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Genetic variation in monoploids of diploid potatoes and detection of clone-specific random amplified polymorphic DNA markers

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Abstract. Randomly amplified polymorphic DNA (RAPD) markers have been used to study the genetic variation among androgenetic monoploids of diploid *Solanum* species. Cluster analysis of pairwise genetic distances was used to construct a genetic relationship among anther donor and anther-derived potato plants. The clustering based on Rogers' distances resembled classifications based on parental origins and hybrid combinations. Six of the 32 RAPD primers used resulted in the selective amplification of DNA fragments which were polymorphic between the two *S. phureja* parental clones, 1.22 and A95. It should be possible to construct a genetic linkage map, without making crosses, using monoploids derived from a single heterozygous diploid clone and RAPD markers.

Introduction

Potato (*Solanum tuberosum* L.) is highly heterozygous and may harbor deleterious recessive alleles at many loci without any apparent effects on growth and performance (Uijtewaal et al. 1987). Such alleles may be expressed in monoploid plants where each chromosome is represented without a pairing homolog. If a monoploid embryoid survived androgenesis in anther culture, it is not only free from lethal alleles but also could be expected to have a favorable gene combination, since selection for viable and vigorous monoploids implies selection for favorable gene combinations, i.e., "monoploid sieve" (Singsit and Veilleux 1989). *In vitro* androgenesis has been shown to be genotype specific in potato (Veilleux et al. 1985) and in maize (Petolino and Jones 1986). The successful transmission of genes involved in androgenesis to F_1 and BC_1 individuals of crosses between androgenetic and non-androgenetic clones (Singsit and Veilleux 1989) may permit the construction of linkage maps from a broad genetic base. It also allows indirect selection for marker-linked specific trait(s) in the resulting monoploid or doubled-monoploid plants. Anther-derived lines have proved to be relevant and convenient materials for classical genetic analysis in rice (Chen et al. 1983) and genetic mapping in potato of genes that control traits of interest and molecular markers (Rivard et al. 1989).

Random amplified polymorphic DNA (RAPD) marker system has been useful for quickly placing markers on linkage groups, even in species with large genomes, as it requires no DNA cloning, Southern blotting or hybridizations with labeled DNA probes (Williams et al. 1990). The RAPD marker system is based on the polymerase chain reaction (PCR) amplification of random DNA segments with single primers of arbitrary nucleotide sequence. The use of RAPD markers and haploid DNA from megagametophytes of spruce demonstrated the unique applications of the RAPD technique for genome mapping in a single tree (Tulsieram et al. 1992). Using arbitrary oligonucleotides, genotype-specific markers have also been isolated in *Lycopersicon esculentum* (Klein-Lankhorst et al. 1991), *Brassica* (Quiros et al. 1991), *Theobroma* (Wilde et al. 1992) and *Pennisetum* (Ozias-Akins et al. 1992).

The objectives of this study were: (i) to identify polymorphic RAPD markers by polymerase chain reaction (PCR) and (ii) to determine the validity of using RAPD markers for the construction of genetic relationships among monoploids, doubled monoploids and anther donor parents of diploid potato based on Rogers' distances.

Materials and Methods

Plant materials. The plant materials included four *S. phureja* clones - PP5 (PI 225669), 1.22 (PI 225682), P20 (PI 320349), A95 (2n micro-

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spore-derived of PP5 via AC) and a single clone (B14) of *S. berthaultii* (265838). Also included were three doubled monoploids: AD-2-4, AD-3-8, AD-29-1 (derivatives of PP5 via AC) and 14 monoploids. Two parental clones, *S. phureja* (P20) and the *S. berthaultii* (B14) were not available and only their monoploid derivatives were used in this study. All monoploids were derived via anther culture of *S. phureja* F_1 hybrids and BC_1s or an interspecific hybrid between *S. phureja* and *S. berthaultii* (Singsit and Veilleux 1989). A bilayer anther culture medium proposed by Wenzel and Uhrig (1981) with some modifications (Veilleux et al. 1985) was used. The doubled monoploids and A95 were derived from a single anther donor clone, PP5. The plant materials have been propagated and maintained *in vitro* on MS media with routine transfer at 6-8 week intervals for approximately 4 years. A schematic presentation of the plant materials and methods used in their derivation is given in Fig. 1.

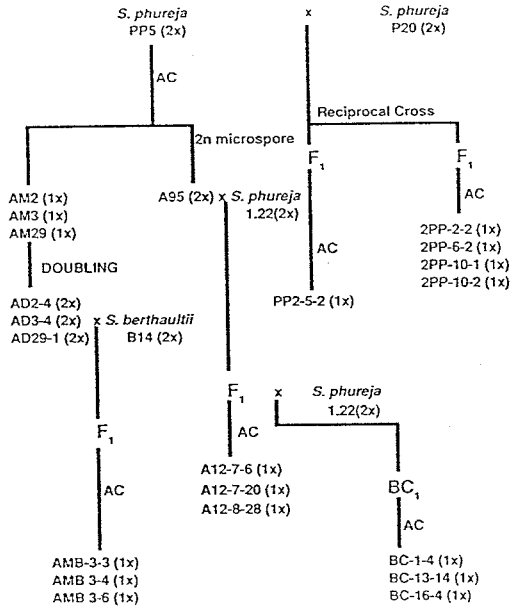

Fig. 1. A schematic presentation of the development of plant materials used in the study of RAPD analysis. Five groups of monoploids and doubled monoploids obtained from intraspecific hybrids of *S. phureja*, interspecific hybrids between *S. phureja* and *S. berthaultii* and a single clone of *S. phureja*. Monoploids were obtained via anther culture (AC) and monoploid chromosomes were doubled *in vitro* following leaf disc culture method (Singsit et al. 1990).

DNA analysis. DNA was isolated from fresh leaves and stems (0.2-2.0 g) of *in vitro*-grown monoploid, doubled monoploid and diploid potato plants following the method of Doyle and Doyle (1987). Larger quantities of tissue (0.4-2 g) were ground in liquid nitrogen with a mortar and pestle. Smaller quantities of tissue (<300 mg) were homogenized in a microcentrifuge tube with a pellet pestle. Vacuum-dried DNA was redissolved in TE (10mM Tris-1mM EDTA, pH 8) containing 10μg/ml RNase. The concentration of DNA was measured using a mini-fluorometer (Hoefer Scientific Instruments, San Francisco, CA).

One hundred forty RAPD primers (Operon Technologies, Alameda, CA) of arbitrary decamer oligonucleotides were surveyed for their ability to reproduce amplified segments of genomic DNA. The sequences of six RAPD primers that amplified clone-specific DNA fragments are: OPC-07 (GTCCCGACGA); OPG-05 (CTGAGACGGA); OPG-12 (CAGCTCACGA); OPH-04 (GGAAGTCGCC); OPH-12 (ACGCGCATGT) and OPH-13 (GACGCCACAC). The amplification conditions were as reported by Williams et al. (1990) with some modifications. The reaction mixtures (25 μl) contained 10 mM Tris-Cl pH 8.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 100 μM each of dATP, dCTP, dGTP and dTTP, 0.25 μM primer, 25 ng genomic DNA, 0.5 unit of *Taq* DNA polymerase (Promega Corporation, Madison, WI) overlayed with a drop of mineral oil. The amplification reactions were carried out using a Perkin-Elmer Cetus thermal cycler under the following conditions: 94 C for 1 min (denaturation), 36 C for 1 min (annealing), 72 C for 2 min (extension), for 30 cycles, followed by a 4 C soak cycle until recovery. The amplified products were assayed by electrophoresis in 1.4% agarose (Seakem HGT) gels run with Tris-borate-EDTA (TBE) buffer. The gels were stained with ethidium bromide, viewed and photographed under UV light with Polaroid film 665 or 107C.

Statistical analysis. Genotypes were scored for the presence or absence of bands. Band-sharing analysis was carried out using Pairwise Rogers' Distances (Messmer et al. 1991) with Average Linkage Cluster Analysis (UPGMA) (SAS 1989). Rogers' distances (RD) were calculated based on the number of RAPD bands for which two genotypes differed divided by the total number of RAPD bands considered. For example, the lower the RD value the greater the similarity between two genotypes.

Results and Discussion

Genetic distances among monoploids

Of the 140 arbitrary primers used to survey 20 genetic lines, 32 (23%) showed bands with polymorphisms ranging in length from 0.26 to 2.46 kb. The number of clearly distinguishable bands varied from 2 to 10 depending on the primer used. A genetic distance matrix was generated based on RD in which each genotype was treated as an independent unit without any assumptions about their assignments. The calculated value of RD across the 171 possible combinations among 19 genotypes ranged from 0.05 to 0.47 with a mean of 0.32, and a standard deviation (sd) of 0.075 (Table 1). Cluster analysis of Rogers' distances segregated monoploids, doubled monoploids and diploid parents into six major groups. The first group of monoploids distinguished by cluster analysis of genetic distance included AMB-3-3, AMB-3-4 and AMB-3-6 with a mean RD value of 0.07 (sd=0.0004). These genotypes displayed a greater genetic homogeneity among themselves and higher proportion of shared fragments (91-95%) than any other group. They were derived from an interspecific hybrid between *S. phureja* (doubled monoploid, AD3-8) and *S. berthaultii* (B-14) (Fig.1). The second group of monoploids (BC-1-4, BC-13-14 and BC-16-4) was obtained from BC_1 individuals between two *S. phureja* clones, A95 and 1.22, using 1.22 as the recurrent parent. This group had an overall mean RD of 0.16 (sd=0.0079). The third group with a mean RD of 0.19 (sd=0.0116) consisted of three monoploids A12-7-6, A12-7-20 and A12-8-28, derived from F_1 hybrid between A95 and 1.22. The fourth group comprised four monoploids derived from the F_1 hybrid between two *S. phureja* clones, P20 and PP5. It included 2PP-2-2, 2PP-6-2, 2PP-10-1 and 2PP-10-2. The RD value of this group ranged between 0.16 to 0.30 (mean=0.22, sd=0.075)

(Table 1). Even though these monoploids were all derived from the same hybrid combination, 2PP-2-2 appeared to be more closely related to the first group of AMB series than the 2PP series (Fig. 1). The fifth group consisted of two doubled monoploids, AD-2-4, AD-3-4 and a diploid clone A95. The last group consist of one monoploid (PP2-5-2, mean RD=0.44) and one doubled monoploid (AD-29-1, mean RD=0.37). Overall, the lowest degree of similarity among monoploids tended to be associated with AD-29-1 and PP2-5-2.

We observed a close resemblance between relationships based on calculated Rogers' distances (Table 1) and those based on parental origins and hybrid combinations (Fig. 1). For example, the three monoploids obtained from BC_1s were closer to the recurrent parent, 1.22 (76% similarity) than those monoploids derived from the F_1 hybrids (64%) between A95 x 1.22 (Table 1). Likewise, greater genetic homogeneity (91-95%) was measured among monoploids (AMB-3-3, AMB-3-4, AMB-3-6) where one of the anther donor parents was a doubled monoploid rather than among those derived from two heterozygous parents (Table 1, Fig. 1).

Table 1. Calculated Rogers' distances among anther donor and anther-derived potato plants based on band sharing analysis.

	A95	1.22	A12-7-6	A12-7-20	A12-8-28	BC-1-4	BC-13-14	BC-16-4	2PP-2-2	2PP-6-2	2PP-10-1	2PP-10-2	PP2-5-2	AMB-3-3	AMB-3-4	AMB-3-6	AD-2-4	AD-3-4	AD-29-1
A95																			
1.22	.32																		
A12-7-6	.25	.35																	
A12-7-20	.27	.35	.07																
A12-8-28	.35	.37	.24	.27															
BC-1-4	.29	.23	.32	.33	.34														
BC-13-14	.31	.26	.35	.36	.37	.06													
BC-16-4	.30	.20	.33	.34	.33	.19	.23												
2PP-2-2	.27	.33	.35	.34	.29	.37	.36	.28											
2PP-6-2	.29	.36	.35	.30	.35	.35	.37	.33	.28										
2PP-10-1	.34	.39	.37	.33	.35	.38	.40	.36	.30	.16									
2PP-10-2	.26	.32	.30	.29	.29	.35	.36	.30	.20	.21	.19								
PP2-5-2	.43	.46	.43	.46	.33	.46	.47	.44	.37	.44	.45	.35							
AMB-3-3	.25	.25	.30	.31	.37	.25	.28	.20	.23	.31	.29	.21	.40						
AMB-3-4	.30	.28	.34	.36	.39	.28	.30	.24	.27	.35	.26	.27	.43	.07					
AMB-3-6	.26	.28	.32	.33	.39	.28	.31	.21	.27	.31	.29	.23	.41	.05	.09				
AD-2-4	.28	.32	.32	.31	.42	.28	.32	.30	.35	.32	.35	.33	.40	.27	.32	.28			
AD-3-4	.25	.36	.34	.33	.42	.36	.39	.31	.32	.29	.32	.27	.39	.23	.29	.22	.16		
AD-29-1	.30	.38	.39	.36	.43	.35	.38	.33	.42	.43	.38	.39	.40	.35	.30	.34	.30	.30	

Note: Overall mean RD=0.32; SD=0.075

Clone-specific markers

The profiles of the amplified products from each anther-derived and anther donor individual were compared to each other for identification of clone-specific markers. Of the 32 RAPD markers showing polymorphisms, a single primer (OPC-07) was found to selectively amplify a fragment of 0.7 kb from *S. phureja* clone 1.22 and four of the 14 monoploids (Fig. 2). Also, five more primers (OPG-05, OPG-12, OPH-04, OPH-12, OPH-13) selectively amplified DNA fragments specific to diploid clone A95 and not 1.22. Amplification of these fragments also was observed in most of the monoploids and the three doubled monoploids (Table 2; Fig. 3a,b). The identification of these markers may be significant in the study of genetic linkage with other traits. Both OPC-07 (0.7kb) and OPH-12 (1.5kb) segregated as single heterozygous markers when a population of F_1 hybrid individuals between A95 and 1.22 was examined. Of the 91 F_1 hybrid individuals examined, OPC-07 segregated as 43 present and 48 absent (χ^2=0.27), and OPH-12 as 53 present and 38 absent (χ^2=2.47) and were found not significantly different from the expected 1:1 ratio indicating heterozygosity at both loci (Fig. 4a,b).

Table 2. Selected primers that amplify DNA fragments polymorphic between *S. phureja* clones, A95 and 1.22

	C-07 (0.7)	G-05 (0.3)	G-12 (0.8)	H-04 (0.8)	H-12 (1.5)	H-13 (1.1)*
A95	−	+	+	+	+	+
1.22	+	−	−	−	−	−
A12-7-6	+	+	+	+	+	+
A12-7-20	+	+	+	+	+	+
A12-8-28	−	−	+	+	+	+
BC-1-4	+	−	−	+	−	−
BC-13-14	+	−	−	−	−	−
BC-16-4	−	−	−	+	−	−
2PP-2-2	−	−	+	−	−	−
2PP-6-2	−	−	+	−	+	+
2PP-10-1	−	−	+	−	+	+
2PP-10-2	−	+	+	−	+	+
PP2-5-2	−	−	−	−	+	+
AMB-3−3	−	−	+	−	−	−
AMB-3-4	−	−	+	−	−	−
AMB-3-6	−	−	+	−	−	−
AD-2-4	−	+	+	+	−	−
AD-3-4	−	+	+	−	+	+
AD-29-1	−	+	−	+	−	−

*Fragment size in kb, + present − absent

RAPD markers have also been used to amplify genotype-specific DNA fragments among interspecific hybrids in *Lycopersicon* using chromosome substitution lines (Klein-Lankhorst et al. 1991), and to map genes for resistance to downy mildew in lettuce (Paran et al. 1991). In rice, linkage relationships among anther-derived populations were observed to be consistent with

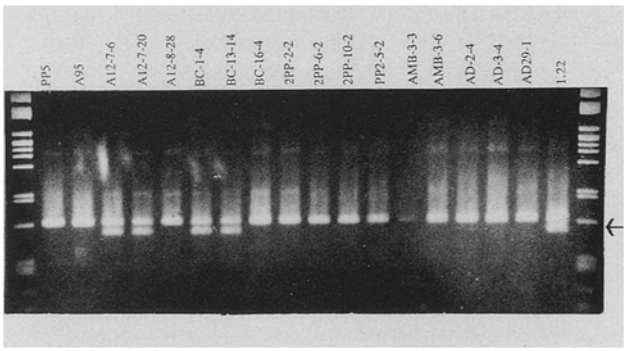

Fig. 2. Amplification of a unique DNA fragment (0.7kb) with RAPD primer OPC-07 from *S. phureja* clone 1.22 and four monoploids obtained from F_1s and BC_1s between two *S. phureja* clones, A95 and 1.22 using 1.22 as the recurrent parent.

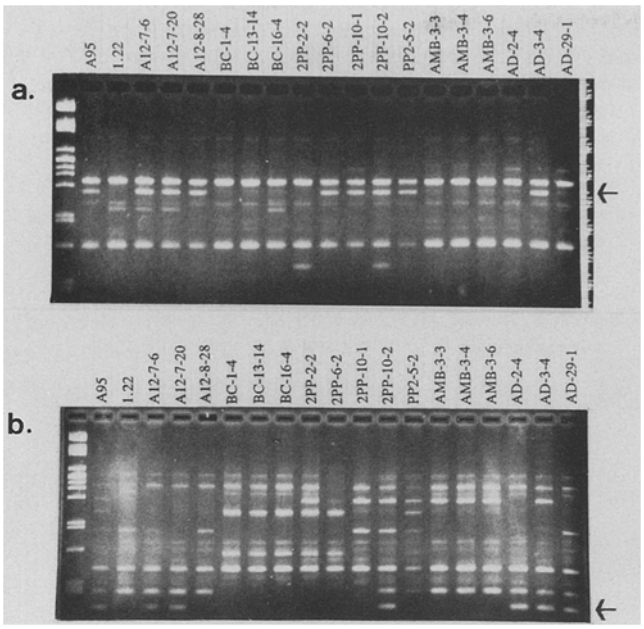

Fig. 3. RAPD primers showing polymorphism and selective amplification of DNA fragments from *S. phureja* clone A95 and not 1.22: (a) amplification of 1.5kb fragment with OPH-12, (b) amplification of 0.3kb fragment with OPG-05.

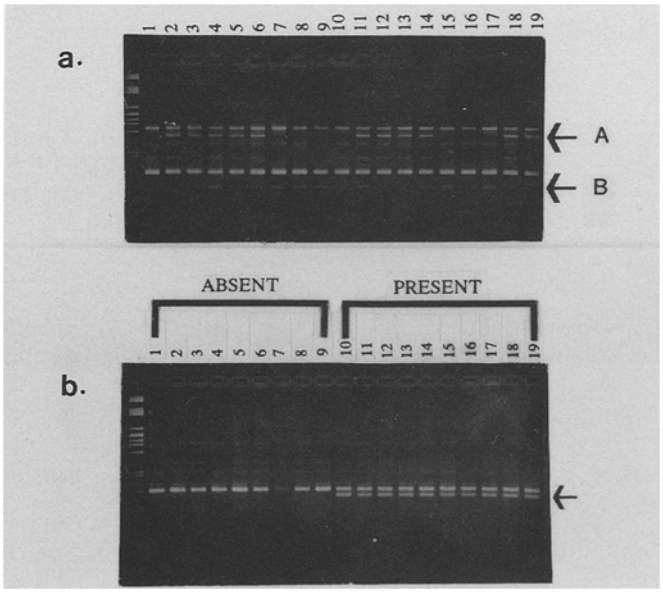

Fig. 4. Segregation of RAPD markers among F_1 individuals between two *S. phureja* clones A95 and 1.22: (a) using primer OPH-12, (b) using primer OPC-07.

linkage in F_2 populations and anther-derived population were suggested to be useful in molecular mapping and gene tagging (Guiderdoni 1991). We identified six RAPD markers, one for parent 1.22 and five for parent A95, that were polymorphic between the two clones. Upon examination of the F_1 hybrid plants these markers were found to be heterozygous. Markers such as these could be used for tagging traits of genetic importance, e.g., the haploid inducing ability of 1.22 or androgenetic trait of A95. One of the limitations of using RAPD markers for genetic linkage or mapping studies is that they are inherited as dominant markers (Williams et al. 1990), and heterozygotes can not be differentiated from homozygous dominant individuals without subsequent crosses. Such problems can be circumvented by using monoploids from heterozygous individuals

A recent application of RAPD technology was the construction of a genetic linkage map from a single spruce tree using haploid DNA from megagametophyte tissue of individual seeds (Tulsieram et al. 1992). However, the advantages of using haploid DNA for genetic studies need not be limited to conifers as haploid microcalli/plants have been produced through anther culture from 85 angiosperm genera (Srivastava and Johri 1988). Evidence of simple genetic control of androgenesis in potato (Uhrig and Salamini 1987) and subsequent transmission of the trait to F_1 and BC_1 individuals (Singsit and Veilleux 1989) may allow the identification of markers linked to genes conferring *in vitro* androgenesis. A preliminary genetic analysis in maize (Cowen et al. 1992) has identified two chromosomal regions, each containing two probes, with highly significant effects on *in vitro* androgenesis.

Conclusion

We have demonstrated that RAPD markers can be used to study the genetic variation in potato monoploids. Polymorphic RAPD markers have been identified for two anther donor clones. Doubled monoploids could be used as test-cross parents for studying inheritance of quantitative trait loci. From both the plant breeders' and the geneticists' viewpoint it is important that the anther-derived plants represent a random array of the microspore population. This assumption held true for most but not all loci in rice (Guiderdoni 1991), possibly due to "monoploid sieve" selection. Using repetitive cycles of anther culture and backcrossing, it is possible to generate recombinant monoploids or doubled monoploids carrying genes from two or more anther donor parents.

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