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Studies on *Glomus aggregatum* with *Allium cepa* at Different Concentration of Soil Phosphate

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Abstract: ***Mycorrhizal association benefits, both the fungi and the host plants. To study the assessment of arbuscular mycorrhizal fungi (AMF) association with the host plant in different concentration of phosphate, Glomus aggregatum spores were collected identified and multiplied in trap culture. Association of a Glomus aggregatum with host plants in 0.01%, 0.02% and 0.03% soil phosphate concentration were used. The percentages of Glomus aggregatum association with Allium cepa were calculated. AMF association with Allium cepa at 0.01% concentration was evaluated.***

Keywords: Mycorrhiza, *Glomus aggregatum*, Phosphate, Mycorrhizal association

1. Introduction

Mycorrhiza is meticulously associated with plant roots to form a unified relationship and the fungi will accept sugars from the plants and in turn the fungi will provide nutrients to the plants (Smith and Read, 1997). The hyphae of the mycorrhizal fungi can transport abundance of phosphate to the plant cells (Mimura T, 1999). The arbuscular mycorrhizal fungi produce a new protein called endomycorrhizins and it lead to the enhancement of symbiotic association (Wyss *et al.*, 1990). The phosphorus availability is one of the most notable matters in the growth of plants (Wang *et al.*, 1998). The availability of phosphorus in the soil is mainly lead to the production of phosphatase enzyme from the mycorrhiza. The phosphate is transferred to the tubular vacuole of mycorrhiza to form polyphosphate (Ezawa *et al.*, 2002). At the time of translocation process the polyphosphate is considered as important phosphorus compound and it translocated to the intraradical hyphae (Ohtomo and Saito, 2005). Concentration of phosphate of soil plays an important role in mycorrhizal association with host plant. In my present study 0.01%, 0.02% and 0.03% concentration of phosphate subjected to check the AMF colonization in host roots.

2. Materials and Methods

2.1 Isolation, Identification and Assessment of AMF Spores

Soil samples were collected from three distinct areas like Mabalipuram, East coast road and Tambaram from the rhizosphere region of Eucalyptus trees. The mycorrhizal spores were isolated by using wet sieving and decanting technique (Gerdemann and Nicholson's, 1963) Trap culture techniques were applied for the multiplication of AMF spores (Morton *et al.*, 1993). *Allium cepa* were used as host plants. AMF spores were sorted under dissecting microscope and identified as *Glomus aggregatum* based on its morphological characters (Walker, 1983). Colonization of *Glomus aggregatum* in trap culture was examined under microscope and colonization was calculated using the formula (Philips and Hayman, 1970).

Percentage of colonization = Total number of root segments colonized / Total number of root segments studied

2.2 Estimation of phosphate

Red soils were collected in and around Chennai, were tested for phosphate content (PWD department, Taramani, Chennai). 0.01%, 0.02% and 0.03% phosphate content soils were labelled as A, B and C was used for present study.

2.3 Assessment of *Glomus aggregatum* association in different concentration of phosphate contain soil

0.01% Phosphate soil sample (A), 0.02% Phosphate soil sample (B) and 0.03% Phosphate soil sample (C) were tested for the symbiotic interaction of *Glomus aggregatum* with *Allium cepa* after 15 days. The association were assessed by Grid line intersect method (Adholeya and Gaur, 1994). The results were tabulated.

2.4 Estimation of proteins and SDS PAGE Analysis

Glomus aggregatum associated *Allium cepa* roots from soil A, B and C were collected and ground with phosphate buffer at pH 7. The content was centrifuged, supernatant were collected and proteins were precipitated by ice cold acetone. Those proteins were estimated by Lowry's method with standard BSA (Lowry *et al*, 1951). 12% acrylamide gel was prepared and the protein samples were loaded along with rainbow protein molecular marker (Laemmli, 1970).

3. Results

Mycorrhizal association may differ in various soils (Porter *et al.*, 1987). AMF spores 48 numbers were obtained from Mahabalipuram area, 64 spores from ECR Road and 22 spores from Tambaram area (Tab.1).

Table 1

Area	*Number of AMF spores / 1Kg*
Mahabalipuram	48
ECR Road	64
Tambaram	22

Figure 1: AMF Spore Identification, *Glomus aggregatum*- 120µm

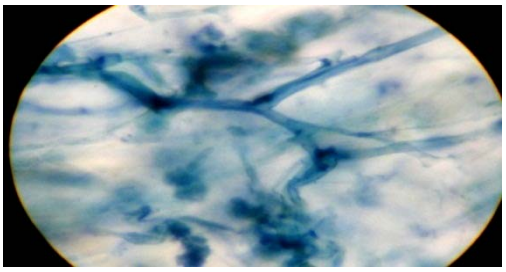

Figure 2: Arbuscules- 100x

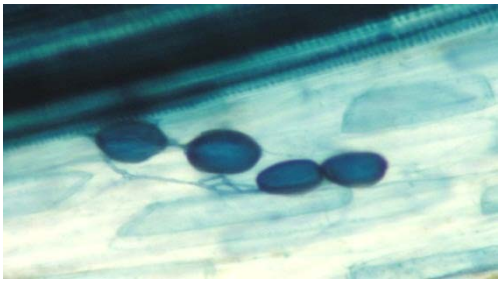

Figure 3: Vesicles- 100x

Based on Walker 1983, *Glomus aggregatum* was identified and multiplied (Fig. 1) (Fig 2 and Fig.3)

***Glomus aggregatum* associated Trap Culture with Different Concentration of Phosphate**

Figure 4: Trap culture of *Allium cepa*

Figure 5: Morphological parameter

Pot 1 – 0.01% phosphate concentration; **Pot 2** – 0.02% phosphate concentration; **Pot 3** – 0.03% phosphate concentration; **Pot C –** Control

Table 2: Morphological Parameters of AMF Associated *Allium cepa*

Treatment of Length of plant (P) mg/100g (cm)	Fresh weight of root (g)	Dry weight of root (g)
C 14.8	6.79	1.21
0.01 15.2	6.90	1.29
0.02 15.4	10.81	2.83
0.03 16.7	12.14	2.97

Assessment of *Glomus aggregatum* Association with *Allium cepa*

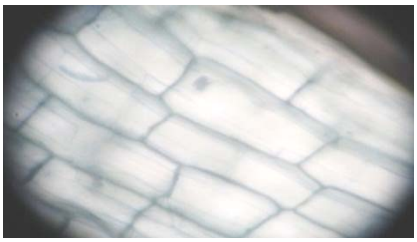

Figure 6: Control

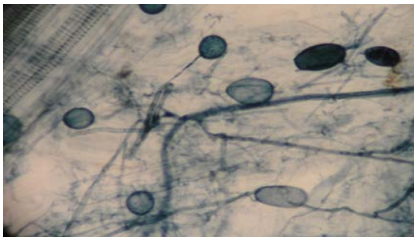

Figure 7: 0.01% phosphate concentration

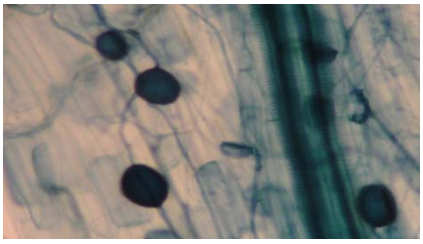

Figure 8: 0.02% phosphate concentration

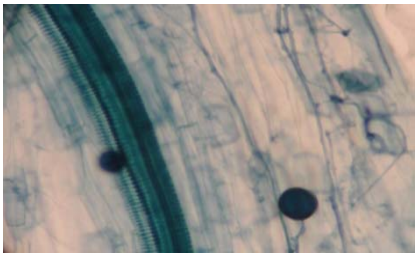

Figure 9: 0.03% phosphate concentration

Table 3: Assessment of *Glomus aggregatum* Association with *Allium cepa* in Different Concentration of Phosphate

Phosphate concentration in trap culture mg/100g	Percentage of *Glomus aggregatum* association
0.01	64%
0.02	52%
0.03	34%

Out of three concentration of phosphate 0.01% (mg/100g) soil proved to be the highest percentage of association, 64% association of *Glomus aggregatum* with host plant were evaluated. 52% at 0.02% of phosphate and the least was 0.03% (Fig. 4, 5,6,7,8 and 9) (Tab. 2 and 3).

Table 4: Protein Profile of *Glomus aggregatum* Associated with *Allium cepa* in Different Concentration of Phosphate

Phosphate concentration mg/L	*Protein concentration µg/µl*
.01	32
0.02	20
0.03	12

Proteins from *Glomus aggregatum* associated host plants at different concentration of phosphate were estimated by Lowry's method followed by SDS PAGE analysis.(Tab. 4) (Fig. 10).

Figure 10: SDS Page Analysis of Proteins from *Glomus aggregatum* Associated with *Allium cepa* in Different Concentration of Phosphate

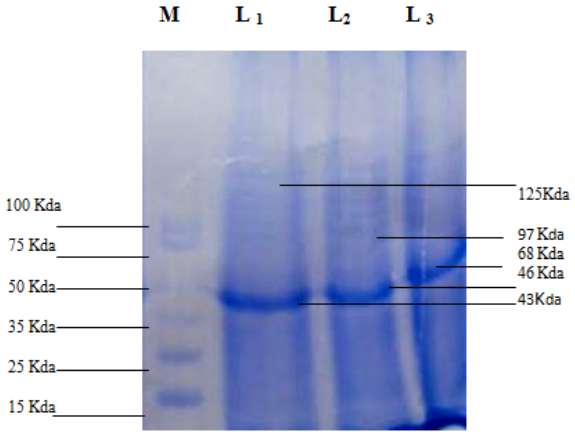

Figure 10: M- Protein marker; **L1**- 0.01% phosphate concentration**; L2**- 0.02% phosphate concentration; **L3**- 0.03% phosphate concentration

4. Conclusion

It concluded from this study that when the concentration of phosphate increases it will result in the decrease of colonization of mycorrhizal fungi. At 0.01% (mg/100g) concentration of phosphate evaluated more percentage of association with *Glomus aggregatum*. 0.01% (mg/100g) phosphate in soil concentration has shown more length and fresh weight of host plant growth and protein concentration also higher in lower level of phosphate.

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