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Effect of NaCl Salinity on Improvement of Nitrogen Metabolism and Some Ions Uptake in Lupine Plants Subjected to Gamma Irradiation

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ABSTRACT

The effect of NaCl salinity in presence or absence of irradiating, the seeds of lupine (*Lupinus termis* L.) with gamma rays, on the nitrogen assimilation and ions uptake was investigated. Protein, amino acids, nucleic acids, and nitrate (NO_3), potassium (K) and phosphorus (P) uptake have been determined to achieve this goal. Significant decreases in the contents of protein, amino acids and nucleic acids were observed upon NaCl exposure (0.0, 500, 1000, 2000 and 3000 ppm). On the other hand, in seeds irradiated with gamma rays (10, 25, 50 and 100 Gy), these nitrogenous fractions were increased after NaCl treatments; the effect was more pronounced particularly with 25 Gy. Furthermore, the absorption rate of NO_3, K and P ions from the growth medium significantly inhibited as a result of treatment with NaCl. Meanwhile, significant increases in the uptake of these ions were obtained in response to γ-rays application, more so with 25 Gy. It is suggested that the salinity-induced metabolic changes in lupine plants might be counteracted by pretreatment the seeds with 25 Gy gamma rays.

Key Words: Gamma rays; Protein; Amino acids; Nitrate reductase; Ion uptake

INTRODUCTION

Two effects of salt stress on plant growth and development are osmotic (water stress) and ionic effect. Osmotic influence of salinity results as a consequence of salt-induced decrease in soil water potential. However, salinity-induced water stress have been reported not to be the limiting factor at cellular (Mansour, 1997) or whole plant level (Munns & Termaat, 1986). In several plant species, salinity resulted in an increase in sodium (Na) and chloride (Cl) levels and a decrease in potassium (K) and calcium (Ca) concentration (Munns, 1993).

Various biological aspects are by now reported to investigate the influence of ionizing radiation on plant growth. γ-rays applied at different doses induce substantial effects on cellular behavior (Sharabash *et al*., 1981; El-Tabbakh *et al*., 1985). As a result of exposure to gamma radiation, considerable changes have been recorded using members of various families or different species of the same family. The genus Lupinus (Papilionaceae) is known to be a rich source for lupine alkaloids (Takamatsu *et al.*, 1990). *Lupinus termis* is cultivated in the Mediterranian region for its edible seeds (Tackholm, 1974). So, lupine is considered as an important plant from medical and nutritional points of view. Therefore, the present work was conducted to study whether γ-irradiation treatment of salt stressed lupine plants could mitigate the deleterious effects of salinity. Nitrogen assimilation (protein, amino and nucleic acids) and NO_3, K and P uptake in seeds treated with NaCl or NaCl plus gamma-rays were investigated to examine the effect of γ-radiation on alleviating the deleterious influences of salinity.

MATERIALS AND METHODS

Seeds of lupine (*Lupinus termis* L.) were obtained from the Crop Institute Agricultural Research Center, Giza, Egypt. Lupine seeds were divided into two sets, one of which was irradiated with γ-rays at (10, 25, 50 and 100 Gy) emitted from cobalt 60 source. Irradiation process was performed at the National Center for Research and Radiation Technology, Nasr City, Cairo, Egypt. The other set was untreated with γ - rays and received NaCl treatment. All seeds (treated and untreated) were germinated in 10 cm Petri-dishes containing moistened filter papers in the dark at 24°C and light intensity 3000 lux 12 h/day for 4 d. The seedlings were grown in three groups: Firstly, non-treated seedlings, which were transplanted in 50% strength Hoagland nutrient solutions (Hoagland & Arnon, 1950) for 30 d that served as control. Secondly, non-treated seedlings were grown in Hoagland nutrient solutions supplemented with 500, 1000, 2000, and 3000 ppm NaCl and serve as salinized plants. Thirdly, seedlings of irradiated seeds were further subdivided to two subgroups: subgroup one were transplanted in Hoagland nutrient solution and received no NaCl treatments, while subgroup two was grown in Hoagland solution and treated with NaCl. All treatments were left to grow in aerated Hoagland solution under the previous growth conditions for 30 d. Each treatment was replicated six times. The data presented in the Tables are the means of the six replicates.

Fresh samples of each treatment were directly subjected to determination of pigments (Chlorophylls *a, b* and carotenoids) according to the method of Metzner *et al*.,

(1965). Photosynthetic machinery was carried out in the Atomic Energy Authority Radioisotopes Department, Cairo, Egypt, following the procedure of Moussa (2001). Sugar fractions content was estimated as described by Naguib (1964). Protein level was determined as given by Lowry *et al*. (1951). DNA content was quantitatively estimated as described by Burton (1965). The method of Schneider (1957) was applied to estimate RNA content.

On the other hand, Amino Acid Analyzer Model Beckman system (7300) and Data system 7000 column No. A/BID 25cm column sample vol.25 µL was used to determine the concentration of amino acids (A.O.A.C., 1984). The estimation of NO_3 was carried out using Beckman Spectrophotometer (Model 35), while K and P were determined using PYE Unicam Sp.1900 Atomic Absorption Spectrophotometer.

Statistical analysis. The data were statistically analysed by the least significant difference test (LSD) at 1 and 5% levels of probabilities according to the method of Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

NaCl-treated lupine plants exhibited significant reduction in NO_3, K and P uptake as compared with control samples (Table I). Ghoulam *et al*. (2001) reported a similar inhibition in uptake of NO_3, K and P in sugar beet. It is likely that salinity induced stomatal closure and reduced transpiration rate as it is indicated by Sultana *et al*. (1999) and Silveira *et al*. (2001). These are two important factors in nutrient acquisition. Nitrate influx is mediated by a NO_3^- transporter protein, which is induced by the ion itself (Compbell, 1999). It is evident that these three ions play an important role in plant metabolism (Cooper *et al*., 1967; Humble & Raschke, 1971; Boyle & Keys, 1987; Hopkins, 1995; Silveira, 2001) and their reduction induced by salinity will markedly affect the plant growth and development.

Several studies have shown a close relationship between NO_3 absorption and NO_3 reduction in higher plants; nitrate reductase (NR) activity is severely influenced by NaCl treatment (Abd-El-Baki *et al*., 2000). NR activity is largely dependent on NO_3 flux from roots (Ferrario *et al*., 1998). Ghoulam *et al*. (2001) demonstrated an inhibition in NR capacity of sugar beet plants submitted to salinity treatment. Application of γ-rays to the stressed lupine plants significantly stimulated the absorption of NO_3, K and P as compared with those received NaCl only (Table I). This increase might be due to the effect of such rays on accelerating the transpiration rays through increasing the stomatal aperture. Moreover, gamma rays application at low doses, have been reported to potentiate the biosynthesis of endogenous phytohormones (e.g. indole acetic acid, gibberellic acid, Cytokinins) and nitrogenous compounds (Farghal & Abd-El-Hamid, 1996b). Fraghal and Abd El-Hamid, 1996b) indicated that gamma irradiation could enhance the growth pattern which was explained by them to the effect of increased K on growth. The stimulative effect of γ-rays on P absorption reported in this study (Table I) is probably due to an interference of these rays with hormonal biosynthesis (Panjie & Jegadees, 1959).

The protein and nucleic acids (Table II) as well as amino acids (Table III) contents of lupine plants showed significant decrease in response to salt stress treatment relative to the control. Salinity has been reported to inhibit drastically the protein biosynthesis in the plant tissues (Lutts *et al*., 1999). Muthukumarasamy *et al*. (2000) demonstrated that salt-induced decrease in protein content was due to an increase in protease activity in radish. DNA replication was ceased with the onset of NaCl stress (Aspinall, 1986), which was suggested to be the result of change in hydrolytic enzymes (Sheoran & Garg, 1978; Reddy & Vora, 1985). Data of the present work suggest that salinity affected protein synthesis in lupine plants through an influence on NO_3 availability. In addition, NaCl salinity retards NO_3 reduction into ammonia which is further incorporated into amino acids, and hence diminishes the rate of protein synthesis. The induced reduction in amino acid content by salinity was explained by Lutts *et al*. (1999) to be due to depression in protein synthesis. On the other hand, γ-irradiation treatment resulted in an increase in the protein, nucleic (Table II) and amino acids levels (Table III) of lupine species. Farg (1996) noticed that low doses of gamma rays (5, 10 and 20 Gy) caused progressive increment in protein content of bean plants, which supports

Table I. Effect of NaCl salinity (ppm) for 30 d in absence or presence of gamma irradiation (Gy) on NO_3, K and P uptake by lupine plants

Salinity levels (ppm)	Gamma Radiation (Gy)														
	0			10			25			50			100		
	NO^{3-}	K	P	NO^{3-}	K	P	NO^{3-}	K	P	NO^{3-}	K	P	NO^{3-}	K	P
0	118	72	36	129	78	42	148	87	55	137 **	88	39	125 **	74	37
500	105 **	67 **	31 **	119 **	74 **	38 **	137 **	81 **	49 **	115 **	76 **	33 **	110 **	72 **	31 **
1000	86 **	60 **	23 **	98 **	68 **	30 **	122 **	74 **	40 **	106 **	67 **	32 **	90 **	68 **	30 **
2000	56 **	50 **	13 **	77 **	60 **	26**	97 **	65 **	32 **	70 **	57 **	24 **	63 **	55 **	19 **
3000	40 **	37 **	4 **	66 **	52 **	19 **	80 **	57 **	27 **	60 **	55 **	16 **	56 **	48 **	13 **
L.S.D 1%	4.3	3.1	2.0	4.3	3.1	2.0	4.3	3.1	2.0	4.3	3.1	2.0	4.3	3.1	2.0
5%	3.0	2.2	1.4	3.0	2.2	1.4	3.0	2.2	1.4	3.0	2.2	1.4	3.0	2.2	1.4

** Highly significant

Table II. Effect of NaCl salinity (ppm) for 30 d in presence or absence of gamma irradiation (Gy) on protein (mg g^{-1} Dry weight), DNA and RNA contents (μg g^{-1} Fresh weight) of lupine plants

Salinity levels (ppm)	Gamma Radiation (Gy)														
	0			10			25			50			100		
	Prot.	DNA	RNA	Prot.	DNA	RNA	Prot.	DNA	RNA	Prot.	DNA	RNA	Prot.	DNA	RNA
0	78.5	63.8	98.6	88.9	71.5	101.7	98.5	80.1	114.5	86.4	73.4	103.2	82.5	72.7	105.5
500	71.2 **	60.0 **	93.7 **	76.5 **	63.4 **	99.1 *	87.9 **	73.2 **	107.6**	77.9 **	66.7 **	96.8 **	74.7 **	60.4 **	95.8 **
1000	60.5 **	51.4 **	85.8 **	70.3 **	58.6 **	87.6 **	79.6 **	64.1 **	100.5**	68.6 **	56.9 **	88.1 **	68.6 **	58.1 **	85.6 **
2000	45.9 **	38.6 **	75.4 **	59.6 **	47.4 **	81.8 **	65.4 **	53.7 **	90.7**	57.8 **	49.2 **	80.0 **	58.2 **	45.7 **	82.0 **
3000	28.8 **	25.8 **	63.8 **	47.5 **	37.1 **	76.4 **	53.5 **	40.6 **	81.6**	50.1 **	36.5 **	71.9 **	48.7 **	35.5 **	70.6 **
L.S.D 1%	2.8	3.0	3.3	2.8	3.0	3.3	2.8	3.0	3.3	2.8	3.0	3.3	2.8	3.0	3.3
5%	2.0	2.1	2.3	2.0	2.1	2.3	2.0	2.1	2.3	2.0	2.1	2.3	2.0	2.1	2.3

** Highly significant; Prot.: Protein content

Table III. Effect of NaCl salinity (ppm) for 30 d in presence or absence of gamma irradiation (Gy) on amino acid contents (mg/100 g protein) of lupine plants

Amino acids (mg/ 100 g protein)	Salinity levels (ppm)					Gamma irradiation (Gy)				25 Gy +	
	0.0	500	1000	2000	3000	10	25	50	100	500 ppm	3000 ppm
Arginine	7.99	7.87	7.65	7.19	7.00	8.12	8.76	8.10	7.99	8.51	7.41
Aspartic acid	11.41	11.31	11.03	10.71	10.21	11.62	12.11	11.73	11.32	11.81	11.07
Serine	6.73	6.51	6.28	6.02	5.75	7.03	7.81	7.50	6.83	7.48	6.62
Glutamic acid	24.00	23.72	23.10	22.72	22.21	25.10	26.18	25.19	24.2	25.30	23.91
Proline	3.75	3.42	3.10	3.00	2.68	4.10	5.08	4.32	4.16	4.62	3.82
Glycine	3.62	3.31	3.00	2.75	2.35	3.95	4.92	4.31	3.75	4.37	3.48
Alanine	3.30	3.12	3.01	2.68	2.41	3.75	4.28	3.69	3.42	4.00	3.18
Cystine	1.33	1.21	1.02	0.81	0.52	1.72	2.51	1.88	1.62	1.98	1.41
Tyrosine	4.52	4.31	4.11	3.71	3.42	4.91	5.79	4.99	4.61	5.21	4.99
L.S.D 1%						2.3					
5%						1.4					

our results that the magnitude of enhancing protein synthesis was much more obvious with 25 GY of γ - rays. Farghal and Abd EL-Hamid (1996a) reached a similar conclusion that low doses of gamma rays accelerated the growth rate, protein and amino acids. It has also been mentioned that γ-rays applied at low doses resulted in obvious increase in IAA, GA_3 of lupine plants (Abbas, 1994). Khan and Srivastava (1998) found that GA_3 and kinetine increase the nitrogen content and the growth of maize plants, which may be the case in our work. The mitigating effect of γ - rays on the nucleic acids level in those plants treated with NaCl (Table II) was also reported by Mostafa *et al.* (2000), who observed a stimulation in RNA synthesis of embryos of castor oil plants and maize grains irradiated with γ - doses that activated plant growth and development.

Findings of the present work indicated that the adverse effect of salinity on lupine plants might be opposed upon exposure the seeds to 25 Gy gamma rays.

REFERENCES

Abbas, S.M., 1994. Some physiological effects of gamma radiation on *Lupinus termis* seedlings. *M.Sc. Thesis*, Bot. Dep., Fac. Sci., Menouf Univ., Egypt

Abd-El-Baki, G.K., F. Siefriitz, H.M. Man, H. Weiner, R. Kaldenhoff and W.M. Kaiser, 2000. Nitrate reductase in *Zea mays* L. under salinity. *Plant Cell Environ.,* 23: 515–21

A.O.A.C., 1984. *Official Methods of Analysis of Association of Official Analytical Chemists*, Benjamin Franklin Station, Washington, BC.

Aspinall, D., 1986. Metabolic effects of water and stress in relation to expansion of the leaf surface. *J. Pl. Physiol.,* 13: 59–63

Boyle, F.A. and A.J. Keys, 1987. The state of activation of ribulose–1,5–bisphosphate carboxylase in wheat leaves. *Photosyn. Res.,* 11: 97–108

Burton, K., 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of DNA. *Biochem. J.,* 62: 315–23

Compbell, W.H., 1999. Nitrate reductase structure, function and regulation: bridging the gap between biochemistry and physiology. *Ann Rev. Plant Physiol. Mol. Biol.,* 50: 277–303

Cooper, R.B., R.E. Blaser and R.H. Brwon, 1967. Potassium nutrition effects on net photosynthesis and morphology of alfalfa. *Soil Sci.,* 31: 231–5

El-Tabbakh, A.E., M.T.M. Sharabash and S.A. Azer, 1985. Effect of gamma rays on sugar beet (*Beta vulgaris*) plant. *In: Proc. Egypt. Bot. Soc.* 4, pp: 1509–22. Ismailia, Egypt

Farg, I.A., 1996. Effect of some environmental factors on certain metabolic products in *Vicia faba* seeds during their development and processing, *M.Sc. Thesis,* Institute of Environ. Studies and Res. Ain Shams Univ., Cairo, Egypt

Farghal, I.I. and K.I. Abd-El-Hamid, 1996a. Physiological studies on the effect of gamma irradiation on certain chemical constituents in Black-Cumin (*Nigella sativa* L.) seedlings II. Carbohydrate and nitrogenous fractions. *Al-Azhar Bull. Sci.,* 7: 499–510

Farghal, I.I. and K.I. Abd-El-Hamid, 1996b. Physiological studies on the effect of gamma irradiation on certain chemical constituents in Black-Cumin (*Nigella sativa* L.) seedlings b. Endogenous growth regulating substances (Auxins, growth inhibitors, Gibberellins and Cytokinins). *Al-Azhar. Bull. Sci.,* 7: 511–27

Ferrario, S., M. Valadier and C.H. Foyer, 1998. Over–expression of nitrate reductase in tobacco delays drought–induced decreases in nitrate reductase activity and mRNA. *Pl. Physiol.,* 117: 293–302

Ghoulam, C., A. Foursy and K. Fares, 2001. Effects of salt stress on growth inorganic ions and proline accumulation in relation to osmotic adjustment five sugar beet cultivars. *Environ. Exp. Bot.,* 47: 39–50

Hoagland, D.R. and D.I. Arnon, 1950. The water culture method for growing plants without soil. *Calif. Agric. Exp. Stat. Circ.,* 347: 32

Hopkins, W.G., 1995. *Introduction to Plant Physiology*. pp: 72–4. John Wiley and Sons, Inc., USA

Humble, G.D. and K. Raschke, 1971. Stomatal opening quantitatively related to potassium transport. *Pl. Physiol.,* 48: 447–53

Khan, M. and H. Srivastava, 1998. Changes in growth and nitrogen assimilation in maize plants induced by NaCl and growth regulators. *Biol. Plant.,* 41: 93–9

Lowry, O.H., J. Rosenbrough, A.C. Fan and R.J. Randal, 1951. Protein measurement with folin phenol reagent. *J. Biol. Chem.,* 193: 265–75

Lutts, S., V. Majerus and J.M. Kinet, 1999. NaCl effects on proline metabolism in rice (*Oryza sativa*) seedlings. *Physiol. Plant.,* 105: 450–8

Mansour, M.M.F., 1997. Cell permeability under salt stress. *In:* Jaiwal, P.K., R.P. Singh and A. Gulati (eds.), *Strategies for Improving Salt Tolerance in Higher Plants.* pp: 87–110. Enfield, USA

Metzner, H., H. Rau and H. Senger, 1965. Untersuchungen zur synchonisierbarteit einzelner pigmentan angel mutanten von chlorella. *Planta*, 65: 186–91

Mostafa, I.Y., M.A. Laila, M.A.S. Aly, M.A. Kord and H.A. Kamel, 2000. Manifestation of some biochemical changes in wheat and Beans due to salinity and /or γ – irradiation. *Arab. J. Sci. Appl.,* 33: 233–41

Moussa, H.R., 2001. Physiological and biochemical studies on the herbicide (DUAL) by using radiolabelled technique. *Ph.D. Thesis.* Ain Shams Univ, Cairo, Egypt

Munns, R., 1993. Physiological processes limiting plant growth in saline soils: Some dogmas and hypotheses. *Plant Cell Environ.,* 16: 15–24

Munns R. and A. Termaat, 1986. Whole plant response to salinity. *Australian J. Plant Physiol.,* 13: 143–60

Muthukumarasamy, M.S., D. Gupta and R. Panneerselvam, 2000. Influence of Triadimefon on the metabolism of NaCl stressed radish. *Biol. Plant.,* 43: 67–72

Naguib, M.I., 1964. Effect of sevin on the carbohydrate and nitrogen metabolism during germination of cotton seeds. *Indian J. Expt. Biol.,* 11: 149–52

Panje, R.R. and P.P.R. Jagadees, 1959. The effect of ionizing radiation on sugarcane. *In: Proc. Int. Soc. Sugarcane Tech.,* 10th, 775.

Reddy. M.P. and A.B. Vora, 1985. Effect of salinity on protein metabolism in Bazra (*Pessisetum typhoides* S. and H.) leaves. *Indian J. Plant Physiol.,* 28: 190–5

Schneider, W.C., 1957. "*Colorimetric analysis of sugars" Methods in Enzymology*. Grossman and Moldave (eds.) XII (Part A), 557.

Sharabash, M.T.M., H.E. Olfat, T.Y. Rizk and A.H.A. Hamed, 1981. Physiological response of sugarcane to gamma irradiaition. *Res. Bull. (Fac. Agric. Ain Shams Univ., Cairo, Egypt),* 1446

Sheoran, I.S. and O.P. Garg, 1978. Effect of salinity on the activities of RNAase and Protease during germination and early seedlings growth of mungbean. *Biol. Plant.,* 20: 392–6

Silveira, J.A.G., A.R.B. Melo, R.A. Viegas and J.T.A. Oliveira, 2001. Salinity–induced effects on nitrogen assimilation related to growth in cowpea plants. *Environ. Exp. Bot.,* 46: 171–9

Snedecor, G.W. and W.G. Cochran, 1967. *Statistical Methods*. 6th Ed. P. 275. Iowa State Univ. Press Ames. Iowa, USA

Sultana, N., T. Ikeda and R. Itoh, 1999. Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. *Environ. Exp. Bot.,* 42: 211–20

Tackholm, V., 1974. *Student Flora of Egypt.* 2nd Ed. P. 224. Cairo Univ. Press, Cairo, Egypt

Takamatsu, S., K. Saito, T. Sekine, S. Ohmiya and H. Kubo, 1990. Glycosidic alkaloids from *Lupinus hirsutus. Phytochem*., 29: 3923–6

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