallanca, 1996).

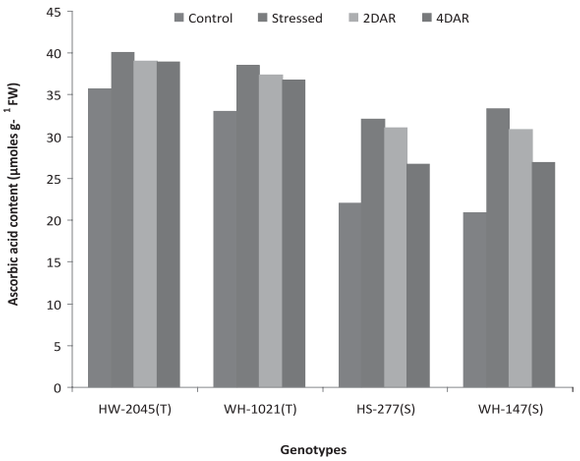

Fig. 1. Effect of heat stress and revival on ascorbic acid content (mmoles g-1 fresh weight) in leaves of heat-tolerant and heat-susceptible genotypes of wheat seedlings

Chlorophyll stability index- (CSI)

The control seedlings of all genotypes had same amount of chlorophyll content in their leaves (100%). Decline in chlorophyll stability in terms of loss of chlorophyll content (%) was noted in all genotypes under high temperature stress conditions. The loss of chlorophyll was less in tolerant genotypes than in susceptible ones (Fig 2). Decrease in chlorophyll stability index under high temperature was least in tolerant genotype HW-2045 (32%) and the highest in susceptible genotype WH-147 (80%). On 2nd and 4th day of revival, the chlorophyll stability increased in all the genotypes as compared to the genotypes grown under stress conditions but chlorophyll content was still lower than the seedlings of control treatment.

Ristic *et al.* (2007) observed decrease in chlorophyll content in response to high temperature treatment in wheat. The cultivar differences in chlorophyll loss observed in present study are in agreement with those of Wardlaw *et al.* (1980) and Blum (1986) who demonstrated decrease in chlorophyll content in wheat cultivar when exposed to high temperature.

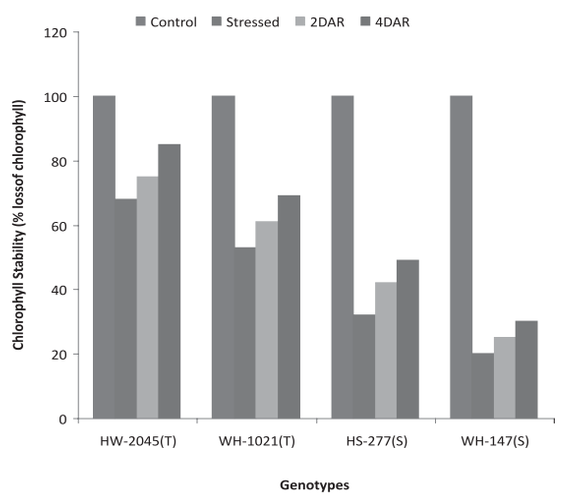

Fig. 2. Effect of heat stress and revival on chlorophyll stability index (%) in leaves of heat-tolerant and heat-susceptible genotypes of wheat seedlings

Protein profile

Water-soluble proteins were extracted from control, stressed and revival seedlings of four wheat genotypes (two tolerant, WH-1021 and WH-2045 and two susceptible, HS-277 and WH-147). The electrophoretic pattern for these proteins in tolerant and susceptible genotypes has been shown in plate 1 and plate 2, respectively.

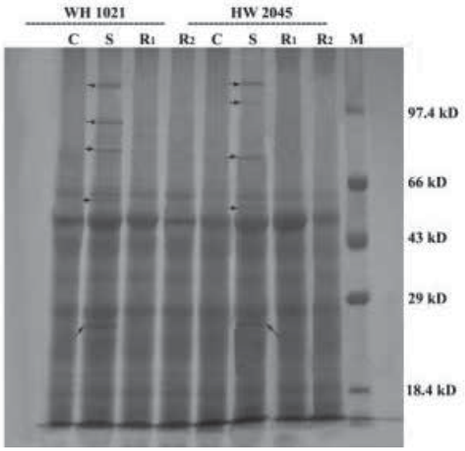

Plate 1: Electrophoretic pattern of leaves of tolerant wheat genotypes viz. WH-1021 and HS-2045 by SDS PAGE
M: Protein marker
C: Control-25°C
S: Stress - 40°C for six hr
R_1: Two days after revival
R_2: Four days after revival

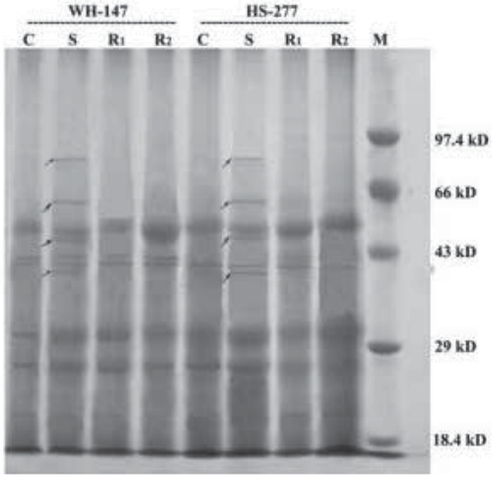

Plate 2: Electrophoretic pattern of leaves of susceptible wheat genotypes viz. WH-147 and HS-277 by SDS PAGE
M: Protein marker
C: Control-25°C
S: Stress - 40°C for six hr
R_1: Two days after revival
R_2: Four days after revival

Tolerant genotype WH-1021 showed five more protein bands with molecular weight (MW) 103.7, 92.2, 83.0, 65.0 and 26.0 kDa under heat stress conditions than control, while the other tolerant genotype HW-2045 under heat stress

gave five more bands with MW 103.7, 98.5, 79.0, 62.0 and 26.0 kDa as compared to control. These newly synthesized HSPs might protect the plant from heat stress. These bands disappeared when stress was removed. In comparison to tolerant genotypes, the susceptible genotypes HS-277 and WH-147 showed four more protein bands with molecular weight 80.0, 57.9, 45.7 and 34.4 kDa under heat stress than control (Plate 2). On 2nd and 4th day of revival, these bands disappeared.

All organisms respond to elevated temperature with the production of a defined set of proteins called heat shocked proteins. These heat shocked proteins (HSPs) protect the cells from detrimental effect of high temperature, and that accumulation of HSPs, leads to increase in thermo-tolerance. A large number of studies reveal a positive correlation between inductions of HSPs and acquisition of thermal tolerance (Bhattacharjee and Mukherjee, 2006).

In wheat seedlings, Joshi *et al.* (1997) linked the acquired thermo-tolerance with 26 kDa plastid localized heat shocked protein. The results are also in accordance with the findings of Mahmoud and Mohamed (2007) who observed the production of HSPs bands of MW 17 kDa and 103.7 kDa in wheat seedlings exposed to differential temperature stress. Ouebbou and Paulsen (1999) found a new set of heat shocked proteins (17-80 kDa) induced within 4 h in wheat when the incubation temperature was raised to 37°C. Del-Aquilla *et al.* (1998) reported that following heat shock treatment, the heat shock response resulted in the production of several HSPs with different MW and specific low MW HSPs (17-14.2 kDa). Heat shocked proteins have been even related to the recovery of protein synthesis after removal of heat stress (Hendrich and Hartle, 1993). The differential thermo-tolerance of wheat cultivars was found related with accumulation and expression of both HMW and LMW heat shocked proteins (Sharma *et al.*, 2007).

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