Indian J. Plant Physiol., Vol. 15, No. 3, (N.S.) pp. 203-212 (July-Sept., 2010)

ETHYLENE EVOLUTION AND MODIFICATION OF ANTIOXIDANT DEFENSE MECHANISM AS INDICES OF SALINITY STRESS TOLERANCE IN *CICER ARIETINUM L.* NODULES

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Received on 12th Feb., 2010, Revised on 26th July, 2010

SUMMARY

Salinity induced changes in the ethylene evolution, antioxidant activity, and membrane integrity in relation to water and mineral status in indeterminate type of chickpea (*Cicer arietinum* L.) nodules in cv. CSG-8962, (National check for salinity tolerance) were studied under natural conditions of screen house. At flowering stage (80-90 DAS) plants were exposed to single saline irrigation (Cl^--dominated) of levels 2.5, 5.0 and 10.0 dSm^{-1} and sampled after 3 d. The control plants were irrigated with canal water. The other set of treated plants were revived after desalinization and the plants were sampled after further 3 d. Water potential (Ψw) of leaf and osmotic potential (Ψs) of leaf and nodules significantly decreased from -0.77 to -0.93 MPa and from -0.86 to -1.35 MPa and from -0.94 to -1.75 MPa, respectively upon salinization. Relative water content (RWC %) of leaf and nodules also reduced from 82.55% to 75.60% and 95.75% to 85.35%, respectively. The decline in (Ψs) of nodules was due to accumulation of proline and total soluble sugars. In comparison to control, the increase in ethylene (C_2H_4) production was 33% to 82% higher and correspondingly increases in 1-aminocyclopropane-1-carboxylic acid (ACC) content (50-162%) and ACC oxidase activity (46-167%) was also noticed. Similarly, 1.42 to 3.08 fold and 1.08 to 1.61 fold increase in H_2O_2 and thiobarbituric acid reactive substances (TBRAS) contents was also observed, respectively. N content of nodules declined after saline irrigation. The induction in specific activity of antioxidant enzymes was confirmed by the increase in specific activity of superoxide dismutase (11-133%), catalase (9-109%), peroxidase (50-227%), ascorbate peroxidase (17-87%), glutathione reductase (69-288%) and glutathione transferase (8-66%). The induced antioxidant enzymes activity was not sufficient to scavenge the oxidative damage of nodules as it is clear from the accumulation of H_2O_2 in nodules. Ascorbic acid (AA) content also declined from 13.24 to 54.50%, whereas Na^+/K^+ ratio and Cl^- content were significanty increased. All the metabolic changes were also correlated to the osmotic status of the nodules. Upon revival, a partial recovery in all above metabolic processes and water relation parameters were noticed. It is concluded that under the cumulative effect of salinity and reduced water status, ethylene, lipid peroxidation and H_2O_2 are playing a key role in the functioning of chickpea nodules.

Keywords: **Antioxidant enzyme, chickpea, ethylene, lipid peroxidation, minerals, proline, water relations**

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INTRODUCTION

Salinity (both soil and water) is one of the major abiotic stresses responsible for deterioration of soil and making it unfit for agriculture. Its deleterious effects, on plant involve osmotic stress, ion toxicity and mineral deficiency (Munns 2002, Flowers 2004, Ashraf *et al*. 2008) and reduction in growth and alterations in several physiological processes (Flowers and Yeo 1986, Nandwal *et al*. 2000, Sharma 1996, Zhu 2001, Flowers 2004, Kukreja *et al*. 2005, Nafazi *et al.* 2007, Tawfic 2008, Grewal 2010) including N_2- fixation (Nandwal *et al*. 2000) etc. As a result of these primary effects secondary stresses such as oxidative damage often occurs (Bartels 2001, Becana *et al*. 2000, Sairam *et al*. 2002, Kukreja *et al*. 2005) under salinity.

Legumes root nodules are especially at risk from oxidative damage by reactive oxygen species (ROS) because they contain an abundance of oxygen labile protein such as leghemoglobin (LHb) and Fe potentially available for catalyzing free radical production (Becana *et al.* 1998). Gogorcena *et al*. (1995) reported that H_2O_2 and O^-_2 radical can be generated by oxidation of nitrogenase, and by autoxidation of oxygenated LHb. In addition to enzymes, superoxide dismutase (SOD) catalase (CAT) and peroxidase (POX), a major antioxidant mechanism operating in nodule cytosol is the ascorbate-glutathione cycle which results ultimately in detoxification of H_2O_2 at the expense of NAD(P)H (Becana *et al*. 2000).

Lipid peroxidation which leads to impairment of membrane is the system most easily ascribed to oxidative damage and also most frequently measured (Sairam *et al*. 2002). In plant organs, secondary effects of salinity like water deficiency/dehydration are equally important in addition to Na^+ toxicity (Munns 2002), the former has been considered as one of the most serious consequences of salinity that results in membrane injury, and hence determination of water relations is therefore also critical for any study of plant under this condition.

The induction of senescence has also been correlated with augmentation in ethylene evolution under various environmental stress conditions (Abeles *et al.* 1992, John 1997). Chickpea is an important crop in semi-arid and arid regions of the world where irrigation is an essential aid to survival of agriculture. The identification of direct causes and primary and secondary responses of inhibition of nodule functioning requires precise measurement of the ethylene-correlated processes under salinity. Hence the present investigations on an indeterminate type of nodules were confined to antioxidant defense system, ethylene evolution and membrane integrity in relation to changes in plant water and mineral status under single saline irrigation of three ECe levels and subsequently on their revival.

MATERIALS AND METHODS

Growth conditions

Chickpea (*Cicer arietinum* L.) cv. CSG-8962, (National check for salinity tolerant) was raised in earthen pots (30 cm dia) filled with 5.5 kg of dune sand (*Typic torrispamments*) under natural condition of screen house, in the Department of Botany and Plant Physiology, CCS Haryana Agricultural University, Hisar-125 004, India. The seeds before sowing were surface sterilized and inoculated with effective *Rhizobium* culture (Ca 181). The crop was supplied with an equal quantity of nitrogen free nutrient solution at regular interval of 15 d. After thinning two plants were retained in each pot. The chloride (Cl^-) dominated salinity was prepared by using a mixture of different salts such as NaCl, $MgCl_2$, $MgSO_4$ and $CaCl_2$ where Na:Ca+Mg was in the ratio of 1:1 and Ca:Mg in the ratio of 1:3, the Cl:SO_4 ratio was 7:3 on a meq basis. At flowering stage i.e. 80-90 days after sowing, the desired salinity was applied to saturate each pot so as to maintain four levels i.e. control, 2.5, 5.0 and 10.0 dSm^{-1} of Cl^-- dominated salinity. The sampling was done at 3 days after treatment. Half of the treated plants were revived after desalinization and were sampled after 3 days to see their revival.

Water potential

The third fully expanded leaf from the top was used to measure water potential (Ψw) with the help of a Pressure Chamber (Model-3005, Soil Moisture Equipment Corporation, USA) and was expressed in '-M Pa'.

Osmotic potential

The osmotic potential (Ψs) of leaf of the same position and nodules was determined separately using psychrometric technique with a Vapour Pressure Osmometer (Model-5100, Wescor, Logan, USA) and was expressed in '-M Pa'.

Relative water content

The relative water content (RWC %) of leaf and nodules was calculated as described by Weatherley (1950). The calculations on dry matter (d.m.) were done on the basis of conversion factor from fresh weight to dry matter under different stress levels. These measurements were made between 10.00 and 12.00 h (local time) during a sunny day having the mean temperature of 20± 2 °C.

The proline content of nodules was estimated by the methods of Trotel *et al.* (1996). The total soluble sugars (TSS) of nodules were determined with the method of Dubois et al. (1956). Ascorbic acid (AA) content of nodules was measured by using the method of Schopfer (1966).

1-Aminocyclopropane-1-carboxylic acid (ACC) content

Free 1-Aminocyclopropane-1-carboxylic acid (ACC) content of nodules was assayed following the method of Miller and Pengelly (1984). One gram of fresh nodules was ground in 2 ml of 5% (w/v) 5-sulfosalicylic acid with a mortar and pestle and the extract was centrifuged at 30,000 x g for 30 min at 4°C. Then 0.4 ml of supernatant and 0.2 ml of 50 mM $HgCl_2$ was added to 0.6 ml of 5% (w/v) 5-sulfosalicylic acid in 15 cm^3 reaction vials. The vials were made airtight with subaseal and 0.1 ml of 2.6% NaOCl in 5 N NaOH was injected into the vials. The vials were then vortexed for 5 s and incubated in ice for 50 min. The ethylene produced was estimated by injected 2 ml of gas sample in to a steel column (2m x2m) filled in a gas chromatograph (Nucon, 5700) using a flame ionization detector (FID). Standard ethylene (110 vpm) was used for quantification of data. The ACC content was calculated in terms of n moles (C_2H_4) g^{-1} (d.m.) min^{-1} x 10^{-3}.

ACC Oxidase

The activity of ACC oxidase was measured by the method described by Fearn and La Rue (1991). One gram of fresh nodules was incubated in 15 cm^3 reaction vials containing 2 ml of 20 mM ACC. The vials were made airtight with subaseal and kept in dark for 4 h at 25°C. Then 2 ml of gaseous sample was taken from each vial and analysed for ethylene using Gas Chromatograph (Nucon, 5700). The activity of ACC oxidase was calculated as n moles (C_2H_4) g^{-1} (d.m.) min^{-1} x 10^{-2}.

Ethylene evolution

The fresh nodules after detaching from roots were placed within 50 ml reaction vials containing wet cotton pad. The vials were made airtight with subaseal and kept in dark for 20 min at 25°C as described by Fearn and La Rue (1991). Then 2 ml of gas sample was taken from each vial and assayed for ethylene production on Gas Chromatograph (Nucon 5700). The dry matter (d.m.) of each sample was recorded and the amount of ethylene evolved was calculated as n moles (C_2H_4) g^{-1} (d.m.) min^{-1} x 10^{-2}.

Extraction of protein for enzymes assay

One g of fresh nodules were washed in chilled distilled water and homogenized with a chilled pestle and mortar in 5ml of extraction buffer (0.1M phosphate buffer, pH 7.0), containing 10 mM KCl, 1 mM $MgCl_2$ and 10 mM EDTA and centrifuged at 10,000 x g at 4 °C for 20 min. The supernatant was used for the following enzymes assay. The enzymatic protein was determined by the method of Lowry et al. (1951).

Enzyme Assay: Catalase (CAT: EC 1.11.1.6) activity was estimated by the UV method of Aebi (1983). The enzyme activity was calculated as µmol (H_2O_2 decomposed) mg^{-1} (protein) min^{-1} by using the H_2O_2 extinction coefficient 36 μM^{-1} cm^{-1}.

Peroxidase (POX: EC 1.11.1.7) activity was assayed by using guaicol as substrate. The enzyme activity was expressed as Units mg^{-1} (protein) min^{-1} x 10^4 by using extinction coefficient 6.39 μM^{-1} cm^{-1}.

Glutathione reductase (GR: EC 1.11.1.9) activity was estimated by the method of Goldberg and Spooner (1983). The enzyme activity was calculated as Units mg^{-1} protein min^{-1} using the molar extinction coefficient of NADH 6.23 μM^{-1} cm^{-1}.

Glutathione transferase (GTase: EC 2.5.1.18) activity was measured by thiolysis method of Habig and Jakoby (1981) by using the extinction coefficient for *p*-nitrophenol at pH 7.0 was taken as 8.79 mM^{-1} cm^{-1}. The enzyme activity was calculated as µmol (*p*-nitrophenol) mg^{-1} (protein) min^{-1}.

Ascorbate peroxidase (ASC-POX: EC 1.11.1.11) activity was measured by the method of Nakano and Asada (1981). Under the assay conditions a decrease in 0.01 absorbance corresponds to 3.6 mmol ascorbate oxidised. The enzyme activity was calculated as µmol (ascrobate decomposed) min^{-1}.

The activity of superoxide dismutase (SOD: EC 1.15.1.1) was estimated by the method of Giannopolitis and Ries (1977). The enzyme activity was calculated as Unit mg^{-1} (protein) min^{-1}.

Hydrogen peroxide (H_2O_2) content of nodules was determined by a modified Patterson *et al*. (1984). H_2O_2 content was calculated using its molar extinction coefficient of 36 mM^{-1} cm^{-1} and calculated as mol g^{-1} d.m.

The level of lipid peroxidation in nodules was measured in terms of thiobarbituric acid reactive substances (TBARS) contents (Heath and Packer 1968). The TBARS content was calculated using its extinction coefficient of 155 $mM^{-1}cm^{-1}$ as µmol g^{-1} d.m. The sodium (Na^+), potassium (K^+) and chloride (Cl^-) contents of nodules were determined from oven dried ground material. 50 mg material was digested in 5 ml of a diacid mixture of H_2SO_4 and $HClO_4$ (9:1) and diluted to the desired volume. Na^+ and K^+ contents were estimated using the Flame Photometer (Elico, India) and further expressed on Na^+/K^+ ratio. The Cl^- content in the digested material was determined by EIL mV meter from Caltex instruments (Model CM 2400A, UK) using Calomel Chloride electrode and expressed as mmol g^{-1} d.m. The total nitrogen (N) was estimated by the Micro Kjeldahl technique using Kjeltac Auto 1030 analyzer (Sweden) and expressed as mg g^{-1} d.m. of nodules.

Statistical analysis: Three replicates consist of three pots and each pot containing two plants was used for each observation under each treatment. The data were analyzed statistically using completly randomized design and the significance was tested at 5% level of critical difference using the table 'ANOVA'.

RESULTS AND DISCUSSION

Water relations: With raising the level of saline irrigation from 2.5 to 10 dSm^{-1} a significant decrease in water potential (Ψw) of leaf from –0.77 to –0.93 M Pa was noticed. Similarly, osmotic potential (Ψs) of leaf and nodules also declined from –0.86 to –1.35 M Pa and from –0.94 to –1.75 MPa, respectively (Table 1). The decrease observed in relation to RWC of leaf and nodules was from 82.53% to 75.60% and from 95.75% to 85.35%, respectively (Table 1). The cumulative effects of reduced osmotic potential of soil solution, ion toxicity in soil/nodules and ionic imbalance (increased in Cl^- content and Na^+: K^+ ratio) within plant system under increased salinity contributed to reduce Ψw of leaf and RWC (%) and Ψs of leaf and nodules. Grewal (2010) also reported similar results in the leaves of various crops under salinity. Substantial variations in Ψs and RWC of leaf and nodules were seen. Nodules showed more negative value of Ψs but higher RWC than leaf. The prepared mechanism for decreasing Ψs is that plants adjust under stress conditions to maintain turgor (Nandwal *et al*. 2000, Sairam *et al*. 2002). The proline content of nodules increased from 1.13 to 4.8 fold whereas TSS moved from 9 to 38% under salinity in comparison to control (Table 1). The low levels of RWC and Ψs of nodules and leaves were apparently adjusted by accumulating sugars and proline which are known for their osmotic influences in plants (Nandwal *et al*. 2000, Sairam *et al*. 2002, Tawfik 2008). After revival, proline and TSS sharply decreased due to their utilization. Ψw of leaf and Ψs of leaf nodules became less negative due to reduced level of these metabolites. These effects were also facilitated by the simultaneous increase in RWC of leaf and nodules.

Table 1. Changes in Ψw, Ψs and RWC of leaf and nodules and TSS and proline content of nodules in chickpea upon salinization and during revival.

Parameters	C	2.5 dSm^{-1}	Revival	5.0 dSm^{-1}	Revival	10.0 dSm^{-1}	Revival	CD at 5%
Ψw leaf [-MPa]	0.77	0.83	0.76	0.89	0.80	0.93	0.87	0.04
Ψs leaf [-MPa]	0.86	0.97	0.86	1.25	0.97	1.35	1.11	0.05
RWC leaf [%]	82.53	81.55	82.95	77.15	80.05	75.60	79.3	2.45
Ψs nodules [-MPa]	0.94	1.05	0.92	1.22	1.00	1.75	1.20	0.06
RWC nodules [%]	95.75	93.35	95.10	88.75	91.75	85.35	0.55	2.19
TSS nodules [mg g^{-1} (d.m.)]	26.20	28.07	23.75	29.20	23.01	36.08	30.69	2.11
Proline nodules [mg g^{-1} (d.m.)]	0.61	0.69	0.64	1.41	0.86	2.92	0.90	0.06

Ethylene production: Salinity stressed nodules showed over production of ACC from 50% to 147% over control (Fig. 1). Similarly, activity of ACC oxidase increased from 41% to 147%. Upon revival these values declined but to a small extent. The increase in ACC content and ACC oxidase activity led to more production of ethylene (C_2H_4) under said conditions. Hence, on exposure to saline irrigation, chickpea nodules showed 13–82% increase in ethylene evolution in comparison to control (Fig. 1). A positive correlation between the levels of salinity and the amount of ACC content, ACC oxidase activity and ethylene production in chickpea nodules was noticed. The complex relationship between stress and ethylene like symptoms is termed as the stress ethylene syndrome (Morgan and Drew 1997). It is now accepted that stress induced enhancement of ethylene synthesis is due to stress promoted synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC) (John 1997). In addition, ACC oxidase, S-adenosyl methionine (Ado-Met) synthetase and the enzymes that conjugate ACC are regulated by stress. Soon after ACC was identified as the immediate precursor of ethylene, it was prepared that variability in ethylene synthesis rates results from changes the activity of ACC synthase and that ethylene forming enzyme (ACC oxidase) was constitutive. Another possibility is that "stress ethylene" is formed as a result of non-enzymatic conversion of ACC to ethylene which is mediated by active oxygen. The active oxygen could be formed by lipoxygenase-catalysed lipid peroxidation. Similar observations were reported in this investigation. It is also proposed that

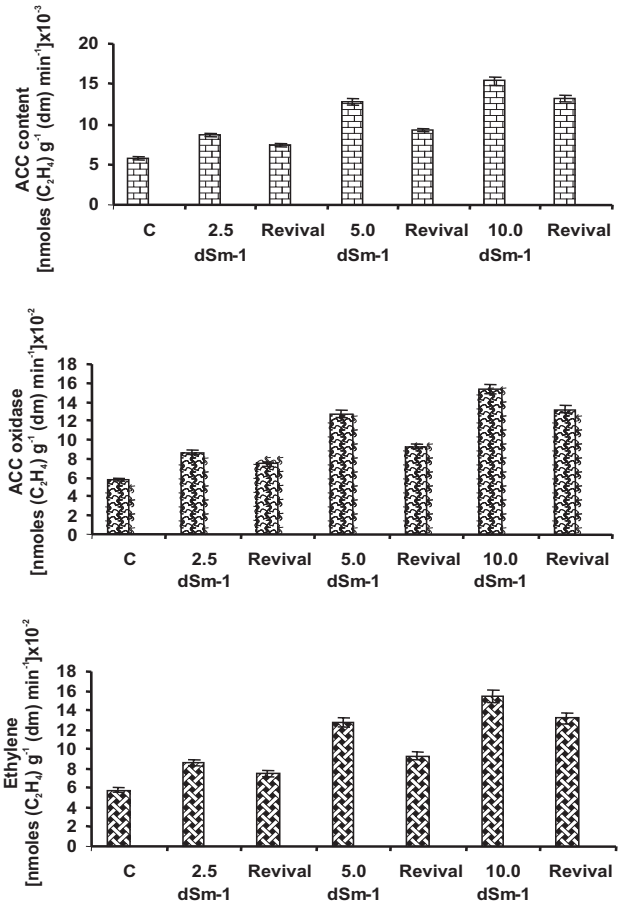

Fig. 1. ACC content, ACC oxidase activity and ethylene evolution in chickpea nodules as affected by 2.5, 5.0 and 10.0 dSm^{-1} levels of salinity and during their revival. C = Control. Vertical Bars indicate ± SE mean values. CD at 5% level = 2.52, 0.64 and 1.36, respectively.

stress induced changes in membrane permeability may activate the enzyme ACC oxidase through increased substrate and /or cofactors availability (Kacperska and Kubacka-Zebalska 1993). Our results are also in agreement of above reports. In the present investigation, it is clear that salinity stress promoted ethylene evolution in chickpea nodules through the disturbance in water status as a result of decrease in Ψs and RWC of nodules in addition to increase in ACC content and ACC oxidase activity. Upon revival, decrease in the level of C_2H_4, ACC content and ACC oxidase activity was noticed along with the increase in Ψs and RWC of nodules, suggesting that ethylene plays a very important role in chickpea nodules metabolism under salinity.

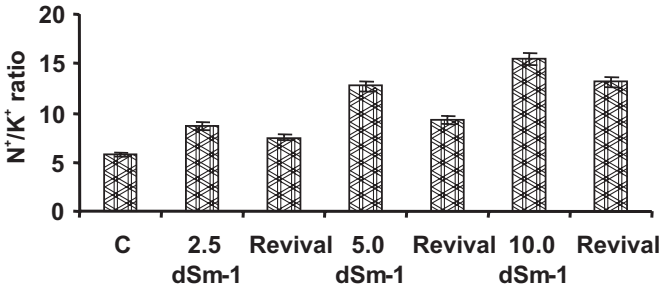

Fig. 2 Sodium and Potassium ratio in chickpea nodules as affected by 2.5, 5.0 and 10.0 dSm^{-1} levels of salinity and during their revival. C = Control. Vertical Bars indicate ± SE mean values. CD at 5% level = 4.48.

Minerals: Analysis of ion accumulation revealed that Na^+/K^+ ratio and Cl^- content were greatly increased in nodules with the rise of saline irrigation level (Fig. 3). About 2.5 times increase in Na^+/K^+ ratio was noticed when a drastic reduction in Ψs and RWC of nodules occurred under the influence of various salinity levels. High Na^+/K^+ ratio in nodules under salinity indicates the existence of competition effects between Na^+ and K^+ ions which most likely share the same transport system at root surface (Ashraf *et al.* 2008). Thus, the cumulative effects of salt and reduction in Ψs and RWC of nodules caused maximum membrane injury, confirming the findings as indicated in terms of TBARS content (Table 2). The changes in Na^+/K^+ ratio also reflected in the changes in Cl^- content (Fig. 3) of nodules. Hence, the Cl^- content increased from 2.33 to 4.72 mmol. Dadkhah and Grrifithis (2006) reported similar changes in sugarbeet. The high Cl^- content in current study

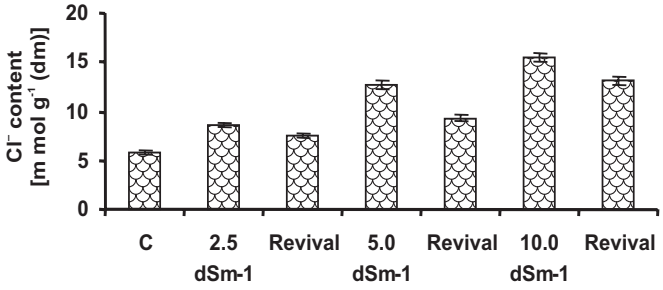

Fig. 3. Chloride content in chickpea nodules as affected by 2.5, 5.0 and 10.0 dSm^{-1} levels of salinity and during their revival. C = Control. Vertical Bars indicate ± SE mean values. CD at 5% level = 0.15.

probably caused Cl^- toxicity to plant roots affecting greater absorption and transportation of Cl^- to roots (Grewal, 2010) and ultimately leading to elevated of Cl^- in nodules. Upon revival, the ratio of Na^+/K^+ and Cl^- content were decreased, however, the values were still higher than control. The N content of nodules declined from 18.35% to 48.88% as the salinity level increased from 2.5 to 10 dSm^{-1} (Fig. 4). Upon revival the N content of nodules increased but the values were still lower than the control. The decrease in N_2-ase activity and leghemeglobin content (Nandwal *et al*. 2000) and an increase in the permeability of nodules under salinity were the reasons for decrease in N content (Fig. 4) of nodules in chickpea.

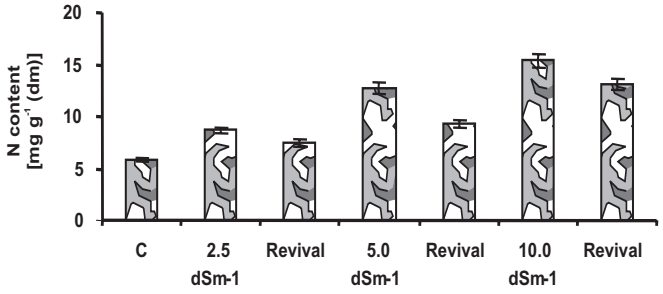

Fig. 4. Nitrogen content in chickpea nodules as affected by 2.5, 5.0 and 10.0 dSm^{-1} levels of salinity and during their revival. C = Control. Vertical Bars indicate ± SE mean values. CD at 5% level = 1.51.

Antioxidant defenses: In the present investigation, attempts were also made to establish a correlation between antioxidative defense system and salinity induced physiological changes in chickpea nodules. Upon salinization, the defense mechanism was activated which

was apparent from increased specific activity of various antioxidant enzymes. The specific activity of SOD increased from 10% to 133% under salinity, whereas for of CAT, it increased from 9% to 109% (Table 2). The specific activity of ASC-POX and GTase was increased from 17% to 87% and 8% to 66%, respectively. The increase in the activity of POX and GR was from 50% to 227% and 69% to 288%, respectively (Table 2). Thus, the effect of saline irrigation on the antioxidant defense mechanism may be characterized by its activation in chickpea nodules. However, activation of these enzymes could not overcome the accumulation of H_2O_2. Upon revival a partial reversibility was observed in the activity of these enzymes. Beneficial effect of higher osmolyte concentration i.e. total soluble sugars and proline was affected in maintenance of higher RWC in nodules and stabilization of essential protective enzymes proteins, resulted in their higher activity under salinity stress than control. However, this induced antioxidant enzymes activity was not sufficient to scavenge the oxidative damage of nodules.

Salinity can induce oxidative damage to the plant by the enhanced production of reactive oxygen species (ROS), such as superoxide ($O_2^{\cdot\cdot}$), hydrogen peroxide (H_2O_2), singlet oxygen and hydroxyl radical. The accumulation of harmful ROS depends on an imbalance between the rates of production and elimination through several biochemical reactions (Cavalcanti *et al.* 2007). These ROS are extremely cytotoxic. As a consequence, a series of cellular degenerative processes are triggered, including peroxidation of membrane lipids and programmed cell death (Cheeseman 2007). H_2O_2 is most stable ROS and most of the H_2O_2 present in the plant

Table 2. The specific activity of antioxidant enzymes and AA, H_2O_2 and TBARS contents in chickpea nodules as affected by salinization and during revival.

Parameters	C	2.5 dSm^{-1}	Revival	5.0 dSm^{-1}	Revival	10.0 dSm^{-1}	Revival	CD at 5%
Superoxide dismutase (Units mg^{-1} protein min^{-1})	3.20	3.55	3.23	5.26	4.58	7.45	5.17	0.43
Catalase (µmol H_2O_2 decomposed mg^{-1} protein min^{-1})	32.78	35.6	25.68	46.77	32.15	68.49	50.15	4.57
Peroxidase (Units mg^{-1} protein min^{-1}) x 10^4	2.26	3.30	2.66	5.26	3.56	7.38	4.12	0.32
Ascorbate peroxidase (µmol ascorbate decomposed min^{-1})	25.6	30.0	23.38	66.44	41.49	47.97	33.12	5.02
Glutathione reductase (Units mg^{-1} protein min^{-1})	33.9	57.1	40.07	84.96	55.55	131.37	89.13	8.08
Glutathione transferase (µmol p-nitrophenol mg^{-1} protein min^{-1})	2.35	2.38	2.33	3.62	2.51	3.91	3.11	0.48
AA content (mg g^{-1} d.m.)	2.33	2.02	3.25	1.70	2.12	1.06	1.54	0.06
H_2O_2 [(mol g^{-1} d.m.) x 10^{-4}]	76.62	109	68.20	176.40	127.97	236.18	177.78	6.33
TBARS content (µmol g^{-1} d.m.)	2.97	3.21	2.50	3.96	3.15	4.78	3.68	0.09

cell is produced by the action of SOD. The enhanced SOD activity led to the accumulation of H_2O_2 from 1.42 to 3.08 fold under salinity (Table 2). H_2O_2 is also known to induce many other non-enzymatic and enzymatic processes in plants. Upon re-irrigation of stressed plants, the H_2O_2 content in nodules declined with simultaneous decrease in the activity of SOD and POX. H_2O_2 is a relatively stable metabolite that may act as a second messenger, since it could diffuse from the site of production and is known to induce several other genes and proteins involved in stress defenses like CAT (Prasad *et al*. 1994a), POX (Prasad *et al*. 1994b) and ASC-POX (Morita *et al*. 1999).

In nodules, the AA content decreased from 13.24% to 54.50% upon salinization (Table 2) in comparison to control. The failure to maintain AA content under said conditions might be an indication of oxidative damage of nodules. AA participate in the removal of H_2O_2 as a substrate of ASC-POX enzyme and directly reduces H_2O_2 and regenerate reduced tocopherol (Foyer 1993), thus provide protection to the integrity of cellular membrane along with α-tocopherol (Menconi *et al*. 1995). The decrease in AA content could be due to the rapid oxidation or its slow rate of synthesis (Gogorcena *et al*. 1995). A complete recovery in AA content of nodules was not seen upon desalinization of the plants.

Lipid peroxidation: The lipid peroxidation (in terms of TBARS) in nodules increased significantly from 1.08 to 1.61 fold over control upon salinization (Table 2). A possible cause of increased TBARS under salinity was the accumulation of H_2O_2 and also as a result of induced nodule senescence. It has been reported that free iron ions (LHb) directly or indirectly participate in lipid peroxidation (Becana *et al.* 2000). The level of TBARS was brought down upon desalinization but values were still higher than that of control.

We observed a progressive increase in nodular injury and reduction in RWC and Ψs with increasing salinity levels. Under such a situation, salinity and water deficiency both caused the damage to the nodules, including injury to the cell membrane which is in present study was up to 1.61 fold in terms of TBARS content (Table 2). The results here propose that lipid peroxidation goes along with ethylene formation. It is concluded that the decrease in functional efficiency of chickpea nodules was due to over production of C_2H_4, increase in H_2O_2 content, Na^+/K^+ ratio and Cl^- content along with decrease in water status and ascorbic acid content. Though the antioxidant defense system was activated by increasing the activity of SOD, catalase, peroxidase and ASC-GSH cycle enzymes under saline conditions, yet, they could not overcome the accumulation of H_2O_2 in nodules. In future, these ethylene correlated processes in relation to antioxidant defense system can be used as key marker while studying the mechanism of salinity tolerance in plants.

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