RESEARCH ARTICLE

NaCl induced oxidative stress in legume crops of Indian Thar Desert: an insight in the cytoprotective role of HO1, NO and antioxidants

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Abstract The manuscript highlights the role of antioxidants in alleviation of salinity stress in two principal legume crops *Cyamopsis tetragonoloba* and *Vigna radiata* of Indian Thar Desert. The study evaluates correlation between the antioxidants of two cultivars in terms of morphological and physiological alterations. Hydroponically acclimatized seedlings of both the crops were subjected to NaCl stress at different concentrations ranges from 10 to 100 mM. After 96 h, the treated legumes were harvested to analyze the cellular homeostasis and salt tolerance mechanism via examining growth, stress parameters, osmoprotectants and enzymatic antioxidants. Differential response in the antioxidants activity was observed in crops. Equal contribution of antioxidants in mitigation of salinity stress was recorded in *C. tetragonoloba* while *V. radiata* shows greater tolerance by accumulating greater amount of proline which is approximately 2.72 folds higher than *C. tetragonoloba*. Moreover, the NR and HO1 activities in *V. radiata* were recorded to be 2.76 and 1.55 folds respectively which is 1.2 times higher in comparison to *C. tetragonoloba*. The detrimental effect of NaCl in terms of MDA content was also higher in *V. radiata* which concluded that *V. radiata* is more reactive towards salinity stress than *C. tetragonoloba*. The study is significant as this is the first report illustrating the sensitivity and tolerance level of NaCl in legumes of Thar Desert.

Keywords Legumes · *Cyamopsis tetragonoloba* · *Vigna radiata* · Indian Thar Desert · Salinity stress · Antioxidants

Introduction

Thar Desert, one of the smallest deserts forms the eastern extremity of the great arid and semi-arid belt of the world and exhibits wide range of habitats (Sharma and Mehra 2009). About 7,20,000 ha area of desert is saline. Due to high salinity in desert soil, plants in this region have acclimatized to cope up with elevated salt concentrations (Arora et al. 2010). Salinity stress is one of the major abiotic factors that limit the yield of agricultural crops by affecting seed germination and crop productivity (Munns and Tester 2008). Among the different resources of salinity, irrigation and poor drainage are the most challenging issue which leads to reduction in productive agricultural area (Zhu 2007). Calcium (Ca^{2+}), magnesium (Mg^{2+}), and sodium (Na^{+}) ions are major constituents of irrigated water in which sodium ions remains in the soil while Ca^{2+} and Mg^{2+} precipitate into carbonates during water evaporation (Serrano et al. 1999). Thus, Na^{+} surpasses other macronutrients and micronutrients present in the soil. Increasing NaCl concentration in soil creates external osmotic potential which inhibit water influx into roots and hinders soil aeration (Singh and Chatrath 2001).

Salinity stress affects cell equilibrium and metabolism by oxidative damage to nucleic acid, protein and lipids (Baxter et al. 2007). Different salt tolerance mechanism adapted by the plant species to provide the cellular protection against NaCl stress (Ramani et al. 2006). Plant defense system comprises of ROS scavenging enzymes including Catalase (CAT), Ascorbate peroxidase (APX), Superoxide dismutase (SOD), Guaiacol peroxidase (GPX),

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Nitrate reductase (NR) as well as various antioxidant molecules namely proline, thiols and other ROS quencher compounds (Mahawar and Shekhawat 2016; Verma et al. 2013; Liu et al. 2009; Demirevska-Kepova et al. 2004). ROS detoxification process commences with the SOD activity, which converts O_2^- radicals to H_2O_2 and O_2 (Alscher et al. 2002). Accumulation of H_2O_2 in the plant cell is reduced by CAT activity or through the ascorbate–glutathione cycle where ascorbate peroxidase reduces it to H_2O so that the deposition of O_2^- and H_2O_2 is effectively prevented (Sharma and Dietz 2009). Beside the discussed antioxidants, heme oxygenase is considered as a novel antioxidant enzyme in mitigating the effects of salt induced oxidative stress by stereospecific degradation of heme to biliverdin IX α , ferrous ion and carbon monoxide (Shekhawat et al. 2019; Mahawar and Shekhawat 2018; Verma et al. 2015; Shekhawat et al. 2011; Shekhawat and Verma 2010).

Legumes, the members of Fabaceae family are economically important and an excellent source of proteins for nutritional and health benefits. *Cyamopsis tetragonoloba* (Clusterbean) and *Vigna radiata* (Mungbean) are the two significant summer-growing legumes, grown predominantly under semi-arid regions of Thar Desert (Thomas et al. 2004; Ashraf et al. 2002). Moreover, *C. tetragonoloba* received great industrial importance due to the presence of galactomannan (Sangwan et al. 2014). The toxic effects experienced by plants due to climate change highlights the requirement for intensive research to develop stress tolerant crop plants for better agronomic performance, yield and survival. This study therefore focuses on the effect of salt concentrations on morphological and biochemical parameters in seedlings of *C. tetragonoloba* and *V. radiata*. The objectives of the current research were to examine the probable role of antioxidants in providing cellular defense against NaCl mediated oxidative stress in crop plants. The present investigation mainly emphasized on the physiological application of antioxidants in various plant parts as well as to compare the correlation between the changes in antioxidant system and degree of tolerance in two cultivars in terms of growth and physiological alterations.

Methodology

Plant material and experimental design

Seeds of *C. tetragonoloba* (RGC 936) and *V. radiata* (PDM 54) were procured from National Bureau of Plant Genetic Resources, Jodhpur (Rajasthan), India and sterilized with 1000 ppm of mercuric chloride for one to two minutes to remove contamination and rinsed thoroughly with

deionized water 4 to 5 times to eliminate the residuals of mercuric chloride. Disinfected seeds were germinated in sterilized glass petri-dishes (10 cm) containing whatmann filter paper No.1 absorbed with autoclaved distilled water (15–20 ml) at 25 °C in a seed germinator (vaiometra) under dark conditions. After a week, the hydroponic cultures were established by transferring the uniformly sprouted seedlings in plastic pots containing one litre of Hoagland medium (pH 6.8 to 6.9, Elico LI 120 pH meter) at 25 ± 2 °C temperature and 50% relative humidity. The nutrient rich medium was stirred twice a day using glass rod to provide ample amount of oxygen and mixing of nutrients.

NaCl treatment in Hoagland medium

The one week old hydroponically adapted seedlings of crop plants were subjected to NaCl stress at different concentrations ranges from 10 mM to 100 mM. A Hoagland medium without salt was considered as a control and used to compare the effect of NaCl on *C. tetragonoloba* and *V. radiata*. After 96 h, the NaCl treated crops were harvested to study different physiological parameters.

Seedlings of *C. tetragonoloba* and *V. radiata* were also treated with sodium nitroprusside (SNP), a NO donor at various concentrations ranges from 0.1 to 20 mM for a period of 96 h. The effect of SNP on nitrate reductase (NR) activity at different concentrations was recorded and the desired concentration was selected for further experimental work at which maximum NR activity was recorded.

Growth parameters

To determine the growth root length, shoot length, fresh weight, dry weight, tolerance index and leaf water content were calculated from both treated as well as control seedlings. For calculating the dry weight, fresh tissue of both the legumes were placed at 65 °C in a hot air oven (Nsico Hicon Oven) for dehydration and the weight (Adair Dutt 125 A SCS) of dried tissue was calculated. Root and shoot length of the seedlings were measured in centimeters. The leaf water content was calculated from the formula (Fresh weight – Dry weight/Fresh weight) $\times$ 100. Tolerance index (TI) of plants was estimated from the formula, (Fresh weight of treated tissue/Fresh weight of control tissue) $\times$ 100 (Wilkins 1978). Total protein content was also determined by the method of Lowry et al. (1951).

Determination of ROS (H_2O_2) level and its toxicity level

H_2O_2 content in seedlings of *C. tetragonoloba* and *V. radiata* was determined by the method given by Alexieva

et al. (2001). While its toxicity level was recorded by measuring the MDA content formed due to oxidation of lipid which was determined according to the procedure of De Vos et al. (1989).

Estimation of antioxidants

Proline content (an osmoprotectant) was estimated according to the procedure of Bates et al. (1973) and was calculated by a standard curve prepared from L-proline ($\mu\text{g g}^{-1}$ fresh weight of tissue).

For antioxidant enzyme extraction 500 mg of fresh plant tissue was homogenized in 5000 μl of 50 mM phosphate buffer (pH-7.0). The homogenate was centrifuged in cold conditions at $5000 \times g$ for 20 min. The supernatant was stored at -20°C for the antioxidant enzymes assay. APX (EC 1.11.1.11) activity was assayed by the procedure given by Chen and Asada (1989). Activity of CAT (EC 1.11.1.6) was measured by the method of Aebi (1974). Guaiacol Peroxidase (GPX) (EC 1.11.1.7) activity was assayed according to the procedure of Putter (1974). Activity of superoxide dismutase was assay by calculating its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) as per the procedure given by Beauchamp and Fridovich (1971). Hemeoxygenase 1 (HO1) activity (EC 1.14.99.3) was measured by the method of Balestrasse et al. (2005). HO1 activity was calculated by biliverdin formation (extinction coefficient $6.25 \mu\text{M}^{-1} \text{cm}^{-1}$). The Nitrate reductase (EC 1.6.6.1) activity was determined by the modified procedure of Datta and Sharma (1999) and its activity was recorded from a standard curve prepared by sodium nitrite.

Statistical analysis

Experimental data were statistically determined by SPSS 16 version software. Results were considered as mean ($\pm$ standard error) of individual replicates ($n = 3$) of every test conducted independently. The significance of

difference amongst control and each treatment were interpreted using one way ANOVA at 0.05% level of significance according to DMRT (Duncan's Multiple Range test).

Results

Growth analysis under NaCl stress in *Cyamopsis tetragonoloba* and *Vigna radiata* seedlings

The overall growth in *C. tetragonoloba* seedlings initially increased (10 mM) and then decreased with higher concentration of NaCl (Table 1). The maximum effect of NaCl on *C. tetragonoloba* was observed at 50 mM where 11.42%, 13.74% and 16.61% increase in fresh weight, root length and shoot length were observed with respect to control. The tolerance index was increased from 90.65 (30 mM NaCl) to 111.51 (50 mM NaCl) over a period of 4 days (Table 1) in *C. tetragonoloba* and after 50 mM tolerance index was found to be reduced with the elevated level of NaCl. While in *V. radiata* the decrease in fresh weight, dry weight, root length and shoot length was observed at initial concentration of NaCl (10 mM). A significant increase in overall growth was observed at 20 mM where 5.01%, 47.69%, 161.97%, 100.32%, 127.25% and 50.52% increase in fresh weight, dry weight, root length, shoot length, tolerance index and leaf water content were recorded with respect to 10 mM NaCl (Table 2).

A noticeable rise in protein content was observed at 50 mM which were 2.04 and 1.51 folds respectively in leaves and roots than that of control in *C. tetragonoloba* (Fig. 1a). While the protein content increased with increase in salt concentration till 60 mM and then decreased with further increase in concentration in leaves of *V. radiata*. At 60 mM NaCl 51.46% increase in total protein content was recorded in leaves than control (Fig. 1b).

Table 1 Effect of NaCl at different concentrations on Fresh weight, Dry weight, Root length, Shoot length, Leaf water content and Tolerance index in seedlings of *Cyamopsis tetragonoloba*. Values are mean $\pm$ standard error ($n = 3$)

Conc. of NaCl (mM)	Fresh weight (g)	Dry weight (g)	Root length (cm)	Shoot length (cm)	Tolerance index (%)	Leaf water content (%)
Control	0.613 ± 0.020^b	0.163 ± 0.008^{ab}	15.33 ± 0.601^b	9.633 ± 0.233^{ab}	100.00 ± 0.00^{ab}	73.370 ± 1.0150^a
10	0.707 ± 0.017^a	0.188 ± 0.007^a	15.40 ± 0.611^b	10.167 ± 0.441^a	115.647 ± 6.64^a	73.323 ± 1.530^a
30	0.573 ± 0.014^{bc}	0.140 ± 0.005^b	11.167 ± 0.601^c	8.433 ± 0.698^b	90.647 ± 2.24^{bc}	75.527 ± 1.438^a
50	0.683 ± 0.023^a	0.163 ± 0.020^{ab}	17.437 ± 0.731^a	11.233 ± 0.376^a	111.507 ± 3.47^a	76.24 ± 2.522^a
70	0.537 ± 0.023^c	0.141 ± 0.005^b	9.633 ± 0.555^c	8.033 ± 0.260^b	87.936 ± 6.68^{bc}	73.577 ± 1.326^a
100	0.467 ± 0.029^d	0.103 ± 0.006^c	5.700 ± 0.145^d	5.1670 ± 0.328^c	76.553 ± 7.25^c	77.447 ± 2.647^a

Data marked with different letters show significant differences ($p < 0.05$) within treatments

Table 2 Effect of NaCl at different concentrations on Fresh weight, Dry weight, Root length, Shoot length, Leaf water content and Tolerance index in seedlings of *Vigna radiata*. Values are mean $\pm$ standard error (n = 3)

Conc. of NaCl (mM)	Fresh weight (g)	Dry weight (g)	Root length (cm)	Shoot length (cm)	Tolerance index (%)	Leaf water content (%)
Control	0.563 $\pm$ 0.038 ^b	0.138 $\pm$ 0.011 ^d	7.30 $\pm$ 0.987 ^c	9.94 $\pm$ 0.658 ^c	100 $\pm$ 0.00 ^b	73.60 $\pm$ 3.18 ^{ab}
10	0.558 $\pm$ 0.040 ^d	0.130 $\pm$ 0.003 ^d	9.40 $\pm$ 0.608 ^c	6.24 $\pm$ 0.742 ^d	99.111 $\pm$ 8.147 ^d	76.70 $\pm$ 8.78 ^d
20	0.586 $\pm$ 0.031 ^{ab}	0.192 $\pm$ 0.003 ^b	24.62 $\pm$ 2.897 ^a	12.50 $\pm$ 0.908 ^{bc}	110.366 $\pm$ 5.813 ^{ab}	66.86 $\pm$ 1.91 ^{bc}
40	0.412 $\pm$ 0.038 ^c	0.172 $\pm$ 0.003 ^c	12.06 $\pm$ 1.480 ^{bc}	13.44 $\pm$ 0.314 ^b	77.704 $\pm$ 7.996 ^c	56.48 $\pm$ 4.79 ^{cd}
60	0.518 $\pm$ 0.030 ^d	0.200 $\pm$ 0.004 ^b	17.34 $\pm$ 1.177 ^{ab}	14.30 $\pm$ 0.889 ^b	97.603 $\pm$ 6.105 ^b	60.90 $\pm$ 2.25 ^{bc}
80	0.442 $\pm$ 0.039 ^e	0.140 $\pm$ 0.007 ^e	12.90 $\pm$ 1.100 ^a	10.16 $\pm$ 0.765 ^a	78.507 $\pm$ 6.763 ^b	68.32 $\pm$ 1.99 ^a
100	0.366 $\pm$ 0.037 ^a	0.126 $\pm$ 0.005 ^a	7.38 $\pm$ 1.037 ^{ab}	7.40 $\pm$ 1.86 ^a	65.00 $\pm$ 7.208 ^a	65.57 $\pm$ 2.52 ^{bc}

Data marked with different letters show significant differences ($p < 0.05$) within treatments

Fig. 1 Total protein and Proline content in *C. tetragonoloba* (a, c) and *V. radiata* (b, d). Seedlings were treated with 10 to 100 mM sodium chloride for a period of 96 h. Values are mean $\pm$ SE (n = 3)

NaCl stress improved proline accumulation

In *C. tetragonoloba* proline content significantly increased with increase in NaCl concentration and maximum proline was recorded at 100 mM which was 1.69 folds higher than control in leaves (Fig. 1c). While in shoots and roots the

and are statistically significant according to Duncan Multiple Range Test ($P < 0.05$). Data points marked with the same letters show insignificant differences ($P < 0.05$) within treatments

highest proline level was recorded at 50 mM NaCl which were 1.18 and 1.39 times than control. Similarly, in *V. radiata* proline content increased with increase in salt concentration and the highest proline was recorded at 100 mM NaCl which were 16.3, 5.8 and 2.49 folds in leaves, shoots and roots respectively than control (Fig. 1d).

Fig. 2 MDA and H₂O₂ content in *C. tetragonoloba* (a, b) and *V. radiata* (c, d) at varying NaCl concentration. Values are mean $\pm$ SE (n = 3) and are statistically significant according to DMRT test ($P < 0.05$)

Effect of salt on ROS and its toxicity

A noticeable increase in MDA content was observed in the crops at initial exposure to NaCl (Fig. 2). However, the NaCl toxicity was greater in *V. radiata* than *C. tetragonoloba*. In seedlings of *V. radiata* a substantial elevation in MDA content was recorded at 10 mM in leaves and roots which were 1.34 and 1.30 times higher with respect to control (Fig. 2c). While in *C. tetragonoloba*, a progressive increase in MDA was found at 30 mM which were 1.14 and 1.63 folds higher than that of control in leaves and roots respectively (Fig. 2a). The level of H₂O₂ was 1.38 times (at 30 mM) in comparison to control and thereafter a significant decrease was recorded at 50 mM NaCl in leaves of *C. tetragonoloba*. The H₂O₂ accumulation gradually decreased from 37.67% (30 mM NaCl) to 10.05% (50 mM NaCl) in leaves over a period of 96 h (Fig. 2b). Conversely H₂O₂ content in *V. radiata* increased at 10 mM NaCl which was 47.11% with respect to control in leaves (Fig. 2d). After this concentration 40.24% (20 mM) decrease in H₂O₂ content was observed in leaves of *V.*

radiata with respect to 10 mM NaCl concentration (Fig. 2d).

Role of antioxidants in detoxification of NaCl stress

SOD activity in *C. tetragonoloba* significantly increased with increase in NaCl concentration in leaves (Fig. 3a). Moreover, a progressive increase in SOD activity was recorded at 50 mM NaCl which was 2.12 folds higher than control (Fig. 3a). Similar results were obtained in *V. radiata* in which the SOD activity increased with initial NaCl concentration till 60 mM and then decreased with higher salt concentration in leaves. At 60 mM NaCl SOD activity was found to be 2.13 times higher than that of control (Fig. 4a). GPX activity was gradually increased with initial salt concentration (10 mM) then decreased at 30 mM and thereafter progressively increased up to 70 mM NaCl then again decreased with higher salt concentration. An increase of 43.68% and 59.68% in Guaiacol peroxidase activity were noticed in shoots and roots respectively at 70 mM NaCl concentration for a period of 96 h in *C. tetragonoloba* (Fig. 3b). While in *V. radiata*

Fig. 3 SOD (a), GPX (b), CAT (c) and APX (d) activity in *C. tetragonoloba* seedlings treated with varying concentrations of NaCl. Values are mean $\pm$ SE ($n = 3$) and are statistically significant

74.69% increase in the GPX activity was observed at 10 mM NaCl treatment in roots with respect to control (Fig. 4b). After 10 mM concentration GPX activity was decreased 59.38% (at 20 mM) with respect to 10 mM NaCl thereafter almost remain constant with further increase in concentration (Fig. 4b). Catalase activity in *C. tetragonoloba* seedlings was found to be decreased with initial exposure of NaCl in leaves (Fig. 3c). A noticeable increase in CAT activity was observed at 50 mM NaCl, but the recorded activity was approximately equivalent to that of control (Fig. 3c). While insignificant effect of NaCl on CAT activity was noticed in seedling of *V. radiata* (Fig. 4c). APX activity in *C. tetragonoloba* was found to be increased significantly ($P \leq 0.05$) at 70 mM NaCl i.e. 65.38%, 44.29% and 54.18% higher than control in leaves, shoots and roots respectively (Fig. 3d). Whereas APX activity in *V. radiata* seedlings was mainly recorded in roots (Fig. 4d). APX activity was increased at initial concentration of NaCl (20 mM = 1.21 folds than control) and then considerably decreased with further increase in concentration (Fig. 4d).

according to Duncan Multiple Range Test ($P < 0.05$). Data points marked with the similar letters show insignificant differences ($P < 0.05$) within treatments

Nitrate reductase activity was greater in roots in comparison to leaves and shoots in both the seedlings. In roots NR activity was significantly increased up to 50 mM NaCl in *C. tetragonoloba*. At 50 mM NaCl 94.03% and 134.67% increase in NR activity were recorded in shoots and roots respectively (Fig. 5a). In contrast to NR, HO1 activity was mainly observed in leaves which progressively increased with elevated NaCl concentration. The highest HO1 activity was recorded at 50 mM NaCl which was 1.87 times higher than control (Fig. 5b). While in *V. radiata*, NR activity was increased with initial salt concentration (20 mM) thereafter decreased with further increase in NaCl concentration (40 mM). Maximum NR activity was recorded at 60 mM i.e. 2.76 folds with respect to control in roots and subsequently decreased with higher concentration (Fig. 5c). Similarly HO1 activity increased with increase in NaCl concentration and the highest activity was recorded at 20 mM (1.94 times) and 60 mM (1.5 times) NaCl than that of control in leaves (Fig. 5d).

A preliminary research on NR with SNP (nitric oxide donor) at different concentrations 0.1, 0.5, 1, 5, 10 and 20 mM was conducted with both the legumes. At 5 mM

Fig. 4 SOD (a), GPX (b), CAT (c) and APX (d) activity in *V. radiata* seedlings treated with varying concentrations of NaCl. Values are mean $\pm$ SE ($n = 3$) and are statistically significant according to Duncan Multiple Range Test ($P < 0.05$)

SNP treatment a significant increase in NR activity was recorded which were 2.57 folds and 3.13 folds higher than control in roots of *C. tetragonoloba* and *V. radiata* respectively (Fig. 6a). To estimate the cytoprotective role of NO in salt induced oxidative stress, the seedlings of both the legumes were treated with two set of concentrations 50 mM NaCl, 50 mM NaCl + 5 mM SNP (*C. tetragonoloba*); 60 mM NaCl, 60 mM NaCl + 5 mM SNP (*V. radiata*) (Fig. 6b). The 50 mM NaCl (*C. tetragonoloba*) and 60 mM NaCl (*V. radiata*) concentrations were favored because the maximum physiological changes were noticed at these concentrations. The seedlings with combined treatment (NaCl + SNP) show maximum NR activity (5.64 and 6.5 folds higher in *C. tetragonoloba* and *V. radiata* than control) than individual treatment of NaCl and SNP (Fig. 6b).

Discussion

The improvement in agronomic production and quality of legume crops of arid and semi-arid region of Indian Thar Desert and their tolerance towards salinity stress is a matter

of concern. HO1 and NO are the important and novel molecule but their role as an antioxidant are less explored in the plant against salinity stress. Thus the current study is primarily focused on the physiological importance of antioxidants in alleviation of NaCl mediated oxidative stress. The present work is beneficial as it signifies the relation of the antioxidants with other parameters including growth and stress parameters that is helpful in understanding the sensitivity and tolerance level of NaCl in two cultivars which will be of great importance in producing salt tolerant varieties in near future.

C. tetragonoloba and *V. radiata* seedlings primarily responds to NaCl toxicity by affecting its growth in terms of fresh weight, dry weight, root length and shoot length. In the present investigation the adverse effect on overall morphology with varying NaCl concentration was higher in *V. radiata* than *C. tetragonoloba* seedlings. The study was supported by the previous work conducted on different plants treated with salinity stress (Liu et al. 2017; Wang et al. 2017; Verma et al. 2015). The reduction in overall plant growth might be due to the decline in photosynthetic pigment and water uptake. Leaf water content is an effective indicator of plant tolerance towards salinity stress

Fig. 5 Nitrate Reductase and Heme oxygenase1 in *C. tetragonoloba* (a, b) and *V. radiata* (c, d) at varying NaCl concentration. Values are mean $\pm$ SE (n = 3) and are statistically significant according to

DMRT test ($P < 0.05$). Data points marked with the different letters show significant differences ($P < 0.05$) within treatments

as leaf water reduction frequently occurs under saline condition. The decrease in LWC in *V. radiata* seedlings signifies the greater degree of salt tolerance than *C. tetragonoloba*. This decrease in LWC might be due to the turgor pressure failure which results in limited accessibility of water for cell expansion processes (Katerji et al. 1997). Increased protein content was recorded at sublethal concentration (50 mM NaCl in *C. tetragonoloba* and 60 mM NaCl in *V. radiata*) in both the legumes, which is probably due to the production of stress proteins at these concentrations (Sanita di Toppi and Gabbrielli 1999).

MDA content is an index of membrane destruction under stress. Higher MDA level at 100 mM NaCl in *V. radiata* indicates greater ROS production than *C. tetragonoloba* (Fig. 2). The findings were supported by the work conducted on halophyte *Centaurea tuzgoluensis* and *Eruca sativa* (Yildiztugay et al. 2011; Mahawar and Shekhawat 2019). Higher H_2O_2 accumulation was recorded in both the crops at elevated concentration of NaCl. But a

sudden decrease was noticed at sub-lethal concentration (50 mM NaCl in *C. tetragonoloba* and 60 mM NaCl in *V. radiata*) which might be due to the increased activity of antioxidants at these concentrations that quenches H_2O_2 . The similar pattern of result was recorded in *B. juncea* treated with sodium chloride at varying concentrations for a period of five days (Verma et al. 2015).

To rectify the damaging effect of NaCl, plant accumulates high level of compatible osmolyte (proline) in cytosol and other cell organelles (Abdel Latef and Miransari 2014) which is positively correlated with the increased tolerance towards stress. Higher proline accretion in *V. radiata* indicates the enhanced salt tolerance than *C. tetragonoloba*. The study was in accordance with the previous research conducted on different crop plants (Mahawar et al. 2018b; Lu et al. 2017; Shankar et al. 2016; Tripathi et al. 2013; Verma et al. 2008) (Fig. 1).

C. tetragonoloba and *V. radiata* treated with NaCl at different concentrations stimulates different antioxidant

Fig. 6 Nitrate reductase activity under different SNP treatments (0.1, 0.5, 1, 5, 10, 20 mM) in seedlings of *C. tetragonoloba* and *V. radiata* (a) and NR activity under different treatments in *C. tetragonoloba* (50 mM NaCl, 5 mM SNP, 50 mM NaCl + 5 mM SNP) and *V. radiata* (60 mM NaCl, 5 mM SNP, 60 mM NaCl + 5 mM SNP).

enzymes to overcome the deleterious effect of ROS produced under stress condition. SOD is the first metalloenzyme in the detoxification process which deactivates the superoxide radicals into H_2O_2 and O_2 . Increase in SOD activity with increased NaCl concentration in both crops (Fig. 3, Fig. 4) probably indicates the enhanced ROS level, which results in the increase expression of gene encoding SOD (Shekhawat et al. 2010). H_2O_2 formed through SOD catalyzed reaction was further reduce to H_2O via other antioxidant enzymes APX and CAT. In our study, APX activity considerably increased with increase in NaCl concentration and decreased at higher treatment in both crops (Fig. 3, Fig. 4). While no noticeable effect of NaCl on CAT activity was recorded in both legumes. The inactivation in CAT activity is possibly due to the photoinactivation of enzyme or may be correlated with destruction caused by peroxisomal proteases (Sandalio et al. 2001). Disparate activity of two H_2O_2 neutralizing enzymes viz APX and CAT were observed probably because both enzymes are functioning on same substrate hydrogen peroxide that display greater activity with APX than CAT. Excluding CAT and APX, Guaiacol peroxidase (GPX) is the other H_2O_2 neutralizing enzyme. The reduction in stress parameters (MDA and H_2O_2) is well associated with improved activity of GPX at sub-lethal concentrations in the legumes. However, at higher salt concentration decrease in GPX activity was directly related with increase in MDA and H_2O_2 content (Maksymiec and Krupa 2006; Mahawar and Shekhawat 2019). In the present study, a considerable increase in NR activity was mainly recorded in crop roots subjected to combined treatment of SNP (NO donor) + NaCl than NaCl alone treatment. SNP positively

Values are mean $\pm$ SE ($n = 3$) and are statistically significant according to DMRT test ($P < 0.05$). Data points marked with the different letters show significant differences ($P < 0.05$) within treatments

affect NR activity which was decreased by NaCl alone treatment. The study was in agreement with other reports on *Ocimum tenuiflorum* reveals that reduction in NR activity was observed under Cr toxicity (Rai et al. 2004).

Our report indicates that both crops produce enhanced level of HO1 in leaves at sub-lethal NaCl concentration (50 mM in *C. tetragonoloba* and 60 mM in *V. radiata*). The study was supported by numerous previous reports that demonstrated the protective role of heme oxygenase 1 against abiotic stress which is strongly related with the HO1 mediated reduction of reactive oxygen species in plants (Mahawar et al. 2018a; Xie et al. 2015; Noriega et al. 2012; Yannarelli et al. 2006). High dosage of NaCl (100 mM) reduces HO1 formation probably due to the destruction of cell metabolism. Attenuation in HO1 activity in leaves at higher salt concentration was supported by the earlier report on *Glycine max* treated with salinity stress (Zilli et al. 2009).

The present study signifies that seedlings of crops treated with sub-lethal salt concentration (50 mM in *C. tetragonoloba* and 60 mM in *V. radiata*) exhibits noticeable changes in antioxidant activity with respect to control. In *C. tetragonoloba*, the antioxidant molecule as well as enzymes including HO1 and NR play equal role in combating stress which leads to the plant survival. These findings were supported by Kauser et al. (2013) in wheat and by Hayat et al. (2012) and Manai et al. (2014) in tomato. However in *V. radiata*, scavenging potential of ROS and inhibition of membrane destruction was greater at sub-lethal concentration (60 mM) in comparison to initial salt concentration (20 mM). Higher activity of antioxidants at 60 mM suggests that various antioxidants at this

Fig. 7 Protective role of antioxidants at sublethal salt concentrations (50 mM in *C. tetragonoloba* and 60 mM in *V. radiata*) to overcome the detrimental effects of NaCl in *C. tetragonoloba* (a) and *V. radiata* (b) with respect to control. Linear correlation among the growth

parameters (c) and stress parameters (d) with antioxidants in seedlings of *V. radiata*. The mean data ($n = 3$) of the measured values under NaCl stress were used for correlation analysis. The correlation values are significant at 0.01 level

concentration work in an assembly for the plant's survival in which the major role was played by proline (Mahawar et al. 2018b; Dixit et al. 2014).

In order to devise new strategies for crop development and improved tolerance, it is important to understand the basic principle of antioxidants in providing cellular protection. The present work signifies that NaCl is the cause of oxidative stress in chosen crops by ensuing overall inhibition in physiological process. The stimulatory action of antioxidants overcomes the negative effect of salinity stress via reducing ROS level. However 50 mM NaCl concentration in *C. tetragonoloba* and 60 mM NaCl concentration in *V. radiata* supports the growth of legume crops. At this concentration highest antioxidants activity including HO, NR, SOD, CAT, APX, GPX which leads to a decrease in the level of MDA and H₂O₂. The present study suggested that elementary response of plant by antioxidants is most prevalent mechanism to the variety of stress responsive pathways. Antioxidants (HO, NR, SOD and proline) play a

central role in salt tolerance by regulating upregulation and downregulation of other antioxidants. *V. radiata* shows greater level of oxidation in terms of MDA as well as tolerance by accumulating higher amount of proline (osmoprotectant) than *C. tetragonoloba* which is further confirmed through linear correlation between antioxidants and growth parameters in *V. radiata*. Differential responses in the antioxidant activity in two legumes demonstrated that *V. radiata* is more reactive towards salinity stress than *C. tetragonoloba* (Fig. 7). The study is beneficial as it has demonstrated the sensitivity and tolerance level of NaCl in two important crops of Indian Thar Desert.

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