Proceedings of the 7th Hungarian Congress on Plant Physiology, 2002
S5-06

Volume 46(3-4):187-188, 2002
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Advantages of application of mycorrhizated plants in environment-friendly agriculture and forestations

Zoltán Bratek[1], Ibolya Vörös[2], Tünde Takács[2], István Parádi[1], Szabolcs Rudnóy[1], Krisztián Halász[1]*

[1]Dept. of Plant Physiology, Eötvös Loránd University, Budapest, Hungary, [2]Research Institute for Soil Science and Agricultural Chemistry, Budapest, Hungary

ABSTRACT Ectomycorrhizal inoculation procedures have been developed to improve forestation practice in areas with unfavourable habitat properties. The mycorrhizal inoculation of cultivated crops with specially developed inocula of arbuscular-mycorrhizal fungi succeeded in reaching a substantial harvest surplus of plant material, and makes environment-friendly, fertilizer-free farming practices around shallow lakes possible.

Acta Biol Szeged 46(3-4):187-188 (2002)

KEY WORDS

mycorrhiza
environment-friendly agriculture
forestry

With the usage of appropriate mycorrhizal fungi the plants' stress tolerance can be raised substantially against unfavourable environmental effects. Natural forest growth of domestic land areas with extreme soil and climate properties, such as salt affected soils, sands, and in mine pits and mine spoils is a slow process. The hastening of this process has been made possible by enhanced stress-tolerant/vitalized mycorrhizal plants has become common practice in many countries. It is remarkable that some studies were carried out by Hungarian researchers in this field (Bokor 1954; Szántó 1994a,b). While growing tree seedlings in nurseries, resistance against different diseases improves in cases of mycorrhizal plants, just as with their growth indicators. The survival of mycorrhizal plants is significantly greater than non-mycorrhizal ones, therefore mycorrhizal inoculation results in avoiding unnecessary replacements by working with a smaller number of plants.

The use of mycorrhiza of cultivated crops (causes) goes along with the growth in uptake of macro- and micro-elements, making it possible to decrease or even stop the usage of fertilizers. Beside this, the quicker emergence of soil life is made possible, and favourably affects the physical properties of the soil on the behalf of plants.

Materials and Methods

The first stage of the work meant the mapping of mycorrhiza-forming fungi with the trees of the territories mentioned being developed, as well as the isolation of their sterile cultures, which was followed by the processing of the cultures which had been maintained on solid agar media and accrued in shake flask cultures (Molina and Palmer 1982; Rudnóy 1999). The molecular identification of the strains was carried out based on RFLP and sequence analysis of the nuclear ribosomal ITS region (Rudnóy et al. 2000).

The greenhouse ectomycorrhizal inoculation was performed with the sterile mycelial cultures fragmented in a blender, or with spores from fruit bodies which had been added to a pasteurised mixture of peat and vermiculite. Host plants such as black-locust tree, oak, poplar, and pine tree species were inoculated in nursery seedbeds. Mycorrhizal colonization was quantified on one- and two-year old mycorrhizal seedlings in the nursery. Two years old tree seedlings were outplanted in field soil and the physiological parameters (CO_2-fixation, chlorophyll content, chlorophyll fluorescence kinetics), the growth and survival characteristics of them were measured continuously.

We isolated monosporic arbuscular-mycorrhizal fungi strains from a number of soil types (brown forest soil and carbonated field soil) in the Balaton drainage basin. We used perennial ryegrass (*Lolium perenne*) and ribwort plantain (*Plantago lanceolata*) as snare plants in sterile soil. We augmented the cultures further under semisterile laboratory conditions, using alfalfa (*Medicago sativa*) and red clover (*Trifolium pratense*). The large scale augmentation of the cultures was performed in the greenhouse with the help of Sudanese grass (*Sorghum sudanense*). Those inoculums which were gained in such a way, which were comprised of the roots of the host plant, the soil, and those mycorrhizal particles present in both, were used for field mycorrhization, where the augmented strains, or their mixtures, so called coctail inoculums were used. The growth of AMF-colonization and the physiological parameters (CO_2-fixation, chlorophyll content, stoma conductance) of crops (winter wheat, corn, alfalfa) used in the field experiments were examined continuously.

Mycorrhizal colonization was quantified by the methods of Phillips and Hayman (1970) and Trouvelot et al. (1986). Dry matters were determined after drying at 80°C until constant weight. Total percentage plant phosphorus, nitrogen and kalium contents (% of d. wt) were measured by an ICP spectrometer (Thermo Jarrell Ash ICAP 61E) after digest according to Buzás (1988). Chlorophyll a+b concentration (Porra et al. 1989) and chlorophyll fluorescence kinetics (Fv/Fm) by a PAM (Pulse Amplitude Modulation) Chlorophyll Fluorometer (Heinz Walz GmbH, Effeltrich) were measured (Schreiber 1986). $^{14}CO2$ fixation capacities of excised leaves were determined using a closed illuminated glass chamber with saturating light intensity (1000 μmol m^{-2} s^{-1}) and with

*Corresponding author. E-mail: bratek@ludens.elte.hu

0.1 % CO_2 concentration (Láng et al. 1985). Radioactivity of the leaf samples was determined by a liquid scintillation apparatus (Beckman LS 5000 TD).

Results and Discussion

Lists of fruit body forming mycorrhizal fungi species living in domestic saliferous and sandy forests as well as the younger forests of mining sites was drawn up, and the typization and identification (where possible) of the uncovered, cleaned and washed ectomycorrhizal roots was performed (Halász et al. 2000; Rudnóy et al. 2000; Barna et al. 2001; Berecz 2001). The isolation of several hundred ectomycorrhizal fungal strains succeeded in the areas mentioned, and the identification of the strains was reinforced by molecular methods (Rudnóy et al. 1999-2000; Kovács et al. 2001), and their maintenance was solved by compiling a collection. Methods for the economical large-scale production of mycelium-based inoculum as well as for their mycorrhizal inoculation procedures were succesfully developed. The mycorrhizal synthesis resulted in many cases of heavy mycorrhizal colonization without the use of mycorrhization helping bacteria (MHBs). The growth of mycorrhizal trees usually surpassed substantially that of non-mycorrhizal ones followed by increased values of CO_2-fixation. The high falling out rate was detected only in the case of non-mycorrhizal white poplars growing under greenhouse conditions. In order to decrease contamination, increase mycorrhizal colonization and select of plant clones with higher mycorrhizal colonization further studies are required.

Among AM-fungi strains isolated from plants used as snares, those ones were selected which had high indicator values for mycorrhization and growth of ploughland crops. During the small parcel field experiments, directed mycorrhizal inoculation provided extraordinary results in the amount of biomass and the growth somewhat in alfalfa but especially in corn. We took physiological characteristics as well as biomass values into consideration when comparing inocula. These values of the artificially mycorrhizated plants varied greatly compared to those experienced with control plants, but in most experimental versions surpassed the controll. Based on the results so far, in the case of certain host plant species mycorrhizal inoculation makes application of fertilizers unnecessary or greatly reducable, which – especially in the vicinity of surface water courses - could become an important factor in environment-friendly farming practise.

Acknowledgments

We wish to express our thanks to the Silvanus Group Ltd. and the Silvanus Nursery Inc. as well as to the NEFAG Inc. for the support they gave to our research.

References

Barna T, Jakucs E, Bratek Z, Szántó M (2001) Az erdősítések eredményességét fokozó közös mikorrhiza kutatások az ESZTK-ERTI-ELTE részvételével. In Erdészeti Tudományos Intézet Kiadványai, Gondolatok az erdővédelemről az ezredfordulón. pp. 169-178.

Berecz B (2001) „Bauxit meddők és bányagödrök mikorrhizagombái". Thesis, ELTE.

Bokor R (1954) A mykorrhiza gombákkal történő talajoltások új agrokémiai eljárása. Erd Kut Bp 4:27-45.

Halász K, Bratek Z, Jakucs E, Albert L, Csiha I. Ökológiailag stabil szikfásítás. In Kutatói Nap 1998-1999. eds., Horváth B, Alföldi Erdőkért Egyesület, pp. 91-93.

Halász Krisztián (2000) Szikes talajú tölgyesek ektomikorrhiza-gombáinak táptalaj-szelekciója és ITS-régiójuk szekvenciaanalízise. Thesis, ELTE

Kovács G, Rudnóy Sz, Vágvölgyi Cs, Lásztity D, Rácz I, Bratek Z (2001) Intraspecfic invariability of the ITS region of rDNA of Terfezia terfezioides in Europe. Folia Microbiol 46:423-426

Láng F, Sárvári É, Szigeti Z.(1985) Apparatus and method for rapid determination of photosynthetic CO2 fixation of leaves. Biochem Physiol Pflanzen 180:333-336.

Molina R, JG Palmer (1982) Isolation, Maintenance, and Pure Culture Manipulation of Ectomycorrhizal Fungi. In: Schenk, N. C., ed., Methods and Principles of Mycorrhizal Research. St Paul, MN: Amer Phytopat Soc 115-129.

Phillips JM, Hayman DS. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. – Trans. Brit. Mycol. Soc. 55: 158-160, 1970.

Porra RJ, Thompson WA, Kriedemann PE. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim Biophys Acta 975:384-394, 1989.

Rudnóy Sz, Bratek Z, Albert L, Barna T, Lásztity D (2000) Kiskunsági fehérnyárasok mikorrhiza viszonyainak feltárása. In Kutatói Nap 1998-1999. Szerk.: Horváth B, Alföldi Erdőkért Egyesület, pp. 94-97.

Rudnóy Sz, Bratek Z, Halász K, Rácz I, Lásztity D (1999-2000) A fehér nyárral (Populus alba L.) ektomikorrhizás kapcsolatban álló két gombafaj táptalaj szelekciója és molekuláris biológiai vizsgálata. Bot Közl 86-87:121-128

Rudnóy Sz (1999) Ektomikorrhizás gombatörzsek táptalaj-optimalizálása és molekuláris biológiai vizsgálata. Thesis, ELTE.

Schreiber U. Detection of rapid induction kinetics with a new type of high frequency chlorophyll fluorometer. Photosynth Res 9:261-272, 1986.

Szántó M (1994a) Mikorrhizált erdei- és feketefenyő (Pinus sylvestris L., Pinus nigra Arn.) csemeték összehasonlító vizsgálata 1. Növekedésvizsgálat, a magasság alakulása, hossznövekedés. Mikol Közl 34:64-74.

Szántó M (1994b) Mikorrhizált erdei- és feketefenyő (Pinus sylvestris L., Pinus nigra Arn.) csemeték összehasonlító vizsgálata 2. A növények tömegviszonyai. Mikol Közl 34:42-47.

Trouvelot A, Kough JL, Gianinazzi-Pearson V. Mesure du taux de mycorhization VA d'un systéme radiculare. Recherche de méthodes d'estimation ayant une signification fonctionelle. In: Mycorrhizae: physiology and genetics-Les mycorhizes: physiologie et génétique. - Proceedings of the 1st ESM/1er SEM, Dijon, 1-5 July 1985-INRA, Paris: 217-221,1986.