CHAPTER 12

IONS AND VARIOUS METABOLITES OF NaCl-SENSITIVE AND RESISTANT CELL LINES

12.1 INTRODUCTION

Advantages of using tissue culture for assessing physiological effects of salt at the cellular level are that complex organ-organ and plant-environment interactions can be removed or controlled. To exploit this advantage, there have been detailed studies of the effects of salt *in vitro* in a number of species (Chandler and Thorpe, 1986). The plant cells must adjust osmotically to the water stress imposed by salts in order to maintain turgor for growth. The osmotic adjustment is achieved by ion uptake, the synthesis of organic solutes in cytoplasm or both. The identification of solutes which accumulate in response to salinity, is an initial step towards elucidating the biochemical and physiological mechanisms which are responsible for and regulate osmotic adjustment. A number of substances, i.e. reducing sugars, proline, glycine betaine, betaine and organic acids have been suggested to accumulate in plant cells during adaptation to salt stress. The assay of such substance(s) could provide a convenient screening procedure for plants with enhanced adaptability to salt stress. Unfortunately, unequivocal evidence that any substance, including those listed above, which accumulate during stress have adaptive value to the plant, is lacking. A comparison of salt-resistant cell lines with that of salt-sensitive for the metabolites that accumulate under stress, will give a clear cut picture, whether the accumulated substance(s) have adaptive role or not. Therefore, in order to study the cellular mechanisms of salt tolerance in glycophytic species, cell line of *Vigna radiata* resistant to 300 mol m^{-3} NaCl was compared with sensitive one for various metabolites which accumulate during adaptation to salt stress.

12.2 MATERIALS AND METHODS

NaCl-resistant (isolated at 300 mol m^{-3}) and salt-sensitive callus lines were grown in petridishes containing 20 ml of modified PC-L2 medium supplemented with 0-300 mol m^{-3} NaCl. The petri dishes were sealed with parafilm and incubated under 16 h photoperiod (80 μ mol m^{-2} s^{-1}) at 25 ± 2°C. For each treatment, 10 callus pieces (each 25 ± 2 mg) per dish and twelve replicate dishes were used. Callus from 3 petridishes of each treatment was removed after 7-d of growth and rinsed with isotonic solution of mannitol, frozen and then stored at -20°C for analysis of various metabolites. This procedure was repeated till 28-d of growth.

Solute analysis

Na^+ and K^+

One hundred mg of dried well ground callus tissues was digested with nitric acid as described in section 8.2. Na^+ and K^+ in the final acid digest extract were determined using an Elico flame photometer.

Reducing sugars and sucrose

Reducing sugars and sucrose were extracted by homogenizing 100 mg fresh weight frozen callus in 2 ml of 80% ethanol. The extract was centrifuged and the supernatant was collected. This procedure was repeated thrice. The reducing sugars were estimated as described by Nelson (1944) using D-Glucose as standard. Sucrose was measured by using the method of Handel (1968) using sucrose as standard.

Protein

A weighed amount of callus was homogenized in pre-chilled pestle and morter with 80% chilled acetone. The homogenate was centrifuged at 12000 xg for 10 min at 4°C. The residue was washed 2-3 times with 80% acetone. The soluble proteins of the residue were precipitated with 10% TCA. The protein precipitates were redissolved in 0.1 N NaOH and the amount of protein was measured by the method of Lowry *et al.*, (1951) using bovine serum albumin as standard.

Soluble amino-nitrogen

The supernatant obtained after centrifugation of TCA precipitated proteins was used for the estimation of soluble amino-nitrogen (in terms of leucine equivalents) by using the method of Rosen (1957).

Proline

Proline was extracted from fresh frozen callus tissue and assayed according to Bates *et al.*, (1973) using L-Proline as standard.

12.3 RESULTS

Ion accumulation

Kinetics of Na^+ and K^+ uptake by NaCl sensitive calli grown on media containing NaCl :

Na^+ and K^+ uptake by the normal calli grown on NaCl was estimated at 7-d intervals over a 28-d culture period (Fig.1).

Na^+ accumulation in the cells increased with increasing concentrations of NaCl in the medium. However, the concentration of Na^+ attained by 28-d in the callus grown at high NaCl concentrations were not markedly different from those grown for 7-d. At low concentrations of NaCl (25-50 mol m^{-3}), Na^+ levels reached a maximum by third week.

K^+ content of the callus declined continuously with increasing NaCl levels. The reduction was pronounced during the fourth week of culture. During first week, however, K^+ content was maintained at all the levels of NaCl.

Comparison of Na^+ and K^+ contents in NaCl-resistant and sensitive calli

The Na^+ concentration in the NaCl-resistant callus remained low and comparable to those of the sensitive line, when both were grown on normal medium. Na^+ levels in both callus lines increased with increasing concentrations of NaCl in the nutrient medium, but not proportionately. The NaCl-resistant callus line accumulated more Na^+ than the sensitive line when exposed to the same degree of salt stress (Fig.2).

The NaCl-resistant callus contained low amount of K^+ than sensitive callus line when grown in the absence of NaCl. However, in the presence of increasing concentrations of NaCl, K^+ levels in sensitive callus line declined continuously, while those of NaCl-resistant line decreased only at and above 100 mol m^{-3} NaCl. Further, the overall decrease in K^+ levels was more pronounced in the sensitive callus line than in the NaCl-resistant callus under identical conditions of NaCl stress (Fig.3).

Reducing sugars

The reducing sugar content of NaCl- sensitive and resistant calli grown on normal and NaCl (0-300 mol m^{-3}) containing medium, was compared at weekly intervals over a period of one month (Table.1).

The reducing sugar content of NaCl-sensitive calli grown on normal medium increased with the growth of callus upto 21 day and, thereafter, decreased non-significantly ($P > 0.05$) on 28-d. Sugar content of sensitive calli at different levels of NaCl also increased with the growth of callus over the entire period of growth. However, compared to the control, non-significant variations in reducing sugar content were observed in stressed calli almost at all salt concentrations upto 14-d of growth and, thereafter, a significant increase was noted at all the salt levels.

Reducing sugar content of resistant calli grown on normal medium increased significantly with the growth of calli till 28-d. On 28-d, a 2.5 fold increase was observed over the initial value recorded on 7-d. Salt treatment caused marked increase in reducing sugar content at all the salt levels throughout the growth period compared to control.

Sucrose

Sucrose content of sensitive and resistant calli grown on normal as well as on NaCl-containing medium increased upto 14-d of culture and decreased, thereafter, till 28-d (Table 2). NaCl treatment caused non-significant increase in sucrose content of NaCl-sensitive line upto 21-d of growth, whereas on 28-d sucrose content decreased considerably especially at higher salt levels compared to control, i.e. in the absence of NaCl. On the other hand, in NaCl-resistant

line, no significant variation in sucrose content was observed during 7 to 21 days of growth, but on 28-d, moderate salt levels caused an increase in the level of sucrose, whereas high salt levels of 250 and 300 mol m^{-3} suppressed such increase.

NaCl-resistant callus under non-stressed conditions contained higher sucrose level than NaCl-sensitive callus. NaCl caused more increase in sucrose content in the latter than the former upto 21-d of growth. However, on 28-d, resistant calli maintained high sucrose than sensitive calli at all the salt levels.

Protein

The changes in protein content of NaCl-sensitive and resistant calli grown in the presence or absence of NaCl are shown in Table 3. The protein content of sensitive calli on normal and salt containing medium gradually increased with the progress of growth. Salt treatment caused an increase in protein content of sensitive calli at NaCl concentrations above 100 mol m^{-3} from 14-d onwards till 28-d of culture. Salt-resistant calli also showed significant increase in protein content under salinity compared to control. At similar level of salt treatments, the salt stressed calli of NaCl-sensitive line had non-significantly higher levels of protein than NaCl-resistant calli.

Soluble amino-nitrogen (SAN)

The SAN content of sensitive calli in the presence or absence of salt was the highest during first week of growth, thereafter, declined significantly and remained almost similar during the last three weeks of growth. The SAN content of sensitive calli increased under NaCl stress throughout the growth period. Resistant calli in the absence of salt showed non-significant variation in SAN content during first three weeks of growth and in the next 7 days four fold increase in SAN was observed. Salt treatment decreased the amount of SAN in resistant calli during the entire growth period except during first 7 days where a significant increase was observed (Table 4).

The salt stressed calli of sensitive line had significantly higher amount of SAN than resistant line at all the salt levels over the period of 28-d.

Proline

The endogenous free proline levels were comparable in NaCl-sensitive and resistant callus lines grown in the absence of NaCl upto 21-d of growth whereas on 28-d, the former contained higher amount of proline than the latter. The salt stressed calli of sensitive line, showed increase in proline content at all the salt levels upto 21-d of growth, thereafter, decreased compared to control. In resistant line too, proline content was increased at all the salt treatments throughout the growth period except at 100 mol m^{-3} during first three weeks of growth. Thus, salt stressed

sensitive callus showed non-significantly greater accumulation of free proline than salt stressed-resistant callus at all the salt concentrations upto 21-d of culture, but during next 7-d, i.e. on 28-d, accumulation of proline was higher in resistant line than sensitive line (Table 5).

12.4 DISCUSSION

The increase of Na^+ and decrease of K^+ content in callus as a function of external NaCl concentrations in the medium is in agreement with the results of other workers (Taleinsk *et al.,* 1983; Pandey and Ganapathy, 1985; Garcia-Reina *et al.,* 1988). The kinetics of Na^+ and K^+ uptake have shown that considerable amount of Na^+ was accumulated by first week, but it further increased by very small amounts with the age of culture. Thus, salt has penetrated the tissue within a few days and have reached to a high concentration in the cells long before the growth processes have started. High Na^+ in tissues are toxic and often manifested by growth inhibition. High K^+ content of stressed callus was maintained during first week of culture in comparison to non-stressed one, despite high Na^+. This probably suggest that K^+ plays an important role in osmotic adjustment during early stages of growth under salt stress (Bernstein, 1977). A comparative study of ions uptake of both cell lines have shown that NaCl-resistant line has accumulated more Na^+ and has maintained higher levels of K^+ than NaCl sensitive line at all external NaCl levels. These results are in accordance with these of Croughan *et al.,* in *Medicago sativa* Watad *et al.,* (1983) in *Nicotiana* and Pandey and Ganapathy, (1984) in *Cicer arictinum.*

The NaCl resistant callus line utilizes uptake of these ions for osmotic adjustment and enhancing turgor. Therefore, accumulation of Na^+ and maintaining K^+ levels could be the mechanism allowing better growth in selected lines at salinity levels (100-300 mol m^{-3}).

Soluble sugars such as sucrose and reducing sugars are osmotically active and accumulate under stress (Handa *et al.,* 1983). Accumulation of soluble sugars under NaCl stress has been correlated with osmotic adjustment and/or their reduced utilization in the absence of growth (Sacher and Staples, 1985). Besides, the activity of some of the enzymes involved in carbohydrate utilization has been reported to be reduced by the imposition of stress (Poljakoff-Mayber, 1982). Moreover, the respiration rate has also been reported to decrease at high salinity levels in plant cells (Lamberts, 1985) which might accumulate sugars - the respiratory substrates - under high salt stress. In the present study, sensitive and resistant calli showed accumulation of reducing sugars at all the salt levels after 14 d of growth but this accumulation being higher in resistant than sensitive calli. Accumulation of reducing sugars provide an advantage to the dividing cells through turgor maintenance and thereby sustained growth (Morgan, 1984). Newton *et al.,* (1986) reported low levels of soluble carbohydrates and

organic acids in drought tolerant cultivars of sorghum with low water potential, but the susceptible cultivar callus had a higher solute level. On contrary in the present study, the accumulation of reducing sugars in resistant calli under higher salt concentrations was higher than sensitive calli. On the other hand, the amount of sucrose of sensitive and resistant calli in presence or absence of NaCl was much lower than their reducing sugar content. The decrease in sucrose does not seem to be due to less absorption of sucrose from the medium as the amount of reducing sugars has increased with the imposition of higher salt stress. However, this decrease may be due to immediate hydrolysis of sucrose after its absorption. Similar results has also been obtained by Handa *et al.,* (1983) in *Lycopersicon esculentum*. At 300 mol m^{-3} NaCl, the sucrose level increased in resistant calli by 29% ($P < 0.05$) than sensitive ones only at 28-d of culture while that of reducing sugars remained almost the same in both the time. Similarly, the accumulation of sucrose is favoured over that of reducing sugars in halophytes in response to salt stress (Briens and Larher, 1982).

NaCl decreases protein synthesis and increase its hydrolysis in many crop plants (Nieman, 1965; Eder *et al.,* 1977). On contrary, our results show increase in protein levels in sensitive and resistant calli grown in the presence of NaCl than their respective controls, i.e. those grown in the absence of NaCl, throughout the growth period. Increase in protein levels under NaCl has also been reported in *Pisum sativum* (Mehta and Vora, 1987), *Vigna unguiculata* (Vyas and Rao, 1987) and *Oryza sativa* (Dubey and Rani, 1989). This increase appears to be possibly due to increased synthesis of new proteins. Such synthesis of new proteins have been shown under various types of environmental stresses like heat, water and salinity (Webster, 1980; Fleck *et al.,* 1982; Ericson and Alfinito, 1984). On contrary to Dubey and Rani (1989), our results show that the protein levels of tolerant calli at different salt concentrations is non-significantly lower than that of sensitive calli.

Salt stress caused a considerable increase in the level of free amino acids in various plants (Stewart and Larher, 1980; Reddy and Vora, 1985). Accumulation of amino acids is due to decreased protein synthesis and/or increased protein hydrolysis due to stimulation of enzyme protease under salinization (Strognov, 1964; Eder *et al.,* 1977; Reddy and Vohra, 1985). In the present study, sensitive calli lies under stress showed increase in SAN concent throughout the growth period compared to control. However, resistant callus line also showed increased in SAN level under stress during the first week of growth. The accumulation of soluble amino nitrogen is not necessarily due to increased protein hydrolysis since protein level was also increased under salinization. The significant high levels of SAN in sensitive line than tolerant under stressed conditions show that increased accumulation of SAN under salinization is not correlated with salt tolerance ability in *Vigna radiata*. These results are not in accordance with

those of Dubey and Rani (1989) in *Oryza sativa*. These differences may be due to genetic and biochemical make ups of the species as salt tolerance ability is ultimately attributed to genetic and biochemical characteristics (Subramanian, 1979).

Proline has been shown to accumulate in plant cells exposed to salt or water stress (Chandler and Thorpe, 1986) and there is evidence that proline act as a compatible cytoplasmic osmoticum (Dains and Gould, 1986; Handa *et al.*, 1983, 1986). On the other hand, proline may even be the pathological consequence of stress-induced damage of the cells (Hanson *et al.*, 1979).

The salt-resistant callus line showed satisfactory growth and accumulated greater proline on a medium containing 250 to 300 mol m^{-3} NaCl. Similar observations have been made for several other plant species (Dix and Pearce, 1981; Watad *et al.*, 1983; Pandey and Ganapathy, 1985). These results indicate that proline accumulation is not symptomatic to stress injury, but accompanies survival and growth in a saline environment. On the other hand, sensitive cells whose growth was inhibited by high salt concentration (250 to 300 mol m^{-3}) produced non-significantly ($P > 0.05$) higher amount of proline than tolerant callus. One explanation for this could be that even though growth was inhibited in unselected callus, production of osmotica still proceeded and that proline was far more important here than it was in resistant callus. An alternative explanation for enhanced proline level in unselected callus could be that excess production was a symptom of stress damage (Hanson *et al.*, 1979). Whatever the cause of the enhanced levels of proline in the unselected callus, it is clear that proline accumulation was not confined to tolerant callus, therefore unlike the situation in other salt-tolerant callus lines (Watad *et al.*, 1983; Pandey and Ganapathy, 1985; Spiegel-Roy and Ben-Hayyim, 1985) cannot be the sole mechanism of salt stress tolerance.

High proline content of sensitive and resistant cells may possibly be due to a decreased rate of synthesis or decreased rate of oxidation of proline (Katz and Tal, 1980). For the high levels of proline observed in NaCl-selected cells of *Nicotiana tabacum*, gene amplification has been suggested as a mechanism for conferring salt resistance in the selected line (Watad *et al.*, 1983). To determine which of these mechanisms are operative in mungbean needs further investigation.

Increased level of protein, proline and soluble amino nitrogen in salt stressed sensitive callus appear to function as compatible cytoplasmic solutes in osmotic adjustment in order to equalize the osmotic potential of the cytoplasmic adverse conditions of salinity (Flowers *et al.*, 1977). This is also true for resistant calli upto 7-d of growth. Thereafter, these solutes are replaced by sugars since the concentration of these compounds are lower than the measured intracellular concentration of sugars.

Table 1. Effect of increasing concentrations of NaCl on reducing sugar content of NaCl-sensitive and resistant callus lines of Vigna radiata cv. K-851 at different days of growth.

Callus line	NaCl concentration mol m^{-3}	Reducing sugar mg $(g\ FW)^{-1}$ Days after growth			
		7	14	21	28
Resistant	0	2.9 ± 0.1	3.7 ± 0.1	4.7 ± 0.1	7.3 ± 0.1
	100	3.6 ± 0.1**	^{b}4.5 ± 0.1**	^{a}8.2 ± 0.1**	8.7 ± 0.1
	200	4.1 ± 0.1**	^{a}5.7 ± 0.1**	^{b}11.0 ± 0.5**	^{a}14.0 ± 0.1*
	250	^{b}5.3 ± 0.2**	^{b}6.6 ± 0.2*	^{a}14.7 ± 0.2*	^{a}15.6 ± 0.1*
	300	3.0 ± 0.1**	^{a}10.3 ± 0.2*	^{a}13.3 ± 0.1*	^{a}17.2 ± 0.1**
Sensitive	0	2.8 ± 0.1	4.0 ± 0.1	4.3 ± 0.1	4.1 ± 0.1
	100	1.7 ± 0.1	3.4 ± 0.1	7.1 ± 0.1	9.1 ± 0.5
	200	3.8 ± 0.1	4.6 ± 0.1	10.0 ± 0.1	11.5 ± 0.1
	250	2.5 ± 0.1	3.0 ± 0.1	8.4 ± 0.1	13.0 ± 0.1
	300	3.0 ± 0.2	6.6 ± 0.1	9.1 ± 0.1	16.1 ± 0.1

Values are mean ± SE based on three independent observations.

Significance of difference between resistant and sensitive callus lines

$^{*}p < 0.05$, $^{**}p > 0.05$.

Significance of difference between control (O salt) and salt treated one of the respective line.

$^{a}p < 0.05$, $^{b}p > 0.05$

Table 2. Effect of increasing concentrations of NaCl on sucrose content (μg (g FW)$^{-1}$) of NaCl-sensitive and resistant callus lines of Vigna radiata cv. K-851 at different days of growth.

Callus line	NaCl concentration mol m^{-3}	Sucrose content, μg (g FW)$^{-1}$ Days after growth			
		7	14	21	28
Resistant	0	852.9 ± 24.5	2204.6 ± 61.3	1662.9 ± 58.9	1083.2 ± 31.1
	100	737.3 ± 6.8	2100.9 ± 38.1	1508.5 ± 81.7	1504.2 ± 82.5
	200	786.5 ± 21.1	2430.9 ± 31.6	1548.7 ± 50.7	1289.8 ± 48.6**
	250	698.7 ± 23.3	2371.7 ± 55.9	1540.2 ± 42.3	744.7 ± 93.5**
	300	667.5 ± 17.9	1741.2 ± 65.9	1313.8 ± 0.0	507.7 ± 54.7**
Sensitive	0	759.0 ± 36.4	1584.6 ± 65.5	1235.5 ± 24.9	924.5 ± 36.8
	100	b801.3 ± 39.5	b2024.7 ± 32.9	b1506.4 ± 8.46	837.8 ± 80.3
	200	b830.4 ± 26.5	b2045.9 ± 78.2	b1667.2 ± 94.2	b981.7 ± 80.4**
	250	b838.3 ± 27.7	b2297.8 ± 31.8	b1436.5 ± 16.9	594.5 ± 9.22
	300	b877.5 ± 25.3	b2185.5 ± 88.3	b1730.6 ± 35.9**	391.5 ± 21.1

Values are mean ± SE based on three independent observations.

Significance of difference between resistant and sensitive callus lines

*$p < 0.05$, **$p > 0.05$.

Significance of difference between control (O salt) and salt treated calli of the respective line.

$^{a}p < 0.05$, $^{b}p > 0.05$

Table 3. Effect of increasing concentrations of NaCl on soluble protein content (mg (g FW)$^{-1}$) of NaCl-sensitive and resistant callus lines of Vigna radiata cv. K-851 at different days of growth.

Callus line	NaCl concentration mol m^{-3}	Soluble protein content, mg (g FW)$^{-1}$ Days after growth			
		7	14	21	28
Resistant	0	8.7 ± 0.2	12.3 ± 0.1	10.8 ± 0.2	18.5 ± 0.4
	100	9.6 ± 0.0	14.6 ± 0.2	^{a}14.5 ± 0.0	16.5 ± 0.1
	200	^{a}12.2 ± 0.0	^{a}16.8 ± 0.1	^{a}16.8 ± 0.1	17.7 ± 0.1
	250	^{a}11.7 ± 0.1	^{a}20.9 ± 0.2	^{a}26.7 ± 0.0	^{a}30.1 ± 0.3
	300	^{a}11.2 ± 0.2	^{a}19.2 ± 0.4	^{a}23.9 ± 0.7	^{a}29.5 ± 0.2
Sensitive	0	11.6 ± 0.1	13.4 ± 0.1	15.7 ± 0.0	18.8 ± 0.1
	100	12.1 ± 0.1*	^{a}20.9 ± 0.2*	14.9 ± 0.1**	19.3 ± 0.3**
	200	13.5 ± 0.3**	^{a}20.5 ± 0.2**	^{a}24.8 ± 0.5*	^{a}27.7 ± 0.1*
	250	12.9 ± 0.1**	^{a}22.7 ± 0.2**	^{a}29.4 ± 0.3**	^{a}30.0 ± 0.1
	300	15.0 ± 0.1*	^{a}26.2 ± 0.7**	^{a}22.3 ± 0.2**	^{a}29.0 ± 0.1

Values are mean ± SE based on three independent observations.

Significance of different between resistant and sensitive lines

$^{*}p < 0.05$, $^{**}p > 0.05$

Significance of difference between control (0 salt) and salt treated ones of the respective callus lines.

$^{a}p < 0.5$, $^{b}p > 0.05$

Table 4. Effect of increasing concentrations of NaCl on soluble amino-nitrogen (mM (g FW)$^{-1}$) of NaCl-sensitive and resistant callus lines of Vigna radiata cv. K-851 at different days of growth.

Callus line	NaCl concentration mol m^{-3}	Soluble amino nitrogen, mM m^{-3} (g fr. wk.)$^{-1}$ Days after growth			
		7	14	21	28
Resistant	0	1.5 ± 0.4	2.3 ± 0.2	1.0 ± 0.1	4.9 ± 0.1
	100	^{a}8.4 ± 0.1	1.8 ± 0.1	0.3 ± 0.1	2.2 ± 0.1
	200	^{a}11.2 ± 0.1	1.8 ± 0.1	1.4 ± 0.1	3.0 ± 0.1
	250	^{a}8.8 ± 0.3	1.8 ± 0.0	1.6 ± 0.1	4.1 ± 0.1
	300	^{a}10.6 ± 0.2	1.8 ± 0.1	^{a}2.8 ± 0.0	^{b}7.1 ± 0.3
Sensitive	0	19.3 ± 0.3	2.5 ± 0.2	1.2 ± 0.1	5.2 ± 0.2
	100	^{b}20.0 ± 0.1*	^{b}6.7 ± 0.3*	^{a}5.3 ± 0.2*	^{b}7.4 ± 0.2*
	200	^{a}27.9 ± 0.1*	^{a}9.5 ± 0.3*	^{a}11.4 ± 0.1*	^{a}10.2 ± 0.2*
	250	^{a}24.9 ± 0.0*	^{a}9.9 ± 0.6*	^{a}12.0 ± 0.3*	^{b}10.7 ± 0.6**
	300	^{a}22.6 ± 0.2*	^{a}13.3 ± 0.2*	^{a}8.0 ± 0.4*	^{b}10.0 ± 0.5**

Values are mean ± SE based on three independent observations.

Significance of difference between resistant and sensitive callus lines

$^{*}p < 0.05$, $^{**}p > 0.05$.

Significance of difference between control (O salt) and salt treated calli of the respective line.

$^{a}p < 0.05$, $^{b}p > 0.05$

Table 5. Effect of increasing concentrations of NaCl on proline μ mole $(g\ FW)^{-1}$ of NaCl sensitive and resistant callus lines of Vigna radiata cv. K-851 at different days of growth.

Callus line	NaCl concentration mol m^{-3}	Proline, μ mole $(g\ FW)^{-1}$ Days after growth			
		7	14	21	28
Resistant	0	31.5 ± 5.1	60.8 ± 2.7	88.1 ± 1.0	47.6 ± 0.1
	100	22.7 ± 1.5	25.4 ± 1.5	38.9 ± 0.1	^{a}60.7 ± 0.5
	200	^{b}72.3 ± 2.3	^{b}81.7 ± 1.8	^{b}94.3 ± 4.4	^{a}100.2 ± 0.7*
	250	^{a}147.6 ± 9.1	^{a}150.5 ± 0.5	^{a}175.2 ± 2.3*	^{a}75.3 ± 0.6
	300	^{b}39.4 ± 1.1	^{b}90.3 ± 0.7	^{a}114.6 ± 1.7	^{a}97.6 ± 2.4
Sensitive	0	29.7 ± 0.7	58.4 ± 1.5	83.3 ± 3.3	118.9 ± 2.3*
	100	^{a}153.7 ± 3.2*	^{a}226.8 ± 4.1*	^{b}143.3 ± 3.5*	108.9 ± 0.9*
	200	^{a}71.0 ± 1.6**	^{b}118.0 ± 5.1**	^{b}128.1 ± 1.5**	65.5 ± 1.1
	250	^{a}133.0 ± 6.1**	^{a}188.4 ± 5.0**	^{a}158.1 ± 1.5	^{a}174.3 ± 1.7*
	300	^{a}168.3 ± 6.0*	^{a}117.1 ± 1.5*	^{a}128.2 ± 1.2**	99.0 ± 0.4**

Values are mean ± SE based on three independent observations.

Significance of difference between resistant and sensitive callus lines

$^{*}p < 0.05$, $^{**}p > 0.05$.

Significance of differences between control (O salt) and salt treated calli of the respective line.

$^{a}p < 0.05$, $^{b}p > 0.05$

EXPLANATION OF FIGURES

Fig. 1 Effect of NaCl on Na^+ and K^+ content of *Vigna radiata* callus cultures. A,0; B,25; C,50; D,100; E,150; F,200; G,250; H,300; I,350 J,400; K,450 mol m^{-3} NaCl. Vertical lines are the minimum and maximum standard error observed with the data in this figure.

Fig. 2 Effect of NaCl on Na^+ content of NaCl-sensitive and resistant callus lines of *Vigna radiata*. Vertical bars represent standard error of the mean. Na^+ of sensitive and resistant line in the absence of NaCl were 4.34 $\pm$ 0.0 μ moles g^{-1} DW.

Fig. 3 Effect of NaCl on K^+ content of NaCl-sensitive and resistant callus lines of *Vigna radiata*. Vertical bars represent standard error of the mean. K^+ in the absence of NaCl were 79.1 $\pm$ 6.0 μ moles g^{-1} DW (sensitive) and 51.2 $\pm$ 3.2 μ moles g^{-1} DW (resistant).

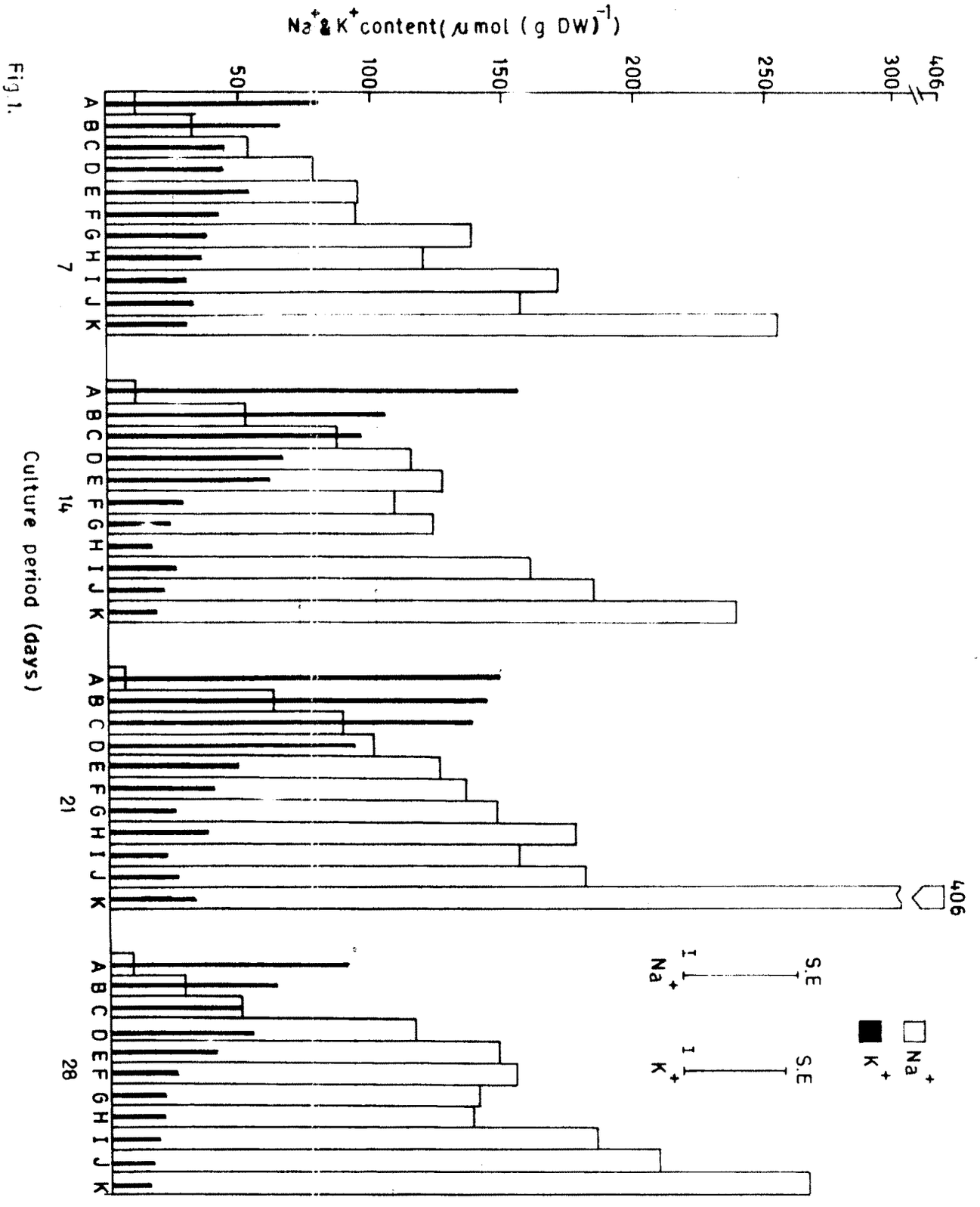
Na+& K+content(μmol (g DW)-1)
50
100
150
200
250
300
406
A B C D E F G H I J K
7
14
21
28
406
S.E
Na+
S.E
K+
Na+
K+
Culture period (days)

Fig. 1.

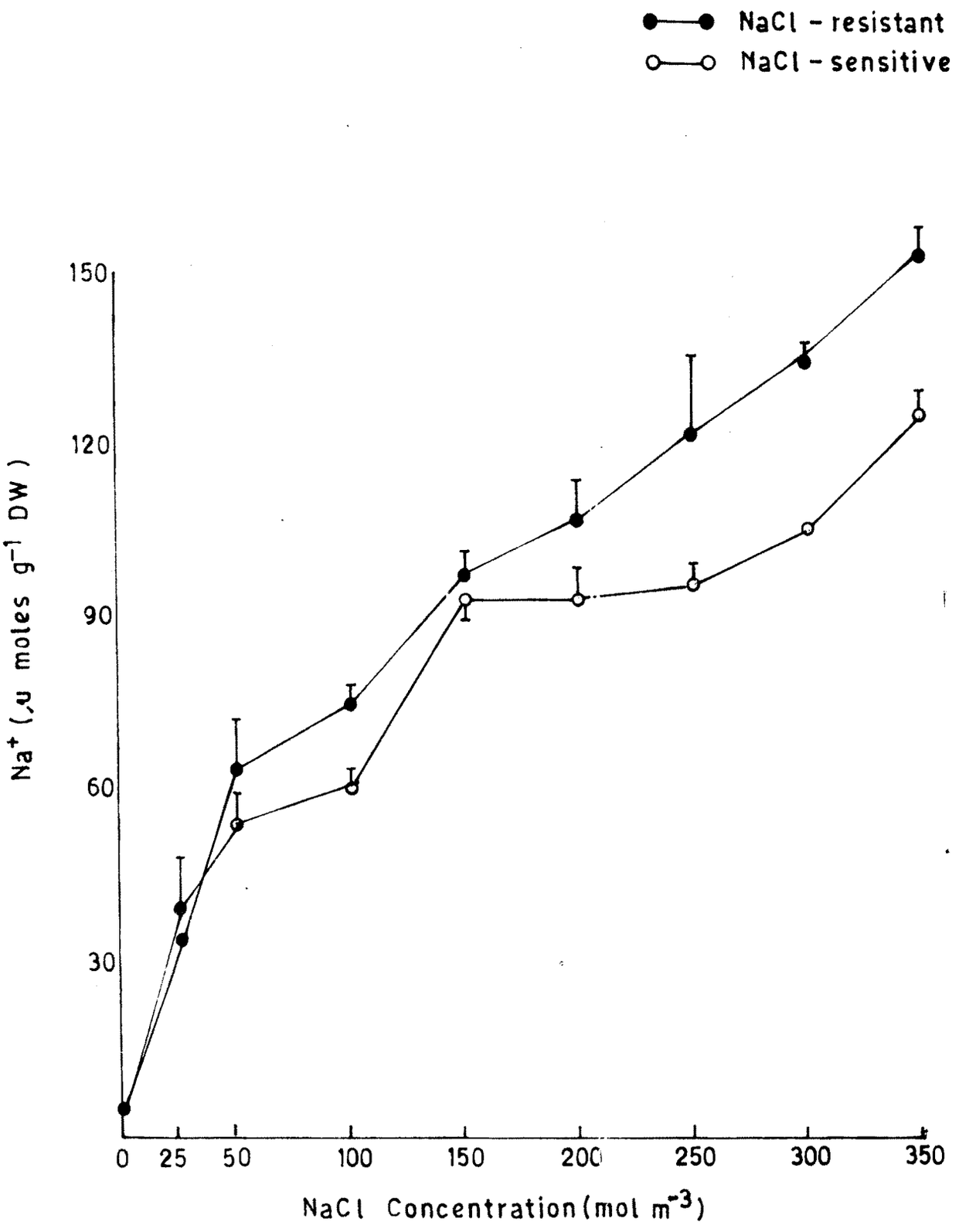
NaCl - resistant
NaCl - sensitive
150
120
90
60
30
Na^+ (,u moles g^{-1} DW)
0
25
50
100
150
200
250
300
350
NaCl Concentration (mol m^{-3})

Fig.2.

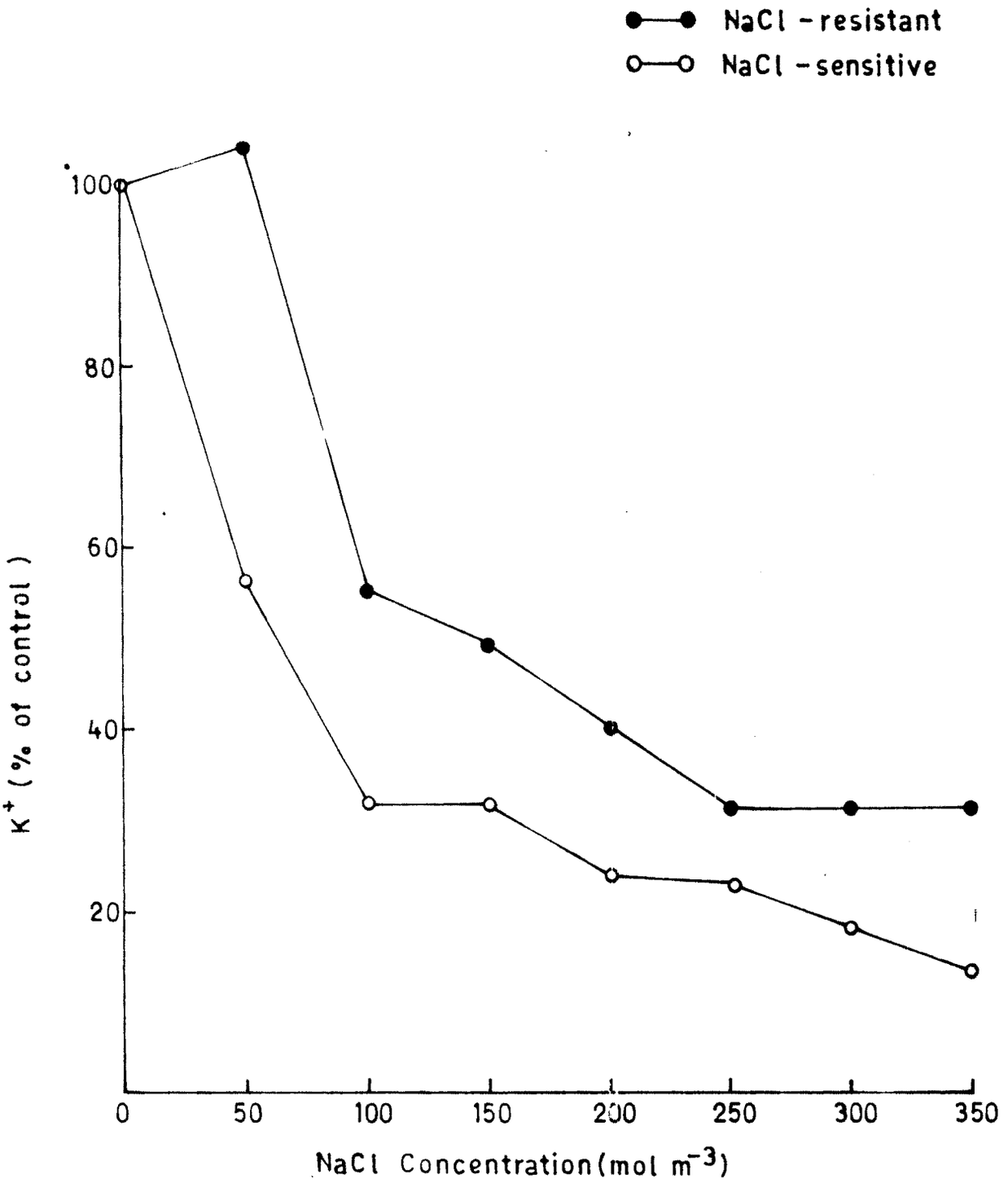
NaCl - resistant
NaCl - sensitive
100
80
60
40
20
K+ (% of control)
0
50
100
150
200
250
300
350
NaCl Concentration (mol m-3)

Fig. 3.