

Assessing genetic variation to predict the breeding value of winter triticale cultivars and lines

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Abstract. The objective of this study was to evaluate the usefulness of amplified fragment length polymorphism (AFLP) analyses for selection of the best parents for breeding of hybrid winter triticale. Phenotypic diversity was measured for 8 agronomic traits in 10 parents and 27 F₁ hybrids. Genotypic diversity was measured by 91 AFLP markers. Coefficients of correlation of genetic similarity (AFLP-GS) with both Euclidean distances and mean values of the traits were generally not significant. A correction of the preliminary binary matrix into trait-specific derived matrices increased the values of 5 correlation coefficients to a significant level. The correlation of AFLP-GS with mid-parent heterosis of grain weight per plant was low but significant ($r = -0.452$). Our study confirms the effectiveness of marker preselection for obtaining AFLP-GS better correlated with heterosis. The use of derived matrices is promising for reducing the number of cross combinations tested for specific combining ability.

Key words: AFLP, genetic diversity, heterosis, phenotypic diversity, triticale.

Introduction

Breeding of hybrid triticale cultivars is justified, as it gives an opportunity of yield improvement by up to 20% (Góral et al. 1999; Góral 2002; Oettler et al. 2001, 2003). Combining ability needs to be tested to select the best parents for the process of hybrid seed production. This is connected both with extension of breeding programmes and high costs. Thus the knowledge of genetic distance (GD) in triticale can improve the effectiveness of production of hybrid cultivars.

Information on descent, morphology, qualitative traits, and biochemical and molecular markers can be exploited as a clue for reducing the extent of field experiments. In wheat, the correlation of yield heterosis with GD measured by morphology amounted to $r = 0.45$ (Cox and Murphy 1990; Samsuddin 1985). Fabrizius et al. (1998) investigated a relationship of heterosis with GD measured by descent, morphology and gliadin

polymorphism. No correlation between GD and heterosis was found. However, for clusters based on morphology and parentage, intergroup crosses yielded better than intragroup hybrids.

It is suggested that DNA analyses may be useful to predict effects of heterosis. Selection of parents that are most genetically distant is expected to give the best effects of heterosis (Stuber et al. 1992; Subudhi et al. 1998). There are no reports on the use of DNA markers for predicting heterosis in triticale hybrids. RFLP-based genetic distance (RFLP-GD) was significantly correlated with yields of maize and oilseed rape hybrids (Smith et al. 1990; Boppenmaier et al. 1993; Bernardo 1994; Diers et al. 1996). Barbosa-Neto et al. (1996) investigated the usefulness of genetic distance based on coefficient of parentage (COP-GD) and RFLP-GD for predicting wheat yield heterosis. They found no correlation of RFLP-GD with yield heterosis (r range: 0.18 to 0.02) and a significant correlation of heterosis with COP-GD in

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a one-year of study (r range: -0.41 to -0.12). No significant correlations were found between genotypic variation for 7 agricultural wheat traits and genetic similarity (GS) measured with RFLP, AFLP and simple sequence repeat (SSR) markers (Bohn et al. 1999), as well as for yield with RFLP-GD and genetic distance based on sequence-tagged sites (STS-GD) (Barbosa-Neto et al. 1996; Martin et al. 1995).

The purpose of this study was to evaluate the usefulness of simplified *PstI*-based AFLP analyses for selection of the best parents for breeding of hybrid winter triticale. Genetic variation of 11 lines and cultivars of winter triticale was measured by both morphology and DNA profile. We tested relationships between genetic similarity (AFLP-GS) and phenotype characteristics on the basis of 8 agronomic traits in parents and F_1 progeny.

Material and methods

Material

Six genetically male-sterile lines: cms Salvo 15/1 B₁₄, cms 19 B₉, cms Grado 2 B₁₄, cms Purdy 5/5 B₈, cms Malno 53/3 B₇, and cms Tm 105/6 B₁₅ were crossed with 5 cultivars: Lamberto, Pawo, Sekundo, CHD 1181/98 and Krakowiak in factorial design. Thirty F_1 hybrids were obtained.

Phenotypic variation

Seeds of parental forms (maintainer lines for cms lines, and cultivars used as pollen donors) and F_1 hybrids were sown (spacing 7.5 cm × 20 cm) in 1-metre-long single-row plots in two replicates in the Prusy Experimental Station near Krakow. All the plants with both neighbours were selected for measurements due to a high level of winter damages. Cms Salvo 15/1 and three cross combinations (cms Tm 105/6 × Pawo, cms Tm 105/6 × Krakowiak and cms Tm 105/6 × Krakowiak) were lost. Tm 105/6 line was represented by a single plant, and 9 plants on average (range: 3 to 17) were measured for the other objects. Eight traits were measured: (1) straw length; (2) ear length; (3) ear number per plant; (4) grain number per ear; (5) peduncle length; (6) grain weight per ear; (7) 1000-grain weight; and (8) grain weight per plant.

Genetic variation

DNA bulks made of 5 leaves sampled separately from 2-week-old seedlings germinated on Petri

dishes were used for genetic analyses. The material was sampled for 11 parental forms analysed in two independent bulks. Cms Purdy was represented by a single sample. DNA was isolated according to Milligan (1992). *PstI*-AFLP assays were run according to Tyrka et al. (2002). 500 ng of DNA was digested, ligated and subsequently purified. Analyses were run using 14 selective primers (P33, P34, P39–P47, P58, P59 and P63) marked according to the rules proposed by KeyGene (<http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html>). The presence or absence of a band were assigned the values 1 and 0, respectively, to prepare the preliminary binary matrix (PBM).

Statistical analysis

Mean values of the agronomic traits for parents and hybrids were standardized and used for computing Euclidean distances between them. Mid-parent (MPH) and better parent heterosis (BPH) was computed according to equations [1] and [2], respectively:

$$[1] \text{ MPH (\%)} = 100 \times (F_1 - \text{MP})/\text{MP}$$

$$[2] \text{ BPH (\%)} = 100 \times (F_1 - \text{BP})/\text{BP},$$

where F_1 = mean value of the trait in the hybrid, MP = mean of the two parents, and BP = value of the trait in the better parent.

Pairwise genetic similarities were computed using the formula of Dice, as in Nei and Li (1979) for all bands in the preliminary binary matrix:

$$F = 2N_{XY}/(N_X + N_Y),$$

where N_{XY} = number of shared bands for genotypes X and Y; while N_X and N_Y = total numbers of bands observed in genotypes X and Y, respectively. Genotypes were clustered with the Unweighted Pair-Group Method with Arithmetic Averages (UPGMA), using NTSYS-pc 2.0 software (Rohlf 2001).

Association between phenotype and genotype was estimated for preliminary and derived matrices. Spearman correlation coefficients (SCs) between the banding pattern and the mean values of each trait for 10 parental forms were initially computed. The preliminary binary matrix (PBM) was subsequently modified for each trait on the basis of SC values. The modification was performed in three stages:

a) Values of correlation coefficients were transformed into directional indices (DIs):

DI = 0 for $-0.3 \leq \text{SC} \leq 0.3$; DI = 1 for $\text{SC} > 0.3$; DI = -1 for $\text{SC} < -0.3$

b) Subsequently, the preliminary binary matrix (PBM) was transformed on the basis of DI values

to derived matrices (DBMs) for each trait according to the following rules:

values (0,1) from PBM were deleted in DBM for $DI = 0$; values (0,1) from PBM were left unaltered in DBM for $DI = 1$; values (0,1) from PBM were transformed into (1,0, respectively) in DBM for $DI = -1$.

c) The derived matrices obtained for each trait were used for computing AFLP-GS, and subsequently their correlations with means for the agronomic traits measured in F_1 , mid-parent heterosis and better parent heterosis effects were assessed.

Results

Phenotypic traits

The dwarf fertility-maintaining line Tm 105/6 differed from the other parental forms in most of the analysed traits. It was characterized by the shortest straw and peduncle, long ear, the low-

est 1000-grain weight, and the highest grain number per ear (Figure 1). Different characteristics of line cms 19 resulted from a linkage of a short straw and ear with a low grain yield. The remaining cultivars were in a single cluster with several subgroups, characterized by a long straw and a high 1000-grain weight. Cultivars composing this group differed in grain weight per ear and ear number per plant. Hybrids with the highest grain weight per plant clustered with Krakowiak.

F_1 hybrids had on average more ears per plant, longer ears and a higher 1000-grain weight, along with a lower grain number and weight per ear, as compared to parents. Average straw length and grain yield per plant were similar in hybrids and parents. Some hybrids showed heterosis in relation to the better parent (BPH), predominantly for ear number per plant and grain weight per plant (Table 1). Eight out of 22 hybrids showed BPH for yield per plant exceeding 10% and 7 combinations showed BPH for ear number per plant. BPH for

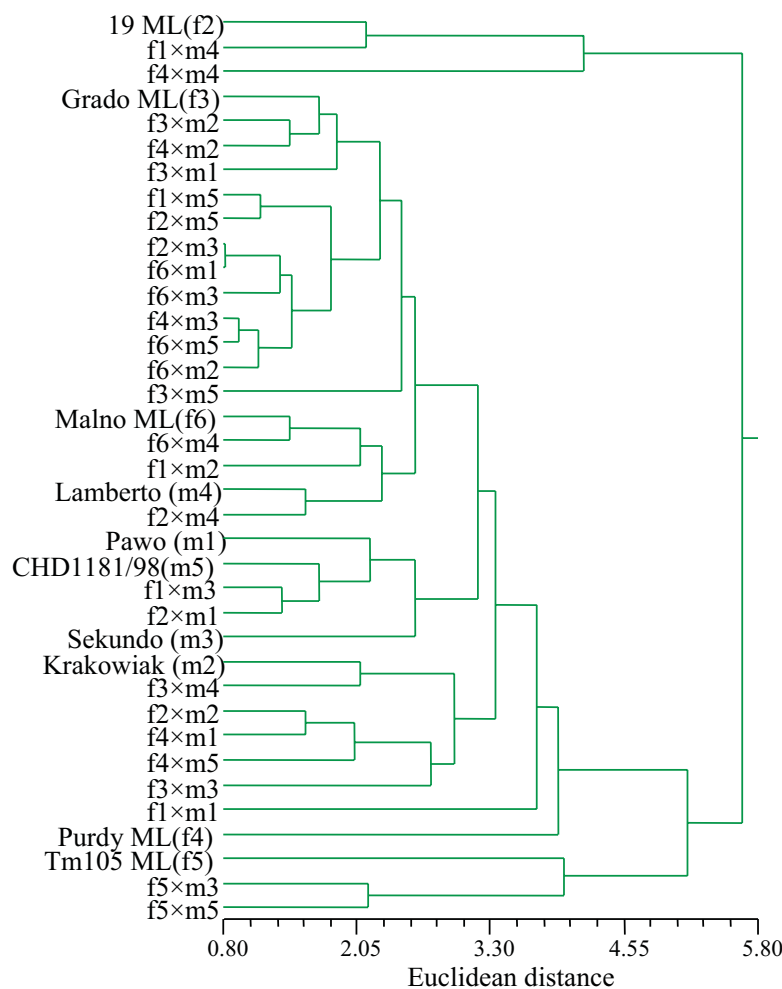


Figure 1. UPGMA dendrogram for wheat cultivars, lines and F_1 hybrids, based on Euclidean distances assessed for mean values of 8 agronomic traits

Table 1. Mean trait values in parents and F₁ hybrids along with heterosis as deviation (%) of F₁ from the mid-parent (MP) and better parent (BP)

Trait	Mean values		Mid-parent heterosis (MPH %)			Best parent heterosis (BPH %)			No. of F ₁ significantly better from	
	parents n = 10	F ₁ n = 27	mean	min	max	mean	min	max	MP	BP
1000-grain weight (g)	45.0	50.7	12.2	-7.5	31.0	1.2	-15.5	15.9	13	9
Ear length (cm)	10.8	11.4	7.9	1.3	17.0	3.0	-4.9	12.5	16	6
Ear number/plant	14.1	14.9	16.2	-41.2	80.9	2.5	-44.4	72.8	5	2
Grain number/ear	61.0	49.5	-16.9	-63.5	25.2	-22.4	-66.8	23.5	1	1
Grain weight/ear (g)	2.8	2.5	-8.2	-56.3	27.5	-18.9	-59.3	16.0	2	0
Grain weight/plant (g)	39.2	39.3	11.7	-69.5	93.8	-5.2	-73.2	83.1	6	2
Peduncle length (cm)	36.3	39.3	5.6	-8.6	14.3	-0.3	-21.1	12.1	10	4
Straw length (cm)	83.2	82.2	-1.0	-13.5	16.6	-8.4	-28.9	2.6	4	0

1000-grain weight was found in a single F₁ combination, and the two hybrids showed good effects in respect to grain weight and number per ear.

PstI AFLP analysis

PstI AFLP with 14 selective primers generated 91 polymorphic bands. The genetic similarity (AFLP-GS) based on all markers reached on average 84.6% and ranged from 77.3% to 91.9%. Genotype-specific bands enabled identification of lines cms Malno, cms 19, cms Salvo, cms Purdy

and cms Tm 105/6 as well as cultivars Pawo, Lamberto and Krakowiak.

Mutual DNA-based relationships between lines and cultivars are presented on a dendrogram (Figure 2). Three groups were formed. Cms Malno 53/3 was separated in the first cluster. The second group comprises cms Salvo, cms 19 and cms Grado. Remaining lines and cultivars formed the third group, with cms Tm 105/6 and cms Purdy as one subgroup and cultivars Lamberto, Pawo, Sekundo, CHD 1181/98 and Krakowiak as the second subgroup. The cluster created by cms

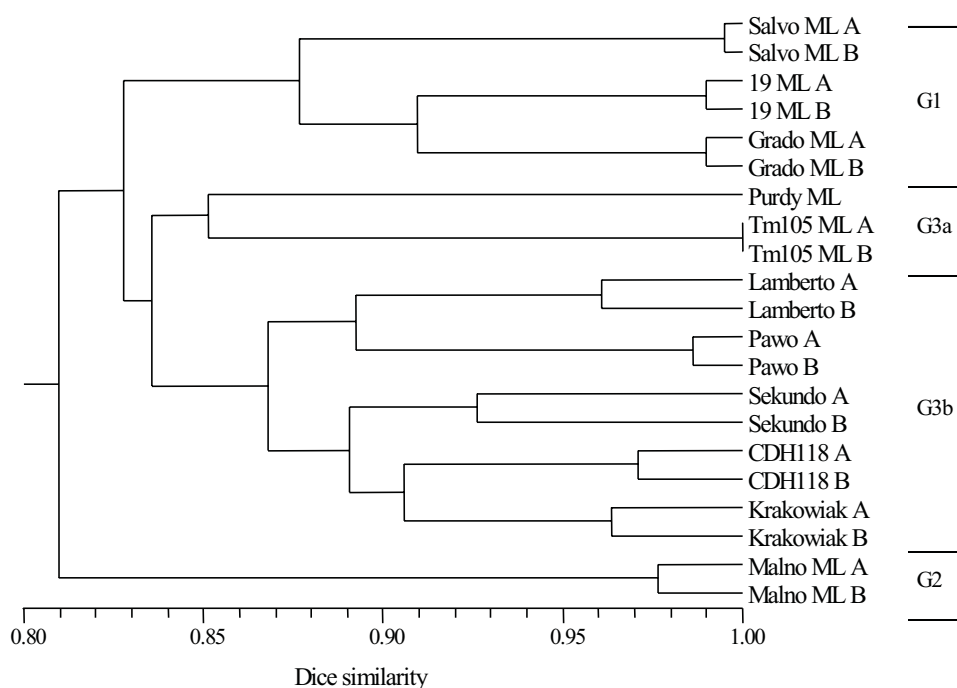


Figure 2. UPGMA dendrogram for 11 wheat lines and cultivars, showing genetic similarity (AFLP-GS) based on 153 AFLP bands

Table 2. Coefficients of correlation of genetic similarity based on AFLP with means, mid-parent heterosis (MPH) and better parent heterosis (BPH) for analysed agronomic traits in preliminary (PBM) and derived binary matrices (DBMs). Number of comparisons in brackets.

Trait	Indices			Mean		MPH (%)		BPH (%)	
	0	+1	-1	PBM	DBMs	PBM	DBMs	PBM	DBMs
1000-grain weight (g)	14	9	14	0.330 (27)	0.091 (27)	0.068 (22)	-0.170 (22)	0.013 (22)	0.250 (22)
Ear length (cm)	18	12	7	0.108 (27)	0.398* (27)	0.314 (22)	-0.339 (22)	0.296 (22)	-0.084 (22)
Ear number/plant	19	10	8	-0.024 (27)	-0.133 (27)	0.294 (22)	-0.413 (22)	0.266 (22)	-0.429* (22)
Grain number/ear	16	12	9	0.145 (27)	-0.136 (27)	0.268 (22)	-0.371 (22)	0.182 (22)	-0.380 (22)
Grain weight/ear (g)	24	6	7	0.298 (27)	0.159 (22)	0.282 (22)	-0.014 (17)	0.200 (22)	0.006 (17)
Grain weight/plant (g)	16	11	10	0.172 (27)	-0.148 (27)	0.381 (22)	-0.452* (22)	0.277 (22)	-0.154 (22)
Peduncle length (cm)	17	8	12	0.123 (27)	0.173 (25)	-0.021 (22)	-0.240 (20)	0.010 (22)	-0.299 (20)
Straw length (cm)	17	8	12	-0.273 (27)	0.554** (27)	-0.054 (22)	0.148 (22)	-0.137 (22)	0.612** (22)

Salvo, cms 19 and cms Grado was stable also after deleting from the preliminary matrix those markers that indicated internal variation, which resulted in 39 polymorphisms. All F₁ hybrids studied represented intergroup crosses, judging by genetic similarities, but only some of them showed heterosis.

DNA vs. phenotype diversity

The coefficient of correlation between AFLP-GS and Euclidean distances was negative: $r = -0.115$ ($n = 42$). No significant correlations were found between mean values of traits measured or F₁ heterosis and AFLP-GS computed for the preliminary binary matrix (Table 2). This suggests a low relationship between yield parameters and DNA diversity estimated by randomly generated markers.

To explore better the potential of AFLP-GS for predicting yield parameters and effects of F₁ heterosis, the preliminary matrix was used to derive trait-specific matrices. Spearman correlation coefficients (SC) between the presence or absence of PstI AFLP markers and values of phenotypic traits in 10 genotypes were computed. Evaluation of SC and respective indices suggests that for the threshold of 0.3 used for marker selection, 48% AFLP markers were neutral in respect to the traits analysed, 26% were correlated positively ($r > 0.3$), and 27% were correlated negatively ($r < -0.3$). This suggests a random distribution of

markers in respect to the analysed loci. After the DI-based transformation of the preliminary matrix (PBM), the calculated similarities based on AFLP were significantly correlated with straw and ear length, with BPH for straw length and ear number, and with MPH for grain weight per plant (-0.452).

Discussion

Molecular markers are believed to be more useful predictors of genetic variation and clues for crosses than morphological traits due to insensitivity to environmental conditions (Liu et al. 1999). However, attempts to predict values of wheat hybrids have not been effective so far (Martin et al. 1995; Barbosa-Neto et al. 1996; Bohn et al. 1999). In the case of RFLP, cDNA probes for genetic distance (GD) estimation can be selected. This gives advantages of RFLP over random systems, such as RAPD and AFLP, where only some markers affect gene expression.

The reasons of low correlation of GD predicted by molecular markers and a positive yield heterosis include unequal genome coverage, random marker distribution, and diversified effects of dominance (Bernardo 1992). Simulations showed that correlation between a single trait and DNA-based distance is reduced by the presence of markers that are not associated with the trait (Charcosset et al. 1991). Thus, only markers corre-

lated with the trait should be selected. Barbosa-Neto et al. (1996) found no increase in correlation after marker preselection. Liu et al. (1999) investigated the connection between RAPD-GD and heterosis in wheat. Molecular data showing discrepancies in comparison with morphological characteristics were removed. Clusters obtained could be used as clues for crosses, as intergroup hybrids showed a higher effect of heterosis than intragroup hybrids.

The present study confirmed previous results on negative, positive and insignificant correlations of DNA markers with the analysed agronomic traits (Tyrka and Mikulski 2003). Therefore, it can be expected that the genetic variability of parents, assessed on the basis of selected markers correlated with the agronomic traits, can help to predict the usefulness of cross combinations in programmes of triticale hybrid breeding. The correction of the preliminary binary matrix raised the values of correlation coefficients in relation to that matrix. However, due to the modest design of the field experiment, results of this study should be treated as preliminary, and a better experimental design should be used for verification.

Conclusions

The present study confirms the usefulness of correcting of the preliminary binary matrix (PBM) to derived binary matrices (DBMs). The genetic similarity (GS) based on DBMs is promising for reducing the number of cross combinations in testing of specific combining ability. Marker preselection improved the 5 correlations between respective genetic similarities based on AFLP (AFLP-GS) and F_1 phenotype of straw and ear length, the better parent heterosis (BPH) for straw length and ear number per plant, and mid-parent heterosis (MPH) for grain weight per plant. The coefficient of correlation between MPH for grain weight per plant and AFLP-GS reached -0.452 .

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