Gene families: Clarifying the role of individual members through real time qPCR expression studies, the case of *Mal d 1/ pr-10* genes in *Malus domestica*

Pagliarani G. [1], Paris R. [2], Tartarini S. [2], van de Weg E. [1]

[1] Plant Breeding, Plant Research International, Wageningen, The Netherlands
[2] Department of Fruit Tree and Woody Plant Sciences, University of Bologna, Bologna, Italy

In plant genomes many genes exist in multiple copies as members of gene families. The high number of similar genes and their co-localization on the same genomic region hamper the elucidation of the genetic base of related traits. We demonstrate how PCR based expression studies may be of help to clarify the role of the individual genes. This qPCR approach is robust, cheap and easy to apply. As case we use the Mal d 1/PR10 family of apple, composed by 31 genes. This family is involved in food allergy (referred to as Mal d 1) as well as in the defence system of the plant (referred to as PR-10) whereby the biological function is still unclear. We developed gene specific primer pairs for all the 31 genes. Specificity was validated both in silico and in vivo. Expression was studied firstly, on young apple leaves from susceptible and resistant genotypes after challenging with the fungus Venturia inaequalis. The observed differences in expression profiles (intensity & time-li nes) indicate differentiation in biological functions. Secondly, expression was studied on peel and flesh of fruits from cultivars that differ in allergenicity. Only part of the family was expressed in fruit and a clear differential expression was found between tissues as between cultivars. This allowed to trim down the number of genes that can be involved in apple allergy. We hereby demonstrate that qPCR based expression studies are useful to further clarify the role of individual members of large gene families.

New SNP markers for raspberry germplasm genotyping

L. Palmieri[1], Giongo L [1]

[1]Research and Innovation Centre. Fondazione Edmund Mach, Via E. Mach, 1 - 38010 San Michele all'Adige, Italy

Since 2003 more then 250 raspberry (Rubus idaeus) accessions were collected at FEM in order to generate a germplsam collection, which needed to be genotyped. To do so, ten new SNP markers were developed on 10 raspberry varieties, starting from SCAR markers transferred from diploid strawberries and apple (Sargent et al.,2007; Sargent et al., 2009). As results of sequencing analysis, new raspberry sequences of anthocyanidin synthase (ANS), lipoxygenase (LOX), ent-kaurene oxydase (EKO), cynnamyl alcohol deydrogenase (CAD), exspansin (EXP), dihydroflavonol 4-reductase (DFR), cytosolic ascorbate peroxidase (APX), spermine synthase (ACL5), alpha amylase (AMY), maltose transporter (MEX), soluble inorganic pyrophosphatase (SIP), polyamine oxidase (PAO), pectate lyase (PL) enzyme regions were obtained and the polymorphisms presence was

assessed. Finally, the germplasm genotyping was done on about half of the accessions number using the new markers. The data obtained were analysed with PowerMarker v.3.25, NTSY and STRUCTURE softwares to determine the genetic diversity of the whole collection and to obtain a preliminary cluster analysis. These molecular markers might be helpful not only for germplasm characterization but also, such as candidate genes, for mapping, synteny studies within the Rosaceae family and as a starting point functional to marker-assisted selection for raspberry breeding programs.

Silencing Mlo-like susceptibility genes to achieve broad-spectrum resistance to powdery mildew in apple

Pessina S. [1,2], Bai Y. [1], Malnoy M. [2], Schouten H. [1]

[1] Wageningen UR Plant Breeding P.O. Box 16, 6700 AA, Wageningen, The Netherlands,
[2] Fondazione Edmund Mach, Centro Ricerca e Innovazione, Department of Genomics and Biology of Fruit Crop, Istituto Agrario San Michele all'Adige–IASMA, Via E. Mach 1, 38010 San Michele all'Adige (TN), Italy

Powdery mildew is a disease caused by about 650 obligate biotrophic fungi species, capable of colonizing around 10,000 plant species, including many crops. A particular kind of resistance to this pathogen, characterized by durability, broad-spectrum effectiveness and recessive inheritance, was obtained for the first time in a barley mlo mutant. Mlo genes encode for susceptibility factors and are therefore called susceptibility genes (S-genes). Loss-of-function mutations in these genes lead to lack of susceptibility, which means resistance. Mlo genes have been found and studied in many plant species, including several crops, while there are no studies on fruit trees yet. Our work aims to study Mlo genes in apple, in order to achieve broad spectrum resistance. Previous works on different species (tomato, arabidopsis, grape) show how the expression levels of some of the Mlo genes increased in response to the inoculation with the pathogen. By means of a bioinformatic approach, we looked for mlo-like genes in apple genome and we tested their expression after inoculation with Podosphaera leucotricha. Three apple cultivars have been tested: Golden Delicious, Gala and Braeburn. Preliminary analysis conducted on Golden Delicious show that 4 Mlo genes are up-regulated because of the interaction with the pathogen; one of these genes co-localize with a QTL. Further we are silencing mlo-like genes using a RNA interference approach, in order to deprive the pathogen of its target and, therefore, to obtain resistance.

Complementary strategies for the breeding of cultivated strawberry for high level of antioxidants

A. Petit[1], Tallès K.[1], Jousseaume G.[1], Chartier P.[1], Denoyes B.[2]

[1]Ciref, Douville, France
[2]INRA, UMR 1332, Université Bordeaux, Villenave d'Ornon, France