Chapter IV

Summary and Conclusions

Among various amino acids occurring in plants, proline is perhaps the most noteworthy, since it has attracted attention of plant physiologists and breeders for last several years. It is a heterocyclic amino acid of glutamate family. A phenomenal accumulation of free proline has been observed in number of plant species under the conditions of different environmental stresses. These environmental stresses include drought, salinity, high and low temperature stresses, water logging, soil acidity, mineral deficiencies and pathogenesis. The water and air pollution stress conditions are also there. At the same time there are few plant species which have been found to opt for some other physiological strategy other than proline accumulation under stress conditions. The free proline accumulation, in proline accumulating species is due to number of biochemical events. These include increased protein degradation, increase in rate of proline synthesis from the precursors like glutamate and arginine, decrease in rate of proline oxidation, decrease in incorporation of proline into proteins and alterations in cytoplasmic pH.

There are several reports which indicate that free proline plays different positive roles in plant cells under stress conditions. Proline being highly water soluble, it increases the bound water content in plant tissues. It is relatively very less toxic and protects enzyme proteins from

denaturation. It is osmotically active and plays a role in osmotic adjustment of the plant tissue. It serves as a storehouse for carbon and nitrogen which can be utilized for growth processes during post-stress recovery. Proline also serves as a precursor for hydroxy-proline a well known component of cell-wall proteins. Review of literature indicates that, the studies of proline accumulation have been mainly performed on the crop species, which are having a short lifespan and the tree species have been paid very little attention in this respect. Hence we throught it worth-while to study this aspect in the tree species. It is now very well realised that agroforestry is a must effective strategy to deal with the problem of problem soils and waste lands. Several tree species have been recommended as the promising candidates in this respect. Among these species Eucalyptus and Acacia are perhaps the most widely accepted tree species. Besides the agroforestry, the social forestry programme is also receiving a great impetus due to increased awareness of the Government and Society, regarding the value of forests in the 'National Economy'. In this programme, besides the conventional tree species, some hardy fruit trees like Anona are also planted. Hence for the present investigation the three tree species namely (1) Acacia auriculiformis, (2) Anona squamosa, (3) Eucalyptus globulus. These trees have been planted in the barren fields of Shivaji University Campus by Social Forestry Department. One year

old plantation was selected for the present investigation. In the present study, an attempt was made to find out the fate of free proline in the leaves of above species growing under natural conditions in relation to various environmental variables such as rainfall, soil moisture and temperature and endogenous factors like sugars, nitrogen and minerals like Ca^{++} and K^{+}. Besides this study an attempt was also made to find out relation between free proline level and nitrate reductase activity in the leaves of three species.

The significant findings of the present investigation can be summerised as follows:

(1) The analysis of average total nitrogen and average free proline content in the leaves of three tree species indicated that, the species differ with respect to total nitrogen status and contribution of nitrogen for free proline synthesis. Anona leaves had relatively higher total nitrogen as well as free proline content. While in Eucalyptus the nitrogen values were lower than either Acacia or Anona and the free proline level was also very low. It was also noticed that Acacia was relatively more efficient in driving nitrogen for proline synthesis than Eucalyptus. These observations clearly indicate that, great variations in free proline level are possible among the three species growing under similar environmental

conditions. Thus the free proline level appears to be partly controlled by genetic factors.

(2) The analysis of free proline content in the leaves during different months revealed that, there were distinct variations in the proline contents in the three tree species, during different months of the experimental time period. In Acacia there was maximum proline accumulation in the month of February, and the proline level was significantly low during July.

In case of Anona, the proline level was highest in the month of February and it was very low in the month of November. In Eucalyptus, the proline level was very high in the month of March and minimum was recorded in August. Thus these observations clearly indicate the influence of different environmental factors prevailing during different months on the fate of free proline in leaves.

(3) An attempt was made to find out the relationship between rainfall, soil moisture and free proline levels in the three free species. The monthly record of rainfall revealed that, November, February and March were the dry months as there was no rainfall. In December 60 mm, April 26 mm, and in May 27.8 mm rainfalls were received by soil. These rainfalls were not regular but were occasional rainfalls. The months like June, July and August were the monsoon months in which appreciable

rainfalls were received by earth in this region, where the experimental study was carried out. It was found that, soil moisture also fluctuated in accordance with the rainfall fluctuations. In February and March severe soil moisture tension was seen. It was noticed that in these two months heavy proline accumulation took place in Anona and Acacia and increase in proline level was also noticed in Eucalyptus leaves. The proline levels in the leaves dropped down during the rainy months (June, July and August). Although in August, there was slight waterlogging due to heavy rainfalls, the proline levels were very low inspite of water logging stress in all the three species. Thus it is evident from the present investigation that, the free proline accumulation in all the three species is mainly linked to very low precipitation and high soil moisture stress.

(4) The analysis of maximal air and soil temperatures during different months revealed that, there were variations in air and soil temperatures during different months. The highest air temperature was recorded in the month of March and in this month Eucalyptus showed a peak of proline accumulation however such situation was not seen either in Acacia or Anona. Similarly the fluctuations in soil temperature were also not relevant to free proline level in any of the three species. These observations indicate that, the fluctuations of air temperature

and soil temperature within a narrow range generally observed in this region of India, do not influence proline accumulation process, in these plants studied in present investigation.

(5) The analysis of leaf moisture status during different months revealed that, the leaf moisture status varied to a marked extent, in different months and there was a great decline in moisture level during summer months when soil moisture tension was very high. A leaf water deficit was found to be associated with proline levels in all the three species. Thus in this respect the perennial tree species show a similar behaviour to that of annual crop species.

(6) The potassium and calcium play a vital role in plant metabolism. There are some reports indicating relation of these elements with proline metabolism. Marked variations in the levels of both K^+ and Ca^{++} in the leaves of three tree species were evident during different months of experimental duration. The calcium levels were fouhd to be relatively low during rainy months as compared to summer months. Among the three species the average Ca^{++} content were highest in leaves of Anona and this species also showed highest degree of free proline accumulation under stress conditions. The K^+ levels showed inconsistant fluctuations during

different months and it was noticed that, all the three species were able to maintain adequate levels of K^+ in their leaves during the months of high soil moisture tention (February and March). However, in April and May K^+ levels dropped down probably because of an acceleration of leaf senescence and transport of this element to other parts of the plant. During the three species, the average K^+ content were highest in the leaves of Eucalyptus and in this species both K^+ accumulation and proline accumulation attained their peak in March. But such relationship was not evident in other tree species. Thus there appears to be very little influence of these two elements on proline levels, when the entire experimental period is taken into consideration.

(7) It has been demonstrated in some experiments that sugars serve as precursor for proline biosynthesis in wilted leaves. The analysis of total sugars and free proline in the leaves of the three tree species revealed that, although there are marked fluctuations in total sugars during different months of the experimental period, they could be hardly correlated with the corresponding alterations in the free proline levels. The only significant observation in this respect was a decline of sugar levels during the period February to March, which was also a period of heavy proline accumulation in all the three species. Thus sugars may be indirectly linked

with proline accumulation during stress conditions rather than during normal and favourable environmental conditions.

(8) It is now very well established that a good stomatal control is an important component of drought resistance process in plants. The analysis of mean transpiration rate during different months of experimental duration, with the help of autoporometer revealed that, there are great variations in the three tree species with respect to transpiration rates during different months. The transpiration rates were found to be high during November (winter) and August (Late rainy monsoon) and a marked decline in transpiration rate was observed during summer months. In July also the transpiration rates were significantly low in all the three species probably because of very high relative humidity. The transpirational water loss was found to be very high in Anona and Eucalyptus. Whereas it was very low in Acacia. Among these species a great stomatal control was shown by Acacia during summer months and this appears to be an important factor contributing for its drought resistance. It was noticed that, there was appreciable proline accumulation in Acacia at the same time it is difficult to correlate proline accumulation with stomatal control because a very high proline accumulation as well as very heavy transpirational water loss was noticed in

Anona under water stress conditions.

(9) All the three species were exposed to severe water stress from the period March to May. Hence an attempt was made to find out an influence of watering some stressed plants periodically on the fate of free proline and enzyme nitrate reductase a key enzyme of nitrogen metabolism for the period of three months. It was evident that in the leaves of three tree species the proline level of the watered plants is considerably lower than that of the stressed plants. On the other hand, exactly opposite behaviour was shown by an enzyme nitrate reductase and there was increase in enzyme activity due to watering of the plants. It was observed that, in contrast to Eucalyptus, the proline levels as well as the nitrate reductase activity were quite high in Acacia, and Anona under the conditions of water stress. These findings suggest the possibility that, proline may offer protection to enzyme nitrate reductase under stress conditions.

In conclusion it can be stated that, there is great variation in the three tree species (1) Acacia auriculiformis (2) Anona squamosa (3) Eucalyptus globulus, with respect to magnitude of free proline accumulation in different seasons. Although an increase in proline level was noticeable in all the three species. Acacia and Anona can be definitely regarded

as proline accumulating type while Eucalyptus probably belongs to the category of proline non-accumulating type of plants. The proline accumulation appears to be controlled by the environmental factors, like rainfall and also the internal factors. Thus proline accumulation may not be an inversal strategy of drought resistance in all three species, and species like Eucalyptus may be adopting some other strategies to combat with environmental stresses. The exact role of proline in the drought resistance of Anona and Acacia needs further experimental probe, which will involve study of effect of exogenous proline application, on various metabolic processes, and the fate of labelled precursors of proline under stress conditions in these two species. Such studies will be undertaken in future.

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