Enabling barley production in arid soils by only exploiting the indigenous microbial biodiversity

Anna Rosa Sprocati [1], Patrizia Paganin[1,2], Chiara Alisi[1], Priscilla Casale[3],Giada Migliore[1], Flavia Tasso[1], Giuseppina Falasca[3], Tayel El- Hasan[4], Khaled Khlaifat[4] , Giovanni De Giudici[3]*

[1]ENEA-Casaccia, Rome, Italy. annarosa.sprocati@enea.it
[2]Cagliari University, Cagliari, Italy
[3]Sapienza University, Rome, Italy
[4]Mutah University, Karak, Jordan

Abstract summary

Mediterranean region is characterized by scarcity of natural resources such as water and productive soils. Agricultural practices, until now, have been based on the intensive use of fertilizers and water resources although limited. A knowledge-based bioaugmentation strategy that exploits the local soil biodiversity may provide microbial toolboxes to enhance target metabolic functions, in order to create optimal conditions for promoting specific crop growth under adverse conditions. This work is carried out under ERANETMED SUPREME project and presents the effect of bacterial bioaugmentation on plant growth under water stress. The bacterial formula JOR is composed by PGP autochthonous strains selected from an arid soil . The site is located in Al-Ghweir, an agricultural station for cereals cultivation, among which barley is the major crop. An exploratory pot experiment was set up to investigate the role of the microbial formula, in comparison with fertilisers, to support the growth of barley during the tillering phase. Results showed that under water stress only bacteria were able to sustain the vitality and the growth of barley. These results are confirmed by a second pot experiment replicated in Jordan, using the same microbial formula. The same bioaugmentation approach is applied in a two-year open-field trial now underway in the Al-Ghweir site.

Keywords: Soil functions, Biotechnology, Environmental sustainability, Resource efficiency.

Introduction, scope and main objectives

Mediterranean region is characterized by scarcity of natural resources such as water and productive soils. Agricultural practices, until now, have been based on the intensive use of fertilizers and of the limited water resources. As a result, local communities are experiencing soil impoverishment and water resources overexploitation. This work is part of the EU project "SUPREME" (Developing tools for sustainable food production in Mediterranean area using microbes) that aims at favouring the set-up of a sustainable agricultural production frame, addressing vulnerable communities living in semi-arid and arid areas in the Mediterranean region. The project objective is to combat impoverishment of soils and to reduce the use of water, fertilizers, and pesticides harnessing the microbiome potential to improve soil functions and promote plant growth. A knowledge-based bioaugmentation strategy that exploits the local soil biodiversity will provide microbial toolboxes to enhance target metabolic functions, in order to create optimal conditions for promoting specific crop growth under adverse conditions. This project addresses local communities distributed over 4 different Mediterranean countries (Italy, Jordan, Cyprus and Algeria) that have been increasingly challenged by water scarcity and by low agricultural productivity due to the scarce bio-geochemical functions of soils. Different soils and crops (tomatoes, legumes, such as fava beans and vetch, barely, wheat or high biomass leading grasses like sorghum, health crops and cereals) are considered in the test sites. The project's scope will be achieved through integration of state-of-the-art biotechnologies and leading-edge characterization, monitoring and modelling tools, accessed through an innovative, interactive web-based observation system. The assessment of soil characteristics, hydrological and climate conditions as well as type of crops will be the basis to measure the efficacy of the geo-biotechnology applied to improve crop production and to reduce negative effects of agricultural practices on the environment.

Microbial biodiversity at the soil level is a key issue to create optimal condition for crop growth. Microbes have already been proven to improve agriculture practice in controlled situations by reducing the need of water demand, fertilizers, and by improving crop resistance to pathogens.

Bacteria and fungi can effectively influence plant physiology, growth, defence mechanisms and nutrient uptake (van der Heijden *et al.,* 2008). Microorganisms associated with plant roots increase the absorption of nutrients, particularly phosphorus and nitrogen, enhancing the growth of crops and trees (Berg and Smalla, 2009; Gosling *et al.,* 2006). Inocula of bacteria can be used to improve soil functions and the resistance of plants to drought periods, and thus can contribute to reducing irrigation needs (Marasco *et al.,* 2012). Especially on arid and bare soils, bacterial inoculants can positively influence biogeochemical element cycles and formation of soil. Thus, they can help to reduce water, N and P requirements by augmenting the soil functions, and to reduce the effect of climate change on crop production.

The use of tailor-made consortia, functionally linking the indigenous microbial community with the chemical characteristics of the native soil and the needs of the target plant species, represents a successful strategy towards a rational selection of stable and reliable inocula (Sprocati *et al,* 2014a).The selection of indigenous strains can overcome the risk of introducing foreign microorganisms that could have a negative influence on the different components of soil biodiversity (Sprocati *et al,* 2014b).

In this work we present the set-up of a bacterial formula composed by some indigenous strains selected from the agricultural research station of Al-Ghweir in Jordan, used for cereals cultivation and especially for barley crop. The performance of the formula to support the growth of barley in conditions of reduced supply of water and fertilizers was tested in pot experiments prior to the field-scale step.

Methodology

Ten endemic botanical species were sampled with the soil in ten different points of the Jordan site. The plant and soil samples were collected in sterile bags and transported to the laboratory for further study. The rhizospheres of the ten samples were then mixed to create a composite sample and immediately processed for microbiological analysis.

Fifty grams of the rhizosphere sample were suspended in 500 ml of Sodium Pyrophosphate 0.1 percent W/V in a sterile flask and placed in an orbital shaker at 180 rpm for 90 minutes at room temperature. Biolog ECOPlates (Biolog Inc.,Hayward, CA, USA) were inoculated with the soil suspension (containing the dissolved bacterial community) in order to analyse the Community-Level Physiological Profile (CLPP).

The cultivable bacterial community was isolated spreading the soil suspension on three different solid agar media (TSA, MM and N-fix) in Petri dishes and incubating them at room temperature. The isolated strains were tested for plant growth promoting (PGP) traits. For strains identification, single colony DNA amplification was performed by polymerase chain reaction (PCR) with the Euroclone Gradient One thermocycler (Euroclone, Milano, Italy) using the universal Eubacteria primers 9bmf (5'- GAG TTT GAT YHT GGC TCA G -3') and 1512r (5'- ACG GHT ACC TTG TTA CGA CTT -3') to amplify the 16S rRNA gene (ca. 1500 bp).

The tailor-made microbial formula (JOR) was composed on the basis of the best-performing PGP traits, and was tested as bioaugmentation agent in a 20 days pot experiment with barley seeds and soil from the Jordanian experimental field. In the first pot experiment, the effects on the barley growth of the addition of the microbial formula JOR or a fertilizer (Diammonium phosphate) in optimal irrigation (100W) or water stress (25W =25 percent of the optimal) were compared.

The pot experiment was replicated in Jordan with the JOR formula (B), testing three irrigation levels: 100, 50, 25, under the same three conditions: just water W, W + DAP, W + B.

Results and discussion

The metabolic activity of the soil measured by CLPP showed high oxidation substrates kinetics as well as a high functional diversity index (94 percent, in terms of number of oxidized substrates), comparable to any agricultural soil.

Forty different colony morphotypes were isolated. The bacterial strains were distributed among the phyla of *Proteobacteria* (52.4 percent) *Actinobacteria* (38.1 percent), and *Firmicutes* (9.5 percent) (Figure 1).

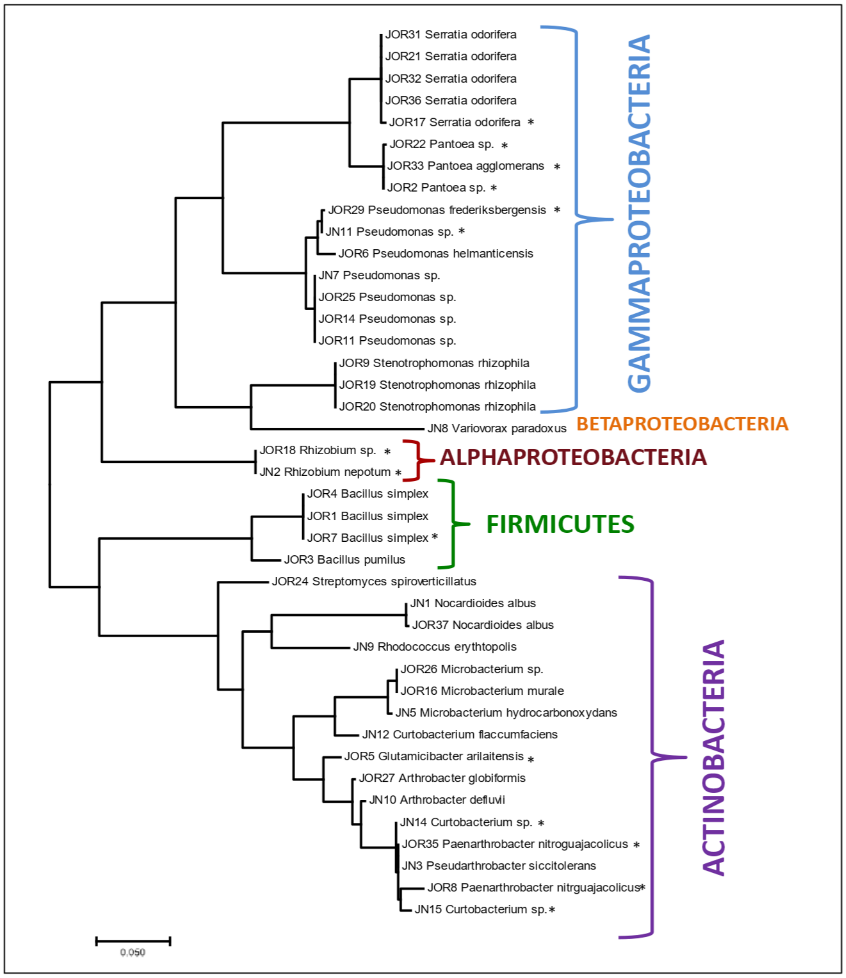

Figure 1: Neighbour-joining tree of 16S rDNA gene sequences showing the phylogenetic relationships of soil strains

The strains marked with * are those selected for JOR bacterial formula.

Some plant growth promoting (PGP) traits of the isolated strains were assessed: 88 percent were able to fix nitrogen, 29 percent to solubilize phosphates, 38 percent to produce siderophores and 16.7 percent to produce auxin. On the basis of these data, 16 of the best performing strains were selected to establish a bacterial formula that encompasses all the PGP functions and reflects the phylogenetic composition of the cultivable indigenous community.

The tailor-made bacterial formula (JOR) was tested as bioaugmentation agent in a 20 days pot experiment with barley seeds grown in soil comung from the Jordanian experimental field. Subsequently the formula was tested in a second experiment replicated in Jordan with Al-Gweir soil.

The effects of the treatments, either the microbial formula (B) or a fertilizer (DAP) in optimal irrigation (100W) and water stress (25W) conditions, were compared during the tillering phase of barley growth.

Both soil (CLPP) and plant parameters (aerial apparatus and roots system) were observed. The soil metabolic activity and the functional diversity were improved in the pots treated with bacteria, in both the conditions of optimal water supply and under water stress.

Under optimal water supply, the growth of plantlets (grams of fresh weight) was statistically equivalent (as induced from the t-test) with both bacteria and fertilizer and was greater than in the 100W control. Under 25W condition, only the presence of bacteria allowed plantlets to survive healthy, while the plantlets grown in the control and with fertilizer showed severe stress symptoms (Figure 2).

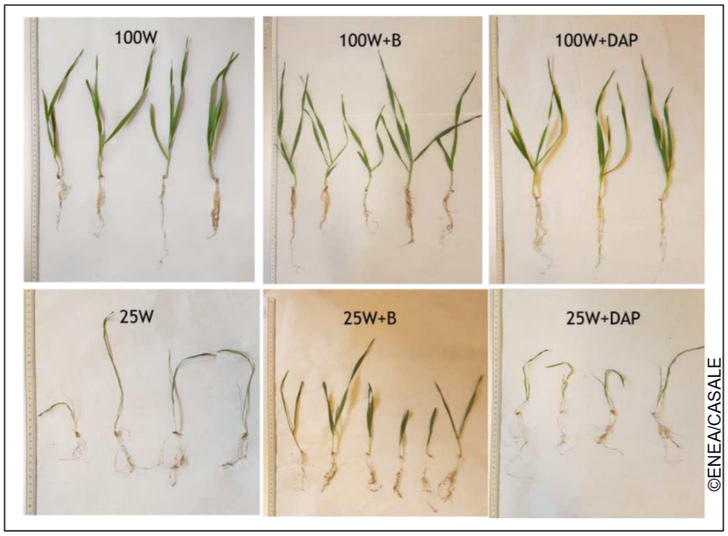

Figure 2: Exp 1-Comparison of the growth of plantlets in different condition: optimal irrigation (100W) and water stress (25W) with the addition of the microbial formula (B) or a fertilizer (DAP)

The root system was analysed by counting the number of adventitious and lateral roots and by measuring the length of the adventitious roots.

Anova and Student's t-test have highlighted as follows: in optimal water conditions the average number of adventitious roots was statistically equal for all treatments while under water stress the bioaugmentation improved the formation of adventitious and of lateral roots, compared to the other treatments; the bioaugmentation induced a different effect on the average length of adventitious roots compared to the other treatments, determining a shorter length in both 100W and 25W.

The second experiment confirmed previous results: under water stress (25W) only bacteria are able to support the growth of barley during the tillering phase; under optimal (W100) and intermediate (W50) water supply the microbial formula JOR can replace fertilizers (figure 3).

On this basis, a field trial for two harvest seasons was planned at the Al-Gweir site. The second year is currently underway.

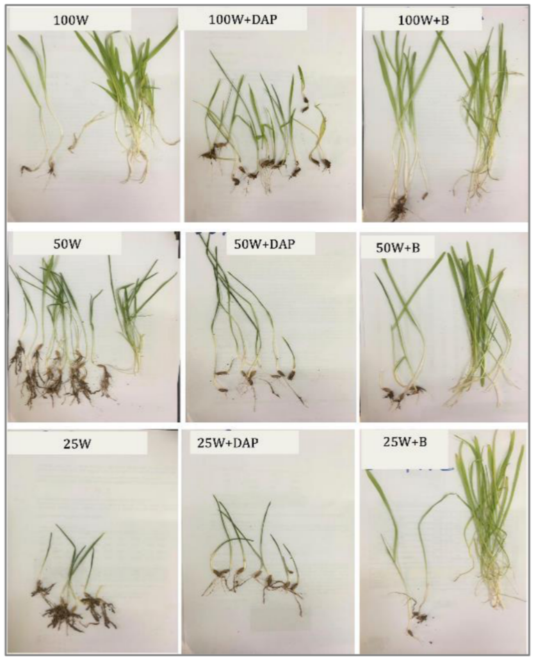

Figure 3: Exp 2. comparison of the growth of barley plantlets in different conditions : optimal water (100W), intermediate irrigation (50W) and water stress (25W), with the addition of the microbial formula (B) or fertilizer (DAP)

Conclusions

1. At Al-Ghweir agricultural research station the soil biodiversity retains an intrinsic microbial potential suitable to be exploited, in order to support the cultivation of barley in semi-arid soil and under water stress.
2. In the presence of optimal water supply, no major differences in the results are observed between the treatments, highlighting that the selected microbial formula JOR can replace DAP fertilizer, commonly used in cereal crops.
3. The bioaugmentation with the native bacteria showed to be the only treatment that allowed barley plantlets to survive under water stress, as well as maintaining good physiological performances.
   The bioaugmentation with JOR microbial pool improved the metabolic activity and the functional diversity of soil.

Acknowledgements

The work is supported by the project ERANETMED2-72-094 “SUPREME” (Developing tools for sustainable food production in Mediterranean area using microbes) co-financed by ENEA, Mutah University and Cagliari University.

References

Berg, G. & Smalla, K. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology*, 68(1): 1–13.

Gosling, P., Hodge, A., Goodlass, G. & Bending, G.D. 2006. Arbuscular mycorrhizal fungi and organic farming. *Agriculture, Ecosystems & Environment*, 113(1-4): 17–35.

Marasco, R., Rolli, E., Ettoumi, B., Vigani, G., Mapelli, F., Borin, S., Abou-Hadid, A.F., *et al.* 2012. A Drought Resistance-Promoting Microbiome Is Selected by Root System under Desert Farming. *PLoS ONE*, 7(10): e48479.

Sprocati, A.R., Alisi, C., Tasso, F., Fiore, A., Marconi, P., Langella, F., Haferburg, G., Nicoara, A., Neagoe, A. & Kothe, E. 2013a. Bioprospecting at former mining sites across Europe: microbial and functional diversity in soils. *Environmental Science and Pollution Research*, 21(11): 6824–6835.

Sprocati, A.R., Alisi, C., Pinto, V., Montereali, M.R., Marconi, P., Tasso, F., Turnau, K., *et al.* 2013b. Assessment of the applicability of a "toolbox" designed for microbially assisted phytoremediation: the case study at Ingurtosu mining site (Italy). *Environmental Science and Pollution Research*, 21(11): 6939–6951.

van der Heijden, M.G.A., Bardgett, R.D. & van Straalen, N.M. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, 11(3): 296–310.