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Nitrogen modifies NaCl toxicity in eggplant seedlings: Assessment of chlorophyll a fluorescence, antioxidative response and proline metabolism

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ABSTRACT

In the present study, effect of different levels [0, 25,75 and 150 kg NO_3^- ha^{-1} sand correspond to N_0 (deprived), N_{25} (sub-optimum), N_{75} (optimum) and N_{150} (supra-optimum)] of nitrate in eggplant (*Solanum melongena* L.) seedlings under NaCl ($NaCl_1$, 0.3 g kg^{-1} sand and $NaCl_2$, 0.5 g kg^{-1} sand, respectively) stress was investigated. Growth, photosynthetic pigments and K^+ content in test plant were declined by both the doses of NaCl. Furthermore, fluorescence parameters (JIP-test): F_m/F_0, F_v/F_0, F_v/F_m or ϕP_0, ϕE_0, Ψ_0, PI_{ABS}, ABS/RC, TR_0/RC, ET_0/RC and DI_0/RC were also affected by NaCl but toxic effects of NaCl on photosystem II photochemistry were ameliorated by N. NaCl increased accumulation of Na^+ and oxidative stress biomarkers: superoxide radical, hydrogen peroxide, lipid peroxidation and electrolyte leakage despite increased activities of superoxide dismutase, peroxidase, catalase and glutathione-*S*-transferase. N addition caused enhancement in NaCl-mediated decline in endogenous proline and activity of Δ_1-pyrroline-5-carboxylate synthetase (P5CS), while the activity of proline dehydrogenase decreased. The results indicate that different levels of N significantly modulated NaCl-induced damaging effects in eggplant. Further, results suggest that after N addition Na^+ content, enzymatic antioxidants, and pool of free proline and activity of P5CS are finely regulated, which might be associated with the mitigation of NaCl stress and this effect was more pronounced with supra-optimum level of N.

1. Introduction

Increased human activities such as repeated irrigation through canal system and heavy crop production practices led to rise in the levels of salts in crop field, which cause substantial decline in crop productivity worldwide (Hu et al., 2011; Howladar, 2014). Evidences reveal that crop productivity has dramatically decreased up to 20–35% due to increased levels of salinity (FAO, 2008). Salinity affects every aspect of physiology and biochemistry of plants and, therefore, altered metabolism decreases crop productivity (Singh et al., 2015a). It alters plant metabolic responses like osmotic adjustment, ions uptake and their exchange, microbial activity in soil, organic solutes accumulation, protein as well as nucleic acid synthesis, hormonal equilibrium, photosynthetic responses and water availability to crop plants (Siddiqui et al., 2012; Koyro et al., 2013; Yildiztugay et al., 2014; Forieri et al., 2016). Sodium chloride (NaCl) salinity arises from combination of osmotic and ion toxicity as it reduces water uptake capacity hence, affecting the physiology of crop. Beside this, such salinity stress also induces toxic levels of Na^+ and Cl^- in cells, thereby restricted K^+ uptake results hampered activity of K^+ requiring enzymes (Munns and Tester, 2008).

Sodium chloride salinity induces interruption in respiratory and photosynthetic electron transport processes thereby electron leakage to O_2 generates reactive oxygen species (ROS) such as superoxide radicals ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), hydroxyl radicals ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2). Excess accumulation of ROS

Abbreviations: ABS/RC, the energy fluxes for absorption of photon per active RC; CAT, catalase; DI_0/RC, energy dissipation flux per active RC; ET_0/RC, electron transport flux per active RC; F_v/F_0, size and number of active reaction center of photosynthetic apparatus; F_0/F_v, Efficiency of water splitting complex; F_v/F_m or Phi_P_0 or ϕP_0, quantum yield for primary photochemistry; GST, glutathione-*S*-transferase; H_2O_2, hydrogen peroxide; K, potassium; MDA, malondialdehyde; N, nitrogen; Na, sodium; NaCl, sodium chloride, Phi_E_0 or ϕE_0, quantum yield of electron transport; PI_{ABS}, performance index of PS II; POD, peroxidase; Pro, Proline; PS II, photosystem II; ProDH, proline dehydrogenase; Psi_0 or Ψ_0, yield of electron transport per trapped excitation; P5CS, Δ_1-pyrroline-5-carboxylate synthetase; Q_A, primary electron accepter of PS II; RC, reaction center; ROS, reactive oxygen species; RWC, relative water content; SOD, superoxide dismutase; SOR, superoxide radical; TR_0/RC, trapped energy flux per active RC

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in cells results into enhanced rate of lipid peroxidation and protein oxidation and thus, causes damage to cellular membrane and enzymes (Parihar et al., 2014). The contents of ROS in cells are regulated by antioxidants such as superoxide dismutase (SOD), peroxidases (PODs), catalase (CAT), glulathion-*S*-transferase (GST) and non-enzymatic antioxidant i.e. proline, cystein and non-protein thiol etc. (Siddiqui et al., 2012; Yildiztugay et al., 2014; Nath et al., 2016). Under stress condition accelerated antioxidant system brings the level of ROS under limit, hence reduces risk of lipid peroxidation and electrolyte leakage linked membrane deterioration (Siddiqui et al., 2012; Koyro et al., 2013). In addition to this, increased biosynthesis of compatible solutes such as proline, ectoine, glycine betaine, sorbitol etc. also play significant role in osmotic adjustment under salinity stress (Wang et al., 2011; Siddiqui et al., 2012; Yang et al., 2013; Singh et al., 2015a). Such solutes at high concentrations inside the cell are involved in maintining turgor pressure and high concentrations of Na^+ and Cl^- in the vacuole (Munns and Tester, 2008; Zakery-Asl et al., 2014). Beside acting as osmolyte, proline is known to contribute in scavenging radicals ($^{\bullet}OH$), stabilizing sub-cellular structures (membranes and proteins), and buffering cellular redox potential under salinity stress conditions (Wang et al., 2011; Mittal et al., 2012; Yang et al., 2013). In plants, proline is synthesized by glutamate or ornithine pathways (Sanchez et al., 2001; Kishor et al., 2005). Under stress condition the levels of proline is maintained by rate limiting biosynthetic enzyme Δ_1-pyrroline-5-carboxylate synthetase (P5CS, EC 2.7.2.11) as well as degrading enzyme proline dehydrogenase (ProDH, EC 1.5.99.8) (Kishor et al., 2005; Naliwajski and Skłodowska, 2014).

In recent years, nutrient management approach is being applied to alleviate toxic/damaging effects in plants growing under abiotic stress (Singh et al., 2014). Nitrogen (N) is one of such nutrients has been used to alleviate the toxicity caused by UV-B (Correia et al., 2005), drought (Wu et al., 2008) and heavy metal stress (Giansoldati et al., 2012). N regulates photosynthetic activity of plant by altering the structural and functional attributes (carboxylating enzymes and membrane proteins involved in electron transport and light harvesting complexes) of chloroplast (Correia et al., 2005). Plants may absorb both inorganic an organic form of N, but nitrate is appeared to be most preferred source of N (Tschoep et al., 2009). In field condition concentration of N varies from 135 to 270 kg N ha^{-1} (Chen et al., 2010) and 75 kg N ha^{-1} was reported to be optimum for growth of *Chenopodium* and Safflower (Nasr et al., 1978; Basra et al., 2014). Further, Siddiqui et al. (2012) have reported an appreciable rise in the activity/levels of enzymatic and non-enzymatic antioxidants upon addition of N source (urea) improved the growth performance of *Brassica* under NaCl stress. However, none of the studies has demonstrated about the detail mechanism of toxicity induced by NaCl salinity stress in vegetables and also toxicity alleviation by the most preferred N source i.e. nitrate.

The vegetables are one of the important components of human diet as they are the major sources of essential minerals, vitamins, antioxidants etc., hence greatly contribute in maintaining good human health. A popular vegetable *Solanum melongena*, known as eggplant is cultivated in more than 1.5 million ha land all around the world, and it was reported to be sensitive to NaCl stress (Abbas et al., 2010). Considering the above facts the efforts are being made to minimise the toxicity induced by salinity stress all over the world. One such approach is nutrient management, and in the present investigation impact of nitrate application on growth, photosynthetic performance, status of Na^+ and K^+, oxidative stress, antioxidants and metabolic enzymes related with proline in eggplant seedlings growing under NaCl salinity stress was analyzed.

2. Material and methods

2.1. Plant material and growth conditions

Seeds of eggplant (*Solanum melongena* L., var. Neelam) were obtained from Nunhems Pvt. Ltd. India. The healthy seeds were surface sterilized with 2% (v/v) sodium hypochlorite solution for 15 min followed by the repeated washing with distilled water. After soaking in distilled water for 1 h the seeds were wrapped in sterilized cotton cloth and kept overnight for germination at 26 ± 1 °C. Sprouted seeds were sown in plastic pots (5 cm diameter and 10 cm depth) containing 150 g acid washed sterilized sand, already mixed with two doses ($NaCl_1$; 0.3 g NaCl kg^{-1} sand and $NaCl_2$; 0.5 g NaCl kg^{-1} sand) of NaCl. Seedlings were placed in a growth chamber (CDR model GRW-300 DGe, Athens) under photosynthetically active radiation (PAR) of 350 μmol photons m^{-2} s^{-1} with 16:8 h day-night regime and 65–70% relative humidity at 26 ± 1 °C.

2.2. Nitrogen treatments

After the emergence of primary leaf (at 15 days of growth) NaCl treated and untreated seedlings were irrigated with full strength Hoagland nutrient medium containing different concentrations (0, 25, 75 and 150 kg N ha^{-1} which correspond to N_0, deprived; N_{25}, suboptimum; N_{75}, optimum and N_{150}; supra-optimum respectevely) of N as nitrate [$Ca(NO_3)_2.4H_2O$]. The experimental set up included 12 combinations: $NaCl_0+N_0$, $NaCl_1+N_0$, $NaCl_2+N_0$, $NaCl_0+N_{25}$, $NaCl_1+N_{25}$, $NaCl_2+N_{25}$, $NaCl_0+N_{75}$, $NaCl_1+N_{75}$, $NaCl_2+N_{75}$, $NaCl_0+N_{150}$, $NaCl_1+N_{150}$, $NaCl_2+N_{150}$. The 75 kg N ha^{-1} (N_{75}; optimum requirement) was selected as control. The seedlings were irrigated with different levels of N at every 3rd up to 12 days and at 4th day of last treatment; seedlings were harvested for the analysis of all the parameters. During the interval periods seedlings were irrigated with DDW.

2.3. Estimation of growth and photosynthetic pigments

For the determination of growth seedlings from each sample were uprooted gently, washed carefully under running tap water and divided into root and shoot. The fresh weight and length of each sample was determined by using digital electronic balance (Model CA 223, Contech, India) and meter scale, respectively. Photosynthetic pigments from fresh leaves (20 mg) of each sample were extracted in 5 ml of 80% acetone and absorbance was read at 663.2 and 646.5 nm spectrophotometrically (Shimadzu double beam UV–Visible spectrophotometer-1700). The amount of total chlorophyll was calculated following the method of Lichtenthaler (1987).

2.4. Estimation of relative water content (RWC)

Relative water content (RWC) of leaf was determine by recording the saturated mass (SM) of 0.5 g fresh leaf mass (FM) samples by keeping in distilled water for 4 h and followed by drying in hot air oven till constant dry mass (DM) is achieved (Weatherley , 1950). RWC was calculated by using an equation: relative water content (RWC)=[(FM-DM)/(SM-DM)] × 100.

2.5. Estimation of $N^{\pm}$ and $K^{\pm}$ contents

For the determination of Na^+ and K^+ contents, dried sample (100 mg) of root and shoot was digested in mixed acid (HNO_3, H_2SO_4 and $HClO_4$ in 5:1:1 ratio, v/v) at 80 °C until a transparent solution was obtained (Allen et al., 1986). After cooling, the digested sample was filtered using Whatman No. 42 filter paper and