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Effects of Mycorrhizal Inoculation and Phosphorus Application on the Nodulation, Mycorrhizal Infection and Yield Components of Faba Bean Grown Under Two Different Watering Regimes

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Abstract: A field experiment was conducted in the 1994/95 and 1995/96 growing seasons to investigate the effects of mycorrhizal inoculation and phosphorus application on infection, symbiotic activity and yield of faba bean (*Vicia faba L.*) grown under two watering regimes. Water stress significantly decreased nodule number, flower set, pod production and seed yield in both seasons. Substantial mycorrhizal infection occurred with low P concentration under both well-watered and dry soil conditions. However, at high soil P, mycorrhizal infection was not suppressed in the dry watering regime as it was in the well-watered treatment. Mycorrhizal inoculation significantly increased nodule number, nodule dry weight, flower set, pod production and seed yield compared to non-mycorrhizal plants under both watering regimes, but P application alone had no significant effect on all the above mentioned parameters. The stimulation of faba bean symbiotic activity and seed yield by mycorrhizal inoculation was suppressed by the application of phosphorus.

INTRODUCTION

The beneficial effect of vesicular arbuscular mycorrhiza (VAM) on plant growth, especially in soils of low fertility and adequate water supply, is well documented (Mahdi and Atabani 1992; Elghandour *et al*. 1996). The increase in growth associated with VAM inoculations have been attributed, among other factors, to the ability of VAM to increase nutrients uptake. Of all the nutrients, P is

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the most studied one in relation to VAM because improved growth by VAM infection is most often correlated with P uptake (Martenson and Carlgen 1994). In faba bean (*Vicia faba*), Elghandour *et al*. (1996) stated that mycorrhizal inoculation could remove the deficiency effect of P on plant growth, particularly in soil of low nutrient content. Preliminary work by Reid and Bowen (1979) indicated that the establishment of mycorrhizal fungi might be impaired by both very wet and extremely dry conditions. However, Subramanian *et al*. (1995) showed that mycorrhizal inoculation improved tolerance to drought in various crops.

In the Sudan, faba bean has traditionally been grown in fertile soils, but with the high demand for the crop and ever-increasing prices, new land of inferior quality has been brought into production. Most such soils are of alkaline pH (>8.0) and with low available phosphorus. It has been suggested that P and water uptake may be improved by mycorrhizal inoculation (Mahdi and Atabani 1992). However, a substantial inference of the interactive effect of moisture stress and soil P levels on the responses of plant to VAM has not been made unequivocally. The objective of this investigation was to assess the effect of mycorrhizal infection on faba bean productivity under limiting conditions of soil moisture and phosphorus. The effects of watering regime and phosphorus application on symbiotic activity and yield of faba bean was also investigated.

MATERIALS AND METHODS

The faba bean cultivar ‘Shambat 75’ and a locally isolated VA mycorrhizal strain from Shambat soil, identified as *Glomus* sp., were used in this study. The method used for fungus isolation was the wet sieving and decanting technique, as described by Hayman (1983). After isolation, the strain was multiplied by growing in pot culture of Sudan grass (*Sorghum bicolor* var. *sudanense*) grown in a sterilized mixture of sand and silt soil (1:1 w/w). The pots were kept in a green house, and frequently irrigated with deionized water until harvest. Pots with only autoclaved soil were used for the non-mycorrhizal treatments.

A field experiment was conducted for two consecutive seasons (1994/95 and 1995/96) in the Demonstration Farm of the Faculty of Agriculture at Shambat (15^{0} 40′ N, 32^{0} 32′ E and 386 m asl). The soil in the experimental plots is montmorillonitic clay with a pH in the range of 7.8-8.5 and low P content (0.03 mg P_2O_5 kg^{-1} soil). The experiment was arranged in split-plot design with four replications. The main plots were allotted to the two watering regimes, with differential watering initiated 30 days after planting (DAP) as follows: Well-watered (plants were irrigated every week) and dry (plants were irrigated every two weeks). The subplots treatments included: control, viable VA mycorrhizal inoculum, P and viable VA mycorrhizal inoculum plus P.

The land was prepared by disc ploughing followed by ridging. Spacing between ridges and holes were 70 cm and 20 cm, respectively. The size of the subplot was 4.2 x 3 m consisting of five ridges of three m length. Between the main plots 1m was left as guard area for water control. Ten grams of mycorrhizal inoculum were placed and spread at the bottom of each hole in the mycorrhizal treatments, whereas 10g of autoclaved soil was used for the non-mycorrhizal treatments. The inoculum consisted of spores, infected roots and soil from Sudan grass culture. Phosphorus, as triple-superphosphate, was applied at a rate of 100 kg P_2O_5 ha^{-1} for the high soil P treatment, whereas the low P treatment is the original P content of the soil (0.03 mg P kg^{-1} soil). According to Mahdi (1993), the indigenous population of rhizobia is high (10^6 cfu g^{-1}), thus only a basal dose of urea was applied at a rate of 40 kg N ha^{-1} to all plots.

The seeds were sown at a rate of three seeds per hole, during the second week of November in the two seasons. The plants were thinned to one plant per hole to achieve a stand of approximately 7×10^4 plants ha^{-1}. The irrigation water was controlled and calculated by a 90^o V-notch. The plants were irrigated every week during the first month to obtain vigorous seedlings. After this, the plants in the dry treatments were irrigated every two weeks. Two hand weedings were done each season.

Two months after planting, three plants per plot, from the inner three ridges, were dug out carefully to avoid root injury. The roots were then separated, washed in a water stream to remove mud and soil particles and examined for the presence of nodules. Nodule dry weight was determined by drying nodule samples after being detached from roots in an oven at 80^oC for 48 h. Root segments (30 segments per plot) from each treatment were stained by lactophenol cotton blue and examined using a light microscope. The percentage of root segments that contained mycorrhizal hyphae was determined for each treatment. Before flowering, five plants per plot were selected randomly and tagged to monitor flower set and pod production. Finally, an overall harvest was made to determine shoot dry weight and seed yield.

RESULTS AND DISCUSSION

Mycorrhizal infection

Though uninoculated plants showed some infection, inoculation with VAM significantly increased mycorrhizal infection under both watering regimes in both seasons, with well-watered plants consistently showing greater mycorrhizal infection than those in the drier treatment. Differences in this parameter were not, however, statistically significant (Table 1). Similar results were reported by Stevens and Peterson (1996) who stated that the overall hyphal and arbuscular colonization levels were significantly higher in the wet treatments than in the dry treatments. On the other hand, Tisdal and Oades (1980) indicated that the presence of VAM also stabilizes soil aggregates and increases plant uptake of water under water deficient conditions. This might explain the nonsignificant effect of watering regime on mycorrhizal infection observed in this study.

Phosphorus application completely inhibited the native VAM in both watering regimes and in both seasons (Table 1). Phosphorus application also suppressed mycorrhizal infection in the VAM-inoculated plants, in each season, particularly in the wet treatment (Table 1). Numerous other studies have indicated that the application of high doses of superphosphate results in less VAM infection (Martenson and Carlgen 1994). The decrease in mycorrhizal infection probably depends upon the increased levels of P in plants resulting in large and healthy root cells that are less liable to infection. Surprisingly, in the present study, mycorrhizal infection was not suppressed by high P concentration in the dry treatment (Table 1). Kwapata and Hall (1985) have also noted substantial mycorrhizal infection at high soil P levels under dry conditions. They suggested that the hypothesized stimulation of mycorrhizal infection by high rates of root exudation could be valid if drought causes plants with high P levels to have high rates of root exudation.

Nodule number and nodule dry weight

Nodule number per plant was greater in the well-watered treatment than in the dry treatment in both seasons, and plants inoculated with VAM were more heavily nodulated than the uninoculated plants in each season (Table 1). Nodule dry weight followed a similar pattern to nodule number in each season (Table 1). Nodule mass was greater in mycorrhizal plants than non-mycorrhizal plants under both watering regimes, and addition of P significantly reduced nodule number and nodule dry weight of mycorrhizal plants, particularly in the well-watered treatment (Table 1). The reductions in the nodule number and the nodule dry weight by water stress, observed in this study, are in harmony with the findings of Hebblethwaite *et al*. (1983) who showed that water stress results in fewer nodules and, therefore, less nitrogen fixation. In addition, water stress can reduce assimilates supply from leaves to nodules by inhibition of transpiration and phloem translocation. Similarly, Venkateswarlue *et al*. (1989) found that water stress caused severe inhibition of nitrogenase activity and nodule respiration in groundnut and cowpea. The improvement in plant symbiotic activity due to the presence of VAM in low soil P condition was probably due to better phosphate acquisition, as has been reported by Mahdi and Atabani (1992).

Dry matter production and seed yield

Watering regimes significantly affected the dry matter production and seed yield, but no interaction was evident (Table 2). Dry matter production was greatly reduced by the dry treatment in both seasons. The reduction in dry matter was attributed to accelerated senescence and shedding of leaves under water stress. The significant reduction in seed yield under dry treatment was associated with low flower and pod retention. However, Turk and Hall (1980) stated that seed yield of cowpea, under water stress, is limited by the source of carbohydrate or by the size of the reproductive sink. In the present investigation, water stress had no effect on seed size suggesting that assimilate supply is not limiting seed yield under conditions of low soil moisture.

Appendix

Appendix

Appendix

Inoculation with VAM has been reported to increase dry matter, yield and seed quality in various crops compared to uninoculated plants (Elghandour *et al*. 1996; Elsheikh *et al*. 2000). In this study, dry matter production and seed yield were significantly greater in mycorrhizal plants than non-mycorrhizal plants particularly under limiting conditions of soil P (Table 3). This is probably due to mycorrhizal enhancement of P uptake, though the addition of P to non-mycorrhizal plants insignificantly increased the above mentioned parameters. This poor response could have been the result of P fixation in the soil or to the slow transformation of P into available forms. In conclusion, the results of the present study indicated that the level of VA mycorrhizal infection is affected by moisture content and phosphorus status of the soil. In addition, the improvement in faba bean growth and yield is thought to have been due to improved P uptake by mycorrhizae suggesting the possibility of using VAM as a low input technology in poor soils.

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