CHAPTER - IV

SUMMARY AND CONCLUSIONS

Hydroxyperoxidases is a group of respiratory enzymes which is an important enzyme system in plant metabolism. This group comprises of enzymes peroxidase and catalase with hydrogen peroxide as a common substrate.

Peroxidase (E.C. 1.11.1.7) (Doner : Hydrogen peroxide oxidoreductase) plays an important role in growth and development of plants through their control in auxin catabolism, H_2O_2 formation and lignin and ethylene biosynthesis. It is an indicator of respiration rate and may be involved in the catabolism of chlorophylls during senescence. Enzyme peroxidase catalayses the oxidation of different substances like $NADH_2$, $NADPH_2$, phenolpyruvate and IAA. It is also able to decarboxylate some aminoacids oxidatively.

Peroxidase, which is generally composed of number of isozymes, is capable of catalyzing several above types of oxidative reactions. The isozymes differ in biochemical properties such as specific activity, substrate affinity, cofactors, sensitivity to inhibitors, pH optima etc.

During aging the metabolic activities rise and continue to increase over a period of several days and among the metabolic changes, the increase in the activities of several enzymes including peroxidase is significant in plants. An increase in peroxidase activity is taken as a reliable indicator of leaf senescence. An inverse relationship between catalase and peroxidase levels has been reported during development and senescence of leaves. Increased level of peroxidase in the tissue has been

correlated with increased respiration which is considered as a 'wasteful' respiration.

The presence of peroxidase has been demonstrated in various subcellular components such as nucleus, mitochondria, ribosomes, cell walls and cell membranes. A possible role of peroxidase in plant resistance during disease development has been attributed to its ability to oxidise important metabolites either of the parasite or of the host plants. The increased peroxidase activity has often been studied in connection with the oxidation of phenolic substances in the diseased plants. Some authors suggest that high peroxidase activity is the result rather than a cause of incompatibility between host and parasite.

Catalase (E.C. 1.11.1.6) (Hydrogen peroxide : Hydrogen peroxide oxidoreductase) protects the cells from being destroyed by hydrogen peroxide. An inverse relationship has been reported between catalase and peroxidase levels in the leaves during their development and senescence. It is pressumed that catalase decreases the H_2O_2 level in the cells, limiting the peroxidative reaction catalysed by peroxidase. Enzyme catalase catalyses decomposition of H_2O_2 to give water and oxygen. Recent cytochemical and biochemical findings indicate that catalase in plant cell is located only in microbodies namely peroxysomes and glyoxysomes. Significant differances in catalase activity are observed between C_4 and C_3 species with 4-fold greater activity in C_3 plants over that in C_4 plants.

IAA oxidase (E.C. 1.16.13) and polyphenol oxydase (E.C. 1.14.18.1) are related to enzyme peroxidase. Both enzymes have oxidative catalytic

action. IAA oxidase is the enzyme involved in the catabolic degradation of IAA. Polyphenol oxidase are copper proteins of wide occurance in nature which catalyse the aerobic oxidation of certain phenolic substances. The activities of polyphenol oxidases are important with regard to plant defence mechanism against pests and diseases and appearance, palatability and use of plant products.

Peroxidase, polyphenol oxidase and IAA oxidase have been considered by some authors as isozymes of the same enzyme.

Eventhough lot of studies have been carried out on these enzymes in several plants, the exact nature of these enzymes has been little understood. There are quite a few attempts in the kinetic studies of these enzymes. Their relationship with photosynthetic carbon metabolism in the plants differing in photosynthetic pathways and particularly in succulents has not been studied. Therefore, in the present investigation an attempt has been made to study these enzymes in *P. oleracea*, a C4 succulent plant and *K. pinnata*, a typical CAM succulent plant. For comparison these enzymes from *S. grandiflora*, a fast growing legume with high levels of hydroxyperoxidases have also been studied.

In the present study kinetics of enzymes peroxidase and catalase from the leaves of these plants have been studied. Under these studies the effect of substrate concentration, pH of the assay medium and temperature on the activities of peroxidase and catalase has been investigated. Activities of enzymes IAA oxidase and polyphenol oxidase in the leaves of these plants have also been measured. Isozyme pattern for peroxidase has

also been determined following polyacrylamide gel electrophoresis. For the study standard methods and techniques have been followed.

The significant findings of the investigation are as follows :

1. Activity of enzyme peroxidase in the leaves of *S. grandiflora* increases linearly with increase in substrate concentration upto 1.8 and 2.0 µmoles of H_2O_2. The enzyme records the maximum activity of 22.8 $min^{-1}g^{-1}$ fresh tissue or 0.396 $min^{-1}mg^{-1}$ protein.
2. Activity of enzyme peroxidase in the leaves of *P. oleracea* increases linearly with increasing the substrate concentration, maximum activity being at 3.0 and 3.5 µmoles H_2O_2. The maximum activity recorded is 2.22 $min^{-1}g^{-1}$ fresh tissue or 0.234 $min^{-1}mg^{-1}$ protein.
3. Km for enzyme peroxidase in the leaves of *S. grandiflora* ranges between 0.230 to 0.235 µmoles, while that for *P. oleracea* ranges from 0.24 to 0.25 µmoles. Thus Km for peroxidase in *P. oleracea* is slightly higher than that in *S. grandiflora.*
4. Enzyme peroxidase from the leaves of *S. grandiflora* records the highest activity at 6.03 to 6.17 pH of the assay medium. pH above 6.88 affects the enzyme activity drastically. pH 6.0 to 6.2 can be taken as pH optimum for peroxidase in the leaves of *S. grandiflora.*
5. 6.3 is found to be the pH optimum for enzyme peroxidase in the leaves of *P. oleracea.* Thus the pH optimum for this enzyme in *P. oleracea* appears to be slightly higher than that in the leaves of *S. grandiflora.*
6. The level of enzyme peroxidase appeared to be more in the leaves of *S. grandiflora* than that in the leaves. of *P. oleracea.*

7. With increase in temperature there is an increase in the activity of enzyme peroxidase in the leaves of *S. grandiflora* and *P. oleracea*, reaching to a maximum and then declining with further increase in temperature.
8. Enzyme peroxidase from the leaves of *S. grandiflora* records the maximum activity at 20°C temperature. However, it is not much affected with further increase in temperature up to 40°C. It appears that peroxidase from this species has some degree of thermotolerance.
9. 20° to 25°C can be taken as optimum temperature for enzyme peroxidase in the leaves of *P. oleracea*. Higher temperatures like 40°C appear to be a strong inhibitory temperature for the enzyme. Thus peroxidase from *P. oleracea* appears to have less degree of thermotolerance.
10. While studying peroxidase in *K. pinnata*, earlier observations made by Upadhye *et al.* (1986) have been confirmed. We could not measure activity of enzyme peroxidase in this species.
11. It is observed that Km for enzyme catalase in the leaves of *S. grandiflora* ranges between 1.162 to 1.18 µmoles.
12. The Km for catalase from the leaves of *P. oleracea* found is arround 0.88 µmoles.
13. The Km value recorded for catalase in the leaves of *K. pinnata* is found to be 1.18 to 1.19 µmoles.
14. Km for catalase in the leaves of *P. oleracea* is slightly lower than that in the leaves of *S. grandiflora* and *K. pinnata*.

15. pH 6.8 to 7.0 can be taken as pH optimum for catalase in the leaves of *S. grandiflora.*
16. 6.92 is the pH optimum recorded for enzyme catalase in the leaves of *P. oleracea.*
17. 6.18 appeared to be pH optimum for enzyme catalse in the leaves of *K. pinnata.*
18. In comparision with *S. grandiflora* and *P. oleracea*, enzyme catalase from *K. pinnata* has pH optimum which is much more acidic. It can be noted that *K. pinnata* is a CAM plant with an ability to synthesize and accumulate organic acids.
19. 15° to 20°C has been found to be optimum temperature for enzyme catalase from the leaves of *P. oleracea.*
20. The optimum temperature recorded for catalase from *S. grandiflora* is 15°C which is lower than that for *P. oleracea.*
21. Enzyme catalase from the leaves of *K. pinnata* also recorded slightly lower temperature optimum i.e. 15°C.
22. Enzyme catalase is found to be dominant in the leaves of *S. grandiflora.*
23. The level of hydroxyperoxidases found in succulents is much below to that of *S. grandiflora.*
24. The level of enzyme IAA oxidase is found to be slightly higher in the leaves of *S. grandiflora* than in *P. oleracea.* We could not measure activity of this enzyme in the leaf tissue of *K. pinnata.*

25. The highest activity of enzyme polyphenol oxidase has been recorded in the leaf tissue of *S. grandiflora* and the lowest being in the leaves of *P. oleracea.*

26. Peroxidase preparation from *S. grandiflora* leaves exhibited 5 isozymes while that from *P. oleracea* leaves 4 isozymes. Some of the isozymes are found common in both the plants. We failed to separate or find the isozymes of peroxidase from *K. pinnata.*