Chapter III

RESULTS AND DISCUSSION

1) Germination Percentage :

The influence of different concentrations of NaCl on the germination of C.juncea and P.aconitifolius seeds is depicted in Table 1, Fig.2,4,5. It is evident that the process of germination is relatively delayed in C.juncea as compared to that in P.aconitifolius. It reaches to its maximum after 96 hours in C.juncea whereas in P.aconitifolius, it is quick and reaching to its maximum within 48 hours. The effect of period on germination under salt influence seems to be insignificant in P.aconitifolius. It is also evident that there is no germination in C.juncea, at higher salt concentrations beyond 150 mM after 24 hours of germination, while in P.aconitifolius germination is recorded in all the concentrations used at the same stage. In C.juncea, however, it was improved during later hours. So far the effect of salt is concerned, all the concentrations of NaCl found to be inhibitory for C.juncea at all the time. The percentage germination is maximum in control and decreases with the increase in salt concentration. The response of P.aconitifolius, however, is different. It can be seen that the germination percentage is improved by lower salt concentrations. It is obivious that in control there is 93% germination while in the media containing from 10 to 80 mM NaCl, it increases to 100% and that within 48 hours. As the concentrations increase from 100 to 300 mM still further,

Table 1 : Effect of NaCl salinity (broad range) on germination* percentage in C.juncea and P.aconitifolius seeds.

NaCl treatment mM	Hours of Germination							
	24	48	72	96	24	48	72	96
	C.juncea				P.aconitifolius			
0 (Con)	92	95	95	95	93	93	93	93
10	56	86	87	87	97	100	100	100
20	50	70	70	90	100	100	100	100
40	40	90	90	90	80	80	90	90
60	40	50	50	60	90	100	100	100
80	30	50	60	60	100	100	100	100
100	24	36	41	52	88	95	95	95
150	0	40	42	50	70	90	90	90
200	0	36	43	43	73	86	90	90
250	0	20	30	40	40	80	90	90
300	0	0	0	20	10	70	80	80

* Average of three experiments.

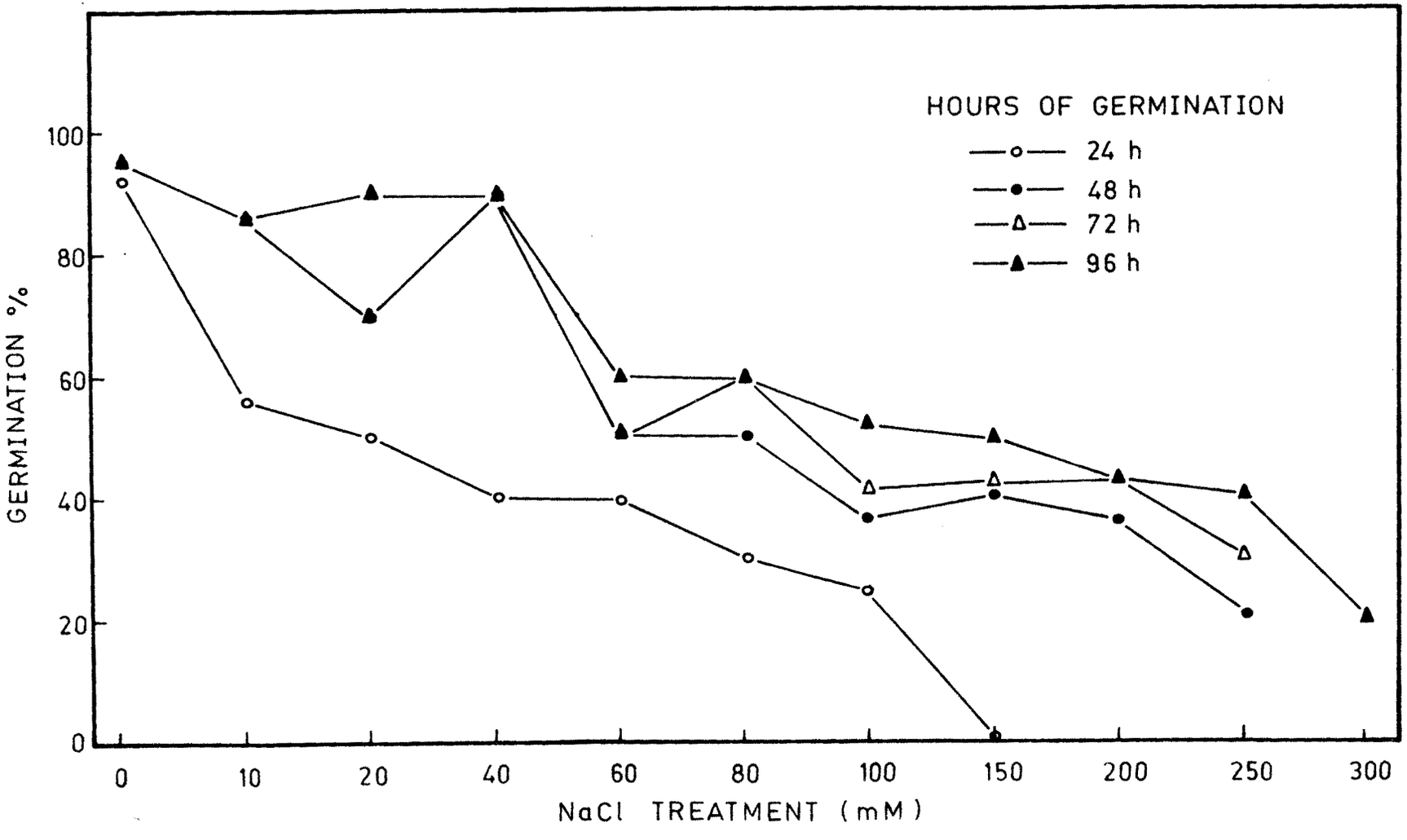

Fig. 4 EFFECT OF NaCl SALINITY (WIDE RANGE) ON GERMINATION PERCENTAGE IN C. JUNCEA SEEDS.

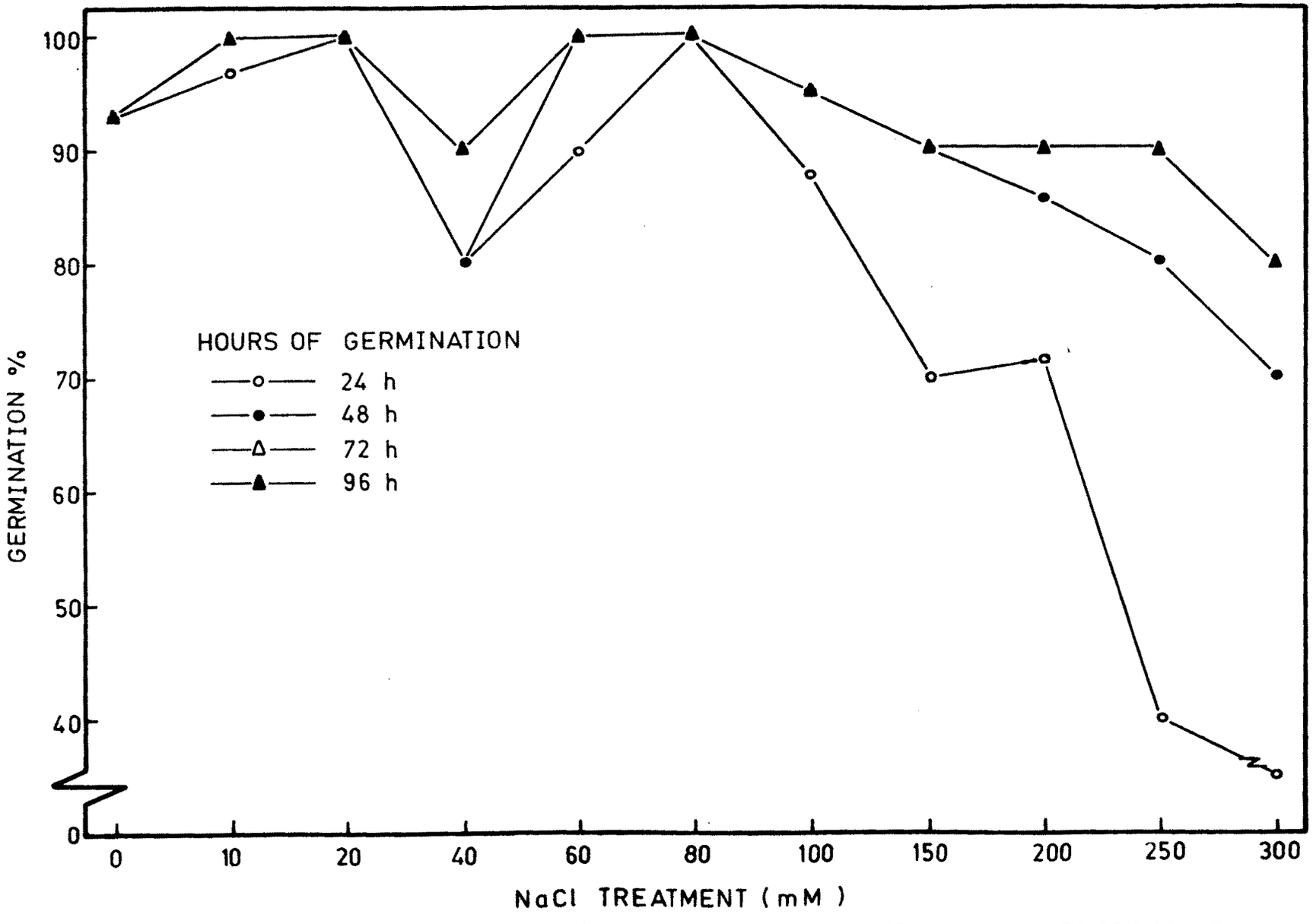

Fig. 5 EFFECT OF NaCl SALINITY (WIDE RANGE) ON GERMINATION PERCENTAGE IN P. ACONITIFOLIUS SEEDS.

germination % falls down from 95 to 70% at 48 hours, with minimum of 70% in 300 mM NaCl. It is interesting to note that the salt concentrations upto 300 mM have not caused much toxic effects on the germination of P.aconitifolius seeds; so far early period of 48 hours of germination is taken into account.

Effect of various concentrations of $CaCl_2$ on germination percentage in C.juncea and P.aconitifolius is depicted in Table 2 and Fig, 3,6,7. It can be seen that when the seeds of both the species were screened for $CaCl_2$ tolerance capacity within wide range of different concentrations of $CaCl_2$, it is observed that in C.juncea there is maximum germination in untreated seeds and it decreases with increasing salt concentration in the medium. However, in Phaseolus, there is maximum germination in 10 mM salt level, even at the early period of 24 h. Germination is improved and increased to the maximum in the concentrations upto 80 mM but rather late. It is also evident that germination percentage is unaffected upto 150 mM $CaCl_2$ concentrations beyond which it is decreased considerably. There is some improvement even in higher concentrations but during the later stages of germination. Therefore, it can be said that between the two species studied, P.aconitifolius has better resistance to $CaCl_2$ also. When the performance of both the species in $CaCl_2$ is compared with that in NaCl (Table 1 and Figs.4,5,6,7), it appears that NaCl is more toxic than $CaCl_2$ during germination.

Table 2 : Effect of $CaCl_2$ salinity (broad range) on germination* percentage in C.juncea and P.aconitifolius seeds.

$CaCl_2$ treatment mM	Hours of Germination							
	24	48	72	96	24	48	72	96
	C.juncea				P.aconitifolius			
0 (Con)	92	93	93	93	98	98	98	98
10	65	82	84	84	100	100	100	100
20	38	60	64	68	92	96	96	96
40	24	52	52	52	96	100	100	100
60	24	48	52	52	92	92	96	96
80	16	45	50	50	92	96	100	100
100	14	45	52	56	89	98	98	98
150	0	28	32	44	72	88	92	96
200	0	16	26	36	32	58	92	82
250	0	2	24	32	4	24	48	56
300	0	2	16	24	0	4	12	14

* Average of three experiments.

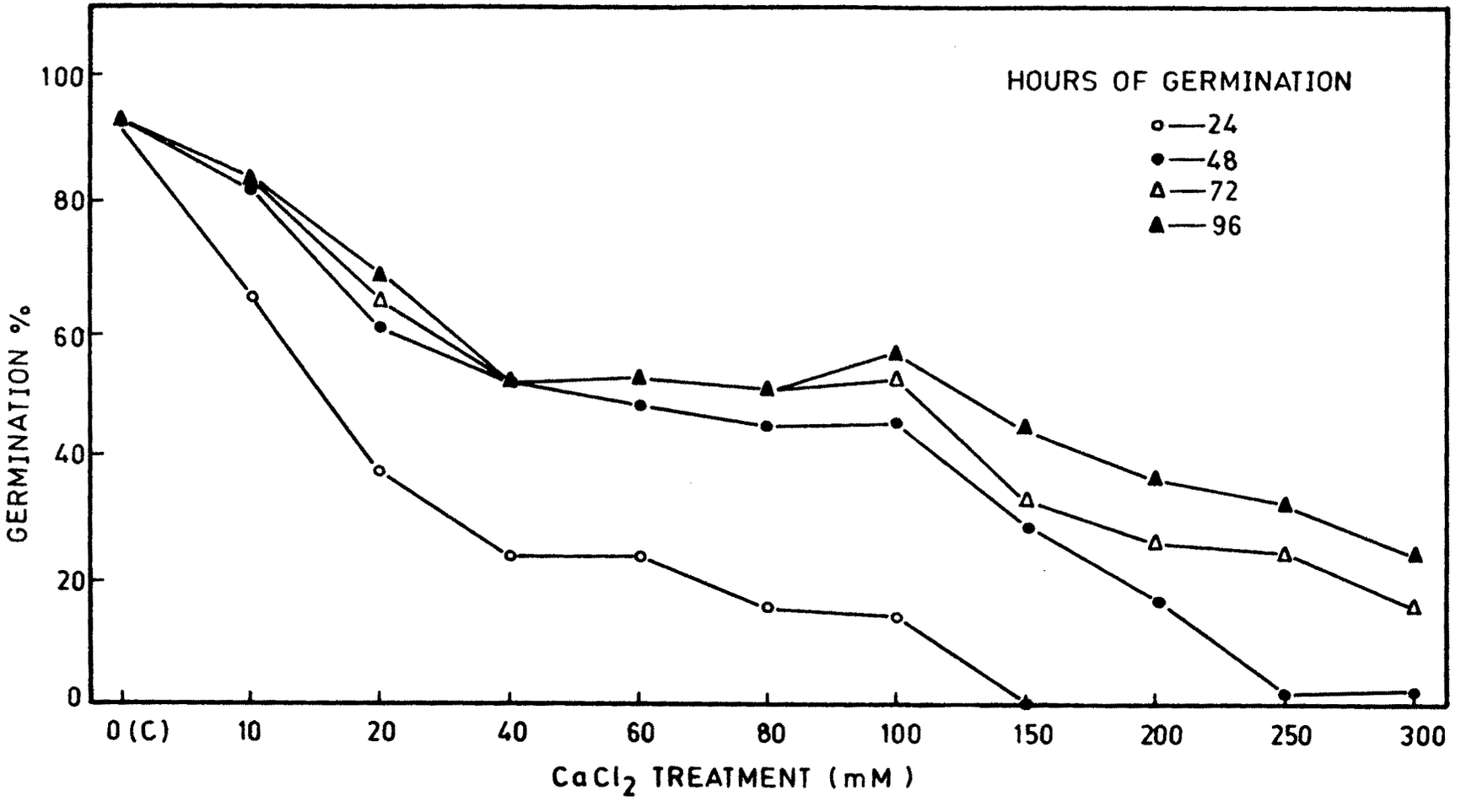

Fig. 6 EFFECT OF $CaCl_2$ SALINITY (WIDE RANGE) ON GERMINATION PERCENTAGE IN C. JUNCEA SEEDS.

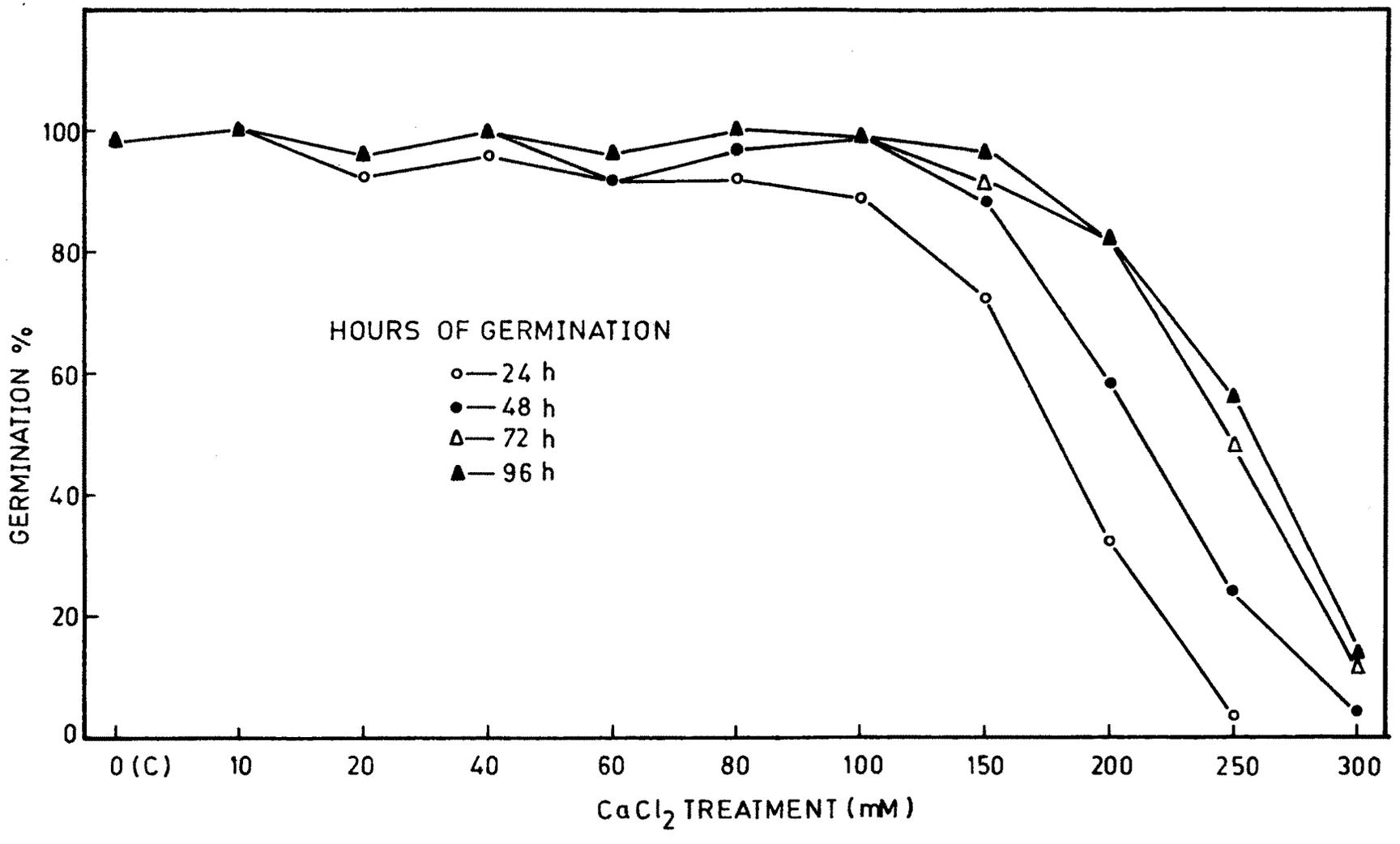

Fig. 7 EFFECT OF $CaCl_2$ SALINITY (WIDE RANGE) ON GERMINATION PERCENTAGE IN P. ACONITIFOLIUS SEEDS.

When both the species were screened within narrow range of concentrations of NaCl (Table 3 and Fig.8), it is observed that germination in C.juncea is inhibited while in P.acnoitifolius, it is stimulated by all the concentrations used. Thus the performance of Phaseolus in NaCl medium is better than that of Crotalaria.

Seeds of both the species when subjected to lower concentrations of $CaCl_2$, germination is inhibited in C.juncea, (Table 4 and Fig.9) during early stages (24 h and 48 h) of germination. But it is improved during the later period and shows maximum germination percentage in 5 and 7.5 mM salt treatments. Higher concentrations, however, are inhibitory. In case of Phaseolus germination is improved appreciably upto 10 mM salt concentration that that in control. Even in the higher concentrations of 50 and 100 mm, it is improved and reaches to its maximum within 48 hours. Since germination is not inhibited even by higher concentrations and as there is no significant delay of germination, P.aconitifolius seems to be salt tolerant particularly to the chloride salts of Na^+ and Ca^{2+}.

It is well established that halophytes are generally more salt-tolerant than glycophytes at the germination stage

Table 3 : Effect of NaCl salinity (narrow range) on germination* percentage in C.juncea and P.aconitifolius seeds.

NaCl treatment mM	Hours of Germination							
	24	48	72	96	24	48	72	96
	C.juncea				P.accnitifolius			
0.0 (Con)	92	95	95	95	93	93	93	93
2.5	77	86	86	86	90	96	96	96
5.0	58	68	83	86	94	100	100	100
7.5	55	85	85	90	95	100	100	100
10	56	86	87	87	97	100	100	100
50	39	55	57	66	97	100	100	100
100	24	36	41	52	88	95	95	95

*Average of three experiments.

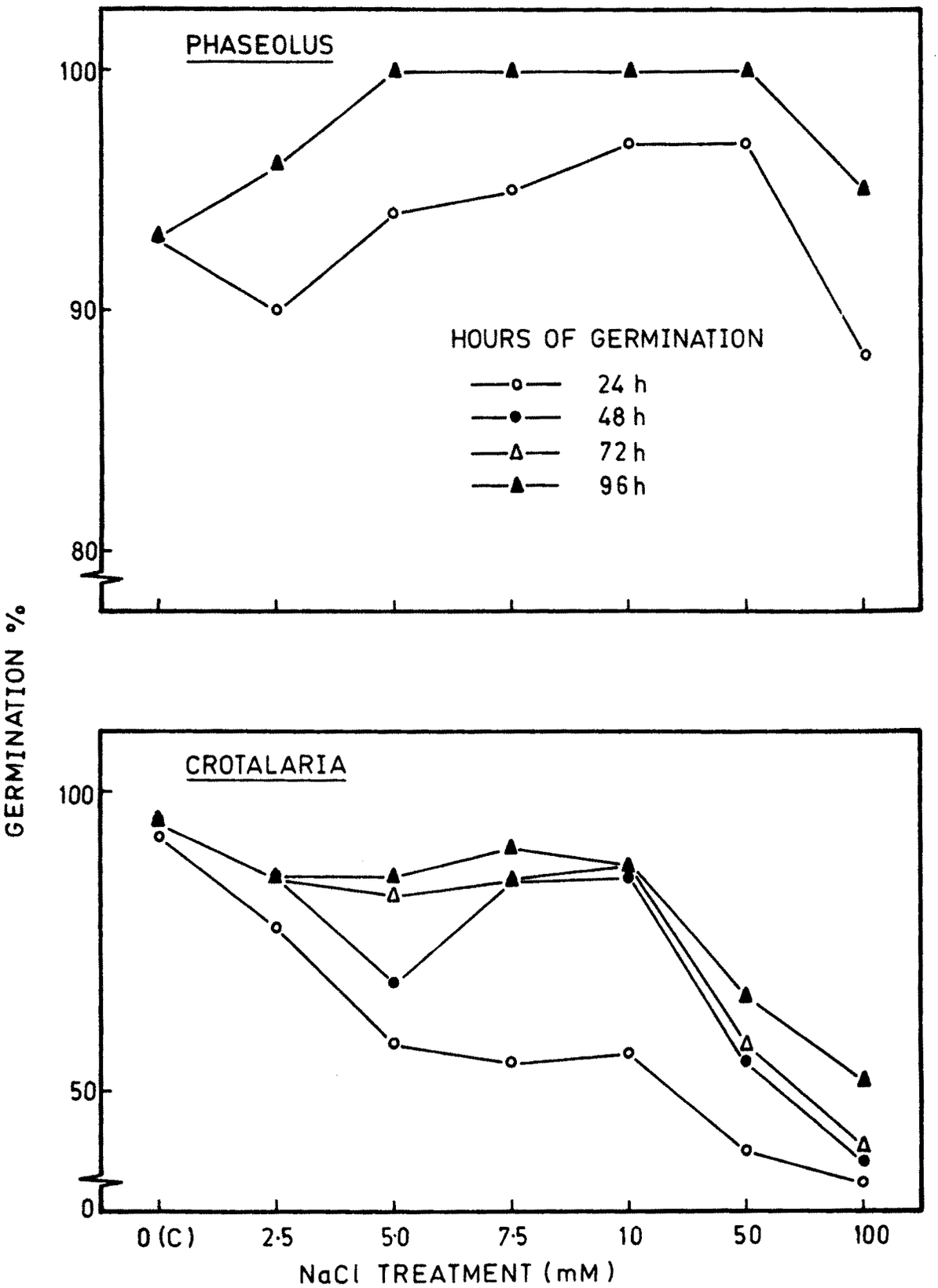

Fig. 8 EFFECT OF NaCl SALINITY (NARROW RANGE) ON GERMINATION PERCENTAGE IN C. JUNCEA AND P. ACONITIFOLIUS SEEDS.

(Ungar, 1978). However, the vascular plants including crop plants so far investigated display both delay and the reduction in the total number of seeds germinated, as the salt stress is increased beyond the optimal level for seed germination. The retarding effects of salt solution on germination of seeds have been shown by many investigators viz. Wahhab, (1961); Bernstein, (1964); Maliwal and Paliwal (1967); Sarin and Narayanan (1968); Macke and Ungar, (1971); Saini (1972); Velankar et al., (1973); Karami, (1974); Sorour et al., (1977); Sheoran, (1980); Sung, (1981); Mahmoud and Hill, (1981); Dubey, (1982); Mukherjee et al., (1982); Joshi and Iyengar, (1982).

Ogasa (1939) found that seed germination of soybean variety KO 561 was inhibited by a 0.2% NaCl solution at 30°C. Ota and Yasue (1957) have demonstrated significant varietal differences in salt tolerance of wheat. Arca and Alvarez (1963) reported that barley is more tolerant than cotton or maize during germination. Abel and Mackenzie (1964) evaluated six soybean cultivars for their reactions to salinized soil during germination and later growth. They found that salinity affected the percentage and rate of emergence. They have suggested that the variations in seed emergence of the varieties at the lower salinity levels may be due to inherited differences. The changes in the relative rates of emergence as salinity increased

Table 4 : Effect of $CaCl_2$ salinity (narrow range) on germination* percentage in C.juncea and P.aconitifolius seeds.

$CaCl_2$ treatment mM	Hours of germination							
	24	48	72	96	24	48	72	96
	C.juncea				P.aconitifolius			
0.0 (Con)	92	93	93	93	98	98	98	98
2.5	88	88	92	92	96	98	98	98
5.0	78	80	100	100	100	100	100	100
7.5	76	88	88	92	100	100	100	100
10	65	82	84	84	100	100	100	100
50	24	66	76	76	96	96	100	100
100	14	45	52	56	89	98	98	98

*Average of three experiments.

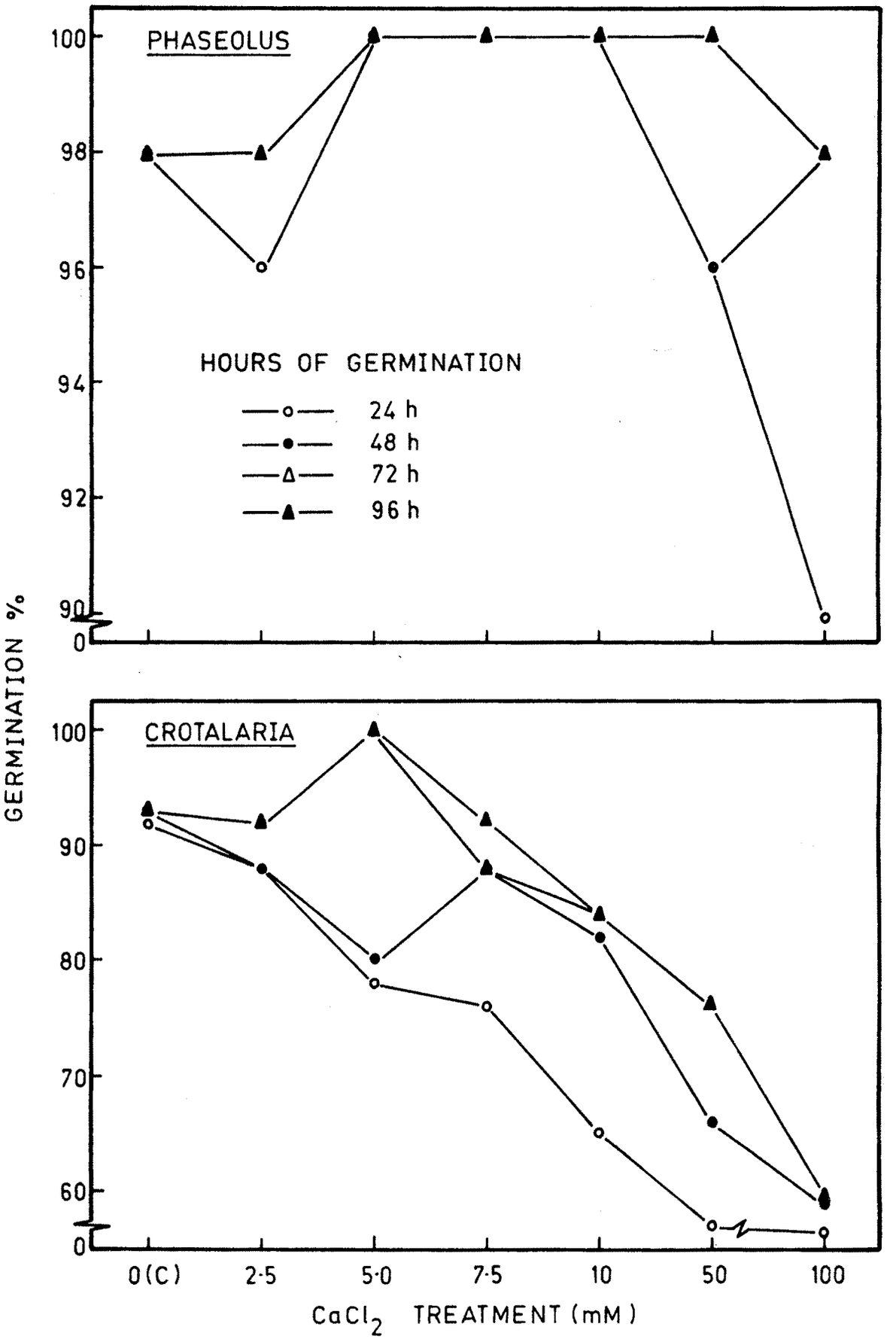

Fig.9 EFFECT OF $CaCl_2$ SALINITY (NARROW RANGE) ON GERMINATION PERCENTAGE IN C. JUNCEA AND P. ACONITIFOLIUS SEEDS.

may be due to differences in the effect of chloride ions on seed germination. Bhumbla and Singh (1965) have observed that the tolerance of crops to salts is not the same at germination and during the later growth. Among the tested crops barley was most tolerant and gram was most sensitive which can be used as an indicator plant to saline and alkali soils.

Lopez (1968) divided plants into following groups based on salinity tolerance at germination (a) slightly resistant plants : Triticum durum, Medicago sativa, Trifolium alexandrium, Solanum lycopersicum, Brassica oleracea and Cinchorium endive, (b) Moderately resistant : Triticum vulgare, Vicia sativa, Lactuca sativa and Beta vulgaris and (c) resistant plants : Hordium sativum. Bhimbla et al., (1966) have observed a difference in germination behaviour in Kharif crops like Sesbania, Cotton, maize and rice and have reported (1968) the satisfactory germination of barley upto an electrical conductivity of 32 m S cm^{-1}. Higher concentrations delayed as well as reduced the germination. Vasudevan and Balasubramanian (1965) found a decrease in germination percentage with increase in osmotic concentration of the solution. Rao et al., (1970) have also made similar observations. They have reported that there was decrease in germination at higher osmotic potential (above 3 atm) in five ragi varieties. Durrant et al., (1974) observed

that low concentrations of NaCl did not affect germination percentage of sugar beet but did affect water uptake and seedling growth. Babu and Kumar (1979) have noticed that P.aureus germinated well under NaCl stress upto 17.63 milli mho/cm with 50% germination followed by Phaseolus mungo and Cajanus cajan. Cicer arietinum was found to be sensitive. Verma (1981) has suggested specific ion effect on early growth in wheat and noticed that Osmotic as well as salt stress decreased tremendously almost all the parameters of growth studied, including germination.

Several workers have shown that individual crop species differ not only with respect to salt tolerance during germination but also exhibit significant varietal difference. Maliwal and Paliwal (1967) have carried out salt tolerance studies at germination stage in six varieties of wheat and barley. They found that germination was delayed and decreased with an increase in salinity and concluded that barley is more tolerant. Paliwal et al., (1970) have shown that varietal differences in salt tolerance exist in case of crops like wheat, paddy and beans at germination stage. Kurian (1969) has studied the effect of sea water on germination and early seedling growth of bajra, sesamum, groundnut, linseed, mustard and safflower. He found that the imbibition of sea water dilutions by various species

and varieties of seeds was inversely proportional to the amount of salt present in the media except for the low concentrations. Treatments with high concentrations of sea water reduced germination of all the crops studied but lower dilutions only delayed it. Habib et al., (1971) have found that in Trigonella foenum-graecum control and 0.02 M concentration of NaCl gave equal germination percentage after 168 hours though there was a delay by 24 hours in the case of 0.02 M concentration. Mohamed and Abdel-Salam (1974) have studied the effect of different salt concentrations on germination and early seedling growth of some vegetable and field crops. They found that germination percentage decreased as salinity level increased. They have also noted that increasing the salinity level also increased the time to maximum germination, decreased plant height and decreased fresh matter yields. Taylor et al., (1975) have studied salt tolerance in 48 cultivars of grain Sorghum and noticed significant difference in cultivars. Recently Kumar and Bharadwaj (1981) have observed a genotypic difference in twenty genotypes of moong (Vigna radiata) in this respect.

Velankar et al., (1973) have reported that salinity reduced the seed germination significantly in wheat and an inverse correlation was noticed between the concentration of

salt and germination percentage. Yadava et al., (1975) have studied salt tolerance of 9 varieties of guar (Cyamopsis tetragonoloba) during seed germination and found that germination percentage decreased with increasing level of salt in the medium. Seed germination of Sesbania acgyptica was inversely related to increasing levels of salinity, alkalinity and water stress (Sinha, 1982). More and Ghonsikar (1982) reported that germination in P.aureus was delayed and decreased with increasing salinity. A sharp decrease in germination was found at 8 mS cm^{-1}. Similar observations have been made by Makhija and Jindal (1983) in papaya seeds.

These studies reveal that salts influence the process of germination to a great extent. Francois et al., (1964) have discussed two main mechanisms. One is the specific salt effect occuring after salts have been entered into the tissue sap and the other is the osmotic effect of the external water potential through which the availability of water is reduced. Hunter and Erickson in as early as 1952 have studied the influence of soil moisture tensions on germination of corn, rice, soyabean and sugarbeets at 25°C. They found that soil moisture tensions less than 12.5, 7.9, 6.6 and 3.5 atm were required to germinate corn, rice, soyabean and sugarbeets respectively. These observations indicate that corn is more

drought resistant than sugarbeet at germination stage. Hadas and Stibbe (1973) predicted that germination rate decreases with decreasing external water potential and for each species there is a critical value of water potential below which germination does not occur. Kaufmann and Ross (1970) observed that the germination in lettuce seeds in soil was totally inhibited when osmotic potential decreases to - 4 bars. Negm and Smith (1978) observed a complete inhibition of lettuce seed germination by polyethyleneglycol (PEG) of osmotic potential -7 bars. In case of Brassica oleraceae and Lepidium sativum, PEG at osmotic potential -12 bars, brought about complete inhibition of germination, (Hegarty and Ross, 1978). Stout et al., (1980) observed a genotypic difference in Sorghum seeds with respect to germination performance under water stress.

According to Paliwal and Gandhi (1968) osmotic pressure predominates the anion effect at higher concentrations. Guerrier (1981) studied the effect of different solutions on germination of Raphanus sativus. He observed that salts have a more toxic effect on germination and suggested that toxic action of the accompanying anion (for organic and mineral salts) or cation (for chloride salts) is greater than the osmotic pressure effect. Singh and Singh (1983) found that cumulative germination percentage and water uptake by germinating

seeds declined progressively in response to decreasing external water potential.

Effect of common salts of sodium and calcium on the germination of different wheat varieties has been studied by Puntamkar et al., (1970) and they have found that relative toxicity of all the salts to germination was in the ascending order of $CaCl_2$, Na_2SO_4, NaCl, $NaHCO_3$ and Na_2CO_3. Sharma et al., (1971) studied the effect of different common salts on germination of P.aureus and concluded that ingeneral germination was the highest in solution containing Na_2SO_4 followed by $CaCl_2$, NaCl, Na_2CO_3 and $NaHCO_3$. Velankar et al., (1973) found reduction in germination percentage with ascending order of 0, 8000, 16000 and 24000 ppm of salt concentrations (NaCl : $CaCl_2$, 1 : 1). $CaCl_2$ was used to prevent theaadverse effect of excessive Na ions. El-Mansy and Bond (1978) have studied the effect of NaCl, Na_2SO_4, $MgCl_2$ and $CaCl_2$ on seed germination of P.antidotale. Germination was found to be decreased with increasing salt concentrations; varying with the salt and specific ions involved. When Cl^- was the anion, Na^+ and Mg^{2+} restricted germination more than did Ca^{2+}. Guerrier (1981) has shown that a NaCl medium is more toxic for germination than that either $CaCl_2$ or KCl. Present results with C.juncea and P.aconitifolius are also confirmatory to the above findings.

Present results show that C.juncea and P.aconitifolius respond differently to various concentrations of NaCl. In P.aconitifolius there is stimulation in germination by some lower concentrations of NaCl. Even there is no delay in the process of germination. Where as, in C.juncea, there is inhibition of germination and also a considerable delay in the process of germination and maximum germination is seen only after 96 hours. However, there is reduction in germination percentage in both the species with increase in salt concentrations.

The results indicate that the effect of NaCl on germination is significant in both the plants tested. The effect of period is insignificant in P.aconitifolius as germination is not delayed. Therefore, it can be suggested that P.aconitifolius has better tolerance capacity than C.juncea and NaCl is more toxic to the seeds tested than $CaCl_2$. Recently Gill and Singh (1985) have studied the effect of salinity on paddy (Oryza sativa L.). Seeds germination and found that tolerant varieties have faster rate of seed germination under stress conditions than the sensitive varieties.

2) Seedling Development :

The effect of different concentrations of NaCl on seedling development after 120 h of germination in C.juncea and P.aconitifolius has been presented in Table 5,6 and Fig.10. It is revealed that in both the species, there is retardation in seedling growth as salinity increases. Further it can be seen that concentrations beyond 80 mM, strongly affect the seedling growth in both the varieties. The higher concentrations beyond 100 mM are found to be remarkably detrimental.

The developmental changes after 120 h of germination in C.juncea and P.aconitifolius, under different concentrations of $CaCl_2$ have been presented in Table 7,8 and Fig.11. The seeds of C.juncea when subjected to different concentrations of $CaCl_2$ ranging from 10 to 300 mM, it is observed that growth of seedlings in 10 mM is more than that of control. However, with the further increase in $CaCl_2$ concentrations, the length of the seedlings decreases. It is clear from the Fig.10,11 that the effect of $CaCl_2$ on seedling development is relatively less inhibitory than NaCl at least upto 60 mM level. The response of P.aconitifolius to various concentrations of $CaCl_2$ (Table 8, Fig.11) reveals that there is slight stimulation in seedling growth at 10 mM salt level and thereafter it is decreased with increasing salt concentrations. The growth of

Table 5 : Effect of NaCl salinity (broad range) on seedling development* in C.juncea (after 120 hours)

NaCl treatment mM	Root length cm	Shoot length cm	Shoot/ Root	Total length cm
0 (Con)	1.83 ±0.52	6.60 ±1.98	3.61 ±0.97	8.43 ±2.32
10	1.11 ±0.55	4.58 ±1.79	4.13 ±1.93	5.69 ±2.07
20	1.50 ±0.68	4.36 ±2.02	2.91 ±1.81	5.86 ±2.38
40	1.78 ±0.73	4.20 ±2.04	2.36 ±0.95	5.98 ±2.64
60	0.94 ±0.58	3.38 ±2.19	3.60 ±3.04	4.33 ±2.60
80	1.13 ±0.45	4.40 ±1.16	3.89 ±2.18	5.53 ±1.01
100	0.53 ±0.3	2.16 ±1.5	4.08 ±2.61	2.69 ±1.70
150	0.24 ±0.11	1.52 ±0.80	6.33 ±2.52	1.76 ±0.90
200	0.24 ±0.05	1.16 ±0.66	4.83 ±1.95	1.40 ±1.62
250	0.20 -	0.50 ±0.2	2.50 ±1.0	0.70 ±0.30
300	0.15	0	0	0.15

*Average of three experiments.

Table 6 : Effect of NaCl salinity (broad range) on seedling development* in P.aconitifolius (after 120 hours)

NaCl treatment mM	Root length cm	Hypocotyl length cm	Epicotyl length cm	Shoot length cm	Shoot / root	Total length cm
0(Con)	3.21 ±0.60	3.72 ±0.44	0.95 ±0.51	4.67 ±0.90	1.45 ±0.32	7.88 ±1.19
10	3.90 ±0.97	3.21 ±0.61	0.67 ±0.25	3.88 ±0.91	0.99 ±0.53	7.78 ±1.47
20	3.05 ±0.47	2.93 ±0.53	0.45 ±0.11	3.38 ±0.72	1.11 ±0.26	6.43 ±0.91
40	2.42 ±0.87	2.38 ±0.76	0.5	2.45 ±0.88	1.01 ±0.38	4.80 ±1.8
60	2.66 ±0.59	1.30 ±0.51	0	1.30 ±0.51	0.49 ±0.22	3.96 ±0.86
80	2.58 ±0.57	1.81 ±0.33	0	1.81 ±0.33	0.70 ±0.31	4.39 ±0.78
100	1.21 ±0.47	0.42 ±0.27	0	0.42 ±0.27	0.35 ±0.38	1.63 ±0.51
150	0.75 ±0.35	0.29 ±0.13	0	0.29 ±0.13	0.39 ±0.16	1.04 ±0.49
200	0.56 ±0.32	0.20 ±0.10	0	0.20 ±0.10	0.36 ±1.65	0.76 ±1.62
250	0.34 ±0.14	0.13 ±0.05	0	0.13 ±0.05	0.38 ±1.00	0.47 ±0.30
300	0.26 ±0.074	0	0	0	0	0.26 ±0.07

*Average of three experiments.

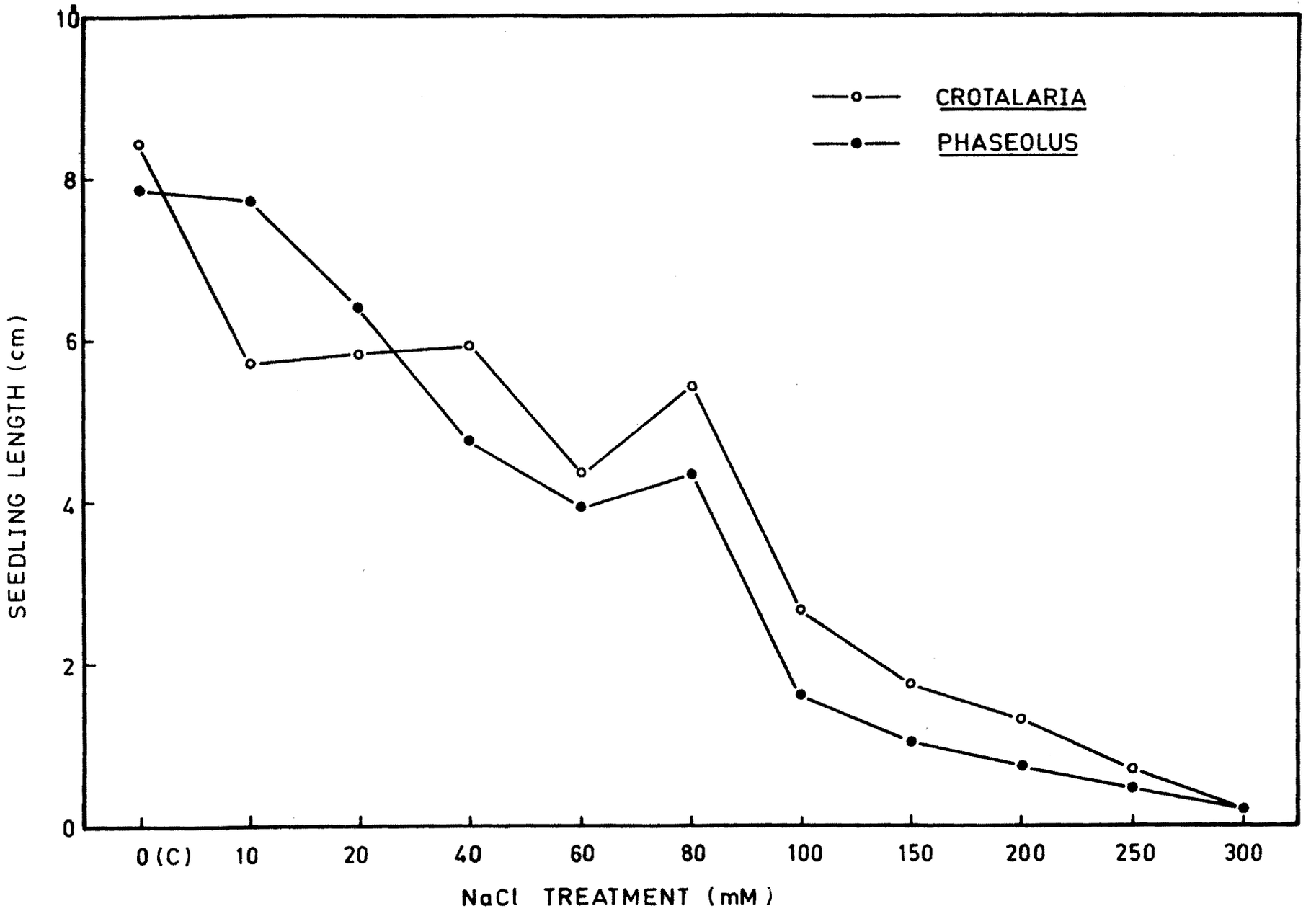

Fig. 10 EFFECT OF NaCl SALINITY (WIDE RANGE) ON TOTAL LENGTH OF C. JUNCEA AND P. ACONITIFOLIUS SEEDLINGS AFTER 120 HOURS OF GERMINATION.

Table 7 : Effect of $CaCl_2$ salinity (broad range) on seedling development* in C.juncea (after 120 hours)

$CaCl_2$ treatment mM	Root length cm	Shoot length cm	Shoot/ Root	Total length cm
0 (Con.)	2.21 ±0.79	9.49 ±2.34	4.29 ±2.03	11.70 ±2.29
10	3.34 ±1.73	11.14 ±2.22	3.34 ±4.18	14.48 ±2.55
20	1.93 ±1.27	7.44 ±3.37	3.85 ±1.79	9.38 ±5.36
40	2.08 ±0.82	8.13 ±2.59	3.91 ±2.53	10.21 ±3.01
60	1.34 ±0.47	4.98 ±3.31	3.72 ±3.07	6.32 ±3.57
80	0.95 ±0.76	3.48 ±0.99	3.66 ±2.49	4.43 ±2.29
100	0.81 ±0.26	2.69 ±1.73	3.32 ±1.89	3.50 ±1.93
150	0.46 ±0.16	1.41 ±0.88	3.07 ±2.67	1.87 ±1.39
200	0.70 ±0.20	0.23 ±0.06	0.33 ±0.08	0.93 ±0.26
250	0.69 ±0.30	0	0	0.69 ±0.30
300	0.43 ±0.16	0	0	0.43 ±0.16

*Average of three experiments.

Table 8 : Effect of $CaCl_2$ salinity (broad range) on seedling development* in P.aconitifolius (after 120 hours).

$CaCl_2$ treatment mM	Root length cm	Hypocotyl length cm	Epicotyl length cm	Shoot length cm	Shoot root	Total length cm
0.0 (Con)	5.04 ±1.49	5.55 ±0.51	3.95 ±1.02	9.50 ±1.14	1.89 ±0.58	14.54 ±2.22
10	6.01 ±1.29	5.80 ±0.93	3.13 ±1.14	8.93 ±1.37	1.49 ±0.55	14.94 ±1.86
20	3.04 ±1.09	5.36 ±0.40	3.78 ±1.36	9.14 ±1.41	3.01 ±1.32	12.18 ±2.38
40	4.17 ±0.95	4.58 ±0.37	3.69 ±1.19	8.27 ±1.25	1.98 ±0.52	12.44 ±1.75
60	2.08 ±0.65	4.56 ±0.77	2.74 ±1.19	7.30 ±1.55	3.51 ±1.25	9.38 ±1.98
80	1.37 ±0.40	3.49 ±0.78	0	3.49 ±0.78	2.55 ±0.97	4.86 ±1.08
100	0.57 ±0.41	0.83 ±0.49	0	0.83 ±0.49	1.46 ±0.79	1.40 ±0.76
150	0.58 ±0.30	0.90 ±0.43	0	0.9 ±0.43	1.55 ±0.93	1.48 ±0.76
200	0.47 ±0.18	0.64 ±0.35	0	0.64 ±0.35	1.36 ±0.37	1.11 ±0.58
250	0.28 ±0.09	0.3	0	0	1.07	0.31 ±0.16
300	0.23 ±0.05	0	0	0	0	0.23 ±0.05

*Average of three experiments.

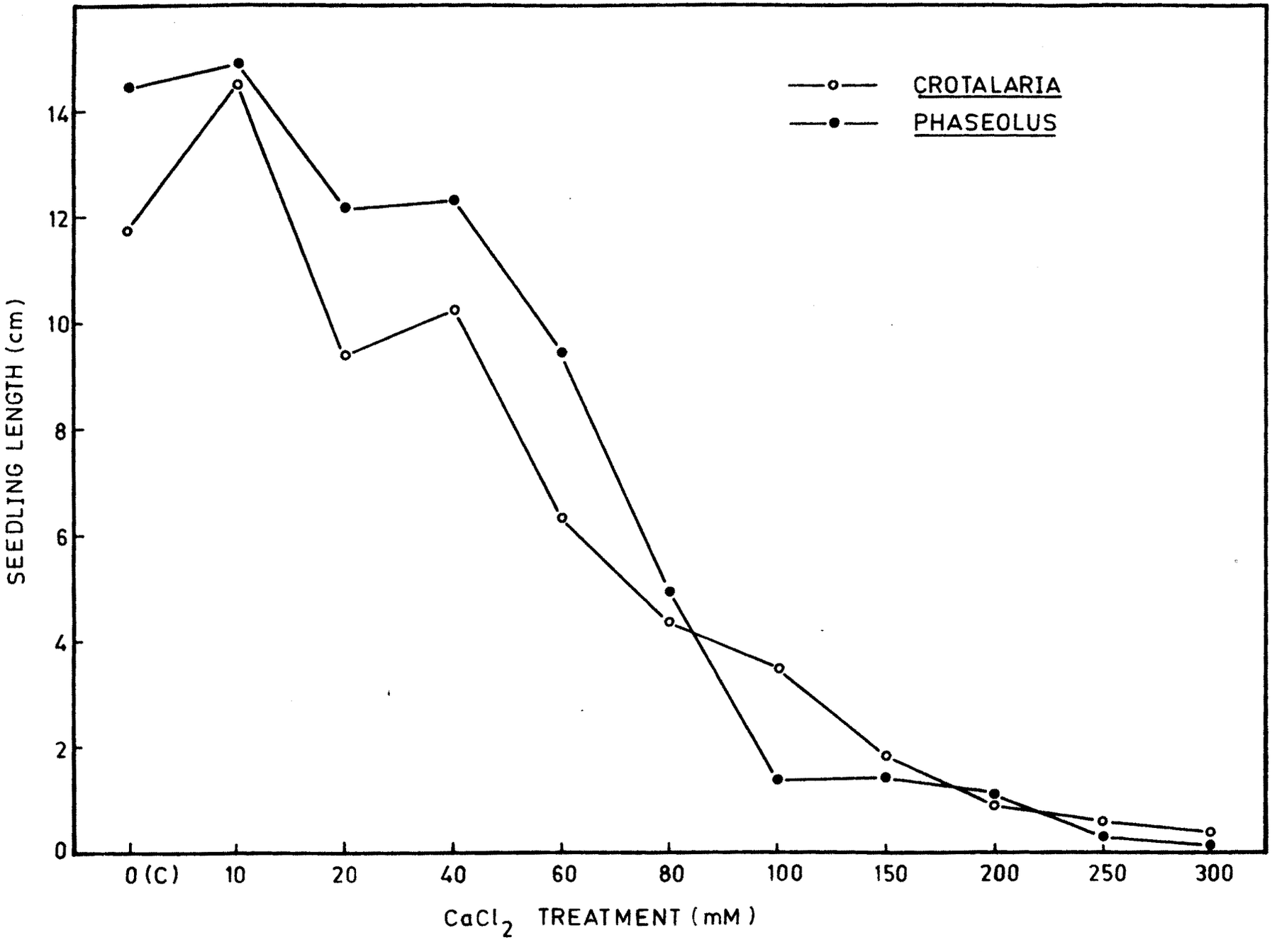

FIG. 11 EFFECT OF $CaCl_2$ SALINITY (WIDE RANGE) ON TOTAL LENGTH OF C. JUNCEA AND P. ACONITIFOLIUS SEEDLINGS AFTER 120 HOURS OF GERMINATION.

seedling beyond 60 mM is hampered drastically. When these results with $CaCl_2$ are compared with those with NaCl, it appears that seedling growth is better in $CaCl_2$ treated seedlings than those grown in NaCl.

When seeds of both legumes were screened within narrow range of NaCl concentrations (Table 9,10 and Fig.12) from 2.5 mM to 10 mM alongwith some higher concentrations, it is found that there is stimulation of the seedling growth in C.juncea only at the lowest salt concentration of 2.5 mM. The further growth pattern shows steady decline with increasing salt concentrations. In P.aconitifolius, however, the length of seedlings, treated with 2.5 to 10.0 mM NaCl is almost equal to that of control. Slight stimulation is observed in some lower concentrations, beyond which there is a rapid fall in the seedling development. From these observations, it appears that C.juncea is more sensitive to salinity than P.aconitifolius.

The seeds of C.juncea treated with narrow range of lower concentrations of $CaCl_2$ (Table 11 and Fig.13) showed that there is stimulation in seedling growth upto 10 mM salt levels and the maximum being that recorded at 2.5 mM. However, higher salt concentrations inhibit the growth. The response of P.aconitifolius to lower concentrations of $CaCl_2$ (Table 12 and Fig.13)

Table 9 : Effect of NaCl salinity (narrow range) on seedling development* in C. juncea (after 120 hours)

NaCl treatment mM	Root length cm	Shoot length cm	Shoot root	Total length cm	Fr.wt. g
0.0 (Con.)	2.06 ±0.93	5.74 ±2.65	2.79 ±1.67	7.81 ±3.24	0.20 ±0.09
2.5	2.26 ±0.68	6.85 ±1.61	3.03 ±1.43	9.11 ±2.11	0.21 ±0.07
5.0	1.57 ±0.74	5.79 ±2.51	3.69 ±1.44	7.35 ±3.68	0.18 ±0.09
7.5	1.30 ±0.74	6.26 ±2.99	4.82 ±2.10	7.58 ±3.85	0.17 ±0.07
10	1.52 ±0.49	5.61 ±2.29	3.69 ±1.50	7.13 ±2.54	0.16 ±0.06
50	1.15 ±0.69	4.46 ±3.01	3.88 ±1.94	5.61 ±3.73	0.13 ±0.09
100	0.43 ±0.22	1.33	3.10	1.76 ±1.25	0.78 ±0.04
200	0.55 ±0.26	1.11 ±0.62	2.02 ±1.74	1.66 ±0.78	0.72 ±0.02

*Average of three experiments.

Table 10 : Effect of NaCl salinity (narrow range) on seedling development* in P.aconitifolius (after 120 hours)

NaCl Treatment mM	Root length cm	Hypocotyl length cm	Epicotyl length cm	Shoot length cm	Shoot/Root	Total length cm	Fr.wt. g
0.0 (Con)	3.63 ±0.59	5.42 ±0.49	1.30 ±0.37	6.72 ±0.65	1.85 ±0.24	10.35 ±1.05	0.114 ±0.05
2.5	3.65 ±1.32	5.36 ±0.065	1.58 ±0.49	6.94 ±0.94	1.90 ±0.49	10.59 ±2.79	0.13 ±0.03
5.0	4.10 ±1.10	4.70 ±0.91	1.52 ±0.43	6.22 ±1.16	1.52 ±0.57	10.32 ±1.74	0.14 ±0.03
7.5	3.59 ±0.73	4.56 ±0.80	1.31 ±0.36	5.87 ±1.12	1.64 ±0.40	9.46 ±1.73	0.12 ±0.1
10	4.18 ±0.97	4.86 ±0.51	1.43 ±0.76	6.29 ±0.85	1.50 ±0.32	10.47 ±1.64	0.13 ±0.02
50	2.61 ±0.51	3.02 ±0.86	0.43 ±0.11	3.45 ±0.98	1.32 ±0.39	6.06 ±1.23	0.11 ±0.01
100	1.43 ±0.57	0.79 ±0.53	0	0.79 ±0.53	0.55 ±0.31	2.22 ±1.01	0.07 ±0.02
200	0.34 ±0.13	0.23 ±0.12	0	0.23 ±0.12	0.68 ±0.20	0.57 ±0.25	0.04 ±0.04

*Average of three experiments.

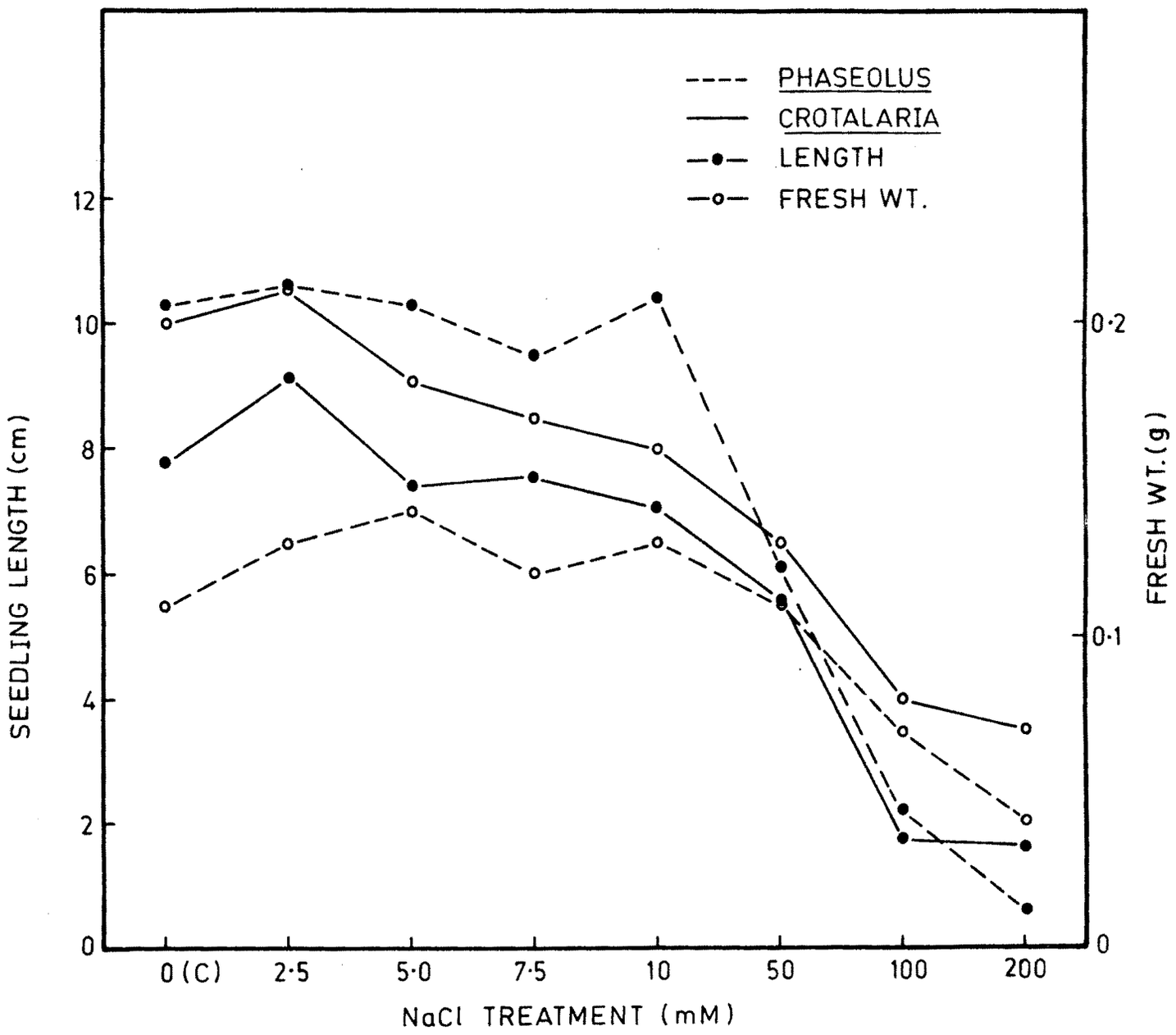

Fig. 12 EFFECT OF NaCl SALINITY (NARROW RANGE) ON TOTAL LENGTH AND FRESH WT OF C. JUNCEA AND P. ACONITIFOLIUS SEEDLINGS AFTER 120 HOURS OF GERMINATION.

Table 11 : Effect of $CaCl_2$ salinity (narrow range) on seedling development* in C.juncea (after 120 hours)

$CaCl_2$ treatment mM	Root length cm	Shoot length cm	Shoot/Root	Total length cm	Fr.wt. g
0.0 (Con.)	2.21 ±0.66	10.26 ±1.52	4.64 ±1.62	12.47 ±1.90	0.37 ±0.06
2.5	3.95 ±1.59	11.80 ±2.07	2.99 ±2.79	15.75 ±3.15	0.36 ±0.06
5.0	3.78 ±1.01	11.72 ±1.73	3.10 ±1.05	15.50 ±2.19	0.35 ±0.05
7.5	4.52 ±1.76	10.99 ±2.08	2.43 ±0.88	15.51 ±3.44	0.36 ±0.08
10	4.64 ±1.12	9.60 ±2.13	2.07 ±2.74	14.24 ±2.74	0.31 ±0.08
50	3.12 ±0.48	7.26 ±2.10	2.33 ±0.65	10.38 ±2.48	0.25 ±0.06
100	1.17 ±0.58	3.37 ±1.67	2.88 ±1.48	4.54 ±2.07	0.17 ±0.03
200	0.46 ±0.13	0.54 ±0.34	1.17 ±0.87	1.00 ±0.47	0.08 ±0.02

*Average of three experiments.

Table 12 : Effect of $CaCl_2$ salinity (narrow range) on seedling development* in P.aconitifolius (after 120 hours)

$CaCl_2$ treatment mM	Root length cm	Hypocotyl length cm	Epicotyl length cm	Shoot length cm	Shoot/Root	Total length cm	Fr.wt. g
0.0 (Con.)	4.33 ±1.01	5.85 ±0.74	2.77 ±0.80	8.62 ±1.15	1.99 ±0.43	12.95 ±1.84	0.20 ±0.02
2.5	6.95 ±1.50	5.57 ±0.65	3.07 ±0.86	8.64 ±1.06	1.24 ±3.99	15.59 ±1.52	0.21 ±0.03
5.0	6.82 ±0.84	5.41 ±0.79	4.32 ±0.98	9.73 ±0.78	1.43 ±0.22	16.55 ±1.09	0.20 ±0.03
7.5	5.92 ±1.01	5.63 ±0.58	3.10 ±0.83	8.73 ±1.16	1.47 ±0.36	14.65 ±1.82	0.18 ±0.02
10	5.13 ±1.32	5.95 ±0.51	3.68 ±1.04	9.63 ±0.84	1.88 ±0.52	14.76 ±1.54	0.18 ±0.01
50	2.78 ±0.90	4.45 ±0.53	1.29 ±0.74	5.74 ±1.14	2.06 ±0.68	8.52 ±1.79	0.13 ±0.02
100	1.30 ±0.66	2.43 ±0.84	0	2.43 ±0.84	1.87 ±0.77	3.73 ±1.34	0.10 ±0.01
200	0.30 ±0.14	0.40 ±0.14	0	0.40 ±0.14	1.33 ±0.18	0.70 ±0.27	0.05 ±0.01

* Average of three experiments.

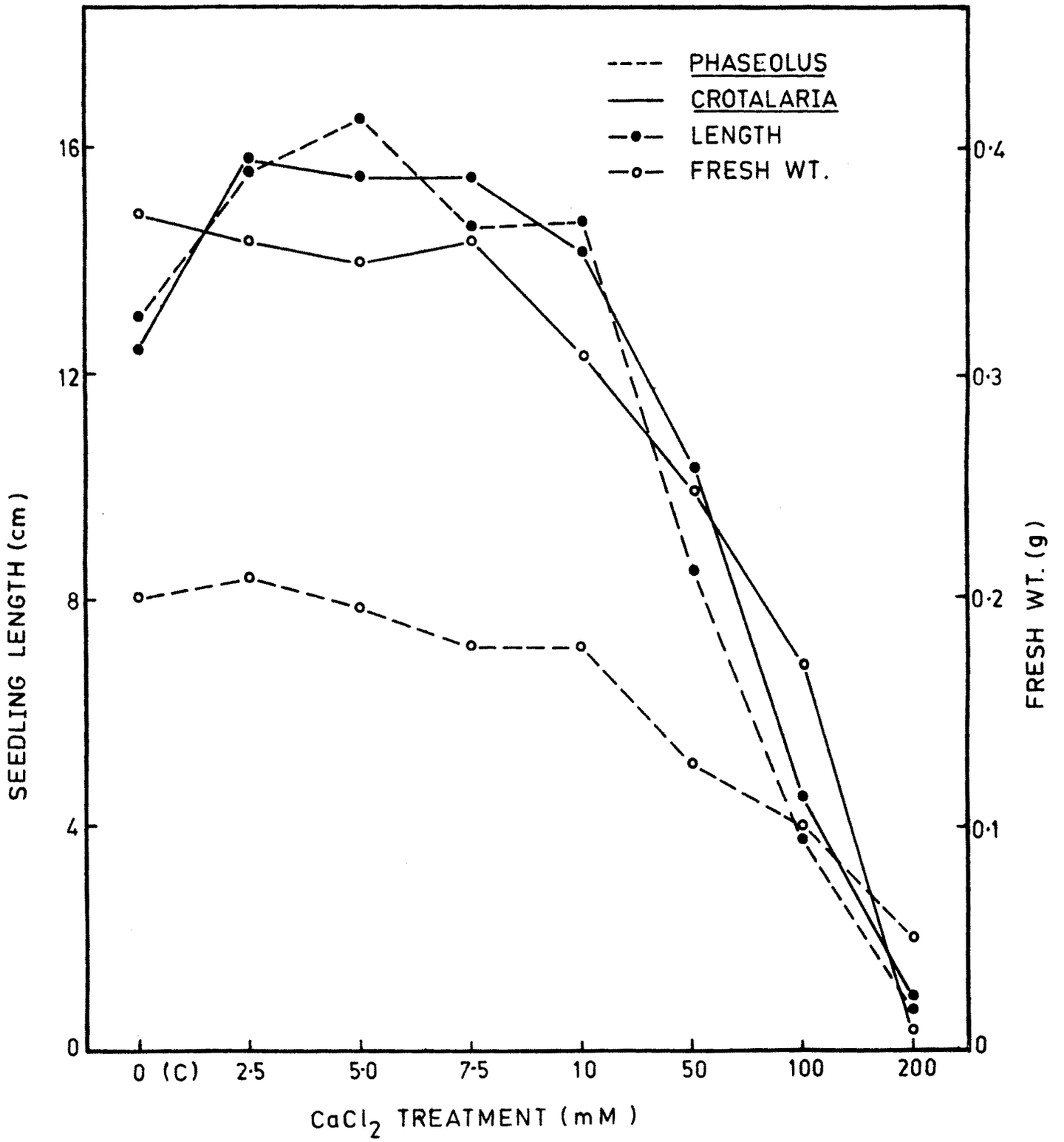

Fig. 13 EFFECT OF $CaCl_2$ SALINITY (NARROW RANGE) TOTAL LENGTH AND FRESH WT OF C. JUNCEA AND P. ACONITIFOLIUS SEEDLINGS AFTER 120 HOURS OF GERMINATION.

indicates that growth of seedlings in 2.5, 5.0, 7.5 and 10 mM salt levels is more than that in control. Maximum growth is shown by seedling treated with 5.0 mM, when the values with $CaCl_2$ are compared with those with NaCl, it appears that, the effect of NaCl on seedling growth is more detrimental than that with $CaCl_2$.

Root and Shoot Growth :

From the Table 5 and Fig.14, it is clear that root and shoot growth in C.juncea is retarded by all the NaCl concentrations used. Similar trend has also been observed in P.aconitifolius (Table 6 and Fig.14) except a slight stimulation in root growth at 10 mM level.

The effect of various concentrations of $CaCl_2$ on seedling development (120 h germination) in both the varieties has been shown in Table 7,8 and Fig.15. It is apparent that in both the plants the root and shoot growth is maximum in 10 mM treated seedlings than that of control. Higher salt concentrations, however, inhibit the growth of seedlings. It can also be seen that beyond 60 mM $CaCl_2$ concentration, root and shoot growth is suppressed.

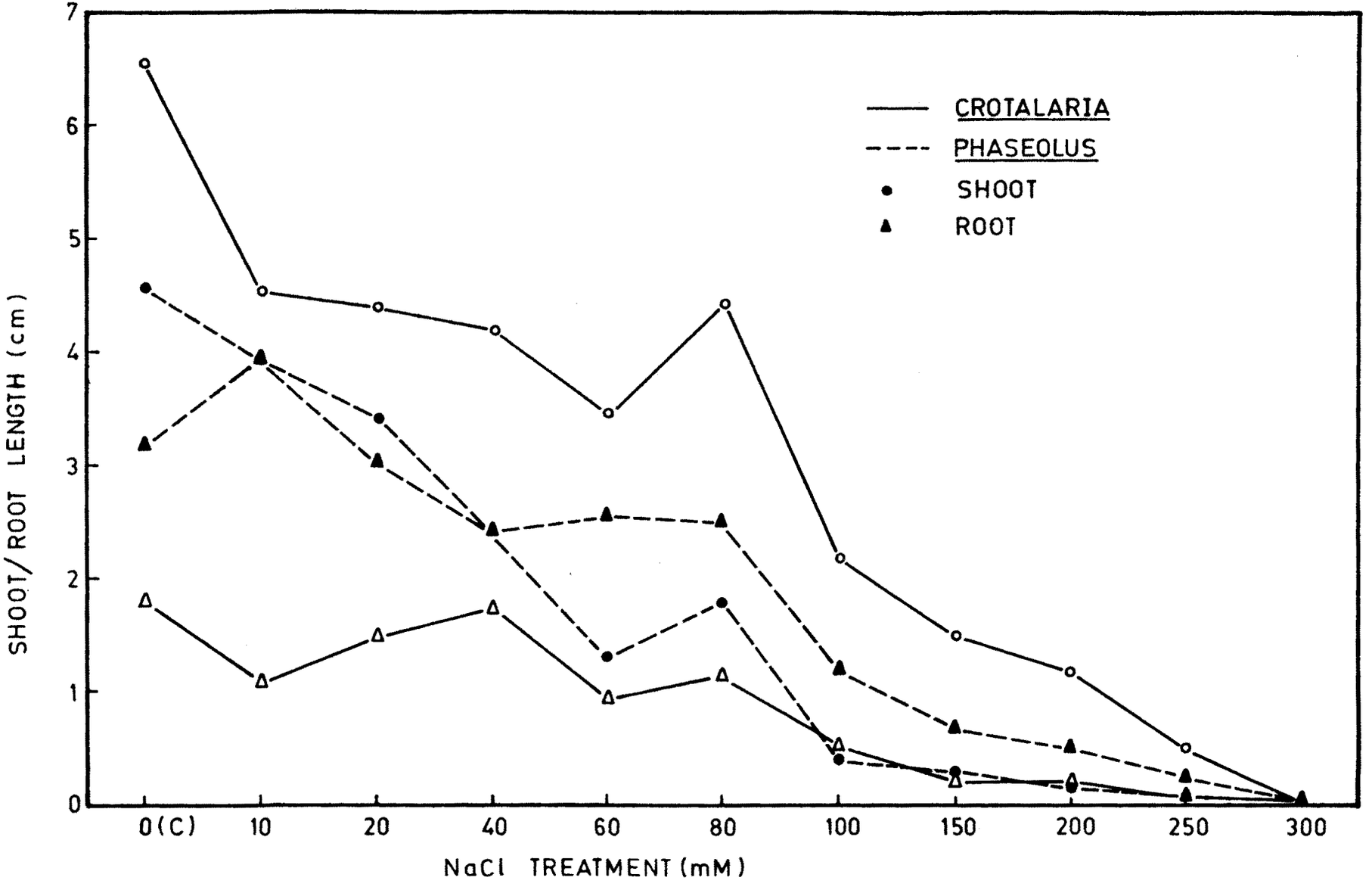

Fig. 14 EFFECT OF NaCl SALINITY (WIDE RANGE) ON SHOOT AND ROOT GROWTH IN C. JUNCEA AND P. ACONITIFOLIUS AFTER 120 HOURS OF GERMINATION.

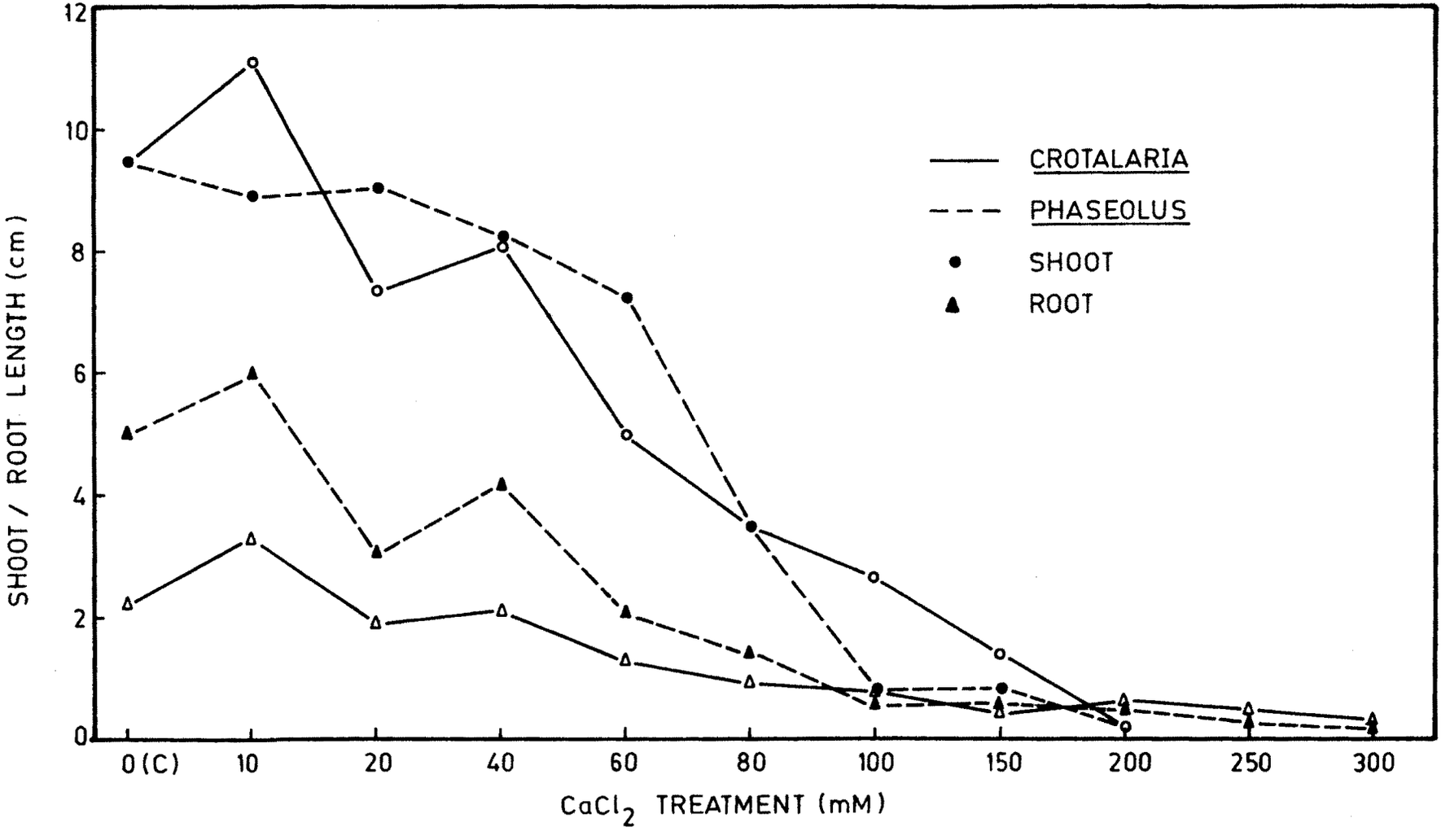

Fig. 15 EFFECT OF $CaCl_2$ SALINITY (WIDE RANGE) ON SHOOT AND ROOT GROWTH IN C. JUNCEA AND P. ACONITIFOLIUS SEEDLINGS AFTER 120 HOURS OF GERMIATION.

When screened within narrow range of NaCl concentrations (Table 9, 10 and Fig.16,17), it can be seen that the root growth in C.juncea is slightly stimulated at 2.5 mM while in P.aconitifolius it is stimulated roughly upto 10 mM NaCl level. In both species root growth goes on decreasing with increaseiin salt concentrations in the medium. The shoot growth at the lowest salinity level is maximum in both the species. However, it is more in C.juncea treated with 5.0 and 7.5 mM concentrations inhibit the shoot growth.

In lower range of $CaCl_2$ concentrations are found to be stimulatory for the root and shoot growth. Maximum root length in Crotalaria has been recorded (Table 11,12 and Fig.18,19) for 10 mM treated seedlings while shoot length is maximum in 2.5 mM treated seedlings. From these observations it appears that C.juncea shows better response but only with the lower concentrations of $CaCl_2$. In P.aconitifolius, on the other hand the root length recorded is higher than that in control in the seedlings treated with salt concentrations upto 100 mM. Beyond this level, however, it decreases with increase in salt concentration in the medium. The shoot length is also better with slight stimulation at the lower treatments upto 10 mM and thereafter it is decreased.

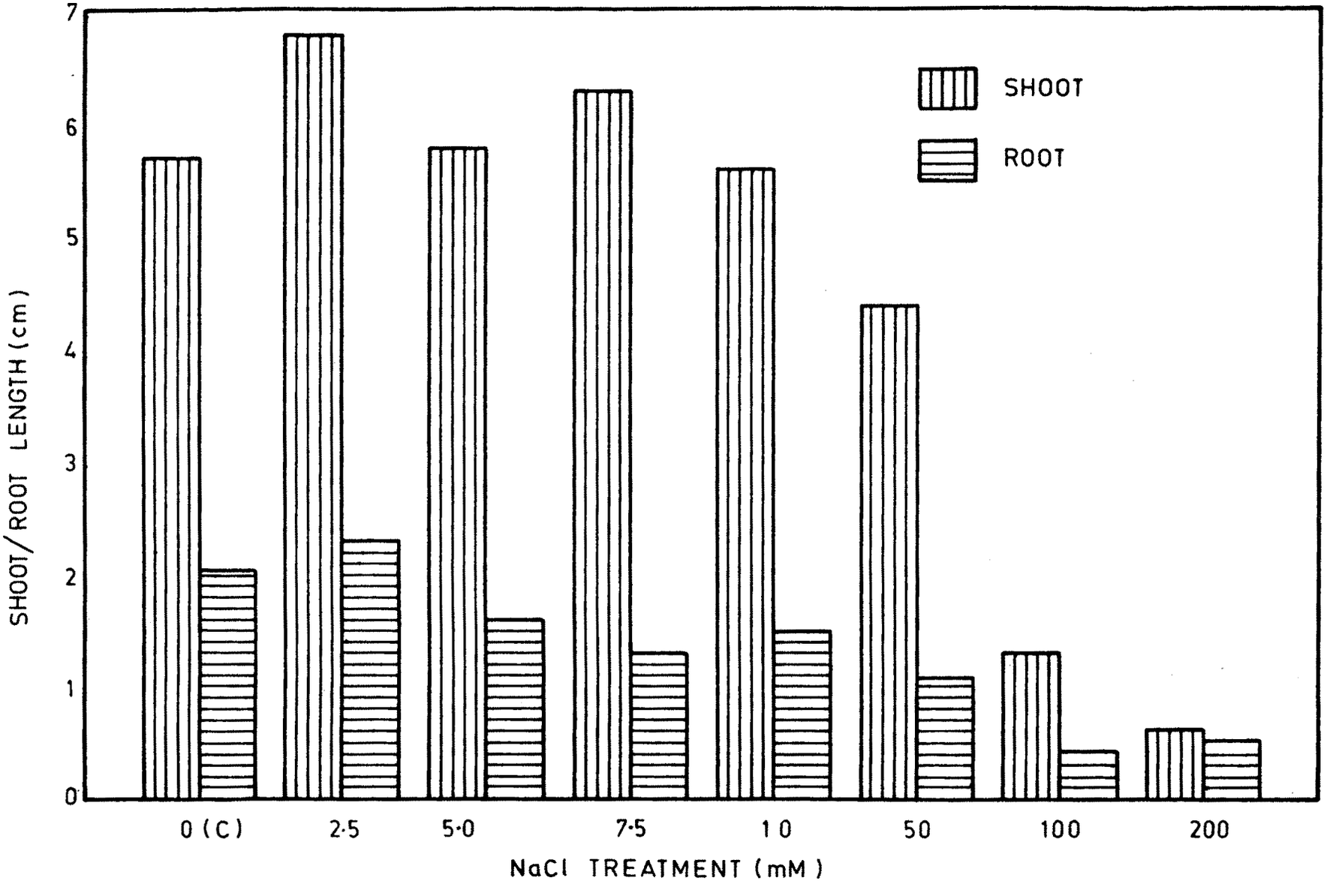

Fig. 16 EFFECT OF NaCl SALINITY (NARROW RANGE) ON SHOOT AND ROOT GROWTH IN C.JUNCEA AFTER 120 HOURS OF GERMINATION.

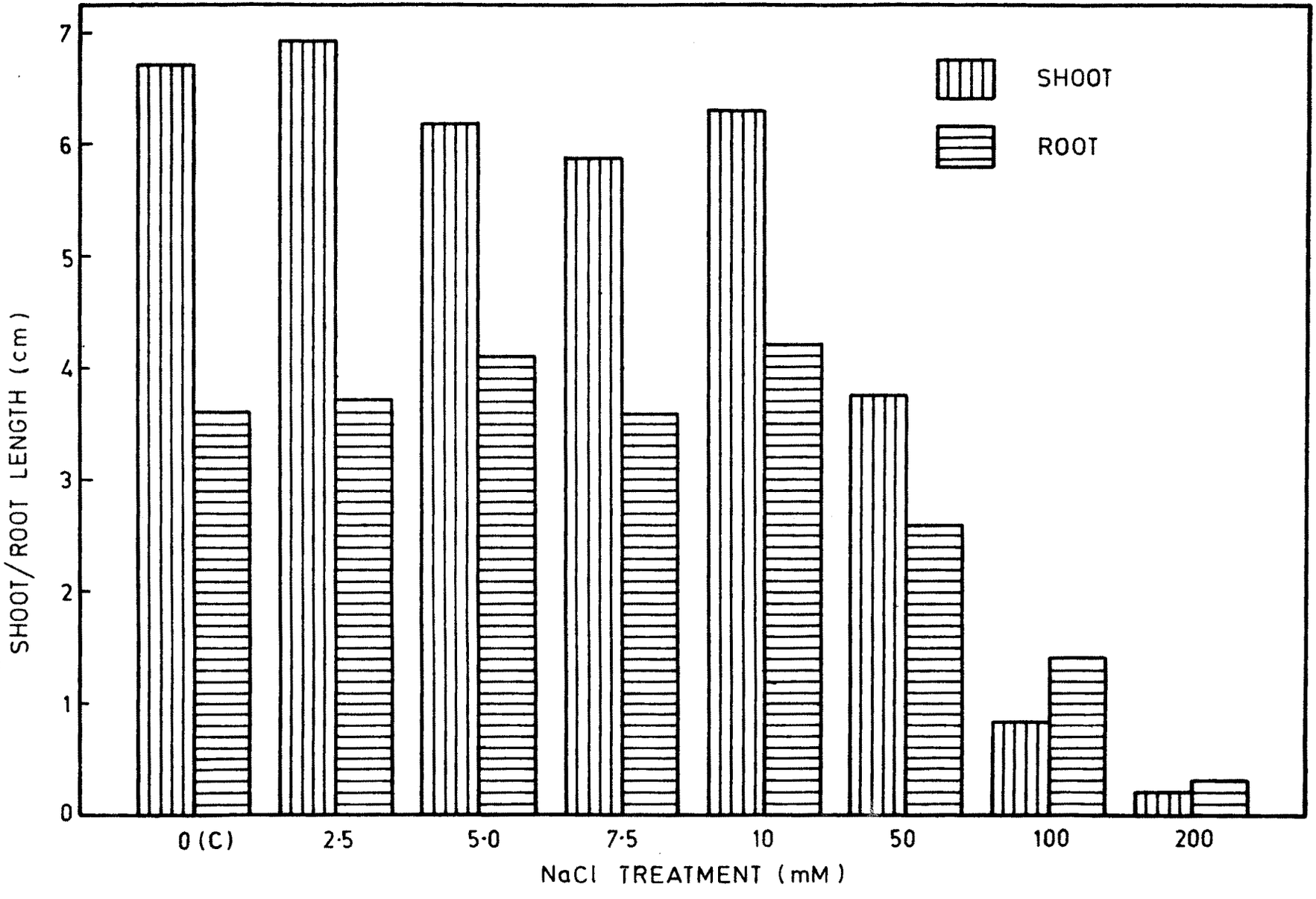

Fig. 17 EFFECT OF NaCl SALINITY (NARROW RANGE) ON SHOOT AND ROOT GROWTH IN P. ACONITIFOLIUS SEEDLINGS AFTER 120 HOURS OF GERMINATION.

Shoot/Root Ratio :

The values (Table 5) of shoot/root ratio in C.juncea are more than unity in the seedlings grown in non-saline as well as saline media, except the highest salinity level of 300 mM, where the shoot growth is totally suppressed. These values which are more than unity indicate more shoot growth. However, values when compared with those of control it is evident that in lower salt concentrations from 20 to 60 mM, the shoot/root ratio is less than that in control except that in 10 mM while in higher concentrations the values are more than those in control indicating that the lower salt concentrations stimulate root growth whereas, the higher salt concentrations inhibit it. In P.aconitifolius (Table 6), shoot/root ratio is roughly equal to the unity indicating the balanced growth of both the root and the shoot. However, in higher salt concentrations from 60 mM onwards, the values are less than unity. This indicates that higher salt concentrations inhibit shoot growth. It is also very clear that shoot/root ratio in C.juncea is higher than that in P.aconitifolius. This suggests that in C.juncea shoot growth is predominant.

The values of shoot/root ratio of C.juncea seedlings grown in different concentrations of $CaCl_2$ (Table 7) are more than unity in control as well as in the seedlings treated with

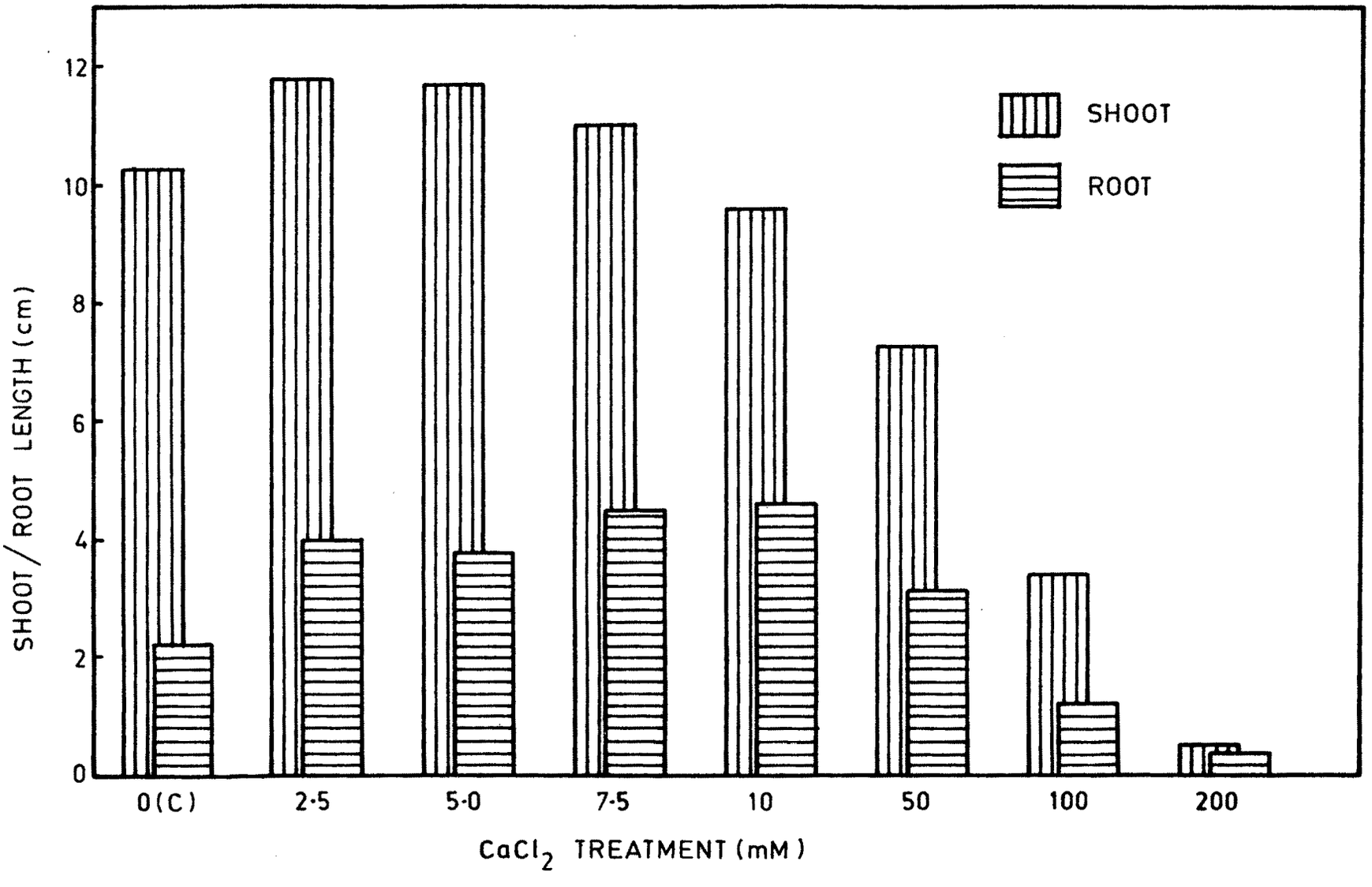

Fig. 18 EFFECT OF $CaCl_2$ SALINITY (NARROW RANGE) ON SHOOT AND ROOT GROWTH IN C. JUNCEA SEEDLINGS AFTER 120 HOURS OF GERMINATION.

salt concentrations from 10 to 200 mM, indicating appreciable growth of shoot than that of root. The response of P.aconitifolius, (Table 8) to different concentrations of $CaCl_2$ also shows more or less the same trend.

The seeds of C.juncea and P.aconitifolius when treated with narrow range of lower concentrations of NaCl (Table 9,10) the values of shoot/root ratio are more than unity both in control as well as in all NaCl treated seedlings. This indicates that there is an appreciable growth of shoot under saline conditions. But root growth is markedly affected. In P.aconitifolius, shoot growth is more than that of root except in 100 and 200 mM salt levels. The high value of shoot/root ratio at 2.5 mM salt level indicates slight retardation of root growth. In general, increasing salt concentrations appear to be inhibitory to the shoot growth.

The values of shoot/root ratio of C.juncea seedlings grown in different concentrations of $CaCl_2$ (Table 11) are more than unity in control as well as in seedlings treated with salt concentrations from 10 to 200 mM indicating appreciable growth of shoot than that of root. The response of P.aconitifolius to different concentrations of $CaCl_2$ (Table 12) also shows the same trend.

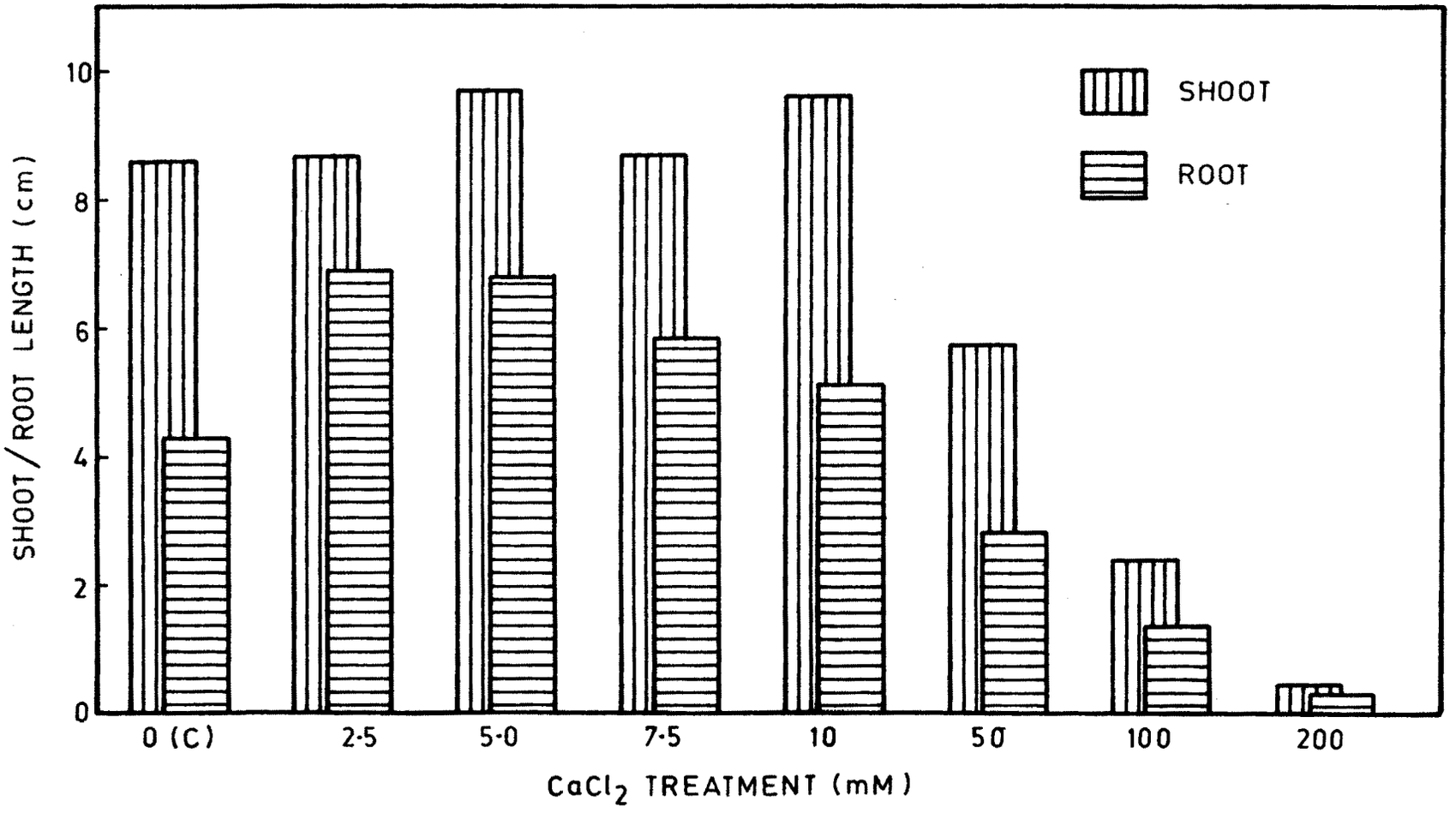

Fig. 19 EFFECT OF $CaCl_2$ (NARROW RANGE) ON SHOOT AND ROOT GROWTH IN P. ACONITIFOLIUS SEEDLINGS AFTER 120 HOURS OF GERMINATION.

Epicotyl Development :

Effect of various concentrations of NaCl (Table 5,6) and $CaCl_2$ (Table 7,8) on epicotyl development in two varieties after 120 h growth showed that in C.juncea neither control nor salt treated seedlings showed the development of epicotyl and unfolding of leaves. However, P.aconitifolius seedlings showed development of epicotyl and unfolding of leaves in control as well as in the seedlings treated with salt concentrations upto 40 mM. However, growth of epicotyl was affected by salt treatment. Total suppression of epicotyl growth in C.juncea is observed. Epicotyl development is seen only in P.aconitifolius in control as well as in $CaCl_2$ treated seedlings upto 10 mM salt level beyond which there is inhibition.

The effect of lower concentrations of NaCl and $CaCl_2$ on epicotyl development in the legumes indicates (Table 9,10,11,12) that there is no epicotyl development in crotalaria after 120 h growth even in the seedlings grown under non-saline conditions. P.aconitifolius, however, showed better epicotyl development upto 50 mM concentration of both the salts. Higher concentrations namely 100 and 200 mM of both salts are inhibitory to the development of epicotyl.

The high values of epicotyl in $CaCl_2$ treated seedlings as compared to those of the seedlings treated with NaCl, indicate that $CaCl_2$ is less toxic than NaCl. Among the two species tested P.aconitifolius seedlings appear to be establishing themselve) much earlier than the seedlings of C.juncea even under the same lower saline regimes.

Lall and Sakhare (1970) have found that 0.5 atm NaCl was beneficial for the seedling growth in Sorghum and also reported a linear reduction in seedling growth as the salinity level increased. Durrant et al., (1974) have studied the effect of NaCl salinity on germination and seedling growth of sugar beet. They found that the radicle length decreased almost linearly when NaCl concentration in the medium was increased from 0 to 0.2 M. Mohamad and Abdel-Salam (1974) reported that the increasing salinity decreased plant height and fresh ma tter yields. Robinson (1974) has also reported that the seedlings of P.aureus become stunted when grown in presence of NaCl.

According to Bernstein (1974) both osmotic and specific ion effects are responsible for growth retardation. The inhibition of seedling growth by chloride salinity has been reported by several workers (Paliwal and Maliwal, 1973;

Panigrahi et al., 1978; Fattah and Asghani Bano, 1980; Gujarathi et al., 1981; Vora and Gopalkrishnan, 1981; Kale & Gupta, 1982; Sing and Kale, 1985). Sheoran and Garg (1978) have studied the effect of chloride and sulphate salinity on germination and early seedling growth of P.aureus. They have reported that the germination, early seedling growth and length of radicle and hypocotyl decreased due to the effect of all the salts tested. Harradine (1982) has seen reduction in germination and growth of Pennisetum macrourum with increasing NaCl concentration in the nutrient culture. Inhibitory effect of sodium chloride on soybean root elongation and O_2 uptake has been studied by Bejaoul (1980) and reported that the salinity effect was significant only if the NaCl concentration was higher than 50 mM. Sung (1981) reported that NaCl treatments significantly inhibited the α- amylase and respiratory activity in germinated seeds and resulted in the reduction of plumule and radicle growth. Significant differences among the cultivars and radicle elongation were observed with increasing NaCl stress intensity. Sharma (1983) observed adverse effect of excess accumulation of Na and Cl in the seedlings. Sheoran and Garg (1983) have reported that the reduction in growth under chloride salinity seems to be due to toxic ion accumulation.

On the contrary, Bhardwaj (1965) and Lall and Sakhare (1970) have observed stimulation of seedling growth in pea and Sorghum seedlings respectively in lower regimes of NaCl salinity. Hassan-Porath et al., (1972) have observed an increase in growth rate of shoots of pea seedlings on exposure to -3 to -4 atm of NaCl and a slight increase in growth of roots exposed to -3 atm of salt. Acharya & Sahu (1974) have studied the salt tolerance of P.aureus variety vaishakhi. They have reported that the hypocotyl length increased from 9.6 to 11.2 cm and that of epicotyl from 7.8 to 10.7 cm with increasing salinity from nil to 4 m S cm^{-1} and then sharply declined after 72 hours of growth with further increase in salinity. Such enhancement of seedling growth in moong at lower doses of salt was evident in the experiments by Kumar and Bhardwaj (1981). Makhija and Jindal (1983) have studied salt tolerance limits in papaya seeds during germination. They have observed that increasing the salt concentration reduced and delayed seed germination and seedling growth . The seedlings become slender and stunted and could not survive beyond 4 m $S \cdot cm^{-1}$ Ece. Larik and Hafiz (1983) have observed the maximum detrimental effect of NaCl concentration of 1.5% on wheat germination and seedling growth. Salt tolerance limit varied with the cultivar.

High salt concentrations in the saline medium create high osmotic pressure reducing the uptake of water. The inhibition of germination by salts may be due to prevention of water uptake. Harris (1915) showed that the relative toxicity of soluble salts at germination is in the following descending order NaCl $CaCl_2$ > KCl > $MgCl_2$ > KNO_3 > $Mg(NO_3)$ > Na_2SO_4. However, such an order is not universal because every plant species responds differently of different salts. Strogonov, (1964) and Yadav et al., (1975) observed that NaCl was more toxic than $CaCl_2$ with respect to germination of nine cultivars of guar (Cyamopsis tetragonoloba). Similar observations were also made for cotton and Medicago species by Eweida et al.,(1978)and Rizk et al.,(1978) respectively. Guerrier (1981) extensively studied the influence of various salinity levels on germination of Raphanus sativus. He reported that among the three chlorides, the toxicity decreased in the order of NaCl > $CaCl_2$ > KCl. It was evident in the experiment by Everitt (1983) that NaCl and $MgCl_2$ were highly inhibitory for the germination of seeds of a grass Sporobolus while $CaCl_2$ was the most suppressive salt for seed germination in case of Leptochloa dubia. It is evident therefore that species differ with respect to their tolerance to specific ions.

Fresh Weight :

The fresh weight of 120 h old seedlings grown under different concentrations of NaCl are depicted in Table 9,10 and Fig.12. It can be seen that the seedlings of C.juncea showed slight gain in weight at 2.5 mM salinity level as compared to that in control and thereafter showed decline in weight with increasing concentrations of NaCl. The seedlings at higher concentrations of 100 to 200 mM salt showed significant reduction in fresh weight. However, in P.aconitifolius, there is stimulation in the fresh matter production at all the concentrations of salt upto 10 mM. Maximum fresh weight is recorded in 0.5 mM treated seedlihgs. At higher NaCl concentrations, namely 100 and 200 mM, however, there is reduction in fresh weight of the seedlings.

Effect of different concentrations of $CaCl_2$ on fresh weight of C.juncea and P.aconitifolius seedlings after 120 h of growth has been presented in Table 11,12 and Fig.13. It is evident that in C.juncea, fresh weight of the seedlings treated with 2.5, 5.0 and 7.5 mM $CaCl_2$ is nearly equal to that in control and thereafter declines with increasing salt concentration in the medium. However, in P.aconitifolius fresh weight of seedlings is more in 2.5 mM salt level than that in control and then reduces with further increase in

salt concentration. These observations indicate that lower levels of $CaCl_2$ stimulate the biomass production while the higher salt concentrations retard the same.

Effect of salt concentration on fresh weight of young seedlings of Lactuca sativa, indicated that increasing salinity levels from 0 to 0.12 M NaCl reduced the fresh weight, (Odegbaro and Smith, 1969). Taylor et al., (1975) took the fresh weight as a measure of growth and found that the average fresh weight of Sorghum seedlings is greatly reduced due to reduction in water uptake. Varma and Poonia (1979) studied germination and early seedling growth of pearl millet under saline conditions and found that the root and shoot growth and their biomass decreased with increasing salinity, except at the low levels. Screening tests for salt tolerance in 7 days old lettuce (Lactuca sativa) seedlings, showed that fresh weight declined as salinity increased, (Shannon et al., 1983). They have also observed that there was an additional 4.5% decrease in biomass for each $ds \cdot m^{-1}$ (ds : decisiemens) increase above 4.6 $ds \cdot m^{-1}$ in 30 and 60 day old seedlings.

Present observations in C. juncea and P. aconitifolius show more or less the same trend. There is slight increase in seedling biomass at the lower salt regimes and a linear decline with increasing concentrations of salt in the medium.

From the foregoing discussion it is certain that the presence of salts beyond the critical level is toxic to the germinating seeds. Salt tolerance limit varies with the species and type of salinity. Present results with C.juncea and P.aconitifolius indicate that there is slight increase in biomass of seedlings treated with lower concentrations and a linear decline with increasing concentrations of salt in the medium. Both species differ considerably in their tolerance limits. Among the two P.aconitifolius, appears to be more salt resistant than C.juncea and NaCl seems to be more harmful.

3) Moisture and Dry Matter Contents :

A) Moisture content :

The metabolic reactions taking place during germination ultimately determine the capacity to germinate. Under stress conditions, the Na and Cl ions absorbed and accumulated will largely influence enzymatic reactions involved in germination. They will have a profound effect on utilization of food reserve and other metabolic sequences. In order to understand these aspects well, the seedlings of both varieties after 120 hours of germination in different salinity levels were analysed for moisture percentage, dry matter contents and organic constituents.

Table 13 : Effect of NaCl salinity on moisture percentage and dry matter content in C.juncea and P.aconitifolius seedlings after 120 hours of germination.

NaCl treatment mM	Moisture %	Dry matter %	Moisture %	Dry matter %
	C.juncea		P.aconitifolius	
0.0 (Con.)	93.87	6.13	91.99	8.01
2.5	93.50	6.50	92.27	7.73
5.00	92.94	7.26	91.96	8.04
10	91.91	7.09	91.43	8.57
50	91.03	9.97	88.91	11.09
100	85.86	14.14	84.17	15.83
Dry seed	8.20	91.80	9.60	90.40

(Average of three experiments)

The effect of different concentrations of NaCl on moisture percentage and dry matter content in the seedlings of C.juncea and P.aconitifolius after 120 hours of germination is shown in Table 13 and Fig.20. It is evident that the moisture level of dry seed of C.juncea is 8.2%. It increase tremendously when germination commences. The moisture percentage in control seedlings grown under non-saline conditions is maximum and declines thereafter with increasing salt concentrations. The seedlings of P.aconitifolius however, differed a little in response to salinity to C.juncea and showed no remarkable effect of lower concentrations of NaCl, but showed the same trend of reduction in moisture content with higher salinity levels. It may be recalled that the germination percentage in P.aconitifolius falls down (to 40% and 10%) in higher salt concentrations (of 250 and 300 mM respectively) (Table 1).

The moisture and dry matter contents of the seedlings after 120 hours of germination in different concentrations of $CaCl_2$, have been presented in Table 14 and Fig.21. It is evident that the moisture percentage of dry seed of Crotalaria is 8.20% and the dry matter is 91.80%. When germination begins moisture content of the seed increases rapidly and dry matter content relative to fresh wt decreases very rapidly.

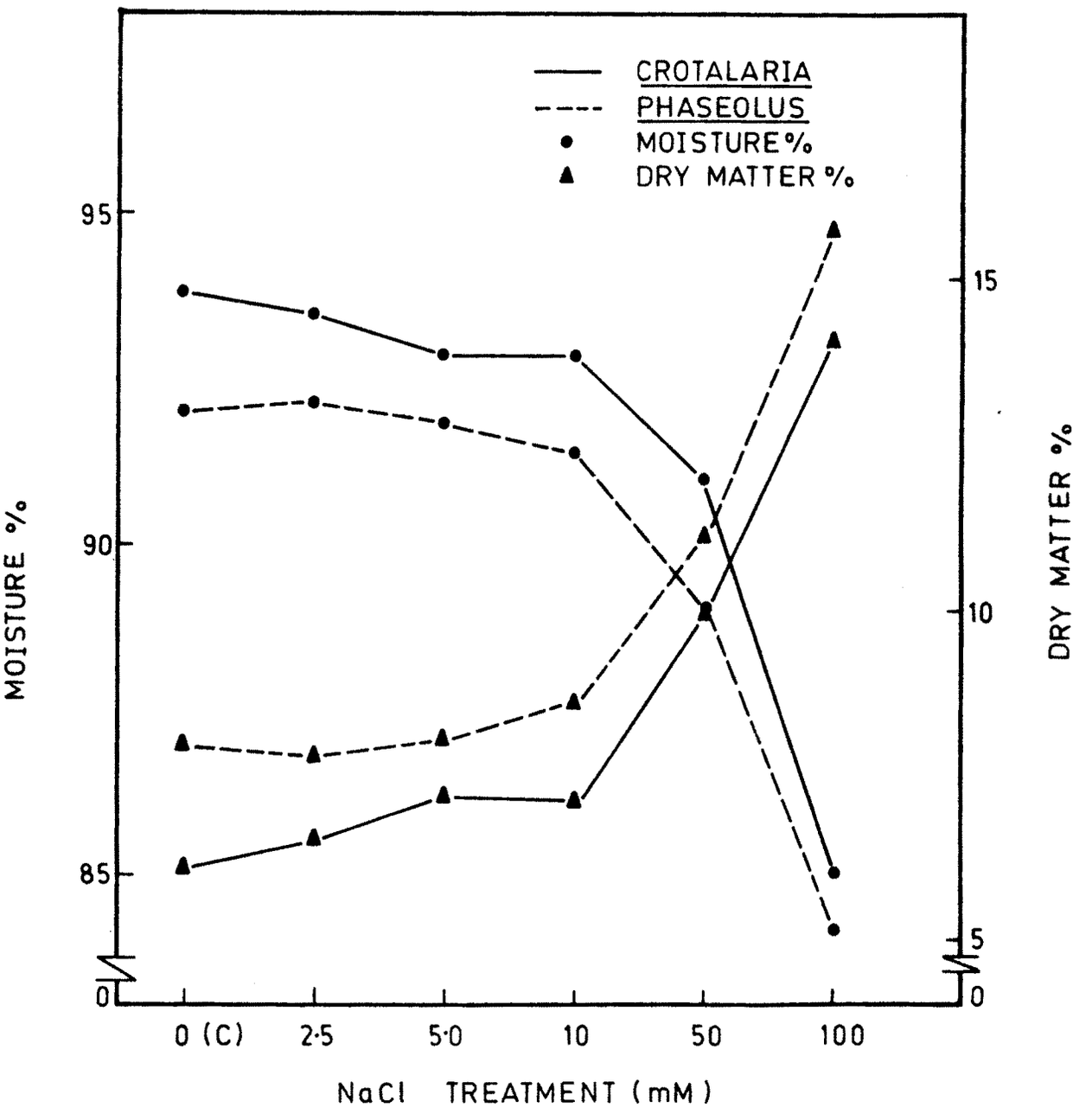

FIG. 20 EFFECT OF NaCl SALINITY ON MOISTURE PERCENTAGE AND DRY MATTER CONTENT IN C. JUNCEA AND P. ACONITIFOLIUS SEEDLINGS AFTER 120 HOURS OF GERMINATION.

It can also be seen that the dry matter content is increased with increase in $CaCl_2$ concentration in the medium. These higher levels of dry matter in seedlings treated with high concentrations are due to inhibition of breakdown of food reserve in respiration and/or inhibition of absorption of water. Phaseolus also shows similar response to $CaCl_2$. Legumes imbibe water much more rapidly than grasses and nearly all of the water needed for germination is imbibed during the first 4-8 hours (Mc William et al., 1970). Water absorbed in seed tissues activates various enzyme systems which serve to break down stored food and also aid to the transfer of nutrients from storage areas to the growing points.

Uhvits (1946) has discussed inhibitional and osmotic components of water absorption by alfalfa seeds. She has shown that inhibition is not significantly affected by increased osmotic pressure of the medium but osmotic water uptake is affected. The minimal amount of water that a seed must absorb for germination to occur varies from species to species (Brown, 1965).

Kahn et al., (1957) have reported an osmotic inhibition in lettuce seeds by 0.15 M mannital. Tailakov (1967) has observed decreased absorption of water by the germinating seeds

of Sorghum and maize due to NaCl treatment. Ramana and Rama Das (1978) have observed that as the concentration of the salt solution increases there is reduction in the absorption of water. Khan et al., (1978) have reported that the higher levels of salinity (15.8 and 63.2 m S cm^{-1}) markedly reduced the uptake of water in Capsicum annum seedlings. Mayeux (1982) has reported that reduced germination in salt solution can be attributed to an osmotic effect rather than a direct effect of ions.

These studies indicate that the presence of NaCl salt in the medium influences the water uptake and thereby the growth of seedlings in early stages. Different crop plants differ in their salinity tolerance capacity. The present studies also support the above observations and it appears that the osmotic pressure due to NaCl salinity probably decreases the uptake of water resulting into decrease in seedling growth. Both species respond to $CaCl_2$ in similar manner as they have responded to NaCl. However, the seedlings show better growth in $CaCl_2$ media.

B) Dry matter content :

The effect of different concentrations of NaCl on dry matter of Crotalaria and Phaseolus seedlings (120 h growth) has been presented in Table 13 and Fig.20. It is evident that

Table 14 : Effect of $CaCl_2$ salinity on moisture percentage and dry matter content in C.juncea and P.aconitifolius seedlings after 120 hours of germination.

$CaCl_2$ treatment mM	Moisture %	Dry matter %	Moisture %	Dry matter %
	C.juncea		P.aconitifolius	
0.0 (Con.)	94.61	5.39	93.32	6.68
2.5	93.73	6.27	92.92	7.71
5.0	94.27	5.73	93.37	6.63
10	94.32	5.68	93.57	6.43
50	88.54	11.46	89.91	10.09
100	89.78	10.22	85.62	14.38
Dry seed	8.20	91.80	9.60	90.40

(Average of three experiments).

the dry seeds of C.juncea, record 9.8% of dry matter. As germination starts the dry matter content falls down to 6.93% in control seedlings. This abrupt decrease of dry matter is due to the utilization of organic substances in oxidation as well as absorption of tremendous amount of water. The dry matter in 2.5, 5, 10, 50 and 100 mM NaCl treated seedlings is 615, 7.26, 7.09, 9.97 and 14.14% respectively. The slight decrease in dry matter content in the seedlings treated with 2.5 mM NaCl indicates that the catabolism in the seedlings is probably stimulated. The dry matter however, in seedlings grown at higher concentrations increases with increase in salt concentration. These results show that as the salinity increase the high values of dry matter of the seedling indicate the slow utilization of organic substances in respiration and their difficulty in absorption of water. Thus the metabolism is inhibited in treated seedlings due to NaCl salinity.

The dry seeds of P.aconitifolius contain 90.40% of dry matter. Pant and Tulsiani (1968) have recorded 89.94 dry matter in these dry seeds. On germination dry matter level falls down to 8.1% in control seedlings after 120 hours. This sudden decrease is due to rapid break down of organic compounds during respiration. The dry matter content slightly decreases to 7.73% in 2.5 mM NaCl treated seedlings. Here this salt

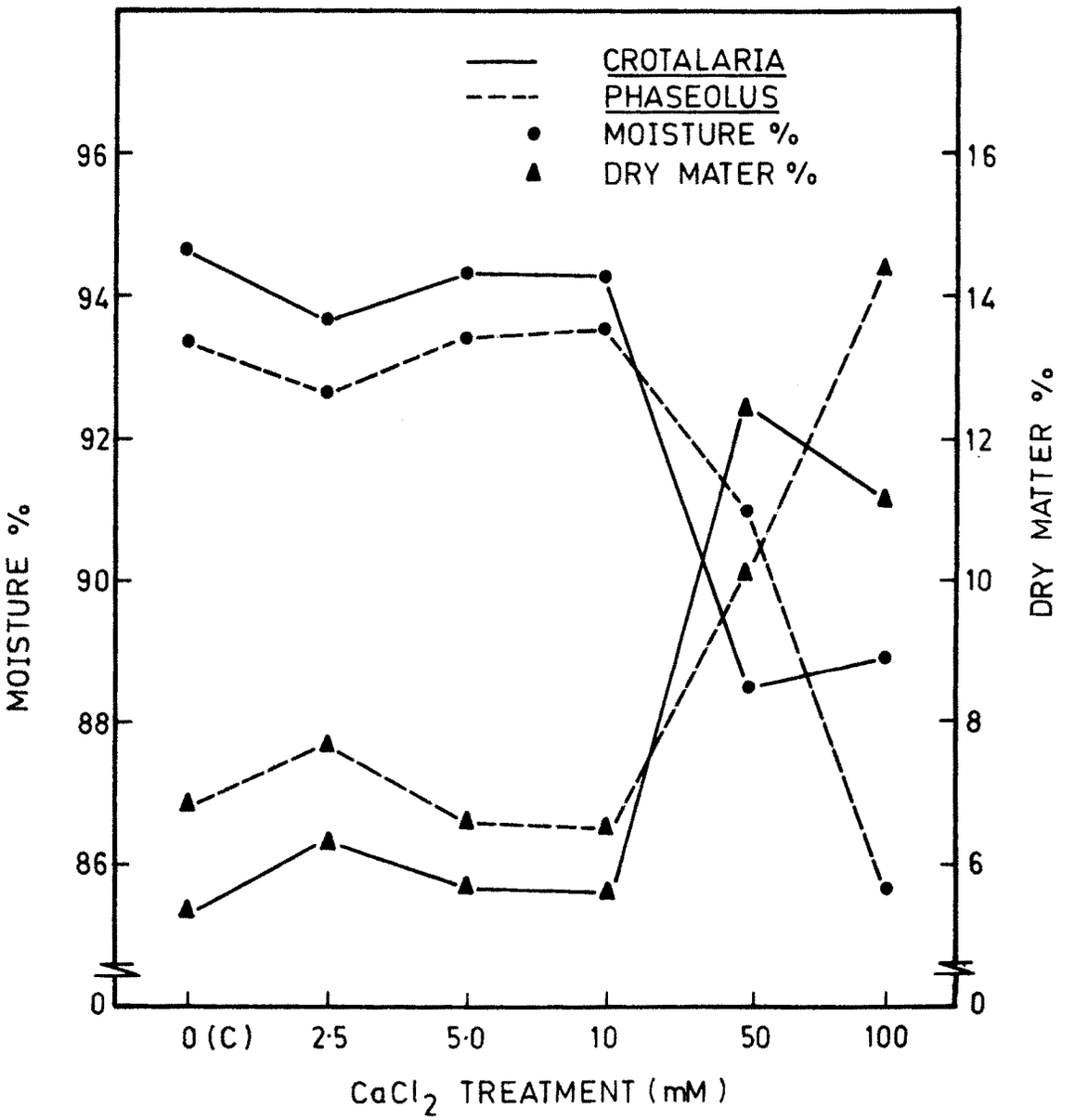

FIG. 21 EFFECT OF $CaCl_2$ SALINITY ON MOISTURE PERCENTAGE AND DRY MATTER CONTENT IN C. JUNCEA AND P. ACONITIFOLIUS SEEDLINGS AFTER 120 HOURS OF GERMINATION.

treatment appears to be stimulating catabolic processes. The dry matter content increases with increase in NaCl concentration and indicates inhibition of catabolism by higher salinity levels in the medium.

The effect of different concentrations of $CaCl_2$ on dry matter in Crotalaria and Phaseolus seedlings after 120 hours of germination has been depicted in Table 14 and Fig.21. It is clear that dry matter of dry seed of C.juncea is 91.8% which on germination falls down to 5.39%. This almost remains constant upto 20 mM $CaCl_2$ treated seedlings. There onwards it increases with increase in salt concentration in the medium. High content of dry matter in higher levels of salinity indicates the less utilization of reserve matter of the seeds.as well as inhibition of the process of water absorption. P.aconitifolius also shows more or less the same trend in $CaCl_2$ media.

Rizk et al., (1979) have studied the effect of varying concentrations of NaCl on germination and seedling growth of two rape (Brassica) varieties. They have reported that there was a progressive increment in mean values of seedling dry weight with increase in the concentration of either NaCl or $CaCl_2$. Masih et al., (1983) have found more hypocotyl dry wt at EC 8 and 12 m.S cm^{-1} eventhough length of hypocotyl was maximum in EC 4 m.S cm^{-1}.

Present studies also indicate that there is a direct relation between the dry matter content and concentrations of the salts. The relations indicate inhibitory effects of salts on the utilization of reserve material of the seed. The response of both the species however, varies with salt. NaCl appears to be more toxic than $CaCl_2$. The values of dry matter of NaCl treated seedling are higher as compared to those of $CaCl_2$ treated seedlings.

4) Carbohydrates :

Organic constituents during germination of seeds are very important as they supply energy to the developing embryo. Among the organic constituents, carbohydrates are the primary source of chemical energy in most of the seeds. Hence carbohydrate constituents of seeds and seedlings of C.juncea and P.aconitifolius and their utilization during germination under normal and salt stressed conditions have been studied.

The effect of various concentrations of NaCl on carbohydrates of seedlings of C.juncea after 6, 48 & 96 hours of germination is presented in the Table 15 and Fig.22,23. The table also records the carbohydrates of dry seeds.

Table 15 : Effect of NaCl salinity on the carbohydrate contents of *C. juncea* seeds during germination.

Germination Hours	Carbohydrates	NaCl Treatment (mM)			
		0.0(C)	5	10	100
0	Total sugars	3.44	-	-	-
	Starch	46.80	-	-	-
	Total carbohydrates	50.24	-	-	-
6	Total sugars	4.59	2.79	3.06	4.08
	Starch	14.12	12.34	13.00	13.51
	Total carbohydrates	18.71	15.13	16.05	17.59
48	Total sugars	0.74	0.75	0.83	1.19
	Starch	5.11	6.24	7.10	7.74
	Total carbohydrates	5.85	6.99	7.93	8.93
96	Total sugars	0.26	0.25	0.41	0.65
	Starch	3.96	4.74	4.28	6.28
	Total carbohydrates	4.22	4.99	4.69	6.93

Values are expressed as g 100^{-1} g fresh tissue.
Each value is a mean of three determinations.

It can be seen that the total carbohydrates in dry C.juncea seeds are 50.24% (fresh wt). Among these carbohydrates starch is the major constituent amounting 46.80% while total sugars are 3.44%. When germination takes place, the total carbohydrate content falls down under normal non-saline conditions from 50.24% to 18.71% (fresh wt) after 6 hours of germination. This sudden fall may be due to their utilization in the process of respiration. With germination the total carbohydrate content continues to decrease further. At 48 h, it falls down from 18.71% to 5.85% and at 96 hours it goes down to 4.22%. This decrease in total carbohydrates with time is indication of their utilization for the development of seedlings where other organic substances are formed. This is rather an usual phenomenon of germinating seeds.

The starch content decreases from 46.80% to 14.12% during the early period, i.e. after 6 hours of germination. It falls down to 5.11% after 48 hours of germination, and after 96 hours, it decreases still further to 3.96%. This decrease with time indicates more and more utilization of this major component of carbohydrates.

The total sugar content, however, shows an increase from 3.44% to 4.59% during the first 6 hours of germination. However,

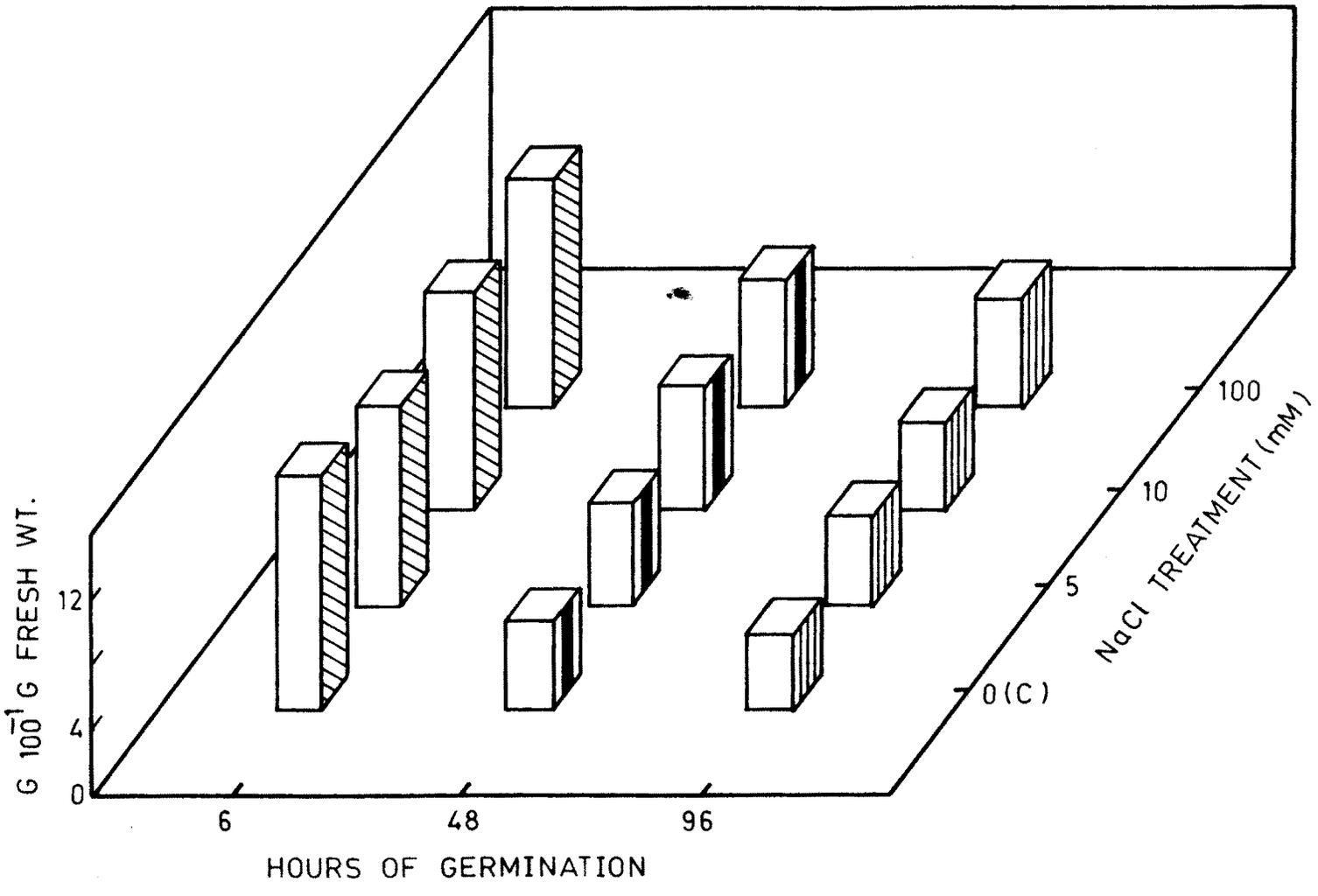

FIG. 22 EFFECT OF NaCl SALINITY ON STARCH CONTENT IN C. JUNCEA SEEDS DURING GERMINATION.

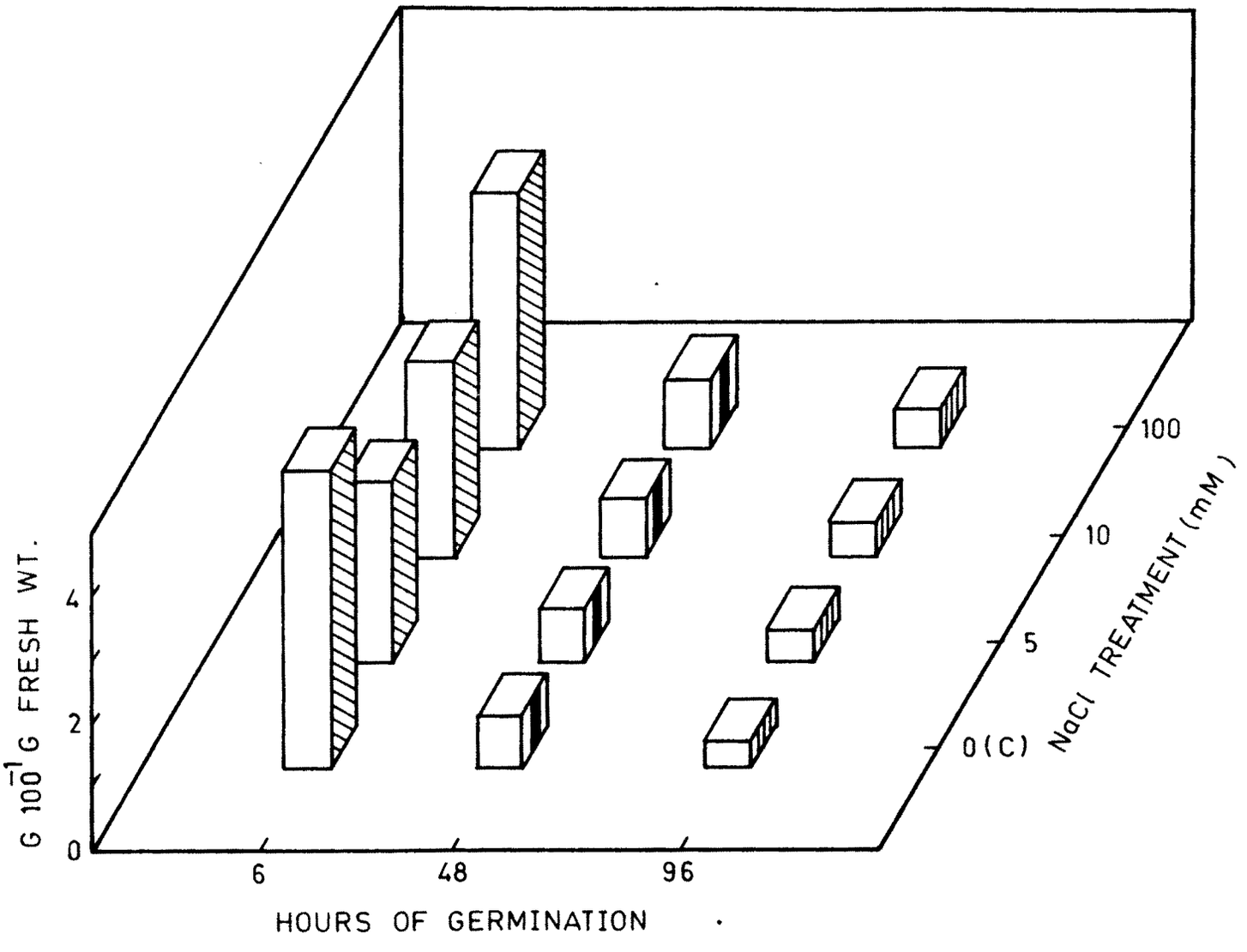

FIG. 23 EFFECT OF NaCl SALINITY ON TOTAL SUGARS IN C. JUNCEA SEEDS DURING GERMINATION.

during later periods it goes on decreasing with time. The total sugar content after 48 hours of germination decreases to 0.74% and then to only 0.26% after 96 hours of growth, This dramatic reduction in total sugar content suggests the rapidity of utilization of different sugars during the later stages of seedling growth and development.

The influence of various concentrations of NaCl on carbohydrate contents of the germinating seeds as revealed by Table 15 indicates that there is decrease in total carbohydrate contents in 5.0, 10 and 100 mM NaCl treated seedlings with maximum decrease being in 5.0 mM treated seedlings. However, in 10 and 100 mM NaCl treated seedlings, the values of total carbohydrates are 16.05% and 17.59% respectively.

These values showing increasing trend with increasing concentration of NaCl, indicate less and less utilization of total carbohydrates, after 6 hours of germinations. The values of total carbohydrates are fairly low at 48 and 96 hours of growth. The low values at 96 hours indicate that the carbohydrates may be utilized in the later periods. It may be recalled that (Table 1,2) germination percentage is fairly good in later stages than that in the early periods. The inhibitory effect of increasing concentrations of NaCl is clear from the higher values of carbohydrate contents in the treated seedlings at all the stages.

Among the carbohydrates, starch is the main carbohydrate which remains unutilized in the germinating seeds under saline conditions. This can be well correlated with the low activity of starch hydrolyzing enzyme, amylase (Table 21 and Fig.22,29) in the seeds germinated under higher salinity levels.

The total sugar content of the germinating Crotalaria seeds decreases with increasing salinity level (Table 15). The total sugars in control seedlings are 4.59% after 6 hours of germination. Under 5 mM NaCl level the treated seedlings, however, exhibit low value for total sugars (2.79%) indicating probable less breakdown of complex carbohydrate molecules (Fig.23). The higher salt concentrations have only a slight effect. During the later stages of development (48 and 96 hours) however, there is increase in total sugar content with increasing salt concentration in the medium. This may be due to their unutilization suggesting inhibition of respiratory activities in the seeds.

The effect of different concentrations of NaCl on carbohydrate contents of seedlings of P.aconitifolius after 6, 48 and 96 hours of growth has been presented in Table 16 and Fig.24, 25. It is obvious from the results that the carbohydrate content of seeds of P.aconitifolius is 65.50% (fresh

Table 16 : Effect of NaCl salinity on the carbohydrate contents of P.aconitifolius seeds during germination

Germination hours	Carbohydrate	NaCl treatment (mM)			
		0.0(C)	5	10	100
0	Total sugars	3.89	-	-	-
	Starch	61.61	-	-	-
	Total carbohydrates	65.50	-	-	-
6	Total sugars	1.93	2.87	1.63	2.39
	Starch	24.27	32.56	29.57	37.08
	Total carbohydrates	26.20	35.43	31.20	39.47
48	Total sugars	0.17	0.17	0.61	1.20
	Starch	16.51	17.37	19.51	22.54
	Total carbohydrates	16.68	17.54	20.11	23.75
96	Total sugars	0.51	0.56	0.43	0.41
	Starch	8.11	9.24	10.96	16.03
	Total carbohydrates	8.63	9.80	11.40	16.46

Values are expressed as g 100^{-1} g fresh tissue.
Each value is a mean of three determinations.

mater.) in which the major contribution is that of starch (61.61% fresh mater.). While that of total sugars is 3.89%. When germination takes place the total carbohydrate level falls down to 26.20% after 6 hours. During further stages of germination it declines still further and the minimum is at 96 hours of germination.

The starch content (Table 16 & Fig.24) decreases from 61.61% to 24.27% after 6 hours of germination and then drops down to 16.51% and 8.11% after 48 hours and 96 hours of growth respectively. The abrupt fall in the total carbohydrate content and particularly that in starch content indicates the rapid breakdown of carbohydrates and their utilization, which appears to be directly related to the maximum germination and seedling growth.

The total sugars (Table 16 and Fig. 25) are comparatively less in the seeds at all the stages of germination and changes are almost insignificant.

The total carbohydrate contents in Phaseolus seedlings increase with increasing concentrations of NaCl after 48 and 96 h of growth. The starch content in NaCl treated seedlings also shows similar trend. This clearly indicates the inhibi-

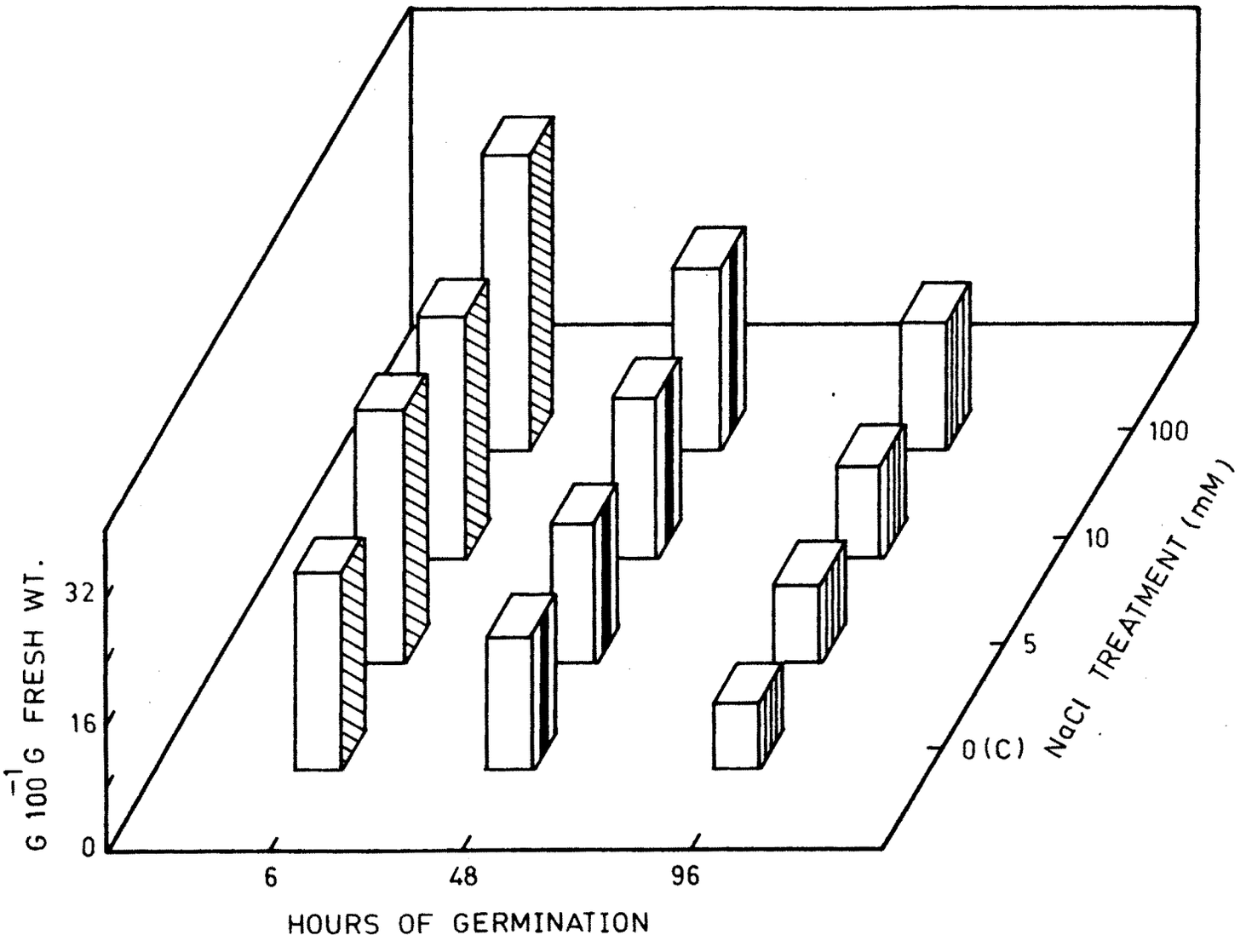

FIG. 24 EFFECT OF NaCl SALINITY ON STARCH CONTENT IN P. ACONITIFOLIUS SEEDS DURING GERMINATION.

tory effect of salt on the utilization of starch probably due to slight inhibition of amylase in the germinating seeds under saline conditions (Table 21 and Fig.24,30), which ultimately can be correlated with decrease in germination percentage and poor growth of seedlings.

The effect of salt stress on total sugars content of seedlings, however, is not remarkable. The lower values of total sugars in Crotalaria after 96 hours and those in Phaseolus after 48 h recall the maximum germination in them.

Present studies indicate that in both the species studied, there is stimulation in hydrolysis of starch only in the seedlings treated with lower concentration of salt (control and 5 mM) and that after 6 hours of germination. It appears that Na and Cl ions stimulate hydrolysis when present in lower concentrations. It can also be suggested that both the species respond in the similar manner to the salinity stress. However, as the carbohydrate (starch) content of Phaseolus seeds is basically higher than that in Crotalaria seeds, the species differ in respect of their ability to germinate even under the higher salinity levels. High starch content of Phaseolus seeds thus appears to be one of the reasons for better salt tolerance in the species.

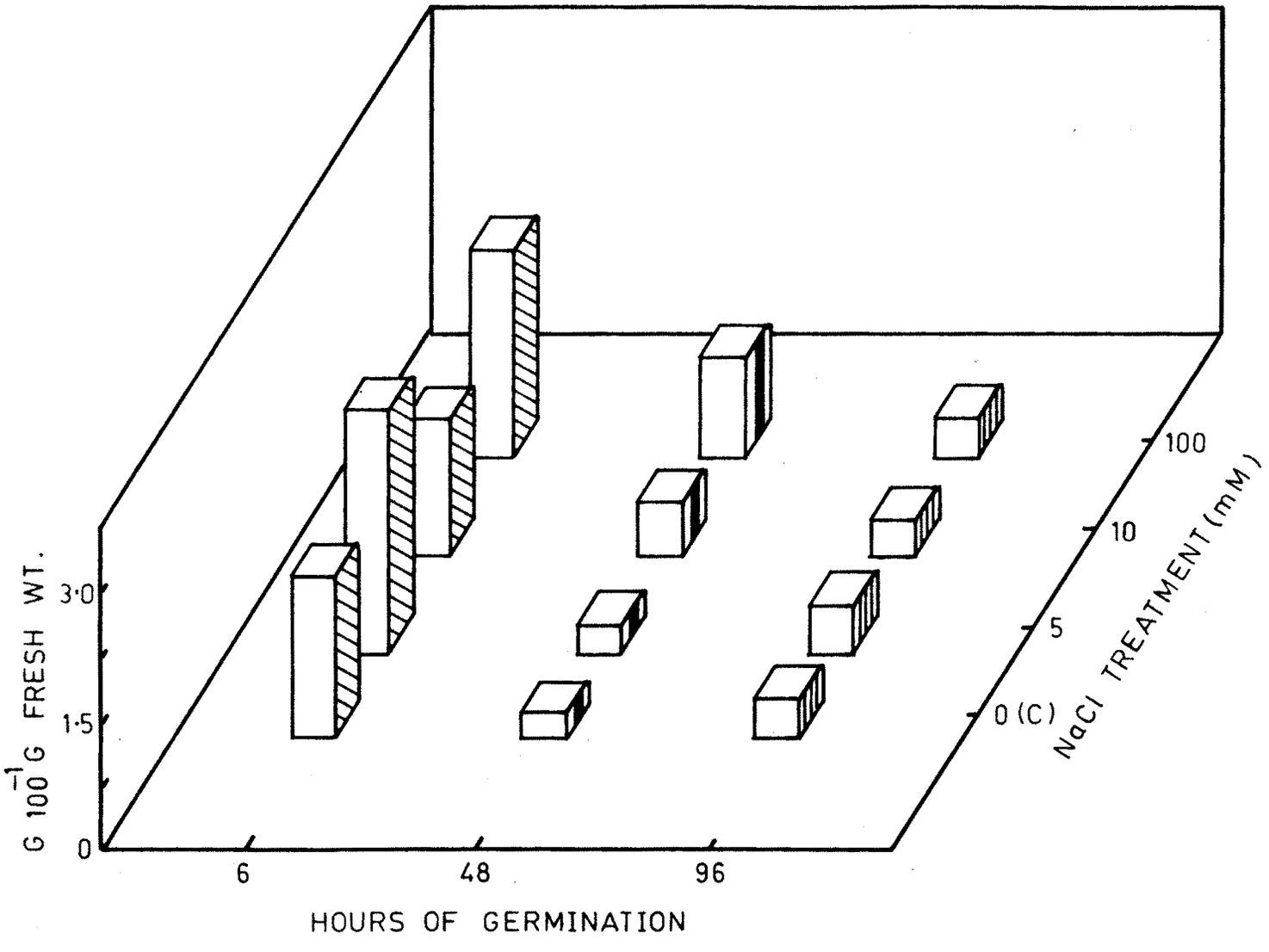

FIG. 25 EFFECT OF NaCl SALINITY ON TOTAL SUGARS IN P. ACONITIFOLIUS SEEDS DURING GERMINATION.

Tewari (1965) reported that the level of reducing sugars and total carbohydrates in the seeds of Phaseolus radiatus fell when they were germinated in light. It was still lower in the seeds which were allowed to germinate in red light. Brown (1965) has mentioned that the nutrition of the embryo depends on the conversion of insoluble reserves to soluble forms. The most prominent of these insoluble substances is starch. Salinity causes delay in the release of reducing sugars in gram (Bhardwaj, 1965) and also in Wheat (Sarin and Narayanan, 1968) during germination. Paul et al., (1970) have found that starch hydrolyzing enzymes ∝- amylases are active in P.aureus in the cotyledon and in the axis from the start of imbibition. They have (1974) noticed the rapid depletion of starch content in the cotyledon as soon as the seeds of P.aureus are soaked in water. After 120 h of germination the starch content in the cotyledon was reduced to a maximum value. Jaya and Venkataraman (1981) have found decrease in the concentration of total carbohydrates during 96 h germination of chickpea and green gram.

Hatata and Farah (1982) have studied the effects of different salts (NaCl and $MgCl_2$ and Na_2SO_4, $MgSO_4$) on carbohydrate metabolism in young corn seedlings and found that there was decrease in the total carbohydrate content with the advancement in time and increase of salt concentrations. Gill

and Singh (1985) have studied the effect of salinity on carbohydrate metabolism during paddy (Oryza sativa) seed germination. They have reported that starch content (mg/grain) decreased with lapse of time of sowing but less at higher salinity levels. From the amylase activity they have further reported that there is rapid decrease in starch and early and greater release of soluble sugars in the tolerant, than in the sensitive variety. Rapid germination, early induction of amylase activity and higher rate of water uptake are the characters of tolerant varieties (Gill and Singh, 1985). From these observations it appears that response of carbohydrate metabolism to salinity varies with species. Higher concentrations of salt in the medium inhibit the breakdown of carbohydrates, possibly by blocking the activity of amylases.

So far salt tolerance of two species under study are concerned, Phaseolus shows faster rate of germination, and early induction of amylase activity under salt stress conditions than Crotalaria and therefore it appears to be more tolerant than Crotalaria.

5) Proline :

Increasing salinity of soil and water threatens agriculture in arid and semi-arid regions. Hence the development of salt tolerant crops will be of great value for using the

Table 17 : Effect of NaCl salinity on proline contents of C.juncea seeds during germination

NaCl treatment mM	Hours of germination					
	6	24	48	72	96	120
0.0 (C)	83.28	149.33	150.33	110.75	180.54	142.18
2.5	71.88	181.34	242.20	136.57	97.71	80.59
5.0	46.64	158.38	190.19	136.02	82.89	66.02
10	77.72	185.41	195.74	105.53	128.33	93.77
50	45.33	167.53	218.95	226.68	162.59	106.79
100	61.76	120.95	184.71	234.19	238.25	142.30

Values are expressed in $\mu g\ g^{-1}$ fresh tissue.
Each value is a mean of three determinations.

problem soils. In order to develop such salt tolerant species, it is essential to analyse the salt tolerance mechanism in those plants.

Accumulation of proline under saline conditions is one of the mechanisms in plants. However, the exact role of proline in the mechanism of salt tolerance is not clearly understood. It may be functioning as a source of solute for intercellular osmotic adjustment under saline conditions (Stewart and Lee, 1974). Schobert (1977) has suggested two mechanisms quite different from osmotic regulation for the regulatory functions of proline. Chu et al., (1976) have suggested that proline has only a minor contribution to osmotic regulation in plants. Palfi et al., (1974) suggested that high concentrations of proline exerts the least inhibitory effects on cell growth. Though the exact role of proline during stress conditions is not yet completely understood it appears that it adds to the plant resistance mechanism.

The effect of different concentrations of NaCl on free proline accumulation in the germinating seeds of C.juncea and P.aconitifolius after 6, 24, 48, 72, 96 and 120 hours of germination has been presented in the Table 17, 18 and Fig.26. It can be seen that the seedlings of C.juncea after 6 hours of

Table 18 : Effect of NaCl salinity on proline contents of *P.aconitifolius* seeds during germination

NaCl treatment mM	Hours of Germination					
	6	24	48	72	96	120
0.0(C)	160.40	193.95	188.71	98.88	87.03	67.74
2.5	106.15	158.35	156.39	103.79	85.81	73.18
5.0	78.87	170.28	108.90	125.02	103.02	68.89
10	65.90	173.83	116.12	121.43	73.22	70.23
50	55.41	123.18	93.90	155.29	143.22	112.40
100	47.57	136.99	139.41	101.71	106.21	106.38

Values are expressed in $\mu g\ g^{-1}$ fresh tissue.
Each value is a mean of three determinations.

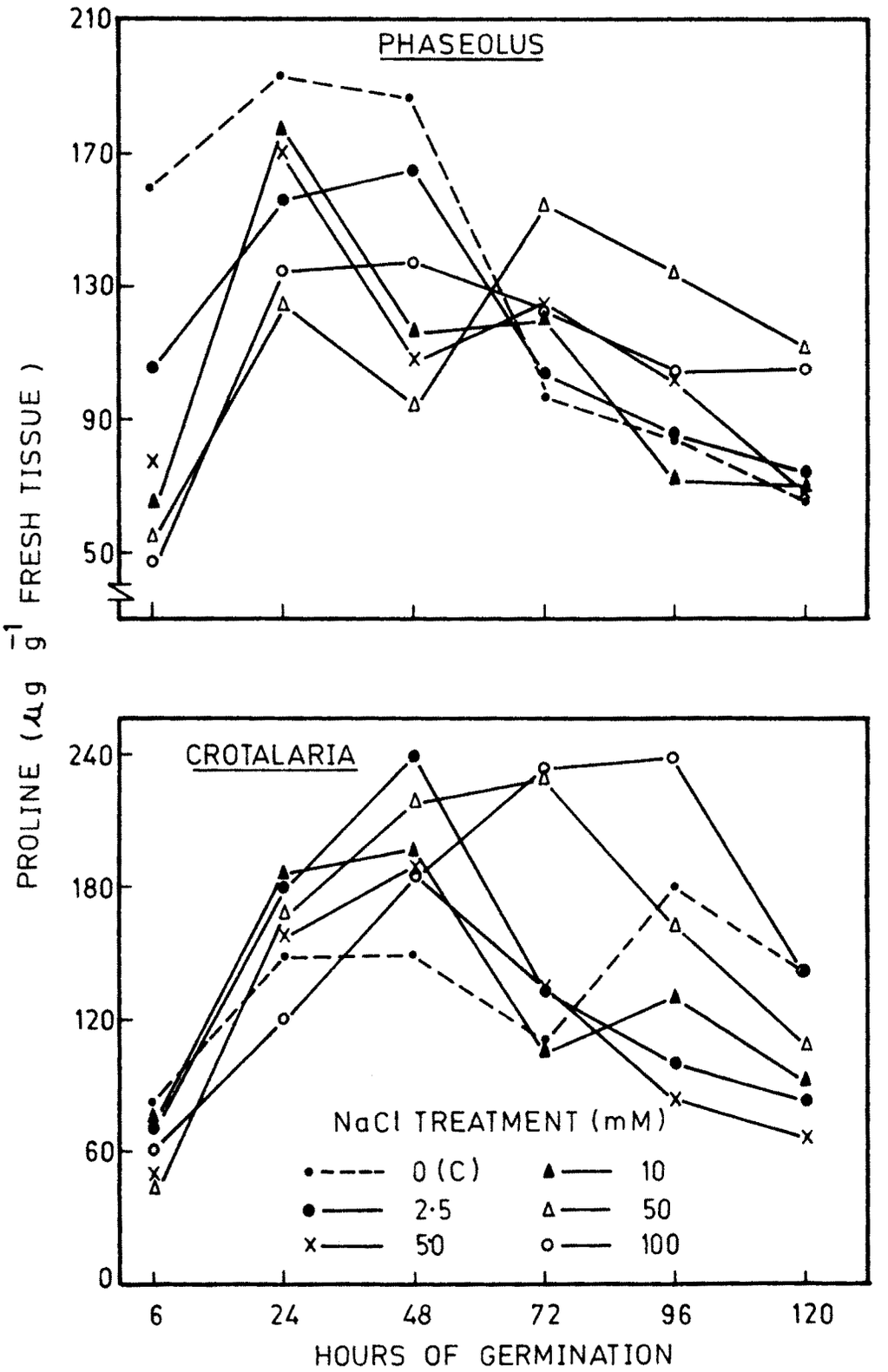

Fig. 26 EFFECT OF NaCl SALINITY ON PROLINE CONTENTS OF C. JUNCEA AND P. ACONITIFOLIUS SEEDLINGS.

germination contain 83.28 µg of proline per g of fresh material. When germination continues further, proline content increases. In control seedlings, proline content roughly appears to be increasing with time. The effect of different concentrations of NaCl on proline content indicates that except in early period of germination of 6 hours, proline content increases with increasing salt concentrations at all the developing stages.

The free proline content of the seedlings of P.aconitifolius is 160.40 µg per g of fresh material, under non-saline conditions. It increases with time of germination and the maximum value recorded is after 48 hours of germination. Thereafter, it is declined. During initial phase of germination the proline content decreases. It is also quite clear that the accumulation of proline takes place in Crotalaria seedlings grown under saline conditions even at the early stages of growth and development (24, 48 and 72 hours of germination). This effect is quite significant at 100 mM NaCl level. However, the response shown by Phaseolus is remarkably different. Proline accumulation in the seedlings of this species takes place only during later stages (72, 96 and 120 h) of growth and development. However, this proline accumulation is not pronounced as it has been observed in Crotalaria.

It has been observed by Goas (1968) that proline content was high in Aster trifolium when grown under saline conditions and when transferred to non-saline medium it developed more slowly with decrease in proline content. Bal (1976) observed salt tolerance through seed treatment with proline and found that seed treatment with 0.02% proline increased germination in rice under saline conditions. Cavalieri et al., (1979) have found that proline accumulation is species specific, and adaptive to the salt marsh environment. Chauhan et al., (1980) have reported that free proline accumulation in barley and wheat crops increased with the salt stress. Prasad et al., (1980) have studied the salt tolerance capacity of some ragi cultivars. They found that the cultivars Kalyani and EA-955 showed higher percentage of germination, greater survival of seedlings and high levels of proline in higher salt solutions showed high degree of tolerance when compared with other cultivars.

Dix and Pearce (1981) are of the opinion that proline accumulation is considered either to be symptomatic of self induced stress or to have a protective role, other than as an osmotic regulation. Gering (1982) has found that high salt stress increases proline level in plants viz. maize, rye and rice plumules. He suggested that free proline accumulation

may serve as a criterion for determining a stress condition in plants. A positive correlation was found between proline and over production and osmotolerance (Riccardi et al., 1983). Dreier (1983) observed that application of NaCl to crop plants in their culture medium leads to an increase in their endogenous content of free proline. He established a relationship between endogenous Na^+ and proline contents. Measurement of proline concentration served as a basis for the analysis of the salt tolerance of crop plants. He verified the test of crop seedlings for the determination of the salt tolerance. There is a typical concentration of NaCl in the culture medium above which an enhancement of the free proline content is induced. In 19 cultivars of arid areas, the critical concentration is 50 to 80 m mol l^{-1} of NaCl. Rao et al., (1981) found that free proline accumulation indicated that some cultivars show high degree of tolerance to 0.4% salinity.

Contrary to the above reports Chu et al., (1976) have observed that NaCl inhibits proline accumulation caused by a reduction in external osmotic potential. Goering and Bui Huy Thien (1978) have studied the influence of increasing concentrations of NaCl and KCl in the culture medium on the proline accumulation in roots and shoots of corn seedlings. They found that at a very high salt stress, proline formation was

inhibited. The reports describing the changes in proline content during seed germination, however, are scanty.

The present studies also confirm the observations made by several workers that salinity stress induces free proline accumulation in plants. However, the extent of free proline accumulation seems to be under the control of magnitude of stress. It also varies from species to species and is dependent on the stage of development of the seedlings. It can be seen that usually salt stress during the later stages of development of the seedling, induces this accumulation. It appears that proline has no role to play during heterotrophic phase of the developing seedling under stress.

Based on the response of the species undertaken to the salinity stress particularly during the autotrophic phase of development (96 and 120 h) it appears that Phaseolus shows some tendency of slow but steady accumulation of proline in the seedlings. In Crotalaria, however, there is no such tendency.

It is also probable that as Crotalaria is yet under heterotrophic condition i.e. without epicotyl and unfolded leaves upto 120 h, the tendency to accumulate proline or otherwise is not yet developed. Thus it can be suggested that

Phaseolus has a better adaptive nature to the salinity stress than that in Crotalaria.

6) Enzymes :

A) Peroxidase :

A close correlation between peroxidase activity and polyphenol accumulation in individual parts of seedling of C.juncea suggested an important role of peroxidase in polyphenol biosynthesis (Shah et al., 1976). The effect of sodium chloride salinity on the activity of peroxidase during germination of C.juncea and P.aconitifolius seeds is recorded in Table 19 and Fig.27. It is evident that peroxidase is probably not activated during first 6 h of germination in both the legumes. Activity of the enzyme increases with time upto 72 hours where it is the maximum and thereafter it declines. However, this inhibition of enzyme activity after 72 h of germination is remarkable in Crotalaria.

Kamboj and Nainawtee (1978) have studied peroxidase isoenzyme changes in germinating soybean varieties and showed that the peroxidase level in seeds decreased as the germination percentage characteristic of the variety increased. In all, peroxidase activity increased between 2 and 6 days of germination but remained constant in the seeds which failed to germinate during 6 days of soaking. The number of isoenzymes increased to two at 48 h of germination in all the

Table 19 : Effect of NaCl salinity on the activity of peroxidase in C.juncea and P.aconitifolius during germination

Plant	NaCl treatment mM	Hours of germination					
		6	24	48	72	96	120
C.juncea	0.0 (Con.)	N.D.	0.26	0.78	1.69	2.37	1.60
	2.5	,,	0.23	0.77	1.29	1.86	1.94
	5.0	,,	0.51	0.81	1.15	2.12	2.26
	10	,,	0.38	0.73	0.86	2.24	1.75
	50	,,	0.03	0.67	0.67	2.59	0.68
	100	,,	0.00	0.23	0.45	2.11	0.55
P.aconitifolius	0.0 (Con.)	N.D.	0.37	1.42	3.35	3.29	4.93
	2.5	,,	0.41	1.14	2.58	4.17	6.43
	5.0	,,	0.42	1.12	2.43	3.11	5.02
	10	,,	0.32	0.99	2.41	2.58	5.10
	50	,,	0.25	0.59	2.78	3.10	4.63
	100	,,	0.26	0.87	2.07	6.38	3.16

Values are expressed as Δ O.D. min^{-1} mg^{-1} protein.
N.D. : Not detected.

varieties. Peroxidase activity increases slowly and intermittently soon after soaking and increases sharply 48-72 h after soaking or in the early stages of germination of wheat seeds. The enzyme activity was much higher in the embryos than in the endosperm throughout the seed development. Increase in peroxidase after 48 h of germination was mainly due to fast migrating isoperoxidases of the embryo (Zairov et al., 1983). The changes in peroxidase activity and peroxidase isozyme pattern were examined by Hong et al., (1983) in different parts of legume seedlings. The enzyme showed a tendency to increase at an early stage and then a gradual decrease as germination continued. However, Asins et al., (1983) have observed a drastic change in peroxidase pattern during only 1st hour of germination in all materials of rye and wheat seedlings.

Present study supports the findings described earlier, that peroxidase is induced remarkably during the later stages of germination only. Further, it can be seen that the level of peroxidase in both the legumes during the later stages of germination is different and indicates vigorous metabolic activities in Phaseolus, particularly during 72 to 120 h germination.

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The effect of NaCl salinity on the peroxidase activity indicates that, in C.juncea it is bit stimulated at 5.0 mM NaCl level after 24 hours of germination and declining in the higher concentrations of salt (50 and 100 mM) levels which seem to be the highly toxic doses. However, in later periods of germination it responds well with all the salt concentrations used. Roughly salt concentrations upto 10 mM level stimulated the activity of peroxidase beyond which it was declined.

Peroxidase, in P.aconitifolius under saline conditions however, shows a very different response as compared to that in C.juncea. Peroxidase activity is maximum in control than those under saline conditions upto 48 h of growth indicating its inhibition by salt stress. However, the activity during later periods of germination (from 72 to 120 h) increases with time and with salt concentrations. This increase appears to be an adaptive feature of this legume to salinity stress.

The inhibitory effects of high concentrations of NaCl and Na_2SO_4, have been demonstrated by Vasile (1963) in case of pea, corn and wheat. El-Fouly and Jung (1970) have observed that peroxidase activity was not affected by lower concentrations of NaCl but decreased at higher salt concentrations. Maliwal and Paliwal (1972) noticed a decrease in peroxidase

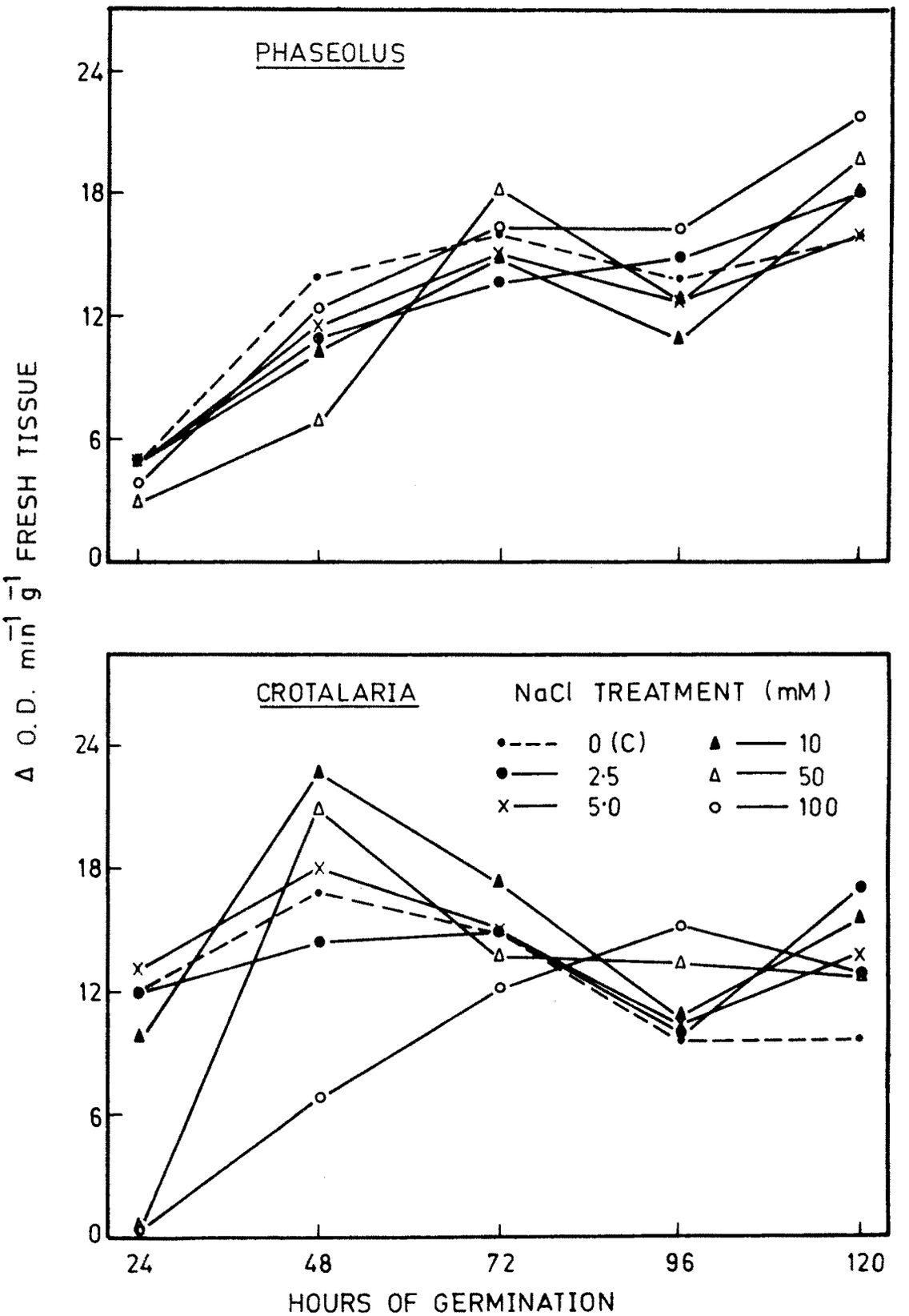

Fig. 27 EFFECT OF NaCl SALINITY ON PEROXIDASE ACTIVITY IN C. JUNCEA AND P. ACONITIFOLIUS SEEDLINGS.

with increase in salinity in Okra (Abelmoschus esculentus) and sponge gourd (Luffa cylindrica). Flowers (1972) has observed a significant inhibition of the enzyme activity by added NaCl (0.34 M) in in vitro studies of peroxidase in Suaeda martima and Pisum sativum. Quantitative and qualitative changes in peroxidase during germination of mung bean under salt stress have been studied by Sheoran and Garg (1979) and found that activity of the enzyme increased in embryo axis and leaves but not in cotyledons and roots with different salt treatments to varying degrees. They have also observed that the number of isoenzymes of peroxidase increased with the time of germination.

On the other hand, Gopalachari (1963) observed steep increase in peroxidase activity in all parts of Sorghum vulgare and in roots and cotyledons of Phaseolus mungo during seed germination and further seedling growth. In several legume seeds an intensification of peroxidase was noticed by Ioana (1979).

Tailakov (1967) found that salt treatment decreased the activity of peroxidase and catalase in germinating seeds of Sorghum and maize and also noticed a difference in responses of cultivars to the salts. Weimberg (1970) studied the levels

of 18 enzymes in pea seedlings grown on highly salinized media. Though seedlings grown in saline media were stunted, the specific activities of the enzymes were the same in the given tissues of all parts (root, stem and leaves). However, the isozyme pattern of peroxidase from roots of salt grown plants was altered.

A number of workers have observed the changes in isozyme composition of peroxidase as influenced by salinization. Aleshin et al., (1971) and Molokov et al., (1973) have observed intensification of peroxidase activity due to both Cl^- and SO_4^- salinities as well as changes in the isozyme pattern. Stevens et al., (1978) investigated in detail the utility of peroxidase as an indicator of salt stress in 11 cultivars of Brassica. Out of these, in seven cultivars the enzyme activity was inhibited due to salt stress while in 4 cultivars there was stimulation. Dias and Costa (1983) have studied the effect of low salt concentrations on peroxidase in the leaves of sugar beet and found an increase in peroxidase activity at 48 h of salt application. Thus it is apparent that there is a genotypic difference with respect to this response. Hence no generalization can be made in this respect.

Peroxidase is involved in various metabolic pathways. Paul and Mukherji (1972) have found a strong correlation between peroxidase and catalase activity and respiration and suggested that these enzymes are acting as a part of respiratory mechanisms of rice seedlings. Hendricks and Taylorson (1974) suggested possible participation of peroxidase in NADPH oxidase and electron transport during dormancy breaking in Amaranthus albus and Lactuca sativa. It is admitted that the role of peroxidase in seed germination can be clearly defined only after finding the location of different isozymes within the seed.

In conclusion it can be suggested that the activity of peroxidase in the germinating seeds can be the indicator of metabolic activities in them. It reflects, therefore, in the level the respiratory activities. High peroxidase during later stages of germination and even under higher salinity levels in Phaseolus is suggestive of maintenance of respiratory activities even under saline conditions exhibiting salt tolerant nature of this plant.

B) Catalase :

Catalase is an oxidative enzyme which catalyzes redox reactions between H_2O_2 as electron acceptor and many kinds of

Table 20 : Effect of NaCl salinity on the activity of catalase in *C.juncea* and *P.aconitifolius* during germination

Plant	NaCl treatment (mM)	Hours of Germination					
		6	24	48	72	96	120
C.juncea	0.0 (Con.)	0.09	0.08	0.19	0.32	0.34	0.21
	2.5	0.10	0.07	0.22	0.36	0.40	0.11
	5.0	0.09	0.20	0.24	0.34	0.49	0.18
	10	0.09	0.16	0.19	0.27	0.39	0.36
	50	0.09	0.11	0.18	0.23	0.46	0.27
	100	0.09	0.08	0.09	0.14	0.42	0.16
P.aconitifolius	0.0 (Con.)	0.25	0.38	0.55	0.98	0.96	0.37
	2.5	0.24	0.41	0.49	0.93	1.20	0.55
	5.0	0.26	0.39	0.45	0.75	0.97	0.73
	10	0.25	0.33	0.46	0.99	1.04	0.30
	50	0.30	0.41	0.43	0.72	1.18	0.60
	100	0.29	0.29	0.39	0.63	2.19	0.72

Values are expressed as mg H_2O_2 broken min^{-1} mg^{-1} protein.

substrates. Kremer (1970) has reported that catalase is able to oxidise other substrates besides H_2O_2. But Grinber (1971) has suggested that since catalase has got more affinity for H_2O_2 it is mainly involved in regulation of H_2O_2 level in plant tissues.

In the process of seed germination catalase has been shown to play a peculiar role. According to Nanda (1950) the activity of catalase is very well correlated with germination capacity of the seeds. Mukherji and Paul (1971) have implicated catalase activity in the respiratory mechinary of germinating rice seeds and have noticed a positive correlation between catalase activity and respiratory rate.

Present investigation shows that catalase activity in germinating seeds of C.juncea and P.aconitifolius (Table 20 and Fig.28), steadily increase and reaches to its maximum after 48 h of germination, and declines during the later periods of growth. Present observations are in agreement with the findings of several workers. Mukherji and Paul (1971) observed that during rice seed germination the rate of increase in catalase was maximum between 24 and 48 hours of germination. The activity went on increasing upto 96 hours and then declined in the next 24 hours. However, continuous increase in catalase

upto 6 days of germination of rice seeds (Palmiano and Juliano, 1972) and that upto 11th day in case of almond seeds (Mihalyfi, 1968) has been found. De Oliveira *et al.*, (1976) found that catalase activity was almost doubled during germination of *Coffea arabica* L. seeds. On the other hand Gopalachari (1963) has noticed a regular decrease in the activity of this enzyme during germination of *Sorghum vulgare* and *Phaseolus mungo* seeds. Hong *et al.*, (1983) found that the activity of catalase and the contents of H_2O_2 in the red bean seedlings decreased in cotyledon, root and epicotyl fractions throughout germination, whereas those of the shoot fraction increased. The enzyme activity was related to the contents of H_2O_2.

Effect of salinity on catalase activity indicates that in *C.juncea*, it is stimulated by salinity levels upto 50 mM at all the time. Beyond this level, activity of the enzyme begins to decrease. Even under saline conditions upto 50 mM salt level it goes on increasing upto 48 h as it has been observed in control and declines thereafter during the later periods of germination.

The response of catalase to salt stress in *P.aconitifolius*, however, is different. It appears that catalase in

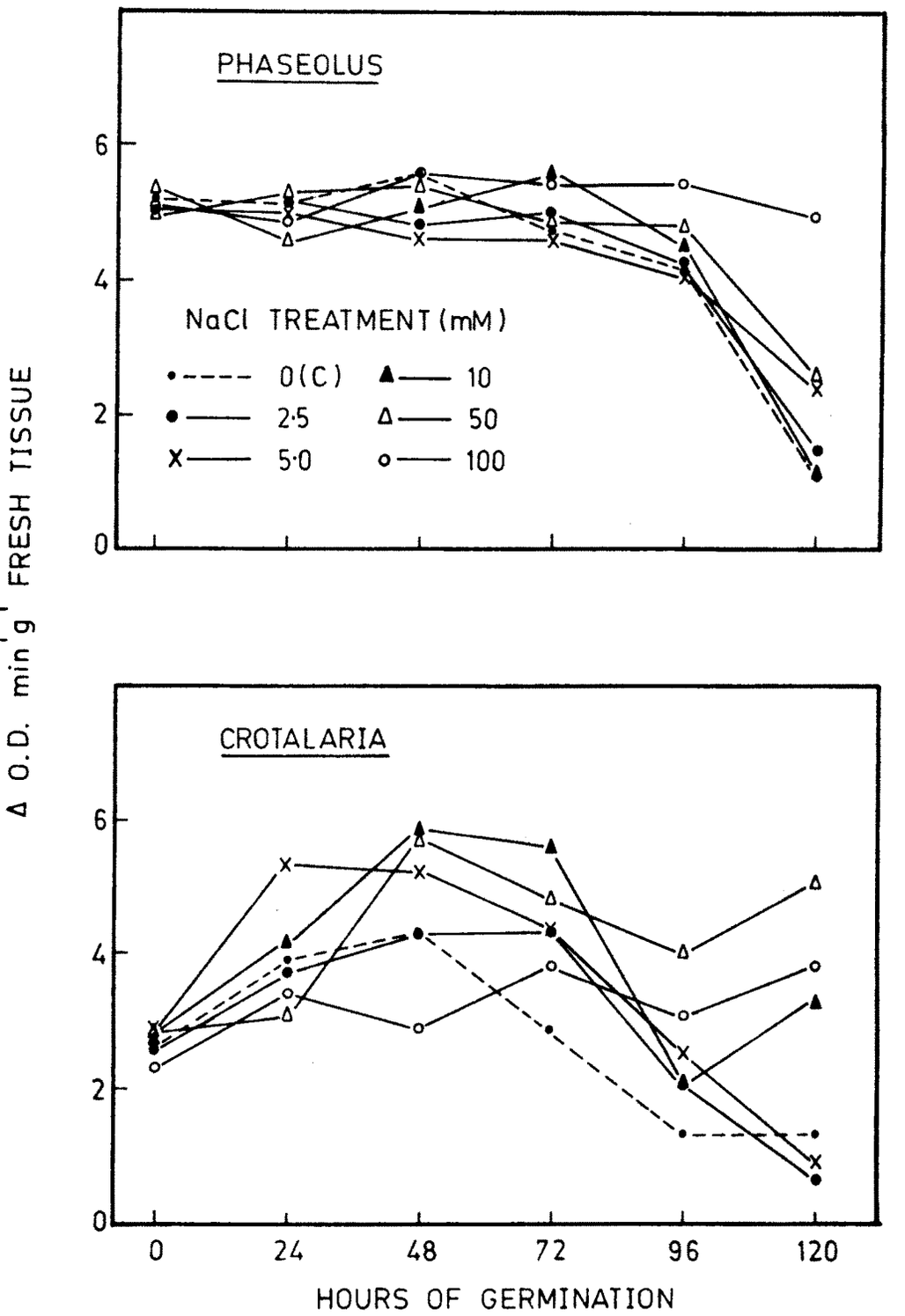

Fig. 28 EFFECT OF NaCl SALINITY ON CATALASE ACTIVITY IN C. JUNCEA AND P. ACONITIFOLIUS SEEDLINGS.

this plant is only a slightly affected and that only at the higher salinity levels. Thus the seedlings of P.aconitifolius are able to maintain their catalase level during germination even under saline conditions. When we compare these results with those of peroxideese it can be said that the level of hydroxyperoxidases is unaffected during germination by the salinity stress. This is also suggestive of a good germination capacity in this species under the stressed conditions (Nanda, 1950).

Tailakov (1967) has observed that salt treatment decreased the activity of catalase and respiratory rates in Sorghum and maize seeds during germination. He has also reported that different cultivars responded differently to the salts and there was more variation in maize than in Sorghum.

Tesu et al., (1978) have observed that the increase of soil salinity enhances the rate of catalase activity in sugar beet, wheat and sunflower leaves and consequently of respiration. However, it decreases with macro and microelements on saline soils.

Parida et al., (1981) have studied the enzymatic changes in gourd (Cucurbita maxima) and bean (Phaseolus vulgaris)

cotyledons during aging and the effect of detopping. They found that catalase activity decreases during the senescence of cotyledon. When cotyledon senescence was retarded by detopping, marked increase was found in the activity of the enzyme. Activity of catalase and the content of H_2O_2 in red bean seedlings was found to be decreased in cotyledon, root and epicotyl fractions throughout germination whereas those of shoot fractions were increased. Thus the catalase activity was related to the content of H_2O_2.

In conclusion it can be said that activity of enzyme catalase increases with the age of seedling upto certain period e.g. 48 to 72 h when it is maximum and falls down during the later stages of development. Catalase system in Crotalaria appears to be highly sensitive particularly at the higher salinity revels of more than 100 mM. On the other hand this enzyme system in Phaseolus is quite resistant and remains unaffected even to the salinity level beyond 100 mM. This signifies a good germinating capacity in Phaseolus under saline conditions. Thus Phaseolus seems to be relatively salt tolerant species.

C) Amylase :

The activity of ∝- amylase during germination of C.juncea and P.aconitifolius under different levels of NaCl

is recorded in Table 21 and Fig.29,30. It is interesting to note that there is a dramatic fall in the activity of this enzyme immediately after 6 h of germination i.e. at 24 h and then slowly it goes on increasing. It is also evident that in C.juncea (Fig.29) there is slow rise in the activity of amylase upto 96 h of growth where it is maximum and declining thereafter. However, in P.aconitifolius (Fig.30) the activity increases steadily and reaches to its maximum little earlier i.e. at 72 h.

According to Chao (1970) and Jacobson et al., (1970), the over all amylase increases during germination of maize and barley and can be attributed largely to α- amylase. Goswami et al., (1977) reported that during first 24 hours of wheat seed germination starch was hydrolysed by a free β - amylase. In next 24 hours some of the inactive form of β-amylase was converted into the active form and this, together with α- amylase synthesized de-novo, resulted in the hydrolysis of starch. Sheoran and Wagle (1981) have found that β-amylase isoenzymes increased in number upto 48 h of germination and then decreased during germination of bajra and barley. Similar type of observations were made by Parvathy and Sadashiwan (1982) in three plants during germination. They have found that the activity of amylase was low at the initial stages of

Table 21 : Effect of NaCl salinity on the activity of amylase in C.juncea and P.aconitifolius during germination

Plant	NaCl Treatment mM	Hours of Germination					
		6	24	48	72	96	120
C.juncea	0.0 (Con)	2.51	0.35	2.08	5.86	8.67	3.34
	2.5	2.29	0.39	0.91	2.32	7.20	2.34
	5.0	1.94	0.37	1.47	2.61	6.14	3.40
	10	1.66	0.82	1.51	2.23	7.72	4.37
	50	1.31	0.92	1.43	4.24	3.86	5.01
	100	2.19	0.31	1.03	3.15	3.34	4.78
P.aconitifolius	0.0(Con)	1.36	0.67	9.92	18.66	11.27	6.08
	2.5	1.94	0.64	9.95	15.57	16.43	7.29
	5.0	1.74	0.52	12.53	18.13	16.25	8.66
	10	2.14	0.36	10.15	20.99	29.83	6.46
	50	3.26	0.26	10.18	18.34	18.01	11.12
	100	3.70	1.14	9.41	18.62	19.98	10.22

Values are expressed as Δ O.D. min^{-1} mg^{-1} protein.

germination but exhibited a subsequent marked increase. They have observed maximum germination on the 5th day (120 h) in Eclainochloa frumentacea and Panicum miliaceum and on the 4th day (96 h) in Setaria italica. They have further reported that β-amylase activity in all 3 species reached maximum levels on the 7th day (168 h) and subsequently declined. Shaw & Chuang (1982) have found that α- amylase is quite active between pH 4 to 10 with the optimum at 4.5 in germinating seeds of rice. Observation made by Artsruni and Ponosyan, (1984) indicate considerable increase in α- amylase activity in aleurone layer of fresh seeds of wheat during germination than that of stored seeds.

The influence of sodium chloride salinity on the activity of enzyme α- amylase during germination of C.juncea and P.aconitifolius is recorded in Table 2 and Fig.29,30. It is evident that after 72 h germination (The optimum period) the activity of this enzyme in Crotalaria is inhibited by the salinity treatments. However, at the optimum period of 96 hours in case of Phaseolus there is only a slight effect of salinity rather the activity of the enzyme is stimulated to some extent. Even at the higher salinity levels and after 120 hours of germination the activity is stimulated considerably in this species. Thus this enzyme in Crotalaria appears to be highly sensitive to salinity stress.

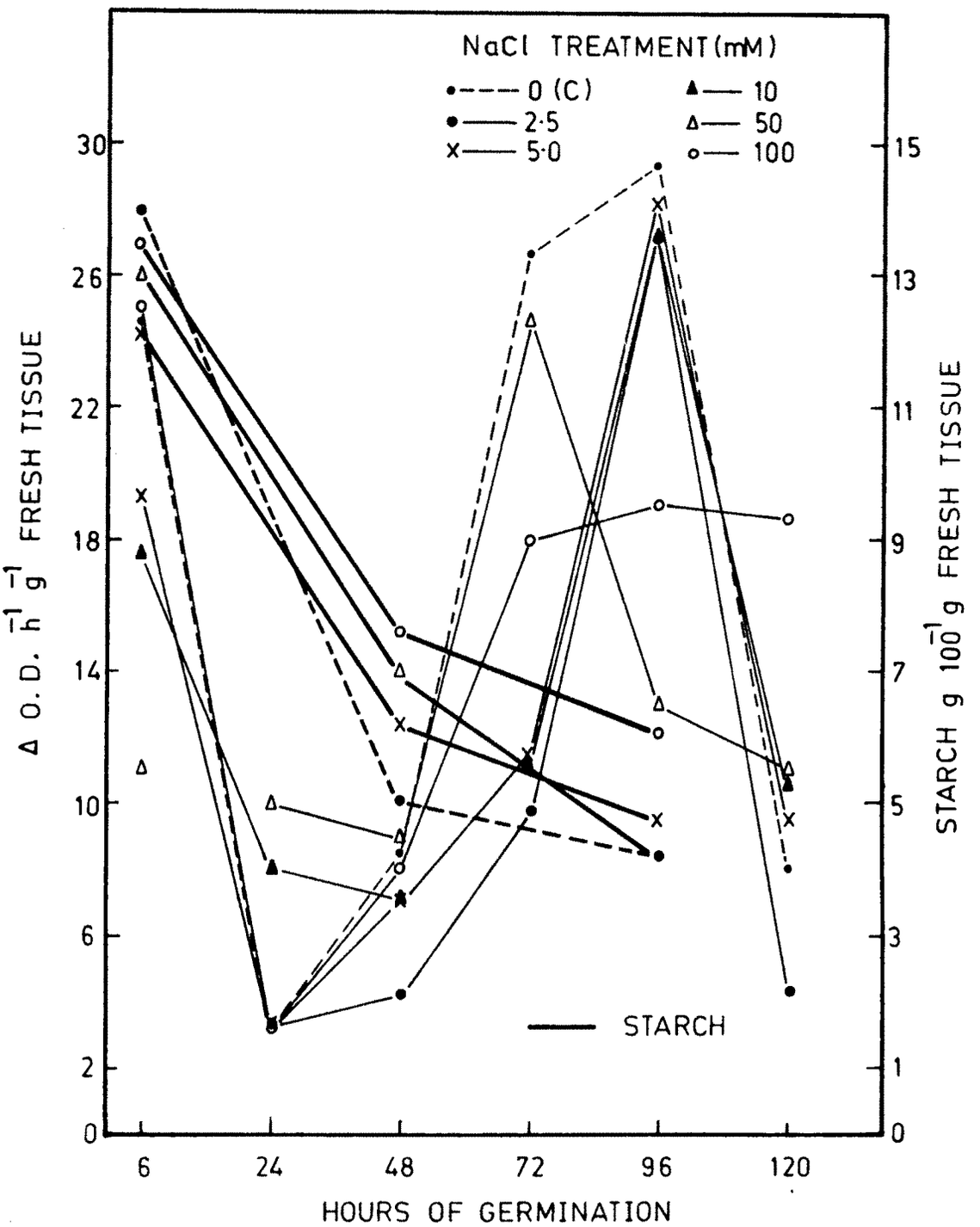

Fig. 29 EFFECT OF NaCl SALINITY ON AMYLASE ACTIVITY AND STARCH CONTENT IN C.JUNCEA SEEDLINGS.

Dixon and Webb (1957) reported that NaCl stimulates the activity of ∝- amylase in wheat seedlings grown in soil containing high levels of NaCl upto 4%. Strogonov (1964) has reported that amylase is activated by NaCl and Na_2SO_4. El-Fouly and Jung (1972) have found an increase in amylase activity in wheat seedlings grown under low levels of NaCl salinity (Sarin and Narayanan, 1968). Dubey (1982) has observed that lower level of NaCl caused an increase in amylase activity but a sharp decrease occured with higher concentration and so was depletion of starch from endosperm in germinating rice (Oryza sativa) seeds under saline stress.

On the contrary to the stimulatory effect, Sarine and Narayanan (1968) have found a delay and decrease in the release of reducing sugars and amylase activity in germinating wheat seeds grown under high concentration of NaCl. Prisco and Vieira (1976) recorded a delay in development of ∝- amylase activity in cowpea seeds during germination under saline conditions. Huber et al., (1974) have noticed similar trend in millet species. Sheoran (1980) has studied changes in amylase during germination and early seedling growth of mung bean (V.radiata) under different salts and observed a decrease in amylase activity due to salt stress in cotyledon, whereas, in embryo axis, roots and leaves an increase was noticed. He

also observed a decrease in number of isoenzymes of amylase in the embryo axis under high salinity. Ogra and Baijal (1982) reported that an increase in salt concentrations in the external medium result in the decrease of oc- amylase activity in both cultivars of Sorghum during germination and seedling growth. They have further noticed that in susceptible variety the decrease was more resulting delayed mobilization of food.

Rodrin and Mercado (1975) observed a varietal difference in rice regarding the behaviour of amylase under saline conditions. According to Sung (1981) comparative salt tolerance in barley cultivars could be evaluated at the germination stage using speed of germination, oc- amylase activity, plumule and radicle elongation as screening indices. In his experiment he found that NaCl treatment significantly inhibited the oc- amylase activity resulting in the retardation of speed of germination and growth of plumule and radicle. Goyal (1985) has tested 4 cultivars of Oat against five saline waters. It was observed that amylase activity declined more in salt sensitive cultivar as compared to that in tolerant one. Jeyachandran and George (1985) have studied relative salt tolerance in four cowpea varieties and have noticed inhibition in amylase activity under salt stress. Gill and Singh (1985) have observed during early induction of oc- amylase in paddy (Oryza sativa L.)

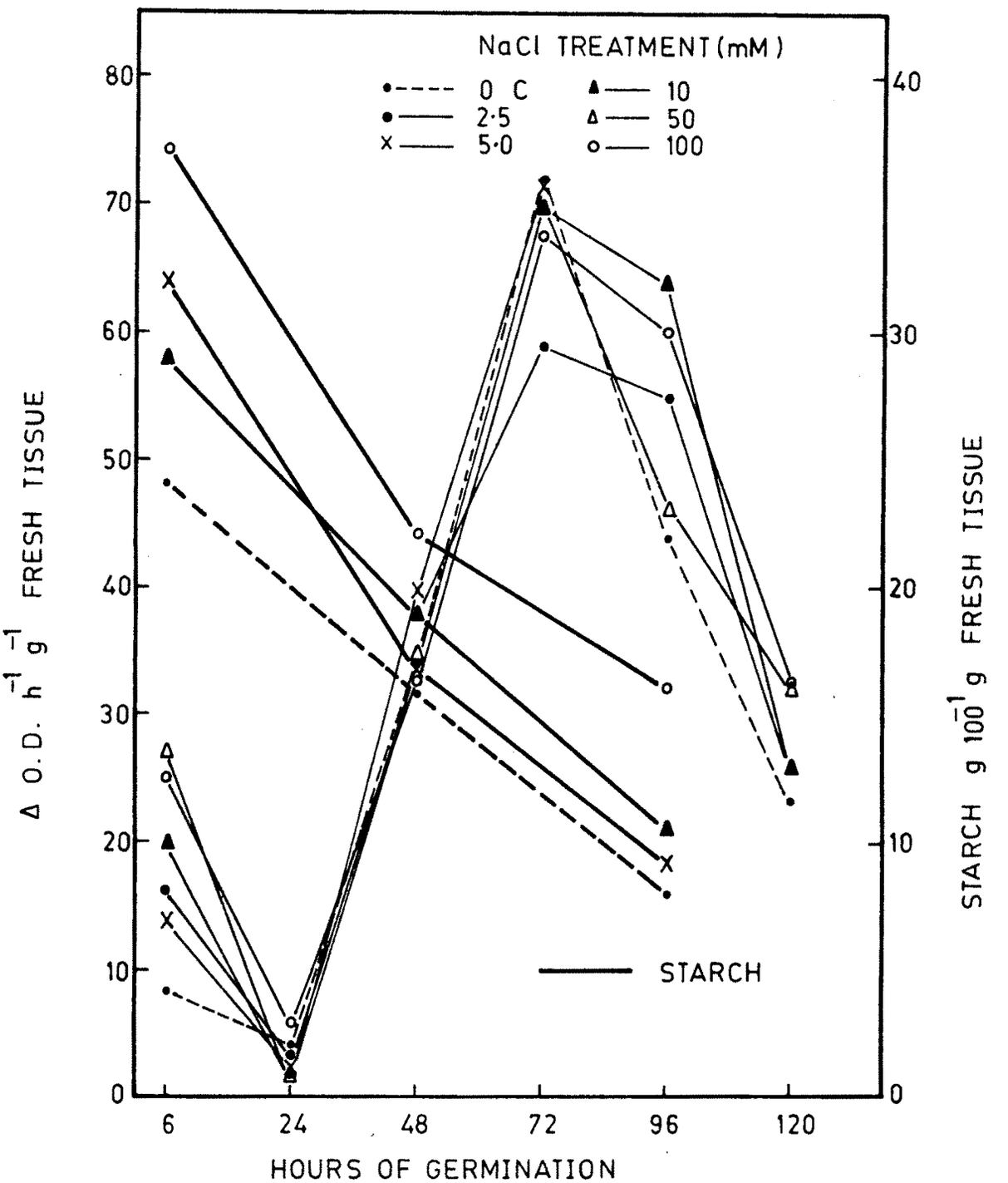

Fig. 30 EFFECT OF NaCl SALINITY ON AMYLASE ACTIVITY AND STARCH CONTENT IN P. ACONITIFOLIUS SEEDLINGS.

seeds during germination under salt stress conditions in tolerant varieties as compared to that in sensitive varieties.

From these reports it appears that the inhibition of α- amylase activity during germination due to salinity is more or less a common feature in most of the seeds. However, the response of amylase to salinity varies from species to species. Higher levels of salinity inhibit the amylase activity. The salinity effects are mainly the osmotic effects affecting the amylase activity (Jones, 1969; Jones and Armstrong, 1971). Irrespective of the cause, amylase inhibition by salinity ultimately leads to the disturbance in carbohydrate metabolism, the inhibition of hydrolysis of reserves and thereby reflecting in the reduction of growth (Sheoran, 1975; Sheoran, 1980 and Ramana & Rama Das, 1978).

Present observations also support the above ideas and further suggest that the salt tolerance in species are well reflected during germination also though the behaviour of the hydrolytic and other enzymes involved in the breakdown and mobilization of reserves in the seeds. Based on the findings with the species under study P.aconitifolius seems to exhibit better tolerance.

D) Acid Phosphatase :

Acid phosphatase is an enzyme involved in non-specific breakdown of a variety of phosphate compounds including ATP (De Leo and Sacher, 1970). According to Flin and Smith (1967) this enzyme plays an important role in the mobilization of nutrient reserves. Besides hydrolytic activities the enzyme is involved in serveral other processes in germinating seeds. Acid phosphatase plays a positive role in germinating seeds and has been correlated with germination capacity by Arbestain Ribas (1977).

The activity of acid phosphatase in the germinating seeds of C.juncea and P.aconitifolius under normal or non-saline and saline conditions is presented in Table 22 and Fig.31. It is clear that the activity of this enzyme goes on increasing and reaches to its maximum at 72 h in C.juncea and then declines rapidly. However, in P.aconitifolius its activity gradually increases with time and continues even upto 120 h.

Popov (1969) recorded stimulation of enzyme acid phosphatase during maize seed germination. Price and Fy (1970) observed that coleorhiza was the most prominent site of enzyme activity in dry wheat embryo. Kulkarni and Rege (1973) have studied the acid phosphatase in seedlings of P.radiatus and

Table 22 : Effect of NaCl salinity on the activity of acid phosphatase in C.juncea and P.aconitifolius during germination

Plant	NaCl Treatment mM	Hours of Germination					
		6	24	48	72	96	120
C.juncea	0.0(Con)	0.93	5.83	31.51	31.83	24.36	22.26
	2.5	0.92	7.35	23.36	32.47	17.28	27.20
	5.0	0.93	8.11	22.63	30.25	15.07	23.16
	10	0.91	6.73	21.42	27.05	24.93	26.11
	50	1.07	5.85	16.93	21.36	29.98	31.90
	100	0.84	4.41	13.39	18.46	27.43	21.16
P.aconitifolius	0.0(Con)	5.40	16.55	43.69	48.29	35.12	61.44
	2.5	3.8	19.06	31.70	36.56	38.32	64.66
	5.0	5.68	16.42	30.22	32.32	40.95	59.62
	10	3.69	15.96	22.98	37.31	66.72	60.77
	50	3.72	16.99	22.70	25.04	41.36	78.54
	100	5.31	16.96	35.49	26.01	28.40	32.32

Values are expressed as Δ O.D. h^{-1} mg^{-1} protein.

suggested the possibility of presence of separate enzymes capable of hydrolysing DPP and AMP specifically. Wieczorek et al., (1975) have found the highest level of acid phosphatase on the 3rd day after barley seed germination. The relation between acid phosphatase content and germinating capacity in different species has been studied by Arbestain (1977). He found decrease in acid phosphatase during the first 24 h (imbibition stage) of germination in all species except sunflower. The acid phosphate content started increasing 24 h after germination. Biswas et al., (1979) have studied acid phosphatase activity in germinating seeds of Vigna sinensis at various hours of germination and observed that activity increases with time. This increase has been attributed by them to de novo synthesis of enzyme protein but not to the activity of the already synthesized protein present in the dry seed. Szczolka and Ewa (1980) found substantial acid phosphatase in the embryo axis of dry resting seeds and its increase in a periodical manner during germination. Klobus (1980) observed relatively high levels of acid phosphatase activity during early phases of cucumber growth. Bansal et al., (1981) have observed continuous increase in acid phosphatase activity upto 7 days in musk melon (Cucumis melo) seedlings.

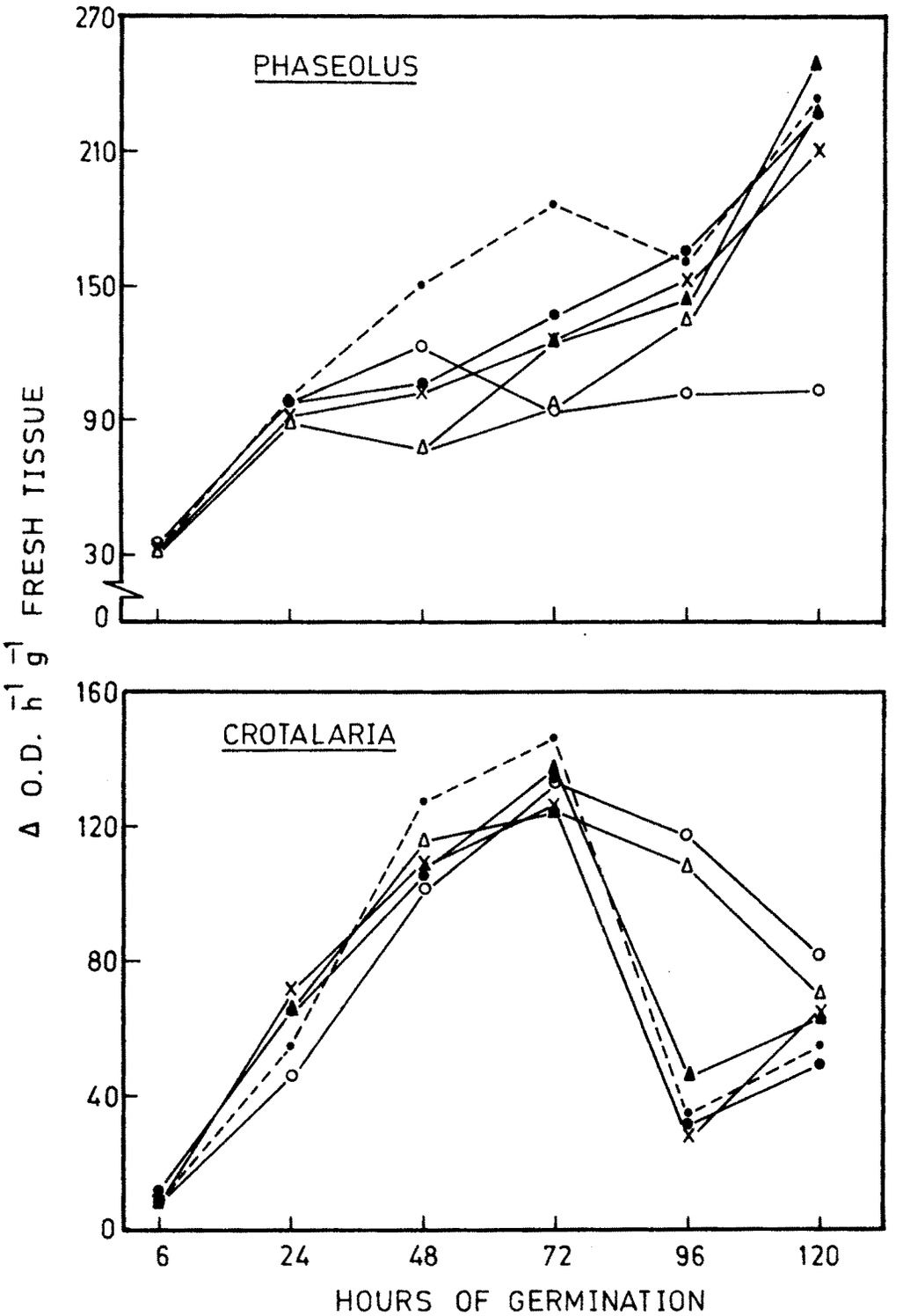

Fig. 31 EFFECT OF NaCl SALINITY ON ACID PHOSPHATASE ACTIVITY IN C. JUNCEA AND P. ACONITIFOLIUS SEEDLINGS.

The effect of NaCl on acid phosphatase activity in germinating seeds of C.juncea and P.aconitifolius is depicted in Table 22 and Fig. 31. It is evident that in C.juncea the activity of acid phosphatase increases linearly with time upto 72 h where it is maximum and declines thereafter. There is slight stimulation in the activity of this enzyme at the lower levels of salinity while it is inhibited at the highest salinity level, during early period of germination i.e. upto 24 h. After 48 h, however, the activity is decreased continuously with increasing salt concentration in the medium upto 72 h germination. After this period there is only a slight effect on this enzyme.

In P.aconitifolius however, there is no remarkable change in acid phosphatase under saline conditions and even it goes on increasing with time upto 120 h. The highest level of salinity (100 mM NaCl) inhibits the enzyme activity and that after 72 h of germination.

Zhukovskaya (1971) observed that both chloride and sulphate salinities activated glucose-6-phosphatase and alkaline and neutral fructose-1-6-diphosphatases in roots and leaves of barley, millet, sunflower and tomato. She further reported that some new phosphatases were induced by salinities

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which were not active in control plants. El-Fouly and Jung (1972) have observed a considerable increase in acid phosphatase under the conditions of NaCl and Na_2SO_4 salinities. Ahmad and Huq (1974) have also noticed an increase in acid phosphatase activity in the leaves of halophytic spinach under saline conditions. Most of these observations indicated a shift towards catabolic activities induced by salt stress. However, Narsagaudar et al., (1979) have observed an inhibition of acid phosphatase due to salinity during germination of Sorghum.

From the present results it appears that acid phosphatase in the germinating seeds of both the species responds in the similar manner to salinity. However, basically, the level of this enzyme seems to be higher in P.aconitifolius and it continues to increase with time even upto 120 h. Probably this basic difference makes the difference in the salinity tolerance in these species. P.aconitifolius thus appears to be better adapted to the saline conditions.

E) Nitrate Reductase (NR) :

Nitrate is the major source of nitrogen available in the soil to the plants. It is reduced to ammonia prior to its assimilation. This process of nitrate reduction takes place in two steps (i) the reduction of NO_3^- to NO_2^- and (ii) the

reduction of NO_2^- to NH_3. The first step is catalysed by the enzyme 'nitrate reductase' (NR) and the second by 'Nitrite reductase' (NiR).

Nitrate reductase (EC 1.6.6.1) was detected for the first time by Evans and Nason (1953) in soybean leaves. This enzyme is a complex of two proteins. The molecular weight is obviously very high. It is present in all parts of the plant e.g. cotyledons, root, shoot leaves (Beevers and Hageman, 1969) and embryos (Rijven, 1958). The NR activity varies with parts and age of the part of a plant.

The control of NR by light through phytochrome was first shown by Jones and Sheard (1972) in Pisum arvense. This has also been shown in seedlings of Sinapis alba (Johnosn, 1976), maize (Duko and Duke, 1978) and wheat (Vijayaraghavan et al., 1979). Kwon and Hung Sik (1981) have found the relationship between enzyme activity and pH and temperature during soybean germination. They have reported 6.5 as the optimal pH and 30° the optimum temperature.

Effect of NaCl salinity on the activity of nitrate reductase (NR) in C.juncea and P.aconitifolius seedlings has been presented in Table 23 and Fig.32. It is evident that NR

Table 23 : Effect of NaCl salinity on the activity of nitrate reductase in C.juncea and P.aconitifolius during germination

Plant	NaCl Treatment mM	Hours of Germination					
		6	24	48	72	96	120
C.juncea	0.0(Con)	0.02	0.07	0.18	0.19	0.25	0.50
	2.5	0.05	0.10	0.12	0.23	0.29	,0.43
	5.0	0.05	0.05	0.21	0.20	0.18	0.23
	10	0.05	0.10	0.14	0.17	0.12	0.26
	50	0.05	0.05	0.10	0.08	0.23	0.19
	100	0.05	0.054	0.05	0.12	0.13	0.25
P.aconi-tifolius	0.0(Con)	0.19	0.05	0.16	0.24	0.31	0.62
	2.5	0.19	0.09	0.13	0.25	0.27	0.60
	5.0	0.12	0.05	0.14	0.25	0.26	0.47
	10	0.12	0.06	0.06	0.17	0.20	1.10
	50	0.12	0.12	0.08	0.29	0.21	2.85
	100	0.10	0.13	0.10	0.34	4.20	5.55

Values are expressed as $\mu g\ NO_2\ h^{-1}\ mg^{-1}$ protein.

activity in C.juncea under non-saline conditions increases with time reaching to its maximum at 48 h stage of germination. In P.aconitifolius, however, the pattern of this enzyme is different. There is a dramatic fall in the activity of the enzyme after 24 h of germination as compared to that after 6 h (from 2.01 to 0.69 $\mu g\ NO_2^-\ h^{-1}\ g^{-1}$ fresh wt). This activity is slowly but gradually recovered through the further development of the seedling and after 120 h it is the maximum (2.6 $\mu g\ NO_2^-\ h^{-1}\ g^{-1}$ fresh tissue).

Chauhan and Srivastava (1980) have studied some aspects of regulation of NR in the seedlings of Cuscuta reflexa. They have observed that enzyme activity varied with seedling age. Schrader et al., (1968) have studied this enzyme from the leaves of higher plants namely maize, marrow and spinach. They have estimated a half-life of approximately 4 h for NR from the inactivation studies with excised corn seedlings. Radian (1974) observed that the activity of NR in roots and cotyledons of cotton seedlings increased rapidly during germination reaching to the maximum after 1 day imbibition and then declined until emergence and greening of the cotyledons when it began to increase steadily.

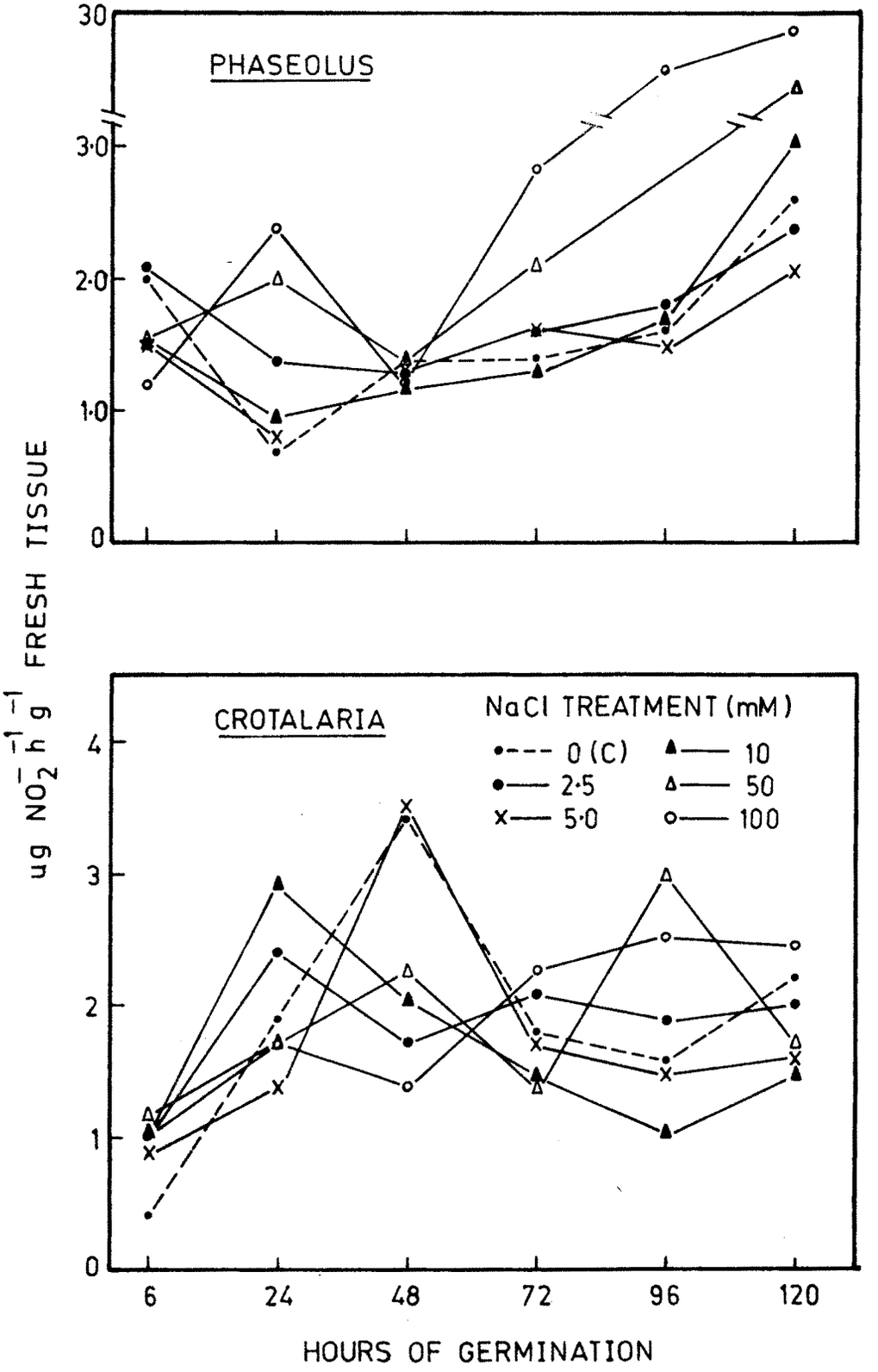

Fig. 32 EFFECT OF NaCl SALINITY ON NITRATE REDUCTASE IN C. JUNCEA AND P. ACONITIFOLIUS SEEDLINGS.

The effect of NaCl on NR in C.juncea (Table 23 and Fig. 32) seedlings indicates that NR activity at 48 h of germination is maximum under 5 mM NaCl treatment. However, in P.aconitifolius seedlings it increases with time and salt concentrations in the medium. It can be seen that rate of NO_2^- liberation is tremendously high enough in the seedlings treated with 50 and 100 mM NaCl and that after 96 h of growth. It appears that the activity of the enzyme is relatively affected more in Crotalaria and that in Phaseolus seems to be insensitive to salinity.

Strogonov (1964) has pointed out that the nitrogen metabolism is the main target of salt injury in plants. Bardzik et al., (1971) have studied the effect of water stress on the activity of enzymes of nitrogen metabolism in maize seedlings. They have measured the changes in the activity of 3 enzymes and found that NR activity decreased markedly with water deficits of 10 to 20.7%. They have also suggested that progressive tissue dehydration reduced both the enzyme synthesis as well as the enzyme inactivating systems.

Plaut (1974) has observed that NR was inhibited as a result of reduced soil moisture potential or application of NaCl to the nutrient solution. The decrease in enzyme activity

in wheat seedlings, exposed to salinity, was found 24 hours after exposure to stress. They have also found a recovery of the enzyme after the removal of seedlings from saline medium. They have suggested that a fraction of NR may be located in the cytoplasm and looses its activity during stress, probably due to inhibited protein synthesis. Balderston et al., (1977) have noticed significant inhibition in NADPH dependent NR activity in crude extracts of T.viride by physiological concentration of NaCl and KCl but not by $(NH_4)_2SO_4$ and Na_2SO_4. The NR inhibition increased in a linear manner with increase in chloride concentration.

Riko et al., (1978) have studied the effect of NaCl and Na_2SO_4 salts on the activity of enzymes involved in the primary assimilation of nitrate and ammonia nitrogen. The response of the enzymes, to the salts added in vitro, did not depend on the salt resistance of a plant. The enzymes are arranged in the following series according to their salt resistance : Glutamine synthetase > Glutamate dehydrogenase > NR. Sahulka (1978) has observed that the presence of Cl in nitrate containing nutrient solution resulted in lowering the NR level in excised pea roots. However, counterions supplied together with Cl^-, tended to modify slightly this general trend. The negative effect of Cl^- was also apparent when Cl^- were applied before

nitrate ions. They have also suggested that more reliable and accurate results can be obtained with the in vitro than with in vivo method.

Kabisheva et al., (1980) have observed a reversible dissociation of molybdate and accumulation of significant quantities of nitrates, inhibiting plant growth and development are the factors responsible for the decline in the activity of pea root NR. Billard and Boucaud (1982) have found that the NR activity extracted from S.maritima is reduced by half in the presence of 0.1 M NaCl. They have reported thatt in Suaeda, the NR activity be the resultant of the effect of NaCl which inhibits the catalytic activity of the enzyme and stimulates the synthesis of the enzyme protein. Kumar et al., (1985) have determined the NR activity in two cultivars of pearl millet (Pennisetum americanum L.) at early seedling stages under saline conditions. They have observed that salinity did not affect NR activity till 72 h but significantly decreased with increasing salinity levels after 96 and 120 h imbibition.

Contrary to these findings, stimulatory effects of salts on NR have also been found by a number of workers. Sankhla and Huber (1975) have observed that both NaCl and ABA (abscisic acid) promoted the in vivo activity of NR in cotyledons and

leaves of 4 day P.aconitifolius seedlings. The effect of salt was much more pronounced than that of ABA. Present studies with the same species also confirm these findings. Sinha and Rajgopal (1975) have studied the effect of moisture stress on NR activity in three drought tolerant Sorghum cultivars and found that the two tolerant cultivars retained NR activity longer under stressed conditions. Smith et al., (1980) found marked stimulation of NR by NaCl in rye grass (Lolium perenne) and timothy (Phleum pratense). They have suggested that this stimulation was more the result of increased uptake of nitrates than a specific effect of this salt on the enzyme. Luque and Bingham (1981) have seen that lowering the osmotic potential of nutrient solutions resulted in decreased concentration of NO_3 in the plant, little or no effect on uptake and increased the NR activity. Increased rates of NO_3^- reduction were in particular associated with Cl^- concentration of the nutrient solution.

From the foregoing discussions it appears that activity of NR varies from species to species, their age and parts involved. Under saline conditions it responds well within lower limits of salt concentrations. Higher concentrations inhibit the activity. According to Shevyakova and Leonova (1975) stimulation or inhibition of protein synthesis under

salinized conditions depends on the activity of functional ribosomes and of the related enzymes.

In the present investigation the NR activity in Phaseolus under saline conditions clearly signifies the salt tolerant nature of the species. It is well established that NR is a substrate inducible enzyme and as in our experiment we have used either distilled water or a salt solution without any nutrients, particularly without any nitrogen source, as the media, it is rather difficult to explain why the activity of the enzyme is elevated remarkably in the seedlings treated with 50 and 100 mM NaCl after 96 or 120 h of germination. It is probable that during germination nitrates are formed during protein breakdown which induces the enzyme. Higher percentage of germination in this species under saline conditions even at higher salinity levels and that inhibition in Crotalaria clearly indicates that in Phaseolus, the rate of breakdown of proteins is higher and continued for longer periods even under higher salinity levels. This undoubtedly suggests the salinity tolerance in Phaseolus.