RAPD Marker Polymorphism among Saskatoon Cultivars, Clones, and Seedlings

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Abstract. Randomly amplified polymorphic DNA (RAPD) markers were used to distinguish among 16 cultivars of saskatoon (*Amelanchier* spp.). Eight 9-base, oligonucleotide primers amplified a total of 98 DNA fragments, of which 29 were useful as reproducible polymorphic markers. Twelve cultivars and two pairs of cultivars were uniquely characterized by these 29 markers. Polymorphism was not detected among five sources of the cv. Thiessen, whereas variability was found among seedlings from self-pollinated 'Thiessen'. Samples of the cvs. Regent and Parkhill were indistinguishable from one of two sources, suggesting that the cultivars were mislabelled.

Amelanchier alnifolia Nutt. is native to western Canada, with a geographic distribution including Manitoba, Saskatchewan, Alberta, British Columbia, Yukon Territories, and the northern United States (Jones, 1946). *A. alnifolia* produces edible fruit which are commonly referred to as "saskatoons" or "saskatoon berries" in western Canada. The name saskatoon is derived from the Cree word "mis-sas-kwa-to-min," which means "tree of much wood" (Harris, 1970). Fruit from related species are known by some 18 different names, such as "serviceberry," "shadbush," and "juneberry" (Harris, 1970). The term "berry" is a misnomer, for although a saskatoon berry resembles a blueberry (*Vaccinium corymbosum* L.), it is actually a pome, and is more closely related to an apple (*Malus* spp.) or pear (*Pyrus communis* L.).

Cultivation and domestication of the saskatoon was initiated on the Canadian prairies in the early part of this century. Selection of superior genotypes began at the Beaverlodge Research Station, Alberta, in 1918 (Harris, 1976; Pruski et al., 1991; Wallace and Graham, 1976). At least 19 different cultivars

have been described by various authors (Darrow, 1975; McConkey, 1979; St. Pierre, 1991b; Stushnoff, 1990), including three from the United States: 'Parkhill', 'Regent', and 'Success' (Wallace and Graham, 1976). The species designation for these three U.S. cultivars is not consistent in the literature. Wallace and Graham (1976) suggested all three cultivars may be either *A. alnifolia* or *A. stolonifera* Wieg. However, 'Success' and 'Parkhill' have been treated as *A. sanguinea* (Pursh.) DC (Davidson and St. Pierre, 1994). Additionally, 'Success' has been designated as *A. oblongifolia* (Torr. & Gray) M. Roemer (McConkey, 1979) and *A. canadensis* (L.) Medic. (Stushnoff, 1990). Nonetheless, they will be referred to as saskatoons in this study.

Although differences among saskatoon cultivars exist (Davidson and Mazza, 1991; Davidson and St. Pierre, 1994), distinguishing among cultivars, even at maturity, is very difficult based on morphological characters (St. Pierre, 1991b). The ability to distinguish among these cultivars using a molecular marker is important in the continuing development and domestication of the saskatoon as a crop plant. Saskatoon cultivars are maintained asexually; thus, there is a need to accurately identify individual cultivars, in order to monitor clones for genetic variation and eliminate duplication. A molecular technique useful to address these problems is randomly amplified polymorphic DNA (RAPD) markers.

RAPD markers were first reported as a means of genetic fingerprinting by Williams et al. (1990) and Welsh and McClelland (1990). The technique is also known as AP-PCR, referring to arbitrarily primed polymerase chain reaction (Welsh and McClelland, 1990) and DAF, indicating DNA amplification fingerprinting (Caetano-Anolles et al., 1991). Regardless of the acronym used, the techniques

are similar in that single, oligonucleotide primers are used in a polymerase chain reaction (PCR) to amplify random segments of genomic DNA. RAPD markers have been used for cultivar or germplasm identification in a number of crops such as rice (*Oryza sativa* L. ssp. *indica* and *O. sativa* L. ssp. *japonica*) (Welsh and McClelland, 1990), corn (*Zea mays* L.), soybean (*Glycine* spp.) (Williams et al., 1990), cocoa (*Theobroma cacao* L.) (Wilde et al., 1992), cauliflower and broccoli (*Brassica oleracea* L.) (Hu and Quiros, 1991), and apples (Koller et al., 1993).

The main objective of this study was to characterize 16 saskatoon cultivars using RAPD markers. The second objective was to characterize the genetic variability among five sources of 'Thiessen' and two sources of 'Parkhill' and 'Regent', and among seedlings of self-pollinated 'Thiessen'.

Materials and Methods

Plant material. Sixteen saskatoon cultivars and seedlings from self-pollinated 'Thiessen' were characterized with RAPD markers (Table 1). Leaf samples were collected from all cultivars and seedlings in 1992. 'Parkhill' and 'Regent' were re-sampled from the Alberta Tree Nursery and Horticulture Centre (ATN) in 1993. Freshly collected leaves were washed, frozen in liquid nitrogen, and stored at -70 °C until DNA was extracted as previously described (Weir et al., 1996). Three separate DNA extractions were performed for each cultivar or seedling.

Amplification conditions and primers. Amplification reactions were carried out in 25 µL volume of solution containing: 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2.0 mM MgCl₂, 0.001% gelatin, 2 units Taq DNA polymerase (Boehringer, Mannheim, Germany), 0.2 mM of a single 9-base oligonucleotide primer, 100 mM each of dATP, dCTP, dGTP, and dTTP, and 2.5 ng of genomic DNA template. The reaction mixture was overlaid with 25 µL mineral oil. Reactions were performed in 0.5-mL microfuge tubes, which were kept on ice during preparation. Amplifications were performed on a Hybaid thermal reactor (Bio/Can Scientific, Mississauga, Ont.) programmed for 1 cycle at 95 °C for 1.5 min, followed by 35 cycles at 95 °C for 1 min, 36 °C for 1.5 min, and 73 °C for 2.25 min, using the fastest possible transition between temperatures. Temperatures were controlled by the plate temperature. The Hybaid thermal reactor was placed in a 4 °C refrigerator to reduce the cooling transition time. Results were replicated at least three times for each primer-cultivar combination (i.e., each of the three DNA extractions for each cultivar was used at least once). Eight primers were used (Table 2).

Amplification products were separated by electrophoresis on a 1.4% agarose gel containing 0.5 mg·mL⁻¹ ethidium bromide, and photographed on a UV transilluminator (Fotodyne, New Berlin, Wis.). Each amplified product was named according to the primer used and its size in base pairs (bp). For example, RC16-500 refers to a 500-bp DNA fragment ampli-

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fied by primer RC-16. Amplification products (bands) were scored as present (1) or absent (0). Only consistently amplified bands (i.e., present in all reactions) were compiled into a diagnostic fingerprint for the cultivars. In addition, if a particular band was consistently amplified for several cultivars, but inconsistently amplified for another, it was excluded as a marker for all cultivars.

Molecular weights for amplification products were determined from the graph of the linear relationship between the log of molecular weight of the standards and their migration distances. Marker VI (Boehringer, Mannheim, Germany) was used as the molecular weight standard. It is a mixture of fragments produced by cleaving plasmid pBR328 with *Bgl* I and pBR328 cleaved with *Hinf* I.

Data analysis. A phenogram was generated using the Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc), version 1.8 (Exeter Software, Setauket, N.Y.). Consistently amplified RAPD markers were used to generate a matrix based on Jaccard's coefficient of similarity. The cultivars were then clustered using the unweighted pair-group method with arithmetic averages (UPGMA) employing the SAHN (sequential, agglomerative, hierarchical and nested clustering) program from NTSYS-pc.

Results and Discussion

Molecular characterization of cultivars. A total of 98 DNA amplification products (bands) were generated using eight primers for the characterization of 16 saskatoon cultivars (Table 3). The bands generated using primer RC-28 are shown in Fig. 1. The bands generated by each primer varied in number and size. The number of bands per primer ranged from eight (RC-37) to 17 (RC-30), with an average of 12.3. Lower (6.6), similar (11), and higher (15.5) average bands per primer have been reported for *Stylosanthes* spp. (Kazan et al., 1993), celery (*Apium graveolens* L. var. *dulce*) (Yang and Quiros, 1993), and *Brassica* spp. (Demeke et al., 1992). These differences in number of bands generated per primer are probably due to differences in primer length, primer sequence, and primer-DNA template interaction among the various reports. Rafalski et al. (1991) observed that the number of bands in RAPD profiles is independent of genome complexity.

Among the 98 bands generated in this study, 29 (29.6%) were consistently amplified and used to generate a phenogram of the saskatoon cultivars (Fig. 2). Twenty-five bands (25.5%) were reproducible but showed no variation among the cultivars, and 44 (44.9%) were considered unstable and therefore excluded (Table 3). In contrast, Yang and Quiros (1993) used 28 primers to identify celery cultivars, which generated 309 bands. Of those 309 bands, only 9.3% were useful as polymorphic markers, 68% were reproducible but uninformative (monomorphic), and 22.7% were unstable. The 3-fold difference in polymorphic markers for saskatoon cultivars suggests a broader genetic base than for celery culti-

Table 1. Saskatoon cultivars fingerprinted using randomly amplified polymorphic DNA (RAPD) markers. Information compiled from Davidson and St. Pierre (1994).

Cultivar	<i>Amelanchier</i> species	Cultivar origin	Year released	Leaf source ²
Thiessen	alnifolia	Waldheim, Sask.	1976	ATN, LSN, PBI, PPS, PAR
Pembina	alnifolia	Barrhead, Alta.	1952	ATN
Moonlake	alnifolia	Moon Lake, Sask.	1974	ATN
Northline	alnifolia	Beaverlodge, Alta.	1960	ATN
Honeywood	alnifolia	Parkside, Sask.	1973	ATN
Smoky	alnifolia	Peace River, Alta.	1952	BRS
Success	sanguinea ¹	Pa.	1878	ATN
Parkhill	sanguinea ¹	Mich.	1974	MRS, ATN
Regent	alnifolia ¹	Regent, N.D.	1977	MRS, ATN
Forestburg	alnifolia	Forestburg, Alta.	1958	MRS
Pearson II	alnifolia	Bowden, Alta.	---	ATN
Altaglow	alnifolia	Lacombe, Alta.	1958	MRS
Bluff	alnifolia	Buffalo Lake, Alta.	1990	PBI
Nelson	alnifolia	Bradwell, Sask.	1992	UofS
Buffalo	alnifolia	Buffalo Lake, Alta.	1990	PBI
Martin	alnifolia	Langham, Sask.	1990	PPS
Thiessen	alnifolia	(seedlings)		LSN

¹Species designation uncertain in literature.

²Leaf sources are: ATN = Alberta Tree Nursery and Horticulture Centre, Edmonton, Alta.; PBI = Pacific Biotechnologies, Vedder Crossing, B.C.; UofS = Dept. of Horticulture Science, Univ. of Saskatchewan, Saskatoon, Sask.; MRS = Agriculture Canada Research Station, Morden, Man.; BRS = Agriculture Canada Research Station, Beaverlodge, Alta.; PPS = Prairie Plant Systems, Saskatoon, Sask.; LSN = Lakeshore Nursery, Saskatoon, Sask.; PAR = R. Parenteau, Langham, Sask.

Table 2. Nucleotide sequence of primers used to generate amplification products and detect polymorphism.

Code	Sequence	Code	Sequence
RC-12	GGG CAA TGA	RC-28	CAC GGC AGT
RC-16	GAG CGT TGT	RC-30	ACC GCT GTG
RC-19	TAC GCA CGG	RC-36	TGC GTG AGA
RC-22	TGG AGC AAG	RC-37	ACC GCC GTT

Table 3. Total number of bands, minimum and maximum band size (bp), and number of stable and unstable bands generated by the primers used to characterize the 16 saskatoon cultivars.

Primer	No. bands	Band size (bp)		Stable bands		Unstable bands
		Min	Max	Poly ¹	Mono ²	
RC-12	14	400	2200	4	1	9
RC-16	13	300	1800	3	3	7
RC-19	13	450	1750	4	3	6
RC-22	10	650	2300	1	4	5
RC-28	11	400	2300	5	2	4
RC-30	17	300	2700	8	2	7
RC-36	12	500	2600	2	7	3
RC-37	8	450	2300	2	3	3
Total	98			29	25	44

¹Polymorphic bands.

²Monomorphic bands.

Fig. 1. Amplification products for 16 saskatoon cultivars generated using standard PCR conditions and primer RC-28. Numbers above each lane correspond to the following cultivars: 1 = Thiessen, 2 = Pembina, 3 = Moonlake, 4 = Northline, 5 = Honeywood, 6 = Smoky, 7 = Success, 8 = Parkhill, 9 = Regent, 10 = Forestburg, 11 = Pearson II, 12 = Altaglow, 13 = Bluff, 14 = Nelson, 15 = Buffalo, and 16 = Martin. Lane M contains molecular weight marker VI with fragment size in base pairs (bp) indicated.

Fig. 2. Phenogram of saskatoon cultivars. Scale indicates Jaccard's coefficient of similarity.

vars. Nearly 45% of the bands generated in the current study were considered unstable, which was twice the value found by Yang and Quiros (1993). This difference in band stability may be due to differences in the stringency of the PCR in each study. Factors such as source of polymerase, DNA quality, cycling temperatures, primer sequence, and magnesium concentration are known to affect PCR stringency (Innis and Gelfand, 1990). It is also possible that the longer (10-base) primers used in the celery study were generally more stable than the 9-base primers used in this study, but there is no indication of this in the literature. Overall, these results indicate the importance of replication to identify reproducible RAPD markers.

Twelve saskatoon cultivars and two pairs of cultivars were uniquely characterized by the amplification products of as few as two primers (Table 4). The cultivars Pembina, Moonlake, Northline, Success, Parkhill, Forestburg, Altaglow, Buffalo, and Bluff were distinguished by primer RC-30, and primer RC-12 distinguished cultivars Regent, Honeywood, and Nelson. The remaining four cultivars form two pairs: 'Smoky' and 'Pearson II', and 'Thiessen' and 'Martin'. Amplification products from the other six primers did not resolve either of these two pairs (Table 4).

The number of primers needed to distinguish among cultivars in other species has been variable. Two primers were sufficient to distinguish 14 cultivars of broccoli (Hu and Quiros, 1991), one primer for 13 genotypes of cocoa (Wilde et al., 1992), 12 primers for 21 celery cultivars (Yang and Quiros, 1993), and 11 primers to distinguish 11 apple cultivars (Koller et al., 1993). In contrast, Williams and St. Clair (1993) could only distinguish nine of 19 tomato (*Lycopersicon esculentum* Mill.) cultivars using 24 primers, and Halward et al. (1992) were unable to detect any polymorphism among 25 unadapted germplasm lines of peanut (*Arachis hypogaea* L.) using 10 primers. Halward et al. (1992) reported that the RAPD results for peanut were consistent with RFLP studies, which indicated a narrow genetic base of the cultivated peanut.

The pairs of saskatoon cultivars, 'Smoky' and 'Pearson II', and 'Thiessen' and 'Martin', were not uniquely characterized by the prim-

ers used in this study. A possible reason is that they originate from a narrower genetic base relative to the other cultivars. 'Pearson II' originated from self-pollinated 'Smoky', and 'Martin' from self-pollinated 'Thiessen'. If 'Smoky' and 'Thiessen' are highly homozygous, then more primers may need to be examined to allow them to be distinguished from their respective offspring. Currently there are no scientifically valid data on morphological characteristics that distinguish 'Pearson II' from 'Smoky', or 'Martin' from 'Thiessen'. The saskatoon is normally self-pollinated, thus the possibility of homozygosity does exist. Near isogenic lines have been distinguished by screening hundreds of primers in tomato (Carland and Staskawicz, 1993), bean (*Phaseolus vulgaris* L.) (Haley et al., 1993), and lettuce (*Lactuca* spp.) (Paran et al., 1991).

Another possible explanation for the identical fingerprints for these pairs of cultivars is that 'Pearson II' and 'Martin' are the products of apomixis. Two separate reports lend some support to this hypothesis. First, studies conducted with *A. alnifolia* indicated that 20% of the flowers set fruit even when pollination was completely prevented (St. Pierre, 1991a). Second, apomictic individuals exist in the related species *A. laevis* Wiegand (Campbell et al., 1985), *A. canadensis* (L.) Medic., *A. stolonifera* Wieg. (Campbell et al., 1987) and *A. × neglecta* Egglet. (Weber and Campbell, 1989). Also, apomixis is not uncommon among other genera of the Maloideae, such as *Cotoneaster*, *Crataegus*, *Pyrus* (Campbell et al., 1985), *Sorbus*, and *Malus* (Robinson, 1982).

Monitoring clones and seedlings for genetic variability. A total of 51 bands were generated by the eight primers used to detect genetic variation among five sources of 'Thiessen', a single source of 'Martin', and eight seedlings from self-pollinated 'Thiessen' (Table 1). Of these 51 bands, no reproducible RAPD markers were found to distinguish among the five 'Thiessen' clones and 'Martin'. However, segregation of bands was detectable among 'Thiessen' seedlings using primers such as RC-19 and RC-28 (Fig. 3). A total of nine bands segregated among the seedlings (data not shown). Using these bands, all the seedlings (except 'Martin') could be distinguished from the parent 'Thiessen'. The

possibilities of screening more primers to detect polymorphism between 'Thiessen' and 'Martin', or the apomictic origin of 'Martin' were discussed above.

Amplification products were generated for all sources of 'Parkhill' and 'Regent' using the five primers: RC-12, RC-16, RC-19, RC-22, and RC-30. These primers generated 29 reproducible bands, with no detectable polymorphism among any of the 'Parkhill' sources and 'Regent' from ATN. In contrast, 10 polymorphic markers distinguished 'Regent' [Agriculture Canada Research Station, Morden, Manitoba (MRS)] from all 'Parkhill' sources and 'Regent' (ATN). In total, 16 reproducible markers were generated using all eight primers, which distinguished between 'Regent' and 'Parkhill', both from the MRS source (data not shown). These results suggest that the ATN 'Parkhill' may be identified as both 'Parkhill' and 'Regent'. 'Parkhill' and 'Regent' were obtained from ATN as part of the recently established saskatoon cultivar trials being conducted by the Dept. of Horticulture, Univ. of Saskatchewan. The practical application of RAPD analysis to screen for duplications among germplasm is illustrated by these results.

RAPD markers, with the proper replication to distinguish between reproducible and nonreproducible bands, are a reliable method to distinguish among most of the available commercial saskatoon cultivars and for monitoring clones and seedlings for genetic variability. This technique could also be used for germplasm collection and preservation, or to identify specific RAPD markers linked to desirable traits, such as disease resistance (Paran et al., 1991).

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Table 4. Consistently amplified RAPD markers scored as present (1) or absent (0) for 16 saskatoon cultivars.

RAPD marker	Cultivar number ^a															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
RC12-1190	0	0	0	0	0	1	1	1	1	0	1	0	0	1	1	0
RC12-1100	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
RC12-670	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
RC12-640	1	0	0	1	1	1	1	0	0	1	1	0	1	1	0	1
RC16-650	1	0	0	1	0	1	1	0	1	0	1	0	0	1	0	1
RC16-600	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
RC16-380	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
RC19-1760	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0
RC19-1450	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0
RC19-1390	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
RC19-540	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
RC22-800	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0
RC28-1500	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0
RC28-1325	0	0	1	0	0	0	1	1	1	0	0	0	0	0	0	0
RC28-1030	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
RC28-640	1	1	0	0	1	1	1	0	0	1	1	1	1	1	1	1
RC28-450	1	0	1	1	0	1	1	0	1	0	1	1	1	1	1	1
RC30-2000	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
RC30-1700	1	0	1	0	1	1	1	1	1	1	1	0	0	1	1	1
RC30-1100	0	1	1	1	1	0	0	1	0	0	0	1	0	1	1	0
RC30-1030	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
RC30-990	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
RC30-950	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
RC30-920	0	1	1	1	1	0	0	0	0	0	0	0	0	1	1	0
RC30-550	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	1
RC36-1100	0	1	1	1	1	1	0	1	0	1	1	1	1	1	1	0
RC36-800	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0
RC37-1050	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
RC37-970	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0

^a1 = Thiessen, 2 = Pembina, 3 = Moonlake, 4 = Northline, 5 = Honeywood, 6 = Smoky, 7 = Success, 8 = Parkhill, 9 = Regent, 10 = Forestburg, 11 = Pearson II, 12 = Altglow, 13 = Bluff, 14 = Nelson, 15 = Buffalo, and 16 = Martin.

Fig. 3. Amplification products generated using standard PCR conditions and primers RC-19 (upper) and RC-28 (lower) for 'Thiessen' (Th), 'Martin' (Ma), and eight 'Thiessen' seedlings (1-8). White arrows indicate segregating bands. Lane M contains molecular weight marker VI with fragment size in base pairs (bp) indicated.

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