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TIME COURSE CHANGES IN IONIC COMPOSITION AND TOTAL SOLUBLE CARBOHYDRATES IN TWO BARLEY CULTIVARS AT SEEDLING STAGE UNDER SALT STRESS

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Abstract

Seeds of two barley cultivars (i.e. Jou-87 and B-00047) were exposed to two levels of NaCl (150 mol m^{-3} and 300 mol m^{-3}) along with control in petridishes. Salt stress severely affected germination rate and percentage. Early growth parameters including plumule and radical length as well as fresh and dry weights of plumules and radicals were severely reduced by increasing salinity level in the growth medium. Total soluble carbohydrates increased in the plumule however, the reverse was true for radicle. Na^+ content increased whereas K^+ and Ca^{2+} content decreased in both plumule and radicle. It was observed that with the passage of time, there was a sharp decline in relative increase in the plumule and radical length. In addition, Na^+ content increased in both plumule and radicle, whereas K^+ content increased only in plumule. In contrast Ca^{2+} contents were not much affected with time in both plumule and radicle.

Keywords: Time-course, ionic composition, soluble sugars, barley, germination

Introduction

Abiotic stresses such as salinity, water logging and temperature etc., drastically affect crop productivity worldwide (Taiz & Zeiger, 2006). Among abiotic stresses, soil salinity more adversely affects plant growth and development (Rus *et al*., 2000). It is estimated that, about one-third of irrigated land has been affected with salinity and saline area is sharply increasing each year.

A general response of plants to salinity may be reduction in growth (Ashraf and Harris, 2004), especially at high concentrations due to its osmotic effects (Munns & Termaat, 1986; Ashraf, 1993). An excess of soluble salts in the soils leads to osmotic stress, which results in specific ion toxicity and ionic imbalances (Munns, 2003). However, at low or moderate salt stress many plants adjust osmotically and thus maintain a potential for the influx of water for continued growth (Ghoulam *et al*., 2002).

Adaptation to salinity stress is associated with metabolic adjustments that lead to the accumulation of several organic solutes like sugars, polyols, betaines and proline (Greenway & Munns 1980; Ashraf & Foolad, 2007). Among these, sugars represent the major reserve in the seeds (Bewley & Black, 1994) which are mobilized and readily transported during germination to various tissues (Jamil *et al*., 2005) in the form of sucrose, glucose and fructose. These carbohydrates are ultimately utilized to maintain growth (Ashraf, 1993) and osmotic regulation of cells (Ashraf, 1997) under stressful environments.

In this present study, we aimed to draw relationship between time course changes in ionic composition and total soluble carbohydrates under salt stress in two barley cultivars.

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Materials and Methods

Seeds of two barley cultivars (i.e. Jou-87 and B-00047) were exposed to two levels of NaCl (150 mol m^{-3} and 300 mol m^{-3}) along with control in petridishes lined with double layer filter paper, containing 10 ml of half strength Hoagland solution (Hoagland, 1950). The experiment was laid down in completely randomized design (CRD) with factorial arrangement with five replicates. The data regarding various parameters in response to salinity was recorded at three different time intervals i.e., 7 (H_1), 14 (H_2) and 21 (H_3) days after treatment. Upon germination of the seeds, days to 50 % germination were counted and germination percentage was calculated. Fresh and dry weights of plumules and radicals and plume and radical length were measured for all three harvest intervals. Ionic (Na^+, K^+ and Ca^{2+}) distribution in plumule and readical was determined in acid digest (NHO_3) using an atomic absorption spectrometer (AAnalyst 300, Perken-Elmer, USA). Total soluble carbohydrates of the plant tissues were determined following Yemm & Willis (1954). Ground dry plant material (0.1 g) was homogenized in hot 80% ethanol and centrifuged at 2900 **x** g. The residue was retained and was repeatedly washed with 80% ethanol to remove all the traces of soluble sugars. The filtrate thus obtained was used for the determination of soluble sugars. The extracts were treated with the anthrone reagent and absorbance was read at 625 nm using a spectrophotometer (Hitachi U-2000).

Results

The results of this study revealed that 50 % germination rate was significantly reduced by increasing extent of salinity stress. Both genotypes had no difference with regards to 50% germination rate. Similarly, the interactive effect of salinity and genotypes was also non-significant (Table 1). Both genotypes showed maximum reduction was at the highest level of salt (300 mol m^{-3}) followed by lower level (150 mol m^{-3}). Although, Jou-87 showed comparatively better response at 150 mol m^{-3}, the response of both the cultivars was similar at 300 mol m^{-3} (Fig. 1).

Germination %age was severely reduced by application of salinity to barley cultivars. Both cultivars showed significantly different response towards salt application. However the interactive effect of salinity with genotypes was non-significant (Table 1). In contrast to 50% germination rate, germination percentage was better in B-00047 compared with Jou-87 (Fig. 1).

Salinity stress significantly reduced plumule length in all intervals. On the other hand, the response of both genotypes was significant at the first interval only and no difference was observed at the second and third interval. Similarly, the interactive effect of salinity with genotypes was significant at the first and second intervals and non-significant at the third interval (Table 2). Jou-87 performed better at the first harvest interval and showed less reduction in plumule length compared with B-00047. However, at the second and third harvest interval, both the genotypes showed equal reduction in plumule length upon exposure to salinity stress (Fig. 2).

Radical length was also significantly reduced by at all harvest intervals. However, the interaction between genotypes and salinity was significant at the second interval only and was non significant at the first and third intervals (Table 2). Jou-87 again showed less reduction in radical length as compared to B-00047 at all the intervals (Fig. 2).

Table 1. Analyses of variance (ANOVA) for germination parameters of the two barley cultivars subjected to salt (NaCl) stress.

Source of Variation	d.f.	Days to 50 % germination	Germination percentage
Salinity	2	60.6 ***	126.17 ***
Genotypes	1	0.50 ns	364.50 ***
Interaction (SxG)	2	0.17 ns	1.17 ns
Error	12	0.17	4.44

*, **, *** = significant at 0.05, 0.01 and 0.001 levels, respectively, ns = non-significant

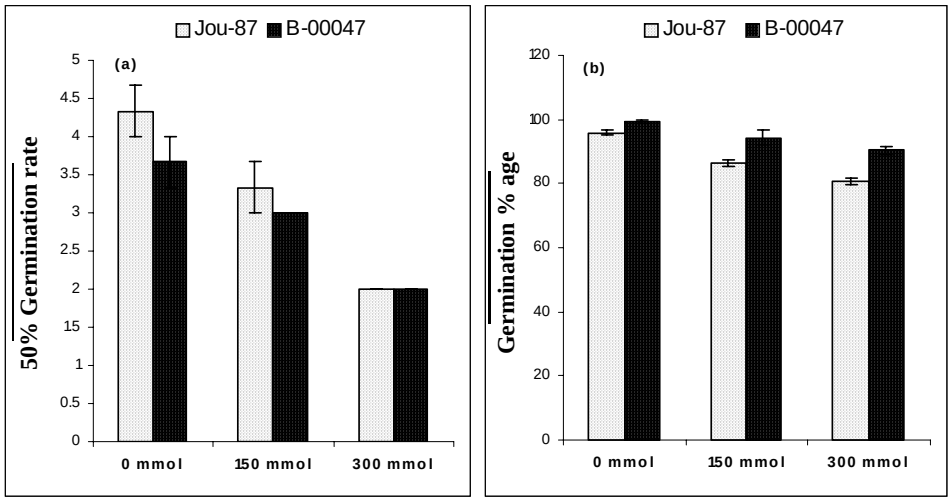

Fig. 1. Effect of different levels of NaCl (mol m^{-3}) on (a) 50% germination rate and (b) germination %age of the two barley cultivars.

Table 2. Analyses of variance (ANOVA) for growth parameters of the two barley cultivars subjected to salt (NaCl) stress.

Source of variation	d.f.	Plumule length			Radicle length		
		7-days	14-days	21-days	7-days	14-days	21-days
Salinity	2	42.96 ***	145.02***	114.73 ***	17.15 ***	89.61***	113.61 ***
Genotypes	1	1.02 *	0.27ns	20.93 ns	2.26 ***	9.00 **	11.89 ***
Interaction (SxG)	2	2.32 ***	89.61*	9.73 ns	1.41 ns	2.94*	1.65 ns
Error	12	0.17	9.00	5.65	0.12	2.46	0.57
		Plumule fresh weight			Radicle fresh weight		
Salinity	2	7043.45***	11016.67***	13400.00***	2357.61***	11016.67***	5016.67***
Genotypes	1	1090.45***	200.00 ns	3472.22***	25.21ns	200.00 ns	1250.00*
Interaction (SxG)	2	326.89***	350.00 ns	955.56**	57.52*	350.00 ns	616.67ns
Error	12	20.95	205.56	88.89	10.71	205.56	161.11
		Plumule Dry weight			Radicle Dry weight		
Salinity	2	43.79 ***	19.13 *	13.50 *	9.67 ***	22.88 ***	68.09 ***
Genotypes	1	21.17 ***	0.50 ns	79.38 ***	8.54 ***	0.29 ns	17.80 *
Interaction (SxG)	2	2.18 ***	3.13 ns	0.35 ns	1.31 *	3.29 **	6.25 ns
Error	12	0.14	3.98	3.61	0.22	0.35	2.03

*, **, *** = significant at 0.05, 0.01 and 0.001 levels, respectively, ns = non-significant

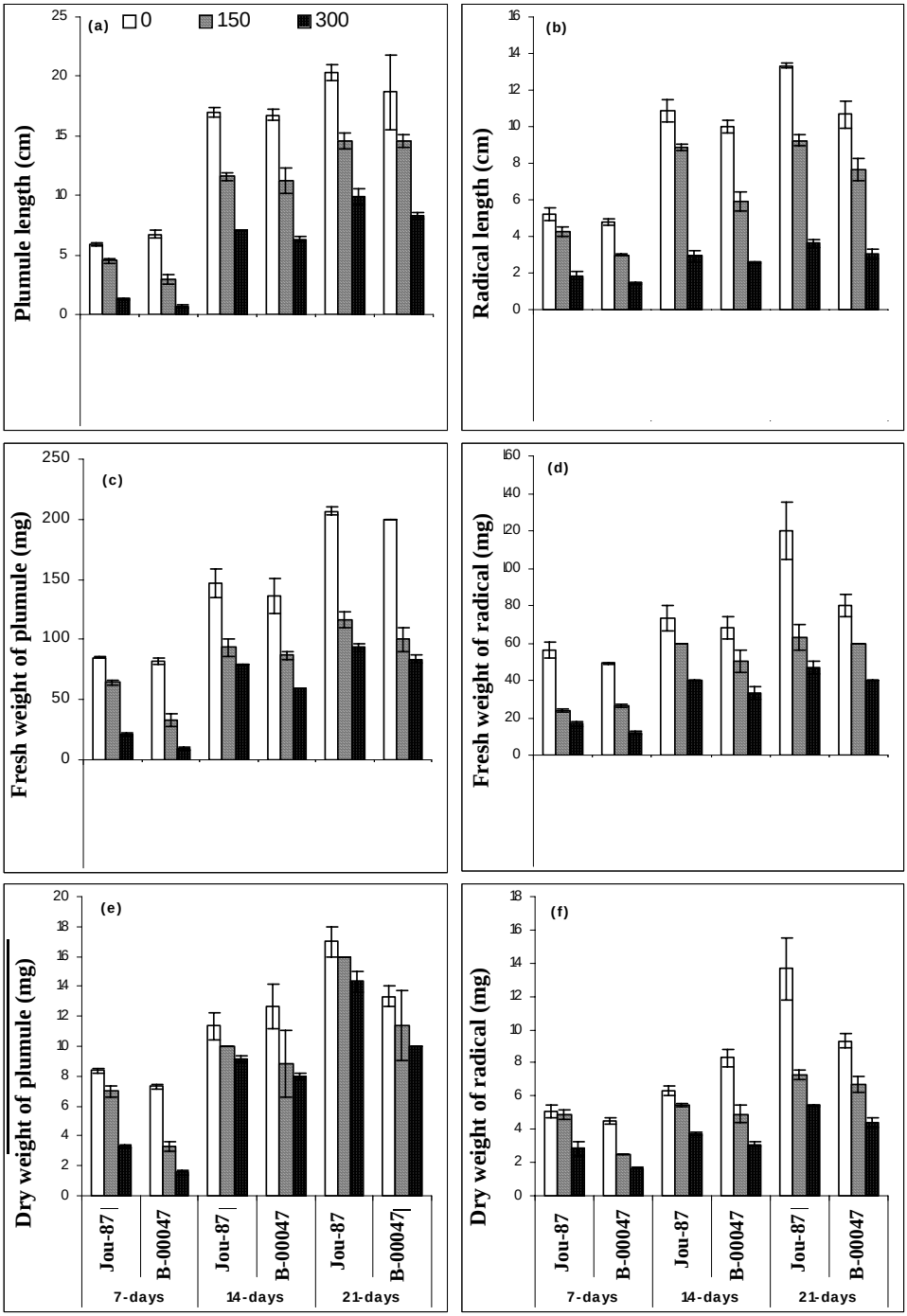

Fig. 2. Time course changes in different growth parameters of the two barley cultivars under the influence of NaCl salinity (mol m^{-3}).

Both levels of salinity significantly reduced plumule fresh weight in both genotypes at the first and third intervals, however, it was not different at second interval. The interactive effect of salinity with genotypes was significantly different at the first interval as well as third interval and non significant in second interval (Table 2, Fig. 2).

Radical fresh weight was significantly reduced by both levels of salinity in both cultivars. The interactive effect of salinity with genotypes was significant at the first interval and non-significant at the second and third interval (Table 2). Barley genotype Jou-87 performed better only at the first interval, whereas the response of both the genotypes was similar at the second and third intervals (Fig. 2).

Plumule dry weight was more reduced as compared to plumule fresh weight. The plumule dry weight was significantly different in both the genotypes at the first and third intervals but it was not statistically different at the second interval. The interactive effect of salinity with genotypes was significant only at the first interval (Table 2). Barley cultivar Jou-87 performed better as compared to B-00047 at the first two intervals, however its response was significantly better at the third interval (Fig. 2).

Radical dry weight was also reduced at both levels of salinity. Radical dry weight was significantly different in both genotypes at first and third intervals. However, no difference was observed in radical length at the second interval. Although the interactive effect of salinity with both cultivars was different in the first and second intervals, no difference was observed at the third interval (Table 2; Fig.2)

Na^+ content in the plumule increased significantly with the increasing concentration of salt in the growth medium. Both the genotypes accumulated significantly different Na^+ contents in the last two intervals however; this difference was less in the first interval. The interaction between salinity and genotypes was non-significant in the first and third interval however; it was significantly different in the second interval (Table 3). B-00047 accumulated more Na^+ as compared to B-87 at all the intervals (Fig. 3).

Table 3. Analyses of variance (ANOVA) for cation accumulation of the two barley cultivars subjected to salt (NaCl) stress.

Source of Variation	d.f.	Plumule Na^+			Radicle Na^+		
		7-days	14-days	21-days	7-days	14-days	21-days
Salinity	2	487.42 ***	484.69 ***	435.50 ***	487.36 ***	478.21 ***	1145.58 ***
Genotypes	1	68.06 *	58.68 ***	84.50 ***	40.50 ns	40.5 **	5.28 ns
Interaction (SxG)	2	9.96 ns	11.39 ***	3.79 ns	24.51 ns	12.32 *	4.57 ns
Error	12	7.63	0.42	3.72	25.24	2.92	6.75
		Plumule K^+			Radicle K^+		
Salinity	2	48.51 ***	36.95 ***	88.59 **	10.01 ***	9.19 ***	14.76 **
Genotypes	1	51.68 ***	15.59 **	174.22 **	5.01 **	5.56 ***	0.28 ns
Interaction (SxG)	2	1.51 ns	5.27 *	33.18 ns	1.67 *	2.82 ***	1.12 ns
Error	12	0.58	1.00	9.67	0.27	0.12	1.28
		Plumule Ca^{2+}			Radicle Ca^{2+}		
Salinity	2	7.38 *	19.48 ***	10.80 *	14.26 *	19.16 ***	12.99 **
Genotypes	1	5.35 ns	0.85 ns	3.10 ns	0.30 ns	0.98 ns	1.25 ns
Interaction (SxG)	2	0.11 ns	0.48 ns	0.11 ns	0.23 ns	0.12 ns	0.44 ns
Error	12	1.13	1.36	1.68	0.95	1.01	1.65
		Plumule total soluble carbohydrates			Radicle total soluble carbohydrates		
Salinity	2	898.14 ***	1770.35 ***	148.19 ***	262.35 ***	745.41 ***	256.43 ***
Genotypes	1	687.59 ***	9.39 ns	145.92 **	618.35 ***	917.35 ***	137.53 *
Interaction (SxG)	2	11.69 ns	1.94 ns	7.88 ns	3.39 ns	364.55 ***	21.76 ns
Error	12	7.09	12.57	10.32	9.05	10.79	19.29

*, **, *** = significant at 0.05, 0.01 and 0.001 levels, respectively, ns = non-significant

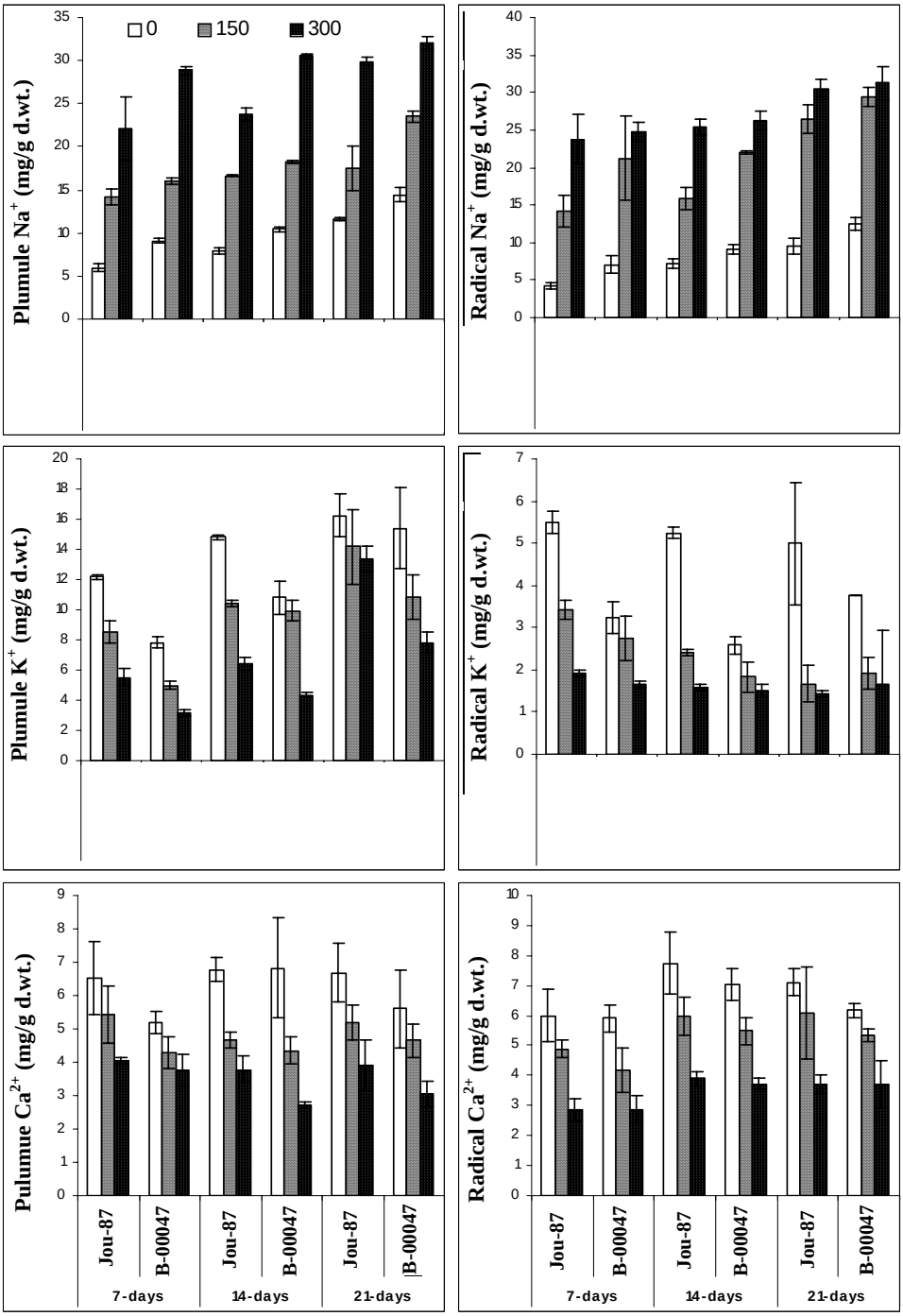

Fig. 3. Time course changes in cation accumulation in the two barley cultivars under the influence of NaCl salinity (mol m^{-3}).

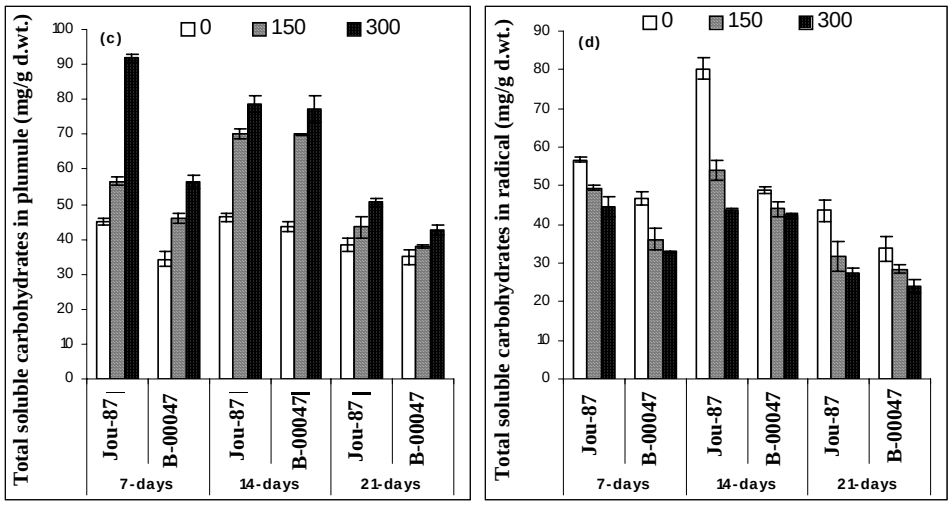

Fig. 4. Effect of different levels of NaCl (mol m^{-3}) on (a) total soluble carbohydrates in plumule and (b) radicle of the two barley cultivars.

Increasing level of salinity significantly increased Na^+ content in radical of barley seedlings. Genotypes accumulated almost same amount of Na^+ in the first and third intervals however, this accumulation was different in the second interval. The interactive effect was significant only in the second interval (Table 3; Fig. 3).

K^+ content of plumule significantly decreased with increasing level of salinity in the growth medium at the first two intervals. The interactive effect of salinity with genotypes was non significant in the first and third interval and significant in second interval (Table 3). Jou-87 accumulated more K^+ content compared with B-00047 and showed less reduction in K^+ content with increasing stress level in the growth medium. There was a gradual increase in K^+ content from first to third harvest interval (Fig. 3).

A similar trend was shown by radical K^+ content where it decreased in the first two intervals. Genotypes showed significantly different K^+ content in the first and second intervals and non significant in the third interval. The salinity x genotype interaction was also significant in all intervals (Table 3, Fig. 3).

Almost same trend was shown by plumule and radicle Ca^{2+} content. NaCl salinity reduced Ca^{2+} content in all intervals. No difference among genotypes was observed with regard to this parameter was observed in any interval. The interaction between salinity and genotypes was also non-significant in the entire three intervals (Table 3, Fig. 3).

Total soluble carbohydrates in plumule were significantly reduced by both levels of salinity at the first interval and third intervals. However, there was no difference between genotypes at the second interval. The interactive effect between salinity and genotypes was non-significant at all the three harvest intervals (Table 3, Fig. 4).

Similarly, total soluble carbohydrates reduced significantly at both the levels of salt in the medium at all the three harvest intervals. The interactive effect of genotypes with salinity was significant at the second interval only and it was non significant in the first and third intervals (Table 3, fig. 4).

Discussion

In This study, germination of seed was extremely sensitive to soil salinity. The seed lings exposed to high ionic concentration showed decline in rate and percentage of germination. The adverse effect of NaCl on germination might be due to toxic or osmotic effect (Kurth *et al*., 1986) of salt in the growth medium. High concentration of NaCl inside the seed has been reported to alter various biochemical activities (Guerier, 1988) and affect mobilization of reserves during germination and ultimately retard growth. In addition, alleviated levels of NaCl in the growth medium are known to inhibits water uptake by seed (Allen *et al*., 1986) and hence suppress seed germination.

Reduction in growth (plumule and radicle length) due to salinity was a common observation of our studies. The reduction may be due to water shortage and ionic toxicity created by salinity (Iqbal *et al*., 1998). In our studies, plant height decreased in both genotypes with increase in salt concentration in growth medium. More reduction occurred in genotype B-00047 than in Jou-87 under both saline conditions at all the three harvests. Although, the plumule and radicle length of both genotypes decreased at all three harvests but its was more prominent in the first and third intervals, which might be due to improper imbibition at first interval and long exposure to salt stress at the third interval. Sodium chloride is well known to inhibit the emergence and elongation of embryonic structures due to accumulation of Na^+ and Cl^- (Marcar, 1987) to toxic level in the germinating seedlings.

The present studies revealed that at germination stage with the passage of time, there was a sharp increase in both K^+ and Na^+ content in both plumule and redicle. Na^+ content increased with the increasing concentration of salt in each time interval. However, the reverse was true for K^+ content. Genotype B-00047 accumulated comparatively less Na^+ and showed less reduction in K^+ than Jou-87. It is now well established that one of the most pronounced effects of salinity is a consistent increase in Na^+ contents along with a consistent decrease in K^+ and Ca^{2+} content.

Calcium plays an important role in the synthesis of new walls, particularly the middle lamellae that separate newly divided cells (Taiz & Zeiger, 2006). Calcium is also used in the mitochondria1 spindle during cell division. All these phenomena take place during seed germination. In addition, calcium is essential for the maintenance of membrane integrity (Grattan & Grieve, 1994). The membrane damage and enhanced permeability may be affected by the displacement of Ca^{2+} by Na^+ from the binding sites of phospholipids of membranes (Zhao & Mingliang, 1988). In our studies, calcium content in plumule was increased in the second interval then decreased in the third intervals. Almost similar trend was observed for radical. It seemed that seedlings were unable to uptake the required quantities of Ca^{2+} from the medium and it ultimately affected growth and development of seedlings.

Under saline condition osmotic adjustment generally result from increase in either the rate of solute uptake by the cell or from decrease in utilization of organic substances. The production of sufficient osmotica is metabolically expensive and potentially limits the plant growth by consuming significant quantities of carbon that could other way be utilized by plants (Greenway & Munns, 1980). In the present study, B-00047 accumulates more total soluble carbohydrate than Jou-87. Carbohydrate changes have direct relationship with physiological processes like photosynthesis, transpiration and respiration. Kerepesi & Galiba (2001) reported that in tolerant genotype sugars tran-

located to the stem was initially metabolized to monosaccharide which cause their contents to rise at first and was followed by a decline. In contrast, our data show a consistent increase in the sugar content, in plumule of both genotypes under both salt concentrations. While under the same conditions, radicals of both the genotypes exhibited reduction as the day of harvest increased.

In conclusion, salt stress severely affected germination rate and percentage, plumule and radical length as well as fresh and dry weights of plumule and radicals. Moreover, total soluble carbohydrates increased in the plumule however, the reverse was true for radicle. Na^+ content increased whereas K^+ and Ca^{2+} content decreased in both plumule and radicle. Barley cultivar Jou-87 tolerated higher levels of salinity as compared to B-00047 and showed reduction in all parameters studies upon exposure to salt stress.

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