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A new allele in a *uzu* gene encoding brassinosteroid receptor

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Brassinosteroids are growth-promoting natural products, found at low levels in pollen, seeds and young vegetative tissues throughout the plant kingdom. They play an essential role in diverse developmental processes including cell expansion, vascular differentiation, etiolation and reproductive development (Clouse and Sasse, 1998). It has been found that semidwarf barley accessions carrying the *uzu* gene are non-responding to brassinosteroids. The synteny suggested that the *uzu* gene may be homologous to rice *D61*, which is a homolog of *Arabidopsis thaliana BR-insensitive1* (*BRI1*), encoding a BR-receptor protein. A barley homolog of *BRI1*, *HvBRI1* (acc. no. AB088206) was isolated (Chono et al., 2003). The sequence analysis showed that uzu phenotype was caused by single-nucleotide substitution A > G at the position 2612 of the *HvBRI1* gene. This mutation resulted in an amino acid change at the highly conserved residue (His-857 to Arg-857) of the kinase domain of BRI1 receptor protein and caused the reduced sensitivity to BRs and reduced plant height (Chono et al., 2003). We have found a semidwarf mutant 093AR, produced by MNU (N-methyl-N-nitrosourea) treatment of variety Aramir, that proved to be allelic to the uzu form. The aim of this work was to determine and compare the sequences of *HvBRI1* gene between spontaneous mutant uzu, induced mutant 093AR and its parent Aramir variety in order to identify the putative mutation(s).

DNA extraction was performed according to “micro C-TAB” protocol (Doyle and Doyle, 1987). Six primer pairs were designed on the basis of *HvBRI1* sequence (GenBank acc. no. AB088206) utilizing the Primer3 program (http://frodo.wi.mit.edu/cgi-bin/primer3). PCR reactions were performed using the “touch-down” strategy. Three independent PCR reactions were performed for each primer pair and amplification products were extracted from agarose gels using QIAEX II Gel Extraction Kit (QIAGEN) and cloned into pGEM-T Easy vector (Promega). Plasmid purification was carried out using QIAprep Spin Miniprep Kit (QIAGEN). Inserts were sequenced using SequiTherm EXCEL II DNA Sequencing Kit (EPICENTRE) utilizing LI-COR IR2 sequencer.

We determined the sequences of *HvBRI1* gene in the genotype uzu, 093AR and Aramir variety. The single-nucleotide substitution has been confirmed at the position 2612 (A-2612 to G-2612) in the spontaneous mutant uzu, which proved to be specific for this genotype. This mutation is localized in highly conserved kinase domain of receptor protein. The comparison of sequences of *HvBRI1* gene between mutant 093AR and Aramir variety, from which this mutant was obtained, led to the identification of the C to A substitutions at the positions 1760 and 1761 in the mutant (Fig. 1).

1 cgcttctcgcatggtctcaaggtagagcgagcgagctcccacatggattg

1701 gatgccgagcaagaagctgtgcaacttcacaaggatgtacatggggagca

1751 cagagtata**cc**ttcaacaagaatggctccatgatatttctggatttgtca

1801 tttaatcagcttgactcggagatacccaaggagcttggcaatatgttcta

…

2551 gtgagtggccagggtgaccgggagttcacagcggaaatggagaccattgg

2601 caagatcaaac**a**ccgcaaccttgttccgctcctcggctactgcaagatcg

2651 gcgaggagcggctgctgatgtatgacttcatgaagtatggcagcttggag

…

3501 gcctctcagatgattgatgatgatgtgatgataccattctgaggttccca

3551 cgcaagct

Fig. 1. The sequence of *HvBRI1* gene (acc. no. AB088206). **cc**- at the positions 1760 and 1761 indicate C > A substitutions in the 093AR sequence; **a-** at the position 2612 indicates A > G substitution in spontaneous mutant uzu.

On the basis of *in silico* translation using Jellyfish program it was postulated that this mutations lead to the replacement Thr-573 by Lys-573 (Fig. 2).

1 mdclrlavaaaalllaalaaaaddaqlldefrmalpsqaplegwtarega

…

501 ippelaeqsgkmtvgliigrpyvylrndelssqcrgkgsllefssirsed

551 lsrmpskklcnftrmymgsteyt**t**fnkngsmifldlsfnqldseipkelgn

601 mfylmimnlghnllsgaiptelagakklavldlshnrlegqipssfssls

…

1101 pgfgvmdmtlkeakeekd

Fig. 2 The sequence of HvBRI1 protein (acc. no. BAD01654). **t** – indicates the Thr-573 to Lys-573 conversion, deduced by *in silico* translation, caused by C > A substitutions at the positions 1760 and 1761.

The threonine residue is localized between last LRR (Leucin-Rich Repeat) domain and pair of cysteines participating in protein dimerization, followed by transmembrane domain. It was shown utilizing ClustalW program that Thr-573 residue is highly conserved among BRI1 homologs from barley, rice, arabidopsis and tomato. As threonine and lysine have different chemical properties, their substitution within the conserved domain may lead to the change in protein activity.

References:

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