Journal of Plant Physiology 164 (2007) 1161–1169

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JOURNAL OF
PLANT PHYSIOLOGY

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Plant water status, ethylene evolution, N_2-fixing efficiency, antioxidant activity and lipid peroxidation in *Cicer arietinum* L. nodules as affected by short-term salinization and desalinization

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KEYWORDS
Antioxidative enzymes;
Ascorbic acid;
Ethylene;
Leghemoglobin;
Lipid peroxidation;
Minerals;
N_2-ase activity;
Water relations

Summary

Salinity induced changes in ethylene evolution, antioxidant defense system, N_2-fixing efficiency and membrane integrity in relation to water and mineral status in chickpea (*Cicer arietinum* L.) nodules were studied under screen house conditions. At vegetative stage (55–65 DAS) plants were exposed to single saline irrigation (Cl^- dominated) of levels 0, 2.5, 5.0 and 10.0 dS m^{-1} and sampled after 3 d. The other set of treated plants was desalinized by flooding 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 –0.44 to –0.56 MPa and from –0.65 to –1.15 MPa and from –0.75 to –1.77 MPa, respectively upon salinization. RWC of leaf and nodules also reduced from 86.05% to 73.30% and 94.70% to 89.98%, respectively. The decline in Ψs of nodules was due to accumulation of proline and total soluble sugar. In comparison to control, the increase in ethylene (C_2H_4) production was 35–108% higher and correspondingly increase in 1-aminocycloprane-1-carboxylic acid (ACC) content (37–126%) and ACC oxidase activity (31–118%) was also noticed. Similarly, marked increase in H_2O_2 (25–139%) and thiobarbituric acid substances (TBRAS, 11–133%) contents was seen. N_2-fixing efficiency i.e. N_2-ase activity, leghemoglobin and N contents of nodules declined significantly after saline irrigation. The induction in specific activity of antioxidant enzymes was confirmed by the increase in activity of superoxide dismutase, peroxidase, ascorbate peroxidase, glutathione reductase and glutathione transferase, whereas reverse

Abbreviations: AA, ascorbic acid; ACC, 1-aminocyclopropane-1-carboxylic acid; ARA, acetylene reduction assay; LHb, leghemoblobin; RWC, relative water content; TBARS, thiobarbituric acid reactive substances; Ψ_s, osmotic potential; Ψ_w, water potential

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doi:10.1016/j.jplph.2006.05.017

was true for catalase. These activated enzymes could not overcome the accumulation of H_2O_2 in nodules. Ascorbic acid content also declined from 20 to 38%, whereas Na^+/K^+ ratio and Cl^- content were significantly enhanced. Upon desalinization, a partial recovery in all above metabolic processes and water relations parameters was noticed. It is suggested that ethylene in relation to water status and lipid peroxidation and along with other metabolic processes has an important role in induced nodules senescence under salinity.

Introduction

In arid and semi-arid regions, salinity (both soil and water) is one of the major factors responsible for deterioration of soil and making it unfit for agriculture. Salinity caused reduction in growth is the consequence of alterations in several physiological processes (Flowers and Yeo, 1986; Kukreja et al., 2005; Nandwal et al., 2000a, b; Sharma, 1996, 1997; Zhu, 2001) including N_2 fixation (Cordovilla et al., 1994; Farnandez-Pascual et al., 1996; Nandwal et al., 2000a, b) etc. As a result of these primary effects secondary stress such as oxidative damage often occurs (Bartels, 2001; Becana et al., 2000; Kukreja et al., 2005; Sairam et al., 2002) 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 catalysing free radical production (Becana et al., 1998). Dalton et al. (1986) and Gogorcena et al. (1995) reported that H_2O_2 and O_2^- radical can be generated by oxidation of nitrogenase, hydrogenase and by autoxidation of oxygenated LHb. Interaction of LHb with H_2O_2 also gives rise to highly toxic hydroxyl ions (OH^-)) which are likely to damage the membrane structure and one or more component of N_2 fixing machinery. In addition to enzymes, super oxide dismutase 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; Dalton et al., 1986).

Lipid peroxidation which leads to impairment of membrane function is the system most easily ascribed to oxidative damage and also most frequently measured (Sairam et al., 2002).

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 and the mechanism by which salinity affects its nodule metabolism is still not completely understood especially the ethylene-correlated processes. Hence, the present investigations on an indeterminate type of nodules are 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 desalinization.

Methods and materials

Growth conditions

Chickpea (*Cicer arietinum* L.) cv. H 96-99 was raised in earthenpots (30 cm dia) filled with 5.5 kg of dune sand (Typic torrispmments) under screen house conditions 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 vegetative stage i.e. 55–65 d after sowing, the desired salinity was applied to saturate each pot so as to maintain four levels [0 (control), 2.5 (S_1), 5.0 (S_2) and 10.0 (S_3) $dS\,m^{-1}$] of Cl dominated salinity. The sampling was done at 3 d after treatments. Half of the treated plants were revived by flooding and were sampled after 3 d to see their revival and were designated as S_1R, S_2R and S_3R, respectively.

Water potential

The third fully expanded leaf from the top was used to measure water potential (Ψ_w) with the help