



Short communication

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



Krzysztof Mikołajczak, Piotr Ogrodowicz, Maria Surma, Tadeusz Adamski, Anetta Kuczyńska *

Institute of Plant Genetics, Polish Academy of Sciences, Strzeszyńska Str. 34, Poznań 60-479, Poland

ARTICLE INFO

Article history:

Received 7 April 2016

Accepted 5 September 2016

Available online 14 September 2016

Keywords:

BC lines

Gene annotation

Semi-dwarf gene

Single nucleotide polymorphism spring barley

Marker selection

Marker-assisted backcrossing

Marker-assisted introgression

DNA markers

Selection in plant breeding

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: BC₆ 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.

© 2016 Pontificia Universidad Católica de Valparaíso. Production and hosting by Elsevier B.V. All rights reserved. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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

* Corresponding author.

E-mail address: akuc@igr.poznan.pl (A. Kuczyńska).

Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

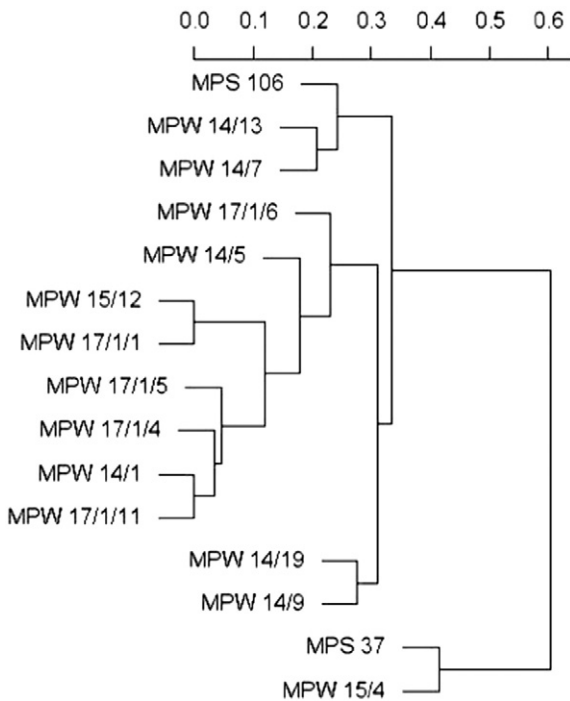


Fig. 1. Dendrogram with a hierarchical relationship among analyzed BC₆ lines.

The approach of present study can result in introgression of *LTP2* 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-dwarfing 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.

BC₆ 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.

References

- [1] Jia Q, Zhang XQ, Westcott S, Broughton S, Cakir M, Yang J, et al. Expression level of a gibberellin 20-oxidase gene is associated with multiple agronomic and quality traits in barley. *Theor Appl Genet* 2011;122:1451–60. <http://dx.doi.org/10.1007/s00122-011-1544-5>.
- [2] Kuczyńska A, Surma M, Adamski T, Mikołajczak K, Krystkowiak K, Ogrodowicz P. Effects of the semi-dwarfing *sdw1/denso* gene in barley. *J Appl Genet* 2013;54:381–90. <http://dx.doi.org/10.1007/s13353-013-0165-x>.
- [3] Kuczyńska A, Mikołajczak K, Cwiek H. Pleiotropic effects of the *sdw1* locus in barley populations representing different rounds of recombination. *Electron J Biotechnol* 2014;17:217–23. <http://dx.doi.org/10.1016/j.ejbt.2014.07.005>.
- [4] Kuczyńska A, Wyka T. The effect of the *denso* dwarfing gene on morpho-anatomical characters in barley recombinant inbred lines. *Breed Sci* 2011;61:275–80. <http://dx.doi.org/10.1270/jsbbs.61.275>.
- [5] Barua UM, Chalmers KJ, Thomas WTB, Hackett CA, Lea V, Jack P, et al. Molecular mapping of genes determining height, time to heading, and growth habit in barley (*Hordeum vulgare*). *Genome* 1993;36:1080–7. <http://dx.doi.org/10.1139/g93-143>.
- [6] Bak-Jensen S, Laugesen S, Roepstorff P, Svensson B. Two-dimensional gel electrophoresis pattern (pH 6–11) and identification of water-soluble barley seed and malt proteins by mass spectrometry. *Proteomics* 2004;4:728–42. <http://dx.doi.org/10.1002/pmic.200300615>.
- [7] Ostergaard O, Finnie C, Laugesen S, Roepstorff P, Svensson B. Proteome analysis of barley seeds: Identification of major proteins from two-dimensional gels (pI 4–7). *Proteomics* 2004;4:2437–47. <http://dx.doi.org/10.1002/pmic.200300753>.
- [8] Kader JC. Lipid-transfer proteins in plants. *Annu Rev Plant Physiol Mol Biol* 1996;47:627–54. <http://dx.doi.org/10.1146/annurev.arplant.47.1.627>.
- [9] Sterk P, Booij H, Scheelekens GA, van Kammen A, de Vries SC. Cell-specific expression of the carrot *EP2* lipid transfer protein gene. *Plant Cell* 1991;3:907–21. <http://dx.doi.org/10.1105/tpc.3.9.907>.
- [10] Stintzi A, Heitz T, Prasad V, Wiedemann-Merdinoglu S, Kauffmann S, Geoffroy P, et al. Plant 'pathogenesis-related' proteins and their role in defense against pathogens. *Biochimie* 1993;75:687–706. [http://dx.doi.org/10.1016/0300-9084\(93\)90100-7](http://dx.doi.org/10.1016/0300-9084(93)90100-7).
- [11] Molina A, García-Olmedo F. Enhanced tolerance to bacterial pathogens caused by the transgenic expression of barley lipid transfer protein LTP2. *Plant J* 1997;12:669–75. <http://dx.doi.org/10.1046/j.1365-313X.1997.00669.x>.
- [12] Kalla R, Shimamoto K, Potter R, Nielsen PS, Linnestad C, Olsen OA. The promoter of the barley aleurone-specific gene encoding a putative 7 kDa lipid transfer protein confers aleurone cell-specific expression in transgenic rice. *Plant J* 1994;6:849–60. <http://dx.doi.org/10.1046/j.1365-313X.1994.6060849.x>.
- [13] Yeats TH, Rose JKC. The biochemistry and biology of extracellular plant lipid-transfer proteins (LTPs). *Protein Sci* 2008;17:191–8. <http://dx.doi.org/10.1110/ps.073300108>.
- [14] Douliez JP, Pato C, Rabesona H, Molle D, Marion D. Disulfide bond assignment, lipid transfer activity and secondary structure of a 7-kDa plant lipid transfer protein, LTP2. *Eur J Biochem* 2001;268:1400–3. <http://dx.doi.org/10.1046/j.1432-1327.2001.02007.x>.
- [15] International Barley Genome Sequencing Consortium. A physical, genetic and functional sequence assembly of the barley genome. *Nature* 2012;491:711–6. <http://dx.doi.org/10.1038/nature11543>.
- [16] Lindorff-Larsen K, Winther JR. Surprisingly high stability of barley lipid transfer protein, LTP1, towards denaturant, heat and proteases. *FEBS Lett* 2001;488:145–8. [http://dx.doi.org/10.1016/S0014-5793\(00\)02424-8](http://dx.doi.org/10.1016/S0014-5793(00)02424-8).
- [17] Stanislava G. Barley grain non-specific lipid-transfer proteins (ns-LTPs) in beer production and quality. *J Inst Brew* 2007;113:310–24. <http://dx.doi.org/10.1002/j.2050-0416.2007.tb00291.x>.
- [18] Surma M, Adamski T, Świecicki W, Barzyk P, Kaczmarek Z, Kuczyńska A, et al. Preliminary results of *in vitro* culture of pea and lupin embryos for the reduction of generation cycles in single seed descent technique. *Acta Soc Bot Pol* 2013;82:231–6. <http://dx.doi.org/10.5586/asbp.2013.021>.
- [19] Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull* 1987;19:11–5.
- [20] Peukert M, Weise S, Röder MS, Matthies IE. Development of SNP markers for genes of the phenylpropanoid pathway and their association to kernel and malting traits in barley. *BMC Genet* 2013;14:97. <http://dx.doi.org/10.1186/1471-2156-14-97>.
- [21] Close TJ, Bhat PR, Lonardi S, Wu Y, Rostoks N, Ramsay L, et al. Development and implementation of high-throughput SNP genotyping in barley. *BMC Genomics* 2009;10:582. <http://dx.doi.org/10.1186/1471-2164-10-582>.
- [22] Hospital F. Selection in backcross programmes. *Philos Trans R Soc B* 2005;360:1503–11. <http://dx.doi.org/10.1098/rstb.2005.1670>.
- [23] Linh LH, Lingham TH, Xuan TD, Ham LH, Ismail AM, Khanh TD. Molecular breeding to improve salt tolerance of rice (*Oryza sativa* L.) in the Red River Delta of Vietnam. *Int J Plant Genomics* 2012;2012:1–9. <http://dx.doi.org/10.1155/2012/949038>.
- [24] Diab AA, Teulat-Merah B, This D, Ozturk NZ, Benscher D, Sorrells ME. Identification of drought-inducible genes and differentially expressed sequence tags in barley. *Theor Appl Genet* 2004;109:1417–25. <http://dx.doi.org/10.1007/s00122-004-1755-0>.
- [25] Mikołajczak K, Ogrodowicz P, Gudyś K, Krystkowiak K, Sawikowska A, Frohberg W, et al. Quantitative trait loci for yield and yield-related traits in spring barley populations derived from crosses between European and Syrian cultivars. *PLoS One* 2016;11:e0155938. <http://dx.doi.org/10.1371/journal.pone.0155938>.
- [26] Mikołajczak K, Kuczyńska A, Krajewski P, Sawikowska A, Surma M, Ogrodowicz P, et al. Quantitative trait loci for plant height in Maresi × CamB barley population and their associations with yield-related traits under different water regimes. *J Appl Genet* 2016;1–13. <http://dx.doi.org/10.1007/s13353-016-0358-1>.
- [27] Ceccarelli S, Acevedo E, Grandio S. Breeding for yield stability in unpredictable environments: Single traits, interaction between traits, and architecture of genotypes. *Euphytica* 1991;56:169–85. <http://dx.doi.org/10.1007/BF00042061>.
- [28] Hoekstra FA, Golovina EA, Buitink J. Mechanisms of plant desiccation tolerance. *Trends Plant Sci* 2001;6:431–8. [http://dx.doi.org/10.1016/S1360-1385\(01\)02052-0](http://dx.doi.org/10.1016/S1360-1385(01)02052-0).
- [29] Ding Y, Virilouvet L, Liu N, Riethoven JJ, Fromm M, Avramova Z. Dehydration stress memory genes of *Zea mays*: comparison with *Arabidopsis thaliana*. *BMC Plant Biol* 2014;14:141. <http://dx.doi.org/10.1186/1471-2229-14-141>.