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ALLELIC DIVERSITY OF THE BETA-AMYLASE GENE *BMY1* IN LATVIAN BARLEY BREEDING LINES

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The endosperm specific beta-amylase, a key enzyme involved in the storage starch degradation during malting process, is encoded by the single copy gene *Bmy1*. Different allelic variants of the structural genes encode enzymes of different thermostability and/or kinetic properties. Genotyping on the trait responsible intron III mutations including 126 bp (MITE) and (1+6) bp insertion/deletion (InDel) events was applied to evaluate genetic diversity of the *Bmy1* gene in covered and hulless spring barley breeding lines of State Stende Cereal Breeding Institute and State Priekuli Plant Breeding Institute. Frequency of MITE indel alleles was similar within breeding lines for both breeding institutes (heterozigosity index 0.4). The diversity of indel (1+6) bp was higher within Priekuli breeding lines compared to Stende material (heterozigosity index 0.03 and 0.48 respectively). Only three of four possible two loci (MITE/(1+6)) haplotypes were observed. Haplotype characterized by the deletion in both loci and associated with high malting quality, was found only in eight Priekuļi lines selected from the cross of 'Latvijas Vietejie' and 'Inari'. *Bmy1* structural gene of most middle malting quality breeding lines was shown to have MITE deletion and (1+6) bp insertion. Insertions in both loci of the gene were detected in the resting breeding material suggesting their low malting quality. Variant with MITE insertion and (1+6) bp deletion was not revealed in our study. 25% from all investigated breeding lines were heterogeneous. Results obtained in the study confirm usefulness of the molecular marker application in the identification of breeding lines perspective for malting and/or feed barley selection.

Key words: spring barley, *Bmy1*, allelic diversity, haplotypes

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INTRODUCTION

Developing of the new cereal varieties is still a multi-step process. Taking into account great diversity of barley grain qualities necessary for different usages of the cereals that range from animal feed to brewing, numerous genes should determine qualities and should be selected in appropriate way during development of new varieties. Understanding of the molecular basis of barley quality was achieved in biochemical studies (Fox et al. 2003). Application of biotechnology has considerably shortened the time necessary for new varieties to reach the market. Marker-assisted selection (MAS) is one of the tools providing easiest way to select useful plant traits, control the transmission of the traits of interest through the generation and finally to reduce time of new variety development (Xu & Crouch 2008).

Strong enzymatic activities in the germinating barley grain, together with protein and starch content, are crucial for high extraction values in the resulting malt and, consequently, in barley (*Hordeum vulgare* L.) malting quality (B.Cullis et al. 2003). The amylolytic enzyme ā-amylase (1,4-į-D-glucan maltohydrolase) catalyses the release of maltose from starch which is an important biochemical pathway during germination and the malting process. The enzyme ā-amylase is encoded by the gene *Bmy1* and located on the long arm of chromosome 4H (M.Erkkila et al. 1998). Differences in extractable ā-amylase activities exist between enzymes from malt and feed barleys. Four alleles (Sd2L, Sd1, Sd2H, Sd3) of the endosperm specific ā-amylase gene *Bmy1* giving rise to proteins with different thermostability and electrophoretic characteristics were identified in cultivated and wild barley. These alleles are distinguished by coding single nucleotide polymorphism (cSNP) (J.Eglington et al. 1998; Erkkila & Ahokas 2001). There is found the association of 126 bp (MITE) and (1+6) bp insertion/deletion (InDel) events in the intron III of the *Bmy1* gene with the differences of ā-amylase thermostability (Erkkila & Ahoka 2001; Sjakste & Roder 2004).

In this study the genotyping of the 126 bp (MITE) and (1+6) bp InDels was applied to evaluate genetic diversity of the *Bmy1* gene in covered and hulless spring barley breeding lines of State Stende Cereal Breeding Institute and State Priekuli Plant Breeding Institute.

MATERIAL AND METHODS

Plant material and DNA isolation

The set of 200 barley accessions used in this study was composed of 100 breeding lines from Priekuli Plant Breeding Institute (Priekuli PBI) and 100 barley lines from Stende Cereal Breeding Institute (Stende CBI). These lines comprise 47 covered and 53 hulless barley lines from Priekuli PBI, 50 covered and 50 barley hulless lines from Stende CBI. Selection material was chosen from different selection nurseries (F_4-F_{11}) at different homogeneity level. Twenty four F_4 lines from Priekuli PBI were originated from cross combination between parent varieties 'Latvijas Vietejie' (LV) and 'Inari'. Leaves of individual plants or of 10 plants per sample (bulk) were taken in the field and transported at 4ŗC to the laboratory. Genomic DNA was extracted according to the previously described procedures (J.Plaschke et al., 1995).

PCR amplification and genotyping

PCR amplification of the 5' and 3' regions of the intron III and genotyping of the polymorphic loci were performed according to the previously described protocols (Sjakste & Roder, 2004; Sjakste & Zhuk, 2006) with slight modifications. Fragment containing 126 bp InDel was amplified with fluorescent forward Bmy126F: 5'-6-FAM-cgggagaattcatcgtgagtg-3' and reversal Bmy126R: 5'-catcacatattcacatgttgagttaca-3' primers correspondingly. Amplification resulted in 229 bp (insertion of 126 bp) or 103 bp (deletion of 126 bp) products. Fragment polymorphic on the (1+6) bp InDel was amplified with fluorescent forward Bamy1F: 5'-HEX-tgaaatagttaaaacaaatgcacga-3' and reversal Bamy1R: 5'-gctgctgctgctttgaagct-3' primers. Presence and absence of (1+6) bp Indel

resulted in the products of 157 bp and 150 bp correspondingly. Size difference was detected using both the agarose gel electrophoresis and fluorescence measurement using ABI Prism 7000 sequence detection system and ABI Prism 7000 SDS software version 10.

RESULTS

Successful extraction of genomic DNA of the high molecular weight was performed for all barley breeding lines. Amplification resulted in the fragments of expected sizes and was scored in terms of insertion or deletion as described above and illustrated in Fig. 1. and Fig. 2.

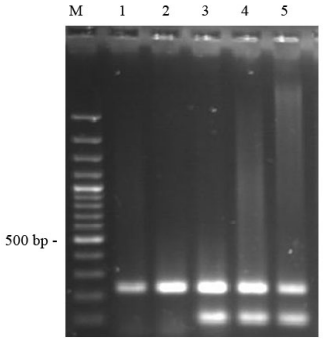

Fig. 1. Genotyping of the 126 bp InDel using agarose gel electrophoresis. M – 100 bp ladder; 1 and 2 – homozygotes on 126 bp insertion; 3, 4, and 5 – heterozygotes on 126 bp both insertion and deletion.

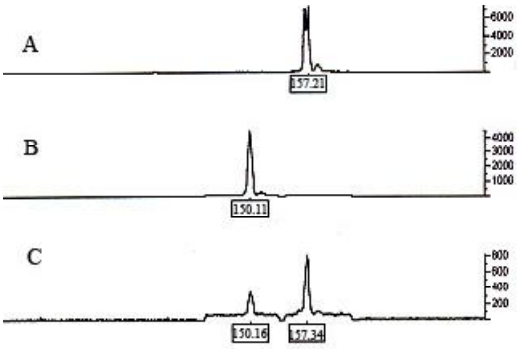

Fig. 2. Genotyping on the (1+6) bp Indel. A – (1+6) bp insertion; B – (1+6) bp deletion; C – heterozygote.

The frequency of occurrence of mutations 126 bp (MITE) and (1+6) bp insertion/deletion (Indel) alleles and heterozigosity indexes in the breeding lines of both breeding institutes are presented in Table 1. Frequency of Indel 126 bp (MITE) alleles was similar within breeding lines for both breeding institutes. The heterozigosity index was 0.40 where allelic frequency of presence indel 126 bp was 0.28 and 0.29, but the absence of these alleles was 0.72 and 0.71 in the material of both institutes respectively. The diversity of indel (1+6) bp was higher within Priekuli breeding lines compared to Stende material. The presence of indel (1+6) pb allele was 0.98 and 0.60 for Stende CBI and Priekuli PBI respectively, but the absence of this allele was 0.02 and 0.40. (heterozigosity index 0.03 and 0.48 respectively).

Data of distribution *Bmy1* haplotypes in the Priekuli PBI and Stende CBI breeding material are presented in Table 2 and Table 3. Only three from four possible two loci (MITE/(1+6)) haplotypes were observed. Haplotype with presence of allele 126 bp and absence 1+6 bp allele was not found in the breeding material analyzed. Haplotype characterized by the deletion in both loci and associated with high malting quality are present in 8 covered and 3 hulless breeding lines from Priekuli material (Table 2). All covered lines with such haplotype selected from the cross of 'Latvijas Vietejie' and 'Inari'. Breeding lines characterized by average/ low malting quality (aB un AB haplotypes) were predominant (75 lines). Part of material (23 lines) was heterogeneous and contains all possible haplotypes.

In the Stende material haplotype characterized by the deletion in both loci was found only in two hulless lines (Table 3). Breeding lines characterized by average/low malting quality (aB and AB haplotypes) were predominant (75 lines). Twenty three lines contain all possible haplotypes.

Table 1. Frequency of *Bmy1* polymorphic locuses and heterozigosity indexes in barley lines of State Stende CBI and Priekuli PBI

Breeding institute	Fenotype	Generation	Number of individual plants	Allelic frequency of *Bmy1* markers			
				Indel 126 bp (MITE)		Indel (1+6) bp	
				presence	absence	presence	absence
State Stende CBI	Covered	F6 - F8	19	0.33	0.67	1	0
		DH	1	0.10	0.90	1	0
		F4	30	0.28	0.72	0.98	0.02
	Hulless	F4	50	0.39	0.61	0.95	0.05
Average allelic frequency				0.28	0.72	0.98	0.02
Heterozigosity index				0.40		0.03	
State Priekuli PBI	Covered	F4 - F14	46	0.32	0.68	0.72	0.27
		DH	1	0.50	0.50	0.20	0.80
	Hulless	F5 - F7	51	0.28	0.72	0.95	0.05
		DH	2	0.05	0.95	0.52	0.48
Average allelic frequency				0.29	0.71	0.60	0.40
Heterozigosity index				0.40		0.48	

Table 2. Characterization of *Bmy1* haplotypes in barley lines of State Priekuli PBI

Marker Presence/absence +/-			Haplotyps	Generation, number of lines			Potential quality for malt production
				F4 (24 lines) Latvijas Vietejie/ Inari	F6 ÷ F9 (76 lines)		
				Covered	Covered	Hulless	
126 bp	-	a	ab	8	-	3	High
1 +6 bp	-	b					
126 bp	-	a	aB	2	17	31	Average/low
1 +6 bp	+	B					
126 bp	+	A	AB	3	2	7	Low
1 +6 bp	+	B					
126 bp	+/-	Aa	All possible haplotypes: AB; aB; ab	11	4	12	Heterogeneous
1 +6 bp	+/-	Bb					
126 bp	+	A	Haplotype not found				

DISCUSSION

Up to now different studies were performed to characterize the registered barley cultivars and genetic resources with respect to one of the relevant thermostability enzymes (*ā*-amylase) that is an essential requirement in the barley breeding for definite end use. The good experience is obtained up to now in the different countries regarding to investigation of allelic diversity of *Bmy1* gene in the barley genotypes. Allelic diversity and inheritance of polymorphic sites of the intron III-exon IV region of the seed specific ā-amylase gene *Bmy1* were studied in a set of 55 barley accessions composed mainly of old Latvian and Scandinavian commercial varieties (Sjakste & Roder 2004). cSNP genotyping of ā-amylase alleles in 79 spring barley varieties were performed (K.Polakova et al. 2003). The set of 21 Latvian spring barley varieties was used in the polymorphisms detection of the *Bmy1* gene (Sjakste & Zhuk 2006). The single nucleotide

Table 3. Characterization of *Bmy1* haplotypes in barley lines of State Stende CBI

Marker Presence/absence +/-			Haplotypes	Generation, number of lines			Potential quality for malt production
				F6 ÷ F8 (20 lines)	F4 (80 lines)		
				Covered	Covered	Hulless	
126 bp	-	a	ab	-	-	2	High
1 +6 bp	-	b					
126 bp	-	a	aB	13	17	25	Average/low
1 +6 bp	+	B					
126 bp	+	A	AB	3	9	8	Low
1 +6 bp	+	B					
126 bp	+/-	Aa	All possible haplotypes: AB; aB; ab	4	4	15	Heterogeneous
1 +6 bp	+/-	Bb					
126 bp	+	A	Haplotype not found				

polymorphisms (SNPs) in the *ā*-amylase coding sequence resulting in different thermostability enzyme across 84 Czech barley cultivars were detected (J.Ovesna et al. 2006).

In this study the genotyping on the trait responsible intron III mutations including 126 bp (MITE) and (1+6) bp insertion/deletion (Indel) events was applied to evaluate genetic diversity of the *Bmy1* gene in covered and hulless spring barley breeding lines of Latvian origin. For the first time in Latvia, the test on the allelic presentation of the endosperm specific barley *Bmy1* gene was applied to the barley breeding material. These results are the first experience in application of marker-assisted selection methods in practice.

As it was suggested by Sjakste & Zhuk (2006) five *Bmy1* haplotypes could be classified according to their microsatellite motif as HN (Haruna Nijo), HS (*H. vulgare* subsp. *spontaneum* NPGS PI 296897), HA (HA 52), AB (Abava), and AD (Adorra) haplotypes. The presence of MITE element is a characteristic of AD haplotype only. Pedigree stories of Latvian barley varieties permit analysis of transmittance of the intron III polymorphisms trough the generations to be performed. Spring barley varieties from State Priekuļi PBI Balga and Linga both descended from the same cross possess the same whole intron III AD-like haplotype (to have MITE and (1+6) insertion). Spring barley variety 'Abava' from Stende CBI posess its haplotype (to have MITE deletion and (1+6) insertion) to variety 'Ruja' (T. Sjakste & Zhuk 2006). The breeding material included in our study contain haplotypes corresponds to AB-like and AD-like haplotypes with middle and low malting quality. Analysis of breeding lines originated from cross combination between Latvian landrace 'Latvijas Vietejais' (contains high malting quality HN-like hyplotype) and 'Inari' showed the transmittance of the different intron III polymorphisms to the progeny. Eight breeding lines from this combination also characterized with HN-like hyplotype will be desirable source of genetic diversity. Therefore results obtained in this study confirm usefulness of the molecular marker application in the identification of breeding lines perspective for malting and/or feed barley selection.

Conclusions

1. Frequency of MITE InDel alleles is similar within breeding lines for both breeding institutes (heterozigosity index 0.4).

2. The diversity of indel (1+6) bp is higher within Priekuli breeding lines compared to Stende material (heterozigosity index 0.03 and 0.48 respectively). Three from

four possible two loci (MITE/(1+6)) haplotypes were observed.

3. Haplotype characterized by the deletion in both loci and associated with high malting quality, was found only in eight Priekuļi lines selected from the cross of 'Latvijas Vietejie' and 'Inari'.

4. *Bmy1* structural gene of most middle malting quality breeding lines was shown to have MITE deletion and (1+6) insertion. Insertions in both loci of the gene were detected in the resting breeding material suggesting their low malting quality. Variant with MITE insertion and (1+6) deletion was not revealed in our study.

5. Fifty lines or 25% from all investigated breeding lines were heterogeneous and contains all possible haplotypes.

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REFERENCES

Cullis B., Smith A., Panozzo J.F., Lim P. 2003. Barley malting quality: are we selecting the best?, Australian Journal of Agricultural Research, 54:1261-1275.

Eglinkton J.K., Langridge P., Evans D.E. 1998. Thermostability variation in alleles of barley beta-amylase. Journal of CerealScience, 28:301-309.

Erkkila M.J., Leah R., Ahokas H., Cameron-Mill V. 1998. Allele-dependent barley grain beta-amylase activity. Plant Physiology, 117: 679-685.

Erkkila M.J., Ahokas H., 2001. Special barley beta-amylase allele in a Finnish landrace line HA52 with high grain enzyme activity. Hereditas, 134:91-95.

Fox G.P., Panozzo J.F., Li C.D., Lance C.M., Inkerman P.A., Henry R.J. 2003. Molecular basis of barley quality, Australian Journal of Agricultural Research, 54:1081-1101.

Ovesna J., Machova Polakova K., Kucera L., Vaculova K., and Milotova L. (2006) Evaluation of Czech spring malting barleys with respect to the ā-amylase allele incidence, Plant Breeding, 125:236-242.

Plaschke J., Ganal M.W., Roder M.S. 1995. Detection of genetic diversity in closely related bread wheat using MS markers. Theor Appl. Genet., 91:1001-1007.

Polakova K., Laurie D., Vaculova K. and Ovesna J. 2003. Characterisation of ā-amylase alleles in 79 barley varieties with pyrosequencing. Plant Molecular Biology Reporter, 21: 439-447.

Rae S.J., Macaulay M., Ramsay L., Leigh F., Matthews D., O'sullivan D.M., Donini P., Morris P.C., Powell W., Marshall D.F., Waugh R., Thomas W.T.B. 2007. Molecular barley breeding. Euphytica, 158:295-303.

Sjakste T.G., Roder, M. 2004. Distribution and inheritance of ā-amylase alleles in north European barley varieties. Hereditas, 141:39-45.

Sjakste T.G., Zhuk A.F. 2006. Nover haplotype description and structural background of the eventual functional significance of the barley ā-amylase gene intron III rearrangements. Theory Applied Genetics, 113:1063-1079.

Xu Y., Crouch J.H. 2008. Marker-assisted selection in plant breeding: from publications to practice. Crop Science, 48:391-407.

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