Parental Bias in Segregation Distortion of RAPD Markers from *Solanum* Anther-Derived Plants

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ABSTRACT

A diploid *Solanum* clone 9507-04 was used to grow anther-derived plantlets that were putatively monoploid. Sixteen RAPD primers were used to observe the RAPD profiles from anther-donor and anther-derived materials. A total of 56 RAPD markers were scored and each marker was identified according to the primer's designation and size (kilo base pairs) of the amplified fragment. Of 56 RAPD markers assayed, 44.6% did not follow the expected 1:1 Mendelian segregation ($\chi^2 > 4.26$, $P < 0.05$) in the anther-derived progeny. The selected six morphological traits also segregated in anther-derived plants including the nine that retained 3-4 of the parental leaf characters. The 12 distortion-causing RAPD markers ($\chi^2 = 8.89-17.64$, $P < 0.003$) with above 84% presence in the progeny were retained in all above nine plants. Such preference of parental markers in potato anther-derived progeny may indicate a linkage with androgenesis.

Key words: Anther culture, parental-type, polymorphism, potato.

Introduction

Anther-culture of diploid potatoes can be used for the production of monoploid plants [1,2,3]. However, the gametic cells respond variedly to the culture conditions leading to different levels of tissue development in anther-derived progeny [1,4]. Such variations in anther-culture response are attributed to actions of gametophytic active genes, sporophytic active genes, or gameto-sporophytic interactions [5]. Therefore, potato anther-derived progeny has been reported to give distorted segregation in 29 to 70% of the molecular markers [6,7,8]. Gamete selection, (sub) lethal alleles, anther-culture technique, and regeneration pressure have been suggested as possible causes of distorted segregation of molecular markers [9,10]. RAPD (random amplified polymorphic DNA) markers can be used without any prior genome information for the genetic analyses of anther-derived monoploids [11,12]. The issue of dominant inheritance of RAPD markers [13] can be circumvented by using gametophytic tissue, since a heterozygous genotype (for an allelic pair) segregates into gametes carrying dominant and recessive markers [14]. RAPDs have been markers of choice for genetic analyses of tissue cultured potatoes [15, 16, 17]. This report aims to examine the segregation of some RAPD markers and morphological traits in anther-derived progeny of the diploid 9507-04 parent.

Materials and methods

Plant materials

The diploid *Solanum* clone 9507-04 (50% *S. tuberosum* and 50% wild: *S. chacoense*, Argentinian wild potato species) was kindly provided by Dr. Hielke De Jong, Potato Research Centre, Agriculture and Agri-Food Canada. *Solanum* clone 9507-04 produced abundant anther-derived plantlets under anther-culture conditions of Aziz et al. [18]. The leaves silhouette and amount of anthocyanin as well as morphological features of primary/terminal leaflet were identified as described [19, 20, 21]. The putative monoploid anther-derived material from the clone 9507-04 was chosen for the RAPD analysis.

DNA isolation and amplification

Up to 50mg of the leaf tissue was homogenized in 300µl of the extraction buffer [22] with a pellet pestle (Mandel Scientific Co.). The mixture was incubated at 65°C for 90 min followed by two chloroform/isoamyl alcohol (24:1) extractions. The DNA was precipitated by an equal volume of cold isopropanol and resuspended in 30µl of distilled water. Quantification of the DNA preparation was made by comparing with DNA Mass Ladder (Life Technologies) under gel electrophoresis conditions. Ten ng DNA template or distilled sterile water as control was used in polymerase chain reaction (PCR) mixtures. The PCR mixture (25µl in 0.2ml tube) also

included 0.2µM primer, 50µM each of dATP, dCTP, dGTP, dTTP, 1 unit Ampli-Taq DNA polymerase (Perkin Elmer) and 1X PCR buffer (10mM TrisHCl, pH 8.3, 50mM KCl, 2mM MgCl₂, 0.001% gelatin) that was overlaid with a drop of fresh mineral oil. After an initial four min denaturation at 94°C in a PTC-100 Thermal Controller (MJ Research), the amplification reactions were carried out using 45 cycles (default ramp time) of 1 min denaturation at 94°C, 1 min annealing at 36°C, and 2 min extension at 72°C. All PCR products were then subjected to 10 min extension at 72°C followed by soak at 4°C until recovery. In each set of amplification reactions one control was a PCR mixture excluding any template whilst the other contained DNA from the anther donor (Figure 1).

The PCR products (10µl of the reaction mixtures) were separated by electrophoresis (3.5Vcm⁻¹ for 3 h) on 1.4% agarose gel stained with ethidium bromide (0.5µgml⁻¹) in 0.5X TBE (0.045M Tris, 0.045M Boric acid, 0.001M EDTA) buffer. The gels were photographed using a UV transilluminator (Fotodyne, Inc.). Sizes of the RAPD markers were determined by comparing with a DNA Mass Ladder.

Statistical analysis

Reproducible RAPD-PCR products in the parent were used as markers. Parental RAPD markers were then scored for presence or absence in the anther-derived progeny. For each RAPD marker goodness-of-fit to expected segregation of 1:1 was tested by Chi-square analysis using SYSTAT version 7.0 (SPSS Inc., USA). Segregation ratios that differed from the expected value (significant at P=0.05 or less) were classified as distorted.

Results and discussion

RAPD markers' identification

Sixteen RAPD primers (Table 1) reported to generate polymorphic markers from potato [22, 23, 3, 24, 25, 8] and an additional primer OPH-08 (selected for producing a segregation pattern) were used in this study. The primers from University of British Columbia (UBC) and Eurofins MWG Operon (OP) have recently been used for genetic diversity studies [26]. These 16 primers generated 56 reproducible RAPD markers from anther-donor clone 9507-04 (Table 1). A similar number of scorable markers have been reported for the same RAPD primers in genetic identity studies of potato [22, 23, 3, 24, 25, 8]. Complete documentation of the sizes of RAPD markers was not reported by the above authors; therefore, accurate comparison for all the RAPD markers was not possible. The present study confirmed the reports of

Sosinski and Douches [25] in which primer OPA-02 reproduced RAPD markers with molecular sizes of approximately 0.67 kbp, 0.76 kbp, 0.93 kbp and 1.11 kbp. Primer UBC131 reproduced the markers of approximately 1.03 kbp and 1.55 kbp sizes similar to those reported under PCR conditions of Demeke et al. [22]. A nomenclature of RAPD markers based on their molecular size and primer name has been proposed to standardize any RAPD assessment of germplasm [23]. Their recommended nomenclature of RAPDs was used in this study. The 56 RAPD markers in this study were designated according to the name of primer and size (kbp) of the amplified fragment (Table 1). Therefore, these markers can conveniently be used for a standard comparison with RAPD profiles from other related genotypes.

Leaf morphology of anther-derived plants

The mature leaves of potato are described as a pinnately compound [21] and six of the leaf characters were used to identify progeny of anther-donor parent (Table 2). The leaf morphological traits of parent segregated in anther-derived plants thus differing from each other. The leaf silhouette was either open or medium. Amount of leaf anthocyanin and pubescence varied from light to medium or heavy. The shape of terminal or primary leaflet was found elliptical, narrowly ovate or broadly ovate. The tip of these leaflet were classified as acuminate, acute or cuspidate. Also the leaflet bases were found as cordate, obtuse, acute or truncate. Such morphological differences reflected the segregation of parental and non-parental type genetic characters in the progeny. The anther-derived progeny revealed assorted non-parental leaf traits. Androgenesis has been reported to induce genomic changes in potato [27]. Out of the 20 anther-derived plants generated, nine were found similar to the parent. These plants showed retention of three or four of the parental leaf characters (Table 2). Segregation studies in monoploid regenerants would also be useful in genetic analysis since they are expected to be developed from individual microspores. Therefore, such studies can also be used to identify extra-chromosomal and/or physiological factors affecting the survival of monoploids or dihaploid.

Segregation distortion of RAPD markers

Parental RAPD markers were scored for presence or absence of a DNA band in the anther-derived progeny. Segregation of the RAPD markers was examined against the expected 1:1 ratio by Chi-square analysis. Of 56 markers assayed (Table 1), 44.6% did not segregate according to expected 1:1 Mendelian ratio

($\chi^2 \geq 4.26$, $P \leq 0.05$). Anther-derived progeny is reported to give much higher percentage of skewed markers as compared to that from the populations of selfed progenies [7]. Segregation distortion can be caused by the gamete selection, i.e., only a few selected microspores may survive in the mature anthers [28]. During anther culture, the genotypes which are culture-responsive could be further selected, causing an even greater distortion in the segregation of molecular markers [7,10]. The marker UBC291-1.4 showed a distorted ratio in anther-derived progeny for 'presence of band: absence of band'=21:2 ($\chi^2=15.696$, $P<0.005$). Interestingly, the same marker also showed the distorted segregation in the pollen population [29]. It appears that UBC291-1.40 is selected against during pollen maturation and selected for anther culture. This marker therefore may be related to alleles essential for the survival of culture responsive microspores.

Segregation distortion can occur due to the selection pressure in the pollen maturation process, and additional selection pressures during anther-culture conditions may contribute further to the distorted segregation of the markers. From 25 distorted markers (Table 1) 13 RAPDs were either unevenly present or absent in anther-derived progeny

($\chi^2=4.26-11.56$, $P=0.001-0.05$). Non-random chromosome segregation during meiosis is another possible explanation for the distorted segregation observed [30]. The other 12 RAPD markers showed distorted segregation by being retained in more than 84% of the progeny ($\chi^2=8.89-17.64$, $P<0.003$). Thus being distortion-causers [31], most of these RAPDs have been used as seed-markers for a linkage mapping approach. Incidentally this group of 12 distortion-causing markers was found intact in the nine plants (Table 2) having mostly parental traits. Segregation distortion during doubled haploid production is likely to be due to a range of factors including selection during the process of gamete culture and plantlet regeneration [32]. Simultaneous presence of group parental markers in anther-derived progeny reported in this study may indicate a linkage with androgenesis in potatoes. Androgenic fitness has an important role in the prevalence of markers with segregation distortion and linkage of such markers to specific genes controlling *in vitro* androgenesis [33]. Further studies of segregation of molecular markers at various pollen maturation and microspore regeneration stages would help to identify the genetic factors involved in these processes.

Table 1: Analysis of the random amplified polymorphic DNA (RAPD) markers for 1:1 segregation in the anther-derived progeny of *Solanum* clone 9507-04.

RAPD markers designation*				Analysis for 1:1 segregation	Categories ¹
OPA002-1.11, OPA005-0.61, OPB010-0.57, OPB010-0.65, OPB010-1.60, OPC007-0.58, OPC007-0.87, OPC013-0.36, OPC013-0.93, OPC013-1.16, OPC013-2.24, OPC013-6.53, OPC015-0.63, OPC015-0.70, OPC015-0.78, OPC015-1.00, OPG015-0.26, OPG015-0.83, OPG015-1.47, OPH008-0.92, UBC131-1.25, UBC184-1.15, UBC291-0.40, UBC308-2.00, UBC504-0.86, UBC504-0.92, UBC504-1.50, UBC504-1.98, UBC504-2.24, UBC559-1.14, UBC559-2.00				$\chi^2 \leq 0.391-3.24$, N.S.	RAPD markers segregating according to the expected 1:1 Mendelian ratio.
OPA002-0.93, OPC007-1.84, OPC013-0.56, OPC015-0.71, OPC015-1.00, OPC015-1.16, UBC131-1.03, UBC131-1.55, UBC184-0.59, UBC291-0.67, UBC308-0.67, UBC504-1.03, UBC533-0.93				$\chi^2=4.26-11.56$ $P=0.001-0.05$	RAPD markers showing distortion from the expected 1:1 segregation
OPA002-0.67, OPA002-0.76, OPB010-0.36, OPB010-0.96, OPC015-0.39, OPC015-1.47, OPH008-1.00, OPH008-1.26, UBC153-0.64, UBC291-1.14, UBC308-0.96, UBC533-1.27				$\chi^2=8.89-17.64$ $P<0.003$	Distortion causers with >84% presence in monolpoids

*The letters on left side of the hyphen represent the name of the RAPD primer (UBC/OP); digits on the right show the size (kbp) of the amplified fragment.

¹Categories for RAPD markers are based on Chi-square test for 1:1 segregation of the parental DNA band presence vs. absence in the progeny.

Table 2: Leaf morphological characters of anther-derived plants* from <i>Solanum</i> clone 9507-04						
Plants	Leaf silhouette	Terminal/primary leaflet:			Anthocyanin in leaves	Leaf pubescence
		Shape	Shape of Tip	Shape of base		
Parent	Open	Narrowly ovate	Acuminate	Cordate	Light	Medium
1	Open	Narrowly ovate	Cuspidate	Obtuse	Heavy	Medium
2	Open	Narrowly ovate	Acuminate	Acute	Medium	Medium
3	Open	Narrowly ovate	Acuminate	Obtuse	Medium	Medium
4	Open	Narrowly ovate	Acuminate	Acute	Medium	Medium
5	Medium	Narrowly ovate	Acute	Cordate	Light	Light
6	Open	Narrowly ovate	Acuminate	Obtuse	Light	Light
7	Open	Narrowly ovate	Acute	Obtuse	Medium	Medium
8	Open	Narrowly ovate	Acuminate	Obtuse	Heavy	Medium
9	Open	Narrowly ovate	Acuminate	Obtuse	Heavy	Light

* The segregating leaf morphological characters [19, 20, 21] of parent in the nine anther-derived plants that showed retention of 3-4 traits of the anther donor.

Figure 1: Groups of four lanes showing PCR amplifications with a random amplified polymorphic DNA (RAPD) primers: the two lanes at the extreme left present the template free reactions and the DNA profiles of the parental clone 9507-04 respectively; the two lanes at the extreme right show the segregation of parental RAPD markers in the selected anther-derived progenies. Lanes a-d, primer OPC-07; lanes e-f, primer OPC-15; lanes i-l, primer UBC184. Lane m presents a 2.0 kbp (200 ng) to 0.1 kbp (10 ng) DNA Mass Ladder (Life Technologies). At the left margin relative positions of the DNA markers are shown. The parental RAPD markers were identified by primer name and their sizes (kbp), to be scored as present or absent in the anther derived progeny.

Acknowledgments

The author acknowledges the greenhouse maintenance of the plants by Andrew Gardner and Steven Allaby. Thanks are due to L. Katheryn Douglass for tissue culture suggestions, Susan Oborn for computer analysis suggestions, Coral Curtis for flow cytometer operation, and Roger Smith for photographic printing. Technical assistance by Drs. Janet E.A. Seabrook and George C. C. Tai is also appreciated. This research was supported by the Department of Biology, University of New Brunswick and Agriculture & Agri-Food Canada.

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