HORTSCIENCE 31(1):130–133. 1996.

Using Polymerase Chain Reaction-based DNA Amplification to Fingerprint North American Potato Cultivars

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Additional index words. Solanum tuberosum, RAPD, cultivar identification

Abstract. **DNA from 46 North American potato (*Solanum tuberosum* L.) cultivars was examined using the polymerase chain reaction (PCR) with 16 arbitrary primers of 10 nucleotide length (10 mers) to determine the efficiency of randomly amplified polymorphic DNA (RAPD) in delineating cultivars, both sexually derived and clonal variants. The 16 primers yielded 43 useful polymorphisms that were evaluated according to the presence or absence of fragments of equal size. All cultivars were discriminated with as few as 10 primers. The russet sport of Burbank was distinguished from a white-skinned clone by one band. More primers (29) were examined to identify a band polymorphism among six Russet Burbank clonal variants. When the cultivars were grouped by tuber type (excluding the russet clonal variants), three to four primers discriminated these commonly grown cultivars. Determination of cultivar integrity was accomplished with PCR amplification, regardless of tissue source (leaf vs. tuber) for DNA extraction. Cluster analysis based on RAPD markers was performed to examine pedigree relationships of the cultivars. Genetic relationships correlated with some pedigrees; however, many exceptions were noted.**

There is a need for a simple, objective means of identifying potato cultivars. Historically, potato cultivars have been discriminated and identified through using various morphological characteristics and precise record keeping. The characteristics typically used for identification and certification include tuber type, leaf type, flower color, sprout appearance, growth habit, and disease reaction. However, many of these morphological traits can vary drastically with environmental conditions. An additional problem with using morphological traits is that they are seldom available for interpretation simultaneously (tuber vs. leaf and flower). Therefore, it is important to continue to develop objective, efficient, rapid, and reliable methods that can assist in discriminating potato cultivars for seed certification purposes.

Molecular markers that aid in identifying potato cultivars, including isozyme markers (Douches and Ludlam, 1991; Oliver and Martinez-Zapater, 1985), general protein banding patterns [polyacrylamide gel electrophoresis (PAGE)] (Desborough and Peloquin, 1968; Stegeman et al., 1973), and restriction fragment length polymorphisms (RFLPs) (Douches et al., 1990; Gebhardt et al., 1989; Gorg et al., 1992), have been characterized. A DNA-based marker has been reported by Williams et al. (1990) and Welsh and McClelland (1990), whereby discrete regions of DNA are amplified using the polymerase chain reaction (PCR) with arbitrary primers to produce randomly amplified polymorphic DNAs (RAPD). RAPDs offer several advantages over RFLPs: 1) the need for only nanogram amounts of DNA as opposed to the milligram quantities for RFLPs, 2) absence of radioisotopes, 3) lower cost, and 4) simpler and quicker laboratory procedures. RAPD technology recently has been used to discriminate cultivars of turfgrasses (Caetano-Anolles et al., 1992), apple (*Malus domestica* Borkh.) (Koller et al., 1993), broccoli and cauliflower (*Brassica oleracea* L. Botrytis Group) (Hu and Quiros, 1991), and Japanese and North American potatoes (Demeke et al., 1993; Mori et al., 1993). Our objectives were to investigate 1) the usefulness of RAPD markers to discriminate North American potato cultivars (sexually derived and clonal variants) and the efficiency of this process when combined with simply observed and stable tuber traits and 2) the ability of RAPDs to group cultivars according to their pedigrees. The value of RAPDs for use by the seed industry also is discussed.

Received for publication 16 Mar. 1995. Accepted for publication 25 Oct. 1995. This study was supported in part by the Michigan Potato Industry Commission and Michigan Agricultural Expt. Station. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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Materials and Methods

Forty-five potato cultivars and breeding lines were selected based on production importance over the past century in North America, along with 84S10, a *S. phureja* Juz. and Buk. diploid species selection, which served as an unrelated comparator species for the phenetic analysis. These cultivars are described according to tuber type and year of release (Table 1). May et al. (1982) reported that cultivar mixtures have occurred in cultivars obtained from potato breeding programs in the United States. To ensure true-to-type cultivars, the potato tubers used in this study were obtained from N.S. Wright, Agriculture Canada, Vancouver, B.C., which is the major repository for virus-free North American cultivars.

DNA from these cultivars was extracted from young leaves of greenhouse-grown plant material using a protocol from Doyle and Doyle (1987). DNA also was extracted from greenhouse-grown tubers of three cultivars to compare the consistency of RAPD patterns when amplified from DNA isolated from different tissue sources. DNA from all cultivars was randomly amplified in a 25-µl volume consisting of 10 mM tris-HCl (pH 8.3); 10 mM

Table 1. Potato cultivars used in randomly amplified polymorphic DNA analysis.

Cultivar	Introduction year	Type[z]
Alaska Frostless	1967	RW
Atlantic	1976	RW
B. O. Hebron	1878	RW
Belchip	1979	RW
Bintje	1910	Y
Bliss Triumph	1878	R
Burbank	1876	L
Butte	1978	L
Chieftain	1966	R
Chipbelle	1981	RW
Conestoga	1982	RW
Desiree	1967	R
Early Rose	1861	L
Early Ohio	1871	RW
Frontier Russet	1990	L
Garnet Chili	1853	R
Gemchip	1989	RW
Goldrush	1992	L
Green Mountain	1885	L
Irish Cobbler	1876	RW
Katahdin	1932	RW
Kennebec	1948	RW
Krantz	1985	L
La Rouge	1962	R
Lemhi Russet	1981	L
Nooksack	1973	L
Norchip	1968	RW
Norgold Russet	1964	L
Norland	1957	R
Onaway	1956	RW
Ontario	1946	RW
Ranger Russet	1992	L
Red Pontiac	1945	R
Red Lasoda	1953	R
Rosa	1981	RW
Rural New Yorker	1888	RW
Russet Burbank	1914	L
Russet Norkotah	1987	L
Saginaw Gold	1988	Y
Sangre	1982	R
Snowden	1991	RW
Spunta	1968	L
Superior	1961	RW
Wauseon	1967	RW
White Rose	1893	L
Yukon Gold	1980	Y

[z]Y = yellow flesh, RW = round white, L = long, and R = red skin.

Reprints from HORTSCIENCE, Vol. 31(1), February 1996
A Publication of the American Society for Horticultural Science, Alexandria, VA 22314

KCl; 5 mM $MgCl_2$; 200 μM each of dATP, dTTP, dCTP, and dGTP; 0.5 unit Ampli*Taq* DNA polymerase Stoffel Fragment (Perkin-Elmer Cetus, Norwalk, Conn.); 25 ng DNA; and 2.5 ng of 10-base oligonucleotide primers from Operon (Alameda, Calif.) in a Perkin-Elmer Cetus (Foster City, Calif.) GeneAmp PCR system 9600 thermal cycler (Table 2). Primers A04, A08, A12, A15, A17, and F05 were previously mapped in potato (Freyre and Douches, 1994). The thermal cycling regime used was adapted from Williams et al. (1990) as follows: 4 min at 94C; three cycles of 15 sec at 94C, 15 sec at 35C, and 75 sec at 72C, which was then followed by 34 cycles of 15 sec at 94C, 15 sec at 40C, 75 sec at 72C; and 7 min at 72C.

The completed reactions were held at 4C until the amplification products could be separated by electrophoresis on a 2% agarose gel stained with ethidium bromide (4.0×10^{-5} mg·ml^{-1}) in 1X TAE buffer (40 mM tris-acetate and 1 mM EDTA). Only those primers that produced distinct, discrete, band patterns were scored (Table 2). The amplification products were scored for presence or absence of the more intensely amplified polymorphic band as recommended by Weeden et al. (1992). Amplifications were replicated at least twice to check for repeatability.

A phenetic analysis of the cultivars, based on the 43 RAPD bands, was determined using Nei's distance index (Nei, 1972). An unweighted group pairs means analysis was used to construct a dendrogram from the distance matrix (Sneath and Sokal, 1973) using a computer program developed by K. Ritland (Univ. of Toronto, Toronto, Canada).

Results

The 46 cultivars were initially screened using 25 arbitrary 10-mer oligonucleotide primers. Of these primers, 16 (64%) displayed useful polymorphic banding patterns (Fig. 1 A and B). Amplification of DNA with each of these primers yielded monomorphic and polymorphic bands. Scorable band sizes ranged from ≈600 to 1700 bp. From the 16 useful primers, 43 distinct band polymorphisms were identified. The frequency of a band of similar mobility among the screened cultivars ranged from 2.6% to 94.9% (Table 2). Of the 43 polymorphic bands present among the cultivars, the average cultivar carried 41% of them, while the band presence ranged from 28% ('Burbank' and 'Russet Burbank') to 56% ('Saginaw Gold' and 'Chipbelle'). Of these 16 primers, as few as 10 identifying 23 polymorphisms were needed to discriminate all cultivars.

The 46 cultivars were divided into four groups based on easily identifiable tuber traits: yellow flesh, red skin, round white, and long (or long russet) tuber shapes. The three yellow-fleshed, nine red-skinned, and 19 round white cultivars could be discriminated using three primers each, while the 16 long-type potatoes required four primers for discrimination. In each of these groupings, the primers identified 3, 9, 11, and 12 polymorphic bands (putative loci) for the yellow-fleshed, red-skinned, round white, and long tuber types, respectively. Therefore, when tuber grouping classification was combined with PCR, only four primers were needed for any single individual to determine its identity. Notably, PCR amplification detected a band difference between Burbank and its russet sport ('Russet Burbank'). The most primers were needed to identify a polymorphic band between clonal variants of 'Russet Burbank'.

Due to missing data, only 39 potato cultivars and one *S. phureja* diploid clone (84S10) were used for the cluster analysis and dendrogram construction (Fig. 2). 84S10 clustered independently from the North American cultivars. The yellow-fleshed 'Saginaw Gold' also clustered independently from the other 38 cultivars. The rest of the cultivars could be separated into two main clusters. The closest relationships were found among the 19th century cultivars: 'Burbank'/'Russet Burbank' and 'Early Rose'/'Beauty of Hebron'. There were no associations between tuber type and presence of RAPD bands.

Discussion

Cultivar identification via marker-based analyses is useful for plant species that have an asexual means of propagation because genetic variation is fixed within a line. If it is assumed that mechanical mixtures are absent, the need for extensive intracultivar sampling is eliminated. In potato, clonal propagation combined with polyploidy and a highly heterozygous genetic state results in a large number of phenotypic classes for fingerprinting. Therefore, it is not surprising that PCR-based markers are able to generate significant levels of DNA

Table 2. Description of 10-mer primers and randomly amplified polymorphic DNA bands used for fingerprint analysis of potatoes.

Primer	Sequence	Approximate fragment size (bp)[z]	Presence (%)[y]
A02A	5'-TGCCGAGCTG-3'	600	69.2
A02B	5'-TGCCGAGCTG-3'	1000	64.1
A03A	5'-AGTCAGCCAC-3'	600	7.5
A03B	5'-AGTCAGCCAC-3'	800	35.0
A03C	5'-AGTCAGCCAC-3'	900	65.0
A04A	5'-AATCGGGCTG-3'	800	28.2
A04B	5'-AATCGGGCTG-3'	1200	79.5
A04C	5'-AATCGGGCTG-3'	1300	17.9
A04D	5'-AATCGGGCTG-3'	1350	33.3
A04E	5'-AATCGGGCTG-3'	1500	43.6
A05A	5'-AGGGGTCTTG-3'	950	40.0
A05B	5'-AGGGGTCTTG-3'	1200	90.0
A07A	5'-GAAACGGGTG-3'	700	57.9
A07B	5'-GAAACGGGTG-3'	950	47.4
A08A	5'-GTGACGTAGG-3'	950	43.6
A08B	5'-GTGACGTAGG-3'	970	46.2
A08C	5'-GTGACGTAGG-3'	1000	48.7
A08D	5'-GTGACGTAGG-3'	1200	5.1
A08E	5'-GTGACGTAGG-3'	1400	56.4
A10A	5'-GTGATCGCAG-3'	600	40.0
A10B	5'-GTGATCGCAG-3'	800	70.0
A12A	5'-TCGGCGATAG-3'	920	12.5
A12B	5'-TCGGCGATAG-3'	950	32.5
A12C	5'-TCGGCGATAG-3'	1100	7.5
A15A	5'-TTCCGAACCC-3'	700	17.9
A15B	5'-TTCCGAACCC-3'	1200	43.6
A17A	5'-GACCGCTTGT-3'	700	35.0
A17B	5'-GACCGCTTGT-3'	900	10.0
A17C	5'-GACCGCTTGT-3'	1300	15.0
F03A	5'-CCTGATCACC-3'	1000	72.5
F03B	5'-CCTGATCACC-3'	1500	70.0
F03C	5'-CCTGATCACC-3'	1600	35.0
F05A	5'-CCGAATTCCC-3'	1000	37.5
F08A	5'-GGGATATCGG-3'	950	2.6
F08B	5'-GGGATATCGG-3'	1200	7.9
F08C	5'-GGGATATCGG-3'	1600	63.2
F09A	5'-CCAAGCTTCC-3'	700	94.9
F09B	5'-CCAAGCTTCC-3'	800	48.7
F09C	5'-CCAAGCTTCC-3'	1000	53.8
F14A	5'-TGCTGCAGGT-3'	1400	70.3
F14B	5'-TGCTGCAGGT-3'	1800	2.7
Z01A	5'-TCTGTGCCAC-3'	1100	25.6
Z01B	5'-TCTGTGCCAC-3'	1700	7.7

[z]Based on EcoRI/HindIII digested lambda marker.
[y]Percentage of cultivars with band.

polymorphism to discriminate potato cultivars. All previous molecular-marker-based analyses also have been effective in generating polymorphisms to discriminate potato cultivars (Demeke et al., 1993; Desborough and Peloquin, 1968; Douches and Ludlam, 1991; Douches et al., 1990; Gebhardt et al., 1989; Gorg et al., 1992; Mori et al., 1993; Stegemann et al., 1973).

May et al. (1982) reported genetic variation within putative clones that was attributed to mislabeling. The issue of cultivar mixtures is not addressed in our study; however, due to seed certification procedures along with the use of Douches and Ludlam's (1991) electrophoretic fingerprinting method, it is an issue of minor consequence among the potato seed industry in North America. In all previous studies (as previously referenced) in which potato identification techniques were examined, intra-cultivar variation was not considered to be the primary issue in determining the value of markers for fingerprint analysis.

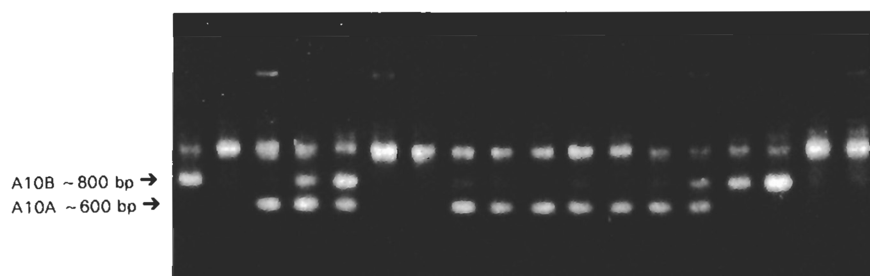

Fig. 1. (**A**) Amplification of potato DNA from a sample of potato cultivars with the A02 primer. The polymorphic fragments (A02A and A02B) were used for the fingerprint analysis to discriminate between red-skinned cultivars. (**B**) Amplification of potato DNA with the A10 primer. The polymorphic fragments (A10A and A10B) were used for the fingerprint analysis.

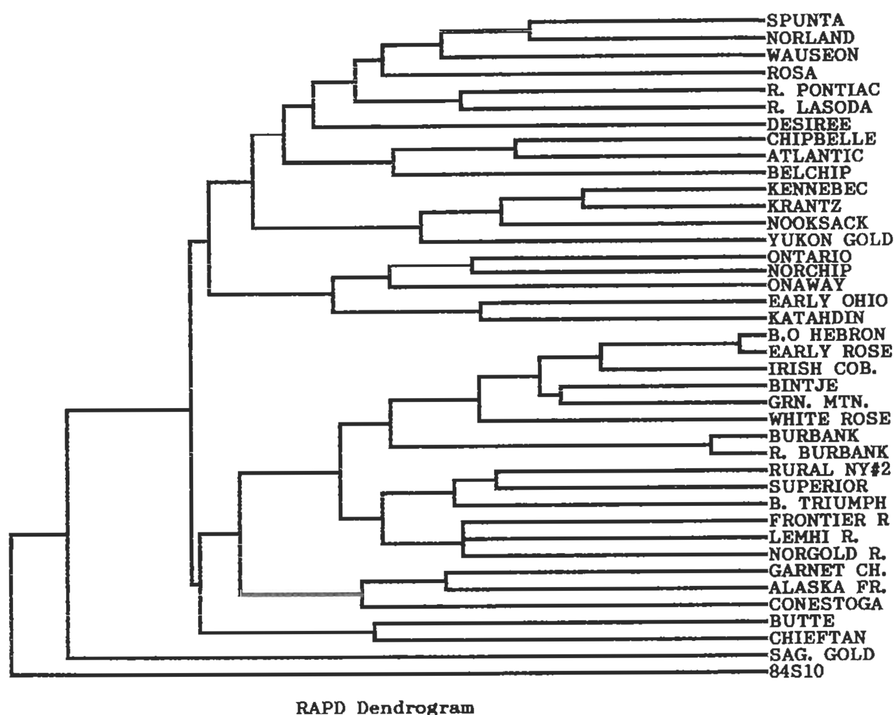

Fig. 2. Dendrogram constructed for phenetic analysis of 39 potato cultivars ($4x$) and a *S. phureja* clone ($2x$) based on 43 randomly amplified polymorphic DNA bands. The maximum distance between cultivars was 0.65.

Within the North American potato industry, numerous clonal selections of previously released cultivars have been selected; these selections have stable, identifiable traits that distinguish them from their original clone (Leever et al., 1994). RAPD marker technology potentially provides many polymorphic markers that can be used to survey the plant genome quickly. With this class of markers, the existence of intracultivar variation in potato now can be examined. Isozyme analysis does not discriminate these clonal selections (Douches and Ludlam, 1991), and this lack of discrimination may likely be due to the small number of loci (14) available for sampling. The discrimination of sports was not addressed by RFLP analyses (Douches et al., 1990; Gebhardt et al., 1989). Our PCR-based DNA amplification study examined one cultivar and its clonal variant. We were able to separate 'Burbank' from its russet sport with one band difference among the 43 bands examined. Using 20 primers, Demeke et al. (1993) also were able to discriminate clonal variants of 'Russet Burbank' and those of 'Viking' but not those clonal variants from 'Superior', 'Norland', or 'Norgold Russet'. The extent to which RAPDs can detect differences between somatic variants has not been fully addressed by our study or that of Demeke et al. (1993); however, we used twenty-nine 10-mer primers to amplify DNA from six tissue culture lines of 'Russet Burbank'. Only one band from one primer (Operon primer A8) showed variation between the six lines (data not shown). We suggest that further studies be conducted to determine the extent to which RAPDs could discriminate somatic variants of potato cultivars and concurrently examine intracultivar variation of true-to-type cultivars.

Using 13 polymorphic isozyme loci, fingerprint analysis provided an opportunity to examine cultivar relationships. Douches and Ludlam (1991) found no relationship between isozyme loci similarity and pedigree. RAPD analysis based on 43 polymorphic bands was more useful than isozyme loci in grouping the same cultivars. For example, full-sibs ('Red Lasoda'/'Red Pontiac' and 'Atlantic'/'Chipbelle'/'Belchip') clustered closely with RAPD analysis. In addition, eight of the eleven 19th century cultivars grouped together within the dendrogram; however, no strong patterns were observed for the 20th century cultivars. This grouping may be due to lower levels of genetic diversity among these cultivars, as suggested by coefficient of parentage (Mendoza and Haynes, 1974) in contrast to the

introgression of *Solanum* species in the 20th century cultivars (Plaisted and Hoopes, 1989). The value of RAPDs compared to isozyme loci for examining genetic relationships may be due to the larger number of putative loci examined in the potato genome. If more RAPDs are surveyed, a closer correlation between pedigrees and genetic relationships might be observed.

For a marker-based cultivar identification technique to be adopted by the seed industry and certification agencies, the technique must be objective, reliable, cost-effective, technically simple, and rapid. PAGE markers have the drawback of tissue specificity, and they often depend on environmental conditions at the time of tissue harvest (Stegemann et al., 1973). Analysis of plant DNA via hybridization of radio-labeled probes has been used successfully to discriminate potato cultivars, but the RFLP technique is time consuming (≤1 week) and has a high tissue requirement (Douches et al., 1990; Gebhardt et al., 1989). The isozyme technique of Douches and Ludlam (1991) has satisfied cultivar identification requirements and is being used by the potato seed industry; however, this technique has the drawback of tissue specificity and a limited numbers of scorable loci. The RAPD technique also satisfies many cultivar identification requirements, and our results, along with those of Demeke et al. (1993) and Mori et al. (1993), demonstrate the potential efficacy of using RAPDs in potato for this purpose. The procedure also is relatively quick (1 to 2 days) and technically more simple than RFLP analysis. The procedure's cost can be reduced by combining the tuber grouping with the RAPD analysis (10 primers reduced to three to four primers).

Most potato fingerprint analyses likely would be based on tuber samples. In this study, leaf tissue was the source of DNA for RAPD analysis. We also were able to isolate DNA from tuber tissue with the same procedure and obtained similar band patterns for 17 Operon primers (G10 and X1 through X16) (data not shown). Based on these limited observations, tissue source for DNA isolation should not be a significant factor in sampling cultivars for RAPD analysis. Another advantage of RAPD analysis over isozyme analysis is the many polymorphisms available, as mentioned earlier. This characteristic, if it can be applied to discriminate clonal variants, will be of significant value for seed certification agencies. If the RAPD analysis technique can be standardized across laboratories, this technology may be used in combination with key morphological characteristics or in conjunction with limited isozyme polymorphisms to discriminate potato cultivars.

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