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# Effect of *Rhizobium* and AM fungi inoculation on soybean

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Symbiotic effectivity of two- and tripartite symbiotic agents was investigated in a pot experiment on three soybean (*Glycine max* L. Merr.) cultivars with special regard to compatibility. Host plants were single and co-inoculated with two *Bradyrhizobium japonicum* strains and two commercial arbuscular mycorrhizal fungal (AMF) products. Significant differences were found in infectivity and effectivity of the microsymbionts. While infectivity of AMF inocula was very poor, the benefits of AMF treatments were more expressed than that of the rhizobial ones. In case of commercial AMF inoculants we should also check the effect of the ingredients of the explain medium.

Keywords: Bradyrhizobium sp., arbuscular mycorrhizal fungi, soybean, host compatibility, biomass

#### 1 Introduction

Soybean (*Glycine max* L. Merr.) is a protein plant and oilseed crop which is important in the food industry, or as a forage. Nitrogen fixing bacterial and arbuscular mycorrhizal fungal (AMF) symbionts are undoubtedly essential in soybean host nutrition and alleviation of plant stress caused by adverse soil conditions (Gosling et al., 2006). Soils where soybean is a non-native plant usually lack *Bradyrhizobium* strains, like in European soils (Albareda et al., 2009), thus in conventional agricultural practices and especially in organic farming the role of microbial inoculation of soybean is essential to provide adequate nitrogen supply and promote maximum yield. In the present study, the effect of inoculation, using single or two symbiotic partners, was investigated on three soybean genotypes in their early developmental stages.

### 2 Material and Methods

#### 2.1 Plants and microbial materials

In a pot experiment three soybean (*Glycine max* L. Merr.) cultivars registered in Hungary -Emese (S<sub>1</sub>), Alíz (S<sub>2</sub>) and Sponsor (S<sub>3</sub>) – were used to investigate the compatibility of soybean with microbial inoculants. A combination of two factors was applied as microbial treatments: (1) AMF inoculation with a commercial product [F<sub>1</sub>: AEGIS Sym Microgranule or F<sub>2</sub>: SYMBIVIT]; and (2) rhizobial inoculation with a commercialy available peat based soybean inoculum [R<sub>1</sub>] or a Bradyrhizobium japonicum strain [R<sub>2</sub>]. Control [ØØ] plants were not inoculated.

### 2.2 Experimental design and growth conditiions

Soybeans (1 seed pot<sup>-1</sup>) were planted in pumice (1.25 dm<sup>3</sup> plastic pots containing 1.15 kg;  $pH_{H2O}$  of 6.5, 1.02 kg dm<sup>-3</sup> bulk density) and were treated with single [S<sub>1-3</sub>F<sub>1</sub>Ø; S<sub>1-3</sub>F<sub>2</sub>Ø, S<sub>1-3</sub>ØR1; S<sub>1-3</sub>ØR2] or co-inoculation [S<sub>1-3</sub>F<sub>1</sub>R<sub>1</sub>; S<sub>1-3</sub>F<sub>1</sub>R<sub>2</sub>; S<sub>1-3</sub>F<sub>2</sub>R<sub>1</sub>; S<sub>1-3</sub>F<sub>2</sub>R<sub>2</sub>] with AMF or rhizobial inoculum. Treated and non-treated plants [S<sub>1-3</sub>ØØ] were cultivated for two months in a growth

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chamber kept at 24/18 °C and 16/8 h day/night temperature and photoperiod respectively, with a photon flux density of 300  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> and relative humidity of 50–80 %. Optimal plant nutritional status was kept up by weekly irrigation with modified Hoagland's solution (100 mL pot<sup>-1</sup>; with 0.5 M KH<sub>2</sub>PO<sub>4</sub>).

## 2.3 Investigations

Biomass production (shoot and root dry weights), leaf area (LA), chlorophyll fluorescence (Fv/Fm) (Solti et al., 2011) and electrical capacitance (EC) as indicators of root system activity were measured in early developmental stages (Cseresnyés et al. 2013; Takács et al. 2014). Root colonization of AMF (Trouvelot et al. 1986), nodulating parameters and acetylene-reduction (Hardy et al. 1968) were tested to estimate the functionality of symbiotic partners.

## 3 Results

After single inoculation with products containing AMF -  $F_1$  (AEGIS Sym Microgranule) and  $F_2$  (SYMBIVIT) - the increase in shoot dry weight (Figure 1A-B) and root dry weights, leaf area and electrical capacitance (EC) was higher than that of the following rhizobial treatments (data not shown). The previous parameters except EC, slightly differs among soybean cultivars. All *Bradyrhizobium* inoculation resulted intensive nodule formation in the root system. No significant differences were found among inoculation treatments or soybean cultivars. The acetylene reduction test resulted a significant difference in the efficiency of the two rhizobium inocula (nitrogen fixing ability  $R_1$ : 596-684nmol  $C_2H_4$  h<sup>-1</sup> pot<sup>-1</sup>,  $R_2$ : 96-259 nmol  $C_2H_4$  h<sup>-1</sup> pot<sup>-1</sup>).



Figure 1 Shoot dry weights of soybean plants (A-B) and photosynthetic activity (Fv/Fm values at 735 nm) of soybean leaves (C-D). A and C: Mean values of the inoculation

treatments (C-control plants;  $R_1$  and  $R_2$  rhizobia;  $F_1$  and  $F_2$  AM fungal products), **B** and **D**: Mean values of soybean cultivars Least significant differences (LSD at P < 0.05) are shown as a result of the two-way ANOVA

Viticultural measurements show a tendency of decreasing yield in case of inter-rows with cover crops, but the difference is significant only at one site. This reduction was measured also in case of the pruning weight. The indices of the must quality were not significantly affected by the applied cover crop. There were no differences in the diameter of the internodes among treatments.

## 4 Conclusions

Successful interaction between host and microsymbionts was indicated by extra biomass production, number of nodules and richness of AMF root structures. Significant benefits of symbiosis were more obvious in case of plants single or co-inoculated with AMF than in case of rhizobium inoculated ones. The higher number of species in Symbivit ( $F_2$ ) may increase the chance to higher infectivity and effectivity. Symbivit can also be more successful in developing a compatible partnership. We suppose that extra biomass production caused by AEGIS Sym Microgranule ( $F_1$ ) can be a result of the high organic material content of the biofertilizer.

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