PU-03 Understanding molecular, metabolic and phylogenomic events underlying Arbuscular Mycorrhizal Symbiosis: Scope for improving crop productivity

Shalini K. Vasan[0], Divya Srivastava[0], Xavier Conlan[1], Leena Johny[0], Pushplata Singh[0], Sangram Lenka[0], David Cahill[1], Alok Adholeya[0]

[1] Deakin University, Waurn Ponds,geelong, Australia
[0] Sustainable Agriculture Division, Teri-deakin Nanobiotechnology Centre, India

Arbuscular Mycorrhizal Fungi (AMF) are mutualists that colonize more than 80% terrestrial plants. This study uses *in vitro* hairy root cultures to analyze the regulatory control exerted over AMF by the plant hosts and non-hosts by integrating transcriptome, metabolome and phylogenomic analysis. We have screened 21 hairy root cultures using bright field microscopy and ink vinegar staining approach to identify a host (Tomato Roma) and a non-host (Tomato Grafter) of AMF *Rhizophagus irregularis*. Bi-compartmental studies comprising of a mycorrhiza established host (*Daucus carota*) was used to re-confirm non-host status of Tomato Grafter. Comparative transcriptomics of mycorrhized host, blank host and blank non- host revealed >2000 differentially expressed genes (DEGs, FDR 0.01). KEGG pathway analysis of DEGs was used as a reference for complete metabolomic profiling aimed at analyzing AMF-specific early signalling patterns distinguishing a host from a non-host tomato root culture. Top 50 DEG hits were functionally characterized *in silico* and among these DEGs most relevant 12 gene-targets were subjected to phylogenetic analysis in 7 hosts and 4 non- host plant species for identifying the pattern of gene convergence and/or divergence which could trace the evolutionary molecular patterns for adaptations favouring AM symbiosis in hosts. Further, we have proposed the concept of conditional non- host (Tomato Grafter) versus absolute Non- host (*Arabidopsis thaliana, Brassica rapa, Beta vulgaris* and *Nelumbo nucifera*). Understanding the molecular basis of AM symbiosis distinguishing a host from non-host might provide scope for introducing crops that are modified to attain better nutrient uptake, crop productivity, drought and pathogen tolerance. This study paves the way for application of mycorrhization in agriculturally relevant host plants.

PU-04 Co-inoculation of rice plants with nitrogen-fixing and indole-3-acetic acid (IAA)-producing endophytes: changes in physiological parameters of the host plant.

Anna Andreozzi[5], Pilar Prieto[1], Jesus Mercado-Blanco[2], Stefano Monaco[3], Elisa Zampieri[3], Silvia Romano[5], Gianpiero Valè[4], Roberto Defez[5], **Carmen Bianco[5]**

[1] Departamento de Mejora Genética, Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), Campus 'Alameda del Obispo', Avd. Menéndez Pidal s/n, 14004 Córdoba, Spain
[2] Departamento de Protección de Cultivos, Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), Campus 'Alameda del Obispo', Avd. Menéndez Pidal s/n, 14004 Córdoba, Spain
[3] CREA – CI, Research Centre for Cereal and Industrial Crops, 13100, Vercelli, Italy
[4] DiSIT, Dipartimento di Scienze e Innovazione Tecnologica, Università del Piemonte Orientale, Piazza San Eusebio 5, I-13100 Vercelli, Italy
[5] IBBR, CNR, Italy

To cope with the growing world population an increase in the production of the main crops for human nutrition, including rice, is now urgently needed. To achieve this goal, the expensive and polluting chemical fertilizers have already been overused. Nitrogen (N) is one of the primary nutrients limiting plant growth in agriculture. Biological nitrogen fixation (BNF) by diazotrophic bacteria, which reduce atmospheric N to ammonium using nitrogenase enzyme systems, accounts for 30-50% of the total N in crop fields. The area of BNF research has been expanded by the discovery of N-fixing bacterial endophytes in non-nodulating plants. In the last few years a wide diversity of bacteria associated with cereals have shown to possess the *nifH* gene coding for dinitrogenase reductase. This gene is genetically conserved and thus traditionally used as a marker gene to study the genetic diversity of diazotrophs in nature. To improve plant growth and yield the use of genetically modified diazotrophs or the co-inoculation with nitrogen-fixing and plant growth promoting bacteria has been proposed. We have previously reported that the strain *Enterobacter cloacae* RCA25-64, engineered to produce and release 36-fold more indole-3-acetic acid (IAA) than the wild type *E. cloacae* RCA25, showed increased *nifH* gene expression and nitrogenase activity in liquid cultures and inoculated rice plants. In the present study we analysed the effect of purified IAA on the nitrogen-fixing ability of *E. cloacae* RCA25. Co-inoculation studies were also carried out to test the ability of different wild type IAA-producing endophytes to enhance the *nifH* gene expression and nitrogenase activity in *E. cloacae* RCA25, preventing the use of engineered strains. Our results showed that *Herbaspirillum huttiense* RCA24 performed best. Improvements in nitrogen-fixation and changes in physiological parameters such as chlorophyll, nitrogen content and shoot dry weight were observed for rice plants (*Oryza sativa L.* cv. Baldo*)* co-inoculated with strains RCA25 and RCA24 in a 10:1 ratio. Based on confocal laser scanning microscopy analysis, strain RCA24 was the best colonizer of the root interior and the only IAA producer located in the same root niche occupied by RCA25 cells. Our data highlight that the assessment of location and distribution of the individual microbial components within the host plant tissues is fundamental to select bio-inoculants containing IAA-producer strains able to enhance nitrogen-fixation.