Development of a TILLING platform in melon

Mireia Gonzàlez-To[1], Cristina Roig[2], Pere Arús[1], Fernando Nuez[2], Antonio Monforte[1], Belén Picó[2], Jordi Garcia-Mas[1]

[1]IRTA, Centre de Recerca en Agrigenòmica (CSIC-IRTA-UAB), Carretera de Cabrils Km 2, 08348 Cabrils, Barcelona, Spain.
[2]COMAV-UPV, Departamento de Biotecnología, Camino de Vera s/n, 46022 Valencia, Spain.

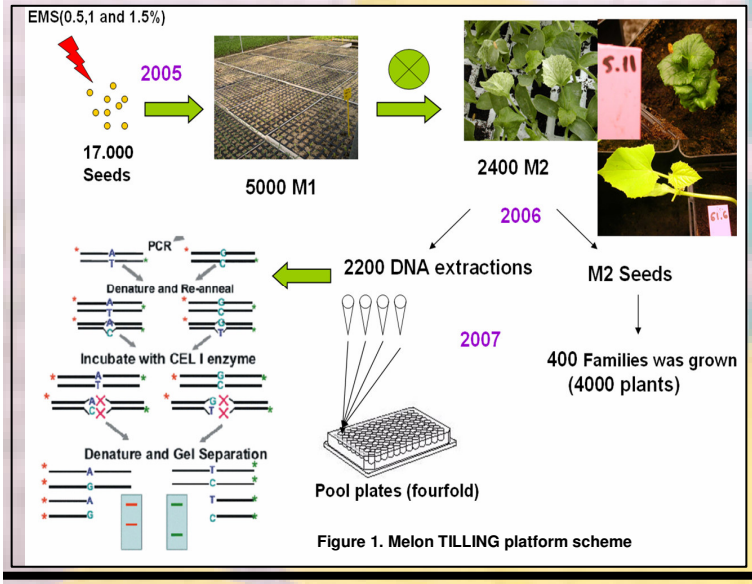

Figure 1. Melon TILLING platform scheme

In 2004 the Spanish Initiative in Genomics funded the MELOGEN project. Central to MELOGEN was the development of a TILLING platform for reverse genetics.
We have mutagenized 17.000 seeds from line M62-113, an inodorus melon type (*Cucumis melo L.*), to develop the TILLING platform (Figure 1). We have obtained 2.400 M2 families with enough seed (Figure 2). DNA extractions were performed for 2.200 M2 families and fourfold pool plates were used for the Tilling protocol with *CELI*. Amplicons from melon *Phytoene Desaturase* (PDS) and the eukaryotic initiation factor 4E (eIF4E) genes will be used to test the efficiency of the mutation procedure. Additionally, 400 M2 families were grown for phenotypic studies (Figure 1).

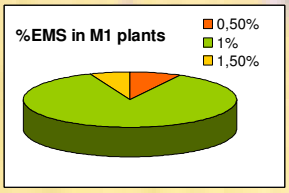

Figure 2. Number of M2 families that were obtained at three different EMS concentrations.

Dwarf. Leaf morphology


Dwarf. Leaf morphology


Leaf color


Color and plant morphology. Sterility

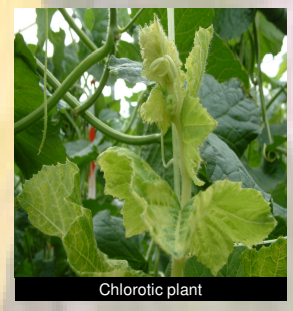
Chlorotic plant

Figure 3. Selected mutant phenotypes observed in M2 families during the phenotypic evaluation in 2007.

In order to test the mutation efficiency of the mutant population, we cloned the melon PDS gene (Figure 4).
An amplicon of 1.169 bp, covering the last three exons of the PDS gene, was selected for setting up the TILLING procedure in our lab using a LI-COR 4XXX instrument (Figure 5).

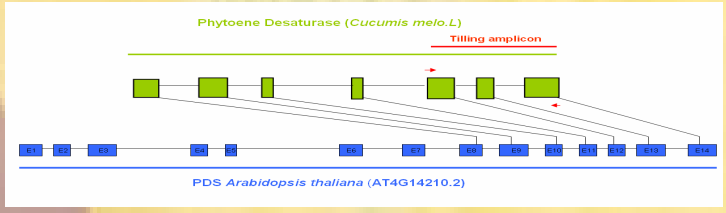

Figure 4. Comparison of the PDS gene structure between melon (green) and *Arabidopsis thaliana* AT4G14210.2 (blue). We cloned a 3' fragment of 4.368 pb from melon containing 7 exons and 6 introns. The three last exons were chosen for amplification for theTILLING procedure(red).

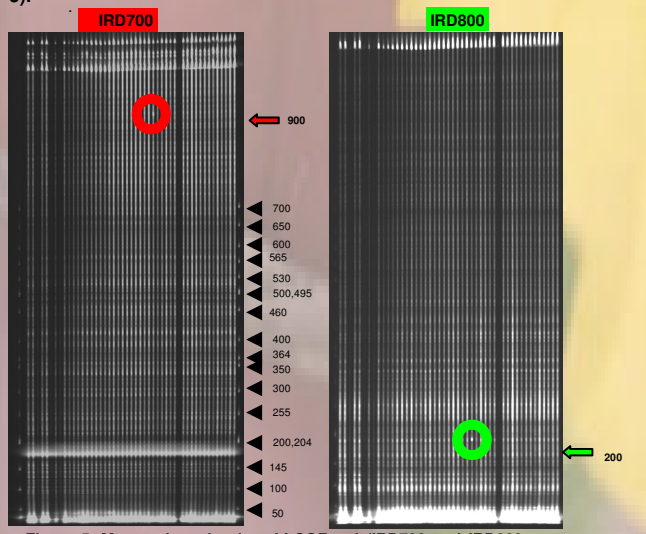

Figure 5. Mutant detection in a LI-COR gel (IRD700 and IRD800 channels) with the PDS amplicon (1169 pb). In each gel a 96-well fourfold pool plate is loaded. A possible mutant is highlighted in each channel (both DNA strands).

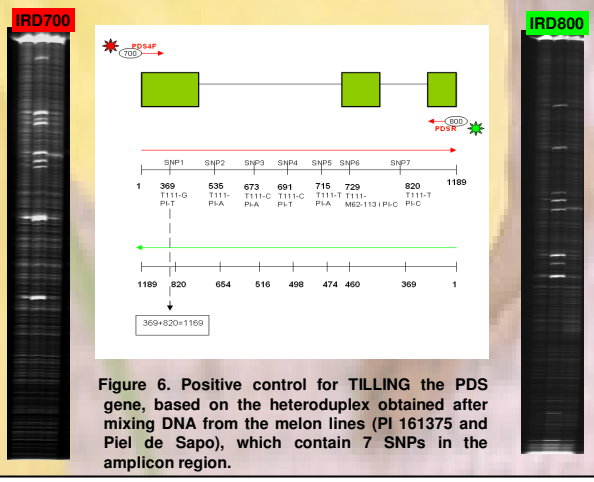

Figure 6. Positive control for TILLING the PDS gene, based on the heteroduplex obtained after mixing DNA from the melon lines (PI 161375 and Piel de Sapo), which contain 7 SNPs in the amplicon region.

FUTURE PERSPECTIVES

- To test all the M2 families with amplicons from different genes (PDS, eIF4E, elf(iso)4E) in order to estimate the mutation frequency. In preliminary experiments we estimated 5 mutants for the PDS gene in 480 M2 families, but we need to verify them.
- Once mutants are verified, check for possible phenotypes