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Thermotolerance and Antioxidant Response Induced by Putrescine and Heat Acclimation in Wheat Seedlings

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ABSTRACT

Following heat acclimation (35°C) and putrescine (Put, 100 μM) pretreatment, seedlings of three wheat (*Triticum aestivum* L.) cultivars namely PBW 343, C 306 (heat tolerant) and WH 542 (heat susceptible) were exposed to heat stress at 45°C for 2 h and then recovered at 25°C for 5 days. Pre-treated seedlings performed better under heat stress than the control. Heat acclimation and Put pretreatment ameliorated heat shock response through reduced electrolyte leakage and increased respiratory activity indicating protection of the cell membrane. Higher activities of POX, DAO and PAO were observed in pre-treated seedlings than control in both roots and shoots. Seedling growth of all three cultivars was dramatically reduced under heat stress but heat acclimation and Put pre-treatment were effective in imparting thermoprotection against the lethal heat shock of both roots and shoots in intact seedlings.

Keywords: antioxidative enzyme, heat acclimation, putrescine, thermotolerance, wheat
Abbreviations: DAO, diamine oxidase; **PA**, polyamine; **PAO**, polyamine oxidase; **Put**, putrescine; **POX**, peroxidase; **TTC**, triphenyl tetrazolium chloride

INTRODUCTION

High temperature is becoming one of the major abiotic stresses limiting plant growth, development and productivity (Houghton *et al*. 2001; Alcazar *et al*. 2010). In higher plants, heat stress is responsible for causing oxidative stress leading to imbalance in production between prooxidants and antioxidants (Blokhina *et al*. 2003). It is known that plants resist to the stress-induced production of ROS by increasing component amounts of their defensive system (Foyer *et al*. 1994a, 1994b; Zabalza *et al*. 2008; Cvikrova *et al*. 2012). In addition, plant cells are normally protected against such effects by a complex antioxidant system such as non-enzymatic and enzymatic antioxidants (Wahid *et al*. 2007; Ali *et al*. 2008; Goyal and Asthir 2010). Heat acclimation, during which the plants develop heat tolerance, is a genetically controlled process that is triggered by exposure plants to high, non-lethal temperatures (Wang and Li 2006). The processes involved in temperature acclimation are initiated by the perception of temperature signals and transduction of these signals into biochemical processes that finally lead to the development of heat tolerance (Xu *et al*. 2006). These adaptation processes include adjustment of metabolism and gene expression at high temperatures, which enables plants to minimize heat injury.

Polyamines (PAs) comprising the diamine putrescine (Put), the triamine spermidine, and the tetramine spermine are ubiquitous in plant tissues and have been implicated in an overwhelming array of plant growth and developmental processes. There is a growing appreciation of the role of PAs in plant stress responses (Hummel *et al*. 2002; Liu *et al*. 2007; Alcazar *et al*. 2011), but their role in heat shock protection in plants is not well understood. The repair of plant cells exposed to heat shock, after having been returned to an optimal temperature has been reported (Bauer and Senger 1979), but the degree of recovery and the time required for recovery depend upon the severity of stress (Berri and Bjorkman 1980).

PAs, being cationic in nature, can associate with anionic components of the membrane such as phospholipids thereby stabilizing the bi-layer surface and retarding membrane deterioration (Velikova *et al*. 2000). PAs also have radical scavenging properties (Singh *et al*. 2002) and antioxidant activity which have been found to confer protection from abiotic stresses (Prabhavathi and Rajam 2007; Yiu *et al*. 2009) but their mode of action is not well understood. Protection of membranes from peroxidation by PAs could involve both their ability to interact with phospholipids (Hussein *et al*. 2006; Cavusoglu *et al*. 2007). Groppa and Benavides (2008) reported that that the rise in Put is mainly attributed to the increase in arginine decarboxylase (ADC) activity as a consequence of the activation of ADC genes and their mRNA level. Improvement of *in vitro* androgenesis in niger using PAs was reported recently (Hema and Murthy 2008; Quinet *et al*. 2010).

Diamine oxidase and PA oxidase are thought to play a major role in the catabolism of PAs in plant tissues (Asthir *et al*. 2002). Diamine oxidase catalyzes the oxidative deamination of the diamine Put (Put) and cadaverine producing the corresponding aminoaldehyde, ammonia and H_2O_2, while PAO preferentially cleaves the amino propyl side-chains at secondary amino groups of PA substrates, such as spermidine or spermine producing H_2O_2, 1,3-diamino propane and 1-pyrroline or 1-(3-aminopropyl)-pyrroline, respectively. Any H_2O_2 generated is not accumulated or if so, then catabolised by peroxidase (POX), in healthy and in stressed plant cells (Ros Barceló 1998; Asthir *et al*. 2010a). It was suggested that H_2O_2 generated by amine oxidation, was important for callose deposition and lignification both in normal and stress conditions (Asthir *et al*. 2001, 2002; Cona *et al*. 2006). Role of POX in formation of diphenyl bridges, cross-linking of hydroxyproline-rich proteins (extension) in the cell wall matrix and during stress related physiological processes is well demonstrated (Low and Merida 1996; Asthir *et al*. 2010b).

Exogenous application of Put might reverse the effect of heat shock and impart thermotolerance. It is therefore imperative to investigate the mechanisms of Put by which

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plant adopts to, get injured, and recovers from the stress imposed. The present study was planned to investigate the mechanisms of Put action and heat acclimation during heat shock in acquired thermotolerance in wheat seedlings utilizing heat-tolerant (PBW 343, C 306) and heat-susceptible (WH 542) cultivars with respect to membrane thermoprotection, TTC cell viability and amine oxidases and POX activities.

MATERIALS AND METHODS

Chemicals and biochemicals

Substrates used to determine DAO and PAO activities were all supplied by the Sigma-Aldrich Company Ltd, Poole, Dorset, UK. All other chemicals used were of analytical grade.

Plant materials and growth conditions

Seeds of three wheat cultivars (*Triticum aestivum* L.) namely PBW 343, C 306 and WH 542 were obtained from Department of Plant Breeding and Genetic, Punjab Agricultural University, Ludhiana. Field trials of previous studies of these cultivars have indicated that WH 542 is a heat-susceptible cultivar whereas PBW 343 and C 306 are relatively heat tolerant cultivars. These cultivars were used for conducting the following investigations. Seeds were surface sterilized with 0.1% $HgCl_2$ for 1 min, rinsed thoroughly with distilled water and germinated in dark for 24 h at 25°C in Petri dishes (9 cm) on filter paper moistened with 5 ml of distilled water in triplicates for each treatment. A seed was considered to have germinated when its radicle emerged at least 5 mm. Heat shock treatment at 45°C for 2 h was applied to germinated seeds followed by recovery growth at 25°C for 5 days in dark. Heat acclimation treatment at 35°C alone and in the presence of Put (100 µM) was given to the germinated seedlings for 2 h prior to heat shock at 45°C, followed by recovery growth at 25°C for 5 days. Seedlings kept entirely at 25°C without subjection to any treatment were taken as control. Twenty seedlings were used in each experiment and each experiment was repeated in triplicate.

Cell membrane stability (CMS)

For each treatment, 10 embryonic axes (root and shoot) were excised and washed with distilled water to remove adhering electrolytes (Blum *et al*. 2001). The tissue was then immersed in test tubes containing 20 ml of distilled water and stirred continuously at 28°C. After 5 h the electrolyte leakage was estimated by conductivity meter. The samples were then boiled for 30 min and conductivity was measured again. The percentage CMS was calculated as:

$$\frac{\text{Conductivity before boiling}}{\text{Conductivity after boiling}} \times 100$$

TTC cell viability measurements

This method is based on the reduction of 2,3,5-triphenyl tetrazolium chloride (TTC) to formazan which is red colored product, indicating respiratory and metabolic activity as described by Asare-Boamah and Fletcher (1983). Primary root tips and hypocotyls (1 cm) were floated in 0.1% TTC (w/v) solution in the dark at 30°C for 3 h. The root tips were homogenised and extracted twice with ethyl acetate and the volume was made up to 4 ml. The extract was centrifuged (2500 × *g*) for 10 min at 0°C. The intensity of red colour was measured at 486 nm using double beam UV-VIS Spectrophotometer 2203, Systronics, India.

Extraction and assay of POX, DAO and PAO

1 g of root/ shoot tissue was homogenised (triplicate samples) at 4°C in 100 mM K-phosphate buffer (pH 6.5) containing 5 mM dithiothreitol and the extract centrifuged at 16,000 × *g* for 20 min at 4°C. The supernatant was used as source of enzyme. POX (EC 1.11.1.7) was assayed by the method of Claiborne and Fridoric (1979). DAO (EC 1.4.3.6) and PAO (EC 1.4.3.4) activities were estimated spectrophotometrically by a method based on the colorimetric assay of Δ-pyrroline using Put (for DAO) and spermidine (for PAO) as substrates (Holmstead *et al*. 1961). For DAO and PAO activities, the reaction mixture of 2.0 ml consisted of 0.1 ml of enzymic extract, 50 units of catalase, 0.1% *O*-aminobenzaldehyde and the reaction started with one of the two different buffer and substrate combinations i.e. 10 mM Put in 50 mM K-phosphate buffer (pH 7.5) for DAO; 10 mM spermidine in 50 mM K-phosphate buffer (pH 6.0) for PAO. The reaction was incubated at 30°C for 3 h, and then stopped with 2.0 ml of 10% (v/v) perchloric acid and the tubes centrifuged at 5000 rpm for 15 min. Formation of the Δ-pyrroline product was determined by reading the absorbance at 430 nm in spectrophotometer. Control reactions were carried out with inactivated enzyme prepared by heating for 20 min in a boiling water bath. Activities are shown as the mean of six determinations and are expressed as $\Delta E\ min^{-1}g^{-1}$ FW for POX, DAO, and PAO activities as pmol Δ-pyrroline $min^{-1}g^{-1}$ FW for DAO and PAO activities.

Statistical analysis

All the values reported in this paper are the means of three replicates. All data obtained was subjected to analysis of variance (factorial experiment in completely randomized design) by using CPCSI software package. In all the tables ± values represent standard error of the means.

RESULTS AND DISCUSSION

Induction of thermotolerance by putrescine on growth parameters

In the present investigation, the role of Put and heat acclimation treatments in induction of thermotolerance was investigated. Heat shock exposure to wheat seedlings caused inhibition of both root and shoot growth measured in

Table 1 Effect of temperature and putrescine (Put) treatments on root/shoot lengths (cm) of wheat seedling.

Temperature ± Put	PBW 343		C 306		WH 542	
	Root	Shoot	Root	Shoot	Root	Shoot
Control	7.8 ± 0.4	7.2 ± 1.1	8.0 ± 0.9^{b}	7.6 ± 0.6^{b}	9.8 ± 1.0^{b}	6.9 ± 0.8^{b}
HS	4.0 ± 0.3^{a}	4.3 ± 0.3^{a}	5.0 ± 0.4^{ab}	4.4 ± 0.5^{a,b}	3.0 ± 0.5^{ab}	4.8 ± 0.3^{a,b}
HA+HS	7.0 ± 0.5^{a}	7.6 ± 0.4^{a}	7.6 ± 0.2^{ab}	6.4 ± 0.5^{a,b}	6.5 ± 0.7^{ab}	6.6 ± 0.3^{a,b}
HA+HS+Put	8.0 ± 0.3^{a}	10.4 ± 0.5^{a}	13.1 ± 1.5^{ab}	10.4 ± 0.6^{ab}	10.0 ± 0.6^{ab}	9.7 ± 0.6^{ab}
Dry weight (mg/seedling)						
Control	6.9 ± 0.3	5.2 ± 0.9	6.4 ± 0.8	3.1 ± 0.7^{b}	4.1 ± 0.6^{b}	3.4 ± 0.4^{b}
HS	4.4 ± 0.5^{a}	2.5 ± 0.5^{a}	3.1 ± 0.3^{a}	2.8 ± 0.9^{a,b}	2.5 ± 0.4^{a,b}	2.8 ± 0.5^{a,b}
HA+HS	5.7 ± 0.7^{a}	5.2 ± 0.3	6.4 ± 0.8^{a}	3.6 ± 0.5^{b}	3.5 ± 0.7^{a,b}	3.6 ± 0.6^{b}
HA+HS+Put	6.2 ± 0.8	5.8 ± 0.9^{a}	7.9 ± 0.7^{b}	4.9 ± 0.5^{ab}	3.7 ± 0.6^{b}	3.7 ± 0.5^{ab}

Values are mean of ± SE of three independent experiments and are significantly different at $P < 0.05$. Significances were tested by factorial CRD. 25°C, Control; 35°C heat acclimation (HA) and 45°C, heat shock (HS) temperature. Control – Seed germinated at 25°C without any treatment. HS – treatment involves direct heat shock at 45°C (2 h) followed by recovery at 25°C for 5 days. HA + HS treatment involves heat acclimation at 35°C (2 h) followed by heat shock at 45°C (2 h) and later recovery at 25°C for 5 days. Significant effects ($P \leq 0.05$) of treatment (^{a}) and cultivar (^{b}) at CD (5%)
Root length: a – 0.57, b – 0.49, Shoot length: a – 0.53, b – NS; Dry wt (root): a – 0.55, b – 0.48, Dry wt (shoot): a – 0.56, b – 0.48

Table 2 Effect of temperature and putrescine treatment on cell membrane stability and TTC cell viability test in root and shoot of etiolated wheat seedlings.

Temperature ± Put	Relative injury of membrane (%)					
	PBW 343		C 306		WH 542	
	Root	Shoot	Root	Shoot	Root	Shoot
Control	76.7 ± 4.2	16.7 ± 4.0	83.3 ± 5.6^{b}	31.7 ± 3.1^{b}	72.5 ± 4.6^{b}	70.7 ± 6.2^{b}
HS	92.0 ± 6.8^{a}	80.0 ± 6.1^{a}	89.0 ± 6.1^{ab}	66.0 ± 6.7^{ab}	89.3 ± 6.4^{ab}	78.0 ± 6.7^{ab}
HA + HS	69.0 ± 6.1^{a}	40.0 ± 6.2^{a}	64.0 ± 4.5^{ab}	44.0 ± 3.6^{ab}	67.1 ± 7.5^{ab}	40.0 ± 3.8^{ab}
HA + HS + Put	38.2 ± 5.8^{a}	14.8 ± 2.7^{a}	49.9 ± 6.5^{ab}	14.3 ± 1.3^{ab}	45.0 ± 7.0^{ab}	14.6 ± 1.2^{ab}
TTC cell viability test						
Control	0.14 ± 0.01	0.13 ± 0.01	0.20 ± 0.04^{b}	0.32 ± 0.01^{b}	0.20 ± 0.02^{b}	0.23 ± 0.01^{b}
HS	0.04 ± 0.01^{a}	0.10 ± 0.03^{a}	0.14 ± 0.01^{ab}	0.19 ± 0.02^{ab}	0.16 ± 0.02^{ab}	0.11 ± 0.01^{ab}
HA + HS	0.10 ± 0.03^{a}	0.13 ± 0.02^{a}	0.20 ± 0.03^{ab}	0.30 ± 0.03^{ab}	0.20 ± 0.04^{ab}	0.20 ± 0.02^{ab}
HA + HS + Put	0.29 ± 0.01^{a}	0.45 ± 0.02^{a}	0.29 ± 0.01^{ab}	0.35 ± 0.02^{ab}	0.28 ± 0.01^{ab}	0.49 ± 0.04^{ab}

Values are mean of ± S.E. of three independent experiments and are significantly different at $P < 0.05$. Significances were tested by factorial CRD. Significant effects ($P \leq 0.05$) of treatment (^{a}) and cultivar (^{b}) at CD (5%)
Relative membrane injury (roots): a – 5.17, b – NS, Relative membrane injury (shoots): a – 4.20, b – 3.64
TTC (Root): a – 0.02, b – 0.17, TTC (Shoot): a – 0.20, b – 0.17

Table 3 Effect of temperature and putrescine on peroxidase activity (ΔE min^{-1} g^{-1} FW), diamine oxidase and polyamine oxidase (U g^{-1} FW) activities in wheat seedlings.

Temperature ± Put	PBW 343			C 306			WH 542		
	Root	Shoot	Endosperm	Root	Shoot	Endosperm	Root	Shoot	Endosperm
Peroxidase activity (ΔE min^{-1} g^{-1} FW)									
Control	107 ± 3.3	150 ± 5.1	182 ± 7.1	210 ± 10.1^{b}	151 ± 11.7^{b}	219 ± 12.0^{b}	160 ± 3.1^{b}	193 ± 5.5^{b}	208 ± 11.1^{b}
HS	301 ± 18.8^{a}	306 ± 13.1^{a}	604 ± 43.1^{a}	218 ± 2.9^{ab}	156 ± 13.3^{ab}	230 ± 0.0^{ab}	241 ± 0.6^{ab}	209 ± 16.3^{ab}	288 ± 29.2^{ab}
HA + HS	509 ± 15.5^{a}	404 ± 6.0^{a}	906 ± 6.1^{a}	241 ± 5.9^{ab}	218 ± 8.3^{ab}	251 ± 3.9^{ab}	259 ± 8.5^{ab}	252 ± 19.9^{ab}	294 ± 11.6^{ab}
HA + HS + Put	928 ± 18.1^{a}	510 ± 16.8^{a}	910 ± 40.2^{a}	294 ± 2.2^{ab}	260 ± 9.1^{ab}	280 ± 0.6^{ab}	277 ± 0.6^{ab}	287 ± 5.2^{ab}	296 ± 9.3^{ab}
Diamine oxidase activity (U g^{-1} FW)									
Control	175 ± 8.0	135 ± 5.0	95 ± 8.0	95 ± 7.0^{b}	50 ± 4.6^{b}	55 ± 5.0^{b}	163 ± 16.3^{b}	170 ± 14.1^{b}	163 ± 12.6^{b}
HS	25 ± 5.0^{a}	35 ± 5.0^{a}	65 ± 5.0^{a}	19 ± 4.2^{ab}	40 ± 4.0^{a,b}	53 ± 4.1^{a,b}	56 ± 7.1^{ab}	106 ± 7.1^{ab}	103 ± 13.6^{ab}
HA + HS	55 ± 5.0^{a}	120 ± 18.7^{a}	85 ± 9.0^{a}	134 ± 4.7^{ab}	84 ± 3.2^{ab}	83 ± 4.6^{a,b}	71 ± 4.1^{ab}	149 ± 7.1^{ab}	109 ± 7.1^{ab}
HA + HS + Put	125± 10.2^{a}	149 ± 18.6^{a}	142 ± 12.0^{a}	192 ± 7.1^{ab}	124 ± 24.8^{ab}	163 ± 7.1^{ab}	124 ± 3.4^{ab}	173 ± 4.2^{ab}	110 ± 13.5^{ab}
Polyamine oxidase activity (U g^{-1} FW)									
Control	130 ± 10.0	90 ± 13.0	143 ± 12.4	208 ± 17.3^{b}	115 ± 13.1^{b}	62 ± 2.3^{b}	164 ± 10.8	196 ± 10.7^{b}	81 ± 3.5^{b}
HS	70 ± 1.3^{a}	55 ± 5.0^{a}	35 ± 5.0^{a}	159 ± 5.5^{ab}	70 ± 6.0^{ab}	43 ± 10.7^{ab}	49 ± 21.1^{a}	120 ± 9.4^{ab}	52 ± 14.5^{ab}
HA + HS	101 ± 10.1^{a}	65 ± 5.0^{a}	85 ± 10.0^{a}	174 ± 8.0^{ab}	86 ± 9.2^{ab}	78 ± 8.0^{ab}	67 ± 7.8^{a}	135 ± 8.6^{ab}	92 ± 6.1^{ab}
HA + HS + Put	145 ± 18.0	120 ± 10.0	95 ± 5.0^{a}	181 ± 3.5^{b}	124 ± 10.6^{b}	88 ± 14.2^{a}	146 ± 22.2	156 ± 7.1^{b}	110 ± 10.7^{ab}

Values are mean of ± S.E. of three independent experiments and are significantly different at $P < 0.05$. Significances were tested by factorial CRD. Significant effects ($P \leq 0.05$) of treatment (^{a}) and cultivar (^{b}) at CD (5%)
POX (root): a – NS, b – 10.38, POX (shoot): a – 10.59, b – 9.17, POX (endosperm): a – NS, b – 16.52
DAO (root): a – 7.68, b – 6.65, DAO (shoot): a – 11.91, b – 10.31, DAO (endosperm): a – 9.06, b – 7.85
PAO (root): a – 12.20, b – 10.57, PAO (shoot): a – 8.19, b – 7.09, PAO (endosperm): a – 8.37, b – 7.25

terms of their lengths and dry weights and the degree of inhibition increased with heat shock treatment (**Table 1**). However, in presence of Put, growth of seedling increased in all the cultivars studied when compared to control values (normal 25°C, HA + HS, HS alone). Likewise heat acclimation at 35°C (2 h) before heat shock also resulted in increase of root/shoot lengths and dry weights in all the cultivars compared with their control. However, Put pre-treatment confer better heat tolerance than heat-acclimation and heat shock treatment and these observations are supported by the studies of Bouchereau *et al.* (1999) and Quinet *et al*. (2010) where they reported enhancement of cell growth and development by PA.

The repair of plant cells exposed to reversible heat damage, after having been returned to an optimal growth temperature has been reported earlier (Liu *et al*. 2007; Goyal and Asthir 2010; Handa and Mattoo 2010). Sarita and Mishra (2005) and Cvikrova *et al*. (2012) reported that PAs are considered to be regulators of plant growth and development owing to their effects on cell division and differentiation. Growth of bean seedlings was also improved with application of arginine which is a precursor of PAs (Zeid 2009). PA accumulation due to increase in arginine decarboxylase activity exhibited enhanced tolerance to multiple abiotic stresses including heat tolerance (Alcazar *et al*. 2011). Thus, the PA biosynthesis can be engineered for producing stress tolerant plants.

Induction of thermotolerance by Put in terms of cell membrane stability and TTC cell viability

To further prove the role of Put in the establishment of thermotolerance, Put (100 μM) pretreatment at 35°C (2 h) was performed to study its effect in terms of relative injury (RI) of membranes and triphenyl tetrazolium chloride (TTC) cell viability (**Table 2**) in root/shoot of three wheat cultivars namely PBW 343, C 306 and WH 542. RI of membranes was significantly high with heat stress as compared to control. Low injury was recorded in root and shoot pre treated with Put than the seedlings subjected to heat acclimation alone. Reports indicate that high temperature damage in plants has been associated with reduced membrane integrity and increased electrolyte leakage (Kochera *et al*. 2005; Shi *et al*. 2006; Almeselmani *et al*. 2006; Bala *et al*. 2010). The alleviation of high temperature damage by growth regulators is therefore related to reversal of these membrane changes. Increase in electrolyte leakage was more pronounced in root as compared to shoot indicating more cell membrane damage in roots. Deshmukh *et al*. (1991) and Reddy *et al*. (2003) have reported that cell membrane stability is used as an index for screening genotypes against heat and drought tolerance.

Heat stress is quantified by mitochondrial electron transport activity, which is determined through reduction of TTC. The TTC cell viability assay is based on the principle of tetrazolium salt reduction to formazan by dehydrogenase respiratory enzymes and hence evaluates the respiratory and metabolic activity in the tissue (Norman *et al*. 2004; Mohapatra *et al*. 2009). During heat shock treatment decline in

the metabolic and respiratory activity of the root was observed (**Table 2**). With reduction in the respiration, the formation of ATP and carbon skeletons for various biosynthetic and transport processes are expected to be curtailed leading to inhibition of the seedling growth. Put pretreatment resulted in a marked increase of the metabolic activity of seedlings compared with the heat shock treatment. Overall, Put and heat acclimation pretreatment conferred protection of membrane integrity through reduced electrolyte leakage and increased respiratory activity.

Induction of thermotolerance by Put in terms of POX, DAO and PAO activities

Exposure to high temperature elevated the POX activity in both root and shoot significantly as compared with control values for all the three cultivars (**Table 3**). Invariably, the level of POX activity was noted to be higher in acclimated seedlings and also with treatment of Put pretreated seedlings. In cvs PBW 343 and C 306, the application of 100 µM Put concentration was found to be most effective for increasing POX activity. It has been reported that the abiotic stresses cause increase in the production of reactive oxygen species, ionic imbalance, damage to membranes and macromolecules and changes in osmolarity (Zhu 2001; Asthir e*t al*. 2010) and PAs play an important role in regulation of these processes (Liu and Huang 2000; Kumar *et al*. 2006). Exposure to high temperature increased diamine oxidase (DAO) and PA oxidase (PAO) activities in both root and shoot significantly as compared with control values for all the three cultivars (**Table 3**). However, DAO and also PAO activities were found to be more in Put treated seedlings as compared to heat stressed tissue alone. The PAO activity was found to be enhanced in PBW 343 and WH 542 with 100 µM Put and in C 306. The relationship between amine oxidases and POX activities can be very interesting in relation to physiological and biochemical effects of these enzymes in plants grown under different temperature regimes. Since DAO and PAO enzymes produces H_2O_2 and POX utilizes this H_2O_2 as substrate, therefore the activities of these enzymes seem to be linked.

The markedly higher amine oxidase activity of etiolated pea seedlings correlates with faster growth requiring processes e.g., faster metabolism of PAs (Luhova *et al*. 2003). The improved performance of eggplant (*Solanum melongena*) transgenic seeds to high temperature tolerance was attributed to enhanced accumulation of PAs and increased activity of DAO (Prabhavathi and Rajam 2007). Further, PAs may be involved in signal transduction pathways associated with abiotic stress tolerance (Gill and Tuteja 2010), probably through the activation of protein kinases and transcription factors (Kasukabe *et al*. 2004; Alcazar *et al*. 2010).

CONCLUSIONS

Put and heat acclimation pretreatment conferred protection of membrane integrity through reduced electrolyte leakage and increased respiratory activity. The induced heat-stress tolerance response may be directly linked to the coordinated response of oxidative and antioxidative enzymes like POX, DAO and PAO activities. The scavenging of reactive oxygen species like H_2O_2 by POX plays a key role in imparting thermotolerance in wheat cultivars.

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