Pak. J. Bot., 38(3): 663-672, 2006.

TIME COURSE OF ION ACCUMULATION AND ITS RELATIONSHIP WITH THE SALT TOLERANCE OF TWO GENETICALLY DIVERSE LINES OF CANOLA (*BRASSICA NAPUS* L.)

M. QASIM AND M. ASHRAF*

Department of Botany,
University of Agriculture, Faisalabad 38040, Pakistan
*Correspondence addressed: email: ashrafm@fsd.paknet.com.pk

Abstract

A greenhouse experiment was conducted to examine the time course of ion accumulation in two genetically diverse lines of canola (*Brassica napus*) viz., Dunkeld (salt tolerant) and Cyclon (salt sensitive) subjected to 2.4 (control), 4, 8, and 12 dS m^{-1} of NaCl. Plants were harvested every 10-day interval after the initiation of salt treatment and harvesting continued until 5^{th} harvest i.e., 50 days after the initiation of salt treatment. An age-dependent accumulation of Na^+ and Cl^- occurred in both lines and the discrimination in the two lines with respect to ion accumulation was discernable at the last three harvests, Dunkeld was significantly lower in shoot Na^+ and Cl^- as compared to Cyclon. The salt tolerant line Dunkeld maintained higher K^+/Na^+ and Ca^{2+}/Na^+ ratios than those of the salt sensitive line Cyclon under saline conditions. However, the discrimination between the two canola lines with respect to K^+/Na^+ ratio was discernable at the later growth stages, whereas that of Ca^{2+}/Na^+ ratio at all stages. Discrimination between the two genetically diverse lines of canola with respect to accumulation of toxic ions was only possible at the later stages of vegetative growth rather than at the early stages.

Introduction

Response of a plant to saline growth substrate varies with its age thereby altering the degree of salt tolerance (Kingsbury & Epstein, 1984; Ashraf, 1994; Ashraf & Harris, 2004; Raza *et al*., 2006). This is true in many plant species in which degree of salt tolerance was found changed with change in plant growth stage e.g., sugar beet (Bernstein & Hayward, 1958), rice (Akbar & Yabuno, 1974), wheat (Kingsbury & Epstein, 1984; Ashraf & McNeilly, 1988; Ashraf & Khanum, 1997), alfalfa (Ashraf & O'Leary, 1994), *Cajanus cajan* (Ashraf, 1994), *Brassica carinata* (Ashraf & Sharif, 1998), although in some other crops the reverse was true since the salt tolerance in them was not age dependent, e.g., *Medicago sativa* (Noble 1984), *Brassica juncea* (Ashraf *et al*., 1994), *Linum usitatissimum* (Ashraf & Fatima, 1994), *Carthamus tinctorius* (Ashraf & Fatima, 1995), sunflower (Ashraf & Tufail, 1995). However, of the various plant responses to salt stress reported in literature, pattern of ion uptake is of prime importance since it determines the means whereby plants maintain water balance and avoid Na^+ and/or Cl^- toxicity under saline conditions (Munns *et al*., 2000). Control of Na^+ uptake by cells and long distance Na^+ transport was considered as an important adaptation of plants to saline substrate (Munns *et al*., 2000). For example, salt tolerance in most plants is associated with low uptake and accumulation of Na^+, which is mediated through the control of influx and/or by active efflux from the cytoplasm to the vacuoles and also back to the growth medium (Jacoby, 1999; Grattan & Grieve; 1999; Blumwald, 2000; Carden *et al*., 2003). Under naturally salt affected field using four *Brassica* species (relatively salt tolerant, *B. napus* and *B. carinata*; salt sensitive, *B. campestris* and *B. juncea*), Haq *et al*., (2002) found a close association between their degree of salt tolerance and ability to exclude both Na^+ and Cl^-. In contrast, while assessing the contribution of single cell cytosolic Na^+ and K^+ to salt tolerance in barley, Carden *et al*., (2003) found that the salt tolerant variety maintained a 10-fold lower cytosolic Na^+ in the root cortical cell than the salt sensitive variety, particularly at day 5 of salt stress, but this difference was masked at day 8 of the stress. Working with two species of *Lupinus*, Van Steveninck *et al*., (1982) showed that the salt tolerant species, *L. luteus* accumulated more Na^+ and Cl^- in the shoots than the salt sensitive *L. angustifolius*. Such pattern of ion accumulation was also found in different plant species, e.g., *Medicago sativa* (Croughan *et al*., 1978, Ashraf *et al*., 1986), *Lolium perenne* (Ashraf *et al*., 1990). Subbarao *et al*., (1990a) found that salt tolerant and salt sensitive lines of *Atylosia* species, did not differ significantly in shoot Na^+ and Cl^- contents. Similarly, in *Lesquerella fendleri*, Dierig *et al*., (2003) have found that a selected salt tolerant line WCL-SL1 did not differ significantly from the original line in mineral ion uptake and accumulation in leaf tissue.

All the afore-mentioned reports depict that glycophytes can use both ion exclusion or inclusion mechanisms in response to saline growth rooting medium. Therefore, full understanding of the ion response mechanism of a particular species would allow to use ion content as selection criterion (Ashraf, 2004). In view of a number of earlier reports on different crops parallels between pattern of ion uptake and growth under salt stress have been drawn at only one particular stage, but this may not reflect the true relationship particularly in that species in which degree of salt tolerance is age dependent (Ashraf & Khanum, 1997). Thus, the primary objective of conducting the present study was to assess as to whether pattern of ion accumulation varies with age of two lines of canola differing in salt tolerance (Qasim *et al*., 2003) to salt stress.

Materials and Methods

The seeds of two canola (*Brassica napus* L.) lines, Dunkeld (originally from Australia) and Cyclon (from Denmark) were obtained from the Oilseed Botanist, Ayub Agricultural Research Institute Faisalabad (latitude 31°30 N, longitude 73°10 E and altitude 213 m), Pakistan. Cv. Dunkeld was found salt tolerant and Cv. Cyclon as salt sensitive among a number of canola lines screened by Qasim *et al*., (2003). All seed samples were surface sterilized with 10 % Sodium hypochlorite solution for 5 min., and washed three times with distilled water. The experiment was conducted in a glasshouse at the Ayub Agricultural Research Institute, Faisalabad, where the maximum photosynthetic photon flux density (PPFD) measured at noon was 1360 μmol m^{-2} s^{-1}, average day/night relative humidity 58/74% and average temperature 24/8 °C. Glazed ceramic pots (26 cm diameter and 32 cm depth) containing 14 kg soil were arranged in a completely randomized design. Twenty seeds were sown in each pot, but after germination the seedlings were thinned to 12 in each pot. The experiment comprised of four treatments with four replications and two canola lines. At the seedling stage (20 days after germination), salt treatment was started by adding appropriate amount of NaCl in half strength Hoagland's nutrient solution to attain electrical conductivity 2.4 (control), 4, 8, and 12 dS m^{-1}. The salt was applied gradually in aliquot of 4 dS m^{-1} every day. Every day 200 cm^3 of distilled water was added to each pot to compensate for evapotranspiration loss.

This was sufficient to moisten the soil but did not cause leaching of salts from the pots. Two plants from each pot were harvested after every 10-day interval after the initiation of salt treatment and harvesting continued until 5^{th} harvest, i.e., 50 days after the initiation of salt treatment. At each harvest, plants were separated into shoots and roots, and washed with distilled deionized water. All plant samples were dried in an oven at 64 ^{o}C until constant dry weight.

Determination of ions in shoots and roots: For the determination of cations (Na^+, K^+ and Ca^{2+}) the dried ground shoot and root material (0.1 g each) of each harvest was digested in 5 mL concentrated HNO_3. Sodium, K^+ and Ca^{2+} were determined with a flame photometer (Jenway, PFP-7). For the determination of Cl^-, shoot and root samples of 50-100 mg each were ground and extracted in 10 ml of distilled water by heating at 80 ^{o}C for 3 h. Cl^- content in the extracts was determined with a chloride meter (Corning Chloride Analyzer, 925).

Statistical analysis: Data for all attributes were subjected to the COSTAT computer package (Cohort Software, Berkeley, USA) for calculating analysis of variance. The mean values were compared with the least significant difference test (LSD) following Snedecor & Cochran (1980).

Results

Salt stress caused a significant reduction in dry weights of shoots and roots of the two canola lines differing in salt tolerance, but the reduction was more pronounced in salt sensitive Cyclon than that in salt tolerant Dunkeld. However, time increase in shoot and root biomass was more in salt tolerant Dunkeld than that in salt sensitive Cyclon (Table 1). Generally, there was an increasing trend in shoot and root Na^+ of both cultivars with increase in salt level of the growth medium (Table 2). The pattern of Na^+ accumulation in the shoots of the two lines varied significantly with age. Maximum accumulation of Na^+ in the shoots of both cultivars occurred at harvest III, which was maintained at the last two harvests. The varietal difference in shoot Na^+ was discernable at the last three harvests at which salt sensitive Cyclon had much higher shoot Na^+ accumulation as compared to that in salt tolerant cv. Dunkeld. Root Na^+ concentration in both cultivars remained almost unaffected until harvest IV, but a pronounced reduction in root Na^+ in both cultivars was found at harvest V (Table 2). However, the varietal difference in root Na^+ was not easily discernable at either harvest.

Maximum accumulation of shoot Cl^- was observed at harvest IV in both cultivars (Table 3), particularly at the two higher external salt regimes (8.0 and 16.0 dS m^{-1}). A marked difference between the cultivars in shoot Cl^- was observed at the two higher salt levels and the last two harvests where shoot Cl^- accumulation was markedly higher in Cyclon than that in Dunkeld (Table 3). Although root Cl^- increased significantly in both cultivars with increase in external salt level, the difference among harvests or between the two cultivars was not discernable except at harvest V, where the root Cl^- accumulation was markedly low in both cultivars as compared to that at other four harvests.

Shoot K^+ concentration in both lines decreased consistently with increase in external salt level (Table 4). Shoot K^+ was significantly higher in salt tolerant Dunkeld than that in salt sensitive Cyclon at all external salt treatments only at harvests III and IV. No marked difference among harvests or between cultivars in root K^+ was found except at harvest V where root K^+ accumulation was significantly lower than that at other harvests (Table 4).

Table 1. Time course of shoot and root dry weights (± SE) in two canola lines.

Salt level (dS m^{-1})	Lines	30 Days*	40 Days	50 Days	60 Days	70 Days
		Shoot [g $plant^{-1}$]				**LSD 5% = 0.12**
2.4	Dunkeld	0.05 ± 0.004	0.18±0.03	0.49±0.07	0.64±0.07	3.57±0.25
	Cyclon	0.07 ± 0.005	0.14±0.01	0.38±0.05	0.56±0.04	2.96±0.14
4.0	Dunkeld	0.06 ± 0.003	0.17±0.04	0.56±0.04	0.92±0.07	2.18±0.16
	Cyclon	0.06 ± 0.005	0.14±0.03	0.25±0.04	0.52±0.05	1.07±0.08
8.0	Dunkeld	0.09 ±0.006	0.19±0.05	0.47±0.07	1.04±0.11	2.73±0.18
	Cyclon	0.06±0.004	0.13±0.04	0.24±0.06	0.37±0.02	0.97±0.04
12.0	Dunkeld	0.09±0.007	0.23±0.06	0.45±0.06	1.19±0.10	2.01±0.12
	Cyclon	0.06±0.005	0.12±0.01	0.22±0.03	0.38±0.04	0.50±0.03
		Root [mg $plant^{-1}$]				**LSD 5% = 7.32**
2.4	Dunkeld	3.10±0.13	12.2±1.07	29.2±1.92	33.4±2.43	127.3±11.2
	Cyclon	3.01±0.16	9.6±0.56	23.3±2.01	29.3±3.01	116.4±9.6
4.0	Dunkeld	3.11±0.19	16.3±1.23	30.3±2.45	47.2±3.24	142.2±12.9
	Cyclon	3.23±0.21	9.7±0.74	16.0±1.23	25.6±2.54	112.6±14.6
8.0	Dunkeld	3.45±0.24	10.2±0.65	24.2±1.43	43.4±3.21	144.3±10.4
	Cyclon	2.47±0.14	9.3±0.32	16.1±1.02	23.3±1.65	96.8±7.8
12.0	Dunkeld	2.92±0.22	10.2±0.76	21.4±1.11	35.4±2.97	96.5±8.6
	Cyclon	2.11±0.13	8.2±0.54	13.3±1.02	24.7±3.21	57.7±4.8

*30 days after planting and 10 days after the start of salt treatment.

Table 2. Time course of Na^+ accumulation [mg g^{-1} d.m. ± SE] in two canola lines.

Salt level (dS m^{-1})	Lines	30 Days*	40 Days	50 Days	60 Days	70 Days
		Shoot				**LSD 5% = 3.42**
2.4	Dunkeld	17.80±0.55	18.47±0.57	20.55±0.98	21.17±1.04	19.15±0.56
	Cyclon	16.02±0.31	15.27±1.11	19.37±0.50	16.42±1.25	26.72±0.75
4.0	Dunkeld	21.15±0.50	21.77±0.68	23.15±0.63	20.10±1.70	23.12±0.73
	Cyclon	19.40±0.25	17.07±0.22	24.65±0.74	22.00±1.63	32.62±1.24
8.0	Dunkeld	25.32±0.67	24.45±0.76	25.57±0.52	23.52±0.97	27.47±0.87
	Cyclon	24.92±0.11	21.15±0.54	28.30±0.61	25.37±1.53	34.47±1.22
12.0	Dunkeld	29.80±0.55	30.62±0.95	34.10±1.04	34.20±1.79	33.45±0.64
	Cyclon	28.50±0.21	29.47±0.51	39.87±0.68	38.37±0.40	39.25±0.73
		Root				**LSD 5% = 2.12**
2.4	Dunkeld	13.15±0.17	8.75±0.10	12.35±0.22	13.25±0.17	8.75±0.39
	Cyclon	12.40±0.17	9.97±0.46	12.80±0.14	11.60±0.08	6.32±0.62
4.0	Dunkeld	14.95±0.13	11.25±0.22	13.92±0.56	14.97±0.11	10.35±0.23
	Cyclon	16.67±0.19	11.15±0.44	14.25±0.30	12.70±0.19	12.25±0.20
8.0	Dunkeld	16.85±0.29	14.12±0.25	15.37±0.22	17.02±0.27	11.32±0.38
	Cyclon	17.80±0.18	14.82±0.27	16.00±0.39	14.57±0.27	10.15±0.27
12.0	Dunkeld	18.95±0.10	18.15±0.26	17.02±0.23	20.62±0.17	12.63±0.17
	Cyclon	21.65±0.61	17.65±0.17	17.40±0.34	18.05±0.15	11.07±0.14

*30 days after planting and 10 days after the start of salt treatment.

Shoot and root Ca^{2+} decreased significantly with increase in external salt level (Table 5). There was a significant age effect on the accumulation of Ca^{2+} in the shoots and roots of both cultivars. In particular, at the last 3 harvests, shoot and root Ca^{2+} was significantly higher than that at the initial two harvests. The cultivars did not differ significantly in shoot or root Ca^{2+} at either harvest or salt treatment.

Table 3. Time course of Cl^- accumulation [mg g^{-1} d.m. ± SE] in two canola lines.

Salt level (dS m^{-1})	Lines	30 Days*	40 Days	50 Days	60 Days	70 Days
		Shoot			**LSD 5% = 3.46**	
2.4	Dunkeld	14.50±0.19	12.82±0.17	17.10±0.18	16.47±2.35	17.35±1.62
	Cyclon	17.85±0.37	13.47±0.22	21.60±0.16	13.42±2.07	19.22±2.08
4.0	Dunkeld	18.70±0.14	18.10±0.21	22.07±0.20	30.05±2.12	33.45±5.11
	Cyclon	21.85±0.29	19.87±0.51	26.00±0.13	13.67±1.19	13.85±1.30
8.0	Dunkeld	29.75±0.27	27.42±0.34	27.95±0.13	30.07±0.86	28.75±5.79
	Cyclon	31.42±0.47	30.95±0.33	29.30±0.21	22.35±1.17	25.75±1.95
12.0	Dunkeld	46.22±0.31	40.02±0.41	39.00±0.21	54.57±4.21	41.32±7.74
	Cyclon	50.22±0.46	42.25±1.96	44.60±0.37	65.15±10.18	60.15±4.15
		Root			**LSD 5% = 2.14**	
2.4	Dunkeld	9.95±0.18	5.57±0.11	12.60±0.21	5.67±0.57	3.27±0.08
	Cyclon	10.70±0.14	10.00±0.17	11.37±0.13	4.45± 0.10	2.80±0.11
4.0	Dunkeld	14.87±0.13	10.70±0.11	17.85±0.19	8.12±0.15	5.72±0.33
	Cyclon	15.25±0.19	14.12±0.19	15.55±0.22	7.05±0.22	5.35±0.12
8.0	Dunkeld	23.90±0.15	15.45±0.19	23.22±0.26	13.12±0.16	11.72±0.20
	Cyclon	23.65±0.17	20.45±0.20	18.45±0.15	10.50±0.20	7.85±0.22
12.0	Dunkeld	31.52±0.17	20.90±0.17	34.42±0.45	14.47±0.17	16.20±0.18
	Cyclon	28.95±0.20	23.95±0.09	25.15±0.26	13.75±0.17	12.85±0.29

*30 days after planting and 10 days after the start of salt treatment.

Table 4. Time course of K^+ accumulation [mg g^{-1} d.m. ± SE) in two canola lines.

Salt level (dS m^{-1})	Lines	30 Days*	40 Days	50 Days	60 Days	70 Days
		Shoot			**LSD 5% = 3.61**	
2.4	Dunkeld	36.35±0.86	33.37±1.10	30.42±2.48	44.17±1.52	26.12±1.16
	Cyclon	41.15±0.61	34.35±1.38	45.85±2.06	38.77±2.30	32.62±0.44
4.0	Dunkeld	31.45±0.34	25.57±0.84	28.97±2.19	32.95±2.45	25.67±0.40
	Cyclon	34.02±0.96	28.42±1.36	26.75±1.26	29.52±1.38	27.15±0.41
8.0	Dunkeld	25.25±0.67	21.47±0.34	27.55±1.32	30.65±2.27	21.17±0.33
	Cyclon	29.25±0.67	21.95±1.26	23.90±0.81	25.17±0.52	22.87±0.98
12.0	Dunkeld	15.82±1.36	20.40±0.85	24.90±1.10	25.02±1.55	19.20±0.66
	Cyclon	20.47±0.70	19.07±0.50	18.10±0.98	22.90±0.58	19.50±0.51
		Root			**LSD 5% = 3.01**	
2.4	Dunkeld	33.00±0.41	27.25±0.48	38.37±1.41	35.00±1.29	26.85±0.17
	Cyclon	32.50±0.29	31.00±0.41	36.87±1.87	33.00±0.41	26.17±0.55
4.0	Dunkeld	28.25±0.25	24.00±0.41	30.90±0.30	30.00±4.41	21.00±1.05
	Cyclon	29.75±0.25	30.00±0.41	29.70±0.30	29.50±0.29	19.80±0.18
8.0	Dunkeld	23.50±0.29	22.50±0.29	28.90±0.07	27.00±0.41	18.50±0.53
	Cyclon	26.50±0.65	27.50±0.29	27.77±0.54	27.50±0.29	19.30±1.07
12.0	Dunkeld	20.50±0.29	22.00±0.41	22.65±0.21	25.25±0.63	14.05±0.17
	Cyclon	20.25±0.25	22.75±0.48	22.70±0.37	24.25±0.48	14.15±0.38

*30 days after planting and 10 days after the start of salt treatment.

Shoot and root K^+/Na^+ ratios decreased linearly in both cultivars with increase in external salt level (Table 6). The maintenance of shoot K^+/Na^+ ratio was changed with age of plants. For example, at the first two harvests salt sensitive Cyclon maintained significantly high shoot K^+/Na^+ ratios at all external salt levels, but the reverse was true at the latter three harvests. There was no age-dependent increase or decrease in root K^+/Na^+ ratio in both lines and the cultivars also did not differ significantly at either harvest or salt treatment.

Table 5. Time course of Ca^{2+} accumulation [mg g^{-1} d.m. ± SE] in two canola lines.

Salt level (dS m^{-1})	Lines	30 Days*	40 Days	50 Days	60 Days	70 Days
		Shoot			LSD 5% = 2.41	
2.4	Dunkeld	17.50±0.96	14.50±1.71	31.50±0.96	29.50±1.85	26.25±0.63
	Cyclon	13.50±0.50	8.50±0.96	31.75±0.25	27.00±0.58	26.00±1.16
4.0	Dunkeld	16.75±0.86	11.25±0.48	29.75±0.25	26.25±2.14	20.00±1.36
	Cyclon	11.00±0.41	7.75±0.48	29.00±1.08	25.00±1.23	22.00±1.96
8.0	Dunkeld	12.50±0.65	9.75±0.25	28.00±0.82	24.75±2.50	18.25±2.96
	Cyclon	9.75±0.25	5.25±0.48	25.00±0.58	23.50±0.29	21.50±0.29
12.0	Dunkeld	11.00±0.41	6.25±0.25	26.50±0.50	21.75±0.75	21.75±0.86
	Cyclon	8.75±0.48	3.50±0.29	23.00±1.29	21.00±0.71	20.25±0.25
		Root			LSD 5% = 2.26	
2.4	Dunkeld	17.50± .50	11.75±0.86	25.50±1.19	21.50±1.90	16.25±0.25
	Cyclon	9.25± .48	10.50±0.29	26.75±0.63	26.00±1.42	19.25±0.48
4.0	Dunkeld	15.50±0.50	10.50±0.29	20.75±0.48	18.50±0.96	15.25±0.48
	Cyclon	12.00±0.82	9.25±0.48	21.25±0.48	23.00±1.29	17.25±0.48
8.0	Dunkeld	22.00±0.82	8.00±0.82	17.50±0.29	15.00±0.58	15.00±0.41
	Cyclon	11.00±0.58	7.50±1.26	16.00±0.41	18.50±0.96	16.25±0.25
12.0	Dunkeld	11.50±0.96	5.75±0.25	12.25±0.25	14.00±1.42	13.25±0.48
	Cyclon	9.50±0.50	3.75±0.25	11.75±0.63	17.50±0.50	15.00±0.41

*30 days after planting and 10 days after the start of salt treatment.

Table 6. Time course of K^+/Na^+ ratio (± SE) in two canola lines.

Salt level (dS m^{-1})	Lines	30 Days*	40 Days	50 Days	60 Days	70 Days
		Shoot			LSD 5% = 0.34	
2.4	Dunkeld	2.05±0.09	1.81±0.08	1.50±0.18	2.10±0.10	1.36±0.03
	Cyclon	2.57±0.04	2.30±0.23	2.37±0.10	2.10±0.23	1.33±0.04
4.0	Dunkeld	1.49±0.05	1.17±0.03	1.26±0.13	1.67±0.17	1.11±0.05
	Cyclon	1.75±0.05	1.60±0.07	1.08±0.04	1.37±0.13	0.83±0.02
8.0	Dunkeld	1.00±0.03	0.88±0.02	1.08±0.06	1.30±0.07	0.77±0.04
	Cyclon	1.17±0.03	1.04±0.08	0.85±0.05	1.00±0.06	0.66±0.03
12.0	Dunkeld	0.53±0.45	0.67±0.03	0.73±0.05	0.73±0.02	0.5±0.01
	Cyclon	0.72±0.03	0.65±0.02	0.45±0.02	0.60±0.29	0.50±0.02
		Root			LSD 5% = 0.44	
2.4	Dunkeld	2.51±0.04	3.11±0.06	3.11±0.16	2.65±0.13	3.09±0.17
	Cyclon	2.62±0.06	3.12±0.12	2.88±0.12	2.85±0.05	4.27±0.45
4.0	Dunkeld	1.89±0.02	2.13±0.02	2.23±0.11	2.00±0.02	2.02±0.09
	Cyclon	2.03±0.03	2.70±0.13	2.09±0.06	2.23±0.36	2.33±0.07
8.0	Dunkeld	1.39±0.01	1.59±0.01	1.88±0.29	1.59±0.02	1.64±0.10
	Cyclon	1.49±0.04	1.85±0.02	1.74±0.02	1.89±0.33	1.90±0.11
12.0	Dunkeld	1.08±0.02	1.21±0.01	1.33±0.03	1.22±0.03	1.11±0.01
	Cyclon	0.94±0.34	1.29±0.03	1.31±0.35	1.34±0.01	1.28±0.38

*30 days after planting and 10 days after the start of salt treatment.

Shoot or root Ca^{2+}/Na^+ ratios were found to be higher at the last 3 harvests than those at the first two harvests (Table 7). A prominent difference in shoot Ca^{2+}/Na^+ was observed between the two lines at all harvests or salt levels, Dunkeld being higher in shoot Ca^{2+}/Na^+ ratio than cv. Cyclon. However, in root Ca^{2+}/Na^+ ratio cv. Dunkeld was higher than cv. Cyclon at the first three harvests, but at the latter three harvests the reverse was true.

Table 7. Time course of Ca^{2+}/Na^{+} ratio (± SE) in two canola lines.

Salt level (dS m⁻¹)	Lines	30 Days*	40 Days	50 Days	60 Days	70 Days
		Shoot			**LSD 5% = 0.18**	
2.4	Dunkeld	0.98±0.05	0.79±0.10	1.54±0.05	1.39±0.06	1.37±0.03
	Cyclon	0.84±0.02	0.56±0.08	1.64±0.04	1.67±0.11	0.98±0.07
4.0	Dunkeld	0.80±0.06	0.52±0.03	1.29±0.04	1.33±0.15	0.87±0.09
	Cyclon	0.57±0.02	0.45±0.03	1.18±0.05	1.16±0.13	0.67±0.04
8.0	Dunkeld	0.49±0.03	0.40±0.01	1.09±0.02	1.05±0.08	0.67±0.05
	Cyclon	0.39±0.01	0.25±0.03	0.88±0.02	0.94±0.07	0.62±0.02
12.0	Dunkeld	0.37±0.02	0.20±0.01	0.78±0.04	0.64±0.06	0.65±0.03
	Cyclon	0.31±0.02	0.12±0.01	0.58±0.04	0.55±0.03	0.52±0.01
		Root			**LSD 5% = 0.12**	
2.4	Dunkeld	1.33±0.10	1.34±0.11	2.06±0.09	1.62±0.16	1.87±0.08
	Cyclon	0.74±0.04	1.05±0.02	2.09±0.06	2.24±0.11	3.12±0.29
4.0	Dunkeld	1.04±0.03	0.94±0.03	1.50±0.09	1.23±0.07	1.47±0.06
	Cyclon	0.82±0.07	0.83±0.05	1.49±0.05	1.81±0.11	2.03±0.10
8.0	Dunkeld	1.31±0.06	0.57±0.07	1.32±0.03	0.88±0.02	1.33±0.45
	Cyclon	0.62±0.03	0.51±0.09	1.00±0.03	1.27±0.07	1.60±0.02
12.0	Dunkeld	0.61±0.06	0.32±0.02	0.72±0.02	0.68±0.07	1.05±0.04
	Cyclon	0.44±0.03	0.21±0.01	0.67±0.02	0.97±0.02	1.36±0.05

*30 days after planting and 10 days after the start of salt treatment.

Discussion

From the data for time course of ion accumulation in two canola lines differing in salt tolerance under saline conditions it is evident that an age-dependent accumulation of Na^+ and Cl^- occurred. For example, shoot Na^+ and Cl^- remained unaffected at the first two harvests (30 and 40 d after planting but 10 and 20 d after the start of salt treatment, respectively). However, higher accumulation of both Na^+ and Cl^- in the shoots of the two lines was found at harvest III and onwards, and the discrimination in the two lines with respect to ion accumulation was discernable at the last three harvests; Dunkeld was significantly lower in shoot Na^+ and Cl^- as compared to Cyclon. A similar age-dependent accumulation of Na^+ and/or Cl^- has earlier been observed in some crops e.g., *Medicago sativa* (Ashraf & O'Leary, 1994), *Brassica carinata* (Ashraf & Sharif, 1998), *Triticum aestivum* (Ashraf & Khanum, 1997). Furthermore, while measuring single cells for assessing the contribution of cytosolic Na^+ and K^+ to salt tolerance of barley, Carden *et al*., (2003) found that the salt tolerant cultivar maintained a 10-fold lower cytosolic Na^+ in the root cortical cell than the salt sensitive cultivar, particularly at day 5 of salt stress. However, this difference was masked at day 8 of the stress.

The uptake and accumulation of ions in plants is considered an important indicator of salinity tolerance, because they are genetically regulated, though also affected by the environment (Mahmood, 1991; Chaubey & Senadhira, 1994). However, the different pattern of ion accumulation in the two canola lines at different growth stages clearly shows that though genes for ion uptake are present in the canola lines, their expression occurs only at the late vegetative growth stage.

It is now well established that plants subjected to saline environments, take up high amounts of Na^+, whereas the uptake of K^+ and Ca^{2+} is significantly reduced. Reasonable amounts of both K^+ and Ca^{2+} are required to maintain the integrity and functioning of cell membranes in plants subjected to saline conditions (Marschner 1995, Davenport *et al*., 1997; Wenxue *et al*., 2003). Thus, high K^+/Na^+ and Ca^{2+}/Na^+ ratios in plants under saline conditions have been suggested as important selection criteria for salt tolerance (Ashraf, 1994, 2004; Gorham *et al*., 1997; Wenxue *et al*., 2003; Chinnusamy *et al*., 2005). Likewise, in the present study the salt tolerant line Dunkeld maintained higher K^+/Na^+ and Ca^{2+}/Na^+ ratios than those of the salt sensitive line Cyclon under saline conditions. However, the discrimination between the two canola lines with respect to K^+/Na^+ ratio was discernable at the later growth stages, whereas that of Ca^{2+}/Na^+ ratio at all stages.

The maintenance of Ca^{2+} acquisition and transport under saline conditions is an important determinant of salinity tolerance (Soussi *et al*., 2001; Unno *et al*., 2002). Although Ca^{2+} uptake in both canola lines in the present study was age-dependent, no difference in this attribute was found between the lines differing in salt tolerance. Such an age-dependent Ca^{2+} uptake was found in two *Brassica* species differing in salt tolerance (He & Cramer, 1993). They found that a relatively salt-tolerant *Brassica napus*, differed from the relatively salt-sensitive *B. carinata*, in the relative reduction in Ca^{2+} at the cellular level in callus, but not in whole plants. Furthermore, the relationship between salt tolerance and Ca^{2+} retention among different plant species was examined by Unno *et al*., (2002) using salt tolerant maize (*Zea mays*) and squash (*Cucurbita maxima*), and salt sensitive reed canary grass (*Phalaris arundinacea*) and cucumber (*Cucumis sativus*). There was a high release of Ca^{2+} from root sections and intact roots of the salt sensitive plants subjected to saline substrate. The distribution of Ca^{2+} in shoot declined to a great extent in the salt sensitive plants under saline conditions. These results suggest that the ability of plants to retain Ca^{2+} is associated with their salt tolerance. Furthermore, Foolad (1997) found that Ca^{2+} maintenance and Na^+ exclusion, which is related to salinity tolerance in *Lycopersicon esculentum*, were genetically controlled with additive major genetic components. The inherent genetic capability to maintain Ca^{2+} in tissue and to exclude Na^+ from shoots were highly heritable traits, suggesting Ca^{2+}/Na^+ ratios as the promising indicators.

Overall, accumulation of Na^+ and Cl^- in the two canola lines differing in salt tolerance was age-dependent. Discrimination between the two genetically diverse lines in ion accumulation was possible at the later vegetative growth stage but not at the early stages.

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(Received for publication 12 August 2005)