Abstracts

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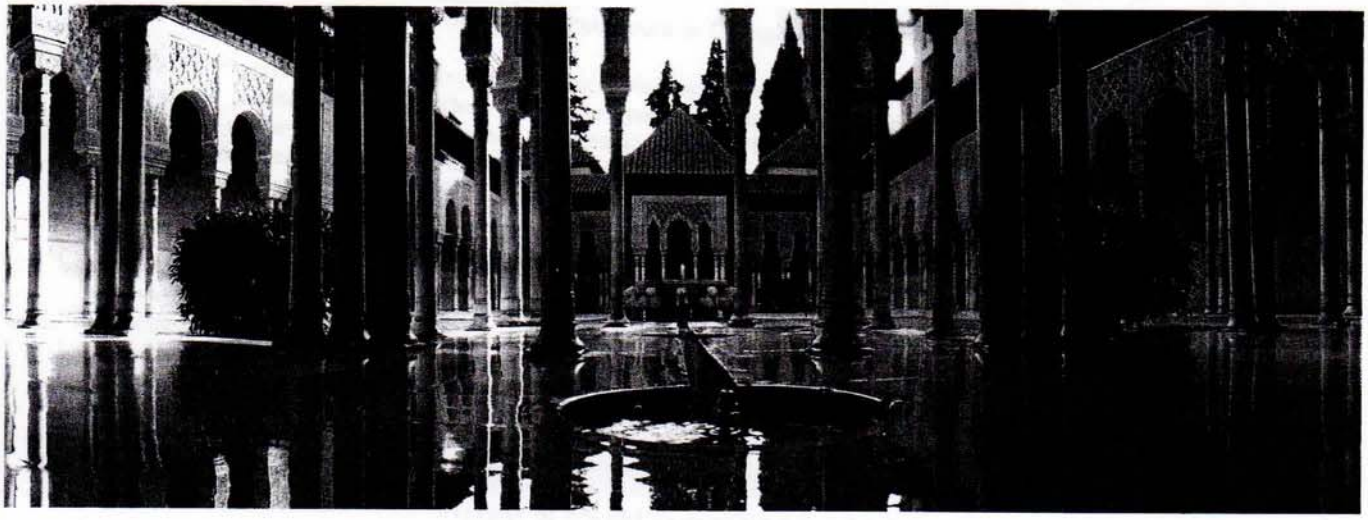

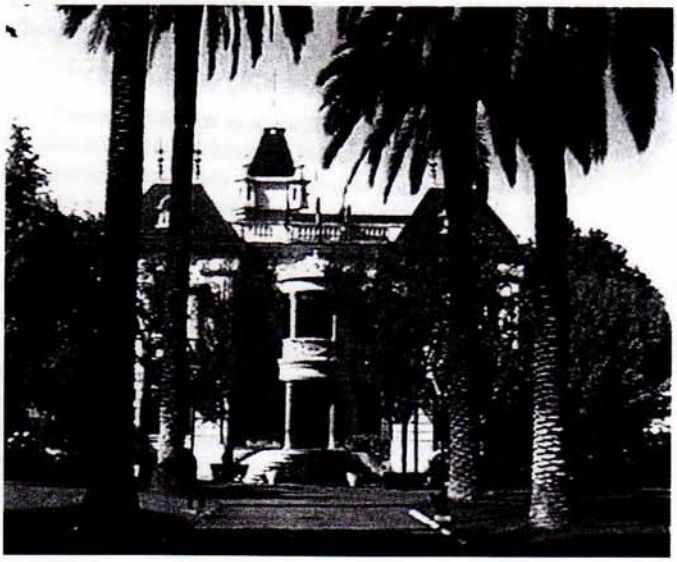

Estación Experimental del Zaidín
CSIC

High-throughput TILLING to identify symbiosis-related plant gene function in arbuscular mycorrhiza

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A large number of plant genes have recently been reported to be modulated during the establishment and development of arbuscular-mycorrhiza (AM). However, knowledge of molecular mechanisms involved in this symbiosis still remains limited. We have identified *M. truncatula* genes that are highly or only expressed during mycorrhizal interactions, using suppressive-subtractive hybridization and cDNA microarrays. A subset of these genes was selected to evaluate their possible role in AM formation and/or function using the reverse genetics approach of high-throughput TILLING (Targeted Induced Local Lesions In Genome), which is based on screening mutagenised plants for mutations in the selected genes. Genomic DNA, extracted from a second self-cross generation of *M. truncatula* plants obtained from EMS-mutagenised seeds, was pooled to screen a wide number of individuals (4500). Genes were PCR-amplified, cleaved by Cell enzyme digestion, products were analysed by LiCor gel-electrophoresis to detect mutations and sequences validated. First screening targeted a gene coding a protease inhibitor, which is uniquely expressed in AM roots. A missense mutation was detected which does not significantly affect the morphological aspects of the symbiosis. Expression of phosphate transporter and H^+ATPase genes are being profiled to assess whether the mutation interferes with symbiotic functions. Other mycorrhiza-related *M. truncatula* genes are presently being screened using the TILLING strategy.

Functional genomics in pea using virus induced gene silencing

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Viruses induce a defence system in plants, which targets the viral genomic RNA for degradation. Insertion of a host gene fragment into the virus genome, results in degradation of both the recombinant virus and the targeted host mRNA. This phenomenon, Virus Induced Gene Silencing (VIGS), has been shown to be a powerful tool for functional genomics as an alternative to transformation by RNAi constructs. It allows the analyses of essential genes and can be employed in plant species, which cannot be transformed. *Pisum sativum* and the tobravirus, *Pea early-browning virus* (PEBV) were chosen as model system, to transfer this technology to legumes. One of the advantages of developing a VIGS system for legumes is that it facilitates functional analyses of genes involved in the legume-rhizobium and legume-mycorrhiza symbioses. The PEBV-VIGS system was initially shown to cause efficient down-regulation of three non-symbiotic genes (1) and the nodulation reference gene, *PsSym35*, in pea (Constantin et al, work in progress). We are currently testing the reference genes, *PsSym9* (dmi3) and *PsSym19* (dmi2) of the common pathway for rhizobial and mycorrhizal symbioses, to study the potential of the PEBV-VIGS system to interfere with plant genes involved in mycorrhization. The PEBV-VIGS system and *PsSym9*, *PsSym19* silencing results will be presented. 1) Constantin et al., 2004, Plant J, 40, 622-631.

Suppression subtractive hybridization as a tool for identifying genetic diversity between mycorrhizal fungal genomes

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The Suppression Subtractive Hybridization (SSH) applied to genome is a technique allowing the isolation of DNA fragments that are present in one genome (tester) but not in another (driver). Genomic SSH has been widely used to detect genetic differences among bacteria. By contrast, to our knowledge, this technique has not yet been applied to mycorrhizal fungi. The aim of this work was to use such a technique to identify genetic diversity between mycorrhizal fungal genomes. First, to identify genomic regions involved in the evolution of the genus *Tuber*, we performed three experiments : 1) SSH experiments with phylogenetically non related species (*T. melanosporum-T. borchii*); 2) SSH experiments with phylogenetically more related species (*T. magnatum-T. borchii*) and 3) SSH experiments with two isolates of the same species (*T. magnatum* from Croatia and Piedmont). Second, to identify specific genomic regions linked to metal resistance in the endomycorrhizal ericoid fungus *Oidiodendron maius* we realized SSH experiments between a metal tolerant isolate and a non metal tolerant isolate. We demonstrate that SSH can be used to identify specific genomic regions between mycorrhizal fungal genomes at inter- and intra-specific level and in different experimental systems. In addition, results suggest that retrotransposons might have played a role in truffle evolution.