Genetic Relationships among Lowbush Blueberry Genotypes as Determined by Randomly Amplified Polymorphic DNA Analysis

Karen L. Burgher, Andrew R. Jamieson, and Xuewen Lu¹

Agriculture and Agri-Food Canada, Atlantic Food and Horticulture Research Centre, 32 Main Street, Kentville, N.S., Canada B4N 1J5

ADDITIONAL INDEX WORDS. DNA fingerprinting, *Vaccinium angustifolium*, genetic similarity

ABSTRACT. Twenty-six genotypes of lowbush blueberry (*Vaccinium angustifolium* Aiton) representing four geographical zones (Maine, United States; New Brunswick, northern Nova Scotia, and western Nova Scotia, Canada) were selected to obtain DNA fingerprints and to estimate genetic similarity by randomly amplified polymorphic DNA analysis. The genotypes were either native accessions or selections from crosses involving native accessions as parents or grandparents. Thirty 10-base RAPD primers were initially screened; 11 proved to be polymorphic, resulting in 73 consistent RAPD bands. All 26 genotypes could be distinguished by their unique RAPD banding patterns and three unlabeled samples were correctly identified. The RAPD band data set was analyzed with Genstat5 to calculate similarity and distance matrices. Average similarity across all genotypes was 56%. Results from average linkage cluster analysis were used to construct a dendrogram which demonstrated six main clusters with an average similarity linkage of 70%. The selection 'Fundy' and its parent 'Augusta' clustered at 77% similarity. The corresponding principal coordinate analysis supported the clusters and identified two distinct outliers. There was a small association by geographic grouping for five genotypes from Maine. It was concluded that RAPD analysis is a useful tool for genotypic identification and estimates of genetic similarity in lowbush blueberry.

Lowbush blueberry (*V. angustifolium*) is a perennial, outbreeding, tetraploid, rhizomatous shrub with a diverse distribution ranging from northeastern to mid-North America and is restricted mainly to acidic soils (Vander Kloet, 1988). The species is an important crop in eastern Canada and northeastern United States that was derived from highly heterogeneous native stands. In Nova Scotia the lowbush blueberry is the leading fruit crop in acreage and value (McIsaac, 1999). In the early 1960s a program of genetic improvement was initiated at the Atlantic Food and Horticulture Research Centre (AFHRC), Kentville, Nova Scotia. Native genotypes selected from commercial lowbush blueberry fields in New Brunswick, Nova Scotia, and Maine formed the basis of a breeding program (Hall, 1983). The program introduced six named cultivars: three from native accessions, two from open-pollinated seedlings and one from cross-pollinated seedlings (Table 1). None of these cultivars have gained commercial success due to the costs of propagation and difficulties in plant establishment. Many original accessions and hundreds of selections are maintained by AFHRC. These accessions and selections represent a vast array of unique genotypes exhibiting diversity in plant form, productivity, and fruit characteristics including chemical composition (Kalt and McDonald, 1996). This germplasm has not been tested by molecular DNA techniques for genetic diversity.

Randomly amplified polymorphic DNA (RAPD) is a polymerase chain reaction (PCR) based on a dominant molecular marker technique (Williams *et al.*, 1990). This technique has been used for genetic fingerprinting apple [*Malus sylvestris* (L.) Mill var. *domestica* (Borkh.) Mansf.] and strawberry (*Fragaria × ananassa* Duchesne) cultivars at AFHRC since 1994 (unpublished) and could provide genetic information for lowbush blueberry. The RAPD technique is

an informative and relatively simple molecular tool that is used to estimate genetic relatedness among genotypes, identify cultivars, and construct genetic linkage maps (Tingey and del Tufo, 1993). RAPD markers have been reported widely for genotypic identification and/or relatedness studies in important fruit crops such as cranberry (*Vaccinium macrocarpon* Aiton) (Polashock and Vorsa, 1997), raspberry (*Rubus* L. sp.) (Parent and Page, 1998), apple (Autio *et al.*, 1998), plum (*Prunus* L. sp.) (Ortiz *et al.*, 1997), strawberry (Hancock and Callow, 1994) and grape (*Vitis*, subgenera *Euvitis* Planch and *Muscadinia* Planch) (Qu *et al.*, 1996). In blueberry (*Vaccinium* L. sp.), RAPD markers have been applied in genetic fingerprinting, estimation of relatedness, verification of parentage and sorting out cultivar ambiguities (Aruna *et al.*, 1993 and 1995; Levi and Rowland, 1997; and Polashock and Vorsa, 1997). The technique has also been used to establish an initial genetic linkage map for diploid *V. darrowi* Camp (Qu and Hancock, 1997; Rowland and Levi, 1994) and RAPD markers have been used in studies to determine the level and mode of heterozygosity transmitted via *2n* gametes in an interspecific cross of diploid *V. darrowi* and autotetraploid highbush blueberry, *V. corymbosum* L. (Qu and Hancock, 1995; Vorsa and Rowland, 1997). No studies regarding the level of genetic relatedness in lowbush blueberry have been reported with RAPD data. Hokanson and Hancock (1998), examined allozyme data to determine the level of genetic variation in three *Vaccinium* sp. and reported that lowbush blueberry has a high level of genetic diversity with an average heterozygosity of 57%.

The purpose of this study was to apply the RAPD technique to explore the level of relatedness among lowbush blueberry genotypes. The objectives were to 1) establish a DNA extraction technique for lowbush blueberry, 2) obtain DNA fingerprints for the cultivars and selections, and 3) estimate genetic similarity among 26 genotypes representing four different geographical origins.

Materials and Methods

PLANT MATERIAL. All plant material for this study was *V. angustifolium* maintained in plots at the Sheffield Farm (Kings

Received for publication 16 Feb. 2001. Accepted for publication 15 Aug. 2001. Contribution 2230. 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.

¹Current address: Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre, 43 McGillivray Street, c/o University of Guelph, Guelph, Ontario, Canada N1G 2W1.

County, Nova Scotia). The 26 genotypes selected had varied origins and parents (Table 1). Nine genotypes were native accessions, i.e., part of a larger collection of genotypes selected from commercial lowbush blueberry fields from 1963 to 1968 (Aalders, 1979). Many of these genotypes were used as parents in a breeding program initiated in 1967. Fourteen genotypes had been selected from this breeding program and three were mystery samples (i.e., identity unknown to the principal investigator) chosen for a blind test of the analysis. Twenty two of the genotypes could also be grouped by geographical origin. Five had originated in Maine (Maine group); six were selected, or had parents selected, in northern Nova Scotia or southern New Brunswick (North group); seven were selected, or had parents selected, in western Nova Scotia (West group); and four genotypes had parents selected from more than one geographical group.

DNA EXTRACTIONS. Dormant plants of the 26 genotypes were dug from the field in Nov. 1997, placed in pots with soil and stored at 2 °C. After 3 months the potted plants were placed in greenhouse conditions (days/nights of 21 ± 1 °C/ 16 ± 1 °C with ≈ 10 h of natural day light) to initiate a break in dormancy for leaf collection and DNA extractions. Terminal and lateral leaf buds with two or three expanded leaves were collected and then immediately placed on ice prior to grinding with liquid nitrogen. Total cellular DNA was extracted from 0.1 g of leaf tissue using a simplified 2% CTAB method adapted from Doyle and Doyle (1990) and Cheng et al. (1997). The protocol is as follows: frozen ground tissue was transferred to 1 mL of extraction buffer (Cheng et al., 1997) in a 1.5 mL Eppendorf microcentrifuge tube and incubated at 65 °C for 60 min. Samples were centrifuged for 10 min at 8944 g_n and the

supernatant transferred to a 2 mL microcentrifuge tube. An equal amount of 24 chloroform : 1 iso-amyl alcohol (v/v) was added and the samples were gently mixed and centrifuged for 10 min at 8944 g_n . The aqueous phase was transferred to a new 1.5-mL centrifuge tube and treated with 1 μ L (500 ng- μ L⁻¹) of preboiled RNase (Boehringer Mannheim GmbH) at 38 °C for 20 min. An equal volume of cold isopropanol was added to each sample and set at 4 °C for 60 min. Samples were centrifuged at 8944 g_n for 20 min at 4 °C. The DNA pellet was washed with 75% cold ethanol, vacuum dried, and dissolved in 100 μ L of TE buffer (10 mM TRIS, 1 mM EDTA). DNA quality and quantity were determined by electrophoresis in 0.8 % agarose gels and by spectrophotometry and then subsamples were diluted to 10 ng- μ L⁻¹ for PCR.

DNA AMPLIFICATION CONDITIONS. Thirty 10-base primers from the Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia, Canada were chosen for this study. Thirteen of these primers had been shown previously to be polymorphic in some *Vaccinium* sp. (Rowland and Levi, 1994). All amplification reactions were in 25 μ L volumes with the following conditions: 1.2 ng- μ L⁻¹ of template DNA; 1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 0.001% v/v gelatin (Sigma PCR buffer, Oakville, Ont.); 100 μ M dATP, dCTP, dGTP, and dTTP (Amersham Pharmacia Biotech, Baie d'Urfe, Quebec, Canada); 0.36 μ M of 10-base primer (Biotechnology Laboratory, UBC) and 0.02 unit/ μ L *Taq* DNA polymerase (Sigma). A control reaction, with no template DNA, was included with each primer and a complete set of the 26 lowbush blueberry DNA samples. Thermal cycling was performed with a thermocycler (PTC-100; MJ Research, Inc., Watertown, Mass.) for 35 cycles. Samples were dena-

Table 1. Code number, lineage, geographical origin, and geographical group for 26 genotypes of lowbush blueberry that were surveyed for genetic relatedness.

No.	Code	Lineage	Geographical origin	Geographical group
1	206	Native accession	Kings or Hants Co. N.S., Canada	West
2	416	Native accession	Pictou Co. N.S., Canada	North
3	537	Native accession	Pictou Co. N.S.	North
4	607	Native accession	Annapolis Co. N.S., Canada	West
5	633	Native accession	Annapolis Co. N.S.	West
6	701	Native accession	Annapolis Co. N.S.	West
Named native accessions				
7	BRN	Brunswick (734)	Albert Co. N.B., Canada	North
8	CHG	Chignecto (508)	Cumberland Co. N.S., Canada	North
9	CMB	Cumberland (510)	Cumberland Co. N.S.	North
Named selections				
10	AUG	Augusta (ME3302 x OP ²)	Maine, United States and unknown	Maine and unknown
11	BLM	Blomidon (451 x AUG)	Cumberland Co. N.S. and Maine	West and Maine
12	FDY	Fundy (AUG x OP)	Maine and unknown	Maine and unknown
Selections				
13	72-3	Unknown	Unknown	Unknown
14	73-7	680 x 670	Yarmouth Co., Canada and Annapolis Co. N.S.	West
15	73-9	680 x 662	Yarmouth Co. and Annapolis Co. N.S.	West
16	73-10	97 x 680	Cumberland Co. and Yarmouth Co. N.S.	North and West
17	73-14	682 x 760	Yarmouth Co. and Albert Co. N.B.	West and North
18	77-10	(97 x 680) x (682 x 794)	Cumberland Co. and Yarmouth Co. N.S.	North and West
19	79-12	510 x 325	Cumberland Co. and Cumberland Co. N.S.	North
20	80-28	(670 x 676) x 676	Annapolis Co. and Yarmouth Co. N.S.	West
21	83-5	ME4161 x 72-3	Maine and unknown	Maine and unknown
24	ME1-H	ME1 (high growth habit)	Maine	Maine
23	ME1-L	ME1 (low growth habit)	Maine	Maine
24-26	MYS- 1,4,5	Mystery samples	Unknown	Unknown

²OP = open-pollinated.

tured for 1 min at 94 °C, annealed for 1 min at 36 °C and then held at 72 °C for 2 min as an extension phase. The last cycle had an extra 5 min for the extension period (modified from Aruna et al., 1995). The products from PCR (20 µL) were separated by electrophoresis with a 1.4% agarose gel in 1× TBA buffer (Sigma) and stained with ethidium bromide at 0.5 µg·mL⁻¹. Band visualization was with ultraviolet light and gels were documented by photograph or the Gel Doc 2000 system (BioRad, Mississauga, Ont.). Band size was determined with a 123 base pair (bp) DNA ladder (Sigma).

BAND SCORING AND DATA ANALYSIS. RAPD bands were scored as present (1) or absent (0) in each genotype for each set of primer and only strong, clear, and reproducible bands were used in this study. Two replications of DNA extraction and PCR done on separate days were scored for each lowbush blueberry genotype. The band scoring data were analyzed with Genstat 5 (Genstat Committee, 1993), to calculate the similarity (*s*) and distance (*d*) matrices with the following equations: $s = (2N_{xy}) / (N_x + N_y)$ and $d = -1 \times \log_{10}(s)$, where *N_{xy}* is the number of shared bands between the genotypes *x* and *y* and *N_x* and *N_y* represent the total number of bands found in each genotype (Nei and Li, 1979). Average linkage cluster and nearest neighbor analysis was performed on the similarity matrix with the Genstat5 HCLUSTER directive to construct a dendrogram and three dimensional principal coordinate (3-D PCO) analysis to demonstrate genetic relationships.

Results and Discussion

Initially 30 primers were used to screen four lowbush blueberry genotypes (‘Cumberland’, ‘Augusta’, 72-3, and 633). Eleven of these primers proved to be polymorphic and consistent for band presence and intensity, therefore, these were used for the complete survey of 26 genotypes. Prescreening of the primers was done because choosing primers that have an increased band polymorphism and reproducibility reduces scoring error and increases consistent replications (Skroch and Nienhuis, 1995). Nine of these 11 primers had been reported previously to be polymorphic in *Vaccinium* sp. (Roland and Levi, 1994). The 11 polymorphic primers resulted in 138 scorable bands and 73 (53%) bands that were polymorphic for the sampled genotypes. From this RAPD data set three bands were found to be unique for specific genotypes. Selections ‘Fundy’ and MYS-5 had a unique band with primer UBC-280 at 1.8 kb and the accession ME1-H had two unique bands with UBC-280 and UBC-293 at 0.4 and 1.0 kb respectively (data not pre-

sented). Plants of ME1-H are tall with large berries, a growth habit more typical of *V. angustifolium* × *V. corymbosum* hybrids, thus unique ME1-H bands may be markers of *V. corymbosum* traits.

The number of polymorphic bands per primer ranged from 2 to 14 (Table 2). The number of unique band patterns per primer ranged from 4 to 20 and primer UBC-287 appeared to be highly polymorphic and produced 20 unique patterns. By combining the primers it was possible to distinguish the 26 genotypes in this study, and the 3 mystery samples had banding patterns similar to 2 of the known genotypes.

The similarity matrix (Table 3) was calculated from the pairwise marker data. The average similarity value (*s*) across all the genotypes in this study was 56% and this was identical to the value reported by Levi and Rowland (1997) for 12 cultivars of *V. corymbosum*. The three mystery samples showed very close identity to known genotypes: MYS-5 and ‘Fundy’ had *s* = 96%, while genotypes MYS-1 and MYS-4 were identical (*s* = 100%) or nearly identical to selection 72-3 (*s* = 99%), respectively. The *s* values for the mystery samples and the closest genotype were not equal to 100% because of scoring and/or sampling error. Skroch and Nienhuis (1995) measured scoring and sampling error in *Phaseolus* L. sp. with a RAPD data set of 192 bands. They reported a scoring error lower than 2% for bold and medium intensity bands (which were only scored in our study) and this type of error is independent of the total number of RAPD bands in the data set. Another source of unavoidable scoring error is that nonhomologous bands can comigrate due to similar band size and are thus scored as homologous. Sampling error may be the main source of error for variation from RAPD data estimates of genetic distance and is inversely related to total band number. Sampling error can be reduced by increasing the number of primers screened, thereby increasing the number of bold RAPD bands scored. Our sampling error was comparable to other studies of ploid species with RAPD data sets of ≈100 bands. (Aruna et al., 1993; Levi and Rowland, 1997; Porebski and Catling, 1998).

Average linkage cluster analysis (Fig. 1) produced a dendrogram with six main groups as defined by percentage similarity. Based on nearest neighbor calculations, the average linkage was 70% (excluding mystery samples which were expected to have *s* values near 100%). The first division was at 50% similarity and resulted in two main clusters. The lower cluster contained 78% of the native accessions and only three of the bred selections. The top cluster contained 79% of the bred selections including all the genotypes that

Table 2. Primer name, sequence, number and percentage of polymorphic RAPD bands and number of unique band profiles for each primer among 26 genotypes of lowbush blueberry.

UBC-primer	Sequence 5'–3'	Polymorphic bands/primer (no.)	Polymorphic bands/primer (%)	Unique band profiles (no.)
UBC-76	GAGCACCAGT	2	22.2	4
UBC-85	GTGCTCGTGC	6	66.7	14
UBC-203	CACGGCGAGT	4	30.8	9
UBC-222	AAGCCTCCCC	6	46.2	17
UBC-239	CTGAAGCGGA	14	77.8	21
UBC-244	CAGCCAACCG	4	28.6	12
UBC-268	AGGCCGCTTA	5	41.7	14
UBC-280	CTGGGAGTGG	7	50.0	16
UBC-287	CGAACGGCGG	8	53.3	20
UBC-292	AAACAGCCCCG	8	72.7	17
UBC-293	TCGTGTTGCT	9	90.0	16

Table 3. Percentage similarity among 26 genotypes of lowbush blueberry.

Genotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1 206	100																									
2 416	55	100																								
3 537	59	63	100																							
4 607	65	68	64	100																						
5 633	62	63	61	75	100																					
6 701	58	49	49	58	57	100																				
7 Brunswick	60	53	50	49	62	40	100																			
8 Chignecto	52	54	54	61	54	60	62	100																		
9 Cumberland	47	52	43	54	68	42	69	52	100																	
10 Augusta	59	55	49	65	65	46	60	48	53	100																
11 Blomidon	53	55	43	60	59	42	54	55	50	73	100															
12 Fundy	53	58	46	64	52	50	51	55	53	77	63	100														
13 72-3	59	49	61	51	55	41	56	57	55	52	49	49	100													
14 73-7	60	60	56	68	68	40	54	62	57	69	57	62	70	100												
15 73-9	56	46	45	48	45	41	47	48	43	53	39	55	55	59	100											
16 73-10	46	61	51	63	62	35	60	56	57	63	61	57	59	69	60	100										
17 73-14	56	48	41	54	45	53	43	51	39	49	45	46	41	50	44	51	100									
18 77-10	54	50	50	61	60	43	58	53	51	71	62	60	50	65	54	59	62	100								
19 79-12	56	55	49	56	58	39	56	58	56	68	63	57	55	71	52	70	43	57	100							
20 80-28	61	62	58	78	63	47	53	60	61	64	59	66	55	75	64	67	46	57	66	100						
21 83-5	49	40	42	51	49	38	50	44	59	52	46	52	76	59	52	56	48	53	49	52	100					
22 MEI-H	59	58	50	57	58	41	54	55	54	59	51	61	58	62	65	65	56	55	61	63	64	100				
23 MEI-L	56	55	42	62	65	45	50	61	59	66	63	49	58	63	41	63	48	57	68	58	61	59	100			
24 MYS-1	61	48	63	53	57	41	55	56	58	52	48	49	99	70	54	58	44	49	54	54	78	60	60	100		
25 MYS-4	61	48	63	53	57	41	55	56	58	52	48	49	99	70	54	58	44	49	54	54	78	60	60	100	100	
26 MYS-5	55	60	46	63	54	46	56	54	52	75	68	96	49	61	55	59	45	63	58	65	51	60	52	48	48	100

The grouping in the 3-D PCO analysis showed similar trends as the dendrogram (Fig. 2). A 3-D PCO plot is useful to distinguish closely related individuals into groups as has been reported with RAPD data from plums (Ortiz et al., 1997); junipers (*Juniperus* L. sp.) (Le Duc et al., 1999); and olives (*Olea europaea* L.) (Gemas et al., 2000). In this study the first three dimensions explained 69% of the variation. The genotypes 'Blomidon' and 'Fundy' have 'Au-

Although there was little grouping by geographical origin for the Nova Scotian native accessions, those genotypes with a Maine origin had a close association (e.g., 'Augusta', 'Blomidon', 'Fundy', ME1-H, and ME1-L). It is unknown how genetically diverse these Maine accessions are, as they were acquired in the 1930s and limited

A 3D biplot illustrating the relationship between various sampling locations and three principal components (PCO 1, PCO 2, and PCO 3). The plot shows vectors for each location, indicating their relative positions along the three axes.

Location	PCO 1 (29%)	PCO 2 (23%)	PCO 3 (17%)
701	-0.8	0.0	0.6
73-9	0.5	0.8	0.5
206	0.0	0.2	0.4
537	0.2	0.1	0.3
73-14	-0.2	0.1	0.2
FDY	-0.3	0.0	0.1
607	-0.4	0.0	0.0
MYS-5	-0.5	0.0	0.0
CHG	-0.2	0.0	0.0
80-28	0.1	0.0	0.0
77-10	0.0	0.0	0.0
AUG	-0.1	0.0	0.0
633	-0.2	0.0	0.0
416	-0.6	0.0	0.0
BLM	-0.5	0.0	0.0
ME1-H	0.3	0.3	0.1
72-3	0.6	0.4	0.1
MYS-1	0.6	0.4	0.1
MYS-4	0.6	0.4	0.1
83-5	0.7	0.5	0.1
73-7	0.4	0.2	0.0
79-12	0.2	0.1	0.0
BRN	0.3	0.1	0.0
73-10	0.4	0.2	0.0
ME1-L	0.3	0.1	0.0
CMB	0.4	0.2	0.0

J. AMER. SOC. HORT. SCI. 127(1):98–103, 2002.

information was available on their specific origin. These Maine accessions may have a common parent or similar place of collection.

The white fruit trait in lowbush blueberry was initially thought to be recessive and follow disomic inheritance as is expected for an allotetraploid species (Hall and Aalders, 1963). Selection 80-28 is easily distinguished as it has albino fruit and it was selected from a backcross of blue and albino fruited native accessions. A unique marker for 80-28 might have been found in this study due to increased double recessive alleles from backcrossing, but no RAPD marker was observed from the data set. Hokanson and Hancock (1993) reanalyzed the data of Hall and Alders (1963) and showed that the white-fruited trait has a higher probability for tetrasomic inheritance. Another marker study using allozymes (Hokanson and Hancock, 1998), reported that lowbush blueberry is an autopolyploid with tetrasomic inheritance, therefore more complex inheritance ratios would be expected (5:1 vs. 3:1 for disomic inheritance). If lowbush blueberry is an autopolyploid, this may explain why a unique band was not found in our data set of only 72 RAPD bands. However, Hokanson and Hancock (1998) reported a lower level of observed mean heterozygosity in lowbush blueberry (57.1%) than in the autotetraploid highbush blueberry (75.6%). This lower level of heterozygosity would be expected in allotetraploid species with a fixed heterozygosity, although lowbush blueberry is probably an autotetraploid as it can freely intercross with highbush blueberry. Further inquiry with segregating molecular marker data may establish ploidy status for lowbush blueberry.

RAPD markers are dominant markers and as a result, homozygotes can not be distinguished from heterozygotes (Lynch and Milligan, 1994; Williams et al., 1990). Polymorphic bands only reflect double recessive alleles and this reduces the accuracy of the estimates of similarity. Therefore, values of s may be higher than the expected 50% relatedness for parent-progeny associations. For this study the average estimates of similarity are close to 78% where relatedness is known to be 50% (e.g., 'Augusta' and 'Fundy' $s = 77\%$) and s values <70% were observed when relatedness was known to be <50% or unknown (e.g., for 'Chignecto' and 701 $s = 60\%$). These findings are in accord with those calculated by Lynch and Milligan (1994). They reported that within a set of 100 RAPD bands, s values would be near 78% when relatedness was equal to 0.5 and s values would be >50% when relatedness is known to be <0.5. Therefore, estimates of similarity and identity can be realized with RAPD band data for *V. angustifolium* (as shown herein), but the relevant distribution of s values within a band data set should be considered. Other studies have reported varied success with RAPD markers used to estimate genetic relatedness in *Vaccinium* sp. Aruna et al. (1993) were able to distinguish rabbiteye blueberry (*V. ashei* Reade, a hexaploid) cultivars. Whereas Levi and Rowland (1997) cautioned against drawing genetic relatedness conclusions using RAPD markers as the cluster analysis in highbush blueberry did not reflect known pedigrees. Perhaps all aspects of the nature of each species (i.e., ploidy level, outcrossing rates, mode of inheritance, and growth type) need to be considered when applying RAPD data analysis for intraspecies relatedness.

RAPD analysis is a useful molecular tool to correctly identify lowbush blueberry genotypes. Average linkage values reflected known pedigrees and were in agreement with expected values in RAPD analysis. Genetic fingerprints of several *V. angustifolium* genotypes have been established. Estimates of similarity among closely related genotypes may be used to explore genetic relationships in further studies of pollination, fruit composition, and morphological traits of the lowbush blueberry.

Literature Cited

- Aalders, L.E., I.V. Hall, and A.C. Brydon. 1979. A comparison of fruit yields of lowbush blueberry clonal lines and related seedling progenies. *Can. J. Plant Sci.* 59:875-977.
- Aruna, M., M.E. Austin, and P. Ozias-Akins. 1995. Randomly amplified polymorphic DNA fingerprinting for identifying rabbiteye blueberry (*Vaccinium ashei* Reade) cultivars. *J. Amer. Soc. Hort. Sci.* 120:710-713.
- Aruna, M., P. Ozias-Akins, M.E. Austin, and G. Kochert. 1993. Genetic relatedness among rabbit eye blueberry (*Vaccinium ashei*) cultivars determined by DNA amplification using single primers of arbitrary sequence. *Genome* 36:971-977.
- Autio, W.R., J.R. Schupp, D.C. Ferree, R. Glavin, and D.L. Mulcahy. 1998. Application of RAPDs to DNA extraction from apple rootstocks. *HortScience* 33:333-335.
- Cheng, F.S., S.K. Brown, and N.F. Weeden. 1997. A DNA extraction protocol from various tissues in woody species. *HortScience* 32:921-922.
- Doyle, J.J. and J.L. Doyle. 1990. Isolation of plant DNA from fresh tissue. *Focus* 12:13-15.
- Gemas, V.J., M.J. Rijo-Johansen, R. Tenreiro, and P. Feveireiro. 2000. Inter- and intra analysis of three *Olea europaea* L. cultivars using the RAPD technique. *J. Hort. Sci. Biotechnol.* 75:312-319.
- Genstat Committee. 1993. Genstat 5 reference manual. Release 4. Clarendon Press, Oxford, United Kingdom.
- Hall, I.V. 1983. Genetic improvement of the lowbush blueberry, *Vaccinium angustifolium*. *Can. J. Plant Sci.* 63:1091-1092.
- Hall, I.V. and L.E. Aalders. 1963. Two-factor inheritance of white fruit in the common lowbush blueberry, *Vaccinium angustifolium* Ait. *Can. J. Genet. Cytol.* 5:371-373.
- Hancock, J.F. and P.A. Callow. 1994. Randomly amplified polymorphic DNAs in the cultivated strawberry, *Fragaria xananassa*. *J. Amer. Hort. Sci.* 119:862-864.
- Hokanson, K. and J. Hancock. 1993. The common lowbush blueberry, *Vaccinium angustifolium* Aiton, may be an autopolyploid. *Can. J. Plant Sci.* 73:889-891.
- Hokanson, K. and J. Hancock. 1998. Levels of allozymic diversity in diploid and tetraploid *Vaccinium* sect. *Cyanococcus* (blueberries). *Can. J. Plant Sci.* 78:327-332.
- Kalt, W. and J.E. McDonald. 1996. Chemical composition of lowbush blueberry. *J. Amer. Soc. Hort. Sci.* 121:142-146.
- Le Duc, A., R.P. Adams, and M. Zhong. 1999. Using random amplification of polymorphic DNA for a taxonomic reevaluation of pfitzer junipers. *HortScience* 34:1123-1125.
- Levi, A. and L.J. Rowland. 1997. Identifying blueberry cultivars and evaluating their genetic relationships using randomly amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR)-anchored primers. *J. Amer. Soc. Hort. Sci.* 122:74-78.
- Lynch, M. and B.G. Milligan. 1994. Analysis of population genetic structure with RAPD markers. *Mol. Ecol.* 3:91-99.
- McIsaac, D. 1999. Wild blueberry production and marketing in Nova Scotia. A situation report. Nova Scotia Dept. Agr. Mktg., Nappan, Nova Scotia.
- Nei, M. and W. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA* 76:5269-5273.
- Ortiz, A., R. Renaud, I. Calzada, and E. Ritter. 1997. Analysis of plum cultivars with RAPD markers. *J. Hort. Sci.* 72:1-9.
- Parent, J.G. and D. Page. 1998. Identification of raspberry cultivars by sequence characterized amplified region DNA analysis. *HortScience* 33:140-142.
- Polashock, J.J. and N. Vorsa. 1997. Evaluation of fingerprinting techniques for differentiation of cranberry and blueberry varieties. *Acta Hort.* 446:239-242.
- Porebski, S. and P.M. Catling. 1998. RAPD analysis of the relationship of North and South America subspecies of *Fragaria chiloensis*. *Can. J. Bot.* 76:1812-1817.
- Qu, L. and J.F. Hancock. 1995. Nature of 2n gamete formation and mode of inheritance in interspecific hybrids of diploid *Vaccinium darrowi* and tetraploid *V. corymbosum*. *Theor. Appl. Genet.* 91:1309-1315.
- Qu, L. and J.F. Hancock. 1997. Randomly amplified polymorphic DNA (RAPD)-based genetic linkage map of blueberry derived from an interspecific cross between diploid *Vaccinium darrowi* and tetraploid *V. corymbosum*. *J. Amer. Soc. Hort. Sci.* 122:69-73.
- Qu, X., J. Lu, and O. Lamikanra. 1996. Genetic diversity in muscadine and american bunch grapes based on randomly amplified polymorphic DNA (RAPD) analysis. *J. Amer. Soc. Hort. Sci.* 121:1020-1023.
- Rowland, L.J. and A. Levi. 1994. RAPD-based genetic linkage map of blueberry derived from a cross between diploid species (*Vaccinium darrowi* and *V. elliotii*). *Theor. Appl. Genet.* 87:863-868.
- Skroch, P.W. and J. Nienhuis. 1995. Impact of scoring error and reproducibility of RAPD data on RAPD based estimates of genetic distance. *Theor. Appl. Genet.* 91:1086-1091.
- Tingey, S.V. and J.P. del Tufo. 1993. Genetic analysis with random amplified polymorphic DNA markers. *Plant Physiol.* 101:349-352.
- Vander Kloet, S.P. 1988. The Genus *Vaccinium* in North America. *Res. Branch Agr. Can. Publ.* 1828.
- Vorsa, N. and L.J. Rowland. 1997. Estimation of 2n megagametophyte heterozygosity in a diploid blueberry (*Vaccinium darrowi* Camp) clone using RAPDs. *J. Hered.* 88:423-426.
- Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18:6531-6535.