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RAPD technique application for intraline evaluation of androgenic carrot plants

Mirosława Staniaszek, Hanna Habdas

Department of Genetic, Breeding and Biotechnology
Research Institute of Vegetable Crops
Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland
e-mail: mstan@inwarz.skierniewice.pl

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ABSTRACT

The aim of this investigation was the evaluation of intraline uniformity of androgenic carrot plants, obtained by the use of anther culture technique. The material studied comprised plants of 30 lines/clones of R_1 generation. The evaluation was carried out using 10 RAPD primers: OPA02, OPA04, OPA07, OPA11, OPB04, OPB11, OPB12, OPB15, OPB17 and OPB18. The RAPD analysis demonstrated significant plant variation within each of the lines studied. This was demonstrated by the presence of quantitative differences in the amplification profiles obtained for individual plants within each line analysed. The results obtained showed that the lines tested were not homozygous and were therefore not DH lines.

INTRODUCTION

In recent years, most carrot cultivars are F_1 hybrids. The basic requirement for the production of hybrid cultivars is the presence of homozygous lines as parental components. Obtaining completely homozygous lines by traditional breeding methods is a strenuous and time consuming process. The breeding cycle for new F_1 hybrids in carrots can be considerably shortened by using androgenesis to yield haploid plants and subsequently doubled haploid lines (Niemirowicz-Szczytt 1997, Szarejko 1997).

The work carried out at Vegetable Crops Research Institute in Skierniewice on the use of anther cultures to yield DH lines of carrots resulted in the production of populations of R_0 and R_1 generations of androgenic carrots plants (Górecka et al. 1999, 2005; Kozik et al. 2002, 2004). The R_1 generation obtained, evaluated on the basis of their morphological traits (Kozik et al. 2002, 2004) should also be evaluated for their homozygosity.

Currently, DNA based methods are increasingly being used for genetic studies in plants. In recent years, the random amplification of polymorphic DNA (RAPD) method based on polymerase chain reactions (PCR) has been one of the most commonly used in studying plant genomes (Williams et al. 1990). In its standard version, a particular feature of this method is the amplification of DNA by the use of a single 10 nucleotide primer of arbitrary sequence, rather than a pair of different primers, as in the classic version of PCR reaction. The number of amplified DNA fragments and their electrophoretic mobility can be different for the genotypes being compared due to their diverse nucleotide sequences or differentiation in their secondary DNA structures (Marczewski 1999). Identification of RAPD markers is unaffected by environmental influences or the stage of development reached by the plant; in many cases, this makes RAPD markers far more useful than morphological traits (Staub et al. 1996, Kumar 1999). The RAPD method has been applied, amongst others, to the identification and mapping of many characteristics caused by single or multiple genes. Instances include studies on the genes responsible for the resistance of carrots to nematodes (Schulz et al. 1994, Simon et al. 1997, Westphal and Wricke 1997), on the Y_2 gene controlling accumulation of carotenoids in carrot roots (Bradeen et al. 1997) and on the restorer gene (Westphal and Wricke 1997). Grzebelus et al. (1997, 2001) and Briard et al. (2001) used RAPD markers for identification and genetic diversity of carrot cultivars and breeding lines. The RAPD technique has also been applied in the evaluation of uniformity in androgenic cauliflower (Stipic and Campion 1997) and cabbage (Kamiński et al. 2003).

The purpose of the present study was to evaluate intraline uniformity of R_1 generation derived from anther culture by using RAPD technique.

MATERIAL AND METHODS

Thirty lines/clones of R_1 generation obtained from four donor cultivars: 'HCM', 'CxC9900 F_1', 'Perfekcja', 'Narbonne F_1' were used in the study. R_1 generation lines/clones were derived by self pollination of R_0 androgenic plants (Górecka et al. 1999, Kozik et al. 2002, 2004). The plant material was obtained in the years 2000 and 2002 (Nowakowska et al. 2006). Plants were grown in field. Young and fresh leaves from each of 10, 6 or 5 plants per line were harvested and stored at -70°C until DNA extraction (Table 1). The RAPD analyses were carried out from 2003 to 2005. Total genomic DNA was isolated individually from plants of each line/clone, according to the procedure of Dellaporta et al. (1983). DNA concentration and purity was determined spectrophotometrically and by visualization on 0.8% agarose gel. PCR reaction was performed in 20 µl volume containing 10 mM Tris-HCL pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 0.1 mM of each deoxynucleotide, 0.25 µM of primer, 0.5 unit of Taq DNA Polymerase (Invitrogen), 0.001% gelatin and 30 ng of DNA. The amplification reaction was carried out in a MJ Research PTC-200 and GeneAmp 9700 (Applied Biosystems) thermal cycler. The cycle parameters were: 94°C for 1 min followed by 45 cycles of 15 s at 92°C, 25 s at 36°C, 74 s at 72°C, and the final extension time of 5 min at 72°C.

DNA amplification was performed with the use of 40 arbitrary 10-mer primers from Operon Technologies Inc. (Alameda, CA), kits OPA – OPB. Amplification products were analysed by electrophoresis in 1.4% agarose gel after staining with ethidium bromide. Each amplification was repeated three times and only strong reproducible bands were considered in this study. Intraline uniformity within each line/clone was evaluated by comparing RAPD profiles of the single plants.

RESULTS AND DISCUSSION

From 40 RAPD primers tested, 10 primers were chosen for the evaluation of intraline uniformity: OPA02, OPA04, OPA07, OPA11, OPB04, OPB11, OPB12, OPB15, OPB17 and OPB18. These primers generated polymorphic bands between individual plants within each of the line studied. Each of ten RAPD primers amplified from 2 to 6 bands, ranging in size from 300 to 2500 bp (base pair), depending on the line tested. Depending on the primer used and the line being evaluated, 1 to 3 polymorphic DNA fragments of lengths 300 to 1700 bp were identified. For each line/clone evaluated, the RAPD primers used and the number and lengths of the polymorphic DNA fragments obtained have been tabulated (Tables 2a and 2b). Clear differences in the amplification profiles for individual plants within each line demonstrate the lack of uniformity within those lines (Figs. 1 and 2).

Table 1. List of analysed carrot lines of R_1 generations

Donor cultivar	Line symbol	Number of plants tested
'HCM'	AA6	10
	AA43	10
	AA50	10
	AA59	5
	AA60	5
	AA62	10
	AA78	10
	AA90	10
	AA91	10
	AA92	10
	AA94	10
	AA95	10
	AA97	10
	AA119	10
'C x C9900 F_1'	BB1	10
	BB2	10
	BB3	10
	BB4	10
	BB5	10
	BB6	10
	BB11	10
	BB12	10
'Perfekcja'	CC4	10
	CC7	6
	CC6	5
	CC8	5
'Narbonne F_1'	EE5	5
	EE6	10
	EE7	5
	EE19	5
Total	30	261

Table 2a. The RAPD primers used for intraline evaluation, the number and size of polymorphic bands

Line symbol	Primer	Number of bands in the RAPD profiles	Number of polymorphic bands	Size of polymorphic bands (bp)
AA50	OPA02	4	1	1000
	OPB04	4	3	850, 1000, 1700
	OPB11	3	1	900
AA62	OPA11	4	2	650,1000
	OPB11	4	2	650, 900
AA6	OPA04	4	3	700, 900, 930
	OPB18	6	1	900
AA43	OPB11	4	2	700, 950
	OPB18	2	1	800
AA59	OPA04	4	2	600, 700
	OPB11	3	1	900
AA60	OPA04	2	1	450
	OPB18	3	1	800
AA91	OPA04	3	1	450
	OPB11	2	1	680
AA92	OPA04	2	1	450
	OPB11	3	1	680
AA95	OPA07	4	2	1150, 1200
	OPB11	3	1	680
AA97	OPA04	2	1	450
	OPB18	2	1	800
AA78	OPB04	3	2	1000, 1700
	OPB11	3	2	650,900
AA90	OPA04	3	2	450, 1000
	OPA07	4	2	1100, 1800
AA94	OPA07	2	1	1100
	OPB18	2	1	800
AA119	OPB04	3	2	1000, 1700
	OPB11	5	2	650, 900

Lines of R_1 generation obtained after selfing R_0 plants are expected to be totally homozygous, which means that RAPD profiles of all plants from the same line should be identical. The absence of genetic uniformity of the R_1 generation plants within each line studied casts doubt on their androgenic origin. The obtained results demonstrate that the lines analysed are not homozygous, so they are not DH lines. Therefore, it can be assumed that R_0 generation plants most likely have originated from somatic anther cells.

Table 2b. The RAPD primers used for intraline evaluation, the number and size of polymorphic bands

Line symbol	Primer	Number of bands in the RAPD profiles	Number of polymorphic bands	Size of polymorphic bands (bp)
BB1	OPA04	4	1	800
	OPB11	3	1	1100
	OPB17	3	1	700
	OPB18	4	1	1200
BB2	OPA04	3	1	450
	OPB11	4	1	900
	OPB12	4	1	1400
BB3	OPA04	4	3	700, 800, 900
	OPB11	3	1	1100
	OPB17	3	1	700
	OPB18	3	1	1200
BB12	OPA04	3	2	700, 800
	OPB12	2	1	900
	OPB17	3	1	700
	OPB18	3	1	1200
BB4	OPA04	3	2	450, 750
	OPB11	3	1	950
BB5	OPA04	4	1	550
	OPB18	3	1	1200
BB6	OPA04	4	2	700, 800
	OPB18	3	1	1200
BB11	OPB11	3	2	900, 1200
CC4	OPA04	3	1	700
	OPA07	2	1	1200
	OPB04	3	2	1000, 1700
	OPB15	2	1	700
	OPB18	3	1	800
CC7	OPA07	2	2	950, 1150
	OPB04	2	1	1000
CC8	OPA04	3	1	700
	OPB04	2	1	1000
CC6	OPB11	5	3	900, 1000, 1500
EE5	OPA04	3	3	700, 800, 900
EE6	OPA04	3	1	700
	OPB11	4	2	800, 950
EE7	OPA07	4	1	1200
EE19	OPA04	3	2	450, 700

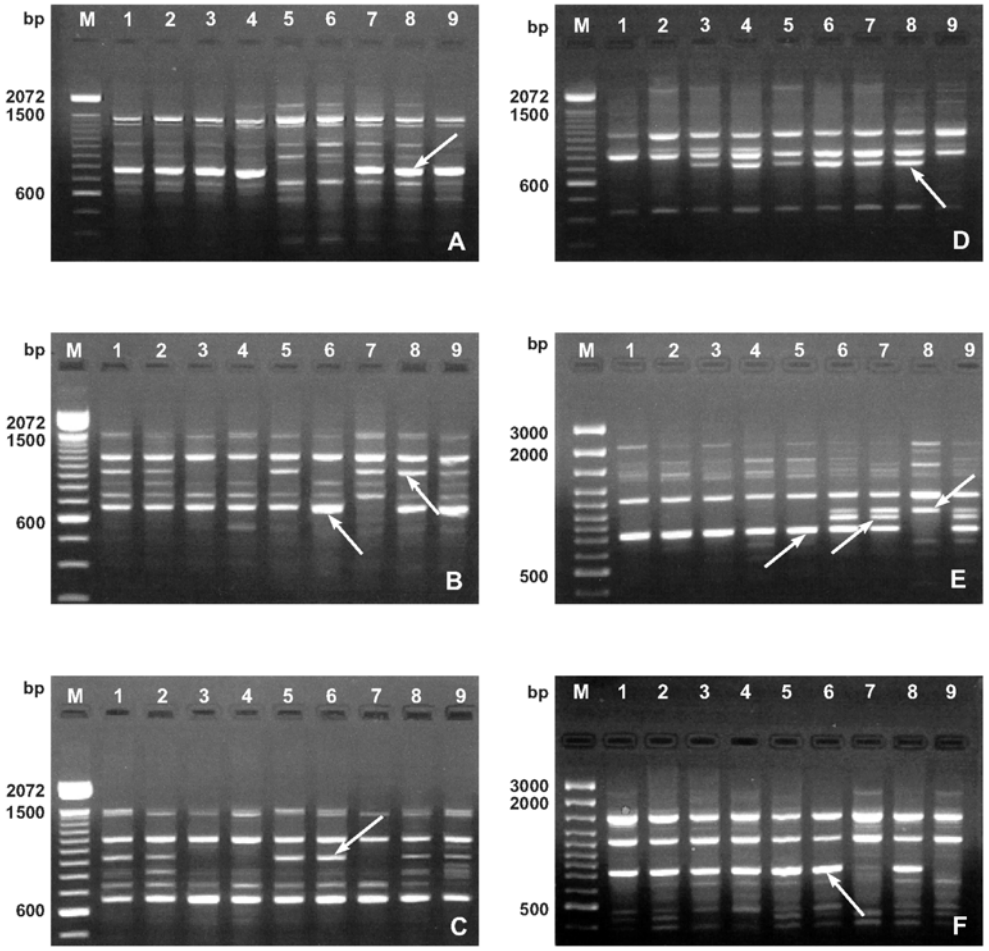

Fig. 1. RAPD profiles of individual plants R_1 generation of six lines/clones: A – line AA94, primer OPB18; B – line AA78, primer OPB11; C – line AA50, primer OPB11; D – line CC4, primer OPA04; E – line AA6, primer OPA04; F – line EE6, primer OPA04.
Lines 1, 2, 3 .....individual plants; M – 100 bp DNA ladder. ← polymorphic bands

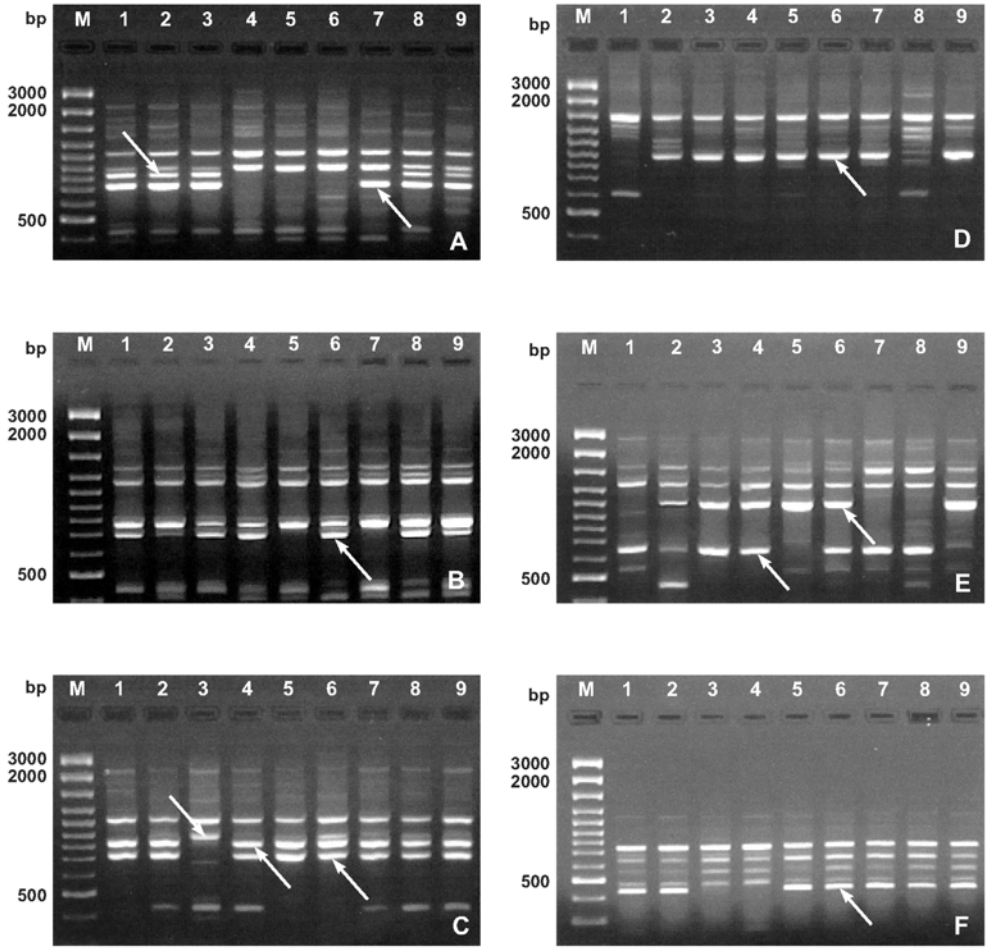

Fig. 2. RAPD profiles of individual plants R_1 generation of six lines/clones: A – line BB6, primer OPA04; B – line BB1, primer OPB17; C – line BB3, primer OPA04; D – line BB12, primer OPB12; E – line AA62, primer OPA11; F – line AA91, primer OPA04.
Lines 1, 2, 3 .....individual plants; M – 100 bp DNA ladder. ← polymorphic bands

In the present study, the RAPD technique was used to evaluate the uniformity of R_1 generation carrot plants. The RAPD technique is particularly useful for genetic testing on plants due to its relatively low cost, simplicity and the possibility of the simultaneous study of a large number of loci. The results obtained by Stipic and Campion (1997) and Kamiński et al. (2003) have confirmed that, for cauliflower and white cabbage, RAPD analysis of DH lines of R_1 generations derived from anther cultures is an effective mean of evaluating homozygosity in breeding materials and as such it should usefully complement other evaluation

methods hitherto used. An essential advantage of the method is the possibility for studying DNA polymorphism at any stage of the plant development, uninfluenced by environmental conditions. The technique used so far for assessing the uniformity of a DH line – making use solely of selected morphological traits of the leaf (length, width, and length of stalk) and the root (length, width, colour) – is not always reliable. Cultivation conditions can markedly influence the development of those characteristics (Kozik et al. 2002, 2004).

Our studies have confirmed that the RAPD technique is an effective method for the identification of intraline polymorphism in R_1 generation carrot plants, obtained by anther culture methods.

CONCLUSION

1. Ten RAPD primers have been identified which gave reproducibly polymorphic band patterns for individual plants within the same line/clone.
2. Differences in the RAPD profiles between R_1 plants of the same line demonstrate that the lines are not homozygous; they are therefore not DH lines.

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WYKORZYSTANIE METODY RAPD DO OCENY WYRÓWNANIA WEWNĄTRZLINIOWEGO ANDROGENICZNYCH ROŚLIN MARCHWI

Streszczenie: Celem badań była ocena wyrównania wewnątrzliniowego androgenicznych roślin marchwi otrzymanych metodą kultur pylnikowych. Materiał badawczy stanowiły rośliny 30 linii pokolenia R_1. Ocenę wykonano przy zastosowaniu 10 starterów RAPD: OPA02, OPA04, OPA07, OPA11, OPB04, OPB11, OPB12, OPB15, OPB17 i OPB18. Analiza RAPD wykazała zróżnicowanie roślin w obrębie każdej badanej linii. Świadczyły o tym ilościowe różnice w profilach amplifikacyjnych otrzymane dla indywidualnych roślin w ramach każdej analizowanej linii. Uzyskane wyniki badań wykazały, że analizowane linie nie są homozygotyczne, a więc nie są to linie DH.

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