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Sodicity-induced antioxidative system in roots of salt-tolerant and salt-sensitive cultivars of Indian mustard (*Brassica juncea* L.)

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Abstract

A differential response of sodicity was observed on enzymatic and non-enzymatic components of antioxidant system in roots of salt-sensitive RH 8113 and salt-tolerant CS 52 varieties of Indian mustard (*Brassica juncea* L.). The antioxidative enzymes *viz*. superoxide dismutase (SOD), glutathione reductase (GR), peroxidase (POX), and ascorbate peroxidase (APX) showed higher basal activities in roots of salt-tolerant CS 52 cultivar as compared to salt-sensitive RH 8113. Imposition of sodicity stress (15-45 ESP) had no effect on SOD and APX activity in roots of CS 52, but it resulted in reduction in activity in salt-sensitive RH 8113. In both varieties, the POX activity decreased progressively with increase in stress level , but the level of decrease was more in RH 8113 as compared to that in salt-tolerant CS 52. Contrarily, GR activity increased in tolerant cultivar CS 52, but showed a continuous reduction with increment in sodic stress in the sensitive cv. RH 8113. The amount of ascorbic acid, reduced glutathione, and ratio of reduced (GSH)/ oxidized glutathione (GSSG) were higher in tolerant than in sensitive ciltivar under sodic conditions. It is inferred that roots of tolerant cv. CS 52 tend to attain greater capacity to perform reaction of antioxidative pathway under sodic conditions to combat sodicity-induced oxidative stress.

Key words: Antioxidant enzymes, ascorbate, *Brassica juncea*, glutathione, reactive oxygen species, sodicity

Introduction

Sodicity is the salt stress having sufficient exchangeable sodium and high pH (more than 9.0) that limits plant productivity and its quality by affecting a number of biochemical processes. The production of toxic reactive oxygen species (ROS) *viz*. O_2^-, H_2O_2, OH^- is a major damaging factor in plants exposed to different environment stresses including drought (Loggini *et al.*,1999), pollutants (Marti *et al.*, 2009), temperature extremes (Rao *et al*.,1995), and herbicides leading to oxidative stress. The enhanced generation of ROS disrupts normal metabolism through peroxidating lipids, denaturing proteins, and nucleic acids (Bor *et al.*, 2003). To protect the plant from deleterious effect of ROS, plant cells possess an antioxidative system consisting of enzymatic and non-enzymatic components (Gill and Tuteja, 2010). Antioxidant enzymes include superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR); antioxidant compounds are both water soluble molecules like ascorbate, glutathione and flavonoids, and lipid soluble antioxidants such as carotenoids and á-tocopherol. Although, plants with high levels of antioxidants, either constitutive or induced, have been reported to possess greater resistance to oxidative damage (Sekmen *et al.*, 2007), the precise mechanism that imparts salt tolerance in plants still remain to be elucidated. However, information is also not available on the effect of sodicity on antioxidant system in crop plants. The present work, therefore, was undertaken to study the sodicity-induced changes in oxidative stress, and antioxidative system in salt-tolerant and salt- sensitive cultivars of Indian mustard [*Brassica juncea* (L.) Czern & Coss.].

Materials and Methods

Seeds of Indian mustard *(Brassica juncea* L.) of salt- sensitive cv. RH8113 and salt-tolerant cv. CS

52 to salt were obtained from the Oilseeds Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar. All the biochemicals used were either from the Sigma Chemical Co. St. Louis, USA, or from Sisco Research Laboratory, India.

Raising of crops

Seeds of uniform size of both the varieties were surface sterilized and sown under natural conditions in a screen house in polyethylene bags (30×40 cm) filled with 10 kg loamy soil supplemented with appropriate amount of $NaHCO_3$ to create sodicity of 15, 30 and 45 exchangeable sodium percentage (ESP). The control plants were sown in the loamy soil that was not treated with $NaHCO_3$. The plants were supplied with modified Hoagland nutrient solution (Singla *et al.*, 2004) at 7-days intervals, and roots of 60-days-old-plants were used for biochemical estimations.

Enzyme extraction and assays

Extraction conditions including type, molarity and pH of buffer, concentration of stabilizing agents, and other constituents of extraction medium were standardized to achieve maximum extraction of enzymes. All the steps of extraction were carried out at 0-4°C. Extraction medium for SOD, APX and GR consisted of 0.1 M phosphate buffer (pH 7.5) containing 5% (*w/v*) polyvinyl polypyrrolidone (PVPP), 1 mM EDTA, and 10 mM â-mercaptoethanol as described by Chawla *et al.* (2013). For POX, the extraction was done in 0.01 M phosphate buffer (pH 7.5) containing 3% (*w/v*) PVPP. The homogenate was prepared by grinding 1 g of tissue in 5 ml of ice cold extraction medium in pre-cooled mortar and pestle. The homogenate thus prepared, was centrifuged at 10,000 x g for 15 min at 4°C. The supernatant was carefully decanted and used as the crude enzyme preparation. All the estimations were carried out in three replicates with two extractions for each replicate. The values reported for each parameter are, therefore, the means of six replicates.

Superoxide dismutase (SOD) activity was determined by quantifying the ability of the enzyme to inhibit light induced conversion of nitroblue tetrazolium (NBT) to formazan (Nishikimi *et al.*, 1972). One enzyme unit was defined as the amount of enzyme which could cause 50 per cent inhibition of the photochemical reaction. Peroxidase activity was assayed at 37°C as described by Shannon *et al.* (1966). Method of Nakano and Asada (1981) was employed to assay APX. The unit of POX activity was defined as 1.0 µmole of H_2O_2 utilized per min while one unit of APX corresponded to 1.0 O.D. change per min. Glutathione reductase activity was determined at 30°C by adding 100 l of enzyme extract to 1 ml of 0.2 M phosphate buffer (pH 7.0) containing 1 mM EDTA, 0.75 ml distilled water, 0.1 ml of 20 mM oxidized glutathione (GSSG), and 0.1 ml of 2 mM NADPH. Oxidation of NADPH by GR was monitored at 340 nm and the rate (nmoles min^{-1}) was calculated using the extinction coefficient of 6.12 mM^{-1} cm^{-1} (Halliwell and Foyer, 1978).

Extraction and estimation of metabolites

For the extraction of ascorbate and glutathione (reduced and oxidized), 1 g of roots from control and stressed plants were ground in 5 ml of chilled 0.8 N $HClO_4$ (Jimenez *et al.*, 1997), and centrifuged at 10,000 x g for 25 min. The clear supernatant was used for the estimation of these metabolites. Ascorbic acid content was estimated according to the method of Roe (1964) which is based on the reduction of 2, 6-dichlorophenol indophenol by ascorbic acid. Method of Smith (1985) was employed for determining the level of oxidized and reduced glutathione.

Results and Discussion

Results presented in figure 1A, demonstrate the activities of SOD that catalyses the oxidation of superoxide radical to H_2O_2 had higher constitutive level (70.5 units/g fresh wt) in tolerant cv. CS 52 as compared to that in sensitive cv. RH 8113 (57.5 units/g fresh wt). Imposition of sodicity stress of 15, 30 and 45 ESP resulted in 16%, 44.4% and 55.7% decrease, respectively, in SOD activity in roots of RH8113. However, SOD activity remained constant in roots of CS 52 under all levels of sodic stress. Results of the present investigations corroborate the previous report (Bor *et al.*, 2003) that SOD activity in salt-tolerant cultivars increased or remained constant whereas in salt-sensitive cultivars

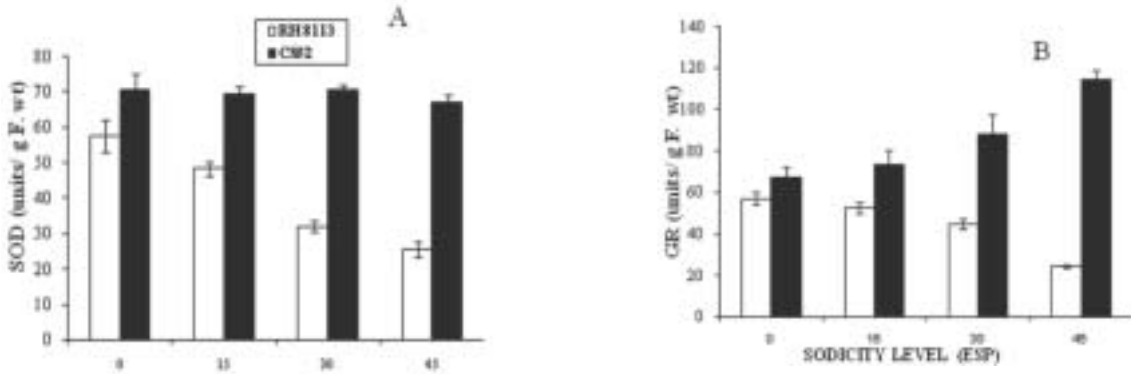

Fig 1: Effect of sodicity on superoxide dismutase (SOD) (A) and glutathione reductase (B) activities in roots of *B. juncea* cultivars RH 8113 and CS 52 (Values are mean ± S.D, RH 8113- no fill, CS 52- black fill).

decreased in response to salinity. The decline in SOD under stress in susceptible cultivar could be due to impaired detoxification of toxic oxygen species (Goggin and Colmer, 2005). The salt-tolerant cultivars of *B. juncea* and *Najas graminia* have also been reported to possess higher basal levels of SOD than the sensitive cultivars (Bor *et al*., 2003). Higher basal level of SOD has been proposed to be the first line of defense, and it signifies the possible involvement of this enzyme in salt tolerance.

As shown in Fig 1B, GR activity increased linearly in roots of tolerant cv. CS 52 (9.0-70%) with the increase in sodicity level from 15 to 45 ESP. On the other hand, in roots of the sensitive cv. RH8113, it decreased at all the levels of stress from 57.1 units in the control to 24.5 units at 45 ESP sodicity level. The tolerant cv. CS 52 not only showed increased GR activity under stress conditions, it also had significant higher basal level (67.1 units/g fresh wt). These results are in agreement with those of Hernandez *et al*. (2001) who reported higher GR in salt-stressed tolerant cv. with no change or decrease in the sensitive cultivar. Higher GR in tolerant cultivar has been considered to indicate that the tolerant plants are endowed with a more active ascorbate-glutathione cycle than the non-tolerant cultivar (Hernandez *et al*., 2001).

The activity of POX (Fig 2A), a H_2O_2 scavenging enzyme, decreased continuously with the increasing level of sodicity stress in both the cultivars. At 45 ESP, it declined to about 19.2% in roots of sensitive cv. RH 8113 compared to 50% in tolerant cv. CS 52. The results on the effect of sodicity on POX are in agreement with those of Cicek and Cakirlar (2008) who reported a significant inhibition in POX at higher NaCl concentration in soybean. In the

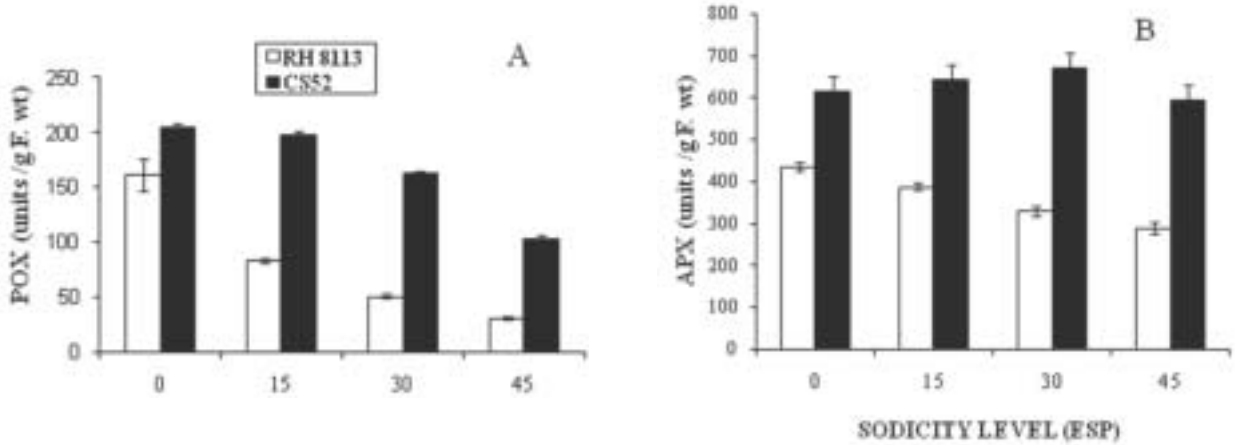

Fig 2: Effect of sodicity on peroxidase (A) and ascorbate peroxidase (B) activities in roots of *B. juncea* cultivars RH 8113 and CS 52 (Values are mean ± S.D, RH8113- no fill, CS 52- black fill).

present investigations, higher level of POX in cv. CS 52 with no significant change in its activity at various stress levels could help in protecting plants from oxidative damage by scavenging H_2O_2 as well as cause the signification and cross linking of cell wall (Dalal and Khanna-Chopra, 2001).

Ascorbate peroxidase (Fig 2B) is another H_2O_2 scavenging enzyme that has very high affinity for H_2O_2 than POX. Imposition of sodicity stress (15-45 ESP) resulted in progressive decrease (11.4-33.8 %) in APX activity in roots of salt sensitive cultivar. Though imposition of varying levels of sodicity had no effect on the APX activity in tolerant cv. CS 52, this cultivar had almost 1.5 fold the activity in roots of non-stressed and stressed plant as compared to that in cv. RH 8113. Increased APX activity with increase in salt stress has also been reported in wheat (Heidari and Mesri, 2008) and pistachio plants, (Abbaspour, 2012) suggesting that high basal level of APX and/or salt-induced increase in APX activity could impart tolerance by detoxifying H_2O_2 generated upon exposure of plants to saline conditions.

Ascorbic acid and glutathione are important ROS scavenging metabolites. Results presented in Fig 3A reveal that although ascorbate content in roots of both *B. juncea* cultivars increased under stress conditions, the basal level, as well as, the magnitude of increment, was higher in tolerant cv. CS 52 than in sensitive cv. RH 8113 under varying levels of sodic

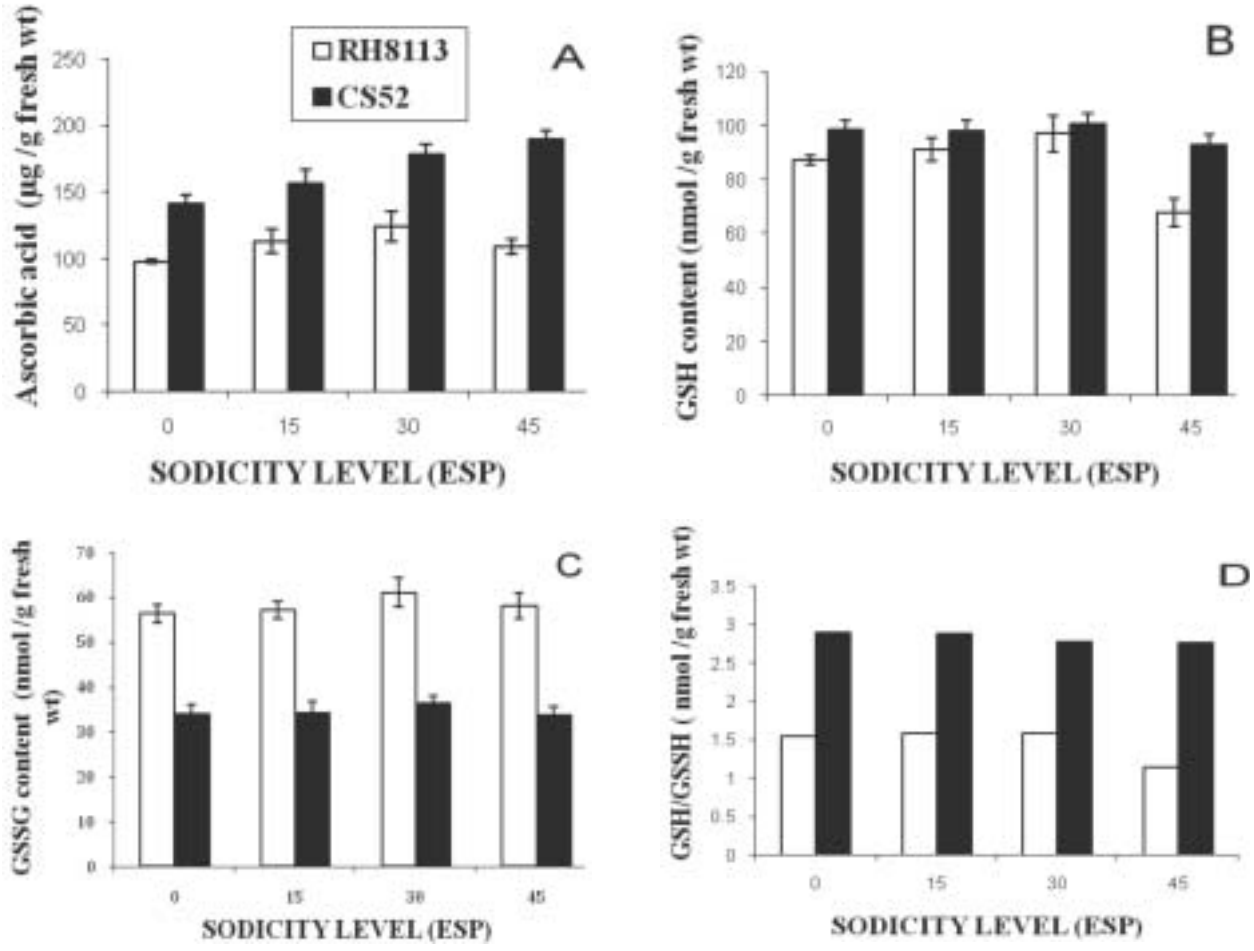

Fig 3: Effect of sodicity on ascorbic acid content (A), reduced glutathione (B), oxidized glutathione (C) and ratio (D) of reduced glutathione (GSH) to oxidized glutathione (GSSG) in roots of *B. juncea* cultivars RH 8113 and CS 52 (Values are mean ± S.D, RH8113- no fill, CS 52- black fill).

stress. The roots of cv. CS 52 of non-treated plants had significantly higher (156.4 µg /g fresh wt) amount of ascorbic acid as compared to that of salt-sensitive cultivar which possessed 113.4 µg /g fresh weight ascorbic acid. In RH 8113, ascorbic acid content increased with increase in sodicity level upto 30 ESP (12.8-26.2%), but in CS 52 a linear increase from 10.7% at 15 ESP, 26.5% at 30 ESP, and 34.7% at 45 ESP was observed. Ascorbate plays an important role in imparting protection against ROS, as it acts as an electron donor for ascorbate peroxidase. Results of our studies are in agreement with those of Chawla *et al*. (2013) who observed more ascorbate content in salt-tolerant than in salt-sensitive cultivars of rice.

Perusal of data on reduced glutathione (GSH; Fig 3B) and oxidized glutathione (GSSG**;** Fig 3C) content reveal no change in roots of both cultivars under sodic stress upto 30 ESP, though the basal level of GSH was found to be significantly higher (98.4 µg /g fresh wt) in roots of the tolerant cv. CS 52 than in the sensitive cv. RH 8113 which had 87.2 µg /g fresh wt. The ratio of reduced glutathione to oxidized glutathione (Fig 3D) was also much higher in CS 52 as compared to cv. RH 8113. Glutathione, a low molecular weight antioxidant, is a powerful regulator of major cell functions (Renenberg and Lamoureux, 1990). Reduced glutathione can react directly with free radicals, hence preventing inactivation of enzymes due to oxidation of essential thiol groups (Wang *et al*., 1991).

The results presented here are similar to those of Amor *et al*. (2007), and Nagesh Babu and Devaraj (2008) who also observed significantly elevated GSH contents in tolerant as compared to these in sensitive cultivars of wheat, potato, Jerba plants, *Cakile maritime*, and French bean, respectively, under saline conditions. Thus, the higher basal levels of antioxidant enzymes and increase in GR activity accompanied by high ascorbate level and GSH/ GSSG ratio may account for better capability of cv. CS 52 to perform better under sodic conditions than the salt-sensitive cv. RH 8113.

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