Ph.D. theses

COMBINATION OF PHENOMICS AND GENOMICS APPROACHES IN THE CHARACTERIZATION OF BARLEY DROUGHT TOLERANCE

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Introduction

In the World and in Hungary the drought stress is a main factor limiting the yield of cereals including barley. Accordingly the development of new barley varieties with improved drought tolerance is one of the main breeding objectives. The probability of a successful breeding for drought tolerance is largely dependent on the understanding and knowing of the genetical factors that regulate this complex and highly quantitative trait.

Genetic improvement of complex traits such as drought adaptation can be advanced by integration of genomic and phenomic approaches. The determination of natural genetic variation in candidate genes can provide valuable information about gene function. We aimed to understanding of the genetic background of the drought tolerance of barley via the combination of phenomic and genomic results. Using genotype-phenotype association studies allows the validation of the involvement of candidate genes in the drought tolerance.

Genomic data were obtained from barley drought related candidate genes using EcoTILLING technology as a polymorphism discovery tool. Phenomic data were generated in the greenhouse by the Complex Stress Diagnostic System.

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) is based on certain PCR steps, such as the formation of heteroduplices and a nuclease cutting of the DNA mismatches. It allows both polymorphism discovery and haplotyping through the sequencing of unique haplotypes.

We have established a set of 96 barley genotypes, which contains drought tolerant and sensitive genotypes /cultivars, ecotypes and wild relatives/ collected worldwide. Candidate genes were selected based on studies dealing with drought tolerance (gene expression and QTL mapping studies, transgenic approaches).

The EcoTILLING reaction included a PCR amplification of targets with fluorescently labelled nucleotides. After heteroduplex formation amplicons was

digested in „mismatch" positions by CEL1 single-strand specific endonuclease and screened on ABI PRISM 377 sequencer. Unique haplotypes were sequenced for each gene is both forward and reverse direction.

Morphological, physiological and drought tolerance related agronomic parameters were monitored by digital photography and thermal imaging using a semi-automated phenotyping platform called Complex Stress Diagnostic System.

Research objectives

1. Our main goal was the analysis of natural variation of drought related candidate genes in barley using the EcoTILLING technology as a polymorphism discovery tool.

2. Developing easily detectable genetic markers (potential „within gene marker") based on overlapping haplotype sequences, which allows distinguishing the main haplotypes showing differences in amino acid sequence.

3. Characterization of drought responses of barley subset by monitoring a set of morphological and physiological and agronomical traits under control and stress conditions using Complex Stress Diagnostic System in greenhouse.

4. We aimed to find potential links between phenotypic stress parameters and the identified haplotype composition in the drought related candidate genes of the tested genotypes. We purposed to establish a basic methodology for combination of phenotyping and haplotyping data in characterization of drought responses of barley.

Materials and Methods

Plant material:

A set of 96 barley genotypes containing drought tolerant and sensitive genotypes were selected for EcoTILLING. Genotypes were obtained from various sources.

Candidate gene selection:

Candidate genes were selected based on studies dealing with drought tolerance. Sequence information was necessary for the primer design.

Genomic DNA isolation:

Genomic DNA was extracted from 10- to 14-day-old seedlings. Following concentration check the aliquots concentrations were normalized and DNAs were mixed equimolar amounts with reference DNA (Which was isolated one of the genotypes: called GK Rezi).

Primer design:

Primers for EcoTILLING were designed using the software Primer3 based on available genomic or mRNA sequences.

PCR reaction and heteroduplex digestion:

The first step of EcoTILLING reaction is the PCR amplification of targets with fluorescently labelled nucleotides. Heteroduplex formation containing the denaturation and reannealing of amplicons and nuclease digestion of DNA mismatches with single-strand specific (mismatch cleavage activity) nucleases either with Cel1 or ENDO-1 enzymes.

Fragment detection:

Treated products were visualized on gel-base sequencer.

Haplotype identification and sequencing:

According to gel images the genotypes were grouped into putative haplotype categories was sequenced to confirm the polymorphisms.

Regenotyping:

Primers for regenotyping were planned based on sequenced haplotypes. Selected InDel polymorphisms were converted into SSLP markers allowing the detection of the potential functional haplotypes. Selected SNPs were detected CAPS assays, Cel1 and ENDO-1 nuclease cutting, enzymatic digestion and SNaPshot assays.

Controlled watering of plants:

Plants were weighted weekly by a computer-controlled balance (GSE model 350, 6000±1g), and the amount of water which was necessary to keep the relative water content at either 60 % or 20 % of the soil was calculated.

Digital imaging and biomass analysis:

Plants were photographed by an Olympus C-7070WZ digital camera from 11 different sideways positions, produced by 32-33° step rotation of the pot. Monitoring of plant growth was performed during the whole growth period once a week.

Thermal imaging of plants:

The efficiency of leaf evaporation was assessed by measuring leaf temperature relative to the surrounding air using a sensitive VarioSCAN 3021 ST thermocamera.

Search of haplotype-phenotype correlations:

A set of tolerant and sensitive genotype categories on the basis of green biomass and grain yield reduction were established and were searched characteristic differences in the frequencies of the previously identified haplotypes.

Results and discussion

I. By using this method approximately 1.5 million basepairs in barley a total number of 94 verified unique haplotypes were identified in 18 amplicons designed for 9 genes. Overall, 185 single nucleotide polymorphisms (SNPs) and 46 insertions/deletions (InDels) were detected with a mean of 92 bp/SNP and 372 bp/InDel in the genomic sequence. The number of haplotypes identified for screened amplicons ranged from 2 to 7.

II. Based on overlapping haplotype sequences of four candidate genes – Hordeum vulgare AR-h gene for aldose reductase (HvARH1), Hordeum vulgare HVA1 gene (HvA1), Hordeum vulgare gene for stress responsive gene protein 6 (HvSRG6), Hordeum vulgare AP2 transcriptional activator gene (HvDRF1) - informative poly-morphisms were converted into genetic markers allowing the detection of the potential functional haplotypes. In addition these easily detectable genetic markers are useful for linkage mapping and Marker Assisted Selection.

III. To testing drought tolerance and agronomic parameters using a complex stress diagnostic system we got up a subcollection of 23 barley genotypes. Large scale of morphological and physiological traits was monitored under optimal (60 % water supply for the whole life cycle) and stress (20 %) conditions. A semi-robotic work station was used for computer controlled watering and digital or thermal imaging. By using imaging technologies such as digital photography and thermal imaging we monitored the biomass production, growth rate and transpirational activity of barley genotypes.

IV. We found significant correlations between grain yield of the genotypes and other important agronomic parameters such as harvest index, water use efficiency and thousand grain weight. This interesting results support the utilization of Complex Stress Diagnostic System.

V. Thermal images showed warmer canopy temperature of drought exposed plants, in selected cases the actual leaf temperature could be related to green mass production.

VI. Based on relative biomass yields, the tested genotypes can be grouped by selection of 45% yield loss as discriminative value between tolerant and sensitive stress responses. Analysis of *HvA1* gene revealed significant differences in haplotype distribution between tolerant and sensitive genotypes. Based on grain yield stability, the genotypes were grouped by selection of 55% grain yield loss as discriminative value between tolerant and sensitive drought stress responses. Comparison of the haplotype composition of genotypes from the tolerant and sensitive groups indicates an essential difference in case of *HvDRF1* and *HvNHX1* genes.

VII. The haplotype/trait association analysis based on the t-test has revealed a positive effect of the haplotype B of the gene encoding the barley fungal pathogen induced mRNA for pathogen-related protein (*HvPPRPX*) on harvest index, thousand grain weight, water use efficiency and grain yield.

VIII. The phenotypic and haplotype dataset created in the frame of this study provides a good starting point to design large scale QTL or association studies aiming to validate the interesting candidate gene variants. The presented pilot study establishes basic methodology for the integrated use of phenotyping and haplotyping data in characterization of genotype-dependent drought responses in barley.

List of publications:

Publications Related to the Theses:

Cseri A., Cserháti M., von Korff M., Nagy B., V. Horváth G., Palágyi A., Pauk J., Dudits D., Törjék O. (2011) Allele mining and haplotype discovery in barley candidate genes for drought tolerance. *Euphytica* (2011), Volume 181, Number 3, 341-356
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Cseri A., Palágyi A., Cserháti M., Pauk J., Dudits D., Törjék O. (2009) EcoTILLING analysis of drought related candidate genes in barley. Plant Abiotic Stress - from signaling to development, 2nd meeting of INPAS (International Network of Plant Abiotic Stress), 14-17 May 2009, Tartu, Estonia

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