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Research Article

Effect of mycorrhizal fungi on growth of *Zea mays* L. Plants

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Abstract

To assess the occurrence and distribution of VAM fungi associated with Rhizosphere soil and roots of *Vigna catjang* and *Phaseolus mungo* which were collected from two different localities Kongarai and Mampattu. To identify the efficient native VAM fungi and their multiplication and influence on the growth nutrition and biochemical compounds of *Glomus* by pot culturing method. *Glomus fasiculatum, Glomus geospore* were most abundant species found in the root zone soils of all study sites. Influence of native strains of VAM fungi namely *Acaulospora* by *Glomus Gigaspora, Margarita, Glomus fasiculatum* were selected for the growth nutrition and biochemical compounds. The genus *Glomus* in general, *Glomus fasiculatum* in particular was the predominant colonizing species followed by *Glomus geosporum* in both test plants. The present study had helped to boundaring the knowledge about VAM in relation to ecological and physiological aspects which could help in developing suitable applications to improved growth of certain oil yielding crop plants in Sandy loam soil.

Keywords VAM fungi ; Rhizosphere soil; *Glomus fasiculatum, Glomus geospore;* Zea Mays Plants.

Introduction

Mycorrhiza is beneficial association between the roots of plants and fungi. It is a distinct morphological structure, which is a result of mutualistic symbiosis between specific root inhabiting fungi and plant root. The term “Mycorrhiza” coined by “Frank”(1885)describes the structure formed by the association of plant root with fungi.

Mycorrhiza can be broadly classfied into ectomycorrhizae, endomycorrhizae and ectoendomycorrhizae. Among the endomycorrhizae types vesicular-arbuscular mycorrhizae is common, wide spread and more important. Mycelia of these fungi invade the roots of the host plant and proliferate within Vesicular-arbuscular (VA). Mycorrhizal infection is characterized by the formation of vesicles and arbuscules in the root cortex, which also contain inter and intracellular aseptate hyphae connected with an external mycelium. The VAM fungi are mainly found in crop plants and they have very ancient origin during back to the early land plants (Simon *et al*., 1998).

Significance of VAM

VAM fungi helps the plants to survive in phosphorous deficient soil. VAM is well adapted to eutrophic soil, and the fungi are especially active in phosphate and zinc uptake from the soil. It is now established that many plants cannot grow adequately without VAM fungi, especially in phosphate deficient soils, VAM fungi promotes plant growth by enhancing the uptake of phosphate through the Mycorrhizal root (Smith and Gianinazzi – Pearson 1988).

The VAM fungi also stimulate the beneficial organisms like *Rhizobium, Azotobacter and* phosphate solubilizer in the Rhizosphere. VAM fungi increase the physiological nature of absorbing surface area of the root system. VAM fungi also increase the availability of roots to plant resulting in more growth under drought conditions. VAM association also induces the phyto hormones like indole acetic acid, gibberellic acid and cytokinemins etc.,

VAM fungi increase the tolerance of plants to droughts, high soil temperature and extremes of soil acidity caused by high levels of metals such as manganese and aluminum. They provide protection from certain plant pathogenic fungi and nematodes that attack roots. (Zak, 1964). VAM fungi modify transpiration rates and the composition of Rhizosphere microflora by excretion of conflating compounds. Ex: Ectoenzymes.

One of the major changes in mycorrhizal plants is the reduced membrane permeability due to increase in nutrition. VAM induction of growth substances such as Indole acetic-acid (IAA) , Indole propionic acid (IPA) and indolic compounds has been demonstrated (Moser 1959); Ulrich, 1960 and Stuzeiczyk *et al.,* 1977). The mycorrhizal infection also induces the production of auxins. These associations might benefit the most plant particularly under limited soil moisture. The mycorrhizal plant responses are result of the increasing the rate of plant physiological process. Those physiological changes are known to induce a potecialy different myco-rhizosphere interactions. The physiological changes that accompany the development of mycorrhizae are undoubtedly extensive and they will after the nutrient balance of the plant tissues. The carbon balance will also change because of increased photosynthetic rate and attested carbon patitioning of mycorrhizal plant (Paul *et al.,*1985)

Mycorrhiza literally means ’fungus root’ of the different kinds of mycorrhizae,vesicular-arbuscular mycorrhizae (VAM) is the most prevalent type. The term VAM fungi denotes the formation of special structures namely, vesicules and arbuscules by the root colonizing fungi inside the host plant tissue, especially in the inner cortex of the root. The VAM are nonspetate, belonging to the families of Glomaceae, Gigasporaceae and Acaulosporaceae of the order glomales of the class Zygomycetes (Morton and Benny,1990). The VAM association is formed by a great variety of plants of different taxonomy groups. It is also geographically ubiquitous occurring in plants from artic and antartic regions over a brode ecological range from aquatic, terrestrial and to desert environments (Mosse 1981).

The role of VAM fungi in plant growth the nutrient uptake is well documented (Jeffries, 1987). The major part of the beneficial effects of VAM is attributed to the role in the uptake and translocation of immobile elements like P, Zn and Cu and also more mobile elements such as S, K, Mg, Ca, Fe and Mn (Tinker ,1984) Enhanced water and nutrient uptake, tolerance to drought, salinity and decreased severity of root diseases are the chief benefits of VAM association in host plants. VAM fungi are not only structurally efficient for expansion of nutrients from exchange sites in soil but also produced enzymes such as phosphatases, nitrogenase compounds (Selvaraj and Subramanian 1995).

Numerous techniques are available for the mass inoculum production of VAM in an almost sterile environment. However, the convenient method of producing large quantities of inoculum is by the traditional ‘pot culture’ technique developed. Several host plants including sudan grass, bahia grass, sorghum, maize and onion have been studied

for the suitability in producing VAM inoculum (Bagyaraj. 1992).

VAM in relation to plant disease

VAM enhance the plant growth through increased nutrient uptake, states tolerance and disease resistance. As an integral part of the root system, they interact, with other microorganisms in soil and result in increased root exudation approaching about 25% of the plants dry mater production. Roots support a multitude of microorganims that in concert, can have profound influence on growth and survival of the plants – VAM fungi can alter the root caudation pattern, exchange chitinolytic activity and alter photosynthetic and respiratory deficiencies. VAM positive plants are known to exhibit varied resistance towards soil borne and doliar pathogens. The known interactions of mycorrhizae into the plant. To improve their productivity pathogens, significant of plant cell walls, changed phosphate nutrition resulting in alter exudation by roots and formation of inhibitory low molecular weight compounds.

Objectives of our work

To isolate the VAM spore from the Rhizosphere region of the two plants namely V*igna catjang .L* and *Phaseolus mungo L*. To study the mass cultivation of spores by the cress inoculation methods in *Allium cepari* seedlings. To screen the phosphotase content in the various type of *mycorrhizal* spores. To asses the growth influencing activity in the *Zea mays* by VAM spores inoculation methods. To asses the growth and yield parameters in *Zea mays* after treated the VAM and Blue green algae in the experimental pots and compared with the control.

Materials and Methods

Study localities

Two different cultivated field localities of Kongarai and Mampattu, Kancheepuram district of Tamil Nadu were select for the study of Mycorrhizal studies of Pigeon pea (*Vigna catjang*.L) and black gram (*Phaseolus mungo*.L) and their colonizing Vesicular Arbuscular Mycorrhizal fungi. The present investigation were made to study the interaction and significance of Vesicular Arbuscular Mycorrhizae in the experimental plants.

Sample Collection

Vigna catjang L. and *Phaseolus mungo* L. are the important pulses crops in Tamil nadu. They were collected from the field of Kongarai and Mampattu.

Soil Characteristics

Soil samples were collected from the two different study sites the collected samples then air dried, mixed thoroughly and analyzed for pH, electrical conductivity, percent organic matter, micro (Fe, Zn, Mn) and macro elements (N, P, K) were done at the Soil Testing Laboratory, Gudimiyanmalai, Pudukkottai. The different elements were analyse as follows:

Elements	Methods
Phosphorus	Olsen *et al. (1954)*
$NaCHO_3$ extractable	Sankaram (1966)
Nitrogen and Potassium	Lindsay and Norvells (1978)
Zinc, manganese, iron	Warkley an Black (1934)
Organic matter	

Collection of root samples

To check the mycorrhizal status of the plant species, young and lateral root samples were collected, washed free from attached soil particles, cut into several small (1 cm) fragments and fixed in FAA (Phillips and Hayman, 1970) in the field itself.

Processing of root samples

The procedure adopted by Phillips and Hayman (1970) was followed by clearing and staining the

roots for rapid assay of VAM colonization. The procedure is as follows.

The roots fixed in FAA were washed thoroughly in tap water but not vigorously enough to detach the external mycelium. The roots were placed in a 250ml beaker and 10% KOH (Potassium hydroxide 10gm in 100ml of distilled water) solution was added to it. This was boiled at 90ºC for 30 min to 45 min depending upon the nature of root samples. The KOH solution clears the host cytoplasm and nucleic acid and readily allows the stain penetration. After boiling was over, the KOH solution was poured off and roots were rinsed with fresh KOH solution for few minutes. Then the roots were thoroughly washed in tap water at least for 3 times. To decolourise the pigments, the roots were immersed in 30% H_2O_2 (Hydrogen peroxide) solution and placed at room temperature for 3-5 min or until the roots get bleached. Then the roots were washed in several changes of water to remove H_2O_2 completely. The samples were then acidified in 5N HCL for 3-5 minutes for proper staining. Finally, the roots were stained in 0.05% tryphan blue stain in Lactophenol for about 30 minutes. The staining time may be reduced or increased depend upon the root samples. The excess stain was removed by using Lactophenol.

The root segments were cut into pieces of 1cm length. One hundred to two hundred root bits of a sample were mounted on slides using lactophenol as a mounting medium and examined in a compound contrast microscope for the presence of VAM hyphae and vesicular structures under low power (10x) and high power (40-60x) for the Arbuscular structures. The slides were made semi permanent by sealing the edges of the cover slip by DPX mountant . The root colonization percentage of each plant was calculated by using the following formula.

$$\text{Percentage of root colonization} = \frac{\text{Number of VAM infected roots}}{\text{Total number of root bits examined}} \times 100$$

Quantification and identification of VAM fungi spores

A small amount of soil from the Rhizosphere region of *Vigna catyung.L* and *Phaseolus mungo* was dug out by a trowel to a depth of 10 to 15 cm cutter scraping away the top 1 to 2 cm soil and collected in a polythene bag. The soil samples in the polythene bags were brought into laboratory and deep-freezer stored at 2 to 5ºC until the endomycorrhizal spores were isolated.

Processing of soil samples by wet sieving and decanting method

100gm of soil was suspended in about 500ml of water. Heavier particles gradually settled down, and the liquid was decanted through a 710µm sieve to remove the larger particles of organic matter and root.

The suspension that passed through 710µm sieve was saved and stirred to re suspend all particles and decanted through 450µm sieve. The re suspended material was again stirred well and decanted through 250µm sieve by adding further 200ml of water. The re suspended material was again passed through a 106µm sieve and re suspended. The residue was taken in another 500ml of water and passed through the sieve and the material was taken from the sieve by the repeated washing. There suspended material was passed through a 75µm sieve. he suspension was passed through 45µm sieve and the residue on the sieve was washed and taken in a small beaker. fter allowing the heavier soil particles to settle down for 5 minutes. The supernatant was filtered through a filter paper. This filter paper was transferred onto a glass plate and observed under a dissection microscope. The spore population was expressed as individuals per 100gm of dry soil samples. Intact spores of subtending hyphae free from debris were transferred to clean microscopic slides with the help of a fine needle and mounted on lactophenol. Semipermanent slides were made by sealing the edges of the coverslips with nailpolish / DPX mountant.

Identificaton of vam fungi

Based upon microscopic characters, the VAM spores were identified, by using the keys and manuals provided by Hall and Fish (1979). Trappe (1982), Walker and Koske (1987), Schenck and Perez (1987), Morton (1988) and Morton and Benny (1990). Microphotographs were taken with the help of Nikon Optiphot No.2 compound microscope.

Pot experiments

Pot Experiments in sterilized soil have been reported to be valuable by providing much useful information on the role of VAM in growth and mineral nutrition. (Krishna and Bagyaraj, 1982; Abbott and Robson 1984) by demonstrating the apparent difference in the affectiveness of the VAM fungi.

VAM inoculum used

For mass inoculum production and selection of an efficient strain of VAM fungal experiments four native VAM fungal inocula viz., *Glomus faciculatum, Glomus Geosporum*, *Agalospora* and *Gigaspora* were used.

Host plant used

For mass inoculum production and influence of native strains of VAM fungal experiments was done using (Zea mays) as the test plant. The following morphological, Biochemical and nutritional parameters were studied after harvest of 65^{th} day. Plant height, dry weight of root biomass. Total chlorophyll,Protein, carbohydrate, amino acids contents. Percent of mycorrhizal colonization in roots, number of VAM spores 100g root zone soil and mycorrhizal effect. Total soluble and reducing sugars, total proteins, total carbohydrates, total amino acids in the leaves were analysed.

Pot culture technique

The pot was filled with red soil and inoculated with the *Zea mays* after the growth the VAM spores are inoculated and incubated for 2 weeks. In the second pot the red soil was filled and inoculated with the *Zea mays* after the growth and inoculated with the VAM spores and onion root fragments are also inoculated. In the third the sandy soil was filled and inoculated with the *Zea mays* after the growth and inoculated with VAM spores and phosphobacteria and incubated for 2 weeks. In the forth the pot was filled with sandy soil and inoculated with *Zea mays* and spinkle with water and add the VAM spores plus cyanobacteria and incubated for 2 weeks and growth was observed. Finally the control was taken and filled with red soil and inoculate the *Zea mays*.

Results

Vesicular Arbuscular Mycorrhizae of beneficial fungi that penetrate and colonize the root of the plant. Then sent out filaments into the surrounding soil. In recent years significance of VAM spores and its enumeration have been fed one by various investigators.

The plant fungal relationship is an elegant association and its development is evidently are regulated are regulated by several factors. The present studies was reported on the Mycorrhizal status and the occurrence of the spores in the roots and Rhizospheric soils of the two economically important pulses crops. Grown in cultivated field of two different sites of the Kongarai and Mampattu.

In the physicochemical analysis of the study soils. In the analysis of the edaphic characters in two different study soils were presented the table1. The physico chemical character of the study soils may be varied. In the two study soils such as Kongarai and Mampattu were chosen for VAM isolations. Among the two sites the Kongarai field showed the red soil and the Mampattu field showed clay loam soil. Among the two sites were showed the neutral to alkaline and generally deficient in phosphorus. The other soil element showed the minor variations.

Significance of the VAM

Vigno Catjang and *Phaseolus mungo* are important pulses variety summer crop plants were selected from two different localities at Kancheepuram district. The enumeration of the VAM colonization present in the Rhizosphere and root give a positive result for the two plants species. The VAM species isolated from the study sites about 12 number of the spore belonging to the three genera namely *Aculospora, Gigaspora* and *Glomus*.

Effect and VAM on plant growth of zea mays

Mycorrhizae inoculated plant shows the significant result of root and shoot length, leaf length, leaf breath and total chlorophyll, protein, than the uninoculated control . The experimental plants of *Zea mays* inoculated VAM along showed a slight increased phonological character like plant height, leaf length, leaf breath and root length (Tables 2,3,4,5).

Phytochemical characters like total chlorophyll, protein, carbohydrates, Ash, increased in VAM +Phosphobacteria treated plants than the uninoculated control plants (Tables 2,3,4,5). The second treatments like VAM plus onion root fragment inoculated with the *Zea mays* shows the significantly increased rate of growth and phytochemical concentrations then the uninoculated plants (Tables 2,3,4,5).

The third method of pot culture treatment in *Zea mays* with VAM spores and phosphobacterium give a promising level of the increased results for the phonological characters like shoot length, leaf length, breath and phytochemical characters . (Tables 2,3,4,5). The last treated plants of *Zea mays* with BGA and VAM spores. They show the partial increasing results of phonological and phytochemical concentration then the uninoculated control (Tables 2,3,4,5).

The VA Mycorrhizal status was considerably higher in all inoculated treatment combined in control. The extent of colonization varied in to the different treatment. The result show that *Zea mays* slightly different their the response to inoculation with VAM and BGA. But they give a better activity only occur in VAM plus phosphobacteria inoculated plants then other treatments.

Discussion

The present study for the VA Mycorrhizae status of an essential for the commertially available plants like *Zea mays* growth and yields. The number of majority of the G. Vesicular Arbuscular Mycorrhizal studies was completed by the previous investigates for the and think about the different in approach. The VAM spores isolate from the two different localities namely Kongarai and Mampattu. In this study the sites selection as well as the selection of commercially available *Zea Mays* were used for the pot culture experiment and mixed with the VAM spore derived from the Rhizosphere soil of *Vigna Catjang* and *Phaseolus mungo*.

In the great interest in VAM in recent years has prompted numerous surveys aimed at enumerating the species and their colonization of host plants in different regions of this country (Singh and Varma, 1981; Mohankumar, 1985 ; Regupathy *etal*, 1988; Selvaraj 1989; Bhaskaran 1997). In our study to isolate and identified the VAM spores from Rhizosphere area of two pulses crops. Agriculture is the main stay in Indian economy related to the functional biodiversity and then ecological survises, soil fertility, crop production and productivity was initially described by Altieri (1995).

In our present investigation also made to anslysis the physicochemical status in the soil and their adaptational ecosystem responsible for the a abadent colconization of Vesicular Arbuscular Mycorrhizae spores in red and clay loam soil. Occur in the two pulses crops. Reena singh and Alok Adholeya (2001) was described the interrelationship between the climatic conditions soil types into the Vesicular Arbuscular Mycorrhizal colonization. In our present studies also were in the climatic condition and soil types also responsible for the increasing number of Vesicular Arbuscular Mycorrhizal colonization in the clone vicinity soil regions two pulses crops.

Table.1 Physico-chemical analysis of Soil samples

S.No	Parameters	Report
1	pH	7.2
2	EC	103
3	Organic matter	2.3 mg
4	Fe	1.8 mg
5	Zn	5.23 mg
6	Mn	3.6 mg
7	Nitrogen	24.8 mg
8	Phosphorus	32.8mg
9	Potassium	15.8 mg

Table.2 Effect of morphological parameters of *Zea mays* plants inoculated with VAM

Treatments	**Number of leaves/plant**	**Length of leaves (cm)**	**Breadth of leaves (cm)**	**Length of plant (cm)**	**Shoot length (cm)**	**Root length (cm)**	**Total length of plant (cm)**
Control	*7.1*	*20.0*	*2.5*	*65.5*	*41.5*	*9.0*	*74.5*
VAM	*9.0*	*26.9*	*3.6*	*69.3*	*49.4*	*10.2*	*79.5*
VAM Onion Plant root	*8.9*	*28.5*	*3.4*	*80.0*	*60.3*	*11.4*	*91.4*
VAM +Phosphobacteria	*12.0*	*31.0*	*3.9*	*82.1*	*69.6*	*11.6*	*93.7*
VAM+BGA	*8.4*	*26.7*	*3.2*	*79.1*	*54.3*	*10.3*	*89.4*

Table.3 Effect on yield concepts of *Zea mays* plants inoculated with VAM

Treatments	**No.of Flowers**	**Root dry weight (Mg)**	**shoot Wet weight (Mg)**
Control	2.4	25	42
VAM	3.4	32	54
VAM Onion Plant root	3.8	27	47
VAM +Phosphobacteria	5.4	43	58
VAM+BGA	4.2	38	46

Table. 4 Effect of biochemical parameters of *Zea mays* plants inoculated with VAM

Treatments	Chlorophyll (mg/g)	Protein (mg/g)	Carbohydrate (mg/g)	Amino acids (mg/g)	Inorganic phosphorus (mg/g)
Control	1.06	0.92	13.14	3.6	2.02
VAM	1.82	0.98	14.80	5.6	2.13
VAM Onion Plant root	1.84	0.97	14.80	8.67	2.32
VAM +Phosphobacteria	3.86	2.0	16.11	19.60	4.18
VAM+BGA	2.30	1.0	15.27	12.24	2.72

Table.5 Effect of biochemical parameters of *Zea mays* plants inoculated with VAM

Treatments	Reducing sugar (mg/g)	Ash (mg/g)	Alkaline phosphatase (mg/g)
Control	1.62	30	0.29
VAM	1.65	45	0.37
VAM Onion Plant root	1.90	45	0.35
VAM +Phosphobacteria	4.78	46	0.59
VAM+BGA	3.33	40	0.45

Krishna and Bagyaraj (1982) was reported the high number of the Vesicular Arbuscular Mycorrhizal spores, present in the Rhizosphere soil region then the Rhizosphere area of oil yielding plants. In our studies also high lights the heavy number of plants spore population present in the Rhizosphere regions than the other area of soil collected from the Kongarai and Mampattu. Gerdemann and Trappe, 1974; mohan and Natarajan 1988, Kannan and lakshmi narasimhan 1988 was recorded the occurrence of VAM spores in oil seedlings plants colonization the genus *Glomus* spores dominant members than the other VAM spores.

In our studies three different type of VAM spores isolated from the two pulses crops inhabiting soil among these type of the VAM spores *Glomus fasiculatum*. Jetterman and Thaper (1974) was described the Bioassay of different VAM spores different VAM species and their infection efficiency in the different plant host. It is helped to oxiformed of taxonomic position of the VAM spores. In our studies also made to the onion and their infection efficiency used as the host plant for the bioassay of VAM spores like VAM spores. Namely *Glomus faciculatum, G. geospora, Acaulospora* and *Gigaspora* play a important role the classify the taxonomic position of the host plant.

The VAM fungi are associated with the plant in a mutually beneficial relationship. The VAM fungi next to reside the root. To expose at to 200 times as the area available to the root alone. The influence of different VAM inoculam in *Zea mays* with a reference to plant height, dry weight, Mycorrhizal spore in root zoon. Leaf length, leaf breath and total chlorophyll, proteins phosphatase enzyme activity.

Conclusion

Vesicular Arbuscular Mycorrhizal fungi – and plant relationship is an elegant association and its development evidently regulated by several factors. Such as the physico – chemical characteristics soil fertility.Vesicular Arbuscular Mycorrhizal fungi can increase the disease resistance against root pathogens, especially, when the VAM fungi can adequately colonize the root before the pathogens. They are important in forming stable soil aggregates by binding soil particles in the filamentous mass as well as producing sticky substances that held the particles together. The present investigations were enlisted the Vesicular Arbuscular Mycorrhizal spores responsible for the influencing the soil physico – chemical characters and increasing the soil fertility. The experimental crops of *Zea mays* L shows the prominent result of VAM and phosphobacteria treated pot and also contained increased level of phosphatase enzyme.

Among the four treatment compared with uninoculated control plants. The VAM and cross inoculated onion root fragments treated plants shows the significant result than the other treated plants. This method influenced the effective colonization of VAM spores in the plant root then the freely VAM spore inoculated plants.

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