CHAPTER - I

INTRODUCTION

Hydroxyperoxidases is a group of respiratory enzymes which is an important enzyme system in plant metabolism. This group is comprised of enzymes, peroxidase and catalase whose substrate is H_2O_2 (Hydrogen peroxide) [Theorell, 1951].

a) **Peroxidase (E.C. 1.11.1.7)** (Donor : Hydrogen peroxide oxidoreductase) :

It has been well established that peroxidases play an important role in growth and development of plants through their control in auxin catabolism (Ray, 1962; Hinman and Lang, 1965), H_2O_2 formation (Gross *et al.*, 1977) and lignin (Halliwell, 1978) and ethylene biosynthesis (Lieberman, 1979). Peroxidase is an indicator of respiration rate (Horovitz *et al.*, 1968). It may be involved in the catabolism of chlorophylls in senescent leaves (Matile, 1980).

Peroxidase and catalase generally catalyse H_2O_2 as electron acceptor and many kinds of substrates (phenolic substances, aromatic amines, ascorbic acid, ferrocytochrome C, $NADH_2$ etc.). Peroxidase differs from catalase only by its efficiency in catalyzing the peroxidative oxidation of certain substances. They differ mutually mainly in their affinity to H_2O_2 as an electron donor (Brill, 1966).

Under certain circumstances, peroxidase also shows oxidative activity besides the peroxidative activity, i.e. it catalyses the oxidation of different substances by atmospheric oxygen under aerobic conditions, without exogenous peroxide e.g. $NADH_2$, $NADPH_2$ (Petrochenko and Kolesnikov, 1966), phenolpyruvate (Jaynes *et al.*, 1972), Indole acetic acid

(Platee *et al.*, 1964; Pilet and Gaspar, 1968; Tuli and Moyed, 1969). Peroxidase is also able to decarboxylate some aminoacids oxidatively, e.g. serine, alanine, phenylalanine, methionine and tryptophan (Mazelis, 1962; Mazelis and Ingraham, 1962).

In the presence of dihydroxyfumarate, peroxidase catalyzes the hydroxylation of various aromatic compounds (Paul, 1963; Daly and Jerina, 1970) and the reduction of nitrate in the presence of some specific electron donors (Peive *et al.*, 1972). Peroxidase is also involved in the formation of ethylene from metinol (β-methylthiopropionaldehyde) or from α-keto ϒ-methylthiobutyric acid (Yang, 1967; Ku *et al.*, 1970a).

Peroxidase, which is generally composed of number of isozymes, is capable of catalyzing several different types of oxidative reactions. Peroxidase isozymes may differ in biochemical properties such as specific activity, substrate affinity, cofactors, sensitivity to inhibitors, pH-optima etc. The reactions catalysed by peroxidase or catalase may also be influenced by various substances having either activator or inhibitor effects (Hare, 1964; Kosuge, 1969; Ku *et al.*, 1970b).

According to Heitefuss *et al.* (1960) during aging the metabolic activities rise and continue to increase over a period of several days, which may be many times more than that in fresh tissue. Among the metabolic changes, the increase in the activities of several enzymes including peroxidase is significant. A quantitative increase in peroxidase activity is reported by Braber (1980) in Bean Leaves.

According to Parish (1968) increase in peroxidase activity can be taken as a reliable indicator of leaf senescence. Shannon *et al.* (1971) reported that the peroxidase content of sweet potato slices increases nearly 100 fold following 84 hours incubation in an atmosphere containing ethylene. Judel (1972) observed low peroxidase activity in young leaves of sunflower while it increased during vegetative growth, reaching its maximum in the yellowing leaves and then decreasing rapidly. Further it has been found that in relation to the whole plant, peroxidase activity was the highest in the basal (older) leaves and decreased with progressive insertion.

Joshi and Karadge (1980) have observed that an increase in peroxidase activity in the senescent leaves of *Portulaca oleracea* is accompanied by a decrease in that of catalase. Braber (1980) reported that in the primary bean (*Phaseolus vulgaris*) leaves catalase and peroxidase levels show an inverse relationship during development and senescence. Lazar and Farkas (1970), Kar and Mishra (1976), Brennan and Frenkel (1977) and Schwenzer and Harte (1980) have also shown an increased peroxidase activity in the senescent leaves.

Fluctuations in the activity of peroxidase during the onset of senescence are also reported. Weston (1969) observed that activities of peroxidase and phenol oxidase fell rapidly after the onset of senescence but later he found a small increase during further development. Patra and Mishra (1979) noted both increasing and decreasing tendancies of peroxidase during senescence which may have some functional

significance during development of leaves and it may be that peroxidase activity follows irregular patterns and the fluctuations of enzyme activities are species specific. According to Baba *et al.* (1964) the increased peroxidase activity is symptomatic of increased respiration but it does not supply more energy, it is a 'wasteful' respiration.

The peroxidase enzyme has several metabolic functions in the cell. In such cases it might suggest their compartmentalization within the cell in which their metabolic functions may vary with their subcellular localization.

The presence of peroxidase has been demonstrated in various subcellular components e.g. in nucleus (Raa, 1973) in mitochondria (Ivanova *et al.*, 1966, 1967; Parish, 1972; Darimont and Baxter, 1973), in ribosomes (Penon *et al.*, 1970; Darimont and Baxter, 1973), in cell walls (Stafford and Bravinder Bree, 1972; Raa, 1973) as well as in cell membranes (De Jong, 1967). It has been suggested that the peroxidase localised in the nucleus might be involved in the structural organization of chromosomes. Some basic isozymes of peroxidase can have a histone like function (Raa, 1973) or may alter the repressor properties of histone, influencing the DNA-dependent RNA synthesis (Stahmann and De-Morest, 1972).

Ivanova *et al.* (1967) indicated that peroxidase in the mitochondria of cabbage and cucumber sprouts has diaphorase activity. These authers suppose peroxidase in the mitochondria to be involved in the electron transfer system from $NADH_2$ to cytochrome C. Plesnicar *et al.* (1967) on

the other hand, suggest that the peroxidase activity in the mitochondria of etiolated mung bean hypocotyl is an artifact from contamination.

Ribosomal peroxidase may catalyze the synthesis of new ribosomes by derepression of genes involved in ribosome synthesis (Stahmann and Demorest, 1972). The lignin formation in cell wall is the function of peroxidase. It is considered that the wall-bound peroxidase may hydroxilate proline to hydroxyproline in the cell wall (Ridge and osborne, 1970). In the plant cells, peroxidase may also have the function of IAA-oxidase (Pilet and Gaspar, 1968; Fric, 1971; Hoyle, 1972). It has been suggested that the oxidative transformation of IAA to other biologically active compounds, e.g. 3-methyleneoxindole (Basu and Tuli, 1972) in the plant cells may contribute to the biological activity of IAA rather than to its inactivation (Meudt, 1967, 1970).

The views concerning peroxidase as a rate limiting enzyme in ethylene synthesis *in vivo* are still contradictory (Pratt and Goeschl, 1969; Ku *et al.*, 1970b; Kowler and Morgan, 1972). On the other hand growth substances such as IAA, Ethylene, Gibberllin and Kinetin, seem to have a function in peroxidase activity regulation by activation of some isozymes of peroxidase and by repressing others (Galaston and Davies, 1969; Ridge and Osborne, 1970).

In most cases, it is difficult to evaluate the role of peroxidase in plant resistance to various diseases. Up to now it has been difficult to obtain direct proof of the participation of peroxidase in the defence reactions and we had to rely mainly on correlations between the biochemical changes in

the diseased tissues and the final disease response. From the view point of the inhibition of pathogen development, the significance of the biochemical changes observed is not always clear.

The increased peroxidase activity in various host parasite combinations may be associated with the disease resistance in the host; however, this is not inevitably so. Results from studying the role of peroxidase in resistance or susceptibility have been summarised in a number of reviews (Farkas and Kiraly, 1958, 1962; Tomiyama, 1963; Sequeira, 1963; Stahmann, 1967; Wood, 1967; Kosuge, 1969). Changes in the activity or in the isozyme composition of peroxidase are not specific reactions of the plant to infection by the parasite, rather they are an accompanying characteristic of the changed metabolic activity of plant cell under the influence of various exogenous or endogenous factors (Filner *et al.*, 1969; Galston and Davies, 1969; Lazer and Farkas, 1970; Benedict, 1971). The role of peroxidase in the plant resistance has been attributed to its ability to oxidise important metabolites either of the parasite or of the host plant, e.g. phenolic substances, enzymes, IAA, toxins etc. (Farkas and Kiraly, 1962; Kuc, 1966; Kosuge, 1969; Moustafa and Whittenbury, 1970).

The increased peroxidase activity has often been studied in connection with the oxidation of phenolic substances in the diseased plants and resistance in the host was attributed to the toxicity of the oxidation products. Such substances, however, may be toxic to the pathogen as well as to the invaded host cells and this question has been discussed further in relation to the role of phenolase in the host parasite complex. The direct

participation of peroxidase in the defence reactions of plants may be supported by the findings of Macko *et al.* (1968) and Lehrer (1969), that peroxidase inhibits the fungal growth.

A number of investigations shows that the peroxidase activity in the host enhances plant resistance (Lovrekovich *et al.*, 1968a, 1968b). These authers have found that heat killed bacteria of *Pseudomonas tabaci* injected into tobacco leaves, increased the peroxidase activity and at the same time induced resistance in the leaves to subsequent inoculation with living bacteria. Development of disease symptoms in the leaves was suppressed not only by infiltrated killed bacteria, but also by a solution of commercial peroxidase.

In numerous incompatible host parasite combinations, peroxidase activity is often several times higher than in compatible ones (Fric and Fuchs, 1970; Simons and Ross, 1970; Daly *et al.*, 1971; Loon and Geelen, 1971). In other cases, however, peroxidase activity is greater in compatible host parasite combinations than in the incompatible ones (Fric, 1969; Grzelinska, 1969; Wood and Barbara, 1971).

It is clear therefore, that the activation of peroxidase in the host parasite complex need not always be accompanied by the incompatible reaction. Recently it has been shown that the strong activitation of peroxidase in rust resistance combinations is not the cause of resistance, but rather a nonseptic response of the host plant. These findings made by Daly and his co-workers using near isogenic lines of wheat, bearing the temperature sensitive sr-6 gene for resistance to *Puccinia graminis*

f.sp.tritici, race 56 (Seevers and Daly, 1970; Seevers *et al.*, 1971; Daly, 1972). Wheat plant showed infection type O when grown and inoculated at 20°C, but type 4 at 24-25°C. Plants grown at 20°C, for 6 days had high peroxidase activity which did not decrease after the plants had been transfered to 26°C, despite a shift of disease reaction from infection type 0 to infection type 3 or 4 (Seevers and Daly, 1970). At 25°C an ethylene induced high peroxidase activity failed to evoke a change of the compatible host parasite reaction to the incompatible. However incompatible reaction (at 26°c) was reverted to the compatible by ethylene, inspite of high peroxidase activity (Daly *et al.*, 1970).

Electrophoretic studies of peroxidase have shown that increased peroxidase activity at incompatible reaction (at 20°C) is caused by the increase of one isozyme only (isozyme no 9) from 14 different ones. After transfering wheat plant from 20° to 25°C or after treating them with ethylene, the disease induced activity of the isozyme 9 did not decrease significantly (Seevers *et al.*, 1971). The results of Daly and co-workers suggest that high peroxidase activity is a result rather than a cause of incompatibility.

b) Catalase (E.C. 1.11.1.6) (Hydrogen peroxide : hydrogen peroxide oxidoreductase) :

Enzyme catalase is very important enzyme system in plant metabolism. It protects the cells from being destroyed by hydrogen peroxide (Grinberg, 1971). Contrary to the increased level of peroxidase during senescence, this enzyme records a decrease (Kar and Mishra, 1976;

Brennan *et al.*, 1977). Braber (1980) has found that catalase and peroxidase levels in primary bean leaves showed an inverse relationship during development and senescence.

Dhindsa *et al.* (1981) reported that in the leaves of *Nicotiana tobacum* L., catalase activity first increased parallel with the chlorophyll content of the leaf and then after full leaf expansion, declined on the basis of both fresh weight and protein content. These changes coincide with the leaf age with the decline in protein and chlorophyll content and in chlorophyll a:b ratio. Leaf senescence may be a consequence of cumulative membrane deterioration due to increasing level of lipid peroxidation, probably controlled by, among other factors, the activities of superoxide dismutase (SOD) and catalase.

As reported earlier, the low level of catalase during senescence of leaves, may be unable to remove hydrogen peroxide protectively produced in excess due to activities of other enzymes like glycolate oxidase (Kar and Mishra, 1976) together with xanthin oxidase (Brennan *et al.*, 1977).

In some studies of host parasite relations catalase has been emphasized as being of importance. Some authers suggest catalase has considerable influence upon the virulence of some pathogens (Rudolph and Stahmann, 1964) as well as upon host parasite coexistance. Enhanced catalase activity may favour compatible host parasite reactions (Farkas and Kiraly, 1958; Fric, 1969). Theoretically it is presumed that catalase decreases the H_2O_2 level in the cells, thus limiting the peroxidative reactions catalyzed by peroxidase.

Catalase has double functions as it catalyze the following reactions.

I) Decomposition of hydrogen peroxide to give water and oxygen.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2 \uparrow$$

II) Oxidation of H donors for example methanol, formic acid, phenol with the consumption of one mole of peroxide.

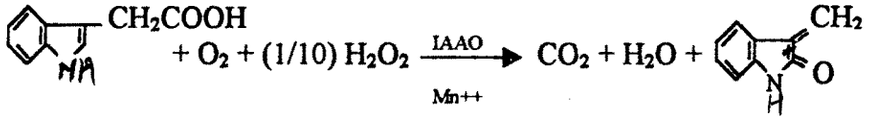
$$ROOH + AH_2 \xrightarrow{\text{catalase}} H_2O + ROH + A$$

Recent cytochemical and biochemical findings indicate that catalase in plant cell is located only in microbodies (peroxisomes, glyoxysomes); catalase activity found in other subcellular organelles may be an artifact, since microbodies are very fragile and during the isolation process the released catalase may be adsorbed to the other subcellular structures. Catalase in peroxisomes and glyoxysomes probably catalyzes the destruction of H_2O_2 generated by the action of flavine oxidases (Tolbert, 1971).

Significant differences in catalase activity are observed between C_4 and C_3 species, the average activity of C_3 plants was 4 fold greater than that of C_4 plants (Du, Shi-Hua, 1983). Catalase plays a key role in mercury vapour uptake by graminaceous C_3 species and lesser role in C_4 species, particularly, sorghum.

c) Indoleacetic acid oxidase (IAA oxidase) (E.C. 1.16.13)

Indoleacetic acid (IAA) is the best known naturally occurring plant auxin. It participates in controlling many phases of plant growth and differentiation. Levels of free IAA are in turn regulated via synthesis binding, esterification and enzyme degradation. Indolyl acetic acid oxidase (IAAO) is the enzyme involved in the catabolic degradation of IAA to 3-methylene oxindole.



[Byrant, and Lane, (1979)].

d) Polyphenol oxidase (E.C. 1.14.18.1)

(Monophenol, dihydroxyphenylalanine : oxygen oxidoreductase E.C. 1.14.18.1)

Phenoloxidases are copper proteins of wide occurrence in nature which catalyze the aerobic oxidation of certain phenolic substrates to quinones which are autoxidised to dark brown pigments generally known as melanins. These enzymes are assumed to be single enzymes with broad specificity although there is some evidence for the presence of more than one phenoloxidases in certain tissues. Each individual enzyme tends to catalyze the oxidation of one particular phenol or phenolic compound more readily than others. The polyphenol oxidase (PPO) comprises of catechol oxidase and laccase. The activities of these enzymes are important with regard to (a) plant defence mechanism against pests and diseases and (b)

appearance, palatability and use of plant products. Fresh fruits, vegetables, mushroom etc. contain these enzymes considerably (Esterbaner *et al.*, 1977).

About the Plants Under Study

1. *Sesbania grandiflora* L.

Sesbania grandiflora (Linn.) poir, also known as *Sesbania formosa* (F. Maller) commonly called Hadga (India), agati (Philipines), August flower (Guyana), turi (Malasiya, Jawa) belongs to sub family papilionaceae of Leguminasae.

In Asian countries, *S. grandiflora* is commonly seen growing on the dikes between the rice paddies, along the road sides and in backyard vegetable gardens. The outstanding feature of the plant is its extremely fast growth rate, especially during the first 3 or 4 years after planting.

It is a soft wooded tree (Plate 1) reaching a height of about 20-30 feet with an average diameter of stem 10 cm or more. The leaves are abruptly pinnate and leaflets are numerous, linear, oblong and deciduous (Plate 2). The flowers are white, 2-3 inches long, in axillary racemes and very showy. Pods are very long (30 cm), narrow, dehiscent, septate transversely between the seeds. The seeds are small, numerous and oblong. The tender leaves, flowers and pods are eaten. Certain parts of tree have repute in native medicine. Prolific nodulation and extremely large nodules are a dramatic feature of *S. grandiflora*.

The propogation of plant is made by cuttings or seedlings. It establishes readily, grows fast and requires little maintenance. The trees

Plate - 1 : *S. grandiflora* seedlings (3-4 months old), growing in plot soil culture

Plate - 2 : Compound leaves of *S. grandiflora* showing their arrangement and leaflets in each leaf

PLATE 1

PLATE 2

are much planted for forage in parts of Jawa, where cattle breading is important and is continually topped to keep it within the animal's reach. It has not been known to have any toxicity to cattle. *S. grandiflora* herbage is fed daily to animals. Foliage of *S. grandiflora* makes an excellent green mannure. Extraordinary nodulation and rapid growth sugest that its soil improvement qualities though unmeasured are exceptional. The stem is soft heart wood. It is used for timber. It is not durable, so it is of little value. It is used as fire wood in S. Asia and is deliberately planted for fuel in several areas in Indonesia.

Recently it has been used as a paper-pulp source for tropical regions. The fibrelength and chemical composition both appear suited to pulping. Australian workers suggested that logs of *S. grandiflora* with bark are unsuitable for the pulping because of gum and resin prevade it. However, they concluded that it should be possible to utilize *S. grandiflora* (without bark) pulps in the production of printing papers. The wood is satisfactorily pulped by the sulphate process and bleaches readily to high brightness. Australian workers suggested that debarked *S. grandiflora* and kenaf can be pulped together satisfactorily and improved the drainage rate of the kenaf pulp without adversely affecting its strength. It is also used for reforesting eroded hill regions in Taiwan and Indonesia. The roots penetrate most soils, thus becomes valuable for reforestation throughout much of tropics.

The leaves, tender pods and giant flowers contain high crude protein percentage. So, *S. grandiflora* is the favourite vegetable used in curries and soups or fried, lightly steamed or boiled. The flowers of *Sesbania*

grandiflora are butterfly like, white or wine red blooms, produced year round and contain a considerable amount of sugar, thus used for food. In some parts of Asia, the long, narrow pods are eaten as a vegetable dish, much like string bean. The seeds are among the richest in protein, with more than 40% by weight, of all legume seeds.

In southeast Asia, the tree is commonly planted along road sides, fence lines and other boundaries for beautification. The continuously dropping leaves of *S. grandiflora* make a thick mulch that adds nutrients to the soils and fertilizes the other crops. The plants are also used as support for pepper and betel and some times for vanilla vines. When cut, bark of the tree exudes a clear gum that has been used as substrate for gum arabic in foods and adhesives.

2. *Portulaca oleracea* L.

Portulaca oleracea (Linn.) sp. pl. 445. 1753; T. Dyer in Hook. F. Fl. Brit. India 1 : 246; 1874; Cooke, Fl. Pres. Bombay 1 : 72, 1958 (Pepr. ed); Rao in Sharma *et al.* (ed.) Fl. India 3 : 4, 1993. 'Ghol;'. It belongs to family. *Portulaceae.*

It is very common along gutters, nallas and as a weed in cultivated fields and gardens (Plate 3). It is an annual succulent prostrate herb or erect or decumbent. Stem is weak and 6-12 inch long, reddish swollen at the nodes, quite glabrous. The leaves are fleshy, subsessile, 1/4-1 inch long, subopposite, cuneiform, rounded and trunket at the apex, spangled when fresh with glistening dots, margin reddish, flowers, few together (in clusters) in sessile terminal heads, sepals unequal, obtuse, petals 5 yellow,

Plate - 3 : *P. oleracea* luxuriantly growing in pot soil in the garden

Plate - 4 : *P. oleracea* leaves and flowers showing a nature of leaves and characteristics of corolla in the flowers

PLATE 3

PLATE 4

oborate (Plate 4). Stamens 8-12 in number, capsules ovoid, circumscis. Seeds many, reniform, shining black, granulate, testa cells stellulate with many tubercles, flowers throughout years.

Whole plant is used as a vegetable and has cooling effect. The leaves are used as local application in swelling and bruises and as a poultice for abscesses and boils; plant juice is used for treating earache and toothache.

3. *Kalanchoe pinnata* (Lamk) pers.

Kalanchoe pinnata (Lamk). Pears. Syn. Pl. 446, 1805. also known as *Cotyledon pinnata* Lamk. J. Encycl. 2 : 141, 1786. *Bryophyllium calycinum* salisb., Cooke, Fl. Pres. Bombay 1 : 494, 1958. (Repr. ed.). It is also known as 'Panphuti'. It is a Crassulaceae member.

It is a native of tropical Africa, grown in gardens, as pot herb (Plate 5). It is succulent, glabrous and erect herb. Leaves are variable, decussate, the lower usually simple, the upper often 3-7 foliate, petiole long, united by a ridge around the stems. Leaves are with crenate margins (Plate 6). Flowers (Jan.– Feb.) occur in dropping terminal panicles. Inflorescence is terminal corymbose cymes. Calyx is inflated, cylindric, 4-fid, corolla reddish purple, swollen at the base, constricted in the middle, stamens 8 in 2 series, epipetalous, carpels 4, narrowed into the long, green styles, ovules many, follicles 4, enclosed in the papery persistent calyx and corolla. Seeds are small.

It is mostly cultivated in the gardens. The propagation of plant is vegetative and most common. The leaves are supposed to be medicinal.

Plate - 5 : *K. pinnata* growing in pot soil in the garden

Plate - 6 : *K. pinnata* leaves exhibiting their characteristics

PLATE 5

PLATE 6

They are used for the treatment of urine stone. The juice of leaf is used in diarrhoea and applied on bruises for its antiseptic and astringent properties.

S. grandiflora, is basically a C_3 plant. Chavan and Karadge (1986) has studied the physiology of salt tolerance in the species. It was observed that the plant showed a luxuriant growth in soil with an electrical conductivity of up to 10 $mScm^{-1}$ (saline soil). Accumulation of Na and Cl in the leaf rachis compared with leaflets appeared to be an adaptive feature of this legume. Maintenance of an optimum K level and accumulation of Ca were indicative of a salt tolerance mechanism in the species. The level of proline, however, remained unaltered in the salt grown plants. Photosynthetic activity was found to be increased in the salt grown plants, particularly at low salt levels in the medium.

P. oleracea has features typical of C_4 species (Hatch *et al.*, 1975; Joshi *et al.*, 1978; Joshi and Karadge, 1980). Joshi and Karadge (1980) reported a shift in photosynthetic carbon metabolism in the leaves during senescence from C_4 to C_3. *P. oleracea* has also been shown to have features of Crassulacean Acid metabolism (CAM) (Karadge and Joshi, 1980) which get much more expressed under stress (Joshi and Karadge, 1979). The activity of enzyme peroxidase in the leaves of this species was found to be increased during senescence which was accompanied by a decline in the level of catalase. Salinity stress also caused an enhancement in the level of peroxidase in the leaves (Karadge, 1981).

Kalanchoe pinnata, a succulent, is a typical Crassulacean Acid Metabolism (CAM) plant. The leaf tissue of the species exhibits a

remarkable diurnal fluctuation in the acidity (3959%) (Bharucha and Joshi, 1958). Upadhye *et al.* (1986) reported that despite the used of several methods and techniques e.g. different assay methods, different isolation media, enzyme purification by sephadex-G column separation, ammonium sulphate precipitation and polyacrylamide gel electrophoresis, the activity of enzyme peroxidase in the leaf tissue could not be detected. However, the presence of this enzyme could be demonstrated *in vivo*. It was suggested by them that there might be some inhibitor liberated during isolation of enzyme, probably binding tightly to the enzyme molecule which interfered the activity of peroxidase in leaf, stem and roots of this succulent.

From this brief report on the physiology of these three species it is evident that except in *K. pinnata*, hydroxyperoxidases have been studied only for their presence and/or level in the tissue in *S. grandiflora* and *P. oleracea*. Besides, these three plants belong to three different catagories of photosynthetic metabolism. *S. grandiflora* is a C_3 plant, *P. oleracea* a typical C_4 succulent plant while *K. pinnata* a typical CAM- succulent. It was thought worthwhile, therefore, to study hydroxyperoxidases in these three plants for some of their characteristics; particularly, enzyme kinetics. In fact *S. grandiflora* leves have a high level of catalase as well as peroxidase, being a lagume species. This plant has been selected, therefore, as a standard or representative of hydroxyperoxidases and the enzyme systems from the succulents, *P.oleracea* and *K. pinnata* have been compared with. In the present investigation, therefore, an attempt has been made to study the effect of substrate concentration, pH of the medium and

temperature on the activities of peroxidase and catalase. The pattern of isozymes of peroxidase from the leaf tissues of these plants has also been attempted by using the gel electrophoresis technique. The activities of enzymes polyphenol oxidase and IAA oxidase in the species have also been determined.

For the convenience and presentation the thesis has been divided in to five different parts. Chapter I of the thesis gives a brief review of the literature on Hydroperoxidases, peroxidase and catalase and other enzymes in plants with reference to their nature, role and importance in plant metabolism. This chapter also includes discription of plants under study, particularly their morphology, economic importance and some physiological aspects. The methodology and techniques used in the present investigation have been given in details in Chapter II, 'Materials and Methods'. The results of the investigation are presented and discussed critically in the light of recent and relevant literature available, in Chapter – III, 'Results and Discussion'. For convenience this chapter has been divided further into sub parts like 1. Enzyme kinetics of hydroxyperoxidases, 2. Study of other related enzymes and 3. Isozymes of peroxidase. The significant findings of the present study are summarized in the last chapter of the thesis Chapter IV 'Summary and Conclusions'. A literature referred to in the form of research papers, research articles, reviews and books has been listed chronologically and alphabetically in the last part of the thesis, Bibliography.