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GROWTH AND IONIC RELATIONS OF *BRASSICA CAMPESTRIS* AND *B. JUNCEA* (L.) CZERN & COSS. UNDER INDUCED SALT STRESS

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Gradient creation of external osmotic potential with NaCl reveals physiological traits of a plant species. *Brassica campestris* (cv. BSA) and *B. juncea* (cv. BARD-1) were grown for six weeks in nutrient solution of various osmotic concentrations (– 0.19, - 0.27, - 0.31 and - 0.42 MPa) using NaCl. Shoot and root length of *B. campestris* and root length of *B. juncea* declined with increasing osmotic potential as compared to control. In *B. juncea*, – 0.19 MPa ψ_s favored accumulation of K^+ and dry mass besides maintaining higher relative water contents. Shoot of *B. juncea* maintained higher concentration of $Ca^{2+}$and K^+ ions than that of *B. campestris* at all levels of external osmotic potential. Sodium ion was in antagonistic relation with K^+, Ca^{2+}, P and S. In shoot of *B. juncea* K^+ and Ca^{2+} were in synergistic relation with Na^+ up to - 0.31MPa, above this, both the nutrients started to decline. Under increasing osmotic conditions relative growth rate, leaf area ratio and relative water contents of both the species were affected.

INTRODUCTION

Besides inherited characters physiological processes of plant species are also a function of environmental factors. During the growth, both micro and macro environmental factors accelerate or retard growth process. Soil water contents control the availability of essential nutrients to plants and also affect nutrient uptake and root growth (Mirrch and Ketcheson, 1973). Excessive soil salinity occurs in many semi-arid to arid regions of the world where it inhibits the growth and yields of crop plants (Tanji 1990). According to Burman *et al.* (2003) there is detrimental effect of salinity and drought on plant water relation, net photosynthesis and leaf metabolite. The primary cause of ion-specific damage is Na^+ (Tester and Davenport, 2003). Therefore, presence of sodium chloride in glycophytes growing system reveals their degree of sensitivity. Besides influencing water storage and plant growth, sodium ion affects mineral compositions because salinity affects water and ionic status in the cells (Hasegawa *et al.*, 2000). Rapeseed (*B. napus* and *B. campestris*) has become the world's third most important vegetable oil crop, after soybean and palm oil (Downey and Rimmer, 1993). Indian mustard (*B. juncea*) maintains higher leaf turgor pressure and longer leaf area and thereby achieves greater dry weight and seed yield under terminal drought than canola (Wright *et al.*, 1997). Plant growth responses to salinity may vary with the degree and duration of stress, plant organ, or due to a specific ion. Salinity imposes two stresses on plant tissues: a water-deficit that results from the relatively high solute concentrations in the soil, and ion-specific stresses resulting from altered K^+/Na^+ ratios (Maris and Eduardo, 2002). Salt tolerance has been associated with preferential accumulation of K^+ against high Na^+ (Badr *et al.*, 2002). Kinraide (1998) reported that main effect of Ca^{+2} on the Na^+ uptake at high Ca^{+2} concentration was the electrostatic displacement of Na^+ from plasma membrane surface. Therefore detrimental effect of sodium ion disturbs the balance of nutrients in a crop. This study was conducted to observe the impact of different solute potential due to NaCl, on growth and ionic concentration of metal nutrients in two *Brassica* species.

MATERIAL AND METHODS

Seeds of *Brassica campestris* (cv. BSA) and *B. juncea* (cv. BARD-1) were germinated using moist quartz sand with distilled water. Full strength nutrient solution (Hoagland and Arnon, 1950) along with 15, 30, 60 and 90 mM NaCl having osmotic concentrations – 0.19, - 0.27, - 0.31 and -0.42 MPa respectively were prepared. The osmotic potential of the nutrient solution was measured by Vapour Pressure Osmometer, VAPRO, Wescor. One week old seedlings were foam-plugged in lids of plastic pots containing continuously aerated 2.5 L of nutrient solution. The fresh nutrient solution was replaced weekly. The light intensity was 450 µmol m^{-2} s^{-1}. Photoperiod was adjusted to 16 h light period and temperature was maintained at 30 ± 2 °C. The pH of the solution was adjusted to 6.0 with HCl or KOH and was monitored regularly. The treatments were applied in quadruplicates. Two harvests were taken on 17th and 42nd day after transplantation to pots. Data for absolute growth attributes pertains to 42nd day. Leaf area was measured on leaf area meter (CI-202, CID, Inc. USA). After recording fresh mass (FM), the plants were rinsed with deionised water, and were separated into shoot and root portions. Plant samples were dried at 65 °C to constant mass. Dry mass (DM) of each sample was recorded and was ground to pass a 40-mesh Wiley Mill. Ground samples of root and shoot were separately digested in 2:1 perchloric-nitric di-acid mixture (Chapman, and Pratt 1961). Relative water contents (RWC) were calculated according to Neelam and Dwievi (2004), Relative growth rate (RGR) and Leaf area ratio (LAR) were calculated as given by Franklin *et al.* (1985). Sodium, potassium and calcium ions in the digested material were determined by atomic absorption spectroscopy. Sulphur and phosphorus in the digested material was determined as given by Verma *et al.* (1977) and Chapman and Pratt (1961) respectively. The data were statistically analyzed according to two factors CRD and treatment means were compared using LSD test (Gomez and Gomez, 1984).

Table 1. *Brassica juncea* and *B. campestris* growth under NaCl stress

Osmotic potential (ψ_s) (-M Pa)	Shoot Length (cm)	Root Length (cm)	Fresh mass (g plant^{-1})		Dry mass (mg plant^{-1})	
			Shoot	Root	Shoot	Root
			B. campestris			
Control	15.6 b	12.3 b	2.90 d	1.90 c	276.3 f	176.3 d
0.19	12.3 c	10.5 d	2.22 e	1.22 e	242.3 g	142.3 e
0.27	10.6 d	8.3 e	1.71 f	0.71 g	213.6 h	113.6 g
0.31	8.5 f	7.3 f	1.30 g	0.38 i	180.3 i	80.3 h
0.42	6.3 g	4.3 h	0.78 h	0.13 j	130.5 j	30.5 i
			B. juncea			
Control	16.0 b	14.2 a	3.95 b	3.25 a	364.3 e	274.3 a
0.19	17.8 a	12.1 bc	4.35 a	2.49 b	388.2 d	228.2 b
0.27	12.8 c	11.8 c	3.81 bc	1.51 c	430.2 b	186.2 c
0.31	10.0 e	8.1 e	3.61 c	1.01 f	462.3 a	132.6 f
0.42	6.8 g	5.1 g	2.69 d	0.51 h	400.6 c	78.6 h
LSD	0.540	0. 435	0.261	0.087	11.73	6.48

Means sharing similar letter(s) in a column do not differ significantly at $p < 0.01$

RESULTS AND DISCUSSION

Brassica campestris and *B. juncea* expressed highly significant ($p < 0.01$) variability in growth and ionic concentrations of the nutrients in the shoot and root portions under external osmotic potential due to NaCl application in the root medium. Shoot and root length of *B. campestris* were declined gradually at ψ_s - 0.19, - 0.27, - 0.31 and – 0.42 MPa over the control. The sequence was same in the root of *B. juncea* however 11% increase in shoot length was observed compared to control at – 0.19 M Pa ψ_s. The same declining trend was recorded for DM of root of both the species and shoot of *B. campestris* at all ψ_s over control. This may be due to the presence of sodium ion, as enormous negative membrane potential across the plasma membrane of plant cells that favored the passive transport of Na^+ into cells. Sodium ion enters plant cells through the high-affinity K^+ transporter HKT1(Rus *et al.*, 2001) and through non-selective cation channels (Amtmann and Sanders,1999). In *B. juncea*, DM and FM of shoot was increased by 7 and 10 percent respectively higher than control at – 0.19 MPa ψ_s. From ψ_s - 0.27 to – 0.42 MPa, DM of shoot for *B. juncea* ranged from 49 to 71% than that of *B. campestris*. (Table 1).

This trend shows that tolerance to NaCl solute potential is also a function of composition and concentration gradient of the growth medium. There is wide variation in relative growth rate (RGR) when plant species are grown under near-optimal conditions (Poorter, 1989). Here both the species were grown under same conditions; RGR of *B. juncea* was higher than *B. campestris* that may be due to tolerance of the former species for sodium ion. Shoot and root of *B. campestris* was declined ranging from 101.4 to 93.5 and 96.2 to 73.9 µg g^{-1} d^{-1}. In *B. juncea* RGR of root declined in the same ψ_s sequence over control.

However, in this species RGR of shoot was increased at ψ_s - 0.19, - 0.27, and - 0.31 MPa but at - 0.42 MPa ψ_s it decreased (Table 2).

Leaf area ratio (LAR) of *B. campestris B. juncea* was decreased with increasing ψ_s. At ψ_s having – 0.19 LAR in both the species were same. Later on at subsequent increase of ψ_s, LAR of *B. campestris* was higher than that *of B. juncea*. Thus *B. juncea* performed better to accumulate metabolite with high efficiency than *B. campestris*. Relative water contents (RWC) of shoot and root in *B. campestris* decreased from 90.5 to 83.3 % and from 90.7 to 76.8 % respectively at elevated osmotic potential. In root of *B. juncea,* it decreased from 91.6 to 84.5 %. In its shoot, RWC was decline at -0.19 MPa ψ_s it was same as in control. Vacuolar sequestration of Na^+ not only lowers Na^+ concentration in the cytoplasm but also contributes to osmotic adjustment to maintain water uptake from saline solutions (Zhu, 2003). Nutrients had variable relations under sodium ion in these species. In both the species sodium ion had antagonistic relations with K^+, Ca^{2+}, P and S. The antagonistic correlation ranged from 0.91 to 0.99 at ψ_s -0.19 MPa, in shoot of *B. juncea* having Na^+ 1.15 %, K^+ concentration was increased slightly. Sodium ion stress disrupts K^+ uptake by root cells when Na^+ enters cells and accumulates to high levels, it becomes toxic to enzymes (Hasegawa *et al.*, 2000).

In shoot of *B. juncea* Mg^{2+}, S and P were in antagonistic relations with sodium ion with increasing solute potential whereas Ca^{2+} and K^+ were in synergistic relation with sodium ion with increasing ψ_s but at – 0.42 MPa both nutrients began to decline (Fig.1).

When under salt stress, plants maintain a high concentration of K^+ and a low concentration of Na^+ in the cytosol, they do this by regulating the expression and activity of K^+ and Na^+ transporters and of H^+ pumps that generate the driving force for transport (Zhu, 2003). Sodium ion current mediated by non-selective cation channels is partially sensitive to calcium, and this correlates with the inhibition of Na^+ entry into roots by calcium (Tester and Davenport 2003). Berthomieu *et al.* (2003) showed in *Arabidopsis*, AtHKT1 mutant is involved in Na^+ recirculation from shoots to roots which causes over accumulation of Na^+ in the shoot. In this study the concentration of Na^+ was higher in the root of *B. juncea* than that of *B. campestris*. There seems to be present accumulation process in the root of *B. juncea* that may be non-circulated.

The negative effects of NaCl created external osmotic potential on growth and higher ionic imbalancing in *B. campestris* compared to *B. juncea*. In *B. juncea*, – 0.19 MPa ψ_s favored accumulation of K^+ and dry mass, besides maintaining higher RWC. Thus gradient creation of external osmotic potential with NaCl revealed physiological traits in both the species.

Table 2. Effect of osmotic potential under NaCl application on relative growth rate, leaf area ratio and relative tissue water contents in *Brassica juncea* and *B. campestris*

Osmotic potential (ψ_s) (-M Pa)	RGR ($\mu g\ g^{-1}\ d^{-1}$)		LAR ($cm^2\ mg^{-1}$)	RWC (%)	
	Shoot	Root		Shoot	Root
B. campestris					
Control	101.4 d	96.2 a	0.280 b	90.5 ab	90.7 ab
0.19	99.5 f	95.4 ab	0.274 c	89.1 abc	88.3 bc
0.27	97.2 g	94.1 abc	0.264 d	87.5 bcd	83.9 e
0.31	94.9 h	82.9 cd	0.257 e	86.1 cde	78.9 f
0.42	93.5 i	73.9 de	0.250 f	83.3 e	76.8 f
B. juncea					
Control	102.5 c	98.1 a	0.298 a	90.8 a	91.6 a
0.19	103.1 c	97.2 a	0.275 c	91.1 a	90.8 ab
0.27	104.4 b	95.3 e	0.236 g	88.7 abc	87.6 c
0.31	105.3 a	83.8 bcd	0.211 h	87.2 cd	86.8 cd
0.42	100.7 e	81.7 d	0.198 i	85.1de	84.5 de
LSD	0.676	11.72	0.0014	3.06	2.58

Means sharing similar letter(s) in a column do not differ significantly at $p < 0.05$.

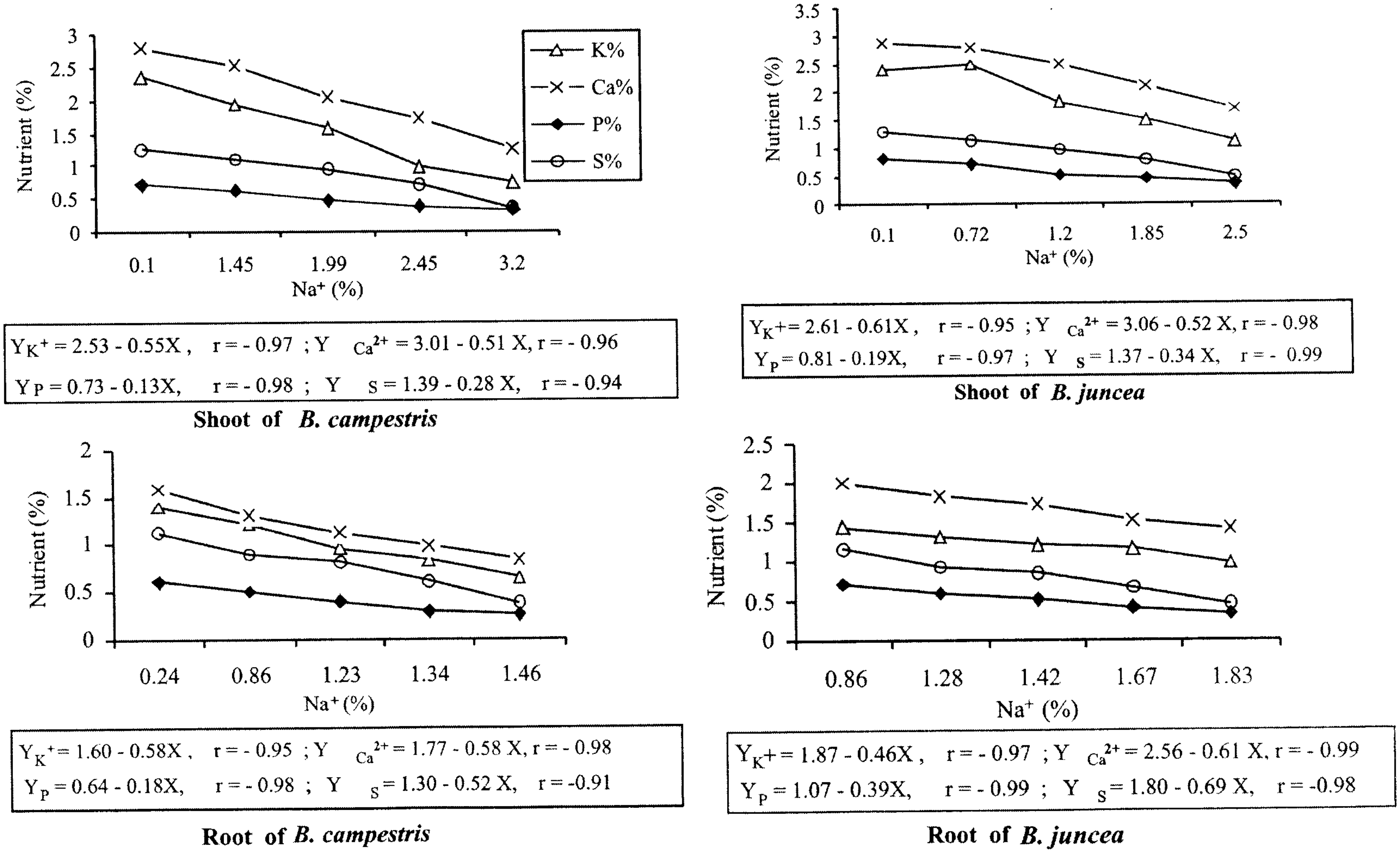

Fig.1. Relation between sodium ion and nutrients in shoot and root of *Brassica campestris* and *B. Juncea* under NaCl stress in the root medium

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