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DNA polymorphism and agronomic traits of revertants from barley (*Hordeum vulgare* L.) mutant *tw*

Donatas ŽVINGILA[1], Virginija VAITKŪNIENĖ[1], Jolanta PATAMSYTĖ[1], Algė LEISTRUMAITĖ[3], Monika STANIŪTĖ[1], Laimutė BALČIŪNIENĖ[2], Tatjana ČĖSNIENĖ[1], Violeta KLEIZAITĖ[1], Raimondas ŠIUKŠTA[1], Vytautas RANČELIS[1]

[1]Vilnius University
M. K. Čiurlionio 21, Vilnius, Lithuania
E-mail: donatas.zvingila@gf.vu.lt

[2]Botanical Garden of Vilnius University
Kairėnų 43, Vilnius, Lithuania

[3]Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry
Instituto 1, Akademija, Kėdainiai distr., Lithuania

Abstract

In the present study, the high polymorphism of quantitative characters in revertants derived from homeotic and pleiotropic genetically unstable *tw*-type barley mutants was confirmed by DNA polymorphism analysis using random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) assays. The possibility to find differences among wild type (WT) (cv. 'Auksiniai II'), original mutants tw_1 or tw_2 and 34 revertants studied in the present work, depended on RAPD and ISSR primers used in PCR. Homeotic *tw*-type mutants have lodicules converted to pistils or stamens while typical revertants (N-type) have normal spike and flower structure. A separate group of revertants (C-type) has normal flower structure and compactoid spike. Our study showed, that all revertants of this group are more resistant to loose smut, but less resistant to lodging, powdery mildew and net blotch in comparison with the standard cv. 'Simba'. C-type revertants tend to cluster together on the UPGMA dendrogram drown on the basis of ISSR markers. Sequencing and BLAST analysis of 6 monomorphic and 3 polymorphic RAPD fragments showed rather high homology of some RAPD fragments with retrotransposons and coding sequences of *Hordeum vulgare.*

Key words: *Hordeum vulgare*, revertants, RAPD, ISSR, DNA polymorphism.

Introduction

Reversions of gene mutations (*missense*, *nonsense* or *frame shift*) in most cases do not mean an exact return to initial WT, but only restore the activity of gene and/or other characteristics of WT phenotype and may be one of the ways to create plant genetic resource collections for quantitative trait locus (QTL) alleles.

The revertant collections may be created as a byproduct of transposon mutagenesis (Bai et al., 2007), chemical and physical mutagenesis (Liu et al., 2008), instability of meiosis (Piffanelli et al., 2004) or newly arisen unstable mutants (Rančelis et al., 2004). This latter approach was used to develop the barley revertant collection on the basis of genetic unstable pleiotropic recessive homeotic barley mutants *tw* (*tweaky spike*) with specific spike structure and homeotic conversion of lodicules to stamens or/and pistils. Three of 12 allelic *tw* mutants were genetically unstable and gave reversions to WT phenotype. Revertants *tw→WT* are easily distinguished by normal spike form and normal flower structure. Some revertants were characterized by incomplete reversion to WT according to quantitative characters, several having economic value. From this point of view, the most interesting are revertants with increased protein content and restored productivity, and also revertants with higher resistance to abiotic stress (e.g., mutagenic factor azaridine) (Rančelis et al., 2004).

The high variation of quantitative characters in *tw* revertants has not been elucidated till now and may have two alternative explanations. First, split of the pleiotropic character complex was supposed. Separate phenotype characteristics of the pleiotropic character complex of initial *tw* mutants remained in the tested revertants. Increased protein content (Rančelis et al., 2004), sensitivity of grains to micromycete infection (Vaitkūnienė et al., 2006) may be attributed to such characteristics. Increased resistance to mutagenic factor azaridine (ethyleneimine) was also observed in mutant *tw* and it was partially retained in revertant lines (Rančelis et al., 2004).

The second explanation of phenotypic variation in *tw* revertants could be multiple minor genetic changes not only in the basic gene, leading to morphological feature of the spike form and variations of the flower structure, but also mutations in unknown number of QTLs, forming different genetic background not only for initial mutant *tw* alleles, but also for their revertants. In the present study, we used two different molecular marker systems (RAPD and ISSR) to detect possible molecular variation in *tw* revertants.

Another goal of this investigation was to estimate the economic value of revertants. Our attention was focused on the type of revertants possessing normal flower structure but compactoid (C) spike. In the preliminary study, several C-type revertants were more resistant to lodging and had increased protein content, but grain yield was comparable with initial for cv. 'Auksiniai II', but not with cv. 'Ūla', used as the standard (Vaitkūnienė et al., 2006).

In this paper, we also studied molecular properties of several fragments from RAPD profiles of analyzed barley accessions.

Materials and methods

Plant material. All revertants tested in the present work were originated from two formerly genetically unstable mutants tw_1 (N_1–N_{25}, C_1, C_3) and tw_2 (N_{26}–N_{52}, C_4, $C_{6\text{-}13}$). Mutants of *tw* type were induced by chemical mutagenesis from cv. 'Auksiniai II', which was used here as wild type (WT). Revertants and initial plant material was grown and preliminary tested in the Botanical Garden of Vilnius University. Plant reproduction and preliminary investigation of hybrids were also performed in the Botanical Garden. As quantitative characters depend significantly on the environmental conditions, the revertants have been examined for specific quantitative character for several years (Rančelis et al., 2004).

Selected revertants were evaluated at the Lithuanian Institute of Agriculture during 2006–2009. The experimental cultivars were grown in two replications with a plot size of 10.0 × 1.5 m^2. The crop was sown in a well-prepared seedbed with a "Hege 80" drill at a rate of 5 million seed ha^{-1}. The soil of the experimental site is *Endocalcari-Epihypogleyic Cambisol* (*CMg-p-w-can*) (55°24′ N, 23°50′ E), with a pH of 7.3, organic matter content of 2.1%, available P_2O_5 150–180 mg kg^{-1} and K_2O 100–150 mg kg^{-1}. At sowing, 90 kg of N, 60 kg of P_2O_5 and 60 kg of K_2O were broadcast-applied, weeds and insects were controlled chemically.

Barley mutants, revertants were grown according to the technology used for breeding. The plots were harvested by a "Wintersteiger" harvester. Combine-harvested grain from each plot was dried and sampled for analyses. One thousand grain weight (ISO 580), total grain protein (total nitrogen by Kjeldahl multiplied by 6.25, ICC 105/2), total starch content (by hydrochloric acid dissolution, ICC 123/1) were determined. Yield data were adjusted to 15% moisture content, grain quality characters were determined in dry grain.

A sample of sheaf was taken from each plot shortly before harvesting for length measurement of individual plants and productivity components.

The diseases were assessed at medium milk development stage (BBCH 75). The disease resistance was measured in scores, using a 1–9 scale. Score 1 – no visible symptoms of diseases, score 9 – plants heavily infected (infection ≥80%). Lodging resistance was measured in scores, using a 1–9 scale (9 – all plants erect).

The data were statistically processed by a software package *ANOVA* (Tarakanovas, Raudonius, 2003) and statistically analysed using Fisher's analysis of variance technique. The least significant difference test at 0.05 probability level was employed to compare the differences among the treatment means.

DNA isolation and analysis. DNA was isolated from leaves of etiolated seedlings using Genomic DNA purification kit ("Fermentas", Lithuania). Leaf samples from 3–4 plants were used for DNA isolation. RAPD-PCR was carried out as described by Williams et al. (1990), ISSR-PCR – according to Hou et al. (2005) with some modifications. For RAPD-PCR, 30 primers from Roth ("KarlRoth", Germany) were applied, but only 9 of them produced polymorphic DNA fragments (Table 1).

For ISSR-PCR, 9 primers ("Metabion International AG", Germany) were used: ISSR E – $(CCA)_5$, annealing temperature 52°C; ISSR F – $(ACACAC)_2ACA$, 48°C; ISSR H – $(GAA)_5$, 38°C; ISSR I-18 – $GTG(CT)_7C$, 56°C; ISSR I-29 – $(GT)_6CA$, 41°C; ISSR I-32 – $(AGC)_4C$, 44°C; ISSR I-34 – $(AGC)_4GG$, 50°C; ISSR I-39a – $(AGC)_4AC$, 47°C; ISSR I-50a – $CCA(GCT)_4$, 52°C. Informative primers are shown in Table 1.

Twenty five accessions were analysed using both assays, 12 additional lines were tested only by ISSR assay. Amplification of each DNA sample was repeated at least twice to ensure reproducible results.

DNA fragments were stained with ethidium bromide ("Sigma-Aldrich", Germany) and documented under UV light using the "BioDocAnalyse System" ("Biometra", Germany). The size of DNA fragments was determined according to the molecular-size standards GeneRuler™ 1 kb DNA Ladder and GeneRuler™ DNA Ladder Mix ("Fermentas", Lithuania) using *BioDocAnalyse Image Acquisition and Analysis* software ("Biometra", Germany).

Data analysis. DNA fragments with length below 400 bp were very variable and were rejected from further analysis. In each sample, the presence and absence of DNA bands were recorded as 1 or 0, respectively. The absent DNA band was considered as a null allele of certain RAPD or ISSR locus. A pairwise comparison of banding patterns was evaluated using the *TREECON* software for *Windows* (Van de Peer, De Wachter, 1994). The genetic distances (GDxy) were calculated according to Nei and Li (1979). The dendrograms were constructed by applying the unweighted pairgroup method of arithmetic average (UPGMA) clustering method and the Nei and Li (1979) distance matrix.

The correlation between the molecular genetic distance matrixes determined by RAPD and ISSR methods was estimated using *Statistica 8* (2008).

Table 1. Informative primers for RAPD-PCR and ISSR-PCR

Primer	Primer sequence 5'→3'	Scored DNA fragments	% polymorphic fragments	Fragment length bp
RAPD-PCR primers				
Roth C05[1]	GATGACCGCC	14	57.1	470–1070
Roth C10	TGTCTGGGTG	14	85.7	570–1030
Roth C14	TGCGTGCTTG	10	70.0	500–920
Roth C20	ACTTCGCCAC	5	60.0	580–680
Roth 470-06	GCACGTGAGG	13	53.8	800–2000
Roth 470-07	CTATCGCCGC	13	61.5	700–2000
Roth 470-09	GCGGGGTTAC	12	66.7	400–2200
Roth C07	GTCCCGACGA	11	54.5	480–1030
Roth C16	CACACTCCAG	8	75.0	400–920
ISSR-PCR				
ISSR F	$(ACACAC)_2ACA$	15	20.0	460–1000
ISSRH	$(GAA)_5$	20	30.0	690–2000
ISSR I-32	$(AGC)_4C$	11	9.1	640–1200
ISSR I-34[2]	$(AGC)_4GG$	24	45.8	380–1300
ISSR I-39a	$(AGC)_4AC$	23	21.7	600–2000
ISSR I-50a	$CCA(GCT)_4$	15	13.3	610–1400

[1] – see Figure 1, [2] – see Figure 2

Nine individual DNA fragments were extracted from agarose gels using *NucleoSpin Extract II* kit ("Macherey-Nagel", Germany) and sequenced with 3130 × l genetic analyzer using *BigDye® Terminator v3.1 Cycle Sequencing* kit ("Applied Biosystems", USA). Similarity search was performed using online program *BLAST* at the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/).

Results and discussion

Significant diversity of revertants was observed not only for quantitative characters (Rančelis et al., 2004), but also for DNA loci revealed by RAPD and ISSR methods. However, the success to identify DNA polymorphism in barley revertants depended significantly on the primers used for DNA amplification. This was equally valid for both, RAPD (Fig. 1) and ISSR (Fig. 2), assays.

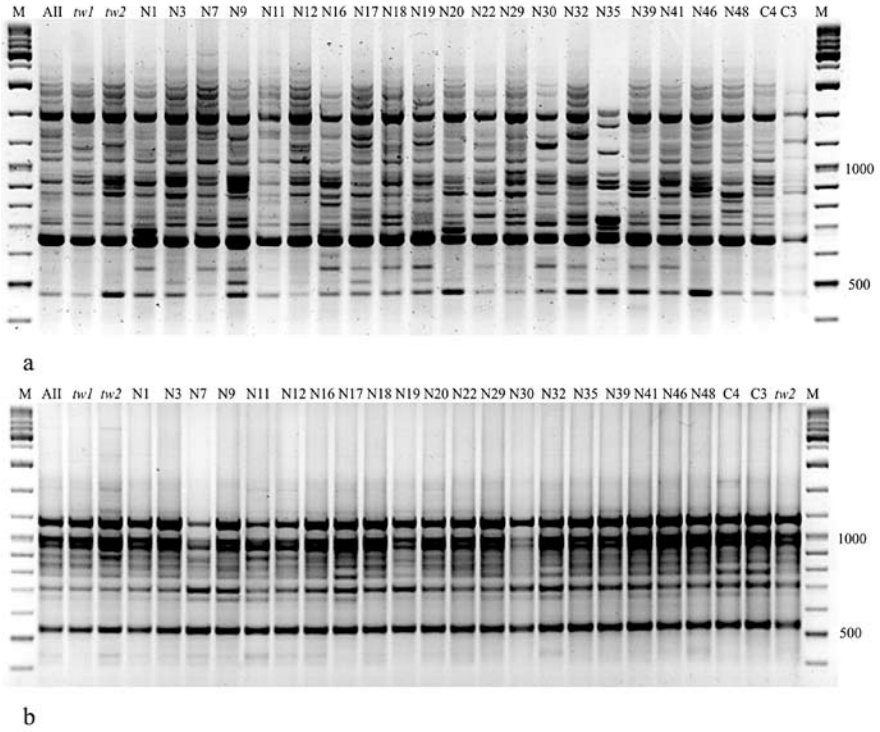


Figure 1. Monomorphic and polymorphic loci revealed with primer Roth C05 (a) and only monomorphic loci – with primer Roth C02 (b), used for RAPD-PCR (M – GeneRuler DNA Ladder)

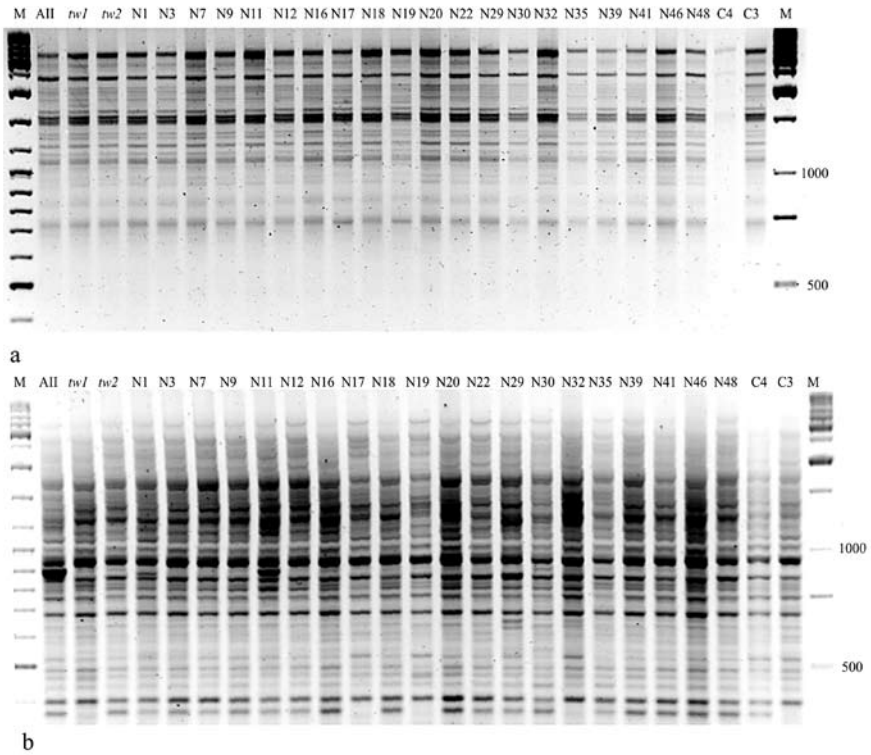


Figure 2. Monomorphic and polymorphic loci revealed with primer ISSR I-34 (a) and only monomorphic loci – with primer ISSR H (b), used for ISSR-PCR (M – GeneRuler DNA Ladder)

Only nine primers of 30 tested for RAPD-PCR produced polymorphic DNA fragments. Percentage of informative primers was higher for ISSR-PCR, five primers of the 9 tested were informative, but the level of polymorphic DNA fragments was only in a range of 9.1–45.8%, while for RAPD-PCR primers it was 53.8–85.7% (Table 1); 113 RAPD loci and 153 ISSR loci were identified in our study.

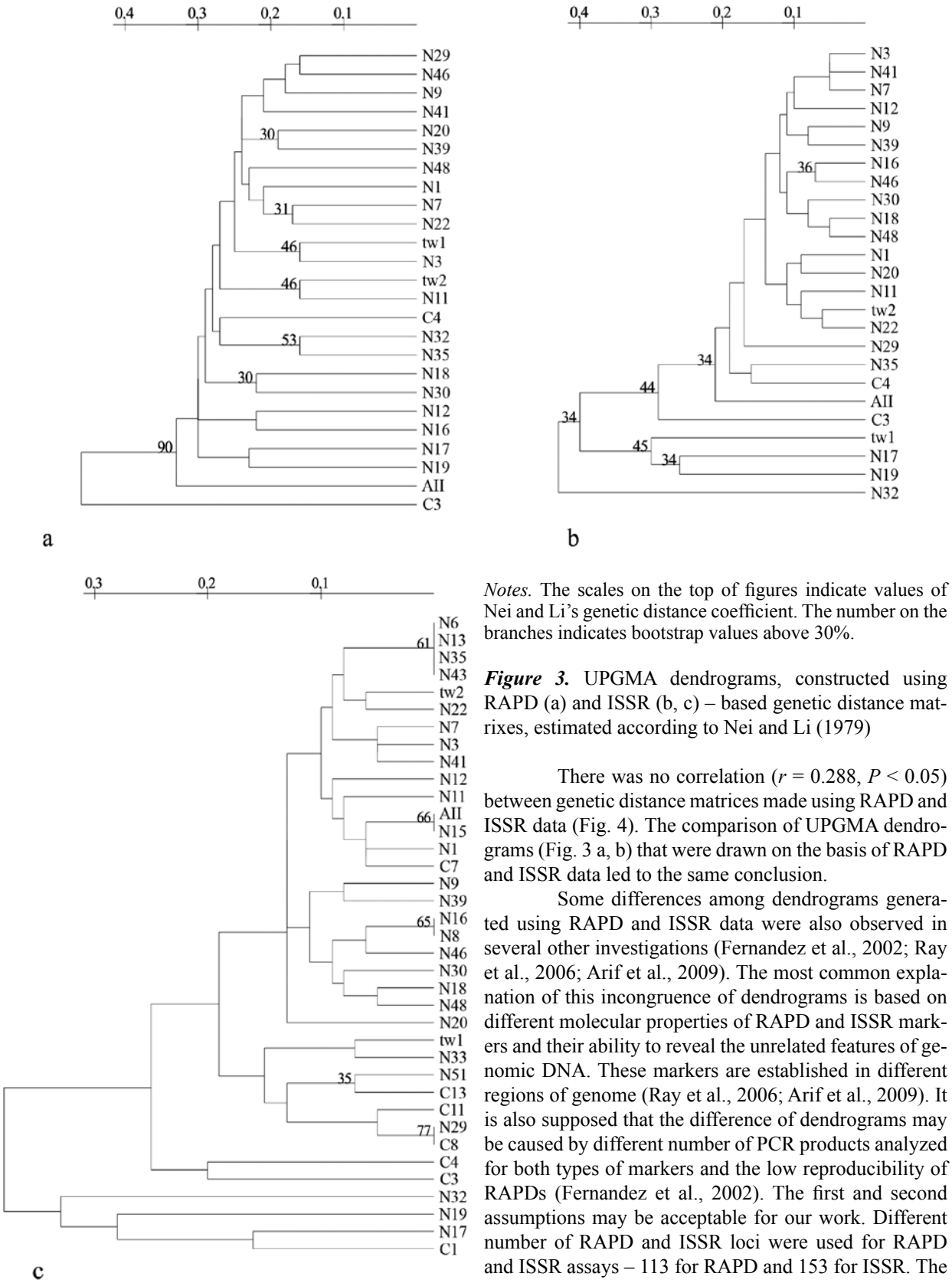
The set of informative primers is common problem for many RAPD and ISSR studies, including studies of barley. For example, only one RAPD primer of 10 tested, and four ISSR primers of 10 tested were effective to distinguish phylogenetic relationships among 16 barley cultivars (Fernandez et al., 2002). In the study of wild barley (*Hordeum vulgare* subsp. *spontaneum*) populations only 55 polymorphic loci were found using 55 RAPD and 10 ISSR primers (Tanyolac, 2003). Two ISSR primers of 12 tested were effective to show genetic diversity of *Pyrenophora teres*, affecting barley crops in different districts of Lithuania (Statkevičiūtė et al., 2010) and as many as 500 RAPD primers were tested for investigation of barley resistance to *Pyrenophora teres* (Molnar et al., 2000). Only 139 (39%) of them produced polymorphic patterns. Nearly the same percentage of informative primers (15 of 40 tested) generated reproducible polymorphism in modern, ancestral and introgressed Finnish six-rowed barley varieties (Manninen, Nissila, 1997). Three primers of 38 evaluated oligonucleotide primers produced reproducible polymorphic bands for several barley species from Turkey – *H. murinum*, *H. bulbosum*, *H. vulgare spontaneum* (Albayrak, Gözükirmizi, 1999). A higher percentage of informative primers was applied for diversity studies of wild barley *H. spontaneum* accessions from Jordan: 27 RAPD primers of 40 tested were informative (Al-Saghir et al., 2007). Successful set of RAPD primers allowed discrimination of *H. vulgare* and *H. bulbosum* species and showed that populations of *H. bulbosum* were significantly more polymorphic in comparison with *H. vulgare* accessions (Okumus, Urzun, 2007). Only five primers of 68 tested generated 48 DNA fragments, but it was enough for genotyping hulless barley varieties from Tibet. Specific RAPD locus was revealed for selection of barley varieties with high and low β-glucan content (Yu et al., 2002).

In one more study, four ISSR primers yielded in total 47 polymorphic loci, but it was enough to determine the main principles of genetic diversity origin of barley landraces and cultivars from Nordic and Baltic regions. Among barley accessions from Southern Scandinavia, Latvia and Lithuania, the highest DNA diversity was established in landraces and cultivars of the oldest origin, in comparison with cultivars of 1931–1970 breeding period and especially with cultivars bred after 1971. In the group of barley cultivars from Northern Scandinavia, Finland and Estonia, the opposite relation was observed – the highest DNA diversity was among cultivars bred after 1971 (Kolodinska-Brantestam et al., 2004). This data shows that ecological conditions have significant influence on the establishment of intraspecific genetic diversity of barley. For wild barley it was experimentally shown by Owuor et al. (2003) using RAPD assay.

To increase efficiency of RAPD and ISSR assays for investigation of barley and other plant genomes, various modifications of both methods are made, inclu-

ding electrophoresis of denaturated DNA (Bahieldin et al., 2006), usage of lengthened primers for RAPD (Sivolap, Kalendar, 1995), treatment of genomic DNA with restriction endonuclease (Roslinsky et al., 2007) or conversion of RAPD markers into STS markers (Hoffman et al., 2003).

The set of ISSR and RAPD primers, used in the present work, is about the same efficiency as in the other works discussed above. UPGMA dendrograms from the RAPD and ISSR data of 25 genotypes were drawn (Fig. 3 a, b).



Notes. The scales on the top of figures indicate values of Nei and Li's genetic distance coefficient. The number on the branches indicates bootstrap values above 30%.

Figure 3. UPGMA dendrograms, constructed using RAPD (a) and ISSR (b, c) – based genetic distance matrixes, estimated according to Nei and Li (1979)

There was no correlation ($r = 0.288$, $P < 0.05$) between genetic distance matrices made using RAPD and ISSR data (Fig. 4). The comparison of UPGMA dendrograms (Fig. 3 a, b) that were drawn on the basis of RAPD and ISSR data led to the same conclusion.

Some differences among dendrograms generated using RAPD and ISSR data were also observed in several other investigations (Fernandez et al., 2002; Ray et al., 2006; Arif et al., 2009). The most common explanation of this incongruence of dendrograms is based on different molecular properties of RAPD and ISSR markers and their ability to reveal the unrelated features of genomic DNA. These markers are established in different regions of genome (Ray et al., 2006; Arif et al., 2009). It is also supposed that the difference of dendrograms may be caused by different number of PCR products analyzed for both types of markers and the low reproducibility of RAPDs (Fernandez et al., 2002). The first and second assumptions may be acceptable for our work. Different number of RAPD and ISSR loci were used for RAPD and ISSR assays – 113 for RAPD and 153 for ISSR. The last assumption is not adequate for our study because

only reproducible and clearly expressed RAPD bands were scored for data analysis. On the other hand, there are several publications, where high correlation between RAPD and ISSR data was established. Good correspondence among RAPD, ISSR and SSR data was observed by Sundaramoorthi et al. (2009) in rice study. Ray et al. (2006) also found high correlation between RAPD and ISSR data for micropropagated banana accessions. Such discrepancy of results obtained by different authors may be explained by differences of studied genomes and peculiarities of primers used. Despite differences of dendrograms, it is obvious that barley mutation tw_1 is more divergent from WT (cv. 'Auksiniai II') than allelic mutant tw_2 (Fig. 3 a, b).

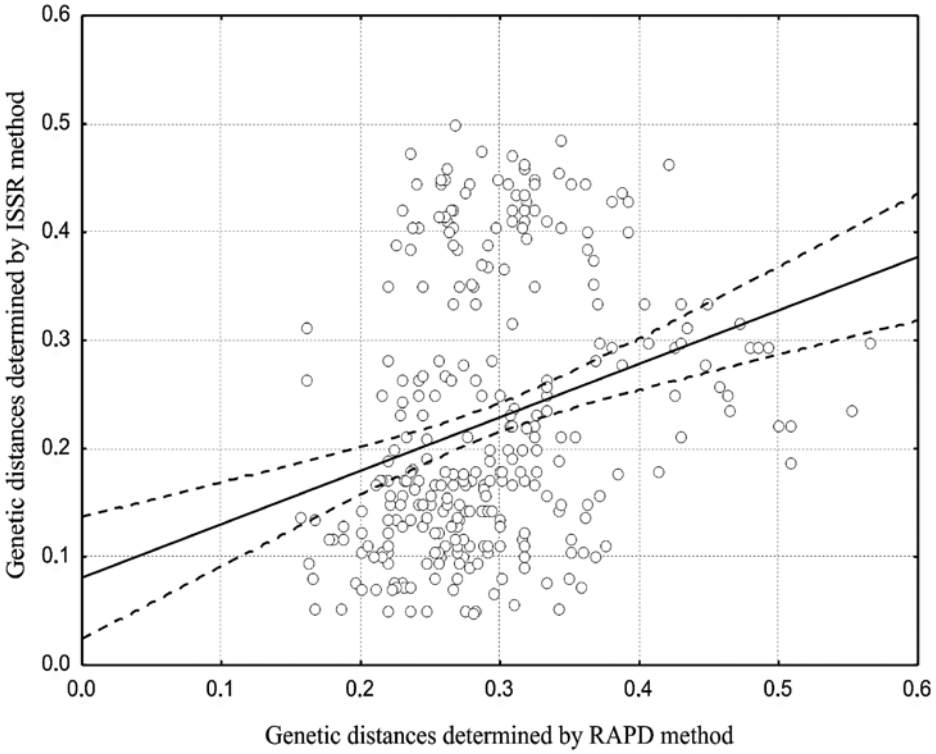


Figure 4. Correlation between genetic distance matrixes determined by RAPD and ISSR methods. Genetic distances among 25 barley genotypes, estimated using data of both assays, were compared

To assess genetic relationships among initial mutant lines tw_1, tw_2 and their revertants more appropriately we increased the number of studied genotypes by 12 additional accessions of revertants and analyzed them using the same set of ISSR primers. In this case the dendrogram representing genetic relationships of 37 barley genotypes was drawn. One more common feature of all dendrograms is the absence of close relationships among initial mutant lines tw_1 or tw_2, and their revertants (Fig. 3). Several revertants (N6, N13, N22, N11, N1, N15, N12, N3) derived from tw_1 are even closer to tw_2 than to tw_1 (Fig. 3 c). All these revertants together with AII and tw_2 compose a common cluster.

In our opinion, revertants of compactoid type (C-type) of spike represent a very interesting group of accessions. These revertants have normal flower structure, while the lodicules of mutants tw_1 or tw_2 are converted to stamens or carpels. Compactoid spike is also distinct form the spike of *tw* mutants. After initial studies at the Botanical Garden of Vilnius University (Vaitkūnienė et al., 2006) selected C-type revertants were tested at the Lithuanian Institute of Agriculture. Only a few of C-type revertants had spike density higher than standard cv. 'Simba' and all of them were less resistant to lodging (Table 2). All C-type revertants had also higher protein content in grains, but produced lower yield. Only C6 was about the same productivity as cv. 'Simba'. It is noteworthy that in our previous experiment C-type revertants showed better resistance to lodging (Vaitkūnienė et al., 2006). Different environmental conditions (soil properties, relative humidity, etc.) may be responsible for the discrepancies in lodging results between the experimental sites.

The study of C-type revertants according to ISSR assay gave more homogeneous results in comparison with N-revertants. The main group of C-type revertants tends to cluster together on the ISSR dendrograms (Fig. 3 c). This result shows some genetic peculiarities of C-type revertants.

As mentioned before, molecular properties of some DNA bands produced using RAPD primers were also studied. Six monomorphic (No. 1–6) fragments common for all tested barley accessions and three polymorphic (No. 7–9) RAPD fragments were extracted from the agarose gel and sequenced (Table 3). Monomorphic loci were included in this analysis because they are represented in RAPD profiles of different barley genotypes

Table 2. Grain yield characteristics of compactoid type revertants, 2009

Revertant	Grain yield		Spike density	1000 grain weight g	Protein % d.w.	Starch % d.w.	Lodging points	Diseases in points			
	t ha^{-1}	difference from S						PM	NB	PhS	LS
S	4.07	0	15.8	43.4	11.9	59.1	9	1	4	5	137
C3	3.88	−0.19	16.9	34.7	12.4	61.2	5	5	3	4	0
C11	3.43	−0.64	16.1	34.5	13.4	59.1	3	5	3	4	0
C13	3.43	−0.64	15.8	33.5	13.3	59.3	4	4	3	4	0
C4	3.15	−0.92	14.5	36.2	13.1	59.0	3	5	3	3.5	0
C6	4.04	−0.03	13.8	46.0	13.3	59.4	9	4	3	3	0
C9	3.66	−0.42	14.7	35.4	14.0	58.3	6	5	3	4	0
C10	3.62	−0.46	14.3	37.3	14.1	58.4	5	5	3	4	0
C14	2.56	−1.52	17.6	38.5	15.0	53.9	9	6	3	3	0

Notes. S – standard cv. 'Simba', C3–C13 – compactoid type revertants, Spike density – number of rachis internodes per 4 cm of rachis length, d.w – dry weight. Diseases: PM – powdery mildew, NB – net blotch, PhS – physiological spots, LS – loose smut. $LSD_{0.5}$ = 0.658, 18.61, $LSD_{0.1}$ = 0.958, 27.07%; S_x = 0.20, S_x% = 5.71.

Table 3. BLAST analysis in NCBI data base of several isolated DNA fragments

No.	Genotype	RAPD primer	Fragment length bp	Similar to	The highest similarity %
1.	N32	Roth A01	535	*Hordeum vulgare* subsp. *vulgare Rpg4*, *Rpg5*, *RGA1*, *PP2C*, *ADF3* genes	84
				H. vulgare subsp. *vulgare* retrotransposon *Derami*_D22C-1; retrotransposons *Vagabond*_D22C-1 and *Lolaog*_D22C-1; transposon *Damocles*_D22C-1, gene *CBF12*; transposon *Tourist*_D22C-1, retrotransposon *BAGY*_D22C-1;	93
				Triticum monococcum BAC clone 116F2 and 115G1 gene	97
2.	N20	Roth A04	583	No similarity found	
3.	N32	Roth A09	438	No similarity found	
4.	N32	Roth D16	686	*Hordeum vulgare* subsp. *vulgare* cDNA clone FLbaf160g12, mRNA sequence;	73
				Triticum aestivum 3B chromosome, clone BAC TA3B63E4;	69
				H. vulgare subsp. *vulgare* cDNA clone FLbaf137j16, mRNA sequence	82
5.	tw_1	Roth D16	633	*Hordeum vulgare Mla* locus;	76
				H. vulgare cv. 'Cepada' *Capa Rym4* and *MCT-1* genes;	79
				H. vulgare subsp. *vulgare eIF4E* gene locus;	79
				H. vulgare tonoplast intrinsic protein 1/2 (*TIP1/2*) and *Rar1* (*Rar1*) genes;	75
				H. vulgare subsp. *vulgare* retrotransposon *Sukkula* N5D-1; transposon *Thalos*_N5D-1; pseudogene *CBF8*; full sequence of transposon *Icarus*_N5D-1; putative glucanase/cellulase	91
6.	N32	Roth D05	715	*Oryza sativa* group Japonica genome DNA – chromosome 1 BAC clone OSJNBa0014K08	100
7.	N18	Roth 470-9	563	*Hordeum vulgare* subsp. *vulgare eIF4E* gene	84
8.	N20	Roth 470-9	563	*Hordeum vulgare* subsp. *vulgare eIF4E* gene;	84
				H. vulgare DNA region of *HvNAS1* gene	83
9.	tw_1	Roth 470-9	703	No similarity found	

by DNA bands of identical size but variable intensity. This kind of variation usually is ignored in genetic diversity studies (Harris, 1999). However, these quantitative variations can be caused also by variation in the copy number of certain locus in plant genome or some changes in primer binding site. This kind of variation may be phenotypically neutral but also can be associated with some quantitative differences among revertants. Monomorphic RAPD fragments No. 1 and No. 5 showed similarity with known barley mobile elements and slightly matched to fragments of the genes encoding resistance to pathogens (*Rpg4/5*, *Mla*), seed maturation (*PP2C*, *ADF3*), tonoplast intrinsic protein (*TIP1/2*) and others (Table 3). The 703 bp length fragment (No. 9) was specific to mutant tw_1, but the BLAST analysis did not reveal homologous sequences in NCBI database. The 563 bp length fragment (No. 7 and No. 8) was specific to accessions of revertants N18 and N20 from the tw_1 group and showed similarity with sequence of *eIF4E* locus and downstream region of gene *HvNAS1*. The *eIF4E* encode subunit of translation initiation factors which recognize cap in mRNA. At the present time, a function of *eIF4E* is more widened. This gene is also involved in response to virus infection and to other stress conditions (Rhoads, 2009). The nicotianamine synthase gene *HvNAS1* of barley takes part in response to metal stress. The *HvNAS1* gene is strongly induced by Fe deficiency in roots and encodes chelator nicotianamine (NA). However, recently it was pointed out that NA makes complexes not only with Fe, but also with other metals (Curie et al., 2009). From this point of view, it is noteworthy that the higher resistance of revertant N18 to Al and Co treatment was also observed (data not shown). According to this aspect, revertant N20 was not investigated.

Conclusion

Evaluation of barley revertants for DNA polymorphism using RAPD and ISSR assays revealed differences among wild type (WT) genotype, *tw*-type mutants and revertants. In general, both mutants tw_1 and tw_2 were induced by chemical mutagenesis, and mutagens could act not only on *tw* locus, but also on many other genomic loci. It could be the possible reason of DNA polymorphism and differences in the quantitative traits among WT and mutants of *tw*-type. Genetic instability of *tw* mutants could also increase the genetic variation of studied genotypes.

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Revertantų iš paprastojo miežio (*Hordeum vulgare* L.) *tw* mutanto DNR polimorfizmas ir agronominiai požymiai

D. Žvingila[1], V. Vaitkūnienė[1], J. Patamsytė[1], A. Leistrumaitė[3], M. Staniūtė[1], L. Balčiūnienė[2], T. Čėsnienė[1], V. Kleizaitė[1], R. Šiukšta[1], V. Rančelis[1]

[1]Vilniaus universitetas
[2]Vilniaus universiteto Botanikos sodas
[3]Lietuvos agrarinių ir miškų mokslų centro Žemdirbystės institutas

Santrauka

Didelė revertantų, gautų iš homeozinių bei pleiotropinių genetiškai nestabilių miežių *tw* tipo mutantų, įvairovė pagal kiekybinius požymius buvo patvirtinta atliekant šiuos DNR polimorfizmo tyrimus RAPD (angl. *random amplified polymorphic* DNA) ir ISSR (angl. *inter-simple sequence repeat*) metodais. Aptiktus skirtumus tarp miežių WT (angl. *wild type*) genotipo 'Auksiniai II', pradinių mutantų tw_1 arba tw_2 ir 34 tirtų revertantų lėmė pradmenys, naudoti DNR pagausinti. Homeozinių *tw* tipo mutantų žiedo lodikulės virtusios kuokeliais ir/arba piestelėmis, o tipiškų revertantų (N tipo) varpos ir žiedai yra normalūs. Atskirą grupę sudaro revertantai su kompaktiška varpa (C tipo), kurių žiedai yra normalūs. Tyrimų metu nustatyta, kad šie revertantai yra atsparesni dulkančiosioms kūlėms, bet mažiau atsparūs išgulimui, miltligei ir dryžligei nei standartinės veislės 'Simba' miežiai. C tipo revertantams būdinga tendencija sudaryti atskirą grupę dendrogramoje, sudarytoje pagal ISSR žymeklius. Sekvenuotų 6 monomorfinių ir 3 polimorfinių RAPD fragmentų analizė parodė kai kurių iš jų panašumą su retrotranspozonais ir kai kuriomis koduojančiomis *Hordeum vulgare* sekomis.

Reikšminiai žodžiai: *Hordeum vulgare*, revertantai, RAPD, ISSR, DNR polimorfizmas.