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Rapid Genetic Assessment of Carrot Varieties Based on AFLP Analysis

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Abstract: It is necessary to regard the biodiversity of carrot as a genetic source of useful and indispensable components for the human diet. Amplified fragment length polymorphism (AFLP) markers were used to discriminate eight carrot genotypes with different root colors. With the combination of enzymes *Tru*9I, *Pst*I, and three sets of primer pairs corresponding to adapters joint to the restricted sites, 92 loci were produced, including 60 polymorphic ones. Each of the three primer sets showed high efficiency, according to estimations of PIC (0.34, 0.34, and 0.41), D (0.36, 0.67, and 0.67), Rp (5.5, 11.3, and 15), and H_E (0.32, 0.49, and 0.49). The genetic distances were calculated using values of Nei's coefficient. The most genetically similar were Chantenay Coeur Rouge and Colmar a Coeur Rouge at a distance of 0.12, whereas the most distant were Saint Valery and Purple Dragon at the highest distance of 0.34. Confirming its genetic identity, Purple Dragon and Gelber Goliath with purple and yellow roots were the most detached varieties from others at distances of 0.23–0.34 and 0.23–0.28, respectively. Male sterile Berlicum breeding accessions were well distinct from other orange-colored varieties at the highest distance of 0.30 from Deep Purple F1. Slight modifications including the facilitation of gel staining enables the wide use of the AFLP method for genetic diversity assessment in carrot breeding accessions.

Keywords: AFLP markers; carrot; genetic diversity; polymorphism; *Daucus carota* L.; polymorphism

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1. Introduction

The quality of humans' vegetable diet directly depends on the use of different genotypes carrying helpful properties. The carrot (*Daucus carota* L. subsp. *sativus* (Hoffm.) Schübl. & G. Martens) is a rich source of carotene that is known for powerful antioxidant activity. The introduction of valuable genotypes is one of the main tasks when realizing a breeding program. Genetically diverse breeding accessions can be regarded as new sources of useful biochemical content that crucially improve the quality of the product. The goals of carrot breeding require studying the biodiversity of handled accessions. The first research work on carrot variation revealed significant genetic diversity between wild and cultivated accessions and was performed on the basis of AFLP analysis [1]. Much deeper genotyping with the application of diversity arrays technology (DArT) defined the genetic features in the process of carrot domestication [2]. Later, owing to improved genomic data, the first simple sequence repeats (SSR markers) were developed and a high level of polymorphism among inbred lines was found with them [3]. Finding, and the further design of, microsatellite markers for carrot enables expanding the study on different accumulation levels of carotene content in the roots and biodiversity among cultivated accessions [4,5]. The use of genomic libraries enriched with SSR repeats with further cloning in bacterial artificial chromosome (BAC) vectors led to the construction of additional 300 SSR markers [6]. The sequence analysis of the transcriptome allowed developing a set of 114 SSR markers using in silico instruments [7]. Similarly, 4000 single-nucleotide polymorphisms (SNPs) that could be effectively used for genetic diversity studies and gene mapping have found in

obtained sequenced data [8]. Western carrot cultivars were characterized and the genetic structure was assessed with 2354 SNP markers and 93 DcS-ILP markers [9].

As has been mentioned, significant study of genetic variation in carrot has been carried out, but the protocols for SSR and SNP markers occasionally require the time- and cost-intensive preparation of choosing markers and conducting many rounds of PCRs to produce a sufficient number of countable polymorphic fragments. AFLP markers, regardless of the laborious protocol, remain indispensable for analyzing the breeding accessions of any crops because of their versatility and high productivity compared to other multilocus DNA fingerprinting techniques that do not require any specific genome sequence information from the plant object. Thus, the advantage of AFLP-PCR is that hundreds of specific and reproducible markers derived from both polymorphisms of restriction and primer annealing sites can be generated quite rapidly to characterize the entire plant genotype. In our research, the slight modifications simplify the procedure of the AFLP technique. Moreover, there have not been enough publications concerning the studies of genetic variation among breeding accessions in carrot. Hence, highly effective AFLP markers are proved to be appropriate for biodiversity estimation. The aim of the study is to analyze genetic distances among different carrot genotypes based on the modified AFLP protocol.

2. Materials and Methods

Five varieties, hybrid F1, and two breeding accessions with male sterility of the Berlicum type of carrot with different root colors were taken for the study (Table 1). Young leaves from five plants of each genotype grown from seeds in the open-field experimental plot at FSBSI FSVC were collected. DNA extraction was performed from fresh leaf material according to the manufacturer's instruction for the DNA extraction kit Sorb-GMO-B (Syntol, Moscow, Russia). The purity and concentration of the extracted DNA were evaluated using a Smart Spec Plus spectrophotometer (BioRad, Hercules, CA, USA).

Table 1. Carrot genotypes selected for the study.

Accession	Flowers	Root Shape	Root Color	Origin
Berlicum	Sterile	Berlicum	Orange	FSBSI FSVC
Berlicum	Sterile	Berlicum	Orange	FSBSI FSVC
Chantenay Coeur Rouge	Fertile	Chantenay	Orange	Graines Baumaux
Colmar a Coeur Rouge	Fertile	Flakkee	Orange	Graines Baumaux
Purple Dragon	Fertile	Danvers	Purple	Graines Baumaux
Gelber Goliath	Fertile/Sterile	Half-long	Yellow	Graines Baumaux
Saint Valery	Fertile	Flakkee	Orange	Graines Baumaux
Deep Purple F1	Sterile	Imperator	Purple	Bejo

AFLP analysis was carried out on the basis of the main protocol developed by Vos et al. (1995) [10] with a slight modification in the staining procedure. Preamplification was carried out using primers that were complimentary to the adapters adjoined to the restriction sites of the corresponding enzymes *Tru*9I and *Pst*I (SibEnzyme Ltd., Novosibirsk, Russia). The PCR products obtained after preamplification served as a template for specific amplification using three combinations of specific primers, P-AGA/T-CAG, P-AAT/T-CAA, and P-ATA/M-CTC. All amplifications were performed in a C1000 Touch Thermal Cycler (BioRad, USA). AFLP fragments were separated using 6% polyacrylamide gel electrophoresis (PAGE) in the Sequi-Gen GT nucleic acid sequencing cell (BioRad, USA) and then stained with SYBR™ Safe DNA Gel Stain (Invitrogen, Waltham, MA, USA). Digital documentation was carried out in the ChemiDoc XRS+ Gel Imaging System (BioRad, USA); fragments that were analyzed were compared with GeneRuler100 bp plus DNA ladder (Thermo Fisher Scientific, Waltham, MA, USA). Digital images of electropherograms were processed with the Image Lab 3.0 software (BioRad, USA).

The estimation of primer pair efficiency was based on such parameters as seven polymorphism indices: heterozygosity index (H_E), polymorphism information content

(PIC), discriminating power (D), effective multiplex ratio (E), marker index (MI), arithmetic mean heterozygosity (H_{avp}), and resolving power (Rp), which were calculated using the iMEC software [11]. Fragment counting was carried out as dominant markers, where "1" means the product is present and "0" means the product is absent. The binary matrix was constructed, where genetic distances were calculated using Nei's coefficient [12]. A graphic representation of the genetic relationship was performed using the method of principal component analysis (PCA) in GenAlEx 6.5 software [13].

3. Results

With three primer combinations, a high level of genetic variation between the studied accessions was obtained, where 60 polymorphic loci out of 92 common loci were generated. On average, 30 AFLP bands representing fragments of less than 500 bp were generated per primer pair. The highest number of bands was produced with the pair P-AAT/M-CAA (Figure 1).

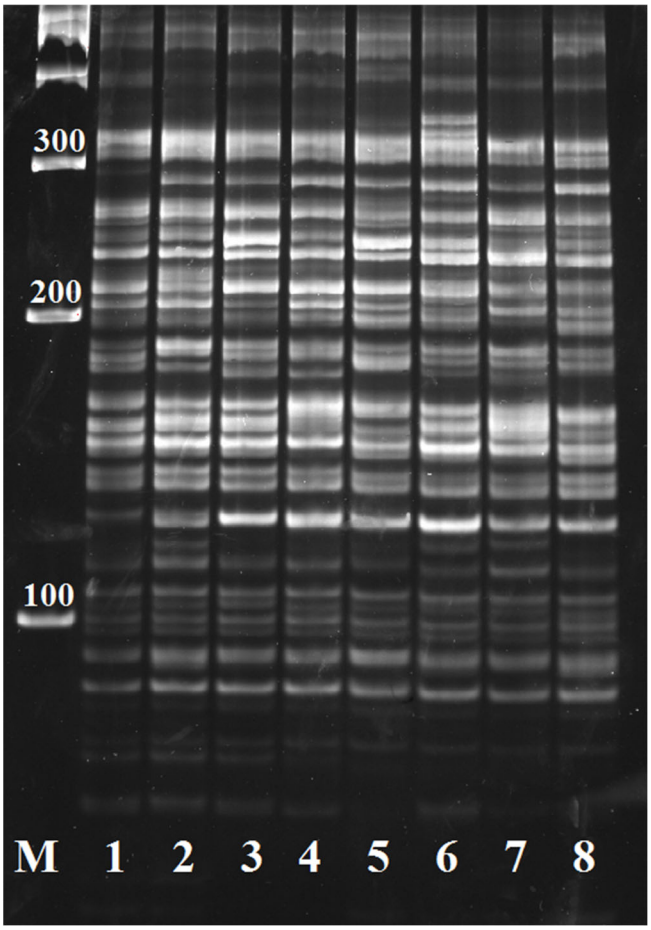

Figure 1. Electropherogram of AFLP fragments produced through the analysis of eight carrot accessions with the primer combination P-AAT/M-CAA showing the highest PIC value (0.41); Berlicum St (1);—Berlicum St (2); Chantenay Coeur Rouge (3); Colmar a Coeur Rouge (4); Purple Dragon (5);—Gelber Goliath (6);—Saint Valery (7);—Deep Purple F1 (8); GeneRuler100 bp plus DNA ladder (M).

Among the calculated parameters, the highest PIC (0.41) value was observed with the primer combination P-AAT/M-CAA, but the discriminating power (D) and resolving power (Rp) values were higher with primer combinations P-AGA/M-CAG (0.66, 15) and P-ATA/M-CTC (0.67, 11.3), respectively (Table 2).

Table 2. Primer pair indices calculated on the basis genetic variation revealed in eight carrot genotypes.

Primer Pair [1]	H_E	PIC	E	H_{avp}	MI	D	Rp
P-AGA/M-CAG	0.49	0.34	17.5	0.002	0.035	0.66	15
P-ATA/M-CTC	0.49	0.34	18.9	0.002	0.035	0.67	11.3
P-AAT/T-CAA	0.32	0.41	23.3	0.001	0.032	0.36	5.5

[1] H_E—expected heterozygosity; PIC—polymorphism information content; E—effective multiplex ratio; H_{avp}—mean heterozygosity; MI—marker index; D—discriminating power; Rp—resolving power.

After binary matrix analysis, the high level of genetic variation according to the values of Nei's indices between 0.09 and 0.34 was revealed. Thus, the highest Nei's coefficient value (0.34) confirmed the greatest genetic distance between Saint Valery and Purple Dragon with purple-colored roots, whereas the closest accessions were Chantenay Coeur Rouge and Colmar a Coeur Rouge according to the Nei's coefficient (0.12) (Table 3). Judging by the close genetic similarity between the sterile Deep Purple F1 with purple-colored roots and the variety Colmar a Coeur Rouge, it can be supposed that the breeding line originated from orange carrot could have been taken as a parental component for the hybrid development. The yellow-colored variety Gelber Goliath was also genetically distant from the main group of accessions. Two male sterile Berlicum accessions were placed from other genotypes at Nei's distances of 0.21 to 0.31 (Figure 2).

Table 3. Matrix of Nei's genetic distance coefficients between studied carrot genotypes.

Accession [1]	1	2	3	4	5	6	7
2	0.09	0					
3	0.26	0.27	0				
4	0.24	0.27	0.12	0			
5	0.31	0.30	0.27	0.31	0		
6	0.28	0.27	0.22	0.26	0.23	0	
7	0.21	0.18	0.21	0.17	0.34	0.27	0
8	0.30	0.29	0.16	0.20	0.23	0.26	0.23

[1] Berlicum St (1);—Berlicum St (2); Chantenay Coeur Rouge (3); Colmar a Coeur Rouge (4); Purple Dragon (5);—Gelber Goliath (6);—Saint Valery (7);—Deep Purple F1 (8).

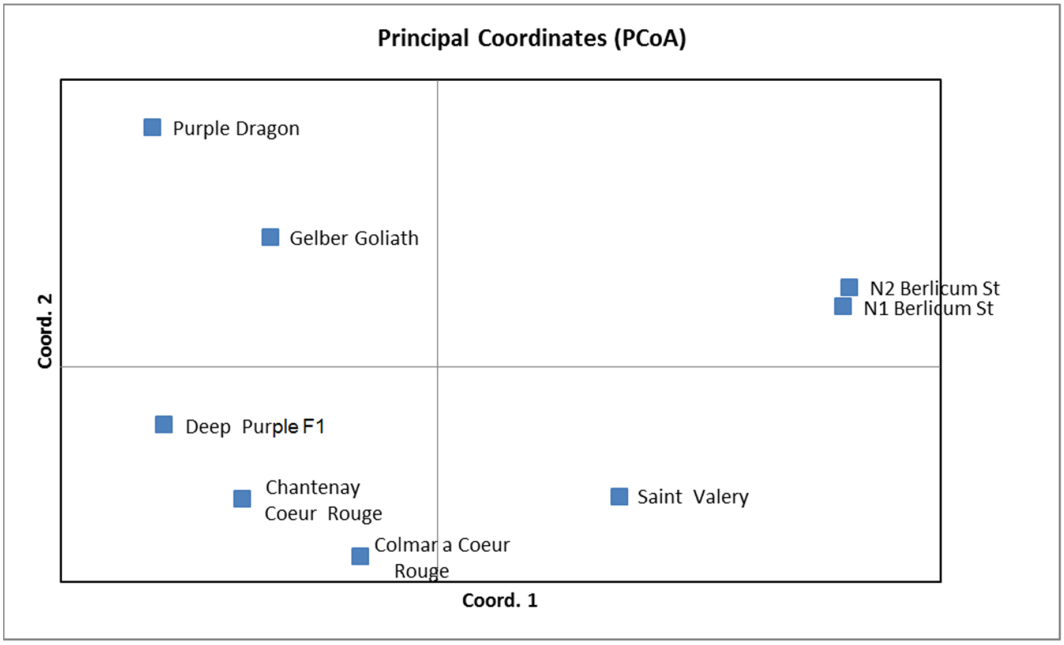

Figure 2. Representation of genetic distance values using principal component analysis (PCA) among eight carrot genotypes.

As was shown, the most distant in accordance with Nei's indices (0.23–0.34) from other accessions was the heirloom variety Purple Dragon. The nutritional value of its roots consists of an accumulation of carotenes (α and β-carotene) in the core together with anthocyanins that cause the exhibition of the typical purple color [14]. It is known that the variety was developed on the basis of Afghan genotypes being the source of the purple root color. The variety Gelber Goliath with typical yellow roots was also genetically distant from other accessions with Nei's distances from 0.22 to 0.27. The yellow-colored carrots descended from such regions of origin as Afghanistan, Pakistan, and Iran [15].

4. Discussion

It is worth mentioning that using the primer pairs with the corresponding enzymes was effective, not only for the studied carrot genotypes, but also for garlic accessions, as was reported earlier [16]. The combination of enzymes *Pst*I/*Mse*I for AFLP protocols was proved to reveal more polymorphism in plant objects, as was demonstrated in sorghum and corn [17]. In the study, a significant level of polymorphism (65%) was achieved that was comparable with that previously produced with highly polymorphic SSR markers in carrot [6]. Some primer evaluation parameters were shown to be higher than the average values, such as PIC and H_E indices that were over 0.3, that confirms the sufficient discriminatory ability of markers. On the basis of the observed AFLP data, the carrot accessions were discriminated in agreement with their origin. In this case, AFLPs confirmed their productivity and effectiveness in the discrimination of carrot genotypes and continued to be the "golden standard" among multilocus-dominant-derived DNA techniques. In different breeding programs for quality characteristics, yielding, and resistance despite the broad genetic variation in genotypes, the genetic uniformity should be assessed in populations and inbred lines required for the development of heterotic hybrids; for this reason, AFLPs can be regarded as a reliable and sufficient tool [18]. In our research, a rapid protocol enabled reducing the number of manipulations, where gel staining was nearly performed as quickly as agarose gels. Previously, to simplify the procedure of the AFLP method and diminish the time of analysis, 3% Synergel™ agarose gels of high resolution—instead of laborious PAGE silver staining—were used to separate fragments that were then simply stained with ethidium bromide [19]. However, it remains difficult to achieve quality and sharp bands in gels through the separation of PCR fragments only in agarose, particularly among those fragments that are shorter than 500 bp. PAGE separation remains very versatile together with the use of sensible SYBR™ Safe DNA Gel Stain for long AFLP gels. Taking a set of endonucleases combined with corresponding primer combinations can be recommended for the assessment of genetic variation among different breeding accessions of carrot. The practical application of these markers, nevertheless, remains very effective and less demanding than many other DNA techniques.

5. Conclusions

The obtained AFLP data confirmed the origin of carrot accessions, where genotypes with purple and yellow root color were genetically distant from ones with orange-colored roots. Thereafter, a sufficient difference was also discovered between genetically closer genotypes with orange roots: Chantenay Coeur Rouge, Colmar a Coeur Rouge and Berlicum type accessions. All 3 primer combinations, according to the calculated parameters, showed efficiency revealing polymorphism among 8 accessions, where 30 fragments per primer pair were produced on average. The slight modifications of the AFLP method, concerning the gel-staining procedure, make it recommended for biodiversity study and polymorphism estimation in different breeding material of carrot.

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