

EARLY STEPS INTO SUNFLOWER TILLING

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Cultivated sunflower (*Helianthus annuus* L.) is one of the four most important annual oil seed crops in the world. Sunflower kernels produce a valuable edible oil rich of unsaturated fatty acids like oleic and linoleic, but it should also been considered as an important crop for biodiesel production, particularly in southern European countries.

The manipulation of fatty acid composition, by means of genetic tools, allows to obtain a product useful for nutritional or industrial purposes (high-oleic mutant line obtained by chemical mutagenesis).

To this aim, TILLING (Targeting Induced Local Lesions IN Genomes) (Comai *et al.*, 2006) represents a powerful tool to identify novel genetic variation in genes that affect key traits.

Seeds of an inbred line of sunflower were treated with four EMS concentrations for different times (6 and 3 hours) in order to establish the acceptable percentage of germination after the treatment. The best results for the germinability test (0.7% EMS) was chosen for the treatment. Mutagenized seeds have been grown to obtain 4211 M₁ plants. To avoid ambiguities caused by chimerism of mutant plants in the first (M₁) generation, M₁ plants were self-fertilized, and M₂ progeny from single seed descent was used for screening. 3899 M₂ plants were regularly observed and screened regarding visible and interesting mutant phenotypes.

For the initial set up of TILLING procedure, two SNP markers were used; different *Cell* concentrations (1:2, 1:4, 1:10 and 1:20 dilutions) and digestion times (15, 30 and 45 min) and different DNAs pooling (2-4-6-8-12-16 fold) were tested.

Heteroduplex analysis for mutation detection has been adapted to sunflower seeds. The DNAs from 771 M₂ plants are going to be analysed for a pilot screen on three genes (3-keto-acyl-ACP synthase II, oxoacyl synthase and acetyl CoA carboxylase).