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Investigation of arbuscular mycorrhizal status and functionality by electrical impedance and capacitance measurement

Tünde Takács*, Anna Füzy, Kálmán Rajkai, Imre Cseresnyés

Institute for Soil Sciences and Agricultural Chemistry, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary

ABSTRACT Applicability of electrical impedance (EI) and electrical capacitance (EC) measurement for the investigation of root colonization of mixed arbuscular mycorrhizal fungi (AMF) and functional diversity of separated AMF strains were studied in two pot experiments. In the first experiment, mycorrhizal and non-mycorrhizal maize cultivars were compared for testing the sensitivity of EI and EC measurement in relation to mycorrhizal status of host plants. In the second one, root colonization, biomass production, EI and EC of *Rhizophagus intraradices* or *Funneliformis mosseae*, inoculated and non-inoculated cucumber and bean hosts were monitored. The mycorrhizal plants showed lower EI and higher EC than control plants for each species. Since fungal colonization did not produce an increase in root surface area of maize, the higher root-soil interface showed by EI and EC values was undeniably due to the increased absorption surface area caused by the growth of AMF hyphae. As for cucumber and bean, the two selected AMF strains differed significantly in infectivity and effectivity, and the measured values also vary with host plants. Measuring EI and EC proved to be well applicable for *in situ* investigation of functional aspects of mycorrhizae.

KEY WORDS

arbuscular mycorrhiza
functional diversity
electrical capacitance
electrical impedance
root-soil interface

Conventional root investigation methods are unsuitable for *in situ* monitoring of root growth and function (Čermák et al. 2006; Cao et al. 2010). Measurement of electrical impedance (EI – basically the resistance against alternating current) and electrical capacitance (EC – charge-storing ability of root membranes) in a plant–soil system can provide a rapid *in situ* assessment about root status without plant damaging. This method was developed by Chloupek (1972). By inserting an electrode at the plant stem and the other one into the soil, and connecting them by a capacitance meter (LCR-bridge), root EI and EC proved to be negatively and positively correlated, respectively, with root fresh or dry weight and root surface area. Since the theoretical background was described (Dalton 1995), the EI and EC method has been modified and optimized for the practice (Rajkai et al. 2005; Čermák et al. 2006; Cseresnyés et al. 2013).

The arbuscular mycorrhizal fungi (AMF) form important symbiotic associations with the roots of nearly all native and crop plants (Brundrett 2009). The plant provides the fungus with carbon compounds, and the fungus, in turn, is beneficial to the host plant by enhancing its capability for extracting water, macro- and micronutrients from the soil (Marschner 1997). The AMF colonization often induces root morphologi-

cal changes in host plants: fungal infection can affect root mass, root length or root architecture (Kothari et al. 1990). It has been suggested that extraradical hyphae may enhance the total surface area of roots and fungus, conducting to an increase in the soil–plant interface (Augé 2004). AM fungi show different degrees of host compatibility, in terms of infectivity of isolates and effectivity of inoculation both.

Standard methods to assess AMF presence are based on investigation of fungal colonization in roots. In most cases, these techniques are destructive, time-consuming and information about AMF functionality is not provided. As root conductivity and permeability are reliable indicators for water uptake activity and absorptive root surface area (Aubrecht et al. 2006), we supposed that differences in root functionality induced by AMF colonization could be monitored by EI and EC measurement. In the present work, the applicability of EI and EC measurement for the investigation of root colonization of mixed arbuscular mycorrhizal fungi (AMF) and functional diversity of separated AMF strains were studied in two pot experiments. We investigated the relationships between the mycorrhizal and non-mycorrhizal root properties (root mass, length and surface area) and the measured EI and EC values. It was assumed that the application of the EI and EC method could contribute to confirming several former observations about AMF presence in host roots and moreover mycorrhizal function.

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*Corresponding author. E-mail: takacs@rissac.hu

Materials and Methods

In the first experiment, mycorrhizal and non-mycorrhizal maize (*Zea mays* L., DK-440 Hybrid) cultivars were compared for testing the sensitivity of EI and EC measurement in relation to mycorrhizal status of host plants. In the second one, root colonization, biomass production, EI and EC of *Rhizophagus intraradices* (R) or *Funneliformis mosseae* (F), inoculated and non-inoculated cucumber (*Cucumis sativus* L. cv. 'Perez-F1') and bean (*Phaseolus vulgaris* L. cv. 'Goldrush') hosts were monitored. Plant cultivation, AMF inoculation, as well as EI and EC measurements were carried out in the same manner in both experiments.

At experiment day 1, germinated seeds were planted into 1.25 l pots containing 1.1 kg of sterile (free of infective AMF propagules) pumice medium with 0.6–1 mm particle size, pH of 6.5, 0.94 kg/l bulk density and 0.26 cm³/cm³ water content at field capacity.

15 replicates of maize [M(RF)] in the first experiment and same number of cucumber [C(R) or C(F)] and bean [B(R) or B(F)] in the second one were treated with 10 g of AMF inoculum that contained pumice medium, and dried root cuttings of white clover (*Trifolium repens* L.) colonized by AMF fungi. 15 non-inoculated plants for each species [M(Ø), C(Ø) and B(Ø)] were used as controls.

In the first experiment, a mixed inoculum [(RF); 1:1 %w/w] of *Funneliformis mosseae* (Schüssler and Walker; syn. *Glomus mosseae* Nicholson and Gerdeman) and *Rhizophagus intraradices* (Schüssler and Walker; syn. *Glomus intraradices* Schenck and Smith) strains were used for inoculation, while in the second one, the same amounts of these strains were separately put in the mycorrhizal fungi treated pots. The inoculum was applied as a thin layer in 5 cm depth below seedlings in pots. Plants were maintained randomly in growth chamber (26/18 °C and 16/8 h for day/night and 300 µmol/m²/s photon flux density) with changing their places daily to avoid position effect. Planting medium was daily irrigated to field capacity. Optimal plant nutritional status was kept up by weekly irrigation with modified Hoagland's solution (with only 10% KH₂PO₄).

Root EI and EC were measured weekly during plant development with a GW-8101G precision LCR instrument (GW Instek, 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 at 15 mm above the substrate level and a second one earthed by a stainless steel rod (6 mm ID, 15 cm long) inserted to the substrate. Electrocardiograph paste was smeared around stem to keep up electrical contact (Rajkai et al. 2005; Cseresnyés et al. 2013).

The parameters of the AMF colonization were measured every second day in the early phase of symbiotic associations and weekly after the 14th or 21st day of the experiments. On each occasion, 2–2 pots were destructively sampled in order

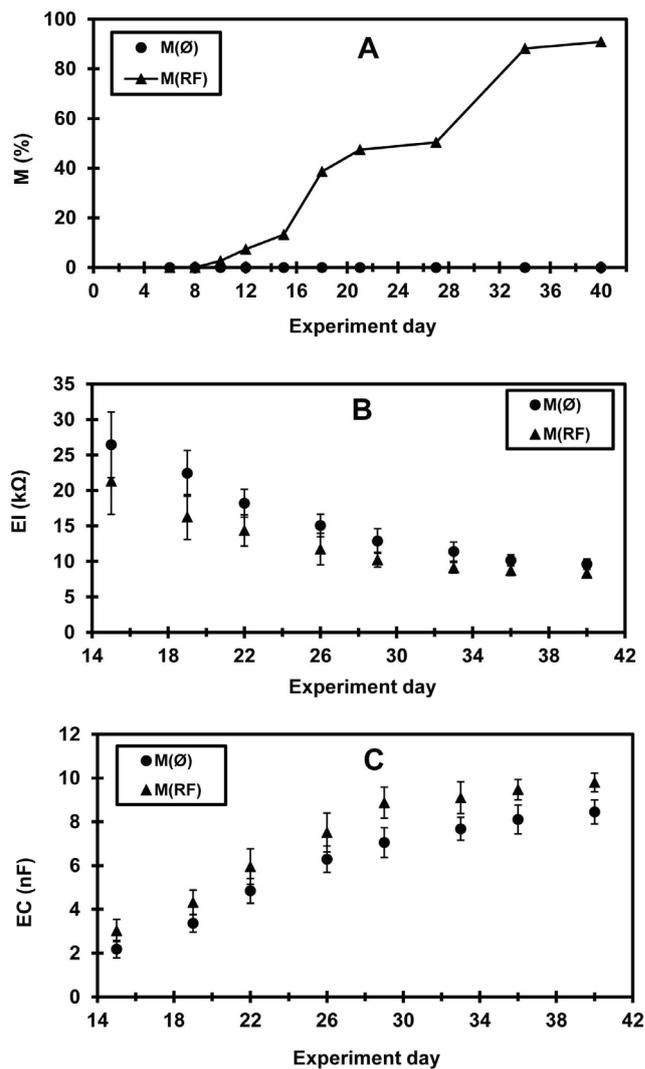


Figure 1A-C. Intensity of fungal root colonization (M%) in AMF-treated maize [M(RF)] (Fig. 1A) and signal intensity of root electrical impedance (EI; Fig. 1B) and electrical capacitance (EC; Fig. 1C) related to time in control (MØ) and mycorrhizal maize [M(RF)]. AM fungi treatments: R – *Rhizophagus intraradices*, F – *Funneliformis mosseae*; RF – mixed inoculum of the two strains

to follow the temporal development of AMF colonization. Roots were stained with cotton-blue (Phillips and Hayman 1970), and frequency (F%) and intensity (M%) of AMF colonization were determined by the method of Trouvelot et al. (1986).

Standard regression analysis was carried out in order to relate root EI and EC to root dry mass and root surface area in case of mycorrhizal and non-mycorrhizal plants. A non-parametric Welch test was applied if the standard deviations of the compared groups (*F* test) were not identical. Statistical significance was assessed at $p < 0.05$ in each case. Computations were performed by using Statistica software (ver. 9., StatSoft Inc., OK, USA).

Table 1. Biomass production of maize (M), cucumber (C) and bean (B) non-treated (control – Ø) or treated with AMF fungal inoculum (R – *Rhizophagus intraradices*, F – *Funnelformis mosseae*; RF – mixed inoculum of the two strains).

Plants and treatments	Root dry weight (g)	Shoot dry weight (g)	Root surface area (cm ²)
MØ	1.756 (±0.262)	3.935 (±0.515)	1704 (±215)
M(RF)	1.795 (±0.281) NS	4.115 (±0.604) NS	1406 (±186) NS
CØ	0.505 (±0.071)	3.159 (±0.323)	665 (±93.5)
C(F)	0.539 (±0.053) NS	4.067 (±0.473) ***	708 (±69.6) NS
C(R)	0.556 (±0.076) NS	4.204 (±0.386) ***	729 (±99.6) NS
BØ	0.702 (±0.088)	1.723 (±0.125)	357 (±44.7)
B(F)	0.956 (±0.115) ***	2.912 (±0.045) ***	487 (±58.6) ***
B(R)	0.924 (±0.110) ***	2.458 (±0.057) **	470 (±55.9) ***

*, **, ***: significantly different from the control plants, at the p < 0.05, 0.01 and 0.001 levels respectively; NS: not significant.

Results

In the first experiment, the electrical measurement proved to be adequate to discriminate the AMF-infected and non-AMF maize plants. AMF infection was not detected in control plants (MØ). AMF root presence (F= 8.3%; M= 1.3%) was first detected in 10 days old maize plants, and AMF colonization parameters (F and M) increased with age of maize. Microscopic examination of harvested roots showed 100% frequency and 90.9% intensity of fungal colonization in mycorrhizal plants (Fig. 1A). Mycorrhizal status of maize had no effect on shoot dry weight, root dry weight, but AMF colonization altered the root morphology: significantly increased the average root diameter (by 22.8%), and decreased the root length (by 33.0%; data not shown) and the root surface area (by 17.4 %) (Table 1).

In relation to plant root expansion through the experiment time, the amplitude of EI and EC showed a continuous decrease and increase, respectively (Fig. 1B–C; EI is basically inversely proportional to EC). The effect of AMF colonization on root electrical properties was clearly reflected by the EI and EC monitoring. Decrease of EI and increase of EC by time correlated strongly with mycorrhizal status of maize during plant development. At inoculated maize plants [M(RF)], root EI and EC were significantly lower and higher, respectively, over the non-inoculated control (MØ), indicating

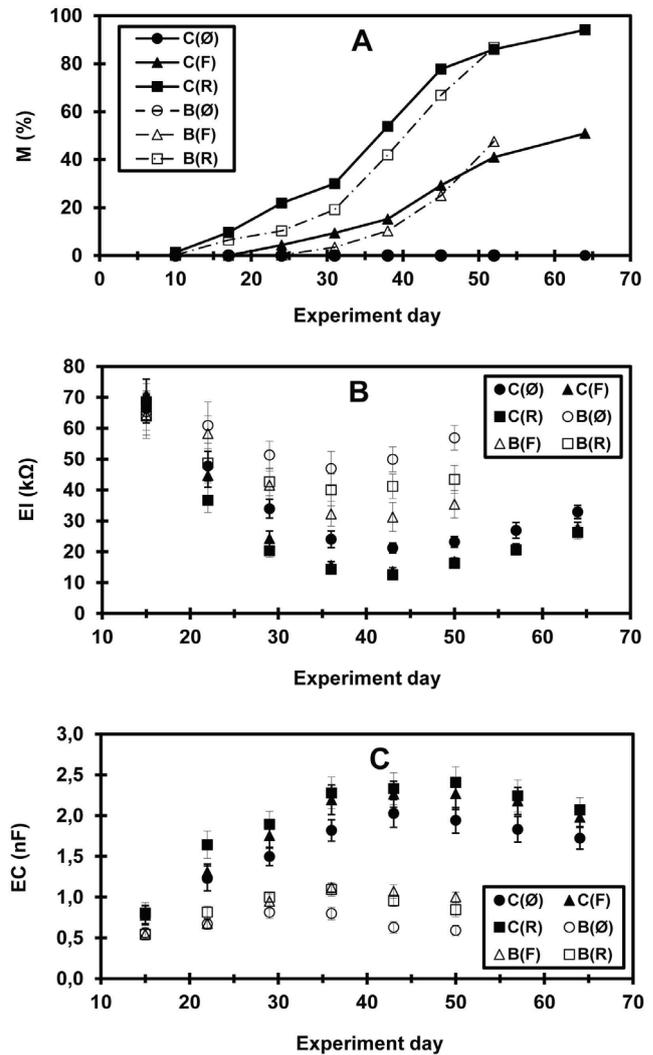


Figure 2A-C. Intensity of fungal root colonization (M%) in cucumber (C) and bean (B) inoculated with *Rhizophagus intraradices* (R) or *Funnelformis mosseae* (F) AMF strain (Fig. 2A) and signal intensity of root electrical impedance (EI; Fig. 2B) and electrical capacitance (EC; Fig. 2C) related to time in control (Ø) and mycorrhizal plants [C(R); C(F); B(R); B(F)].

the expansion of the absorbing root–soil interface caused by AMF colonization.

In the second experiment, root EI and EC measurement were used for *in situ* monitoring of functional differences that appears due to the specific colonization strategy of AMF strains in cucumber and bean hosts.

R. intraradices strain colonized both plants more effectively than *F. mosseae* (Fig. 2A). In the root of 10 days old plants, the presence of *R. intraradices* was detected in each host. In contrary, the structures of *F. mosseae* were found in host roots not earlier than the 24th experimental day. The colonization parameters (F and M) increased with the age

of plants. The maximal colonizations were similar at the two host plants, but early colonization was higher in case of cucumber. Mycorrhizal colonization exerted a significant influence on biomass production of both crop species. Post-harvest biomass measurement showed higher shoot dry weight and root dry weight at AMF-plants compared with the non-infected ones (Table 1). The effect of the AMF strains differed more significantly on host response in case of the high mycorrhizal-dependent bean. 29–33% and 43–69% relative shoot dry weight increments caused by AMF colonization were calculated for cucumbers and beans, respectively. Considering root growth, the effect of AMF colonization proved to be significant only for beans, in which 32–37% difference in root dry weight or root surface area was observed, as opposed to cucumbers, where AMF infection had just an insignificant effect on root development (Table 1).

EI decreased continuously with growing of both mycorrhizal and non-mycorrhizal plants (Fig. 2B). EC values had an upward trend during the vegetative stage of plant development, culminated at the flowering stage then reduced continuously through the fruit setting (Fig. 2C). Cucumbers expressed 1.5 to 3 fold higher EC than was detected for beans. The values of EI and EC are very similar at control (CØ; BØ) and colonized plants [C(R), C(F); B(R), B(F)] in early stage, when colonization has not occurred, yet. After the first mycorrhizal structures appeared, EI and EC values of colonized plants became significantly lower and higher, respectively, than those of control plants (Fig. 2B–C). At late colonization, higher M% values are not in relation to higher capacitance values. The differences in the root colonization of the two AMF strains were well expressed by root EI and EC. The infectivity of *R. intraradices*, that was higher than that of *F. mosseae*, caused higher decrease in EI and higher increase in EC values compared to control plants.

Discussion

AMF fungi are one of the key components of soil organisms; therefore, it is important to understand the physiological and ecological role of AMF in host plant community. Methods to detect and estimate AM fungi colonization and structures in roots are essential tools in mycorrhizal research. The basic techniques to detect the AMF infection and colonization degree are stainings and light-microscopic observations. Numerous destructive – both vital and non-vital – staining methods for observation of intraradical structures of AMF has been reported by the detailed overview of Vierheilig *et al.* (2005). In most cases they do not provide information about AMF functionality. The challenge of methodological developments is to establish routine and cost-effective techniques to measure the quantitative and qualitative AMF properties simultaneously to providing information about the AMF functionality.

In the presented work, significant effect of AMF colonization on root EI and EC values, as a consequence of the increased root–soil interface, was shown in maize, cucumber or bean cultivars. AMF colonization caused significant decrease in the root EI and increase in the root EC, respectively. The degree of host growth responses to AMF colonization is expressed as mycorrhizal dependency (MD). The AMF-induced relative increment not only in total plant biomass but also in root EC proved to be higher in bean than in cucumber (respectively for the last EC measurement), in relation to the higher degree of MD generally observed in legumes (Muleta 2010). The root surface area of slightly mycorrhiza-dependent hosts (maize or cucumber) was similar to that of control plants, indicating the expansion of the absorbing root–soil interface. Changes in electrical properties could be due either to the increased area of plant root surface or to the enhanced absorption surface area by extraradical fungal hyphae (possibly to the complex interaction of the two phenomena). AMF external mycelial network is mostly dynamically developing and functioning component of plant–AMF symbiosis. The rate of intraradical AMF colonization is correlated with the extent of extraradical hyphae which promotes host supply (Luciano *et al.* 2006). Symbiotic efficiency can be assessed through host biomass production due to the ability of AMF isolates or species to promote stress tolerance and to improve mineral nutrition.

In the second experiment, the functionality of infective and absorptive AMF hyphae were visualized with EI and EC measurements, concurrently monitoring the stage of root colonization. The AMF isolates were characterized by the EI, EC and biomass production of host plants. In our case, the effectivity of inoculation depended strongly on AMF species, especially in the highly mycorrhiza-dependent bean host. The EI and EC measurement is a suitable method to detect little differences among various plant–fungi symbiosis, such as variations in the beginning of symbiotic phase or the spreading of intra- or extraradical hyphae.

We can conclude, that the introduced non-destructive and simple electrical impedance and capacitance measurement is an adequate technique for *in situ* monitoring of AMF colonization and function. Synthesizing the methods and knowledge of plant physiology, soil physics or soil microbiology sciences (in our case the EI and EC applications in detection of AMF colonization or stress diagnostic of terrestrial plants) could be contribute to understanding of functions and relationships in soil–plant–symbiont systems.

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