Plant Physiology and Biochemistry 109 (2016) 72–83

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Responses of photosynthesis, nitrogen and proline metabolism to salinity stress in *Solanum lycopersicum* under different levels of nitrogen supplementation

Madhulika Singh, Vijay Pratap Singh [1], Sheo Mohan Prasad*

Ranjan Plant Physiology and Biochemistry Laboratory, Department of Botany, University of Allahabad, Allahabad, 211002, India

ARTICLE INFO

Article history:
Received 9 July 2016
Received in revised form
29 August 2016
Accepted 29 August 2016
Available online 31 August 2016

Keywords:
Chlorophyll fluorescence
Nitrogen metabolism
Nitrogen
NaCl
Proline metabolism
Solanum lycopersicum

ABSTRACT

In the present study, effect of different levels of nitrogen (N_0, deprived; N_{25}, sub-optimum; N_{75}, optimum and N_{150}, supra-optimum) in *Solanum lycopersicum* L. seedlings under NaCl ($NaCl_1$, 0.3 g kg^{-1} sand and $NaCl_2$, 0.5 g kg^{-1}sand) stress was investigated. Biomass accumulation, pigments, K^+ concentration, nitrate and nitrite contents were declined by NaCl in dose dependent manner. As compared to control (N_{75} without NaCl), fresh weight declined by 4% and 11%, and dry weight by 7 and 13% when seedlings were grown under N_{75}+$NaCl_1$ and N_{75}+$NaCl_2$ combinations, respectively. Furthermore, fluorescence parameters (JIP-test): the size and number of active reaction centres of photosynthetic apparatus (F_v/F_0), efficiency of water splitting complex (F_0/F_v), quantum yield of primary photochemistry (φP_0 or Phi_P_0), yield of electron transport per trapped excitation (Ψ_0 or Psi_0), the quantum yield of electron transport (φE_0), and performance index of PS II (PI_{ABS}) and parameters related to energy fluxes per reaction centre (ABS/RC, TR_0/RC, ET_0/RC and DI_0/RC) were also affected by NaCl. However, toxic effect of NaCl on photosystem II photochemistry was ameliorated by N. The lower dose ($NaCl_1$) of NaCl exerts damaging effect on oxidation side of PS II, while higher dose ($NaCl_2$) damages PS II reaction centre and its reduction side. Moreover, control seedlings (N_{75} without NaCl) when exposed to $NaCl_1$ and $NaCl_2$ exhibited a significant enhancement in respiration rate by 6 and 16%, Na^+ accumulation by 111 and 169% in shoot, and 141 and 223% in root and ammonium contents by 19 and 34% respectively. Nitrate and ammonium assimilating enzymes such as nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS) and glutamate synthase (GOGAT) were adversely affected by NaCl stress while glutamate dehydrogenase (GDH) showed reverse trend. N addition caused further enhancement in free proline, and activity of Δ_1-pyrroline-5-carboxylate synthetase (P5CS), while activity of proline dehydrogenase (ProDH) decreased. The results indicate that different levels of N significantly modulated NaCl-induced damaging effects in tomato seedlings. Furthermore, the results suggest that after N addition Na^+, nitrite, nitrate, ammonium contents, nitrogen metabolic enzymes, proline content, and activity of P5CS are favourably regulated, which might be associated with mitigation of NaCl stress and effect was more pronounced with supra-optimum level of N (N_{150}).

Abbreviations: ABS/RC, the energy fluxes for absorption of photon per active reaction centre; 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 centre 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; GDH, glutamate dehydrogenase; GOGAT, glutamine 2-oxoglutarate aminotransferase or glutamate synthase; GS, glutamine synthetase; H_2O_2, hydrogen peroxide; K, potassium; MDA, malondialdehyde; N, nitrogen; Na, sodium; NaCl, sodium chloride; NH_4^+, ammonium; NO_3^-, nitrate; NO_2^-, nitrite; NR, nitrate reductase; NiR, nitrite reductase; Phi_E_0 or φE_0, quantum yield of electron transport; PI_{ABS}, performance index of PS II; 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 centre; TR_0/RC, trapped energy flux per active RC.

* Corresponding author.
E-mail addresses: madhulikasingh1090@gmail.com (M. Singh), vijaypratap.au@gmail.com (V.P. Singh), profsmprasad@gmail.com (S.M. Prasad).
[1] Present address: Govt. Ramanuj Pratap Singhdev Post Graduate College, Baikunthpur, 497335, Koriya, Chhattisgarh, India.

http://dx.doi.org/10.1016/j.plaphy.2016.08.021
0981-9428/

1. Introduction

Salinity is a major environmental factor responsible for decreasing crop productivity in many arid and semi-arid regions of the world. According to the FAO (2011), approximately 34 Mha (11% of the irrigated land) land is affected with salinity worldwide. Pakistan, China, United States and India contribute more than 60% of the total (34 Mha) salinity affected lands. Soil salinity is resulted due to natural weathering of saline rocks as well as anthropogenic activities such as repeated irrigation practices, excessive application of chemical fertilizers, deforestation, overgrazing, intensive cropping, etc. (Singh et al., 2015). Excess accumulation of salt at the upper layer of land severely affects morphology, physiology and metabolic attributes of plants which result into reduced crop yields (Siddiqui et al., 2012; Singh et al., 2014; Taïbi et al., 2016).

Sodium (Na) being non-essential mineral nutrient for most of the plants gets accumulated in plant tissues under saline condition hence, alters ratios of Na^+/Ca^{+2}, Na^+/K^+, Ca^{+2}/Mg^{+2} and Cl^-/NO_3^-, and thereby affects plant growth and productivity (Grattan and Grieve, 1999; Maathuis, 2014; Singh et al., 2014). Moreover, excess salt accumulation alters electron transport systems taking place in the chloroplast, mitochondria and biological membranes, whereby electrons are leaked from normal routes and quickly taken up by oxygen molecules, resulting into formation of reactive oxygen species (ROS) (Singh et al., 2015; Taïbi et al., 2016). Overproduction of ROS alters synthesis of essential amino acids, proteins, lipids, photosynthetic pigments, enzymatic activities and their regulations (Singh et al., 2015).

Nitrogen (N) is considered as one of the essential macronutrients required for plant growth and development (Correia et al., 2005; Singh et al., 2016; Giagnonia et al., 2016). N is main constituent of all amino acids, proteins and a number of nitrogen containing compounds which protect plants against salinity stress. In plants, N also acts as a signaling element that affects expression of numerous genes, regulates process of nitrogen assimilation, rate of photosynthesis, carbohydrate metabolism, antioxidant systems, the cell cycle, etc. (Siddiqui et al., 2012; Singh et al., 2014, 2016). Studies have reported that the addition of nitrogen alleviates toxicities of abiotic stresses in plants such as drought (Wu et al., 2008), UV-B radiation (Correia et al., 2005) and heavy metal (Giansoldati et al., 2012). On the other hand, deficiency/deprivation of N result into declined biomass accumulation and accumulation of soluble sugars and starch in leaves as compared to sufficient N condition (Cai et al., 2012; Hussain et al., 2016). Generally, in natural condition plants are affected by multiple stresses at a time. N deficiency under Cd stress decreased chlorophyll, protein and nitrate contents as well as lowers chloroplast antioxidant capacities which result into greater ROS formation (Lin et al., 2011). Similarly, Foyer and Noctor (2003) reported that chilling and nutrient deprivation condition enhanced level of intracellular reactive oxygen intermediates (ROIs). Basra et al. (2014) have reported that 75 kg N ha^{-1} was adequate for the optimum growth, yield and biomass production in crops. It is taken up by plants from soil in the form of charged ions such as nitrate (NO_3^-) and ammonium (NH_4^+) (Giagnonia et al., 2016), finally converted into amino acids, involving several enzymes: nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS), glutamine 2-oxoglutarate aminotransferase (GOGAT) and glutamate dehydrogenase (GDH).

Among vegetable crops, *Solanum lycopersicum* L. (tomato) is an important vegetable grown in every part of the globe, and possesses a central position in the human diet. It is a rich source of vitamins, carbohydrates, proteins, mineral nutrients and other several important chemicals: carotenoids (lycopene, β-carotene and lutein), tocopherols, and polyphenols. Since salinity is a global scientific problem which affects crop productivity including vegetables. Therefore, methods are needed which can restrict and/or minimize salinity mediated loss to the crop productivity. In recent years, some studies showed that application of nitrogen may alleviate toxicities of abiotic stresses (Correia et al., 2005; Wu et al., 2008; Giansoldati et al., 2012; Siddiqui et al., 2012; Singh et al., 2016). To ascertain this, whether exogenous N addition has a role in the regulation of salinity stress, this study was undertaken taking tomato as a test plant. In this study, possible mechanism of nitrogen-mediated alleviation of NaCl stress was investigated by analyzing photosynthetic performance and metabolism of nitrogen and proline in tomato seedlings.

2. Material and methods

2.1. Plant material and growth conditions

Seeds of *Solanum lycopersicum* L., Var. Lakshmi (tomato) were obtained from Nunhems Pvt. Ltd. India. The detail of growth conditions and treatments is described in Singh et al. (2016). Briefly, the healthy seeds were surface sterilized with 2% (v/v) sodium hypochlorite solution for 15 min followed by repeated washing with distilled water. Thereafter, seeds were soaked in distilled water for 1 h. Further, seeds were wrapped in sterilized cotton cloth and kept overnight for germination at 25 ± 1 °C and then sprouting seeds were sown in 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 in plastic pots (5 cm in diameter and 10 cm in depth) containing 150 g acid washed sterilized sand. 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 25 ± 1 °C. After emergence of primary leaves, seedlings were irrigated with different levels of nitrogen (N), which were prepared in full strength Hoagland nutrient medium.

2.2. Nitrogen treatments

After primary leaf emergence (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}, suboptimal; N_{75}, optimal and N_{150}; supra-optimal, respectively) of N as nitrate ($CaNO_3$. $4H_2O$). The experimental set up includes 12 combinations: $NaCl_0$+ N_0, $NaCl_1$ + N_0, $NaCl_2$ + N_0, N_{25}, $NaCl_1$ + N_{25}, $NaCl_2$ + N_{25}, N_{75}, $NaCl_1$+ N_{75}, $NaCl_2$ + N_{75}, N_{150}, $NaCl_1$+ N_{150}, $NaCl_2$+N_{150}. The 75 kg N ha^{-1} (N_{75}; optimal requirement of soil) was selected as control. The seedlings were irrigated with different levels of N at every 3 day up to 12 days and at 4th day of last treatment; seedlings were harvested for the analysis of various parameters.

2.3. Estimation of growth and photosynthetic pigments

Growth was measured in terms of fresh and dry weight, leaf area and relative water content. Treated and untreated seedlings were selected randomly; their fresh weights and leaf area were determined by using digital electronic balance (Model CA 223, Contech, India) and leaf area meter (Systronics, India), respectively. For dry mass estimation, plant material was dried at 80 °C for 48 h and weighed by using electronic balance.

Leaf relative water content (RWC) was estimated 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 (Whetherley, 1950). RWC was calculated by using an equation: relative water content (RWC) = [(FM-DM)/(SM-DM)] × 100.