Colonization of Arbuscular Endomycorrhizal Fungi on Maize Affected by Various N Rates in Long-term Field Experiments

T. TAKÁCS and I. VÖRÖS

Research Institute for Soil Science and Agricultural Chemistry (RISSAC) of the Hungarian Academy of Sciences, Budapest

Introduction

Nutrient balance of Hungarian soils

During the period between the early sixties and mid-eighties, when intensive agricultural systems were in practice in Hungary, excess quantities of nutrients were often applied at various N, P and K rates. Due to this fact, the nutrient balance of most arable fields became positive with an improved nutrient supplying capacity. This was favourable for soils which originally contained less nutrients (weak nutrient supply). Fertilization therefore played a significant role in increasing yields and in sustainability.

Under certain environmental conditions the positive nutrient balance might change not only the availability of P and K, but also that of N (SARKADI et al., 1986), having a positive influence on soil biological characteristics. The danger of overfertilization is more pronounced in the case of nitrogen. Unfortunately, the estimation of soil organic-C content does not serve reliable information about the easily available N fractions. The effect of N fertilization on soil N content can be characterized, however, by measuring the mineral-N (exchangeable NH_4^+ and NO_3^-) content of the soil (WEHRMANN & SCHARPF, 1979).

In the case of overfertilization, under Hungarian conditions, excess P and K rates are mainly left in the ploughed layer (SARKADI et al., 1986), while the surplus N (as NO_3-N) may accumulate along the profile, get leached to deeper soil layers or to the groundwater (CAMPBELL et al., 1983; LINVILLE & SMITH, 1971; NÉMETH et al., 1987-1988) as a function of the physical characteristics of the soil and of the environmental – mainly meteorological – conditions.

The mineral-N content of the upper 60 cm soil layer and the seasonal dynamics of the N forms were investigated in a long-term experiment on calcareous chernozem soil after 11 years' application of four N fertilization rates (SARKADI et al., 1986). After 11, 12 and 17 years of N fertilization the movement, accumulation and distribution of NO_3-N in the profiles down to 6 m was also examined (NÉMETH, 1995; NÉMETH et al., 1987-1988). On the basis of these results two N fertilization experiments were carried out to compare the residual effect of previously – in long-term – and the freshly applied (in spring) nitrogen. Results of the 8-year experiment regarding the measurements, calculations and calibrations of mineral-N and its role in a better utilization of these nitrogen forms were presented earlier (NÉMETH, 1996; NÉMETH & BUZÁS, 1991a,b).

Role of AM fungi in the nutrient supply

Mycorrhizas are the most widespread associations between microorganisms and higher plants in both natural and agricultural ecosystems. The roots of most terrestrial plants have mycorrhizal connection. Arbuscular endomycorrhizal (AM) fungi (Glomales) probably belong to the most common soil fungi (GERDEMANN, 1968) and are associated with about 80% of the plant species of the world (MARSCHNER, 1995). The Glomales symbionts have been shown to increase the uptake of mineral elements from soil, e. g. nitrogen, phosphorus, potassium, sulphur, calcium, copper and zinc, especially when these elements are in low concentration or relatively immobile forms in the soil (BOLAN, 1991; GILDON & TINKER, 1983). The symbiosis of AM may also improve the resistance of plants to root-infecting pathogens and tolerance to soils contaminated by heavy metals, saline or acidic soils, high soil temperature or dry conditions (BARRAGAN et al., 1996; GEORGE et al., 1992). Much research effort has also been directed to the agricultural significance of AM, regarding that the symbiosis is considered to be beneficial to the growth of arable crop and other economically important plants (SIEVERDING, 1991; SIEVERDING & TORO, 1987; SCHENCK & PÉREZ, 1990; ABBOTT et al., 1992; BAGYARAJ, 1992).

The symbiosis between endomycorrhizal fungi and plants depends on the plant and the fungal species and also on biotic and abiotic environmental factors e. g. soil properties. A general observation made in several studies is that under optimal environmental conditions, when soils are sufficiently supplied with nutrients, the symbiosis is not frequent (FABER et al., 1992; GEORGE et al., 1992). Due to the optimal nutrient level of host plants, the activity of fungal symbiont seems not to be necessary. Considering that the growth and development of hosts are also limited at a low nutrition level, it may also suppress the fungal symbiosis. There must be an optimal level of nutrients therefore, that is favourable for the symbiosis in a certain environment. When colonization has developed, the AM fungi is capable of providing not only the water but also most of the macro- and micronutrients for the host, especially if the nutrients are in a very limited concentration in the soil. The development of symbiosis is inhibited by the high concentration of phosphorus, nitrogen, other nutrients as well as by high organic matter content (BIRÓ et al., 1993; VÖRÖS et al., 1995).

DAVIS & YOUNG (1985) have found that among the mineral nitrogen forms the inhibitory effect of nitrate is higher than that of ammonium.

Root samples were taken from maize (*Zea mays* L.) in two long-term fertilization experiments (started 13 years ago) and the arbuscular endomycorrhizal root colonization on maize was studied on calcareous chernozem soil of Nagyhörcsök and a humous sandy soil of Őrbottyán.

The aim of this study was to characterize the soil mineral-N content via investigating the effect of different N treatments on the colonization of arbuscular mycorrhizal fungi as a function of two soil types.

Materials and Methods

Field experiments mentioned above were set up in autumn 1984 on a calcareous chernozem soil (Nagyhörcsök) and a calcareous sandy soil (Őrbottyán) at the experimental stations of the Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences.

Soil and meteorological characteristics

Őrbottyán. – Annual mean precipitation is 500 mm in the area characterized by continental climate. The soil type – according to the Hungarian Soil Classification – is a calcareous sandy soil, with 3–6% $CaCO_3$, 0.9–1.1% organic matter and 800–1200 mg total-N in the ploughed layer, and a C/N ratio of 7.5–8.0. The thickness of the humus layer is 20-60 cm. According to the soil analyses, $pH_{(KCl)}$ is 7.0-7.3; the ammonium-lactate (AL) soluble P_2O_5 and K_2O content is 70-80 and 50-80 mg/kg, respectively. These results show that the original nutrient supply of the soil is medium–to–weak. The average level of the groundwater table is around 270-320 cm.

Nagyhörcsök. - This site has an annual mean temperature of 10.9 °C and an average precipitation of 550-580 mm; the climate is also continental. The soil type is a calcareous chernozem, the parent material loess, and the whole profile is loamy. The main characteristics of the ploughed layer are: $CaCO_3$ content: 5%; organic matter: 3%; total-N: 2100–2200 mg/kg; C/N ratio: 7.7–8.5. The thickness of the humus layer is 75-90 cm. According to the soil analyses the $pH_{(KCl)}$ is 7.2–7.3; the AL-soluble P_2O_5 and K_2O content is 60-80 and 140-160 mg/kg, respectively. The original P supply of the soil is weak, while the K supply is medium. The average level of the groundwater table is around 13–15 m.

Treatments

Large (250 m²) plots were set up with four N fertilizer rates (0, 150, 300 and 450 kg N/ha/year) in four replications in autumn 1984. The rate of P (100 kg P_2O_5/ha) and K (200 kg K_2O/ha) were identical in all N treatments. The mineral-N content of the profiles of these 16 large plots were checked in spring and autumn each year just before fertilization. The mineral-N analyses were carried out according to the methods of Bremner & Keeney (in NÉMETH, 1996)

Investigation of the colonization of arbuscular mycorrhizal fungi

Root samples of maize plants were taken from all treatments in autumn 1997 at Nagyhörcsök and Őrbottyán, respectively, from the different N treatments. After sampling the roots were washed, cleared and stored in ethanol (70%) preceding the AMF investigations. During the elaboration process root samples were boiled in a potassium hydroxide solution (15% KOH) for 30 minutes. After washing, the samples were stained in aniline blue for 30 minutes, and the excess dye was removed by shaking in concentrated lactic acid solution for 30 minutes. The final step during the preparation was submerging the sample in glycerine solution (80%) for long storage. Arbuscular mycorrhiza (AMF) colonization was observed after aniline blue staining of roots by using a stereomicroscope.

The rate of AM colonization was evaulated according to the method of TROUVELOT et al. (1986), where the infection frequency (F%), the intensity of mycorrhization (M) and the frequency of arbuscules (A%) were evaluated

Results and Discussion

N content of the investigated soil

In previous studies it was found that under similar experimental conditions the surplus N could only be detected in the form of NO_3 (NÉMETH et al., 1987-1988). The NH_4-N content of the soils did not vary following the different N

Table 1

Average nitrate-N concentration (mg/kg) in the 0-100 cm soil profile after different N application rates (on average of different sampling times)

Soils	Treatments (kg N/ha/year)			
	0	150	300	450
Sandy soil	4.2	4.9	6.3	9.1
Chernozem soil	7.8	11.7	30.8	44.5

rates applied throughout the 11 years. Similar results were obtained in the soil profiles of these two experiments (NÉMETH, 1996). In the calcareous sandy soil, differences in NO_3-N concentration were only measured below 50 cm in the profiles, while in the calcareous chernozem soil, such differences were observed from the top of the profiles (Table 1).

Colonization of endomycorrhizal fungi

Infection frequency (F%) and the intensity (M) of mycorrhization on maize root did not differ significantly in the calcareous chernozem soil (Nagyhörcsök). There was a significant difference, however, in infection frequency (F%) and the intensity (M) of AM fungi between the treatments ($P < 5\%$) in the sandy soil (Őrbottyán) (Table 2). The highest value of AM-infection (F%) was found at the 150 kg N/ha/year rate. With higher annually applied N rates (300, 450 kg N/ha/year) the AM-infection decreased in the Őrbottyán soil.

The highest amount of arbuscules were found in the chernozem soil, which indicated greater efficiency of symbiosis under this condition as compared to the sandy soil. The highest quantity of arbuscules (A%) was found in the 150 kg N/ha/year nitrogen treatment in Nagyhörcsök (chernozem soil), while the 300 kg N/ha/year treatment gave the best results for arbuscularity in case of the sandy soil (Őrbottyán). The maximal efficiency of symbiosis was detected at the higher N doses in case of the sandy soil, which was due to the relatively low concentration and supply of essential elements, and other soil properties,

Table 2

Root colonization of maize by arbuscular mycorrhizal fungi (Nagyhörcsök, July 1997 and Őrbottyán, September 1997)

Replication	Treatment	N-rate kg/ha/year	Infection frequency (F%)	Intensity of mycorrhization (M)	Arbuscularity (A%)
Nagyhörcsök, July, 1997					
			N.S.	N.S.	$LSD_{5\%}$ 16.4
I.	1.	0	94.15	48.19	13.70
II.	2.	150	96.66	62.86	43.73
III.	3.	300	84.16	49.59	24.05
IV.	4.	450	95.00	53.26	29.50
Őrbottyán, September, 1997					
			$LSD_{5\%}$ 8.23	$LSD_{5\%}$ 19.11	$LSD_{5\%}$ 24.73
I.	1.	0	90.,0	43.37	12.14
II.	2.	150	92.50	56.33	26.72
III.	3.	300	84.16	51.00	32.32
IV.	4.	450	60.80	21.28	6.530

rather than to the rate of N application. It was concluded that arbuscular mycorrhizal fungi (AMF) may develop an efficient association on maize at optimal soil nutrient levels, while higher doses of nitrogen fertilization may cause inhibition.

Summary

The root colonization of indigenous arbuscular mycorrhizal fungi on maize was studied in two long-term nitrogen fertilization experiments of Hungary. In both locations, on two different soil types (calcareous chernozem soil, Nagyhörcsök, and calcareous sandy soil, Őrbottyán) four annual N fertilizer rates (0, 150, 300 and 450 kg N/ha/year) were applied from 1984. The abundance and effectivity of arbuscular mycorrhizal fungi (AMF) were tested as a function of the above-mentioned N fertilizer rates and two different soil types.

Mycorrhizal infection (F%) was high and did not really differ in the various treatments. The amount of arbuscules, however – which show the real efficiency of arbuscular mycorrhiza symbiosis – decreased with increasing N levels in both soil types to a certain level.

The infection frequency of AM fungi was only slightly influenced by the different N fertilizer rates. Arbuscularity, however tended to be a more reliable indicator of the functioning and effectivity of AM fungi under the varying environmental conditions (such as different N supply and soil types).

Acknowledgement

Authors greatly appreciate the financial and instrumental support received from the Hungarian National Scientific Research Fund (OTKA) under grants No. 17647, 17648 and 21275.

References

ABBOTT, L. K., ROBSON, D. & GAZEY, C., 1992. Selection of inoculant vesicular-arbuscular mycorrhizal fungi. In: Methods in Microbiology. Vol. 24. (Eds.: NORRIS, J. R., READ, D. J. & VARMA, A. K.) 1-21. Academic Press. London.

BIRÓ, B. et al., 1993. Symbiont effect of Rhizobium bacteria and VAM fungi on pisum sativum in recultivated mine spoils. Geomicrobiol. J. **11.** 275-284.

BAGYARAJ, D. J., 1992. Vesicular-arbuscular mycorrhiza: application in agriculture. In: Methods in Microbiology. Vol. 24. (Eds.: NORRIS, J. R., READ, D. J. & VARMA, A. K.) 359-373. Academic Press. London.

BARRAGAN, A. T. et al., 1996. The use of AM fungi to control onion white root under field conditions. Mycorrhiza. **6.** 253-257.

BOLAN, N. S., 1991. A critical review on the role of mycorrhizal fungi in the uptake of P by plants. Plant and Soil. **134.** 189-207.

CAMPBELL, C. A. et al., 1983. The first 12 years of a long term crop rotation study in south-western Saskatchewan – Nitrate-N distribution in soil and N uptake by the plant. Can. J. Soil Sci. **63.** 563-578.

DAVIS, E. A. & YOUNG, J. L., 1985. Endomycorrhizal colonization of glasshouse grown wheat as influenced by fertilizer salts when banded or soil mixed. Canad. J. Bot. **63.** 1196-1203.

FABER, B. A. et al., 1991. A method for measuring hyphal nutrient and unter uptake in mycorrhizal plants. Can. J. Bot. **69.** 87-94.

GEORGE, E., MARSCHNER, H. & JAKOBSEN, I., 1992. Role of AM fungi in uptake of P and N from soil. Crit. Rev. in Biotechnol. **15.** 257-270.

GEORGE, E. et al., 1992. Water and nutrient translocation by hiphae of *Glomus mosseae*. Can. J. Bot. **70.** 2130-2137.

GERDEMANN, J. W., 1968. Vesicular-arbuscular mycorrhiza and plant growth. Annu. Rev. Phytopathol. **6.** 396-418.

GILDON, A. & TINKER, P. B., 1983. Interactions of VA mycorrhizal infection and heavy metals in plants. I. Development of VA mycorrhizas. New Phytol. **94.** 247-261.

LINVILLE, K. E. & SMITH, G. E., 1971. Nitrate content of soil cores from corn plots after repeated nitrogen fertilization. Soil Sci. **112.** 249-255.

MARSCHNER, H., 1995. Mineral Nutrition of Higher Plants. Academic Press. London.

NÉMETH, T., 1995. Nitrogen in Hungarian soils - nitrogen management relation to groundwater protection. J. Cont. Hidr. **20.** 185-208.

NÉMETH, T., 1996. Long-term N-fertilization calibration experiments - environmental aspects. In: Nitrogen Economy in Tropical Soils. (Ed.: AHMAD, N.) Developments in Plant and Soil Sciences. **69.** 371-377. Kluwer Academic Publishers. The Netherlands.

NÉMETH, T. & BUZÁS, I., 1991a. Long-term nitrogen fertilization experiment on humous sandy soil and on calcareous chernozem soil. (In Hungarian) Agrokémia és Talajtan. **40.** 399-408.

NÉMETH, T. & BUZÁS, I., 1991b. Calibration experiment on nitrogen fertilization using winter oilseed rape as indicator plant. (In Hungarian) Agrokémia és Talajtan. **40.** 409-418.

NÉMETH, T., KOVÁCS, G. & KÁDÁR, I., 1987-1988. Nitrate, sulphate and water soluble salt accumulation in the soil profile of a long-term fertilization experiment. (In Hungarian) Agrokémia és Talajtan. **36-37.** 109-126.

SARKADI, J., NÉMETH, T. & KÁDÁR, I., 1986. Heterogeneity of the readily soluble nutrient content of the soil. (In Hungarian) Agrokémia és Talajtan. **35.** 295-306.

SCHENCK, N. C. & PÉREZ, Y., 1990. Manual for the Identification of VA Mycorrhizal Fungi. Third Edition. Synergistic Publications. Gainesville, USA.

SIEVERDING, E., 1991. Vesicular-arbuscular Mycorrhiza Management in Tropical Agrosystems. Atelier Niederjes. Friedland, Germany.

SIEVERDING, E. & TORO, S., 1987. Acaulospora denticulata sp. nov. and Acaulospora rehmii sp. nov. (Endogonaceae) with ornamented spore walls. Angewandte Bot. **61.** 217-223.

TROUVELOT, A., KOUGH, J. L. & GIANINAZZI-PERSON, V., 1986. Mesure du taux de mycorrhisation VA d'un systeme radiculaire. In Physiological and Genetical Aspects of Mycorrhizae. (Eds.: GIANINAZZI-PERSON, V. & GIANINAZZI, S.) 217-221. INRA.

VÖRÖS, I. et al., 1995. Effect of various organic additives on humus accumulation and mycorrhizae recolonization of dump spoils in Hungary. In: AM Fungi in Sustainable Soil-plant Systems. (Ed.: TURNAU, K.). 34-44. Krakow, Poland.

WEHRMANN, J. & SCHARPF, H. C., 1979. Der mineralstickstoffgehalt des Bodens als Mass-stab für den Stickstoffdüngerbedarf (N_{min}-methode). Plant and Soil. 52. 109-126.