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# #257

## Association of microsatellite and AFLP markers with traits of agronomic importance in winter oilseed rape (*Brassica napus* L.)

ADDRESS

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ORALS

POSTERS

WORKSHOPS

The aim of this work was to analyze phenotype-genotype associations among canola-type winter oilseed rape cultivars and breeding lines of agronomic value collected at the Plant Breeding and Acclimatization Institute-NRI, Research Division in Poznań, Poland.

Plant material included 25 winter oilseed rape breeding lines and cultivars. Field trials were conducted in six environments (3 years at two locations) using a randomized complete block design, with four replicates. The traits assessed included: seed yield (YLD), seed yield components, seed quality parameters: oil, protein and glucosinolate (GLS) content, acid (ADF) and neutral (NDF) fiber content. Genotyping was done using 85 microsatellite (SSR) loci and 10 AFLP primer combinations, as well as allele-specific CAPS and SNP markers for non-mutated and mutant FAD2 and FAD3 desaturase genes, respectively (Falentin et al., 2007; Mikołajczyk et al., 2010). In addition, SCAR markers for the ogura male-sterile cytoplasm and the Rfo restorer gene were applied (Mikołajczyk et al., 2011). In total, 779 polymorphic DNA markers were analyzed. Association analyses were performed both, for each of the six environments separately, and for the calculated mean value of the six environments using the GenStat 18 statistical software.

As a result, the range of phenotypic variation regarding the mean value of six environments accounted by particular marker equaled, respectively, 20.3 – 33.2% for seed yield, and 19.9 – 36.8% for yield component traits. For seed quality traits, phenotypic variation ranged from 20.9 to 34.5%, for total GLS content, 16.9 – 37.5% for fatty acid composition, and 17.9% – 64.8% for total fiber content.

Genotype/environment association was determined. Groups of markers associated with economically important traits as seed yield, yield structure elements and seed quality parameters were identified with statistical significance. As the next step, NG high-throughput genotyping will be performed to determine SNPs useful for effective marker-assisted breeding of agronomically important winter rapeseed genotypes.

Keywords: winter oilseed rape (*Brassica napus* L.), microsatellite markers, AFLP, field trials, association study



# Association of microsatellite and AFLP markers with traits of agronomic importance in winter oilseed rape (*Brassica napus* L.)

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The aim of this work was to analyse phenotype-genotype associations among canola-type winter oilseed rape cultivars and breeding lines collected at the Plant Breeding and Acclimatization Institut-NRI, in Poznań.

## Material

- Plant material included 25 winter oilseed rape (WOSR) cultivars, breeding lines, DH and resynthesized lines (PB 17-20 collection).
- The material was genotyped with 85 microsatellite (SSR) loci, 10 AFLP primer combinations, allele-specific CAPS and SNP markers for non-mutated and mutant FAD2 and FAD3 desaturase genes (Falentin et al., 2007; Mikołajczyk et al. 2010) and in addition, SCAR markers for the *ogura* male sterile cytoplasm and the *Rfo* restorer gene (Mikołajczyk et al. 2011).

## Field experiments

- Field trials have been carried out in two locations (Borowo - B, Łagiewniki - Ł) using a randomized complete block design, with four replicates. The traits assessed included: seed yield, seed yield components, seed quality parameters: oil, glucosinolates (GLS), acid (ADF) and neutral (NDF) fiber content and fatty acid composition in seed oil.



## Associations

Association analyses were performed on selected 779 polymorphic DNA markers using GenStat 18 statistical software (sign. at  $p=0.05$ ).

Selected markers associated with phenotypical traits (PB 17-20 collection)

Marker	Estimates of regression coeff.	P-value	The proportion of total phenotypic variance explained by the marker
<b>Seed yield</b>			
SSRG02_328	30.04/B	<.001	58.2
E-ACCned:M-CAG-61.7	30.75/L	0.002	30.9
E-ACCned:M-CAC_91.3			
<b>Oil content</b>			
SSRG12_315	2.06/B	0.02	31.1
	1.92/L	0.02	18.0
<b>Length of silique</b>			
E-AGGjoe:M-CAT_96.1	-10.6/B	<.001	40.4
<b>Number of seeds per silique</b>			
E-AGGjoe:M-CAG_140.0	6.82/L	<.001	44.9
<b>Weight of 1000 seeds</b>			
E-ACCned:M-CTC_458.3	-0.43/B	0.005	26.3
	-0.47/L	0.006	25.5
<b>C18:1</b>			
SSRG36_266	9.29/B	<.001	46.8
	11.97/L	<.001	50.5
<b>C18:2</b>			
SSRG36_266	-7.06/B	<.001	47.8
	-8.63/L	<.001	49.2
<b>C18:3</b>			
SSRB02_23	4.68/B	<.001	67.3
	5.74/L	<.001	63.5
SSRG05_652	-4.68/B	<.001	67.3
SSRkl_n_155	-5.74/L	<.001	63.5
<b>Total GLS content</b>			
SSRG28_202	-13.27/B	<.001	53.7
	-11.35/L	<.001	41.6
SSRG28_218	13.27/B	<.001	
	11.35/L	<.001	
<b>ADF fiber content</b>			
E-ACCned:M-CAC_71.04	7.02/L	<.001	70.6
E-ACCned:M-CAC_263.5			
<b>NDF fiber content</b>			
E-ACCned:M-CAC_80.8	4.57/L	<.001	58.3

In previous studies made for the collection PB 14-17 WOSR the range of phenotypic variation regarding the mean value of six environments accounted by particular marker equaled, respectively:

- 20.3 - 33.2% for seed yield /11 markers: 8 SSR and 3 AFLP
- 19.9 - 36.8% for yield component traits /8 markers: 3 SSR and 5 AFLP
- 20.9 - 34.5% for GLS content /8 markers: 4 SSR, 4 AFLP
- 16.9 - 37.5% for fatty acid composition
- C18:1/ 7 markers: 1 SSR, 6 AFLP
- C18:2/ 8 markers: 1 SSR, 7 AFLP
- C18:3/ 16 markers: 12 SSR, 4 AFLP
- 17.9 - 64.8% for total fiber content
- ADF/ 34 markers: 21 SSR, 13 AFLP
- NDF/ 31 markers: 18 SSR, 13 AFLP

## References

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Characteristic of seed yield, seed yield components and seed quality parameters of PB 17-20 collection in field trials

Genotype	Seed yield (dt/ha)	Oil content (%)	Length of silique (mm)	Number of seeds per silique	Weight of 1000 seeds (g)	C18:1 (%)	C18:2 (%)	C18:3 (%)	Total GLS content (μmol·g <sup>-1</sup> )	ADF (%)	NDF (%)
Mean (2 localities)	36.48	44.34	62.95	16.37	5.34	68.20	16.36	8.39	13.93	19.11	24.01
Minimum	11.67	41.57	56.40	12.23	4.70	57.09	7.46	3.69	5.51	10.98	17.25
Maximum	74.66	47.98	72.53	20.43	6.20	78.19	24.18	11.93	28.76	22.63	26.53
F calc.	40.52**	14.61**	16	1.25	8.61**	41.55**	50.91**	33.15**	64.96**	33.51**	33.86**
LSD 0.05	5.39	1.24	6.22	3.30	0.25	1.48	1.09	0.47	1.14	1.64	1.31

## Conclusions

- Group of markers associated with economically important traits as seed yield, yield component traits and seed quality parameters were identified with statistical significance

## Perspectives

- As the next step, NG high-throughput genotyping will be performed to determine SNPs useful for effective marker-assisted breeding of agronomically important winter oilseed rape genotypes