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P-505. Association of microsatellite and AFLP markers with yield and seed quality in winter oilseed rape (*Brassica napus* L.)

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The aim of this work was to analyze phenotype-genotype associations among the selected 25 breeding lines and cultivars of rapeseed. Field trials including the analyzed 25 objects were performed in four replicates of a randomized complete block design in six environments. Seed yield was estimated for harvest from a total plot. Seed protein and oil content, fatty acid composition in seed oil as well as glucosinolates content and the ADF and NDF fiber fractions were analyzed. Genotyping was done using 80 microsatellite 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; Mikolajczyk et al., 2010), and also SCAR markers for the *Ogura* male-sterile cytoplasm and the *Rfo* restorer gene (Mikolajczyk et al., 2011). In total, 685 polymorphic DNA markers were analyzed. Association analyses were performed both, for each of the six environment separately, and for the calculated mean value of the six environments using the Genstat 17 statistical software. DNA markers determining particular phenotype traits with statistical significance level of $p=0.05$ were estimated using five parameters: estimate of regression coefficients, standard error of estimation, t-test value, P-value, and the range of phenotypic variation accounted by particular marker; estimation was done using the regression analysis. The range of phenotypic variation accounted by particular marker equaled, respectively: for seed yield, 22.4 - 36.1 %, for oil content 20.9 - 42.9 %, total glucosinolates 23.5 - 36.7 %, protein content 20.1 - 35.3 %, and for fiber, ADF 29.8 % - 73.2, NDF 25,5 - 71,2 %. As a result, DNA markers associated with seed yield and agronomically important grain quality traits were identified. The obtained results will be used for further designing of markers specific for particular genotypes revealing agronomically important traits.

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

References

- Falentin et al., (2007) International Patent Publication WO 2007/138444
Mikolajczyk et al., (2010) *Plant Breeding*, 129: 502-507
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ASSOCIATIONS OF MICROSATELLITE AND AFLP MARKERS WITH YIELD AND SEED QUALITY IN WINTER OILSEED RAPE (*BRASSICA NAPUS* L.)



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Field trials including the analyzed 25 objects were performed in four replicates of a randomized complete block design in six environments. Seed yield was estimated for harvest from a total plot. Seed protein and oil content, fatty acid composition in seed oil as well as glucosinolates (gls) content and the ADF and NDF fiber fractions were analyzed (Fig. 1A, 1B). Genotyping was done using 80 microsatellite 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; Mikolajczyk et al., 2010), and also SCAR markers for the *ogura* male-sterile cytoplasm and the *Rfo* restorer gene (Mikolajczyk et al., 2011). In total, 685 polymorphic DNA markers were analyzed. Association analyses were performed both, for each of the six environment separately, and for the calculated mean value of the six environments using the Genstat 17 statistical software.

Table 1. Selected markers associated with phenotypical traits

Marker	The proportion of total phenotypic variance explained by the marker	Estimates of regression coeff.	Standard error of estim.	P-value	Marker	The proportion of total phenotypic variance explained by the marker	Estimates of regression coeff.	Standard error of estim.	P-value
Seed yield									
E-AAC-M-CAC-169	27.9	11.83	3.69	0.004	E-AGG-M-CAC-121	27.6	10.11	3.17	0.004
E-ACC-M-CAC-97.8	26.9	-8.72	2.78	0.005	SSRE04_236	27.6	-10.11	3.17	0.004
E-ACC-M-CAC-91.1	24.8	-12.70	4.25	0.007	SSRE04_242	32.1	11.54	3.28	0.002
E-ACC-M-CTC-139	36.1	10.83	2.84	<.001	SSRG08_318	35.3	10.72	2.85	0.001
Oil content									
E-AAC-M-CAC-70	42.9	-1.37	0.31	<.001	SSRE01_398	24.0	0.991	0.34	0.008
E-AGG-M-CAG-270	22.2	0.85	0.31	0.01	SSRG31_242	24.4	0.858	0.29	0.007
C18:1					C18:2				
E-AAC-M-CAG-241	30.5	6.07	1.79	0.002	E-AAC-M-CAC-165	31.7	-4.94	1.42	0.002
SSRG02_219	30.5	-6.76	1.99	0.002	E-ACC-M-CAG-267	39.6	-5.42	1.32	<.001
SSRG53_160	30.2	7.08	2.10	0.003	E-AGG-M-CTA-228	31.7	-4.94	1.42	0.002
FAD2 H4-mutant	22.0	6.60	2.37	0.01	SSRG53_160	27.5	-5.39	1.7	0.004
C18:3									
E-AAC-M-CAC-438	35.7	3.96	1.05	<.001	FAD2 H3-wild	30.6	2.23	0.66	0.002
E-ACC-M-CAC-429	31.7	2.01	0.58	0.002	FAD2 H3-mutant	32.8	-1.87	0.52	0.002
E-AGG-M-CAG-157	35.7	-3.96	1.05	<.001	FAD2 H4-wild	35.7	3.96	1.05	<.001
SSRG02_219	43.0	1.88	0.43	<.001	FAD2 H4-mutant	35.7	-3.96	1.05	<.001
SSRG57_352	31.4	-2.70	0.78	0.002	FAD3A-wild	35.7	3.96	1.05	<.001
SSRG05_356	30.3	1.45	0.43	0.003	FAD3A-mutant	35.7	-3.96	1.05	<.001
Total gls content					Seed protein content				
E-ACC-M-CAG-77	35.5	10.25	2.72	0.001	E-AAC-M-CAC-67.8	31.9	0.76	0.22	0.002
E-AGG-M-CTA-152	35.5	6.20	1.64	<.001	E-ACC-M-CAC-306	35.3	0.97	0.26	0.001
E-AGG-M-CAG-140	36.9	6.63	1.71	<.001	SSRG09_210	33.7	0.89	0.25	0.001
SSRG52_202	31.9	-8.17	2.33	0.002	SSRG19_225	30.4	-0.99	0.29	0.003
ADF fiber content					NDF fiber content				
E-ACC-M-CAC-223	73.2	6.58	0.81	<.001	E-ACC-M-CAC-263	52.1	3.05	0.59	<.001
E-ACC-M-CAG-68	55.9	3.50	0.62	<.001	E-ACC-M-CAC-223	71.2	5.86	0.75	<.001
E-AGG-M-CTA-163	73.2	6.58	0.81	<.001	E-AGG-M-CAT-163	71.2	5.86	0.75	<.001
SSRG31_366	66.2	4.53	0.65	<.001	SSRG15_366	65.9	4.08	0.59	<.001
SSRG18_217	73.2	6.58	0.81	<.001	SSRE04_242	46.0	2.34	0.51	<.001
SSRR93_154	73.2	6.58	0.81	<.001	SSRG89_217	71.2	5.86	0.75	<.001
SSRG41_224	43.1	3.72	0.85	<.001	SSRE05_138	71.2	5.86	0.75	<.001

DNA markers determining particular phenotype traits with statistical significance level of $p=0.05$ were estimated using four parameters: estimate of regression coefficients, standard error of estimation, P-value, and the range of phenotypic variation accounted by particular marker. Estimation was done using the regression analysis.

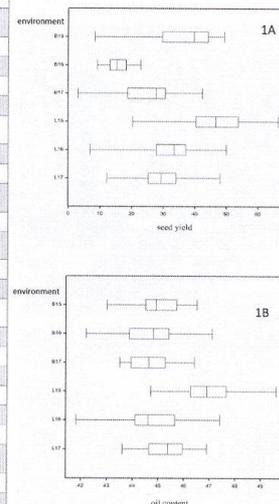


Figure 1A, 1B. Boxplot of seed yield and oil content of 25 genotypes of winter oilseed rape in six environments

The range of phenotypic variation accounted by particular marker equaled, respectively: for seed yield 24.8 – 36.1%, for oil content 22.9 – 42.9%, total glucosinolates (gls) 31.9 – 36.9%, protein content 30.4 – 35.3%, and for fiber, ADF 43.1 – 73.2%, NDF 46.0 – 71.2% (Tab. 1). As a result, DNA markers associated with seed yield and agronomically important grain quality traits were identified.

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