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# EcoTILLING analysis of candidate genes for drought tolerance in barley

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The development of new barley varieties with improved drought tolerance is one of the main breeding objectives in Hungary, because drought is a main factor limiting the yield of cereals including barley. The development of stress-tolerant varieties with yield stability will help to reduce the risk in barley cultivation. The probability of a successful breeding for drought tolerance is largely dependent on the understanding and knowing of the genetical factors that regulate this highly quantitative trait.

In this project drought tolerance related candidate genes were analyzed by using the EcoTILLING (Comai et al. 2004) technology. EcoTILLING is a high throughput, low cost technique for rapid discovery of polymorphisms in natural populations. It is a variant of TILLING (Targeting Induced Local Lesions IN Genomes), (Colbert et al. 2001) is based on certain PCR steps, such as the formation of heteroduplexes and a nuclease cutting DNA mismatches. It allows both SNP discovery and haplotyping through the sequencing of unique haplotypes.

We have established the EcoTILLING technology in order to identify putative SNPs and small INDELs in a set of 96 barley cultivars and wild germplasm containing drought tolerant and sensitive genotypes (cultivars and landraces and wild relatives) collected worldwide. Target genes were selected based on studies dealing with drought tolerance. Candidate genes are dedicated as potentially involved in the variation of key agronomic traits. The identification/determination of natural genetic variation in candidate genes can provide valuable information about gene function.

In this pilot study 7 drought related barley candidate genes were screened. In the case of 4 genes overlapping amplicons were designed, trying to cover the whole gene in the genetic diversity screens. For these 4 genes also more easily detectable markers were created after the evaluation of the obtained haplotypes sequences allowing distinguishing the main haplotypes. In the case of 3 candidate genes only one primer pair was planned based on the available mRNA sequences.

EcoTILLING reactions were performed in one-well format using fluorescently labeled nucleotides and after heteroduplex formation ENDO-1 and Cel-1 treated products were visualized on an ABI PRISM 377 sequencer.

Untill now more than one hundred unique haplotypes identified for 9 genes (HvARH1, HvDREB1, HvDRF1, HVA1, HvNHX1, HVP1, HvPPD-H1, HvNUD and HvPRPX) in 18 EcoTILLING screens. It's including more than 1.5 million base pairs sequence. The number of haplotypes identified for screened amplicons ranged from 2 to 9. Overall, 185 single nucleotide polymorphisms and 46 insertions/deletions were finded with a mean of 1SNP/92 bp and 1INDEL/372 bp genomic sequence.

In four candidate genes (HvARH1, HVA1, HvDRF1, HvSRG6) a set of informative polymorphisms were converted into easily detectable genetic markers, which are useful for marker assisted selection.

The obtained sequence/haplotype information will be used for development of further easily detectable genetic markers (potential „within gene marker") useful for linkage mapping and Marker Assisted Selection. Functional alleles can be directly integrated in barley breeding programs for improvement of drought tolerance.

Colbert, T, Till BJ, Tompa R, Reynolds S, Steine MN, Yeung AT, McCallum CM, Comai L, Henikoff S (2001) High-throughput screening for induced point mutations. Plant physiology 26:480-484.

Comai L, Young K, Reynolds SH, Codomo C, Enns L, Johnson J, Burtner C, Henikoff JG, Greene EA, Till BJ, Henikoff S (2004) Efficient discovery of nucleotide polymorphisms in populations by EcoTILLING. Plant Journal 37:778-786.

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# Application of synthetic antisense oligodeoxynucleotides in higher plants

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Antisense oligonucleotides *i.e.* short, synthetic strands of DNA or analogs that are complementary to a target DNA or RNA along with short interfering RNAs (siRNAs), 21-25 bp dsRNA with dinucleotide 3' overhangs became a powerful tool for the functional genetics. These structures are designed to interfere with nucleic acid metabolism, most preferentially with transcription, translation or splicing. Sequence-selective inhibition of gene expression is applied extensively for elucidation of complex gene expression patterns or validation of results gained from high throughput genomic experiments such as DNA-arrays. Common and attracting features of both antisense oligo and siRNA are that they act in a dose-dependent reversible manner, while no genetic transformation is required.

Though the sequence-selective gene-silencing by these synthetic oligonucleotides is quite general phenomenon for all organisms, only few applications are described for plant systems. We elaborated several methods for the introduction of oligonucleotides into monocot and dicot plants. By fluorescent labeling, we examined the uptake efficiency and inner traffic of these molecules, and determined the optimal conditions of treatment.