6. SUMMARY AND CONCLUSIONS

Mangroves and halophytic species, which grow in coastal areas, have varied economic potential as well as capacity to withstand salt concentrations. Furthermore, integrated information on physiological, biological, chemical and morphological characters of such species provides clues for understanding the basic mechanism of salt tolerance in plants. Some of them not only tolerate high degree of salinity but also show vigorous growth under salt stress. Biological reclamation to increase productivity of saline wastelands would be possible by using halophytes. Nevertheless, before introducing any salt tolerant species one has to collect information on its means of propagation and its optimum levels of salt tolerance at seedlings and juvenile growth stages.

Such an approach would be beneficial to those countries which have to increase their forage production to support ever increasing cattle heads. Salt tolerant species are also considered as possible sources of germplasm for increasing salt tolerance capacity of salt sensitive crop species.

The above facts prompted us to study the following aspects in salt tolerant forage halophytes.

Attempts were made to examine the germinability of seeds of *Aeluropus lagopoides*, *Sporobolus madraspatanus* and *Salvadora persica* in different concentrations of NaCl, KCl, MgCl$_2$, Na$_2$SO$_4$, MgSO$_4$ and natural seawater in order to evaluate their limit of salt tolerance.

Complementary data on variations in pH, salinity and ionic composition of barren saline wastelands are also necessary for
arriving at conclusion whether the selected salt tolerant species would be able to establish at a new location. Such details for five barren saline sites selected for this investigation have been collected.

Survival and establishment of seedlings and young plants is equally important for biological reclamation of saline soils. Seedlings of two forage yielding halophytic grasses viz., A. lagopoides and S. madraspatanus were raised in control, 16-, 32- and 48 dS.m\(^{-1}\) seawater concentrations for 70-days. Efforts were made to examine effects of seawater salinity on important eco-physiological parameters such as growth, accumulation of free amino acids and sugars in the seedlings. These grasses were also collected from three natural habitats in monsoon, winter and summer to study the trace elements composition in their vegetative organs.

In the next phase of experimental work, sand culture experiments were designed to grow young plants of A. marina in 8-, 16-, 32- and 48 dS.m\(^{-1}\) seawater concentrations at controlled light and temperature. Observations were also made on growth and accumulation of proteins, free amino acids, sugars and mineral ions including trace elements in 52-day-old plants of A. marina.

The experimental design was further expanded for S. persica, which was grown in various concentrations of seawater, NaCl and Na\(_2\)SO\(_4\) at controlled light and temperature conditions.

The results of germination experiment of A. lagopoides showed that 99 per cent seeds germinated in distilled water. The process was remarkably inhibited at 5,000 ppm concentrations of NaCl, KCl and MgCl\(_2\). A few seeds could germinate in 25,000 ppm Na\(_2\)SO\(_4\) and no germination was observed in similar concentra-
tions of MgSO₄ or seawater. Further, a gradual decrease in germination percentage was noted in relation to increased concentrations of the salts. The seed germination was more adversely affected by seawater than sulphates and chlorides of Na, K and Mg. Recovery experiments also suggested greater toxic effects of seawater on seed germination than individual salts. However, the data collectively showed inhibition of seed germination due to a combination of osmotic and specific ion effects of salts. It was further observed that the germination percentages were always greater at alternate temperature than at constant temperature of 25 °C.

Results of seed germination of the second grass species namely, S. madraspatanus showed that the seeds failed to germinate in 10,000 ppm NaCl, 15,000 KCl and 36 per cent of seeds germinated into 25,000 ppm MgCl₂ reflecting the maximum adverse effects of chlorides of Na than those of K and Mg. Likewise, Na₂SO₄ caused greater inhibition of the process than MgSO₄ although germination was more in sulphates than corresponding concentrations of chlorides. Furthermore, germination at constant temperature of 25°C was remarkably less as compared to the results of alternate temperature. Seawater concentrations created maximum inhibitory effects on seed germination.

The findings of the present study indicated greater degree of salt tolerance by seeds of shrub species namely, S. persica, which also grows on saline soils. Germination was totally inhibited in 25,000 ppm NaCl, KCl and 40 dS.m⁻¹ seawater concentrations. Moreover, a high rate of recovery suggested that salts created only osmotic effects on germination in this species.
Observations on growth of 70-day-old seedlings of *A. lagopoides* and *S. madraspatanus* in control, 16-, 32- and 48 dS.m⁻¹ seawater concentrations reflected adverse effects on shoot length and fresh weight. Nevertheless, a slight increase in dry matter production was noticed under saline conditions.

The seedlings of *A. lagopoides* accumulated greater amounts of proline and phenylalanine, whereas those of *S. madraspatanus* contained greater concentrations of proline and glutamic acid under seawater stressed conditions. On the other hand, concentrations of glutamic acid and threonine in *A. lagopoides* and that of alanine, glutamine, glycine, leucine and serine in *S. madraspatanus* decreased in response to increase in seawater concentrations in growth media. Accumulation of remaining amino acids did not show any specific response to seawater salinity. It should be noted here that the proline content in these grasses increased manifold under seawater salinity.

Effects of salinity on accumulation of sugars showed greater concentrations of glucose and galactose in seedlings of *A. lagopoides* and that of glucose, galactose, arabinose and rhamnose in seedlings of *S. madraspatanus* raised in seawater concentrations than those grown in non-saline condition. Nevertheless, accumulation of galactose in the former species and that of galactose and arabinose in latter species progressively decreased with increased in seawater concentrations.

Variations in accumulation of trace elements namely, Fe³⁺, Mn²⁺, Cu²⁺ and Zn²⁺ showed that concentrations of Fe³⁺ in vegetative organs of both the grass species growing in nature was greater than remaining three elements. It was further observed that more than often roots contained maximum amounts of
these trace elements as compared to stems and leaves and that the accumulation of microelements was not affected by seasonal changes.

Results of effects of seawater on growth and accumulation of organic and inorganic metabolites in 52-day-old plants of A. marina grown in 8 to 48 dS.m⁻¹ seawater concentrations showed that growth and the proteins content were adversely affected by salinity. Nevertheless, greater accumulation of alanine, r-amino butyric acid, aspartic acid, cystine, glycine, methionine, phenylalanine, serine, threonine and valine in vegetative organs of the plants grown in seawater than those raised in non-saline condition was observed. Interestingly, concentrations of some of these amino acids positively increased in response to increase in seawater concentrations in growth media. It should be added here that proline, which has been considered as an important osmoregulatory amino acid in halophytes, was not detected in young plants of A. marina.

Amounts of total and reducing sugars in vegetative organs of A. marina were adversely affected by seawater concentrations. In contrast, leaves and roots of seawater treated plants contained greater amounts of glucose and rhamnose than those raised in control condition.

These results further showed that plants grown in saline conditions accumulated greater amounts of salts, Na⁺ and Cl⁻ in vegetative organs than those raised in non-saline condition. Likewise, a positive relationship between accumulation of ions in leaves, stems and roots and increase in seawater concentrations in growth media was observed.

Furthermore, plants subjected to seawater concentrations
showed greater accumulation of Fe$^{3+}$ and Cu$^{2+}$ than those raised in control condition. Again, the Fe$^{3+}$ content in vegetative organs progressively increased in response to seawater concentrations up to 32 dS.m$^{-1}$. However, such relationship was not observed in case of Cu$^{2+}$ and Zn$^{2+}$. The relative concentrations of trace elements showed greater amounts of Fe$^{3+}$ in vegetative organs followed by that of Cu$^{2+}$ and Zn$^{2+}$. On the other hand, amongst vegetative organs, roots accumulated greater amounts of Fe$^{3+}$ than stems and leaves.

Observations on growth and other parameters recorded for 60-day-old plants of *S. persica* grown in 8 to 48 dS.m$^{-1}$ seawater, NaCl and Na$_2$SO$_4$ concentrations indicated an increase in dry matter production in saline conditions. Leaves accumulated maximum proteins in plants grown in saline conditions as compared to those raised in control condition, whereas the opposite trend was observed in case of stems and roots. Accumulation of proteins was not severely affected under salt stressed conditions but it did not show any specific relation to varying degree of salinities.

Findings of amino acids in vegetative organs of *S. persica* grown in various concentrations of seawater, NaCl and Na$_2$SO$_4$ showed that alanine, asparagine, aspartic acid, glutamic acid, glycine, methionine, phenylalanine, serine and threonine, constituted a major pool of amino acids. These data collectively showed that accumulation of many amino acids was greater in plants subjected to salt stressed conditions. Nevertheless, it was difficult to generalise the uniform effects of seawater, NaCl and Na$_2$SO$_4$ on concentrations of amino acids. Comparative amounts of amino acids in vegetative organs indicated their
greater accumulation in above ground parts. Surprisingly, proline which has been considered as osmoregulatory solute in salt tolerant plants, was not detected in *S. persica* raised in seawater, NaCl and Na$_2$SO$_4$.

Maximum amounts of total and reducing sugars were observed in vegetative organs of salt free plants as compared to salt-treated ones. With a few exceptions, concentrations of the sugars in leaves, stems and roots further decreased with increased salt concentrations in growth media. These observations did not reflect any specific effects of varying concentrations of three different salts on accumulation of ethanol soluble sugars namely, glucose, galactose and arabinose. Moreover, plants contained greater accumulation of glucose, galactose than that of arabinose in vegetative organs under salt stressed conditions.

A progressive transport of inorganic ions from roots to shoots was noticed in *S. persica* under salt stressed conditions. Although the Na$^+$ and Cl$^-$ content showed a positive response to concentrations of seawater and NaCl in growth media, the Cl$^-$ content successively decreased in Na$_2$SO$_4$ treated plants.

Findings of accumulation of trace elements showed greater concentrations of Fe$^{3+}$, Cu$^{2+}$ and Zn$^{2+}$ in leaves, stems and roots of plants subjected to various concentrations of seawater, NaCl and Na$_2$SO$_4$ than those grown in control condition. Nevertheless, amounts of these elements did not show any specific relationship with various concentrations of the salts in growth media. Comparatively the Fe$^{3+}$ content in vegetative organs was greater than amounts of Cu$^{2+}$ and Zn$^{2+}$.

The physico-chemical characteristics suggested that soils
of the selected sites were saline and their pH was very close to pH variations in soils supporting halophytic vegetation. The electrical conductivity of (1:2) soil extracts fluctuated between 12.6 to 136.2 dS.m\(^{-1}\) and marshy habitat at Madhia was more saline than remaining four sites. The barren soils had a greater degree of salinity as compared to those supporting halophytes. The dominance of Na\(^+\) and Cl\(^-\), followed by lesser concentrations of Mg\(^{2+}\) and Ca\(^{2+}\) was noted for the soils. Variations in inorganic ions resulted in high SAR and ESP, which are known to interfere with uptake of ions and water from soils.

Remarkable conclusions which emerged out of the findings of present study are presented in the following paragraphs.

Various concentrations of Na\(_2\)SO\(_4\), MgSO\(_4\) and MgCl\(_2\) created less inhibitory effects on seed germination of two grass species namely, A. lanopoides and S. madraspatanus than those of KCl, NaCl and seawater. Maximum recovery of ungerminated seeds in higher concentrations upon their transfer to distilled water indicated osmotic effects on the process. Additionally, seawater created greater degree of toxic effects on germination than remaining salts. It can safely be concluded that the germination of these grass species was always better at alternate temperature than at constant temperature of 25°C.

A shrub species, S. persica exhibited better germination than grasses and the degree of adverse effects on the process followed the trend : seawater > chlorides > sulphates. Moreover, the seeds remained viable for long time even after exposure to high salt concentrations. These findings lead to a significant conclusion that this species would survive successfully if it is introduced in saline wastelands having appropriate levels of
salinity changes.

No adverse effects of seawater concentrations up to 48 dS.m\(^{-1}\) on root-length, dry weight and dry matter production of 70-day-old seedlings of *A. lagopoides* and *S. madraspatanus* obviously suggest that the seedlings of these two grass species are able to withstand seawater stress at early growth stage. Greater accumulation of proline, phenylalanine and glutamic acid and lesser concentrations of glutamic acid, threonine, alanine, asparagine, glutamine, glycine, leucine and serine observed in the seedlings of these halophytic grasses, suggest that in addition to proline, some amino acids may have osmoregulatory role or protective effects on cell membranes. However, further research is required to explain eco-physiological role of amino acids in grass species. Increased accumulation of glucose, galactose, arabinose and rhamnose in the seedlings reflects their role in osmotic adjustment.

Findings of concentrations of trace elements in vegetative organs in mature plants of *A. lagopoides* and *S. madraspatanus* indicated that roots contained maximum amounts of Fe\(^{3+}\), Mn\(^{2+}\), Cu\(^{2+}\) and Zn\(^{2+}\) as compared to shoots. Similarly, plants growing at Ghogha site often showed greater amounts of Fe\(^{3+}\) and Cu\(^{2+}\) than those plants growing at two other sites. No consistent seasonal variations in accumulation of trace elements were observed. These results suggest that halophytic grasses may function as indicators for micronutrients in salt-affected soils.

Better growth in terms of dry matter production of 52-day-old plants of *A. marina* under seawater stressed conditions supports the conclusion that this mangrove species possesses
high degree of salt tolerance. However, the proteins content in young plants was adversely affected by salinity. On the other hand, greater accumulation of 7-amino butyric acid, aspartic acid, alanine, cystine, glycine, methionine, phenylalanine, serine, threonine and valine under seawater stressed conditions prompts us to infer that some of these amino acids would have osmoregulatory function. Declining trend of total and reducing sugars as well as that of rhamnose and arabinose and slight increase in amounts of glucose and galactose in vegetative organs of young plants support the suggestion that sugars do not have prominent role in osmotic adjustment in A. marina.

Quite high concentrations of Na+ and Cl− in vegetative organs and their progressive increase in response to increase in concentrations of seawater in growth media show that A. marina is able to withstand high degree of salinity even during early growth stage. It may be noted here that the Fe3+ and Cu2+ content in plants raised in saline conditions was often greater than those grown in non-saline condition.

Observations on growth of 60-day-old plants of S. persica grown in various concentrations of salts viz., seawater, NaCl and Na2SO4 reflected an increase in the root-length and dry matter production. Thus, this species is able to withstand and grow successfully under salt stressed conditions. Furthermore, the proteins content in leaves was more in plants raised in various salts as compared to those of grown in non-saline condition.

Results of amino acids in vegetative organs of the plants grown in four concentrations each of seawater, NaCl and Na2SO4 reflected the fact that accumulation of alanine, asparagine,
aspartic acid, glutamic acid, glycine, methionine, phenylalanine, serine and threonine increased under salt stressed conditions. It is safe to conclude that some of these amino acids would be helpful in osmotic adjustment in young plants of the species. A noticeable absence of proline, an important osmoregulatory solute in salt tolerance plants, suggests that S. persica does not belong to a proline accumulating group.

The experimental findings further showed that accumulation of total and reducing sugars in young plants was adversely affected by salinity and no consistent effects of various concentrations of three salts were observed. Nonetheless, concentrations of glucose and galactose were often greater than those of arabinose in vegetative organs. This fact suggests that a care should be taken in deciding osmoregulatory function of sugars in plants subjected to salt stressed conditions.

A relative increase in amounts of Na⁺ and Cl⁻ in vegetative organs in response to increase in concentrations of salts in growth media accompanied by their progressive transport from roots to shoots evidently suggests that the young plants of this species possess a well adapted mechanism of salt tolerance. Vegetative organs of salt-treated plants contained greater concentrations of Fe³⁺, Cu²⁺ and Zn²⁺ as compared to those grown in non-saline condition. But no specific relationship existed between their amounts and various concentrations of salts in growth media. However, young plants of S. persica are able to absorb and accumulate considerable amounts of trace elements.

Variations in physico-chemical characteristics of saline soils collected from five barren sites indicated high salinity and dominance of Na⁺ and Cl⁻, which resulted in higher SAR and
ESP values than soils supporting vegetation. On the other hand, seeds of grass species namely A. lagopoides and S. madraspatanus failed to germinate even in low concentrations of 5,000 ppm of salts. This suggests that seeds of these species will not be able to germinate successfully, if directly sawn at these sites. But if they are germinated in lesser concentrations and then transferred to barren saline soils when their salinity is at minimum levels in monsoon, then species may establish. Results of three experiments further indicate that seedlings of these grass species and plants of A. marina and S. persica withstand high degree of salinity. Therefore, if these species are introduced in barren saline soils when salinity is low, they can successfully established. Thus, if planned scientifically, forage production on saline wastelands can be increased by using the plant species included in the present study.