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Molecular aspects of arbuscular mycorrhiza: research for the roots finally blossoms

The readers of *Mycorrhiza* are well aware of the crucial role played by universal mycorrhizal symbioses, present in most natural and agricultural ecosystems, in many key processes, including nutrient cycling, conservation of soil structure and plant health. A remarkable review of current knowledge on several important topics is provided in the recent book *Mycorrhizal Symbiosis* edited by Smith and Read (1997). However, there are fields of research where progress can be so fast as to require update sources that are different from books or reviews. Molecular biology is one of these fields, and the recent progress that has been made in mycorrhizal research using molecular approaches provided impetus for COST ACTION 821 (a European network devoted to the study of arbuscular mycorrhizas – see Gianinazzi, *Mycorrhiza,* 1996) to organise a workshop meeting on "Gene Expression in Arbuscular Mycorrhizas". The main aim of the meeting was to bring together scientists from European laboratories which have developed molecular approaches to study arbuscular mycorrhizas and to provide an update on the progress achieved so far. A meeting had never previously been organised around this theme, but the development of molecular techniques based on the polymerase chain reaction, such as RT-PCR and mRNA differential display, together with more traditional techniques has opened possibilities for researchers to study molecular events involved in mycorrhizal establishment.

The meeting was held in Torino (22–25 May 1997) in Villa Gualino, located on the top of a hill with a splendid view of the mountains surrounding the city. About 60 participants attended, the majority from Europe (15 EU countries were represented), but some also from Israel and the United States. A significant proportion of the scientific community currently involved in molecular aspects of arbuscular mycorrhizas was therefore present. This short report summarises some of the many new or recently published findings that were presented in different sessions.

In the first sessions ("Towards the identification of symbiotic genes"; "Changes in gene expression during the establishment of arbuscular mycorrhizas"), evidence for similarities between legume-*Rhizobium* and mycorrhizal symbioses was provided at the genetic and molecular level. In collaborative research using promoter-GUS-transformed plants, David Barker and Vivienne Gianinazzi-Pearson (Toulouse and Dijon, France) have demonstrated that the early nodulin genes *MtENOD*11 and *MtENOD*12, which are expressed in *Medicago truncatula* within 24 h of *Rhizobium* inoculation or within 1 h after exposure to purified Nod factor, are also expressed in arbuscular mycorrhizas, although with a different pattern and level. Yoram Kapulnik (Israel) reported how two genes which are expressed early in alfalfa nodule development (*MsENOD*40 and *MsENOD*2) are also activated during mycorrhization, and how their expression seems closely related to a higher cytokinin level in tissues (van Rhijn et al., *Proc Natl Acad Sci USA,* 1997). Identification of legume mutants on the basis of their symbiotic phenotype is an important approach not only in the study of nitrogen fixation, but also in the investigation of gene expression in arbuscular mycorrhizas (Gianinazzi-Pearson, *Plant Cell,* 1996 ; Eliane Dumas-Gaudot, Dijon, France). The necessity to screen for other mutants was stressed by Martin Parniske (Norwich, UK) who showed that about 64% of Nod− mutants of *Lotus japonicus* also turned out to be Myc−. Another group of presentations focussed on strategies for identifying genes whose expression changes with arbuscular mycorrhiza development. In the first, Maria Harrison (Ardmore, Okla.) gave examples of two approaches. One was based on heterologous probes to identify phosphate transporters in *M. truncatula* colonised by *Glomus versiforme.* One of the transporters identified was of fungal origin (*Nature,* 1996), whereas two others were of plant origin. In the second approach, differential cDNA screening revealed six genes whose expression changed during the symbiosis, one of which resembled a phosphate-starvation-inducible gene (Burleigh and Harrison, *Plant Mol Biol,* 1997). Several other speakers followed who discussed the advantages and

the drawbacks of different molecular biology strategies (e.g. differential mRNA display, RT-PCR, cDNA screening) (Lucy Harrier, UK; Juan Manuel Ruiz-Lozano, Hélène Roussel, Vivienne Gianinazzi-Pearson, France; Michael Kaldorf, Germany). These techniques have also led to the identification of a number of plant genes expressed in arbuscular mycorrhiza, including those coding for important functions like nitrate reductase and channel-like proteins (Roussel et al., *Plant Sci*, 1997; Martin-Laurent et al., *Mol Gen Genet,* in press).

What is happening on the fungal side? Screening of the fungal genome is revealing interesting new results: Philipp Franken (Marburg, Germany) summarised some of the specific problems related to the study of the glomalean genome, where the amount of fungal material is limited or mixed with plant tissue. Using differential mRNA display, Nathalia Requena from the same group studied events that take place in the presymbiotic phase of the fungus, when germinating spores interact with other micro-organisms (e.g. *Bacillus subtiles*), while Laurence Lapopin took advantage of pea mutants which stop arbuscule formation to study fungal interactions with the plant. Researchers in Turin have used genomic libraries to investigate multiple- and single-copy genes in *Gigaspora margarita* and *Glomus versiforme* by PCR-based techniques. Three different chitin synthase gene fragments (Luisa Lanfranco, Marianne van Buuren) have been cloned and sequenced. The genomic library of *G. margarita* contains bacterial DNA, corresponding to an endosymbiotic *Burkholderia* living within spores (Bianciotto et al., *Appl Environ Microbiol,* 1996), and Daniela Minerdi presented preliminary results on the molecular analyses of these enigmatic endofungal bacteria. PCR-based techniques also allowed Nuria Ferrol (Granada, Spain) to identify fungal genes coding for plasma membrane H^+-ATPases which probably play an important role in nutrient transfer between the symbionts. There was great interest in a report by Lucy Harrier (Aberdeen, UK) on attempts to transform *Gigaspora rosea* using the gold particle bombardment technique; first results suggested that it was possible to introduce constructs carrying the GUS reporter gene into spores. An intense debate followed the presentation. Given the wide range of host plants, the use of transformed arbuscular mycorrhizal fungi could encounter problems with EU regulations on genetically modified organisms.

Lively and useful discussion among all the participants characterised the meeting. Everybody stressed the need to continue along the path of molecular studies of arbuscular mycorrhizas and their fungi and to move from a descriptive to a functional phase with the help of a coordinated and multidisciplinary approach.

Some concluding remarks. The comparison between arbuscular mycorrhizas and nodules is clearly very fruitful, and recent data suggest that there may be conservation of signal transduction pathways (van Rhijn et al., *Proc Natl Acad Sci USA,* 1997). In addition, it was interesting to see that *M. truncatula,* which has been recently proposed as a model system for nodulation (Cook et al., *Plant Cell,* 1997), seems to have all the prerequisites to become a good plant model for the mycorrhizal system. The gap between the knowledge acquired for the plant and the fungus is, however, striking: we still know very little about the genome and the functioning of glomalean fungi. The increasing efforts of many groups across the globe will hopefully throw more light onto the genetic make-up of these fascinating and enigmatic fungi.

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