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SHORT COMMUNICATION

SUPEROXIDE DISMUTASE IN CHICKPEA GENOTYPES UNDER HIGH TEMPERATURE

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Superoxide dismutase (SOD) isozymes and activity in chickpea genotypes was examined under high temperature stress. Two chickpea (*Cicer arietinum* L.) genotypes, Pusa 1103 and Pusa 261 were grown in pot culture and transferred to BOD incubator for high temperature (35^{0}C) treatment for 4 h and 6 h at 95 days after sowing. The SOD activity was estimated using native PAGE in fresh leaf sample collected from control and high temperature exposed plants. The activity of this enzyme was found significantly higher under high temperature in Pusa 1103, known to have greater thermotolerance compared to Pusa 261.

Key words: **Reactive oxygen species, superoxide dismutase, thermotolerance**

The Inter-Governmental Panel on Climate Change of the United Nations in its recent report has confirmed the global warming trend and projected that the global average temperature of the air above the earth's surface would rise by 1.4-5.8^{0}C over the next 100 years (IPCC 2007). In chickpea, the greater part of the reproductive phase is exposed to high temperature affecting seed yields upto 50% (Dua 2001). The terminal flower abort up to the extent of 40-50% in different varieties under high temperature prevailing at the end of flowering. Several reports indicate that heat stress leads to the formation of reactive oxygen species causing damage to the cell membranes. Plants can counteract this effect by producing reactive oxygen scavengers such as superoxide dismutase, peroxidase and catalase (Sairam and Saxena 2000). For maintaining the productivity of chickpea under late planting condition it is, therefore, important to analyse the role of antioxidant system.The present study analysed the response of chickpea (*Cicer arietinum* L.) genotypes to high temperature stress in relation to superoxide-dismutase (SOD) activity and its isoforms.

Two chickpea genotypes Pusa 1103 and Pusa 261 (both *desi* types) were grown in earthen pots (diameter 30 cm) containing 10-12 kg sandy loam soil mixed with farmyard manure (FYM) in the ratio of 1:4. All the pots were kept under natural light and atmospheric conditions. NPK was applied in the ratio of 1:2:1 in each pot at the time of sowing. Ten seed per pot were sown by dibbling method at 2 to 2.5 cm depth. Thinning was done to four plants per pot at 20 days after sowing. One set of plants were raised in plastic pots containing one plant per pot. These pots were shifted to BOD incubator for a period of 4 h for high temperature (35^{0}C) treatment at 95 days after sowing. After exposure to high temperature, leaf samples were collected for bio-chemical estimation along with the plants raised under ambient temperature under natural environment. To identify the different isoforms of superoxide dismutase, native PAGE analysis was done in the samples treated with high temperature (35^{0}C) for about 4 h and 6 h (95 DAS). Enzyme extract for superoxide dismutase, was prepared by freezing the leaf samples (1 g) in liquid nitrogen followed by grinding with 10 ml extraction buffer (0.1 M phosphate buffer, pH 7.5,

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containing 0.5 mM EDTA and 1 mM ascorbic acid). Superoxide dismutase activity was estimated by recording the decrease in absorbane of formazone made by superoxide radical and nitro-blue tetrazolium dye by the enzyme (Dhindsa *et al*. 1981). Native PAGE was carried out to analyse the superoxide dismutase activity and the staining was done as per Beauchamp and Fridowich (1971). Inhibitors were used to distinguish various SOD isoforms as per Sandalio *et al*. (1987). Leaf protein (200 µg) were loaded in native PAGE having resolving gel (7.5%), stacking gel (6.0%). Gels were constantly shaken during their incubation in water 89 ml, 50 µl 0.1M potassium phosphate buffer, pH 7.8. KCN (0.2M) was used to inhibit Cu/Zn SOD and 0.2M KCN + 0.5M H_2O_2 was used to inhibit Cu/Zn SOD and Fe-SOD. The effect of high temperature stress (35^{0}C for 4 h 95 DAS) on SOD activity is shown in (Table 1). The results showed that the SOD activity was higher in control and increased under high temperature treatment in both the genotypes. Genotype Pusa 1103 showed 28 per cent more SOD activity compared to Pusa 261 under high temperature. Under many abiotic stresses like drought, desiccation, salt and heatshock, most of the subcellular compartments like chloroplasts, mitochondria, peroxisome, plasma membranes and apoplast produces reactive oxygen species (ROS). Polle (2001) and Sairam *et al.* (2004) showed that ROS causes damage to lipids, proteins and DNA. Peroxidation of membrane lipid occurs when ROS react with unsaturated fatty acids, which lead to leakage of cellular contents, rapid desiccation and cell death. To control the levels of ROS and to protect the cells from injury under stress conditions plants posses very efficient enzymatic and non enzymatic defence systems. Results of the present investigation indicated that high SOD activity in Pusa 1103 under high temperature may impart tolerance to this genotypes.

Table 1. Effect of high temperature stress (35^{0}C for 4 h at 95 DAS) on superoxide dismutase activity (units min^{-1} g^{-1} fw) in chickpea genotypes

Genotypes	Control	Treatment	Mean %	Decrease/ Increase
Pusa 1103	12.58	26.81	19.69	113.12
Pusa 261	10.69	19.79	15.24	85.13
Mean	11.63	23.30		
CD at 5%				
Genotype (G)	0.86			
Treatment (T)	0.86			
G x T	1.2			

Native PAGE analysis revealed three SOD isoforms in the form of three active bands, SOD I, SOD II, SOD III in both the genotypes (Fig. 1). Using KCN and H_2O_2 as selective inhibitors, these isoforms have been identified as Mn-SOD, Cu/Zn-SOD I, and Cu/Zn-SOD II. Fe-SOD is found to be lacking in both the genotypes under control and treatment. In control plants (23^{0}C), both the genotypes showed three isoforms Mn-SOD, Cu/Zn SOD I, Cu/Zn SOD II, which were less visible (Fig. 1), but prominent bands of all isoforms (Mn SOD, Cu/Zn SOD I, Cu /Zn SOD II.) were seen in treatments (35^{0}C for 4 h) (Fig. 2). Prolonged treatment (35^{0}C for 6 hr) led to variations in banding patterns in tolerant and susceptible genotypes. In tolerant genotype again all the three isoforms were seen (Fig. 2), but in the susceptible genotype Mn SOD is found to be completely lacking and also bands of Cu/Zn SOD I and Cu/Zn SOD II were less prominent. Within a cell, the SOD constitutes the

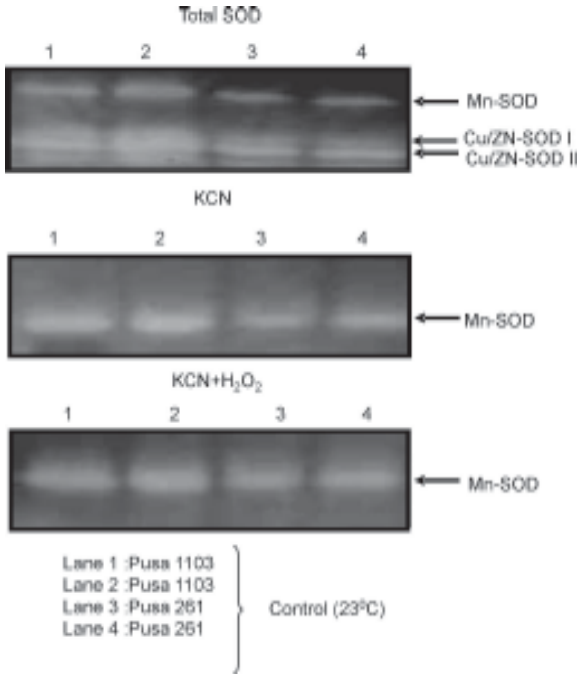

Fig. 1. Effect of temperature (23^{0}C, 95 days after sowing) on superoxide dismutase (SOD) isozyme patterns in chickpea genotypes. Lane 1 = Pusa 1103, Lane 2 = Pusa 1103, Lane 3 = Pusa 261, Lane 4 = Pusa 261

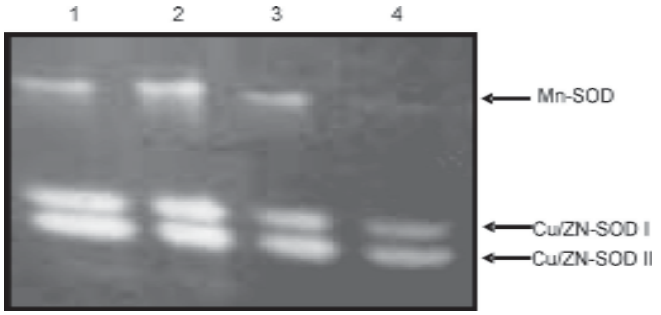

Fig. 2. Effect of temperature stress (35^{0}C for 4 h and 35^{0}C for 6 h, 95 DAS) on superoxide dismutase (SOD) isozyme patterns in chickpea genotypes. Treatment 1 (35^{0}C for 4 h): Lane 1 = Pusa 1103, Lane 2 = Pusa 261, Treatment 2 (35^{0}C for 6 h) Lane 3 = Pusa 1103, Lane 4 = Pusa 261

first line of defense against ROS. Based on metal cofactor SOD are classified in to three groups namely iron SOD (Fe-SOD), Manganese SOD (Mn SOD) and copper zinc SOD (Cu-Zn SOD) and these SODs are located in different compartments of the cell. Tsang *et al*. (1991) reported that under abiotic stress the SOD genes showed differences in expression. Wu *et al*. (1999) showed that under drought stress, spring and winter wheat possessed enhanced expression of SOD 3 (encoding Mn SOD) and SOD I (encoding Cu/ZN SOD). Under stress condition there was a rapid induction of Mn SOD than other. Almeselmani *et al*. (2006) showed the amelioration of high temperature stress induced oxidative stress by antioxidant enzymes in wheat genotypes. Chakraborty and Tongden (2005) reported that the induced heat-stress tolerance response in *Cicer* seedlings may be directly linked to the coordinated response of antioxidative enzymes like POX, APOX and CAT. The scavenging of reactive oxygen species like H_2O_2, superoxide radical by these enzymes play a key role in imparting heat tolerance. On the basis of results obtained from the present investigation, it is concluded that the genotype Pusa 1103, known to have greater thermotolerance possessed more SOD activity and isoforms compared to Pusa 261.

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