ANTIOXIDANT ENZYMES AND PROTEIN PROFILES IN WHEAT SEEDLINGS UNDER ABIOTIC STRESS

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Abstract

Objective: To find out the early responses of antioxidant enzymes which play a major role in protection of germinating wheat seeds against oxidative stress. Furthermore, the effect of chronic stress on protein contents and profiles were also analyzed.

Method: The response of antioxidant enzymes peroxidase and catalase to dehydration stress was imposed at low (4°C) and high (38°C) temperature, salinity (0.2M NaCl), sucrose (0.5M), ABA (30µM) and H_2O_2 (10mM) in 72 hours old wheat seedlings. Three genotypes namely LU-26, Siren and Anmol-91 were used for the experiment.

Result: Analysis of variance showed non-significant differences for peroxidase while catalase depicted significant differences among the treatments. For total protein contents significant differences of total protein contents were found among the treatments, genotypes LU-26 and Siren reflected decreased protein contents at all the stresses whereas Anmol-91 exhibited increased protein content at low temperature, NaCl and sucrose. Correlation coefficient results elicited that negative and significant correlation between total protein contents and peroxidase under optimal, NaCl and ABA whereas in total protein contents and catalase reflected negative and significant correlation by exogenous

stress of ABA only. Electrophoretic profiles of total proteins in endosperm of wheat seedlings germinated under optimal and stress conditions were also investigated.

Conclusion: Results showed variation in the presence or absence of the bands that arose under optimal and stress conditions.

Key words: Abscisic acid, correlation coefficient, dehydration, hydrogen peroxide, wheat

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Introduction

Environmental stresses exert their effects directly or indirectly through the formation of reactive oxygen species (ROS) [1]. ROS are produced in both unstressed and stressed cells[2]. The production and destruction of ROS under normal conditions is well balanced. However, under adverse environments such as extreme temperatures, salinity, drought or intense light, the formation of ROS is more rapid than scavenging and detoxifying, and this misbalance creates oxidative stress [3, 4.] It is now known that ROS are involved in seed development and the completion of seed germination, and the generation of ROS during seed dessication, germination and ageing has been proven[5]. Therefore, antioxidant compounds and enzymes scavenging and detoxifying ROS are of particular importance for

the survival of plants under stress[6-8]. Numerous studies shows that antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and ascorbate peroxidase (APX) and their relative compositions change during exposure to stress [3,9,7,10]. Plant peroxidases have been used as biochemical markers for various types of biotic and abiotic stresses[11,12]. Catalase is the most efficient antioxidant enzyme[13]. The expression of catalase is important and critical against oxidative stress induced by a given environmental stress[14]. Proteins are compounds of fundamental importance for all functions in the cell [15]. 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 [16-18]. The objective of this study was to find out the early response of antioxidant enzymes against oxidative stress and the effect of chronic stress on protein profile subjected to dehydration, hydrogen peroxide and abscisic stress in wheat seedlings.

Research methods

Three genotypes namely LU-26, Siren and Anmol-91 were used for the experiment. Seeds were germinated in petriplates underline filter paper with distilled water at optimal (24°C control), dehydration (4°C, 38°C, 0.2M NaCl and 0.5M sucrose), 30µM ABA and 10mM H_2O_2. After 72 hours sowing wheat seedlings were harvested.

Seedlings subjected to various treatments were analyzed for antioxidant enzymes (peroxidase and catalase), total protein contents and electrophoretic protein profile. POX activity was monitored at 470 nm[19]. CAT was assayed by monitoring the decrease in

absorbance due to hydrogen peroxide at 240 nm as described by Aebi[20]. Protein content was determined according to the method of Lowry et al [21]. SDS PAGE was conducted on 12% acrylamide gels according to the description of Laemmli [22].

Experiment was carried out in factorial design with two replications and the differences among means were determined by Duncan's multiple range test (DMRT) at 5% level and correlation coefficient studies were done using SPSS for Windows Version 11.0 (SPSS, Inc., Chicago, IL).

Results and discussion

Analysis of variance showed non-significant differences for peroxidase while catalase depicted significant differences among the treatments (Table 1).

Table 1. Mean squares for POX, CAT and Total Protein Contents among different treatments in wheat genotypes

Sources of Variation	df	POX	CAT	Total Protein Contents
Replications	1	5.72	0.36	200
Treatments	6	12.01	1.89*	298.52**
Genotypes	2	15.42	1.74	60.76
T×G	12	15.08	1.47	165.57**

LU-26 showed increase in catalase activity under most of the stresses except high temperature and sucrose that illustrated decline of the CAT activity (Figure 1). Genotype

Siren reflected the similar pattern as in LU-26 with the exception of H_2O_2 condition. Anmol-91 exhibited decline in CAT activity among all the stress factors applied with a reverse effect in H_2O_2 treatment.

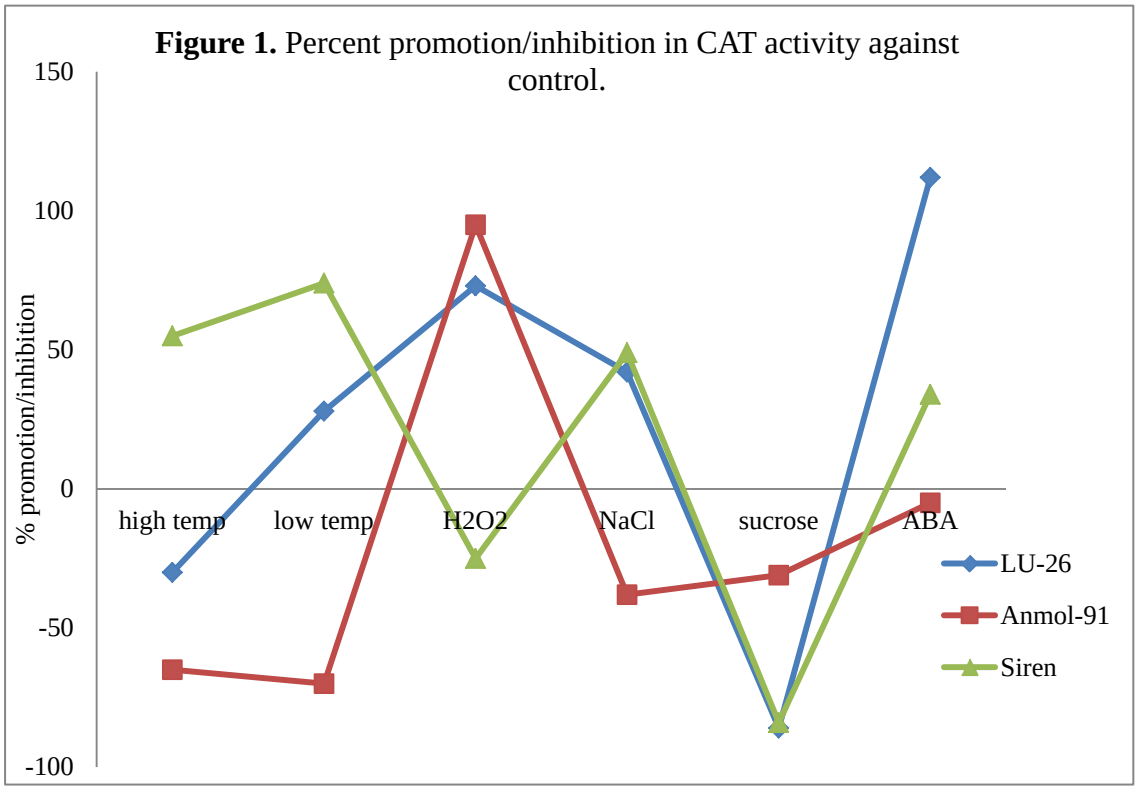

Figure 1. Percent promotion/inhibition in CAT activity against control.

A prolonged decrease in catalase expression under the influence of low temperature, ABA and sucrose (hyperosmoticum) treatment has been observed by Baek and Skinner [4, 23]. An increase in CAT activity has been observed in wheat tolerant genotypes by exogenous stress of ABA[24].

The results obtained for total protein contents showed significant differences among the treatments. Genotypes LU-26 and Siren reflected decreased protein contents at all stresses whereas Anmol-91 had increased protein content at low temperature, NaCl and sucrose (Figure 2). Increased in protein levels have also been reported in wheat[25]. One way of plants to tolerate abiotic stresses is synthesis of dehydrins that accumulate in plants in response to ABA, low temperature, drought, salinity or chilling[26].

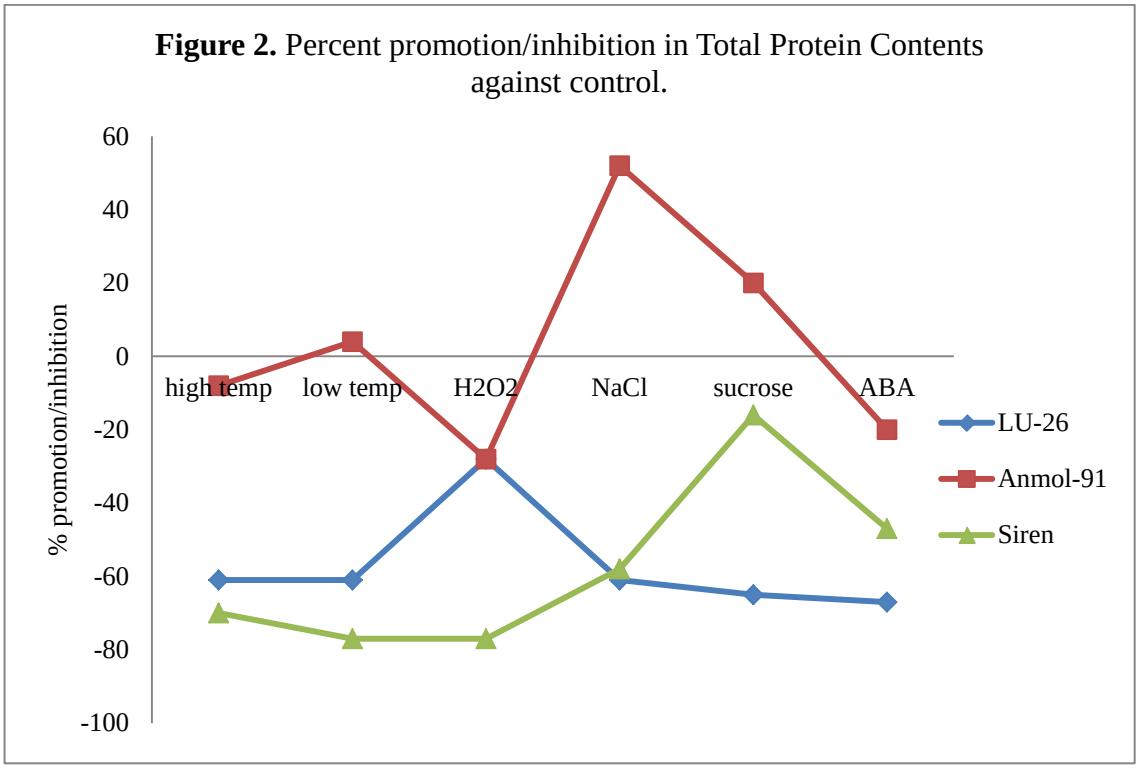

Figure 2. Percent promotion/inhibition in Total Protein Contents against control.

Table 2. Correlation coefficient of total protein contents, peroxidase and catalase activity among optimal and different treatments

Control	**TP**	**POX**	**CAT**
TP		-0.936**	-0.725
POX			0.823*
High temperature	**TP**	**POX**	**CAT**
TP		-0.718	-0.926*
POX			0.388
Low temperature	**TP**	**POX**	**CAT**
TP		-0.680	-0.832
POX			0.359
H_2O_2	**TP**	**POX**	**CAT**
TP		-0.814	-0.985*
POX			0.916*
NaCl	**TP**	**POX**	**CAT**
TP		-0.918*	-0.682
POX			0.805
Sucrose	**TP**	**POX**	**CAT**
TP		-0.995	-0.776
POX			0.669
ABA	**TP**	**POX**	**CAT**
TP		-0.850*	-0.932**
POX			0.837*

** significant at 5% probability

Table 2 illustrated negative and significant relationship between total protein contents and antioxidant enzymes under different treatment conditions. It showed that if protein contents had decreased then antioxidant activities increased or vice versa. Furthermore, activity of POX and CAT reflected positive and significant relationship under optimal, H_2O_2 and abscisic acid. It exhibited that both enzyme activities dependent on each other.

Banding profile segregated into ten groups based on molecular weight. Anmol-91 banding patterns could be ordered as follows: NaCl = sucrose >ABA >control = low temperature = high temperature = H_2O_2 (Figure 3). A newly induced band has been noticed in high and low temperature, NaCl, sucrose and ABA within the range of 120-91 kDa.

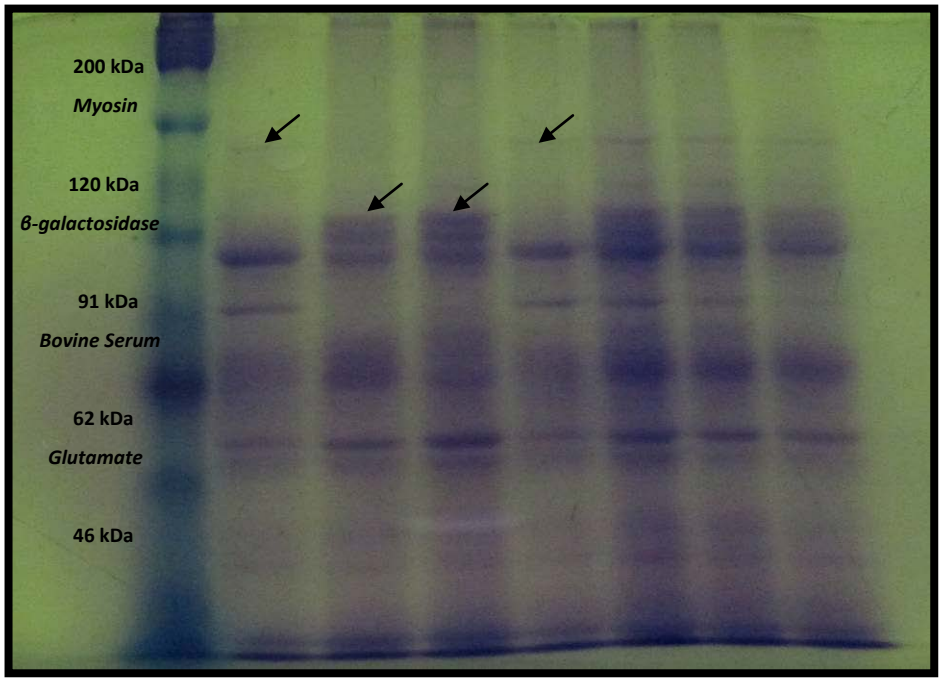

Figure 3. SDS PAGE of total proteins in the wheat endosperms of Anmol-91.

Lane 1- *Molecular weight markers (indicated on the left)*, Lane 2-*control*, Lane 3-*high temperature*, Lane 4 -*low temperature*, Lane 5 -*10 mM* H_2O_2, Lane 6-*0.2 M NaCl*, Lane 7- *0.5 M sucrose*, Lane 8 -*30 µM ABA*

LU-26 band patterns could be ordered from greatest to lowest as follows: NaCl >control = sucrose = ABA >low temperature >high temperature >H_2O_2 (Figure 4). Similar variant band has been noted with the same position as in Anmol-91.

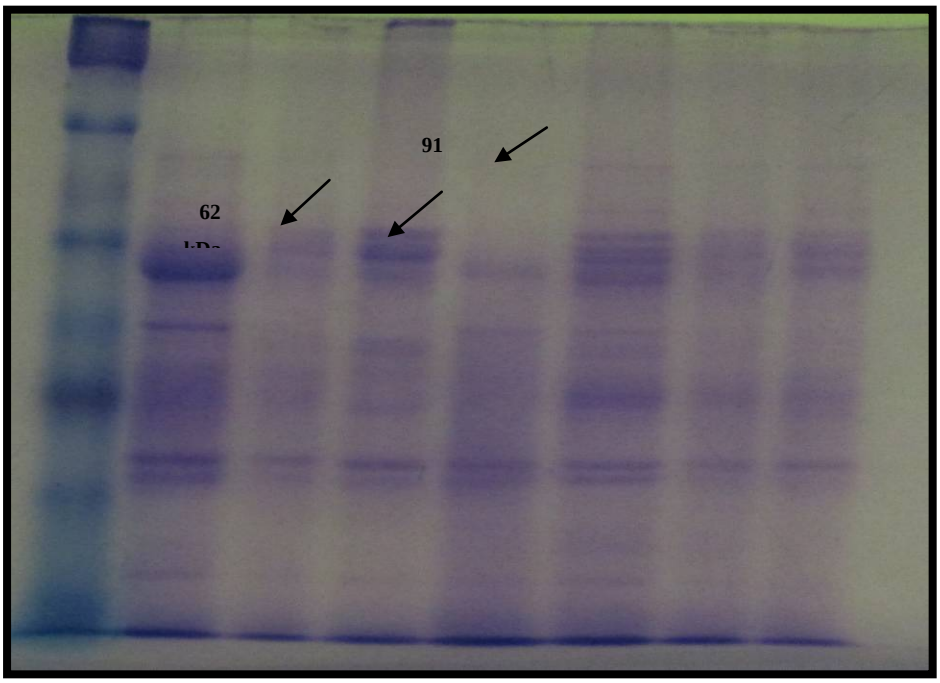

Figure 4. SDS PAGE of total proteins in the wheat endosperms of wheat genotype LU-26.

Banding profile for Siren could be arranged from greatest to lowest as: Sucrose >ABA >control = high temperature = low temperature = NaCl >H_2O_2 (Figure 5). Similar banding profile has been detected with respect to variant band and absence of band as of genotypes Anmol-91 and LU-26.

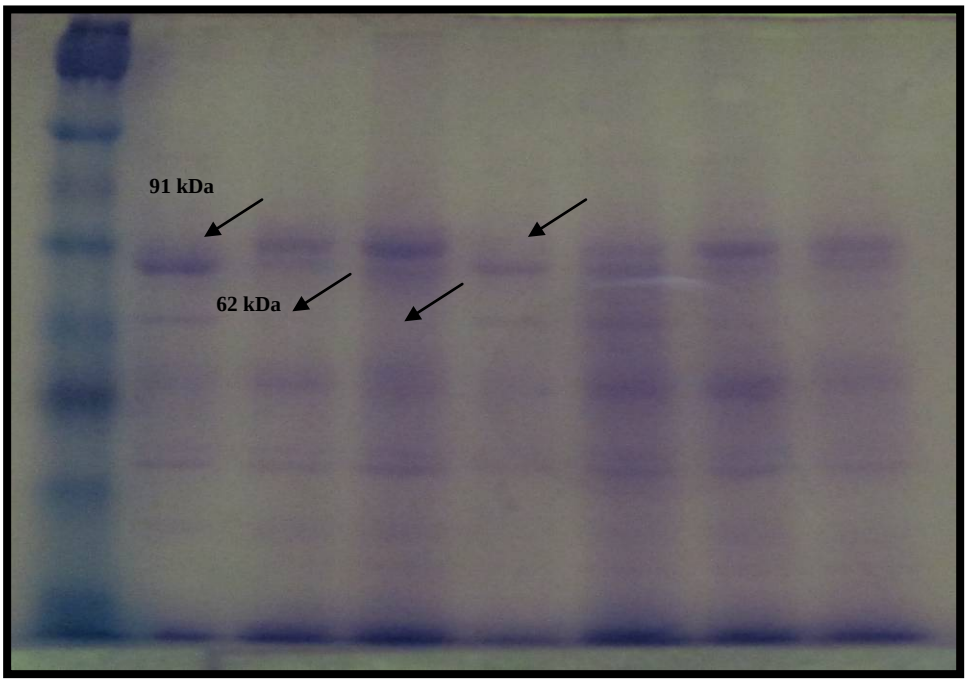

Figure 5. SDS PAGE of total proteins in the wheat endosperms of wheat genotype Siren.

Conclusion

Based on the results presented in this study the following conclusions could be drawn:

Genotypes LU-26 and Siren revealed decreased levels of total protein content among stress conditions. The variable response of CAT activity among treatments in three different genotypes. LU-26 and Siren demonstrated a similar effect of CAT activity with the few exceptions. In contrast, Anmol-91 exhibited decline activity of CAT under all stresses that was applied on wheat seedlings except H_2O_2 condition. It can be concluded that these two genotypes i.e. LU-26 and Siren reflected more tolerance with respect to CAT activity under different treatments compared with Anmol-91.

SDS PAGE profile of three genotypes reflected erratic pattern under optimal and stress conditions. Genotypes LU-26 and Anmol-91 illustrated more variation in stress conditions regarding newly induced bands that were absent in control condition. However the genotype Siren showed one newly induced band at low and high temperature. It can be concluded that these stress factors had greater influence on wheat seedlings that produce new type of bands this indicated stress proteins may play a protective role against oxidative damage/stress.

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