ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Hortorum Cultus, 18(3) 2019, 53–69
www.acta.media.pl ISSN 1644-0692 e-ISSN 2545-1405 DOI: 10.24326/asphc.2019.3.6

ORIGINAL PAPER

Accepted: 12.09.2018

THE RELATIONSHIP BETWEEN RAPD MARKER-BY-MARKER INTERACTIONS AND QUANTITATIVE TRAITS OF CARAWAY (*Carum carvi* L.)

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ABSTRACT

Application of molecular markers makes the selection process much more effective. Marker assisted selection is an important tool for plant breeders to increase the efficiency of a breeding process, especially for multigenic traits, highly influenced by the environment. Epistasis is the interaction between alleles from two or more loci determining the complex traits, and thus plays an important role in the development of quantitative traits of crops. In this paper, the relationships between RAPD marker-by-marker interactions and 22 quantitative traits of caraway (*Carum carvi* L.) were analyzed. Significant associations of 116 epistatic markers with at least one trait in 2004 as well as 112 in 2005 were found on the basis of multivariate regression analysis. The proportion of total phenotypic variances of individual trait explained by the marker-by-marker interactions ranged from 25.3% to 96.0%.

Key words: caraway, interaction, molecular markers, RAPD-PCR, morphological traits

INTRODUCTION

Caraway (*Carum carvi* L.), a biennial medicinal plant of the family *Apiaceae* is widely cultivated in northern Europe, Russia, India, Canada and the United States and the world production of fruit may reach 18−25 thousand tones a year. In Poland, caraway is the one of the important medicinal plant that is cultivated on the area of 3500 ha and the fruit production is four thousand tones a year. Dried caraway (*Carvi fructus*) is used mainly as a spice in food industry [Seidler-Łożykowska and Bocianowski 2012]. In medicine, caraway is used for its digestive, carminative, spasmolytic and stimulant properties. Limonene and carvone are the most important compounds of essential oil, which is the main active substance of caraway fruit. Caraway is also the source of essential oil for cosmetic industry. Moreover, caraway could be a component of animal forage to enhance their well-being [Sadowska and Obidoska 1998]. Development of high yielding cultivars characterized by high content of active substances and better adapting to abiotic and biotic stress is the main aim of caraway breeding programs.

Genetic variation of quantitative traits is usu-

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ally controlled by a number of genes (quantitative trait loci, QTLs) with main effects as well as with epistatic and gene by environmental interactions [Bocianowski and Krajewski 2009, Krajewski et al. 2012, Bocianowski 2013]. With the development of modern molecular biology, it has become possible to dissect the genetic mechanism of quantitative trait and to identify the associated genes and their interacting network. Application of molecular markers makes the selection process much more effective. Marker assisted selection is an important tool for plant breeders to increase the efficiency of a breeding process, especially for multigenic traits, highly influenced by the environment. Epistasis is the interaction between alleles from two or more loci determining the complex traits, and thus plays an important role in the development of quantitative traits of crops. In many studies, genes with small effects do not come into the final model and thus the total epistasis interaction effect is biased. Many loci may not have a significant direct effect on the trait under consideration, but they may still affect the trait expression by interacting with other loci [Bocianowski 2014]. In this case, all loci are used for estimation of the epistatic effects, not only the loci with significant main effects [Bocianowski 2014].

Statistical approaches of two-way analysis of variance and multiple regression have been applied to analyze the epistasis for quantitative traits based on genetic markers [Wu et al. 1995, Li et al. 1997a, b, Yan et al. 2006, Bocianowski 2012b]. Some statistical models and methods based on marker linkage maps have been suggested for analyzing the QTLs with epistatic effects: nested test framework [Sen and Churchill 2001], omnibus test [Chatterjee et al. 2006], family-mixed model [Borecki and Province 2008], allelic scoring method [Jung et al. 2009], generalized linear model [Clarke et al. 2009, Bocianowski and Nowosad 2015], principal component analysis [Li et al. 2009].

The aim of this paper is to estimate the marker-by-marker interactions effects of 22 quantitative traits of the selected populations and cultivars of caraway using RAPD markers.

MATERIALS AND METHODS

Plant material. Material for the study covered caraway collection consisted of 22 genotypes: 12 populations from botanical gardens of: Cambridge (GBR), Lillehammer (NOR), Geneva (CHE), Glasgow (GBR), Żalec (POL), Arhus (DEN), Strasburg (AUT), Nancy (FRA), Rouen (FRA), Klagenfurt (AUT), Berno (CHE), Padwa (ITA), cultivars: 'Rekord' (CZE), 'Trojica' (SLO). Moreover, cultivar 'Kończewicki' (POL) was represented by three strains: 2, 6, 7, and five newly bred strains, which were obtained from the breeding program carried out in the Institute of Medicinal Plants of Poznań. Strains of cultivar 'Kończewicki' originated from the maintenance breeding, which is being performed in the Institute.

Field test. The field plots of caraway collection were established in 2004 and 2005 in the Institute of Medicinal Plants in Plewiska (52°21'59"N, 16°48'32"E) near Poznań. Each year in April, seeds of all collected genotypes were sown in a greenhouse to obtain 5–8-leaf plantlets, which were planted on the field at the beginning of May. The collection was established with four repetitions and number of plants in the genotypes oscillated from 25 to 100.

Morphological traits. Morphological measurements were done in 2004 and 2005 on plants in the second year of plant growing. During flowering time, 10 random plants were chosen in each genotype of collection and the following traits were measured: plant height, number of lateral shoots, number of branches on main stem, number of leaves on main stem, leaf length, diameter of main umbel, number of umbelets in main umbel, diameter of primary umbel, number of umbelets in primary umbel, number of umbel on main stem branches and number of umbels per plant.

The seeds were collected from the same selected plants and after drying and cleaning the fruit weight per plant and weight of 1000 seeds were estimated.

Estimation of content of essential oil and its composition. The content of essential oil was estimated after hydro-distillation of caraway fruit in Dering's apparatus following the methods recommended by European Pharmacopoeia VI [2008].

Gas chromatography data. The hexane solution of the oil (1 : 10) was analyzed with gas chromatography using Perkin Elmer Clarus 500 system in the following conditions: chromatographic column Elite 1 (30 m × 0.32 mm × 0.25 μm), volume of each injected sample = 1 μL, injector temperature – 200°C, carrier gas – helium, helium flow

= 1 ml · min^{-1}, FID detector temperature – 220 C. Time of components retention of the tested solution: carvone – 45.17 min, limonene – 15.69 min, α-pinene – 9.16 min, β-pinene – 11.83 min, α-felandrene – 13.79 min, dihydrocarvone – 38.04 min, dihydrocarveol – 45.50 min, carveol – 49.91 min, α-thujone – 25.25 min.

DNA extraction. Genomic DNA was extracted from five young leaves (each leaf from a different plant) collected from leave-rosette of each collection genotype in the first year of cultivation in 2004 and 2005. DNA was isolated using the CTAB extraction method by Doyle and Doyle [1990].

Random amplified polymorphic DNA assays. The RAPD-PCR reactions were carried out using a *Taq* PCR Core Kit (QIAGEN Inc.) in a total volume of 12.5 μl. The reaction mixture contained 0.2 μM primer DNA, 10 mM Tris-HCl, pH 8.3, 2.3 mM $MgCl_2$, 2.5 μg BSA, 0.1 μM of each dNTP and 0.4 U of *Taq* DNA polymerase (Fermentas, GmBH, St Leon-Rot, Germany). Thirty random 10-nucleotide primers (Genset Oligos) were used to screen the cultivars for polymorphism [Bocianowski and Seidler-Łożykowska 2012]. Amplification was carried out in a PTC-200 thermocycler (MJ Research) using the following program: an initial denaturation at 95°C for 30 s, followed by 45 cycles of denaturation at 94°C for 30 s, primer annealing at 35°C for 1 min and at 72°C for 2.5 min. The amplification was ended with an additional extension at 72°C for 5 min. Each reaction was carried out in three replications. To increase the reliability of RAPD markers, only repeated products were selected for analysis.

The electrophoresis conditions. The PCR products were separated by electrophoresis for 90 min at 100 V in 1.5% agarose gels containing TBE buffer and visualized under UV light after staining with ethidium bromide. A Gene Ruler[TH] 100 bp DNA Ladder Plus (Fermentas, GmBH, St Leon-Rot, Germany) was used as a molecular size standard for PCR products.

STATISTICAL ANALYSIS

The normality of residuals from the regression model was tested using Shapiro-Wilk's normality test [Shapiro and Wilk 1965]. A two-way analysis of variance (ANOVA) was carried out to determine the effects of genotype, year and genotype×year interaction on the variability of studied traits. For the detection of interaction between markers, a two-locus interaction model was fitted:

$$y_j = \mu + a_1 \cdot m_{1j} + a_2 \cdot m_{2j} + b \cdot m_{1j} \cdot m_{2j} + \varepsilon_j,$$

where y means observed trait, μ – general mean, a_1 and a_2 are the regression coefficients for the main effects of markers m_1 and m_2, respectively, b is the regression coefficient for the epistatic interaction between marker m_1 and marker m_2, m_{1j} and m_{2j} are indicator variables of the marker genotypes, ε is an error of observations. Above model was tested against the model considering the lack of interaction ($b = 0$), for all markers, not only for markers that showed association with traits.

The total percentage of phenotypic variation (coefficient of determination, R^2) was estimated. The coefficient of determination was used to measure how the model fits the data and, in this study, the amount of the phenotypic variance explained by the marker-by-marker interaction effects. Data analyses were performed using the statistical package GenStat 18.

RESULTS

ANOVA indicated that the main effects of genotype, year as well as genotype by year interaction were significant for all observed traits. Coefficient of variation ranged from 6.84% (for carveol content) to 78.98% (for number of lateral shoots). Differences between the years were large, therefore the association analyses between molecular markers and observed traits were made separately for years.

Significant associations of 116 pairs of markers with at least one trait in 2004 as well as 112 in 2005 were found on the basis of multiple regression analysis (Tabs. 1–22). Different number of significant marker-by-marker interactions in the first and the second year for individual traits was caused by environment effect. Number of significant epistatic effects ranged from 2 for dihydrocarveol content (Tab. 20) to 27 for diameter of primary umbel (Tab. 8). The proportion of total phenotypic variance of individual trait explained by the marker-by-marker interaction ranged from 25.3% for GS-1 750 bp by GS-3 1300 bp interaction (for leaf length) and GS-21 1200 bp by GS-3 1400 bp

Table 1. Significant epistasis interactions affecting plant height at 0.01 level

Marker 1 symbol	Marker 2 symbol	Interaction effect	*P*-value	The proportion of total phenotypic variance explained by the markers interaction
		2004		
GS-22 800 bp	GS-43 500 bp	–13.91	0.002	63.8
GS-43 700 bp	**GS-53 850 bp**	**13.47**	**0.009**	**59.5**
GS-22 700 bp	GS-5 350 bp	–14.31	0.002	52.2
GS-5 350 bp	GS-5 1031 bp	–14.31	0.002	52.2
GS-1 750 bp	GS-5 350 bp	–17.35	0.005	45.0
GS-3 1300 bp	GS-5 350 bp	–17.35	0.005	45.0
GS-22 800 bp	GS-38 700 bp	–7.72	0.008	38.7
GS-1 750 bp	GS-38 800 bp	–14.9	0.007	35.4
GS-21 900 bp	GS-38 800 bp	–14.9	0.007	35.4
		2005		
GS-43 800 bp	GS-5 1031 bp	–13.56	0.008	66.5
GS-43 700 bp	**GS-53 850 bp**	**12.01**	**0.007**	**61.9**
GS-21 1200 bp	GS-5 1031 bp	–13.24	0.002	52.3
GS-21 1200 bp	GS-38 700 bp	–13.11	0.004	45.4
GS-12 1200 bp	GS-53 850 bp	10.66	0.002	43.9
GS-3 1400 bp	GS-5 1031 bp	–12.98	0.006	43.5
GS-22 700 bp	GS-39 550 bp	12.86	0.010	31.0
GS-21 1200 bp	GS-22 700 bp	–10.91	0.004	30.8
GS-22 700 bp	GS-3 1400 bp	–10.41	0.007	27.7

Estimates of regression coefficients for marker-by-marker interaction

Bold font – common significant interacted markers in both years

Table 2. Significant epistasis interactions affecting number of lateral shoots at 0.01 level

Marker 1 symbol	Marker 2 symbol	Interaction effect	*P*-value	The proportion of total phenotypic variance explained by the markers interaction
2004				
GS-19 600 bp	GS-3 1400 bp	5.15	<0.001	39.8
GS-19 600 bp	GS-21 1200 bp	5.15	0.001	37.9
GS-3 500 bp	GS-3 1400 bp	5.03	0.002	35.9
GS-21 1200 bp	GS-3 1450 bp	4.81	0.002	35.1
GS-22 700 bp	GS-3 1450 bp	4.81	0.003	34.1
GS-3 1400 bp	GS-3 1450 bp	4.6	0.004	30.8
GS-3 1400 bp	GS-8 550 bp	5.73	0.006	29.2
GS-1 750 bp	GS-3 1400 bp	4.58	0.008	26.8
GS-1 750 bp	GS-21 1200 bp	4.74	0.009	26.2
2005				
GS-39 550 bp	GS-41 550 bp	–1.725	<0.001	65.3
GS-1 1500 bp	GS-41 550 bp	–1.944	0.001	50.8
GS-3 500 bp	GS-41 550 bp	–1.944	0.001	50.8
GS-41 550 bp	GS-5 400 bp	–2.543	0.008	46.8
GS-3 500 bp	GS-53 450 bp	–1.728	0.009	33.6
GS-19 600 bp	GS-22 700 bp	1.238	0.007	28.1

Table 3. Significant epistasis interactions affecting number of branches on main stem at 0.01 level

Marker 1 symbol	Marker 2 symbol	Interaction effect	*P*-value	The proportion of total phenotypic variance explained by the markers interaction
2004				
GS-38 600 bp	GS-5 1031 bp	–2.837	0.006	57.9
GS-22 1300 bp	GS-5 350 bp	–2.42	0.002	52.9
GS-21 1200 bp	GS-5 350 bp	–2.067	0.007	41.9
GS-3 1400 bp	GS-5 350 bp	–2.067	0.007	41.9
2005				
GS-21 1200 bp	GS-38 600 bp	–1.8	0.001	52.6
GS-3 1400 bp	GS-38 600 bp	–1.668	0.003	46.0
GS-22 800 bp	GS-38 600 bp	–1.588	0.006	41.0
GS-38 550 bp	GS-38 600 bp	–1.52	0.010	36.9
GS-19 600 bp	GS-21 1200 bp	–1.407	0.006	28.5
GS-21 1200 bp	GS-3 1400 bp	–1.335	0.008	26.6
GS-22 800 bp	GS-3 1400 bp	–1.316	0.008	26.4

Table 13. Significant epistasis interactions affecting thousand seed weight at 0.01 level

Marker 1 symbol	Marker 2 symbol	Interaction effect	*P*-value	The proportion of total phenotypic variance explained by the markers interaction
2004				
GS-1 1500 bp	GS-53 850 bp	0.47	0.004	40.5
GS-12 1200 bp	GS-3 500 bp	0.409	0.002	34.4
2005				
GS-21 1200 bp	GS-5 350 bp	–0.543	0.006	44.0
GS-3 1400 bp	GS-5 350 bp	–0.543	0.006	44.0

Table 14. Significant epistasis interactions affecting essential oil content at 0.01 level

Marker 1 symbol	Marker 2 symbol	Interaction effect	*P*-value	The proportion of total phenotypic variance explained by the markers interaction
2004				
GS-12 1200 bp	GS-5 350 bp	0.855	0.006	44.3
GS-12 1200 bp	GS-19 200 bp	0.668	0.009	25.9
2005				
GS-43 800 bp	GS-5 350 bp	1.143	0.010	65.0
GS-38 800 bp	GS-53 450 bp	1.022	0.007	48.2
GS-12 1200 bp	GS-38 800 bp	1.008	0.002	46.2
GS-19 200 bp	GS-22 800 bp	0.855	<0.001	40.3

Table 15. Significant epistasis interactions affecting α-pinene content at 0.01 level

Marker 1 symbol	Marker 2 symbol	Interaction effect	*P*-value	The proportion of total phenotypic variance explained by the markers interaction
2004				
GS-1 750 bp	GS-53 850 bp	–0.02357	0.001	47.7
GS-1 750 bp	GS-3 1400 bp	–0.0161	0.006	28.3
2005				
GS-43 500 bp	GS-53 850 bp	0.0325	<0.001	84.3
GS-19 200 bp	GS-5 400 bp	0.02	0.006	43.4
GS-21 400 bp	GS-5 400 bp	0.02	0.006	43.4
GS-21 400 bp	GS-3 1450 bp	0.019	0.003	33.6
GS-19 200 bp	GS-53 850 bp	0.01833	0.009	33.5

Table 16. Significant epistasis interactions affecting β-pinene content at 0.01 level

Marker 1 symbol	Marker 2 symbol	Interaction effect	*P*-value	The proportion of total phenotypic variance explained by the markers interaction
		2004		
GS-38 600 bp	GS-38 700 bp	–0.021	0.003	45.8
GS-1 750 bp	GS-38 550 bp	–0.02167	0.003	32.5
		2005		
GS-21 1200 bp	GS-43 700 bp	0.016	0.007	52.6
GS-3 1400 bp	GS-43 700 bp	0.016	0.007	52.6
GS-22 1300 bp	GS-38 800 bp	0.01619	0.003	42.7
GS-19 600 bp	GS-41 550 bp	0.01714	0.006	38.8
GS-21 1200 bp	GS-41 550 bp	0.01714	0.006	38.8
GS-3 1400 bp	GS-41 550 bp	0.01714	0.006	38.8
GS-3 1450 bp	GS-41 550 bp	0.01714	0.006	38.8
GS-21 1200 bp	GS-38 800 bp	0.01308	0.009	33.2
GS-3 1400 bp	GS-38 800 bp	0.01308	0.009	33.2

Table 17. Significant epistasis interactions affecting limonene content at 0.01 level

Marker 1 symbol	Marker 2 symbol	Interaction effect	*P*-value	The proportion of total phenotypic variance explained by the markers interaction
		2004		
GS-22 700 bp	GS-5 350 bp	–4.91	0.007	42.9
GS-5 350 bp	GS-5 1031 bp	–4.91	0.007	42.9
GS-1 1500 bp	GS-3 1400 bp	–4.71	0.002	34.9
GS-3 500 bp	**GS-3 1400 bp**	**–4.65**	**0.004**	**30.9**
GS-22 1300 bp	GS-3 1400 bp	–4.3	0.007	27.2
GS-12 1200 bp	GS-22 800 bp	4.18	0.009	26.1
GS-22 700 bp	GS-3 1400 bp	–4.13	0.009	25.7
		2005		
GS-3 500 bp	**GS-3 1400 bp**	**–5.2**	**<0.001**	**49.5**
GS-3 1400 bp	GS-38 600 bp	–3.99	0.003	45.6
GS-21 1200 bp	GS-3 1400 bp	–4.55	0.001	38.7
GS-21 1200 bp	GS-3 500 bp	–4.12	0.005	29.3

Table 18. Significant epistasis interactions affecting α-thujone content at 0.01 level

Marker 1 symbol	Marker 2 symbol	Interaction effect	*P*-value	The proportion of total phenotypic variance explained by the markers interaction
		2004		
GS-22 700 bp	GS-43 500 bp	–0.0567	0.006	54.2
GS-22 800 bp	GS-53 850 bp	0.0371	0.008	34.7
GS-12 1200 bp	**GS-22 700 bp**	**–0.0318**	**0.006**	**28.7**
GS-12 1200 bp	**GS-21 1200 bp**	**–0.0312**	**0.008**	**27.1**
GS-19 200 bp	**GS-22 700 bp**	**–0.0312**	**0.008**	**27.1**
GS-19 200 bp	GS-38 550 bp	–0.0325	0.010	25.5
		2205		
GS-41 550 bp	GS-5 400 bp	0.0518	0.009	46.1
GS-38 550 bp	GS-53 850 bp	0.03	0.005	38.7
GS-12 1200 bp	**GS-22 700 bp**	**–0.02636**	**0.001**	**37.4**
GS-12 1200 bp	GS-19 200 bp	–0.025	0.003	32.8
GS-21 400 bp	GS-38 550 bp	0.0425	0.004	31.5
GS-19 200 bp	**GS-22 700 bp**	**–0.0245**	**0.004**	**31.3**
GS-12 1200 bp	**GS-21 1200 bp**	**–0.02267**	**0.009**	**26.1**

Table 19. Significant epistasis interactions affecting dihydrocarvone content at 0.01 level

Marker 1 symbol	Marker 2 symbol	Interaction effect	*P*-value	The proportion of total phenotypic variance explained by the markers interaction
		2004		
GS-38 700 bp	**GS-38 800 bp**	**0.35**	**<0.001**	**93.0**
GS-21 400 bp	**GS-38 800 bp**	**0.3437**	**<0.001**	**83.1**
GS-22 1300 bp	GS-38 800 bp	0.15	0.005	38.2
		2005		
GS-38 700 bp	GS-38 800 bp	0.305	<0.001	96.0
GS-21 400 bp	**GS-38 800 bp**	**0.2994**	**<0.001**	**83.6**
GS-38 600 bp	GS-41 550 bp	0.03333	0.004	53.0
GS-22 1300 bp	GS-38 800 bp	0.1438	0.001	48.0
GS-39 550 bp	GS-41 550 bp	0.04	0.006	46.6
GS-22 700 bp	**GS-38 800 bp**	**0.1135**	**0.007**	**35.5**
GS-21 1200 bp	GS-38 800 bp	0.1102	0.009	33.1
GS-3 1400 bp	GS-38 800 bp	0.1102	0.009	33.1
GS-21 400 bp	GS-38 550 bp	0.1355	0.009	25.7

Table 20. Significant epistasis interactions affecting dihydrocarveol content at 0.01 level

Marker 1 symbol	Marker 2 symbol	Interaction effect	*P*-value	The proportion of total phenotypic variance explained by the markers interaction
2004				
Lack of markers with interaction effects				
2005				
GS-22 700 bp	GS-5 350 bp	0.106	0.002	54.3
GS-5 350 bp	GS-5 1031 bp	0.103	0.002	54.3

Table 21. Significant epistasis interactions affecting carveol content at 0.01 level

Marker 1 symbol	Marker 2 symbol	Interaction effect	*P*-value	The proportion of total phenotypic variance explained by the markers interaction
2004				
GS-19 200 bp	GS-53 450 bp	0.0581	0.005	37.4
GS-1 400 bp	**GS-19 200 bp**	**0.0572**	**0.002**	**36.9**
GS-19 200 bp	GS-22 300 bp	0.0533	0.004	31.4
GS-1 400 bp	GS-21 900 bp	0.0635	0.004	31.2
2005				
GS-3 1450 bp	GS-43 700 bp	0.0463	0.010	49.0
GS-19 200 bp	GS-3 1450 bp	0.035	0.005	39.7
GS-21 1200 bp	GS-53 450 bp	0.0444	0.010	32.6
GS-3 1450 bp	GS-53 450 bp	0.0444	0.010	32.6
GS-1 400 bp	**GS-19 200 bp**	**0.0481**	**0.005**	**30.5**
GS-12 1200 bp	GS-21 1200 bp	0.0315	0.008	27.1

Table 22. Significant epistasis interactions affecting carvone content at 0.01 level

Marker 1 symbol	Marker 2 symbol	Interaction effect	*P*-value	The proportion of total phenotypic variance explained by the markers interaction
2004				
GS-22 700 bp	GS-5 350 bp	5.05	0.008	41.0
GS-5 350 bp	GS-5 1031 bp	5.05	0.008	41.0
GS-1 1500 bp	GS-3 1400 bp	4.65	0.003	33.2
GS-3 500 bp	**GS-3 1400 bp**	**4.56**	**0.006**	**29.1**
GS-22 1300 bp	GS-3 1400 bp	4.44	0.006	28.7
2005				
GS-3 500 bp	**GS-3 1400 bp**	**5.2**	**<0.001**	**50.0**
GS-3 1400 bp	GS-38 600 bp	4.01	0.003	47.4
GS-21 1200 bp	GS-3 1400 bp	4.47	0.001	37.4
GS-21 1200 bp	GS-3 500 bp	4.07	0.006	28.7

interaction (for diameter of main umbel) to 96.0% for GS-38 700 bp by GS-38 800 bp interaction for dihydrocarvone content in 2005 (Tab. 19).

Seventeen marker-by-marker interactions performed significant epistatic effect on the least one tested trait in both years of experiment (Tabs. 1, 7, 8, 17, 18, 19, 21 and 22). Most of all, six marker-by-marker interactions, were significant for diameter of primary umbel. The agreement of signs of regression coefficients in both years study was observed for all 17 cases. Fifty three marker-by-marker interactions significantly determined at least two traits, while 14 traits had no marker-by-marker interactions, which would determined them in both years.

DISCUSSION

In this study, the results of estimation of marker-by-marker interactions effects of 22 quantitative traits of caraway, using selected populations and cultivars, were demonstrated. Our study allowed to obtain information about the epistatic gene action by a model-based analysis of first-order marker–marker interaction effects. Ma et al. [2007], Imtiaz et al. [2008] and Peng et al. [2011] used a similar statistical approach to study the epistatic effects. More often, the molecular genetics tools are used in breeding programs and selection. Molecular markers are an effective and powerful method for determining the interspecific genetic variation. Random amplified polymorphic DNA (RAPD) has the capacity to generate markers that span the genome without prior knowledge of their sequence [Bocianowski and Seidler-Łożykowska 2012]. RAPD is kind of molecular marker based on PCR. This technique has been applied to a wide range of organisms. There are some medicinal plants, variability of which was tested by polymorphic markers: artichoke, chamomile, marjoram, peppermint, quinoa, sweet basil [Arnholdt-Schmitt 2002, Klöcke et al. 2002, Messmer et al. 2002, Wetzel et al. 2002, Liersch et al. 2013, Skuza et al. 2013, Kiełtyka-Dadasiewicz et al. 2017, Lema-Rumińska et al. 2018].

Recently, several experiments using molecular markers indicated that marker-by-marker interactions might play an important role in controlling the expression of quantitative traits [Zhao and Meng 2003, Bocianowski 2012a, Rakoczy-Trojanowska et al. 2017]. Several marker-by-marker interactions associated with the content of essential oil and oil components content as well as morphological traits were found using multiple regression analysis. Some of these interactions influenced on more than two examined traits, which can be well understood considering the polygenic background of the investigated traits. In our research, 17 RAPD marker-by-marker interactions, which were repeated in both years of analysis, are the most promising markers for future breeding program, because they make possible selection with regard on many traits simultaneously.

CONCLUSIONS

RAPD markers are able to produce many markers in a single PCR without prior sequence knowledge of the genomes of interest. These techniques are powerful and able to amplify many loci within a single reaction and are useful for assessing the genetic diversity or in the creation of genetic maps. Molecular maps are being continuously enriched by new markers and are being increasingly used in genetic studies. The relationships between marker-by-marker interactions and phenotypic traits such as plant height, thousand seed weight, essential oil content, fruit weight per plant, carvone content may be important diagnostic tool in breeding selection of caraway. The interacted markers explaining the highest percentage of phenotypic variation (from 25.3% to 96.0%) in quantitative traits could be transformed into codominant specific markers, such as sequence characterized amplified regions (SCARs). The molecular markers defining epistatic interaction will be effective tools for selection and genetic improvement of caraway in the future.

ACKNOWLEDGEMENTS

The research was financially supported by Ministry of Science and Higher Education, found No. N310 029 32/1557.

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