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Short communication

Genetic diversity among cultivars of spring barley revealed by random amplified polymorphic DNA (RAPD)

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Abstract. RAPD (random amplified polymorphic DNA) polymorphism was studied in 23 malting and non-malting spring barley cultivars included in the official list of Polish cultivated varieties. Twenty-four 10-mer primers were tested in each cultivar, giving altogether 149 amplification products, 45% of which were polymorphic. The number of polymorphic bands revealed by one primer ranged from 1 to 6, with an average of 2.8. Genetic distance for all pairs of compared varieties was estimated and a dendrogram was constructed using unweighted pair group method of arithmetic means. The genetic distance between cultivars ranged from 0.11 for cvs. Apex and Bryl to 0.62 for cvs. Orthega and Madonna. Of the seven malting cultivars only two (Brenda and Stratus) formed one group at D = 0.25. The genetic distance between cvs. Brenda and Scarlett, especially recommended for brewery, was equal to 0.34. The detected polymorphism appeared to be sufficient for assessing genetic distances between cultivars, but on the basis of this polymorphism groups of malting and non-malting cultivars were not clearly distinguished.

Key words: barley cultivars, genetic distance, RAPD polymorphism.

The gene pool used in barley breeding is very limited, which results in low genetic diversity among cultivars. Additionally, many of the current breeding programmes in barley involve crosses among lines/varieties with common ancestry. For this reason determination of genetic distances between genotypes on the basis of morphological or physiological traits is difficult.

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Knowledge about genetic similarity/distance among varieties is important both from the genetic and breeding point of view. In genetic research connected with construction of genetic linkage maps a high level of polymorphism is required between individuals/lines in a studied population. A high frequency of polymorphic loci in a segregating population is expected when crossed parents are unrelated (HEUN et al. 1991). In barley, choice of genetically distant varieties may be based on polymorphism of molecular markers such as RFLP (restriction fragment length polymorphism) and RAPD (random amplified polymorphic DNA) (HUEN et al. 1991, GRANER et al. 1991, TINKER et al. 1993, BARUA et al. 1993, de BUSTOS et al. 1998).

The aim of this study was to detect genetic differences among cultivars of spring barley using RAPD.

RAPD polymorphism was studied in a set of twenty-one elite spring barley cultivars included in the official list of Polish cultivated varieties. Of the tested genotypes, seven were malting cultivars (Brenda, Maresi, Mobek, Orlik, Rasbet, Scarlett, Stratus), and fourteen were non-malting (Atol, Bies, Boss Bryl, Edgar, Gwarek, Madonna, Nagrad, Othega, Rabel, Rambo, Rastik, Rodion Start). Additionally, two older cultivars: Apex and Roland were included in the experiment because they were in a pedigres of some studied genotypes. All the studied cultivars are two-rowed. The seeds were kindly supplied by the Research Centre for Cultivar Testing at Słupia Wielka (Poland).

DNA was extracted from leaves of 3-week-old seedlings grown in a growth chamber. Ten 2 mm^2 discs of leaf tissue were soaked for 15 min at 95°C in 200 µL of TPS buffer (10 mM TRIS-HCL pH 9.5, 1M KCl, 10 mM EDTA) (THOMPSON, HENRY 1995). DNA concentration was determined using a Versa-Fluor Fluorometer (Bio-Rad). Amplification was performed in a reaction volume of 25 µL containing 35 ng of 10-mer primer (GENSET), 1.5-4.5 ng DNA, 10 mM Tris-HCL, pH 8.3, 2 mM $MgCl_2$, 2.5 µg BSA, 100 µM of each dNTP, and 1.5 U of Taq DNA polymerase (Fermentas). PCR reactions were carried out in a PTC-200 thermocycler (MJ Research) and the cycling was performed as follows: 95°C/5 min, followed by 45 cycles of 94°C/1min, 36°C/2 min and 72°C/2 min, with a final extension of 72°C/5 min.

Twenty-four 10-mer primers (GENSET) containing 60-70% (G + C) were used for RAPD analysis of each studied cultivar. Amplification products were analysed by electrophoresis for 2.5 h in 1.5% agarose gels containing TBE buffer and ethidium bromide. 100 bp DNA Ladder Plus (Fermentas) was used for fragment length determination.

Genetic similarities (GS) for all pairs of compared varieties were estimated according to the formula given by NEI and LI (1979): $GS = 2N_{ij}/(N_i + N_j)$, where N_{ij} is a number of alleles present in both compared lines, while N_i and N_j are numbers of alleles in line *i* and *j*, respectively. Genetic distances (D) were calculated by transformation of GS as follows: $D = 1 - GS$. The matrix of D values was the basis

Table 1. Sequences of primers detecting polymorphism in barley and characteristics of the amplification products

Primer	Sequence 5'→ 3'	Number of amplified DNA fragments	Fragment size (bp) min-max	Polymorphic fragments no. (%)
1	GTGCCTAACG	5	200 – 1031	5 (100.0)
2	CTGACGTCAC	7	200 – 1200	2 (28.5)
3	CAGCTCACGA	6	200 – 1500	4 (66.6)
4	CTACTGCCGT	5	300 – 1200	1 (20.0)
5	ACGGCGTATG	5	200 – 900	3 (60.0)
6	GGTCGGAGAT	6	100 – 1500	4 (66.6)
7	CACTCTCCTC	7	200 – 1500	3 (42.8)
8	AAAGCTGCGG	4	300 – 900	2 (50.0)
9	TCATCCGAGG	5	300 – 1500	2 (40.0)
10	TGCCCAGCCT	8	100 – 1500	4 (50.0)
11	CAATCGCCGA	6	100 – 900	4 (66.6)
12	TCGGCGATAG	7	100 – 1500	4 (57.1)
13	AGCCAGCGAA	8	200 – 2000	6 (75.0)
14	TGATCCCTGG	6	200 – 900	2 (33.3)
15	TGCTCTGCCC	6	300 – 900	1 (16.6)
16	ACCCCCGAAC	6	100 – 1031	3 (50.0)
17	GGTGATCAGG	6	200 – 800	1 (16.6)
18	TGCTGCAGGT	7	400 – 1500	2 (28.5)
19	TTCCCGGGTT	6	100 – 900	2 (33.3)
20	CCGGACACGA	7	100 – 1031	4 (57.1)
21	GGACTGGAGT	7	400 – 2000	2 (28.5)
22	GTCGCGGTCA	6	300 – 800	2 (33.3)
23	CAGGCGCACA	6	200 – 800	1 (16.6)
24	GGTGACGCAA	7	300 – 1200	3 (42.8)

on which the dendrogram was constructed using unweighted pair group method of arithmetic means (UPGMA) (SNEATH, SOKAL 1973).

Twenty-four primers were tested in each variety to detect RAPD polymorphism (Table 1). In total, the primers used gave 149 amplification products, from which 67 (45%) were polymorphic. The number of polymorphic bands revealed by one primer ranged from 1 to 6, with an average of 2.8. The size of amplification products ranged from 100 to 2000 bp. For the study of similarity between cultivars, 21 distinguishable polymorphisms revealed by primers Nos. 1, 2, 3, 5, 11, 16 and 17 (Table 1) were used.

Genetic distance was computed for all 253 pairwise comparisons. D values ranged from 0.11 to 0.62. The lowest genetic distances were found for cvs. Bryl and Apex (0.11), Stratus and Atol (0.13), and Rasbet and Madonna (0.15). Among

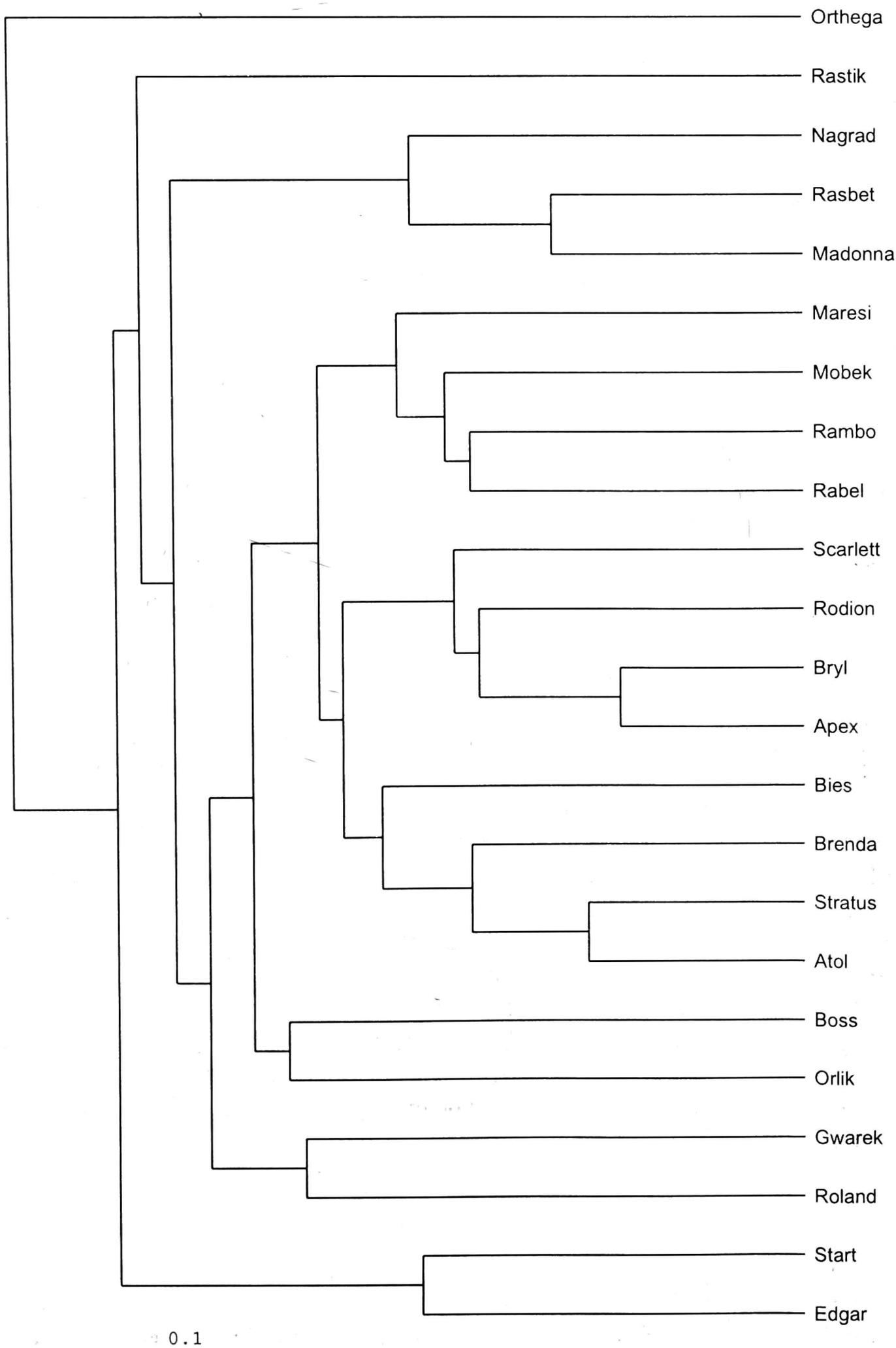

Figure 1. Dendrogram illustrating relationships among spring barley cultivar

these pairs of cultivars, Bryl and Apex both have cv. Aramir in their pedigree, and Stratus and Atol were selected from the same cross (Vista × Grosso), but Rasbet and Madonna are unrelated. The highest genetic distance was revealed between cultivars Orthega and Madonna (D = 0.62), and Orthega and Rambo (D = 0.60). Cv. Orthega alone formed a separate group on the dendrogram, which suggests its distinct genetic composition (Figure 1).

Of the seven malting cultivars only Brenda and Stratus formed one group at D = 0.25. Cultivars Brenda and Scarlett, especially recommended for brewery, are unrelated and the genetic distance between them is equal to 0.34.

Results presented here indicate that RAPD technique may be applied in studies of genetic diversity of spring barley cultivars grown in Poland. TINKER et al. (1993) showed that RAPD markers were suitable to gain information on genetic distance between barley inbred lines adapted to the environmental conditions of Canada. Results presented by those authors revealed that RAPD polymorphism divided two- and six-rowed genotypes into two separate groups. In the present study only two-rowed genotypes were examined and the detected polymorphism appeared to be sufficient for assessing similarity among cultivars. However, on the basis of this polymorphism, groups of malting and non-malting cultivars were not clearly distinguished.

In the breeding of self-pollinated species a high frequency of transgression effects is desirable. As was shown by POWELL and THOMAS (1992), and SURMA et al. (1998), the frequency of transgressive segregants depends on gene distribution between parental genomes; the frequency is high when favourable alleles underlying a given trait are dispersed between parents, and low when they are associated. As it is known from the formula given by JINKS and POONI (1976), lines with a high phenotypic similarity but different genetically should be crossed in order to obtain transgressive recombinants. For that reason information about genetic diversity among phenotypically similar cultivars/lines is needed for producing appropriate crosses, from which transgressive homozygous lines could be derived. Our results indicate that in malting barley breeding, crosses between cvs. Scarlett, Brenda and Rasbet should be more promising than, for example, between Brenda and Stratus.

REFERENCES

BARUA U.M., CHALMERS K.J., HACKETT C.A., THOMAS W.T.B., POWELL W., WAUGH R. (1993). Identification of RAPD markers linked to a *Rhynchosporium secalis* resistance locus in barley using near-isogenic lines and bulked segregant analysis. Heredity 71: 177-184.

BUSTOS De A., CASANOVA C., SOLER C., JOUVE N. (1998). RAPD variation in wild populations of four species of the genus *Hordeum* (Poaceae). Theor. Appl. Genet. 96: 101-111.

GRANER A., JAHOOR A., SCHONDELMAIER J., SIEDLER H., PILLEN K., FISCHBECK G., WENZEL G., HERRMANN R.G. (1991). Construction of an RFLP map of barley. Theor. Appl. Genet. 83: 250-256.

HEUN M., KENNEDY A.E., ANDERSON J.A., LAPITAN N.L.V., SORRELLS M.E., TANKSLEY S.D. (1991). Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34: 437-447.

JINKS J.L., POONI H.S. (1976). Predicting the properties of recombinant inbred lines derived by single seed descent. Heredity 36: 253-266.

NEI M., LI W.H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. (USA) 79: 5269-5273.

POWELL W., THOMAS W.T.B. (1992). A comparison of the phenotypic distribution of single seed descent families and second cycle hybrids in barley. J. Genet. & Breed. 46: 91-98.

SNEATH P.H.A., SOKAL R.R. (1973). Numerical Taxonomy. Freeman, San Francisco.

SURMA M., ADAMSKI T., KACZMAREK Z., KAPAŁA A. (1998). Frequency of transgression and gene distribution in barley doubled haploid populations from first and second cycle hybrids. J. Appl. Genet. 39: 237-247.

TINKER N.A., FORTIN M.G., MATHER D.E. (1993). Random amplified polymorphic DNA and pedigree relationships in spring barley. Theor. Appl. Genet. 85: 976-984.

THOMPSON D., HENRY R. (1995). Single-step protocol for preparation of plant tissue for analysis by PCR. BioTechniques 19: 394-400.