The use of Molecular Markers to Determine Wheat Lines with Chasmogamic Type of Flowering

Danuta KURASIAK-POPOWSKA [1]
Zbigniew BRODA [1]
Piotr TOKARSKI [1]
Jan BOCIANOWSKI [2] (✉)

Summary

Results of the genetic distance between two cultivars and seven lines of spring wheat are presented. Another objective of the presented paper was to find an amplification product that could be a marker of a faster growth stage and of the type of flowering. Random amplified polymorphic DNA (RAPD) was used in the studies. Forty selected primers were tested. Fourteen primers were used to develop a dendrogram presenting similarities among the cultivars and lines. Numerical analysis showed two similarity groups. The first group included KOH 5560, KOH 5637, KOH 5674, KOH 5884, KOH 5930 as well as ‚Nawara' and ‚Zadra'. The second group consisted of: KOH 5561 and KOH 5718. Results showed the presence of some specific amplification products: OPA 09f, OPF 13k, OPF 14j, OPC 11k in wheat lines KOH 5884 and partially in KOH 5561 which indicate a fast rate of growth and chasmogamic type of flowering.

Key words

RAPD markers, flowering, spring wheat, association

[1] Department of Plant Genetics and Breeding, Poznań University of Life Sciences, Dojazd 11, 60-632 Poznań, Poland
[2] Department of Mathematical and Statistical Methods, Poznań University of Life Sciences, Wojska Polskiego 28, 60-637 Poznań, Poland
✉ e-mail: jboc@up.poznan.pl

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Introduction

Molecular markers based on Polymerase Chain Reaction (PCR) are used in the search for new and favorable features in wild and cultivated species, in the estimation of genetic differentiation, in genotype identification and genetic fingerprinting, in the estimation of genetic distance between populations, inbred lines and breeding materials and in Marker Assisted Selection (MAS) (Hernándes, 2000; Gupta, 2002; Korzun, 2002; Tambasco-Talharii et al., 2005). The technique RAPD-PCR (Random Amplified Polymorphic DNA) has shown to be effective in the studies on the determination of the genetic differentiation in wheat and it can be used in fingerprinting of cultivars (Vierling and Nguyen, 1992; Gupta et al., 1999; Liu et al., 1999; Iqbal, 2007).

Wheat belongs to selfpollinating plants. It is characterized by cleistogamic type of flowering - flowering takes place in closed flowers leading to a complete pollination with own pollen (Campbell et al., 1983; Maeng et al., 2006). Chasmogamy, where flowering takes place in an open flower, is less frequently observed. Many scientists (Diaz and Macnair, 1998; Porras and Munoz, 2000; Masuda et al., 2004; Gotelli et al., 2006) stress the advantages resulting from good familiarity with cleistogamy and chasmogamy. It permits a better understanding of the morphological differentiation of flowers and to utilize this knowledge in the breeding work.

The presented studies utilized the RAPD technique in order to determine the genetic similarities between the studied cultivars and lines of spring wheat and to find influence of the occurrence of frequency of amplification products in the course of developmental phases and the differentiated types of flowering.

Material and methods

Plant material included the cultivars: ‚Nawra', ‚Zadra' and the breeding lines: KOH 5561, KOH 5560, KOH 5637, KOH 5674, KOH 5718, KOH 5884, KOH 5930 of spring wheat produced in the Plant Breeding Strzelce Ltd., Co. - IHAR Group.

Genomic DNA was isolated by the modified method of Thompson and Henry (1995) from combined 30 seedlings originating from each object. Leaf fragments (2 mm^2) taken from 10-day old spring wheat seedlings were treated with 200 µl TPS buffer with the following compositions: 100 mM Tris HCL with pH=9.5; 1 M KCl; 10 mM EDTA. Incubation was carried out in a water bath at 95°C for 15 minutes.

Polymerase chain reaction (PCR) was performed in two replications, and the mixtures of 12.5 µl contained 1M Tris HCl with pH=8.3; 25 mM $MgCl_2$; BSA; 2 mM dNTP; primer – 5 pmol/µl; Tag polimerase – 5U/ul; DNA – 25 ng/µl; deionized water. DNA amplification was carried out using thermocycler T3 BIOMETRA of POLYGEN Co. and subsequently, to each amplification l µl dye (0.25% bromophenol blue; 40% saccharose, deionized water) was added.

Electrophoresis of amplification products was performed in 1,5% agarose gel in 1 x TBE buffer, stained with ethidium bromide, and visualized and photographed under UV. The coefficients of genetic similarity (GS) of the investigated species were calculated using the formula of Nei and Li (1979):

$$GS_{ij} = \frac{2N_{ij}}{N_i + N_j}$$

where N_{ij} indicates the number of alleles present at *i*th objects and *j*th objects, N_i – the number of alleles present at the *i*th objects, N_j – the number of alleles present at the *j*th objects, $i, j = 1, 2, ..., 9$. The coefficients were used to group objects hierarchically according to the unweighted pair group method of arithmetic means (UPGMA). The relationship among objects was presented in the form of a dendrogram. The package GenStat v. 7.1 was used to archive band pattern after electrophoresis and to analyze amplification product.

The association between amplification products and the values of the particular features of analysed cultivars and lines was estimated using analysis of regression (Bocianowski et al., 2011). The amplification products observations were treated as independent variables and considered in individual models (Bocianowski and Seidler-Łożykowska, 2012). For products exerting a significant effect on the values of features, the percent of the variability of the feature explained by the given amplification product was calculated. Critical values were read out from tables (Fisher and Yates, 1963).

Additionally, in the year 2007, observations of the developmental phase of the studied plant material were carried out in the experimental garden and in the breeding greenhouse of the Department of Plant Genetics and Breeding, Poznan University of Life Sciences. The determination of developmental stages was done according to the Zadoks scale (Zadoks, 1974).

Results

In the period of flowering and heading of the studied two cultivars and seven lines of wheat in the experimental garden there followed the number of rainfalls and an increase of temperature that caused that all analyzed genotypes were characterized by a closed type of flowering (Table 1). In the greenhouse three lines (KOH 5674, KOH 5884, KOH 5930) had an open type of flowering. Cleistogamic type of flowering had two genotypes (‚Zadra', KOH 5718), while a mixed type of flowering we found in four studied genotypes (‚Nawra', KOH 5560, KOH 5561, KOH 5637).

Table 1. Weather conditions at the experimental field in 2007

Month	March	April	May	June	July
Rainfall [mm]	65	74	82.2	44.3	39.6
Temperature [°C]	6.9	10.9	15.7	20.1	20.4

In genotypes with chasmogamic flowering type: KOH 5674, KOH 5884, KOH 5930, it was observed that they entered into the particular developmental phases in the fastest rate and they also finished the flowering stage in the fastest way (Table 2).

Table 2. Course of heading and flowering in experimental garden and in the greenhouse

Zadoks scale	Nawra	Zadra	KOH 5560	KOH 5561	KOH 5637	KOH 5674	KOH 5718	KOH 5884	KOH 5930
				Heading					
50-51	79*	73	81	86	82	79	84	94	87
	64	*57*	*81*	*64*	*66*	*71*	*70*	*57*	*61*
52-53	82	74	83	89	84	83	85	97	90
	66	*64*	*84*	*68*	*73*	*73*	*72*	*60*	*64*
54-55	84	77	86	92	87	86	87	100	91
	71	*67*	*89*	*71*	*76*	*76*	*81*	*64*	*67*
56-57	86	78	89	95	90	88	90	105	93
	73	*71*	*91*	*76*	*81*	*81*	*83*	*68*	*71*
58-59	89	81	92	98	92	90	93	107	94
	81	*81*	*93*	*81*	*87*	*84*	*87*	*71*	*81*
				Flowering					
60-61	93	75	83	88	84	81	86	110	90
	86	*83*	*95*	*86*	*89*	*88*	*93*	*76*	*84*
64-65	96	78	89	97	93	88	93	112	97
	88	*87*	*97*	*88*	*93*	*92*	*95*	*81*	*87*
68-69	98	87	97	104	98	97	99	115	106
	94	*89*	*101*	*94*	*95*	*94*	*100*	*94*	*92*

Underlined values indicate the number of days from the day of sowing to the date of entering into the developmental phase on the experimental plot. Values marked with slanted fonts indicate the number of days from the day of entering into the given phase recorded in the breeding greenhouse

Table 3. Characteristics of primers and detected polymorphism (for two replications)

Primer	Sequence of primer 5'→3'	Proportion of bases		Number of paths showing		Total number of paths
				Polymorphism	Monomorphism	
OPA 01	CAGGCCCTTC	A:T 1:2	G:C 2:5	9	2	11
OPA 07	GAAACGGGTG	A:T 3:1	G:C 5:1	4	3	7
OPA 09	GGGTAACGCC	A:T 2:1	G:C 4:3	5	2	7
OPA 12	TCGGCGATAG	A:T 1:1	G:C 2:1	2	5	7
OPA 13	CAGCACCCAC	A:T 3:0	G:C 1:5	2	4	6
OPA18	AGGTGACCGT	A:T 1:1	G:C 4:2	3	5	8
OPA 19	CAAACGTCGG	A:T 3:1	G:C 1:1	12	4	16
OPB 10	CTGCTGGGAC	A:T 1:2	G:C 4:3	9	0	9
OPB 17	AGGGAACGAG	A:T 4:0	G:C 5:1	1	9	10
OPC 11	AAAGCTGCGG	A:T 3:1	G:C 2:1	13	2	15
OPF 08	TGGACCGGTG	A:T 1:1	G:C 5:1	7	0	7
OPF 13	GGCTGCAGAA	A:T 3:1	G:C 2:1	10	2	12
OPF 14	TGCTGCAGGT	A:T 1:3	G:C 4:1	7	1	8
OPF 20	GGTCTAGAGG	A:T 1:1	G:C 5:1	5	2	7
Total				86	39	125

In laboratory experiment, with the use of RAPD-PCR technique, 40 primers generated a polymorphism permitting to determine the genetic similarity of the studied plant materials (Table 3).

On the basis of results obtained from the electrophoretic separation of RAPD-PCR products, genetic similarity coefficients were calculated among the studied cultivars and lines of spring wheat.

The highest similarities, from the genetic aspect, were revealed between KOH 5560 and ‚Zadra' (0.7586) and between KOH 5561 and ‚Nawra' (0.7407). The least similar were KOH 5561 and KOH 5637 (0.3824), as well as KOH 5561 and KOH 5930 (0.3836). The difference between the highest and the lowest coefficient of genetic similarity for the analysed form was rather significant (0.3762). Additionally, one can distinguish similarities of two clusters at the level of 60% (Fig. 1). One of them was created by the objects of Nawra, Zadra, KOH 5637, KOH 5560, KOH 5674, KOH 5884 and KOH 5930. The other cluster included KOH 5561 and KOH 5718 (Fig. 1).

In reference to the obtained polymorphism genetated by 14 studied primers, we tried to find such an amplification product obtained by the RAPD technique whose presence would indicate that the given genotype with that particular product had the possibility of chasmogamic way of flowering (Table 4).

Comparisons carried out indicated that the amplification products: OPA 09f, OPF 13k as well as OPF 14j that occurred in the genotype marked as KOH 5884 and partially in the genotype KOH 5561 were potentially connected with the heading and flowering phases.

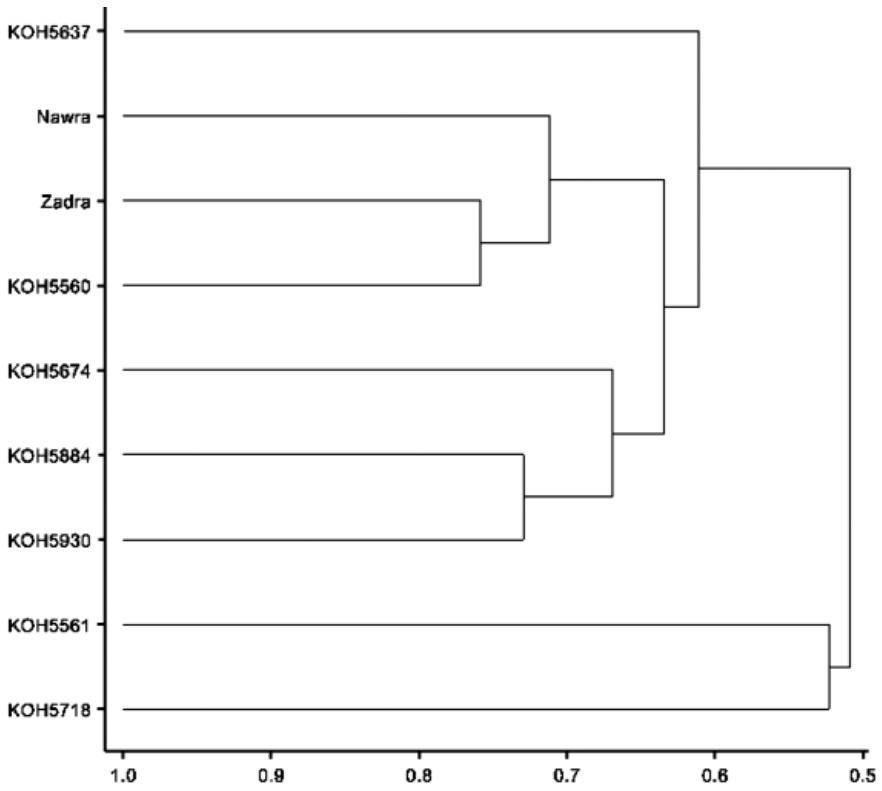

Figure 1. Dendrogram of the similarity of the studied plant material. Dendrogram was constructed using the unweighted pair group method of arithmetic means (UPGMA)

Discussion

Taking into consideration the number of anthers thrown outside of the flowers, three types of flowering can be distinguished: (a) chasmogamic flowering (when anthers are outside of the flower), (b) cleistogamic flowering (when all three anthers remain inside the flower) and (c) mixed flowering (when one or two anthers are on the outside of the flower) (Kociuba and Kranek, 2004). The number of thrown out anthers is very variable. Maksimow (1964) observed 61.9% of open flowers in durum wheat, while Joppa et al. (1968) found only 22-31% of thrown out anthers (cited after Chhabra and Sethi, 1991). Gorin (1968) found that the majority of bread wheat showed 80-90% of open flowers. Improvement of the frequency of open

Table 4. Amplification fragments connected with heading and flowering of the studied wheat genotypes

Zadoks scale	Amplification product	Estimation of regression coefficient	*P*–value	Percentage variation accounted	Standard error of observations
		Heading			
50	OPA 09f*	12.63	0.033	42.8	4.5
50	OPC 11k	8.15	0.028	45.2	4.41
50	OPF 13k	12.63	0.033	42.8	4.5
50	OPF 14j	9.29	0.041	39.8	4.62
52	OPA 09f	13.25	0.038	40.8	4.89
52	OPF 13k	13.25	0.038	40.8	4.89
52	OPF 14j	10	0.038	40.7	4.9
54	OPA 09f	13.75	0.026	46.6	4.59
54	OPF 13k	13.75	0.026	46.6	4.59
54	OPF 14j	10.57	0.022	48.7	4.5
56	OPA 09f	16.38	0.02	50.2	5.13
56	OPF 13k	16.38	0.02	50.2	5.13
56	OPF 14j	12.29	0.021	49.3	5.17
58	OPA 09f	15.87	0.019	50.9	4.91
58	OPF 13k	15.87	0.019	50.9	4.91
58	OPF 14j	12.36	0.014	54.8	4.71
		Flowering			
60	OPA 09f	26.38	0.001	77.7	4.63
60	OPF 13k	26.38	0.001	77.7	4.63
60	OPF 14j	16	0.029	45	7.27
64	OPA 09f	21.87	0.014	54.5	6.33
64	OPF 13k	21.87	0.014	54.5	6.33
64	OPF 14j	15.36	0.028	45.1	6.96
68	OPA 09f	16.75	0.027	46	5.65
68	OPF 13k	16.75	0.027	46	5.65
68	OPF 14j	12.07	0.039	40.5	5.93

Small letters at primer numbers indicate the different DNA fragments

flowering in cereals aims at the increasing of cross-pollination with a foreign pollen and thereby at the heterozygote that may contribute to a significant yield increase regarding the potential ability and stability (Abdel-Ghani et al., 2005).

Effect of environmental factors as well as of the genetic background on chasmogamy or cleistogamy, both in wheat and in other cereal plants are the subjects of many studies, but the results are not unambiguous (Clay, 1982; Kandaurov and Belkovskaja, 1966; Chhabra and Sethi, 1991; Turuspekov et al., 2004; Masuda et al., 2004; Gilsinger et al., 2005). De Vries (1970) and Waines and Hegde (2003) argue that a low temperature and the increase of rainfalls cause flowering with closed flowers. Chhabra and Sethi (1991) had completely inverse point of view. According to the latter authors, throwing out of anthers is a stable feature and it does not depend on climatic changes. Chhabra and Sethi (1991) reported that cleistogamy is caused by a recessive allele of one gene, while chasmogamy is caused by the dominant allele. They also noticed that cleistogamy is connected with poorly developed lodicules.

In our studies, the closed flowering system was observed in spring wheats in the didactic garden of the Poznań University of Life Sciences in the year 2007. In the period of growth and development, there was observed a temperature increase with a drop of rainfalls, in relation to the mean value from many years. Such weather course probably was responsible for the flowering in closed flowers. In the breeding greenhouse of the Poznań University of Life Sciences, the growth and development took place at a constant temperature and constant air humidity. The studied wheat showed a mixed type of flowering (‚Nawra', KOH 5560, KOH 5561, KOH 5637), the percentage of cleistogamic flowering was negligible (‚Zadra', KOH 5718) and the chasmogamic flowering was low (KOH 5674, KOH 5884, KOH 5930). It must be stressed that the lines of KOH 5674, KOH 5884, KOH 5930 that were characterized by chasmogamic flowering type created a common subgroup of similarity.

Statistical analyses indicated that there was a distinct effect of certain genes detected be amplification products determined as OPA 09f, OPF 13k, OPF 14j on the phases of heading and flowering. These DNA fragments occurred in the KOH 5884 genotype and partially in the genotype KOH 5561. During observations in the field and in the greenhouse, the genotypes KOH 5884 and KOH 5561 were distinctly characterized by an accelerated growth rate, in comparison with the remaining genotypes. This difference oscillated from 3 to 10 days. In the field and in the greenhouse both genotypes entered definitely faster into the heading and flowering phases. This may suggest that the above mentioned DNA fragments can be responsible for quicker growth and faster entering into the phase of heading or flowering.

In the presented studies, one can also notice that the chasmogamic type of flowering occurred in of the studied wheats that showed distinctly marked awn in the ear (KOH 5674, KOH 5884, KOH 5930). The presence of dorsal awn and the chasmogamic flowering type require further ionvestigation.

Conclusions

1. On the basis of analyses, it was found that genetic similarity between two cultivars and seven lines of spring wheat ranged from 38.24% to 75.86%.
2. Three of the obtained DNA fragments (OPA 09f, OPF 13k, OPF 14j) were potentially connected with the phases of heading and flowering of the genotype determined as KOH 5884 and partially with the genotype KOH 5561.
3. In the genotypes KOH 5884 and KOH 5561 an accelerated growth rate was noticed in the phase of flowering and heading both in the greenhouse and in the field.

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