SALINITY STRESS EFFECTS BEING MEDIATED VIA NITRIC OXIDE IN COMMON BEAN (*PHASEOLUS VULGARIS*) SEEDLINGS

Sonal Dhamgaye and Rekha Gadre

School of Biochemistry, Devi Ahilya University, Khandwa Road, Indore- 452 017, India.

ABSTRACT

Present study analyses salinity effects on growth and *in vivo* nitrate reductase (NR) activity in bean seedlings to work out the mechanism. Bean seedlings were cultivated in plastic pots containing acid washed sand for 4 d and transferred to the test tubes with 10-200 mM NaCl containing 5 mM NH_4NO_3 as nitrogen source for 24 hr. Decrease in growth parameters, like root weight, shoot weight and total seedling weight, root length and shoot length were observed. NR activity was prominently increased at high salt concentrations in root as well as in shoot. Biochemical parameters, like protein, RNA and proline content in the shoot tissue were enhanced. Nitric oxide (NO) content of the root tissue was increased with NaCl treatment. Reduction in growth but increased NR activity and NO content suggest the involvement of NR mediated NO stress during high salinity. Thus, the study reveals an insight into the formation and function of NO under salinity stress in common bean seedlings.

Keywords: NaCl effects . *Phaseolus vulgaris*. nitrate reductase . nitrate assimilation . nitric oxide

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Introduction

Salinity in soil or water is one of the major abiotic stresses experienced by plants, especially in arid and semi arid regions which represent around 40% of earth's area (Qadus, 2011). According to FAO (2000), world's 831 million hectares of arable land is affected by salinity. It has become a great challenge for agriculture as this area is growing very rapidly with time. Salinity leads to a number of physiological, biochemical and molecular changes that adversely affect plant growth and productivity (Wang et al., 2001). Various processes involved at different stages of plant development, such as, seed germination, seedling growth and vigour, vegetative growth, flowering etc. are adversely affected by high salt concentrations, ultimately reducing quantity and quality of crop yield. Salinity effects on seedling growth in terms of fresh weight, root length, shoot length etc. have been reported in various systems (Debouba et al., 2007, Hussain et al., 2009; Amirjani, 2010; Kurum et al., 2013; Kavas et al., 2015; Nasri et al., 2015; Amukali et al., 2015). .

Nitrate assimilation is one of vital process of plant metabolism through which nitrogen is utilized as a nutrient by plant which is used in synthesis of main biomolecules, like nucleic acids, amino acids, proteins including enzymes, photosynthetic pigments etc. The major form of soil nitrogen available to the plants is nitrate. Nitrate is assimilated to nitrite and ammonia by sequential action of the cytosolic nitrate reductase (NR) and nitrite reductase (NiR). The study of NR is important as it is the first regulatory enzyme of nitrate assimilation and it is influenced by environmental factors including salinity. Salinity has been reported to decrease NR activity in several plant species like linseed genotypes (Khan et al., 2007), tomato seedlings (Debouba et al., 2007), cucumber seedlings (Sacala et al., 2008) and jatropha cotyledons (Gao et al., 2013). Apart from nitrate assimilatory function, NR has been reported to be involved in the synthesis of nitric oxide (NO) in plants (Rockel et al., 2002). Recently, NO production by NR has been confirmed in *Chlamydomonas reinhardtii* (Chamizo-Ampudia et al., 2016). Now a days, scientists has got the attention towards NO as it is an important bioactive signaling molecule which play a beneficial role in ameliorating negative stress effects (Uchida et al., 2002; Salahuddin et al., 2017). In Panax notoginseng herb, production of endogenous NO by NR is reported under cadmium stress (Kan et al., 2016). However, studies involving NO effects mediated by NR under salinity stress are lacking.

Common bean or Rajmah is a major crop with very high nutritional value which accounts for high consumption, demand and economical importance worldwide. It is salt sensitive crop grown for green vegetable as well as dry seeds (Cokkizgin, 2012). In India, this crop is grown mainly in saline regions with poor irrigation. Although, a number of studies have been carried out related to salinity effects in plants, no specific study has

been reported in common bean showing NR and NO correlation under salinity stress. Hence, the present study is planned to investigate the effects of NaCl on growth and NR activity in relation to NO formation with an insight to elucidate the possible mechanism for adaptation of common bean to salinity stress.

Material and methods

Plant material and treatments: Seeds of *Phaseolus vulgaris* cv. Rajmah purchased from Pahuja seeds, New Delhi, were surface sterilized with 0.1 % $HgCl_2$ for 5 min followed by thorough washing with distilled water. The seedlings were raised in plastic pots containing acid washed sand for 4 d in continuous light of intensity 30 Wm^{-2} supplied by fluorescent tubes at 28 ± 3°C. They were watered with ½ strength Hoagland's solution (pH 6.0) containing no nitrogen. Uniformly grown seedlings were used for treatment of NaCl at concentrations 0, 10, 50, 100 and 200 mM. For this, the seedlings were uprooted and washed with tap water to remove excess sand. After rinsing with distilled water, seedlings were placed on the test tubes (Fig. 1) filled with ¼ strength Hoagland's solution having 5 mM NH_4NO_3 in the presence of desired concentration of NaCl for 24 hr in continuous light supplied by fluorescent tubes. The shoot and/or root tissue of the treated seedlings were used for various analyses.

Analytical Procedures:

Growth measurements: Root, shoot and total weight of seedling after 24 hr NaCl treatment was taken by using electrical balance. Root and shoot length were measured by centimeter scale.

Estimation of protein, RNA and proline content: Total soluble protein extracted from the treated material was estimated according to Lowry's method (Lowry, 1951). Total RNA content was extracted and estimated according to the method of Webb and Levy (1958). The proline content of the tissue was estimated by the method of Bates et al., (1973).

Assay of peroxidase activity: The peroxidase activity was assayed by the method of Putter (1974) with some modifications. The enzyme was extracted by homogenizing with phosphate buffer (0.1 M, pH 7.0), 10 ml for shoot and 5 ml for root using mortar and pestle in cold room. The extract was centrifuged at 10,000 rpm for 20 min at 4°C in cooling centrifuge and the supernatant was used for enzyme assay. The assay mixture contained 2.5 ml of phosphate buffer (0.1 M, pH 7.0), 1 ml of freshly prepared guaicol (10 mM), 0.1 ml of enzyme extract and 0.03 ml of H_2O_2 (12.3 mM having extinction of 0.436 at 240 nm). Increase in absorbance was monitored at 436 nm at the interval of 30 sec for 3 min. The enzyme activity was expressed as ΔA min^{-1} g^{-1} fresh weight.

Estimation of NO content: NO content extracted from the treated tissue was determined involving Griess reagent as described by the method of Kumar et al., (2010). Treated root was ground in a mortar and pestle in 2.0 ml of cold acetate buffer (0.05 M containing 4 % zinc acetate, pH 3.6) in cold room. The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant

was collected and the pellet was washed with 0.5 ml of extraction buffer and both the supernatants were pooled. Activated charcoal (0.05 g) was added to the supernatant. The suspension was vortexed and filtered through whatmann filter paper and then centrifuged. To 0.5 ml of supernatant, 0.5 ml of Greiss reagent was added and incubated at room temperature for 30 min. Absorbance of the solution was measured at 540 nm.

Assay of *In vivo* NR activity: *In vivo* NR activity of the treated material was assayed by colorimetric estimation of nitrite according to the method of Srivastava (1975). Whole shoot or root system of the seedling was cut into small segments and suspended in 10.0 ml of incubation mixture (8.0 ml of 0.1 M phosphate buffer, pH 7.4; 1.0 ml of 0.2 M KNO_3 and 1.0 ml of 25 % isopropanol) inside a tightly stoppered dark vial. The reaction mixture was incubated in dark for 30 min at 30°C. After incubation, 2.0 ml of the incubation medium was added with 2.0 ml of acidic sulphanilamide (1 % in 1 N HCl) and 2.0 ml napthylethylenediamine (NED) (0.02 % in distilled water). After 10 min, developed color was read for absorbance at 540 nm. The enzyme activity was expressed as nmoles of nitrite formed $hr^{-1}g^{-1}$ fresh weight.

Statistical analysis: Results expressed are the average values of at least six independent experiments with ± S.E. Difference between means obtained for various treatments was tested by Student's *t* test at level of significance - *p < 0.05, **p < 0.01. The compound correlation values are determined by Graph Pad Prism 5 software.

Result and Discussion

Salinity effects on growth and biochemical parameters

The common response of plants towards salinity stress is growth inhibition. To evaluate growth effects under salinity stress, the parameters, like root fresh weight, shoot fresh weight, total seedling fresh weight, root length, shoot length, total seedling length etc are studied. In this investigation, 10-200 mM NaCl treatment in the presence of 5 mM NH_4NO_3 caused reduction in total weight, root length and shoot length of the seedlings. Amongst these parameters, fresh weight was reduced by 10-19 %, root length by 8-24 % and shoot length by 11-21 % in the range of 10-200 mM NaCl. Strong negative correlation of NaCl treatment with fresh weight and root length was observed with R^2 values, 0.760 and 0.824, respectively (Table 1). Reduction in root and shoot weight also resulted with NaCl treatment exerting strong correlation with R^2 values of 0.902 and 0.641, respectively (Table 1). The negative effect of NaCl on growth has been reported in several studies (Debouba et al., 2007; Hussain et al., 2009; Amirjani, 2010; Kurum et al., 2013; Kavas et al., 2015; Nasri et al., 2015; Amukali et al., 2015). Salinity effects on growth were found to involve different processes, like unbalanced nutrient uptake, decreased water absorption, reduced turgor pressure, inhibition of

cytokinesis and cell expansion etc. In our previous study, fresh weight and root length of 4 d old common bean seedling was not altered by 10-200 mM NaCl when 10 mM NH_4NO_3 was used as nitrogenous source, whereas the percentage of reduction in shoot length at high salinity was 14 % as compared to control (Dhamgaye & Gadre, 2015). In this study, the percentage of reduction with 5 mM NH_4NO_3 at high salinity was found to be in the range of 21-24 % being higher than that of 10 mM NH_4NO_3. Thus, the inhibitory effect of NaCl on growth of common bean seedlings depends upon the tissue as well as NH_4^+ and NO_3^- concentrations.

Salinity leads to a number of physiological, biochemical and molecular changes in plants (Wang et al., 2001). Plants respond to salinity by maintaining cell osmotic potential through accumulation of ions and osmolytes like proline. Proline plays a highly beneficial role in plants exposed to various stress conditions. The accumulation of free proline has been reported under various abiotic stresses like, drought (Choudhary et al., 2005), low temperature (Naidu et al., 1991) and heavy metal exposure (Sharma & Dietz, 2006). Increased proline concentration under salt stress has been reported in root of common bean (Babu & Devraj, 2008), in shoot of peanut (Kavas et al., 2015) and in seedling of bean (Kaymakanova & Stoeva, 2008). Similarly, in our study, proline content significantly increased in shoot at 100 mM NaCl (Table 2). Increased proline content reflects its involvement in osmotic adjustment under high salinity. Decrease in peroxidase activity with NaCl treatment suggests the involvement of oxidative stress. The other biochemical parameters in shoot, like, protein and RNA content were increased, however, in the root, all parameters were marginally changed (Table 2). Thus, depending upon the organ involved, differential effect of NaCl on metabolic status of the system is observed showing enhancement in shoot tissue whereas no alteration in root tissue.

Table 1. Effect of supply of NaCl in the presence of 5 mM NH_4NO_3 on Total weight, Root length, Shoot length, Root weight and Shoot Weight of the seedlings

NaCl (mM)	Growth Parameters				
	Total weight (mg)	**Root length (cm)**	**Shoot length (cm)**	**Root Weight (mg)**	**Shoot Weight (mg)**
0	1053±49 (100)	8.6±0.6 (100)	3.8±0.2 (100)	153±26 (100)	811±112 (100)
10	950±33 (90)	7.9±0.5 (92)	3.2±0.2* (84)	140±15 (92)	802±82 (99)
50	949±143 (90)	7.3±0.6 (85)	3.0±0.2* (79)	127±24 (83)	868±141 (107)
100	926±41* (88)	7.4±0.4 (86)	2.9±0.2** (76)	101±10 (66)	776±97 (96)
200	855±108 (81)	6.5±0.4* (76)	3.0±0.2* (79)	89±8* (58)	696±87 (86)
R^2 values	**0.760**	**0.824**	**0.376**	**0.902**	**0.641**

Values relative to control are given in parentheses.
Level of significance, *p value ≤ 0.05, **p ≤ 0.01.

Table 2. Effect of supply of NaCl in the presence of 5 mM NH_4NO_3 on Protein, RNA, Proline content and Peroxidase activity in root and shoot tissue of the seedlings.

NaCl (mM)	Protein content (mg g^{-1} fresh weight)		RNA content (mg g^{-1} fresh weight)		Proline content (mg g^{-1} fresh weight)		Peroxidase activity ($\Delta A\ min^{-1}\ g^{-1}$ fresh weight)	
	Root	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**
0	7.8±1 (100)	45±4.5 (100)	1.9±0.1 (100)	9.2±0.5 (100)	0.023±0.003 (100)	1.41±0.2 (100)	0.161±0.03 (100)	0.192±0.03 (100)
10	8.8±1 (112)	51±2.6 (113)	2.1±0.1 (111)	9.9±0.6 (108)	0.019±0.002 (83)	1.63±0.2 (116)	0.134±0.02 (83)	0.143±0.02 (74)
100	8.1±1 (104)	54±6.0 (120)	1.9±0.1 (100)	11.8±0.8* (128)	0.022±0.004 (96)	2.44±0.2* (173)	0.163±0.02 (101)	0.133±0.02 (69)

Values relative to control are given in parentheses. Level of significance, *p value ≤ 0.05.

Table 3. Effect of supply of NaCl in the presence of 5 mM NH_4NO_3 on NR activity and NO content in the bean seedlings.

NaCl (mM)	NR Activity (nmoles NO_2^- hr^{-1} g^{-1} fresh weight)			NO Content (nmoles NO_2^- g^{-1} fresh weight)
	Root	Shoot	Total	
0	194 ± 23 (100)	40 ± 2 (100)	234 (100)	29 ± 10 (100)
10	196 ± 44 (101)	52 ± 11 (130)	248 (106)	27 ± 10 (90)
100	153 ± 11 (79)	51 ± 7* (143)	204 (87)	57 ± 19 (190)
200	251 ± 43 (129)	45 ± 4 (113)	296 (126)	182 ± 97 (628)

Level of significance, *p value ≤ 0.05.

Table 4. The compound correlation analysed by using Graphpad Prism 5 software between salt treatment and total weight, total NR activity and NO content

NaCl	Total Weight	Total NR activity	NO content
	0.88	0.60	0.95
		0.57	0.80
			0.81

Salinity effects on NR activity and NO content

NR is regulated by various factors such as nitrate, carbon and nitrogen metabolites, light etc (Kaiser et al., 1999). Apart from these factors, various environmental stresses including salinity influence NR activity. In the present study, supply of NaCl with 5 mM NH_4NO_3 for 24 hr showed no effect on *in vivo* NR activity at 10 mM, but decreased at 100 mM and increased substantially at 200 mM in root tissue, though the observed effect was not significant (Table 3). In the shoot tissue, the activity was increased significantly at 100 mM. However, various studies report inhibitory effect of salt on NR activity (Omrav et al., 1998; Maighany, 2004; Khan et al., 2007; Garg & Singla, 2005; Rathore & Varshney, 2005; Sacala et al., 2008). The salinity effects on NR may involve the processes, like osmotic effects, (Meloni et al., 2004), nitrate deficiency (Albassam, 2001; Sacala et al., 2008), reduced nitrate transporter activity (Aslam et al., 1984) etc. Other studies have reported stimulation of NR activity under salinity stress such as in cucumber (Reda et al., 2011) and bean, lentil and chickpea (Salha & Chabaane, 2016). In cucumber, the observed increase in NR corresponds to dephosphorylation of NR protein (Reda et al., 2011). Increased NR activity along with increased shoot fresh biomass revealed salt tolerance of bean, lentil and cowpea (Salha & Chabaane, 2016). However, in our previous study, with 10 mM NH_4NO_3 as nitrogenous source *in vivo* NR activity was inhibited in root tissue but enhanced in shoot tissue by 10 and 100 mM NaCl (Dhamgaye & Gadre, 2015). Thus, the effect of salinity on NR activity in root of common bean seedlings depends upon the concentration of NH_4^+ and NO_3^-.Here, it is worth to mention the dual role of NR in formation of nitrite as well as synthesis of NO in plants (Rockel et al., 2002). Further, production of NO by NR has been shown to play a central role in controlling amount of NO in plant cell (Chamizo-Ampudia et al., 2016). NO formation by NR has been studied in the model system, Chlamydomonas (Chamizo-Ampudia et al., 2017). The beneficial role of NO has also been reported, as Sodium nitroprusside, exogenous NO donor, ameliorates negative growth effects of salinity in mung bean (Salahuddin et al., 2017) and oxidative stress in tomato plants (Manai et al., 2014). In the present study, the NO content of the NaCl treated root tissue was slightly reduced at low NaCl concentration but substantially enhanced at high NaCl concentration (Table 3). Hence, it seems that increased NR activity at high salinity is responsible for NO formation. The compound correlation analyzed by Graph Pad Prism 5 software for NaCl with total weight and NO content was very strong being 0.88 and 0.95, respectively (Table 4). Similarly, the correlation value for total weight and NO content was 0.80. With total NR activity, the observed correlation was strong for NO content (0.81), lesser for NaCl and total weight being 0.60 and 0.57, respectively (Table 4). Thus, strong correlation of NO content for NaCl treatment, total weight and total NR activity supports NO mediated effect under salinity stress through the involvement of NR at least partially. Further studies related with nitric oxide

synthase (NOS), NR and NO are in progress to elucidate the involvement of the three in common bean under salinity stress.

CONCLUSION

NaCl stress negatively affected overall growth of common bean seedlings with combination of inorganic nitrogenous sources, NH_4^+ and NO_3^-. The differential effect of NaCl stress on metabolic status was observed through enhancement in shoot tissue, but no change in root tissue. Further, increased NR activity and NO content at high salinity suggest that NR has a role in mediating salinity stress effects via NO synthesis in common bean seedlings.

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REFERENCES

Albassam B.R. (2001). Effect of nitrate nutrition on growth and nitrogen assimilation of pearl millet exposed to sodium chloride stress. Journal of Plant Nutrition. 24(9):1325-1335.

Amirjani, M.R. (2010). Effect of salinity stress on growth, mineral composition, proline content, antioxidant enzymes of soyabean. Am. J. Plant Physiol., **5(6):**350-360.

Amukali O., Obadoni B.O. and Mensah J.K. (2015). Effects of different NaCl concentrations on germination and seedling growth of Amaranthus hybridus and Celosia argentea. African Journal of Environmental Science and Technology. 9(4):301-306.

Aslam M., Huffaker R.C. and Rains D.W. (1984). Early effects of salinity on nitrate assimilation in barley seedlings. Plant Physiology. 76:321-325.

Babu, N.R. and Devraj, V.R. (2008). High temperature and salt stress response in French bean (*phaseolus vulgaris*). Australian J. crop sci., **2(2):**40-48.

Baki, G.K., Siefritz, F., Man, H.M., Weiner, H., Kaldenhoff, R. and Kaiser, W.M. (2000). Nitrate reductase in *Zea mays* L. under salinity. Plant Cell Env., 2000, **23:**515-521.

Bates, L.S., Waldren, R.P. and Teare, I.D. (1973). Rapid determination of free proline for water- stress studies. Plant Soil, **39:**205–207.

Chamizo-Ampudia, A. (2016). A dual system formed by the ARC and NR molybdoenzymes mediates nitrite-dependent NO production in Chlamydomonas. Plant Cell Environ., **39:**2097–2107.

Chamizo-Ampudia, A., Sanz-Luque, E., Llamas, A., Galvan, A. and Fernandez, E. (2017). Nitrate reductase regulates plant nitric oxide homeostasis. Trends in Plant Science, **22:** 2.

Choudhary, N. L., Sairam, R. K. and Tyagi, A. (2005). Expression of delta1-pyrroline-5-carboxylate synthetase gene during drought in rice (*Oryza sativa L.)*. Indian J. Biochem. Biophys., **42:**366–370.

Cokkizgin, A. (2012). Salinity stress in common bean (*Phaseolus vulgaris* L.)

seed germination. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, **40(1)**:177-182.

Debouba, M., Dghimi. H.M., Suzuki, A., Ghorbel, M.H. and Gouia, H. (2007). Changes in growth and activity of enzymes involved in nitrate reduction and ammonium assimilation in tomato seedlings in response to NaCl stress. Ann. Bot., **99:** 1143-1151.

Dhamgaye, S. and Gadre, R. (2015). Salinity stress effects on growth and nitrate assimilation in bean seedlings likely to be mediated via nitric oxide. J. Stress Physiol. Biochem.,**11:**4.

FAO 2000. Global Network on Integrated Soil Management for Sustain-Able Use of Salt-Affected Soils. Rome, Italy. http://www.fao.org/ag/agl/agll/spush.

Gao, S., Liu K., Chung, T. and Chen, F. (2013). The effects of NaCl stress on Jatropha cotyledon growth and nitrogen metabolism. J. Soil Sci. Plant Nutri., **13(1):** 99-113.

Garg, N. and Singla, R. (2005). Nitrate reductase activity in roots and leaves of chickpea cultivars under salt stress. Spanish J. Agri. Res., **3(2):** 248-252.

Ghoulam C., Foursy A., Fares K. (2002). Effect of salt atress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugarbeet cultivars. Envion. Exp.Bot., **47**: 39-50.

Hussain, K., Majeed, A., Nawaz, K., Khizar, H.B. and Nissar, M.F. (2009). Effect of different levels of salinity on growth and ion contents of black seeds. Curr. Res. J. Biol. Sci., **1(3):**135-138.

Kavas, M., Akca, O.E., Akcay, U.C., Peksel, B., Eroglu, S., Oktem, H.A. and Yucel, M. (2015). Antioxidant responses of peanut (*Arachis hypogaea* L.) seedlings to prolonged salt-induced stress. Arch. Biol. Sci., **67(4):**1303-1312.

Kaymakanova, M. and Stoeva, N. (2008). Physiological reaction of bean plants (*Phaseolus vulg*. L.) to salt stress. Gen. Appl. Plant Physiol., **34 (3-4):** 177-188.

Kan, Q., Wenwei, W., Wenqian, Y., Jiarong, Z., Jin, X., Zed R., Limei, C., Xiuming, C. and Qi C. (2016). Nitrate reductase-mediated NO production enhances Cd accumulation in Panax notoginseng roots by affecting root cell wall properties. J. Plant Physiol., **193:** 64-70.

Kaiser W.M., Weiner H. and Huber S.C. (1999). Nitrate reductase in higher plants: A case study for transduction of environmental stimuli into control of catalytic activity. Physiologia Plantarum, **105**, 385-390.

Khan, M.N., Siddiqui, M.H., Firoz, M., Mansoor, M., Khan, A. and Naeem, M. (2007). Salinity induced changes in growth, enzyme activities, photosynthesis, proline accumulation and yield in Linseed genotype. WJAS, **3(5):** 685-695.

Kumar, P., Tewari, R.K. and Sharma, P.N. (2010). Sodium nitroprusside-mediated alleviation of iron deficiency and modulation of antioxidant responses in maize plants. AoB Plants, plq002.

Kurum, R., Ulukapi, K., Aydinsakir, K. and Onus, A.N. (2013). The influence of salinity on seedling growth of some pumpkin varieties used as rootstock. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 41(1): 219.

Lowry, O.H., Roseborough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with Folin-phenol reagent. J. Biol. Chem., **193**, 265-275.

Maighany, F. and Ebrahimzadeh, H. (2004). Intervarietal differences in nitrogen content and nitrate assimilation in wheat (*Triticum aestivum* L.) under salt stress. Pak. J. Bot., **36**(1):31-40.

Manai, J., Kalai T., Gouia, H. and Corpas, F.J. (2014). Exogenous nitric oxide (NO) ameliorates salinity induced oxidative stress in tomato (*solanum lycopersicum*) plants. J. Soil Sci. Plant nutri., **14**(2), 433-446.

Meloni D.A., Gulotta M.R., Martínez C.A. and Oliva M.A. (2004). The effects of salt stress on growth, nitrate reduction and proline and glycinebetaine accumulation in *Prosopis alba*. Brazilian J. Plant Physiol., **16**(1), 39-46.

Naidu, B.P., Paleg L.G., Aspinall, D., Jennings, A.C. and Jones, G.P. (1991). Amino acid and glycine betaine accumulation in cold stressed wheat seedlings. Phytochem., **30**, 407-409.

Nasri N., Saïdi I., Kaddour R. and Lachaâl M. (2015). Effect of salinity on germination, seedling growth and acid phosphatase activity in lettuce. American J. Plant Sci., **6**(1), 57.

Ndakidemi, P. and Makoi, J. (2009). Effect of NaCl on the productivity of four selected common bean cultivars (*Phaseolus vulgaris* L.). Sci. Res. Essay, **4**(10), 10661072.

Omarov, R.T., Sagi, M. and Lips, S.H. (1998). Regulation of aldehyde oxidase and nitrate reductase in roots of barley by nitrogen source and salinity. J. Exp. Bot.,**49**(322), 897-902.

Putter, J. (1974). Methods of enzymatic analysis. In Peroxidases, (ed. Bergmeyer, H.U.), Verlag Chemie-Academic Press, New York, **2**, 685-690.

Qadus, A.M.S.A. (2011). Effect of salt stress on plant growth and metabolism of bean plant *Vicia faba* (L.). J. Saudi Soc. Agri. Sci., **10**, 7-15.

Rathor, S. and Varshney, K.A. (2005). Effect of Salinity and Sodicity on Nodulation and Nitrate Reductase Activity in Black Gram (*Vigna mungo* L. Hepper). Vegetos-An International J. Plant Res., **18**(1and2), 117-121.

Reda M., Migocka M. and Kłobus G. (2011). Effect of short-term salinity on the nitrate reductase activity in cucumber roots. Plant Science, **180**(6),783-788.

Rockel, P., Strube, F., Rockel, A., Wildt, J. and Kaiser, W.M. (2002). Regulation of nitric oxide production by plant nitrate reductase *in vivo* and *in vitro*. J. Exp. Bot., **53**(366), 103-110.

Sacala, E., Demczuk, A., Grzys, E. and Spiak, Z. (2008). Effect of salt and water stresses on growth, nitrogen and phosphorus metabolism in *cucumis*

sativus L. seedlings. Acta Soc. Bot. Pol., 77(1), 23-28.

Salahuddin, M., Nawaz, F., Shahbaz, M., Naeem M., Bilal Z., Shabbir, R.N., Hussain, R.A. (2017). Effect of exogenous nitric oxide (NO) supply on germination and seedling growth of mungbean (cv. Nm-54) under salinity stress. Legume Res., 2017, **40**(5), 846-852.

Salha B.and Chabaane R. (2016). Effect of molybdenum and nitrogen on *Phaseolus vulgaris*, *Cicer arietinum* L. and *Lens culinaris* M. seedlings grown under salt stress. Int. J. Biosci., **8** (6), 34-42.

Sharma, S.S., Dietz, K.J. (2006). The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. J. Exp. Bot., **57**, 711-726.

Srivastava, H. S. (1975). Distribution of nitrate reductase in bean seedlings. Plant Cell Physiol., **16**, 995-999.

Srivastava, H.S. (1980). Regulation of nitrate reductase in higher plants. Phytochem., **19**, 725-733.

Uchida, A., Jagandorf, A.T., Hibino, T., Takabe, T. and Takabe, T. (2002). Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. Plant Sci., **163**, 515-523.

Wang W.X., Vinocur B., Shoseyov O. and Altman A. (2001). Biotechnology of plant osmotic stress tolerance: physiological and molecular considerations. Acta Horticulture, **560**, 85-292.

Webb, J.M. and Levy, H.B. (1958). In: Methods in Biochemical analysis (ed. Glick, D.), Interscience, New York, 61.