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TILLING in tomato : A reverse genetic approach for tomato crop improvement – The French Platforms

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A limiting step in the characterisation of DNA sequences derived from genome sequencing projects as well as genes identified in transcriptome projects is the ability to study the function of these genes in a cellular context to relate the gene sequence to a phenotype. Such studies cannot be carried out only in model plants in which some agronomic characters cannot be characterised.

To bridge the gap between genomics and functional genomics in tomato, INRA has developed a high throughput gene knockout system as a reverse genetics tool. This system is based on the TILLING concept. This strategy has many advantages; first it permits the identification of mutations in target genes without the production of transgenic plants. Second, it can be automated in a high throughput system. Third, it is an efficient way to identify mutants in a specific gene with a leaky phenotype and consequently with a higher potential agronomic value.

Two EMS-mutant populations of 10 000 M2 families are exploited in this project. Genomic DNA was extracted, normalised, pooled and used routinely in TILLING screens. Mutations in targeted genes are identified through mismatch cleavage with the EndoI system (Triques et al., 2007)

We screened genes controlling flavonoid, carotenoid and lycopene content in the fruit, genes controlling fruit ripening and softening and virus resistance. For virus resistance, we screened for mutations in the eukaryotic translation initiation factors and identified alleles that confer immunity to potyviruses. Detailed analysis of a few examples will be presented.