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HIGHER GLYCINEBETAINE AND ANTIOXIDANT ENZYMES ACTIVITY ARE ASSOCIATED WITH HIGH TEMPERATURE TOLERANCE IN POTATO

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SUMMARY

Potato is well adapted to temperate climate and predicted increase in temperature under current climate change scenario and this may have a profound effect on potato growth and productivity. A field study was conducted to analyse physiological and biochemical adaptations in potato cultivars for high temperature tolerance. Two potato cultivars *viz*. Chipsona-3 (sensitive) and Kufri Surya (tolerant) were grown under three temperature environments *viz*. control (E1), early planting (E2) and inside polyhouse tunnel (E3). The level of temperature under E2 and E3 treatment environments was nearly 5 and 10°C higher than the control during both tuber initiation (S1) and bulking (S2) stages. The results revealed that total sugar concentration in leaves and tubers increased but tuber starch content decreased under high temperature. Membrane stability index (MSI) and proline content decreased with increasing temperature levels, while glycinebetaine (GB) increased in both the cultivars and K. Surya maintained higher GB than Chipsona-3. Activities of antioxidant enzymes *viz*. superoxidase dismutase (SOD) and ascorbate peroxidase (APX) increased under high temperature in both the cultivars and K. Surya showed higher increase than Chipsona-3. Catalase activity increased in K. Surya but decreased in Chipsona-3 under high temperature. The study suggests that high temperature tolerance in K. Surya cultivar may be associated with increased antioxidant enzymes activities and higher GB concentration compared with Chipsona-3.

Keywords: **Antioxidative enzymes, heat tolerance, membrane stability index, osmolytes accumulation, potato.**

INTRODUCTION

Rising atmospheric temperature is one of the key variables of current climate change and may cause significant effects on agricultural productivity. IPCC (2007) has projected 1.6 to 3.8°C increase in global average air temperature by the year 2100. High temperature stress directly affects crop yield by reducing flowering, fertilization and seed formation. By increasing the rate of plant development, high temperature also reduces the length of the growing period, thereby reducing the yield potential. However, the direct effects of high temperature stress depend on the crop species and its adaptability.

Potato is grown under different environments and best adapted to temperate climates. Optimum temperature is 20-25°C for foliage growth, 16-25°C for net photosynthesis and 20°C for tuberization (Levy 1992). Temperatures above 29°C can reduce leaf area and dry weight sufficiently to decrease tuber production. In most potato cultivars, tuber initiation and bulking are favoured

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by temperature below 20°C (Ewing 1981). Tuberization in potato is most sensitive to high temperatures. Generally, reduction in potato tuber yield occurred at high temperature due to reduced production of assimilates, tuber initiation and partitioning of assimilate to tubers. Without adoption of mitigation options under the climate change scenario, especially due to rise in temperature may affect productivity of potato crop worldwide by 18-32% (Hijmans 2003). It has been estimated that potato production in India may decline by 3.16 and 13.72 % by the year 2020 and 2050, respectively. However, adoption of measures like breeding of heat tolerant varieties and use of efficient agronomic and water management practices can significantly arrest the proposed decline in potato production (Singh *et al*. 2009).

High temperature stress can directly affect physiological processes and alter the growth and development but the response depends largely on the sensitivity of growth stage exposed (Wahid *et al.* 2007). Plants develop physiological and biochemical adaptations like higher membrane stability and accumulation of compatible solutes to cope up with any environmental stress including high temperature. Plants have also evolved an elaborate system of antioxidants and enzymatic scavenging systems to detoxify the harmful levels of reactive oxygen species (ROS) produced in the plant cell during stress. Various studies have demonstrated the role of antioxidant enzymes in protecting plant against high temperature and other abiotic stresses (Larkindale and Kinght 2002; Suzuki and Mittler 2006). In addition, accumulation of a variety of osmolytes like sugars, proline and glycinebetaine (GB) in plants plays significant role in imparting tolerance to high temperature and other stresses (Sakamoto and Murata 2002). Higher level of GB in plants in response to abiotic stress and its correlation with stress tolerance has been reported in various crops (Chen and Murata 2008). Like many other crops, antioxidant system and GB may be involved in high temperature tolerance in tuber crops like potato. Therefore, the present study was carried out to evaluate physiological and biochemical mechanisms for high temperature tolerance in two promising potato cultivars exhibiting variable sensitivity to temperature.

MATERIAL AND METHODS

A field study was carried out during 2008-09 at Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi. The experiment was planned in a randomized complete block design under factorial arrangement with three temperature environments *viz*. E1 = control, E2 = early planting and E3 = planting in polyhouse tunnel. The dimensions of plots were 2.6 x 2 m with four rows and space between rows and plants were 65 and 25 cm, respectively. Potato tubers Kufri Surya (tolerant) and Chipsona-3 (sensitive) were used as study material and obtained form Central Potato Research Station, Modipuram, Meerut. Prior to planting, the sprouted tubers were treated with 2% fungicide (Bavistin) solution. Fertilizers @ 150 kg N, 100 kg P, 150 kg K and FYM (10 tons per hectare) were applied. Full dose of P and FYM and half of the N and K were applied at planting and the rest of N and K at 50 days after planting as side dressing. Furrow irrigation was given throughout the crop cycle and all recommended cultural practices were followed (Singh *et al.* 2005).

For high temperature exposure, plants were grown inside the temperature polytunnel by staggered sowing. Temperature data for daily maximum and minimum temperatures, during the experimental period for E1 and E2 treatments was obtained from the IARI agrometeorological station. For the polytunnel (E3) data on daily temperature were recorded during the experimental period. Data on maximum and minimum temperature during tuberization and bulking stages are shown in Table 1.

For estimation of concentration of sugars in the leaves, 1.0 g of fully expanded fresh leaves were boiled in 80% ethanol and the extract was clarified following the method of Mc Cready *et al.* (1950). The aliquot of clarified sugar extract was used for determination of sugar content using Nelson's arsenomolybdate method (Nelson, 1944) and improved copper reagent of Somogyi (1952). The concentration of total sugars was expressed as mg g^{-1} dry weight (DW) of leaves. Free sugars in tubers were extracted in 10.0 g samples using 200 ml

Table 1. Average daily maximum, minimum and mean temperature (°C) at tuber initiation and bulking stages of potato under different temperature environments

Temperature environment	Date of sowing	Tuber initiation			Bulking		
		Max. (°C)	Min. (°C)	Mean (°C)	Max. (°C)	Min. (°C)	Mean (°C)
E1	28.10.10	25.3	10.5	17.9	20.7	6.7	13.7
E2	23.09.10	31.9	14.1	23.0	24.8	9.2	17.0
E3	23.09.10	39.4	18.2	28.8	38.1	11.2	24.5

E1: Control; E2: Early planting; E3: Polyhouse tunnel

isopropanol (80%) and refluxing for 4 h and the extract was clarified following the above method of Nelson (1944). The concentration of total sugars was expressed as mg 100 g^{-1} fresh weight (FW). After extraction of sugars, the boiled tuber material was used for starch estimation following the method of Hedge and Hofreiter (1962) and data was expressed as mg 100 g^{-1} DW.

Free proline content was determined using rapid colorimetric method, developed by Bates *et al.* (1973). 0.5 g fully expanded leaf tissues were homogenized in sulphosalicylic acid (3%) and then 2.0 ml of extract was mixed with equal amount of glacial acetic acid and ninhydrin. The content was boiled in water bath at 100°C, reaction mixture was extracted with toluene and the absorbance was recorded at 520 nm using spectrophotometer. The concentration of proline was expressed as mg per g FW of the sample.

GB concentration in the leaves was determined following the method of Grieve and Grattan (1983). Dried fine powder of leaf (0.5 g) was mechanically shaken in 20.0 ml of double-distilled water for 48 h at 25°C. Filtered extracts were diluted with 2 N H_2SO_4 (1:1). Aliquot (0.5 ml) was cooled in ice water for 1 h and cold potassium iodide-iodine reagent (0.2 ml) was added and mixed gently using vortex mixture. The samples were stored at 0-4°C for 16 h and centrifuged at 10,000 g for 15 minutes at 0°C. The supernatant was aspirated with micropipette and periodite crystals were dissolved in 9.0 ml of 1, 2-dichloro ethane and after 2.5 h the absorbance was measured at 365 nm with UV-visible spectrophotometer. The standard of GB (50-200 µg ml^{-1}) was prepared using 2 N sulphuric acid and following the above procedure.

Enzyme extract for superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) was done using 0.1 M phosphate buffer (pH 7.5, 0.5 mM EDTA and 1 mM ascorbic acid). Total SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of nitro-blue tetrazolium (NBT) at 560 nm following the method of Dhindsa *et al.* (1981). APX activity was assayed by recording the decrease in absorbance at 290 nm, due to reduction in H_2O_2 content (Nakano and Asada 1981). CAT activity was determined following the initial rate of disappearance of H_2O_2 at 240 nm (Bergmeyer 1970). Membrane stability index (MSI) was determined following the modified method of potato by Nagarajan and Bansal (1986). 100.0 mg of fresh leaf samples were incubated in 20.0 ml deionized water at 52°C for half an hour. Initial electrical conductivity (IC) was measured using conductivity meter. Same tubes were boiled at 100°C for 10 minutes and final conductivity (FC) was measured. Membrane stability index was calculated using the formula: MSI = [1 – (IC/FC)] x 100.

Data was statistically analysed using MSTAT-C statistical program and mean values were compared using Duncan's Multiple Range Test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Exposure of plants to unfavourable environments like high temperature leads to increased production of reactive oxygen species (ROS). To protect themselves against ROS, plant cells and organelles like chloroplast, mitochondria and peroxisome employ antioxidant defense system, comprising mainly of superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase

(CAT) enzymes. In this study, we analysed the activity of above antioxidant enzymes in two potato cultivars, which showed variable response under different temperature environments. Effect of high temperature on SOD, APX and CAT activities was highly significant ($p \leq 0.05$) (Table 2). SOD activity showed increasing trend in both the cultivars with increase in temperature levels, but the increase in activity was higher in K. Surya, whereas in Chipsona-3, increase in SOD activity was significant only under E3 treatment (Fig. 1). On the other hand APX activity increased significantly under both E2 and E3 treatment, while in Chipsona-3, it increased significantly only under E2 treatment (Fig. 1). This indicate that high temperature induced the activities of both the antioxidant enzymes (SOD and APX) in K. Surya and suggested that higher enzyme activity may be involved in protecting against the high temperature stress. Besides this, marginal induction in the activities of SOD and APX enzymes in Chipsona-3 pointed out towards its sensitivity of this cultivar to high temperature. CAT enzyme activity showed increasing trend in K. Surya at higher temperatures under both E2 and E3 treatments but in Chipsona-3 the activity decreased and reduction was higher under E2 (Fig. 1). Similar increase in the activity of antioxidant enzymes under thermal conditions has been reported and correlated with tolerance in sweet potato (Rui *et al.* 1990), wheat (Sairam *et al.* 2000; Almeselmani *et al.* 2006) and mulberry (Chaitanya *et al.* 2002).

The membrane of cell organelles play a vital role in their functioning and adverse effect of high temperature or other stresses may lead to disruption of membrane integrity. Thus, heat susceptibility in the form of electrolyte leakage has been used to examine the genotypes against various abiotic stresses including high temperature in potato (Nagarajan and Bansal 1986; Arvin and Donnelly 2008). In this study, we analyzed the effect of high temperature on MSI in potato cultivars during tuber initiation stage and found highly significant differences ($p \leq 0.05$) under different temperature environments (Table 2). High temperature treatments caused considerable reduction in MSI of both K. Surya and Chipsona-3 cultivars with maximum reduction observed under E3 temperature environment. However, the reduction was significantly lower in K. Surya when compared with Chipsona-3 (Fig. 2).

Table 2. Summary of analysis of variance (ANOVA) for physiological and biochemical parameters of potato cultivars grown under different temperature environment.

Mean sum of square (MSS) values for parameters	Source of variance			
	Cultivar (C)	Temperature environment (E)	C x E	Error
Leaf total sugars at 25 DAE (S1)	328.53^{NS}	1011.72**	201.12^{NS}	79.82
Leaf total sugars at 45 DAE (S2)	533.34**	1158.16**	226..09*	33.91
Tuber total sugars	5582.7*	5761.0*	2093.7^{NS}	1087.9
Tuber starch content	73.2^{NS}	210.2*	50.9^{NS}	50.98
Proline concentration	0.003^{NS}	23.55**	0.942^{NS}	0.593
Glycine betaine concentration	25455.2**	163653.6**	8016.7**	583.9
Superoxide dismutase activity	28.0**	61.8**	2.94^{NS}	1.46
Ascorbate peroxidase activity	4.29**	6.5**	2222.2**	138.4
Catalase activity	50668.0**	315.4**	11.5^{NS}	43.5
Membrane stability index (MSI)	118.6*	732.1**	39.9^{NS}	24.23

**Significant at 1%; * Significant at 5% level; NS = Non significant; DAE = Days after emergence
S1 = 25 DAE; S2 = 45 DAE

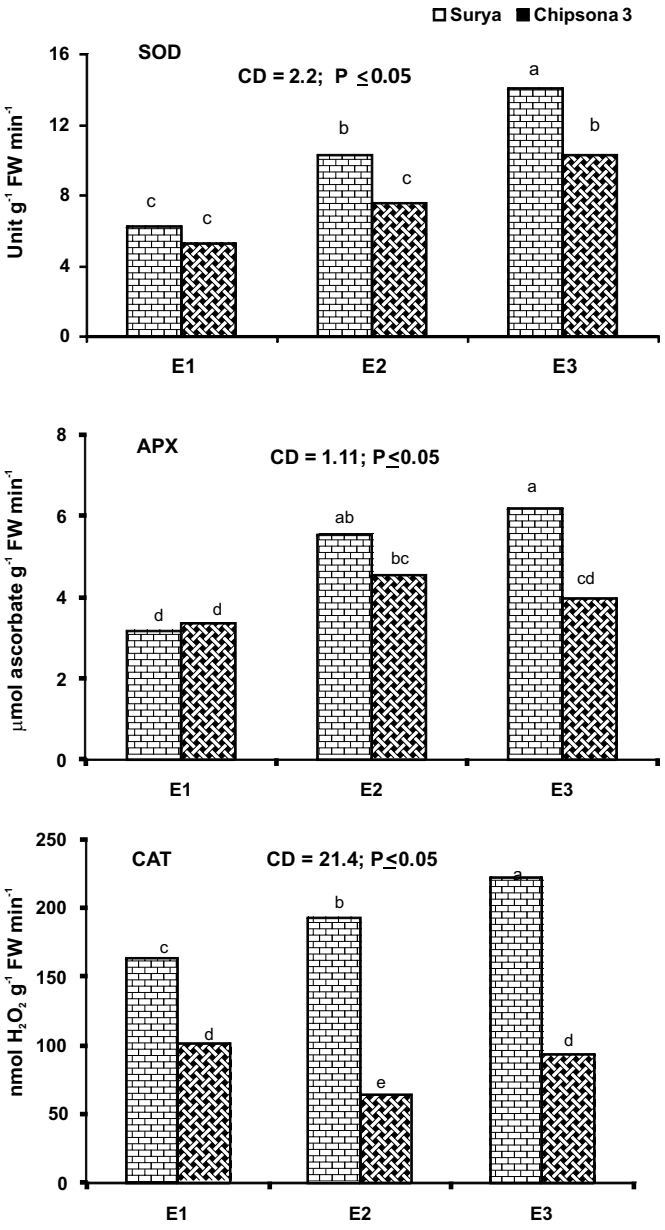

Fig. 1. Effect of different temperature environments on SOD (superoxide dismutase) activity; APX (ascorbate peroxidase) activity and CAT (catalase) activity in the leaves of two potato cultivars at tuber initiation stage. Bars indicated with same letters are not significantly different. E1= Control; E2= Early planting; E3= Polyhouse tunnel and FW= fresh weight.

Accumulation of osmolytes like soluble sugars, proline and glycine betaine is considered as one of the key adaptive mechanisms in plants grown under abiotic stresses, including temperature, salinity and drought (Sairam and Tyagi 2004). In the present study, effect of high temperature was highly significant ($p \leq 0.01$) on total sugars concentration in potato leaves at both tuber initiation (25 DAE) and bulking (45 DAE) stages, (Table 2). Total sugars increased under high temperature environments (both E2 and E3) in cultivar K. Surya. On the other hand in Chipsona-3, total sugar decreased marginally (5%) under E2 treatments and increased under E3 treatment during both tuberization and bulking stages (25 and 45 DAE) (Table 3). K. Surya showed 52 to 64 percent increase in total sugars under E3 treatments, whereas in Chipsona-3, the increase was 18 and 34 percent under similar treatment. Lafta and Lorenzen (1995) have reported increased sucrose and decreased starch accumulation under heat stress in mature leaves of potato while no effect on glucose concentration was noticed. Such changes in sugar concentration have been attributed to increased sucrose-6-phosphate synthase (SPS) activity in mature leaves of plants under high temperature (Lafta and Lorenzen 1995). Similarly, accumulation of soluble sugars under heat stress has been reported in sugarcane (Wahid *et al.* 2007).

High temperature treatments caused increase in total sugars in tubers of both the potato cultivars and highest increase was recorded in the tubers of plants grown under polyhouse tunnel but the increase was not significant in Chpsona-3 (E3). Among the two cultivars, K. Surya exhibited higher accumulation of soluble sugars than Chipsoana-3 (Table 3). In contrast, tuber starch content declined under high temperature in both the cultivars but reduction was not significant in K. Surya (Table 3). Similarly, reduction in starch content at high temperature has been reported by Krauss and Marschner (1984) due to inhibition of conversion of sugars into starch. Lafta and Lorenzen (1995) reported that the activity of sucrose synthase and ADP glucose pyrophosphorylase (the main enzymes associated with synthesis of starch in tubers), reduced under high temperature condition. Similar reduction in tuber starch has been reported under high temperature by Thompson *et al.* (2008).

In the present study high temperature exposure altered the concentration of proline and glycinebetaine in the leaves of both the cultivars. The effect of high temperature and its interaction with cultivars on leaf

Table 3. Effect of different temperature environments on carbohydrates concentration in the leaves and tubers of two potato cultivars.

Treatment	Leaves		Tubers	
	Total sugars at 25 DAE (mg g^{-1} DW)	Total sugars at 45 DAE (mg g^{-1} DW)	Total sugars (mg $100g^{-1}$ FW)	Starch (mg $100g^{-1}$ DW)
V_1 (E1-E3)	72.87[a]	58.9[b]	262.1[b]	52.91[a]
V_2 (E1-E3)	80.08[a]	69.36[a]	297.3[a]	56.94[a]
CD at 5%	10.19	6.12	34.7	7.49
E1 (V_1-V_2)	70.19[b]	54.61[c]	250.7[b]	61.62[a]
E2 (V_1-V_2)	74.99[b]	62.36[b]	276.2[ab]	50.37[b]
E3 (V_1-V_2)	94.69[a]	81.60[a]	312.3[a]	52.80[b]
CD at 5%	11.49	7.49	42.4	9.18
V_1 x E1	72.43[bc]	55.76[c]	241.3[b]	62.83[a]
V_1 x E2	68.76[bc]	51.37[c]	271.7[b]	47.53[b]
V_1 x E3	85.86[b]	75.11[b]	273.3[b]	48.37[b]
V_2 x E1	67.95[c]	53.46[c]	260.0[b]	60.40[ab]
V_2 x E2	81.22[c]	73.35[b]	280.7[b]	53.20[ab]
V_2 x E3	103.53[a]	88.08[a]	351.3[a]	57.23[ab]
CD at 5%	16.25	10.59	60.0	12.99

*Means indicated with the similar letters are not significantly different. V_1= Chipsona-3; V_2= K. Surya; E1= Control; E2= Early planting; E3= Polyhouse tunnel; DAE = Days after emergence; FW= Fresh weight and DW = Dry weight

proline content was not significant (Table 2). There was considerable reduction in leaf proline content in both the potato cultivars and reduction was greater in Surya as compared to Chipsona-3 (Fig. 3). These findings are in contrast to the hypothesis that the proline accumulates in plants in response to environmental stresses including high temperature (Velasquez *et al.* 2005). Moreover, Rahnama and Ebrahimzadeh (2004) reported clear relationship between accumulation of proline and salt stress in potato. Conversely, Rizhsky *et al.* (2004) have suggested that heat stress may inhibit proline accumulation in the plants when they experience it as an additional stress factor. However, no clear evidences are given in above report.

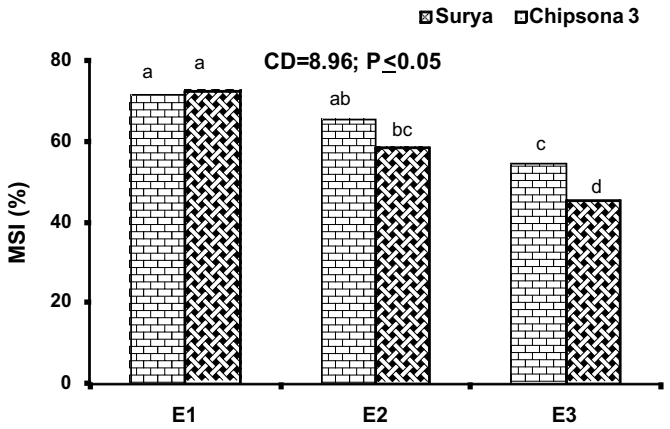

Fig. 2. Effect of high temperature environments on membrane stability index (MSI) in two potato cultivars at tuber initiation stage. Bars indicated with similar letters are not significantly different. E1= Control; E2= Early planting and E3= Polyhouse tunnel.

Glycinebetaine (GB) is well known to play an important role for tolerance to either high temperature or other stresses in plants but the capacity to synthesize GB differs from species to species (Ashraf and Foolad 2007). In this study, concentration of GB increased in the leaves with increasing temperature levels in both the potato cultivars during tuber initiation, and K. Surya showed higher accumulation than Chipsona-3. Highest concentration of GB was observed in K. Surya grown

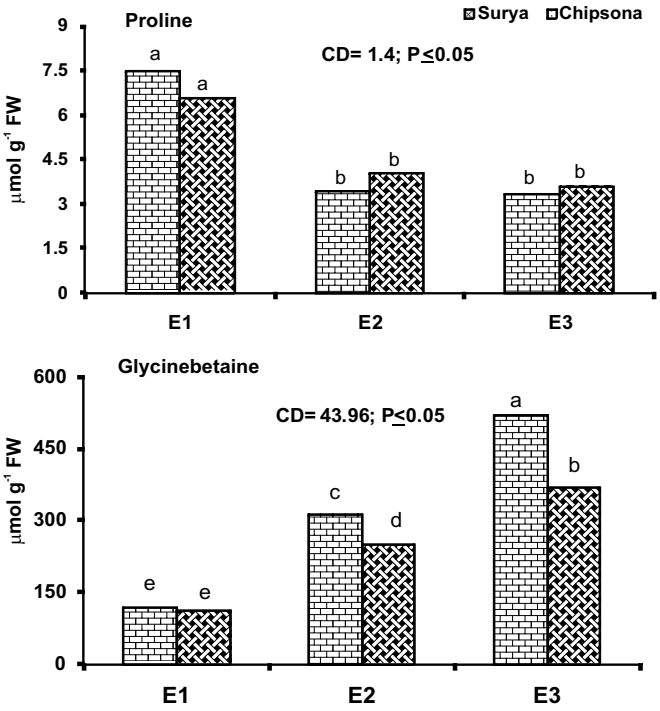

Fig. 3. Effect of high temperature on proline and glycinebetaine concentration in the leaves of two potato cultivars at tuber initiation stage. Bars indicated with the similar letters are not significantly different. E1= control; E2= Early planting; E3= Polyhouse tunnel; FW= Fresh weight.

inside polyhouse (E3 treatment) (Fig. 3). The magnitude of increase in GB in K. Surya was 2 and 3 fold higher under E2 and E3 treatments, respectively compared to control, while in Chipsona-3 the increase in GB was one and two fold of the control under similar temperature environments. Desingh and Kanagaraj (2007) and Kholova *et al.* (2009) have reported similar increase in GB concentration under salinity and high temperature in cotton and maize, respectively. Shirasawa *et al.* (2006) reported that rice transgenic plants with higher synthesis of GB indicated enhanced tolerance to salt and temperature stress at seedling stage. In view of above, our findings suggest that higher increase in glycinebetaine under high temperature in K. Surya might be associated with its tolerance to high temperature in comparison to Chipsona-3.

In conclusion, high temperature induced the activity of SOD, APX and CAT antioxidant enzymes significantly in K. Surya whereas, in Chipsona-3 induction of SOD and APX was lower (when compared with K. Surya), while CAT activity decreased under high temperature. On the other hand, GB concentration increased at tuber initiation stage in both the cultivars and comparatively K. Surya accumulated higher GB than Chipsona-3 under high temperature. Proline content and MSI decreased in both the cultivars with the increase in level of temperature. This study suggests that increase in the activities of SOD and APX enzymes along with the accumulation of GB in K. Surya may be involved in imparting as high temperature tolerance characteristics to this cultivar in comparison with Chipsona-3 (a senstive cultivar).

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