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Short communication

Introgression of *LTP2* gene through marker assisted backcross in barley (*Hordeum vulgare* L.)

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ABSTRACT

Background: Marker-assisted introgression currently represents the most widely spread application of DNA markers as an aid to selection in plant breeding. New barley germplasm should be supplemented by genes that facilitate growth and development under stressful conditions. The homology search against known genes is a fundamental approach to identify genes among the generated sequences. This procedure can be utilized for SNP search in genes of predicted function of interest and associated gene ontology (GO).
Results: Backcross breeding enhanced by marker selection may become a powerful method to transfer one or a few genes controlling a specific trait. In the study, the integrated approach of combining phenotypic selection with marker assisted backcross breeding for introgression of *LTP2* gene, in the background of semi-dwarf spring barley cultivar, was employed. This study discusses the efficiency of molecular marker application in backcrossing targeted on the selected gene.
Conclusions: BC_6 lines developed in this study can serve as a unique and adequate plant material to dissect the role of *LTP2* gene. Due to its role in lipid transfer, the *LTP2* may be crucial in lipidome modification in response to abiotic stress.

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1. Introduction

Backcross (BC) breeding enhanced by marker selection (marker-assisted backcrossing, MAB) may become a powerful method to transfer one or a few genes controlling a specific trait from one line into another, usually elite breeding line. One of the traits of interest in breeding programs leading to yield improvement in barley is plant height. Among a few genes affecting plant height in barley the semi-dwarfing *sdw1/denso* gene (chromosome 3H) is one of the most important and it has been incorporated into many cultivars. The semi-dwarf cultivars had improved lodging resistance, higher harvest index and increased grain yield as a consequence [1,2,3]. The juvenile plant stature has been reported to be a morphological marker of the *sdw1/denso* gene [4,5]. Considering the *sdw1/denso* pleiotropic (putative) effects, it may be a good candidate for further analysis and genetic manipulation in barley breeding. However, due to the increasing effects of the abiotic stresses (*e.g.* drought, salinity, soil acidity) breeding programs targeted on development of semi-dwarf cultivars are insufficient. New barley germplasm should be supplemented by genes that facilitate growth and development under stressful conditions.

The mature barley seed proteome is dominated by proteins involved in stress response including Pathogenesis-Related proteins (PRs) [6,7]. Group 14 of PRs comprises lipid transfer proteins (LTPs). LTPs have been recognized to be involved in several biological processes. They transfer different lipids between membranes *in vitro* [8], play role in cutin and wax deposition [9] as well as in pathogen defense [10,11]. They are also involved in plant growth and development [12,13]. One of them – LTP2 – has the ability to bind not only linear lipid molecules, but also sterols (of a great importance to human, including the total cholesterol and LDL reduction, without HDL decrease). Due to its role in lipid transfer LTP2 may be crucial in lipidome modification in response to abiotic stress [14]. According to the International Barley Sequencing Consortium [15] SNP marker 954-1377 has been mapped to the gene MLOC_53422 by Ensembl Plants and annotated as *LTP2* gene. The search for stress-induced genes has led to the characterization of genes encoding LTP proteins. A set of LTP-like proteins has been induced in salt or drought stress in barley [13,16,17]. Therefore *LTP2* mapped on chromosome 4H [15] may be proposed as a candidate gene to be incorporated into modern barley cultivars for secured crop yield in a changing climate. It would be important to combine both the genes in one genotype, *i.e.* conditioning semi-dwarf plant stature and resistance to stress. Such aim would be achieved by the evaluation of a large

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Table 1
Similarity matrix for analyzed barley BC lines.

MPS 37	0.1724															
MPW 14/1	0.6897	0.3793														
MPW 14/13	0.7586	0.0345	0.5862													
MPW 14/19	0.6552	0.3103	0.7241	0.4483												
MPW 14/5	0.6552	0.5172	0.8621	0.4483	0.6552											
MPW 14/7	0.7586	0.1724	0.7586	0.7931	0.5862	0.6207										
MPW 14/9	0.7241	0.2069	0.7241	0.6207	0.7241	0.5862	0.6897									
MPW 15/12	0.7931	0.2759	0.8966	0.6897	0.6897	0.7586	0.8621	0.7241								
MPW 15/4	0.5172	0.5862	0.5862	0.3103	0.3793	0.5862	0.3793	0.4483	0.4828							
MPW 17/1/1	0.7931	0.2759	0.8966	0.6897	0.6897	0.7586	0.8621	0.7241	1.0000	0.4828						
MPW 17/1/11	0.6897	0.3793	1.0000	0.5862	0.7241	0.8621	0.7586	0.7241	0.8966	0.5862	0.8966					
MPW 17/1/4	0.7241	0.3793	0.9655	0.5517	0.7586	0.8621	0.7241	0.6897	0.8621	0.5862	0.8621	0.9655				
MPW 17/1/5	0.6552	0.3448	0.9655	0.5517	0.6897	0.8276	0.7241	0.6897	0.8621	0.6207	0.8621	0.9655	0.9310			
MPW 17/1/6	0.6552	0.3103	0.8276	0.5517	0.5517	0.6897	0.6207	0.6897	0.7241	0.5517	0.7241	0.8276	0.7931	0.7931		
	MPS 106	MPS 37	MPW 14/1	MPW 14/13	MPW 14/19	MPW 14/5	MPW 14/7	MPW 14/9	MPW 15/12	MPW 15/4	MPW 17/1/1	MPW 17/1/11	MPW 17/1/4	MPW 17/1/5		

number of recombinant inbred lines derived from hybrids between appropriately selected parents. An alternative can be conventional backcrossing connected with an analysis of plant materials by markers identifying the target genes. The pre-selection procedure associated with genotype data might be a helpful tool for the efficiently and time-effective selection breeding strategy in crop species. The aim of the present study was to introduce allele of *LTP2* gene present in the genome of spring barley line characterizing by tall plants into the genome of semi-dwarf line.

2. Materials and methods

Plant material for the study consisted of spring barley lines. Both MPS lines (MPS 37 and MPS 106) were derived by single seed descent technique from the hybrids between variety Pomo and Maresi. Mentioned barley varieties were chosen for the sake of large phenotypic diversity. Maresi is a two-rowed, hulled and brewing cultivar which possesses the semi-dwarfing *sdw1/denso* gene. For the purpose of our studies, hybrids MPS 37 × MPS 106 were backcrossed with the MPS 106 line up to BC_6 generation. Line MPS 37 was characterized by erect and line MPS 106 by prostrate growth habit. The BC technique was accelerated *via in vitro* culture of immature embryos [18] resulting in the development of at least three generations per year. The selection of individuals to produce BC lines was based on both phenotype and genotype criteria: the type of juvenile growth habit (erect/prostrate) and by the SNP marker 954-1377. Extraction of genomic DNA of barley samples was conducted according to Doyle and Doyle [19], whereas PCR, DNA sequencing and SNP detection were carried out according to Peukert et al. [20] with modifications.

Parental genotypes and BC_6 lines were analyzed by 1536 SNP markers (Single Nucleotide Polymorphism) using the "Illumina Golden Gate SNP detection Bead Array platform" (BOPA1) [21] at the University of California, Los Angeles (UCLA).

Similarity matrix was created using the simple matching coefficient. The distance was calculated as the 1-SMC. Hierarchical cluster analysis (with method "average") using the set of dissimilarities given by the distance was performed and visualized by dendrogram. Calculation was done in R system.

3. Results and discussion

After six rounds of backcrossing with respect to juvenile plant, stature and allele of SNP 954-1377 13 BC_6 lines were distinguished and the backcrossing effectiveness was validated by high-throughput genotyping. Molecular analysis showed polymorphism for parental genotypes (MPS 37 and MPS 106) in the case of 368 (23.9%) markers only, which is probably the effect of their common origin from Maresi × Pomo hybrids. BC_6 lines had the same alleles as MPS 106 (recipient parent) for 344 SNP markers, *i.e.* 93.4%. The residual polymorphic SNP markers were mapped in genes MLOC and six of them had no annotation. Besides mentioned SNP marker 954-1377 worth noting are the markers: 2036-1027 (biological process: lipid metabolic process), 272-944 (molecular function: heat shock protein binding), and 4070-386 (biological process: response to light stimulus; jasmonic acid biosynthetic process; regulation of lipid metabolic process; defense response to bacterium; oxidation–reduction process).

Similarity matrix of studied BC_6 lines revealed the greatest similarity of MPW 17/1/1 and MPW 15/4 to MPS 106 and MPS 37, respectively (Table 1). The results of the hierarchical cluster analysis are shown on the dendrogram of genetic distance (Fig. 1). Among 13 BC_6 lines 11 have allelic form of *LTP2* gene compatible with donor line MPS 37 introduced onto the genetic background of the line MPS 106. Hence, these lines seem to be a promising plant material for further investigations. Two lines MPS 15/4 and MPS 14/7 appeared to have allelic form of *LTP2* gene the same as the initial parent MPS 106.

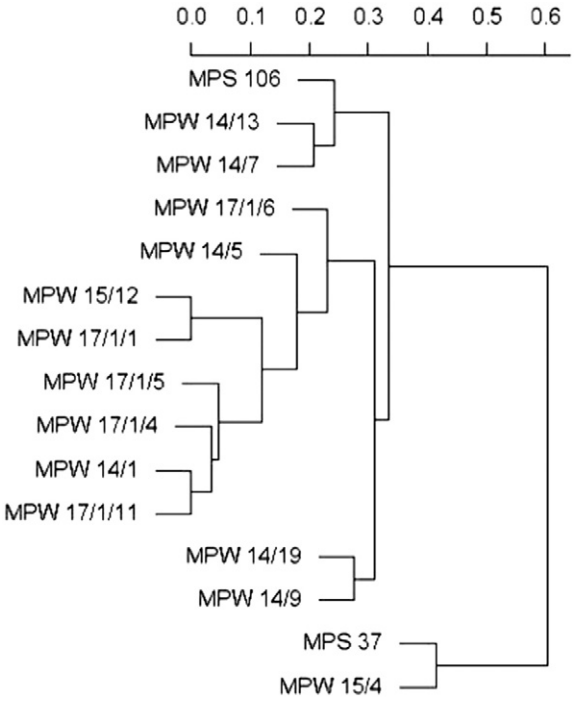

Fig. 1. Dendrogram with a hierarchical relationship among analyzed BC_6 lines.

The approach of present study can result in introgression of *LPT2* allele into the genetic background of prostrate parent. It has been assumed that the expression of *LTP2* gene (MLOC_53422) may be related to plant response to abiotic stress and the encoded protein which is involved in lipid transport mechanism might be an important clue facilitating the stress resistance incorporation into crops through BC process. On the other hand the introgression causing a phenotypic effect relative to recurrent parent (prostrate stature, semi-dwarf plants) may contribute to stress tolerance in the developed BC lines. In sixth generation of backcross, it is expected that recovery of the recurrent parent genome would be 99.2% [22,23].

Diab et al. [24] detected the QTL controlling osmotic potential co-segregates with *denso* locus under irrigated conditions. In the same region Mikołajczak et al. [25] and Mikołajczak et al. [26] found the strongest QTL for plant height under both well-watered and drought stress conditions. Those studies were performed on recombinant inbred lines derived from cross between Syrian line adopted to drought conditions and cv. Maresi being the source of semi-dwarfing *sdw1/denso* gene for BC lines developed in the present study. Plants possessing the *sdw1/denso* gene are characterized by prostrate growth habit which allows to cover the ground around the plant and according to Ceccarelli et al. [27] the high ground cover is associated with better resistance to drought conditions.

BC_6 lines developed in this study can serve as a unique and adequate plant material to dissect the role of *LTP2* gene in the response to different abiotic stresses, *e.g.* drought, salinity and heat [28,29]. The integrative analysis of gene expression profiling, proteomic, lipidomic and phenotypic data will be coordinated in the research topic in the future.

Conflict of interest

The authors declare that they have no conflict of interest.

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