

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Growth and Some Physiological Attributes of Pea (*Pisum sativum* L.) As Affected by Salinity

¹F. Najafi, ¹R.A. Khavari-Nejad, ²F. Rastgar-jazii and ³M. Sticklen

¹Department of Biology, Tarbiat Moallem University, P.O. Box 15815-3587, Tehran, Iran

²National Research Center for Genetic Engineering and Biotechnology,
P.O. Box 14155-6343, Tehran, Iran

³Department of Crop and Soil Science, Michigan State University, Plant and Soil Science Building,
East Lansing, Michigan 48824, USA

Abstract: The effects of salt stress were studied on growth and physiology of pea (*Pisum sativum* L. cv. Green Arrow) in a pot study. Pea plants were treated with NaCl at 0, 10, 30, 50 and 70 mM in Hoagland solution. Plants were harvested after 21 days for measurements of physiological parameters. The highest NAR and RGR were found in 10 mM NaCl. However, in 70 mM NaCl, RGR and RLGR were significantly decreased in respect of other concentrations of NaCl. In 50 and 70 mM NaCl, chlorophylls contents and photosynthetic rate, were significantly decreased and CO₂ compensation concentration and respiration rate increased in comparison with control. In 10 and 30 mM NaCl gas exchanges and chlorophyll contents were not significantly decrease in respect of control. Results indicated that *Pisum sativum* L. cv. Green Arrow can tolerate to 70 mM NaCl, also growth of plants in 10 and 30 mM NaCl was better than that of those in 0 mM NaCl.

Key words: Growth, *Pisum sativum* L., Photosynthetic rate, salinity, respiration

INTRODUCTION

Plants are influenced by a variety of biotic or abiotic stresses, such as drought, salt loading, and freezing as changes in their development, growth and productivity. One of the major abiotic stresses that affect plant productivity is water stress resulting from drought or salinity (Gueta-Dahan *et al.*, 1997).

There is increasing evidence that salinity changes photosynthetic parameters, including osmotic and leaf water potential, transpiration rate, leaf temperature and relative leaf water content. Salinity can seriously change the photosynthetic carbon metabolism, leaf-chlorophyll content, as well as photosynthetic efficiency (Seemann and Critchley, 1985; Sharkey, 1985). Changes in these parameters depend on the severity and duration of stress (Lakshmi *et al.*, 1996; Misra *et al.*, 1997) and on plant species (Dubey, 1994).

The effect of salinity on growth, nodulation and N₂ fixation of legumes plants has been studied (Lauter and Munns, 1986; Elsheikh and Wood, 1990). The adaptation to salinity stress is accompanied by alternations in the levels of numerous metabolites, proteins and mRNA (Serrano, 1996). Various genes, expression of which is activated in response to salt stress, have been identified (Shinozaki, 1999; Kawasaki *et al.*, 2001). Some of these

genes encode for protective proteins such as osmotin (Zhu *et al.*, 1995), late embryogenesis abundant (LEA) proteins (Espelund *et al.*, 1992) and ion transporters (Blumwald, 2000) others code for enzymes that participate in metabolic processes specifically triggered by salinity stress (Cushman, 1992; Gong *et al.*, 2001).

In this research, physiological changes of pea plants in saline environment are studied. Results indicated that, in 10 and 30 mM NaCl, pea plants have more growth rate than control (0 mM NaCl) plants and also they can tolerant to 70 mM NaCl.

MATERIALS AND METHODS

Pea (*Pisum sativum* L. cv. Green Arrow) seeds were prepared from Agricultural Research Center, IRAN. Pea seeds were surface-sterilized in 70% (v/v) ethanol (1 min) followed by 1% (w/v) sodium hypochlorite (20 min) and washed five times with sterile distilled water (Schroeder *et al.*, 1993).

Seeds germinated in pots containing perlite in a growth chamber under a 24°C temperature and at a relative humidity of 70%. Germinated seeds were translated to pots in growth chamber with 17 h light periods and 300 $\mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ light intensity, day/night temperatures of 25/18°C and irrigated with a Hoagland's

solution. After 10 days, the seedlings were transplanted in the saline nutrient solutions containing 0, 10, 30, 50 and 70 mM sodium chloride, with pH 6.5 and fresh nutrient solution replaced the old every week. The plants were grown under controlled environment (17 h light periods, 300 $\mu\text{mol quanta m}^{-2} \text{sec}^{-1}$ light intensity, day/night temperatures of 25/18°C) in a greenhouse. After 21 days of experimental duration, for per physiological analysis from per treatment four plants were harvested.

Chlorophylls a and b and total chlorophylls were estimated as described by Arnon (1949). Photosynthetic rate (P_n), respiration rate and CO_2 compensation concentration (Γ) were determined from intact plants, employing an Infrared Gas (CO_2) Analyzer (IRGA) as described by Khavari Nejad (1980, 1986). Growth analyses were carried out using the equations of Watson (1952) and Evans and Hughes (1962). The research was conducted using completely randomized design with four replications. Data were analyzed statistically using SAS software.

RESULTS

Growth: Fresh and dry weight of the stem in 50 and 70 mM NaCl were significantly lower than those of plants treated with 0, 10 and 30 mM NaCl. Increasing fresh and dry weight of leaf in 10 and 30 mM NaCl were not significant in respect of control.

NAR, RLGR and SLA were not significantly decreased in different treatments of NaCl. However, LWCA in 50 and 70 mM NaCl was higher than that of plants in 0, 10 and 30 mM NaCl also RGR was significantly decreased in 70 mM NaCl (Table 1).

Gas exchange: In 70 mM NaCl, P_n was significantly decreased in respect of other treatments. However, P_n was not significantly decreased in 10, 30 and 50 mM NaCl in respect of control. Respiration rate in 50 and 70 mM NaCl was higher than that of plants in 0, 10 and 30 mM NaCl. CO_2 compensation concentration with increasing concentration of NaCl was not significantly enhanced (Table 2).

Chlorophylls contents: Concentration of chlorophylls of leaves in 50 and 70 mM NaCl were significantly decreased in respect of 0, 10 and 30 mM NaCl. In 70 mM NaCl Chl.a/ Chl.b ratio was decreased in respect of other treatments (Table 3).

DISCUSSION

Decreasing effects of NaCl on growth have been reported in legumes, as well as the chick-pea (Dua, 1992), soybean (Singleton and Bohlool, 1984) and faba bean (Zahran and Sprent, 1986; Cordovilla *et al.*, 1996). In the present work, the effect of NaCl on pea plants were investigated and indicated that *Pisum sativum* L. cv. Green Arrow was tolerant to 70 mM NaCl and growth in 10 and 30 mM were increased in respect of control.

Gas exchanges were more sensitive to salinity than growth parameters. Amongst damages caused by salinity stress in plants, the reduction of photosynthetic processes is one of the most important (Delfine *et al.*, 1999). Our results indicated that in 70 mM NaCl, P_n was significantly decreased and respiration rate and Γ were increased in respect of control. However, 10, 30 and 50 mM NaCl had a low effect on gas exchanges (Table 2).

Table 1: Effects of NaCl on growth parameters, Means (\pm SE) of four replicates, Numbers followed by the same letter are not significantly different ($p>0.05$).

Growth parameters	NaCl (mM)				
	0	10	30	50	70
Mean final values					
LFM (g)	1.892 \pm 0.12a	2.355 \pm 0.35a	2.420 \pm 0.03a	2.572 \pm 0.23a	2.465 \pm 0.15a
SFM (g)	0.894 \pm 0.04a	0.773 \pm 0.11ab	0.603 \pm 0.02bc	0.492 \pm 0.04c	0.422 \pm 0.02c
RFM (g)	1.292 \pm 0.33a	1.236 \pm 0.10a	1.432 \pm 0.13a	1.120 \pm 0.08a	1.260 \pm 0.22a
LDM (g)	0.214 \pm 0.01a	0.275 \pm 0.04a	0.275 \pm 0.00a	0.280 \pm 0.03a	0.201 \pm 0.01a
SDM (g)	0.093 \pm 0.01a	0.090 \pm 0.01a	0.062 \pm 0.00b	0.050 \pm 0.01b	0.048 \pm 0.01b
RDM (g)	0.074 \pm 0.02a	0.073 \pm 0.00a	0.070 \pm 0.00a	0.050 \pm 0.00a	0.052 \pm 0.00a
LA(cm^2)	102.9 \pm 13.6a	91.45 \pm 14.4a	81.05 \pm 5.44a	74.18 \pm 5.9a	75.38 \pm 2.05a
NAR ($\text{g m}^{-2} \text{day}^{-1}$)	5.48 \pm 0.45a	6.07 \pm 0.41a	5.95 \pm 0.47a	5.00 \pm 0.42a	5.00 \pm 0.18a
RGR ($\text{g kg}^{-1} \text{day}^{-1}$)	85.3 \pm 1.95ab	93.7 \pm 3.6a	89.1 \pm 2.9ab	84.8 \pm 5.5ab	81.7 \pm 2.4b
RLGR ($\text{cm}^2 \text{m}^{-2} \text{day}^{-1}$)	1491 \pm 56.9a	1410 \pm 44.7ab	1424 \pm 45.7ab	1400 \pm 54.5ab	1332.0 \pm 30.0b
SLA ($\text{m}^2 \text{kg}^{-1}$)	31.9 \pm 2.50a	33.9 \pm 1.33a	31.9 \pm 0.73a	32.3 \pm 2.60a	35.7 \pm 1.22a
LWCA ($\text{g (H}_2\text{O) m}^{-2}$)	189.5 \pm 15.8c	228.6 \pm 8.7b	247.4 \pm 5.0ab	270.0 \pm 18.0a	276.3 \pm 15.0a

Table 2: Effects of NaCl on photosynthetic rate (P_n), respiration rate, CO_2 compensation concentration (Γ), Means (\pm SE) of four replicates, Numbers followed by the same letter are not significantly different ($p>0.05$).

Gas exchanges	NaCl (mM)				
	0	10	30	50	70
Photosynthesis rate ($\mu\text{mol (CO}_2\text{) dm}^{-2} \text{sec}^{-1}$)	18.25 \pm 0.80a	18.15 \pm 0.80a	16.50 \pm 0.86a	18.17 \pm 0.31a	12.80 \pm 1.40b
Respiration rate ($\mu\text{mol (CO}_2\text{) dm}^{-2} \text{sec}^{-1}$)	16.0 \pm 3.20b	14.8 \pm 1.20b	21.0 \pm 1.70ab	26.3 \pm 0.78a	27.0 \pm 2.30a
CO_2 compensation concentration ($\mu\text{L L}^{-1}$)	155 \pm 5.60a	165 \pm 3.70a	158 \pm 12.4a	161 \pm 7.50a	178 \pm 11.0b

Table 3: Effects of NaCl on chlorophylls content and chl.a/chl.b ratio, Means (\pm SE) of four replicates, Numbers followed by the same letter are not significantly different ($p>0.05$)

Chlorophylls content	NaCl (mM)				
	0	10	30	50	70
Chl.a (mg g ⁻¹ dry matter)	10.47 \pm 0.25a	9.95 \pm 0.35ab	8.94 \pm 0.70bc	8.12 \pm 0.40c	8.06 \pm 0.42c
Chl.b (mg g ⁻¹ dry matter)	3.94 \pm 0.12a	3.78 \pm 0.13ab	3.47 \pm 0.3abc	3.13 \pm 0.16c	3.3 \pm 0.2bc
Chl. (a+b) (mg g ⁻¹ dry matter)	14.4 \pm 0.37a	13.7 \pm 0.46ab	12.4 \pm 0.98bc	11.27 \pm 0.55c	11.37 \pm 0.62c
Chl.a/Chl.b ratio	2.65 \pm 0.03a	2.63 \pm 0.045a	2.58 \pm 0.03a	2.58 \pm 0.03a	2.45 \pm 0.05b

Growth parameters are ultimately a function of total photosynthetic capacity and comparison of photosynthetic and growth responses to salinity illustrate that with decreasing photosynthetic rate in 70 mM NaCl, RGR and RLGR are significantly decreased in respect of another concentrations of NaCl.

LWCA was significantly enhanced in all treatments. As described earlier (Tester and Davenport, 2003) an ability to grow in saline conditions has been attributed to an ability to close stomata. In fact both glycophytes and halophytes tend to show reduced stomatal conductance in high NaCl conditions (Ball, 1988; Robinson *et al.*, 1997; James *et al.*, 2002). The productivity of crops in saline soils is limited to a large extent by the low rate of gas exchange imposed by the drought component of NaCl stress and further reduction in stomatal conductance and photosynthetic rate is unlikely to improve plant productivity (Tester and Davenport, 2003).

In this study, in plants treated with 50 and 70 mM NaCl, chlorophylls contents were significantly decreased as compared with control. However, decreasing chlorophylls contents in plants grown in 10 and 30 mM NaCl were not significant, the decline in chlorophyll a/b ratio of plants treated with 70 mM NaCl was significantly higher than that of plants treated in others treatments. Decrease in chlorophyll concentration in salinized plants could be attributed to increased activity of chlorophyll-degrading enzyme chlorophyllase (Reddy and Vora, 1986).

It is concluded that pea plants (*Pisum sativum* L. cv. Green Arrow) are resistant to 50 and 70 mM NaCl and their growth is higher in 10 and 30 mM NaCl than that of plants grown in 0 mM NaCl.

REFERENCES

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol., 24: 1-15.
 Ball, M.C., 1988. Salinity tolerance in the mangroves, *Aegiceras corniculatum* and *Avicennia marina*. 1. Water use in relation to growth, carbon partitioning and salt balance. Aust. J. Plant Physiol., 15: 447-464.
 Blumwald, E., 2000. Sodium transport and salt tolerance in plants. Curr. Opin. Cell Biol., 12: 431-434.

Cordovilla, M.P., F. Ligerio and C. Lluch, 1996. Growth and nitrogen assimilation in nodules in response to nitrate levels in *Vicia faba* under salt stress. J. Exp. Bot., 47: 203-210.
 Cushman, J.C., 1992. Characterization and expression of a NADP-malic enzyme cDNA induced by salt stress from the facultative crassulacean acid metabolism plant, *Mesembryanthemum crystallinum*. Eur. J. Biochem., 208: 259-266.
 Delfine, S., A. Alvino, M.C. Villani and F. Loreto, 1999. Restrictions to carbon dioxide conductance and photosynthesis in spinach leaves recovering from salt stress. Plant Physiol., 119: 1101-1106.
 Dua, R.P., 1992. Differential response of chick pea (*Cicer arietinum*) genotypes to salinity. J. Agric. Sci., 119: 367-371.
 Dubey, R.S., 1994. Protein Synthesis by Plants Under Stressful Conditions. In: Handbook of Plant and Crop Stress. Pessaraki, M. (Ed.), Marcel Dekker, New York, pp: 277-299.
 Elsheikh, E.A.E. and M. Wood, 1990. Effect of salinity on growth, nodulation and nitrogen yield of chickpea (*Cicer arietinum* L.). J. Exp. Bot., 41: 1263-1269.
 Espelund, M., S. Saeboe-Larssen, D.W. Hughes, G.A. Galav, F. Larsen and K.S. Jakobsen, 1992. Late embryogenesis-abundant genes encoding proteins with different numbers of hydrophilic repeats are regulated differentially by abscisic acid and osmotic stress. Plant J., 2: 241-252.
 Evans, G.C. and A.P. Hughes, 1962. Plant growth and the aerial environment. III on the computation of unit leaf rate. New Phytol., 61: 322-327.
 Gong, Z., H. Koiwa, M.A. Cushman, A. Ray, D. Bufford, S. Kore-eda, T.K. Matsumoto, J. Zhu, J.C. Cushman, R.A. Bressan and P.M. Hasegawa, 2001. Genes that are uniquely stress regulated in salt overly sensitive (SOS) mutants. Plant Physiol., 126: 363-375.
 Gueta-Dahan, Y., Z. Yaniv, B.A. Zilinskas and G. Ben-Hayyim, 1997. Salt and oxidative stress: Similar and specific responses and their relation to salt tolerance in citrus. Planta, 203: 460-469.
 James, R.A., A.R. Rivelli, R. Munns and S. Von Caemmerer, 2002. Factors affecting CO₂ assimilation, leaf injury and growth in salt-stressed durum wheat. Functional Plant Biol., 29: 1393-1403.

- Kawasaki, S., C. Borchert, M. Deyholos, H. Wang, S. Brazille, K. Kawai, D. Galbraith and H.J. Bohnert, 2001. Gene expression profiles during the initial phase of salt stress in rice. *Plant Cell*, 13: 889-905.
- Khavari Nejad, R.A., 1980. Growth of tomato plants in different oxygen concentrations. *Photosynthetica*, 14: 326-336.
- Khavari Nejad, R.A., 1986. Carbon dioxide enrichment preconditioning effects on chlorophylls contents and photosynthetic efficiency in tomato plants. *Photosynthetica*, 20: 315-317.
- Lakshmi, A., S. Ramanjulu, K. Veeranjanyulu and C. Sudhakar, 1996. Effect of NaCl on photosynthesis parameters in two cultivars of mulberry. *Photosynthetica*, 32: 285-289.
- Lauter, D.J. and D.N. Munns, 1986. Salt resistance of chickpea genotypes in solutions salinized with NaCl or Na₂SO₄. *Plant and Soil*, 95: 271-279.
- Misra, A.N., S.M. Sahu, M. Misra, P. Singh, I. Meera, N. Das, M. Kar and P. Shau, 1997. Sodium chloride induced changes in leaf growth and pigment and protein contents in two rice cultivars. *Biol. Plant.*, 39: 257-262.
- Reddy, M.P. and A.B. Vora, 1986. Changes in pigment composition, hill reaction activity and saccharides metabolism in bajra (*Pennisetum typhoides* Sand H) leaves under NaCl salinity. *Photosynthetica*, 20: 50-55.
- Robinson, M.F., A.A. Very, D. Sanders and T.A. Mansfield, 1997. How can stomata contribute to salt tolerance?. *Ann. Bot.*, 80: 387-393.
- Schroeder, H.E., A.H. Schotz, T. Wardley-Richardson, D. Spencer and T.J.V. Higgins, 1993. Transformation and regeneration of two cultivars of pea (*Pisum sativum* L.). *Plant Physiol.*, 101: 751-757.
- Seemann, J.R. and C. Critchley, 1985. Effects of salt stress on the growth, ion content, stomatal behaviour and photosynthetic capacity of salt-sensitive species, *Phaseolus vulgaris* L. *Planta*, 164: 151-162.
- Serrano, R., 1996. Salt tolerance in plants and microorganisms: Toxicity targets and defense responses. *Int. Rev. Cytol.*, 165: 1-52.
- Sharkey, T.D., 1985. Photosynthesis in intact leaves of C₃ plants: Physics, Physiology and rate limitations. *Botanic. Review*, 51: 53-105.
- Shinozaki, K., 1999. Plant response to drought and salt stress: Overview, *Tanpakushitsu Kakusan Koso.*, 44: 2186-2187.
- Singleton, P.W. and B.B. Bohlool, 1984. Effect of salinity on the nodule formation by soybean. *Plant Physiol.*, 74: 72-76.
- Tester, M. and R. Davenport, 2003. Na⁺ tolerance and Na⁺ transport in higher plants. *Ann. Bot.*, 91: 503-527.
- Watson, D.J., 1952. The physiological basis of variation in yield. *Adv. Agron.*, 4: 101-145.
- Zahran, H.H. and J.I. Sprent, 1986. Effects of sodium chloride and polyethylene glycol on root-hair and nodulation of *Vicia faba* L. plants by *Rhizobium leguminosarum*. *Planta*, 167: 303-309.
- Zhu, B., T.H. Chen and P.H. Li, 1995. Expression of three osmotin-like protein genes in response to osmotic stress and fungal infection in potato. *Plant Mol. Biol.*, 28: 17-26.