PLANT STRESS RESPONSE DETECTION BY *IN SITU* MEASURED ROOT ELECTRICAL PROPERTIES – FINAL REPORT 2016–2019

Results in the last year

Workplan of the project for 2019 was fully accomplished.

1) For AMF inocula production of *Rhizophagus intraradices* (BEG 144), the pot cultures of infected white clover (*Trifolium repens* L.) hosts were grown in rhyolite-vermiculite (1:1) mixture for three months in a growth chamber. The AMF infectivity was then estimated microscopically according to Trouvelot's five-class system. *R. intraradices* showed high root colonization rate infection (F=96.6–100%; M=64–72%; A=60–68%), so it was applied for the microbial inoculation in the pot experiment devoted to study cadmium (Cd) stress.

2) As the third stress factor, the effect of Cd contamination on plant growth parameters and root electrical properties was investigated. A pot experiment was conducted with wheat (*Triticum aestivum* L.) cv. 'TC33', non-inoculated or inoculated with *Rhizophagus intraradices* AMF strain, and exposed to different Cd levels (1, 2.5 and 5 mg Cd per kg medium) in 15 replicates. Rhyolite-vermiculite (1:1) mixture was used as growth medium. The increasing Cd content caused a progressive reduction in root capacitance (by 7–44%), in relation to the decreasing root (by 3–35%) and shoot dry mass (by 21–52%). Shoot proved to be more affected by Cd than root, leading to an increased root-to-shoot ratio in the stressed plants (from 0.26 to 0.42). Leaf area drastically decreased by the stress (by 15–62%). Photosynthetic efficiency (Fv/Fm and Fv/F0) reduced only by the highest Cd level, whereas leaf chlorophyll content decreased in all treatments. The increasing Cd load provoked a decreasing trend in root membrane stability index (MSI). Significant positive linear correlations were found between the root capacitance and the root dry mass both for AMF-inoculated (R^2=0.688) and non-AMF plants (R^2=0.782). The microscopic investigation revealed high variability in the AMF root colonization indices in all treatments with the mean values of F=21.1–45.8%, M=12.9–20.2%, a= 38.6–61.5%, A=7.3–13.1%. Each index showed a decreasing trend with the increasing Cd content, however – due to the highly variable colonization – significant difference was only found in F% between the control and high Cd-level treatment. AMF colonization caused an increase both in root electrical capacitance and in root dry mass, but the effect was significant only for the low and medium Cd level. The contents of Cd, Cu, Fe, K, Mg, P and Zn in shoot and root were measured by ICP-OES instrument. Cd stress increased shoot and root Cd content, shoot Mg content and root Mg content (in non-AM plants), whereas reduced shoot and root Cu content, as well as shoot and root K and P content under high stress level. AMF colonization increased root Cd and P content and shoot Fe content under stress, whereas reduced root Cu, Fe and Mg content.

3) The evaluation of measurement results, and the preparation of research articles about our findings were continued during the year and is being in progress.

Summary of the project results (2016–2019)

1) The main physical and chemical properties of the two planting media (pumice and rhyolite-vermiculite mixture) were determined according to the current Hungarian and international standards and methods. The measured parameters were: $pH_{(H2O)}$, $pH_{(KCl)}$, cation exchange capacity (CEC), humus content, lime content, total N, P and K content, bulk density. The volumetric water contents of the substrates at total water saturation (pF=0), at field capacity (pF=2.5) and at permanent wilting point (pF=4.2) were also determined. This investigation aimed to adjust the water conditions for plant cultivation (including optimal water regime and drought stress) and root electrical measurement.

2) The mycorrhizal status and dependence of wheat cv. 'Mv. Hombár' and 'TC33' were examinated for AMF strain selection in a pot experiment. The previously propagated BEG11 (*Funneliformis geosporum*), BEG12 (*Funneliformis mosseae*), BEG47 (*Glomus versiforme, Diversipora epigaea*) and BEG144 (*Rhizophagus intraradices*) AMF inocula were used for inoculation of white clover host. The plants were grown in pumice. Differences in root colonization, biomass production and mycorrhizal dependence (MD) were found between the two wheat cultivars. Biomass production of cv. 'Mv Hombár' was not affected or decreased by AMF inoculation. 'Mv Hombár' roots were not infected by BEG11. BEG47 showed the highest infectivity in both cultivars. Therefore, the BEG47 strain was selected for the further pot experiments aimed to examine abiotic stress conditions. MD of the cultivars proved to be small, and cv. 'Mv. Hombár' was less mycorrhizal-dependent than 'TC33'.

3) Three main pot experiments were designed and carried out during the project to evaluate the potential of the root electrical measurement to study plant responses to stress factors, including drought (*Exp.1*), substrate alkalinity (*Exp. 2*) and Cd contamination (*Exp. 3*). As a preliminary experiment, the effect of salt (Na_2CO_3) or Cd addition on the electrical properties of the substrates and on the above- and belowground plant biomass was investigated. Six doses of Na_2CO_3 (from 0.05 to 0.6 m/m%) or Cd (from 1 to 100 mg kg^{-1}) were applied in both wheat cultivars. Salt and Cd addition progressively decreased the root capacitance (except at the lowest salinity level). At some high-dose Cd treatments (10–50 mg kg^{-1}), cv. 'TC33' showed lower root capacitance compared to cv. 'Mv. Hombár'. Root dry mass decreased as salinity or Cd stress increased. 'Mv. Hombár' generally produced higher root biomass under Cd stress. The treatments reduced the shoot dry mass. Cultivar 'TC33' had significantly higher shoot biomass. Based on these results, 0.1, 0.2 and 0.3 m/m% Na_2CO_3 and 1, 2.5 and 5 mg Cd per kg substrate were considered as efficient doses for further alkalinity and Cd-stress experiments, respectively.

4) Seeds of the two wheat cultivars were subjected to germination test to evaluate their salinity tolerance. The germination ability was studied in distilled water (control) and in 0.1–1.0 w/w% aqueous Na_2CO_3 solution (pH 9.1–11.2), involving 25 seeds in four replicates placed on filter paper in Petri dishes. The seeds were incubated in dark at 25±1 °C. The number of germinated seeds was daily counted, and the germination stress index (GSI) was calculated after 10 days. Salinity decreased the percentage of germination. The stress was more pronounced for cv. 'TC33', which showed a lower GSI, than cv. 'Mv. Hombár'.

5) Three sets of AMF inocula production were completed to ensure the sufficient amount of inocula for the main pot experiment each (10 g inocula per pot). For the propagation of *Funneliformis mosseae* (BEG 12), *Diversipora epigaea* (BEG 47) and *Rhizophagus intraradices* (BEG 144), the pot cultures of infected white clover hosts were grown in pumice or rhyolite-vermiculite mixture for three months in a growth chamber (25/17 °C, 18/6 hours light/dark). The AMF infectivity, such as the frequency (F%) and intensity (M%) of the mycorrhizal infection and the quantity of the arbuscula (a%, A%) were estimated microscopically according to Trouvelot's five-class system. High infection was detected in pumice, whereas lower infectivity was found in rhyolite-vermiculite mixture. After verifying AMF infectivity, the infected roots and the soil-born spores of the species were homogenized with the growth medium. The drought-stress (*Exp.1)* experiment was carried out with *Diversipora epigaea* (BEG 47), while in the alkalinity- and heavy metal stress (*Exp.2.,3.)* experiments *Rhizophagus intraradices* (BEG144) was applied for AMF inoculation.

6) The drought-stress experiment (*Exp. 1*) was carried out with a complete randomized block design with two wheat cultivars ('TC33' and 'Mv. Hombár'), two water treatments (well-watered and drought-stressed) and two AMF inoculations (non-inoculated and inoculated with *Diversispora epigaea*) in 15 replicates. Pumice was used as rooting media. Throughout the plant growing period, cv. 'TC33' showed significantly lower root capacitance (by 12–23%) than cv. 'Mv. Hombár'. Interestingly, the AMF inoculation resulted in a significant (10–23%) decrease in root capacitance; the difference were higher for cv. 'TC33'. Drought led to a 14–20% reduction in root capacitance. Microscopic investigation of roots showed high intensity and arbuscularity of AMF colonization with no significant differences among the treatments. Root dry mass of cv. 'TC33' was 37–52% smaller compared to cv. 'Mv. Hombár' in all treatments. The AMF inoculation and drought caused significant reduction in root dry mass by 29–42% and 9–35%, respectively, and in shoot dry mass by 26–45% and 6–37%, respectively. A strong linear relationship was found between root electrical capacitance and root dry mass for cv. 'Mv. Hombár' (R^2=0.792) and for cv. 'TC33' (R^2=0.865). Average mycorrhizal dependence (MD) showed negative values. Under drought conditions, the mean MD was higher for both cultivars. According to ICP-OES analysis, P, Cu and Mg content in plants generally increased by AMF colonization, but no differences were found for K and Fe.

7) The alkalinity-stress experiment (*Exp. 2*) was conducted using wheat cv. 'TC 33'. The plants were non-inoculated or inoculated with *Rhizophagus intraradices* (BEG 144) AMF strain, and exposed to different levels of alkalinity, such as control, and low, medium and high salt level with 1, 2 and 3 g Na_2CO_3 kg^{-1} substrate, respectively, in 15 replicates. A rhyolite-vermiculite (1:1) mixture was applied for plant cultivation. The substrate pH under alkaline stress was 8.58, 9.45 and 10.12 with corresponding ECe of 2.62, 4.18 and 5.27 dS m^{-1}. Alkalinity caused significant reduction in root capacitance (by 29–57%), root dry mass (by 25–69%) and shoot dry mass (by 20–70%), and the rate of decrease was related to the stress level. The AMF colonization had no significant effect on root capacitance and root and shoot biomass. The colonization indices proved to be strongly varied (from 0.1% to 47.4%), and decreased significantly with the increasing salt content. The low colonization intensity (M=12.8%; A=7.0%) in non-salinized plant roots indicated the low AM susceptibility of the cultivar. The ICP-OES analysis showed decreasing trends in shoot Ca content, shoot and root K content, and shoot Mg content by increasing alkalinity, whereas shoot and root Na content increased by salt exposure. No clear effect of AMF-inoculation on mineral contents were revealed, but shoot Ca and Cu content showed a reduction by AMF, presumably due to the increased uptake of Na-ions.

8) For Cd-stress experiment (*Exp. 3*), see the "Results in the last year", paragraph 2.

9) Root capacitance technique was concurrently applied with other conventional, non-destructive plant physiological methods, including the measurement of maximum quantum efficiency of PS II (Fv/Fm and Fv/F0), SPAD chlorophyll content and stomatal conductance in the youngest fully expanded leaves. In some cases, cultivar-specific differences (cv. 'TC33' vs. 'Mv. Hombár') in the photosynthetic parameters were revealed, particularly under stressed conditions, and this alterations were related to the changes in root dielectric properties. The AMF inoculation provoked a significant increase in stomatal conductance in well-watered plants, but no effect was detected under drought. AMF caused a significant increase in SPAD value only in well-watered cv. 'TC33'. We found that root capacitance method had the potential to complement plant physiological investigations to provide more information on plant root stress *in situ*.

10) Pot experiments were conducted to demonstrate that a complex dielectric characterization of root system, including the parallel, single-frequency detection of electrical capacitance, impedance phase angle and electrical conductance was an adequate method for monitoring root growth and some aspects of stress response. The capacitance progressively decreased with the increasing stress level due to impeded root growth. The stress resulted in a reduction in impedance phase angle. This effect was presumably due to physicochemical and morpho-anatomical changes of roots, which led to the alteration of apoplastic and symplastic current pathways and thus dielectric response. Principally the enhanced lignin and suberin deposition to exo- and endodermis was thought to be responsible for the dielectric changes.

By exposing plants to alkalinity stress, electrical conductance of the whole root system was unchanged in spite of the reduced root size, implying increased symplastic conductivity, which derived from higher electrolyte leakage through damaged root membranes. The stress-induced membrane injury was supported by determining root membrane stability index (MSI) from 0.1 g of root samples taken at the end of the experiment. Alkalinity stress decreased the MSI due to enhanced membrane permeability. MSI proved to be also appropriate for distinguishing wheat cultivars of different salinity tolerance, in accordance with root electrical and above- and belowground biomass measurements.

11) Glomalin related soil protein (GRSP) content of growth medium was measured according to the easy extractable (EEG) and total glomalin (TG) content methods. We were able to extract more GRSP with the TG method, but the values were highly variable in the experiment and did not differentiate from the blank sample. We can conclude that this method is not compatible with pumice and rhyolite-vermiculite mixture. We also evaluated the potential of triphenyl tetrazolium chloride (TTC) test for detection of stress-related damage of plant root in pot experiments. Two root sampling procedures, such as random fine-root sampling and root-segment sampling were applied. Drought or alkalinity stress decreased the TTC absorbance of root in some cases, but the differences were usually insignificant, chiefly due to the high variance of the measurement data. In other cases, increased TTC reduction was detected in roots of plants exposed to stress. Therefore, the TTC method was considered inadequate for discrimination of stressed and non-stressed roots.

12) The methodological development of root capacitance technique was an important goal of the project. We carried out pot experiments involving different plant species (bean, cucumber, maize, soybean, tomato and wheat) and soil types (pumice, arenosol and chernozem). The application of various statistical models supported that consideration of the detected dissipation factor (which indicates the efficiency of the capacitive charge-storage) in evaluation of root capacitance data was important for more reliable estimation of root mass, root length and root surface area. It was also verified that moistened substrates and soils exhibited much higher electrical capacitance and conductance than the roots. Consequently, impedance response (including parallel capacitance) of the root–substrate system is mainly determined by the roots and is not considerably influenced by the substrate (soil). Therefore, capacitance data are comparable even when soil electrical properties are different among the treatments, owing to *e.g.* addition of salt to the soil.

13) Our development also aimed to study the effect of soil water content on the detected root capacitance. This was an essential prerequisite for adaptation of the capacitance method to monitoring root activity directly in the field. At first, we conducted pot experiments with maize, soybean and wheat to establish functions between root capacitance and actual soil water content for the field soil. We showed an exponential increase in root capacitance with soil water content, and the relationships proved to be species-specific. The root activity of field-grown crops increased until flowering, and then decreased during maturity. This was consistent with data obtained with other methods. The results demonstrated that the root capacitance method could be useful for time-course studies on root activity under field conditions, and for comparing single-time capacitance data collected in areas with heterogeneous soil water status. We managed to show the positive effect of AMF inoculation on soybean root activity and biomass production in the field. Furthermore, the capacitance method proved to be useful to evaluate the efficiency of microbial (*Bradyrhizobium japonicum* nitrogen-fixing bacteria and AMF strains) colonization in soybean plants grown in pots under well-watered and drought-stressed conditions.

14) Several plant growth parameters, plant physiological properties, phenology, visual stress symptoms, photosynthetic activity and root vitality were measured in pot experiments involving wheat and salt-stress treatments. The results were evaluated with principal component analysis (PCA) to classify the parameters, which were effectively separate plants according to salt-stress treatments. Beside plant biomass or plant height, the electrical capacitance was the most reliable stress indicator. Impedance phase angle was among the medium strong indicators, similarly to root MSI.

15) The main conclusions are: (***i***) the root capacitance method is adequate for detection of root responses to stress factors; (***ii***) the technique can be integrated with other conventional plant physiological methods; (***iii***) complex dielectric characterization of roots is more informative about root stress response; (***iv***) detection of the dissipation factor contributes to an enhanced reliability of capacitance data; (***v***) the capacitance method is suitable for monitoring root activity in field-grown plants.

16) Dissemination of the results: The project results were published in nine original research articles (cumulative IF=16.895) in scientific journals, such as *Acta Physiologiae Plantarum*, *Agrochemistry and Soil Science*, *Biosystems Engineering*, *Frontiers in Plant Science*, *International Agrophysics*, *Plant and Soil*. The main findings were presented (orally and in posters) in international conferences, including: *4th International Conference on Biohydrology* (Almería, Spain – 2016), *3rd International Conference on Plant Nutrition, Growth and Environment Interactions* (Vienna, Austria – 2017), *Plant Biology Europe* (Copenhagen, Denmark – 2018), *General Assembly of European Geosciences Union* (Vienna, Austria – 2018), *12th International Conference on Agrophysics* (Lublin, Poland – 2018), *4th Wageningen Soil Conference* (Wageningen, The Netherlands – 2019). Two BSc thesis (University of Óbuda) has been written within the scope of the project.