Proceedings of the 53rd Italian Society of Agricultural Genetics Annual Congress
Torino, Italy – 16/19 September, 2009
ISBN **978-88-900622-9-2**

Poster Abstract – 7.58

SunTILL: A SUNFLOWER TILLING PLATFORM FOR FUNCTIONAL ANALYSIS OF GENES INVOLVED IN FATTY ACID BIOSYNTHESIS

SABETTA W.*, MONTEMURRO C.*, ALBA V.*, ALBA E.**, SUNSERI F.***, BLANCO A.*

*) Department of Agro-Forestry and Environmental Biology and Chemistry, section of Genetics and Breeding, University of Bari, Via Amendola 165/a, 70126 Bari, Italy
**) Department of Biology, Plant Protection and Agro-Forestry Biotechnology, University of Basilicata, Potenza, Italy
***) Department of Management of Agro and Forestry Systems, Mediterranean University of Reggio Calabria, Italy

sunflower, TILLING population, fatty acids

Plant oils represent renewable natural resources, useful in several economic sectors, from food to specialty chemicals and high added value products. Most marketed plant oils contain the most common C:18 fatty acids, i.e. stearic, oleic, linoleic and linolenic acids. Sunflower oil is considered a polyunsaturated oil for its high linoleic acid and low saturated fatty acid contents. The highly polyunsaturated nature of this oil makes it an interesting multi-purposes product. Until now, sunflower breeding programs aimed to the identification of genetic variants have been carried out and improved lines for stearic and oleic contents have been selected. Anyway, the narrow genetic variability, as a consequence of selection programs for high oil yield developed in the last century, suggests the need of innovative techniques to obtain new genotypes with a modified lipidic composition. To this aim, TILLING (Targeting Induced Local Lesions IN Genomes) represents a powerful tool to identify novel genetic variation in genes that affect key traits.

A kill-curve analysis was carried out on 8 seeds batches in order to discover the optimal experimental conditions (different EMS concentrations and exposure times). According to the germination rate, a treatment with 0.7% EMS for 6 hours was chosen and applied to the whole M0 population. Thereby, a stock of 30.000 seeds was mutagenized. To avoid ambiguities caused by the chimerism of M1 generation, the obtained 4211 plants were self-fertilized; finally, an M2 population of 3553 plants was developed and used to create the DNA TILLING library. Obtained plants were previously analysed for the main phenotypic characters, following the guidelines arranged by IPGRI (International Plant Genetic Resource Institute, Italy), and a photographic catalogue was created. From each plant, three adult leaves were collected, dried, stored and used for subsequent DNA extraction. Since this step represents a critical point of the TILLING technique, different extraction methods were tested. Moreover, a microsatellite analysis was carried out in order to evaluate the presence of any Taq polymerase inhibitors. To set up the TILLING technique on sunflower genome and to reduce the background from LiCOR gel imagines, a preliminary CelI-nuclease mismatch cleavage assay, with different enzyme concentrations (1:2, 1:4, 1:10, 1:20 dilutions) and different digestion times (15-30-45 minutes), has been performed. The best CelI activity resulted for the highest dilution and the longest incubation time, so these conditions will be applied for the screening of the whole population. Expressed Sequences TAGs (ESTs) of genes involved in fatty acids biosynthesis (Acetyl CoA carboxilase, 3-chetoacyl-ACP synthase type II and III and so on) have been website-selected and analysed by bioinformatic tools, in order to

reconstruct the gene models and to identify the genomic regions most suitable for TILLING screening. PCR conditions for each primer pairs were optimized to obtain an extremely specific product with optimal length for TILLING analysis (700-1500 bp).

Thereby, based on the production of a mutagenized population and on an high throughput identification of EMS-induced point mutations in specific target genes, TILLING represents a powerful tool to produce novel genetic variation and to study the gene function in genotypes of agronomical importance, without the creation of transgenic material.