Using of polymorphism in cluster of Dhn3, 4 and 7 genes as possible selection criteria in barley breeding for higher level of frost tolerance

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Two pairs of Dhn4 specific primers were used for assessment of polymorphism of Dhn4 gene sequences in 24 barley varieties with different level of freezing tolerance expressed by LT50. Almost whole sequence of the Dhn4 gene was amplified from the first pair of primers and only sequence of the first exon of this gene was amplified from the second pair of the primers. In both reactions, a specific pattern of PCR products was identified in six-rowed winter varieties with lower level of LT50.

The length polymorphism of the PCR products in reactions with the first pair of the primers revealed two specific products, which differentiated the varieties as follows :

1. Six-rowed (seven winter and two intermediate varieties) were characterised by the products A1/B2 and by LT50 from -14.3 to -15.1°C (in average -14.8°C). LT50 of this group differed significantly (P = 0.001) from the following group.
2. The others (six-rowed - one spring and one intermediate, two-rowed - both spring and winter varieties) were characterised by the revealed B1-product and different combinations of the A-products (A1, A2, A3). Their LT50 varied from -10.0°C to -15.3°C, in average -12.3°C.

The combination of the products of the dehydrin gene Dhn4 named as A1/B2 was therefore typical of the six-rowed winter (or intermediate) varieties of barley and was related to higher frost tolerance.

In reaction with the second pair of primers, the common pattern of PCR products, named as Okal type of „marker" (**O**, tolerant), was typical of all tested varieties with combination of PCR products A1/B2. The rest of the tested varieties demonstrated other common patterns named as Akcent type of „marker" (**A**, sensitive). The sequence analyses of the PCR products in reactions with the second pair of the primers in the five contrast genotypes (showing PCR pattern **O** or **A**) confirmed known mutation (deletion of 6 bp) in the first exon of Dhn4 already observed in the genotypes Dicktoo and Morex (GenBank). Our results, however, indicated also existence of other mutation typical of the tested winter and intermediate six-rowed varieties (except var. Dicktoo) probably in the other sequences near Dhn4 gene (cluster genes Dhn3, 4 and 7). To prove possible utilization of presence of the specific PCR pattern **O** (tolerant) as a selection criterium of freezing tolerance in field conditions, we selected F3 and F4 family from reciprocal cross between the spring variety Akcent (**A**, sensitive) and the winter six-rowed variety Okal (**O**, tolerant) and F_4 and F_5 family of reciprocal cross from the winter two-rowed variety Monaco (Akcent type, sensitive) and the winter six-rowed variety Okal (O, tolerant).

The results showed, that 83% of progeny of Akcent x Okal and 68% of progeny of reciprocal cross of Okal x Akcent survived in field conditions of winter 2004/2005, provided the parental „lines" have the marker O (tolerant), but only 0% of progeny Akcent x Okal or 39% progeny of reciprocal cross Okal x Akcent survived in the same conditions, if selected parental „lines" have molecular marker A (sensitive). It means that O genotypes of these crosses showed higher winterhardiness than A genotypes. It was also confirmed in the cross between varieties Monaco x Okal and Okal x Monaco, but differences were only 7 and 18% respectively. Winterhardiness of the crosses between winter varieties were, of course, higher, without respect to the marker S or T. These findings will be studied in detail.

The research was supported by the Czech government agencies (NAZV QF3191 and MŠMT 1M0570) and by Mendel University of Agriculture and Forestry (IGA 250121).

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