Tripartite symbiosis: A promising mitigation and an efficient adaptation strategy for less nitrogen depended agricultural systems

M. Omirou, D. Fasoula, I.M.Ioannides
Agricultural Research Institute, Nicosia, Athalassa, 1516, Cyprus
Keywords: Nitrogen Fixing Bacteria, Arbuscular Mycorrhizal Fungi, tripartite symbiosis
Presenting author email: michalis.omirou@ari.gov.cy

Globally, agricultural soils are a major source of anthropogenic N_2O and NO emissions, and there is little doubt that the use of nitrogen (N) fertilizer and manure is driving the increase in atmospheric N_2O. Emissions can be reduced by reducing nitrogen-based fertilizer applications and applying fertilizers more efficiently, as well as following better manure management practices (Davidson, 2009). The inclusion of legumes in crop rotation schemes results in the reduction of nitrogen chemical fertilizers since part of the nitrogen needed for the subsequent crop is available through legume biomass incorporation. In addition, less nitrogen derived from chemical fertilizers is needed for legume crops since they can form symbiotic association with both arbuscular mycorrhizal fungi (AMF) and nitrogen fixing bacteria (NFB). The symbiosis between legumes and NFB involves the formation of plant organs called nodules where the reduction of N_2 into NH_4 take place and subsequently assimilated by the host plant. At the same time, legumes are associated with AMF under a complex hyphal network that affects the growth and plant development mainly by increased nutrient uptake. The efficiency of the system relies on complex interactions between the three major components of the symbiosis: plant, fungi and the bacteria. Several studies showed that both AMF and NFB are significantly interacted either positively or negatively and is crucial to develop specific and pre-tested inocula to enhance plant productivity. The aim of our study was to test the compatibility of a local *Bradyrhizobium* strain and two different mycorrhizal species in cowpea performance without nitrogen supply. In addition, we described the impact of dual inoculation on AMF assemblage structure in cowpea roots using molecular approaches to test whether this strategy is environmental friendly. Our hypothesis was that dual inoculation alters the AMF communities in cowpea roots as well as plant performance thus mitigating climate change by reducing the chemical fertilizers usage.

To test our hypothesis a factorial randomized design (inoculation x soil sterilization) was established including control plants (without inoculation), inoculated plants with AMF, NFB, their combination (AMF+NFB) and soil sterilization resulting in eight combined treatments replicated 5 times each. Total N, P, K, Ca, Mg, Cu, Zn and Fe where monitored in cowpea leaves in a 75 days experiment. In addition, we evaluated AMF colonization and nodule formation whereas AMF assemblage diversity was evaluated using PCR-DGGE approach.

Root and shoot dry weight biomass were significantly affected ($p<0.05$) by the treatments. Overall, shoot and root biomass of inoculated plants was significantly higher compared to the non-inoculated control plants. The different nutrient measured showed similar trend regarding the inoculation treatment. The general trend noticed was an increase of the majority of nutrients examined in the current study. In particular, inoculated plants showed significantly higher N (%), P (%) and K (%) content compare to control plants. A synergistic effect between AMF and NFB inoculation regarding K (%) content was noticed in plants grown under unsterilized soils. Similar trend was observed for Ca, Cu, Zn and Fe. None of the treatments established in this study had a significant effect on Mg content of cowpea leaves. Under unsterile conditions a threefold increase of root colonization was noticed in dual inoculated plants compare to control plants. Mycorrhizal colonization of control plants suggests an active local AMF community in the soil used. Dual inoculated plants also showed significantly higher colonization compare to AMF and NFB plants indicating a synergism between AMF and the *Bradyrhizobium* isolate. Molecular fingerprints showed a relatively rich profile in all treatment showing that native AMF colonized cowpea plants. Multivariate analysis indicated that sterilization was the main factor introducing alterations in AMF assemblage.

The implementation of dual inoculation showed a positive response of cowpea plants under N free fertilization schemes. Under these conditions plants exhibited higher plant biomass and N content suggesting increased assimilation of both soil and atmospheric N into plant biomass. At the same time dual inoculation increased the diversity of the local AMF community colonized cowpea roots, further supporting the environmental friendly character of such kind of strategies. We proposed tripartite symbiosis as a valuable and promising tool for mitigating climate change be part of a broader strategic approach for a Climate Smart Agriculture.

Davidson EA (2009) The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860. Nat Geosci 2(9):659–662.