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Effect of short-term heat stress on total sugars, proline and some antioxidant enzymes in moth bean (*Vigna aconitifolia*)

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Abstract Heat stress leads to an array of physiological, biochemical, and molecular changes in plants affecting its growth and development. An experiment was conducted to find out the effect of short-term heat stress on osmo-protectant and antioxidants in 37 genotypes (32 mutants and five varieties) of moth bean (*Vigna aconitifolia*). Seeds were grown in plastic pots containing sterilized vermiculite. Heat stress conditions were created by exposing seven days old seedlings at 42 °C for one hour in hot air oven. Analysis of various parameters was carried out at three days after heat stress. A significant over-accumulation of total sugar and proline along with an increased activity of CAT, GPOX and SOD was observed in most of the genotypes under heat stress. However, correlation analysis among heat stress induced biochemical parameters suggests that none of these traits were associated with the level of thermo-tolerance except GPOX activity. Among 37 genotypes, on the basis of number of fresh plants with least wilting symptoms, six were categorized as tolerant, 13 as moderately tolerant and 18 as susceptible accession. Eventually, it is evident that thermo tolerance and biochemical parameters can be efficiently altered and improved through mutagenesis, though this altered tolerance level could not be associated with the parameters studied in the present investigation.

Abbreviations: CAT, catalase; GPOX, guaiacol peroxidase; SOD, superoxide dismutase.

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Introduction

High temperature is one of the most important constraints for agriculture. There is a risk that increased global temperature will change the optimum sites and conditions for crop production and thus affect agriculture (Poli et al., 2013). This condition is more tragic in the arid and semi-arid environments like in the Thar desert where crop production is highly unstable and unsustainable due to inhospitable climate and poor soil fertility status. Moreover, under hot arid situations high temperatures are associated with water stress and make abiotic stresses more compound consequently physiological and biochemical changes occur in as adaptive strategies. As accumulation of low-molecular-weight chaperones, compatible solutes such as proline, sugars, polyols are often regarded as a basic strategy for the protection and survival of plants under abiotic stress (Chen et al., 2007). Such unfavorable conditions also elicited oxidative metabolism in plants through overproduction of reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydroxyl (OH^-), singlet oxygen, and hydrogen peroxide (H_2O_2).

To counteract the injurious effects of over-produced ROS under abiotic stresses, plants have evolved complex antioxidative detoxification system which includes antioxidant enzymes such as superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), guaiacol peroxidase (GPOX; EC 1.11.1.7), ascorbate peroxidase (APX; EC1.11.1.11), peroxidase (POX; EC 1.11.1.7) and glutathione reductase (GR; EC 1.8.1.7) and non-enzymatic antioxidants such as flavonoids, anthocyanins, carotenoids and ascorbic acid. The removal of O_2^- by SOD generates H_2O_2, which is removed by peroxidases and CAT.

Moth bean (*Vigna aconitifolia*) grows throughout the tropic, sub-tropic and warm temperate areas of different countries. It is cultivated on marginal lands, under rain fed conditions mainly in the arid and semi-arid zones like in the Thar desert where temperatures rise beyond 45 °C, heating the soils up to 65 °C during summer crop growing season, though productivity of moth bean remains low due to inherited bottlenecks of low variability, slow growth, longer maturity period (> 100–110 days), drooping growth habit and poor response to agronomic practices. Thus, there is need of genetic improvement in this legume to make it more productive particularly in marginal and sub marginal arid areas with limited resources. While harboring the limited variation, mutagenesis in moth bean may allow recovery of wider spectrum of mutants through selection. In moth bean, like other crops, a few early maturing mutants (~60 d) have been produced in recent years (Khadke and Kothekar, 2011) that are capable of taking advantage of lesser rains phenomenon climate change scenario. However, these mutants vary for tolerance to abiotic stresses including elevated temperature. Previous study has indicated an inducible nature of thermo-tolerance in moth bean with genetic variations for level of induction (Sharma et al., 2014).

Amalgamation of tolerance to high temperature in moth bean might provide an alternative crop for summer cultivation. Keeping in view, the present study was varied out *in vitro* through biochemical characterization of moth bean mutants (M_5) to identify genotype (s) with tolerance to high temperature at seedling stage and to identify biochemical and antioxidative defense mechanisms associated with high temperature tolerance in moth bean.

Materials and methods

Plant material and experimental details

Seeds of four varieties of moth bean (*V. aconitifolia*) viz., Jwala, RMO 40, RMO 225 and RMO 423, were procured from Agricultural Research Station, Beechwal, of Swami Keshwanand Rajasthan Agricultural University (SKRAU), Bikaner, Rajasthan. The air-dried seeds of moth bean were exposed to 100, 200 and 300 Gy doses of gamma radiation during July 2006 at Board of Research in Nuclear Sciences (BRNS), Trombay, Mumbai, and soon after the treatments seeds were sown to raise M_1 generation. For M_2 and subsequent generation several single plants were selected and harvested separately in each radiation dose and raised for next subsequent generations. Eventually, the experimental material for the present investigation consisted of 37 moth bean genotypes, comprising five varieties (Jadia SN-1 & 2, RMO 40, RMO 423 and RMO 225) and their 32 mutant lines selected for enhanced abiotic stress tolerance (Table 1), developed at Plant Biotechnology Centre, SKRAU, Bikaner. Seeds were grown in plastic pots containing sterilized vermiculite and were watered with measured amount of 1× Hoagland solution (20 ml/pot) every day, in growth chamber at 27 °C and a 12-h light cycle. These seedlings were grown for seven days under normal condition, and then exposed at 42 °C for one hour in growth chamber to create the heat stress conditions. This temperature was decided as per our previous report on moth bean (Sharma et al., 2014).

Stress induced visual observations

All observations in control and temperature treated plants were taken after three days of treatment. A scoring procedure including number of plants showing wilting symptoms and degree of damage viz. leaf drooping, stem lodging, leaf margin or tip burning was developed to enumerate stress induced. A scale of 0–7 was developed 0 means complete drying (non-viable) of the plants and seven with most fresh plants.

Proline content and total soluble sugars

Leaf samples (0.01 g) were homogenized in five ml of 3% aqueous sulfosalicylic acid and centrifuged at 5000*g* for five min (Bates et al., 1973). Supernatant was used, and equal volume of glacial acetic acid and ninhydrin solution was added to it. A tube with two ml of sulfosalicyclic acid was served as blank. The samples were heated to 100 °C for 1 h and after cooling, five ml of toluene was added. The absorbance of the toluene layer was measured at 528 nm on spectrophotometer. The quantity of proline was calculated using standard curve.

The Soluble Sugar was estimated by Anthrone method (Dubois et al., 1956). Leaf sample (0.1–0.5 g) was homogenized in a mortar and pestle containing hot (90 °C) aqueous ethanol (v/v 80%). After centrifugation, 0.2 ml of supernatant was taken in separate test tubes and volume was made up to one ml by adding 0.8 ml of deionized water. After that 0.2% of

Table 1 Effect of short term water stress on proline and total soluble sugar in varieties and mutants of moth bean.

Genotypes	Proline		Percent increment/ decrement of proline under stress	Total soluble sugar		Percent increment/ decrement of sugar under stress	Survived seedlings (Out of 7)	Remark*
	Control	Stress		Control	Stress			
J-100-10-12-5	3.63	4.00	10.19	0.16	0.48	200.00	5	MT
J-100-10-1-4	0.38	2.13	460.5	0.13	0.43	230.76	3	S
J-100-15-6-1	0.38	1.25	228.9	0.32	0.32	0.000	5	MT
J-100-10-12-2	2.38	3.25	36.55	0.13	0.18	38.46	5	MT
J-100-10-1-8	2.13	2.88	35.21	0.27	0.35	29.63	5	MT
J-100-10-1-1	1.50	2.63	75.33	0.22	0.32	45.45	3	S
J-100-10-8-10	0.25	1.13	352.0	0.18	0.24	33.33	3	S
J-100-10-9-4	1.50	4.38	192.0	0.18	0.31	72.22	3	S
J-100-10-11-1-1-11	0.75	4.88	550.67	0.42	0.51	21.42	3	S
J-100-10-10-2	1.13	2.63	132.7	0.2	0.32	60.00	3	S
40-300-17-20-7	0.50	5.88	1076.0	0.32	0.39	21.87	3	S
40-300-17-11-1	1.13	2.88	154.87	0.29	0.32	10.34	3	S
40-300-17-14-7	0.38	2.00	426.32	0.59	0.59	0.00	5	MT
40-300-17-14-6	0.13	0.13	0.000	0.23	0.41	78.26	7	T
RMO-40 5-1	0.50	1.38	176.0	0.28	0.35	25.00	7	T
RMO-40 SN 3-3	0.75	0.38	−49.33	0.25	0.43	72.00	7	T
40-300-17-10-3	3.25	0.63	−80.62	0.23	0.36	56.52	7	T
40-200-11-5-2-4	0.63	0.50	−20.63	0.25	0.31	24.00	5	MT
40-300-17-14-9	2.63	0.88	−66.54	0.18	0.08	−55.55	3	S
423-8-9-10-3	5.50	10.75	95.45	0.38	0.69	81.57	5	MT
423-8-8-1-1	0.50	1.00	100.0	0.18	0.28	55.55	3	S
423-2-3-2-4	0.25	0.25	0.00	0.46	0.56	21.73	5	MT
423-8-9-10-1	0.63	1.13	79.37	0.31	0.28	−9.67	7	T
423-2-15-1-3	0.75	0.75	0.00	0.16	0.26	62.50	3	S
423-8-9-10-6	1.50	2.38	58.67	0.34	0.33	−2.94	5	MT
423-2-15-3-1-1	1.25	4.50	260.0	0.19	0.4	110.52	3	S
423-8-9-6-1	3.25	5.50	69.23	0.34	0.27	−20.58	5	MT
225-9-14-18-1-2	1.50	1.50	0.00	0.2	0.33	65.00	3	S
225-9-9-9-2-2	1.00	1.50	50.00	0.34	0.34	0.00	3	S
225-9-9-1-1	1.00	0.75	−25.00	0.29	0.31	6.89	3	S
225-9-9-17-1-2	0.25	1.13	352.0	0.44	0.68	54.54	5	MT
225-9-14-13-1	0.88	3.63	312.50	0.13	0.23	76.92	5	MT
SN-1-1 Jadia#	1.50	7.00	366.67	0.29	0.39	34.48	3	S
SN-2-1 Jadia#	0.63	1.50	138.10	0.39	0.5	28.20	3	S
RMO-225#	1.00	0.63	−37.00	0.2	0.07	−65.00	3	S
RMO-423#	0.75	3.00	300.00	0.38	0.58	52.63	5	MT
RMO-40#	0.75	4.88	550.67	0.24	0.44	83.33	7	T
Standard error Within Treatment	0.19	0.37	–	0.19	0.22	–	–	–
Standard Error Between control and stress	–	0.22	–	–	0.02	–	–	–

* T-Tolerant; MT-Moderately tolerant; S-Susceptible; characterization is on the basis of number of fresh plants with least wilting symptoms.
\# These genotypes are varieties.

anthrone reagent (Merk, India) was added in tubes. The samples were heated for eight min in a boiling water bath and were cooled rapidly; the intensity of green to dark green color was measured at 620 nm on spectrophotometer.

Antioxidant enzyme essays

Leaf samples (0.5 g fresh mass) were homogenized in ice-cold 50 mM sodium phosphate buffer (pH 7.0) containing 0.1 m methylenediaminetetra acetic acid (EDTA) and 1% polyvinylpoly-pyrrolidone (PVP). The homogenate was filtered and centrifuged at 4 °C for 20 min at 15,000*g* and supernatant was used for enzyme activity measurements by considering protein amounts. The protein amount was determined by the Bradford method (Bradford, 1976) using bovine serum albuminas as a standard.

The CAT and GPOX activities were assayed as per the protocol of Bergmeyer (1974). CAT activity was determined as decrease in absorbance at 240 nm (coefficient of absorbance, $\varepsilon = 39.4\ mM^{-1}\ cm^{-1}$) for two min following the decomposition of H_2O_2 (Merk, India) in a reaction mixture containing 50 mM phosphate buffer (pH 7.0), 15 mM H_2O_2. Enzyme activity was expressed as µmol of H_2O_2 decomposed mg^{-1} (protein) min^{-1}. For GPOX, the oxidation of guaiacol was

measured by following the increase in absorbance at 470 nm (ε = 26.6 mM^{-1} cm^{-1}) for one min. The assay mixture contained 50 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 10 mM guaiacol and 10 mM H_2O_2. GPOX activity was expressed as μmol (tetraguaiacol formed) mg^{-1} (protein) min^{-1}.

SOD assay was performed as per the protocol of Dhindhsa et al. (1981). Enzyme extract for SOD was prepared by grinding 0.5 g leaf material with 10 ml of chilled 0.1 M potassium phosphate buffer (pH 7.5) containing 0.5 mM EDTA was filtered through cheese cloth and centrifuged in a refrigerated centrifuge for 15 min at 20,000*g*. The 3.0 ml reaction mixture contained 13 mM methionine, 25 mM nitrobluetetrazolium chloride (NBT), 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.8), 50 mM sodium bicarbonate and enzyme extract. Reaction was started by adding 2 μM riboflavin and placing the tube below 2 × 15 W fluorescent lamp for 15 min. Reaction was stopped by switching off the light and covering the tube with black clothes. Tubes without enzymes develop maximum color. A non-irradiated complete reaction mixture did not develop color and served as control. Absorbance was recorded at 560 nm and one unit of enzyme was taken as that quantity of enzyme, which reduced the absorbance reading 50% in comparison with the tubes lacking enzyme.

Isoenzyme analysis

In addition to antioxidant activity analysis, isoenzyme pattern of antioxidant CAT under high temperature was examined. Two accessions each from three categories i.e. tolerant, medium tolerant and susceptible were used to develop isoenzyme patterns for control and treated plants. CAT isoenzymes were separated by native 10% PAGE according to Davis (1964). CAT activity was visualized after soaking the gel in 3.3 mM H_2O_2 for 30 min, washing in double distilled water and stained with a mixture of 1% (w/v) potassium ferricyanide and 1% (w/v) ferric chloride (Woodburry et al., 1971). Afterward complete staining gel was destained in destaining solution on a slowly rocking platform and changed the destaining solution 3–4 times, till the background became quite clear. The clearly visible isoenzyme bands were recorded against corresponding proteins/polypeptides of known molecular weight markers.

Experimental design and data analysis

All experimental data recorded were averaged for at least three independent assays with three replicates each. Arithmetic mean, standard errors and Pearson's correlation were calculated for each trait using the standard formula given in Chandel (1997)

Results and discussion

Screening of genotypes

Crop production in the arid and semi-arid environments is highly unstable and unsustainable due to uncongenial climate (Sharma et al., 2014). Inhospitable climate such as heat stress due to increased temperature is an important agricultural problem in many areas of the world. Due to endowed morphological and physiological features of moth bean, one of the most important arid legumes in India, it is a key grain legume of arid and semi-arid ecosystem and dry land crop husbandry. A set of 37 moth bean genotypes developed through mutagenesis were raised in stress along with control for estimating thermotolerance of genotypes by assigning a score for tolerance level based on number of seedlings wilted. All seedlings remained healthy without stress (control) at 27 °C temperature. However, significant genotypic differences were observed among genotypes 42 °C temperature for one hour screening (Fig 1). Out of 37 genotypes six mutants/genotypes i.e. 40-300-17-14-6, SN 5-1, SN 3-3, 40-300-17-10-3, 423-8-9-10-1, RMO-40 showed high rates of survival at 42 °C of temperature for one hour. Thirteen genotypes showed average survival and eighteen genotypes showed less survival at 42 °C of temperature for one hour. A score for tolerance level based on number of seedlings wilted and wilting symptoms developed was assigned to each genotype tested (Table 1). The genotypic effect on the ability to resist lethal temperature stress has been indicated previously in many crops including moth bean (Mullarkey and Jones, 2000; Sharma et al., 2014). Thus, results indicated that mutants are valuable resources for genetic variations in crop improvement. Recently, Poli et al. (2013) have also been reported that rice mutant has more tolerance to the temperature stress compared to parental genotype.

Proline content

To avoid water loss under stress, plants accumulate compatible solutes such as proline which act as low-molecular weight chaperones (Gupta et al., 2013). These osmolites stabilize and protect the structure of enzymes and proteins, maintain membrane integrity and scavenge reactive oxygen species ROS. Due to its zwitter ion character, under stress conditions, proline accumulates to high-concentration in cell cytoplasm. However, it has also been suggested that over-accumulation of proline could be toxic to plant cells (Rizhsky et al., 2004). There was 10.75% (J-100-10-12-5) to 1075% (40-300-17-20-7) increment in proline accumulation under heat stress conditions (Table 1). It was noticed that average proline content enhanced under heat stress condition in 28 genotypes. However, in five genotypes (SN 3-3, 40-300-17-10-3, 40-200-11-5-2-4, 225-9-9-1-1, RMO-225) a decrement from 20% (40-200-11-5-2-4) to 81% (40-300-17-10-3) in proline content was observed under stress treatment. Though, in four genotypes viz 40-300-17-14-6, 423-2-3-2-4, 423-2-15-1-3 and 225-9-14-18-1-2 no change in proline content was observed under heat stress. Initial level of proline in non-stressed plants was ranging from 0.13 to 5.50 (mg g^{-1} fw), while under stress condition it was ranging from 0.13 to 7 (mg g^{-1} fw). Genotypic variations for increase in proline level due to stress have been known earlier in sunflower (Amutha et al., 2007). However, its level has not always been associated with tolerance level. It has been suggested that proline functions as an indicator of plant water status but not a measure of level of tolerance (Silvente et al., 2012).

Total soluble sugar content

It has shown previously that soluble sugars increase under osmotic stress for osmotic adjustment in many legumes and non-legumes (Sassi-Aydi et al., 2014). Data herein in Table 1

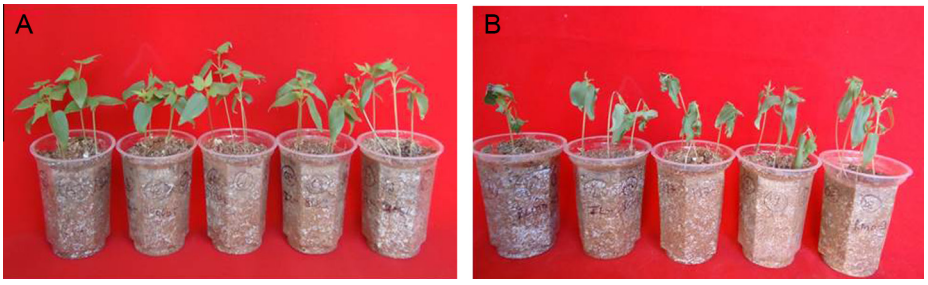

Fig. 1 Differential phenotypic variation in two moth bean varieties (A) RMO 40 and (B) RMO 225 under heat stress.

showed that all studied genotypes varied significantly between 0.13 and 0.59 (mg g^{-1} fw) for initial level of soluble sugar in non-stressed plants, while osmotic stress accumulated sugar contents under stress condition ranging from 0.07 to 0.69 (mg g^{-1} fw). It was noticed that total sugar content enhanced under heat stress condition in 32 genotypes and maximum and minimum increment was observed in J-100-10-1-4 (227.34%) and 40-300-17-14-7(0.69%), respectively. This increment in total sugars may be due to inhibition of sucrose synthase or invertase activities. Astonishingly, result here showed decreased sugar contents in five genotypes namely 40-300-17-14-9, 423-8-9-10-6, 423-8-9-10-1, 423-8-9-6-1, RMO-225 (maximum 66.50%) after stress treatment. Sassi-Aydi et al. (2014) also reported mild and moderate osmotic stress decreased sugar in *Phaseolus vulgaris*. Decreased sugar content could be the consequence of osmotic stress-induced reduction in photosynthesis and the subsequent shortage of photoassimilates in the aerial parts (Hussin et al., 2013; Tejera et al., 2006).

CAT activity and isoenzyme analysis

CAT scavenges H_2O_2 by breaking it down directly to form water and oxygen, and an increase in its activity is related with increase in stress tolerance (Kraus et al., 1995). CAT in non-stressed control plants was ranging from 17.77 to 111.97 µmol mg^{-1} (protein) min^{-1}, while under stress it varied between 20.53 and 149.98 µmol mg^{-1} (protein) min^{-1}. Under heat stress conditions, levels of the CAT varied considerably among the genotypes (Fig. 2). Of 37 genotypes under stress, 28 genotypes showed enhanced activities of CAT, whereas in nine genotypes CAT activity dropped. Application of heat stress caused enhanced CAT activity ranging between 333.33% (423-8-8-1-1) and 2.15% (40-300-17-10-3). A significant reduction in CAT activity was observed at high temperature treated plant, that is, from 4.33% (423-8-9-10-6) to 45.89% (J-100-15-16-1) decrease in the activity after exposure to 42 °C temperature. Similar results were previously observed by Gür et al. (2010) in cotton. Increased CAT activity was reported in chickpea under draught stress (Mafakheri et al., 2010) whereas decreased CAT activity was observed in heat stressed wheat leaves (Hameed et al., 2012) and Kentucky bluegrass (He and Huang, 2010). The observed diminish activity of enzyme could be explained by either increased level of enzyme degradation or decline in synthesis of this enzyme (Gür et al., 2010).

A single band of about 258 KD was observed for CAT isoenzyme whose intensity was observed to correlate with enzymatic activity except in 40-300-17-11-1 and increase in band density was also observed for heat treated plants of each accession (Fig 3).

GPOX activity

Like CAT, GPOX is an important enzyme which plays role in regulation of intracellular H_2O_2 level by converting H_2O_2 into H_2O along with regeneration of $NADP^+$ (Sairam et al., 2000). Under control condition GPOX activity varied from 181.55 to 1960.78 µmol mg^{-1} (protein) min^{-1} while under stress it ranged between 529.94 and 2787.88 µmol mg^{-1} (protein) min^{-1}. The GPOX activity was maximum in 423-2-3-2-4 (1311.79) and minimum in J-100-10-9-4 (585.66) in stressed plants (Fig. 2). The heat stress treatment invariably enhanced GPOX activity in all the genotypes except 40-300-17-10-3 and 40-300-17-14-7 in which 48.25% and 8.91%, respectively, reduction was noted. Many studies have shown that GPOX activity increases during exposure to high temperatures (Rached-Kanouni and Alatou, 2013). Kaur et al. (2009) while working with seedlings of *Brassica* spp. found that peroxidase activity increased in all the four genotypes under high temperature stress. Almeselmani et al. (2006) also reported increase in peroxidase activity in wheat plant under high temperature stress.

SOD activity

Heat stress is known to accompany with overproduction of reactive oxygen species such as O_2^-, H_2O_2 and OH^-, which destruct essential cellular components and structural elements (Mittler, 2002). SOD is usually considered as the first line of defense against oxidative stress (Gupta et al., 2013). SOD, another important antioxidant was also measured in all the genotypes and data have been presented in Table 1. SOD activity under normal condition was ranging from 17.76 to 105.04 µmol mg^{-1}(protein) min^{-1} while it varied between 21.15 to 111.02 µmol mg^{-1}(protein) min^{-1} under stress condition (Fig. 2). An increase in activity of SOD was observed only in 20 genotypes subjected to heat stress. Ostrovskaya et al. (2009) observed increased SOD activity in grape cultivars resistant to lime-induced chlorosis compared with less resistant cultivars. Camejo et al. (2006) found that heat treatment in tomato decreased the total specific SOD activity, similar to this study where decrement in SOD activity was conserved in 17 genotypes. The highest fold increase in SOD activity over the control was recorded in 225-9-14-18-1-2 (184.87%) compared to rest of the genotypes. On the other hand J-100-10-11-1-1-11 and 40-200-11-5-2-4 showed maximum (51.87%) and minimum reduction

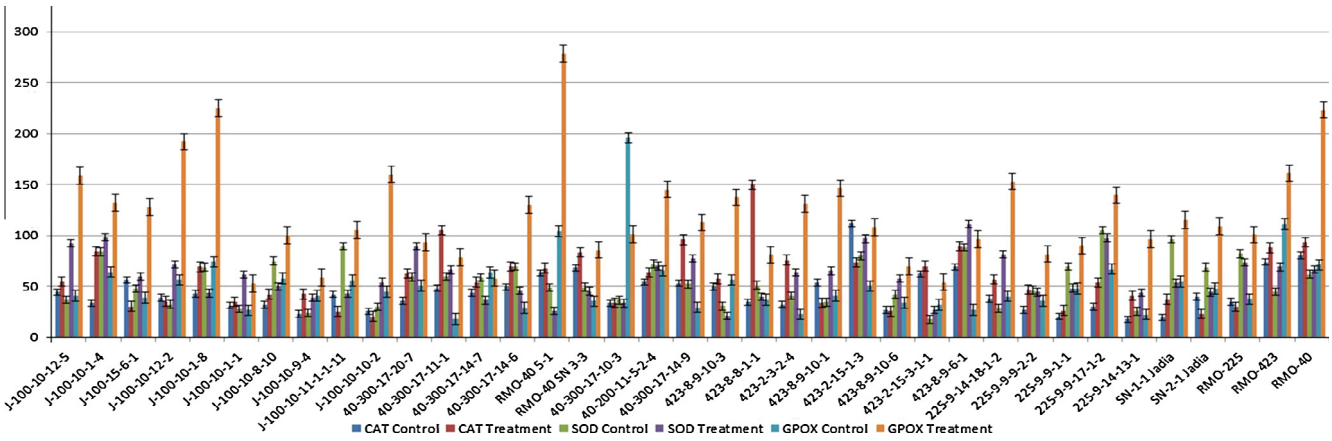

Fig. 2 Effect of heat stress on antioxidant enzyme (CAT, SOD and GPOX) in moth bean seedlings; values are mean ± SE.

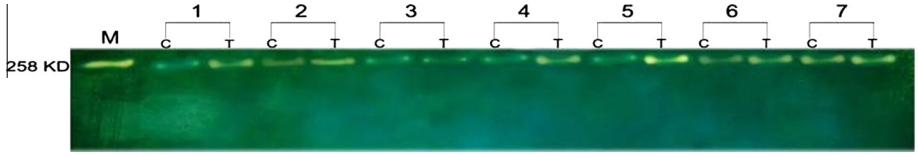

Fig. 3 Isoenzyme profiling of catalase extracted from seedling heat stressed at 42 °C for one hour in moth bean; M-protein weight marker; C: Control; T:Treatment; 1:RMO 40; 2: 40-300-17-20-07; 3: 40-300-17-11-1; 4: 40-300-17-14-07; 5: SN 3-3; 6: 40-300-17-10-03; 7: 40-200-11-5-2-4.

(2.57%), respectively. Variable activity of SOD across genotypes indicates their differential ability to acquire thermotolerance (Gosavi et al., 2014). The result is in agreement with Li et al. (2015) where activities of both superoxide dismutase and catalase were decreased in abiotic stress.

Correlation analysis

Overall, the present study has clearly demonstrated an increase in the three antioxidant enzymes (GPOX, SOD and CAT) along with osmo-protectants proline and sugars. However, their activity level or content was not directly associated with level of tolerance to heat stress as depicted by non-significant correlations with tolerance level (scores). Correlation studies among all studied under control and stress conditions revealed that none of the trait was associated with heat stress tolerance (on the basis of number of fresh plants with least wilting symptoms) except GPOX activity, both under control (0.369) and stress (0.452) condition (Table 2). Correlation among control and treated conditions was highest for sugar (0.662) followed by proline (0.572). The basal level of the three antioxidants and osmoprotectants was significantly correlated with the level in treated plants suggesting genotypes with higher level of enzyme activity under unstressed conditions are more likely to enhance enzyme activity on exposed to heat. However, increase in enzyme activity to a great extent in certain cases

Table 2 Correlation among various characteristics imparting heat tolerance in moth bean under heat stress.

Biomolecule	CAT control	CAT stress	GPOX control	GPOX stress	SOD control	SOD stress	Proline control	Proline stress	Sugar control	Sugar stress	Number of fresh plants
CAT control	1.000	0.435**	0.099	0.273	0.100	0.230	0.005	–0.024	–0.013	0.047	0.299
CAT stress		1.000	–0.084	0.080	0.088	0.181	–0.044	–0.357*	–0.112	0.037	0.060
GPOX control			1.000	0.356*	0.046	–0.194	0.152	–0.055	0.077	0.220	0.369*
GPOX stress				1.000	0.050	0.072	0.056	0.014	–0.090	0.087	0.452**
SOD control					1.000	0.388*	–0.284	–0.086	0.244	0.086	–0.159
SOD stress						1.000	–0.031	–0.093	–0.140	–0.148	–0.165
Proline control							1.000	0.572**	–0.124	0.029	0.095
Proline stress								1.000	0.102	0.320	–0.081
Sugar control									1.000	0.662**	0.167
Sugar stress										1.000	0.252
Number of fresh plants											1.000

* Significant at 0.05 probability level.
** Significant at 0.01 probability level.

indicated stress inducibility of promoter. Varying capacity of promoters to induce under stress and express under control conditions is also reflected in isoenzyme pattern developed for CAT. Tolerant and moderately tolerant plants showed higher activity and induction compared to susceptible types except in one case.

Similar to the present study, poor and negative correlation between antioxidants and osmo-protectants has also reported by Rai et al. (2015) in hyacinth bean (*Lablab purpureus* L.) under high temperature stress. Poor correlation of heat tolerance level with both antioxidants and osmo-protectants (proline and TSS) could be indicative of involvement of various other mechanisms involved in imparting tolerance. In general antioxidants are implicated more with desiccation tolerance and are induced under high temperature because of increased transpiration leading to drop in osmotic potential in leaves (Ahmad et al., 2010). Probably heat shock proteins are more important in imparting heat tolerance to plants. However, significant correlation of GPOX level with tolerance level and positive correlation with sugar content indicate some positive role of these parameters in confirming tolerance to moth bean genotypes.

Conclusion

From the present study it is evident that thermo tolerance and associated osmoprotectants and antioxidative enzymes can be efficiently altered and improved through mutagenesis. However, altered level of these parameters independently could not be associated with the altered tolerance level in the present investigation. In order to find out real tolerance mechanism expression analysis of various gene products including heat stroke protein (HSPs) would be needed.

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