

GROWTH AND IONS (Na⁺, K⁺ AND Cl⁻) ACCUMULATING PATTERN OF SOME BRASSICA GENOTYPES UNDER SALINE – SODIC FIELD CONDITION

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Abstract

The growth and ionic uptake pattern of some Brassica genotypes i.e., Rainbow, Wester, Durr-e-NIFA, Abaseen 95 (*Brassica napus*) and NIFA raya (*Brassica juncea*) under saline-sodic field conditions was studied. Two sets of experiments on normal and saline-sodic site were conducted at NIA experimental farm, Tandojam, Pakistan during Rabi 2006-07. The salinity of the experimental site ranged between 11.0–22.9 dS/m and the pH was alkaline (8-8.6). The dominant cation was sodium (Na). The growth performance was recorded at the time of crop harvest in terms of plant height, grain weight / plant, grain yield and 100 grain weight. It was observed that the performance of Wester was better followed by NIFA- raya. The ionic uptake pattern, of leaves, stem and roots showed that the accumulation of Na was less in leaf as compared to stem and roots. However, the genotypes having better performance were found to have accumulating type of behavior showing comparatively higher Na contents in all plant parts than other genotypes. This suggests that these genotypes might adjust their osmotic potential through the accumulation of sodium in vacuole. On the other hand trend in case of K accumulation was reverse i.e. high in leaves and stem as compared to roots. Higher accumulating pattern of K in leaves might be helpful for reducing the toxic effects of sodium. However, no correlation was observed between K-Na selectivity or K/Na ratio among the genotypes tested. It is therefore concluded that better selective mechanism for Na uptake and strict control of intercellular Na influx for cellular osmotic adjustment could be selected for saline environment.

Introduction

Brassica is an important oil seed crop. In Pakistan, after cotton seed, rape-seed and mustard are the second most important sources of edible oil, (Haq *et al.*, 2002) It has been cultivated under both irrigated and non-irrigated areas of Pakistan. In Sindh province it is mostly cultivated as Zaid Kharif (September) crop on residual moisture of rice on both northern and southern rice tracks (Bhatti & Soomro, 1996). However, the presence of soil salinity on these tracks affects Brassica yield considerably. Brassica is classified as tolerant to salinity (Mass & Hoffman, 1977). It has also been reported that though it has higher threshold values, but rate of yield decline above the thresholds was much greater than most other crops in the tolerant category (Maas, 1990). However the variability in tolerance limits also exists within the genotypes and species. Variation in salt tolerance within the genotypes mainly depends upon the ion uptake pattern of genotypes. The tolerance of plant to Sodium chloride is commonly, but not uniquely, related to the concentration of sodium in the shoot (Flowers, 2004). It has been reported that plants adopt avoidance mechanism by restricting the higher uptake of Na ions in active parts (i.e. leaves), and accumulate them in roots and stem. According to Ashraf & Leary (1995), salt tolerance is related to exclusion of Na ion in leaves from the all ages. The efflux of Sodium at the plasma membrane of root epidermis and cortical cells, and resorption of Na⁺ from xylem sap and its accumulation by xylem parenchyma cells are the processes involved in Na exclusion (Gorham *et al.*, 1986). Sensitive cultivars accumulate ions more quickly than

tolerant cultivars and this ion accumulation leads to leaf death and progressively death of the plant (Munns, 2002).

On the other hand, plants of halophytic nature, adopt the mechanism of accumulation and compartmentalizing ions in leaf vacuole. Van Steveninck *et al.*, (1982) found that the salt tolerant species of *Lupinus luteus* accumulated more Na⁺ and Cl⁻ in the shoot than sensitive *L. angustifolius*. Similar results were also reported by Ashraf *et al.*, (1990), who reported higher concentration of Na in leaves of a selected salt tolerant line of *Lolium perenne* than unselected base population. Keeping in view these adaptive mechanisms, a field trial was conducted to study the growth and ionic uptake pattern in some high yielding genotypes of Brassica.

Material and Methods

Two sets of experiments (Site-1 and Site-2) were conducted at NIA experimental farm, Tandojam during Rabi 2006-07. The salinity of the Site-2 was ranging from medium to highly saline (ECe =11 – 22.9 dS/m), however it was tried that saline-sodic patch must have more or less similar salinity levels ranges (11 – 15 dS/m). Other physio chemical properties are presented in Table 1. Five varieties of brassica, (i). Rainbow (ii) Waster (iii) Durr-e-NIFA (iv) Abaseen-95 (*Brassica napus*) and (v) NIFA raya (*Brassica juncea*) were tested. The experiment was laid out according to randomized complete block design (RCBD) in subplots of size 1x 1.5m² having row spacing of 30cm. The growth observations were recorded at the time of maturity of crop in terms of Plant height (cm), grain wt. / plant, grain yield/ plot and 100 grain weight.

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Table 1. Physio-chemical analysis of the experimental sites at surface layer (0 – 30cm).

S.#	Analysis	Site-I	Site -II
1.	Saturation % age	35-36	31-36
2.	E Ce (dS/m)	1.3-1.36	11.1-22.9
3.	PH	8.0-8.5	8.0-8.6
4.	Na (meq /L)	5.0-11.30	84.78-271 .74
5.	Ca + Mg (meq /L)	4.0-35.5	37.5-78.5
6.	Sodium adsorption ratio (SAR)	4.85 --- 15.45	47.93 --- 147.96
7.	Soil category	Non saline	Saline-sodic

To study the ionic (Na^+ , K^+ and Cl^-) uptake pattern of brassica varieties, Samples of leaves, stem and roots were collected at the time of flowering/ pod formation. After thorough washing with distilled water, the plant samples were dried in hot air oven ($\pm 70^\circ\text{C}$) for 72 hours. For the determination of (Na^+ , K^+ and Cl^-) in plant solute extraction was done in (0.5%) toluene water, according to Weimberg *et al.*, (1981). Sodium (Na^+) and Potassium (K^+) were determined by flame Photometer and Cl^- were determined titrimetrically by Silver nitrate (0.0098N) solution, according to the standard methods as reported by Jackson (1962). The data was analyzed statistically, Analysis of Variance (ANOVA) according to Mstat-C computer package.

Results

Growth performance: The growth performance was recorded at the time of crop maturity. It was observed that there was a decrease in plant height with the increase in soil salinity (Table 2). Under non- saline patches the plant height ranged between 168 to 192cm, whereas under saline field condition, it ranged from 136 to 190cm. The genotype NIFA raya had the maximum plant height under both soil types (i.e. saline and non-saline). Almost all the varieties exhibited less than 25% reduction in plant height, with NIFA raya having the minimum decrease i.e. only 1% followed by Rainbow and Wester. Maximum reduction in plant height was observed in Durr-e- NIFA variety (i.e. 25%).

Table 2. Growth performance of some Brassica varieties under saline condition (2006-07).

Genotypes	Plant height (cm)		Grain yield/plant (g)		Grain yield/plot (g)		100 grain weight (g)	
	Non saline	Saline	Non saline	Saline	Non saline	Saline	Non saline	Saline
Rainbow	168.3	160.0 (4.93)	12.17	5.04 (58.58)	422.7	108.3 (74.45)	0.457	0.442 (3.28)
Wester	179.0	169.3 (5.59)	13.51	9.73 (27.98)	439.2	397.7 (9.44)	0.410	0.399 (2.68)
Durre NIFA	180.3	141.7 (21.41)	16.78	6.47 (61.44)	537.6	249.8 (53.53)	0.424	0.295 (30.42)
Abaseen-95	168.3	136.7 (18.78)	24.96	6.09 (75.56)	439.7	284.3 (35.34)	0.341	0.332 (2.64)
NIFA-ray	191.7	190 (0.9)	29.75	15.34 (48.44)	404.6	299.4 (25.99)	0.346	0.336 (2.89)
LSD (0.05)	0.970		0.5505		0.364		0.0540	

Values in the parenthesis are relative decrease (%) over control

The increase in salinity had a significant effect on grain weight/plant in brassica varieties, showing more the 50 % reduction in Rainbow, Durre-NIFA and Abaseen-95. The only variety, which has exhibited comparatively less reduction, is Wester (i.e. 28%). It was also observed that although the variety NIFA raya has exhibited 48% reduction in grain weight but was found comparatively better in their absolute grain weight production as the variety have produced maximum grain weight (i.e.15.34g/plant) among all the varieties tested. This finding supports the general concept that salt tolerance should always be associated with high yield.

The trend in case of grain yield was also more or less similar in both the varieties (Wester and NIFA- raya), showing less than 50% reduction at saline-sodic site. The variety Wester had maximum grain yield (397g/plot), with minimum reduction under saline condition i.e., 9.44%, followed by NIFA-ray (299g/ plot i.e., 25.77% decrease). Maximum decrease (74.45%) was observed in variety Rainbow having minimum yield (180.0g/ plot).

The effect of salinity on 100-grain weight were not so pronounced as most of the genotypes had shown nominal relative reduction in 100 grain weight (i.e. 2-3%), except in Durr-e-NIFA where 30% reduction was observed.

Ionic contents and uptake pattern: Plant samples analyzed for ionic contents showed a general trend of increasing sodium in plant under saline condition (Table 3). There were almost more or less similar values (0.53 – 0.64%) for Na in all plant parts (leaf, stem and roots), under non-saline condition. On the other hand the mean values under saline-sodic condition, ranged from 0.81 to 1.12%. Comparatively low sodium contents were observed in leaves than stem and roots. The pattern of sodium uptake varied among the genotypes. It was observed that under saline-sodic condition, the better performing genotypes (i.e. Wester and NIFA-ray) have accumulating type of behavior showing comparatively higher Na^+ contents in all plant parts than other genotypes. This suggests that these genotypes might maintain their osmotic potential through the accumulation of sodium in vacuole.

Table 3. Ionic contents % (Na⁺, K⁺ and Cl⁻) in brassica varieties under saline condition.

Genotypes	Sodium ((Na ⁺))		Potassium (K ⁺)		(K ⁺ /Na ⁺) ratio		Chlorides (Cl ⁻)	
	Non saline	Saline	Non saline	Saline	Non saline	Saline	Non saline	Saline
Leaf								
Rainbow	0.38	0.55	2.91	1.65	7.66	3.00	3.04	3.50
Wester	0.56	1.05	2.84	3.30	5.07	3.14	2.54	4.79
Durre NIFA	0.32	0.58	2.83	3.40	8.84	5.86	3.20	4.03
Abaseen-95	0.44	0.60	2.81	2.10	6.39	3.50	3.33	5.61
NIFA-raya	0.67	1.45	2.68	2.83	4.00	1.95	2.87	3.27
Mean	0.53	0.81	2.47	2.68	4.66	3.31	3.06	4.07
LSD (0.05)	0.0542		0.8064		0.0543		0.0939	
Stem								
Rainbow	0.63	1.28	2.83	1.45	4.49	1.13	1.82	2.58
Wester	0.61	1.13	2.15	1.78	3.52	1.58	1.55	3.17
Durre NIFA	0.61	0.95	2.93	2.38	4.80	2.51	1.85	2.64
Abaseen-95	0.85	0.88	2.55	1.25	3.00	1.42	1.95	2.34
NIFA-raya	0.64	1.60	1.30	0.98	2.03	0.61	1.78	2.94
Mean	0.64	1.12	2.41	1.69	3.77	1.51	1.83	2.54
LSD (0.05)	0.0545		0.0543		0.0543		0.0543	
Roots								
Rainbow	0.63	0.80	1.23	1.28	1.95	1.60	1.45	2.94
Wester	0.55	1.55	1.63	1.58	2.96	1.02	1.06	3.00
Durre NIFA	0.60	1.13	1.34	1.33	2.23	1.18	1.16	4.62
Abaseen-95	0.73	0.88	1.05	0.85	1.44	0.97	1.12	2.31
NIFA-raya	0.43	1.15	0.85	2.85	1.98	2.48	1.22	2.74
Mean	0.61	1.10	1.21	1.59	1.98	1.45	1.12	3.01
LSD (0.05)	0.0543		0.0543		0.0543		0.0543	

The pattern for K⁺ uptake was reversed i.e., comparatively high K⁺ accumulation in leaf samples than stem and root both under normal and non-saline conditions. Potassium values in leaves, stem and in roots were (2.47, 2.41 and 1.21%) and (2.68 1.69 and 1.59% n) under normal and saline conditions, respectively. It was also observed that root samples had comparatively less potassium contents than leaf and stem under normal and saline conditions, respectively. This indicates the active transport of potassium towards photosynthetic parts of plant to cope the toxic effects of sodium. Potassium contents with respect to individual varieties showed that K⁺ uptake in leaf samples in Wester, Durre-e-NIFA and NIFA-raya increased under saline sodic soil than under normal conditions. On the other hand, in stem samples almost all the varieties showed a decreasing trend with the increase in salinity. The potassium contents in root samples, with respect to individual varieties were bit different i.e., increased in Rainbow and NIFA-raya and decreased in Wester, Durr-e-NIFA and Rainbow with increasing salinity.

The uptake pattern of chloride was found high in leaf as compared to stem and roots. The chloride uptake increased under saline-sodic soil as compared to non-saline. Almost all the varieties had high Cl⁻ contents under salinity as compared to non-saline condition. Chloride content also varied among the genotypes, comparatively higher values of Cl⁻ in leaf were observed in Abaseen-95, Wester and Durr-e-NIFA genotypes. The stem samples of variety Wester had the maximum Cl⁻ contents, whereas, root samples of Durr-e-NIFA had higher values for Cl⁻ than others.

Discussion

Plant growth is ultimately the direct result of massive and rapid expansion of the young cells produced by meristematic division (Neumann, 1977). The growth

performance was recorded at the time of crop harvest showed that the performance of Wester was better followed by NIFA-raya. Munns *et al.*, (1995), suggest that any varietal diversity in plant growth responses to salinity appears slowly and caused by genotypic differences in rates of salt accumulation. The analytical data of plant samples showed that there was higher accumulation of salts under saline-sodic patches as compared to non-saline soil. When the plant parts were compared for different ions it was observed that salt stress leads to higher accumulation of Na and Cl and less uptake of K in all parts of plants. It was also observed that the accumulation of Na was less in leaf as compared to stem and roots. This net accumulation of Na into root cells might be due to a balance between influx through ion channels and efflux through a probable Na/H antiporter, as reported by Tester & Davenport (2003). The rapid expansion of the growing cells would also help to stop the salt building up to high concentrations (Munns, 2002). According to Pitman (1984), high shoot: root ratio and high intrinsic growth rates will reduce the rate at which salt enters the transpiration stream and accumulates in the shoot. The extent of an apoplastic pathway in roots will also influence the movement of salts across the root and to the xylem (Garcia *et al.*, 1997). It was also observed that the genotypes having better performance were found to have accumulating type of behavior showing comparatively higher Na contents in all plant parts than other genotypes. This suggests that these genotypes might adjust their osmotic potential through the accumulation of Sodium in vacuole. According to Rus *et al.*, (2001), strict control of intercellular Na influx is likely essential in environments of high external Na, as this cation is an osmolyte required for cellular osmotic adjustment but it must be compartmentalized into the vacuole to prevent cytotoxicity.

The data regarding the Chloride showed that the uptake increased under saline patches as compared to non-saline ones. The uptake was found high in leaf samples as compared to stem and roots. Rauf *et al.*, (1989) also reported the increase in leaf Cl⁻ concentration with

increase in salinity. The increase in Cl⁻ concentration was attributed to massive uptake of Cl⁻ ion by the plants as well as reduced growth under adverse environment. Chloride content also varied among the genotypes. Comparatively higher values were observed in Abaseen-95, Wester and Durr-e-NIFA genotypes. Higher accumulation of Cl⁻ in better performing genotype (i.e., Wester) is an exception but can be expected as also reported by Croughan *et al.*, 1978, who produced a salt tolerant line of *Medicago sativa* using tissue culture technique and found this line to be Cl⁻ accumulating. Similar results were reported by Ashraf *et al.*, (1986), in a *M. sativa* salt tolerant line, produced through conventional selection and breeding

The trend for K⁺ accumulation was also same i.e., high in leaves and stem as compared to roots. Higher accumulating pattern of K⁺ in might be helpful for reducing the toxic effects of Na⁺. The mechanism for maintenance of adequate K⁺ uptake in plant tissue under salt stress seems to be dependent upon selective K⁺ uptake and selective cellular K⁺ and Na⁺ compartmentation and distribution in the shoots (Poljokoff-Mayber & Lerner, 1999; Munns *et al.*, 2000; Carden *et al.*, 2003). On the other hand, He & Cramer (1993) did not find any relationship between K/Na ratio and salt tolerance in *Brassica* species. They concluded that neither K⁺/Na⁺ ratio nor K⁺-Na⁺ selectivity was correlated with relative tolerance of six rapid cycling *Brassica* species either at whole plant or callus level, suggesting that these parameters are unreliable selection criteria. As far as our results are concerned the above findings are true, where genotype Durre-e-NIFA, which also had high K⁺ contents and high K/Na ratio in leaf and Stem but could not perform well under saline-sodic environments. It is therefore concluded that the better selective mechanism for Na⁺ uptake and strict control of intercellular Na⁺ influx for cellular osmotic adjustment (which must be compartmentalized into the vacuole to prevent cytotoxicity) might be a selective criteria for the selection of brassica species for saline environments.

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References

- Ashraf, M. and J.W.O. Leary. 1995. Distribution of cations in leaves of salt-tolerant and salt-sensitive lines of sunflower under saline conditions. *J. Pl. Nut.*, 18: 2379-88.
- Ashraf, M., T. Mcnelly and A. D. Brashaw. 1986. Response and ion distribution in selected salt tolerant and unselected lines of three legumes species. *New Phytol.*, 104: 463-472.
- Ashraf, M., T. Mcnelly and A.D. Brashaw. 1990. Patterns of ion distribution in selected NaCl tolerant and normal lines of four grass species. *Biol. Plant.*, 32: 302-312.
- Bhatti, I.M. and A.H. Soomro. 1996. Rapeseed and Mustard, In: *Agricultural inputs and field crops production in Sindh.* (pp. 127-133): XXVI-310p.
- Carden, D.E., D.J. Walker, T.J. Flowers and A.J. Miller. 2003. Single cell measurement of the contributions of cytosolic Na⁺ and K⁺ to salt tolerance. *Plant Physiol.*, 131: 676-683.
- Croughan, T.P., S.J. Stavarek and D.W. Rains. 1978. Selection for NaCl tolerant line of cultured alfa alfa cells. *Crop Sci.*, 18: 959-963.
- Flowers, T.J. 2004. Improving crop salt tolerance. *J. Exp. Botany*, 55: No. 396, 307-319.
- Garcia, A., C.A. Rizzo, J. Ud-Din, S.L. Bartos, D. Senadhira, T.J. Flowers and A.R. Yeo. 1997. Sodium and Potassium transport to the xylem are inherited independently in rice and mechanism of sodium: potassium selectivity differs between rice and wheat. *Plant Cell and Environment*, 25: 1167-1174.
- Gorham, J., B.P. Forster, R.G. Wyn Jones, T.E. Miller and C.N. Law. 1986. Salt tolerance in the triticeae: Solute accumulation and distribution in an amphidiploid derived from *Triticum aestivum* L. cv. Chinese Spring and *Thinopyrum bessarabicum*. *J. Exp. Bot.*, 37: 1435-49.
- Haq, T.U., J. Akhtar, A.U. Haq and M. Hussain. 2002. Effect of soil salinity on the concentration of Na, K and Cl in the leaf sap of four *Brassica* species. *Int. J. Agric. Biol.*, 4: 385-388.
- He, T. and G.R. Cramer. 1993. Salt tolerance of rapid cycling *Brassica* species, in relation to potassium/Sodium ratio and selectivity at whole plant and callus level. *J. Plant Nutr.*, 16: 1263-1277.
- Jackson, M. L. 1962. Soil Chemical analysis. Constable and Company Ltd. England.
- Maas, E.V. 1990. *Crop salt tolerance*. p. 262-304. In: (Ed.): K.K. Tanji. ASCE manuals and reports on engineering 71. ASCE, New York.
- Mass, E.V. and G.J. Hoffman. 1977. Crop salt tolerance—current assessment. *J. of Irrigation and Drainage Division, ASCE*, 103: 115-134.
- Munns, R. 2002. Comparative physiology of salt and water stress. *Plant Cell Environ.*, 16: 15-24.
- Munns, R., D.P. Schachtman and A.D. Condon. 1995. The significance of a two phase growth response to salinity in wheat and barley. *Aust. J. Plant Physiol.*, 22: 561-569.
- Munns, R., R.A. Hare, R.A. James and G.J. Rebertuske. 2000. Genetic variation for salt tolerance of durum wheat. *Aust. J. Agric. Res.*, 51: 69-74.
- Neumann, P. 1997. Salinity resistance and plant growth revisited. *Plant Cell Environ.*, 20: 1193-1198.
- Pitman M.G. 1984. Transport across the root and shoot/ root interactions. In: salinity tolerance in plants: strategies for crop improvement. (Ed.): R.C. Staples. pp. 93-123. Wiley, New York.
- Poljokoff-Mayber, A. and H.R. Lerner. 1999. Plants in saline environments. In: Handbook of plant and crop physiology, (Ed.): M. Pessarkali. Marcel Dekker, New York, 125-154.
- Rauf, A., M. Aslam, J. Akhtar and M.K. Abasi. 1989. Salt tolerance studies on canola (*Brassica napus*.L) varieties in response to salinity. *Canadian J. Pl. Sci.*, 74: 797-799.
- Rus, A., S. Yokoi, A. Sharkhuu, M. Reddy, B.H. Lee, T.K. Matsumoto, H. Koiwa, J.K. Zhu, R.A. Bressan and P.M. Hasegawa. 2001. *AtHKT1* is a salt tolerance determinant and controls Na entry into plant roots. *Proc. Natl. Acad. Sci. U.S.A.* 98: 14150-14155.
- Tester, M. and R. Davenport. 2003. Na tolerance and Na transport in higher plants. *Ann. Bot.*, 91: 503-507.
- Van Steveninck, R.F.M., M.E. Van Steveninck., R. Stelzer and A. Lauchli. 1982. Studies on the distribution of Na and Cl in two species of *Lupinus luteus* and *Lupinus angustifolius* differing in salt tolerance. *Physiol. Plant.*, 56: 465-473.
- Weimberg, R, H.R. Lerner and A. Poljakoff-Mayber. 1981. Kinetics of Toluene-induced leakage of low molecular weight solutes from excised sorghum. *Tissues' Plant Physiol.*, 68: 1433-1438.