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IDENTIFICATION AND CHARACTERIZATION OF INDUCED MUTATIONS IN A SUNFLOWER TILLING PLATFORM

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The TILLING strategy has been successfully applied to the sunflower genome in our laboratory ("sunTILL platform"). The interest was first focused on some key enzymes of the fatty acid pathway, because of the interest in increasing the nutritional value of sunflower oil by the reduction of the ratio of saturated to unsaturated fatty acids. Moreover, *P. halstedii* is one of the most dangerous pathogens that affects sunflower cultivation in the Mediterranean area. Therefore the availability of a stable and effective system, as genetic resistance, for the pest-control results of prime importance. Thereby, a pilot assay on 1,152 sunflower M_2 lines was carried out by the reverse genetic screening of four genes: the *kasII* and *kasIII* genes, respectively codifying the isoforms II and III of the β-keto-acyl-ACP-synthetase; the *fad2-1* gene, encoding the enzyme responsible of the converting reaction of oleic acid to linoleic acid; the *AY490791* gene, involved in *P. halstedii* resistance.

Since few genomic sequences are publicly available for sunflower, the reverse genetic screening was preceded by an accurate reconstruction of candidate gene models, by the amplification and the subsequent sequencing of short overlapping fragments. For each candidate gene the most promising region for TILLING analysis was thereby identified. In this way, new primer pairs flanking this region were set on the intronic sequences, with the aim to improve the screening efficiency on the coding regions.

In the pilot assay, nine mutant lines have been totally identified. The four mutations scored in the *kasII* gene were homozygous; three of them were localized in introns, while one caused a G/T transversion, resulting in a premature stop-codon (E139*). No mutant lines were identified for the *kasIII* gene. In the case of *fad2-1* gene, three mutations were identified: one resulted in a missense change (F26L), a second caused a silent change (R46=) and a third was situated in the non-coding region. The *AY490791* gene screening revealed two mutations, both localized in non-coding sequence. Each mutant line was then confirmed by sequencing and genotyped by microsatellite markers to exclude any individuals originating from cross-pollination events. The results of this first reverse genetic screening translated into an average mutation frequency of 1/475 kb.