CHAPTER-III

Results and Discussion

1. GERMINATION STUDIES :

A. <u>Germination</u> :

Effect of NaCl salinity on the germination of the seeds of S.italica CV SIC-1 and CO-5 has been shown in Table 1 and Fig.2 & 3. From the results, it is clear that the germination percentage under saline conditions is increased above control value at 24 h in both the cultivars (upto 200 mM NaCl in SIC-1 and 100 mM NaCl in CO-5). Germination is completely inhibited even upto 72 h at 300 mM NaCl in SIC-1 while it is considerably delayed (upto 96 h) in CO-5. In CV SIC-1 the germination percentage is decreased by 12-20% upto 100 mM, by 52% at 200 mM and by 92% at 300 mM NaCl level. On the other hand in CV CO-5 the germination percentage is increased by 10-70% upto 100 mM NaCl and then decreased by only 10% at 200 mM NaCl and by 90% at 300 mM NaCl. Maximum germination is recorded in control of SIC-1 while it is maximum at 10 mM NaCl treatment in CO-5. Thus it is clear that in CV SIC-1 germination is considerably affected even at the lower salt concentrations while in CO-5 it is stimulated. Only the higher salt concentrations i.e. 200 and 300 mM NaCl appear to be inhibitory for CO-5. Thus, it seems that CV CO-5 is salt tolerant and that SIC-1 appears to be moderately salt tolerant so far as germination percentage is considered.

El-Shourbagy et al. (1979) recorded a decrease in germination percentage with increase in salinity and the % reduction

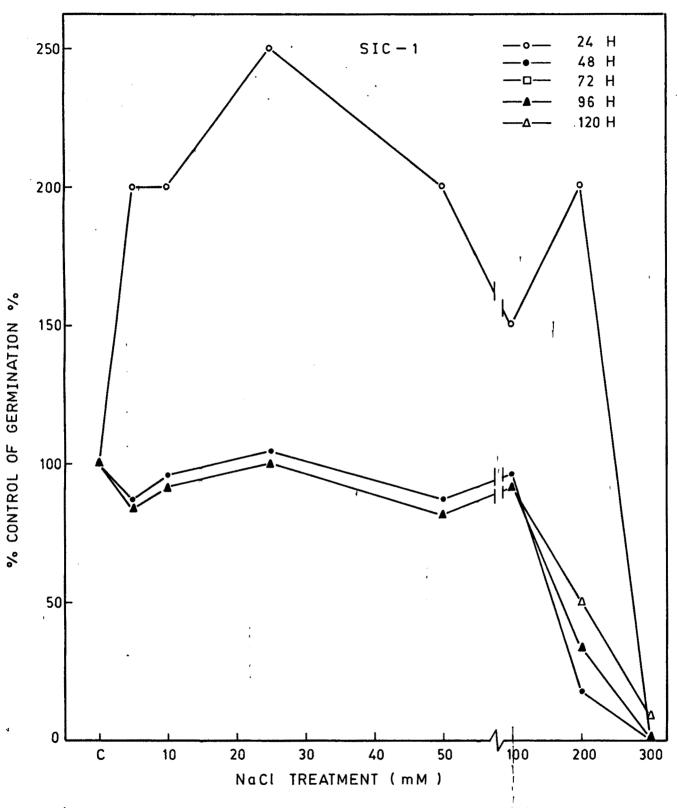
Table 1 : Effect of NaCl salinity on germination in the cultivars of S. italica (SIC-1 and CO-5) differing in salt tolerance.

NaCl	••							ტ	Germination	tion	R					
Treatment mM					SIC-1			Hours of	: germination	nati(, a	co-5	5			
	: 24	••	48	•	72 :	96	••	•• 1		: 48	••	72	••	. 96	120	
0 (Control)	80		92		96	. 96	10	96	80	32	∧ı	40		40	04	
ۍ.	16		80		80	80	0	80	20	64		64		64	68	
10	16		88		88	88	m	88	36	56	10	64		64	72	
25	20		96		96	96	10	96	20	32	0	32		40	44	
50	16		80		84	84	***	84	12	32	0	40		40	44	t 1
100	12		88		88	88	m	88	50	44		48		48	48	
200	16		16		32	48	m	48	1	20	0	24		24	36	
300	۱		t		1	08	m	08	1	١		١	-	1	04	

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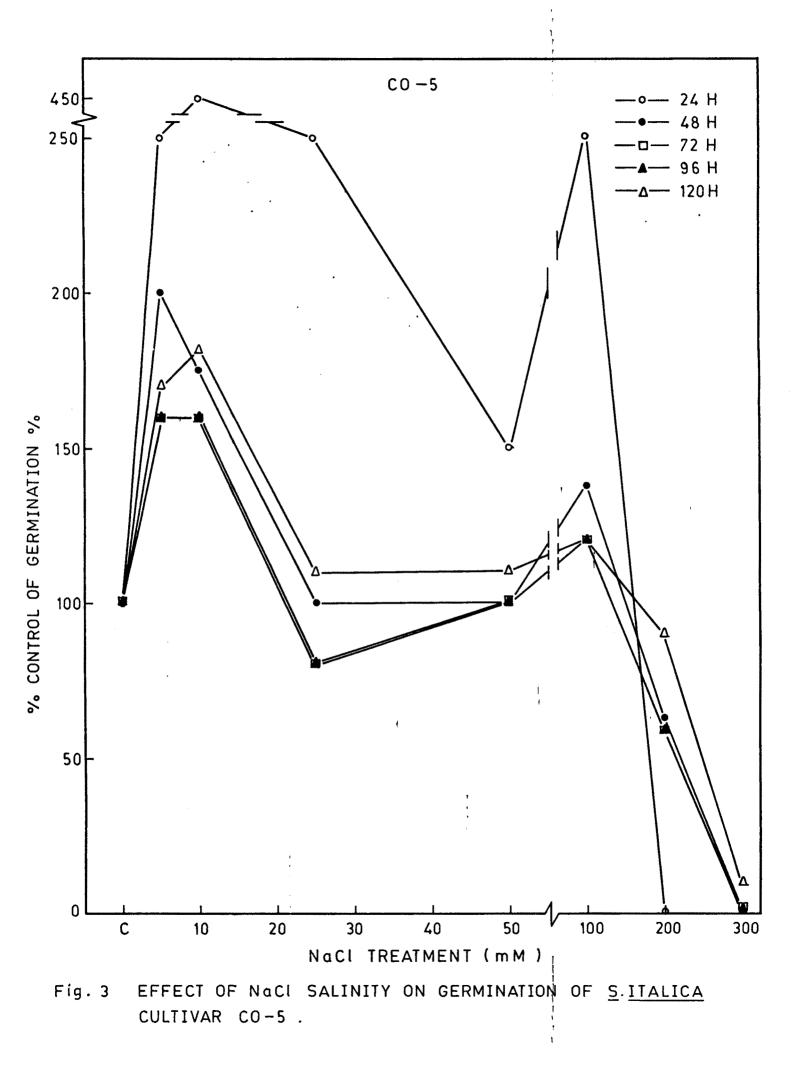
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is different in different varieties of Hordeum vulgare. They also observed that addition of mixture of amino acids enhances general growth and protein content of the seedlings exposed to salt stress. Ungar et al. (1979) observed inhibition of germination of Salicornia under a natural saline conditions. Germination and seedling vigour in Medicago species under saline condition are studied by Rizk et al. (1979). They found a decline in germination % and even in rate of germination under salt stress. According to them the germination depends on the interaction between salt concentration and Medicago species. Karadge and Chavan (1980) studied the effect of NaCl salinity on the germination percentage and the seedling growth in a number of groundnut varieties. According to them germination percentage in some varieties of groundnut is considerably influenced by salt, in some varieties it is not influenced by both, lower as well as higher salt concentrations, while in some other varieties salinity enhances germination and it is inhibited only at the higher salinity level. These studies show that groundnut has a varietal difference in response to salinity even at the germination stage. Singh and Chandra (1980) observed a 50% decrease in germination at salinity level beyond ECe 5 in Pennisetum cultivars and also indicated a varietal difference in salinity tolerance. Manga and Saxena (1981), have observed a decrease in germination under saline habitat, in different millet cultivars. Miyamoto et al. (1982)



Fig, 2 EFFECT OF Naci Salinity on Germination of S.Italica CULTIVAR SIC-1 .

have also observed a 25% reduction in germination in Parthenium due to salt stress. Similar inhibitory trend has been reported by Kumar et al. (1982) in barley, Kumar and Malik (1984) in Brassica juncea, Nerson and Paris (1985) in melons, Verma and Yadava (1986) in Avena sativa, Setia and Narang (1986) in pea and Maliwal and Paliwal (1986) in paddy, maize sorghum, cotton and tobacco. According to Khot (1986) germination is delayed due to salinity in Crotalaria juncea as compared to Phaseolus aconitifolius. It was found that salinity completely inhibits germination beyond 150 mM NaCl in C. juncea. Murumkar (1986) observed that salinity not only affects the germination percentage but also delays the process in chickpea. Joshi and Bhoite (1987) found that the salinity level upto 5.6 m S cm⁻¹ is not toxic for germination in Aeluropus lagopoids, but the higher salinities delay and reduce the process of germination. According to Sheoran and Garg (1979) germination in moong is independent of the type of salinity. However, salt affects the germination by delaying it and also affects the seedling growth. Mahmoud and Hill (1981) observed very little effect of salinity on seedling emergence at 10-15°C in salt tolerant sugarbeet, however, if temperature is increased above 25°C, germination declines with salt concentration. Sung (1981) suggested that speed of germination in barley is retarded by external NaCl but germination percentage is apparently independent of NaCl. However, he suggests that NaCl inhibits ∞ amylase and respiratory activity in barley cultivars. Curtis



and Lauchli (1985) reported that germination is only slightly impaired by salinity upto 200 mM NaCl, in <u>Hibiscus cannabinus</u> and thus it is tolerant to salt. Reddy and Vora (1985,b) while studying the effect of salinity on germination, suggested that the germination is not generally affected by salt, but only the process is delayed in <u>P.typhoides</u> accompanied by accumulation of proline.

Subramanian (1979) has recorded a good performance of rice variety - IR-20 in saline media upto 0.2 M NaCl. Misra and Singh (1982) observed that pretreated seeds of <u>Acacia</u> <u>arebica</u>, <u>A.catechu</u> and <u>Albizzia lebeck</u> show stimulation of germination under saline conditions. Gill and Singh (1985), based on their studies on germination of <u>Oryza sativa</u> seeds under salt stress, suggested that tolerant varieties of paddy have faster rate of seed germination under saline conditions. According to Khot (1986) <u>Phaseolus aconitifolius</u> shows an increase in germination percentage at low salt concentrations, however, salinity concentrations above 100 mM upto 300 mM NaCl inhibit the germination but not to produce any toxic effects.

From the above discussion, it is clear that salinity inhibits and delays germination in most of the plants. This may be due to the fact that osmotic potential of seed cell sap is lower than the external salt solution restricting the seeds to absorb sufficient amount of water necessary for germination.

High concentrations of NaCl inhibit germination also due to toxic effects of Na⁺ and Cl⁻. Apart from this, accumulation of salts in germinating seeds may lead to metabolic disturbances. However, the response given to salt during germination varies from species to species and variety to variety and depends upon the genetic make up of a plant. The environmental factors like light and temperature also affect germination under stress conditions. Addition of mixture of amino acids to germinating seeds increases seedling growth and protein content, which otherwise was hampered due to salinity, during germination. Thus, it appears that salinity inhibits the activity of hydrolysing enzymes like amylase, the activity of which under normal condition increases during germination. However, induction of amylase activity leads to better water absorption and faster rate of germination and this is considered as an adaptive feature. Besides the varietal difference, the responses given to different salts like NaCl, Na2SO4, CaCl2, KCl, MgCl2 etc. are also different. Generally NaCl is considered to be more toxic, than other salts.

From the present studies, it appears that the cultivar SIC-1 is unable to tolerate toxic effects of Na^+ or Cl⁻ or both even at the low salt levels and may not be able to absorb water which results in decrease in germination at all the levels of salinity. On the other hand cultivar CO-5 seems to be able to tolerate toxic effect of Na^+ or Cl⁻ similar to

many other salt tolerant plants or may be able to liberate more hydrolytic enzymes resulting from better water uptake and better osmotic adjustment leading to enhanced germination. However, at the high salt concentration (300 mM NaCl), the seeds are unable to absorb water resulting in inhibition of germination. Thus on the basis of germination percentage, cultivar CO-5 may be considered as a tolerant and cultivar SIC-1 as moderately tolerant plant.

B. Biomass production :

Effect of NaCl salinity on the biomass production by the seedlings of the cultivars of <u>S.italica</u>, namely SIC-1 and CO-5, is shown in Table 2 and Fig.4. It is evident that the biomass production in both the cultivars decreases with increase in salinity level from O (control) to 100 mM NaCl. However, the decrease is more pronounced in SIC-1. In this cultivar, medium and high salt levels (25 to 100 mM) NaCl causes about 25% reduction in the fresh weight of seedlings. On the other hand only 1-8% decrease in fresh weight is observed for CV CO-5.

Kumar and Bhardwaj (1982), while studying early seedling growth of moong, observed a decrease in fresh and dry weight of embryo axis due to salinity. Murumkar (1986) also noted a decrease in fresh weight of chickpea seedlings under salt stress, probably due to failure in imbibition

Table	2	:	Effect of NaCl salinity on biomass production*
			in the seedlings of S.italica cultivars (SIC-1
			and CO-5) differing in salt tolerance

NaCl	:	Biomass (fresh m	atter, mg seedling)-1
Treatment 	:	SIC-1	CO-5
C (Control)		7•30 <u>+</u> 1•67	6.43 <u>+</u> 1.88
5		6•91 <u>+</u> 1•30	6 _• 24 <u>+</u> 2•39
10		6.98 <u>+</u> 1.77	6.11 <u>+</u> 1.69
25		5•45 <u>+</u> 1•24	6.40 <u>+</u> 1.58
50		5.47 <u>+</u> 1.51	5.97 <u>+</u> 1.65
100		5•57 <u>+</u> 1•29	4.81 <u>+</u> 2.04

* From each set 20 seedlings (120 h growth) were analysed.

process. Decrease in dry weight of seedlings under salt stress is observed by Rizk <u>et al.</u> (1979) in <u>Medicago</u> species, Mahmoud and Hill (1981) in sugarbeet, Curtis and Lauchli (1985) in <u>Hibiscus cannabinus</u>; Setia and Narang (1986) in <u>Pisum sativum</u> and by Verma and Yadava (1986) in <u>Avena sativa</u>.

Contrary to these observations, Khot (1986) observed an increase in biomass in <u>Crotalaria juncea</u> and <u>Phaseolus aconiti-</u><u>folius</u> seedlings due to salinity at the low salt concentrations. Higher salt concentrations, however, reduced the fresh weight of seedlings of both the species. Similar increase in biomass production in <u>Atriplex</u> seedlings is observed by Mahmood and Malik (1987) upto EC 15 m S cm⁻¹. Further increase in salinity, however, resulted in reduction of biomass, even though the plants could servive at 50 m S cm⁻¹ salt concentration.

Salinity affects root and hypocotyl elongation in moong (Kumar and Bhardwaj, 1982) and also decreases root and shoot lengths as observed by Reddy and Vora (1985 b), Setia and Narang (1986), and Verma and Yadava (1986). Salinity also affects the respiratory metabolism and the activities of enzymes like protease and amylase. The external NaCl influences the growth by two ways; osmotic effect and specific ion effect. Salt affects the imbibition process and thereby decreases the vegetative growth of the seedlings leading to a decrease in fresh weight of the seedlings. In <u>S.italica</u> CV CO-5 decrease in biomass production appears to be insignificantly influenced

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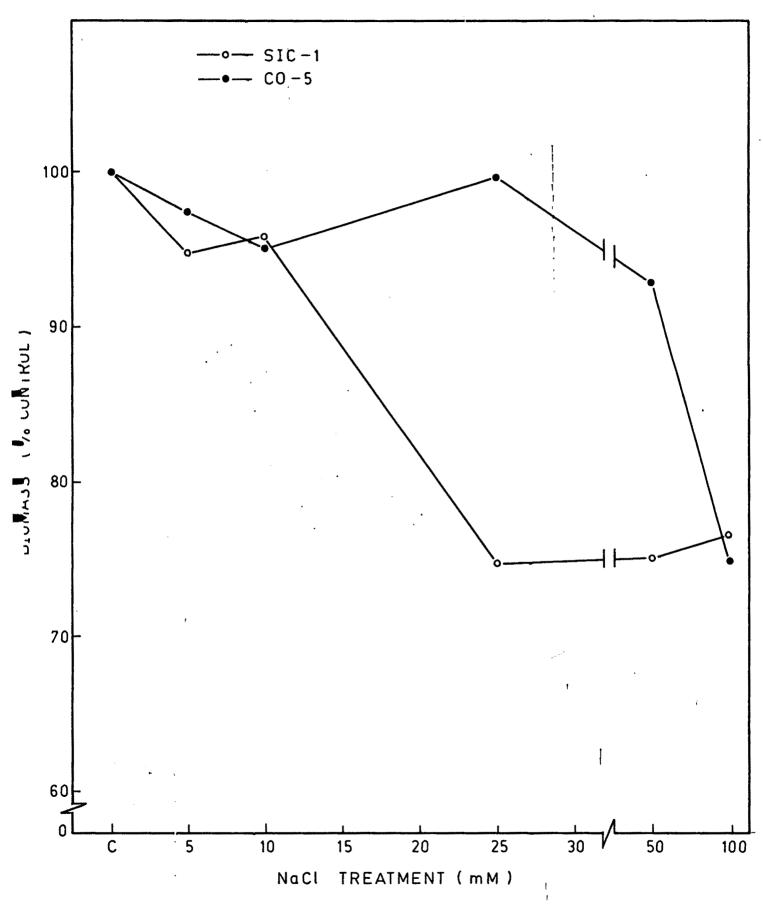


Fig.4 EFFECT OF NaCL SALINITY ON BIOMASS PRODUCTION IN THE SEEDLINGS OF <u>S.ITALICA</u> CULTIVARS (SIC-1 & CO-5) DIFFERING IN SALT TOLERANCE

by salt upto 50 mM and then decreased by 25% only at the high salt concentration i.e. 100 mM NaCl indicating salt tolerance capacity of this cultivar at the early seedling stage. Cultivar SIC-1, however, shows about 25% reduction in biomass production even at the low salt concentration i.e. 25 mM NaCl, indicating its sensitive nature.

C. <u>Nitrate Reductase</u> (NR) :

Effect of salt stress on the activity of nitrate reductase and NO₃-N and NO₂-N contents in the seedlings of <u>S.italica</u> cultivars has been shown in table 3 and Fig.5. It is evident that with increasing salinity level there is a decrease in NR activity in CV SIC-1. This decrease is more pronounced at the salt level of 10 mM NaCl, which shows about 25% decrease in enzyme activity, while at the other salinity levels 10-20% reduction is recorded. Cultivar CO-5 shows exactly opposite trend. In this case the nitrate reductase activity is stimulated at all the salt levels studied. The stimulation is more pronounced, however, at 10 and 100 mM NaCl which shows about 2.25 to 2.5 fold increase as compared to that in control. At the other salinity levels (5, 25 and 50 mM NaCl) 63 to 83% increase in NR activity is recorded.

The NO₃-N content in SIC-1 increases with increasing levels of salinity except 25 and 100 mM NaCl at which a slight decrease in it is observed. Contrary to this NO_3 -N content in

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Table 3 : Effect of NaCl salinity on the activity of nitrate reductase and nitrite reductase in the seedlings (120 h growth) of S.italica cultivars (SIC-1 and CO-5) differing in salt tolerance

T montmont		817-4		•	SJPAT THA	a'la	200	
	NR-n mole: N NO2 libe: n -rated :NO h-1 mg-1:su protein h	iR - :	NO ₃ - N: Mg mg -1: protein:	NO2 - N LE mg -1 protein	NR - nM : NO ₂ libe: -rated : h-1 mg -1: protein :	NiR - n e:mole NO2: consumed :h-1 mg-1 :protein	- n :NO ₇ -N NO ₂ : µg mg -1 umed protein mg -1 ein :	NO2-N- LE mg protein
0 (Control)	1.83	3.71	108.0	0-0317	866 • 0	6.53	100.1	0.0379-
Ŋ	1.64	1.58	125.4	0.0316	1.63	2.64	100.0	0.0501
10	1.37	3•02	120.6	0.0387	2•25	1.01	089-5	0-0560
25	1.60	2.07	096.17	0.0325	1.82	5.06	143.0	0,0663
50	1.54	1.63	110.3	0.0371	1.76	2•28	6•960	0.0552
100	1.47	2.89	097.2	0.0416	2.45	2.57	133.6	0.0671

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Each value is mean of three determinations.

CV CO-5 decreases with increasing levels of salt from 0 to 100 mM NaCl except an increase at 25 and 100 mM NaCl treatment.

 NO_2 -N content in SIC-1 is increased with increasing salinity level from 10 to 100 mM NaCl while at 5 mM salt treatment it remains more or less constant. Maximum NO_2 -N is observed in the seedlings treated with 100 mM NaCl. In cultivar CO-5 also it increases with salinity and reaches to its maximum at 100 mM salinity level (Table 3).

Very few reports are available regarding the effect of salt stress on the activity of NR during germination. Influence of different salts on the activity of NR during germination of <u>Crotalaria juncea</u> has been studied by Desai (1986). According to her, activity of NR is decreased by salts and among the different salts, NaCl is most effective in this respect. She has further suggested that the decrease may be due to inhibition of enzyme-protein synthesis under salt stress. According to Murumkar (1986) the NR activity is markedly influenced by salt in cotyledons and embryoaxis of germinating seeds of <u>Cicer</u> at 120 h growth. Similar observations are made by Khot (1986) in germinating <u>C.juncea</u> seeds.

Sankhla and Huber (1975) however, observed that increasing salinity level brings about a corresponding increase in the activity of NR in the cotyledons of <u>Phaseolus</u>. On the other hand in the germinating seeds of <u>Pennisetum americanum</u>, NR

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activity is not affected by salinity levels till 72 h but later decreased due to salinity. However, % reduction of NR activity differs with genotypes (Kumar <u>et al.</u>, 1985). Khot (1986) observed an increase in NR activity with time of germination and salt concentration in <u>P.aconitifolius</u>. At 50 and 100 mM NaCl and 96 h growth the NR activity is maximum. In this case it appears that NR is insensitive to salt stress, which may indicate its salt tolerant nature.

Decrease in NR activity either in leaves or roots or both is observed by Plaut (1974) in wheat, Heuer and Plaut (1979) in sugarbeet, Billard and Boucaud (1982) in Suaeda maritima, Aslam et al. (1984) in barley, Safaralliev (1984) in Pisum sativum, Glycin max, Medicago falcata and Petrosimonia brochiata and Bottacin et al. (1985) in Pennisetum americanum. The probable reasons for inhibition of NR, given by these workers are different. It is suggested that a shift of ribosomes from polymeric to monomeric form due to salinity is responsible for decreasing NR level since the amount of NR present is related to the amount of polysomes present. Salinity enhances degradation of NR by inactivating the system. NaCl may interfere the synthesis of NR also. When salt is added in vitro, it induces noncompetitive inhibition for all the substrates or co-factor concentration, lowering Km and Vmax. It is also suggested that uptake of NO_3^- (substrate) is inhibited due to salt stress. Dissociation of FAD & molydenum

from apoprotein, due to salt stress may also be responsible for decrease in NR activity.

Contrary to this, increase in the NR activity either in leaves, roots or both has been observed by number of workers. Austenfeld (1974) noticed that the in vitro activity of NR from a halophyte Salicornia is not affected by salt at the low concentration, though it is decreased at the high salt concentration. However, in vivo activity was found to be stimulated by salt at all the concentrations studied. Sankhla and Huber (1975) recorded about seven fold increase in the activity of NR in the leaves of P.aconitifolius. Dias and Costa (1983) reported that NR in sugarbeet is stimulated by low concentrations of salt. Dixit et al. (unpublished) also observed an increase in NR activity due to salt stress in Crotalaria species indicating that the enzyme is fairly stable under saline conditions. The stimulation of enzyme is particularly more prominent in <u>C.verrucosa</u> which grows naturally in coastal regions. Stimulation of the enzyme is related to the ability of intracellular ionic balance under saline conditions. It is also suggested that the in vivo enzyme activity is protected from salt injuries. The stimulation can be caused by modified rates of enzyme synthesis. Activity of the enzyme also depends on the concentration of NO_3^- . Changes in carbohydrate content and photosynthesis may also influence the synthesis of NR.

Aslam <u>et al</u>. (1973) suggested that the activation of the enzyme depends upon the respiration and photosynthesis for its maintenance. The amount of NR present seems to be under control of this turnover system. Hence any change, either in photosynthesis or respiration may reflect in the NR activity. Under saline conditions many times the respiratory activity decreases which may be responsible for decrease in NR. It is suggested by Lin and Kao (1980), that activity of NR is a function of a reaction time or tissue weight. According to Benito and Campbell (1980) the NR activity is parallel with cotyledonary development. As reserved protein decreases, the NR activity increases.

In <u>S.italica</u> CV SIC-1 the NR activity is inhibited due to salinity. It can be suggested that this decrease may be due to reduction of growth, resulting in low protein content which may affect synthesis of enzyme-protein. Na⁺ and Cl⁻ may compete with NO₃⁻ thereby decreasing substrate availability to the enzyme. Salinity also alters the water potential of the cells which may inhibit enzyme activity. However, in CV CO-5 increase in NR activity is recorded. This increase may be considered as an adaptive feature. This increase may be due to successful osmotic adjustment and better protein content as well as proteolytic activity. As seen earlier, the biomass accumulated in this cultivar is more than that in control even

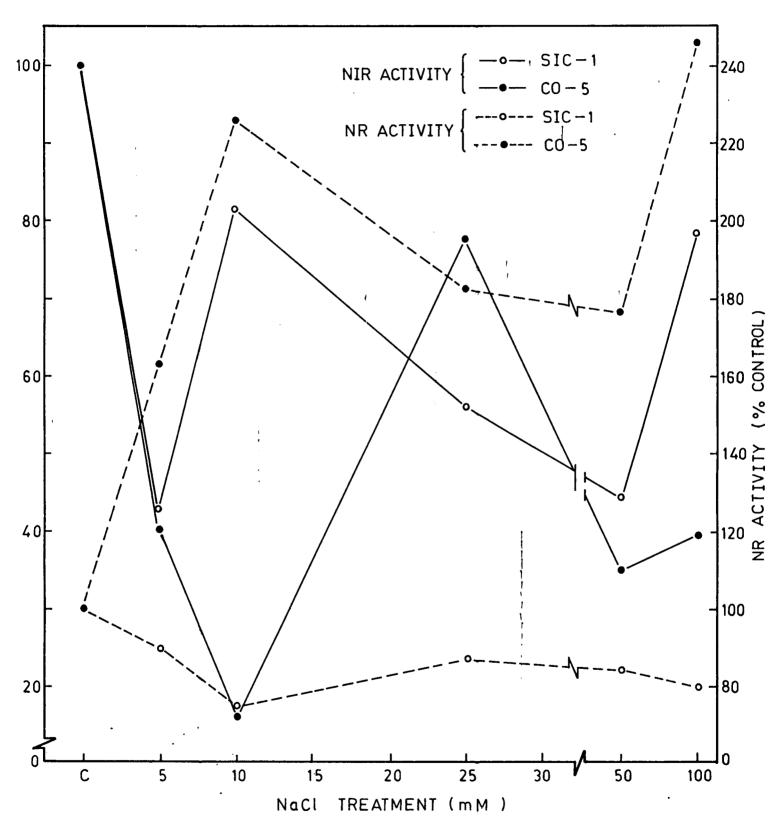


Fig. 5 EFFECT OF NoCL SALINITY ON THE ACTIVITY OF NITRATE REDUCTASE AND NITRITE REDUCTASE IN THE SEEDLINGS (120 h GROWTH) OF <u>S.ITALICA</u> CULTIVARS (SIC-1 & CO-5) DIFFERING IN SALT TOLERANCE. under salinity stress which indicates better growth pattern and stimulation of enzyme.

These results are well supported by the content of NO_3 -N in both the cultivars. In SIC-1, NO_3 -N is increased with salinity but the activity of NR is decreased. This suggests that the enzyme is unable to utilize NO_3^- . The NO_2^-N concentration increases at the same time with salinity indicating an adverse effect of salt on NiR also.

Opposite to this NO_3 -N content is decreased with salinity in CO-5 accompanied by increase in NO_2 -N. This indicates the better uptake of NO_3 -N and stimulation of the activity of NR in CO-5. Thus, cultivar CO-5 may be considered as tolerant than SIC-1. However, high activities of NR in both the cultivars suggest a high nitrate assimilation potential. This is in accordance with the observations of Venkataramana and Das for C₄ plants (1986).

D. <u>Nitrite Reductase</u> (NiR) :

Effect of NaCl salinity on the activity of NiR in the seedlings (120 h growth) is shown in Table 3 and Fig.5. It is clear that the activity of this enzyme decreases with increasing salinity levels in SIC-1 along with an increase in NO_2 -N. The decrease is more pronounced at 5 and 50 mM NaCl which is about 58% over control, while at the other salt levels (10, 25 100 mM NaCl), 20 to 45% decrease is recorded. Cultivar CO-5 also shows same trend with maximum decrease (85%) being at 10 mM NaCl, while at the other levels (5, 25, 50 and 100 mM NaCl), 25 to 65% decrease is recorded.

Very few reports are available regarding the effect of salinity stress on NiR activity during germination. Heuer and Plaut (1979) observed a decrease in NiR in sugarbeet due to inhibition of enzyme-protein itself. Paul <u>et al</u>. (1985) studied the effect of water stress on photosynthetic nitrite reduction in isolated <u>Spinach</u> chloroplasts and

observed more than 39% reduction in the activity of NiR due to low osmotic potential. <u>In vitro</u> assay of NiR showed that the interaction of enzyme with nitrite is not affected by the changes in the concentrations of ions or molecules that might be caused by water stress conditions. Thus they indicated that the interaction of ferridoxin with enzyme is responsible for reduction of enzyme activity. Rajmane (1984), while studying the effect of salt on NiR activity in winged bean, also observed similar decrease in NiR activity, probably due to unavailability of the substrate. Murumkar (1986) also recorded a slight decrease in NiR from germinating seeds of <u>C.arietinum</u>. However, Bottacin <u>et al</u>. (1985) do not found any change in NiR activity in <u>P.americanum</u> genotypes when exposed to salt conditions.

Activity of NiR in both the cultivars of <u>S.italica</u> decreases with increasing salinity level. However, NO_2 -N content is increased with salinity. This indicates that nonavailability of substrate- NO_2 , is not responsible for this inhibition. The reduction is probably due to inhibitory effect encountered by the enzyme itself. The disturbed protein synthesis may also affect the enzyme synthesis or salinity may be affecting the relationship between enzyme and reducing agent that is ferridoxin or NAD(P)H. However, based on the observations made in the present investigation, it is difficult to arrive at a particular conclusion.

2. FIELD STUDIES :

A. Growth

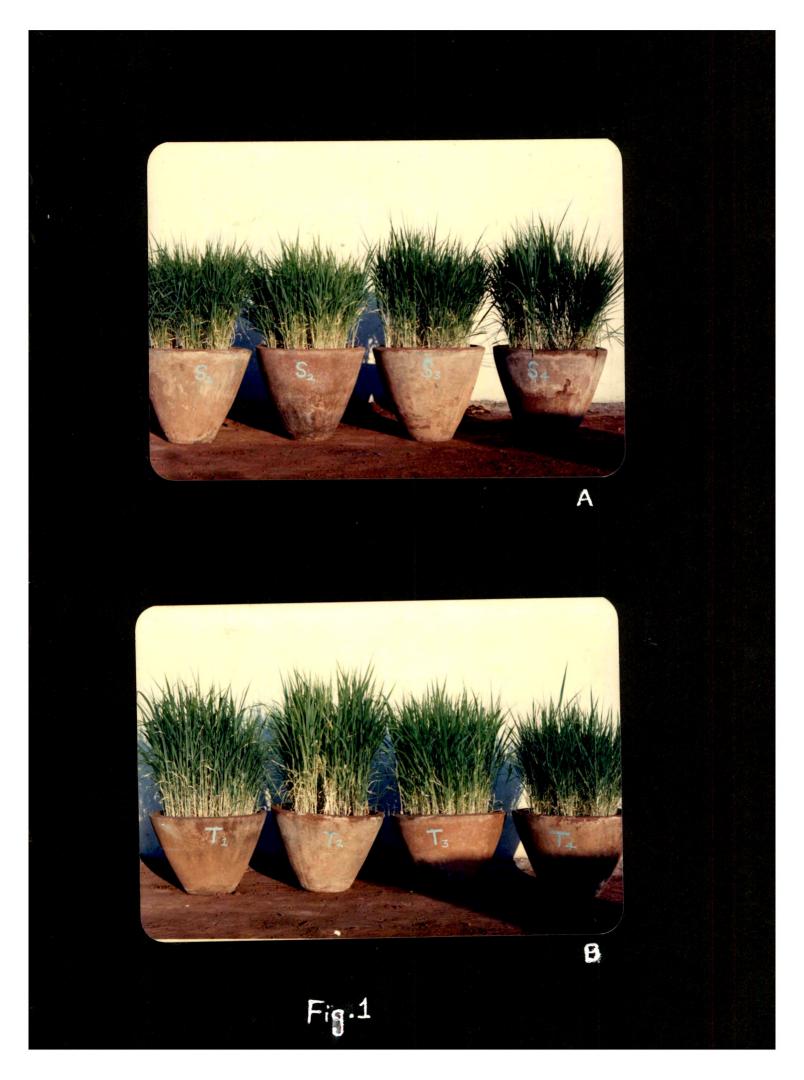
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Effect of salinity on fresh weight, dry weight and moisture content of <u>S.italica</u> cultivars SIC-1 and CO-5 (72 days growth with 32 days salt treatment) is shown in Table 4 and Fig.1&6.It is evident that (20%) decrease in fresh wt. at 50 mM NaCl is seen in SIC-1 while salt concentrations (100 and 200 mM NaCl) increase it by 20 and 53% respectively. In cultivar CO-5 a linear increase in fresh weight is observed with increasing salt concentration. Dry weight in SIC-1 decreases at 50 and 100 mM by about 25 and 65% respectively, while it shows a sudden increase by 44.4% at the highest salt concentration. Moisture % is inversaly related to dry weight and in SIC-1 it increases upto 100 mM and then again

Fig.1 : Photograph showing the effect of NaCl salinity on growth and development of <u>Setaria italica</u> cultivars SIC-1 (A) & CO-5 (B).

> $S_1/T_1 = Control (O mM NaCl).$ $S_2/T_2 = 50 mM NaCl.$ $S_3/T_3 = 100 mM NaCl.$ $S_4/T_4 = 200 mM NaCl.$

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decreases at 200 mM NaCl. However, % moisture percentage at all the salinity levels is more than 100 (control). In cultivar CO-5 dry weight of the plants decreases due to saline conditions and this effect is more pronounced at the low salinity level i.e. 50 mM NaCl. The % reduction ranges from 4.2 to 27.6%. The moisture content increases at all the salinity levels. The maximum dry weight and minimum moisture % under saline conditions is recorded at 100 mM salt.

Decrease in both fresh as well as dry weights is observed in groundnut cultivars by Karadge and Chavan (1980). They observed a considerable decrease in biomass production at the high salt level i.e. 200 mM NaCl. They have also observed that moisture content of the seedlings is not much affected by salinity which indicates a good potential of groundnut for water absorption even in the saline media. Yousef and Sprent (1983), while studying the effect of NaCl on nitrogen fixing and nitrogen fertilized Vicia faba, suggested that the fresh and dry weights of shoot and root are reduced in relation to salt dose and the effect is independent of nitrogen source. Salt does not affect the distribution of dry matter between shoot and root. Similar decrease in fresh and dry weights with increasing external NaCl concentration is observed by Seemann and Christa (1985) in Phaseolus vulgaris. The root/shoot ratio was also found to be increased with salinity indicating disturbed distribution of dry matter.

convent in the Delivered curtivers (picel, curb) dillering in sait	CO-5 : Dry wt.1 : Moisture %	1.5324 42.175	1.1091 63.03	1.4674 60.869	1.3003 68.285
	: Moisture %: Fresh wt : 8 plant-1	2.65	3.00	3.75	4.10
sugar, Tub	Moisture ?	63•999	65.38	71.88	66.130
	: Dry wt-1 : g plant-1	0.7740	0.5955	0.7312	1.1177
tolerance.	: Fresh wt g plant-1	2.15	1.72	2.60	3.30
	NaCl Treatment mM	0 (Control)	50	100	200

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Table 4 : Effect of NaCl salinity on the fresh weight, dry weight and moisture content in the S.italica cultivars (SIC-1. CO-5) differing in salt

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According to them effective compartmentation of intracellular ions is lacking in Phaseolus. Sharma and Garg (1985) observed a graded reduction in all growth parameters that is fresh weight, dry weight, height, leaf number and area due to salinity in wheat. Decrease in fresh weight is recorded by Coughlan and Wyn Jones (1980) in Spinacia oleracea, Murumkar (1986) in Cicer and by Sharma and Kaul (1986) in rice cultivars due to salt stress. The decrease may be due to decrease in water uptake under saline conditions. Decrease in dry weight accumulation is observed by Nukaya et al. (1982), Curtis and Lauchli (1985), Skeffington et al. (1985) in soybean, H.cannabinus and Plantago maritima respectively. Decrease in growth due to salinity is reported by number of workers like Khan (1979) in Zea mays, Hocking (1980) in Typha domingensis, Hoffman et al. (1980) in Capsicum annum, Singh and Singh (1980) in <u>Cicer</u>, Chavan (1980) in ragi, Kumar et al. (1982) in barley, Patil and Patil (1983) in Syzygium cumini, Sharma et al. (1984) in wheat, Gaikwad et al. (1985) in Setaria italica sensitive cultivar, Ahmad et al. (1986) in Aradirachta indica and Melia azedarach, and Kingsbury and Epstein (1986) in wheat. The various parameters studied by these workers are height of the plants, number of tillers, grain yield, diameter of stem and fresh weight of various plant parts like leaves, stem and roots etc.

On the other hand stimulation of growth is also observed

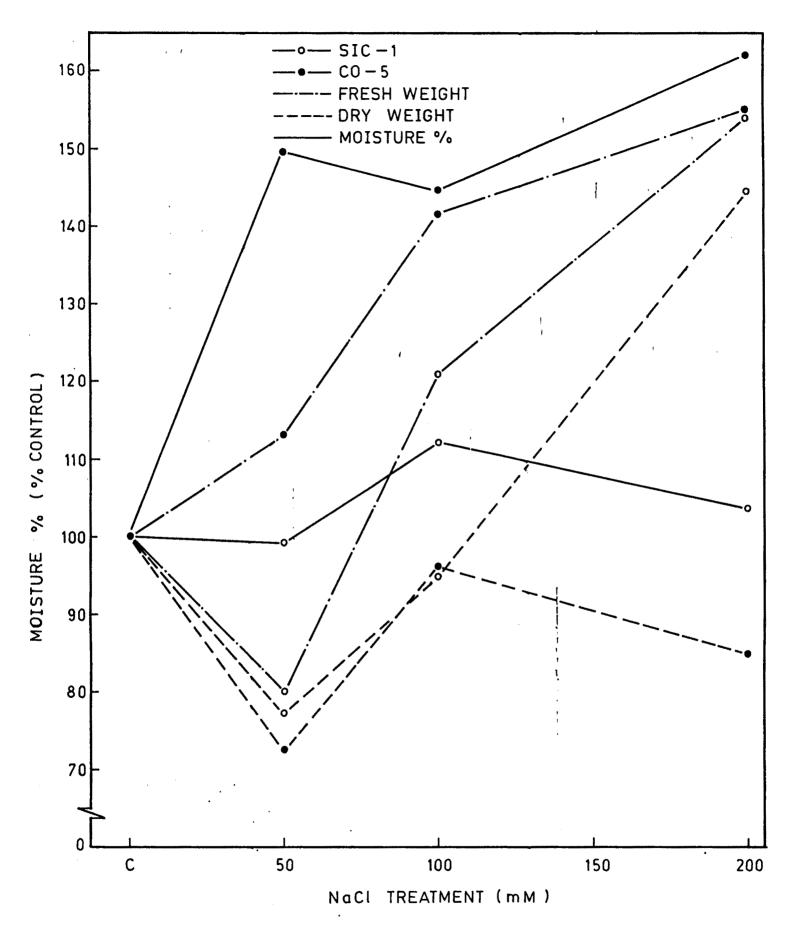


Fig. 6 EFFECT OF NaCL SALINITY ON THE FRESH WEIGHT, DRY WEIGHT & MOISTURE CONTENT OF <u>S.ITALICA</u> CULTIVARS (SIC-1 & CO-5) DIFFERING IN SALT TOLERANCE.

by number of workers in different plants. Yeo and Flowers (1980) observed that the halophyte Suaeda maritima grown in highly saline medium (340 mM NaCl) had more fresh weight than the controls. This may be due to increased cell size. In this case the increased organic growth with increasing salt concentration may be the result of a relationship between turgor pressure and extension growth. Tolerance even at the extreme salinities may be a separate phenomenon in Suaeda. Karadge and Chavan (1982) observed that salt stress causes an increase in growth in terms of height, biomass and dry matter production in Sesbania aculeata and S.grandiflora upto ECe 10 m S cm⁻¹ NaCl. A considerable decrease in growth is observed only at the highest salt level. (ECe 15 m S cm⁻¹). The decrease in weight at this stage may be due to decrease in foliage production and stem growth. Increase in biomass at the low levels of salt is accompanied by an increase in height, number of leaves and leaf area. Increase in dry weight under saline conditions is observed by Ahmed et al. (1979) in oil producing crops upto moderate salinity level (40 meq NaCl). Okusanya (1980) observed that diluted sea water significantly enhances growth in terms of dry weight, leaf area and relative growth rate of Lavatera arborea. However, high salt regimes retard the growth and development. He observed that the succulence of petiol is also enhanced with increasing salinity level. Mahmood and Malik (1987) reported that in salt tolerant Atriplex the biomass yield increases with salinity treatment

upto EC 15 m S cm⁻¹ and above this level, it is adversely affected. However, plants can survive even at the high salt levels upto 50 m S cm⁻¹. Accumulation of ions against a concentration gradient and selective K^+ uptake may be responsible for better growth performance in this plant.

According to Kingsbury and Epstein (1986) the salt composition of external solution had little effect on the growth of salt-resistant wheat line and superior compartmentation of Na⁺ may be responsible for this. Gaikwad <u>et al.</u> (1985) also observed that salinity does not affect growth in salt tolerant cultivar of <u>Setaria</u> and <u>Eleusine</u> suggesting their salt tolerance capacity. Similar observations are made by Smith <u>et al.</u> (1980) in rye grass.

Growth stimulation due to salinity is recorded by Marschner <u>et al.</u> (1981) in sugarbeet. However, they observed that the high salt concentrations inhibit the growth of this plant. Similarly growth of <u>Halimione portulacoides</u> is stimulated by salt upto 85-170 mM NaCl and then decreases at 410 to 690 mM NaCl (Jensen Arne, 1985).

In both the cultivars of <u>S.italica</u> the fresh weight increases with increasing salinity level except at 50 mM in the cultivar SIC-1. However, the dry weight of both the cultivars decreases due to salinity, by about 27, 4 and 15% at 50, 100 and 200 mM NaCl respectively in CO-5 while by about 23 and 5% at 50 & 100 mM NaCl in SIC-1 respectively. However, CV SIC-1 plants show sudden increase in their dry weights at 200 mM NaCl, which may be due to disturbed or irregular pattern of mineral nutrition and other metabolic activities. The decrease in dry weight is accompanied by an increase in moisture content. This may be due to accumulation of Na⁺, Cl⁻ and water with increasing salinity. This is considered as an adaptive feature, a succulent character especially releated to halophytes which are salt tolerant. However, sudden increase in dry weights at the highest salinity level can not be explained on the basis of present studies. From the present studies, therefore, it can be concluded that both the <u>Setaria</u> cultivars are rather salt tolerant even at the vegetative phase of their development: However, cultivar CO-5 appears to be more tolerant than CV SIC-1.

B. Pigment composition

1) Chlorophylls

Effect of NaCl salinity on chlorophyll contents of the leaves of <u>Setaria</u> cultivars is depicted in Table 5 and Fig.7. It is clear that chlorophyll, a, b and total chlorophylls decrease with increasing salinity level in the young leaves of CV SIC-1, while chlorophyll a/b ratio is on increase. In the mature leaves, however, chlorophyll a, b and total chlorophylls decrease only at the low salinity level (50 mM) and then increase at the higher salinity (100, 200 mM NaCl) regimes.

salinity on chlorophyll content* of the leaves of S. italics cultivars (SIC-) and CO-5) differing in Table 5 : Effect of NaCl salt tolerance

Treatment : <u>Young leaves</u> MM :Chl.a : Chl.b : Total ;	aves : Total : a/b : Chl. :					•••				5-2			
:Chl.a 135.1 091.59		•	Mature leaves	BAVBB			Young leaves	BAVeb			Mature leaves	leaver	
135.1 091.59		: Chl.a : Chl.b : Total : : : : : : Chl.	। व.मि	: Total : : Chl. :	a/b	: Chl.a : Chl.b : Total : : : : : Chl.b : Total	d.Ido		: a/b	: Chl.a : Chl.b : Total : : : : : Chl.	d.Id.	: Total	a/b
091.59	85.1 2.69	082.18	30.62 112.8		2.68	101.4	38.88 140.2	140.2	2.61	100.5	42.28	42.28 142.7	2.38
	24.9 2.75	060.79	24.48	085.3	2.48	120.5	48.06 168.5	168.5	2.51	088.3	38.44	38.44 126.7	2.30
100 123.4 45.66 16	169.0 2.70	128.0	50.10	178.1	. 2•56	109.0	42.21 151.2	151.2	2.58	087.4	35.70	35.70 123.1	2.45
200 129.5 47.80 17	177.3 ² .71	<i>LT</i> .120	44.18	135.9	2.08	7.9LL	46.14	165.8	2.60	6.ILL	46.85	46.85 158.8	2.39

Each value is mean of three determinations.

* Values are expressed as mg 100⁻¹ g fresh tissue.

Chlorophyll a/b ratio in the mature leaves decreases. However, cultivar CO-5 shows a different trend where chlorophyll a, b and total chlorophylls increase with increasing salinity level in the young leaves. In the mature leaves the chlorophyll content decreases with salinity (50 & 100 mM) and again increases at the higher salt level (200 mM NaCl). Chlorophyll a/b ratio slightly decreases in both young and mature leaves except a slight increase at 100 and 200 mM NaCl treated mature leaves.

Decrease in chlorophyll content due to salinity is observed by number of workers like Karadge and Chavan (1980) in Arachis hypogea, Ahmed et al. (1980) in leguminous plants, Malakondaiah and Rajeswara Rao (1980) in groundnut, Rao and Rao (1981) in <u>Pigeon</u> pea and gingelly, Hegde and Patil (1982) in Parthenium hysterophorus, Tahahata and Shigesaburo (1982) in Brassica species, Karadge and Chavan (1982) in Sesbania, Singh and Jain (1983) in Chickpea, Patil and Patil (1983) in Syzygium, Patil (1984) in Sesbania, Krishnamoorthy and Siddique (1985) in cowpea, Murumkar and Chavan (1985) in chickpea, Ahmed et al. (1986) in Azadirachta indica and Melia azedarach, Reddy and Vora (1986) in Pennisetum, Ball (1986) in Avicenia and Pisum sativum, Doering and Luedder (1986) in Puncia granatum, El-Sharkawi et al. (1986) in cotton, Hibiscus sabdariffa and sorghum, Karadge et al. (unpublished) in some cultivars of Pennisetum, Panicum, Eleusine and Setaria and Dixit et al. (unpublished) in Crotalaria species. From their observations

it is clear that chlorophyll a is more sensitive to salinity than chlorophyll b resulting in decreasing chlorophyll a/b ratio. According to Strogonov et al. (1970) salinity affects the strength of the forces binding the pigment - protein - lipid complex, which in turn reduces chlorophyll content. However, the response variation depends on the salt tolerance capacity of the plant. The tolerant plants usually show little or no variation and even they increase their chlorophyll content under saline conditions while the salt sensitive plants fail to maintain their chlorophyll content under salt stress. The response given to different salts are also different. It is also suggested that the biosynthesis of chlorophylls is severaly affected by salinity. The plants exposed to salinity show decrease in chlorophylls which can be recovered by the application of phosphorus (Malakondaiah and Rajeswara Rao, 1980) indicating that disturbed ionic balance is responsible for reduction of the chlorophylls, especially due to toxic effect of Na⁺. It is recorded that this decrease in chlorophyll content is associated with an increase in the activity of chlorophyllase which hydrolyses the pigment. NaCl also induces the changes in the fluorescence characteristics of chlorophyll a, reverses characteristics of thylakoids indicating that the water-oxidising site is sensitive to NaCl. It also induces depletion of proteins from PS - II. It is also suggested that chlorophyll metabolism is affected by stress through abscisic acid.

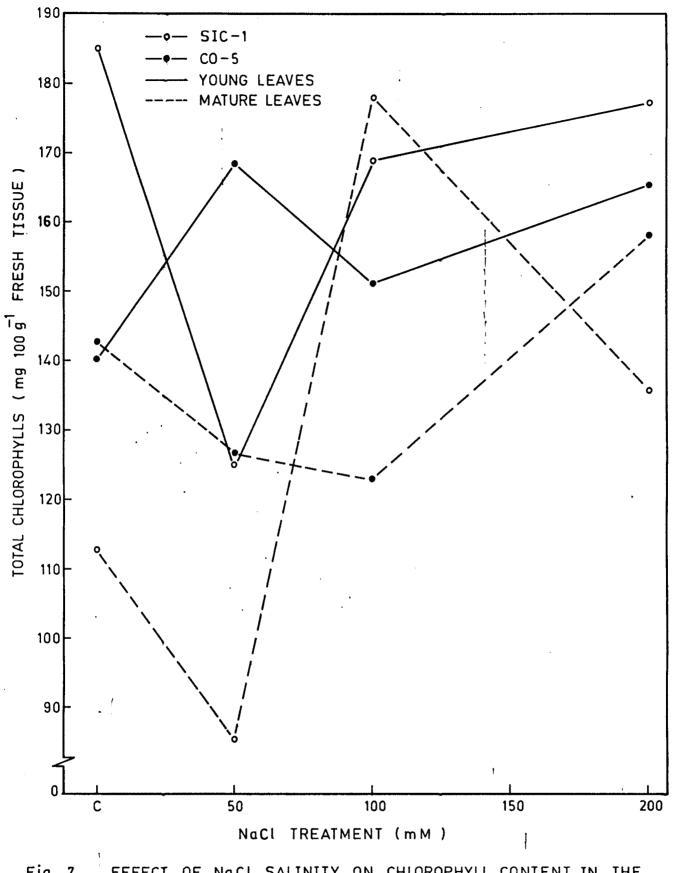


Fig. 7 EFFECT OF NaCL SALINITY ON CHLOROPHYLL CONTENT IN THE LEAVES OF <u>S.ITALICA</u> CULTIVARS (SIC-1 & CO-5) DIFFERING IN SALT TOLERANCE.

Increase in chlorophyll content of the plants grown under saline conditions has also been recorded by number of workers. Ahmed <u>et al</u>. (1979) observed an increase in chlorophyll content in castor bean, flax and sunflower. Chavan (1980) in <u>Eleusine</u>, Dixit <u>et al</u>. (unpublished) in <u>Crotalaria verrucosa</u>, a plant naturally growing on coastal lines, have also made similar type of observations. There are certain reports where chlcrophylls are found to be insensitive to salinity (Kale & Singh, 1987; Karadge <u>et al</u>., unpublished). The capacity to retain or accumulate chlorophylls under saline conditions is considered as an adaptive feature. Decrease in chlorophyll content in such plants at the higher salt concentrations, however, suggests that this pigment - protein - lipid complex is little bit affected by salinity especially at the higher salt levels.

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In CV SIC-1 it appears that young leaves are more affected by salt and 50 mM NaCl appears to be more effective at which there is a sharp decrease in chlorophyll content. An increase in chlorophyll a/b ratio indicates that chlorophyll b is more affected by salt in the young leaves. In case of mature leaves chlorophyll a, b and total chlorophylls are decreased at 50 mM NaCl treatment and then again increased above control values indicating that the pigment-protein-lipid complex of mature leaves is more stable. However, a decrease in chlorophyll a/b ratio in these leaves indicates that accumulation of chlorophyll 'a' is slower than chlorophyll 'b'. In

case of CV CO-5 the young leaves appear to be more stable to salinity. Chlorophyll a/b ratio is decreased at 50 mM NaCl even though the total chlorophylls accumulate at the maximum extent, especially chlorophyll 'a' accumulates more than chlorophyll 'b'. However, at the higher salt concentration chlorophyll a/b ratio is remained some what constant indicating the synthesis of both chlorophyll a and b at the same rate. In mature leaves both chlorophyll a, b and total chlorophylls are decreased at the low and medium salt levels (50 & 100 mM NaCl) and then again increased at the highest salt concentration (200 mM). However, chlorophyll b is less affected than chlorophyll a. Chlorophyll a is affected more at low and medium salt levels i.e. at 50 and 100 mM NaCl. Chlorophyll a/b ratio is decreased only at the 50 mM salt concentration and then increases.

Thus it appears that young leaves of SIC-1 are more sensitive to salinity while mature leaves are some what able to retain chlorophylls. While CO-5 shows stimulation of chlorophyll synthesis in both young as well as mature leaves. However, a slight decrease in chlorophylls in mature leaves at 50 and 100 mM salt levels cannot be explained.

ii) <u>Carotenoids</u> :

The effect of NaCl salinity on the carotenoid content of the leaves of <u>Setaria</u> cultivars SIC-1 and CO-5 has been depicted in Table 6. It can be seen that carotenoid content

Table 6 : Effect of NaCl salinity on the carotene content* in the leaves of <u>S.italica</u> cultivars (SIC-1 and CO-5) differing in salt tolerance

NaCl	1	SIC-1	:		C0-	-5
reatment mM	Young leaves	: Mature : leaves	:	Young leaves	:	Mature leaves
0 Control)	30.80	23•44		24.48		24•40
50	24.40	18.40		29.76		19.52
100	28.00	30.80		26.64		21.92
200	27.28	28.08		26.88		,26 • 9 6

* Values are expressed as mg 100⁻¹ g fresh tissue.

Each value is mean of three determinations.

slightly decreases in the young leaves of SIC-1. In mature leaves, however, it decreases only at the 50 mM salt level and again increases at the higher concentration. In case of CO-5, carotenoid level increases in both young and mature leaves except a slight decrease at 50 and 100 mM NaCl in mature leaves.

Carotenoids play a secondary role in photosynthetic light reactions. There are only a few reports available which describe the effect of NaCl salinity on carotenoid content of a plant. Those include both negative (Kim, 1958; Koverga, 1959; Garter and Myers, 1963), as well as positive (Novikov, 1948; Shakov, 1956; Siegel and Bjarsch, 1962)reports. Strogonov (1970) suggests that the effect of salinity on carotenoid content in plants depends on the salinizing agents and the rate of synthesis of carotenoids under saline conditions, comparable to that in control plants. Increase in carotenoid content is mainly due to abundance of its oxidized from viloxanthin.

Rao and Rao (1981) have observed a decrease in carotenoid level due to salinity in pigeon pea and gingelly. This reduction was found to a greater extent in gingelly, suggesting a varietal difference, in the response given to salinity. Reddy and Vora (1986) have observed a reduction in carotenoid content in <u>Pennisetum</u> leaves due to salinity. According to them carotenes show a similar change as that of chlorophylls. According to

Dixit <u>et al</u>. (unpublished) salinity lowers the carotene content in the leaves of <u>C.juncea</u> and <u>C.retusa</u> while in salt tolerant <u>C.verrucosa</u> it is slightly elevated under saline conditions indicating that the photosynthetic pigment complex of this species is more stable under saline conditions. Decrease in carotenoid level due to salinity is observed by Murumkar and Chavan (1987-a) in chickpea which is considered to be a sensitive species.

From the present studies it appears that both the cultivars of <u>Setaria</u> i.e. SIC-1 and CO-5 are tolerant to salinity in this aspect also. However, CV CO-5 shows some superiority over SIC-1 indicating more salt tolerant nature of the cultivar CO-5.

C. Polyphenols :

The changes in polyphenol content of the leaves of <u>Setaria</u> cultivars as influenced by salinity are recorded in Table 7 and Fig.8. It is evident that salinity does not induce considerable changes in the polyphenol contents in both the varieties. In CV SIC-1, polyphenol content increases with increasing salinity level in both young and mature leaves and only insignificantly decreases in young leaves at 100 mM NaCl treatment. However, neither increase nor decrease is considerable as compared to control. In CO-5 polyphenol content decreases slightly in young leaves. The mature leaves, however, exhibit almost no effect of salinity on polyphenols and only a

Table 7 : Effect of NaCl salinity on the polyphenol content* of the leaves of <u>S.italica</u> (SIC-1 and CO-5) differing in salt tolerance

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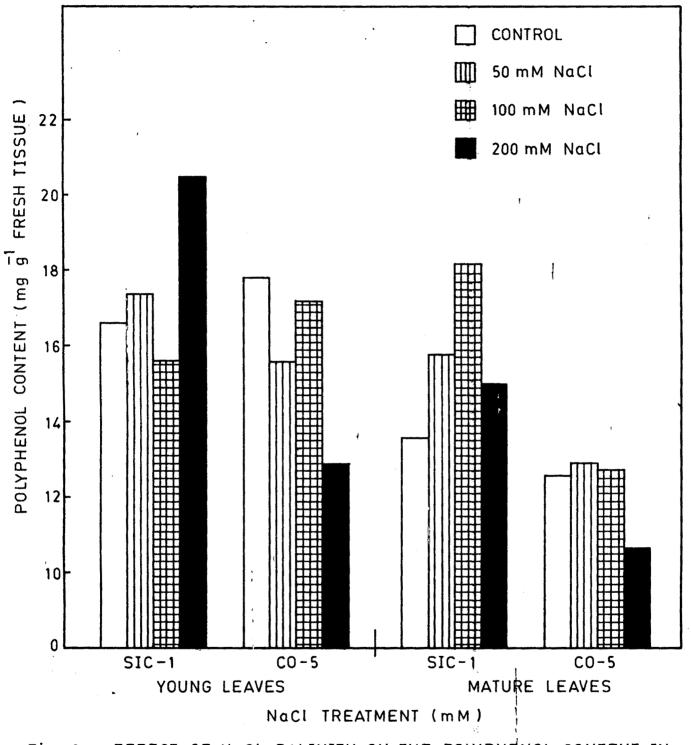
NaCl		SIC	-1	:		C0-	5
Treatment : mM :	Youn		Mature leaves	:	Young leaves	:	Mature leaves
0 (Control)	16.60	D	13.56		17.79		12.61
50	17.3	9	15.80		15.64		12.93
100	15.64	4	18.19		17.23		12.77
200	20.5	1 .	15.00		12.93		10.69
	•						

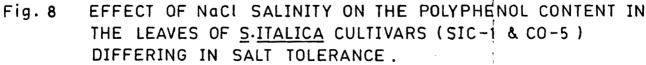
* Values are expressed as mg g^{-1} fresh tissue. Each value is mean of three determinations.

slight decrease is observed and that at the highest salt concentration (200 mM NaCl) only.

Very few reports are available which discuss the effect of salinity on polyphenol metabolism. Generally polyphenols are considered as aromatic compounds produced during secondary metabolism and this may be reason for its ignorance. Decrease in the level of leaf polyphenols is recorded by Karadge (1981) in <u>P.oleracea</u>, Patil (1984) in groundnut and Krishnamoorthy and Siddique (1985) in cow pea due to saline conditions. Similar observations are made by Karadge <u>et al</u>. (unpublished) in <u>Panicum</u> CV MS-1307 and <u>Pennisetum</u> CV GHB-81, which are considered by them as salt sensitive cultivars. Dixit <u>et al</u>. (unpublished) observed that the polyphenol content is decreased in <u>C.verrucosa</u> and <u>C.juncea</u> leaves.

On the other hand an increase in polyphenol level due to salinity in groundnut CV TMV-10 leaves to a considerable extent is observed by Karadge and Chavan (1980), while it decreases in CV SB-11. They have suggested that probably NaCl salinity induces secondary metabolism in the leaves of CV TMV-10 resulting in synthesis and accumulation of polyphenolics. An increase in polyphenol content has also been noted by Chavan (1980) in ragi, exposed to salinity. Stimulatory effect of NaCl on polyphenol accumulation is observed by Patil (1984), in young leaves of <u>Sesbania</u>, however, the highest salt concentration was found to be inhibitory. Opposite trend, however, is observed





by him in the mature leaves. Marked increase in polyphenols especially at the low salt regimes is observed in <u>Crotalaria</u> <u>retusa</u> by Dixit <u>et al</u>. (unpublished). Murumkar (1986) also observed an increase in polyphenols with salinity and the maximum accumulation was found at the highest salt concentration (150 mM NaCl).

The insignificant effect of salinity on the polyphenol content in the leaves of both the cultivars of <u>Setaria</u> is suggestive of stability of the plant under stress conditions and probably the plant has no necessity of induction of secondary metabolism in both the cultivars, with respect to synthesis of phenolics.

D. <u>Nitrogen fractions</u> :

Effect of NaCl salinity on the various nitrogen fractiviz. NO_3 -N, NO_2 -N, Protein-N and insoluble-N, is shown in Ta 8 and Fig.9,10,11 & 12. It appears that NO_3 -N content of you and mature leaves, roots and stems increases with salinity in both the cultivars except in the stem of SIC-1 where it is slightly decreased at 50 and 100 mM NaCl and in roots of CO-5 at 50 mM salt treatment. Increase in the NO_2 -N content is observed in all the plant parts of CV SIC-1. The trend shown by CV CO-5 is reverse to that in CV SIC-1. The level of protinitrogen in the CV SIC-1 decreases with the intensity of salt stress in the young leaves, roots and stems except an apparent.

Table 8 : Effect of NaCl salinity on the fractions of nitrogen in the different parts of S.italica cultivars (SIC-1 and CO-5) differing

in salt tolerance.

Toung leaves Imature leaves Kours Koots Koots<	5									i		SIC-1									
NO ₃ -N : NO ₂ -N : Prot.: Insol.: Total : NO ₃ -N : NO ₂ -N : Prot.: Insol.: Total : NO ₃ -N : NO ₂ -N : Prot.: Insol.: Total : NO ₃ -N : NO ₂ -N : NO ₂ -N : Prot.: Insol.: Total : NO ₃ -N : NO ₂ -N : NO ₂ -N : Prot.: NO ₂ -N : NO ₃ -N : NO ₃ -N : NO ₂ -	tment			aves				Mature	Leaver					Roots					Sten		
xrol) 0.1141 0.00023 3.16 1.183 4.457 0.1725 0.00015 2.52 0.1753 3.446 0.1320 0.00022 1.64 0.550 2.122 0.1301 0.00048 2.55 0.894 3.577 0.2140 0.00045 2.64 0.482 3.377 0.1527 0.00037 1.59 0.499 2.242 0.2404 0.00029 2.01 0.972 3.223 0.2027 0.00040 2.72 1.235 4.158 0.1499 0.00040 1.78 0.366 2.316 0.2408 0.00026 2.59 0.745 3.272 0.00040 2.72 1.235 4.158 0.1499 0.00040 1.78 0.366 2.316 0.2498 0.00026 2.59 0.745 3.585 0.2272 0.00049 2.87 0.832 3.930 0.1452 0.00040 1.83 0.421 2.400		*** **	: NO ₂ -N	Prot.	Insol.	Total:			Prot.	. Tosol.	Total	N- ² ON:		Frot.	Insol.	Total	NO ₃ -N		Prot.	Insol.	Total
0.1301 0.00048 2.55 0.894 3.575 0.2140 0.482 3.377 0.1527 0.00037 1.59 0.499 2.242 0.1980 0.00018 1.77 0.394 0.2404 0.00029 2.01 0.972 3.223 0.2027 0.00040 2.72 1.235 4.158 0.1499 0.00040 1.78 0.366 2.316 0.1527 0.00019 1.75 0.412 0.2404 0.00029 2.01 0.972 3.2027 0.00040 2.72 1.235 4.158 0.1499 0.00040 1.78 0.4215 0.00019 1.75 0.412 0.2408 0.00036 2.59 0.745 3.585 0.2272 0.00049 2.87 3.930 0.1452 0.00040 1.83 0.421 2.400 0.00040 2.45 0.368	0 mtrol)	0.1141	0.00023	3.16	1.183	4.457		0.00015	2.52	0*753	3.446	0.1320			0.350		0.2140	0.00011	1.92	0.315	2.449
0.2404 0.00029 2.01 0.972 3.223 0.2027 0.00040 2.72 1.235 4.158 0.1499 0.00040 1.78 0.386 2.316 0.1527 0.00019 1.75 0.412 0.2498 0.00036 2.59 0.745 3.585 0.2272 0.00049 2.87 0.832 3.930 0.1452 0.00040 1.83 0.421 2.400 0.2121 0.00040 2.45 0.368	o	0.1301			0.894			0.00045	2.64	0.482	3.337	0.1527	0.00037			2.242	0.1980	0.00018	1.77	0.394	2.362
0.2498 0.00036 2.59 0.745 3.585 0.2272 0.00049 2.87 0.832 3.930 0.1452 0.00040 1.83 0.421 2.400 0.2121 0.00040 2.45 0.368	0	0.2404	0.00029	2.01	0.972	3.223	0.2027	0.00040	2.72	1.235	4.158	0.1499	0.00040	1.78	0•386	2.316	0.1527	0.00019	1.75		2.315
		0.2498	0*00036		0.745	3.585		0.00049	2.87	0.832	3.930	0.1452	0.00040	1.83	0.421	2.400	0.2121	0.00040	2.45	0.368	3.031
				•															•		

Total = Sum of NO₃-N, NO₂-N, Protein-N and Insoluble-N. Each value is mean of three determinations.

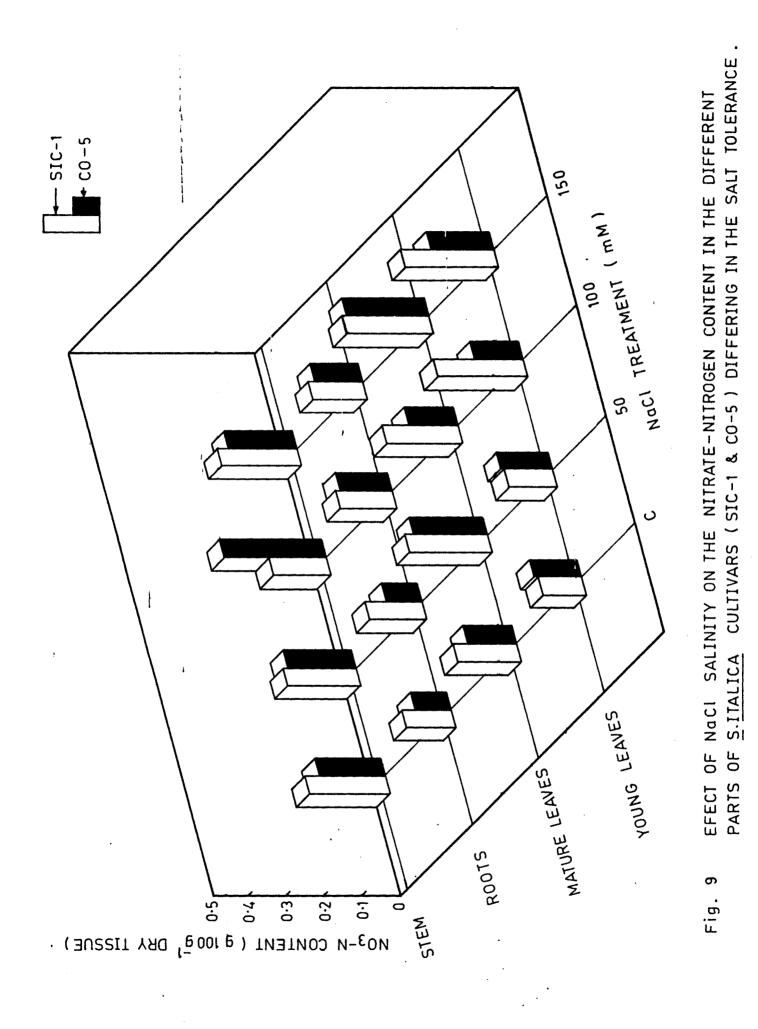
Values are expressed as g 100 g⁻¹ dry matter.

NaCl										C0-5										
Treatment mM		Young	Young Leaves	6		••	Matu	Mature Leaves	Yes				Roots		-			Stem		
•	NO ₃ -N	NO ₃ -N : NO ₂ -N	: N	:Prot.:Insol.:Total :NO ₇ -N : N : N :	Total		: NO ₂ -N :Prot.:Insol.:Total : NO ₃ -N	:Prot.	Insol.	:Total :	1	* NO ₂ -N	Prot.	Insol.	Total :	:Prot.:Insol.:Total :NO ₇ -N : NO ₂ -N : N : N : : : : : : : : : : : : : : :	1	: Prot.	:Prot.:Insol.: Total : N : N :	Total
0 (Control)	0.1216	0.1216 0.00117 3.82 0.999 4.942 0.1310 0.00098 3.45 1.253 4.835 0.0999	3,82	666•0	4.942	01210	96000 • 0 -	3.45	1.253	4.835	6660*0	0.00055 2.46 0.456 3.017 0.1772 0.00074 2.24 0.552 2.970	2.46	0.456	3.017	0.1772	0.00074	2.24	0.552	2.970
50	0.1376	0.00044	2.87	0.972	3.980	0.1989	2.87 0.972 3.980 0.1989 0.00044 2.94 1.481 4.620 0.0905	2.94	1.481	4.620	0*0905	0.00030 2.05 0.920 3.061 0.1838 0.00064 2.14 0.552 2.876	2.05	0.920	3.061	0.1838	0.00064	2.14	0.552	2.876
100	0.1631	0.00041	3.14	3•14 1•349 4•653	4.653	0.1320	0.00033 3.08 1.279 4.491	3.08	1.279	4.491	0.1376	0.00019 2.04 1.078 3.256	2.04	1.078	3.256	0.2734	0.2734 0.00094	2.05	0.526	2.850
200	0.1669	0.1669 0.00026 2.83 0.491 3.488 0.2200 0.00026 2.89 1.121 4.231 0.1263	2.83	0.491	3.488	0.2200	0•00026	2.89	1.12	4.231	0.1263	0.00034 2.II 1.025 3.262 0.1875 0.00052 2.I2 0.421 2.727	2.11	1.025	3.262	0.1875	0•00052	21.2	0.421	2.727

Table No.8 : Contd....

increase in the roots at 100 and 200 mM NaCl and in the stem at 200 mM NaCl. In the mature leaves, however, protein-nitrogen is on increase. Decrease in the level of protein-nitrogen is observed in all the parts of CV CO-5, due to salinity. Insoluble-N decreases in young leaves of SIC-1 and at low salt level in mature leaves while it increases at all the salt concentrations in other parts. In CV CO-5 it decreases in young leaves except at 100 mM treatment. In mature leaves and roots it increases with salinity but shows a little decrease at 200 mM in mature In stem it seems to be stable to salinity and decreases leaves. only at the highest - 200 mM NaCl, concentration. These effects are reflected in the total of all these fractions. In SIC-1, young leaves show a decrease in total of nitrogen fractions due to salinity. In mature leaves it declines at the low salt levels and then again rises up at the higher salt concentra-Both in roots and stems it increases with salinity. tions. In case of CV CO-5, young and mature leaves show a decrease in total nitrogen while roots showed an increase due to salinity. In the stem the values are little bit constant and decline slightly at the highest salt concentration.

According to Huq and Larher (1983) NO_3 plays a key role in osmoregulation of non-nodulated <u>Phaseolus aureus</u>. Aslam <u>et al.</u> (1984) while studying the effect of salinity on NO_3 assimilation in young barley seedlings, observed a severe inhibition of NO_3 uptake by salinity. On the basis of osmola-



lity of the sclution, it was found that the Cl salts are more inhibitory than the SO_A salts. Gorham <u>et al</u>. (1984) suggest that in Leymus sabulosus when treated with NaCl, Clpartially replaces the high levesl of NO_3 which was found in the leaves of the control plants. Bottacin et al. (1985) studied the nitrogen absorption and assimilation in salt resistant and susceptible millet genotypes (P.americanum) and observed that in presence of 300 mM NaCl in the moting medium, uptake of NH_4^+ and NO_3^- was inhibited, and the inhibition is to a greater extent in susceptible genotype. The inhibition of uptake may be due to the antagonism between NO_3 and CI as reported by Abdul-Kadir and Paulsen (1982) and Papadopulos and Rending (1983). Gorham et al. (1985) observed a decrease in nitrate level in Thinopyrum bessarabicum, which could withstand prolonged expsoure to 350 mM NaCl. Eddin and Henk Doddema (1986) observed that NH_4^+ and NO_3^- in the halophyte Arthrocnemum fruticosum decrease with time more or less independently of the salt concentration. However, more NO_3 appeared at the flowering stage.

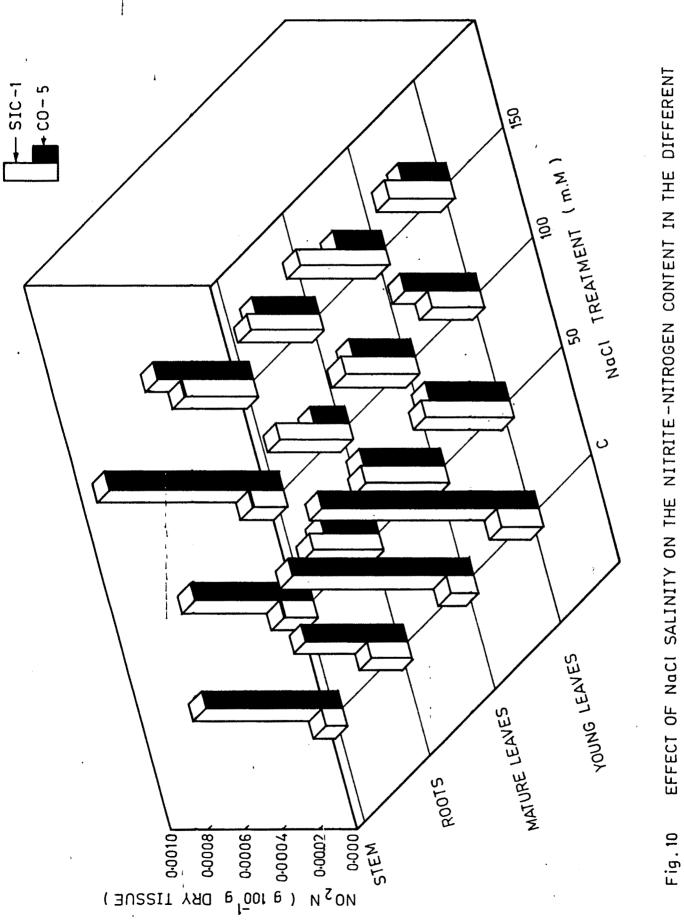
It is suggested by Menary & Allen (1979) that chloride accumulation in leaves and roots of <u>Carica papaya</u> is related to the high levels of NO_3 in the tissue and NO_3 accumulation increases in presence of chloride. Smith <u>et al.</u> (1980) reported that NaCl increased the uptake of total N and NO_3 -N in <u>Lolium</u> <u>perenne</u> and <u>Phleum pratense</u> and this is related to an increase in NR activity. Dias and Costa (1983) reported an increase in

NO3-N in sugarbeet leaves and petiols due to salinity. Murumkar (1986) observed that the NO_3 content increases in the leaf and stem and decreases in roots of Cicer when treated with NaCl. It was also found that the NO_3 from the metabolic pool in the leaves decreases with increasing salinity. Usually accumulation occurs when the utilization of NO3 decreases than uptake. NO_3 accumulation has also been reported by Rajmane (1984) in winged beans grown under saline conditions. According to him the decreased NO_3^- level from the metabolic pool demonstrates that salt induces accumulation of NO_3 in the vacuoles, lowering its amount in the metabolic pool and thus NO_3^- remains unavailable for further metabolism. Maghrabi et al. (1985) pointed out that the absorption of NO_3 is achieved by the efflux of OH^- which shifts the pH of media alkaline. According to them only a minor fraction of the absorbed NO_3^- is accumulated in the tissue and the major is utilised for protein synthesis. Enrico et al. (1981) demonstrated that the accumulated NO_3^- is stored in a large central valuele of the mesophyll cells of barley. This is suggestive of the fact that NO_3^- is not readily available for further utilisation, as it is not found in free condition in cytoplasm.

In case of <u>Setaria</u>, salinity increases the NO_3 -N content in all parts of both the cultivars. This is an accordance with the results obtained by Menary and Allen (1979). It appears that the salt does not affect the uptake of NO_3 , however,

affects its utilisation and hence there is accumulation of NO3-N in Setaria. The rate of accumulation in different plant parts also seems to be influenced by salinity. The roots of the plants grown under non-saline condition show a minimum value of NO3-N while it increases due to salinity in treated plants. It appears, therefore, that salinity influences the translocation of NO_3 . Decrease in NO_3 from stems of SIC-1 supports this. Thus, no generalisation can be made because of fragmentory and scanty information. We can however, definitely suggest that salinity influences the uptake, translocation and distribution of NO3-N in SIC-1. Maximum accumulation in the young leaves of SIC-1 suggests that the young leaves are more sensitive to salt. Decrease in concentration of NO_3^- in stem and its subsequent accumulation in roots indicates disturbed translocation and utilization of it in SIC-1. In CV CO-5 more accumulation is observed in the mature leaves. The stem shows an abrupt increase, at 100 mM salt treatment which cannot be explained. However, at the low and high salt concentrations (50 and 200 mM NaCl) accumulation is negligible indicating that translocation is not affected in CO-5. High concentration of NO_3^- at the higher salinity levels indicates that it is not. utilised for further processes.

No information is available regarding NO_2^- content of the plants under saline conditions. Usually, NO_2^- under normal conditions is readily converted into NH_4^+ and hence it is not



PARTS OF S.ITALICA CULTIVARS (SIC-1 & CO-5) DIFFERING IN SALT TOLERANCE

accumulated in the tissue. The values obtained for NO_2 are very low and support that usually NO_2 does not accumulate in the plant tissue.

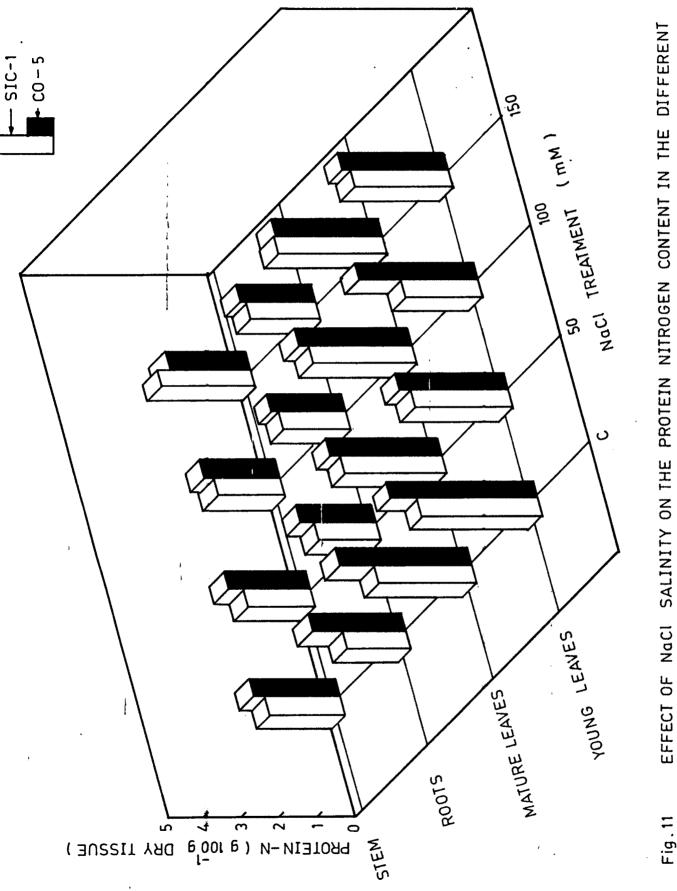
From the results obtained (Table 8 and Fig.10) it appears that NO_2 -N accumulates in SIC-1 which indicates that assimilation of NO_2^- is disturbed in SIC-1. In CV CO-5, on the other hand, the NO_2^- N is on decrease in all plant parts and at all the salinity levels except a slight increase in stem of plants exposed to 100 mM NaCl. These results suggest that the assimilation of NO_2^- N is not affected by salt in CO-5. The accumulated NO_2^- N in case of SIC-1 may create toxic effects on its metabolism.

Strogonov (1970) observed a decrease in protein-nitrogen content in maize and soybean plants subjected to salinity. This inhibition of protein synthesis, as suggested by him, may be due to deficiency of low molecular weight components of nitrogen metabolism. The ratio of non-protein and protein nitrogen increases sharply indicating a break-down of protein or the inhibition of its synthesis. Strogonov also suggested that upon salination, the synthesis of proteins is stimulated in the nucleus, however, inhibited in the cytoplasm, demonstrating that different mechanisms of protein synthesis are developed under saline conditions. Pessarakli and Tucker (1985) studied the ammonium metabolism in cotton under salt stress and observed an enhancement in protein content at the low salinity level

(-0.4 M Pa osmotic potential), its relative stability at the moderately salinized level (-0.8 M Pa) and significant decrease at the higher (-1.2 M Pa) salt concentrations. They suggest that ionic effect probably contributes the inhibition of protein synthesis.Ahmad <u>et al.</u> (1986) also observed similar decrease in protein synthesis in <u>Azadirachta indica</u> and <u>Melia azedarach</u> irrigated with sea water. Murumkar and Chavar (1987-b) pointed out that salinity decreases soluble proteins in chickpea leaves at all the salinity levels studied.

A little variation in soluble proteins is observed in <u>Pennisetum typhoides</u> leaves under saline conditions (Reddy and Vora, 1985-a). Similar results were obtained by Longstreth et al. (1984) in the leaves of <u>Alternanthera philoxeroides</u> based on mesophyll cell area.

Contrary to this, Maas <u>et al</u>. (1979), observed an increase in protein released, with increasing salinity level and this increase is related to the decrease in phosphate uptake. In <u>Brassica juncea</u> and <u>B.compestris</u> exposed to water stress, protein content was higher than that of irrigated plants (Gupta and Sheoran, 1979). Kumar <u>et al</u>. (1982) noted a significant increase in protein level under salt stress in barley. The ability of a plant maintaining higher protein contents at the higher salinity level reveals a greater potentiality of salt tolerance of the plant. Parihar and Baijal (1983) studied



PARTS OF S.ITALICA CULTIVARS (SIC-1 & CO-5) DIFFERING IN SALT TOLERANCE . Fig.11

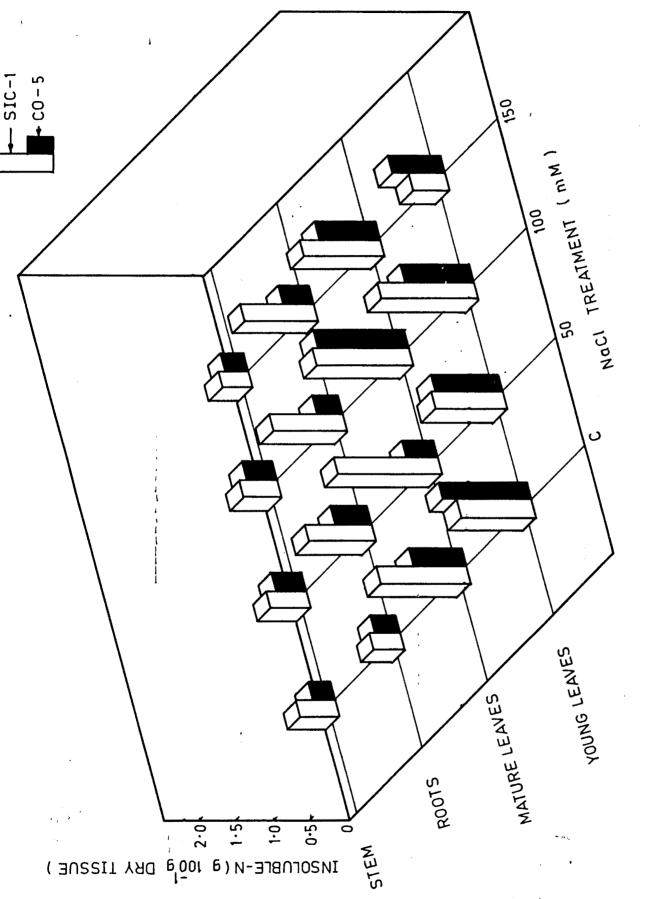
the effect of salinity on nitrogen metabolism in <u>Trifolium</u> <u>alexandrium</u> cultivars and observed an increase in total proteins but without marked difference in total nitrogen. According to them increased protein content is due to slower depletion rather than synthesis of proteins. Ericson and Alfinito (1984) have shown that there is an increase in the level of particular three proteins which are unique to cultured tobacco cells growing in presence of salt. According to them these proteins are involved in the adaptive processes of salt tolerance.

The protein content of different parts of a plant in both the cultivars of <u>Setaria</u> decreases due to salinity except the mature leaves of cultivar SIC-1, (Table 8 and Fig.11). From the above discussion it appears that the inhibition of protein synthesis may be the probable reason for the observed decrease in total protein content. The inhibition may also be due to toxic effects of Na⁺ and Cl⁻ accumulated or probably due to inhibition of protein synthesizing enzymes. However, the reasons for the inhibition is necessary. The increase in protein content observed in mature leaves of SIC-1 may be due to slower utilization of protein rather than its synthesis. In root and stem of the plants exposed to the highest salinity level (200 mM NaCl) the protein content is abruptly increased indicating more disturbed metabolism at this concentration.

Thus in general the protein content in both the cultivars decreases in all plant parts at all the salinity levels. However, the decrease is not much significant and both the cultivars are able to retain the protein level at least to some extent during salt stress.

Decrease in nitrogen (mainly total nitrogen and rarely soluble nitrogen) is reported by number of workers like Karadge (1981) in <u>Portulacha oleracea</u>, Patil and Bhambota (1980) in citrus root stocks, Wahab and Zabran (1981) in <u>Vicia faba</u>, <u>Medicago sativa, Glycine max and Vigna sinensis</u>, Mashhady <u>et al</u>. (1982) in wheat and <u>Triticale</u>, Patil and Patil (1983) in <u>Syzygium</u> root, Papadopulos <u>et al</u>. (1985) in tomato, Patil (1984) in the leaves of <u>Sesbania</u>, Dixit <u>et al</u>. (unpublished) in the mature leaves of <u>Crotalaria</u> species. It is suggested by these workers that the decrease in total N content is due to disturbed metabolism and NaCl is more dentrimental in this respect, Cl probably interferes with nitrogen uptake. The uptake of nitrogen seems to be severely affected by salt.

Helal and Mengel (1979) in barley, Karadge and Chavan (1982) in <u>Sesbania</u>, Parihar and Baijal (1983) in <u>T.alexandrium</u> Patil and Patil (1983) in the leaves of <u>Syzygium</u>, Seemann and Christa (1985) in <u>P.vulgaris</u>, Dixit <u>et al</u>. (unpublished) in young leaves and stem of <u>Crotalaria</u> species have found no significant effect of salinity on total nitrogen content.



EFFECT OF Naci Salinity on the insoluble-nitrogen content in the different PARTS OF S. ITALICA CULTIVARS (SIC-1 & CO-5) DIFFERING IN SALT TOLERANCE. Fig. 12

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Chavan (1980) has reported an increase in total nitrogen in the stem and leaves of ragi when exposed to NaCl salinity. Higher nitrogen concentration in shoot, root and rhizome at the higher salinity treatments is observed by Gallagher (1979) in <u>Sporobolus virginicus</u>. Similar observations are made by Jefferies <u>et al</u>. (1979) in halophytes, Ravikovitch and Yoles (1971) in <u>S.italica</u>, Patil (1984) in stem and roots of <u>Sesbania</u>, Rajmane (1984) in winged bean, Pessarakli and Tucker (1985) in cotton and Murumkar (1986) in chickpea. High levels of nitrogenous compounds along with sugars, proline and quaternary ammonium compounds help to maintain an internal osmotic potential and better salt tolerance potential.

In the present study it is observed that both insoluble fraction and total of fractions of nitrogen decrease in young and mature leaves of CV SIC-1 under saline conditions except at the higher salt concentrations (100 & 200 mM NaCl) in the mature leaves where they are on increase. Both insoluble N and total of nitrogen fractions increase in roots of SIC-1 while in stem insoluble fraction increases and that of total decreases with salinity. From these findings it appears that salinity probably inhibits the translocation of N which is accumulated in the roots and stem.Salinity also influences the distribution of N into various plant parts. Accumulation of N in the mature leaves is indicative of incomplete utilization of N especially at the higher concentrations. In CV CO-5 both

insoluble and total of all fractions decrease in young and mature leaves except a small rise in young leaves at 100 mM and mature leaves at 50 mM NaCl concentrations. In roots both of them increase while in stem they remain almost constant. The accumulation of N in the roots is suggestive of disturbed translocation. By comparing the figures it appears that the effect of salinity is more pronounced in variety SIC-1. The earlier work done in our laboratory also indicates a varietal difference in <u>S.italica</u> suggesting nitrogen metabolism in sensitive cultivar ISe-769 is more affected than the tolerant cultivar CO-4.

E. Proline :

The effect of NaCl treatment on proline content in <u>Setaria italica</u> cultivars is recorded in Table 9 and Fig.13. It can be seen that salinity causes linear increase in proline content of the young leaves of SIC-1. In the mature leaves of this cultivar it dramatically increases even at the low salt level and then decreases sharply below the control value. In roots it increases at the lowest (50 mM) and the highest (200 mM) level of salinity. In case of cultivar CO-5, proline content increases in young leaves only at 50 mM NaCl and then decreases sharply. In mature leaves and roots a decrease is observed only at 100 mM and at the low and high levels of salinity it increases to a considerable extent.

of S.italica cultivars (SIC-1 and CO-5) differing in salt tolerance. Table 9 : Effect of NaCl salinity on proline content* of the leaves and roots

NaC1 :			SIC-1					C0-5			
Treatment: mM :	Young leaves	•••••	Mature leaves	••••	Roots	¦ •• ••	Young leaves	: Mature : leaves	••••	Roots	
0 (Control)	37.5		058.6		4 1 •1	Ý	0678.0	0387.0		081 • 6	•
50	42.5		157.7		56•1	-	1.988.0	1994.0		270.8	
100	47.8		051.7		27.5		0322.0	0194.0		066.7	•
200	55.3		056.7	•	59.4	-	0:575.0	1125.0		087.5	
	1 m − 1 m −					1					

* Values are expressed as ug g⁻¹ fresh tissue.

Each value is mean of three determinations.

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Increase in proline content is observed in a number of plants by several workers. Recently Weimberg <u>et al</u>. (1982) observed accumulation of proline in <u>Sorghum bicolor</u>, Dreier (1983) in <u>Triticum</u> species, <u>H.vulgare</u>, <u>Zea mays</u> and other crop plants, Gorham <u>et al</u>. (1985) in Triticeae, Reddy and Vora (1985 b) in <u>Pennisetum</u> and Chandra & Chauhan (1985) in barley, pearl millet and chickpea. The earlier work done in our laboratory also indicated accumulation of proline in <u>Crotalaria</u> <u>retusa</u>, groundnut, winged bean, <u>Eleusine</u> and other <u>Setaria</u> cultivars. Accumulation of proline is also reported from a number of halophytes.

It is suggested that accumulation of proline is dependent on the concentration of monovalent cations like K^+ . The accumulated proline acts as a osmoticum and helps the plant to osmotically adjust during salt stress.

Karadge (1981) has observed no significant change in proline content of <u>Portulaca</u>. Decreased level of proline due to salinity is also observed by Singh & Jain (1983) in chickpea, Coughlan and Wyn Jones (1982) in spinach leaves and Dixit <u>et al</u>. (unpublished) in <u>Crotalaria juncea</u>. These observations indicate that probably proline has only a minor or no role to play in salt tolerance in the plants studied.

Chandra and Chauhan (1985) observed that free proline accumulated in barley, pearl millet and chickpea does not show

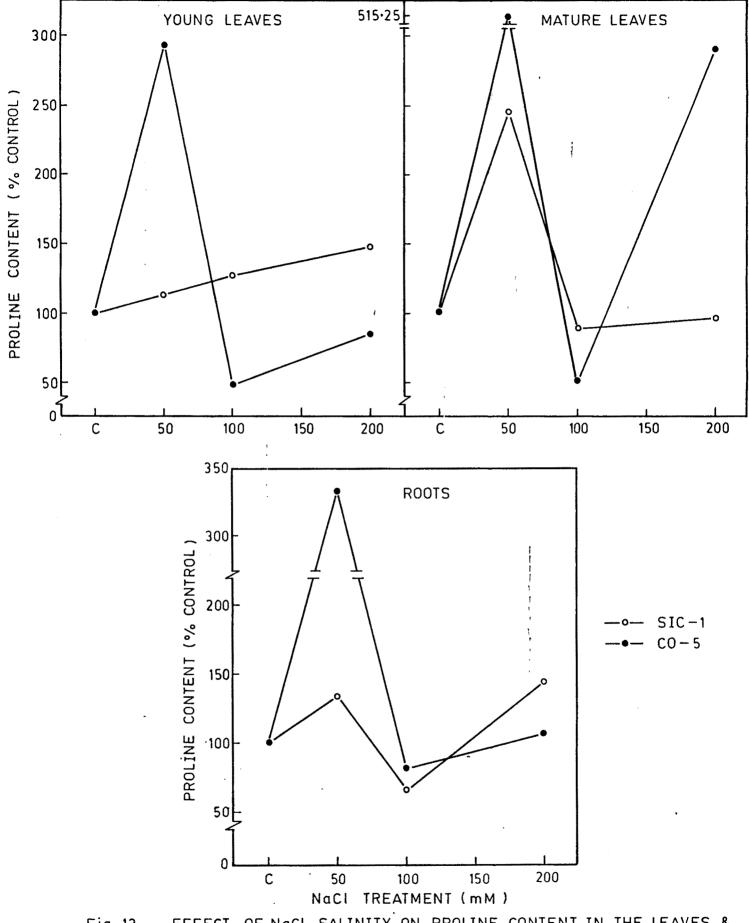


Fig.13 EFFECT OF NoCL SALINITY ON PROLINE CONTENT IN THE LEAVES & ROOTS OF <u>S.ITALICA</u> CULTIVARS (SIC-1 & CO-5) DIFFERING IN SALT TOLERANCE .

any positive corelation with growth and yield under saline conditions. According to them proline in the leaves is not related to salinity resistance and hence generalisation regarding proline accumulation is not possible.

In <u>Setaria</u>, it is observed that proline is accumulated in the young leaves of CV SIC-1 when treated with NaCl. In mature leaves, however, different picture is obtained. In mature leaves of SIC-1 and young leaves of CV CO-5 the level of proline increases only at 50 mM NaCl level and then declines at higher salinity levels. From these observations it appears that 100 mM NaCl level seems to be more dentrimental in both the cultivars and the abrupt change at this concentration may be related to specific ion effect in addition to osmotic effects. However, no definite pattern is observed and hence proline content and salt tolerance capacity of <u>Setaria</u> cannot be correlated.

F. Enzymes :

i) <u>Nitrate Reductase</u> (NR) :

Effect of NaCl salinity on the activity of nitrate reductase - NR is depicted in Table 10 and Fig.14. In SIC-1, the activity of the enzyme is stimulated at 50 mM NaCl and then decreases at 100 and 200 mM NaCl. In mature leaves the activity decreases to some extent due to 50 and 100 mM NaCl treatments and again increases at 200 mM NaCl, however, the

10 : Effect of NaCl salinity on the activity of nitrate reductase and nitrite reductase in the leaves and roots of S.italica cultivars (SIC-1 and CO-5) differing in salt

tolerance

Table

		N-2	3494	0276	1620	0412	
		N = NC	0.0	0 <u>•</u> 0	<u> </u>	<u> </u>	
	ts	Fon	176.	122.1	142.(182.	
	Roots	NR : NAR : NOJN: NO2-N	1.391 139.6 176.9 0.03494	1.190 146.9 122.8 0.0276	1.158 147.1 142.0 0.0291	1.472 124.1 182.3 0.0412	~~~. ^{\$} *
		8 8 8	291 1	1 1 1 1	L58 1	172 1	
	-						
		NR : N1R :NO ₃ -N: NO ₂ -N :	• 0386	.0292	.0510	-0511	
	Mature-leaves	N	0 1.0	3.90	5.90	5.7 0	
CO-5	re-1	NO.	6 10	2000	<u></u>	2 10	
ğ	Matu	: NIF	129 .	072.	149.	<u>– – – – – – – – – – – – – – – – – – – </u>	
		NR ,	7.765 129.9 100.1 0.0386	4.276 072.5 063.9 0.0292	6.445 149.3 115.9 0.0510	113.5 105.7 0.051	
	*	2-N		the second s			
	8	N : NO	0.0		90.9 0.0374	00.5 0.0438	
	.eaves	NO ₃ -1	112•(I	099.8 0.0351	•060	100.	
	Young le	NAR NO₅-N: NO₂-N	4.652 126.8 112.0 0.0321	5.717 125.5	5.524 121.9 0	5.878 109.2 1	
	Υc	NR : ;	652 1	ר גע	524]	878	
	**						
		: N1R :NO ₂ -N: NO ₂ -N :	1.246 124.2 177.1 0.0378	1.657 159.1 102.1 0.0404	1.279 116.0 137.7 0.0292	1.534 137.0 140.1 0.0358	
	8	3-N:	ייר	5•1 (21.7	0.10	
	Roots	R :NC	.2 11			-0-14	**
		TN .	5 124	1 159	9116	137	
		NAR NOZ-N: NOZ-N INR	1.24	1.65	1.27	1•53 ⁴	
		N-2					
-		N INO	90 . 8 0.0464	97.0 0.0457	20.0	5.0.0	
SIC-1	BUUB	NO ₃ -			.611	120.	
	: Mature leaves	N1R	.06.2	20.0	133.0	9 . 41	•
			8.519 106.2	6.189 120.0	6.209 133.0 119.5 0.0406	7.426 114.9 120.2 0.0479	
		N : NR					
		N02-	0, 05(0505	0.045	0•036	
	VOS	NR : NIR : NO ₃ N: NO ₂ -N	7.265 112.6 101.4 0.0509	8.529 136.3 107.1 0 0505	6.920 123.7 115.2 0.0452	4.601 110.4 110.4 0.0360	
	Young leaves	1R :	2.6 1	6.31	3.7 1	0.4 1	
	Youn	K : N:	22 FT	59 13	50 12	1	
			1,26	8.5	6.9	4.6(
	+		rol .				

NR = Activity is expressed as n moles of NO₂ liberated h⁻¹ mg⁻¹ protein. NiR = Activity is expressed as n moles of NO₂ consumed h⁻¹ mg⁻¹ protein. NO₃-N = is expressed as $\mu \in mg^{-1}$ protein. NO₂-N = is expressed as $\mu \in mg^{-1}$ protein. value is still less than that in control. In roots, however, the activity increases with increase in salt concentration, and it is more pronounced at 50 and 200 mM NaCl. On the other hand in case of CV CO-5, the NR activity increases with salinity in young leaves only. Mature leaves and roots, however, show a considerable decrease in the activity of this enzyme especially at 50 and 100 mM NaCl and a slight increase at 200 mM NaCl.

Plaut (1973) has recorded an inhibition of NR in wheat under saline conditions. However, it was recovered after removing the stress. According to him induction of enzyme is inhibited by salinity and this is related to shift of ribosomes from polymeric to monomeric form and subsequent effect on protein synthesis. The recovery of the enzyme is attributed to restoration of polyribosomes. He further suggested that there may be two sites of enzyme; one in cytoplasm and another associated with chloroplasts. Cytoplasmic fraction probably looses its activity due to inhibition of protein synthesis while chloroplastic fraction inhibits activity due to conformational changes and partial denaturation of NR. Rakova et al. (1979) studied the effect of NaCl and Na₂SO₄ on <u>in vitro</u> activity of NR in different plants. He suggested that the response of NR to salts added in vitro did not depend on the salt resistance and enzymes even from halophytes are sensitive to salt. Decrease in NR activity due to water stress is

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reported by Gupta and Sheoran (1979) in <u>Brassica</u> juncea and <u>B.compestris</u>. However, decrease is more pronounced in <u>B.juncea</u>, a drought susceptible plant.

Heuer and Plaut (1979) observed a decrease in NR activity in sugarbeet due to salinity which is attributed by them to the enzyme activity inhibition and synthesis of Billard and Boucaud (1982) observed a decrease in enzyme. NR activity in a halophyte Suaeda macrocarpa. They considered that in vitro the salt induces noncompetitive inhibition and NaCl stabilizes the NR substrate complex. Dissociation of a molybdofactor from the enzyme apoprotein takes place which is responsible for decrease in NR activity (Kabisheva et al. 1981). Decrease in NR activity is associated with leaf relative water content (Sairam and Dube, 1984) in rice genotypes subjected to water stress. Safaralliev et al. (1984) studied the causes of decrease in NR activity in legumes under salt stress and found that it may be due to dissociation of FAD (in leaves) and Mo (in roots). Aslam et al. (1984) observed a severe inhibition of NR when salt was added in vitro while in vivo it was only slightly affected. This indicates that in situ NR activity is protected from salt injury. Decrease in NR is also reported by Patil (1984) in <u>Sesbania</u>, Rajmane (1984) in winged bean, Bottacin et al. (1985) in Pennisetum and Murumkar (1986) in chickpea which is considered by them to be due to the unavailability of substrate. A shift of NR activity from root to shoot

by salt stress is reported in a halophyte <u>Arthrocnemum</u> fruticosum (Eddin and Doddema, 1986).

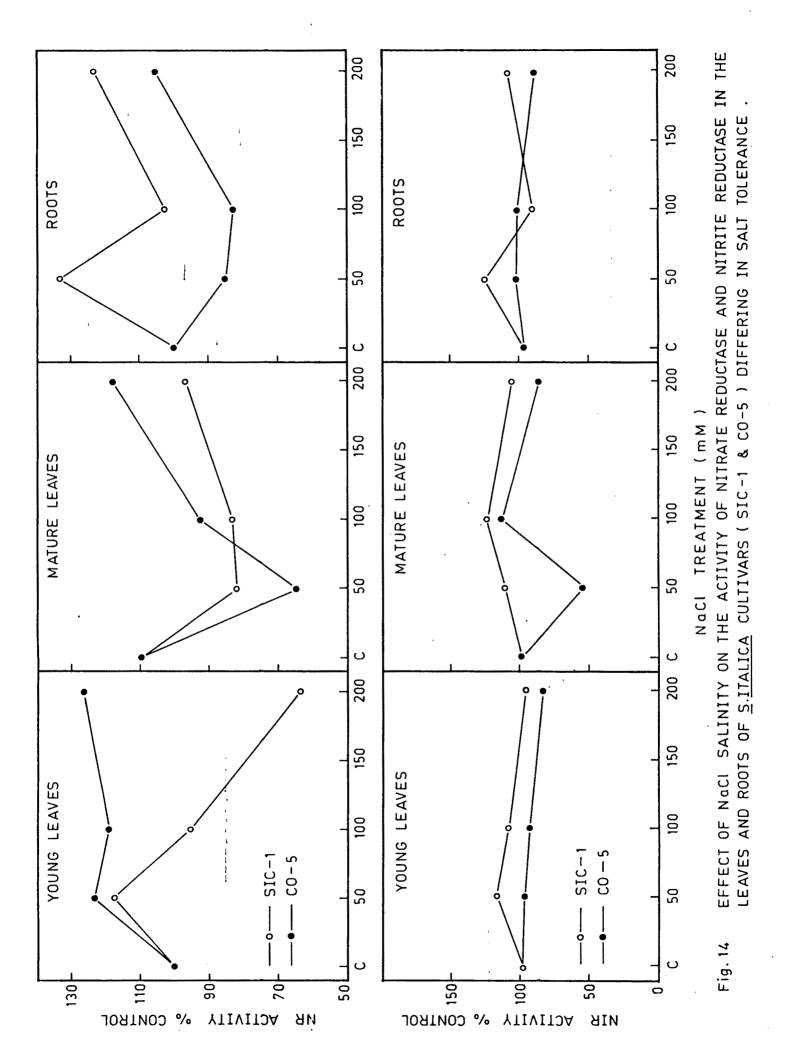
On the other hand stimulatory effect of salt on NR is also reported by number of workers. Sankhla and Huber (1975) observed an increase in in vivo activity of NR in P.aconitifolius seedlings exposed to saline conditions indicating the capacity of the plant to adjust intracellular ionic balance. Chavan (1980) also observed similar trend in ragi and suggested that this increase enables the plant to cope with changes in nitrogen metabolism induced by the salinity. Dias and Costa (1983) studied the effect of salinity on NR in sugarbeet leaves and observed that NR is stimulated by low salt concentration, however, this is the indirect effect of salt treatment on the rate of enzyme synthesis or metabolic regulation. The stimulatory trend is also noticed by Krishnamoorthy and Siddique (1985) in cowpea, Karadge et al. (unpublished) in the leaves of Setaria and Eleusine cultivars and Dixit et al. (unpublished) in Crotalaria species. In Panicum (CV Varada) leaves and the roots of <u>Pennisetum</u> (GHB - 134) the enzyme activity is fairly stable under saline conditions (Karadge et al., unpublished).

NR is considered as a substrate inducible enzyme. Dias and Costa (1983) suggested that the activity of NR is dependent upon the concentration and rate of NO_3^- supply to the tissue. They reported that maximum NR activity in sugarbeet is related to increased NO_3^- at that time. However, a lack

of relationship between the leaf NO_3^- content and NR activity is found by several workers. It is also suggested that most of the NO_3^- is accumulated in cells in the vacuoles (storage pool) and a small, rapidly metabolising inducing pool in cytoplasm.

In the present study the activity of NR decreases in the leaves of SIC-1 accompanied by a decrease in NO2-N. However, at the same time NO3-N content increases, which indicates that substrate unavailability is not responsible for the observed inhibition of NR. It appears, therefore, that the inhibition is probably due to partial dissociation of molybdofactor of the enzyme (Kabisheva et al., 1981) or conformational changes of chloroplast enzyme (Plaut, 1974) or decrease in enzyme synthesis (Heuer and Plaut, 1979). However, the decrease in enzyme activity in the leaves may probably be related to photosynthetic activity under saline conditions. At 50 mM NaCl, the activity is increased in young leaves. In this case it appears that low salt level probably enhances synthesis of NR. The activity of NR is increased in roots at all the concentrations, accompanied with subsequent decrease in NO3-N and increase in NO2-N. At 100 mM NaCl, a slight decrease in NR activity is observed. The increase observed may be associated with indirect effect of salinity or disturbed translocation of NO_3^- .

CO-5 shows an opposite trend. The activity of NR is stimulated in young leaves. It appears that in young leaves



salinity induces enzyme synthesis. In mature leaves, however, NR activity decreases sharply even at the low level but again is recovered at the higher salt concentrations. However, this decrease is not accompanied by an increase in NO_3 -N content and this observation suggests that unavailability of NO_3^- may be responsible for this decrease. However, at higher salt concentrations, NO_3 -N content increases similar to NR activity. In roots of CV CO-5 the activity decrease at 50 and 100 mM NaCl level to some extent and increases at higher concentrations. NO_3 -N content also follows the similar trend with that of NR activity. Thus roots appear to be little bit stable to salinity.

From these observations it appears that in CV CO-5 the NR is somewhat stable to salinity. The activity of this enzyme is more in the leaves than in the roots. In general we can say that the nitrate reduction is not much affected by salt in CV CO-5 and this may be considered as an adaptive feature.

ii) <u>Nitrite Reductase</u> (NiR)

The influence of NaCl salinity on the activity of NiR in the leaves and roots of <u>Setaria</u> cultivars is recorded in Table 10 and Fig.14. It can be seen that the NiR activity is several fold higher than that of NR. It is also clear that the activity increases both in the leaves and roots of CV SIC-1 except a small decrease in the roots at 100 mM NaCl.

106

In CV CO-5 the activity appears to be little bit stable to salinity in young leaves and roots, however, it decreases in mature leaves at 50 and 200 mM while increases to some extent at 100 mM NaCl treatment.

Very few reports are available on the influence of NaCl on NiR activity. Heuer and Plaut (1979) observed a decrease in NiR activity under saline conditions in sugarbeet probably due to adverse effect of salinity on the enzyme itself. Rajmane (1984) has reported inhibition of NiR in the leaves of winged bean, however, the inhibition is not comparable with that of NR. According to him the observed decrease in NiR activity is due to lack of substrate - NO_2 . Murumkar (1986) also observed inhibition of NiR with increasing salinity. Unavailability of substrate, disturbances in enzyme protein and decrease in supply of reducing power are some of the probable reasons for the inhibition that was observed. Paul et al. (1985) have studied the effect of a reduced osmotic potential due to water stress on NiR in isolated spinach chloroplasts and observed inhibition of NiR due to decreased osmotic potential is probably due to the effect on the interaction of NiR with ferridoxin. Bottacin et al. (1985) however, observed no effect of salinity on NiR in P.americanum genotypes.

Helal and Mengel (1979) suggested that salinity impaires incorporation of labelled nitrogen into protein and accumulates labelled inorganic-nitrogen. This may be related to synthesis of enzyme protein. The chloroplastic location of NiR suggests that any change in photosynthetic activity is directly related to NiR activity. The enzyme also depends on photophosphorylation for supply of reduced ferridoxin.

The increase in NiR activity in the leaves and roots of SIC-1 is associated with the decrease in NO2-N content. NO_2 -N concentration increases little above to that in the control plants in the mature leaves at 200 mM NaCl and in roots at 50 mM NaCl. The increase in NiR in SIC-1 is well co-related with increase in NR activity. It is a well known fact that activity of NiR is more than that of NR to avoid accumulation of NO_2 . Due to increased NR activity, NO_2 concentration is increased, to avoid accumulation of NO2, NiR also increases. In case of CV CO-5, in young leaves NR activity increases but NiR activity remains little bit stable or decreases slightly which results in accumulation of NO_2 . Decrease in reducing power may probably be repsonsible for this observed decrease. Mature leaves do not show any definite pattern. At 50 mM NaCl treatment, NiR decreases sharply and this is in accordance with decrease in NR and NO2-N, at the same time. However, at 100 mM NaCl the activity of NR increases to some extent but NiR activity increases to a considerable extent with an increase in NO_2 content. But again at 200 mM, NR activity increases but NiR decreases. It seems that the fluctuations are not related to substrate available but probably related to disturbed enzyme-

protein and supply of ferridoxin. NiR of the roots of CV CO-5 appears to be stable under saline conditions except a decrease at the highest salinity level i.e. at 200 mM NaCl.

iii) <u>Glutamate oxaloacetate transaminase</u> (GOT) :

The influence of NaCl salinity on the activity of GOT is shown in table 11 and Fig.15. It is clear that the activity of this enzyme decreases due to salinity in the leaves and roots of SIC-1. However, a considerable increase of it in young leaves at 100 mM NaCl is noted in the same cultivar. In CV CO-5 the activity of GOT increases in both young and mature leaves except a decrease in both at 50 mM NaCl treatment. In roots the activity of this enzyme decreases at all the salinity levels.

Decrease in GOT activity is recorded by Karmarkar and Rangnathan (1971) in <u>Bryophyllum</u> under saline conditions. Similar decrease is also observed by Gupta and Sheoran (1979), while studying the effect of water stress on <u>Brassica juncea</u> and <u>B.compestris</u>. The decrease is more pronounced in <u>B.juncea</u>, which is susceptible. Krishnamoorthy and Siddique (1985) also observed a decrease in GOT activity in <u>Vigna unguiculatu</u> exposed to salt stress.

Sharma and Garg (1985), however, have observed stimulatory effect of NaCl on the activity of GOT in the leaves and roots of wheat. Murumkar (1986) observed an increase in GOT

		: Roots		Protein:Chloro-:Protein:Chloro-:Protein:Protein:Chloro-:Protein:Chloro-:Protein: : : :phyll : : :phyll : : : :phyll :	0•0941	0.0453	0.0613	0.0404	
- eo 1	C0-5	leaves	-1 mg-1)	:Chloro- :phyll	0.2975	0.4297	0.7040	0.6773	
		: Mature leaves		:Protein :	0.0298	1910.0	0.0468	0•0513	
			activity (mg OAA formed h	:Chloro- :phyll	0.3400	0.4657	LT91.0	0.7723	
differing in salt tolerance.	••	: Young leaves	(mg OAA	:Protein :	0.0335	0.0294	0•0327	0.0537	
in salt		Roots	ctivity	:Protein	1470.0	0•0388	0.0302	0.0504	
ffering		: Mature leaves:	GOT a	:Chloro- :phyll	0.8180	0.6229	0.3917	0.3539	e .
and CO-5) di	SIC-1	: Matur		Protein:	0.0424	0.0362	0.0378	0.0320	
and (:Chloro- :phyll	0,1992	7121.0	0.6057	6171.0	
	••	Toung leaves	••	Protein	0.0127	0600*0	0.0504	0.0134	
	NaCL	Treatment: mM			(Control)	50	100	200	

transaminase in the leaves and roots of S.italica cultivars (SIC-1

Table 11 : Effect of NaCl salinity on the activity of glutamate oxaloacetate

Each value is mean of three determinations.

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activity in chickpea when exposed to salt stress. Similar increase is also reported by Joshi (1976) in mangroves and by Rao Gururaj <u>et al</u>. (1981) in peanut under saline conditions. According to these workers the increase in GOT activity may be due to greater availability of carbon skeleton for amino acid synthesis and this increase may help to decrease toxic effects of NH_A^+ .

In the present study, in CV SIC-1 the activity of this enzyme decreases both in the leaves and roots except a sudden increase at 100 mM NaCl. This decrease in the activity may be due to the effect of salinity on enzyme itself or decrease in the supply of carbon skeleton and donor amino acids. Abrupt increase at 100 mM NaCl cannot be explained. In CV CO-5, in young leaves, activity of the enzyme increases at 200 mM, it is stable at 100 mM and decreases slightly at 50 mM NaCl concentration. In mature leaves, it decreases only at 50 mM salt level and then increases. The increase observed may be due to greater availability of carbon skeleton and this increase is helpful to decrease toxic effect of NHA. The enhanced synthesis of amino acids is considered as an adaptive feature in osmotic adjustment. However, such study is not conducted in Setaria. In roots, however, the activity is decreased at all salinity levels. The results obtained on chlorophyll basis are more or less similar to those obtained on protein basis.

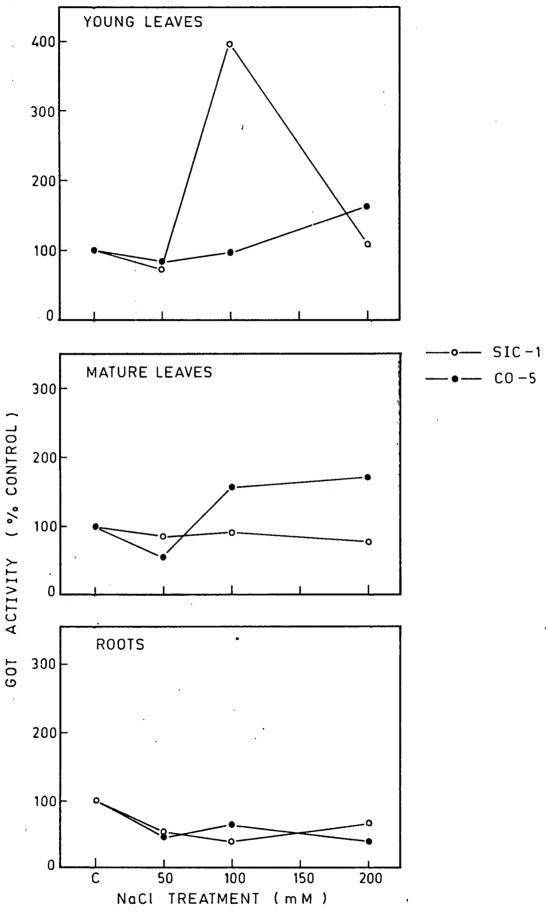


Fig. 15 EFFECT OF Naci Salinity on the activity of glutamate OXALOACETATE TRANSAMINASE IN THE LEAVES & ROOTS OF <u>S ITALICA</u> CULTIVARS (SIC-1 & CO-5) DIFFERING IN SALT TOLERANCE.

iv) Alanine Amino Transferase (AAT) :

The changes in the activity of AAT in the roots and leaves of <u>Setaria</u> cultivars are recorded in Table 12 and Fig.16. It is evident that the activity of AAT in both the leaves and roots increases in SIC-1 while at the same time it decreases in CV CO-5 with salinity stress.

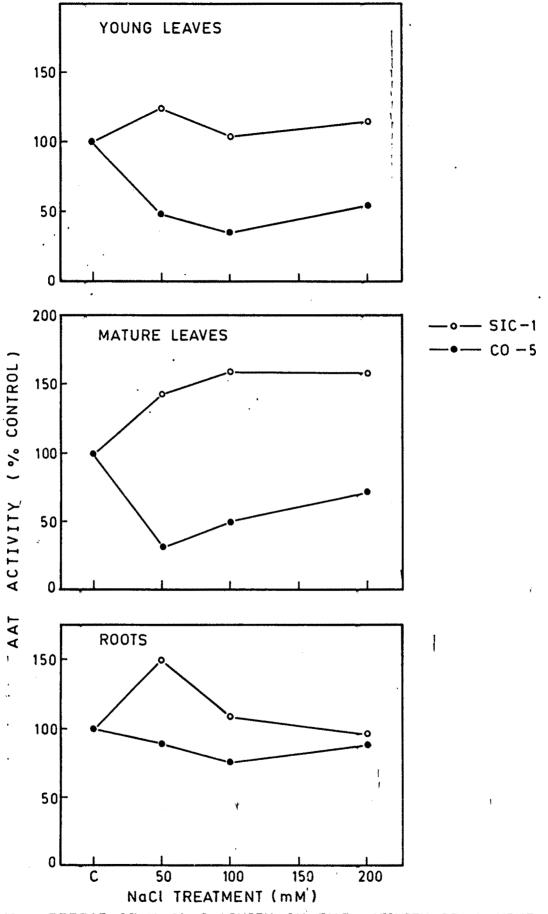
Decrease in AAT activity is recorded by Gupta and Sheoran (1979) in <u>Brassica juncea</u> and <u>B.compestris</u> when exposed to water stress and by Krishnamoorthy and Siddique (1985), in cowpea under salt stress. On the other hand increase in AAT activity is recorded by Joshi <u>et al.</u> (1962). According to them stimulation of amino-transferases along with inhibition of malate dehydrogenase suggests a shift from organic acid to amino acid synthesis. Similar stimulatory trend is also reported by Karmarkar and Rangnathan (1971) in CAM plant, <u>Bryophyllum</u>, Joshi (1976), in mangrooves, Rao Gururaj <u>et al</u>. (1981) in peanut, Sharma and Garg (1985) in wheat, Murumkar (1986) in chickpea and Dixit <u>et al</u>. (unpublished) in <u>Crotalaria</u> species. According to these workers the increase in AAT activity is useful for conversion of pyruvate to amino acids in presence of NH⁺₄ and thus avoid accumulation of toxic ammonia.

In the present study the activity of AAT in CV SIC-1 increases in young leaves, mature leaves and roots. This may be related to synthesis of amino acids. However, in CO-5

Table 12 : Effect of NaCl salinity on the activity of alanine amino transferase in the leaves and roots of S. italica cultivars (SIC-1 and CO-5) differing in salt tolerance

		Roots		tein	0.2608	0.2343	0.1984	0.2338	
,		24 *		:Pro	0.2	0.2	L.0	0•2	
			-1)	:Chloro-	2.515	2.165	1.925	2.417	ţ
	C0-5	: Mature leaves	d h ⁻¹ mg ⁻¹)	:Protein :	0.2516	0• 0809	0.1279	0.1830	
		leaves	tte forme	:Chloro- :phyll	2.062	1.920	1.686	1. 596	
	••	: Young	g pyruva	:Protein :	0.2034	1121.0	0.0723	0111.0	
		Mature Leaves : Roots : Young leaves	AAT activity (mg pyruvate formed h ⁻¹	Protein:Chloro-:Protein:Chloro-:Protein:Protein:Chloro-:Protein:Chloro-:Protein :phyll : :phyll : : :phyll : :phyll : :phyll : :phyll : :phyll :	0.2252	0.3364	0.2450	0.2180	
		Leaves	AAT act	:Chloro- :phyll	2.695	3.461	2.269	2.433	
	SIC-1	: Mature		·:Protein :	0.1396	0.2009	0.2192	0.2202	
		leaves		:Chloro- iphyll	2.430	2•597	1.932	2.266	
	••	: Young leaves	• •	Protein	0.1545	0.1926	0.1608	0.1773	
	NaCl	Treatment:			0 (Control)	50	100	200	

Each value is mean of three determinations.





EFFECT OF NGCI SALINITY ON THE ACTIVITY OF ALANINE AMINO TRANSFERASE IN THE LEAVES & ROOTS OF <u>S.ITALICA</u> CULTIVARS (SIC-1 & CO-5) DIFFERING IN SALT TOLERANCE. the activity decreases in both the leaves and roots at all the salinity levels. The decrease may be probably due to unavailability of carbon skeleton. It is also probable that the another mechanism of amino acid synthesis, namely transamination (GOT) may be dominant in this species.

v) Glutamine Synthetase (GS)

The influence of salinity on the activity of GS in the cultivars of <u>S.italica</u> is shown in Table 13 and Fig.17. It appears that the activity of GS increases both in the young and mature leaves of CV SIC-1, while decreases in both type of leaves in CV CO-5 due to salinity stress.

Very little information is available about the effect of salinity on the activity of GS. Rakova <u>et al</u>. (1979) studied the effect of NaCl and Na₂SO₄ on <u>in vitro</u> activity of GS in different plants having different salt tolerance potential. According to them the GS either from the halophytes or from the glycophytes is equally susceptible to salt stress. According to them GS is more salt sensitive than GDH and NR. Boucaud and Billard (1979) reported inhibitory action of salt on GS activity and suggested that NaCl interferes the enzyme synthesis process in <u>Suaeda</u> a halophyte. Larher <u>et al</u>. (1977) also observed suppression of enzyme activity due to salinity in a halophyte, <u>Triglochin maritima</u>. Similar decrease is observed by Krishnamoorthy and Siddique (1985) in cowpea and Dixit <u>et al</u>.

Table 13 : Effect of NaCl salinity on the activity of glutamine

synthatase in the leaves of S. italica cultivars

(SIC-1 and CO-5) differing in salt tolerance.

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				L	-		_	_
		leaves		Chloro- phyll	0.2181	0.2452	0.1639	0.0319
Cultivars	SIC-1 *(Mature leaves	ng-1		L600°0	0.0126	0.0075	0*00 1 5
		Young leaves :		: Chloro- : Protein : phyll :	0.8598	0.2873	0.5607	0.4432
		: Young	GS activity \$ 0D min ⁻¹	Chloro- : Protein phyll :	0.0328	0.0144	0.0315	0•0205
		Mature leaves	GS acti	: Chloro- : phyll	0•0933	0-8690	0.4102	0.4931
		Mature	Mature	Protein	0.0058	0.05613	0•0193	0.0231
		Young leaves :		Chloro- : phyll :	0.1702	0.1315	0.4487	0.2251
		Young		Protein :	5 TTO•0	0.0104	0.0279	0.0181
	NaCl :	Treatment:	•••	•• ••	0 (Control)	50	100	200

Each value is mean of three determinations.

115

(unpublished) in <u>Crotalaria</u> species. NaCl may act as a noncompetitive inhibitor of GS. Similarly decrease in supply of ATP or Mg²⁺ or Mn²⁺ may be responsible for the decrease of GS activity.

On the other hand increase in GS activity is reported by Bottacin <u>et al</u>. (1985) in <u>P.americanum</u> a resistant genotype, however, the activity in sensitive genotypes decreases with increasing salinity. They further suggested that the increase in GS activity in resistant genotypes under salt stress is related to a shift of nitrogen assimilation pathway towards glutamine route (GS - GOGAT pathway). Rajmene (1984) reported a slight increase in GS activity in winged bean under saline conditions. Murumkar (1986) also reported stimulatory effect of salinity on GS activity in chickpea.

In case of <u>Setaria</u>, the activity in CV SIC-1 increases both in young and mature leaves. At 50 mM NaCl treatment the activity decreases slightly by about 10%, however, at the same time the activity in mature leaves is increased by about ninefold that of the control. The increase in GS activity in SIC-1 may be related to a shif towards glutamine route. However, this needs further studies. In CV CO-5 the activity is decreased both in the young and mature leaves except an increase in mature leaves at 50 mM NaCl. The decrease observed may be due to decrease in enzyme synthesis or decrease in the supply of ATP.

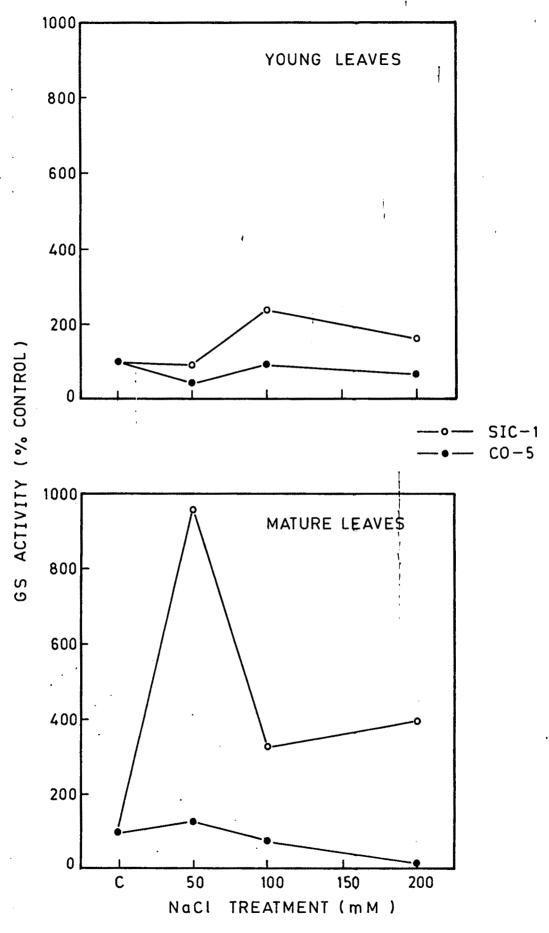


Fig:17 EFFECT OF NaCL SALINITY ON THE ACTIVITY OF GLUTAMINE SYNTHETASE IN THE LEAVES OF <u>S.ITALICA</u> CULTIVARS (SIC-1 & CO-5) DIFFERING IN SALT TOLERANCE.

vi) Glutamate Dehydrogenase (GDH) :

The effect of NaCl salinity on the activity of GDH in the young and mature leaves of <u>Setaria</u> cultivars is shown in Table 14 and Fig.18. It appears that the activity of GDH is stimulated in SIC-1 and that inhibited in CV CO-5 by salinity.

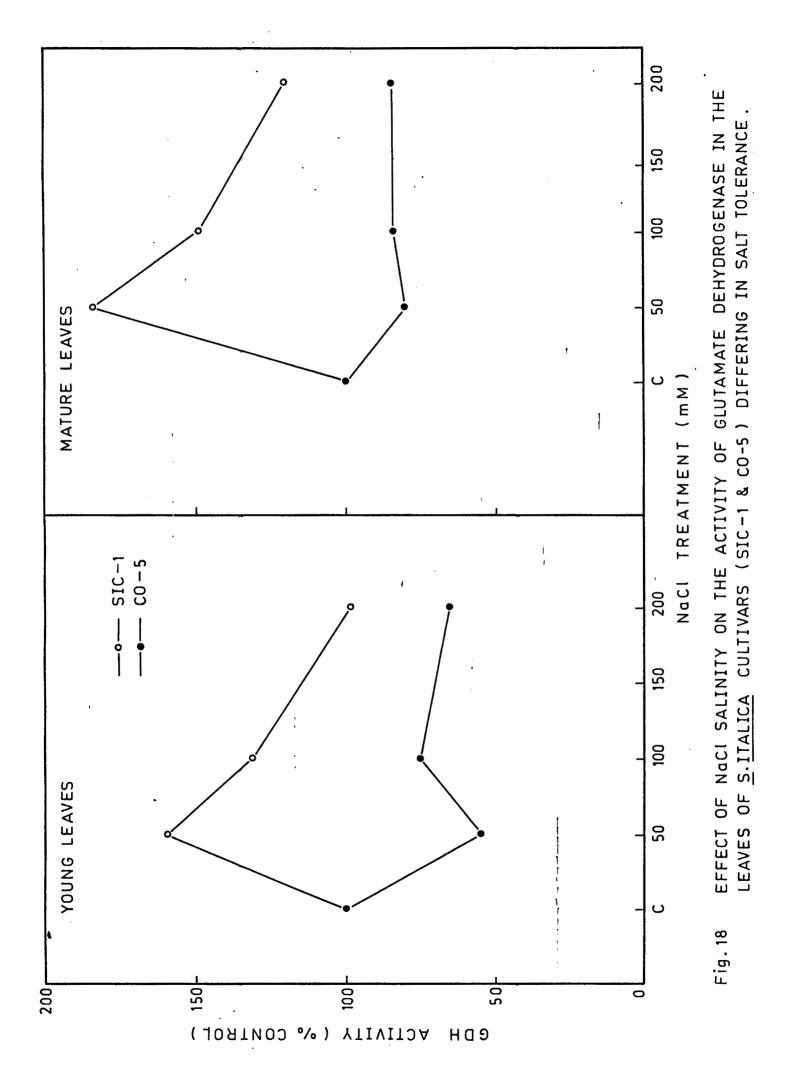
Rakova <u>et al</u>. (1979) observed that <u>in vitro</u> GDH, isolated from tolerant and sensitive plants, is susceptible to NaCl and that GDH is medium tolerant to salt. Gupta and Sheoran (1979) observed a decrease in GDH in <u>Brassica juncea</u> and <u>B.compestris</u> due to water stress. The inhibition is more pronounced in <u>B.juncea</u>, a susceptible species. Ahmad <u>et al</u>. (1979-a) observed that partially purified GDH is inhibited by C1⁻. NaCl changes Km and appears to behave like a noncompetitive inhibitor of this enzyme. The inhibition is pH dependent. According to Kulikov (1983), the behaviour of this enzyme also depends upon the mineral nutrition.

Tur and Skazhenic (1980) observed that salinization produces a sharp increase in GDH activity in seedlings and roots of rice. Increased GDH in roots and leaves of wheat is reported by Sharma and Garg (1985). Bottacin <u>et al.</u>, (1985) observed an increase in GDH by salt stress in <u>P.americanum</u>. However, the increase is more pronounced in susceptible cultivars and it is related to shift of nitrogen assimilation pathway. Similar increase is observed by Dixit

Table 14 : Effect of NaCl salinity on the activity of glutamate dehydrogenase in the leaves of S.italica cultivars (SIC-1 and CO-5) differing in salt tolerance

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Each value is mean of three determinations.



et al. (unpublished) in <u>Crotalaria verrucosa</u> and <u>C.juncea</u>. The increase is indicative of increase in catabolic activity.

The activity of GDH increases in young and mature leaves of SIC-1. This indicates an increase in catabolic activity leading to synthesis of ∞ - ketoglutarate, under saline conditions. In CO-5 the activity decreases which is indicative of stimulation of GS - GOGAT pathway in this cultivar.

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