

PHYSIOLOGICAL RESPONSES OF PEA (*Pisum sativum* cv. Meteor) TO IRRIGATION SALINITY

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The effects of irrigation water or soil salinity on physiological aspects of pea (*Pisum sativum* cv. Meteor) plants were contrived. Ten weeks old pea plants were treated with NaCl at 0, 40, 90 and 140 mM in nutrient solution. Plants were grown in controlled environment and harvested at each 3 days interval for decisiveness of physiological parameters. Photosynthetic rate, relative water content, stomatal conductance and chlorophyll contents reduced by increasing the NaCl concentration while CO₂ concentration and free proline content intensified. By experiment it was adumbrated that high salinity level along with prolonged accentuate duration is more drastic to pea plants physiology. Results also exhibited that pea plants could indulge 40 and 90 mM NaCl but are sensitive to 140 mM.

Keywords: Pea; salt stress; sodium chloride; stomatal conductance; chlorophyll

INTRODUCTION

Many parts of the world are facing two of the most serious soil problems i.e. salinity and water logging. Salt-affected soils exist mostly under arid and semi arid climate and cover about 955 million hectares (Szabolcs, 1991). Despite the various control measures, about 20 million hectares of land go out of production (Malcolm, 1993). Nearly 10% of the total land surface of the world is covered with different types of salt-affected soils (Passarakli *et al.*, 1991). It has been appraised that salinity decreases crop production on 40 x 10⁶ hectares, one -third of the world's irrigated land (Mass and Hoffman, 1977) however, El-Ashry *et al.*, (1985) reported that on 20 x 10⁶ hectares salinity is reducing the crop yield seriously while millions of hectares of the irrigated land lie idle due to high salinity. Pakistan occupies a total geographical area 79.61 x 10⁶ hectares out of which 35.27 x 10⁶ hectares are considered suitable for cultivation while only 20.36 x 10⁶ hectares are actually under cultivation (Anonymous 2004-05). An area of 16.23 x 10⁶ hectares is irrigated through canals and tube wells and the remaining 4.13 x 10⁶ hectares is dependent on rain (Rafiq, 1990). The estimates of salt affected land in Pakistan ranges between 4.85 x 10⁶ hectares (Muhammad, 1988). Salinity affects two plant processes: water relations and ionic relations. During initial exposure to salinity, plants experience water stress which in turn reduces leaf expansion. The long period of salinity cause ionic stress, it results in leaf senescence and thus reducing the photosynthetic rate (Ahmad, 2005; Cramer and Nowak 1992). Reduced photosynthetic rate with increasing salinity level is also to be related with stomatal closure. Salinity excutes the osmotic potential, leaf water potential, transpiration,

leaf temperature along with photosynthetic components such as enzymes, chlorophyll and carotinoids. All these changes depends upon the salinity level and on stress period (Lakshmi *et al.*, 1996; Misra *et al.*, 1997). Most of the water applied to vegetables in Pakistan is abundant with different salts which not only reduce the quality but also limit our yield. So there is a need to produce and recognize the salt resistant vegetable cultivars. Pea is a member of family leguminosae. Peas are excellent human food, either eaten as vegetable or in soup. Most of the vegetables are irrigated with saline water which not only effect the yield but also on plant physiology. Due to pea importance it is necessary to study salt effect on its physiology. In this experiment physiological change of pea plants in saline environment were explored. Results showed that with decreasing relative water content of leaves, stomatal conductance and photosynthetic rate decreases. In same way by increasing salinity level free proline content in plants was increased.

MATERIAL AND METHOD

Pea seeds (*Pisum sativum* cv. Meteor) were surface sterilized by dipping them in 80% ethanol for 2.5 min and in 4% sodium hyper chlorite for 12 min and grown with Hoagland solution. After 12 days seedlings were treated with sodium chloride at 0, 40, 90, and 140 mM in nutrient solution at pH of 6.5. The plants were grown in totally controlled environment (18h light period and day/night temperature 25/18 °C) in a green house. Four plants were harvested after 4, 7, 10, 13 and 16 days of growth for physiological analyses. Relative Water Content was estimated by recording the saturated mass of 0.5g fresh leaf samples. This was

done by keeping the leaves in water for 3.5h and then dried in hot air oven until content by mass achieved as described by (Whetherley., 1950). Chlorophyll content were examined by method described by Arnon (1949). Free proline concentration was determined by Bates *et al.* (1973). Photosynthetic rate and carbon dioxide compensation were evaluated by Khavari-Nejad method (1986). Stomatal conductance was determined with delta-T porometer.

RESULTS

As relative water content and water potential decreased, photosynthetic rate decreases (Lawlor, 1995). At 40mM and 90mM salinity levels photosynthetic rate was significantly decreased at the 10 days sampling, but in 4 and 7 days sampling it was not significantly decreased. At 140mM salinity level photosynthetic rate decreased to very low level after 13 days sampling and relative water content at this level were decreased to 83, 80, and 78% respectively after 7, 10, and 13 days sampling (table 1). Salinity levels 40

and 90 mM after 10 days sampling showed 85.5% relative water content. Carbon dioxide compensation concentration increased as relative water contents decreased (Lawlor, 2002) and this increase occurred in all the treatments but was maximum at 140mM (table 2). Stomatal conductance was low at 140mM salinity level as compared to other two levels. Salinity levels 40mM and 140mM at 7 days sampling gave significantly reduced stomatal conductance (table 1). Proline content of leaves was increased at all salinity levels but 140mM give maximum as compared to 40mM and 90mM levels. Chlorophyll contents of leaves at 90mM and 140mM salinity levels after 7 days sampling were significantly decreased as compared to 40mM salinity level (table 1).

DISCUSSION

Stomata often close in response to drought (Socias *et al.*, 1997). In this experiment stomatal conductance was decreased by increasing the sodium chloride concentration in culture medium. Stomatal

Table 1. Effect of NaCl on Photosynthetic rate (P_n), Carbon dioxide conc. (Γ), Stomatal conductance (g_s), Relative water content (RWC) and Proline content salt stress pea plants during experimental period

NaCl (mM)	Time of Sampling (Day)	P_n ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ S}^{-1}$)	Γ ($\mu\text{L L}^{-1}$)	g_s (mm s^{-1})	RWC (%)	Free praline ($\mu\text{g g}^{-1}$ fresh leaf)
0	4	3.4cde	111d	3cde	90abc	2.5g
	7	3.4cde	100d	5b	92ab	2.2g
	10	4.4c	97d	5b	92ab	1.7g
	13	5.2b	108d	6.7a	93ab	2.1g
	16	6.1a	103d	6.7a	95a	4.6g
40	4	3.1cde	143c	3.7de	93ab	4.3g
	7	2.9def	147c	4.2cd	89abc	14.5f
	10	2.7ef	147c	4.9c	90abc	20.2f
	13	2.1fg	144c	4.6cd	82ef	41.1e
	16	0.5h	174b	0.4ij	85cde	50.3d
90	4	4.2cd	164b	3.5df	89abc	20.5f
	7	3.5cde	158bc	3.1efg	89abc	38.7d
	10	1.6g	166b	2.0gh	87abc	60.0d
	13	1.6g	177b	2.0gh	81def	83.1bc
	16	0.6h	210a	0.2ij	80ef	92.0bc
140	4	3.1cde	204a	2.4fgh	89ef	26f
	7	1.9efg	169b	1.3hd	84def	60d
	10	2.1fg	198g	0.8fg	80ef	100b
	13	0.3h	205g	0.7g	78f	236a
	16	0.0.	00.0	0.0	00.0	00.0

Table 2. Effect of NaCl on chlorophyll a&b, total chlorophyll, chlorophyll a/b ratio of salt stress pea plants

NaCl (mM)	Time of Sampling (Day)	Chl.a (mg g ⁻¹ fresh metter)	Chl.b(mg g ⁻¹ fresh metter)	Chl.a+ Chl.b (mg g ⁻¹ fresh metter)	Chlorophyll a/b ratio
0	4	1.45defg	0.70cde	2.20def	2.05bcd
	7	1.78cde	0.78abc	2.70cd	2.21bcd
	10	1.85cd	0.87ab	2.78c	2.14bcd
	13	2.40b	0.91a	3.18b	2.46abc
	16	2.75a	0.91a	3.73	3.14a
40	4	1.27fgh	0.60def	1.90efgh	1.93bcd
	7	1.62cdef	0.69cd	2.37cde	2.27bcd
	10	1.68cdef	0.63cde	2.35cde	2.38abcd
	13	1.95bcd	0.73abc	2.71c	2.49abcd
	16	1.97bc	0.78abc	2.75c	2.59ab
90	4	1.04gh	0.52ef	1.58gh	1.93bcd
	7	0.93h	0.54cd	1.51h	1.68d
	10	1.26fgh	0.69bcd	1.99efgh	1.83bcd
	13	1.30gh	0.75f	2.08efg	1.72cd
	16	1.03gh	0.51f	1.59gh	2.06bcd
140	4	1.01gh	0.51ef	1.63gh	1.99bcd
	7	1.10gh	0.56def	1.71fgh	1.93bcd
	10	1.17fgh	0.62fgh	1.75fgh	1.79bcd
	13	1.3efgh	0.6def	1.9efgh	2.01bcd
	16	0.0	0.0	0.0	0.0

conductance in plants grown at 40mM and 90mM sodium chloride levels after 7 days sampling was decreased but at 140mM sodium chloride after 4 days sampling decreased (table 1) .In this research relative water content in plants treated with 40 and 90 mM sodium chloride after 13 days sampling was significantly decreased, however in plants treated with 140 mM sodium Chloride after 4 days, relative water content was reduced which leads to loss of Turger pressure. Infact leaf water status interacts with stomatal conductance and transpiration .The major effect of salinity is reduction in photosynthetic processes (Delfine *et al.*,1999).Results showed that with decrease in stomatal conductance , photosynthetic rate was also decreased .At 40, 90, and 140mM salinity levels photosynthetic rate was significantly decreased after 4, 7, and 13 days sampling respectively(table I). By this study it was also come to know that a high degree of coregulation between stomatal conductance and photosynthetic rate was found (Farquhar *et al.*, 2001).Chlorophylls contents were decreased with increase in salinity level as compared with control. The decrease in chlorophyll

a/b ratio of plants treated with 90 and 140mM salinity level. Oxidative stress in Pea leaves chloroplasts was occurred under the effect of salinity(Gomez *et al.*, 2004).The carbon dioxide compensation at all sodium chloride levels was increased (table I) .A significant increase of free proline at all salinity levels in all plants was observed. It was noted that disturbances in photosynthetic metabolism cause proline accumulation and this accumulation is beneficial as it protects membranes and proteins as relative water content decreases particularly against increased ionic concentrations. Results in agreement with the results of Fedina and Popova (1996).

CONCLUSION

By this research work it was finally concluded that pea plants (*Pisum sativum* cv.Meteor) are more resistant to 40 and 90 mM sodium chloride at the initial stages of salinity application, but more sensitive with increasing duration of salinity. This cultivar is strongly sensitive to 140mM NaCl and salinity damages were significantly observed in initial sampling.

REFERENCES

- Ahmad, P. and J. Riffat. 2005. Effect of salt stress on growth and biochemical parameters of *Pisum sativum*. Archives of Agronomy and Soil Science, 51: 665-672.
- Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta Vulgaris*. Plant Physiol. 24: 1-15.
- Anonymous. 2004. Agriculture Statistics of Pakistan. Food and Agriculture Division, Ministry of Agriculture and Livestock, Govt. of Pakistan, Islamabad.
- Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water stress studies. Plant Soil. 39: 205-207.
- Cramer, G.R. and R.S. Nowak. 1992. Supplemental manganese improves the relative growth, net assimilation and photosynthetic rate of salt stressed barley. Physiologiae Plantarum 84: 600-605.
- 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.
- El-Ashry, M.T., J.V. Schilfgaarde and S. Schiffman. 1985. Salinity pollution from irrigated agriculture. Soil Water Conserv. 40: 48-52.
- Farquhar, G.D., S.V. Caemmerer and J.A. Berry. 2001. Modles of photosynthesis. Plant Physiol. 125: 42-45.
- Fedina, I.S. and A.V. Popova. 1996. Photosynthesis, photorespiration and proline accumulation in water stressed pea leaves. Photosynthetica 32: 213-220.
- Gomez, *et al.* 2004. Location and effects of long term NaCl stress on superoxide dismutase and ascorbate peroxidase isoenzymes of pea (*Pisum sativum* cv. Puget) Chloroplasts. J. Exp. Bot. 55: 119-130.
- Khavari-Nejad, R.A. 1986. Carbon dioxide enrichment preconditioning effects on chloroplasts contents and photosynthetic efficiency in tomato plants. Photosynthetica 20: 315-317.
- Lakshmi, A., S. Ramanjuhu, K. Veeranjanyulu and C. Sudhakar. 1996. Effect of NaCL on photosynthesis parameters in two cultivars of mulberry. Photosynthetica 32: 285-289.
- Lawlor, D.W. 1995. The effects of water deficit on photosynthesis. In: Smirnoff, N (Ed). Environment and plant metabolism. Oxford: BIOS Scientific Publishers, pp. 129-160.
- Lawlor, D.W. 2002. Limitation to photosynthesis in water stressed leaves: Stomata vs. Metabolism and role of ATP. Arn. Bot. 89: 871-885.
- Malcolm. C.V. 1993. The potential of halophytes for rehabilitation of degraded land in: productive use of saline land. [N. Davidson and R. Galloway. (Eds.)]. ACIAR Pro. 42: 8-11.
- Mass, E.V. and G.J. Hoffman. 1977. Crop Salt tolerance Current assessment. J. Irrig. Drainage Div. Amer. Soc. Civil Engg. 103: 115-134.
- Muhammd, S. 1988. Salt-affected soils and their reclamation. Pak. Sci. Conf. Karachi. Dec. 26-30, Pak. Assoc. Adv. Sci. 6-B, Gulberg II, Lahore II.
- Misra, A.N., S.M Sahu and M. Misra. 1997. Sodium chloride induced changes in leaf growth and pigment and protein contents in two rice cultivars. Biol. Plant. 39: 257-262.
- Passarakli, M., T.C. Tucker and K. Nakabayashi. 1991. Growth response of barley and wheat to salt stress. J. Plant Nutr. 14: 331-340.
- Rafiq. N.M. 1990. Soil resources and soil related problems in Pakistan. In soil physics application under stress environments. [M. Ahmed. M.E. Akthar and M.I. Nizami (Eds.)]. pp. 315-324. Kluwer Acad. Pub., The Netherlands.
- Socias, F.X., M.J. Correia, M.M. Chaves and H.M. Medrano. 1997. The role of abscisic acid and water relations in drought responses of Subterranean Clover. J. Exp. Bot. 48: 1281-1288.
- Szabolcs, I. 1991. Desertification and salinization. In: plant salinity research. New challenges. [R. Choukr-allah (Ed.)]. Proc. Conf. on agriculture management of salt-affected areas. Ajgadir. Morocco.
- Whetherley, P.E. 1950. Studies in the water relations of cotton plants. The field measurement of water deficit in leaves. New Phytol. 49: 81-87.