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## SHORT COMMUNICATION

# Utilization of a Molecular Marker for Evaluation of Hybridization Success in Spring Barley Genetics and Breeding

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**Abstract**: The success in hybridization of two-rowed spring barley genotypes of various origins was verified using the codominant SSR (Simple Sequence Repeat) marker HVWAXY which is located in the coding sequence of the *waxy* gene. The primer pair F 5' AAG ACG TGG TGT TCG TGT G 3' and R 5' ATG GTT CCA GGG GTA AGT TC 3' generated the PCR product of approximately 200 bp in the varieties/breeding lines Maridol, Bojos, Malz, Xanadu, Isotta, Josefin, Native, Sebastian, Conrad, KM 2436, KM 2439, KM 2629, KM-H-1320, whereas the product of about 250 bp was amplified in the lines KM 2416 and Br 7571h33. The parental genotypes possessing different alleles of the marker were crossed and the segregating lines of the $F_3$ generation were characterized employing molecular methods (marker-assisted selection). This prescreening enabled to work with a lower number of individuals (selfed individuals were excluded) at the initial stages of the breeding process leading to the acceleration of selection as a particular phase of breeding.

**Keywords**: barley; *Hordeum vulgare* L.; hybridization; marker-assisted selection; SSR marker

Breeding of a new cereal variety is a long-term process that requires considerable invention and experience of the responsible breeder. This process, from the beginning of the breeding work to the registration and recommendation of the variety to practical farming, lasts 10–15 years. The effort of each breeder is to shorten the time for breeding of a new variety and to transfer it to practical utilization as soon as possible. Although new progressive methods are used in breeding, e.g. genetic modifications, the majority of the new genotypes of all cereals originates from hybridization between parental genotypes. Hybridization is the beginning of breeder's long activity with the aim to develop a new variety with higher productivity than the old one. The consecutive steps are the multiplication of progenies and selection. Even though the weak and average progenies are excluded from the breeding process based on phenotypic assessment, there are still enough genotypes to be evaluated without the knowledge whether the hybridization really passed or not. The effective negative selection of progenies with an undesired set of alleles can be realized according to Ovesná *et al.* (2004) by means of molecular techniques. The procedures using molecular markers, particularly for targeted selection in the early filial generation, are indicated as marker-assisted selection (MAS). Genetic markers are unambiguously rapidly detectable properties of organisms which are in close correlation with a

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complex of agronomic parameters e.g. grain quality, resistance to biotic and abiotic stresses. Progress in the field of biochemistry and molecular biology has enabled to apply another type of biochemical-genetic markers based on the DNA polymorphism (Williams *et al.* 1990). The historically latest DNA markers include the markers using polymorphism in microsatellite regions of chromosomal DNA or repetition of individual sequences (Becker & Huen 1995; William *et al.* 1997). SSRs provide a valuable source of polymorphism because they are abundant, hypervariable, multi-allelic and equally distributed throughout the nuclear genomes of the majority of organisms (Varshney *et al.* 2006).

The objectives of our investigation were (*i*) to find and verify a marker that is polymorphic among parental genotypes and therefore suitable for marker-assisted selection of a segregating progeny and (*ii*) to implement molecular techniques into the methodology of classical plant breeding in order to hasten and clarify the production of new barley varieties.

The polymorphism of the SSR HVWAXY marker was analyzed in 15 spring barley varieties/breeding lines of various origins (Table 1). The parental genotypes possessing different alleles of the HVWAXY marker were hybridized and 36 lines of the $F_3$ generation were screened. The emasculation was carried out on female plants by removing all three immature anthers. The emasculated spike was wrapped with the leaf sheath and isolated with a paper bag. The third day after emasculation, three anthers from parental male plants were used to pollinate female carpels and the spike was isolated again.

Six plants of each variety/line were grown in a germination box for two weeks. Genomic DNA was extracted from the frozen leaf tissue (2–6 bulked seedlings per accession) using the DNeasy plant mini kit (Qiagen) according to the manufacturer's protocol. The concentration and quality of DNA was checked by Lambda DNA/HindIII Marker (Fermentas) on 1% agarose gel. The polymorphism of parental genotypes, as well as the success in hybridization of the selected $F_3$ generation lines was verified using the HVWAXY marker which is located in the coding sequence of the *waxy* locus (X07931). PCR products were generated using the primers F 5' AAG ACG TGG TGT TCG TGT G 3' and R 5' ATG GTT CCA GGG GTA AGT TC 3' (William *et al.* 1997). PCR amplification was performed according to Špunarová and Kraus (2000) with slight modifications. PCR reaction

Table 1. A list of tested varieties/lines of spring barley (the polymorphism in the *waxy* locus was detected using the HVWAXY marker)

| Variety/line | Origin | HVWAXY allele (bp) |
|---|---|---|
| Maridol | CZE | 200 |
| Sebastian | DNK | 200 |
| Xanadu | DEU | 200 |
| Bojos | CZE | 200 |
| Malz | CZE | 200 |
| Josefin | FRA | 200 |
| Isotta | DEU | 200 |
| Native | FRA | 200 |
| Conrad | USA | 200 |
| KM 2439 | CZE | 200 |
| KM 2416 | CZE | 250 |
| KM 2629 | CZE | 200 |
| KM-H-1320 | CZE | 200 |
| Br 7571h33 | DEU | 250 |
| KM 2436 | CZE | 200 |

was performed in 20 µl volumes comprising 50 ng of genomic DNA, 0.2µM of each primer and 1× PPP Master Mix (Top-Bio). The samples were amplified in the thermocycler TC-512 (Techne): denaturation at 94°C for 2 min followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min and elongation at 72°C for 1 min. The reaction was completed at 72°C for 5 min. The products of amplification were separated on 2% agarose gel (ethidium bromide staining) and visualized under UV light.

The polymorphism of the HVWAXY marker was analyzed in 15 spring barley varieties/lines. Two haplotypes were determined in the analyzed set (Table 1, Figure 1a). Thirteen varieties were characterized by the PCR fragment of about 200 bp. The lines KM 2416 and Br 7571h33 (PCR product of 250 bp) were very simple to distinguish from the other genotypes on agarose slab gel.

The success in hybridization of the progeny can be evaluated by the combination of parents which are polymorphic for a particular locus/marker (various haplotypes).

The results of hybrid grain analyses obtained after the crossing of parental genotypes are presented in Table 2. Six parental combinations were selected for the verification of hybridization in

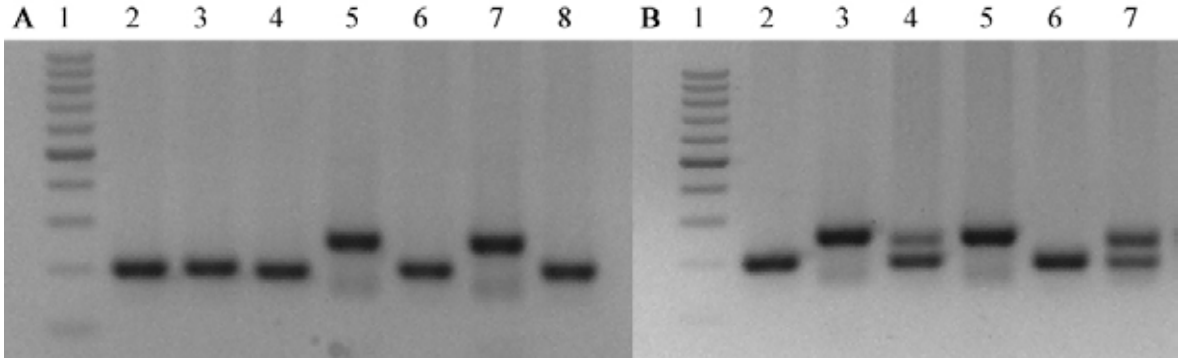


Figure 1. Evaluation of hybridization success in spring barley genotypes using the molecular marker HVWAXY

(A) Assessments of polymorphism of the HVWAXY marker in selected parental genotypes; as described in the text, PCR products of 200 bp (lanes 2, 3, 4, 6 and 8) and of 250 bp (lanes 5 and 7) were generated
1 – 100 bp ladder; 2 – Isotta; 3 – Native; 4 – Conrad; 5 – KM 2416; 6 – KM 2629; 7 – Br 7571h33; 8 – KM 2436

(B) Assessments of hybridization of $F_3$ lines in the cross: Native (♀) × KM 2416 (♂)
1 – 100 bp ladder; 2 – Native; 3 – KM 2416; 4–7 – $F_3$ lines

spring barley by means of the molecular marker. Six $F_3$ generation lines from each combination of parental genotypes were investigated; a total of 36 lines were analyzed. The successful hybridization was confirmed only in the case of male allele occurrence in the segregating progeny. The hybridization was confirmed for 25 out of 36 $F_3$ lines. Negative results in line 24 are rather ambiguous as only the bulk of two plants was evaluated due to the low germinability of seeds.

It is desirable to test the hybridization success already in plants of the $F_1$ generation because these plants are uniformly heterozygous if the crossing was successful. When we verify the hybridization in the $F_2$ generation or higher, expecting segregation in the investigated locus (gene, marker), it is necessary to test single plants from each line, or we can isolate DNA from bulked plants for each line and, hence, conveniently utilize the markers of a codominant character, as in our study.

Figure 1b shows the analysis of the cross Native (♀) × KM 2416 (♂) employing the HVWAXY marker. The female component was characterized by the PCR product of 200 bp, the male component generated the product of 250 bp. Hybrid lines were identified by the presence of 250 bp allele in homozygotic (lane 5) or heterozygotic variant (lanes 4 and 7). The pattern of unsuccessful hybridization (lane 6), which arose from selfing, corresponded to the female genotype (200 bp). Similar utilization of a codominant DNA marker was described by Weising *et al.* (1995) and Paltridge *et al.* (1998).

The utilization of the microsatellite HVWAXY marker for the evaluation of hybridization success in spring barley was verified. The advantage of this marker is the complete linkage with the gene encoding starch synthase (*waxy* locus), therefore it is not necessary to calculate the strength of linkage between the gene of interest and the marker. The appropriate tools for the assessment of the transfer of particular traits from parents to progeny are markers, which are closely linked to genes controling these traits. Czembor and Talbert (1997) and Schwarz *et al.* (1999) described the utilization of RFLP (Restriction Fragment Length Polymorphism) and AFLP (Amplified Fragment Length Polymorphism) markers, respectively, for the *Mla* locus determining the resistance to *Blumeria graminis*. Manninen *et al.* (1997) identified an RAPD (Random Amplified Polymorphic Region) marker closely linked to the *mlo* locus, which also confers the effective resistance to the same pathogen. Molecular and classical breeding techniques were employed to develop cultivars with resistance to five different fungal diseases in the Minnesota Barley Improvement Program (Steffenson & Smith 2006). Šíp *et al.* (2004) exploited the diagnostic CAPS (Cleaved Amplified Polymorphic Sequence) marker for identification of the *Yd2* resistance gene to BYDV (Barley Yellow Dwarf Virus) in spring barley breeding.

MAS can be used to study important malting quality parameters, such as β-glucan content, Kolbach number, friability, viscosity and extract (Poulsen *et al.* 1996). These perspectives of a more

Table 2. Evaluation of hybridization success in spring barley $F_3$ generation lines using the HVWAXY marker

| No.* | Female | Male | $F_3$ generation lines | |
|---|---|---|---|---|
| | | | HVWAXY allele (bp)** | hybridization*** |
| 1 | native | KM 2416 | H | + |
| 2 | native | KM 2416 | 250 | + |
| 3 | native | KM 2416 | 200 | – |
| 4 | native | KM 2416 | H | + |
| 5 | native | KM 2416 | 250 | + |
| 6 | native | KM 2416 | 200 | – |
| 7 | KM 2416 | Isotta | 250 | – |
| 8 | KM 2416 | Isotta | 200 | + |
| 9 | KM 2416 | Isotta | H | + |
| 10 | KM 2416 | Isotta | 200 | + |
| 11 | KM 2416 | Isotta | 250 | – |
| 12 | KM 2416 | Isotta | H | + |
| 13 | KM 2629 | Br 7571h33 | H | + |
| 14 | KM 2629 | Br 7571h33 | 200 | – |
| 15 | KM 2629 | Br 7571h33 | 250 | + |
| 16 | KM 2629 | Br 7571h33 | 200 | – |
| 17 | KM 2629 | Br 7571h33 | H | + |
| 18 | KM 2629 | Br 7571h33 | H | + |
| 19 | Conrad | KM 2416 | 250 | + |
| 20 | Conrad | KM 2416 | 200 | – |
| 21 | Conrad | KM 2416 | H | + |
| 22 | Conrad | KM 2416 | 250 | + |
| 23 | Conrad | KM 2416 | 250 | + |
| 24 | Conrad | KM 2416 | 200 | – |
| 25 | Br 7571h33 | KM 2436 | 250 | – |
| 26 | Br 7571h33 | KM 2436 | 250 | – |
| 27 | Br 7571h33 | KM 2436 | H | + |
| 28 | Br 7571h33 | KM 2436 | H | + |
| 29 | Br 7571h33 | KM 2436 | 200 | + |
| 30 | Br 7571h33 | KM 2436 | H | + |
| 31 | Br 7571h33 | Isotta | H | + |
| 32 | Br 7571h33 | Isotta | H | + |
| 33 | Br 7571h33 | Isotta | H | + |
| 34 | Br 7571h33 | Isotta | H | + |
| 35 | Br 7571h33 | Isotta | 250 | – |
| 36 | Br 7571h33 | Isotta | H | + |

*number of line; **H – heterozygote; ***+ successful, – unsuccessful

effective selection of malting barley parameters by means of DNA markers were also confirmed by other authors (Han *et al.* 1997; Lee & Penner 1997). According to the recent works of Paris *et al.* (2002), Malysheva *et al.*(2004), Malysheva and Roder (2006) it is possible to detect alleles of *Bmy1* locus, encoding β-amylase enzyme with different thermostability, using CAPS analysis.

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