

TILLING STRESS-RELATED GENES IN BARLEY

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Targeting-induced local lesions in genomes (TILLING) is a generally applicable reverse-genetics strategy providing an allelic series of induced point mutations from a population of chemically mutagenized individuals (McCallum *et al.* 2000). At DiSTA-University of Bologna, a sodium azide- mutagenized population of barley (variety “Morex”) has been developed for identifying mutations in specific genes using the TILLING procedure. The population is currently constituted of ca. 5,000 M₃ families. A protocol based on DNA heteroduplex detection on Licor DNA sequencers was successfully implemented. DNA samples were so far isolated from ca. 3,200 M₂ plants organized in 8-fold pools. Molecular screening for mutations has already been completed for four agronomically important genes (*HvCO1*, *Rpg1*, *eIF4E* and *NR*) with the identification of an average of ca. 6 mutants per gene and an extrapolated rate of 1 mutation every 470 kb over the population. Several (ca. 68%) of the identified TILLING mutations were missense implying a change in amino acid sequence of the protein and therefore possible effects on the phenotype.

Phenotypic screening of whole field-grown M₃ population showed a high frequency of morphological alterations (ca. 25% of the total number of plants) which were briefly described and preliminarily classified. We particularly addressed the classification of necrotic and ear morphology mutants which represented ca. 11% and 28%, of the total number of morphological variants observed, respectively. M₄ seeds are being collected and will be properly stored for future phenotypic rescue and/or for forward genetic screening. Furthermore, a web-based database for easy information storing and retrieving, external-users accessibility and easy cross reference with molecular data produced with the TILLING procedure is being prepared.