Plant Stress ©2008 Global Science Books

Physiological Variation of *Brassica* Cultivars/Landraces during Seed Germination and Early Seedling Growth under Salt Stress

Taek-Ryoun Kwon[1,3*] • Zamin Shaheed Siddiqui[1,2] • Phil J. C. Harris[3]**

[1] National Institute of Agricultural Biotechnology/RDA, Suwon, South Korea
[2] Department of Botany, University of Karachi, Karachi, Pakistan
[3] Department of Geography, Environment and Disaster Management Coventry University, Coventry, UK

Corresponding authors: * trkwon@rda.go.kr ** zaminsiddiqui@yahoo.co.in

ABSTRACT

Physiological variations of fifteen *Brassica juncea* cultivars/landraces and a *Brassica rapa* landrace during germination and early seedling growth under salt stress were investigated. Percent germination, rate, vigour and threshold were evaluated. Salinity decreased the percent germination, rate, and vigour of two *Brassica* species, with considerable variation among cultivars/landraces. Relatively high thresholds, between 300 to 250 mM NaCl for inhibition of total germination were observed in *B. juncea* cultivars of Indian origin i.e. 'RH 30', 'RH 8606', 'RH 9021', 'RH 9011' and 'Prakash'. The lowest thresholds were recorded in 'Common green', 'Ndakupuka' and 'Sani'. From the germination study, a relatively salt-tolerant *B. juncea* cv. 'Prakash', two *B. juncea* landraces 'Common green' and 'Ndakupuka' and a relatively salt-sensitive *B. rapa* landrace 'Sani' were selected for salinity tolerance assessment at the seedling stage. The growth of 'Common green', 'Ndakupuka', 'Prakash', and 'Sani' was retarded with increasing salinity reducing the leaf area of all tested cultivars. Salinity caused an accumulation of Na^+ ions and a decrease of K^+, Ca^{++}, and Mg^{++} ions in the shoots of the tested cultivars. Na^+ accumulation was highest in 'Sani' and lowest in 'Common green' at 175 mM NaCl. The K^+ content of the shoots in 'Sani' was lowest in treated and control samples. Likewise, the Ca^{++} content of shoots of 'Sani' seedlings treated with NaCl was low but higher than "Common green". The effect of NaCl on Mg^{++} was similar to that on Ca^{++}. The results suggest that the higher relative growth reduction of 'Sani' may be correlated with higher Na^+ accumulation and lower K^+, Ca^{++} and Mg^{++} uptake under a saline environment. A physiological explanation is provided for salt stress tolerance in all tested cultivars and landraces.

Keywords: ion accumulation, physiology, resistant and sensitive, salinity, screening
Abbreviations: xx, yy, zz

INTRODUCTION

Abiotic stress like salinity is a ubiquitously important constraint to crop production, affecting 95 million ha worldwide (Szabolcs 1994). From 1994 to 2004, it is estimated that 20% of all cultivated land and nearly half of irrigated land is affected by salt, greatly reducing the yield of crops (Munns 2002; Flowers 2004).

Although some crops are moderately tolerant to saline conditions, many others are adversely affected by even low levels of salt. Salinity impairs seed germination, reduces nodule formation, retards plant growth and reduces crop yield (Bayuelo-Jiménez *et al*. 2002). One approach to reducing the deleterious effects of soil salinity on crop production is the development of salt-tolerant cultivars. In certain species, this may be achieved by exploiting intraspecific variability. However, when such variability is limited, as occurs in many crop species, genes may be transferred from closely related wild species adapted to high salinity (Hasterok *et al*. 2005; Snowdon *et al*. 2006).

Brassica are considered a relatively moderate salt sensitive genus (Francois 1994; Hayat *et al*. 2007) within which limited variability for salinity tolerance has been detected (Ashraf and Sharif 1997; Beltrao *et al*. 2000; Maggio *et al*. 2005). In contrast to the cultivated *Brassica*, the genetic diversity of its relatives may provide useful genes for improving tolerance. For example, there are several wild relatives of *Cajanus cajan* (L.) Millsp. belonging to the genera *Atylosia*, *Dunbaria* and *Rhynchosia* (Subbarao *et al*. 1991), which exhibit wide variation in their salinity tolerance and may represent genetic resources for the improvement of salinity tolerance in cultivated *C. cajan* (pigeonpea). Similarly, salinity tolerance is found in wild species of tomato like *Lycopersicon cheesmanii* Riley, *Solanum pennellii* Corr. (Tal and Shannon 1983), and *Lycopersicon pimpinellifolium* (Jusl.) Mill. (Foolad and Lin 1997).

Intraspecific variation of salinity tolerance has been reported in *Brassica juncea* (Uma *et al*. 1992; Sharma and Gill 1994) and interspecific variation in *Brassica* species has also been reported by Ashraf and McNeilly (1990), He and Cramer (1992). Many salt-tolerant *B. juncea* cultivars or lines, all of them of oilseed type, have been developed by field screening with the application of saline water (Kumar 1995). However, selection for salinity tolerance was based on seed yield or seed emergence even though there was no clear physiological understanding of the tolerance mechanism. A genotype with physiological tolerance may display a clear tolerance mechanism maintaining its growth even at an unfavorable salinity. Growth reduction in response to salinity, relative to growth in non-saline condition, was used for comparative physiological studies of salinity tolerance in *Brassica* species (He and Cramer 1992), *Lycopersicon* species (Pérez-Alfocea *et al*. 1994) and *Sorghum* species (Yang *et al*. 1990). Salinity tolerance may vary throughout the life of a plant (Richards 1995). For instance, germination phase is more tolerant to salt stress than the seedling stage in *Atriplex patula* (Ungar 1996), *Brassica rapa* (François 1994) and *Brassica napus* (Redmann *et al*. 1994). In contrast, the seedling stage showed more salt tolerance than seed germination in *B. juncea* (Kumar and Kumar 1990). Likewise, salinity tolerance at the seed germination stage was positively correlated with that of the seedling stage in

Received: 7 June, 2008. Accepted: 6 September, 2008.

Original Research Paper

Cicer arietinum and *Lens culinaris* (Mamo *et al*. 1996) but not in *C. cajan* (Dua and Sharma 1996). Physiological criteria are able to supply more objective and quantitative information than visual assessment when screening for component traits of complex characters (Yeo 1994). For instance, salinity tolerance in *Brassica* species has been associated with ion accumulation or selectivity (He and Cramer 1992; François 1994; Sharma and Gill 1994). Therefore, in this study, an evaluation was based on (1) the salt tolerance response of 15 *B. juncea* cultivars/landraces and a *Brassica rapa* landrace at seed germination and seedling stages, (2) the liaison between salinity tolerance at seed germination and seedling stages, (3) relative growth reduction and ion accumulation at the seedling stage in response to a series of NaCl concentrations.

MATERIALS AND METHODS

Seed germination

Fifteen *Brassica juncea* cultivars/landraces and a *Brassica rapa* landrace were investigated. Seeds from 13 from *B. juncea* cultivars i.e., 'Prakash', 'RH30', 'RH8606', 'RH9011', 'RH9021', 'RH8816', 'RH8814', 'RH9131', 'Kranti', 'Varuna', "RH819", 'RH9132' and 'ISH9152' were kindly supplied by Dr. S. Arya, Haryana Agricultural University, Haryana, India. Two *B. juncea* landraces i.e., 'Common green' and 'Ndakupuka' and a *B. rapa* landrace 'Sani' were provided from Dr. D. Astley, Germplasm Centre, Horticultural Research International (HRI), Wellesbourne, United Kingdom. All seeds used in the experiment are the same age- less than a year from harvest and 90-95% viable.

The percent germination, rate and germination vigour were evaluated. Four replicates of 25 seeds of each cultivar were germinated in 9-cm Petri dishes on two layers of Whatman No. 1 filter paper moistened with 5 ml deionized water as a control and a series of NaCl concentrations from 25 to 400 mM at 25 mM intervals. The Petri dishes were arranged in a completely randomized design in an incubator at 25°C in the dark. Total germination percentage was determined from the number of seeds germinated at 10 days after sowing. The rate of seed germination was determined as the number of days to reach 50% of the final germination at 10 days after sowing. Germination vigour was determined as the ratio between the number of seeds germinated 3 days after sowing and the final germination number.

The arcsine transformed total germination data and germination rate and vigour data were subjected to analysis of variance and a Duncan's multiple range test (DMRT) at $p = 0.05$ using SAS software (SAS Institute Inc., USA) and 95% confidence intervals were calculated using Microsoft Excel. The threshold was defined as the highest NaCl concentration at which the germination parameter does not differ significantly from the control. 50% inhibition was defined as the interval between the highest concentration which permits significantly more than 50% of the control value and the lowest concentration which results in significantly less than 50%.

Seedling stage

A salt-tolerant *B. juncea* cv. 'Prakash', two *B. juncea* landraces, 'Common green' and 'Ndakupuka' and a relatively salt-sensitive *B. rapa* landrace 'Sani' obtained from the germination phase, were selected for salinity tolerance screening at the seedling stage. Plants were grown in a greenhouse at Coventry University, 52° 24′ N 1° 31′ W between 30 January and 27 February, using a continuously aerated hydroponic culture system at a temperature of 23-28°C with a photoperiod of 12 h natural daylight supplemented with 600 ± 50 $\mu mol\ m^{-2}\ s^{-1}$ PAR from 400 W high pressure Son-T sodium lamps.

Seeds, sterilized with 1% sodium hyphochlorite for 5 min, were washed with tap water and then germinated as described earlier. The germinated seeds were planted in trays of horticultural sand (Croxden Horticultural Products Ltd., England) which had been washed thoroughly with tap water and finally with 0.25-strength Hoagland solution (Hoagland and Arnon 1938). After 10 days in the sand tray, young seedlings were transplanted to the hydroponic culture system, which consists of low-density polythene containers (220 × 165 × 150 mm) and was painted black to prevent photosynthetic algal growth. 0.25-strength Hoagland solution was placed in each container. The solutions were changed every day throughout this experiment. A continuous aeration system was established with a pump (General Electric, Model No. 5HCE-10-501X, USA) and a series of 9 mm diameter polyvinyl chloride (PVC) tubes providing 80 ± 5% oxygen saturation in the culture medium. Polystyrene racks, consisting of 1 × 1 cm perforated cells, floating on the surface of the culture medium, were used to hold the plants. The seedlings were inserted into the cells and secured at the hypocotyl axis with a non-absorbent cotton wool plug covered with a layer of black polypropylene beads to prevent algal growth on the surface.

Experimental design

This experiment used a split-plot design with three replicates. Main plots were NaCl treatments. Sub-plots were the six cultivars. NaCl treatments consisted of a control (0.25-strength Hoagland solution), and 25, 75, 125 and 175 mM NaCl dissolved in the control. The treatments were started 3 days after transplanting when the plant root had recovered from any damage due to the transplanting and leaves 1 and 2 were expanding. The treatment solutions were changed every 2 days throughout the experiment to maintain stable salt stress.

Sampling

Five plants per replicate were sampled after 14 days NaCl treatment. Leaf area was determined using a leaf area meter (Delta Area Meter MK2, Delta-T Devices Ltd., England). Shoot and root dry weights were determined after drying at 75°C for 45 h.

Ion analysis

Dried shoots of each replicate were ground separately and 100 ± 10 mg sub-samples taken for ion analysis. The dry matter was digested with 2 ml concentrated HNO_3 using a high performance microwave digestion unit (mls 1200 mega, Milestone, Germany). The extracts were diluted to 25 ml with deionized water and then filtered through Whatman No. 42 filter paper. The concentrations of Na^+, K^+, Ca^{++} and Mg^{++} were determined using an inductively coupled plasma (ICP) emission spectrometer (Plasma 400, The Perkin Elmer Corporation, U.S.A.).

Statistics

Analysis of variance for a split-plot design was performed for total dry weight, shoot and root dry weights, leaf area, Na^+, K^+, Ca^{++}, Mg^{++}, K^+/Na^+, and Ca^{++}/Na^{++} ratio using the SAS programme. NaCl treatment effects within a cultivar were determined on the biomass parameters and ion parameters with LSD at $p = 0.05$ using the same software. Statistical differences in relative growth reduction (percentage of control) in shoot, root dry weights and leaf area were determined with 95% confidence intervals of the means of three replicates using Microsoft Excel.

RESULTS

Seed germination

Salinity decreased the percentage, rate, and vigour of seed germination in the two *Brassica* species, with considerable variation among cultivars/landraces. The threshold for inhibition of total germination after 10 days was recorded (**Table 1**). Relatively high thresholds, from 300 to 250 mM NaCl for inhibition of total germination were found in the Indian originated *B. juncea* cultivars i.e., 'RH 30', 'RH 8606', 'RH 9021', 'RH 9011' and 'Prakash'. The lowest thresholds were found in *B. juncea* landraces i.e., 'Common green' and 'Ndakupuka' and a *B. rapa* 'Sani' the total germination of which was significantly affected by the addition of even 25 mM NaCl. The NaCl concentration causing 50% inhibition in total seed germination after 10 days ranged

Table 1 NaCl concentrations causing threshold and 50% inhibition of seed germination parameters of fifteen *B. juncea* cultivars/landraces and *B. rapa* 'Sani' treated with 0-400 mM NaCl at 25 mM intervals.

Cultivar	Total germination		Germination rate	Germination vigour	
	Threshold	50% Inhibition	Threshold	Threshold	50% Inhibition
	(mM NaCl)				
Prakash	250	375 - 400	150	275	325 - 375
RH 30	300	325 -350	175	300	325 - 350
RH 8606	275	375 - 400	50	250	325 - 350
RH 9011	250	250 - 325	275	275	275 - 325
RH 9021	275	275 - 350	250	175	275 - 325
RH 8816	225	325 - 375	75	225	300 - 350
RH 8814	225	325 - 350	225	225	275 - 325
RH 9131	225	325 - 350	100	225	300 - 325
Kranti	225	300 - 350	150	225	300 - 325
Varuna	225	275 - 300	75	225	325 - 375
RH 819	175	325 -375	0	200	300 - 350
RH 9132	150	275 - 325	250	175	275 - 325
ISH 9152	125	225 - 275	250	125	225 - 325
Common green	0	25 - 75	125	0	50 - 75
Sani	0	125 - 150	0	50	100 - 125
Ndakupuka	0	25 - 75	75	0	50 - 75

Table 2 Ranking of salinity tolerance of fifteen *B. juncea* cultivars/landraces and *B. rap* Sani at the seed germination stage. High score indicates the most salt-tolerant cultivar.

Cultivar	Total germination		Germination rate	Germination vigor		Sum of ranks
	Threshold	50%	Threshold	Threshold	50%	
Prakash	12.5	15.5	9.5	14.5	15.5	67.5
RH 30	16	11	11	16	13.5	67.5
RH 8606	14.5	15.5	3	13	13.5	59.5
RH 9011	12.5	5	16	14.5	6	54
RH 9021	14.5	8	14	5.5	8	50
RH 8816	9	13.5	5	10	11	48.5
RH 8814	9	11	12	10	6	48
RH 9131	9	11	7	10	11	48
Kranti	9	9	9.5	10	9	46.5
Varuna	9	6	5	10	15.5	45.5
RH 819	6	13.5	1.5	7	11	39
RH 9132	5	7	14	5.5	6	37.5
ISH 9152	4	4	14	4	4	30
Common green	2	1.5	8	1.5	1.5	14.5
Sani	2	3	1.5	3	3	12.5
Ndakupuka	2	1.5	5	1.5	1.5	11.5
Sum	136	136	136	136	136	

from 375-400 mM NaCl in *B. juncea* cvs. 'Prakash' and 'RH 8606' and 25-75 mM NaCl in *B. juncea* cvs. 'Common green' and 'Ndakupuka'.

The NaCl thresholds effects on the germination rate were 275 mM in 'RH 9011', 0 mM in 'RH 819' and 'Sani'. Relatively high thresholds, from 250 to 275 mM NaCl, were found in 'RH 9011', 'RH 9021', 'RH 9132' and 'ISH 9152'. While relatively low thresholds, 50 mM NaCl, were shown by 'RH 819' and 'RH 8606' and 'Sani'.

Germination vigour also was affected by the NaCl treatments (**Table 1**). The highest threshold for inhibition, 300 mM NaCl, was displayed by 'RH 30' while the lowest, 0 mM NaCl, was shown by 'Common green' and 'Ndakupuka'. The 50% inhibition points for germination vigour ranged from 325 375 mM NaCl for 'Prakash' and 'Varuna' and 50 75 mM NaCl for 'Common green' and 'Ndakupuka'.

Salinity tolerance at the seed germination stage differed among the 16 cultivars/landraces and these were ranked for each of the five germination parameters scored and presented in **Table 2**. The highest sum of ranks was found with 'Prakash' and 'RH 30', suggesting that these are the most salt-tolerant cultivars at the seed germination stage. The lowest was found with 'Ndakupuka' suggesting that this is the most salt-sensitive entry. The order of the salt tolerance at the seed germination stage based on the sum of ranks was 'Prakash' = 'RH 30' > 'RH 8606' > 'RH 9011' > 'RH 9021' > 'RH 8816' > 'RH 8814' > 'RH 9131' > 'Kranti' > 'Varuna' > 'RH 819' > 'RH 9132' > 'ISH 9152' > 'Common green' > 'Sani' > 'Ndakupuka'.

Seedling growth

The total dry weight (TDW), shoot dry weight (SDW), root dry weight (RDW), leaf area, shoot root ratio (SRR), absolute growth rate (AGR) and relative growth rate (RGA) were tested in four selected cultivar/landraces from germination study. Selections of salt sensitive and tolerant cultivars were made by the sum of ranked in germination studies which are based on five germination parameters. For instance, highest sum of ranks in 'Prakash' suggesting that the most salt-tolerant cultivars while the lowest in 'Ndakupuka', 'Sani' and 'Common green' suggesting that these were the salt-sensitive.

The total dry weight of 'Common green' at 25 and 125 mM NaCl did not differ significantly compared to control. The dry weights of 'Prakash' and 'Ndakupuka' were not significantly reduced by 75 mM NaCl, but were by 125 mM NaCl. The dry weights of 'Sani' were reduced even at 75 mM NaCl.

Salinity significantly reduced the leaf area of all tested cultivars (**Table 3**). The thresholds, which may be defined as the highest concentration showing no significant difference from the control, for leaf area reduction were 75 mM NaCl for 'Ndakupuka', 25 mM NaCl for 'Common green', 'Prakash' and 'Sani'. The reduction of leaf area, as a percent of control, in response to salinity revealed that 'Sani' was the most salt sensitive of the four tested cultivars.

The results from the relative growth reduction did not agree with the absolute biomass production (**Table 3**) under

Table 3 Plant growth responses to salinity in *Brassica* cultivars/landraces. Total dry weight (TDW), shoot dry weight (SDW), root dry weight (RDW), and leaf area were measured after 14 days. Shoot-root ratio (SRR), AGR (absolute growth rate) and RGR (relative growth rate) were calculated from dry weights. For each parameter, means with the same letter do not differ significantly at p = 0.05 within a cultivar.

Cultivar	NaCl (mM)	TDW (mg)	SDW (mg)	RDW (mg)	Leaf area (cm^2)	SRR (ratio)	AGR (mg day^{-1})	RGR (g g^{-1} day^{-1})
Common Green	Control (0)	294 a	257 a	37.3 a	184.67 a	6.98 ab	19.16 a	0.0754 a
	25	303 a	273 a	30.0 ab	188.07 a	9.11 a	19.83 a	0.0763 a
	75	217 ab	191 ab	26.0 ab	120.33 b	7.46 a	13.64 a	0.0642 ab
	125	257 a	229 a	28.0 ab	122.70 b	8.93 a	16.50 ab	0.0713 a
	175	139 b	119 b	20.7 b	52.50 c	5.79 b	8.12 b	0.0522 b
Ndakabuka	Control (0)	297 a	267 a	30.7 a	162.33 a	8.90 bc	19.74 a	0.0821 a
	25	235 ab	217 ab	18.7 ab	142.27 a	13.62 a	15.31 ab	0.0749 a
	75	243 ab	219 ab	23.3 ab	122.67 a	10.14 ab	15.83 ab	0.0749 a
	125	173 bc	151 bc	22.0 ab	77.00 b	7.24 bc	10.88 bc	0.0633 ab
	175	116 c	99 c	16.7 b	39.13 b	5.83 c	6.79 c	0.0509 b
Sani	Control (0)	542 a	483 a	59.3 ab	263.13 a	8.30 a	36.34 a	0.0866 a
	25	570 a	491 a	78.7 a	285.00 a	6.41 a	38.34 a	0.0881 a
	75	385 b	344 b	40.7 bc	175.60 b	9.09 a	25.10 b	0.0754 a
	125	192 c	169 c	22.7 c	98.93 c	7.26 a	11.34 c	0.0519 b
	175	172 c	147 c	28.0 c	49.10 c	5.44 a	9.91 c	0.0508 b
Prakash	Control (0)	497 ab	435 ab	61.3 ab	253.27 a	7.02 a	32.54 a	0.0761 ab
	25	514 a	450 a	64.0 a	265.13 a	6.97 a	33.78 a	0.0777 a
	75	362 bc	311 bc	50.7 bc	170.2 b	6.23 a	22.92 b	0.0663 bc
	125	341 cd	294 cd	47.3 cd	159.07 b	6.27 a	21.45 bc	0.0653 c
	175	205 d	169 d	36.7 d	72.93 c	5.11 a	11.73 c	0.0493 d

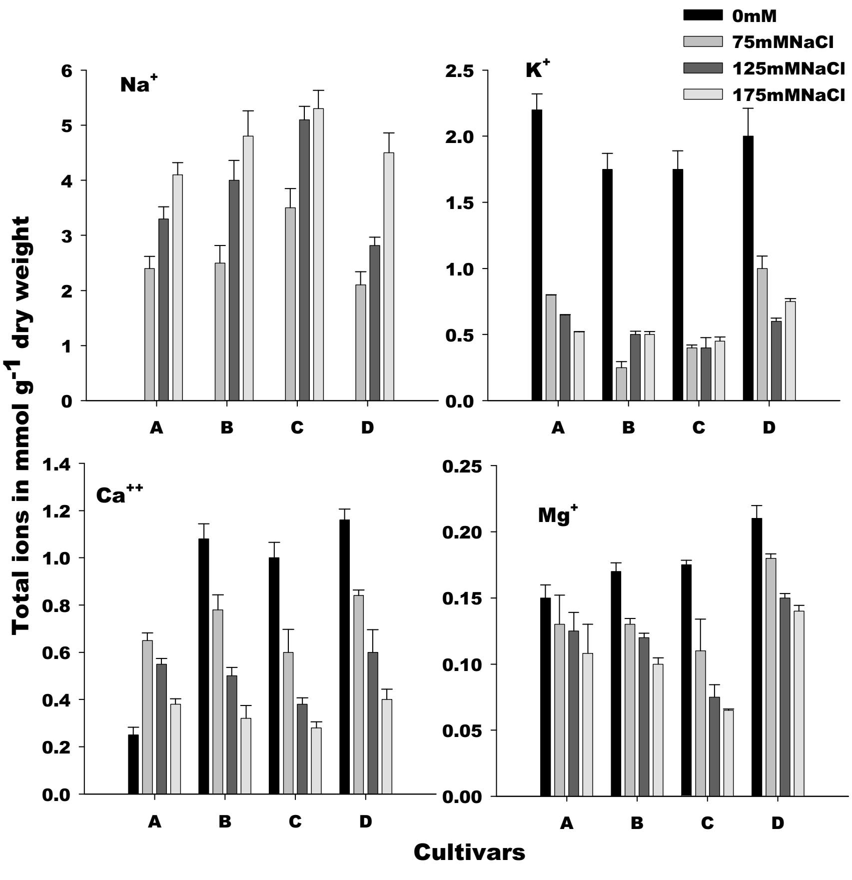

Fig. 1 Accumulation of Na^+, K^+, Ca^{++} and Mg^{++} ions in shoots of *B. juncea* cultivars/ landraces and *B. rapa* in response to salinity. Each point represents the mean of three replicates of five plants. Vertical bars represent standard errors. Symbols on X-axis: A = Common green, B = Ndakabuka, C = Sani and D = Prakash.

the NaCl treatments. 'Common green' showed lower total dry weight than 'Sani' over the whole range of NaCl treatments and maintained a higher relative growth. The smallest relative growth reduction under all the NaCl treatments was found in 'Common green', indicating a relatively salt-tolerant cultivar. On the other hand, 'Sani' showed the greatest relative growth reduction among the selected cultivars, indicating its relative salt-sensitivity. The ranks based on the relative growth reduction gave an order of salt tolerance: 'Common green' > 'Ndakupuka' > 'Prakash' > 'Sani'.

Ion accumulation

Salinity caused an accumulation of Na^+ and a decrease of K^+, Ca^{++}, and Mg^{++} in the shoots of the four cultivars (**Fig. 1**). Na^+ accumulation was highest in 'Sani' and lowest in 'Common green' at each of the NaCl concentrations. However, Na^+ accumulation was not significantly differed in 'Sani' at 125 and 175 mM NaCl. The K^+ content of shoots over the whole NaCl concentration range tested including the control was lowest in 'Sani' and 'Ndakupuka'. Since the Na^+ content of plants grown in optimal conditions (0 mM) were near zero, i.e. at the level of the blank, those data did not have any meanings and thus were excluded from **Fig. 1**. The Ca^{++} content of shoots of seedlings treated with NaCl was lowest in 'Sani' although with plants grown in the NaCl-free control, 'Sani' had a higher Ca^{++} content than did 'Common green'. The Mg^{++} content of the shoots was much lower than that of the other cations. The effect of NaCl on Mg^{++} was similar to that on Ca^{++}. 'Sani' showed a

Table 4 Analysis of variance of biomass, leaf area and ion parameters and F values. ***, **, * and ns: significant at $p < 0.001$, $p < 0.01$, $p < 0.05$ and not significant, respectively.

Source	F values (significance level)									
	Total dry weight	Shoot dry weight	Root dry weight	Leaf area	Na^+	K^+	Ca^{++}	Mg^{++}	K^+/Na^+	Ca^{++}/Na^{++}
Block (A)	0.33 ns	0.56 ns	1.82 ns	0.47 ns	1.21 ns	0.26 ns	7.43 **	5.22 **	1.17 ns	0.81 ns
NaCl treatment (B)	49.07 ***	45.36 ***	27.15 ***	62.65 ***	661.9 ***	518.4 ***	379.5 ***	77.79 ***	124.8 ***	188.5 ***
A X B	0.63 ns	0.78 ns	1.21 ns	1.68 ns	0.95 ns	1.06 ns	1.21 ns	1.14 ns	0.84 ns	0.18 ns
Cultivar (C)	30.62 ***	23.33 ***	52.24 ***	17.31 ***	23.68 ***	18.84 ***	21.54 ***	31.85 ***	24.95 ***	19.92 ***
B X C	2.5 ***	2.18 *	3.34 ***	1.30 ns	3.93 ***	2.33 *	6.92 ***	4.24 ***	5.06 ***	3.23 **

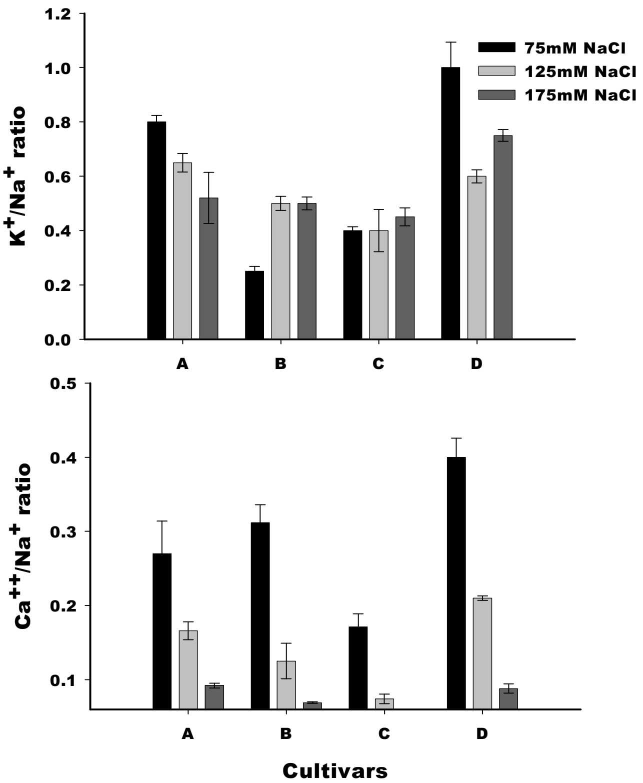

Fig. 2 **K^+ / Na^+ ratio (A) and Ca^{++} / Na^+ ratio (B) in the dried shoots of four *B. juncea* and *B. rapa* cultivars/landraces in response to salinity.** Each point represents the mean of three replicates of five plants. Vertical bars represent standard errors of means. Symbol on X-axis: A = Common green, B = Nadakabuka, C = Sani and D = Prakash.

significant reduction in the shoot Mg^{++} content at 175 mM NaCl compared to the tested cultivars.

Analysis of variance revealed highly significant differences in Na^+, K^+, Ca^{++} and Mg^{++} contents of shoots among NaCl treatments and among cultivars (**Table 4**). There were highly significant interactions between the NaCl treatments and the cultivars.

All cultivars showed a large decrease in their K^+ / Na^+ and Ca^{++} / Na^+ ratio with an increase of NaCl concentrations (**Fig. 2**). At 75, 125 and 175 mM NaCl, 'Prakash' showed the highest K^+ / Na^+ and Ca^{++} / Na^+ ratio while 'Sani' and 'Nadakabuka' displayed the lowest ratios among the four cultivars. However, the K^+ / Na^+ ratio was not significantly differed in 'Sani' at all concentrations compared to the Ca^{++} / Na^+ ratio where a significant difference was observed. Analysis of variance for the K^+ / Na^+ and Ca^{++} / Na^+ ratios indicated that there were very highly significant ($p < 0.001$) differences among cultivars and among NaCl treatments (**Table 4**). There was a highly significant interaction between cultivars and NaCl treatments.

DISCUSSION

Whilst seeds were germinated in the presence of NaCl solutions, an increase in NaCl concentration reduces total germination, germination rate and germination vigour of *Brassica* cultivars/landraces. A NaCl-induced reduction in rate and total germination has also been reported in *Brassica napus* (Redmann *et al.* 1994), *Allium* species (Gutterman *et al.* 1995), *Lycopersicon esculentum* Mill. (Foolad and Jones 1992) and *Sorghum bicolor* (L.) Moench (Esechie 1994). Alteration in the physiological and biochemical responses of seeds during germination and early seedling growth under saline medium is not uncommon. A number of reports suggest that a hyper-saline environment causes delay in final percent germination and germination velocity (Prado *et al.* 2000) due to a reduction in hydrolytic enzyme activities and the retardation in seed reserves mobilization which are considered to be an important event during germination and required for the completion of germination (Prado *et al.* 2000; Ashraf *et al.* 2002). In addition, It has been suggested that salinity reduces the germinability of seed and delays germination through ion effects (Khan and Ungar 2001; Maggio *et al.* 2005; Siddiqui *et al.* 2006), osmotic effects (Yokoish and Tanimoto 1994), or combined osmotic and ion effects (Ashraf *et al.* 2002).

In this study, the highest thresholds for the germination parameters were recorded in *B. juncea*, that was 300 mM NaCl (27.4 dSm^{-1}) for total germination, 275 mM NaCl (25.1 dSm^{-1}) for germination rate and 300 mM NaCl (27.4 dSm^{-1}) for germination vigour. These values indicate that *B. juncea* has a high salinity tolerance at the seed germination stage compared to other plant species. The thresholds of inhibition of total seed germination in the presence of salinity varied among plant species: 6 dSm^{-1} for *B. napus* ICIOLA 41 (Gutierrez *et al.* 1994), 11.6 dSm^{-1} for *B. rapa* L. Rifera group (Francois 1994), 16.2 dSm^{-1} for *Cucumis sativus* (Chartzoulakis 1992), 100 mM NaCl (9.14 dSm^{-1}) and 134 mm NaCl (12.2 dSm^{-1}) for *Stylosanthes humilis* (Lovato *et al.* 1994).

Total germination showed a relationship with germination vigour but not with germination rate. Under salt stress, germination rate was independent of the final germination percentage. For instance, in this study, the threshold of inhibition of total germination of *B. juncea* 'Prakash', the most salt-tolerant cultivar, was 250 mM NaCl but the threshold of inhibition of germination rate was 175 mM NaCl unsteady correlation between the total germination and germination rate was found in several plants (Khan and Ungar 2001; Siddiqui *et al.* 2006). Germination rate and vigour within three days after sowing was highly significantly correlated with final percent germination. This relationship suggests that the maintenance of germination rhythm under salt stress within 3 days after sowing could determine their final germination. Therefore, it is presumed that a delay in seed germination can allow the accumulation of excess salts in the seed to reach a toxic level which could affect on final percent germination. Conclusively, the study suggest the germination responses of fifteen *B. juncea* cultivars/landraces and a *B. rapa* landrace to salinity has a wide range of tolerance at the seed germination stage, indicating that this salt tolerance diversity could be used for the tolerance enhancements in cultivated salt sensitive *Brassica* cultivars.

Salinity significantly reduced the growth of the *Brassica* cultivars/landraces when compared with the control plants. The extent of the NaCl-induced reduction caused by increasing NaCl concentrations differed among the four cultivars/landraces, indicating that there was variation for salinity tolerance at the seedling stage. The response to salinity has revealed a wide range of salinity tolerance among *Brassica* species, cultivars or strains (Kumar and Kumar 1990; Chopra and Chopra 1992; Sharma and Gill 1994).

In this study, we used reduction of dry weights as a percentage of a control as the physiological tolerance criterion for assessing the response to salinity adduced by Rawson *et al*. (1988). *B. juncea* 'Common green' maintained the highest relative dry weight, expressed as percent of control, at all NaCl treatments, suggesting that it was the cultivar with greatest 'physiological tolerance' among the four selected cultivars/landraces. In contrast, *B. rapa* 'Sani' showed the greatest relative reduction in total dry weight under salinity, indicating that it was the most salt-sensitive, being 'physiologically sensitive' to salt stress.

There was a negative relationship between intrinsic absolute growth and relative growth in response to salinity. Generally, *B. rapa* 'Sani' displayed higher absolute growth than *B. juncea* 'Common green' while 'Sani' showed higher relative growth reduction than *B. juncea* 'Common green' in response to salinity, in agreement with the response of *Trifolium repens* cultivar with the highest yield under non-saline conditions showed greater growth reduction than the lowest yielding cultivar (Rogers *et al*. 1993).

Salinity caused an accumulation of Na^+ and a decrease of K^+, Ca^{++}, and Mg^{++} in the shoots of the four cultivars. Na^+ accumulation was highest in 'Sani' and lowest in 'Common green' at each of the NaCl concentrations. However, K^+ content of shoots over the whole NaCl concentration range tested including the control was lowest in 'Sani' and 'Ndakupuka'. The Ca^{++} content of shoots of seedlings treated with NaCl was lowest in 'Sani'. The Mg^{++} content of the shoots was much lower than that of the other cations. The effect of NaCl on Mg^{++} was similar to that on Ca^{++}.

The ion relations of plants under salinity have been considered to be related to salinity tolerance, differing between salt-sensitive and salt-tolerant plants (Gorham *et al* 1990; Noble and Rogers 1992). For all entries, the NaCl treatments significantly increased the Na^+ concentration in the shoots accompanied by a decrease in K^+, Ca^{++}, Mg^{++}, K^+ / Na^+ ratio and Ca^{++} / Na^+ ratio, giving results similar to those of other studies on the salinity tolerance of *Brassica* species (He and Cramer 1992) and other crop species, such as *Sorghum* (Boursier and Läuchli 1990), *Hordeum* (Suhayda *et al*. 1992) and *Phaseolus* (Cachorro *et al*. 1993). In addition, Kumar (1984) found that salt-tolerant *B. juncea* cultivars showed higher seed yields with higher Ca^{++}, K^+ and K^+ / Na^+ ratios at a series of salinity levels than did salt-sensitive cultivars. Sharma and Gill (1994) concluded that the minimum reduction in seed yield of salt-tolerant *B. juncea* genotype was associated with a relatively lower Na^+ and higher K^+ accumulation in leaves of *B. rapa* 'Sani', which was the most salt-sensitive, showed the highest Na^+ accumulation and greatest K^+, Ca^{++}, Mg^{++}, K^+ / Na^+ and Ca^{++} / Na^+ ratio reduction among the four cultivars. *B. rapa* 'Sani' has the least ability of Na^+ exclusion from the shoots and of K^+, Ca^{++} and Mg^{++} maintenance in the shoots. This result suggests that the higher relative growth reduction of *B. rapa* 'Sani' may be correlated with higher Na^+ accumulation and lower K^+, Ca^{++} and Mg^{++} uptake under salinity. Na^+ exclusion from the shoot has been recognized as a salt-tolerance mechanism in glycophytes (Bayuelo-Jiménez *et al*. 2002). Ability to maintain a high shoot K^+ concentration determines the salinity tolerance of glycophytes (Siddiqui *et al*. 2006). Ca^{++} was found to be the only ion whose change was highly correlated with the relative salt-tolerance of six *Brassica* species (He and Cramer 1992). Therefore it has been suggested that salt sensitivity of *B. rapa* 'Sani' is due to its inability to exclude Na^+ ions from the shoot or leaf which may not only effect the maintenance of leaf turgor but also its related physiological process which results in retardation of early plant growth under saline environment.

REFERENCES

Ashraf M, Sharif R (1997) Does salt tolerance vary in a potential oil seed crop *Brassica carinata* at different growth stages? *Journal of Agronomy and Crop Science* **181**, 103-115

Ashraf M, Afaf YR, Qureshi MS, Sarwar G, Naqvi MH (2002) Salinity induced changes in α-amylase and protease activities and associated metabolism in cotton varieties during germination and early seedling growth stages. *Acta Physiologia Plantarum* **24**, 37-44

Ashraf M, McNeilly T (1990) Responses of four *Brassica* species to sodium chloride. *Environmental and Experimental Botany* **30**, 475-487

Bayuelo-Jiménez JS, Graig R, Lynch JP (2002) Salinity tolerance of *Phaseolus* species during germination and early seedling growth. *Crop Science* **42**, 1584-1594

Beltrao J, Faria J, Miguel G, Chaves P, Trindade D (2000) Cabbage yield response to salinity of trickle irrigation water. *Acta Horticulture* **537**, 641-645

Boursier P, Läuchli A (1990) Growth responses and mineral relations of salt-stressed sorghum. *Crop Science* **30**, 1226-1233

Cachorro P, Ortiz A, Cerdà A (1993) Growth, water relations and solute composition of *Phaseolus vulgaris* L. under saline conditions. *Plant Science* **95**, 23-29

Chartzoulakis KS (1992) Effect of NaCl salinity on germination, growth and yield of greenhouse cucumber. *Journal of Horticultural Science* **67**, 115-119

Chopra N, Chopra NK (1992) Salt tolerance of raya (*Brassica napus* var. *glauca*) varieties. *Indian Journal of Agronomy* **37**, 93-96

Dua RP, Sharma PC (1996) Physiological basis of salinity tolerance in pigeon pea (*Cajanus cajan*) and method of testing materials under highly variable soil conditions. *Indian Journal of Agricultural Science* **66**, 405-412

Esechie HA (1994) Interaction of salinity and temperature on the germination of *Sorghum*. *Journal of Agronomy and Crop Science* **172**, 194-199

Flowers TJ (2004) Improving crop salt tolerance. *Journal of Experimental Botany* **55**, 307-319

Foolad MR, Jones RA (1992) Parent-offspring regression estimates of heritability for salt tolerance during germination in tomato. *Crop Science* **32**, 439-442

Foolad MR, Lin GY (1997) Genetic potential for salt tolerance during germination in *Lycopersicon* species. *Horticultural Science* **32**, 296-300

Francois LE (1994) Growth, seed yield and oil content of canola grown under saline conditions. *Agronomy Journal* **86**, 233-237

Gorham J, Bristol A, Young EM, Wyn Jones RG, Kashour G (1990) Salt tolerance in the Triticeae: K/Na discrimination in barley. *Journal of Experimental Botany* **41**, 1095-1105

Gutierrez BFH, Scheiner JD, Lavado RS (1994) Some effects of soil salinity on growth, development, and yield of the rapeseed (*Brassica napus* L.). *Journal of Agronomy and Crop Science* **172**, 182-187

Hasterok R, Wolny E, Kulak S, Zdziechiewicz A, Maluszynska J, Heneen WK (2005) Molecular cytogenetic analysis of *Brassica rapa-Brassica oleracea* var. *alboglabra* monosomic addition lines. *Theoretical and Applied Genetics* **111**, 196-205

Hayat S, Ali B, Hasan SA, Ahmad A (2007) Effect of 28-homobrassinolide on salinity-induced changes in *Brassica juncea*. *Turkish Journal of Biology* **31**, 141-146

He T, Cramer GR (1992) Growth and mineral nutrition of six rapid cycling *Brassica* species in response to seawater salinity. *Plant and Soil* **139**, 285-294

Hoagland DR, Arnon DI (1938) The water-culture method for growing plants without soil. *Agricultural Experimental Station, University of California, Circular* **347**, 1-39

Khan MA, Ungar IA (2001) Alleviation of salinity stress and the response to temperature in two seed morphs *of Halopyrum mucronatim* (Poaceae). *Australian Journal of Botany* **49**, 777-783

Kumar D (1984) The value of certain plant parameters as an index for salt tolerance in Indian mustard (*Brassica juncea* L.). *Plant and Soil* **79**, 261-272.

Kumar D (1995) Salt tolerance in oilseed *Brassicas* present status and future prospects. *Plant Breeding* **65**, 1439-1447

Kumar V, Kumar D (1990) Response of Indian mustard to saline water application at different growth stages. *Trans Indian Society Desert Technology* **15**, 121-125

Lovato MB, Martins PS, de Lemos Filho JP (1994) Germination in *Stylosanthes humilis* populations in the presence of NaCl. *Australian Journal of Botany* **42**, 717-723

Maggio A, Pscale SD, Ruggiero C, Barbeieri G (2005) Physiological response of field-grown cabbage to salinity and drought stress. *European Journal of Agronomy* **23**, 57-67

Mamo T, Richter C, Heiligtag B (1996) Salinity effects on the growth and ion contents of some chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* Medic.) varieties. *Journal of Agronomy and Crop Science* **176**, 235-247

Munns R (2002) Comparative physiology of salt and water stress. *Plant, Cell and Environment* **25**, 239-250

Noble CL, Rogers ME (1992) Arguments for the use of physiological criteria for improving the salt tolerance in crops. *Plant and Soil* **146**, 99-107

Pérez-Alfocea F, Guerrier G, Estañ MT, Bolarin MC (1994) Comparative salt responses at cell and whole-plant levels of cultivated and wild tomato species and their hybrid. *Journal of Horticultural Science* **69**, 639-644

Prado FE, Boero C, Gallardo M, Gonzalez JA (2000) Effect of NaCl on germination, growth, and soluble sugar content in *Chenopodium quinoa* Willd. seeds. *Botanical Bulletin of Academia Sinica* **41**, 27-34

Rawson HM, Richards RA, Munns R (1988) An examination of selection criteria for salt tolerance in wheat, barley and *Triticale* genotypes. *Australian Journal of Agricultural Research* **39**, 759-772

Redmann RE, Qi MQ, Belyk M (1994) Growth of transgenic and standard canola (*Brassica napus* L.) varieties in response to soil salinity. *Canadian Journal of Plant Science* **74**, 797-799

Richards RA (1995) Improving crop production on salt-affected soils: By breeding or management? *Experimental Agriculture* **31**, 395-408

Rogers ME, Noble CL, Nicolas ME, Halloran GM (1993) Variation in yield potential and salt tolerance of selected cultivars and natural populations of *Trifolium repens* L. *Australian Journal of Agricultural Research* **44**, 785-789

Sharma PC, Gill KC (1994) Salinity-induced effect on biomass, yield, yield-attributing characters and ionic contents in genotypes of Indian mustard. *Indian Journal of Agricultural Science* **64**, 785-788

Siddiqui ZS, Shaukat SS, Zaman AU (2006) Alleviation of salinity-induced dormancy by growth regulators in wheat seeds. *Turkish Journal of Botany* **30**, 321-330

Snowdon RJ, Lühs W, Friedt W (2006) Oilseed rape. In Kole C (Ed) *Genome Mapping and Molecular Breeding (Vol 2) Oilseeds*, Springer-Verlag, Berlin, pp 55-114

Suhayda CG, Redmann RE, Harvey BL, Cipywnyk AL (1992) Comparative response of cultivated and wild barley species to salinity stress and calcium supply. *Crop Science* **32**, 154-163

Subbarao GV, Johansen C, Jana MK, Kumar R (1991) Comparative salinity responses among pigeonpea accessions and their relatives. *Crop Science* **31**, 415-418

Szabolcs I (1994) Soils and salinization. In: Pessarakli M (Ed) *Handbook of Plant and Crop Stress*, Marcel Dekker, New York, pp 3-11

Tal M, Shannon MC (1983) Salt tolerance in the wild relatives of the cultivated tomato: Responses of *Lycopersicon esculentum*, *L. cheesmenii*, *L. peruvianum*, *Solanum pennellii* and F1 hybrids to high salinity. *Australian Journal Plant Physiology* **10**, 109-117

Uma MS, Srinivas S, Hebbar M, Patil SG (1992) Screening of Indian mustard genotypes for saline vertisols of Tungabharda area of Karnataka. *Indian Journal of Agricultural Science* **62**, 486-488

Ungar IA (1996) Effect of salinity on seed germination, growth, and ion accumulation of *Atriplex patula* (Chenopodiaceae). *American Journal of Botany* **83**, 604-607

Yang YW, Newton RJ, Miller FR (1990) Salinity tolerance in *Sorghum*. I. Whole plant response to sodium chloride in *S. bicolar* and *S. halopense*. *Crop Science* **30**, 775-781

Yeo AR (1994) Physiological criteria in screening and breeding. In: Yeo AR, Flowers TJ (Eds) *Monographs on Theoretical and Applied Genetics 21, Soil Mineral Stresses, Approaches to Crop Improvement*, Springer-Verlag, Berlin, pp 37-60

Yokoish T, Tanimoto S (1994) Seed germination of the halophyte *Suaeda japonica* under salt stress. *Journal of Plant Research* **107**, 385-388