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The Effect of Inoculation by Mycorrhizae and *Rhizobium* on the Growth and Yield of Wheat in Relation to Nitrogen and Phosphorus Fertilization as Assessed by ^{15}N Techniques

Y.G.M. GALAL[1*], I.A. EL-GHANDOUR[1], M.E. OSMAN[2], and A.M.N. ABDEL RAOUF[1]
[1]*Atomic Energy Authority, Nuclear Research Center, Department of Soil & Water Research, Abou-Zaabl 13759, Egypt, Tel. +20-2-5414195, Fax. +20-2-2876031, Email. Glal1954@yahoo.com;*
[2]*Faculty of Science, Botany Department, Helwan University, Cairo, Egypt*

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Abstract

Wheat (*Triticum aestivum* L.) was cultivated in pots of sandy soil and inoculated with *Rhizobium* (Rh), mycorrhizae (VAM) or both. The object was to verify the potential of these inocula on wheat production, nutrient acquisition and microbial biomass N (MBN) in relation to N and P fertilization. Nitrogen and phosphorus fertilizers were applied at rates of, 0 (N_0P_0); 25 mg kg^{-1} soil N and 3.3 mg kg^{-1} soil P (N_1P_1) and 50 mg kg^{-1} soil N and 6.6 mg kg^{-1} soil P (N_2P_2) in the form of $(^{15}NH_4)_2SO_4$ and super-phosphate, respectively. The results revealed that the highest dry matter and N uptake by wheat shoot were obtained with the dual inoculation (Rh+VAM) at N_2P_2 rates of N and P fertilizers. Maximum grain yield was obtained with single inoculum of AM fungi while N and P uptake were with dual inoculation under N_2P_2 treatment. Inoculation with Rh either alone or in combination with AM had an enhancing effect on wheat growth and N and P uptake. Exception for soil treated

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*The author to whom correspondence should be sent.

with the lowest level of fertilizer, there was an increase in microbial biomass N following bio fertilization. The impact of bio and/or chemical fertilizers on soil microbial biomass-N now needs trials on a field scale.

Keywords: Inoculation, microbial biomass, N and P levels, ^{15}N isotope dilution, wheat yield

1. Introduction

Rhizobium (Rh) bacteria are common soil inhabitants, distinguished by their ability to nodulate leguminous plants and fix atmospheric nitrogen. The inoculation of legume seeds with rhizobia, is a frequent agricultural practice and represents a renewable source of nitrogen unlike fertilizer N (Somani and Bhandari, 1990). The use of *Rhizobium* has been recently extended to non-leguminous crops such as wheat (Amara and Dahdoh, 1997; Galal et al., 2001; Sabry et al., 2000), lettuce and maize (Chabot et al., 1996). Inoculation of wheat with *Azorhizobium* solely or mixed with vesicular arbuscular mycorrhizae (VAM) and 50% N-fertilizer level increased shoot and root dry weights and was attributed to N_2-fixation and/or certain growth promoting substances (Sabry et al., 2000; Abd El-Ghaffar, 1996). Similar stimulation effects have been detected using *Rhizobium* on wheat, rice, maize, lettuce and radish (Antoun et al., 1998; Saleh et al., 2000; Webster et al., 1997; Yanni et al., 1995 and 1997).

The roots of most plants form symbiotic associations with mycorrhizal fungi. The fungal partner plays a particularly important role when the supply of available mineral nutrients in the soil is low. In these conditions the net flux of nutrients from the soil into the plant is greater in mycorrhizal than in comparable non-mycorrhizal plants (Smith and Read, 1997), but little is known about the processes controlling the transfer of material between the symbionts (Ayling et al., 1997). The positive effects of nutrient availability (organic C, farmyard manure, N and P fertilizers) on microbial biomass have been studied (Houot and Chaussod, 1995). Microbial biomass may thus serve as a reliable index of soil productivity (Huaiying et al., 1999; Hassik, 1994). Microbial biomass is significantly correlated with soil organic C, total N and available N, and with dry matter yield and N uptake by plants. Available P and organic matter applications increase microbial biomass N and C while at the same time increasing mineralizable N enhances microbial biomass N (Shibahara and Inubushi, 1997).

The response of microbial biomass N to high fertilizer rates at early stages of the growing season on sandy clay loam and clay soils indicates that there are

N limitations with normal fertilizer rates (Liang and MacKenzie, 1996). Thus, there is competition for soil N between microbial populations and maize plants during these periods and this will likely reduce levels of available N for maize growth, and eventually affect grain yields (Liang and MacKenzie, 1996). The maximum increase in the microbial biomass was observed under the farmyard manure and inorganic fertilizer together, followed by manure alone, and then fertilizer alone (Ghoshal and Singh, 1995). Although microbial biomass-N is a small proportion of soil organic N, experiments using ^{15}N have shown that, microbial biomass N is a major source of labile N in most soils (Myrold and Tiedje, 1986).

The object of the present research was to study the impact of inoculation with *Rhizobium* and endomycorrhizae on wheat growth with various nitrogen and phosphorus fertilization.

2. Materials and Methods

Experimental

A series of pot experiments were carried out under greenhouse conditions at The Soil and Water Research Department, Nuclear Research Center, Egyptian Atomic Energy Authority. Sandy soil with total-N 0.008%; organic matter 0.03%; available-P 2.1 mg kg^{-1} soil; CEC 1 meq 100 g^{-1} soil and pH 8 was placed in 36 pots (height 21 cm and diameter 18.5 cm) at 8 kg soil pot^{-1}. Seeds of wheat (*Triticum aestivum* cv. Sakha 8) obtained from Field Crops Research Institute, Agriculture Research Center, Giza, Egypt, were sown at a rate of 6 seeds pot^{-1}. The experimental design was a completely randomized block comprised of three fertilizer treatments as follows: (1) without N or P fertilizers (N_0,P_0); (2) addition of N at rate of 0.2 g pot^{-1} as $(^{15}NH=)_2SO_4$ (5% atom excess) and P at rate of 0.4 g pot^{-1} as superphosphate (N_1,P_1); (3) addition of N at rate of 0.4 g pot^{-1} and P at rate of 0.8 g pot^{-1} (N_2,P_2).

Labelled ammonium sulphate in proper doses was dissolved in 200 ml of bi-distilled water then added to soil surface according to the required amounts which were calculated on the base of mg N kg^{-1} soil. After harvest, the plant samples were collected and prepared for $^{14}N/^{15}N$ ratio analysis using emission spectrometer model NOI-6 PC. The data of ^{15}N% atom excess was used to estimate the portions of nitrogen derived from fertilizer (%Ndff) and those derived from soil (%Ndfs) using the following equations:

$$\%Ndff = {}^{15}N\% \text{ atom excess in sample} / {}^{15}N\% \text{ atom excess in fertilizer} \times 100$$

$$\%Ndfs = 100 - \%Ndff$$

Inoculum and inoculation treatments

A mixture of endomycorrhizea (AMF) spores was isolated from corn field near to the site of Inshas area using a wet sieving technique (Gerdemann and Nicolson, 1963). Peat-based inoculum of *Rhizobium leguminosarum* biovar. *vicia* strain ICARDA 36 was kindly provided by the Agriculture Research Center, Giza, Egypt. The inoculation treatments were as following (1) uninoculated; (2) inoculated with AMF; (3) inoculated with *Rhizobium* (Rh); (4) inoculated with mixture of AMF+Rh.

Analyses

Soil and plant samples were chemically analyzed for total N and available P as described by Page et al. (1982). Infection percentage of roots by AM fungi was estimated using the method of Philips and Hayman (1970). Soil microbial biomass N was determined by the fumigation-extraction method (Brookes et al., 1985).

Statistical analysis of data was done by analysis of variance (ANOVA) and least significant difference (LSD) followed by Duncan's Multiple Range Test (applied at 0.05 level) according to SAS User's guide (SAS computer system 1987).

3. Results and Discussion

Dry matter accumulation and grain yield

The effects of the different treatments on dry matter accumulation in wheat shoots and the grain yield is shown in Figs. 1 and 2.

The different inoculation treatments, N and P fertilizer levels led to highly significant differences in the dry weight of shoots and yield of wheat plants. The maximum productivity was obtained in response to bio-inoculations coupled with the highest levels of N and P fertilizers. The effect on shoot dry weight was greatest with dual inoculation of Rh+VAM and high levels of N and P. With respect to wheat grain, the yield was largest with VAM inoculation coupled with the highest level of N and P fertilizer. This indicates a positive interaction between cereals yield and VAM fungi.

The results of these pot experiments have been confirmed in preliminary field trials, which have shown that wheat shoot and grain yields were significantly increased by inoculation combined with N fertilizers (198 kg N ha^{-1}) (Galal et al., 2000).

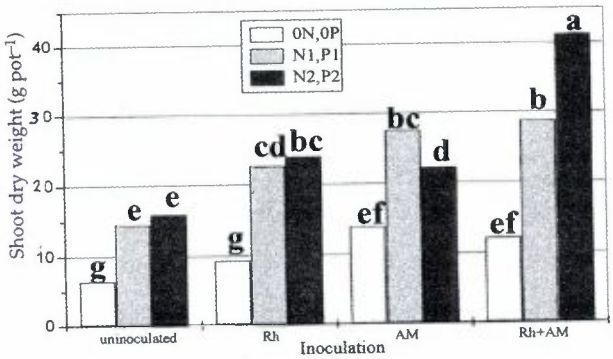

Figure 1. Effect of biofertilizers, nitrogen and phosphorus levels (g pot^{-1}) on wheat dry matter accumulation (shoot dry weight) (g pot^{-1}) (LSD=4.86).

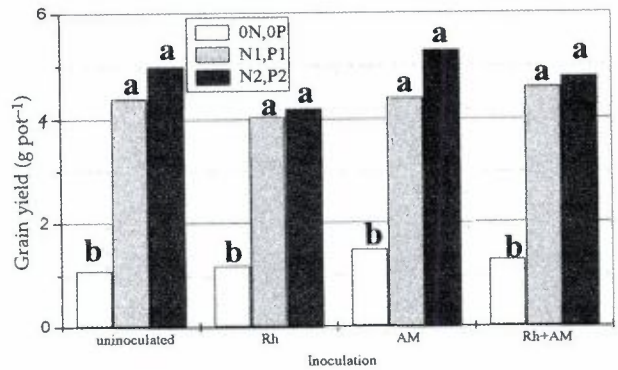

Figure 2. Effect of biofertilizers, nitrogen and phosphorus levels (g pot^{-1}) on wheat grain yield (g pot^{-1}) (LSD=1.62).

N uptake by shoot and grains of wheat

Nitrogen levels in wheat shoots were maximal following dual inoculation with Rh+VAM and at the highest level of N and P fertilizers (Fig. 3). Similar trend was noticed with wheat grain (Fig. 4). It is noteworthy that the use of Rh with wheat (a non-legume) gave positive results either applied alone or in combination with VAM as found in our previous study (Galal et al., 2002). These bacteria have the ability to invade the lateral roots and to localize in cellular or intercellular spaces of the cortex of cereals like wheat, rice and corn through the crack-entry mechanism. Yanni et al. (1997) demonstrated that rhizobia endophytes significantly promoted growth of rice shoots, roots and

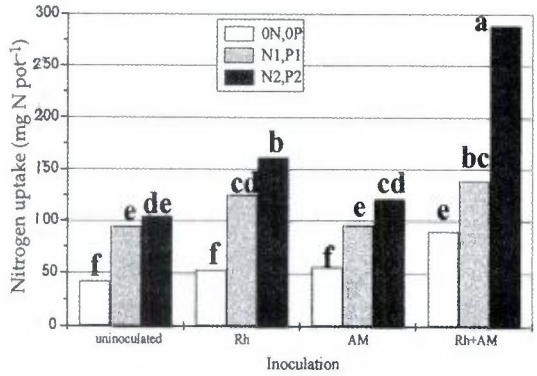

Figure 3. Nitrogen uptake by wheat shoot (mg N pot^{-1}) as affected by inoculation, nitrogen and phosphorus levels (g pot^{-1}) (LSD=22.78).

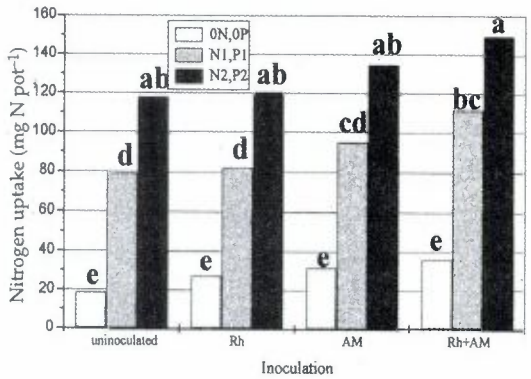

Figure 4. Nitrogen uptake (mg N pot^{-1}) by wheat grain as affected by inoculation, nitrogen and phosphorus levels (g pot^{-1}) (LSD=32.08).

yield. The mechanisms responsible for these effects are unknown. More studies are needed to assess whether the rhizobia fix N_2 in association with cereals. Webster et al. (1997) grew wheat plants in pots in growth chambers using aseptic precautions. They observed high levels of acetylene reduction activity in plants inoculated with *Azorhizobium caulinodans*, indicating the rhizobia colonization and endophytic nitrogen fixation.

The high levels of root colonization following microbial inoculants by VAM fungi may be attributed to changes in root-morphology and physiology (Singh and Singh, 1993). The increased dry matter yield after dual inoculation (VAM+Rh) compared with inoculation by just one organism reflects an active

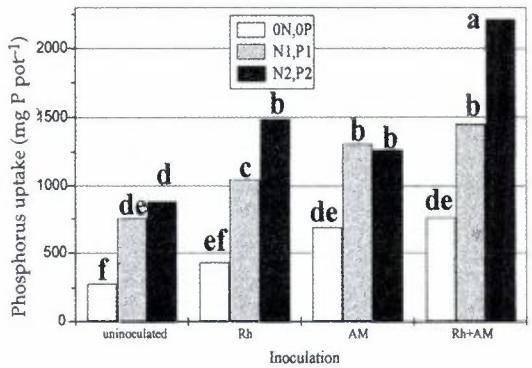

Figure 5. Phosphorus uptake of wheat shoot (mg P pot^{-1}) as affected by inoculation, nitrogen and phosphorus levels (g pot^{-1}) (LSD=336.6).

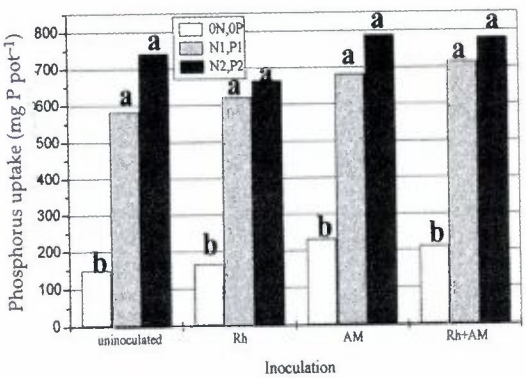

Figure 6. Phosphorus uptake (mg P pot^{-1}) of wheat grain as affected by inoculation, nitrogen and phosphorus levels (g pot^{-1}) (LSD=252.9).

role of rhizobia and an interaction with VAM fungi. Rhizobia may produce root exudates, which enhance the growth of the soil flora and/or the permeability of root cells to fungal invasion (Azcon-Aguilar et al., 1982; Hayman, 1982). Our results support those of Badr El-Din and Moawad (1988) who concluded that the dual inoculation with both rhizobia and mycorrhiza resulted in optimal plant dry weight, N and P levels in lentil and faba bean; as well as the highest seed yield in soybean. Daft and El-Giahmi (1974) also found that infection of phaseolus with Endogone and *Rhizobium* compared to *Rhizobium* alone increased growth, acetylene reduction rate, the number and weight of nodules, and the total protein content.

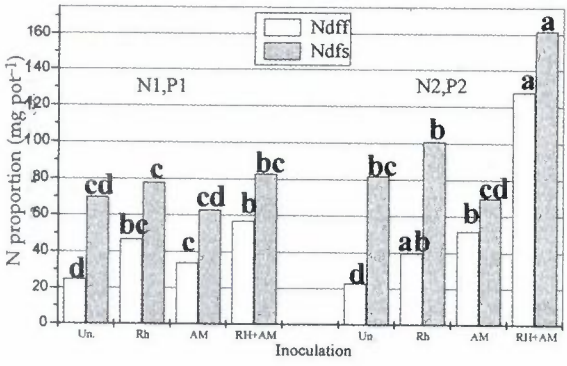

Figure 7. Effect of inoculation, nitrogen and phosphorus levels on N derived by wheat shoot.

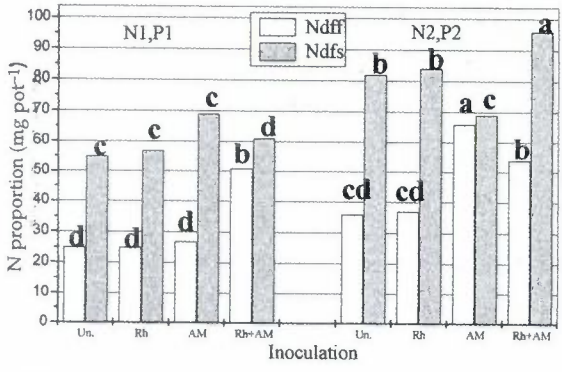

Figure 8. Effect of inoculation, nitrogen and phosphorus levels on N derived by wheat grain.

The interaction between inoculation and N, P levels (Fig. 5) showed maximum P uptake in wheat shoot in Rh+VAM, N_2, P_2 (2217 mg P pot^{-1}) and Rh, N_2, P_2 followed by Rh+VAM, N_1, P_1. The interaction of N and P levels with inoculation (Fig. 6) gave the highest three values for P uptake of wheat grains at N_2, P_2 level in the sequence of VAM, Rh+VAM, uninoculated with no significant difference.

The beneficial role of VAM particularly on P uptake, appears to be related to the nutrients depletion zone that surrounds the root because the fungal mycelia can be extend beyond this area and P uptake becomes more efficient beyond this depletion zone (Hopkins, 1999). About 53% of total P uptake and 24% of total N

located in mycorrhizal plants were transferred to the plant by external hyphae (Marschner and Dell, 1994).

It is clear that proportions of N derived from fertilizer and utilized by shoot (Fig. 7) or grain (Fig. 8) of wheat was promoted by inoculation treatments. In this regard, the inocula followed the descending order: Rh+VAM > Rh >VAM > uninoculated, when Ndff was utilized by shoot under the two levels of N and P application. Under high level of N and P, the following order was obtained: VAM > VAM+Rh > Rh; uninoculated.

Microbial biomass N

The data presented in Table 1 reflected the effect of biofertilizers and N and P levels on microbial biomass N in wheat soils. The values of microbial biomass N fluctuated. With the exception of soil treated with N_1, P_1 level, there was an increase in microbial biomass N, in the soil under wheat, following biofertilization treatments. The data from these pot experiments did not give a clear correlation between total N uptake by wheat and N remaining in microbial cells (biomass N).

Microbial biomass is important in nutrient cycling as well as soil aggregation. The microbial biomass may account for as much as 2% of the total soil C (Somani and Bhandari, 1990). The organic N and C in microbial components represent the active phase of the soil organic matter. As a general rule, from 1–6% of the soil organic N resides in the microbial biomass (Stevenson and Cole, 1999). The data available on the effect of biofertilizers on the microbial biomass are very limited. However, many investigations have documented the effects of mineral and organic fertilization on soil microbial biomass in arable and grasslands under short and long-term experiments (Ghoshal and Singh, 1995; Houot and Chaussod, 1995; Joergensen et al., 1995; Patra et al., 1995). Due to its dynamic character, the biomass responds much more rapidly than soil organic matter to changes in management (Powlson and Jenkinson, 1981). Therefore, our study aimed to evaluate the responses of the microbial biomass in temperate agroecosystem to exogenous soil inputs like biofertilizers and mineral N.

It is clear that microbial biomass does not increase, although the crop yield does, when NPK fertilization is initiated (Ghoshal and Singh, 1995). Although, in our results, soil microbial biomass N fluctuated, we found that in some cases, the microbial fractions were positively affected by both mineral and bio-fertilization treatments. The observed microbial biomass N of wheat soils reflected changes in soil biomass activity and this in turn was influenced by biofertilization and fertilizer N added. The microbial biomass (C, N, P, C: N, C:P and N:P) may have been influenced by fertilizer N additions, crop type

Table 1. Microbial biomass N (μg N g–1 soil) in wheat rhizosphere as affected by inoculation, nitrogen and phosphorous levels.

N and P levels (g pot^{-1})	Inoculation			
	Un	Rh	AM	Rh+AM
N_0, P_0	10.38 c	10.30 b	20.67 b	20.67 b
N_1, P_1	20.67 b	31.11 a	41.50 a	51.80 a
N_2, P_2	31.10 a	10.38 b	20.70 b	22.00 b

Means in the same column followed by the same letter are not significantly different at P 0.05.

Table 2. Mycorrhizal infection (%) of wheat roots as affected by inoculation, nitrogen and phosphorus levels.

N and P levels (g pot^{-1})	Inoculation			
	Un	Rh	AM	Rh+AM
N_0, P_0	0	0	40 c	64 c
N_1, P_1	0	0	60 b	72 b
N_2, P_2	0	0	72 a	84 a

Means in the same column followed by the same letter are not significantly different at P 0.05.

and season. The reasons of biomass increments could be attributed to the stimulating action of the inorganic fertilizer, variations in temperature, or promotion of plant growth (Patra et al., 1995). So, soil texture plays an important role in biomass N turnover. Clay soil with a greater organic matter content compared to a sandy clay loam was more reactive to added fertilizer N, having a greater microbial biomass N immobilization early in the season, and subsequently a greater release of N at late season (Liang and MacKenzie, 1996).

Although microbial biomass is a small proportion of soil N, the application of ^{15}N technique showed that microbial biomass N is a major source of labile N in most soils (Myrold and Tiedje, 1986). Using ^{15}N techniques, Liang and MacKenzie (1996) found that increasing fertilization rates enhanced microbial biomass N immobilization under maize. There was a subsequent greater N release and a reduced fertilizer N recovery. There was a negative effect of

increasing fertilizer rates, on soil microbial biomass N, late in the growing season. In general, our results on biomass N under wheat coincided with these conclusions.

Fungal infection as affected by inoculation and fertilization

The infection percentage caused by mycorrhizal fungi differed with inoculation and fertilizer levels (Table 2). Infection increased with increasing N and P levels under VAM and VAM+Rh treatments. The data revealed the superiority of dual inoculation over the single inoculation with VAM.

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