

A short term scientific mission (STSM) within the COST863 (Euroberry)

STSM Scientific Report 2010

TITLE: Comparison of linkage maps obtained on the octoploid cultivated strawberry and QTL identification using these maps.

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Place: "Churrriana" (Málaga), Malaga – Spain

COST report

I spent four days at the IFAPA, Churrriana near Málaga and one day at the University of Málaga, which works with IFAPA on strawberry.

The working plan has been followed for comparison of linkage maps and QTL. However, considering the large genetic resources of IFAPA, time has been spent on this thematic.

Monday and Friday

GENETIC RESOURCES

Visit of greenhouse

Large *Fragaria* genetic resources are available. They include accessions from wild species, including diploid species and segregating populations.

Each accession is present in greenhouse with three copies planted in pots. These plants are renewed according to the development of the plant and the apparition of virus or fungus symptoms. In general, the removing of the plants and the plantation of new runners occur about every 3-4 years.

In parallel to this collection, the entire collection is duplicated by in vitro plants which are renewed once a year.

Discussion on the management of genetic resources

Traceability of the plant/genotype

After visiting the genetic resources, we discussed with Jose Sanchez Sevilla on the traceability of the plant/genotype, which is critical since strawberry is multiplied mainly by runners. Indeed, in a genetic resource collection, the same genotype can be introduced twice, or it can be multiplied along years. In order to distinguish the different introductions of a genotype, a number is given to each introduction whatever the genotype at IFAPA institute. Traceability has also to be considered with segregating populations, which are considered as genetic resources.

In addition, when seeds are obtained, it is important to consider that seedlings that come from these seeds can be genetically different because of heterozygosity. Therefore, a different number has to be given to each seedling. In IFAPA, they conserved only one seed from 'seed introduction' and then they multiplied the one plant they kept by runnering.

Detection of synonyms:

We were checking different lists from actual COST action 863 and previous 836 in relation to locate previous established synonyms and misspellings. Dr Sánchez Sevilla analysed new accession from Californian and Spanish breeding projects and we were checking main references as

- The book “*The Strawberry: History, Breeding and Physiology*” by George M. Darrow, (<http://www.nal.usda.gov/pgdic/Strawberry/darpubs.htm>).
- Brooks and Olmo Register of Fruit and Nut Varieties 3rd Edition by Michael W. Neff (Editor)
- The Strawberry Varieties list released from 1980 to 2007 at the web site from previous ISHS working group F12 “Strawberry culture and management” at <http://www.agraria.it/isf/attivita/fragola/ishs/index.htm>
- Searching US Patent Collection database for: “strawberry plant” with 292 patents at <http://patft.uspto.gov/>
- Searching the Community Plant Variety Office Website for: “*Fragaria*” as taxon in the database of “Applications and titles in force” with 320 registers at <http://www.cpvo.europa.eu/>
- The NCGR-Corvallis *Fragaria* Germplasm Catalogs at <http://www.ars.usda.gov/Main/docs.htm?docid=11324>

Tuesday and Wednesday

COMPARISON OF LINKAGE MAPS OBTAINED ON THE OCTOPOLOID CULTIVATED STRAWBERRY and QTL IDENTIFICATION

1/ Comparison of linkage maps obtained on the octoploid cultivated strawberry

In order to analyze if common QTL have been detected, we identified as first step the common markers.

Since different names were given to the same SSR, we first homogenized the name of the markers. This step was time consuming and it would be important in future to have similar Excel file for allowing quicker comparison.

Then we started to compare the linkage map. We identified a total of 21 common markers. In order to have better comparison, we plan to develop more common markers.

2/ QTL identification

Common traits have been analyzed into two different segregating populations (one population per country). These common traits were linked to fruit quality such as the total antioxidant. Some QTL seemed to be orthologous. However, we need better saturation of linkage maps with common markers to precise this point.

As an example: For firmness QTL detected in the HGII, it is difficult to conclude on the orthology of QTL between both segregating populations because we miss common markers around the QTLs.

Different QTL software can be used such as QTLcartographer (free) or MCQTL or MapQTL (both expensive). We did not fix the software that has to be chosen for comparison.

Thursday

VISIT AT THE UNIVERSITY OF MALAGA (UMA)

With Iraida Amaya, I visited Victoriano *Valpuesta* (valpuesta@uma.es), who is Professor in the “Departamento de Biología Molecular y Bioquímica, Facultad de Ciencias, Universidad de Málaga”, 29071 Málaga, Spain.

Professor Victoriano Valpuesta works on strawberry fruit development. Among his work, he isolated and characterized a fruit-specific promoter, which is critical for the manipulation of the nutritional value and quality of fruits by genetic engineering.

The activity of the *GalUR* promoter is restricted to the fruit, being strictly dependent on light.

I discussed with three PhD students or post docs of his lab.

Karen Merchante

She is working on two MADS involved in fruit development (ripening) and belonged to AGL2: Phylogenetical analyses were conducted.

Expression analyses were carried out on cv. Camarosa in transgenic plants using the construction: 35S-FaMADS5. No silencing lines using RNAi were constructed since there is a risk to silence all the AGL2 and to obtain a not viable line (or very weak line).

Three overexpressed lines were studied.

She has also worked on the role of ethylene in strawberry. This role has been well established in tomato but is there a role of this hormone in strawberry? The biosynthesis of the hormone starts with conversion of the amino acid methionine to S-adenosyl-L-methionine (SAM, also called Adomet) by the enzyme Met Adenosyltransferase. SAM is then converted to 1-aminocyclopropane-1-carboxylic-acid (ACC) by the enzyme ACC synthase (ACS).

She used the gene *etr1-1* (*etr* ethylene insensitive mutant in *Arabidopsis*) which is an ethylene receptor in *Arabidopsis* to obtain transgenic lines in strawberry. They showed bigger fruits and a faster regeneration.

To have better understanding of the ACC, transgenic lines insensitive to ethylene were obtained.

Finally, a role of ethylene in the achene development has been highlighted.

Fabiana Csukasi

She works on auxin and gibberellins.

Auxin/indoleacetic acid (Aux/IAA) family

Auxin promotes the degradation of Aux/IAA transcriptional repressors, thereby allowing auxin response factors (ARFs) to activate the transcription of auxin-responsive genes. Auxin enhances the binding of Aux/IAA proteins to the receptor TIR1, which is an F-box protein that is part of the E3 ubiquitin ligase complex SCF (TIR1). Using the TIR1 gene, Fabiana Csukasi obtained one allele in F x ananassa : FaTIR1 which is highly similar to AtTIR1. She studies the interaction between Aux/IAA and TIR1 in different conditions such as with auxine (+NAA) or without (-NAA) and with different constructions (using FaTIR1 and AtTIR1).

For gibberellins, she studies the expression of two genes: FaGID1b and FaGIDc (letters were given according to the phylogenetic tree she obtained using various sequences from GenBank). In expression analyses, these two genes showed similar patterns but FaGIDc was more expressed in receptacle than FaGIDb. Using treatment with GA3 (from 0 to 200 μ M) she showed that GID1b was very down regulated while GID1c and FaRGA were less down regulated.

Irene Aragüez Rey

She is working on Phenylpropenes such as chavicol, *t*-anol, eugenol, and isoeugenol, which are produced by plants as defense compounds against animals and microorganisms and as floral attractants of pollinators.

Two genes that presented the NADPH site were identified: FaMb and FaMa that represent two alleles and FaC. Transformants carrying these genes were analysed.

In a second step of analysis, transformed lines were analysed with CHS silenced in order to increase the substrate p-coumaryl.

OTHER POINT

Bin-set in octoploid segregating population

We discussed about the choice of a bin set for a segregating population of type is “pseudo test cross” that belongs to an octoploid species. The construction of a bin set needs a saturated linkage map which was not the case for the Spanish segregating population. For the French segregating population, this approach has already been started before the scientific mission. Validation of the bin set is running.

CONCLUSION

The week I spent in IFAPA was very exciting. The main result is the collaboration we initiated for detecting common QTL. Another important result is the procedure we managed for detecting synonyms. Discussion about traceability of vegetative material regularly multiplied as strawberry was also very useful.