Chapter - IV

Summary and Conclusions

Increasing soil salinity has become one of the threatening problems of modern agriculture, alarming particularly in developing countries like India, Pakistan and Arab, where poor irrigation management and traditional methods of cultivation are practiced with unwise application of fertilizers. In India this problem is confined to arid and semiarid regions more severely. More than 12 million hectares of land in our country is saline and unsuitable for crop production (Sharma and Gupta, 1986).

To overcome this problem for better agricultural yields, two solution approaches have been suggested. An engineering approach have been suggested. An engineering approach which leads with minimizing the soil salinity level, but, is highly costly and unaffordable for Indian farmers. The strategy of screening and identifying crop species for their salt tolerance, a biological approach, seems to be practicable in Indian conditions. To achieve this, however, it is highly essential to understand the physiology of crop plants which will help to understand the mechanism of salt tolerance in them.

Salinity puts various problems for a plant growing in such a medium. It profoundly influences growth and yield of a plant. Plants growing under such conditions are typically stunted. Besides this, anatomical and morphological changes are also brought about. Salinity is also known to affect metabolic processes such as photosynthetic CO_2 assimilation,

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protein synthesis, respiration etc. Glycophytes are generally sensitive to salinity which show drastic changes, while halophytes show a better growth, under saline conditions. Accordingly the plants are categorised for their salt tolerance into salt tolerant, moderately salt tolerant and salt sensitive species.

Most of the adverse effects of salinity are related to the decreased osmotic potential of saline root media or caused mostly by the specific effects of individual ions. The reduction in plant growth under salt stress conditions may be regarded as a consequence of the requirement for osmotic adjustment and the primary mechanism for affecting it. The change in ionic balance in salt stressed plants is the shift in ionic interactions with the enzymes and intermediates of metabolism at several levels.

Salinity influences growth and development of a plant during every phase of its life cycle. It is well known that the salinity tolerance of a plant varies with stage of development also. Seed germination is the first and the most important event in the life cycle of a higher plant. It is the sum total of all the physiological processes, occuring inside the seed which starts with the imbibition of water and ends with the protrusion of embryonic root. It is a very complicated dynamic process involving several biochemical pathways.

Water brings about the hydration of the cell wall and protoplasm and the enzymes in the cotyledon cells are activated to digest the stored food material which is converted into soluble form and transported to the radicle and plumule tips. In this process stored carbohydrates are converted into soluble sugars, the fats are digested into glycerol and fatty acids. These are partly used in respiration and partly in constructing cell wall. The digestion of storage proteins results in the production of amino acids which synthesize the structural proteins from which new protoplasm is built up in the cells.

Environmental factors like light, water, temperature and soil have marked influence on the germination of seeds. Both physical and chemical properties of the soil as well as composition and moisture level influence the seed germination as well as seedling growth. Saline soil is non-alkali soil containing soluble salts in large quantities and having pH 8.5 which adversally affects the growth of a plant.

The phase of germination and seedling growth is a critical one, as the ability of a crop to germinate and establish the seedlings, frequently becomes a limiting factor in crop production. But, there is not always a positive correlation between salt tolerance at germination and during the later stages of growth. Still the knowledge of salt and sodium tolerance of germination and early seedling growth is of primary importance as the initial stand determines the final production of a crop to a large extent.

Usually soil salinity delays and inhibits germination in a number of plants. The time and rate of germination under saline conditions, however, are severely affected. Salinity causes several metabolic disorder in the germinating seeds that can be recognized immediately by changes in the pattern of key enzymes and metabolites, ultimately leading to the retardation of growth and failure of emergence of normal seedlings.

Most of the studies with respect to salinity tolerance in crops are centered around the cereal crops. Considerable work has been done on pulse crops also at various research centers, throughout the world. The extensive work especially, has been done on the legumes like pea, soybean, gram etc. However, most of the legumes have been relatively less investigated for their salt tolerance. There are numerous minor grain legumes which are cultivated on smaller scales and inspite of their high nutritive value and wide range of adaptability, they have received, however, scant attention from the scientific community and therefore, in the present investigation an attempt has been made to test the local cultivars for some of the legumes for their salt tolerance during germination, particularly, the effect of NaCl salinity at different concentrations on germination percentage, linear growth of seedlings (epicotyle, hypocotyle and root length), biomass (fresh and dry wt) production and the rate of moisture uptake, soluble proteins, carbohydrates and the

activities of hydroxyperoxidase i.e. peroxidase and catalase, under saline conditions has been determined. Similarly, the behaviour of acid phosphatase in the germinating seeds under saline conditions at different developmental phases, has also been studied.

The significant findings of the present investigation are as follows.

1) Germination :

P.aconitifolius and P.aureus seem to be salt tolerant as the percent germination in them is not much affected by salinity. However, there is decrease in germination percentage with increasing salt concentration in the medium and even there is no germination at 300 mM NaCl in D.biflorus. In L.esculenta also, the germination is delayed and inhibited with increasing salt concentration. It appears that D.biflorus and L.esculenta are moderately salt tolerant while T.foenum-graecum exhibits salt sensitivity during germination.

2) Linear Growth :

Horse gram has shown successful development of epicotyle and unfolding of leaves only upto 100 mM NaCl. In case of 25 mM NaCl treated seedlings, growth of the seedlings is equal to that in control seedlings (1.5 cm) at 120 h. However, the epicotyle formation in this species is strongly affected by salinity beyond 50 mM NaCl level. In case of lentil it is

observed that, there is more than 50% reduction in the epicotyle length of the seedlings grown at 25 mM NaCl level and at higher salt concentrations it is completely inhibited. Moth bean seedlings have shown better development and unfolding of leaves in control as well as under saline conditions upto 50 mM NaCl level. 100 and 200 mM NaCl levels, however, seem to be inhibitory. P.aureus has also given a good response upto 50 mM NaCl level. Concentration higher than this, however, induces reduction in epicotyle growth. The green gram probably shows better adaptation to adverse conditions at least during seedling establishment. As there is no unfolding of leaves, fenugreek seems to be very slow in development, as even in the non-saline conditions, there is no leaf formation upto 120 h germination.

It is observed in general that, in plants the seedlings are well established before 72 h germination except in P.aureus. It is also observed that the length of the hypocotyle increases with time while decreases with increase in salinity level. The maximum hypocotyle length has been recorded in P.aureus seedlings. While, the fenugreek seedlings produce the shortest hypocotyles and hence P.aureus seems to be sensitive as there is no seedling formation even upto 120 h at 200 and 300 mM NaCl. From 25 mM onwards, it is decreased and strongly suppressed at 300 mM NaCl. Lentil is found to be relatively sensitive, where in hypocotyle length is increased slowly upto 96 h of germination. However, 100, 200 and 300 mM salt concentration are found to be inhibitory.

At lower salt regimes, the hypocotyle development in P.aconitifolius is stimulated. However, the higher salt concentrations suppress the hypocotyle length in this plant. In horse gram, at 300 mM NaCl, there is no root emergence even upto 120 h germination. It is observed that the root growth is affected by more than 15% at 200 mM NaCl in D.biflorus and P.aureus, while, in case of L.esculenta and T.foenum-graecum it is of still lower salt concentration (100 mM). The seedlings of P.aureus has shown the highest root length after 5 days of growth while the shortest root produced after this period is that by fenugreek. D.biflorus and P.aconitifolius have shown almost similar pattern in the rate of growth of root system.

From the observations it is concluded that P.aureus and D.biflorus have a better performance under saline conditions.

3) Biomass Production :

In general, with increase in the period of germination, the fresh wt of a seedling in all plants investigated is increased, but decreased with increasing salt concentration in the medium.

In P.aureus the highest fr.wt of a seedling is reported under saline conditions. P.aconitifolius also exhibited the salt tolerance nature as the biomass of the seedling increases under saline conditions. In horse gram, fresh wt of a seedling slowly increases with time but decreases with salt concentration exhibiting rather salt sensitive nature. In fenugreek the process

of biomass (fresh wt) production is very slow and it is only slightly affected under saline conditions.

As far as the dry wt is concerned, it is observed that, in all plants studied, the dry wt of a seedling grown under saline conditions remains unaffected. It appears that salinity probably affects the water uptake, thereby affecting the fresh wt of a seedling.

4) Moisture (water) Uptake and Dry Wt of Seedlings :

The relationship between moisture uptake and the dry wt of a seedling at 72 and 96 h of age indicate the critical concentration of salt for the growth and development of a seedling. It is observed that 100-200 mM NaCl in the medium is probably the critical concentrations of salt upto which level D.biflorus seeds can tolerate the salinity. L.esculenta seeds seem to be highly sensitive to salinity during germination and the salt concentration beyond 25 mM NaCl seems to be toxic or inhibitory for the process. In case of P.aconitifolius 80 mM NaCl is found to be the critical concentration for seed germination indicating its moderately salt tolerant nature.

Though P.aureus shows three different critical concentrations, 100 to 120 mM NaCl can be taken as the critical concentration. Thus, P.aureus is a moderately salt tolerant species particularly during germination. Fenugreek also showed three critical concentrations (50, 180 and 200 mM NaCl). However, 96 h old seedlings showed about 80 mM as the critical level of salt for seed germination.

5) Carbohydrate Metabolism :

It is observed that, in all the species investigated, starch is remarkably utilized for the development of seedling as with the advancement of stage of germination in all the species, the level of starch falls down regularly. It is also observed, that, the salinity profoundly affects the process of breakdown of starch almost in all species.

It has been found that, the soluble sugar concentration in the germinating seeds of D.biflorus falls down markedly at 200 mM NaCl level, however, upto 100 mM NaCl there is no significant change in the accumulation of soluble sugars in the seeds. The process of starch degradation is severely affected at 200 mM NaCl level indicating its sensitivity at this concentration.

With the advancement of germination more and more sugars are released and accumulated in the seedlings of P.aconitifolius. This species can tolerate the NaCl salinity upto 50 mM NaCl (in case of 96 and 120 h old seedlings), and 100 mM salt concentration strongly affects the level of soluble sugars.

In case of lentil, a linear increase in soluble sugars is observed in the seedlings growing under saline conditions. However, it is also observed that, 100 mM NaCl level seems to be strongly inhibitory at 120 h stage. These observations indicate that, the utilization of starch for germination and seedling formation is continuous and is less affected.

P.aureus shows accumulation of soluble sugars upto 72 h of germination at which stage, the seeds contain the highest amount of soluble sugars. Release of soluble sugars and their accumulation is affected by both 100 and 200 mM NaCl. However, there is no regular pattern of starch degradation.

In fenugreek, the soluble sugar content of the germinating seeds considerably increases after 72 h of germination and then falls down and remains constant at 96 and 120 h. It is also observed that, 100 mM and above concentrations of salt restrict the accumulation of soluble sugars in the seedlings. Thus, it can be suggested that the response of carbohydrate metabolism to salinity varies from species to species. Higher concentration of salts in the medium, however, inhibits the breakdown of carbohydrates.

Soluble Proteins :

It is found that most of the legume species investigated respond equally (except fenugreek) to salinity with respect to protein metabolism. It is observed that, 200 mM NaCl is toxic for the release of soluble proteins in the germinating seeds of all the species. This situation prevails even at 100 mM NaCl level in lentil and horse gram showing the sensitivity of these species to saline conditions during germination.

6) Enzymes :

A. Peroxidase :

The highest activity of enzyme peroxidase has been observed in the dry seeds of lentil followed by finugreek and the least being in the D.biflorus and P.aureus.

In D.biflorus activity of this enzyme is less influenced under saline conditions giving no definite pattern in the germinating seeds. 200 mM NaCl level seems to be inhibitory, particularly after 48 h germination where it is completely suppressed. It has been observed that, the behaviour of peroxidase in the germinating seeds of P.aconitifolius is irregualr and indefinite under saline conditions. Activity of this enzyme in the seeds of lentil increases upto 24 h of germination and later on falls down regularly in the non-saline conditions. The lowest activity is recorded in the seedlings at 120 h stage. Almost the same pattern is observed in the seeds germinating under saline conditions. 100 mM NaCl inhibits the activity, while 200 mM NaCl level is highly toxic. Activity of the enzyme increases with the stage of development of a seedling in P.aureus, the highest being observed in the seedling (120 h old) grown under non-saline conditions. Same pattern has been observed in the seeds germinating under saline conditions upto 100 mM NaCl while, it is disturbed at 200 mM level. Enzyme behaviour observed in fenugreek is quite interesting. The activity of the enzyme increases with the advancement of the period upto 48 h under non-saline conditions.

However, it is strongly inhibited by 200 mM NaCl level upto 48 h and once again it is stimulated to some extent by 200 mM NaCl.

B. Catalase :

It is observed that, in all the species investigated (except P.aureus), the activity of catalase increases during germination at one or the other stages. In case of P.aureus activity of this enzyme is decreased slowly with the advancement of germination period, but has regained after 120 h growth.

It is observed that, the activity of this enzyme at all developmental stages starting from 24 h is strongly affected, particularly by higher salt concentration (100 and 200 mM) in all legume species.

Activity of the enzyme peroxidase is stimulated in all the species under saline conditions, probably compensating the H_2O_2 metabolism but leading to more catabolic activities.

C. Acid phosphatase :

It is observed that, the activity of enzyme acid phosphatase increases with the stage of development in case of moth bean, green gram and fenugreek. However, in other two species namely horse gram and lentil, the activity of the enzyme increases slightly after 24 h of germination (in case of horse gram) and after 96 h (in lentil). Acid phosphatase in the legume species

investigated thus shows a wide range of salinity tolerance and hence the legume species also exhibit a differential response to salinity during germination and seedling growth. Lentil seems to be highly sensitive while moth bean and green gram show a tolerant nature. Horse gram and fenugreek can be considered as moderately tolerant.

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