HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON FIVE EXPERIMENTAL PLANTS
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Introduction

Histochemical processes is essential to study the structural organization of the vesicular-arbuscular mycorrhiza in cells and tissues. Histochemical preparations enable the identification and localization of certain classes of chemical substances. Morphologically the fungus penetrate the plant cell wall and develops an intimate contact with the plant protoplast in response to this infection, the fungus modifies its behavior to control and exploit its own advantage. Visualization of fine structural details in cells interacting in mycorrhizal system is of major importance structure-functional relationships can be understood when the structural organization is known. Conventional techniques could be used to complement other findings. Knowledge of the special distribution of macromolecular components in tissue and cells is another requisite for understanding of VA-mycorrhizal interactions. The recent progress in development of preparation techniques designed for other biological samples. Besides, mycorrhiza should be exploited for the investigation of mycorrhizal systems.

Present study deals with the localization of metabolites in the experimental plants colonized by *G. fasciculatum* and *Glomus mosseae*. 
Selected enzymatic tests were undertaken to define activities in the fungi and association of host tissues.

**Review of Literature**

VA-mycorrhizal fungi have required that anatomical morphological and some physiological processes of the host-parasite relationship be examined with histochemical techniques. Number of workers have found that localization of different chemical substances formed during physiological processes in the VAM symbiosis. Basic staining at first was done by immersing roots in preparation of Aniline blue, Cotton blue in lactic acid (Neill, 1944; Brien *et al.*, 1928). Phillips and Hayman (1970) had given improved procedure for clearing roots staining parasitic and vesicular mycorrhizae for rapid assessment of infection. Kessler and Bland (1972) recognized stained endogone sporocarps associated sugar maple in soil after soaked overnight in an acidified acid fuchsin solution.

Tandy (1975) processed fungus fructifications through formaline acetic acid (FAA) followed by dehydration in a graded alcohol series and infiltration in paraffin. Microtomed or freehand cut sections were routinely stained in ammonical Congo red stain and he used the roots of *Cerococarpus montanus*. Marx *et al.*, (1971) examined Endogone infection in citrus seedlings that was fixed in paraffin, sectioned, and
stained. In recent years particular attention has been given to the mechanism of phosphorus uptake by the plant and utilization of host photosynthate by the fungus. Ling-Lee et al., (1975) found that using histochemical techniques polyphosphate granules, in three different kinds of tree Mycorrhiza. Ling-Lee Ashford and Chilvers (1977) described a histochemical study of the distribution of polysaccharides in *Eucalyptus fastigata* roots. The occurrence of some acid phosphates and dehydrogenases in the vesicular arbuscular mycorrhizal fungus *Glomus mosseae* was reported by MacDonald and Lewis (1978). Acid phosphatase using sodium β-glycerophosphate (B,D,H) as a substrate were detected by the modified Gomori technique (Pearse, 1968).

Nutrients taken up by the fungus and then transferred to the host are experimentally determined by (Sanders and Tinker, 1973; Pearson and Tinker, 1975; Cox et al., 1975). Studied the histological observations on VA-mycorrhizas have shown that, hyphae contain small granules which stain with lead and react meta-chromatically with Toluidine blue. Detection and estimation of polyphosphate in VAM was reported by Callow et al., (1978).

Number of workers have found that localization of different chemical substances formed during physiological process in the VAM symbiosis (Mosse, 1973; Cox and Sanders 1974; Kinden and Brown; 1975; Bonfante, Fosola 1977; Dexheimer et al., 1985; Ling – Lee et
al., 1977; MacDonald and Lewis, 1978; Gianinazzi et al., 1979; Bonfante-Fasolo 1984; Ronald and Soderhall, 1985; Gianinazzi and Gianinazzi-Pearson, 1992; Daft and Okusanya, 1973) examined the effects of mycorrhizal infection of Tomato, Maize, strawberry and Petunia, and observed that the increased amount of vascular tissue; lignifications of the xylem in tomato and petunia plants. Intracellular hyphae and vesicles in the roots of yellow poplar was investigated by Kinden and Brown (1975). They observed the accumulation of carbohydrates (glycogen) and lipids in hyphae and vesicles. Infected cortical cells showed the increased cytoplasmic volume enlarged nuclei and reduction of starch reserves.

In a study of polyphosphate granules in different kinds of mycorrhizae, Ling-Lee et al. (1975) found that polyphosphate granules occurred in hyphae and vesicles further it was indicated that occurrence of similar granules in several diverse mycorrhizal types in Endomycorrhizae. There was an increasing volume of host cytoplasm and the area of interphase between the fungus and the host (Coks and Tinker 1976). In a cytological study, the root colonized by Glomus fasciculatum in Ornithogalum umbrellatum mycelia and vesicles accumulated with fat (Bonfante-Fasolo and Scannerini, 1977). Interfacial matrix and arbuscular were filled with polysaccharides and proteins (Bonfante-Fasolo 1977). Gianinazzi-Pearson et al. (1981) studied ultra
structural organization and some cytochemical features (Proteins and polysaccharide distribution) of the *Glomus tenneus* mycorrhizae in raspberry roots.

**Materials and Methods**

Roots of experimental plants infected with VA mycorrhizal fungus identified as *Glomus fasiculatum* from seedlings grown in beds at the greenhouse and fixed as follows.

**Fixative**  
F.A.A. (Ethyl alcohol = 50ml, Glacial acetic acid = 05ml, Formadehyde = 10ml, Water = 35ml)

The fixed material were dehydrated using a series of ethyl alcohol grades from 50% to 100% alcohol mixture of alcohol and xylol or N-butanol and finally they were treated with butanol.
Processed the fixed material in the following grades. The interval in each of the solutions in the series is minimum 1hr.

**Step 1.** 50% alcohol - 2hrs
2. 70% alcohol - 2hrs
3. 80% alcohol - 2hrs
4. 90% alcohol - 2hrs
5. 100% alcohol - 2hrs
6. 3:1 alcohol + butanol - 2hrs
7. 1:1 1 part of 100% alcohol + 1 part of butanol
8. 1:3 1 part of 100% alcohol and 3 part of butanol
9. Pure butanol – twice 2hrs each.

**Infiltration and Embedding**

After dehydration the materials were transferred to small vials containing a small quantity of pure butanol into which pieces of paraffin were added until the solvent (butanol) reached a saturation point, first at room temperature and later under the table lamp (40W). Finally changes were given with pure paraffin in the temperature controlled oven (normally 55-60°C) to eliminate the last stress of butanol. While giving changes remove the solvent part by part. The interval between each paraffin change is normally three hours (Six to eight changes are required). Later, material were embedded in paraffin. Embedding done by employing paper boat method.
For microtome sectioning small paraffin bits with material were taken, fixed to the wooden blocks and cut, serial sections of 6 μm thickness were cut by Erma rotary microtome. Care was taken to get uniform sections. Paraffin ribbon with serial sections cut into bits of convenient size with a blade and arranged on the microslides flooded with gelatin adhesive. (The adhesive was prepared by dissolving 1 gm of granular gelatin in 100ml of warm distilled water. A pinch of potassium dichromate was added to this). The slides were warmed gently on hot plate to stretch the sections to their original size. Then the slides were labeled and kept for 72 hours in a dust free box for drying. The sections were subsequently processed for staining.

**Preparation of Slides for staining**

The slides were deparafinized in xylene. Then hydrated for localization of total insoluble polysaccharides, starch, nucleic acids, proteins etc. The staining procedure was followed by Phillips and Hayman (1970). Where as anatomical studies of host and symbionts was followed by the method of Johnson, (1940); Pearse, (1962); Feder et al., (1968).
Test for Polysaccharides

Localization of total insoluble polysaccharides was made by employing periodic acid Schiff's (PAS) method. The reaction is based on the oxidation of 1,2-glycol groups of polysaccharides into aldehyde groups with periodic acid (HIO₄). Aldehyde groups formed react with leucobasic fuschin of Schiff's reagent to produce visible magenta-red colour. Periodic acid Schiff's method is highly recommended for the localization of total insoluble polysaccharides because of the following characteristics.

i) The reaction does not cause the breakage of polysaccharide chains.

ii) It is specific to polysaccharides.

iii) It offers least interference and gives no false localization.

iv) It results in a colour complex which was intense as well as stable.

Deparaffinised sections were brought to water level, hydrated sections were treated with 0.5% Periodic acid for 15 minutes at room temperature and then rinsed in water for 30 minutes. Washed sections were incubated in Schiff's reagent for 15-20 minutes at room temperature. Stained sections were washed in distilled water and treated with bleach to remove Super flous. Sections were washed in distilled water, dehydrated in absolute alcohol, cleared in xylene and mounted on
clean slides with DPX. Magenta red colouration in the arbuscules indicating the presence of polysaccharides.

**Control Test**

Hydrated sections were directly inoculated in Schiff's reagent without pre-treatment with Periodic acid. Arbuscules do not produce any colour complex.

**Test for total Proteins (Mazia et al., 1953)**

Deparafinised sections were brought to water. Sections were passed into mercuric bromophenol blue for 5-10 minutes and sections were passed in 7% acetic acid for 1-2 minutes. Finally sections were air dried cleaned in xylene and mounted in DPX. Blue colouration in hyphae and arbuscules indicates the presence of total proteins.

**Control test**: Deparafinised sections were incubated for one hour at 37°C in 0.05 phosphate buffer at 8.9 pH containing 1 mg/ml of pure trypcin washed in water and dehydrated in 95% alcohol and stained with mercuric bromophenol blue. Sites of the proteins do not show blue colouration.
Test for total lipids

Free hand sections of fresh material were used. Root sections were treated with ethylene glycol for five minutes. Ethylene glycol was blotted out and treated with Sudan black-B for ten minutes. Slides were washed 3-4 times in distilled water and sections were mounted on cleaned slides with glycerine jelly.

Control test

Fresh free hand sections have been treated with heat cold alcohol in the reaction mixture.

Test for Polyphosphates

Fresh mycorrhizal infected root section were used to localize polyphosphates. Sections were passed in to 0.1% Toludine blue at pH 1-2 at room temperature. Sections rinsed in distilled water and mounted on clean glass slides with glycerine jelly. Red coloured granular deposition in hyphae, arbuscules and vesicles gives the presence of polyphosphates.

Control test

Fresh free hand sections have been treated with heat cold alcohol in the reaction mixture.
Test for Cytochrome Oxidase

For histochemical studies of enzymes free hand sections of fresh roots were taken. These sections were made use for localization of polyphosphates, peroxidases and cytochrome oxidases.

Sections were treated with stock solution A and B for one hour washed in distilled water and mounted on cleaned slides with diluted glycerine jelly.

Stock solutions consists of a) 3.15gms of Sodium Phosphate dibasic dissolved and made it one litre and b) 3.026 gms of Potassium phosphate monobasic dissolved and made it 1 litre. Mixed as shown below to get a particular pH.

<table>
<thead>
<tr>
<th>pH</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.2</td>
<td>143.0</td>
<td>57.0ml</td>
</tr>
<tr>
<td>7.4</td>
<td>161.0</td>
<td>39.0ml</td>
</tr>
<tr>
<td>7.6</td>
<td>172.0</td>
<td>27.0ml</td>
</tr>
</tbody>
</table>

25ml of stock solution No.1(i.e Buffer Solution ) to the stock solution No.2 which contained 1ml of (alpha solution ) mixed it with the vial containing 1% solution of dimethyl- para-phenylene diamine hydrochloride. This was kept in the working solution at room temperature for about 5-15 minutes. Removed and rinsed the sections in water. Mounted on slide with glycerine jelly. Cytochrome Oxidase appeared blue colour.
**Control test**

Fresh free hand sections have been treated with heat cold alcohol in the reaction mixture.

**Test for Peroxidase**

Many different peroxidase enzymes are present in tissues. A special type of peroxidase is catalase which breaks down hydrogen peroxide. Sections were incubated at room temperature in a stock solution for 5 minutes, then rinsed in distilled water and mounted on cleaned slides with diluted glycerine jelly.

Stock solution was freshly prepared & consists of one part of NH$_4$Cl plus one part of 5% EDTA plus 6 parts of saturated benzidine solution plus one part of 3% H$_2$O$_2$. Blue–brown colouration in the arbuscules indicate the presence of peroxidase.

**Control test**

Fresh free hand sections have been treated with heat cold alcohol in the reaction mixture.

**Test for Nucleic acid**

Deparaffinised sections were brought into water and rinsed in HCL at room temperature for one minute and sections were placed in HCL at 60°C then treated with Schiff’s reagent at room temperature for 45 minutes. The sections were counter stained with fast green (1% FCF in
water for 10-20 minutes). Rinsed 2-3 times in bleach and finally in distilled water and hydrated through alcohol series to xylene and mounted with DPX. The purple colouration indicates the presence of nucleic acid in the arbuscules. DNA: Feulgen method (Gomori, 1952).

This method is based on Skiff's reaction for staining aldehydes, released from deoxyribose sugar, after the removal of purine-deoxyribose glucosid bonds of DNA by hydrolyzing in 1N HCl.

**Staining procedure**

i) Hydrated sections were hydrolysed in 1N HCl at 60° c for 13 minutes.

ii) Sections are rinsed in water and incubated with Skiff's reagent at room temperature for 30 minutes.

iii) After rinsing in water sections are treated with bleach and again rinsed in water dehydrated, cleared in xylol and in DPX.

**Colour indication**

DNA appeared magenta red (Plate – 13, Fig. 6, 7 and 8)

RNA : Toluidine Blue method (Chayen et al., 1973)
Staining Procedure

i) Hydrated sections were immersed in (0.50 mg toluidine blue dissolved in 100ml of 0.05 M citrate phosphate buffer at pH 4.4)

ii) Sections were rinsed in distilled water, air dried, cleared in xylol and mounted with DPX.

Colour indication

RNA stains purple and blue-green

Control test for RNA

Ribo nuclease extraction method (Pears, 1960)

Deparaffinized and hydrated sections were incubated for one hour at 370 c in 0.1 mg/ml solution of ribonuclease in distilled water, the sections are stained with azure B sites of RNA do not stain.

Esterases (Gomori 1950)

Esterases are enzymes which are capable of hydrolyzing esters, with in this group there are many different types of esters, acting upon a number of different substances. The method can be carried out by employing the diazonium salt Fast blue B as the coupling agent.

Reagent required

1) K Naphthyl acetate  2) Acetone  3)0.2N phosphate buffer pH 7.4  Diazonium salt Fast blue B
Knapthyl acetate is dissolved in the acetone and the phosphate buffer is added and thoroughly mixed. The fast blue is added and the solution filtered and used immediately.

Procedure

Sections were brought down to water placed incubating medium for 15-30 minutes at room temperature. Washed the sections in tap water for three minutes and mounted in glycerine jelly. Reddish brown colour stained sections shows the presence of esterase’s.

Preparation of phosphate buffer (Cytochrome Oxidase)

Preparation of Stock solution a) 3.15 gms of Sodium Phosphate dibasic dissolved and made it 1 litre.

Preparation of Stock solution (b) 3.026gms of Potassium Phosphate monobasic dissolved and made it 1 litre. Mixed as shown below to get a particular pH.

<table>
<thead>
<tr>
<th>pH</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.2</td>
<td>143.0</td>
<td>57.0ml</td>
</tr>
<tr>
<td>7.4</td>
<td>161.0</td>
<td>39.0ml</td>
</tr>
<tr>
<td>7.5</td>
<td>172.0</td>
<td>27.0ml</td>
</tr>
</tbody>
</table>
**Buffer preparation:** (Peroxidase)

Stock solution (a) 3.16gm of Sodium Phosphate dibasic dissolved and diluted at 500ml.

Stock solution (b) 3.03 gm of Potassium phosphate monobasic dissolved and diluted to 500ml. Mixed as shown below to get particular pH.

<table>
<thead>
<tr>
<th>pH</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.0</td>
<td>122.0</td>
<td>70.0ml</td>
</tr>
</tbody>
</table>

**Preparation of reagents** (Carbohydrates)

Periodic acid solution 0.5% : 500mg of Periodic acid was dissolved in 100ml of distilled water.

Preparation of Schiff's reagent : One gm of basic fuschin was dissolved in 100ml of 0.15N HCL agitated for 3 hours and then added with 1 gm of Sodium metabisulphate and kept overnight. Treatment of this solution with activated charcoal possessed straw colour schiffs reagent.

Preparation of Bleach : 5ml of 10% Sodium metabisulphate was mixed with 5ml of 1N HCL plus 90ml of distilled water was added.

**Proteins**

1) Preparation of Mercuric bromophenol blue

10gm of bromophenol blue crystals were dissolved in 100ml of 10% mercuric chloride solution in 95% alcohol.
Lipids

1) Preparation of Sudan Black –B.

0.7gm of Sudan black-B powder was dissolved in 100ml of ethylene glycol between 100°c-110°c. The solution was filtered in hot condition and stored in refrigerator for further use.

1) Preparation of Toludine blue (Polyphosphate)

5ml of Toludine solution dissolved in 95ml of distilled water gives 0.05% toludine blue solution.

Results and Observations

The localization of different chemical substances such as polysaccharides, Nucleic acids, total proteins, total lipids and polyposphates in mycorrhizal roots. (Table.18). Magenta red colouration in arbuscules of mycorrhizal root section in Jatropa curcas, Jatropa gossifolia and Madhuca indica showed the presence of polysaccharides. Mercuric bromophenol/blue test conducted for total proteins reacted with fungal components turned blue colour which is positive test for proteins. Total proteins appear to be most common type observed in the immature arbuscules, hyphae and cytoplasm (Plate-13 Fig.1-2 ).

Lipids were present in the form of droplets of various sizes in hyphae and vesicles in Jatropa curcas and Jatropa gossifolia (Plate-14Fig.1&4) Lipid droplets were observed in hyphae of Jatropa curcas
some vesicles appear to be full of lipid droplets (Plate-14 Fig.3) in Madhuca indica. Similarly Polyphosphate granules were determined in the vesicles of Madhuca indica (Plate-15 Fig.1) Localization of three different enzymatic activity presented in (Table. 19).

Polyphosphate granules are detected in hyphae (Plate-15 Fig.2) Orange red granules were detected only at benzoate buffer of pH 4.9 (Plate -15 Fig.6). The localization of Polyphosphate granules indicates the accumulation of phosphate absorbed during the metabolic process of the fungus. These observations suggest that Polyphosphates are of fundamental importance to the phosphorus nutrition of mycorrhizal associations. Purple blue colouration in different areas of hyphae and vesicles indicate the presence of cytochrome Oxidase in Jatropha curcas and Jatropha gossifolia (Plate -16 Fig.1and2).

Peroxidases were located in arbuscules which was confirmed by the formation of brown colour in Jatropha gossifolia, Madhuca indica, and Ricinus communis (Plate-16 Fig. 5 and 6.). Reddish brown colour hyphal structures were observed with the presence of esterases in Ricinus communis and Madhuca indica (Plate-16 Fig.3&4). Semi-thin sections stained with toludine blue revealed the presence of phenolic substances in the roots epidermal and endodermal cells of both inoculated and short
Table-18. Localization of different chemical substances by histochemical techniques in fungal components present in five hydrocarbon plants inoculated with *Glomus fasciculatum* and *G.mosseae*.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Metabolic localised</th>
<th>Test reagent</th>
<th>Colour indication</th>
<th>Localization in fungal structures</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Polysaccharides (total insoluble)</td>
<td>PAS (Periodic acid)</td>
<td>Magenta red</td>
<td>Arbuscules   +</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Nucleic acids DNA</td>
<td>Schiffs reagent</td>
<td>Magenta</td>
<td>Hyphae       -</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>RNA</td>
<td>Toluidine blue</td>
<td>Purple</td>
<td>Vesicles     -</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Total Proteins</td>
<td>Mercuric bromophenol blue</td>
<td>Blue</td>
<td>Arbuscules   +</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Total lipids</td>
<td>Sudan black B</td>
<td>Blue-Black</td>
<td>Hyphae       +</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Polyphosphate</td>
<td>Toluidine blue</td>
<td>Blue-Purple</td>
<td>Vesicles     +</td>
<td>+</td>
</tr>
</tbody>
</table>

Plus- indicates Positive reaction
Minus- indicates Negative reaction
<table>
<thead>
<tr>
<th>S.No.</th>
<th>Type of Enzyme</th>
<th>Substrate in reagent</th>
<th>Temperature</th>
<th>Duration</th>
<th>Colour of reaction product</th>
<th>Localisation in fungal structures</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cytochrome Oxidases</td>
<td>Nadi reagent</td>
<td>Room temp</td>
<td>15 min</td>
<td>Bluish purple</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Esterases</td>
<td>alpha-Naphtyl acetate</td>
<td>Room temp</td>
<td>3 min</td>
<td>Reddish brown</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Plus- indicates Positive reaction
Minus- indicates Negative reaction
Histology and Histochemical studies of VAM colonized experimental plants

Fig-1. L.S. of *Jatropa curcas* roots showing the presence of proteins in immature arbuscules (Ia) (X 1350).

Fig-2. L.S. of *Madhuca indica* roots showing total proteins in matured Arbuscules (Ma) (X 1350).

Fig-3. L.S. of *Jatropa gossifolia* roots showing total polysacharrides (PA) in hyphae (HY) and cytoplasm (X 1350).

Fig-4. The degerating arbuscules (DA) in roots of *Madhuca indica* (X 150).

Fig-5. Polysaccharides in root cytoplasm (PS) of *Jatropa gossifolia* (X 1450).

Fig-6. Root L.S. of *Madhuca indica* showing D.N.A (Da) in arbuscules (X 225).

Fig-7. *Jatrops gossifolia* root L.S. showing D.N.A in arbuscules (X 225).

Fig-8. L.S. of *Jatropa curcas* root possessing D.N.A in arbuscules (X 225).

Fig-9. *Jatropa curcas* root L.S showing R.N.A (RA) in arbuscules (X 225).

Fig-10. Intercellular hyphae (Ia) and arbuscules of *Jatrops curcas* roots showing R.N.A. (X 1400).

Fig-11. L.S. of root of *Jatropa gosifolia* showing R.N.A in arbuscules (X 1400).

Fig-12. Root L.S. of *Jatropa curcus* showing R.N.A arbuscules (X 1400).
PLATE – 14

Histochemical Studies of VAM colonized plants

Fig-1. Lipid (LI) droplets in vesicles (Ve) of Jatropha gossifolia.

Fig-2. Macerated root of Jatropha curcas showing lipid droplets In hyphae.

Fig-3. Roots of Madhuca indica shows lipids accumulation in Vesicles.

Fig-4. Lipids in vesicles in roots of Jatropha curcas.
PLATE - 15

Fig 1. Polyphosphate in vesicles of *Madhuca indica*. Root L.S( x 1450)

Fig 2. Polyphosphate in hyphae of *Jatropha gossifolia*. Root L.S( x 350)

Fig 3. Polyphosphate in arbuscules of *Jatropha curcas*. Root L.S( x 1200)

Fig 4. Polyphosphate in arbuscules of *Ricinus communis* (Mysore local) Root L.S( x 1350)

Fig 5. Polyphosphate in arbuscules of *Jatropha gossifolia*. Root L.S( x 150)

Fig 6. Polyphosphate in hyphae of *Ricinus communis* (Rosa) Root L.S( x 1150)
PLATE-16

Fig. 1  Cytochrome oxidase in hyphae of *Jatropa gossifolia* Root L.S. (x 350 )

Fig. 2  Cytochrome oxidase in vesicles of *Jatropa curcas* Root L.S. (a = x 50 b = x 1550 )

Fig. 3  Esterase in hyphae of *Ricinus communis* (Mysore local) Root L.S (x 450)

Fig. 4  Esterase in hyphae of *Madhuca indica*. Root L.S (x 50)

Fig. 5  Peroxidases in arbuscules of *Ricinus communis* (Rosa) Root L.S (x 650)

Fig. 6  Peroxidases in arbuscules of *Jatropa curcas* Root L.S (x 550)
roots. It was observed that the mycorrhizal roots were considerably large and the cells were densely PAS positive compared to non mycorrhizal roots indicating higher polysaccharide deposition in the cell walls of VA-mycorrhiza (Glomus fasciculatum). It has been detected cytoplasm containing polysaccharides in Jatropha gossifolia (Plate-13 Fig. 3&5). DNA was detected based on magenta red colour in the arbuscules of Jatropha gossifolia and Jatropha curcas (Plate-13 Fig. 7 and 8). Purple blue colouration indicated the presence of RNA in arbuscules of Jatropha curcas and Madhuca indica (Plate-13 Fig. 9-12).

Discussion

Histochemical techniques provided powerful tools for studying the structure and function of symbionts. The results of the conducted experiments revealed that the series of chemical transformation between host and VA-mycorrhiza. It was determined that the arbuscules play an important role in the translocation of material from the host to the fungus and from fungus to host. Microscopic examination revealed that the hyphae appeared Knobby and interwoven closed Vesicles which were globose with simple Knobby and undulated subtending hyphae. Internal vesicles were more in number and showed differential staining and size variation.
Histochemical techniques to study of Polysaccharides shown the nature of the arbuscular wall material and the Osmophillic nature. The acidic property and PAS reactivity suggests that wall is primarily glycolipid composition. This contracts with walls of vesicles and hyphae which are Chitinous. The absence of starch grains in arbuscules and the presence of starch grains in the host cells leads to conclude that the Carbohydrate material might have been taken by the arbuscules in other soluble form but not as starch direct. Similar findings have been observed by Weete (1974). This confirms the view of Ling Lee et al., (1977), the Harris et al., 1970; Juniper et al., 1966; Scannerini et al., 1978; Scannerini et al., 1979; Soderhall, 1985). It was observed that mycorrhizal roots were considerably large and the cells were densely PAS positive compared to non-mycorrhizal roots indicating higher polysaccharide deposition in the cell walls.

Protein components occurred in the interfacial matrix of the host and the Mycorrhiza is evident from the positive reaction to the reagent. The accumulation of lipids in hyphae, vesicles and arbuscules have been observed (Mosse 1959; Sanders and Tinker, 1973; Read and Stribley 1975; Kindern et al., 1976; Spanuan Bonfante-fosolo, 1988; Gianinazzi; and Gianinaxxi-Pearson; 1992). Lipid accumulation more in vesicles get transformed into different forms of chemicals such as triglycerides, monoglycerides, fats, sterols etc. Such accumulation of
lipids in fungal structures is encountered in wide range of host parasitic system. (Brannian and Lose, 1978). Lipids served the fungus as a reserved food and other biochemical process. In a study of phosphorus transport and host growth, the phosphorus released by the arbuscules must be absorbed through the host plasmalemma surrounding the branches. Like wise, the bound carbon given to the fungus probably passes through the same membrane. There is a possible reason why mycorrhizal plants utilized more Phosphate than non-mycorrhizal plants. Polyphosphate is a major P reserve in VA-mycorrhizae. Root cortical cells of mycorrhizal plants contained arbuscules oftern contained high ‘P’ concentration. Where polyphosphate granules are commonly found in young proliferating arbuscules. Polyphosphates may be translocated from the soil in to the mycelium. There were at least two explanations for the differences in peroxidase activity between mycorrhizal and non-mycorrhizal plants. Mycorrhizas are a complex system; two organisms co-exist and interact with each other resulted the plants nutritional status and root growth patterns altered. The localization of peroxidase activity in walls of mature epidermal and hypodermal cells did not seem to be altered when VA-mycorrhizal infection is present. On the other hand, there peroxidase deposition on the middle lamella appeared much more intense in mycorrhial roots
around leading hyphae. The hydrolyzing activity of esters clearly demonstrated by the presence of esterases in hyphal structures.

In conclusion, two different events can be recognized during the establishment of the symbiosis; at first the peroxidase activity in the cell was irritated by the fungus is increased; after this, when the infection was well established, the cell wall bound peroxidase activity, seems to be at levels comparable to those of non-infected plants. These studies support that arbuscules has a distinct specialized highly mutulistic terminal haustorial organs. The changes in the enzymatic activity in the arbuscules life revealed potential nutrient transfer from the host to the fungus and fungus to the host during arbuscular degradation. In general VA-mycorrhizae are now recognized as a potential source of new secondary metabolites.