Industrial Crops and Products 36 (2012) 135–139

Contents lists available at SciVerse ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

The relationship between RAPD markers and quantitative traits of caraway (*Carum carvi* L.)

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ARTICLE INFO

Article history:
Received 20 December 2010
Received in revised form 26 July 2011
Accepted 21 August 2011
Available online 29 September 2011

Keywords:
Caraway (*Carum carvi* L.)
Molecular markers
RAPD-PCR
Morphological traits

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. In this paper the relationships between RAPD markers and 22 quantitative traits of caraway (*Carum carvi* L.) were analyzed. Significant associations of 44 markers with at least one trait in 2004 as well as 48 in 2005 were found on the basis of regression analysis. The proportion of total phenotypic variances of individual trait explained by the marker ranged from 13.8% to 47.6%.

1. Introduction

Caraway (*Carum carvi* L.) is widely cultivated all over the world and its fruit (*Carvi fructus*), as a raw material, is mentioned in many European and the U.S. pharmacopoeias. Caraway fruits affect on digestive system by improving digestion and appetite, have carminative and spasmolytic effects and promote milk secretion (Foster and Duke, 1990). Ground caraway fruits are used as a component of teas and herbal mixtures. Caraway essential oil has medium antibacterial activity (Sadowska and Obidoska, 1998; Toxopeus and Bouwmeester, 1993) by inhibiting development of the following bacteria: *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Vibrio cholerae*, *Mycobacterium tuberculosis*, and also growth of yeast and moulds (Youself and Tawil, 1980). Antibacterial properties of caraway oil are used in medicine, food conservation and cosmetic production (Dachler, 1998). Zheng et al. (1992) mentioned about anticancer activity of caraway oil.

There is a great variability among (and in) different caraway populations and cultivars. The variability is estimated based on morphological and yielding traits, essential oil content and its composition (Seidler-Łożykowska and Bocianowski, 2012). Most of the valuable caraway traits is genetically determined (Heine, 1998; Németh, 1998; Roon and Bleijenberg, 1964; Šmirous and Kocourková, 2006; Toxopeus et al., 1995; Toxopeus and Lubberts, 1998). The main aims of caraway breeding programs are raw material yield and quality, also better resistance to abiotic and biotic stress.

There are many alternative analytical methods used to determine content of active substances: image analysis (Franke et al., 1996), Supercritical Fluid Extraction (SFE) (Sedlàkova et al., 2003), near infrared spectroscopy (NIRS) (Fehrmann et al., 1996) or FT-Raman spectroscopy (Seidler-Łożykowska et al., 2010).

More often, the molecular genetics tools are used in breeding programs. Random amplified polymorphic DNA (RAPD) is an effective and powerful method for determining interspecific genetic variation. RAPD has the capacity to generate markers that span the genome without prior knowledge of their sequence (Irzykowska and Bocianowski, 2008). This technique has been applied to a wide range of organisms. There are some medicinal plants which variability was tested by polymorphic markers: St. John's wort, marjoram, artichoke, sweet basil, chamomile (Arnholdt-Schmitt, 2002; Eckelmann, 2002; Klöcke et al., 2002; Messmer et al., 2002; Osińska, 2004; Wetzel et al., 2002). There is no bibliography regarding molecular markers analysis of caraway, although there are a lot of papers about other species of Apiaceae family (*Daucus carota* L.) (Barański et al., 2004; Bradeen et al., 2002; Grzebelus et al., 2001, 2002).

The aim of this research was finding the molecular markers associated with 22 quantitative traits of caraway. Observations of all traits of the selected populations and cultivars were compared with their DNA profiles to see if any relationships existed.

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doi:10.1016/j.indcrop.2011.08.019

2. Materials and methods

2.1. 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 new breeding strains, which were obtained in the Institute of Medicinal Plants of Poznań. Strains of cultivar 'Kończewicki' originated from the maintenance breeding which is done in Institute.

2.2. 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 greenhouse to obtain 5–8 leaves plantlets, which were planted on the field in the beginning of May. The collection was established with four repetitions and number of plants in the genotypes oscillated from 25 to 100.

2.3. Morphological traits

Morphological measurements were done in 2005 and 2006 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.

2.4. Estimation of content of essential oil and its composition

The content of essential oil was estimated after hydrodistillation of caraway fruit in Dering's apparatus following the methods recommended by European Pharmacopoeia (2008).

2.5. 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, 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.

2.6. 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).

Table 1
RAPD primers used in PCR reaction of caraway collection.

Number	Primer	Sequence 5′–3′
1	GS-1	GTGCCTAACG
2	GS-3	CAGCTCACGA
3	GS-4	CTACTGCCGT
4	GS-5	ACGGCGTATG
5	GS-8	AAAGCTGCGG
6	GS-9	TCATCCGAGG
7	GS-10	TGCCCAGCCT
8	GS-12	TCGGCGATAG
9	GS-13	AGCCAGCGAA
10	GS-14	TGCTCTGCCC
11	GS-15	GGTGATCAGG
12	GS-16	TGCTGCAGGT
13	GS-17	CCGGACACGA
14	GS-18	GGACTGGAGT
15	GS-19	GTCGCGGTCA
16	GS-21	GGTGACGCAA
17	GS-22	GTCTGACGGT
18	GS-30	CCTTGACGCA
19	GS-31	CTCTCCGCCA
20	GS-32	AGACGTCCAC
21	GS-33	GGTGACGCAG
22	GS-34	CTATGCCGAC
23	GS-35	CCTGCTCATC
24	GS-36	CTGCGCTGGA
25	GS-37	GAGGTCCACA
26	GS-38	CAGGGGACGA
27	GS-39	TGGTCCAGCC
28	GS-41	GTGGCTTGGA
29	GS-43	GTGGCCGATG
30	GS-53	ACGCCCAGAG

2.7. 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 containing 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 (Table 1). Amplification was carried out in a PTC-200 thermocycler (MJ Research) using the following programme: 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.

2.8. 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 RulerTH 100 bp DNA Ladder Plus (Fermentas, GmBH, St Leon-Rot, Germany) was used as a molecular size standard for PCR products.

2.9. 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.

The association between molecular markers and observed traits of 22 genotypes was estimated using regression analysis. The molecular marker observations were treated as a independent