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DEVELOPMENT OF A TILLING POPULATION IN DURUM WHEAT CV. AUREO

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During the last two decades, DNA sequencing has led to availability of gene sequences in key species encouraging the development of alternative strategies to create novel alleles in specific genotypes of crop species. One of these new emerging technologies is the TILLING (Targeting Induced Local Lesions In Genomes) that combines random chemical mutagenesis with high-throughput discovery of the induced mutations in target genes.

In the present study we developed a new wheat TILLING population by treating seeds (cultivar Aureo) with ethyl methanesulfonate (EMS) for mutagenesis. The first experiment was conducted to determine a suitable dose of the EMS needing to achieve at least the 50% of seed survival. Eight sets of 100 seeds of cv. Aureo were treated with eight different doses (0.45, 0.50, 0.55, 0.60, 0.65, 0.70, 0.75, and 0.80) of EMS. It was observed that the survival rate of plants with EMS doses 0.60% was closest to the targeted percent of survival. Thus, the 0.60% treatment was chosen for the development of the TILLING population. To evaluate the mutation densities of the TILLING population represented by 4608 M_2 progenies, we used both a phenotypic and molecular approaches. The phenotypic analysis of M_2 families has been conducted for the determination of germination index, mean time of germination, coleoptiles length and for some morphologic characters (colour of coleoptiles, absence of roots and absence of coleoptiles). Of the total of 4608 M_2 progenies, a first screening was carried out on 200 families (10-20 seeds for each family). An high percentage of the mutant phenotypes was scored because of EMS treatment, indeed comparing to the wild-type genotype, most of the M_2 families presented a delay in germination, reduced power of germination, reduced coleoptiles length, different colour of coleoptiles, variable roots number, and absence of coleoptiles. The molecular approach for revealing the mutation density of TILLING library consisted, instead, in the characterization of two genes involved in carotenoid biosynthesis: *LYC-ε* and *LYC-ß* genes. Both enzymes are involved in the different cyclization of lycopene molecules: *LYC-ε* catalyzes the formation of ε-ε rings in a side of lycopene chain producing α-carotene molecule, while *LYC-ß* generates ß-ß rings to the end of chain producing β-carotene molecule. Non functional genes of *LYC-ε* gene is expected to block or reduce the metabolic flux into the ε branch leading to lutein. While null or less efficient alleles of *LYC-ß* gene is expected to block the next steps of oxygenation, thus leading to accumulation of upstream compounds lycopene towards α-carotene production. The sequences of the two genes has been well characterized and genome-specific primers were designed in order to screen the whole wheat TILLING population.