



Symbiotic Effectivity of Dual and Tripartite Associations on Soybean (*Glycine max* L. Merr.) Cultivars Inoculated With *Bradyrhizobium japonicum* and AM Fungi

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OPEN ACCESS

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Specialty section:

This article was submitted to
Plant Abiotic Stress,
a section of the journal
Frontiers in Plant Science

Received: 13 August 2018

Accepted: 19 October 2018

Published: 13 November 2018

Citation:

Takács T, Cseresnyés I, Kovács R,
Parádi I, Kelemen B, Szili-Kovács T
and Fűzy A (2018) Symbiotic
Effectivity of Dual and Tripartite
Associations on Soybean (*Glycine
max* L. Merr.) Cultivars Inoculated
With *Bradyrhizobium japonicum*
and AM Fungi.
Front. Plant Sci. 9:1631.
doi: 10.3389/fpls.2018.01631

Soybean (*Glycine max* L. Merr.) is regarded worldwide as indisputably one of the most important crops for human food and animal feed. The presence of symbiotic bacteria and fungi is essential for soybean breeding, especially in low-input agricultural systems. Research on the cooperation between different microbial symbionts is a key to understanding how the health and productivity of the plant is supported. The symbiotic effectivity of dual and tripartite symbiotic agents was investigated in two pot experiments on different soybean cultivars with special regard to compatibility. In the Selection experiment, two out of sixteen soybean cultivars (Aliz, Emese) were chosen on the basis of their drought tolerance and used in all the other investigations. In the Compatibility experiment, the compatible coupling of symbiotic partners was selected based on the efficiency of single and co-inoculation with two *Bradyrhizobium japonicum* strains and two commercial arbuscular mycorrhizal fungal (AMF) products. Significant differences were found in the infectivity and effectivity of the microsymbionts. The rhizobial and AMF inoculation generally improved plant production, photosynthetic efficiency and root activity, but this effect depended on the type of symbiotic association. Despite the low infectivity of AMF, inocula containing fungi were more beneficial than those containing only rhizobia. In the Drought Stress (DS) experiment, co-inoculated and control plants were grown in chernozem soil originating from organic farms. Emese was more resistant to drought stress than Aliz and produced a bigger root system. Under DS, the growth parameters of both microbially inoculated cultivars were better than that of control, proving that even drought tolerant genotypes can strengthen their endurance due to inoculation with AMF and nitrogen fixing bacteria. Root electrical capacitance (C_R) showed a highly significant linear correlation with root and shoot dry mass and leaf area. The same root biomass was associated with higher C_R in inoculated hosts. As C_R method detects the absorptive surface increasing due to inoculation, it may be used to check the efficiency of the microbial treatment.

Keywords: arbuscular mycorrhizal fungi, *Bradyrhizobium japonicum*, drought stress, functional diversity, root electrical capacitance, soybean, symbiotic compatibility

INTRODUCTION

Soybean (*Glycine max* L. Merr.) is one of the most widely cultivated crops throughout the world under various climates. Future scenarios show that soybean production will expand by 30% for the coming decade (OECD-FAO, 2018), so optimizing the cultivation of this crop has important economic and political implications.

Achieving this purpose, however, is complicated by the extreme weather conditions caused by global climate change. Crop growth and yield are impaired by both abiotic and biotic stress conditions, of which drought has been identified as the most important factor in limiting the productivity of grain legumes (Farooq et al., 2016). Drought sensitivity coupled with high nutrient demand may seriously restrict soybean yields due to weak development and poor competitiveness against weeds. Therefore, the development of resistant cultivars and site-specific selection from the available genetic resources should be major objectives in any breeding program. The significance of nitrogen-fixing bacteria and arbuscular mycorrhizal fungal (AMF) symbionts is indubitable not only in soybean host nutrition, but also in the alleviation of plant stress caused by adverse soil conditions (Ku et al., 2013).

Nitrogen (N) and phosphorus (P) are critical limiting elements for crop growth (Miller and Cramer, 2004; Menge et al., 2012). Legumes require nitrogen-fixing rhizobial symbiotic partners, mostly *Bradyrhizobium japonicum* bacteria, to achieve their maximum yield potential. Soils in areas where soybean is not native usually lack these bacteria, e.g., in Europe (Albareda et al., 2009), so the microbial inoculation of soybean is essential to provide adequate nitrogen supplies and maximum yields both in conventional agricultural practices and more especially in organic farming.

Leguminous plants are highly dependent also on mycorrhizal fungi (Muleta, 2010), symbionts that can resolve the problem of phosphorus limitation. AM fungi live in a mutualistic symbiosis with 80–90% of terrestrial plants and form the most ancient and prevalent type of mycorrhizae (Brundrett, 2009). The improved productivity of AM plants and the physiological and biochemical changes caused by AM can result in greater stress resistance in the host (Cameron et al., 2013; Rapparini and Peñuelas, 2014; Domokos et al., 2018). The extraradical hyphal network of AMF provides more effective water and nutrient (especially plant-unavailable phosphorus) uptake for the host plants (Marschner, 1997; Hodge and Storer, 2015; Wahbi et al., 2016; Wang et al., 2016; Balog et al., 2017). Furthermore, the host plants benefit from the special composition of the microbial community in the mycorrhizosphere (Barea, 1997; Artusson et al., 2006).

Recent studies confirm that there is a common genetic basis for plant root endosymbioses with both rhizobia and AM fungi

(Denison and Kiers, 2011; Ossler et al., 2015). AMF colonization has an influence on the development and function of rhizobial nodules and vice versa, but the multiple mutualistic effect on the host participating in the rhizobia–AM fungi–legume interaction is very fertilizer-dependent. Numerous publications reported that the co-inoculation of legumes with AMF and rhizobial strains resulted in greater benefits for the plants and symbionts alike, due to a synergistic effect (Bethlenfalvay et al., 1987; Biró et al., 2000; Meghvansi et al., 2008; Wang et al., 2011). Neutral or negative responses to co-inoculation indicated that the advantage of tripartite symbiotic associations depended greatly on the compatibility and susceptibility of the partners (Lisette et al., 2003; Wang et al., 2011). As a principal effect, AM fungi improve nodulation in the legume host by enhancing phosphorus uptake (Ossler et al., 2015). In addition, AMF root colonization leads to changes in the nitrogen transfer, microelement uptake and phytohormone production of the plants, which play an important role in nodulation and nitrogen fixation (Behie and Bidochka, 2014). The multi-factorial evaluation of tripartite functional diversity could provide a theoretical basis for optimizing the application of selected biofertilizers for soybean production.

The varying efficiency of biological nitrogen fixation and fungal colonization both show that the compatibility of the mutualistic partners depends on their genotype. Due to intra- and interspecific variability, several possible partners are in the race for the formation of symbiosis, but the actual outcome of colonization is also influenced by the environmental conditions (Johnson et al., 1997; Kiers et al., 2002; Herrera-Peraza et al., 2011). In general, associations of rhizobia and host plants have narrow specificity during nodule development (Fauvert and Michiels, 2008). The association between the bacteria and the soybean may be host-specific, to such a degree that some rhizobial species nodulate plants only in a certain genus (Neves and Rumjanek, 1997; Appunuu et al., 2008). The variability of bradyrhizobial effectiveness on different soybean varieties was reported by Okereke et al. (2001). In contrast, the AMF-host relationship is not strictly specific; the high intra- and interspecific variability of AM fungi creates great functional diversity (Jakobsen et al., 1992; Munkvold et al., 2004; Cavagnaro et al., 2005).

The great functional diversity and non-host specific association of AM fungi give a chance to produce biofertilizers that can establish fungi–host combinations under diverse environmental conditions. Despite the increasing attention paid to AMF as an advantageous symbiotic partner, a number of difficulties have so far prevented the large-scale application of AMF inoculation (Cely et al., 2016). In low-input organic systems, compatible plant–fungus–rhizobium associations may play a more prominent and critical role in the optimal nutrition of the host plants than in conventional agricultural systems (Gosling et al., 2006; Bhardwaj et al., 2014; Schneider et al., 2015). In organic farming, choosing a suitable cultivar is essential to avoid the damaging effects of environmental stress. The metabolism of drought-tolerant and non-tolerant cultivars has been compared (Purcell et al., 2000; Silvente et al., 2012), but few data are available on cultivars with similar tolerance and on the role of symbionts in endurance.

Abbreviations: AMF, arbuscular mycorrhizal fungi; ARA, acetylene reduction assay; C_R , root capacitance; DSS, drought stress symptoms; F_v/F_m , maximum quantum efficiency of photosystem II photochemistry; GSI, germination stress index; LA, leaf area; NN, node number; RDW, root dry weight; RMID, relative microbial inoculation dependence; RSR, root shoot ratio; RWC, leaf relative water content; SDW, shoot dry weight; SH, shoot height.

Well known, that there are differences in the drought tolerance of the registered soybean cultivars (Bousslama and Schapaugh, 1984; Cseresnyés et al., 2016). It is assumed, that even drought tolerant soybeans benefit from inoculation, but because of the various compatibility cultivars show different responses to microbial treatments. For the rational use of commercial inocula, their efficiency should be checked before application in local soils under different environmental conditions. It is also assumed that AMF will add to drought tolerance more than rhizobium considering the active surfaces. The main objective of the experiments was to show the significance of the compatibility of symbiotic partners in a soybean tripartite inoculation and provide the theoretical basis for the application of commercial products in the soybean field production. To assess the effect of symbioses, it is practical to measure multiple growth, physiological and morphological parameters related to all the partners. In the present study, commercial products containing *Bradyrhizobium japonicum* and AM fungal inocula were tested for symbiotic efficiency on soybean in different growth media. The host susceptibility to inoculation and the impact of the soil biotic context (belowground interactions) were investigated to reveal their effect on the compatibility of the symbiotic partners. The functionality of the symbiotic partners was also checked in relation to genotypic differences in the soybean cultivars under drought stress.

MATERIALS AND METHODS

The Sequence of the Experiments

In the Selection experiment 16 soybean (*Glycine max* L. Merr.) genotypes of diverse origin (Table 1) were evaluated for drought tolerance by means of polyethylene glycol (PEG) – induced drought stress. The Compatibility pot experiment was then performed to test two bradyrhizobia and two AM fungi (alone

and in pairs) by inoculating two selected soybean cultivars grown in pumice to identify the most effective combinations of symbiotic partners. Finally, the Drought Stress pot experiment was performed to study symbiotic efficiency under optimal water supplies and water deficit in soil from organic farms. The test plants, methods of plant cultivation and tests for symbiotic effectiveness were the same in both pot experiments.

Selection Experiment

The seeds were selected for size homogeneity, surface-sterilized for 0.5 min in 70% (v/v) ethyl alcohol, rinsed and soaked in sterile distilled water. Seed germination was studied in distilled water (control) and in a 25 w/w% solution of polyethylene glycol (PEG, Karbowax 6000 Fluka AG) generating an osmotic potential of -0.82 MPa (Michel and Kaufmann, 1973). For each genotype, four replicates ($n = 4$) of 25 seeds were placed in Petri dish (\varnothing 9 cm) on a filter paper that covered a piece of cotton wool (4 g). The dishes were filled with 40 cm³ of distilled water or PEG solution. The seeds were incubated in a dark, temperature-controlled chamber at $25 \pm 1^\circ\text{C}$. Seeds were considered germinated when the radicle had reached at least 2 mm in length. The number of germinated seeds was counted daily. After 6 days, the GSI₆ was calculated according to Bousslama and Schapaugh (1984). In the Compatibility and Drought Stress experiments the two cultivars with the highest GSI₆ (Aliz and Emese, indeterminate growth habit, maturity group 0) were used as host for the microsymbionts.

Compatibility Experiment

The Aliz and Emese cultivars were investigated for their compatibility with AM fungi and rhizobial inoculants. Biomass production (shoot and root dry weight; SDW and RDW), LA, photochemical efficiency (F_v/F_m) and root electrical capacitance (C_R) as an indicator of root system activity, were measured in

TABLE 1 | Characteristics of soybean cultivars tested in the germination experiment.

| Cultivar (Year of state registration) | Maturity group | 1000-grain weight ^a (g) | GSI ₆ ^b (%) |
|--|----------------|------------------------------------|-----------------------------------|
| BAGERA (2007, Switzerland) | [00] | 148.7 (7.0) | 29.4 (11.4) |
| ES MENTOR (2011, France) | [00] | 189.0 (3.5) | 13.3 (11.2) |
| ALIZ (2007, Hungary) | [0] | 182.2 (5.2) | 60.3 (4.5) |
| JOHANNA (2011, Hungary) | [0] | 208.3 (6.2) | 19.2 (11.6) |
| MARTINA (2006, Hungary) | [0] | 202.3 (5.2) | 23.8 (10.2) |
| SPLENDOR (2006, EU) | [0] | 180.0 (2.7) | 0.0 |
| ES GLADIÁTOR (2014, France) | [0-I] | 180.9 (3.0) | 10.77 (6.11) |
| EMESE (2006, Hungary) | [I] | 176.4 (3.3) | 54.7 (10.8) |
| ISIDOR (2005, EU) | [I] | 219.0 (5.6) | 17.9 (15.9) |
| PANNÓNIA KINCSE (2008, Hungary) | [I] | 130.1 (3.3) | 1.0 (2.0) |
| SPONSOR (2005, France) | [I] | 156.7 (3.2) | 43.9 (10.7) |
| ZELMA (2008, Hungary) | [I] | 168.7 (7.7) | 29.5 (9.6) |
| GROWPRO (United States) | [III] | 223.1 (3.3) | 11.8 (7.9) |
| HIPRO 15/MN 1505 (2010, United States) | [III] | 198.1 (4.6) | 17.1 (8.0) |
| ROYALPRO (2006, United States) | [III] | 247.5 (4.1) | 11.0 (1.0) |
| PRESTOPRO (United States) | [III] | 117.7 (1.5) | 27.4 (12.0) |

^aBased upon weighing 5×100 seeds; standard deviations are given in brackets. ^bGSI₆, Germination stress index after 6 days of germination.

the early developmental stages. Root colonization with AMF, nodulating parameters and acetylene reduction were tested to estimate the functionality of the symbiotic partners.

AMF inoculation with a commercial product (either F₁ or F₂) and rhizobial inoculation with a commercially available soybean inoculum (R₁) or a *Bradyrhizobium japonicum* strain (R₂) were applied as microbial treatments. Control (C) plants were not inoculated.

F₁ is a 1:1 mixed inoculum of *Rhizophagus intraradices* (syn. *Glomus intraradices*) and *Funneliformis mosseae* (syn. *Glomus mosseae*) with 50 spores g⁻¹. It is characterized by high organic matter content (20%), pH of 6.5–7.0, ammonium lactate (AL)-K₂O 8364 mg kg⁻¹, AL-P₂O₅ 4987 mg kg⁻¹ and 10⁷ colony-forming units of rhizosphere bacteria (CFU g⁻¹) in microgranular form (1–2 mm). F₂ is an infective propagule mixture of six AMF strains, characterized by pH of 6.5–7.0, AL-K₂O 6550 mg kg⁻¹ and AL-P₂O₅ 1402 mg kg⁻¹. It is produced on natural clay carriers and naturally degradable granules of a water-retaining gel. 10 g pot⁻¹ AMF inoculum was layered uniformly at a depth of 5 cm below the seeds in the mycorrhizal treatments.

The R₁ microbial treatment was carried out with a peat-based soybean inoculum of *Bradyrhizobium japonicum* containing 10⁶ CFU g⁻¹. A liquid culture of a *B. japonicum* strain from the strain collection of the Research Institute for Soil Sciences and Agricultural Chemistry was used for the R₂ treatment. R₂ *B. japonicum* strain was incubated on yeast extract mannitol agar (YMA) for 5 days (Vincent, 1970). After incubation, one loopful of the culture was suspended in 10 mL of sterile tap water. 100 mL of YMB (Yeast Extract Broth) culture medium was inoculated with this bacterial suspension and incubated for 5 days under continuous shaking at 28°C. In the case of rhizobial inoculation, the seedbed was inoculated with 1 mL suspension of R₁ (2 g 200 mL⁻¹ sterile tap water) or 1 mL liquid culture of R₂. Each planting combination was represented by four replicates (*n* = 4).

Drought Stress Experiment

The Aliz and Emese cultivars were tested for symbiotic effectiveness in chernozem soil treated with the previously selected microbial treatments. Besides the control, both cultivars were exposed to two treatments, co-inoculation with R₁ *B. japonicum* chosen in the Compatibility experiment and F₁ or F₂ AMF inoculum. Plants growing with different water supplies were investigated in five-five replications (*n* = 5).

Experimental Design and Growth Conditions

In the Compatibility experiment, the soybean seeds were planted in 72 1.25 dm³ plastic pots containing 1.25 kg of soil-analog ground pumice (porous vitroclastic perlite) medium with 0.7–1.1 mm particle size and pH_{H2O} 6.5. Two seeds were planted in each pot and thinned to one after emergence. The pumice, which lacks indigenous rhizobia and infective propagules of AM fungi, was treated with AMF or rhizobial inoculums, alone or in combination, except for the controls. The plants were cultivated in a random arrangement in a growth chamber

for 65 days with day/night temperature and photoperiod of 26/18°C and 16/8 h respectively, at a photon flux density of 600 μmol m⁻² s⁻¹ and relative humidity of 50–70%. Optimal plant nutritional status was maintained by weekly irrigation with 100 ml of modified Hoagland's solution (0.5 M KH₂PO₄) per pot.

The Drought Stress experiment was designed to check the results of the Compatibility experiment in organically managed haplic chernozem soil (IUSS Working Group WRB, 2015) that contains an indigenous AMF community. Pre-germinated soybean seeds were transplanted into 3.5 dm³ plastic pots filled with 3.9 kg of soil in five replicates (*n* = 5). The experimental soil, collected in an organic farming system in Martonvásár, Hungary (N47°18'41'', E18°46'48'', 109 m asl.), has a clay loam texture (USDA, 32.8% sand, 42.2% silt, 25.0% clay) with pH_{H2O} 7.69, pH_{KCl} 7.14, ammonium lactate acetate (AL) extractable P₂O₅ 371 mg kg⁻¹, AL-K₂O 402 mg kg⁻¹, NH₄⁺-N 4.06 mg kg⁻¹, NO₃⁻-N 17.47 mg kg⁻¹, humus 2.93%, and 0.332 and 0.156 cm³ cm⁻³ water content at field capacity and permanent wilting point, respectively.

The cultivars were grown for 65 days with the chosen highly effective combinations of microbial partners (F₁R₁; F₂R₁) under climatic and light conditions identical to those in the Selection experiment. Half of the plants were watered adequately (200 ml per pot three times a week) while the others were put through two drought cycles during 23–35 and 40–60 days after planting (DAP). At the beginning of the drought cycles, water was withheld till the soil moisture decreased near to wilting point (requiring 3–6 days, depending on plant size), after which this water status was maintained by daily irrigation during the rest of the drought cycle.

Pre-harvest Investigations

The effects of non-lethal water deficit during the growth of soybean cultivars prior to the reproductive phase were assessed by recording the number of nodes (NN) on the main stem, measuring the stem height (SH) and the relative water content (RWC) of the leaves, and by examining DSS on the leaves (symptoms of wilting and leaf loss/shedding on a 0–3 scale). Sampling was performed every 7th day from the 14th day after planting (DAP) until the beginning of the reproductive growth stage (R₁). Data representing the functional aspects of the AMF-rhizobia-soybean symbiotic systems were obtained *in situ* by measuring chlorophyll fluorescence induction and root electrical capacitance (C_R).

Chlorophyll Fluorescence Induction

A FMM Chlorophyll-A fluorometer (Barócsi et al., 2009) was used to measure the fluorescence induction parameters *in situ*. The internal light source was a 635 nm laser diode (QL63H5SA, Roithner Lasertechnik GmbH, Wien, Austria) with 20 mW maximum optical power. Traditional Kautsky induction kinetic curves were detected simultaneously at 690 nm (red) and 735 nm (far-red) wavelengths, where Chl-A fluorescence shows two maxima in leaves. Minimal (F₀) and maximal fluorescence (F_m) values were detected, after which the light-adapted, steady-state quantum efficiency of photosynthetic electron transport, F_v/F_m = (F_m - F₀)/F_m, was calculated for both emission

maxima. The F_v/F_m values showed a similar tendency at both wavelengths, so only the results for 690 nm are shown. The F_v/F_m data were measured on all the plants on 63 DAP.

Root Electrical Capacitance (C_R) Measurement

The extension and activity of the root system (rhizosphere) was assessed simply *in situ* by C_R measurement (Chloupek, 1972; Cseresnyés et al., 2016). When an alternating current passes through the root tissue, charge accumulation, i.e., polarization, occurs. The amount of electric charge stored by the root system can be expressed as electrical capacitance (in nanofarads) which is proportional to the active root surface area. The method is only valid for the comparison of plants of the same species grown in the same substrate, at the same moisture level (Chloupek et al., 2010). C_R measurements were carried out on all the plants on DAP 64 (before harvest and after the second drought stress period in the Drought Stress experiment) using a GW-8101G LCR instrument (GW Instek Co., Ltd., Taiwan) at 1 kHz frequency with 1 V terminal voltage. One terminal of the instrument was connected to the plant stem with a spring tension clamp fixed 10 mm above the substrate level, while the second was grounded by a stainless steel rod (6 mm ID, 15 cm long) inserted into the substrate. Electrocardiograph paste (Vascotasin®; Spark Promotions Co., Ltd., Budapest, Hungary) was smeared around the stem to maintain electrical contact, and the substrate was irrigated to field capacity before measurement.

Leaf Relative Water Content (RWC)

In the Drought Stress experiment, the relative water content (RWC) of the leaves was measured during drought exposure on DAP 22 and DAP 63 (González and González-Vilar, 2007).

Post-harvest Measurements

Both the inoculated and control soybeans were harvested and sampled for microbial investigations after 65 days of cultivation (R1, R2 flowering stage and R3 green pod formation) (Ohyama et al., 2013). LA was measured using image processing. In both experiments, SDW and RDW were determined after drying the samples at 80°C for 48 h. The susceptibility to symbiotic associations and the dependence of soybean cultivars on microbial treatments were characterized by the index of RMID. RMID is an extension of the relative field mycorrhizal dependence (RFMD) index (Plenchette et al., 1983), taking into consideration all symbionts rather than just AMF, and is defined as $RMID = 100 * [(dry\ weight\ of\ inoculated\ plant) - (dry\ weight\ of\ non-inoculated\ plant)] / dry\ weight\ of\ inoculated\ plant$.

Quantification of AMF Root Colonization, Nodulation and Functionality of Rhizobia

After harvest, the soybean response to rhizobial inoculation was characterized by a number representing the density of nodules found on the primary roots and lateral root zones.

The nitrogenase enzyme activity of the nodules was measured by means of ARA (Hardy et al., 1968). After removing them from the pots, the 65-day-old plants were carefully washed, then the whole, nodulated roots were placed in a 500 ml glass bottle capped with a rubber septum. After adding 50 cm³ of

acetylene gas (C₂H₂, >99.95% purity, Lindegas) to the bottle, the roots were incubated for 30 min at room temperature (22°C). A gas sample (500 µl) was removed using a gas-tight syringe and analyzed on a gas chromatograph (GC 8000, Fisons Instruments) with the following specifications: flame ionization detector (FID) and Porapak T column to separate ethylene from acetylene, carrier gas N₂ = 175 kPa, hydrogen at 50 kPa, air at 80 kPa, injector temperature 100°C, oven temperature 80°C, isotherm, detector temperature 150°C. The area under the peaks was evaluated with Chrom-Card software and a standard of 10 ppm ethylene (C₂H₄) in N₂ (Scotty 14, Supelco) was used for calibration.

After ARA, the roots were randomly sampled for AMF root colonization measurement performed after staining the separated root (≤1 mm indiameter) sub-samples with lactic acid-aniline blue according to Phillips and Hayman (1970). AMF colonization was estimated using a BX51 microscope (40-200X; Olympus Corp., Tokyo, Japan). The frequency (F%), the intensity of colonization (M %) and the arbusculum richness (A%) in the roots were calculated using a five-class system (Trouvelot et al., 1986) after observing 30 fine root segments, each 1 cm in length.

Concentration of Nitrogen (N), Phosphorus (P) and Potassium (K) in Leaves

The N (%), P and K (mg kg⁻¹) concentrations of the plants were measured after harvest. The P and K macroelement concentrations of the plants were assessed after wet digestion of air-dried plant samples with cc. HNO₃ + cc. H₂O₂. The nitrogen content in the leaves was determined by the Kjeldahl method (Kjeldahl, 1883) after digesting the samples in sulfuric acid (cc. H₂SO₄). The leaf element contents were measured with an ICP-AES instrument (Jobin-Yvon, ULTIMA2).

Statistical Analysis

Results were analyzed using two-way ANOVA or Kruskal–Wallis non-parametric test if the prerequisites of ANOVA did not fulfill. Bartlett test of homogeneity of variance and Shapiro–Wilk normality test with model residuals were carried out before ANOVA. *Post hoc* tests were also carried out: LSD (least significant differences) values were calculated after ANOVA analysis, while a pairwise *t*-test with the Holm method was applied after the Kruskal–Wallis test. The comparisons between means were performed at the significance level of $p < 0.05$. The relationship between C_R and RDW or LA were evaluated using simple regression analysis ($p < 0.05$ for each treatment).

RESULTS

Selection Experiment

The negative osmotic potential significantly decreased seed germination (Table 1). The PEG solution retarded germ development, to the greatest extent for the cultivar Splendor. The GSI₆ values ranged from 0.0% (Splendor) to 60.3% (Aliz). Cultivars Aliz and Emese had the highest GSI₆ values, so these were assumed to have high drought stress tolerance in the early stages of plant development. There was no relationship between

the 1000-grain weight or maturity group of the cultivars and the GSI levels.

Compatibility Experiment

In the Compatibility experiment all the AMF and dual inoculations resulted in higher SDW and RDW (Figures 1A,C), stem length and number of nodes (data not shown), especially in the F_1 AMF treatments. The biomass production of AM plants was significantly higher (by 50–70%) than that of the controls or plants inoculated only with rhizobium. No significant differences in SDW and RDW were found between the soybean cultivars, though the SDW of Aliz was slightly higher than that of Emese. Aliz grew taller and were spindlier. The LA of the differentially inoculated soybean plants showed a tendency similar to their shoot biomass and dry weights (Figure 1B), except that Emese had higher LA than Aliz. No AMF structures were detected in the control or rhizobium inoculated plants (C and $R_{1,2}$). Microscopic examination of the harvested roots showed a low frequency (F%) of fungal colonization in mycorrhizal plants irrespective of the AMF products.

A comparison of F_1 and F_2 showed that the former caused a greater increase in host growth, while the latter resulted in a higher colonization rate [F_1 max(F% = 1.67); F_2 max(F% = 19.84)]. AMF root colonization decreased in plants co-inoculated with both rhizobial and AMF products. No significant differences in AMF infectivity were found for the different host plant cultivars.

Bradyrhizobium inoculation resulted in intensive nodule formation on both the main root and lateral roots in all cases (100–150 nodules plant⁻¹), but there were no significant differences between either the rhizobial treatments or the soybean cultivars. The ARA revealed that R_1 had 2–5 times higher nitrogen-fixing capability (R_1 : 685 nm C₂H₄ h⁻¹ pot⁻¹; F_1R_1 : 633 nm C₂H₄ h⁻¹ pot⁻¹; F_2R_1 : 623 nm C₂H₄ h⁻¹ pot⁻¹) than R_2 (R_2 : 252 nm C₂H₄ h⁻¹ pot⁻¹; F_1R_2 : 144 nm C₂H₄ h⁻¹ pot⁻¹; F_2R_2 : 199 nm C₂H₄ h⁻¹ pot⁻¹; LSD = 198, $P < 0.05$). There were no significant differences in the specific activity of the nitrogenase in the rhizobium-inoculated cultivars. The RMID of rhizobium-inoculated soybeans ($R_{1,2}$) was lower than that of AMF- or co-inoculated plants, and Emese showed higher dependence than Aliz (except in F_2R_2) in well-watered pumice (data not shown).

The N content of plants treated only with rhizobium (R_1 ; R_2 in the case of Aliz) was significantly higher than that of control (C) and AMF-inoculated (F_1 ; F_2 ; F_1R_1 ; F_1R_2 ; F_2R_1 ; F_2R_2) hosts (Table 2). The leaf N content of Aliz was significantly higher, than that of Emese in the F_1 , R_2 , F_1R_1 and F_2R_2 treatments. The P concentration was generally higher in the leaves of Emese, except for the F_1 treatment where a significant increment occurred in Aliz (Table 3).

Root electrical capacitance (C_R) was the highest in AMF-treated plants (Figure 1D) for both Emese and Aliz. Significant differences in C_R were found between the mycorrhizal and non-mycorrhizal (C, R_1 , R_2) plants. Rhizobium treatment caused a lower, but still significant increase in C_R .

The photochemical efficiency (F_v/F_m) differed only slightly among the microbial treatments. R_1 , F_1R_1 and F_1R_2 inoculation

resulted in significantly higher F_v/F_m ratios than the control (C: 0.827; R_1 : 0.839; $F_1R_{1,2}$: 0.840, 0.843 respectively; LSD_{5%} = 0.011). Photochemical efficiency also differed between the two varieties (Aliz: 0.836; Emese 0.827; LSD_{5%} = 0.006).

Drought Stress Experiment

Microbial inoculation caused a significant increment in SDW, LA, RDW (Figures 2A–C) and NN (except in F_1R_1) (Figure 2D) under both well-watered (WW) and drought stress (DS) conditions. The beneficial efficiency of the F_1 was mostly higher than that of F_2 (Figure 2). The microsymbionts induced a more than two-fold increase in LA (WW: 126–128%; DS: 113–115%) (Figure 2B). The LA of Emese was generally higher than that of Aliz. RDW was enhanced by 41–58% (WW) and 24–30% (DS) by co-inoculation (Figure 2C). Greater changes were observed in shoot than in root biomass production: inoculation increased SDW by 60–69% (WW) and 54% (DS).

The RSR values of microbially treated and control plants were statistically similar under WW conditions (Figure 2E).

Shoot dry weight decreased by 41% in the control and by 41–45% in the co-inoculated plants (Figure 2A). LA was 40% smaller in the control and 44% smaller in the co-inoculated plants (Figure 2B), while RDW was reduced by 22% in the control and by 31–36% in the co-inoculated plants (Figure 2C). NN also decreased significantly under drought stress (Figure 2D). SH was 21–24% lower after inoculation in the case of DS.

Combined inoculation with AM fungi and rhizobia was significantly more beneficial for Emese than for Aliz, as shown by differences in various plant growth parameters (LA: 10%, RSR: 15%) (Figures 2B,E). By contrast, the SDW of Aliz was higher than that of Emese. DS induced a greater decrease in SDW (35% Emese and 45% Aliz) than in RDW (18% Emese and 40% Aliz). No differences were found between the effects of F_1 and F_2 AMF inocula on plant growth. The drought-induced decline in LA ranged from 36% (Emese F_2R) to 50% (Aliz F_2R). Water deficit increased the RSR values in all the treatments and led to significant differences between the RSR values of inoculated and control plants, which were absent under WW conditions. Cultivar Emese exhibited significantly higher RSR than Aliz (Figure 2E). The growth parameters of F_1 and F_2 treated plants were statistically similar under DS.

There were no significant differences in RWC between microbially treated and control plants under WW conditions, irrespective of the cultivar (Figure 3A). The exposure of plants to drought led to a noticeable decrease in leaf RWC from 90.4–93.4% to 44.7–78.2% with significantly lower values for inoculated cultivars than for non-inoculated ones. Cultivar Aliz generally had higher RWC values in the vegetative phenophase, especially when inoculated before DS.

No differences were found in the DSS of the two cultivars in any treatment, though plants inoculated with F_1 exhibited pronounced wilting symptoms (Figure 3B).

Fluorescein diacetate (FDA) hydrolysis revealed that the activity of soil microbes was significantly inhibited by DS in

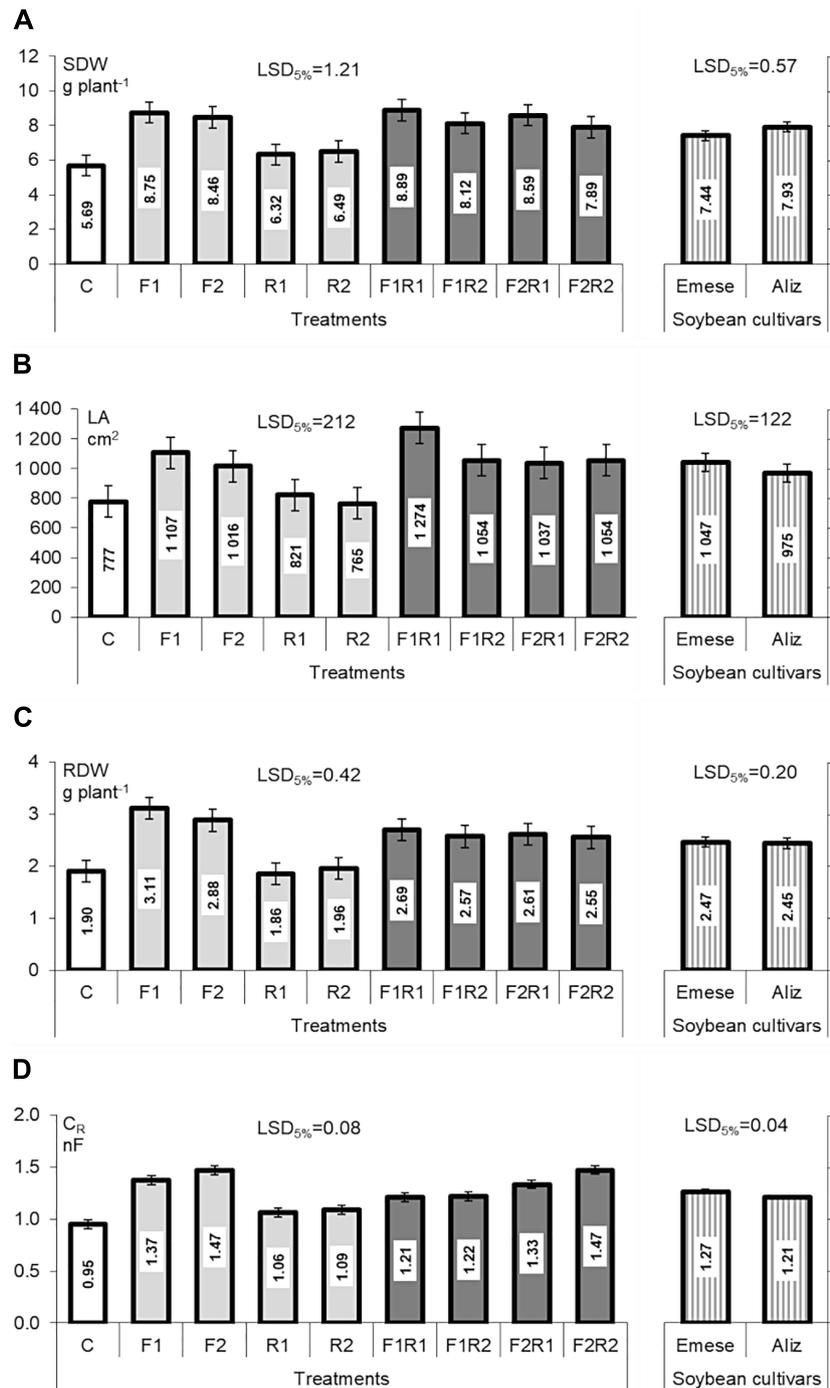


FIGURE 1 | (A) Shoot dry weight (SDW), **(B)** leaf area (LA), **(C)** root dry weight (RDW), and **(D)** root electrical capacitance (C_R) of Emese and Aliz soybean cultivars in the Compatibility experiment. Mean values of the microbial inoculation treatments (left side in the Figure: C, control plants; R₁ and R₂ rhizobia; F₁ and F₂ AM fungal products) and mean values of soybean cultivars (right side in the Figure). Error bars represent LSD ($n = 4$; $p < 0.05$).

all the treatments. No significant effect of co-inoculation was observed for either WW (28.9–30.8 $\mu\text{g Fl g}^{-1}\text{h}^{-1}$; LSD = 4.2, $P < 0.05$) or DS (20.0–22.8 $\mu\text{g Fl g}^{-1}\text{h}^{-1}$). The FDA activity was also independent of the cultivar (Emese 24.2 $\mu\text{g Fl g}^{-1}\text{h}^{-1}$; Aliz 26.5 $\mu\text{g Fl g}^{-1}\text{h}^{-1}$; LSD = 2.4, $P < 0.05$) in both watering regimes.

The number of nodules on the roots of co-inoculated plants was much higher than on non-inoculated, control plants. Nodulation was independent of both the application of mycorrhizal fungi and the cultivar under both watering regimes.

TABLE 2 | Nitrogen (N) content (%) of soybean leaves at the end of the Compatibility experiment.

| N (%) | Cultivar: Emese | Cultivar: Aliz | LSD _{5%} 0.30 |
|-------------------------------|-----------------|----------------|------------------------|
| C | 2.48 ± 0.44 | 2.50 ± 0.34 | 2.49 |
| F ₁ | 2.24 ± 0.51 | 2.69 ± 0.29 | 2.47 |
| F ₂ | 2.27 ± 0.21 | 2.22 ± 0.25 | 2.25 |
| R ₁ | 2.96 ± 0.19 | 2.85 ± 0.12 | 2.91 |
| R ₂ | 2.45 ± 0.03 | 3.09 ± 0.11 | 2.77 |
| F ₁ R ₁ | 2.47 ± 0.15 | 2.94 ± 0.35 | 2.71 |
| F ₁ R ₂ | 2.69 ± 0.11 | 2.79 ± 0.39 | 2.74 |
| F ₂ R ₁ | 2.70 ± 0.39 | 2.51 ± 0.43 | 2.61 |
| F ₂ R ₂ | 2.47 ± 0.29 | 2.73 ± 0.24 | 2.60 |
| LSD_{5%} 0.14 | 2.53 | 2.70 | Mean values |

Means and standard errors of four replicates. Two-way analysis of variance was performed. LSD_{5%} values indicate the least significant difference at the $p = 0.05$ level. Microbial treatments: C, non-inoculated control plants; F_{1,2}, commercial AMF inocula; R_{1,2}, *Bradyrhizobium japonicum* inocula; Soybean cultivars: Aliz and Emese.

TABLE 3 | Phosphorus (P) concentration (mg kg⁻¹) of soybean leaves at the end of the Compatibility experiment.

| P (mg kg ⁻¹) | Cultivar: Emese | Cultivar: Aliz | LSD _{5%} 298 |
|-------------------------------|-----------------|----------------|-----------------------|
| C | 1629 ± 126 | 1593 ± 245 | 1611 |
| F ₁ | 1889 ± 307 | 1907 ± 361 | 1898 |
| F ₂ | 1599 ± 326 | 1325 ± 343 | 1462 |
| R ₁ | 1825 ± 138 | 1466 ± 137 | 1645 |
| R ₂ | 1465 ± 137 | 1512 ± 188 | 1488 |
| F ₁ R ₁ | 1649 ± 320 | 1634 ± 227 | 1641 |
| F ₁ R ₂ | 1703 ± 206 | 1644 ± 513 | 1674 |
| F ₂ R ₁ | 1522 ± 272 | 1259 ± 343 | 1390 |
| F ₂ R ₂ | 1365 ± 373 | 1396 ± 443 | 1381 |
| LSD_{5%} 141 | 1627 | 1526 | Mean values |

Means and standard errors of four replicates. Two-way analysis of variance was performed. LSD_{5%} values indicate the least significant difference at the $p = 0.05$ level. Microbial treatments: C, non-inoculated control plants; F_{1,2}, commercial AMF inocula; R_{1,2}, *Bradyrhizobium japonicum* inocula; Soybean cultivars: Aliz and Emese.

The intensity of colonization (M%) in the roots ranged from 35.2 % to 60.8% in WW soil, with the lowest rate for F₂ (Figure 3C). The M% of roots colonized by indigenous AMF exhibited a 9% increase in response to DS, while those colonized by AMF species from commercial products had suppressed or unchanged infectivity. Indigenous AMF alone generated a higher colonization in roots than it was observed after the inoculation with F₁ or F₂. The intensity of AMF colonization in the roots was slightly higher for Aliz than for Emese. The characteristics of and changes in arbusculum richness (A%) were similar to those of M%, again proving the better natural infectivity and responsiveness of Aliz. The increment in A% caused by DS was significant (data not shown). The RMID values for mycorrhizal and rhizobial treatments of the cultivars were greater under WW than under DS conditions. Under WW conditions, Emese had slightly higher susceptibility to microbial inoculation than Aliz, but DS inverted this relationship.

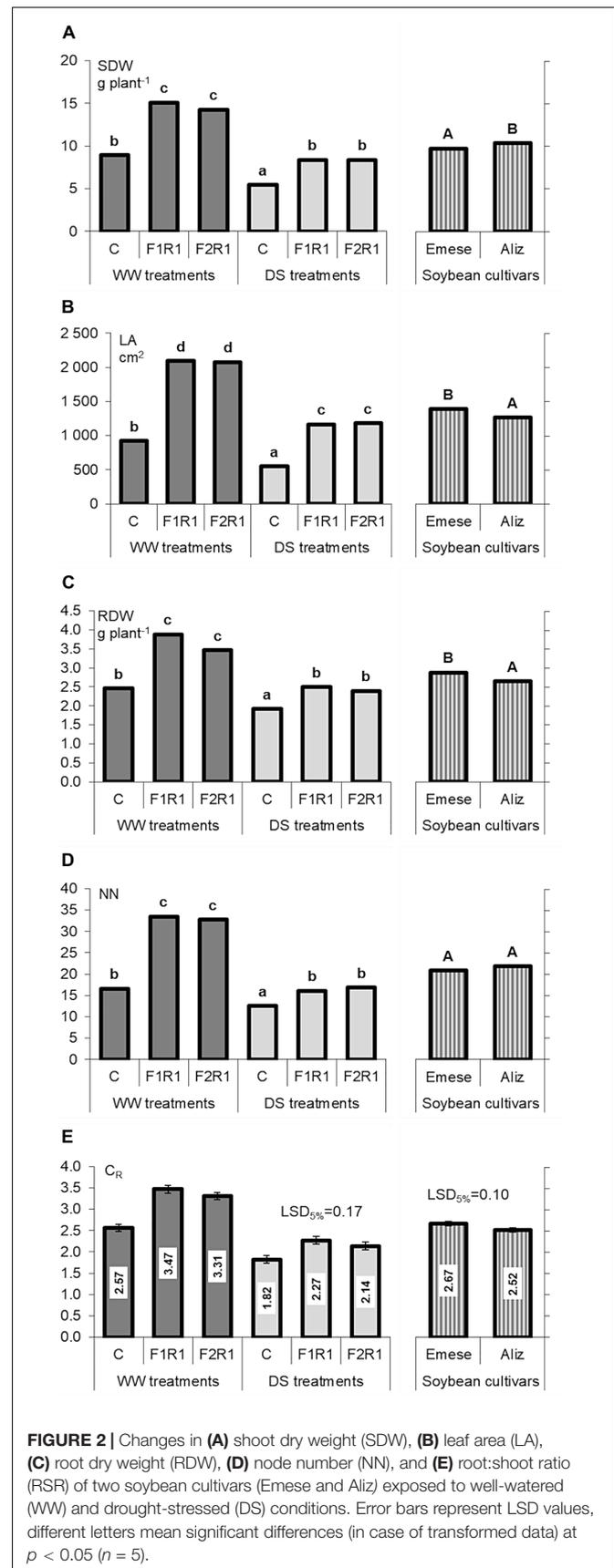
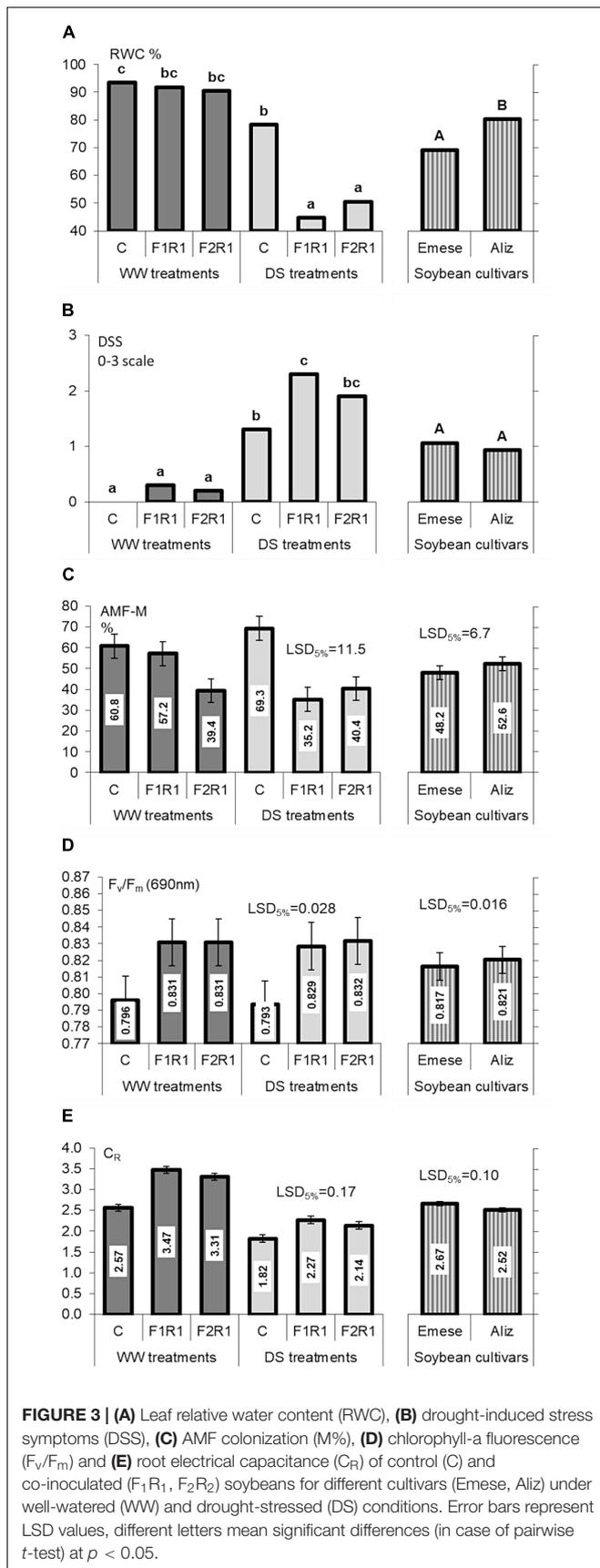


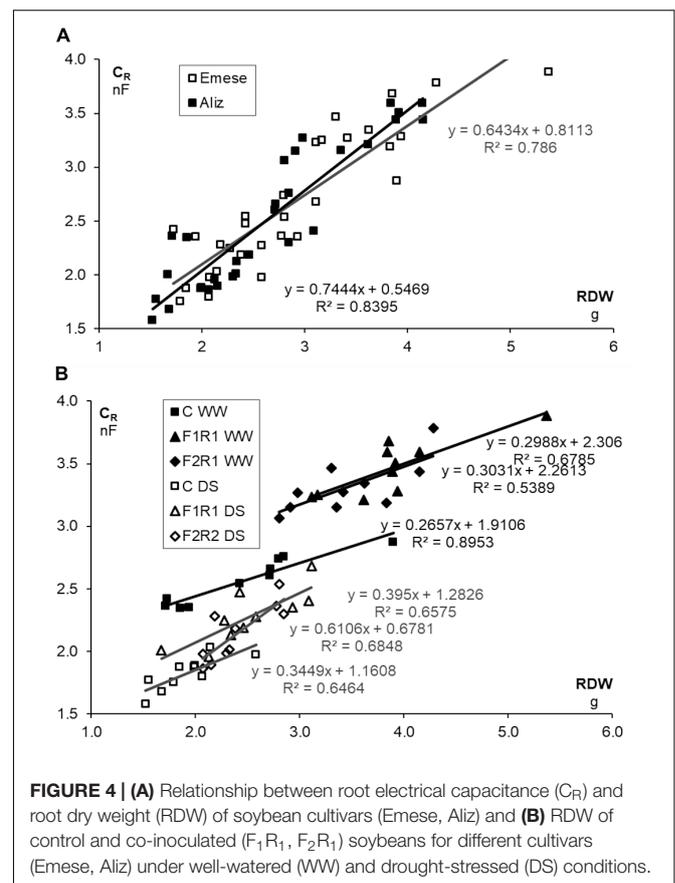
FIGURE 2 | Changes in (A) shoot dry weight (SDW), (B) leaf area (LA), (C) root dry weight (RDW), (D) node number (NN), and (E) root:shoot ratio (RSR) of two soybean cultivars (Emese and Aliz) exposed to well-watered (WW) and drought-stressed (DS) conditions. Error bars represent LSD values, different letters mean significant differences (in case of transformed data) at $p < 0.05$ ($n = 5$).



F_v/F_m values were increased by dual inoculation under both water regimes (Figure 3D). Drought did not significantly affect the photosynthetic activity, and the quantum efficiency of PSII was independent of the cultivar.

Microbial inoculation significantly enhanced the C_R values of the cultivars, irrespectively of watering, particularly in the case of Emese (Figure 3E). The effect of F_1 on C_R (36% for Aliz, 34% for Emese under WW; 21% for Aliz and 28% at Emese under DS) was greater than that of F_2 (26% for Aliz, 32% for Emese under WW; 14% for Aliz and 20% for Emese under DS) (Figure 3D). C_R decreased significantly in response to DS, to a greater extent for Aliz (31–37%, as a percentage of WW) than for Emese (27–33%). Cultivar Emese exhibited significantly higher C_R than Aliz at the end of the experiment. A good linear correlation ($P < 0.01$) was obtained between C_R and both SDW ($R^2 = 0.829$) and LA ($R^2 = 0.716$) by pooling the data of the six plant groups. Strong linear correlations were found between both C_R and RDW and C_R and LA (Aliz $R^2 = 0.672$; Emese $R^2 = 0.758$). Figures 4A,B illustrate an increase in C_R due to inoculation and a decrease due to drought stress. Regression analysis demonstrated linear correlations between C_R and RDW for each treatment. The same root biomass was associated with higher C_R in inoculated treatments and with lower values in the case of drought stress (Figure 4B).

The beneficial effect of rhizobial nodulation on the leaf nitrogen concentration was detected, as in the Selection



experiment. The leaf N percentage of inoculated plants significantly exceeded the controls under both WW and DS conditions (Table 4), but no significant differences were found either between the cultivars or between the microbial treatments. The leaf P concentration was decreased by drought and by inoculation under WW conditions, although DS led to higher leaf P concentration in inoculated plants compared to controls (Table 5). The total P content in the leaves was significantly higher in inoculated plants than in control plants. The P concentrations in Emese were greater than in Aliz.

DISCUSSION

For the future of mycorrhizal biotechnology and industry, it is crucial to incorporate the scientific knowledge derived from fundamental and applied research into the innovation of microbial inoculants (Vosátka et al., 2012). Although various commercial microbial products are already in use, few data have been published on their effect on plant development, nutrition and yield under controlled environmental conditions (Bona et al., 2016). Most studies have been primarily focused on the

investigation of qualitative and quantitative yield indicators in horticulture, fruit or ornamental production (Albrechtova et al., 2012; Bona et al., 2016; Engel et al., 2016).

In the present study symbiotic effectiveness was investigated with 20–20 measured parameters on drought-selected soybean hosts during their vegetative and early reproductive stages, with special regard to the compatibility of dual and tripartite symbiotic agents. Most of the measured parameters confirmed the beneficial effect of inoculation with symbionts on plant development and drought tolerance (Figures 1–4). Similar to other observations (Okereke et al., 2001; Meghvansi et al., 2008), the responses of soybean to microbial inoculation depended considerably on the rhizobial strains, on the fungal products and also on the cultivars. The present investigations showed that the benefits of symbiosis were more obvious in the case of plants singly or co-inoculated with AMF than for those inoculated solely with rhizobium (Figure 1 and Tables 1, 2). After single inoculation with products containing AMF fungal species (F₁ and F₂) the increase in shoot and RDW, LA and C_R was higher than in rhizobium only treatments (Figures 1A–D). With the exception of C_R these parameters differed slightly between soybean varieties. Such functional differences also occurred between the AMF treatments as reported earlier (Louis and Lim, 1988; Antunes et al., 2006; de Varennes and Goss, 2007). Infection with *Bradyrhizobium* clearly enhanced the leaf nitrogen content, but AMF colonization reduced the concentration of nitrogen and phosphorus due to the dilution effect of higher plant biomass (Tables 1, 2). In the Compatibility experiment, the results obtained for ARA and plant nitrogen content revealed clear differences in the effect of the two rhizobial inoculants on plant nutrition. Despite their lower colonization values, plants treated with the AMF inoculum F₁ had a higher growth rate than those inoculated with F₂ (Figures 1A–C). No AM fungal colonization was observed during the experimental period on the great majority of the roots in F₁-treated pots. The extra biomass production caused by F₁ could be due to the high organic content of the biofertilizer. The higher number of species in the F₂ product could lead to higher infectivity and effectiveness, so F₂ could be more successful in developing a compatible relationship.

Numerous field and pot trials proved that AMF inoculation significantly increased soybean yields (by 20–50%); however, the response varied with soil type, fertilization and the origin of the symbiotic partners (Porcel and Ruiz-Lozano, 2004; Meghvansi et al., 2008; Kaschuk et al., 2010; Wang et al., 2011). It was found that inoculation with *Bradyrhizobium* sp. or *Glomus mosseae* in pot culture was able to inhibit both pathogenic infection with *Cylindrocladium parasiticum* and the development of the parasite in soybean roots, while co-inoculation was more effective than the use of any of the microsymbionts alone (Gao et al., 2012). The positive interaction between the microsymbionts was indicated by the extra biomass production, the increased number of nodules and the richness of AMF root structures (Louis and Lim, 1988; Xie et al., 1995). However, the carbon cost of mycorrhizal fungi requires the delivery of 4–20% of the photosynthetically fixed carbon from the host plants to the symbiotic partner (Grimoldi et al., 2006; Kaschuk et al., 2009). According to Minchin et al. (1981),

TABLE 4 | Nitrogen (N) content (%) of soybean leaves at the end of the Drought stress experiment.

| N (%) | | Cultivar: Emese | Cultivar: Aliz | LSD _{5%} 0.36 |
|------------------------------|-------------------------------|-----------------|----------------|------------------------|
| WW | C | 1.33 ± 0.33 | 1.51 ± 0.81 | 1.42 |
| | F ₁ R ₁ | 3.12 ± 0.27 | 2.96 ± 0.24 | 3.04 |
| | F ₂ R ₁ | 3.08 ± 0.26 | 3.27 ± 0.32 | 3.18 |
| DS | C | 1.49 ± 0.39 | 1.20 ± 0.19 | 1.34 |
| | F ₁ R ₁ | 3.04 ± 0.23 | 2.93 ± 0.47 | 2.99 |
| | F ₂ R ₁ | 3.06 ± 0.26 | 3.10 ± 0.54 | 3.08 |
| LSD_{5%} 0.21 | | 2.52 | 2.49 | Mean values |

Means and standard errors of five replicates. Two way-analysis of variance was performed. LSD_{5%} values indicate the least significant difference at the $p = 0.05$ level. Microbial treatments: C, non-inoculated control plants; F_{1,2}, commercial AMF inocula; R_{1,2}, *Bradyrhizobium japonicum* inocula; Soybean cultivars: Aliz and Emese; WW, well-watered conditions; DS, drought stress conditions.

TABLE 5 | Phosphorus (P) concentration (mg kg⁻¹) of soybean leaves at the end of Drought stress experiment.

| P (mg kg ⁻¹) | | Cultivar: Emese | Cultivar: Aliz | LSD _{5%} 333 |
|-----------------------------|-------------------------------|-----------------|----------------|-----------------------|
| WW | C | 3871 ± 780 | 3155 ± 345 | 3513 |
| | F ₁ R ₁ | 2693 ± 350 | 2300 ± 126 | 2496 |
| | F ₂ R ₁ | 2523 ± 131 | 2360 ± 118 | 2441 |
| DS | C | 3091 ± 334 | 3342 ± 616 | 3217 |
| | F ₁ R ₁ | 2744 ± 344 | 2714 ± 271 | 2729 |
| | F ₂ R ₁ | 2968 ± 103 | 2682 ± 226 | 2825 |
| LSD_{5%} 192 | | 2981 | 2759 | Mean values |

Means and standard errors of five replicates. Two-way analysis of variance was performed. LSD_{5%} values indicate the least significant difference at the $p = 0.05$ level. Microbial treatments: C, non-inoculated control plants; F_{1,2}, commercial AMF inocula; R_{1,2}, *Bradyrhizobium japonicum* inocula; Soybean cultivars: Aliz and Emese; WW, well-watered conditions; DS, drought stress.

as much as 25% of net photosynthates may be appropriated for biological nitrogen fixation. Furthermore, the cost of two independent microsymbionts in tripartite associations may be cumulative (Harris et al., 1985). Although multiple inoculation may result in decreased biomass production or unfavorable microbial parameters, the present results indicated that microbial inoculation can improve photosynthetic efficiency both in well-watered soils and under drought stress, though this effect depends on the inoculants (**Figure 3D**). The increased photosynthetic activity and root functionality of bacteria or AMF treated hosts resulted in higher biomass production compared to control plants. However, co-inoculation with rhizobial and AM fungal strains did not produce significantly further more biomass in pumice (**Figure 1A**); moreover, in the case of dual inoculation, AMF root colonization exhibited a slight decrease.

Fluorescence kinetics and the root electrical capacitance methods proved to be useful tools for the *in situ* monitoring of the effect of several stress factors and for the selection of stress-tolerant cultivars or effective microbial strains (Solti et al., 2014; Cseresnyés et al., 2016, 2018), but less research has targeted the characterization of differences between different genotypes of crop species (Barócsi, 2013). The rate of photosynthesis is influenced by environmental conditions (e.g., water, temperature, nutrients, light and CO₂) and internal factors, such as the nutrient concentration of the tissues and the sink strength stimulated by the carbon cost of symbiotic associations (Kaschuk et al., 2009). In the present experiments significant differences were found both in the chlorophyll fluorescence and C_R values of different microbial treatments and soybean cultivars. Of the two varieties, the Aliz had higher photochemical efficiency, which represented an inverse tendency compared to the LA values. Both in pumice and organic soil C_R was closely correlated with root biomass (Cseresnyés et al., 2016). However, the measured values were the joint result of root activity, the activity of root-associated symbionts and the soil. This means that at the same stage of development, under similar soil conditions, both rhizobial nodules and AMF extraradical hyphal network may have a beneficial effect on active root surface area. The same root biomass is associated with higher C_R in the case of inoculated plants and with lower values in response to drought stress (Cseresnyés et al., 2013). The increased water uptake of inoculated plants is presumably due to the enhanced root-soil interface caused by the external fungal hyphae and the root nodulation. It was found earlier that eight soybean cultivars could be classified into different drought-tolerance groups on the basis of C_R showing a strong correlation with cultivar-specific root growth and biomass production under both well-watered and drought conditions (Cseresnyés et al., 2016). Several studies proved that advanced root properties such as greater depth and larger root system with more root hairs are advantageous under water deficit (Ku et al., 2013; Kumagai and Sameshima, 2014; Cseresnyés et al., 2016). It was also found that the effects of co-inoculation were related to the root morphology of soybean genotypes (Wang et al., 2011, 2016). For example, deeply rooted hosts benefited more than shallowly rooted ones. DS impeded plant growth in all

the treatments for both cultivars (**Figures 2A–D**) (Porcel and Ruiz-Lozano, 2004; Masuda and Goldsmith, 2009). Similar to other investigations (Ruiz-Lozano et al., 2001; Porcel and Ruiz-Lozano, 2004), mycorrhizal associations seem to have played an important role in the drought stress tolerance of soybean. Strong linear C_R-RDW and C_R-LA relationships were revealed in the Drought Stress experiment, (**Figures 4A,B**). As the leaves vaporize the water taken up by the roots, LA should be proportional to the absorptive surface area of the root system involved in the mycorrhizosphere, being manifested as a correlation between C_R and LA. In the case of drought stress, the beneficial effect of inoculation was mostly more pronounced in Emese than in Aliz. Emese produced a bigger root system and was more resistant to drought stress than Aliz, where the levels of RSR and C_R were lower (**Figure 2E**). Although Emese lost more water from its leaves due to the larger biomass, it did not differ from Aliz in terms of DSS (**Figure 3B**). The loss of biomass caused by drought was also lower in the case of Emese, while the mycorrhizal dependence of Aliz increased under drought. These results suggest that the functional properties of the roots may significantly affect the susceptibility of cultivars to microsymbionts, which could also influence the development of cultivar-specific stress tolerance (Wang et al., 2016). It has been reported that grasses having hairy and bushy fibrous roots with a large surface for uptake are less mycotroph than the tap-rooted plants in mycotrophic plant families (Muleta, 2010). In a tripartite symbiotic association, AM fungi respond more sensitively to differences in the (functional) properties of the root between species and varieties than rhizobia (Wang et al., 2016). The establishment of symbiosis enlarges the active root surface area responsible for water and nutrient uptake, which can be detected by measuring C_R. C_R measurements are sufficiently sensitive to detect differences in the infectivity of AM strains (Takács et al., 2014). No data on the C_R of nodulated roots have yet been published. In the present experiments plants infected with rhizobium had significantly higher C_R than the control despite the similar RDW (**Figure 4B**). Nodules and the extraradical hyphal network add to C_R by enlarging the absorbing surface.

It should be noted that when evaluating the beneficial effects of inoculation one should allow for the carriers of the commercial products, which may contain nutrients. The extra biomass production caused by the F₁ AMF biofertilizer could be the result of its high organic material content. Multifactorial investigations are needed for the determination of host compatibility and the efficacy of microbial inocula on plant production, including the measurement of plant growth and functional parameters, with both *in situ* and destructive methods.

In the field, the symbiotic effectiveness of rhizobial strains or AMF inoculants and their competitiveness can be achieved by superior microsymbionts originated from natural or managed selection. However, no single AMF or rhizobium strain is likely to be effective in all soils under any conditions and on any plant host, therefore no commercial fertilizer can be expected to be ideal for every field, despite containing multiple strains or species.

All the products need to be tested before application and it is possible that no suitable commercial fertilizer will be adequate for a specific purpose. In this case, the managed selection of strains originating from the indigenous microbial community could be the best solution.

In low-input agricultural systems, research on cooperation between different microbial symbionts is a key to understanding how the health and productivity of the plant is supported (Mahdi et al., 2010; Bhardwaj et al., 2014). Increasing the fitness and vitality of host plants to environmental stress by means of site-adapted, compatible symbiotic partnerships could be a new strategy for mitigating the impacts of environmental stress factors on plant production (Gianinazzi et al., 2010). Little differences in the root properties of drought tolerant cultivars may cause significant differences in the growth and physiological parameters that are used to describe symbiotic relationships. Endurance of even drought tolerant cultivars can be improved by inoculation with AMF or nitrogen fixing bacteria. In well-watered conditions, the tripartite association did not show the synergistic effect on the plants, so the benefits produced by commercial AMF products may have been partially originated from their carrier. The efficiency of these biofertilizers should therefore be checked before large scale application. The present results show the potential of C_R measurements to monitor the effect of symbiotic factors influencing root growth and biomass production. This *in situ* technique provides an opportunity to follow the temporal

changes in root activity and to select efficient plant-microbe partnerships.

AUTHOR CONTRIBUTIONS

TT supervised the project, conducted the pot experiments, discussed the results, and wrote the paper. IC was responsible for C_R measurements and data evaluation. TT, RK, IP, BK, TS-K, and AF designed and carried out the investigations on the symbiotic microorganisms and on the plants. All authors read the manuscript and approved the submission.

FUNDING

This study was funded by the Research Institute of Organic Agriculture, the National Research, Development and Innovation Fund of Hungary (Project Nos. 115714, financed under the K-16 funding scheme) and Bolyai János Research Scholarships of the Hungarian Academy of Sciences.

ACKNOWLEDGMENTS

The authors thank the reviewers for helpful comments, and Barbara Harasztos for improving the English in this paper.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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