Frequency of arbuscular mycorrhizal fungi in the rhizosphere of soybean roots and possibility of the mass reproduction of spores

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Abstract

Arbuscular mycorrhizal fungi coexist with most land plants and these funguses are able to increase the absorption of sedentary nutrients, especially phosphorus, Zn and Cu. Therefore, in this experiment, arbuscular mycorrhizal fungi spore isolation is done through 15 fields of soybean in Golestan province by wet sieve method. The results of this study showed that in all soils studied, there is the potential infection of soybean roots by indigenous mycorrhiza fungi. For further study of the isolated spores, monospore and mass culture were done. Genuses of the collected samples (Glomus, Gigaspora and Scutellospora) were identified. For each gram of soil, respectively, the maximum and minimum number of spores was observed in Azadshahr (284) and Walsh abad-Gorgan (44).

Minimum and maximum root colonization percentage was observed, respectively, 20 and 75% in soybean roots and 10% and 85% of sorghum roots in the culture mass. The results indicate that among all the sampling points, in the spores isolated from soil and root colonization percentage of soybean roots were significant at the 1% level. But there was no significant correlation between them.

Keywords: Arbuscular mycorrhizal; spores; colonization; mono spore, wet sieve

Introduction

Soybean culture, among crops, is important in Golestan province. The soybean plant is legume plants. Soybean oil and protein is applied to human and animal nutrition. Also it is used as the raw material for factories in the industrial products. Soybean plants are suitable for placement with other crops (Bahadori, 2006). Mycorrhiza is obligatory symbiotic relationship between soil beneficial fungi and higher plant roots. Arbuscular mycorrhiza symbiosis is the most common type of mycorrhiza symbiosis (Korade and Fulekar, 2009; Quilambo, 2003). Vesicular-arbuscular mycorrhizal fungi (VAMF) are one of the most interesting soil microorganisms which can solubilize fixed phosphate and retentive phosphate in soil (Mala, 2000). Vesicular-arbuscular mycorrhizal (VAM) fungi form symbiotic association which enhances water and nutrient transport particularly phosphorus (P) and thereby increase growth and yield of many a crop plants (Jaluddin, 2005). In this kind of relationship, the fungal structures such as vesicles and arbuscular are produced in the cortex -root. The main part of beneficial soil microbes are mycorrhiza fungi that help the growth of the plants and increase the plant resistance in unsuitable conditions (Chandra et al, 2010). Fungi help the absorption of phosphorus and other ions. Soybean plant requires 14 nutrients, including phosphorus (Kandel, 2010). Symbiosis between soybean plants and mycorrhiza fungi is important from two aspects: The first, supply of soybean plant needs and second, the supply of phosphorus required for the system symbiotic. This study examined the effects of various conditions (pH,
electrical conductivity, concentrations of phosphorus, organic carbon, nitrate, potassium, manganese, iron, zinc and copper) on a soybean plant root colonization and spore density by mycorrhizal and mass production of them as the first material bio-fertilizers.

Materials and Methods

Collection of rhizosphere soil

In order to check the presence or absence of native fungi, 15 rhizosphere soil samples with roots of soybean (variety Katool) were collected from 15 farms located in Golestan province of 0-30 cm soil depth and 2 kg amount, were put in plastic bags and transferred to the research center of Agriculture and Natural Resources of Gorgan. Sampling from each region was done in stage of 20 percent flowering of the plants of the field.


Isolation of spore

Common technique for isolation of the arbuscular mycorrhiza spores from soil is based on wet sieve which clay, sand and organic matter are removed, while spores and other particles similar soil remain on the sieve with different sizes and stainless steel (Thenmozhi et al, 2011). In order to isolate the fungal spores, a series of sieves and wet sieve method were used (Utobo et al, 2011). The shape and frequency of spores were observed using binocular microscope and were preliminarily identified on the INVAM website and research results Oehl and et al (2011). For this purpose, 50 g of rhizosphere soil samples was air dried, and passed through a 2 mm sieve, were weighed and poured into a liter flask. Then, 500 ml of the city water was added and a magnet was moved inside it and the flask containing rhizosphere soil, water, magnets were placed on a magnetic stirrer for 5 minutes to be mixed completely. The flask was kept in stasis for 45 seconds until the heavier particles are deposited and immediately, the supernatant was poured (3 times to completely drain the contents of the flask) on a series of sieves, with mesh sizes of (10, 18, 70, 100, 140 and 450). The slags on the sieve were washed with sufficient amount of water. Eventually, the remainder of the top sieve 70, 100 and 140 micrometers were collected separately. After their volume had reached 100 cc, 10 cc of each one (3 replicates) was taken and poured onto the graded filter paper No. 1 and spores were counted (Utobo et al, 2011).

Monospore culture and mass culture

For monospore, initially, spores were separated on the basis of size (70, 100 and 140) and color (1. white and yellow; 2. red and orange). After the isolation of spores, single spores were selected from each soil sample, and were added to 5 cc syringe containing a mixture of sterilizing sand and perlite ratio (V:V 1:1) and 2 sterilized sorghum seeds were planted in them. After 10 days, the entire content of the syringe along with sorghum plant was transferred to glass plastic pots (100 mL) containing 80 cm³ mixture of sterile sand and perlite. After one month, 15 sterilized and germinated seed for mass cultivation were sown in the same pots containing fungal inoculum. After 3 months, all seedlings were headed. Then the water was cut for 2 weeks. Roots and content of the pots were air-dried on the newspaper and under a biological hood for a week. Finally, to check the roots infection by fungus, roots were stained according to the Phillips and Hayman (1970) method (Feldmann and Idczak, 2000). During the growth, plants were fed by Hoagland solution (½ p). After harvesting the plants, a gram of ready beds were weighed for determination of the population and preliminary detection of spores.

Staining of sorghum root and determining the colonization percentage

At first, the roots of the sorghum plant were divided into pieces of 1 cm. Then they were washed with tap water and were immersed into 70% alcohol for 10 seconds. Then, they were washed five times with sterile tap water. They were placed in 10% potassium hydroxide (with freshly prepared KOH) in water bath at 95 °C for 1 h until the roots cell content are removed and the roots are discolored steadily. After heating, we pour off the KOH solution until roots are bleached and rinse the root samples well with tap water. Then, the roots were placed in lactophenol solution added to 5% trypan blue at 90 °C in a water bath for 30 min. Following staining, the roots were rinsed with acidified tap water (add several drops of acetic acid to the water) several times for more than 20 min. If water for rinsing is not acidified and has a high pH (neutral is high), roots will distain (Utobo et al, 2011).
Then stained roots were observed with an optical microscope with a magnification of 10, 40 and 100, and the percentage of root colonization was determined according to the following formula (Manimegalai, 2011).

\[
\% \text{colonization } AM = \frac{\text{the total number of infected mycorrhiza}}{\text{the total number of root segments observed}} \times 100
\]

**Chemical analysis of soil**

Soil chemical characteristics including pH, electrical conductivity, concentrations of phosphorus, organic carbon, nitrate, potassium, manganese, iron, zinc and copper were measured (Emami, 1993).

**Statistical Analysis**

Analysis of variance (ANOVA) of data was performed using the SAS 9.1 software.

**Results and Discussion**

In the present study, rhizosphere samples were collected from 15 different soybean farms of Golestan province. The natural presence of fungi in rhizosphere of soybean was studied. The percentage of the colonization, the number of spores, vesicles and arbuscular were determined. Dominant mycorrhiza spores in soil samples belonged to the genera Glomus, Gigaspora and Scutellospora. In Different fields, different levels of colonization and number of spores were observed. In the 15 studied area, in each 100 g soil, maximum 284 spores was observed in Azadshahr and minimum 44 spores in Walsh Abad - Gorgan. Figure 1 indicates the existence of vesicles, hyphae, spores and the mass proliferation of mycorrhizal fungi.
different or limit access to nutrients and mass cultivation of (Chandra et al., 2010). Also, Chandra et al., 2010, stated that the plant’s effectiveness in inoculums the plant’s effect on the mass cultivation of sorghum depends on the soil fertility and, the inoculums which effect on both the host and the colonization AM. -

The possible reason of the low spore in most parts of the sample can be: Destruction of soil structure, nutrient lack or exceed, unfavorable environmental conditions and use of pesticides and fungicides.

For example, sequential and continuous cultivation and tillage can cause the decrease of the fine roots, which are suitable for mycorrhiza infection. Probably the reason of the higher number of spores in the Azadshahr region is due to the low concentration of phosphorus compared to Walsh Abad.- Gorgan (Table 1). The range of organic carbon of soils is usually 0.6 to 2.2 in the depth of 0-20 cm (Choudhary et al., 2010). The difference in the amount of soil organic carbon can be due to frequent use, improper crop management (overuse of fertilizers and pesticides), harvesting straw and sometimes burning straw and stubble. pH or soil reaction has a great effect on the establishment of mycorrhiza and plant growth. It may enhance or limit access to nutrients and effect on the pattern of nutrient uptake and ultimately distribution of microorganisms. The reaction of different qualities and species of arbuscular mycorrhizain response to pH is different. Therefore, changes in soil pH may effect on the abundance of the mycorrhiza fungi (Choudhary et al., 2010). Also, Chandra et al. (2010), stated that the plant’s dependence on mycorrhiza depends on the soil fertility and, the inoculums which effect on both the host and the percentage of the root colonization. In soils that legumes face with a shortage of phosphorus, mycorrhiza fungi play an important role in P absorption. In case of low population density or in effectiveness of the mycorrhiza...
fungi in such soils, we can use plants inoculated with the fungus mycorrhiza. It has been reported that soybean inoculated with *Glomus mosse* in a soil with deficient phosphorus can increase the performance and nitrogen fixation (Khavarzi, 2003). Minimum and maximum root colonization was observed to be, respectively, 20% and 75% in soybean roots and 10% and 85% in sorghum roots after culture mass. In this study, it has been observed that among all parts of sampling, there is a significant difference of 1% level in the number of spores isolated from soil and the percentage of colonization of soybean roots. But, there was no significant correlation among them (Camargo-Raicalde and Dhillion, 2003; Li et al, 2007). However, in some cases, a significant and positive correlation has been observed between the number of spores isolated from the soil and colonization percentage (Songachan et al, 2011). All isolated monospores were able to reproduce and all of them could infect their host plant roots (sorghum). In other words, all of them had the potential of polluting the sorghum roots.

![Figure2: Spore density values (in 100 g of soil) and the percentage of colonization soybean roots by arbuscular mycorrhizal fungi.](image)

**Conclusion**

Arbuscular mycorrhiza fungi play an important role in the survival and stability of plant communities in natural ecosystems. The coexistence of these fungi is more effective among biological factors which are important in re-settlement and growth of the plant. Generally, it can be said that there is a relationship among soil, quantity, type of soil nutrient and mycorrhiza formation. Concerning that the isolated fungi were able to infect sorghum plant roots, so, we can produce a large amount of one kind or quality of fungus in the subsequent amplification of each monospore and replace some of fertilizers, especially, Phosphate fertilizers with it. However, each of these factors, alone or together, will have a different effect.

**References**


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