Chapter IV

SUMMARY AND CONCLUSIONS

Salinity has posed a problem to crops either on salt affected soils or under saline water irrigation. Millions of hectares of land throughout the world is saline and unproductive. In India there are about 12-15 million hectares of saline land distributed in the states of Punjab, Rajasthan, U.P. and Deccan and Coastal areas. About one third of irrigated soils in India, amounting to almost 77 million hectares are said to be sufficiently affected by salinity so as to adversally affect crop growth. Unfortunately no cheaper methods of desalination of soil or water have been achieved to overcome the salinity hazards. According to Epstein et al., (1980) besides an engineering approach, the development of crops tolerant to salinity is a better strategy for meeting the challange of salinity problem. To achieve this, it is of prime importance to understand the performance and the physiology of plant species and the cultivars under saline conditions.

Salinity puts various problems to plants at the population the individual, the physiological and molecular level. The overall reflection of all the problems occurs in the reduction of growth. The work on salinity for the last several decades has lead to the formation of two different schools of thoughts regarding the nature of salt injuries. The champions of osmotic school (Bernstein and Hayward, 1958) claim that most of the adverse effects of salinity are related to the decreased osmotic potential of saline root media. On the contrary the champions of specific ion school (Eaton, 1942; Strogonov, 1964), propose that the adverse effects of salinity are caused mostly by the specific effects of individual ions.

Salinity has far reaching effects on the plant metabolism which ultimately results in impairement of growth and loss in overall productivity. This is true for most of the crop species. At the same time it can be noticed that at salt concentrations injurious to conventional strains, certain plants perform well. Survey made by Maas and Hoffman (1977) indicated that the crop plants differ in their tolerance capacity and broadly be classified into sensitive, moderately sensitive, moderately tolerant and tolerant groups. The varietal differences are also noticed by a number of workers with respect to salt tolerance potential.

The plant species exhibit varying degrees of salt tolerance with respect to the stage of development. It is generally believed that the plants are more sensitive to salinity at germination than at later stages of growth. However, according to West and Taylor (1981) generally there is a poor correlation between salt tolerance at germination and that at later stages of growth.

The First phase of plant growth the seed germination and seedling growth is critical under saline conditions. The ability of a given variety to germinate and establish the seedling is frequently the limiting factor in crop production. This process being the first and the most important phase, it is of utmost importance to study the effect of salts on this process.

Salinity causes several metabolic disorders in the germinating seeds which can be immediately recognized by changes in the pattern of Key enzymes and metabolities. The disorders ultimately reflect in growth retardation and failure of emergence of normal seedlings. The literature on metabolic processes taking place during germination is voluminous. Yet it is not possible to explain all the aspects of this process. It is noteworthy that most of the germination studies are centred around the cereal crops like rice, wheat, maize, barley etc. Considerable work has been done on pulse crops by various research centres throughout the world. There are intensive investigations on the metabolic processes of pea, mung bean soybean gram etc. Among the legumes relatively little attention has been paid to <u>Phaseolus aconitifolius</u> and <u>Crotalaria juncea</u>, so far their salt tolerance ability is considered. In the present investigation, therefore, an attempt has been made to have preliminary idea about the response of these plants under the salt stressed conditions during germination.

For the present studies the seeds were subjected to various concentrations of NaCl and $CaCl_2$. The concentrations used are ranging from 2.5 to 300 mM. Control was maintained under non saline conditions. The seedlings were grown in Petridishes under sterilized conditions. The treatments were continued for 120 hours. The effect of salts on germination percentage was studied by making the observations after every 24 hours. The seedlings were analysed for their linear growth and biomass production and moisture percentage after 120 hours of growth. Germination percentage proline, some enzymes like peroxidase, catalase, amylase, acid phosphatase and nitrate reductase have been studied after every 24 hours of growth till 120 hours. However, the carbohydrates were estimated from the seedlings after 6, 48, and 96 hours of germination at the levels of 5, 10 and 100 mM NaCl concentrations. The significant findings of the present investigation can be summarised as follows :

1. GERMINATION :

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 h. All the concentrations of NaCl used are found to be inhibitory for *C.juncea* during germination at all the time. The response of P.aconitifolius, however, is different. Lower salt concentrations (10 to 80 mM NaCl) stimulate the germination in this species and that within 48 hours. The higher concentration, however, slightly affect the germination. The effect of $CaCl_2$ on germination in these two species is almost similar to that by NaCl.

2. SEEDLING GROWTH :

It is found that in both the species there is retardation in seedling growth as salinity increases. It is also observed that salt (NaCl) concentrations beyond 80 mM strongly affect the seedling growth in both the species. NaCl concentrations upto 10 mM seem to be without any effect on the growth and development of P.aconitifolius seedlings. On the other hand C.juncea shows some improvement at the lowest (2.5 mM) salinity level. Under $CaCl_2$ salinity, stimulation in growth of seedlings of both the species is observed till 10 mM salt concentration. However, higher concentrations inhibit the growth and development of seedlings in both cases. When the results with $CaCl_2$ with respect to seedling growth are compared with those with NaCl, it appears that NaCl effect is more detrimental than that of $CaCl_2$.

It is also observed that root growth is affected by salinity in <u>C.juncea</u>, particularly at the higher salt concentrations beyond 10 mM. However, it is the shoot growth, which is affected by salinity and that after 60 mM salt level in the medium.

<u>C.juncea</u> indicates slight gain in biomass (fresh wt) of the seedlings at 2.5 mM NaCl treatment. However, in <u>P.aconitifolius</u> an appreciable gain in fresh wt is observed upto 10 mM NaCl concentrations. Under $CaCl_2$ stress, there is inhibition of biomass production in case of <u>C.juncea</u> while in <u>P.aconitifolius</u> it is bit stimulated or almost equal to in lower levels of salinity upto 5 mM. It appears that <u>P.aconitifolius</u> does well under both the saline conditions.

3. <u>WATER UPTAKE AND DRY MATTER CONTENT</u> :

Moisture uptake increases very rapidly when germination commences, under normal conditions. However, due to the presence of salt in the medium, water uptake is hindered in both the legumes. Higher levels of salinity (of NaCl and $CaCl_2$) inhibit the water uptake by seeds.

A direct relationship of dry matter content of germinating seeds of <u>C.juncea</u> and <u>P.aconitifolius</u> with salt concentrations used has been observed. Due to salinity, moisture uptake is retarded which in its turn is reflected in inhibition of breakdown of food reserves of the seed. The results indicate that whenever, the values of dry matter are more in any treatment (NaCl or $CaCl_2$) the germination is reduced. It appears that both the legumes behave almost in the same way to both the salts. Only the difference is that, the dry matter values in seedlings treated with $CaCl_2$ are comparatively higher.

4. <u>ORGANIC CONSTITUENTS</u> :

A) <u>Carbohydrates</u> : Carbohydrate content decreases very rapidly during germination. This indicates rapid breakdown of food reserve during germination. Under saline conditions, there is progressive utilization of carbohydrates with time but not with salt concentrations, suggesting inhibition of hydrolyzing enzymes by salinity. Starch is rapidly degraded during germination. This rapid depletion of starch, however, is inhibited by the salt concentrations. Higher the salinity more is the starch in both the germinating legumes. Total sugars are fairly low in both the seeds and showed no significant changes due to salt stress.

It is suggested that both the species respond in the similar manner to the salinity stress. However, <u>P.aconitifolius</u>

shows early induction of amylase activity even under salt stress conditions suggesting that it has a better tolerance capacity than C.juncea.

B) Proline :

Proline accumulation takes place appreciably in C.juncea as compared to that in P.aconitifolius seedlings. In C.juncea its accumulation increases with increasing salt concentration at all the time except at 6 hours stage. However, in P.aconitifolius proline accumulation takes place during the later stages of growth. It appears that Phaseolus shows some tendency of slow but steady accumulation of proline in the seedlings during the autotrophic phase of development (96 and 120 hours). 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.

5. ENZYMES :

A) Peroxidase :

Peroxidase is not activated during the first 6 hours of germination in both the species. In C.juncea, the activity of the enzyme increases with time upto 72 hours and declines thereafter. However, in P.aconitifolius it goes on increasing with time.

Peroxidase in C.juncea is bit stimulated at the lower salt concentrations (2.5 and 5.0 mM) after 24 hours of germination and decreases in the higher concentrations. Salt concentrations beyond 10 mM inhibit the activity of this enzyme. In P.aconitifolius, it is inhibited under salinity stress. However, under any given treatment it increases with time except in case of seeds treated with 100 mM NaCl. This increase in the activity of peroxidase with time even under saline conditions appears to be an adaptive feature of P.aconitifolius to salinity stress.

B) Catalase :

The activity of catalase increases steadily upto 48 h of germination and declines thereafter in both the legumes under study. In C.juncea it is stimulated by salinity levels upto 50 mM at all the time and it appears that increasing levels of salinity stimulate the activity of this enzyme. It is relatively less affected in P.aconitifolius under salt stress conditions. From the results it appears that P.aconitifolius is able to maintain the catalase level high enough even under saline conditions during germination.

C) Amylase :

Activity of amylase in *C. juncea* seedlings increase upto 96 hours of germination except at 6 hours stage and declines thereafter. Whereas in *P. aconitifolius* it increases steadily and reaches to its maximum little earlier i.e. at 72 hours stage and declines thereafter. Under the influence of NaCl salinity the activity of α- amylase in *C. juncea* is inhibited even at the lower salt concentrations. In case of *P. aconitifolius* the activity is stimulated by the salt treatment at all concentrations of salt and at all the periods of development, except a dramatic fall at 6 hours stage of germination. Thus this enzyme in *Crotalaria* appears to be highly sensitive to salinity stress showing inhibitory effect of salt on breakdown of stored food and its mobilization for the growth of seedlings.

D) Acid Phosphatase :

Activity of enzyme acid phosphatase in *C. juncea* increases with time upto 72 hours and declines thereafter, under non-saline conditions. In *P. aconitifolius*, however, it increases with time even upto 120 hours of germination. In *C. juncea* the activity is slightly stimulated by lower levels of salt during early period of germination. During later periods also salt has produced no significant effects. In *P. aconitifolius*, however, there is no remarkable change in acid phosphatase activity under saline conditions and even it goes on increasing with time upto 120 hours.

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. Probably this difference makes the difference in the salinity tolerance in these species.

E) Nitrate reductase :

The activity of nitrate reductase in C.juncea increases with time upto 48 hours of germination under non-saline conditions. However, in P.aconitifolius there is a dramatic fall in the activity of the enzyme after 24 h of germination as compared to that after 6 h. However, it is slowly recovered through further development of seedlings after 24 h of germination.

There is no adverse effect of different salinity levels during early stages (6 and 24 h) of germination in C.juncea. However, later almost all the treatments appear to be inhibitory. On the contrary, NRA in P.aconitifolius increases with salt concentrations during later periods of germination.

It is concluded that the salinity effect on the physiology of germinating seeds varies from salt to salt as well as from species to species. NaCl seems to be more toxic than $CaCl_2$. Salinity affects the seedling growth variously in *C.juncea* and *P.aconitifolius* and from the overall performance and the changes in the physiology of the seedlings under saline conditions, *P.aconitifolius* appears to be a salt tolerant species while *C.juncea* a salt sensitive one.