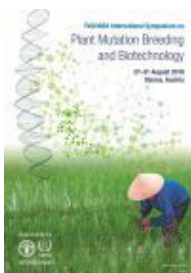


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## BARLEY MUTANT RESOURCES FOR FUNCTIONAL ANALYSIS OF GENES INVOLVED IN RESPONSE TO DROUGHT STRESS

TILLING (Targeting Induced Local Lesions IN Genomes) is a reverse genetics strategy that combines the classical mutagenesis and a high-throughput identification of mutations within a gene of interest. We have created a TILLING platform, HorTILLUS (*Hordeum vulgare* – TILLING – University of Silesia) and used it for functional analysis of genes involved in ABA-related response to drought stress in barley. Double-treatment of spring barley cv. 'Sebastian' with sodium azide and MNU (N-methyl-N-nitrosourea) was used for generation of HorTILLUS population. Mutation identification in target genes: HvCBP20 (Cap-binding protein20), HvERA1 (Enhanced response to ABA1) and HvABI5 (ABA insensitive5) was performed in M2 generation, using specific endonuclease and LI-COR Sequencer or Fragment Analyzer. All three target genes are related to ABA-directed response to abiotic stresses. The identified mutants were evaluated for physiological parameters and transcriptome activity under drought stress. The HorTILLUS platform comprises more than 9,600 M2 plants from which DNA was isolated, seeds harvested and deposited in seed bank. The average mutation density in the HorTILLUS was found to be 1 mutation per 477 kb. Here, we report three drought-tolerant barley mutants carrying missense mutations in target genes: hvcbp20.ab, hvera1.b and hvabi5.d. All of them were characterized by a higher relative water content (RWC) after drought stress compared to 'Sebastian'. Additionally, hvcbp20.ab showed a changed epidermal pattern and lower stomatal conductance, hvera1.b exhibited better photosynthesis efficiency, while hvabi5.d showed faster stomatal closure and better membrane protection. Transcriptome analysis integrated with observed phenotypic traits allowed to draft possible mechanisms in regulation of drought response. The HorTILLUS population proved to be a useful resource, both for functional genomic studies and generation of barley mutants with required phenotypic changes, including tolerance to drought.

### Country or International Organization

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