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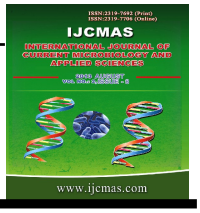


**Original Research Article**

# Biochemical changes in finger millet variety TNAU-914 inoculated with AM fungus *Rhizophagus fasciculatus*

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**A B S T R A C T**

Rhizosphere is inhabited by variety of microorganisms, among, them mycorrhizal fungi are one of the predominate group of microorganisms. The present study was undertaken to understand the effect of mycorrhization on changes in passive physiology and biochemical substances associated with mycorrhizal roots of finger millet. Finger millet (*Eleusine coracana* Gaert.) is the third most important millet crop in India and it is highly nutritious with 0.33% calcium and 12.7% protein. Finger millet was grown with inoculation of AM fungus *Rhizophagus fasciculatus*. The visualization of fine structural components and chemical substances such as polysaccharides, total proteins, total lipids and different type enzymes within the mycorrhizal system and finger root tissue was analyzed. The results revealed that, greater amount of polysaccharides and proteins were observed in mycorrhizal roots compared to non-mycorrhizal once. Cytochrome oxidase enzyme was observed in both hyphae and vesicles but not in arbuscules. In contrast to this alkaline phosphatase, acid phosphatase and peroxidase were localized in both arbuscules and hyphae but not in vesicles. The total lipid was observed in both hyphae and vesicles. These results suggested that, further research is needed to explore activities associated with hidden wealth of soil.

**K e y w o r d s**

*Rhizophagus fasciculatus;* Cytochrome oxidase; *Eleusine coracana;* poly-saccharides; Histochemistry

## Introduction

Soil microorganisms play a pivotal role in the maintenance of plant health and soil fertility. The area around the root, i.e., rhizosphere, is inhabited by variety of microorganisms. Among, these microbes, mycorrhizal fungi are one of the predominate group of microorganisms associated with 90% of the terrestrial plants (Allen, 1192). The symbiotic association of mycorrhizal fungi with roots of higher plants can survive under low temperature, low soil fertility, periodic droughts and other natural stresses. Hence, mycorrhizal research has become one of the most exciting areas of agricultural microbiology in plant sciences.

Millets are the astonishing food grains with rich nutrient content. Among these finger millet (*Eleusine coracana* Gaert.) is

the third most important millet crop in India. Finger millet is highly nutritious and richest cereal with 0.33% calcium and 12.7% protein. Histochemical technique is one of the useful tools in understanding the basic biology of AM fungi and their interactions at the anatomical and physiological levels with host cells/tissue. The visualization of fine structural components and chemical substances within the mycorrhizal system and root tissue is considered as an essential component of passive physiological /histochemical studies. Several such studies have shown a spectrum of chemical constituents in different components of mycorrhizae. Many workers are of the opinion that the localization of different chemical substances formed during physiological processes are mainly due to AMF symbiosis (Mosse, 1973; Cox and Sanders 1974). The present study was undertaken to localize carbohydrates, proteins, RNA, lipids and various enzymes and their metabolites in finger millet variety TNAU-914. This study will enable us to understand the passive physiological and biochemical changes that have occurred between finger millet and mycorrhizal fungi.

## Materials and Methods

### Collection and surface sterilization of Seeds

Seeds of finger millet (*Eleusine coracana* Gaert.) variety TNAU-914 were procured from Agricultural Research Station, Krishi Vignana Kendra (KVK) Hanumanamatii, Haveri district in North Karnataka (INDIA) (unit of University of Agricultural Sciences, Dharwad, Karnataka). These seeds were surface sterilized with 1% sodium hypochlorite solution and washed in distilled water thrice to remove trace amount of sodium hypochlorite adhered to the seed surface. Experimental Design Earthen pots containing 8 kg of growth media (sterilized garden soil and sand in 3:1 ratio V/V) were used for the experiment. The sterilized seeds (4 - 6seeds/pot) of finger millet (*Eleusine coracana* Gaert.) Variety TNAU-914 were sowed on the thin layer of AM fungal inoculum, which was placed just 5cm below the surface of the growth media.

Sorghum root bits infected with Rhizophagus *fasiculatus* (earlier called as *Glomus fasciculatum*) and dried soil sample containing 200-250 spores/ 25 g soil served as AM fungal inoculum. The control treatment was not provided with any AM fungal inoculum.

Experimental pots were arranged in randomized complete block design (RCBD) with triplicate per treatment. Both the treatments were maintained under green house conditions and they were watered thrice in week and Hoagland nutrient solution without phosphorous was given at every fortnight till to harvest.

### Harvest and processing of finger millet roots

Finger millet roots were harvested at 50 days after sowing (DAS). The complete root system was washed with distilled water to make them free from adhered soil particles. Then the roots were fixed in Conroy's fluid (6:3:1- Ethyl alcohol: Chloroform: Acetic acid) for over night and later washed again with distilled water before subjected to further processing. The fixed root bits were then dehydrated in a series of alcohol grades for 2 hour interval

between each grade {50%-70%-90%-100 %-( 3:1-Alcohol: Xylene) –(1:1-Alcohol: Xylene)- (1:3-Alcohol: Xylene) and pure Xylene}. The dehydrated roots were subjected to infiltration and embedded in paraffin wax at 56-58$^0$ C (Johansen, 1940). Later, ultra thin sections were made with help of microtome for the localization of different biochemical components and enzymes in the finger millet roots infected with AM fungus *Rhizophagus fasiculatus*.

**Histochemical localization of biochemical compounds in mycorrhizal roots**

**Localization of total Proteins**

Total proteins in the dehydrated and deparafinized root bits of finger millet was localized by using standard mercuric Bromophenol blue method as described by Mazia *et al*., (1953) with slight modifications. The deparafinized sections were brought to water level and then immersed in mercuric Bromophenol blue solution (10g mercuric chloride and 100mg Bromophenol in 100 ml of absolute alcohol) at room temperature for about 5 min and it was followed by 0.5% acetic acid treatment for 5 -10 min to remove excess dye.

**Localization of Polysaccharides**

Total insoluble polysaccharides were localized by adapting the method described by Feder and O'Brien (1968). Deparafinized root sections were incubated in 0.5% periodic acid (500mg periodic acid in 100 ml distilled water) for 15 min at room temperature and it was followed by 5 min washing under running water. Then these sections were bleached with 2% Sodium meta bisulphate for a minute. Then dehydrated and cleared in xylene and mounted with DPX for the microscopic observations.

**Localization of RNA**

RNA was localized in roots of finger millet by following Toluidine blue method as described by O'Brien and Mc Cully (1981) with minor modification. Localization of different enzymes The root sections were subjected for localization of Peroxidase with help of Diaminobenzidinete trichloride (DAB) method (Graham and Karnovasky, 1966). For the localization of acid phosphatase, sections were incubated for 4-5 hrs in a substrate consisting of 0.1 M acetate buffer and 1mg ml$^{-1}$ sodium 2 naphthyl acid phosphate, 1 mg ml$^{-1}$ fast green, 0.05% $MgCl_2$ at pH 4 to 5 rinsed in distilled water. To test Alkaline Phosphatase enzyme, freshly colonized mycorrhizal sections were incubated overnight in a substrate. It consists of 0.05 tris-citric acid buffers at pH 8.5-9.2, along with 1mg ml$^{-1}$ sodium 2-naphthyl acid (alkaline), 1 mg ml$^{-1}$ fast blue and 0.05% $MgCl_2$. Test for Cytochrome Oxidase Enzyme was undertaken by taking free hand sections of fresh mycorrhizae colonized roots of finger millet and they were treated with stock solution A (315g of Sodium phosphate dibasic dissolved and made it one liter) and stock solution B (3.026 g of Potassium phosphate monobasic dissolved and made it 1 liter ) for one hour washed in distilled water and all the slides were bleached and rinsed with distilled water to remove excess stain.

**Localization of neutral lipids**

Fresh roots of finger millet infected with AM fungus were washed thoroughly with

tap water and then with distilled water. These roots were macerated with KOH and rinsed with distilled water and then treated with ethylene glycol for 5 min. These roots were stained with Sudan black-B (0.7 g Sudan black-C in 100 ml warm ethylene alcohol) for 10 minutes and they were washed to remove excess stain thrice. Then roots were observed under microscope for lipid localization in AM fungal components.

**Localization of 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 to cleaned slides for further microscopic analysis.

## Results and Discussion

The AM fungal colonization in finger millet root was observed at 45 days after sowing. These colonized roots were harvested for the analysis of various biochemical substances and enzymes. It was noticed that, in the present study various biochemical compounds were observed in all the AM fungal components of mycorrhizal roots in greater amount over the non-mycorrhizal finger millet root sections. Localization of RNA, insoluble polysaccharides and proteins was observed in deparafinized and dehydrated sections whereas, lipids and cytochrome oxidase, alkaline phosphatase, acid phosphatase and peroxidase were localized in the hand cut sections of fresh roots (plate 1&2). In the present study, matured arbuscules stained darkly for protein and remained negative in vesicles (plate 1 fig. D). Inter/intracellular hyphae and matured arbuscules showed positive test for PAS indicating high concentration of insoluble polysaccharide (plate 1, fig. C).

Accumulation of lipid in the mycorrhizal roots imparts black colour, which have strong affinity for the general lipid stain called Sudan black-B. Accumulation of lipids in the form of very thick dark droplets was observed in hyphae and vesicles of mycorrhizal finger millet roots (plate 1&2, Fig. A, E and F). Arbuscules in the finger millet roots showed localized Peroxidase (plate 2, Fig. B) and remain absent in vesicles. Striking increase of biochemical substances was due to metabolic activities between finger millet roots and mycorrhizal fungus *Rhizophagus fasciculatus*. The present experimental results enable us to understand the transformation of series of chemical substances due to host and symbiont interactions. The results clearly demonstrated that, the translocation of materials from finger millet to *Rhizophagus fasciculatus* and vice-versa. Cytochrome oxidase was observed in both hyphae and vesicles but no significant results were observed in arbuscules but remaining enzymes such as Alkaline Phosphatase, Acid Phosphatase, Peroxidase were localized in both arbuscules and hyphae but they were remains negative for vesicles (Table 1).

Basic protein appeared to be most common type detected in immature arbuscules, vesicles and hyphal cytoplasm. These results are in consistence with the reports of Nemec (1981). The periodic acid Schiff's (PAS) reaction of polysaccharides in the Arbuscular walls shows osmophilic and acidic properties and it was suggested from the experimental results that, the wall is primarily made of glycolipid. The absence of starch grains in arbuscules and presence

**Table.1** Localization of different biochemical components and enzymes in the finger millet root colonized with *Rhizophagus fasciculatus.*

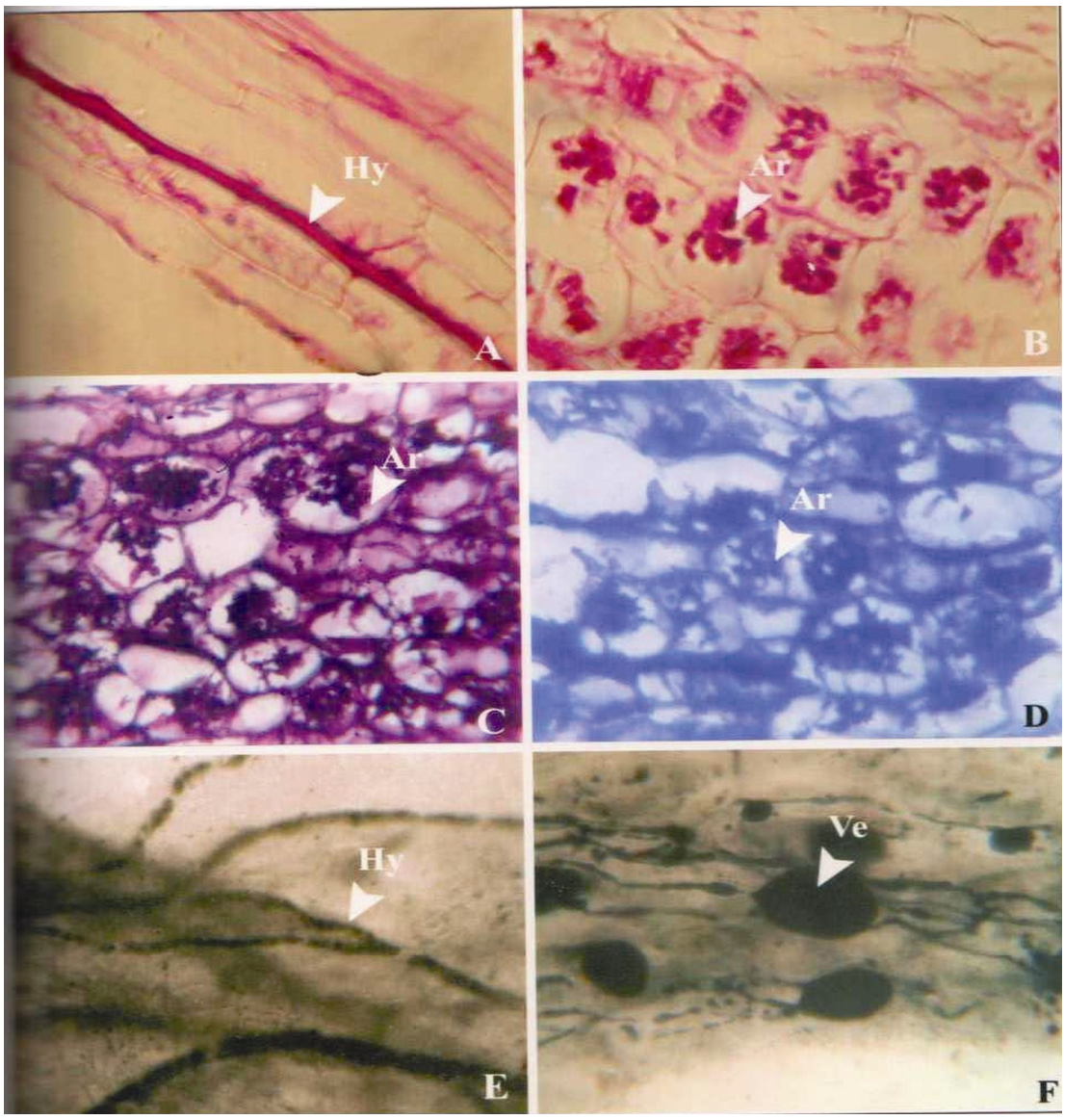
| Sl. No. | Metabolic localized | Colour indication | Localization in fungal structure | | |
|---|---|---|---|---|---|
| | | | Arbuscules | Hyphae | Vesicles |
| 1. | Polysaccharides | Magenta Red | ++ | - | - |
| 2 | Nucleic acids (DNA) | Purple blue | + | + | + |
| 3 | RNA | Purple | + | - | - |
| 4 | Total Protein | Blue | + | ++ | + |
| 5 | Total Lipids | Black | + | + | + |
| 6 | Neutral Lipids | Black | + | + | + |
| 7 | Cytochrome oxidase | Bluish Purple | - | + | + |
| 8 | Peroxidase | blue turns brown | ++ | - | - |
| 9 | Acid Phosphatase | violet black | + | + | - |
| 10 | Alkaline Phosphatase | violet black | + | + | - |

(+): indicates presence and (-): indicates absence

in millet root cells/tissue indicated that, the carbohydrate has taken by arbuscules but not in the form of starch grains. Similar findings were reported by earlier workers in Eucalyptus tree (Ling-Lee *et al*., 1977; Weete, 1974) but not in the agricultural crops.

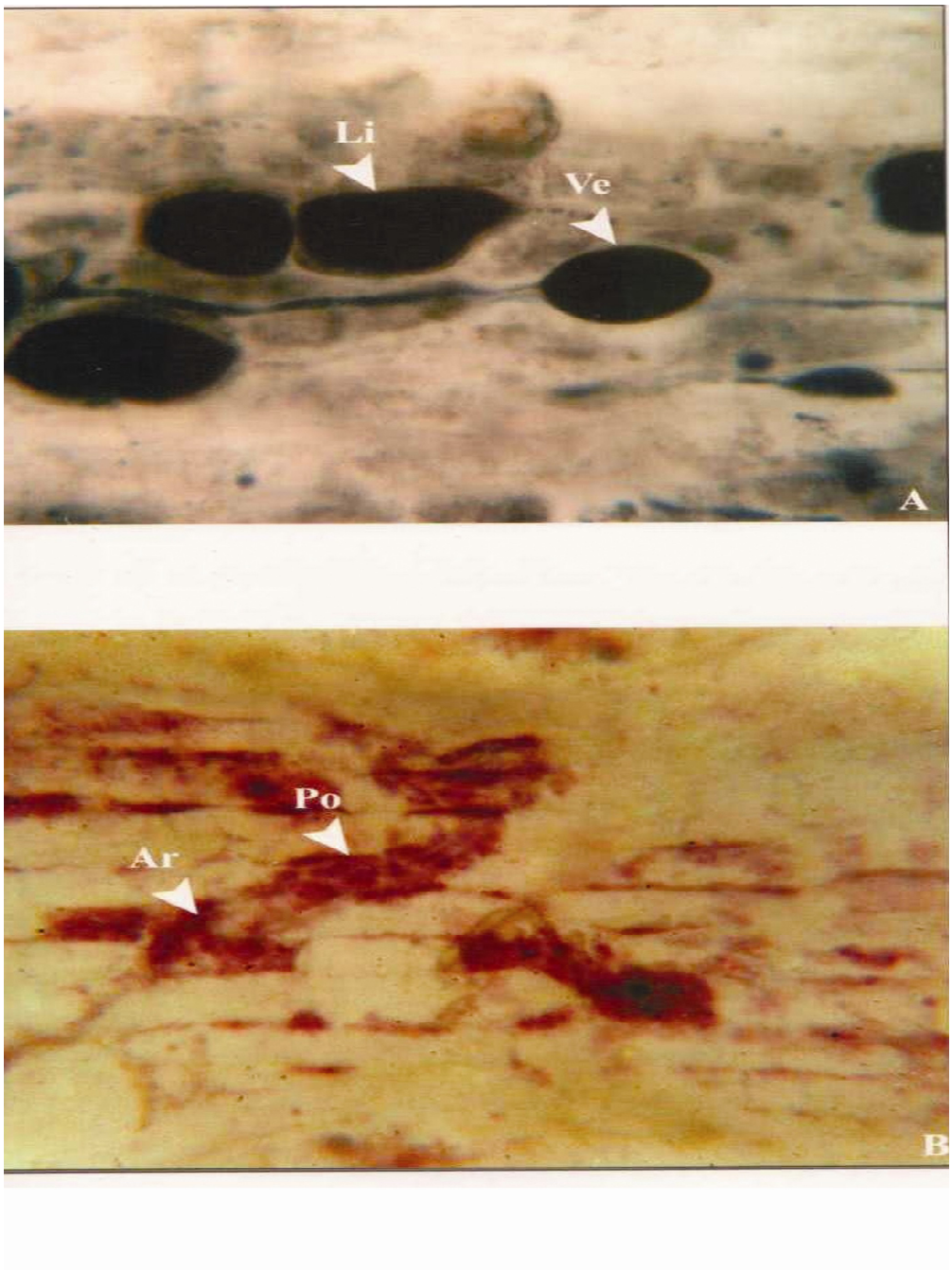
The present study indicated that, the mycorrhizal roots were considerably large and cells were densely stained with PAS than non-mycorrhizal roots of finger millet, this suggests that, they are rich in polysaccharides (Juniper and Robert, 1966; Harrris and Northcote, 1970; Lakshman, 1996 and Mulla, 2002). It was pointed out that, AM fungal hyphae and vesicles were rich in osmophilic substances (Bevenge *et al*., 1975; Cox *et al*., 1975). The accumulation of lipid content in hyphae, vesicles and arbuscules has been observed and these findings are inconsistence with reports of Mosse (1975) in apple and Ericaceous members. More amount lipid accumulation in vesicles resulted in the formation of triglycerides, fats, sterols etc. in finger millet roots and the potential role of phosphate uptake by hyphae was well documented by Callow *et al*., (1978). Peroxidase activity was detected only in senescing arbuscules of the experimental plant roots. It seems that, mycorrhizal fungi colonize the host tissue by combination of mechanical interaction and enzymatic mechanism (Gianinazzi-Pearson *et al*., 1996). Thus, AM fungi play an important role in developing the

**Plate.1** Localization of different biochemical substances in mycorrhizal components of finger millet (variety TNAU-914) roots




A. Insoluble polysaccharides in hyphae (HY) (450X).
B. Insoluble polysaccharides in Arbuscules (Ar) (400X).
C. Higher accumulation of RNA in Arbuscules (Ar) (400X).
D. Rich protein content in arbuscules (Ar) (450).
E. Hyphae (Hy) showing discontinuous lipid in finger millet roots.
F. Vesicles (Ve) showing lipid accumulation in macerated finger millet root.

**Plate.2** Localization of lipids and Peroxidase enzyme in mycorrhizal components of finger millet (variety TNAU-914) roots




A. Vesicles and Hyphae showing lipid accumulation in finger millet root infected with AM fungus *Rhizophagus fasciculate*. (450X)

B. Peroxidase accumulation in vesicles of finger millet root infected by AM fungus *Rhizophagus fasciculate*. (450X)

resistance to host plant against pathogens and abiotic stresses. It can be concluded from the experimental results that, the different biochemical components were accumulated in AM fungal components and they were transformed to the host tissue. The finger millet variety TNAU-914 AM fungal association was indicated with increased accumulation of various biochemical's indicated the passive physiological process between AM fungus and host tissue. Further research is needed to be undertaken to illustrate the genetical/molecular aspects involved in the passive physiology of AM fungal and host root tissue interaction.

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