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RESEARCH ARTICLE

EFFECTS OF AM FUNGI ON SPORES DENSITY AND ROOT COLONIZATION OF CHILLI (CAPSICUM ANNUM) AT DIFFERENT LEVELS OF ROCK PHOSPHATE

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ABSTRACT

The present experiment was carried out in pot culture at department of microbiology, faculty of agriculture, Annamalai University, Tamil Nadu. The main purpose of this research was to find out the effects of Arbuscular Mycorrhizal fungal (AMF) inoculation (with and without Mycorrhiza) along with application of various levels (0%, 25%, 50% and 100%) of rock phosphate (RP) fertilizer on spores density and percent root colonization of selected chilli (*capsicum annum*) hybrids in P-deficient soil. It was observed that spore density and AMF root colonization was higher in the soil of control (RP0) plants, which decreases progressively with increasing fertility level. Less number of spores and percent root colonization was found at high RP level (RP100) in all hybrids. Higher P doses declined the sporulation and colonization. There was total seven AMF species that were observed and recorded. The dominant genus was *Acaulospora* which was followed by *Glomus*, *Sclerocystis* and *Gigaspo- ra*. The average AMF spore density ranged from 66-266 spores/100g soil while root colonization ranged from 34-100%. Mycorrhizal enhancement regarding AMF spores density and root colonization ranked as-RP0>RP1>RP2>RP3 in all hybrids i.e 0%>25%>50%>100%.

Key words: AMF, AMF spores density, AMF root colonization.

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INTRODUCTION

A mycorrhiza is a symbiotic association between a fungus and the roots of a vascular plant. In this association, the fungus colonizes the host plant s roots, either intracellular as in arbuscular mycorrhizal fungi or extracellularly as in ecto mycorrhizal fungi. They are an important component of soil life and soil chemistry. Arbuscular mycorrhizal (AM) fungi are ubiquitous in soil habitats and form beneficial symbiosis with the roots of angiosperms and other plants (Gerdemann, 1968). This AM fungi belong to the family Endogonaceae, of the order Muccorales, of the class Zygomycetes (Gerdemann and Trappe, 1974). The AM forming genera of the family includes Acaulospora, Entrophospora, Gigaspora, Glomus, Sclerocystis and Scutellospora. Pepper (Capsicum spp.) belongs to the Solanaceae family, a year round crop used in variety of ways (Erinle, 1989; Akinyosoye, 1977). There are many varieties of pepper and among these C. annum are the sweet or bell peeper while C. frutescences are hot pepper. Hot peppers (Chilies) have high content of alkaloid capsaicin (C18H2703) which is responsible for pungency or heat (Udoh et al., 2005). It is known that nutritional and visual quality of fruit depends both on variety and growing conditions (Pivovarov et al., 2009).

**Corresponding author:* Tamil Pradha, K. Annamalai University, Faculty of Agriculture, Department of Microbiology, Chidambaram, Tamil Nadu, India. Arbuscular mycorrhizal fungal association is one among these adaptations (Khade and Rodrigues, 2009; Coline et al., 2011). Due to scarcity of phosphorus reserves in the soil and their rapid utilization, efforts are being made to supplement plants with low grade rock phosphate. Mycorrhizal inoculation can help plants by solubilizing rock phosphate into available forms, which helps in plant growth (Sabanavar and Lakshman, 2009).

MATERIALS AND METHODS

Experimental Site

The present study was conducted at the department of microbiology, faculty of agriculture, Annamalai University, Tamil Nadu.

Plant Material

Authentic seeds of four hybrids of chilli i.e CO1,CO2, PKM1 and PLR1 were obtained from vegetables research programme, palur.

Soil

The soil used was sandy loam with pH 7.8, electric conductivity 0.675 dSm⁻¹, Nitrogen 0.032% and Phosphorus 0.8

mgkg⁻¹ with low organic matter (0.6%). All 96 pots having 89 cm diameter and 48 cm depth were filled with 6 Kg of this nutrient deficient soil.

Experimental Design and Treatments

The experimental work was carried out in a randomized complete block design (RCB) along with eight treatments; each treatment was replicated three times with five plants in each pot.

Application of AMF Inoculums

In the experimental work, mixed constorium of different AMF species i.e. *Glomus fasciculatum, G.mos- seae, G. aggregatum, Sclerocystis pakistanica, Gigaspora gigantea* along with roots of maize infected with arbuscular mycorrhiza were used as rhizobase inoculum.

The roots were cut into 1 cm pieces, which along with soil base inoculum (rhizospheric soil) were spread uniformly in pots, at a depth of 3 cm and 6 cm in layers before sowing. Inoculum for each pot consisted of 160 g of mycorrhizal infected roots and adhering soil. Mycorrhizal inoculum preparation, placement and application were done by the method given by Brundrett *et al.* (1996).

Fertilizer Application

Fertilizers were applied by following Krishna and Bagyaraj (1982) method. Rock phosphate fertilizer was obtained from Hazara deposits. Four levels of P fertilizer treatments 0 mg P_2O_5/kg soil, 100 mg P_2O_5/kg soil, 200 mg P_2O_5/kg soil and 500 mg P_2O_5/kg soil in form of RP were applied. The recommended dose was 80 kg P_2O_5/ha . These treatments were applied in combination with or without AMF.

Table 1. Effect of mycorrhiza on AMF spores in the roots of chilli (capsicum annum)levels of phosphate

Rock Phosphate Levels	Spores density / 100g soil			
	CO1	CO 2	PKM1	PLR1
RPO(0%)	208	266	257	251
RP1(25%)	176	209	189	177
RP2(50%)	108	176	145	129
RP3(100%)	65	109	95	86

Table 2. Effect of mycorrh	za on AMF spores	species in chilli hybrd
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Percent rock phosphate level	AMF spores	Spores density in Col	Spores density in Co2	Spores density in PKM1	Spores density in PLR1
RPO(0%)	Acaulospora	+++	+++	+++	+++
~ /	Sclerocystis	++	++	++	++
	Glomus	+++	+++	+++	+++
	Gigaspora	-	+	-	-
RP1(25%)	Acaulospora	++	+++	+++	+++
	Sclerocystis	-	++	+	-
	Glomus	+	++	++	+
	Gigaspora	-	-	+	-
RP2(50%)	Acaulospora	+	+++	++	+
	Sclerocystis	-	+	+	-
	Glomus	-	+	+	+
	Gigaspora	-	-	+	-
RP3(100%)	Acaulospora	-	+	-	-
	Sclerocystis	-	-	-	-
	Glomus	-	+	-	-
	Gigaspora	-	-	-	-

+:Present; - :Absent; +++:100 %; ++:75 %; +:25

 Table . 3 Effect of various level of rock phosphate fertilizer on am infection morphologies, external hyphae, internal hyphae, arbuscules, vesicles, (%) in roots of mycorrhizal chilli hybrid. Percent AMF infection (%)

Hybrids	treatments	External hyphae(%)	Internal hyphae (%)	Vesicals (%)	Arbuscules (%)
	RP0	26	25	25	8
CO1	RP1	21	24	24	5
	RP2	7	14	14	0
	RP3	0	4	7	0
	RP0	42	45	51	15
	RP1	34	34	37	7
CO2	RP2	14	21	21	4
	RP3	0	11	14	0
	RP0	41	44	51	8
	RP1	24	24	24	4
PKM1	RP2	11	17	21	0
	RP3	0	7	11	0
	RP0	37	38	41	14
	RP1	21	25	25	5
PLR1	RP2	4	11	11	0
	RP3	0	4	4	0

Extraction of Spores

The spores were isolated from the soil samples by wet-sieving and decanting technique (Gerdemann and Nicolson, 1963).

Spore Density Calculation

Density of spores in each soil sample was calculated by following Stahl and Christensen (1982) standard method.

Spores Identification

Spores were identified with the help of keys following Hall and Fish (1978), Trappe (1982) and Schenck and Perez (1990).

Assessment of Root Colonization

Roots were carefully dug out and washed thoroughly with water and stored in FAA (Formalin:Acetic acid:Alcohol) solution. The roots were stained and processed following the procedure of Phillips and Hayman (1970). For the assessment of root colonization + slide method of Giovannetti and Mosse (1980) was followed. Total of 25 segments of roots of individual plant each approximately 1 cm long were randomly selected for microscopic study. Morphology of AM entophyte was studied and expressed in percentage (%). The infection percentage was calculated by using the following formula (Giovannetti and Mosse, 1980):

% age mycorrhizal infection = $\frac{\text{No. of infected segments}}{\text{Total No. of segments studied}} \times 100$

RESULTS AND DISCUSSION

AMF Spores Density

The results in Tables 1 and 2 shows the effect of various rock phosphate levels on the AMF spores density in the rhizosphere soil of selected chilli hybrids. Mycorrhizal enhancement regarding AMF spores density followed RP0>RP1>RP2>RP3 trend in all hybrids .It has been observed that spores density was higher in the soil of control (RP0) plants, which decreased progressively with increasing fertility level. Less number of spores was found at RP3 level in all hybrids. Generally the population of AMF spores and soil phosphorus are inversely related to each other (Hao et al., 1991). Chandrasekara et al. (2005) and Panwar and Tarafdar (2006) also found that interaction of mycorrhiza and phosphorus fertilizer had no significant effect on AMF spores density. Guillemin et al.(1995), Antunes et al.(2007) and arumugam et al.(2010) reported that the spores density got declined sharply at high P level but these results negate the findings of sharathbabu and manoharachary(2006) who reported that dual inoculation of AMF (Glumous fasciculatum) and rock phosphate significantly enhance percentage of mycorhizal colonization then in single inoculation or in control Tylophora indica plants. Average number of spores counted per 100 g of soil was different from hybrid to hybrid at various levels of treatments (Table 1). The AMF spore densities ranged from 66-266 spores/ 100 g soil in se- lected chilli hybrids. It was found that among control plants, the highest number of spores was re- corded for CO2 (266/100 g of soil) followed by PKM1 (257/100 g of soil), PLR 1 (251/100 g of soil) and CO1 (208/100 g of soil). The combined effect of AMF+ RP results showed that AMF spores

density followed CO2 >PKM1, PLR 1> CO1 trend at all RP levels (Table 1). AMF spores are ubiquitous in most ecosystems (Marleau *et al.*, 2011), and are essential component of soil micro biota (Hindumathi and Reddy, 2011) AMF exists in soil as spores, hyphae, as vegetative propagules or infected root pieces for infecting plants, but mostly inoculation of plants is brought about by extraradical mycelium (ERM) (Sylvia and Jarstfer, 1992). Occurrence or distribution of AMF varies with host ranges (Sarwade *et al.*, 2011).

AMF Species

Table 2 shows that the following species were identified in rhizosphere soil of selected chilli hybrids at different rock phosphate levels.

- Acalospora mellae
- Acaulospora laevis
- Glomus fasiculatum
- Glomus mosseae
- Glomus aggregatum
- Sclerocystis pakistanica
- Gigaspora gigantea

Nasim et al. (1998) showed that spores are the means of identification of these fungi. In the present work soil was collected from different pots with plants at reproductive stages of growth. Four genera of endogonaceous spores were identified which were Acaulospora (2 spp. i.e. A. mellae, A. laevis), Glomus (3 species i.e. G. mosseae, G. aggregatum and G. fasiculatum), Sclerocystis 1 specie i.e. (S. pakistanica) and Gigaspo- ra (1 spp. G. gigantean). In this research we found that the species of Acaulospora were most common and predominant followed by Glomus, Sclerocystis, and Gigaspora. Our findings are further supported by the work of other researchers (Lovelock et al., 2003; Wongmo, 2008; Tchabi et al., 2008; Charoenpakdee et al., 2010; Gao and Guo, 2010; Songachan and Kayang, 2011) who investigated that there is higher number of Acaulospora in the soil followed by Glomus species. The predominance of Acaulospora species might be due to their adaptation to wide variety of soil types, host species and pH and nutrient availability etc., (Jefwa et al., 2006; Straker et al., 2010). It suggests that AMF strains are biological specific for the host plant as reported by Bever et al. (1996). The large number of AMF spores may be attributed to the deficiency of low phosphorus in the soil. Generally, the population of VAM spores and soil phosphorus are inversely related to each other (Hao et al., 1991). The presence of small number of Gigasporaceae might be due to the fact that they are usually found in sandy dunes (Lee and Koske, 1994) and are usually large sized, which requires long period for their development than the small sized spores species (Hepper, 1984). Moreover, Gigaspora are very common in wild plants than field crops (Gai et al., 2006).

AMF Colonization in Roots

The results given in Tables 3 show the effect of various rock phosphate levels on the percent root colonization in the rhizospheric soil of selected chilli hybrids. AMF root colonization was determined by the presence of external hyphae, internal hyphae, vesicles and arbuscules. The general AMF infection in chilli hybrids at various rock phosphate levels was low as compared to control (RP0) viz 100% (Table 3). Vesicular infection was common and maximum at all RP levels in all hybrids as reported by Iqbal and Bareen (1986) in *Narcissus poeticus* and Burni *et al.* (1993) in *Targionia hypophylla*. Moreover, Al-Raddad (1995) observed that the type of crop and harvesting greatly affect the root colonization. The comparision revealed that highest number of vesicles Was recorded in was recorded in CO2 followed by PKM1, PLR 1 and CO1 at all RP levels shown in Table 3, as reported by Linderman and Davis (2004) in marigold, Janoušková *et al.* (2007) for tobacco and Sensoy *et al.* (2007) for *Capsicum annuum* L. The results shows that AMF inoculated plants had significant positive effects on AMF root colonization.

However, this positive effect of AMF inoculated plants decreased with increasing RP level; lowest root colonization was found at RP3 level in all hybrids. Redecker (2005) found that high concentration of phosphate seems to induce low fungal colonization level by the plants. These results agreed the results of Soleimanzadeh (2012) who showed that positive effect of AMF colonization decreases with increasing P levels. Similar results were reported by Chandrashekara et al. (1995), Mohammad et al. (2003) and Pragatheswari et al. (2004). result in exudation of certain chemicals from the root which enhances AMF colonization and spore germination but such exudations does not take place when phosphorus level is high (Juniper and Abbott, 2006; Murkute et al., 2009). Sharif et al. (2011) and Manske (1990) showed that low availability of soil phosphorus increases AMF colonization.But,results of Satpal and Kapoor (2000) showed that dual inoculation of Vigna radiata plants with rock phosphate and AMF stimulated root colonization as compared to those without rock phosphate.

The results also shows that at control and low level of rock phosphate the internal hyphae and arbuscular infection were moderately frequent and scattered throughout the cortex hich is actually the sites of nutrients exchange (Table 3) .The external hyphae and arbuscules were not seen in any of the studied root segments at high RP level (RP3) as shown in Table 3. High soil phosphate level has direct effect on reduction of hyphal growth and spore production.

Conclusion

This study clearly indicates the potential of using indigenous biofertilizer such as AMF for vegetable crops in low fertility soils, to achieve adequate production level with least utilization of synthetic fertilizers for sustainable agriculture practice. The use of biofertilizer is not only eco-friendly but also economical as it reduces our dependence on expensive chemical fertilizers.

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