

EFFECT OF PHOSPHORUS, NITROGEN AND SOME CULTURAL PRACTICES ON MYCORRHIZAL COLONIZATION OF FABA BEAN AND MAIZE

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Abstract: A set of field experiments was conducted to study the effect of phosphorus and nitrogen fertilizers on mycorrhizal colonization of faba bean and maize plants. In addition, the effect of irrigation and ploughing treatments on spore numbers were assessed in fallow soil. The identification of mycorrhizal fungi was also conducted. The results showed that mycorrhizal colonization was reduced by the addition of phosphorus and nitrogen fertilizers. Number of spores and mycorrhizal colonization were also affected by plant species and management practices. In this respect, the number of spores was greater in maize compared to faba bean rhizosphere and the highest spore numbers were found in unploughed non irrigated fallow land. Under all treatments, the identified fungi belonged to the genus *Glomus* suggesting that VAM species can be considered as universal biofertilizer due to their broad host range.

Keywords: Mycorrhizal colonization; cultural practices; *Glomus*; biofertilizer.

INTRODUCTION

Mycorrhiza is a mutualistic symbiosis between certain groups of soil fungi and most plant root systems (Schüßler *et al.*, 2001). The most publicized benefit of mycorrhiza is the improved growth rate mainly due to improved phosphorus nutrition. Various mechanisms (e.g. exploration of large soil volume, faster movement of mycorrhizal hyphae and solubilization of soil phosphorus) have been suggested for the increase in the uptake of phosphorus by mycorrhizal plants. Non-nutritional benefits to plant such as changes in water relations, phytohormone levels, carbon assimilation, etc. have been reported, but they are difficult to interpret. Other important roles of mycorrhiza in ecosystems include nutrient cycling (Andrade *et al.*, 1998).

In Sudan, as a result of crop diversification, new lands of inferior quality are coming into production. In these lands, which are alkaline in reaction, a very small amount of phosphorus

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is available to the plant because phosphate ions are almost immobile. Mahadi (1993) suggested that VAM fungi have great potentiality as a biofertilizer especially in soils which are very poor in available phosphorus. In addition to nutrient status, however, many researchers reported that VAM infection could be influenced by plant species and certain cultural practices (Amijee *et al.*, 1989; Mozafar *et al.*, 2000; Galvez *et al.*, 2001; Bedini *et al.*, 2013). Therefore, the objectives of this study were to investigate the effect of phosphorous and nitrogen fertilizers on mycorrhizal colonization of faba bean and maize plants. In addition, the effect of irrigation and ploughing treatments on spore number was assessed in fallow soil. The identification and classification of fungi were also conducted.

MATERIALS AND METHODS

Mycorrhizal colonization experiment

Two separate field experiments were conducted, one on faba bean (cv. Houdeiba 93) and the other on maize (cv. Mogtamaa 45). The experiments were conducted in the Demonstration Farm of the Faculty of Agriculture at Shambat (Latitude 15°40'N, Longitude 32°32'E and Altitude 386 m *asl*). The soil in the experimental area is montmorillonitic clay with alkaline pH. In both experiments, the treatments consisted of two types of fertilizers namely: nitrogen and phosphorus. In the faba bean experiment, two levels of nitrogen (0N and 1N) and Phosphorus (0P and 1P) fertilizers were used, whereas in the maize experiment, an additional dose of nitrogen was applied (0N, 1N and 2N). Nitrogen was applied in the form of urea at the rate of 0 kg/ha (0N), 40 kg/ha (1N) and 80 kg/ha (2N). Phosphorous was applied as triple superphosphate (P_2O_5) at the rate of 0 kg/ha (0P) or 100 kg/ha (1P). In both experiments, the treatments were arranged in a randomized complete block design with three replications in the faba bean experiment and four replications in the maize experiment. The soil was prepared by ploughing, leveling and ridging 70 cm apart. Sowing was carried out in the first week of December on the top of the ridges at a rate of 3seeds/hole. The seedlings were then thinned to one plant/hole two weeks after sowing. Plot size was 4X4 m with 5 ridges 4 m in length. Irrigation water was applied every two weeks and no weeding or insect control was done.

Irrigation and ploughing experiment

This experiment was designed to study the effect of irrigation and ploughing on spore number using a one-year fallow soil. The treatments were as follows: L1 (without irrigation and ploughing), L2 (without irrigation but ploughed), L3 (irrigated but not ploughed) and L4 (irrigated and ploughed). The treatments were arranged in a randomized complete block

design with three replications.

Root staining

Root staining to visualize root colonization was carried out by modification of the Kormanik and McGraw (1982) method. Root specimens from each treatment were stained by acid fuchsin (prepared by mixing water, glycerin and lactic acid in 1:1:1 volume proportions) through the following steps: first the cytoplasmic contents of the cell were removed using hot 10% KOH for 10 minutes. Roots were acidified by washing in water and then immersed in 2% HCl for 15-20 minutes. The roots were then stained with acid fuchsin (0.05%) and incubated for 3-4 minutes.

Estimation of mycorrhizal colonization

Mycorrhizal colonization was estimated by the grid intersection method (Newman, 1966) using Petri dishes with filter paper divided into small squares of 1cm² each. The stained roots were spread over the filter paper and percent infection was computed as the ratio of the number of dark parts of the roots which cross the lines of the squares to the total number of the roots crossing the lines.

Spore extraction and characterization

Spore extraction was achieved by the wet sieving and decanting method (Gerdemann and Nicolson, 1963). Representative samples of soils, 400 g each, were taken from the rhizosphere. One liter of water was added to the soil samples and placed in a shaker for 15 minutes. The material was then immediately poured through graded sieves (250, 112, 50 and 36µm). The materials in the bottom sieves were collected in 100 ml beakers and then transferred into 10 ml tubes and centrifuged (approximately 4000 revolutions per minute) for 15 minutes. Soil and other particulates were sediment, whereas spores and fine organic detritus suspended in water. The spores were counted by using a counting chamber (0.1mm, 0.0025 mm² FEIN.OPTIK BAD-BLANKENBURG).

Statistical analysis

Analysis of variance for randomized complete block design was used according to Gomez and Gomez (1984). Mean separation was done using Duncan Multiple Range Test for the different characters.

RESULTS AND DISCUSSION

Effect of phosphorus and nitrogen fertilizers on percent mycorrhizal colonization

VAM being obligate symbiotic fungi, their presence in a particular soil depends on abiotic factors such as soil nutrient status. In the present study, phosphorus application decreased the

percentages of mycorrhizal colonization in the two crops compared to the control (Table 1). Supporting evidences were reported by many workers who found that application of high doses of superphosphate result in less VAM infection (Amijee *et al.*, 1989; Ahmed *et al.*, 2000). Similarly, increasing the levels of nitrogen fertilizer decreased the percentages of mycorrhizal infection particularly in maize (Table 1). This is in agreement with the results reported by Hayman (1983). The reduction in mycorrhizal infection under high phosphorus levels may be due to robust root cell walls which are less liable to infection (Menge *et al.*, 1978). Similarly, Schenck and Smith (1982) hypothesized that the level of infection is influenced by phosphorus concentration through its negative effects on the level of root exudates.

The greater mycorrhizal infection of faba bean, although insignificant, compared to maize observed in the present study may be attributed to the high degree of legume dependency on mycorrhizal association. The same conclusion was reached by Hayman (1983) who stated that the dependency of plant on mycorrhizal association has often been related to the physical characteristics of the root. For example, plants with few short root hairs, such as many legumes, show a high degree of mycorrhizal dependency, whereas those with extensive fine root system, such as grasses, have low degree of dependency.

Table 1: Effect of phosphorus and nitrogen fertilizers on mycorrhizal infection (%) in faba bean and maize plants

Treatment	Faba bean			Maize			
	0N	1N	Mean	0N	1N	2N	Mean
0P	62.6	54.2	58.4 ^a	62.7	60.2	42.7	55.2 ^a
1P	48.2	52.2	50.2 ^a	54.2	52.2	39.3	48.6 ^b
Mean	55.4 ^a	53.2 ^a		58.5 ^a	56.2 ^a	41.0 ^b	

For each row and column, means followed by similar letters are not significantly different at 5% level of probability

Effect of phosphorus and nitrogen fertilizers on number of spores in plant rhizospheres

Phosphate fertilization increased the mean number of mycorrhizal spores in faba bean and decreased it in maize but the differences were not statistically significant. In contrast, nitrogen fertilization increased the mean number of spores in faba bean and decreased it in

maize but the differences being statistically significant (Table 2).

Table 2: Effect of phosphorus and nitrogen fertilizers on mean number of spores/g of soil in faba bean and maize rhizospheres

Treatment	Faba bean			Maize			
	0N	1N	Mean	0N	1N	2N	Mean
0P	57.5	85.0	72.5 ^a	87.5	100.0	75.0	87.5 ^a
1P	75.0	85.0	80.0 ^a	102.5	65.0	67.5	80.0 ^a
Mean	67.5 ^b	85.0 ^a		95.0 ^a	82.0 ^b	72.5 ^c	

For each row and column, means followed by similar letters are not significantly different at 5% level of probability

It should be remembered that spore counts might not give comprehensive values for the actual abundance of the fungi in the soil (Clapp *et al.*, 1995). This might explain why in faba bean rhizosphere, although the infection percentages were higher, the number of spores was lower compared to maize rhizosphere. Similar results were reported by Simpson and Daft (1999) who stated that more spores were produced on cereals than legumes.

Effect of cultural practices on mycorrhizal number of spores in fallow soil

Cultural practices investigated in this study (e.g., ploughing and irrigation) statistically had no significant effect on mean number of spores in fallow soil (Table 3). The highest spore numbers were found in unploughed non irrigated fallow land. The lower number of spores observed in irrigated treatment may be attributed to the loss of top soil as reported by Brundrett (1991). Also, the propagules of mycorrhizal fungi, although insignificant, were slightly reduced under ploughed treatment compared to unploughed treatment. This is supported by the finding of Galvez *et al.*, (2001) who showed that spore populations and colonization of maize roots by VAM fungi were higher in no-tilled than in moldboard plowed or chisel-disked soil. Similarly, Mozafar *et al.*, (2000) reported that tillage reduces the inoculum potential of the soil and the efficacy of mycorrhizae. This might be due to disturbance of AMF hyphae integrity by tillage, changes in nutrient content of the soil, changes in microbial activity, or changes in weed populations as reported by Jansa *et al.*, (2003).

Table 3: Effect of tillage and irrigation on mean number of spores/g of soil in fallow land

Treatment	Ploughed	Not ploughed	Mean
Irrigated	84.4	85.0	83.8 ^a
Not irrigated	91.3	92.6	90.0 ^a
Mean	85.0 ^a	88.8 ^a	

For each row and column, means followed by similar letters are not significantly different at 5% level of probability

Since VAM fungi are obligatorily dependent on living plant roots for continued growth and multiplication, Thompson (1987) suggested that clean fallowing reduced VAM propagules. This contradicts the results obtained in the present study where the number of spores in fallow soil was higher than their number in both faba bean and maize rhizospheres. This is probably due to the high dependency of the previous crops on mycorrhizal association and to the greater number of spores (57.5-100 spores/g soil) and weeds normally present in Shambat soils.

Classification and identification of the fungi

Generally mycorrhizae are classified according to the character of the spores, sporocarps and hyphae, particularly spore size. Fungi with spore size less than 200 μm and with vesicles belong to suborder Glomineae. In both crops, spores shape was globose or reniform and the diameter at maturity ranged between 0.76 and 4.5 μm . In addition, roots examined in this study contained vesicles. The spore color after staining ranged from dark with longitudinal hyphae. Sporocarps were amorphous to tightly packed aggregation of spores. The sporocarps size ranged from 66 to 123 μm and the color of the surface was white, yellow or brown. However, in maize the size of the sporocarps was slightly less (54-90 μm) compared to faba bean. All these characters indicated that the VAM fungi investigated in this study belonged to the family Glomaceae and genus *Glomus*, with probably different species of that genus in faba bean and maize rhizospheres as indicated by many workers (Smith *et al.*, 2000).

Conclusions

Mycorrhizal colonization was reduced by the addition of phosphorus and nitrogen fertilizers. Number of spores and mycorrhizal infection was also affected by plant species and management practices. Under all treatments, the identified fungi belonged to the genus *Glomus* suggesting that VAM species can be considered as universal biofertilizer due to their

broad host range.

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