

EFFECTS OF NaCl SALINITY ON SHOOT GROWTH, STOMATAL SIZE AND ITS DISTRIBUTION IN *ZEA MAYS* L.

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Abstract

The effects of NaCl induced salinity on shoot growth, stomatal size and its distribution in *Zea mays* c.v. Sunahry were studied in nutrient solution. Shoot growth significantly reduced with increasing salinity stress whereas relative growth rate, leaf area, stomatal number, stomatal size and stomatal index showed inverse relationship. The relative growth rate was slightly greater between 1st and 3rd harvest than between 1st and 2nd and 2nd and 3rd harvests.

Introduction

While millions of hectares of non-productive saline land throughout the world already exists more non-productive land is added each year due to salt accumulation. Salinity is a problem in agriculture generally confined to arid and semi-arid regions comprising about 25% of the earth surface (Thorne & Peterson, 1954) or about 50 million hectares of irrigated area (Anon., 1970). Salinity is known to affect the time and rate of germination, growth, and reproduction and induces changes in anatomy and morphology of plants. Waisel (1972) and Strognov (1964) reviewed the research carried out on these aspects.

Stomata display variable resistances in the soil-plant-atmosphere continuum (Weatherley, 1976), thus controlling plant water status and gas exchange. Even though a good deal of work has been done on stomatal structure and their taxonomic significance (Van Cothem, 1973), only a few workers have reported stomatal studies with reference to ecological conditions such as drought (Heuser, 1915; Rippel, 1919; Clemens & Jones, 1978). This study reports the effects of chloride associated salinity on *Zea mays* c.v. Sunahry.

Materials and Methods

Corn seeds obtained from Agricultural Research Institute, Quetta, were surface sterilized with 1% HgCl solution for 30 seconds. NaCl salinity with osmotic potential of 0.00, -4.23, -8.46, -12.69 bars were made in full strength Hoagland solution. Each treatment was replicated 3 times.

Seeds were germinated in dark in paper sandwiches wrapped in polyethylene sheets in an incubator at $30^{\circ}\text{C} \pm 1.0$. Seven day old seedlings of approximately equal size were transferred to polyethylene jars 7 cm diam., containing 250 ml Hoagland solution with or without NaCl. Seedlings were randomly placed in an incubator at $30^{\circ}\text{C} \pm 1.0$ with 14 h photoperiod. The culture solutions were pump aerated daily for 20 min., and replaced after every 3 days.

Three harvests each after 7 days for shoot growth studies and 2 harvests each after 10 days for stomatal studies were taken. At the time of each harvest 3 plants from different pots of each treatment were harvested. Growth of shoot was determined by the direct measurement of length and dry weight. Relative growth rate (RGR) and leaf area (A) were calculated using the formulae:

$\text{RGR} = (\log W_2 - \log W_1)/t$ (Osmond *et al.*, 1980), $A = K.L.W.$ where A = Leaf area, K = Correlation co-efficient. L = Length of leaf. W = Width of leaf.

For stomatal studies the upper epidermis of plant (3rd leaf from top) was removed with forcep. Peels were fixed in F.A.A. and stained in hematoxyline. The stained slides were examined under the microscope for number, size of stomates, number of epidermal cells. Stomatal index was calculated using the following formula (Salisbury, 1927).

$$\text{Stomatal index} = \frac{\text{No. of stomates}}{\text{No. of stomates} + \text{No. of epidermal Cells}} \times 100$$

Results

Growth: All growth parameters decreased with an increase in salinity stress of the medium (Table 1). The length and dry weight were ($P = 0.01$) lower than control with significant differences within treatments. The length of shoot reduced from 181.6, 213.3, 255.6 mm in the control to 56.6, 60.9, 66.6 mm and dryweight from 393.4, 753.0, 1577 mg in the control to 55.3, 90.0, 150.0 mg of plant grown in media of osmotic potential — 12.69 bars, at the 1st, 2nd and 3rd harvest, respectively. Reduction in leaf area was also significant with respect to control and within treatments. At the time of 3rd harvest the leaf area reduced from 18.9 Cm^2 in the control to 9.98 Cm^2 in plant grown in nutrient solution having osmotic potential — 12.69 bar. Dry weight per unit length also significantly reduced in all harvests. Shoot length, dry weight and leaf area showed increase with the passage of time in all treatments except in — 12.69 bars. The increase in dry weight was greater than shoot length and leaf area.

The relative growth rate (RGR) was inversely proportional to the salinity of the medium and directly to the passage of time, relative growth rate being greater between 2nd

Table 1. Effect of NaCl on growth of *Zea mays* shoot.

Parameters	Osmotic Potential, bars	Harvest time after days			LSD P = 0.05
		7	14	21	
Length, mm	0.0	181.6	213.3	255.6	5.2
	—4.23	122.6	139.9	160.0	5.1
	—8.46	92.6	100.5	110.6	3.1
	12.69	56.6	60.9	66.6	5.7
LSD P = 0.05		1.9	2.2	5.4	
P = 0.01		2.9	3.4	8.2	
Dry weight (mgs)	0.0	393.4	753.0	1577.0	16.0
	—4.26	185.0	335.0	650.0	52.2
	—8.46	108.3	190.0	355.0	7.8
	12.69	55.3	90.0	150.0	69.3
LSD P = 0.05		4.8	14.2	45.7	
P = 0.01		7.3	21.6	69.2	
Dry wt./Length	0.0	2.17	3.53	6.1	0.6
	—4.26	1.5	2.3	4.1	0.6
	—8.46	1.1	1.8	3.2	0.1
	—12.69	0.9	1.4	2.3	0.3
LSD P = 0.05		0.4	0.4	0.3	
P = 0.01		0.6	0.7	0.4	
Leaf area (cm ²)	0.0	11.3	15.0	18.9	2.1
	—4.26	9.0	11.7	14.6	0.5
	—8.46	7.0	9.9	12.8	1.2
	—12.69	7.8	8.5	9.9	0.8
LSD P = 0.05		0.6	2.6	1.5	
P = 0.01		0.9	3.9	2.4	

and 3rd harvest than 1st and 2nd harvest (Table 2). The dry weight RGR was higher, lower in leaf area and least in terms of shoot length. The RGR in case of leaf area reduced from 0.2509 in the control to 0.1827 at the time of 3rd harvest whereas the corresponding figures for shoot length and dry weight were 0.2010 and 0.5727 in the control and 0.1680 and 0.3896 in — 12.69 bars.

Stomatal size and Distribution: Number, size of stomates and stomatal index significantly reduced in salinity treatments at the time of both harvest (Table 3). At osmotic potential — 12.69 bars, the stomatal number, stomatal size and stomatal index decreased at 1st and 2nd harvest time.

Table 2. Effect of NaCl on relative growth rate of corn shoot.

Parameters	Osmotic Potential, bars	Relative Growth Rate		
		Between 1st & 2nd Harvest	Between 2nd & 3rd Harvest	Between 1st & 3rd Harvest
Length	0.0	0.1678 ± 0.0063	0.1711 ± 0.0077	0.2010 ± 0.008
	— 4.23	0.1630 ± 0.0024	0.1633 ± 0.0026	0.1856 ± 0.004
	— 8.46	0.1542 ± 0.0034	0.1580 ± 0.0065	0.1706 ± 0.005
	—12.65	0.1537 ± 0.0081	0.1562 ± 0.005	0.1680 ± 0.006
Dry weight	0.0	0.2661 ± 0.0048	0.2991 ± 0.0089	0.5727 ± 0.002
	— 4.36	0.2586 ± 0.0008	0.2686 ± 0.0004	0.4864 ± 0.005
	— 8.46	0.2505 ± 0.004	0.2669 ± 0.0081	0.4680 ± 0.002
	12.69	0.2337 ± 0.0269	0.2380 ± 0.0024	0.3896 ± 0.008
Leaf area	0.0	0.1891 ± 0.0074	0.1895 ± 0.0073	0.2509 ± 0.008
	— 4.23	0.1857 ± 0.0046	0.1782 ± 0.0082	0.2317 ± 0.009
	— 8.46	0.1795 ± 0.0073	0.1687 ± 0.0087	0.2300 ± 0.0163
	—12.69	0.1556 ± 0.0045	0.1677 ± 0.0062	0.1827 ± 0.016

At equal osmotic concentration the increase in stomatal number with the passage of time was not significant ($P = 0.05$) at all levels of salinity, whereas stomatal size and stomatal index showed significant ($P = 0.05$) increase except the stomatal index at — 12.69 bars osmotic potential.

Discussion

Growth: High salt concentration retarded shoot growth of corn. This may be due to the accumulation of Na and Cl ions. Na ion maintains the osmotic potential of the plant sap (Molliard, 1921) but Cl ion causes toxicity (Hayward & Wadleigh, 1949). The salt toxicity influences the metabolic changes such as nucleic acid and protein metabolism (Strognov, 1964) enzymes (Ahmad & Hewitt, 1971; Dhingra & Varghese, 1986) and growth hormones e.g., cytokinin (Itai *et al.*, 1968), abscisic acid (Mizrahi *et al.*, 1970). All these changes are responsible for regulating and stimulating the growth processes. At high NaCl concentration the decrease in growth may be due to the low osmotic potential (Khan & Khan, 1978) and consequently low water potential. An increase in salt concentration of the medium may reduce the osmotic potential of the cell sap (Gale *et al.*, 1967) to neutralize the external osmotic potential. However, even if osmotic balance is achieved

Table 3. Effect of NaCl on stomatal size and its distribution on *Zea mays* L.

Parameters	Osmotic Potential, bars	1st harvest	2nd harvest	LSD P = 0.05
No. of stomates per mm.	0.0	110.65	120.03	n.s.
	—4.23	72.80	80.76	n.s.
	—8.46	72.80	78.74	n.s.
	—12.69	47.88	53.01	n.s.
LSD p = 0.05		8.16	1.91	
Stomatal size, mean of 30 stomates	0.0	11.78	15.66	1.42
	—4.26	9.98	14.39	1.83
	—8.46	8.33	13.72	1.23
	—12.69	8.33	12.32	0.58
LSD p = 0.05		0.66	1.18	
Stomatal Index	0.0	21.41	25.72	0.56
	—4.26	20.32	22.82	2.09
	—8.46	15.97	21.44	0.86
	—12.69	16.00	20.00	n.s.
LSD p = 0.05		0.81	0.64	

growth remained suppressed (Slatyer, 1961). It may be due to ion accumulation (Khan & Khan, 1978) or because of inability of some subcellular organelles to adjust themselves to low osmotic potential prevailing in the cell sap. (Bernstein, 1961).

The increase in dry weight per unit length may be due to the adjustment of subcellular organelles. The major factor in salt tolerance is protoplasmic resistance (Repp, 1964) and with the age of plant salinity resistance increases (Pantanelli, 1937), which may account for an increase in relative growth rate.

Stomatal size and Distribution: Decrease in number of stomates, stomatal size and stomatal index with an increase in the salinity stress of the medium (Table 3) were expected. Stomates act as variable resistance in the soil-plant-atmosphere continuum (Weatherley, 1976), thus control the gas exchange and water vapours between the plant and atmosphere. A significant decrease in number of stomates, stomatal size and stomatal index with an increase in the salinity stress seems to be an adaptation which reduce the transpiration rate and enables the plant to survive in water stress conditions. Generally salt stress also produces water stress conditions in plants. A change in number of stomates and stomatal size has been observed in case of wheat (Heuser, 1915), *Sinapis alba*

(Rippel, 1919) and *Pelargonium zonale* (Amer & William, 1958) when these plants were moved from wet to dry conditions. Ali (1983) reported that the value of stomatal index varies not only within the different species of the same genus but also within the same species collected from different ecological conditions.

Acknowledgements

The authors are grateful to Dr. M.A.K. Lodhi, Botany Department, Cornell University, USA for reviewing the manuscript.

References

- Ahmad, R., E.J. Hewitt. 1971. Studies on the growth and phosphatase activities in *Suaeda fruticosa*. *Plant and Soil*, 34: 691-696.
- Ali, R.A. 1983. A study of epidermal morphology in some members of the family Lamiaceae in Baluchistan. *M.Sc. Thesis*, Bal. Univ., Quetta.
- Amer, F.A. and W.T. William. 1957. Leaf area growth in *Pelargonium zonale*. *Ann. Bot. Lond. N.S.*, 21: 239-242.
- Anonymous. 1970. *Irrigation statistics of the world*, ICID Bull., Int. Commission on Irrigation and Drainage 48, Nyaya Marg, New Delhi-21, India, pp. 76-78.
- Bernstein, L. 1961. Osmotic adjustment of plants to saline media. I. Steady State. *Amer. J. Bot.*, 48: 909-918.
- Clemens, J.I. and P.G. Jones. 1978. Modification of drought resistance by water stress conditions in *Acacia* and *Eucalyptus*. *J. Exp. Bot.*, 29: 895-905.
- Dhingra, H.R. and T.M. Varghese. 1986. Effect of NaCl salinity on the activities of amylase and invertase in *Zea mays* L. pollen. *Ann. Bot.*, 57: 101-104.
- Gale, J., H.C. Kohl and R.M. Hagan. 1967. Changes in water balance and photosynthesis of onion, bean and cotton plants under saline conditions. *Physiol. Plant.*, 20: 408-420.
- Hayward, H.E. and C.H. Wadleigh. 1949. Plant growth in saline and alkali soils. *Bot. Rev.*, 24: 584-635.
- Heuser, W. 1915. Untersuchungen fibr den anatomischen Bau des Weizenblattes fe nach der Hohe scienes Standortes am Halme Und Unts dem Einfluss susserer Bedingungen. *Kuhn Archiv.*, 6: 391.
- Itai, C., A. E. Richmand and Y. Vaadia. 1968. The role of cytokinins durring water and salinity stress. *Israel J. Bot.*, 17: 187-195.
- Khan, M.A. and M.I. Khan. 1978. Effect of light and temperature on seedlings raised under NaCl salinity. *Pak. J. Bot.*, 10: 167-172.

- Mizrahi, Y., A. Blumenfeld and A. E. Richmond. 1970. Absciscic acid and transpiration in leaves in relation to osmotic root stress. *Plant Physiol.*, 40: 169-171.
- Molliard, M. 1921. *Nutrition de la plante*, Vol 1: Echanges d'eau et de substances minerales. Paris, Editions Doin, PP. 372.
- Osmond, C.B., O. Bjorkman and D.J. Anderson. 1980. *Physiological Processes in Plant Ecology*. Springer Verlag, Berlin. pp. 380.
- Pantaneli, E. 1939. Irrigazione con acqua salmastre, *Staz, Agrar, Sper, Bari*, 26, pp. 80.
- Repp, G. 1964. *The salt tolerance of plants. Basic Research Tests*. (UNESCO-Tehran symposium on salinity problem in the arid zones) paper no. 4, pp. 153.
- Rippel, A. 1919. Der Einfluss der Bodentrockenheit auf den anatomischen Ban der flenzen. *Beih bot. Zbl. bi.*, 36: 187-259.
- Salisbury, E.J. 1927. On the causes and ecological significance of stomatal frequency with special reference to wood land flora. *Philos. Trans. Roy. Soc. B.*, 217: 1-65.
- Slatyer, R.O. 1961. Effects of several osmotic substrates on water relationships of tomato. *Aust. J. Biol. Sci.*, 14: 519-540.
- Strognov, B.P. 1964. Physiological basis of salt tolerance of plants. Translated from Russian original (1962) by Poljakoff-Mayber, A. and Mayer, A.M. Jerusalem: Israel program for Sci. Trans.
- Thome, D.W. and H.B. Peterson. 1954. *Irrigated Soils Their Fertility and Management*. New York: The Blakistan Co. Inc. 392. Van Cothem, W.R. J. 1970. A classification of stomatal types. *Bot. J. Linn. Soc.*, 63: 235-246.
- Van Cothem, W.R.J. 1973. Stomatal types and systematics. In: *Phylogeny and Classification of the Ferns. Bot. J. Linn. Soc.*, 67: 59-70.
- Waisel, Y. 1972. *Biology of Halophytes*. pp. 000. Academic press, New York.
- Weatherley, P.E. 1976. Introduction: Water movement through plants. *Phil. Trans. Royol Soc. Lon.*, 273: 435-444.

(Received for publication 19 April 1987)