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ptgc POLSKIE TOWARZYSTWO GENETYKI CZŁOWIEKA

PROGRAM I MATERIAŁY KONGRESOWE

39. AFLP and STR markers - a valuable tool for genetic diversity analysis of winter rapeseed (B. napus L.)

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Molecular methods have been introduced into breeding programs to ensure quick evaluation of breeding material variability. Yield is the most important breeding goal and its significant increase is ensured by the development of hybrid cultivars displaying heterosis effect. The potential of heterosis in hybrid cultivars depends on parental lines combinations belonging to distant gene pools.

The objective of this study was the evaluation of genetic distance among 64 winter rapeseed breeding lines and cultivars included in a breeding program of hybrid varieties based on CMS ogura hybridization system.

AFLP and STR analyses were performed using capillary electrophoresis of fluorescently labeled PCR products. Genetic distance among the genotypes was assessed using ten AFLP primer-enzyme combinations and primer pairs for 48 STR loci. Genetic distance (GD) of the investigated lines was calculated according to Nei and Li (1979) coefficient of dissimilarity, the UPGMA method was applied for the creation of the dendrogram. The results indicate that both AFLP and STR markers are suitable for assessing genetic relationship among rapeseed breeding lines with high level of accuracy.

The approaches combining markers such as AFLP and STR may provide more accurate information on genetic diversity and relationships of B. napus genotypes as compared to selection classical methods for parental components of winter rapeseed hybrid varieties.

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40. Characterization of Chinese cabbage doubled haploid plants

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In the study 14 populations of Chinese cabbage (Brassica campestris spp. pekinensis) doubled haploids were characterized. In total 104 DH plants form three genotypes (YU breeding line, Kilakin F1 and Hilton cultivars) were obtained in vitro, via isolated microspore cultures. At the turn of 2014/2015, the plants were subjected to vernalization in a growth chamber at 4°C. In 2015, while growing in a greenhouse, analysis of plant morphology and pollen viability was conducted. In order to obtain DHR1 generation, DH plants were self-pollinated in the bud stage or at the open flower, after treatment stigma with 8% solution of NaCl.

The mean height of flowering DH plants was 87 cm (the observed range of variation was 40-130 cm). All DH plants produced a flower stem. During stereo-microscopic observations no irregularities in the flower formation were observed. Pollen viability of DH populations was: 78% for Kilakin F1 variety, 85-98% for YU breeding line and 82-98% for Hilton cultivar. Within the examined population, 12% of plants had reduced pollen viability (50-70%), 8% had an average pollen viability (71-80%), and most (80%) of DH plants had high pollen viability (81-90%).

After self-pollination of 104 DH plants about 10 000 of DHR1 seeds were collected. The efficiency of binding DHR1 seeds after self-pollination in the bud stage and at the open flower after treatment stigma with 8% solution of NaCl was similar and amounted to 51 and 49%, respectively. The mean weight of 1000 DHR1 seeds was 3.4 g and was in the range of the mean weight of thousand seeds of Chinese cabbage (2.5-4.1 g)

This research was supported by the Polish Ministry of Agriculture and Rural Development (No. HOR hn-801-PB-11/15).

AFLP AND STR MARKERS – A VALUABLE TOOL FOR GENETIC DIVERSITY ANALYSIS OF WINTER RAPESEED (*B. NAPUS* L.)

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INTRODUCTION

Molecular method have been introduced into breeding programs to ensure quick evaluation of breeding material variability. Yield is the most important breeding goal and significant increase is ensured by the development of hybrid cultivars displaying heterosis effect. The potential of heterosis in hybrid cultivars depends on parental lines combinations belongind to distant gene pools.

The objective of this study was the evaluation of genetic distance among 64 winter rapeseed breeding lines and cultivars included in a breeding program of hybrid varieties based on CMS *ogura* hybridization system.

METHODS

The plant materials used in the study consisted of 64 genotypes of winter oilseed rape and included 30 CMS *ogura* inbred lines, 9 restorer lines (*Rfo*) for CMS *ogura* system and 25 genotypes of Polish and European origin. DNA was extracted from eight-day-old leaves using a modified CTAB procedure according to Doyle and Doyle (1990). AFLP and STR analyses were performed using capillary electrophoresis of fluorescently labeled PCR products (Applied Biosystems). Electrophoretograms were analysed with PeakScanner 1.0 (Applied Biosystems). Genetic distance among the genotypes was assessed using ten AFLP primer-enzyme combinations and primer pairs for 48 STR loci. Genetic distance (GD) of the investigated lines was calculated according to Nei and Li (1979) coefficient of dissimilarity, the UPGMA method was applied for the creation of the dendrogram.

RESULTS

Tab. 1. Genetic information for ten AFLP primer combinations in a study of 64 winter oilseed rape genotypes: total number of fragments scored, number and percentage of polymorphic bands

Primer combinations	No. of bands	No. of polymorphic bands	Degree of polymorphism %
E-AAC NED: M-CAC	50	34	68.0
E-ACC NED: M-CAC	45	23	51.1
E-ACC NED: M-CAG	38	26	68.4
E-ACC NED: M-CTC	15	10	66.7
E-ACT FAM: M-CTC	43	13	30.2
E-ACT FAM: M-CAT	39	27	69.2
E-ACA FAM: M-CTT	51	17	33.3
E-AGG JOE: M-CTA	40	32	80.0
E-AGG JOE: M-CTC	50	34	68.0
E-AGG JOE: M-CAT	57	48	84.2
Total	428	264	61.7

Tab. 2. Minimum and maximum of genetic diversity values calculated using the Nei and Li (1979) measure of genetic dissimilarity

Minimum genetic diversity values			Maximum genetic diversity values		
1326DHR 8	1326DHR 3	0.099	932R-5-15	ATORA	0.612
MS 320/15	MS 416/15	0.115	MS 473/15	ATORA	0.597
MS 299/15	MS 416/15	0.126	932R-5-15	SWO 2075	0.594
MS 299/15	MS 320/15	0.128	743R-2-15	ATORA	0.589
MS 416/15	MS 463/15	0.133	743R-2-15	MH09CM044	0.588
MS 438/15	MS 299/15	0.134	932R-5-15	MH09CM044	0.582
LE 14/280	LE 12/252	0.145	309 MS	ATORA	0.58
MS 476/15	MS 463/15	0.148	743R-2-15	MH07BD018	0.576
MS 438/15	MS 416/15	0.157	743R-2-15	ECS 13017	0.573
MS 463/15	RNX 3521	0.159	932R-5-15	SLM 307	0.571
CWH 361	LE 15-298	0.16	MS 473/15	SWO 2075	0.566

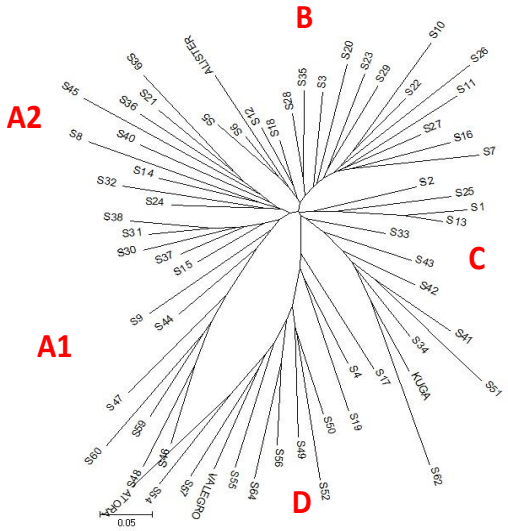

Fig. 1. Genetic dissimilarity dendrogram of 64 winter oilseed rape genotypes on the basis of AFLP and STR markers

AFLP and STR fingerprinting of the 64 genotypes of winter oilseed rape with ten *Eco*RI/*Mse*I primer combinations and few STR loci detected a substantial level of polymorphism (Tab. 1). The size of the AFLP polymorphic fragments ranged from about 50 to 600 nucleotides, with 90% of the markers between 50 to 400 bp. The dissimilarity data matrix on the Nei and Li (1979) coefficients showed the lowest diversity value, 0.099, between two restorer lines (*Rfo)* 1326DHR 8 and 1326DHR 3, and the highest value was 0.612 for restorer line (*Rfo*) 932R-5-15 and cultivar ATORA (Tab. 2). The dendrogram obtained by cluster analysis of the Nei and Li (1979) coefficients of genetic dissimilarity separated the investigated 64 genotypes into four main groups A (A1, A2) - D (Fig. 1). The first (A1 and A2) and second (B) groups included CMS *ogura* lines and few Polish and European origin breeding lines. The third (C) and fourth (D) group was formed generaly by restorer lines (*Rfo*), cultivars: Kuga, Valegro and Atora and breeding lines (Polish and European origin). The results indicate that both AFLP and STR markers are suitable for assessing genetic relationship among rapeseed breeding lines with high level of accuracy.

CONCLUSIONS

The approaches combining markers such as AFLP and STR may provide more accurate information on genetic diversity and relationships of *B. napus* genotypes as compared to selection classical methods for parental components of winter rapeseed hybrid varieties.

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