DIVERSITY ANALYSIS WITHIN A COLLECTION OF WILD CRANBERRY CLONES AND CULTIVARS

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Diversity Analysis within a Collection of Wild Cranberry Clones and Cultivars

by

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Abstract

The cranberry (*Vaccinium macrocarpon* Ait.) is a commercial fruit in Canada with great potential for health benefit. However, the paucity of application with the multifarious DNA markers has hampered the advance of study on cranberry. Therefore, genetic variation and relationship were studied among 102 wild cranberry clones collected from four Canadian provinces (Newfoundland and Labrador, New Brunswick, Prince Edward Island, and Nova Scotia) and five cranberry cultivars using inter simple sequence repeat (ISSR), expressed sequence tag-polymerase chain reaction (EST-PCR), and EST-simple sequence repeat (SSR) markers. Although all three markers discriminated 107 cranberry genotypes effectively, ISSR markers generated the highest number of polymorphic bands and showed the highest values of polymorphic information content (0.97), expected heterozygosity (0.97), and marker index (1.20). These ISSR index values were followed by those of EST-PCR (0.56, 0.60, and 0.56, respectively) and EST-SSR (0.74, 0.77, and 0.77, respectively). The co-dominant markers, EST-PCR (0.54) and EST-SSR (0.35) showed higher major allele frequencies than the dominant ISSR marker (0.08). The unweighted pair-group method with arithmetic averages (UPGMA) analysis depicted the relationships among the genotypes in dendrogram topologies of three DNA markers, solely and in combination. Cluster analysis by the UPGMA separated the 102 wild clones and 5 cultivars into four main clusters with ISSR markers, three main clusters and one outlier with EST-PCR markers, six main clusters with EST-SSR markers, and three main clusters with an outlier with the combination of three markers. With solely DNA markers and the combination of three markers, principal co-ordinates (PCo) analysis

confirmed the UPGMA analysis, although some differences were observed. Analysis of molecular variation detected a sufficient variation among genotypes within communities and among communities within provinces with ISSR (66.29% and 35.50%, respectively), EST-PCR (71.52% and 33.87%, respectively), and EST-SSR (71.76% and 33.60%, respectively) markers, and with the combination of the three markers (70.96% and 34.54%, respectively). Insignificant variation was observed among provinces with all markers (-1.79%, -5.40%, -5.36%, and -5.50% of total variation for ISSR, EST-PCR, and EST-SSR markers, and for combination of three markers, respectively). Combined use of three molecular markers revealed a sufficient degree of variation to differentiate among cranberry genotypes, making these technologies valuable for cultivar identification and for the more efficient choice of parents in the current cranberry breeding program.

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List of Abbreviations

AAFC: Agriculture and Agri-Food Canada, AFLP: amplified fragment length polymorphism, A_i : assay efficiency index, AMOVA: analysis of molecular variance, BE: 'Ben Lear', CCC: cophenetic correlation coefficient, CPGS: Canada's Plant Germplasm System, DNA: deoxyribonucleic acid, EST-PCR: expressed sequence tag-polymerase chain reaction, FAO: Food and Agriculture Organization, FR: 'Franklin', GRIN-CA: genetic resource information network of Canada, He: expected heterozygosity, ISSR: inter simple sequence repeat, IUCN: International Union for Conservation of Nature, MI: marker index, MR: multiple ratio, mRNA: messenger ribonucleic acid, NB: New Brunswick, n_e : the effective number of alleles per locus, N_e : the total number of alleles, NL: Newfoundland and Labrador, NS: Nova Scotia, NTSYS: numerical taxonomy system, PCo: principle co-ordinate, PE/PEI: Prince Edward Island, PI: 'Pilgrim', PIC: polymorphism information content, RAPD: random fragments of genomic DNA, RFLP: restriction fragment length polymorphism, Rp: resolving power, SCAR: sequence-characterised amplified region, SSR: simple sequence repeat, ST: 'Stevens', UPGMA: unweighted pair-group method with arithmetic averages, WI: 'Wilcox'.

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1.1 Cranberry botany and health benefit of cranberries

1.1.1 Cranberry botany

The cranberry (*Vaccinium macrocarpon* Ait; Figure 1), also called American cranberry, is a member of the family *Ericaceae*. It is a slender, woody, creeping, evergreen, and perennial plant, native to North America (Vander Kloet, 1983). Cranberry is one of the commercially valuable fruits in *Vaccinium* species, along with blueberry and lingonberry. Cranberry plantations in Canada is located in British Columbia, Quebec, Newfoundland, New Brunswick, and Nova Scotia (Roper and Vorsa, 1997). The anthocyanin, which contributes the red color to cranberries, is valued for nutrition. The European cranberry variety, known as *Vaccinium oxycoccus* L., is mainly cultivated in parts of central Europe, such as in Finland and Germany. The fruit is smaller with slightly different acid and anthocyanins profiles campared with the North American variety (Girard and Sinha, 2006).

Figure 1: Greenhouse-grown NB4 cranberry clone collected from New Brunswick.

1.1.2 Cranberry industry and production

After getting through uncontrolled flood or drought, fire, uncontrollable pests, and isolation (Roper and Vorsa, 1997), approximately 500–700 million pounds of American cranberries are commercially harvested annually, primarily across the northern United States (85%) and Canada (15%), as well as smaller amounts produced in Chile (Cunningham et al., 2004).

Production of cranberry in Canada is expanding. Cranberry products include fresh fruit, dried fruit, sauces, juices, and ingredients. Among them, juice covers about 60% of the market; sauces, dried fruit, and ingredients covers 35%, and fresh fruit market accounts for other 5% (Zuo et al., 2002).

1.1.3 Cranberry health benefits and medical functions

The cranberry has for centuries been considered as a health food and as a medicine to treat diverse ailments (Pappas and Schaich, 2009). Cranberries and cranberry constituents have shown therapeutic activities such as anti-bacterial (Leitao et al., 2005), anti-carcinogenic (Sun and Liu, 2006), anti-viral (Weiss et al., 2005), anti-mutagenic (Vattem et al., 2006), anti-angiogenic (Roy et al., 2002), anti-tumorigenic (Seeram et al., 2004), prevention of cardiovascular disease (McKay and Blumberg, 2007), and prevention of stomach and oral ulcers (Weiss et al., 2002; 2004), as well as in the prevention of urinary tract infection (Howell, 2007).

1.2 Wild germplasm

Germplasm is a set of plant parts from which entire plants can be regenerated and representing a desired set of genetic resources (i.e. genes, gene frequencies, or genetic combinations), from which a whole plant can be regenerated (What is germplasm, 2013). Plant germplasm carries genetic information for the plant's hereditary makeup. An accession in a gene bank is similar to a book in a library, containing a title (species, population, parental line), editor (collector or breeder) and brief summary (phenotypic information, date of collection, etc) accessible in a database. For most crops, there is a very wide gene pool in the wild species in comparison with the limited range of genetic variability in collections at the existing gene centers (Hawkes, 1977).

From a pragmatic view point, a breeder should obviously consider principally working with collections made ancient and current cultivars and breeding stocks to achieve a genetic traceability to avoid problems in cross compatibility required before considering to wild species. Wild germplasm is also defined as that is not grown as a crop in the most simple way. Knowledge of the phylogeny, taxonomy, and geographical distribution of wild species is required for their best use in a breeding program. Most importance of all, wild species may possess a whole set of favourable (e.g. pest resistance) or unfavourable agronomic features (eg. low yield or poor flavour). Hawkes (1977) gave a general review of the importance of wild germplasm and evaluated its potential in plant breeding research.

The genetic study for major crops has been a concern for breeder. Therefore, the need of broadening the genetic background of crops has been widely recognized (Chang, 1985; Duvick 1984). Major crops differ from their wild ancestors by a group of characters (Purseglove, 1981). For most plants, an extremely large gene source exists in wild species. For decades, the goal has been increasing for additional and improved existing characters which increasing accessibilities for breeding crops (Brown and Marshall, 1986). This need has strong recommendation for wider collection, more resource for conservation and utility of wild germplasm (Goodman, 1985; Hawkes, 1977). Wild genetic resources provide benefits for plant breeders for several reasons

(Lenné and Wood, 1991). The geographical and environmental expansion of range that changed ecological preference and increased susceptibility to diseases and pests are two examples (Lenné and Wood, 1991).

1.3 Genetic diversity

1.3.1 Introduction to genetic diversity

A general view on genetic diversity is that it refers to the sum of all genetic information carried by all living organisms on earth. However, genetic diversity commonly referred to intraspecific genetic diversity. Genetic diversity is one of the three types of biodiversity along with ecosystem diversity and species diversity. Genetic diversity is recognized by the International Union for Conservation of Nature (IUCN) as important focus of its conservation effort (Reed and Frankham, 2003). Genetic uniformity decreases the crops resistance and increases their vulnerability to new pests and stresses. Genetic diversity offers us the sustained ability to improve new plant cultivars that can protect themselves from diseases, pests, and environmental stresses. The need to keep genetic diversity within populations relies on two arguments: one is the necessity of genetic diversity for evolution to occur; and the other is the expected relationship between heterozygosity and population fitness (Reed and Frankham, 2003). Gain of genetic diversity can provide information to inbreeding, since genetic diversity increases reproductive fitness. Since the rate of inbreeding is determined by the mating system, and the amount of inbreeding determines the

observed heterozygosity. Therefore, a correlation is expected between population fitness and heterozygosity.

In the last few decades, much work has been centered around the importance of wild species and primitive cultivars in plant breeding. Considerable progress in this area has been accomplished through the efforts of the FAO (Food and Agriculture Organization) of the United Nations in its pilot work on genetic resources of crops, and the Eucarpia's (European Association for Research on Plant Breeding) promotion of gene bank activities in Europe in the 1960s and 1970s (Hawkes, 1977). In response to the convention on biological diversity, an international treaty to maintain the rich diversity of life on earth, Agriculture and Agri-Food Canada (AAFC) has committed to a Canadian biodiversity strategy in maintaining the Canada's Plant Germplasm System (CPGS) to representing the genetic diversity of crop plants, their wild ancestries and relatives, and plants unique in the Canadian biodiversity. Germplasm is evaluated for a number of desirable agronomic traits; earliness or winter hardiness, screened for resistance to pests, diseases, and environmental stress as well as quality factors such as colour and flavour. The results of germplasm collection are made available through the national database, GRIN-CA (genetic resource information network of Canada). Canadian agriculture is based on crop plants that originated from areas outside of Canada. However, cranberry is among the crops of commercial importance that are native to Canada although with limited biodiversity representation.

Unfortunately the landbase where wild plants grow continues to shrink, and many plant species and variants are disappearing. Hence, there is an urgent need to maintain and increase the catalogue and utilization of germplasm of plants, such as cranberry, that might otherwise be lost.

1.3.2 Measurement of genetic diversity

1.3.2.1 Morphological and allozyme markers

Genetic diversity can be studied by morphological characters. However, this is not always accurate as morphological characters are influenced by environment (Debnath 2007a). During the last decades, some strategies on detection of genetic variation, such as morphology, embryology, and physiology, have been complemented by molecular analytic techniques (Debnath, 2008).

Prior to the introduction of molecular biology techniques, the technique using isozyme (or allozyme) markers has been widely employed to study the genetic variation and population structure of a large range of plant species (Fady-Welterlen, 2005). This technique detects only the variation in protein coding genes, and consequently provides fewer markers compared to DNA-based methods.

1.3.2.2 DNA markers

The introduction of DNA markers for the investigation and exploitation of DNA polymorphism is one of the most critical developments in molecular biology. Data on molecular markers are increasingly applied in crop breeding programs around the world to investigate genetic diversity in and among the *Vaccinium* species (Debnath, 2008). This evaluation would promote the high efficient utilization of genetic variation in crop plant improvement programs (Paterson et al., 1991). Genotype identification and genetic variation are increasingly essential in cranberry for proprietary-rights protection, practical breeding purposes, and germplasm characterization (Debnath, 2009).

DNA markers are classified as hybridization-based markers and PCR based markers. The former may include DNA-DNA hybridization and some way of restriction fragment length polymorphism (RFLP). Random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter simple sequence repeat (ISSR), simple sequence repeat (SSR) etc. are PCR based markers. DNA markers may be dominant, like RAPD, AFLP, ISSR or co-dominant like RFLP, SSR, expressed sequence tag (EST)-PCR, etc.

1.3.2.2.1 Restriction fragment length polymorphism

RFLP was developed for high density genomic mapping (Botstein et al., 1980) in

order to overcome the limitations of allozyme. RFLP was the first widely applied DNA marker and has been applied in identification of species, evaluation genetic diversity and mapping genes of interest in many crops (Debnath, 2008). RFLP is an crucial tool in genome mapping and localization of genes for genetic disorders, determination of risk for disease and paternity testing (Debnath, 2008; Debnath et al., 2012). In RFLP analysis, the DNA sample is digested into pieces with specific restriction enzymes and the resulting restriction fragments are separated based on their lengths via gel electrophoresis. Although RFLP is unlimited, they require elaborate laboratory techniques, including the development of specific probe libraries, use of radioisotopes, Southern blot hybridization procedures, and autoradiography, which make them labour intensive, time consuming, and costly (Kesseli et al., 1994).

1.3.2.2.2 PCR- based techniques

DNA profiling techniques, using a variety of DNA-based markers, allows direct and precise comparison of types genetic material which is independent of environmental influences (Weising et al., 1995). Genetic markers are utilized as genetic finger-prints for the purpose of identification an individual species, DNA fingerprinting, genome mapping, and population genetic studies (Raina et al., 2001). These studies usually apply polymerase chain reaction (PCR) devised techniques. PCR is a technique for reproducing target fragments from a DNA molecule. The target DNA fragments can be amplified thousands of times during the cycles of reaction (Ribinow, 1996). In

recent decades, PCR based techniques using DNA markers have been playing important roles in the study of finger-printing, genetic variation and sequencing. Because PCR amplifies the regions of DNA that it targets, PCR can be used to analyze extremely small amounts of sample. The primary reagents used in the PCR amplification are: DNA nucleotides, the building blocks for the new DNA; template DNA, the DNA sequence that is to be amplified; primers, single-strended DNAs between 20 and 50 nucleotides long that are complementary to a short region on either side of the template DNA; DNA polymerase, a heat stable emzyme that drives the synthesis of new DNA; and $MgCl_2$, a commonly used reaction buffer for facilitating enzymatic reactions that helps in the binding of primer (and the subsequent target DNA) to the template DNA. Different volume of Mg^{2+} exert different complex-forming capabilities, and thus affects the end product of PCR. With these reagents, PCR is based on three main steps: denaturation of the template into single strands; annealing of the primers to each original strand for new strand synthesis; and extension of the new DNA strands from the primers (Delidow et al., 1993). After cycles of repeating these steps, the templates can be amplified thousands times.

1.3.2.2.2.1 Random amplified polymorphic DNA

The RAPD technology was introduced in 1990 (Welsh and McClelland, 1990). RAPD marker is DNA fragments from PCR amplification of random fragments of genomic DNA using a single primer of arbitrary nucleotide sequence. These dominant markers

use short 10-base primers of arbitrary nucleotide sequences (>50% guanine-cytosine) for simultaneous amplification of multiple segments of genomic DNA. The amplified DNA fragments applied are clearly visualized on ethidium bromide-stained agarose gels (Debnath, 2008). The advantages of RAPD include its rapidity, simplicity, the requirement for only a small amount of DNA, and the ability to generate numerous polymorphisms. No prior knowledge of the genome is required, and the procedure does not require the use of radioactive probes (Debnath, 2008). Its reproducibility has been achieved via the improvements on laboratory techniques and the band scoring procedures (Nybom and Bartish, 2000).

1.3.2.2.2.2 Amplified fragment length polymorphisms

AFLP technology was introduced by Vos et al. (1995), and is a method in which total genomic DNA is digested with two restriction enzymes. Double-stranded nucleotide adapter fragments are ligated to the digest fragments to serve as primer sites. These primers are binding sites for PCR amplification. A group of the restriction fragments are chosen to be amplified. This selection is achieved by using primers complementary to the adapter sequence, the restriction site sequence, and a few nucleotides inside the restriction site fragments (Vos et al., 1995). A major drawback for AFLP is that the investigated biallelic loci are worked in a dominant fashion, like RAPDs; heterozygotes cannot be distinguished from homozygotes (Nybom, 2004).

The reproducibility problem appears to be smaller for AFLP than for RAPD (Vos et al. 1995). AFLP employs longer primers and higher annealing temperatures, but needs more steps, and the cost is higher than RAPD. However, since more polymorphic information was detected by a single AFLP reaction, the relative cost is less. The speed and accuracy of detection are also higher also in AFLP than RAPD (Polaschock and Vorsa, 1996).

1.3.2.2.2.3 Inter simple sequence repeat

ISSR primers target microsatellites, repeating sequences of 2-6 base pairs of DNA (SSRs), that are abundant throughout the plant genome (Wang et al., 1994). The primers used in ISSR analyses can be based on any of the SSR motifs (di-, tri-, tetra-, penta- or mixed-nucleotides) found at microsatellite loci, providing a wide array of possible amplification products which can be anchored to genomic sequences flanking either side of the targeted SSR (Gupta et al., 1994; Zietkiewicz et al., 1994). With this technique, primers that are anchored at the 3' or 5' terminus of the repeat are synthesized by extending into a hypothetical flanking sequence by two to four bases. This marker has proved to be more reproducible than RAPD marker, and generally indicates higher levels of polymorphism due to the nature of the locus ISSR detects, longer sequences of ISSR primers, and the higher annealing temperatures used (Debnath, 2005). ISSR is easier to use and cost less than AFLP and does not require prior knowledge of flanking sequences, similar to SSR (Reddy et al., 2002). However,

ISSR marker is an dominant marker. Compared with RAPD and AFLP, ISSR overemphasizes differences between closely related populations and attribute less variation to differences over large geographical distances (Qian et al., 2001). Despite these drawbacks, ISSR marker is very useful tool for detecting genetic variation (Zietkiewicz et al., 1994). ISSR has been put forward as a new type of genetic marker that overcome the technical limitations of RFLP analyses (Rafalski et al., 1991), including in plants (Tsumura et al., 1996).

1.3.2.2.2.4 Simple sequence repeats

SSR and microsatellite markers are repeats of short nucleotide sequences, usually 1-5 base pairs in length, that vary in number (Rafalski et al., 1996). SSRs are abundant in plant genomes, co-dominantly inherited, multi-allelic and highly polymorphic, and reproducible (Debnath et al., 2012; Georgi et al., 2012; Zhu et al., 2012), could be suitable for fingerprinting and parental identification. The advantages of applying SSR markers for detecting genetic variation and fingerprinting have been reported in many plant species (Erfani et al., 2012; Palombi and Damiano, 2002; Panwar et al., 2010; Potts et al., 2012; Ravi et al., 2003; Zhang et al., 2012). However, SSR also have some drawbacks, like expensive to design primers and DNA sequencer required.

1.3.2.2.2.5 Expressed sequence tag (EST)-PCR and EST-Simple Sequence Repeat

ESTs are short DNA molecules (300 - 500 base pairs) reverse-transcribed from a cellular messenger RNA (mRNA) population (MacIntosh et al., 2001). They are generated by large-scale single-pass sequencing of randomly picked complementary cDNA clones and have proven to be efficient and rapid means to identify novel genes. ESTs were originally intended as a way to identify gene transcripts, but have since been instrumental in gene discovery, for obtaining data on gene expression and regulation, sequence determination, and for developing highly valuable molecular markers, such as EST-based PCR and SSR (Debnath et al., 2012). For the EST-SSR, a subclass of repeated sequences containing iterations of short motifs (1-5 base pairs) are commonly referred to as SSR or microsatellites (Weber and May, 1989). PCR primers comprised of SSR motifs have been shown to be effective for the production of molecular markers in animal and plants (Zietkiewicz et al., 1994). Moreover, SSRs are exceedingly abundant in plant genomes, and the loci of SSRs are polymorphic (Wang et al., 1994). EST-PCR and EST-SSR markers detect the expressed genes and they are co-dominant, which are different from ISSR. The selected markers have been developed for blueberry studies and found effective (Debnath, 2011; Boches et al., 2006).

1.4 Use of allozyme markers in *Vaccinium* genetic diversity studies

Isoenzyme loci were investigated among *Vaccinium* species to study allozymic diversity. Mahy et al. (2000) studied 19 isoenzyme loci in diploid and tetraploid populations of *V. Oxycoccos*. They reported that autotetraploids had more polymorphic loci, mean number of alleles, and heterozygosity than those of the diploids. Hokanson and Hancock (1998) reported on the levels of allozymic diversity in the native Michigan populations of diploid *Vaccinium myrtilloides*, and its tetraploids, *V. corymbosum* and *V. angustifolium*. Number of alleles per locus and the level of heterozygosity were noticeably lower in the diploid *V. Myrtilloides* (21.7%; 2.9) than in the tetraploids, *V. corymbosum* (75.6%; 3.6) and *V. angustifolium* (57.1%; 3.4); note that the average level of heterozygosity, in this study, was almost 20% higher in *V. corymbosum* than *V. Angustifolium* (Hokanson and Hancock, 1998).

1.5 Use of DNA markers in *Vaccinium* genetic diversity studies

For the studies of genetic diversity among *Vaccinium* plants, several types of DNA markers such as RAPD, AFLP, ISSR, SSR, EST-PCR and EST-SSR have been applied.

1.5.1 Random amplified polymorphic DNA

Aruna et al. (1993) investigated the extent of genetic relatedness among 15 improved cultivars and four wild selection by RAPD marker. Cluster analysis of genetic distance assessment grouped siblings with each other and with one or both parents. Aruna et al. (1995) also clarified the genetic identity of two wild selections of rabbiteye blueberry, 'Ethel' and 'Satilla' by RAPD technique (Aruna et al., 1995). The probable identity of two cultivars in a commercial blueberry field was verified by RAPD with comparing their amplified DNA patterns with those of standard cultivars (Aruna et al., 1995).

Novy et al. (1994) identified and assessed genetic diversity in 22 cranberry varieties using RAPD-PCR technology. On the basis the 66 polymorphic silver-stained RAPD (ssRAPD), 17 unique ssRAPD-DNA profiles were identified rather than the expected 22; fourteen varieties had unique ssRAPD profiles, while the other eight varieties were represented by three ssRAPD profiles (Novy et al., 1994). Working with 'McFarlin'cranberry cultivar, Novy et al. (1996) reported that genetic heterogeneity was high among 12 bogs; 30% RAPD profile showed to represent the 'true' 'McFarlin'. They observed an association between RAPD profiles and reproduction characteristics (Novy et al., 1996). A unique RAPD profile which exhibited high yield characteristics did not appear to be related to 'McFarlin' (Novy et al., 1996).

Stewart and Excoffier (1996) reported a gradient of molecular diversity between central and marginal cranberry populations (Stewart and Excoffier, 1996). Burgher et al. (2002) showed an average similarity across all lowbush blueberry genotypes of 56%. This study demonstrated that results from average linkage cluster analysis can be applied to construct a dendrogram of six main clusters with an average similarity linkage of 70%. For example, the selection 'Fundy' and its parent 'Augusta' were grouped at 77% similarity (Burgher et al., 2002).

RAPD and sequence characterized amplified regions (SCAR) markers were used to study genetic relatedness among 27 cranberry germplasm accessions (Polashock and Vorsa, 2002). Both markers detected comparable levels of variation. They concluded that sequence-characterised amplified region (SCAR) and RAPD markers can be used to identify closely related genotypes. However, SCAR marker produced more polymorphism than RAPD on a per reaction basis and were more powerful to separate closely progeny (Polashock and Vorse, 2002).

Debnath (2007a) assessed genetic variability in forty-three wild cranberry clones collected from four Canadian provinces and five cranberry cultivars, using RAPD-PCR. A significant degree of genetic diversity were revealed. AMOVA indicated that 10% of total variation was affected by geographical distribution and 90% of total variation was found among the clones (Debnath, 2007a).

1.5.2 Amplified fragment length polymorphism

The AFLP technique has been applied to determine the genetic sources of newly found population of huckleberry (*V. Membranaceum* Ait.) and to characterize the genetic variation between the new and original populations (Yang et al., 2008). It has also been used in the fingerprinting of blueberry and cranberry species (Polaschock and Vorsa, 1996).

Albert et al. (2003) applied AFLP to analyze 112 samples from a bilberry population and identified 32 clones and their geographical distribution. The results of this study obtained with RAPD and AFLP markers showed that both molecular markers worked effectively in bilberry. Albert et al. (2003) reported that genotypic diversity and evenness were observed in *V. myrtillus* and were similar to other species of *Ericaceae*. The observed relationship between the mean similarity index calculated between clones in this study and the outcrossing rate of *Ericaceous* species indicates that the mean value of similarity index could be estimated as a rough estimator of the mating system of plant species (Albert et al., 2003).

1.5.3 Inter simple sequence repeat

Working with ISSR primers, Debnath (2007b) detected a significant degree of genetic

diversity among the 43 wild lingonberry clones; 10% of total variation was revealed by AMOVA and explained by geographical distribution.

Debnath (2009) reported that 43 wild lowbush blueberry (*Vaccinium angustifolium* Ait.) clones obtained from four Canadian provinces and the cultivar 'Fundy' were detected genetic similarity and variation using ISSR markers. A high degree of genetic similarity among wild genotypes was detected with ISSR; the 41 genotypes were clustered in two main clusters by UPGMA as well as three genotypes as outliers (Debnath, 2009). 27% of total variation revealed by AMOVA was contributed by the geographical distribution (Debnath, 2009).

1.5.4 Expressed sequence tag (EST)-polymerase chain reaction

EST-PCR markers from the EST library of the *Vaccinium* genus were developed by Rowland et al. (2003a; 2003b). These markers were found suitable for genetic diversity analysis in *Vaccinium* species (Debnath, 2008; Debnath, 2011). EST-PCR primer pairs which were derived for blueberry were used with cranberry genotypes (two wild selections of *V. oxycoccus* L. and two cultivars of *V. macrocarpon* Ait.). Rowland et al. (2003a) tested many of the EST-PCR primer pairs developed for blueberry (*V. spp*) and investigated whether they were capable of amplifying DNA fragments in other members of the family Ericaceae.

Rowland et al. (2003a) reported that twenty-three out of 26 primer pairs successfully produced amplification and eight out of the 26 available primer pairs produced polymorphic fragments among the cranberry genotypes. Rowland et al. (2003b) reported EST-PCR primers were used to distinguish 19 blueberry (*V. spp*) genotypes, including two wild selections (the original parents of a mapping population), and 17 cultivars (Rowland et al., 2003b). The polymorphic EST-PCR marker developed in this study discriminated all the blueberry genotypes; similarity value was calculated based on molecular marker data, the dendrogram was contributed based on similarity matrix, and coefficients of coancestry were calculated from complete pedigree information for each pair of genotypes (Rowland et al., 2003b).

Similarly, Bell et al. (2008) studied genetic relationship and fingerprinting in lowbush blueberry using EST-PCR. As part of this interspecific genetic relationship study, 14 genotypes, including more than two specimens of each of four closely related *Vaccinium* L. species (*V. corymbosum*, *V. myrtilloides* Michx., *V. pallidum* Ait., and *V. boreale* Hall & Aald.) and the only four pedigreed cultivars of *V. angustifolium*, grouped as expected in a genetic similarity dendrogram (matrix "r" correlation = 0.91) (Bell et al., 2008).

1.5.5 EST- simple sequence repeat (SSR) and SSR markers

EST-SSR marker has been utilized in the genetic profiling of several plant species of

the *Vaccinium* species such as rabbiteye blueberry (*V ashei* Reade), highbush blueberry (*V corymbusm* L.) cultivars (Levi and Rowland, 1997) and cranberry (Fajardo et al., 2013). Boches et al. (2006) detected a total of 627 alleles via 28 SSRs. Unique fingerprints were observed for all 69 accessions representing wild and domesticated highbush blueberry germplasm. Pyrosequenced SSR has been applied in cranberry genotypes (Zhu et al., 2012). Forty-eight polymorphic SSR loci with 2-15 alleles per locus for a total of 323 alleles were detected within 25 cranberry genotypes (Zhu et al., 2012). Fajardo et al. (2013) applied 12 SSR markers and ran genetic cluster analysis to assess the genetic diversity within 21 cranberry cultivars, 11 experimental hybrid and six representative accessions of wild species. The most prospective clonal representatives of some essential cranberry cultivars were identified by consensus genetic profiles detected via SSR alleles detected (Fajardo et al., 2013).

1.6 Overview of thesis

Cranberries has been studied with RAPD (Novy et al. 1994; Stewert and Excoffier 1996; Polashock and Vorsa 2002; Debnath 2007a), AFLP (Polashock and Vorsa 2002), and SSR markers (Zhu et al. 2002; Fajardo et al. 2012). However, there was no report on cranberry diversity analysis using ISSR, EST-PCR, and EST-SSR. ISSR, EST-PCR, and EST-SSR have been studied in blueberry (Debnath, 2009; Debnath, 2011; Boches et al., 2006), and found effective in genetic diversity studies with Vaccinium species. They can be assumed to be available in cranberry study. It is good

to apply more than one marker in a study. There was no comparison among ISSR, EST-PCR, and EST-SSR markers in previous studies with *Vaccinium* species. This study was the first study to use these three markers in Vaccinium species. This thesis was organized with introduction, material and methods, results, discussion, summary, literature cited, and appendix.

1.7 Hypothesis of this project

ISSR, EST-PCR and EST-SSR markers are supposed to be available for the genetic study of cranberry species.

Each type of DNA markers is supposed to be capable to investigate enough variation to completely separate 102 cranberry clones collected from four Canadian provinces and five cranberry cultivars.

1.8 Objectives of this project

The present study was conducted with 102 cranberry clones and five cultivars with the following objectives:

(1) To identify ISSR, EST-PCR and EST-SSR primers suitable for fingerprinting cranberry,

(2) To assess the level of genetic variations among wild cranberry clones and cultivars using these three types of markers and

(3) To assess the level of genetic polymorphism and relative importance among these three genetic markers in molecular diversity analysis of cranberries.

2. Materials and methods

2.1 Materials

2.1.1 Plant material

This study included a total of 102 wild cranberry clones and five cultivars. The cranberry clones were collected in August, 2001 from four Canadian provinces: Newfoundland and Labrador (NL; clones 'NL1' to 'NL71' with 21 location), New Brunswick (NB; clones 'NB1' to 'NB10' with two location), Prince Edward Island (PEI; clones 'PE1'to 'PE20' with two location), and Nova Scotia (NS; 'NS1') (Table 1). Each clone represented a single plant selected from the wild field based on plant vigour, berry colour, berry size, and berry yield per plant and apparent free from disease, insects or other pests. The five cultivars were 'Franklin', 'Stevens', 'Wilcox', 'Pilgrim', and 'Ben Lear'. The origin of the cultivars is presented in Table 2.

3.1.2 Plant maintaining conditions

The wild clones and cultivars were grown and maintained in the greenhouse of Atlantic Cool Climate Crop Research Center in St John's, NL, Canada, under natural light conditions at a maximum photosynthetic photon flux of 90 μmol m^2 s^{-1} at

℃, 85% relative humility. Irrigation and fertilization were applied when necessary. In winter, the plants were maintained at or below 6 ℃ for 12 weeks to meet chilling requirements.

Table 1: Wild cranberry clones collected from Canadian provinces: Newfoundland and Labrador (NL), New Brunswick (NB), Nova Scotia (NS), and Prince Edward Island (PEI)

Clone	Province	Community	Latitude(N)	Longitude(W)
'NL1'	NL	Bell Island East	47°38'	52°56'
'NL2'	NL	Soldiers Pond	47°20'	53°04'
'NL3'	NL	Bell Island West	47°38'	52°58'
'NL4'	NL	Lamaline	46°51'	55°48'
'NL5'	NL	Bell Island East	47°38'	52°56'
'NL6'	NL	Bell Island East	47°38'	52°56'
'NL7'	NL	Bauline Line/ Portugal Cove	47°37'	52°51'
'NL8'	NL	Bell Island West	47°38'	52°58'
'NL9'	NL	Bell Island West	47°38'	52°58'
'NL10'	NL	Bell Island East	47°38'	52°56'
'NL11'	NL	Bell Island West	47°38'	52°58'
'NL12'	NL	Lords Cove/Pump Cove	46°52'	55°40'
'NL13'	NL	New Melbourne	48°03'	53°09'
'NL14'	NL	Bell Island East	47°38'	52°56'
'NL15'	NL	Bell Island West	47°38'	52°58'
'NL16'	NL	Bell Island West	47°38'	52°58'
'NL17'	NL	Bell Island West	47°38'	52°58'
'NL18'	NL	New Melbourne	48°03'	53°09'
'NL19'	NL	Peters River	46°45'	53°36'
'NL20'	NL	Bell Island East	47°38'	52°56'
'NL21'	NL	Port Kirwam	46°58'	52°55'
'NL22'	NL	Bell Island East	47°38'	52°56'
'NL23'	NL	Bell Island East	47°38'	52°56'
'NL24'	NL	Bell Island East	47°38'	52°56'
'NL25'	NL	Bell Island East	47°38'	52°56'

'NL26'	NL	Bell Island West	47°38'	52°58'
'NL27'	NL	Bell Island West	47°38'	52°58'
'NL28'	NL	New Melbourne	48°03'	53°09'
'NL29'	NL	Logy Bay	47°37'	52°40'
'NL30'	NL	Bell Island East	47°38'	52°56'
'NL31'	NL	Lords Cove/Pump Cove	46°52'	55°40'
'NL32'	NL	Bell Island East	47°38'	52°56'
'NL33'	NL	Soldiers Pond	47°20'	53°04'
'NL34'	NL	Bell Island East	47°38'	52°56'
'NL35'	NL	Bell Island East	47°38'	52°56'
'NL36'	NL	New Melbourne	48°03'	53°09'
'NL37'	NL	Ferryland	47°02'	52°52'
'NL38'	NL	The Beamer Flatrock	47°42'	52°42'
'NL39'	NL	Bell Island East	47°38'	52°56'
'NL40'	NL	Cape Spear	47°31'	52°37'
'NL41'	NL	Freshwater	47°45'	53°11'
'NL42'	NL	Soldiers Pond	47°20'	53°04'
'NL43'	NL	New Melbourne	48°03'	53°09'
'NL44'	NL	Freshmans Cove	47°12'	55°24'
'NL45'	NL	NL	47°50'	59°19'
'NL46'	NL	Point La Haye	46°52'	53°36'
'NL47'	NL	Cape Spear	47°31'	52°37'
'NL48'	NL	St. Brides	46°55'	54°10'
'NL49'	NL	Corbin	46°58'	55°14'
'NL50'	NL	Mobile	47°14'	52°50'
'NL51'	NL	Bell Island West	47°38'	52°58'
'NL52'	NL	Bell Island West	47°38'	52°58'
'NL53'	NL	Bell Island East	47°38'	52°56'

'NL54'	NL	Bell Island East	47°38'	52°56'
'NL55'	NL	Soldiers Pond	47°20'	53°04'
'NL56'	NL	Bell Island West	47°38'	52°58'
'NL57'	NL	Bell Island East	47°38'	52°56'
'NL58'	NL	Bell Island East	47°38'	52°56'
'NL59'	NL	Bell Island West	47°38'	52°58'
'NL60'	NL	Bell Island West	47°38'	52°58'
'NL61'	NL	Bell Island West	47°38'	52°58'
'NL62'	NL	Bell Island West	47°38'	52°58'
'NL63'	NL	Bell Island West	47°38'	52°58'
'NL64'	NL	Biscay Bay	46°44'	53°17'
'NL65'	NL	Bell Island East	47°38'	52°56'
'NL66'	NL	Bell Island East	47°38'	52°56'
'NL67'	NL	Bell Island West	47°38'	52°58'
'NL68'	NL	Bell Island West	47°38'	52°58'
'NL69'	NL	Bell Island East	47°38'	52°56'
'NL70'	NL	Bell Island East	47°38'	52°56'
'NL71'	NL	Bell Island East	47°38'	52°56'
'NB1'	NB	Little Shemogue	46°06'	64°01'
'NB2'	NB	Clifton	47°43'	65°22'
'NB3'	NB	Little Shemogue	46°06'	64°01'
'NB4'	NB	Clifton	47°43'	65°22'
'NB5'	NB	Little Shemogue	46°06'	64°01'
'NB6'	NB	Little Shemogue	46°06'	64°01'
'NB7'	NB	Clifton	47°43'	65°22'
'NB8'	NB	Clifton	47°43'	65°22'
'NB9'	NB	Little Shemogue	46°06'	64°01'
'NB10'	NB	Little Shemogue	46°06'	64°01'

'NS1'	NS	Canso	45°20'	60°59'
'PE1'	PEI	Blooming Point	46°23'	62°58'
'PE2'	PEI	Harrington	46°21'	63°10'
'PE3'	PEI	Harrington	46°21'	63°10'
'PE4'	PEI	Harrington	46°21'	63°10'
'PE5'	PEI	Harrington	46°21'	63°10'
'PE6'	PEI	Harrington	46°21'	63°10'
'PE7'	PEI	Harrington	46°21'	63°10'
'PE8'	PEI	Harrington	46°21'	63°10'
'PE9'	PEI	Harrington	46°21'	63°10'
'PE10'	PEI	Harrington	46°21'	63°10'
'PE11'	PEI	Harrington	46°21'	63°10'
'PE12'	PEI	Harrington	46°21'	63°10'
'PE13'	PEI	Blooming Point	46°23'	62°58'
'PE14'	PEI	Blooming Point	46°23'	62°58'
'PE15'	PEI	Blooming Point	46°23'	62°58'
'PE16'	PEI	Harrington	46°21'	63°10'
'PE17'	PEI	Blooming Point	46°23'	62°58'
'PE18'	PEI	Harrington	46°21'	63°10'
'PE19'	PEI	Blooming Point	46°23'	62°58'
'PE20'	PEI	Blooming Point	46°23'	62°58'

Table 2: Origin or parentage of cranberry cultivars sampled for inter simple sequence repeats (ISSR), expressed sequence tag (EST) - polymerase chain reaction, and EST-simple sequence repeats analysis

Cultivar	Origin/parentage	Year of introduction	Reference
'Franklin'	'Early Black'×'Howes'	1961	Dana, (1990)
'Stevens'	'McFarlin'×'Potter'	1950	Dana, (1990)
'Wilcox'	'Howes'×'Searles'	1950	Dana, (1990)
'Pilgrim'	'Prolific'×'McFarlin'	1961	Janick and Moore, (1996)
'Ben Lear'	Selection from a wild population in Wisconsin, USA	1961	Janick and Moore, (1996)

2.2 Methods

2.2.1 DNA extraction

The genomic DNA of cranberry clones and cultivars was isolated from leaf tissues using the Wizard® Genomic DNA Purification Kit from Promega, with some modifications. Fresh leaves, 2-3 g per individual, were harvested in May 2011 from individual genotypes, placed into 1.5 ml Eppendorf tubes, and stored at -80℃ until used for DNA isolation. For DNA extraction, 400 mg leaves samples were grounded with 600 µl lysis solution containing polyvinylpyrrolidone (sigma PVP-40; 2g PVP in 10ml lysis solution). After incubation at 65℃ in water bath, each sample was

centrifuged to remove large debris. After the DNA molecule was washed by 70% ethanol several times, the purified DNA was resuspended in 100 µl DNA rehydration solution.

The DNA concentration was determined at 260 nm using an Ultrospec® 2000 spectrophotometer (Pharmacia Biotech, Cambridge, UK), and the DNA purity of each sample was measured by the ratio of the UV absorbance at 260 nm (A_{260}) over 280 nm (A_{280}). A pure DNA solution has an A_{260}/A_{280} ratio of 1.8-2.1 (Debnath, 2009), and a ratio below 1.8 can indicate protein contamination, which can lower reaction efficiency. For quality control, we only used template DNA with an A260/A280 ratio of 1.8-2.1 in a dilution of 10 ng/ µL for the PCR amplification (Debnath and Ricard, 2009).

2.2.2 PCR amplification

Thirteen ISSR primers (Table 3), ten EST-PCR primers (Table 4) and thirteen EST-SSR primers (Table 5) were selected from previous papers on *Vaccinium* species (Debnath, 2007b; Bell et al., 2008; Boches et al., 2006). The procedures were also as derived by Debnath (2007b), Bell et al. (2008), and Boches et al. (2006) with a few modifications. Different concentrations of template DNA, primer, $MgCl_2$, and *Taq* polymerase were tested for optimal amplification. The optimized amplification reaction mixture (25 µL) contained PCR grade distilled water (dH_2O), 10 ng of DNA template, PCR buffer {50 mM KCl, 10 mM Tris–HCl pH 8.3, 1.5 mM $MgCl_2$, and

0.001% (w/v) gelatin}, 200 μM of each dNTP, 25 pmol primer and 0.05 U of *Taq* DNA polymerase (Sigma, Oakville, ON, Canada; Sigma). The PCR reaction were conducted in a PTC-100® Programmable Thermal Controller (MJ Research, Watertown, MA). Each reaction was performed using an initial 'hot start' of 94℃ for 10 min, followed by 45 cycles of 1 min at 94℃, 1 min at annealing temperatures of each primer, and 2 min on 72℃. The reaction was terminated with a final extension at 72 ℃ for 10 min before to holding the sample at 4℃ for analysis.

Table 3: Identity and sequence for ISSR primers tested on cranberry wild clones and cultivars, with annealing temperature of 45 ℃ for all primers

Primer	Sequence (5'→3')
UBC801	ATATATATATATATATT
UBC807	AGAGAGAGAGAGAGAGT
UBC808	AGAGAGAGAGAGAGAGC
UBC809	AGAGAGAGAGAGAGAGG
UBC810	GAGAGAGAGAGAGAGAT
UBC816	CACACACACACACACAT
UBC817	CACACACACACACACAA
UBC826	ACACACACACACACACC
UBC827	ACACACACACACACACG
UBC835	AGAGAGAGAGAGAGAGYC
UBC867	GGCGGCGGCGGCGGCGGC
UBC890	VHVGTGTGTGTGTGTGT
UBC891	HVHTGTGTGTGTGTGTG

Table 4: Identity, sequence, and annealing temperature for EST-PCR primers tested on cranberry wild clones and cultivars

Primer	Forward/reversed primer sequence (5'→3')	Annealing temperature
CA1590	AACCCAGCACCTCCTTTCTT	54℃
	CTCTGTTGCTGGCTGTGTGT	
CA1029	GAAGTTTTCCGTTCTCTGCAA	52℃
	CTGCAGCTAGGACCGAAGAG	
NA353	GGAAGGGTATGCTGAGCTTG	48℃
	CAGAATCATGAGGCCCACTT	
NA27	CGCTCGCTCCATTGTTTC	60℃
	TATGCATGAAGCTTGCCGTA	
NA1068	CCGGAAGGAATGGTGACTAA	54℃
	ATCCCCACACAAACAAAAGC	
CA54	CCGGTGAACTTCCACTTGTT	48℃
	AGATACTACTGGGGGTGGGG	
CA1423	TCATAGCCAATACACTCGAACC	46℃
	GCCCCACCTTTAGCAAACTC	
CA227	TGGAGACTGGAGTGATGCAA	52℃
	TTTGCAAGAACCATGCTGAG	
CA231	CCAAAATGCCCAAACTCATC	52℃
	AAGGAAAAGGAAACGGGAAA	
CA21	TCCGATAACCGTTACCAAGC	52℃
	TATACAGCGACACGCCAAAA	

Table 5: Identity, sequence, and annealing temperature for EST-SSR primers tested on cranberry wild clones and cultivars

Primer	Repeats motif	Annealing temperature
CA421F	$(CT)_{25}$	52°C
CA794F	$(GA)_{12}$	56°C
NA1040	$(TC)_{11}$	48°C
NA800	$(TC)_{13}$	52°C
NA961	$(TAC)_5$	61°C
CA483F	$(TC)_8$	48°C
CA112F	$(AG)_7$	48°C
CA169F	$(GAT)_4$	48°C
CA236F	$(TG)_{17}$	48°C
NA741	$(TC)_9$	58°C
VCC_I2	$(CT)_{14}$	56°C
VCC_J5	$(TC)_{17}$	47°C
VCC_K4	$(TC)_{16}...(TC)_{12}$	52°C

2.2.3 Gel electrophoresis

Gel electrophoresis is a method for separation and analysis of macromolecules (DNA, RNA and proteins) and their fragments, based on their size and charge. It applies a gel as an alti-convective medium during electrophoresis. When the electric current is running, the larger molecules move more slowly through the gel; whereas, the smaller molecules move faster. The different sized molecules form distinct bands on the gel allowing the determination of the presence or absence of PCR products and size quantification (length of the DNA molecule) of the products (Carle and Olson, 1984).

In the present study, after PCR was performed, amplified fragments, along with a 1 kb or 10 kb DNA ladder (Invitrogen, Burlington, ON), used as a molecular weight standard, were resolved by 2.0% agarose gels electrophoresis in tris-borate-EDTA (TBE) buffer and then stained in an ethidium bromide solution {0.5 μg mL^{-1} of TBE} for 25 min, and washed in distilled water for 20 min. DNA banding patterns were visualized and recorded using a GeneGenius gel documentation system (Syngene, Beacon House, Cambridge, UK). Among the primers tested, twelve ISSR (Table 7), ten EST-PCR (Table 8) and five EST-SSR (Table 9) primers were proven to work well.

Each primer-clone combination was repeated at least twice, and congruence between replicates was verified (Adams and Rieseberg, 1998). All gels were scored for both

polymorphic and monomorphic bands. Non replicated bands were eliminated from analyses. Bands of similar molecular weight and migration distance across individuals were assumed to be homologous (Adams and Rieseberg, 1998).

2.2.4 Data collection

Presence or absence of each ISSR, EST-PCR and EST-SSR fragment was coded as '1' and '0', respectively, where '1' indicating the presence and '0' the absence of a specific allele (ISSR) or bi-allele (EST-PCR and EST-SSR). The presence or absence of fragments was adjusted according to Guichoux et al. (2011). Since ISSR markers are typically dominant, it was assumed that each band represented the phenotype at a single bi-allelic locus (William et al., 1990). The presence of a band indicates either a heterozygote or a "dominant" homozygote, and the absence of a band a recessive "homozygote". EST-PCR and EST-SSR markers are typically co-dominant, so allow the analysis of only a locus at a time. However, they are more informative because the allelic variations of that locus can be distinguished by examining the bands. For EST-PCR and EST-SSR, the presence of a band represents a homozygote, and the absence of a band as either recessive homozygote or a dominant homozygote (Debnath, 2008). The basic data structure finally consisted of a binomial (0/1) matrix, representing the scored ISSR, EST-PCR, and EST-SSR markers.

The polymorphic bands of each of the primers of three markers were counted. The average number of polymorphic bands were also counted. For the number of loci, the three markers had different conditions. For ISSRs the number of loci was referred to the number of polymorphic bands since ISSR is a dominant DNA marker. EST-PCR and EST-SSR had the numbers of loci referred as the number of assay units since they are co-dominant DNA markers (Belaj et al., 2003; Schubert et al., 2001). The assay unit referred as the primer of each DNA marker (Belaj et al., 2003).

2.2.5 Statistical analysis

The ability of the most informative primers to distinguish between clones was assessed by calculating their resolving power (Rp; Prevost and Wilkinson, 1999), which has been shown to be strongly correlated with their ability to distinguish between genotypes. The Rp value was calculated using the following (Gilbert et al., 1999):

$$Rp = \sum Ib \text{ . where, band informative, } Ib = 1-(2\times|0.5-p|), \qquad \text{(Eq. 1)}$$

where p is the proportion of the 107 genotypes (102 wild cranberry clone from four Canadian provinces and five cultivars). The average value of Ib for each marker was calculated.

Major allele frequency, expected heterozygosity and polymorphism information content (PIC) were computed using Powermarker V 3.25 from the pattern table of

each assay unit (Lui and Muse, 2005). The index, allele frequency, is applied to estimate the richness of the gene pool. The measure of the rate of heterozygosity across loci can be applied as a general indicator of the level of genetic variability (Lynch, 1990). The deviations between these values indicate the richness of the genetic dynamics in population, and the observed heterozygosity can be compared to the expected heterozygosity (Reed and Frankham, 2003).

The expected heterozygosity (He) of the polymorphic locus for each genetic marker was computed using Equation 2 (Bejal et al., 2003):

$$He = 1 - \sum p_i^2 \qquad \text{(Eq. 2)}$$

where p_i is the allele frequency for the ith allele.

The PIC was calculated with Equation 3 (Botstein et al., 1980):

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 p_i^2 p_j^2 . \qquad \text{(Eq. 3)}$$

where p_i is the allele frequency for the ith allele.

For each assay unit, the effective number of alleles per locus (n_e) was calculated with Equation 4 (Morgante et al., 1994):

$$n_e = 1 / \sum p_i^2 \quad , \qquad \text{(Eq. 4)}$$

where p_i is the frequency of the ith allele;

For each DNA marker, the total number of alleles (N_e; Pejic et al., 1998) was calculated using Equation 5:

$N_e = \sum n_e$ (Eq. 5)

Assay efficiency index (A_i) was calculated using Equation 6 (Pejic et al.,1998):

$A_i = N_e$ / number of assay units (Eq. 6)

Since ISSR is a dominant marker, its multiplex ratio (MR) was computed, by dividing the average number of DNA polymorphisms by the number of genotypes (Belaj et al., 2003). For co-dominant EST-PCR and EST-SSR are co-dominant markers, the multiplex ratio is 1.00 (Bejal et al., 2003; Varshney et al., 2007).

Since there was no monomorphic band, the fraction of polymorphic loci remained to be 1.00 for each DNA marker (Belaj et al., 2003). The marker index (MI) for each sample was calculated using Equation 7:

$MI = PIC \times MR$ (Eq. 7).

2.2.6 Jaccard's coefficient and cophenetic correlation coefficient

The *Jaccard's coefficient, Sij,* was computed as follow for all DNA marker types:

$Sij = a/(a+b+c)$ (Eq. 8)

where *Sij* is the similarity between two individuals, i and j, a = number of bands shared by both individuals, b = number of bands present in i, but not in j, c = number of bands present in individual j but not in i.

The cophenetic correlation coefficient (CCC) correlates the level of distortion between the similarity matrix and cluster analysis (Sokal and Rohlf, 1962). A higher CCC value indicates a better fitness. The cophenetic correlation coefficient is given by Equation 9 (Sokal and Rohlf, 1962):

$$c = \frac{\sum_{i<j}(x(i,j) - x)(t(i,j) - t)}{\sqrt{[\sum_{i<j}(x(i,j) - x)^2][\sum_{i<j}(t(i,j) - t)^2]}}.$$

(Eq. 9)

where: $x(i, j) = | X_i - X_j |$, the ordinary Euclidean distance between the ith and jth observations of the original data $\{X_i \text{ and } X_j\}$, $t(i, j)$ = the dendrogrammatic distance between the dendrogram points T_i and T_j, x = average of $x(i, j)$, and t = average of $t(i, j)$, this distance is the height of the node at which these two points are first joined together.

2.2.7 Unweighted pair-group method with arithmetic averages (UPGMA) and the principle co-ordinate (PCo)

Dendrograms were generated by cluster analysis from the similarity matrices created by applying the three association coefficients (Debnath et al., 2008). The similarity matrix was employed as the input data for cluster analysis by applying unweighted pair-group method with arithmetic averages (UPGMA), and to compute a principal coordinate (PCo) analysis (Gower, 1996) using NTSYS-pc (Version 2.1) software (Rohlf, 1998). The SAHN option was employed to cluster the data according to the method of UPGMA clustering procedures. Co-phenetic matrices were generated from the dendrogram and compared with the similarity matrix via the Mantel matrix comparison function in NTSYS to test whether clusters in the dendrogram agreed with information from the similarity matrix.

2.2.8 Analysis of molecular variance (AMOVA)

The DNA patten tables were also used to perform a hierarchical analysis of molecular variance (AMOVA; Excoffier et al., 1992) by using Arlequin Software version 2.001 (Schneider et al., 2000). In this study, the wild genotypes were divided into four groups: Newfoundland and Labrador (NL), New Brunswick (NB), Nova Socia (NS), and Prince Edward Island (PEI). The fifth group consisted of five cultivars (Table 6).

Table 6: Communities or cultivars included in groups for AMOVA analysis

Group	Communities/ Cultivars
Newfoundland and Labrador (NL)	Bell Island East, Biscay Bay, Bell Island West, Soldiers Pond, Cape Spear, Point La Haye, Lamaline, Bauline Line/ Portugal Cove, Mobile, Corbin, St. Brides, Freshmans Cove, Ferryland, New Melbourne, Logy Bay, Peters River, Lords Cove/Pump Cove, Port Kirwam, Freshwater, Newfoundland and The Beamer Flatrock
New Brunswick (NB)	Little Shemogue and Clifton
Nova Scotia (NS)	Canso
Prince Edward Island (PE)	Harrington and Blooming Point
Cultivars	'Franklin', 'Stevens', 'Ben Lear', 'Pilgrim', and 'Wilcox'

3. Results

3.1 DNA polymorphism

3.1.1 DNA polymorphism of ISSR primers

Twelve informative ISSR markers produced a total of 133 polymorphic bands with a mean number of 11 bands per primer (Table 7). Identical banding patterns were observed from repeated runs on all samples. The number of bands produced ranged from a maximum of 15 for the primer UBC 827 to a minimum of 7 for the primer UBC809. The survey of 12 primers revealed the presence of polymorphisms in the amplified DNA fragments in a range from 300 to 5,000 base pairs (Table 7). A representative figure for primer UBC801 is presented in Figure 2. All other gel electrophoresis images for primer UBC801 are presented in Appendix I (Figure 17-19). The resolving power values ranged from 3.8 for primer UBC817 to 11.7 for primer UBC867, with a mean value of 7.9 (Table 7).

3.1.2 DNA polymorphism of EST-PCR primers

A total of 31 polymorphic bands were produced by ten EST-PCR primers with a mean numbers of bands of 3.1. The identity, number of polymorphic bands and resolving power were listed in Table 8. Identical banding patterns were observed from repeated

runs on all samples. The number of bands produced ranged from a maximum of 5 for primers CA1423 and CA231 to a minimum of 1 for primer NA27. The survey of 10 primers revealed the presence of polymorphisms in the amplified DNA fragments in a range from 110 to 1900 base pairs (Table 8). The resolving power values ranged from 0.4 for primer NA27 and CA1590 to 3.3 for primer NA1068, with a mean value of 1.46. A representative banding pattern for primer CA231 is presented in Figure 3. All other gel electrophoresis images for primer CA231 are presented in Appendix I (Figure 20, 21, and 24).

Table 7: Identity, polymorphism, band size, and resolving power for 12 ISSR primers tested on 102 wild cranberry clones and five cultivars

Primer	Polymorphic bands (no.)	Size range (bp)	Resolving power
UBC801	10	400-1,500	6.3
UBC808	11	350-1,350	8.3
UBC809	7	550-1,500	4.7
UBC810	11	350-1,450	7.4
UBC816	10	400-6,000	8.3
UBC817	8	550-1,650	3.8
UBC826	9	850-6,000	5.6
UBC827	15	450-1,950	11.3
UBC835	10	300-2,050	9.2
UBC867	16	300-1,850	11.7
UBC890	14	300-1,750	12.3
UBC891	12	400-1,450	7.9

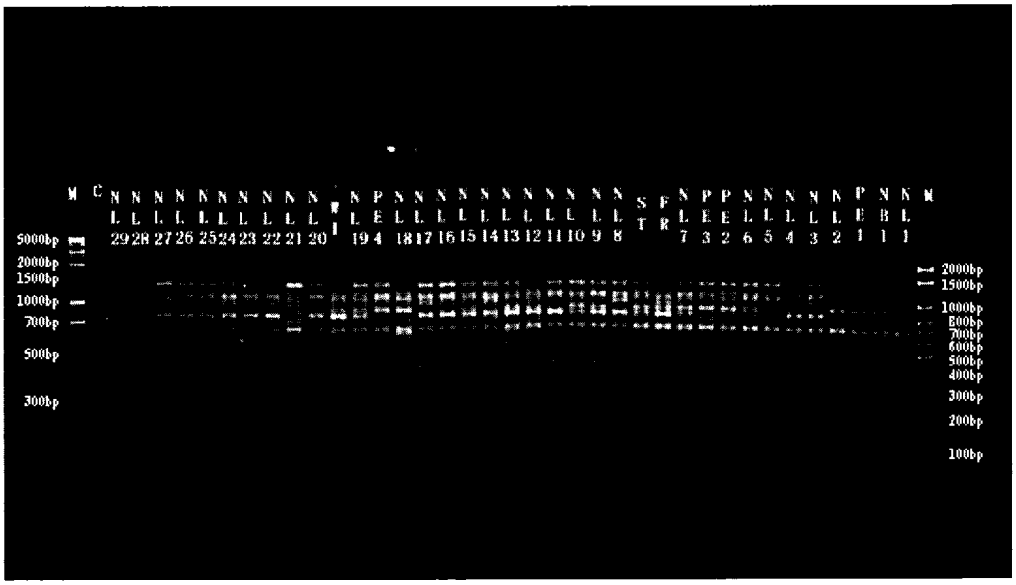

Figure 2: Inter simple sequence repeat (ISSR) banding patterns of 29 Newfoundland and Labrador (NL1-29), one New Brunswick (NB1), and four Prince Edward Island wild cranberry clones (PE1-4), and cultivars, 'Franklin' (FR), 'Stevens' (ST), and 'Wilcox' (WI), generated by primer UBC 801. M-standard molecular sizes: 1kb ladder (right) and 10kb (left).

Table 8: Identity, polymorphism, band size, and resolving power for 10 EST-PCR primers tested on 102 wild cranberry clones and five cultivars

Primer	Polymorphic bands (no.)	Size range (bp)	Resolving power
CA1590	3	450-1,250	0.4
CA1029	2	150-1,250	0.9
NA353	2	250-1,000	0.8
NA27	1	450	0.4
NA1068	4	350-850	3.3
CA54	4	300-1,900	2.4
CA1423	5	200-1,050	1.6
CA227	2	125-1,050	0.9
CA231	5	110-1,050	2.6
CA21	3	850- 1,350	1.3

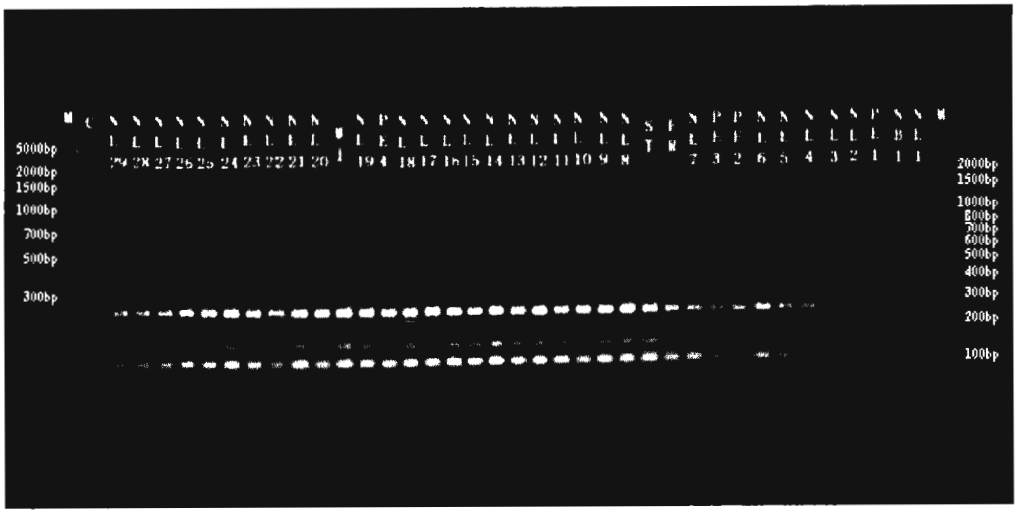

Figure 3: Expressed sequence tag- polymerase chain reaction (EST-PCR) banding patterns of 29 Newfoundland (NL1-29), one New Brunswick (NB1), and four Prince Edward Island wild cranberry clones (PE1-4), and cultivars, 'Franklin' (FR), 'Stevens' (ST), and 'Wilcox' (WI), generated by primer CA 231. M - standard molecular sizes: 1 kb ladder (right) and 10kb (left).

3.1.3 DNA polymorphism of EST-SSR primers

Five out of thirteen EST-SSR primers used produced polymorphic bands. A total of 21 polymorphic bands with a mean numbers of bands 4.2 were produced. The selected primers and the maximum number of polymorphic bands produced by each primer are listed in Table 9. Identical banding patterns were observed from repeated runs on all samples. The number of bands ranged from 3 for primer CA794 and NA961 to 6 for primer NA800. The survey of five primers revealed the presence of polymorphisms in the amplified DNA fragments in a range from 100 to 900 base pairs (Table 9). The resolving power values ranged from 1.0 for primer CA794 to 4.7 for primer NA800; with a mean value of 2.42 (Table 9). A representative banding pattern for primer

NA800 is presented in Figure 4. All other gel electrophoresis images for primer UBC801 are presented in Appendix I (Figure 22-24).

Table 9: Identity, polymorphism, band size and resolving power for 5 EST-SSR primers tested on 102 wild cranberry clones and five cultivars

Primer	Polymorphic bands (no.)	Size range (bp)	Resolving Power
CA421	4	150-900	1.1
CA794	3	100-275	1.0
NA1040	5	165-275	3.3
NA800	6	150-800	4.7
NA961	3	125-450	2.0

Figure 4: Expressed sequence tag- simple sequence repeats (EST-SSR) banding patterns of 29 Newfoundland cranberry wild clones (NL1-NL29), one New Brunswick wild cranberry clone (NB1), and four Prince Edward Island cranberry clones (PE1-PE4), and cultivars, 'Franklin' (FR), 'Stevens' (ST), and 'Wilcox' (WI), generated by primer NA 800. M-standard molecular sizes: 1kb ladder (right) and 10kb (left).

3.2 Polymorphism information content (PIC) and other measure of informativeness for ISSR, EST-PCR, and EST-SSR markers

3.2.1 Levels of informativeness for the ISSR marker system

For the ISSR primers, the major allele frequency ranged from a minimum of 0.03 for primers UBC827, UBC867, and UBC891 to a maximum of 0.20 for primer UBC817, with a mean of 0.76. The allele number ranged from 32 (UBC816) to 101 (UBC867, UBC827), with a mean number of 65. The expected heterozygosity and PIC value had a range each, from 0.93 for primer UBC817 to 0.99 for primers UBC827, UBC867, UBC890, and UBC891, with a mean value of 0.97 (Table 10).

Table 10: Variation patterns of 12 ISSR markers assayed in 107 cranberry genotypes

Markers	Major allele frequency	Allele no.	Expected heterozygosity	Polymorphic information content
UBC801	0.09	59	0.97	0.97
UBC808	0.07	60	0.97	0.97
UBC809	0.08	41	0.96	0.96
UBC810	0.06	60	0.97	0.97
UBC816	0.10	32	0.94	0.94
UBC817	0.20	40	0.93	0.93
UBC826	0.11	44	0.96	0.96
UBC827	0.03	101	0.99	0.99
UBC835	0.08	64	0.97	0.97
UBC867	0.03	101	0.99	0.99
UBC890	0.04	94	0.99	0.99
UBC891	0.03	89	0.99	0.99
Mean	0.08	65	0.97	0.97

3.2.2 Levels of informativeness for the EST-PCR marker

For EST-PCR primers, the major allele frequency ranged from a minimum 0.18 for primer CA231 to a maximum 0.84 for primer CA1590, with the mean value of 0.54. The allele number ranged from 2 (primer NA27) to 15 (primer NA1068), with a mean number of 8.1. The expected heterozygosity ranged from 0.28 for primer CA1590 to 0.89 for primer CA231, with a mean value of 0.60. The PIC value was lowest for the

primer CA1590 (0.26). The primer CA231 had the highest value (0.89) and the mean PIC value was 0.56 (Table 11).

Table 11: Variation patterns of 10 EST-PCR markers assayed in 107 cranberry genotypes

Markers	Major allele frequency	Allele no.	Expected heterozygosity	Polymorphic information content
CA227	0.67	4	0.51	0.47
CA231	0.18	18	0.89	0.89
CA21	0.50	5	0.67	0.62
CA54	0.30	11	0.81	0.78
CA1029	0.63	4	0.54	0.49
CA1423	0.64	14	0.57	0.56
CA1590	0.84	4	0.28	0.26
NA27	0.80	2	0.32	0.27
NA353	0.63	4	0.52	0.44
NA1068	0.25	15	0.86	0.85
Mean	0.54	8.1	0.60	0.56

3.2.3 Levels of informativeness for EST-SSR marker system

For EST-SSR primers, the major allele frequency ranged from a minimum 0.11 for primer NA800 to a maximum 0.61 for primer CA421, with the mean value of 0.35. Primer NA800 had the highest allele number (31), and the primer CA794 had the lowest (7). The mean allele number was 15.2. The expected heterozygosity ranged

from 0.57 for primer CA794 to 0.94 for primer NA800, with a mean value of 0.77. The PIC value ranged from 0.50 for primer CA794 to 0.94 for primer NA800 with a mean value of 0.74 (Table 12).

Table 12: Variation patterns of five EST-SSR markers assayed in 107 cranberry genotypes

Markers	Major allele frequency	Allele no.	Expected heterozygosity	Polymorphic information content
CA421	0.6075	10	0.5910	0.5609
CA794	0.5701	7	0.5711	0.5029
NA800	0.1121	31	0.9419	0.9389
NA961	0.3271	8	0.8151	0.7939
NA1040	0.1308	20	0.9187	0.9130
Mean	0.3495	15.2	0.7675	0.7419

3.3 Comparison of ISSR, EST-PCR, and EST-SSR markers for polymorphism and informativeness

Among the three DNA marker types, ISSR had the most polymorphic bands (133) followed by EST-PCR (31) and EST-SSR (21). However, EST-SSR markers had a higher average number of polymorphic bands per assay units (4.2) than those of EST-PCR markers (3.1). The highest mean number of polymorphic bands was observed with ISSR markers (11).

The ISSR marker detected the most locus and most locus per assay units (133 and 11, respectively) and it was followed by EST-PCR marker (10 and 1, respectively) and EST-SSR marker (5 and 1, respectively).

ISSR had the highest mean value of resolving power and PIC. These indexes with ISSR were followed by those with EST-SSR and EST-PCR (Table 13).

ISSR detected highest mean number of alleles per unit assays (65.4) followed by EST-SSR (15.2) and EST-PCR (8.1). Total number of effective alleles was highest for ISSR markers (570), and it was followed by EST-SSR markers (40) and EST-PCR markers (36.3). However, because of the high number of ISSR locus detected, EST-SSR had highest number of effective number of alleles per locus (7.94); followed by ISSR (4.28) and EST-PCR (3.63). EST-SSR had the highest mean major allele frequency (0.54), and it was followed by EST-PCR (0.35) and ISSR (0.08).

The ISSR had highest mean value of expected heterozygosity (0.97), and for EST-SSR and EST-PCR, these values were much lower, 0.77 and 0.6, respectively. Assay efficiency index was highest for the ISSR markers (48), and it was followed by EST-PCR markers (3.63) and EST-SSR markers (7.94). The highest multiplex ratio and marker index were observed for ISSR, which were 1.24 and 1.2, respectively.

Because there were no monomorphic bands observed, EST-PCR and EST-SSR multiplex ratios for were 1.00 (Belaj et al. 2003; Schubert et al. 2001). Also, EST-SSR had a higher marker index (0.74) than that of EST-PCR (0.56) (Table 13).

Table 13: Levels of polymorphism and comparison of informativeness obtained with ISSR, EST-PCR, and EST-SSR markers in 102 cranberry clones and five cultivars

Indexes	Marker system		
	ISSR	EST-PCR	EST-SSR
Number of assay units	12	10	5
Number of polymorphic bands	133	34	21
Average number of polymorphic bands/ assay units	11	3.4	4.2
Number of loci	133	10	5
Number of loci/ assay units	11	1	1
Average of resolving power	8.06	1.46	2.42
Average of polymorphism information content	0.97	0.56	0.74
Average of allele number detected	65.4	8.1	15.2
Average of major allele frequency	0.08	0.54	0.35
Total number of effective alleles	569.9	36.3	39.7
Effective number of alleles per locus	4.28	3.63	7.94
Assay efficiency index	47.5	3.63	7.94
Average of expected heterozygosity	0.97	0.60	0.77
Multiplex ratio	1.24	1.00	1.00
Marker index	1.20	0.56	0.74

3.4 The similarity matrix and the cophenetic correlation coefficient value

Cophenetic correlation was used as a measure of goodness of fit for each cluster analysis. The cophenetic correlation coefficient (CCC) relates the level of distortion between the UPGMA and Jaccard's coefficient. A higher CCC indicates a better fit. The matrix correlation (r) values based on ISSR, EST-PCR, and EST-SSR markers were 0.91225, 0.81260, and 0.67578, respectively. The value based on the combination of ISSR, EST-PCR, and EST-SSR markers was 0.90830.

3.5 The unweighted pair-group method with arithmetic averages (UPGMA) clustering analysis

3.5.1 The ISSR UPGMA clustering based on Jaccard's similarity coefficient

The UPGMA clustering based on the Jaccard's coefficients obtained with ISSR marker is shown in Figure 5, and contains four main clusters. Cluster I contained 29 NL clones, one NB clone, four PEI clones, and three cultivars ('Franklin', 'Stevens',

and 'Wilcox'). This cluster was resolved into two sub-clusters (Cluster I-1 and Cluster I-2) at the similarity coefficient of about 0.483, leaving 'NL21' as the outlier at the similarity coefficient of 0.482. Cluster I-1 was further divided into two sub-sub-clusters: Cluster I-1-1 and Cluster I-1-2, at the similarity coefficient of 0.51. Cluster I-1-1 contained 16 NL clones, three PEI clones, one NB clone, and one cultivar. The Cluster I-1-1 was divided into two groups at the similarity coefficient of approximately 0.53: (1) 'NL1', 'NB1', 'PEI', 'NL2', 'NL3', 'NL4', 'NL5', 'NL6', 'PE2', 'NL7', and 'PE3'; and (2) cultivar 'Wilcox' (WI), 'NL20', 'NL23', 'NL24', 'NL25', 'NL22', 'NL26', 'NL27', 'NL28', and 'NL29'. Among them, 'NL23' and 'NL24' showed 0.89 similarity coefficient in the UPGMA analysis of ISSR. Cluster I-1-2 contained twelve NL clones and one PEI clone. Cluster I-1-2 was resolved into two groups: (1) 'NL8', 'NL9', 'NL13', 'NL10', 'NL11', 'NL14', and 'NL12'; and (2) 'NL15', 'NL16', 'NL19', 'NL17', 'NL18', and 'PE4'. Cluster I-2 contained two cultivars: 'Franklin' and 'Steven'.

Cluster II contained twelve NL clones, twelve PEI clones, two NB clones, and the cultivar 'Pilgrim'. This Cluster II was divided into two sub-clusters: Cluster II-1 and Cluster II-2, at a similarity coefficient of 0.41. Cluster II-1 included seven NL clones, two NB clones, and seven PEI clones and divided into two groups at a similarity coefficient of 0.60, leaving 'PE11' as an outlier at a similarity coefficient of 0.54. The first group included four NL clones ('NL30', 'NL32', 'NL31', and 'NL33') and the

second three NL clones ('NL34', 'NL35', and 'NL36'), two NB clones ('NB3', and 'NB2'), and six PEI clones ('PE9', 'PE10', 'PE7', 'PE5', 'PE6', and 'PE8'). The Cluster II-2 included five NL clones ('NL39', 'NL37', 'NL40', 'NL41', and 'NL38'), five PEI clones ('PE12', 'PE14', 'PE13', 'PE16', and 'PE15'), and cultivar 'Pilgrim'.

Cluster III included 22 NL clones, two PEI clones, five NB clones, and one NS clone. This cluster was resolved into two sub-clusters (Cluster III-1 and Cluster III-2) at the similarity coefficient of 0.57. Cluster III-1 included eight NL clones: 'NL42', 'NL47', 'NL48', 'NL49', 'NL45', 'NL46', 'NL43', and 'NL44'. Cluster III-2 included fourteen NL clones, two PEI clones, five NB clones, and one NS clone. Cluster III-2 was divided into two sub-sub-clusters (Cluster III-2-1 and Cluster III-2-2) at the similarity coefficient of 0.62. Cluster III-2-1 included eight NL clones and was divided into two groups : (1)'NL50', 'NL54', 'NL51', 'NL52', and 'NL53'; and (2) 'NL55', 'NL56', and 'NL57'. Cluster III-2-2 included six NL clones, two PEI clones, five NB clones, and one NS clone and was resolved into two groups: (1) 'NL58', 'NL60', 'NL62', 'NL61', 'NL63', 'NB5', 'NL59', and 'NB4'; and (2) 'NB6', 'PE17', 'NB8', 'NS1', 'NB7', and 'PE18'.

Cluster IV included eight NL clones ('NL64', 'NL67', 'NL68', 'NL69', 'NL70', 'NL71', 'NL65', and 'NL66'), two NB clones ('NB9' and 'NB10'), two PEI clones ('PE19' and 'PE20'), and the cultivar 'Ben Lear'. Among them, 'NL64', 'NL67',

'NL68', 'NL69', 'NL70', and cultivar 'Ben Lear' formed one group at the similarity coefficient 0.733. 'NB9', 'NL71', 'NB10', 'PE19', 'PE20', and 'NL65' formed another group at the similarity coefficient 0.712 (Figure 5).

Coefficient

Figure 5: UPGMA (unweighted pair-group method with arithmetic averages) dendrogram estimating the genetic distance among 102 Canadian wild cranberry clones (NL1-71, NB1-10, PE 1-20) and five cultivars, 'Franklin' (FR), 'Stevens' (ST), 'Wilcox' (WI), 'Ben Lear' (BE), and 'Pilgrim' (PI), using the Jaccard's similarity matrix of ISSR band profiles.

3.5.2 The UPGMA clustering based on EST-PCR marker Jaccard's similarity coefficient

The UPGMA clustering based on Jaccard's coefficients with the EST-PCR marker is shown in Figure 6. Three main clusters were found, including 101 wild cranberry clones and five cultivars, leaving 'NL38' as an outlier at 0.21 similarity coefficient index. All genotypes, except 'NL38', shared 0.52 similarity coefficient in clustering with EST-PCR DNA profiles.

Cluster I contained nine clones and was divide into two groups with a similarity coefficient of 0.70: (1) 'NL1', 'NB1', 'PE1', and 'NL3'; and (2) 'NL2', 'NL4', 'PE3', 'PE2', and 'NL6'.

Cluster II contained 61 NL clones, nine NB clones, thirteen PEI clones, one NS clone, and four cultivars 'Wilcox', 'Franklin', 'Stevens', and 'Ben Lear'. The genotypes of the Cluster II shared the similarity coefficient of 0.59 and was resolved into two sub-cultivars (Cluster II-1 and Cluster II-2). Cluster II-1 was resolved into two

sub-sub-clusters (Cluster II-1-1 and Cluster II-1-2) with a similarity coefficient of 0.62. Cluster II-1-1 was resolved into two sub-sub-sub-clusters (Cluster II-1-1-1 and Cluster II-1-1-2) and shared a similarity coefficient of 0.63. Cluster II-1-1-1 was divided into two sub-sub-sub-sub-clusters (Cluster II-1-1-1-1 and Cluster II-1-1-1-2) showing a similarity coefficient of 0.64. Cluster II-1-1-1-1 was resolved into two sub-sub-sub-sub-sub-clusters (Cluster II-1-1-1-1-1 and Cluster II-1-1-1-1-2) with similarity coefficient of 0.68. The Cluster II-1-1-1-1-1 was resolved into two sub-sub-sub-sub-sub-sub-clusters (Cluster II-1-1-1-1-1-1 and Cluster II-1-1-1-1-1-2) sharing a similarity coefficient of 0.71.

Cluster II-1-1-1-1-1-1 contained sixteen NL clones, one PEI clone, and three cultivars. Cluster II-1-1-1-1-1-1 was resolved into two groups with a similarity coefficient of 0.74: (1) 'NL5', 'NL18', 'NL7', 'NL25', 'NL8', 'NL22', 'NL24', and cultivar 'Wilcox'; and (2) cultivar 'Franklin', 'NL12', 'NL11', 'NL19', 'NL17', 'PE12', 'NL15', 'NL10', cultivar 'Stevens', 'NL23', 'NL9', and 'NL64'.

Cluster II-1-1-1-1-1-2 included 24 NL clones ('NL13', 'NL21', 'NL59', 'NL61', 'NL62', 'NL63', 'NL60', 'NL16', 'NL36', 'NL26', 'NL56', 'NL57', 'NL71', 'NL30', 'NL54', 'NL58', 'NL55', 'NL50', 'NL53', 'NL14', 'NL29', 'NL27', 'NL28', and 'NL20'), eight NB clones ('NB4', 'NB5', 'NB6', 'NB8', 'NB7', 'NB9', 'NB10', and 'NB2'), five PEI clones ('PE18', 'PE17', 'PE19', 'PE5', and 'PE20'), the NS clone

'NS1', and cultivar 'Ben Lear'. Among them, genotypes of 'NL61' and 'NL62' showed 1.00 similarity coefficient in the EST-PCR clustering.

Cluster II-1-1-1-1-2 contained one PEI clone ('PE4') and six NL clones ('NL42', 'NL67', 'NL68', 'NL69', 'NL70', and 'NL66').

Cluster II-1-1-1-2 contained five PEI clones and one NL clone. This Cluster II-1-1-1-2 was divided into two groups with a similarity coefficient of 0.71: (1) 'PE6', 'PE11', and 'NL37'; and (2) 'PE9', 'PE10', and 'PE13'.

Cluster II-1-1-2 contained clones of 'NL34' and 'NL35'.

Cluster II-1-2 contained ten NL clones. This Cluster II-1-2 was resolved into three groups: (1) 'NL33', 'NL43', and 'NL44'; (2) 'NL45', 'NL46', 'NL47', 'NL48', and 'NL49'; and (3) 'NL51' and 'NL52'.

The Cluster II-2 contained four genotypes: 'NL31', 'NL32', 'PE7', and 'NB3'.

'NL65' standed alone as an outlier outside the Cluster II with a similarity coefficient of 0.554.

The Cluster III contained four PEI clones ('PE8', 'PE14', 'PE15', and 'PE16'), three NL clones ('NL39', 'NL41', and 'NL40'), and the cultivar Pilgrim (PI) at the similarity coefficient of 0.53. Clones 'PE14', 'NL39', 'PE15', 'PE16', 'NL41' formed one group with a similarity coefficient of 0.61. Clone 'NL40' and cultivar 'Pilgrim' formed another group at the similarity coefficient of 0.70(Figure 6).

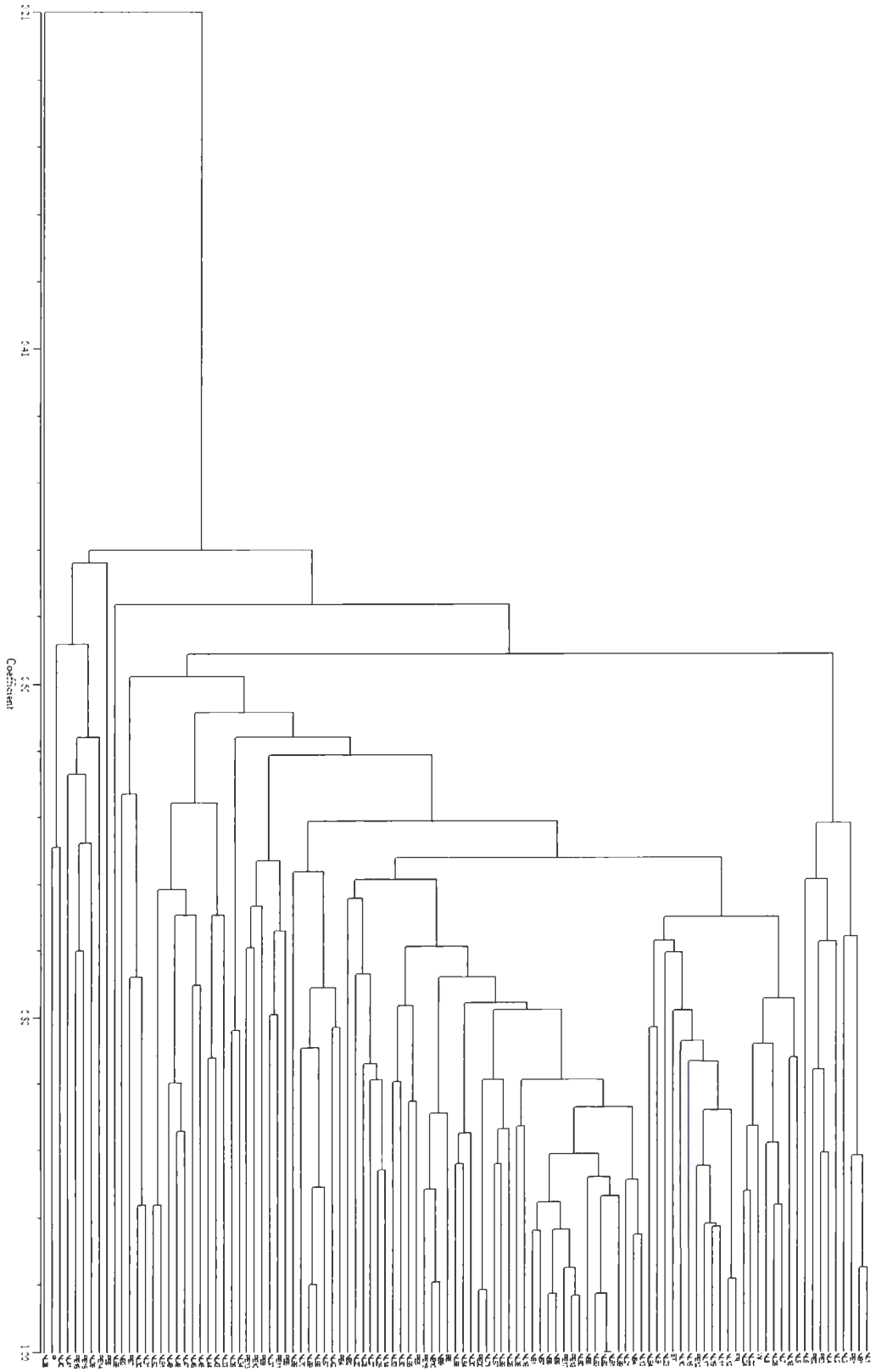
Coefficient
1.25
1.00
0.75
0.50
0.25

Figure 6: UPGMA (unweighted pair-group method with arithmetic averages) dendrogram estimating the genetic distance among 102 wild cranberry clones from four Canadian provinces (NL1-71, NB1-10, PE 1-20) and five cultivars, 'Franklin' (FR), 'Stevens' (ST), 'Wilcox' (WI), 'Ben Lear' (BE), and 'Pilgrim' (PI), using the Jaccard's similarity matrix of EST-PCR band profiles.

3.5.3 The UPGMA clustering based on EST-SSR markers Jaccard's similarity coefficient

The UPGMA clustering based on Jaccard's coefficients using EST-SSR marker is shown in Figure 7. Six main clusters were identified.

Cluster I included twelve NL clones, two NB clones, three PEI clones, and cultivar 'Ben Lear'. Cluster I was resolved into two sub-clusters (Cluster I-1 and Cluster I-2) with a similarity coefficient of 0.58, leaving 'PE1' as an outlier at the similarity coefficient of 0.56. Cluster I-1 included twelve NL clones, two NB clones, three PEI clones, and one cultivar. Cluster I-1 was resolved into two groups at the similarity coefficient of 0.652: (1) 'NL1', 'NL3', 'NB1', 'NL70', cultivar 'Ben Lear', 'NL66', 'NL69', and 'NL71'; and (2) 'NL67', 'NB10', 'PE19', and 'PE20'. Cluster I-2 included five NL clones 'NL2', 'NL4', 'NL5', 'NL33', and 'NL65', sharing the 0.61 similarity coefficient.

Cluster II included 23 NL clones, five NB clones, one NS clone, and one PEI clone; they were grouped together at the similarity coefficient of 0.54. Cluster II was resolved into two sub-clusters (Cluster II-1 and Cluster II-2) at the similarity coefficient of 0.55. Cluster II-1 was divided into two sub-sub-clusters (Cluster II-1-1 and Cluster II-1-2) at the similarity coefficient of 0.60, leaving the clone of 'NL60' as an outlier at the similarity coefficient of approximately 0.55. This Cluster II -1-1 was resolved into two sub-sub-sub-lusters: Cluster II-1-1-1 ('NL42', 'NL50', and 'NL44') and Cluster II-1-1-2 at the similarity coefficient of 0.65. The Cluster II-1-1-2 contained fifteen NL clones and one NB clone and was resolved into four groups: (1) 'NL43', 'NL47', 'NL49', 'NL51', and 'NL46'; (2) 'NL57', 'NL58', 'NL62', 'NL63', 'NL59', 'NL61', and 'NB4'; (3) 'NL52', and 'NL53'; and (4) 'NL55', and 'NL56'. Among the genotypes in Cluster II-1-1-2, 'NL58', 'NL62', and 'NL63' showed 1.00 similarity coefficient in EST-SSR UPGMA analysis. Cluster II-1-2 included four NL clones: 'NL45', 'NL48', 'NL54', and 'NL64'. This sub-cluster consisted of 'NB5', 'PE17', 'NB6', 'NB7', 'NB8', and 'NS1'. Among them, 'NB7' and 'NB8' showed 1.00 similarity coefficient value in EST-SSR UPGMA clustering.

Cluster III included ten NL clones, fourteen PEI clones, three NB clones, and cultivars 'Franklin', 'Stevens', and 'Pilgrim', at the similarity coefficient of 0.47, and was divided into two sub-clusters (Cluster III-1 and Cluster III-2). Cluster III-1 was divided into two sub-sub-clusters (Cluster III-1-1 and Cluster III-1-2) at the similarity

coefficient of 0.52. The Cluster III-1-1 was resolved into two sub-sub-sub-lusters (Cluster III-1-1-1 and Cluster III-1-1-2) at the similarity coefficient of 0.59. This Cluster III-1-1-1 consisted of five NL clones, seven PEI clones, two NB clones, and two cultivar, and was divided into three groups at the similarity coefficient of 0.62: (1) 'PE2', cultivar 'Franklin', 'NL7', 'PE15', and 'PE18'; (2) 'NL9', 'NL68', and 'NL10'; (3) 'NL35', 'PE5', 'PE12', 'PE10', 'NB3', 'PE9', cultivar 'Pilgrim', and 'NB9'. Cluster III-1-1-2 included 'PE3', 'PE13', 'PE7', cultivar 'Stevens', 'NB2', 'PE6', 'NL32', 'PE8', and 'NL34', grouping together at the similarity coefficient of 0.49. Cluster III-1-2 included three clones: 'NL36', 'NL37', and 'PE14'. Cluster III-2 included two clones: 'NL39' and 'PE16'.

Cluster IV included 22 NL clones, one PEI clone, and cultivar Wilcox (WI); and was resolved into two sub-clusters (Cluster IV-1 and Cluster IV-2) at the similarity coefficient of 0.53, leaving 'NL12' as outlier at the similarity coefficient of 0.49. Cluster IV-1 was divided into two sub-sub-clusters Cluster IV-1-1 ('NL6', 'NL30' and 'NL31') and Cluster IV-1-2 at the similarity coefficient of 0.54. Cluster IV-1-2 contained 15 NL clones and one PEI clone. This Cluster IV-1-2 was divied into three groups: (1) 'NL8', 'NL17', 'NL11', 'NL22', 'NL29', 'NL24', 'NL27', and 'NL25'; (2) 'NL14', 'NL15', and 'NL26'; and (3) 'NL16', 'NL19' 'NL20', 'PE4', and 'NL21'. Cluster IV-2 contains three NL clones ('NL13', 'NL28', and 'NL18') and the cultivar 'Wilcox', with the similarity coefficient of 0.57.

Cluster V contained three clones 'NL23', 'NL40', and 'NL41' with the similarity coefficient of 0.39. Cluster VI contained 'PE11' and 'NL38' with the similarity coefficient of 0.59 (Figure 7).

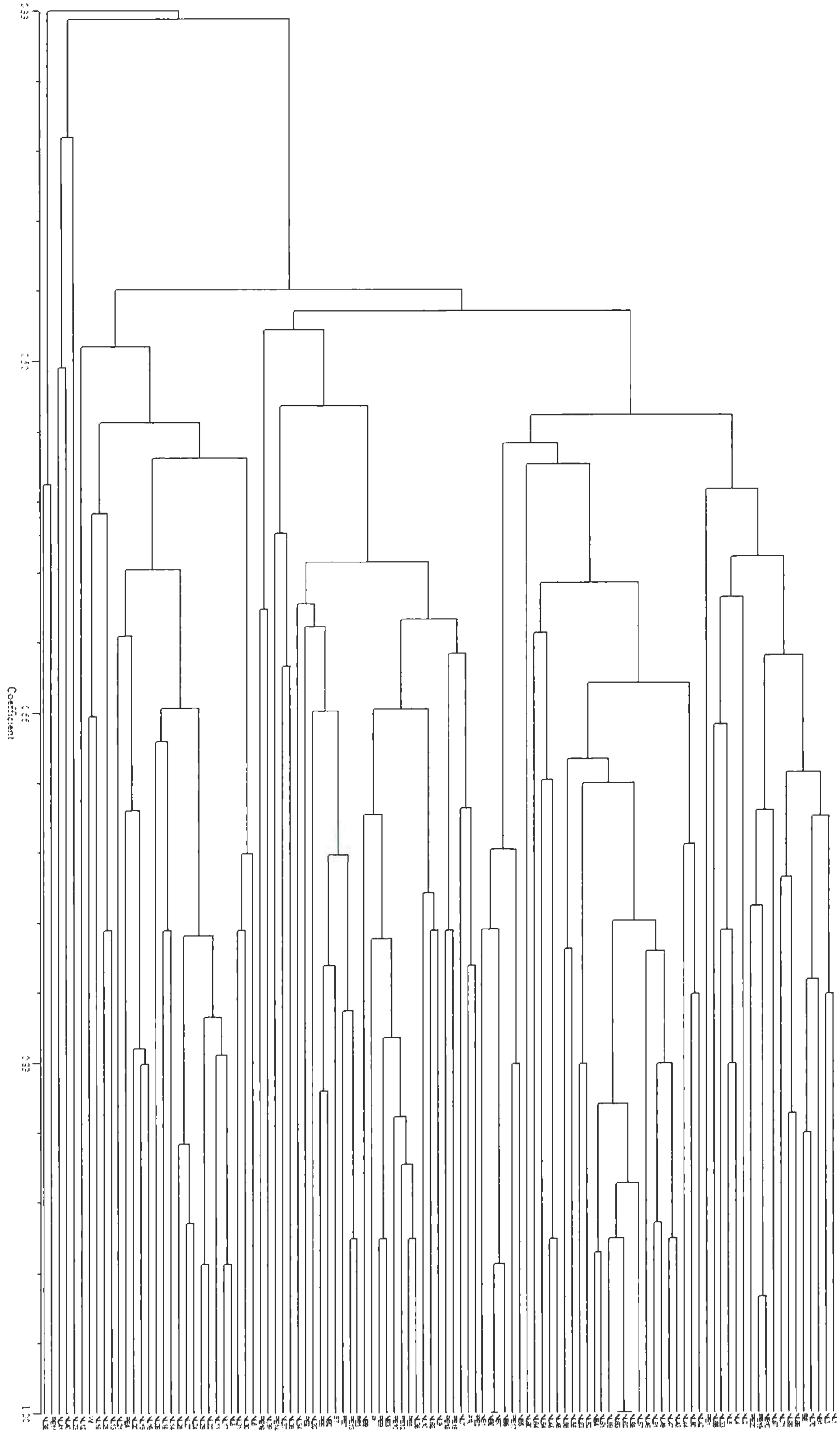

Figure 7: UPGMA (unweighted pair-group method with arithmetic averages) dendrogram estimating the genetic distance among 102 wild cranberry clones from four Canadian provinces (NL1-71, NB1-10, PE 1-20) and five cultivars, 'Franklin' (FR), 'Stevens' (ST), 'Wilcox' (WI), 'Ben Lear' (BE), and 'Pilgrim' (PI), applying the Jaccard's similarity matrix of EST-SSR band profiles.

3.5.4 The UPGMA clustering based on the combination of ISSR, EST-PCR, and EST-SSR markers Jaccard's similarity coefficient

The UPGMA clustering based on Jaccard's coefficients with the combination of ISSR, EST-PCR, and EST-SSR markers is shown in Figure 8. Figure 8 identified three main clusters, including 101 wild cranberry clones and five cultivars, leaving 'NL38' as an outlier at 0.31 similarity index.

Cluster I contained 29 NL clones, one NB clone, four PEI clones, and three cultivars 'Franklin', 'Stevens', and 'Wilcox'. Cluster I was divided into two sub-clusters (Cluster I-1 and Cluster I-2) at a similarity coefficient of 0.531. Cluster I-1 contained seven NL clones, one NB clone, and three PEI clones; and was divided into three groups at the similarity coefficient of 0.59: (1) 'NL1', 'NB1', 'PE1', 'NL3', and 'NL2'; and (2) 'NL4', 'NL5', and 'NL6'; and (3) 'PE2', 'PE3', and 'NL7'. Cluster I-2 contained 22 NL clones, one PEI clone, and three cultivars. This sub-cluster was divided into two sub-sub-clusters (Cluster I-2-1 and Cluster I-2-2) at the similarity coefficient of approximately 0.54. Cultivars 'Franklin' and 'Stevens' were grouped together at the similarity coefficient of 0.60 in Cluster I-2-1. Cluster I-2-2 was

resolved into four groups at 0.57 similarity coefficient, leaving 'NL21' as an outlier of Cluster I-2-2 at the similarity coefficient of 0.56. The four groups included 21 NL clones, one PEI clone and one cultivar: (1) 'NL8' and 'NL9'; (2) 'NL10', 'NL11', 'NL12', 'NL13', 'NL14', 'NL15', 'NL16', 'NL19', and 'NL17'; (3) 'NL18', 'PE4', cultivar 'Wilcox', 'NL20', 'NL22', 'NL23', 'NL24', and 'NL25'; and (4) 'NL26', 'NL27', 'NL28', and 'NL29'.

Cluster II contained 30 NL clones, seven NB clones, one NS clone, four PEI clones, and the cultivar 'Ben Lear'. Cluster II was divided into two sub-clusters (Cluster II-1 and Cluster II-2) at the similarity coefficient of approximately 0.48. Cluster II-1 included 21 NL clones, five NB clones, one NS clone and two PEI clones, and was resolved into two sub-sub-clusters (Cluster II-1-1 and Cluster II-1-2) at the similarity coefficient of 0.50. The genotypes of Cluster II-1-1 was grouped at 0.67 similarity coefficient. Cluster II-1-1 consisted of 'NL42', 'NL43', 'NL45', 'NL46', 'NL47', 'NL48', 'NL49', and 'NL44'. Cluster II-1-2 contained thirteen NL clones, five NB clones, one NS clone, and two PEI clones. This Cluster II-1-2 was resolved into two sub-sub-sub-clusters (Cluster II-1-2-1 and Cluster II-1-2-2) at the similarity coefficient of 0.64. Cluster II-1-2-1 was resolved into three groups: (1) 'NL50', 'NL51', and 'NL52'; (2) 'NL53' and 'NL54'; and (3) 'NL55', 'NL56', and 'NL57'. Cluster II-1-2-2 was resolved into two groups: (1) 'NL58', 'NL63', 'NL61', 'NL62', 'NL60', 'NL59', 'NB4', and 'NB5'; and (2) 'NB6', 'NB8', 'NB7', 'NS1', 'PE17',

and 'PE18'. Cluster II-2 included eight NL clones, two NB clones, two PEI clones, and one cultivar, and was resolved into two sub-sub-clusters (Cluster II-2-1 and Cluster II-2-2) at the similarity coefficient of 0.62. Cluster II-2-1 was consisted of 'NL64', 'NL67', 'NL68', 'NL69', 'NL70', the cultivar 'Ben Lear', 'NB9', 'NL71', 'NB10', 'PE19', and 'PE20'. Clones 'NL67', 'NL68', 'NL69', 'NL70', and cultivar 'Ben Lear' formed a group at the similarity coefficient 0.685. Clones 'NB9', 'NL71', 'NB10', 'PE19', and 'PE20' formed another group at the similarity coefficient 0.687. Among them, 'NB10' and 'PE19' showed 1.00 similarity coefficient at the clustering analysis based on the combination of ISSR, EST-PCR, and EST-SSR markers. Cluster II-2-2 was consisted of 'NL65' and 'NL66'.

Cluster III included eleven NL clones, two NB clones, twelve PEI clones, and one cultivar 'Pilgrim', and was resolved into two sub-clusters (Cluster III-1 and Cluster III-2) at the similarity coefficient of 0.49. Cluster III-1 contained eight NL clones, two NB clones, and nine PEI clones and was resolved into two sub-sub-cultivars (Cluster III-1-1 and Cluster III-1-2) at the similarity coefficient of 0.54. Cluster III-1-1 consisted of 'NL30', 'NL35', 'NB3', 'NL34', 'NB2', 'PE7', 'NL36', 'PE5', 'PE6', 'PE9', 'PE10', 'NL31', 'NL32', 'NL33', and 'PE8'. Cluster III-1-2 was consisted of 'PE11', 'PE12', 'PE13', and 'NL37'. Cluster III-2 contained three PEI clones, three NL clones, and cultivar 'Pilgrim', and was divided into two sub-sub-lusters (Cluster III-2-1 and Cluster III-2-2) at the similarity coefficient of 0.55. Cluster III-2-1

consisted of 'PE14', 'NL39', 'PE16', and 'PE15'. Cluster III-2-2 consisted of 'NL40', the cultivar 'Pilgrim', and 'NL41' (Figure 8).

Coefficient
1.31
1.44
1.57
1.71
0.15

Figure 8: UPGMA (unweighted pair-group method with arithmetic averages) dendrogram estimating the genetic distance among 102 wild cranberry clones from four Canadian provinces (NL1-71, NB1-10, PE 1-20) and five cultivars, 'Franklin' (FR), 'Stevens' (ST), 'Wilcox' (WI), 'Ben Lear' (BE), and 'Pilgrim' (PI), applying the Jaccard's similarity matrix of ISSR, EST-PCR, and EST-SSR combined band profiles.

3.6 Principle co-ordinate (PCo) analysis

3.6.1 Principle co-ordinated analysis based on Jaccard's similarity coefficients generated by ISSR markers

The PCo analysis based on the frequencies of occurrence of polymorphic ISSR marker among 102 wild clones and five cultivars showed four distinct groups among the genotypes (Figure 9). Most of the genotypes showed similar grouping as the UPGMA with the marker of ISSR. For example, 27 genotypes 'NL30', 'NL32', 'NL31', 'NL33', 'NL34', 'NL35', 'NB3', 'NL36', 'PE9', 'PE10', 'NB2', 'PE7', 'PE5', 'PE6', 'PE8', 'PE11', 'PE12', 'PE14', 'NL39', 'PE13', 'NL37', 'PE16', 'NL40', cultivar 'Pilgrim', 'NL41', 'PE15' and 'NL38' grouped in Cluster II were also grouped together in plotting of PCo analysis. The PCo analysis showed that the genotypes clearly formed four groups including all wild clones and cultivars except 'NL17' as outlier. In Figure 9, the two-dimensional plot of the principal coordinates analysis of

distance on ISSR marker considered the first two components 12.77% and 10.96% of the total variation for the first and second components, respectively. In Figure 10, the three-dimensional plot of the principal coordinates analysis of distance on ISSR markers considered the first three components 12.77%, 10.96%, and 7.05% of the total variation for the first, second, and the third component, respectively.

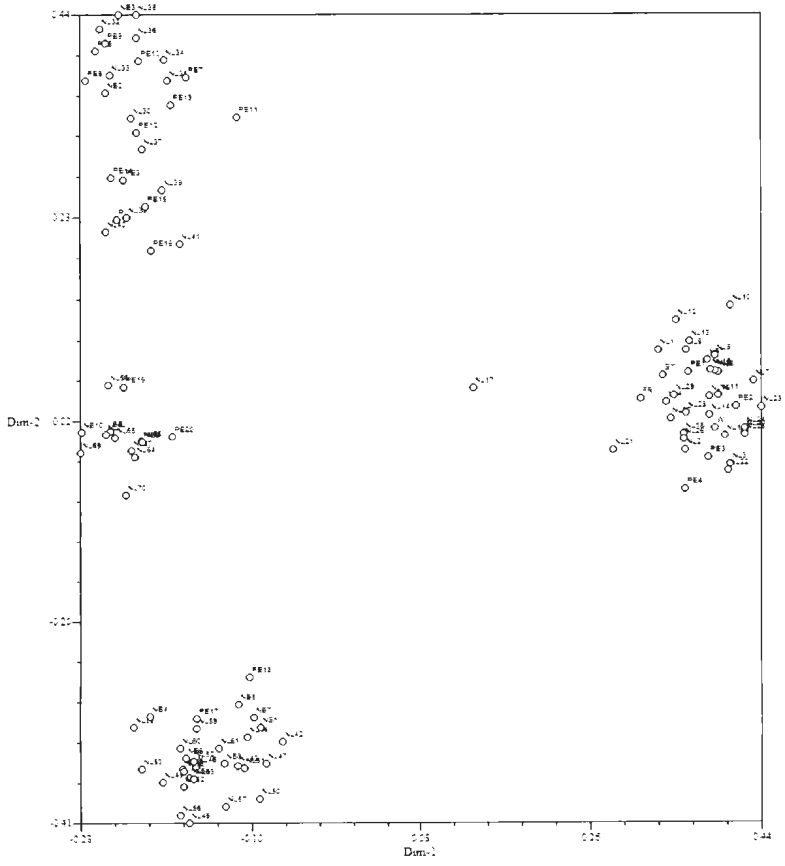

Figure 9: Two-dimensional plot for the first two components of the principal co-ordinates analysis of distance among 102 wild cranberry clones collected from four Canadian provinces and five cultivars, on ISSR marker.

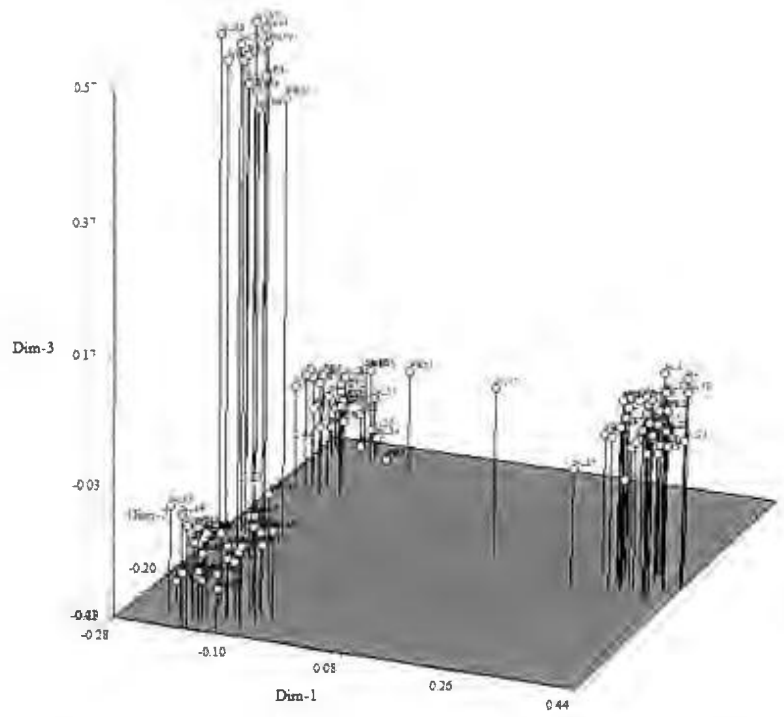

Figure 10: Three-dimensional plot for the first three components of the principal co-ordinates analysis of distance among 102 wild cranberry clones collected from four Canadian provinces and five cultivars, on ISSR marker.

3.6.2 Principle co-ordinate analysis based on Jaccard's similarity coefficients generated by EST-PCR markers

The PCo analysis based on the frequencies of occurrence of polymorphic EST-PCR marker among the wild clones and cultivars showed that the plotting of the first three components, represented 13.96%, 9.66%, and 7.11% of the total variation for the first,

second, and the third component, respectively. In the Figure 11 and Figure 12, wide variation is observed among the cranberry genotypes. The Figure 11, the two-dimensional plot of the principal coordinates analysis of distance on EST-PCR marker considered the first two components 13.96% and 9.66% of the total variation for the first and second components, respectively. In Figure 10, the three-dimensional plot of the principal coordinates analysis of distance on EST-PCR marker considered the first three components 13.96%, 9.66%, and 7.11% of the total variation for the first, second, and the third component, respectively. PCo analysis based on EST-PCR marker confirmed clustering analysis. For example, the genotypes of Cluster I in clustering analysis, 'NL1', 'NB1', 'PE1', 'NL3', 'NL2', 'NL4', 'PE3', 'PE2', and 'NL6', also grouped together in plotting of PCo analysis.

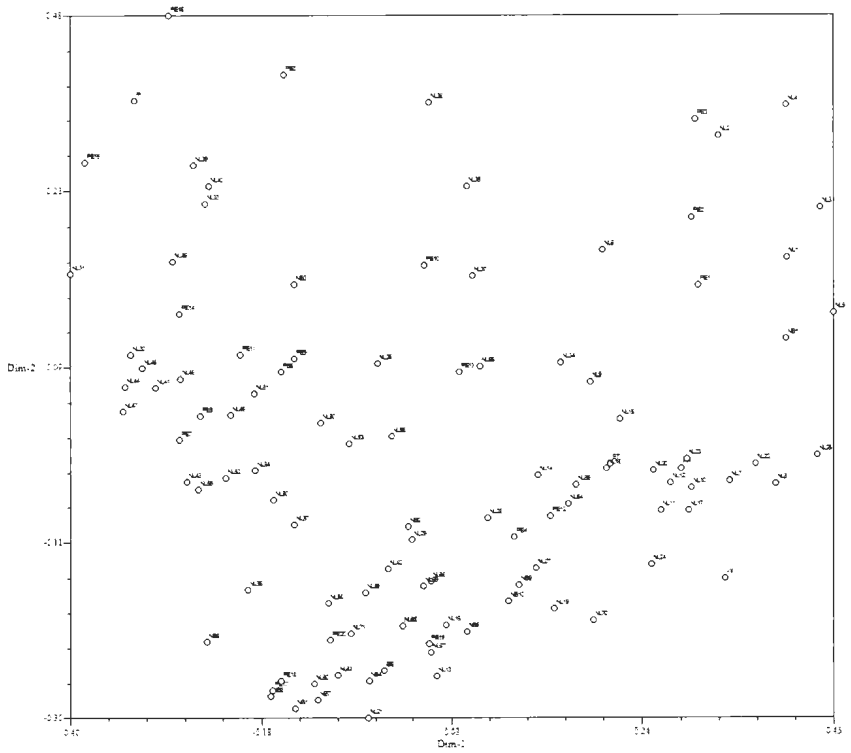

Figure 11: Two-dimensional plot for the first two components of the principal co-ordinates analysis of distance among 102 wild cranberry clones collected from four Canadian provinces and five cultivars, on EST-PCR marker.

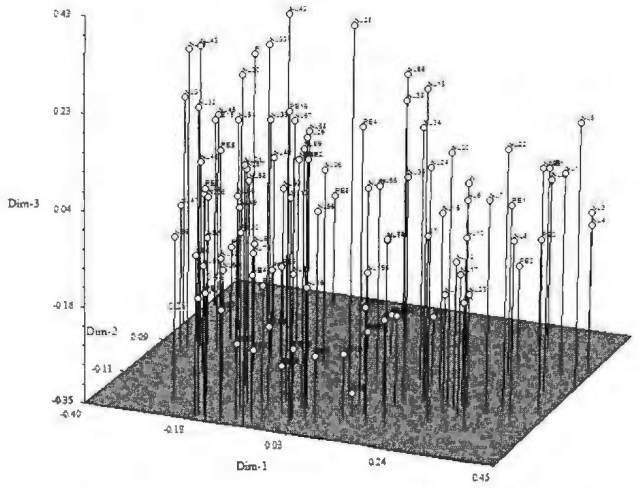

Figure 12: Three-dimensional plot for the first three components of the principal co-ordinates analysis of distance among 102 wild cranberry clones collected from four Canadian provinces and five cultivars, on EST-PCR marker.

3.6.3 Principle co-ordinate analysis based on Jaccard's similarity coefficients generated by EST-SSR markers

The PCo analysis based on the frequencies of occurrence of polymorphic EST-SSR marker among the 102 wild clones and five cultivars showed that the plotting of the first three components represented 13.32%, 9.37%, and 8.03% of the total variation for the first, second, and the third component, respectively. PCo analysis indicated an exitance of considerable variation among the genotypes. The Figure 13, the two-dimensional plot of the principal coordinates analysis of distance on EST-SSR

marker considered the first two components 13.32% and 9.37% of the total variation for the first and second components, respectively. In Figure 14, the three-dimensional plot of the principal coordinates analysis of distance on EST-PCR marker considered the first three components 13.32%, 9.37%, and 8.03% of the total variation for the first, second, and the third component, respectively. The way that genotypes grouped in PCo conformed the grouping of the analysis of UPGMA. For example, in PCo analysis, cultivars 'Franklin', 'Stevens', and 'Pilgrim', and clones 'PE12', 'NB2', 'PE6', 'NL7', 'PE9', 'PE10', 'NB3', 'PE5', 'PE13', 'PE3', 'NL34', 'NL36' were grouped together in 2D and 3D plotting; whereas, they were also grouped together in clustering (Figure 13; Figure 14).

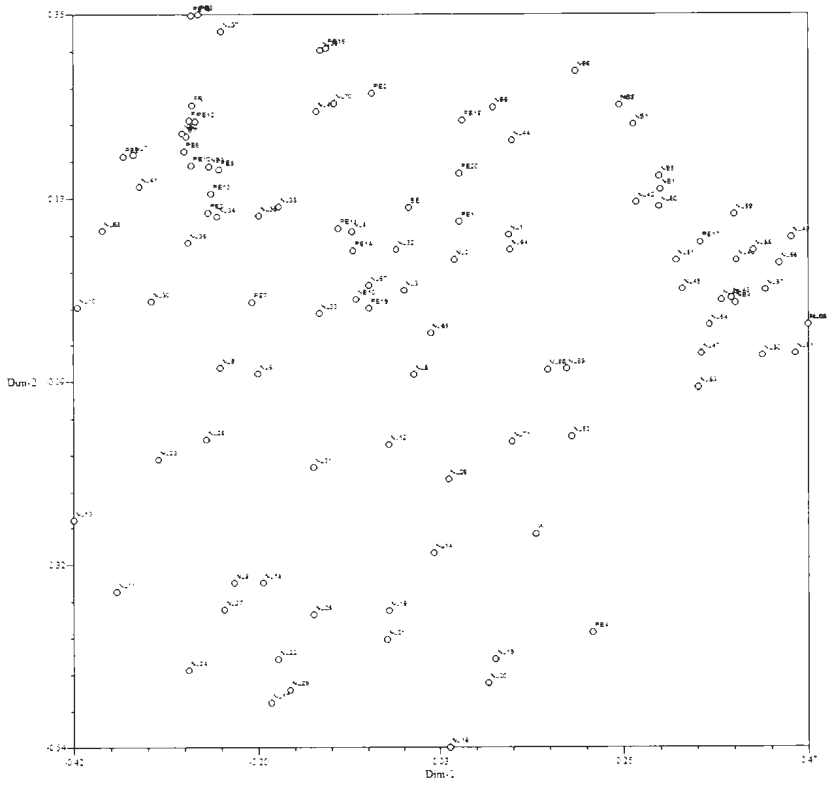

Figure 13: Two-dimensional plot for the first two components of the principal co-ordinates analysis of distance among 102 wild cranberry clones collected from four Canadian provinces and five cultivars, on EST-SSR marker.

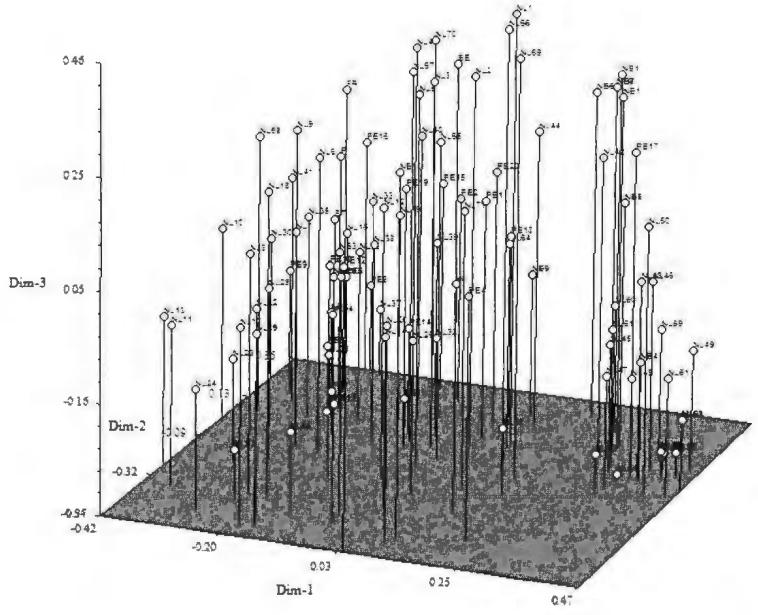

Figure 14: Three-dimensional plot for the first three components of the principal coordinates analysis of distance among 102 wild cranberry clones collected from four Canadian provinces and five cultivars, on EST-SSR marker.

3.6.4 Principle co-ordinate analysis based on Jaccard's similarity coefficients generated by the combination of ISSR, EST-PCR, and EST-SSR markers

The principle co-ordinate (PCo) analysis based on frequencies of occurrence of the combination of ISSR, EST-PCR, and EST-SSR markers among the 107 genotypes, showed that the plotting of the first three components represented 11.64%, 10.05%, and 6.30% of the total variation for the first, second, and the third component, respectively. Figure 15, the two-dimensional plot of the principal coordinates analysis of distance on combination of ISSR, EST-PCR, and EST-SSR markers considered the

first two components 11.64% and 10.05% of the total variation for the first and second components, respectively. In Figure 16, the three-dimensional plot of the principal coordinates analysis of distance on combination of ISSR, EST-PCR, and EST-PCR markers considered the first three components 11.64%, 10.05%, and 6.30% of the total variation for the first, second, and the third component, respectively. This plot of PCo analysis indicated that the genotypes clearly formed four groups including all wild clones and cultivars except 'NL17' as outlier. The way of genotypes grouping showed a similar way as grouping of UPGMA analysis for combination of all three types of markers. For example, 37 genotypes in Cluster I in clustering analysis, 'NL1', 'NB1', 'PE1', 'NL3', 'NL2', 'NL4', 'NL5', 'NL6', 'PE2', 'PE3', 'NL7', 'NL8', 'NL9', 'NL10', 'NL11', 'NL12', 'NL13', 'NL14', 'NL15', 'NL16', 'NL19', 'NL17', 'NL18', 'PE4', 'NL20', 'NL22', 'NL23', 'NL24', 'NL25', 'NL26', 'NL27', 'NL28', 'NL29', 'NL21', and cultivars 'Franklin', 'Stevens', and 'Wilcox' were also grouped together in PCo analysis (Figure 15; Figure 16).

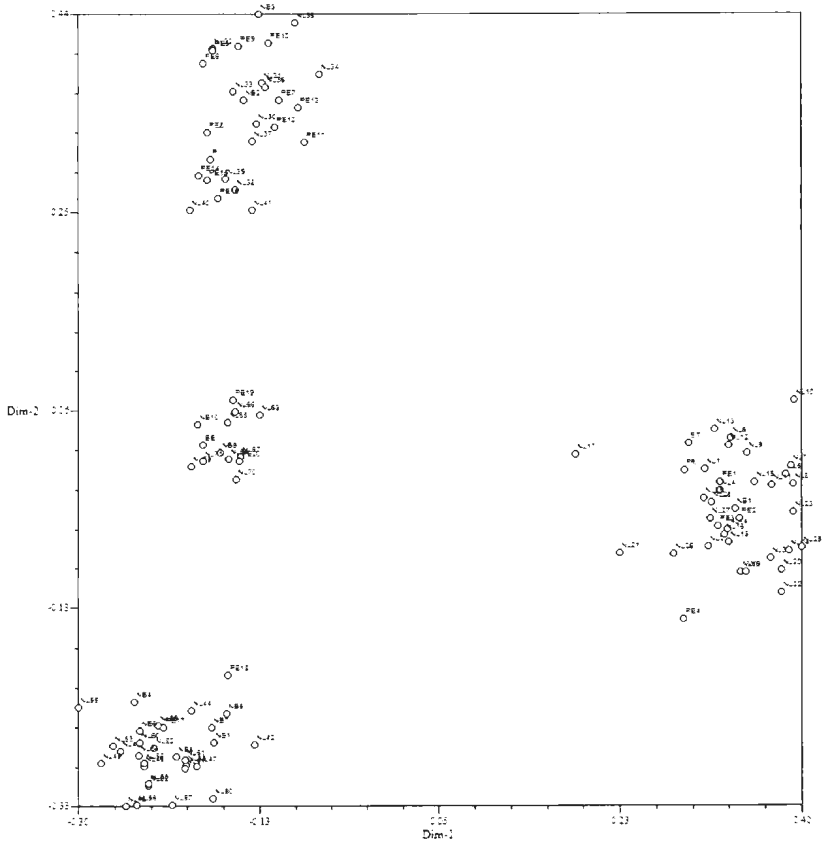

Figure 15: Two-dimensional plot for the first two components of the principal co-ordinates (PCO) analysis of distance among 102 wild cranberry clones collected from four Canadian provinces and five cultivars, on the combined markers from ISSR, EST-PCR and EST-SSR analysis.

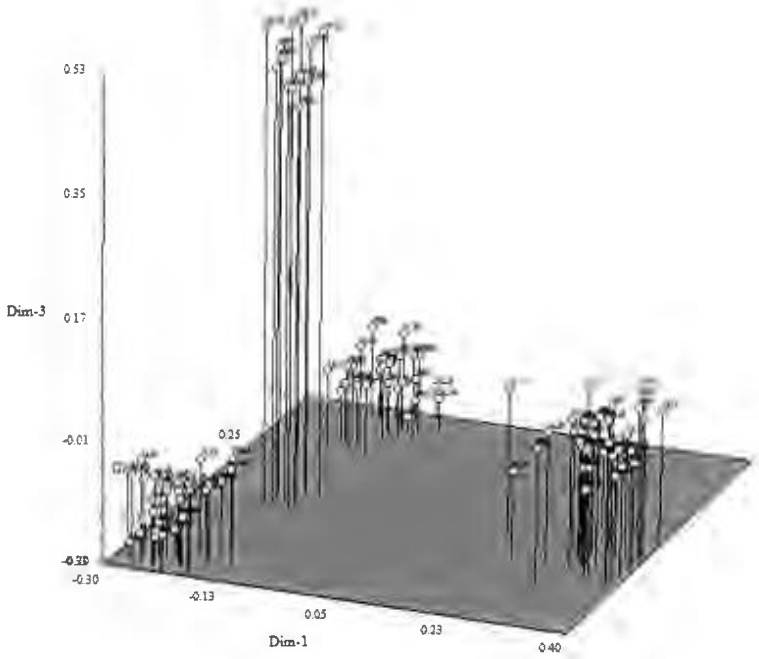

Figure 16: Three-dimensional plot for the first three components of the principal co-ordinates analysis of distance among 102 wild cranberry clones collected from four Canadian provinces and five cultivars, on the combined markers from ISSR, EST-PCR and EST-SSR analysis.

3.7 AMOVA analysis among the 102 wild cranberry clones and five cultivars

3.7.1. AMOVA analysis with ISSR marker

AMOVA analysis, based on the ISSR similarity matrix, indicated that the variation among genotypes within communities accounted for 66.29% of the total variation. The variation among communities within four provinces (NL, NB, NS, and PEI) accounted for 35.50%. The variations for both levels were significant ($p<0.001$). The variation among provinces based on DNA profile with ISSR marker is -1.79%, where the variation was not significant ($p>0.310$) (Table 14). Pairwise distance for all genotypes in AMOVA analysis with ISSR markers in shown in Appendix II (Table 18-25).

Table 14: Analysis of molecular variation for 107 cranberry genotypes with ISSR marker

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P values
Among provinces	4	25030.098	-0.54090	-1.79	P>0.310
Among communities within provinces	26	107217.666	10.72484	35.50	P<0.001
Among genotypes within communities	14200	284375.358	20.02643	66.29	P<0.001
Total	14230	1227414.280	30.21037	100	

3.7.2. AMOVA analysis with EST-PCR marker

The variation among genotypes within communities, among communities within provinces, and mong provinces, based on EST-PCR similarity matrix, were 71.52%, 33.87%, and -5.4%, respectively. The variation among provinces was not significant ($p>0.43$), whereas, the variation within communities and the variation among communities were significant at $p<0.001$ (Table 15). Pairwise distance for all genotypes in AMOVA analysis with EST-PCR marker in shown in Appendix II (Table 26-33).

Table 15: Analysis of molecular variation for 107 cranberry genotypes with EST-PCR marker

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P values
Among provinces	4	62	-0.02704	-5.4	P>0.430
Among communities within provinces	26	403	0.16977	33.87	P<0.001
Among genotypes within communities	3286	1178	0.35849	71.52	P<0.001
Total	3316	1643	0.50122	100	

3.7.3 AMOVA analysis with EST-SSR marker

AMOVA analysis based on the EST-SSR marker, indicated that the variation among genotypes within communities was 71.76% of the total variation. The variation among genotypes within groups accounted for 33.60%. The variations for these two levels

were significant ($p<0.001$). The variation among provinces based on data with EST-SSR marker is -5.36%. This level of variation was not significant ($p>0.402$) (Table 16). Pairwise distance for all genotypes in AMOVA analysis with EST-SSR marker in shown in Appendix II (Table 34-41).

Table 16: Analysis of molecular variation for 107 cranberry genotypes with EST-SSR marker

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P values
Among provinces	4	42.100	-0.02684	-5.36	P>0.402
Among communities within provinces	26	273.809	0.16836	33.60	P<0.001
Among genotypes within communities	2216	796.895	0.35961	71.76	P<0.001
Total	2246	1112.804	0.50113	100	

3.7.4 AMOVA analysis with combination of ISSR, EST-PCR, and EST-SSR markers

AMOVA analysis based on combination of three markers indicated that the variation among genotypes within communities was 70.96% of the total variation. The variation among genotypes within groups accounted for 34.54%. The variations for these two levels were significant ($p<0.001$). The variation among provinces based on data with the combined markers from ISSR, EST-PCR, and EST-SSR analysis is -5.5%. This level of variation was not significant ($p>0.422$) (Table 17). Pairwise distance for all genotypes in AMOVA analysis with the combination of ISSR, EST-PCR, and

EST-SSR markers in shown in Appendix II (Table 42-49).

Table 17: Analysis of molecular variation for 107 cranberry genotypes with combination of ISSR, EST-PCR, and EST-SSR markers

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P values
Among provinces	4	370	-0.02758	-5.5	P>0.422
Among communities within provinces	26	2450	0.17313	34.54	P<0.001
Among genotypes within communities	19764	7030	0.35570	70.96	P<0.001
Total	19794	9850	0.50125	100	

4. Discussion

4.1 DNA polymorphism

In this study, ISSR, EST-PCR, and EST-SSR markers have been applied to evaluate molecular variation among 102 wild cranberry clones collected from 26 communities in four provinces of Canada, and the cultivars 'Franklin', 'Stevens', 'Ben Lear', 'Wilcox', and 'Pilgrim'. This is the first report on cranberry diversity analysis using ISSR, EST-PCR, and EST-SSR markers. Out of 13 ISSR, 10 EST-PCR, and 13 EST-SSR primers, 12 ISSR, 10 EST-PCR, and five EST-SSR primers, respectively, produced polymorphic bands. The sufficient polymorphism discovered via the three marker systems in this study was similar to those of previous studies with RAPD marker in cranberry (Debnath, 2007a), and with ISSR marker in lowbush blueberry (Debnath, 2009) and lingonberry (Debnath, 2007b). EST-PCR and EST-SSR markers were also found effective in diversity analysis of blueberry (Boches et al., 2006) and cranberry (Rowland et al., 2003). Bell et al. (2008) have used 24 EST-PCR primers in lowbush blueberry and found that 17 producing polymorphic bands.

The higher mean level of polymorphism per primer detected in cranberry genotypes by ISSR compared with EST-PCR and EST-SSR demonstrates the superior discriminating capacity of the former. The ISSR, EST-PCR, and EST-SSR primers

varied in their ability to produce polymorphism and to diagnosing genotypes. These results confirmed the previous studies with other *Vaccinium* species. For example, twelve ISSR primers used in the present study also worked well in lowbush blueberry (Debnath, 2009), where 12-26 polymorphic bands were observed. In the present study, 7-16 polymorphic bands were found using these primers. However, there are some differences between the result of the present study and the previous ones, which might be due to the number of primers and the different materials (blueberry and lingonberry) used in the experiments.

Rp value shows a comparative value for diagnostic effectiveness of primers. The higher Rp value generally represents the higher ability of a primer to distinguish the genotypes (Gilbert et al., 1999). The collective value of ISSR marker (a total of Rp values of 12 ISSR primers) was 96.8; whereas that of 10 EST-PCR primers was 14.6; and that of five EST-PCR primers was 12.1. In the present study, the primers UBC835, UBC867, and UBC890 had higher Rp values compared to other ISSR primers. Similar results have been also reported by Debnath (2009) for blueberry.

4.2 Informativeness obtained with ISSR, EST-PCR, and EST-SSR markers

In the present study, the ISSR, EST-PCR, and EST-SSR primers show different discriminating capacities on genetic variation. The capacity of detecting genetic

polymorphism and variation was shown with several indexes. PIC and expected heterozygosity are two common estimation for the degree of polymorphism and variation (Shete et al., 2000). A higher PIC value of a marker system means a higher capacity for detecting polymorphism. The higher expected heterozygosity (also refer as diversity index) value for a marker system means a higher capacity of detecting genetic variation. For calculation, the values of PIC and expected heterozygosity are very close since they have very similar formulae (Shete et al., 2000). In the present study, each primer was calculated with PIC value and expected heterozygosity value in order to obtain the average values of the marker systems, respectively. The two values were very close, in agreement with some previous studies in other species, such as the study of genetic diversity on Turkish native chicken using microsatellites by Kaya and Yildiz (2008).

In the present study, the ranges and mean values of twelve ISSR primers' PIC values and expected heterozygosity demonstrate a significant capacity of detecting polymorphism and variation of ISSR markers. The values of PIC and expected heterozygosity were the same for some ISSR primers. The PIC value and expected heterozygosity were expected to be very close as mentioned above. In ISSR marker system of the present study, this was more obvious than previous studies with other DNA markers developed from ISSR sequences (Gürcan and Mehlenbacher, 2010). They reported the genetic variation among 50 diverse European hazelnut accessions

using 72 SSR marker loci from ISSR fragments, where close mean values of expected heterozygosity and PIC were observed (0.62 and 0.58). However, they were not as close as the results of the present study. Zhao et al. (2007) reported low mean PIC value of ISSR primers. This difference on results between this present study and those of previous study might be due to the material and the different primers applied.

4.3 Comparison of informativeness among ISSR, EST-PCR, and EST-SSR marker analyses

The number of primers applied is a factor to influence the polymorphism detected. The nature of marker applied is another essential factor to influence the polymorphism detected and informativeness levels. These informativeness levels show considerable capacities of detecting variation. The ISSR produced 133 polymorphic bands, with the highest mean values of resolving power, PIC, and expected heterozygosity. The higher polymorphism detected by ISSR marker, compared to EST-PCR and EST-SSR markers, indicated a higher discriminating capacity of ISSR than EST-PCR and EST-SSR (Bejal et al., 2003). Although ISSR is a dominant marker, in the present study, it was found more informative than EST-PCR and EST-SSR markers which are codominant in nature. EST-PCR and EST-SSR markers only detect one locus. However, they can also detect co-dominant alleles in the same locus and amplifying different size of bands to show the co-dominant alleles. In contrary, ISSR detects loci

randomly throughout the genome, but it can only detect dominant alleles. The results of highest expected heterozygosity value with dominant marker showed a disagreement with some previous studies. Bejal et al. (2003) found that expected heterozygosity value was higher for SSR markers than for other dominant markers (SSR>RAPD>AFLP). Zhao et al. (2007) found that PIC value was much higher for SSR marker than for ISSR marker in wild and cultivated *Morus* species. This might be due to the different markers, the different systems of primers applied, and the materials used in the different experiments.

The differentiation between the ISSR and ESTs on mechanism of amplifying bands is the main reason why ISSR markers detected much more loci per assay unit than EST-PCR and EST-SSR markers. The amplification of ISSR is more random than EST-PCR and EST-SSR. These results confirmed those of Belaj et al. (2003), where AFLP marker detected more loci per assay unit than SSR marker. That explains the higher values of Rp and PIC, and the higher number of alleles detected of ISSR than those of EST-PCR and EST-SSR. Additionally, the higher average value of expected heterozygosity of ISSR is also correlated to the higher extent of genetic variation, than EST-SSR and EST-PCR (Belaj et al., 2003).

EST-PCR and EST-SSR are co-dominant markers, that detect the bi-allele throughout the genome (Debnath, 2008). The co-dominant mechanism allows EST-PCR and

EST-SSR to obtain high effective numbers of alleles per locus, which are much higher than those of ISSR. EST-PCR and EST-SSR detected alleles to obtain higher major allele frequencies than ISSR with the similar reason. EST-PCR had the highest average major allele frequency, which means EST-PCR detects the alleles least randomly in this study. ISSR was the marker with the least major allele frequency, which also confirmed the random mechanism of ISSR amplifying bands throughout the genome. Thus, EST-PCR obtained greater major frequency than EST-SSR and ISSR (EST-PCR> EST-SSR> ISSR) (Table 13).

The marker index is related to the multiplex ratio for the three DNA markers. The ISSR's multiplex ratio is dependent on the number of alleles detected for each genotype. However, EST multiplex ratios were 1.00. Thus, multiplex ratio and maker index were both greater for ISSR markers than those of the other codominant markers (ISSR>EST-SSR>EST-PCR) (Table 13).

4.4 UPGMA and PCo analyses

In this study, genetic structure was assessed using UPGMA, PCo and AMOVA analyses. Two methods of multivariate molecular analysis, UPGMA and PCo, were employed to cluster the genotypes. The results of the present study provided abundant sources of genetic variability as well as significant level of genetic relatedness among

the 102 wild cranberry clones and five cultivars. The ISSR-based UPGMA analysis showed a range of 0.34 to 0.89 similarity coefficient values, and the genotypes were grouped into four main clusters (Figure 5). A range of 0.72 to 0.91 similarity coefficient values was observed by Debnath (2009) in the dendrogram of an ISSR-based UPGMA analysis, where 44 lowbush blueberry were grouped two main clusters with three outliers. UPGMA analysis for EST-PCR, EST-SSR, and the combination of the three markers showed similarity coefficient values of 0.21 to 1.00, 0.33 to 1.00, and 0.31 to 0.85, respectively, and grouped the genotypes into three to six main clusters (Figures 6-8). In a previous study, the RAPD-based UPGMA analysis showed a range of 0.19 to 0.56 similarity coefficient values, where 43 cranberry clones and five cultivars were grouped into two main clusters with two outliers (Debnath 2007a). The differences of the results between the present study and previous studies might due to the materials used in the experiments and the different DNA markers applied.

Although the three kinds of primers worked differently in detecting variation among the 107 genotypes, many genotypes were grouped together in UPGMA dendrograms similarly in all three kinds of markers and their combined analysis. Eighty genotypes out of total 107, showed the same clustering in all individual and combined analyses. For example, 'NL42', 'NL43', 'NL44', 'NL45', 'NL46', 'NL47', 'NL48', 'NL49', 'NL50', 'NL51', 'NL52', 'NL53', 'NL54', 'NL55', 'NL56', 'NL57', 'NL58', 'NL59', 'NL60', 'NL61', 'NL62', and 'NL63' were grouped together in dendrograms of all

types of markers and the combination of markers. To the contrary, 27 genotypes did not group together in each of the three marker and the combined analyses. For example, 'NL4', 'NL5', 'NL6', 'PE2', 'NL7', and 'PE3' were grouped together in the dendrogram of ISSR-based analysis; however, they were separate from each other in that of EST-SSR-based analysis.

The results of PCo analysis provided a clear understanding on how each genotype related to any other genotype by the pairwise distance of each other in plotting (Figures 9, 10, 11, 12, 13, 14, 15, and 16). Two-dimensional PCo and three-dimensional PCo were applied. In the present study, the UPGMA and the PCo analyses showed similar grouping for most of the genotypes for all three markers, and for the combination of three markers. However, there were some differences between UPGMA analysis and PCo analysis. Clone 'NL17' was isolated in ISSR-based PCo analysis and in the combination of ISSR, EST-PCR, and EST-SSR marker analysis. However, 'NL17' was grouped with 'NL15', 'NL16', and 'NL19' at the similarity coefficient of 0.63 in ISSR-based UPGMA analysis and of 0.675 in the combination of three marker analysis. Clones 'NL5' and 'NL18' were separate from each other in plotting of PCo analysis based on EST-PCR marker; whereas, they were grouped together at similarity coefficient approximate 0.84 in the UPGMA analysis. Clones 'NB5', 'NB1', 'NL60', and 'NL42' were grouped together in PCo analysis based on EST-SSR marker; however, they were separated in EST-SSR-based UPGMA analysis.

From the result of UPGMA analysis, and PCo analysis, it is evident that some genotypes from different provinces were also grouped together. The genotypes more clearly grouped with ISSR markers and combination of three DNA marker in PCo analysis. The distribution of population on the plotting was also confirmed with the first three components.

4.5 AMOVA analysis

In the present study, the AMOVA results did not indicate significant differentiation among provinces. Similar results were also observed by Debnath (2007a) in cranberry clones and cultivars, who observed 10% of total variation due to geographic distribution.

The AMOVA analysis detected abundant variation among the communities within provinces and among genotypes within communities. High degree of variation among the communities within provinces (35.50% with ISSR marker, 33.87% with EST-PCR marker, 33.60% with EST-SSR marker, and 34.54% with combination of three markers) can be explained by the geographic distribution among these communities, i.e. the zones within provinces.

High level of variation was observed among genotypes within communities (66.29%

with ISSR marker, 71.52% with EST-PCR marker, 71.76% with EST-SSR marker, and 70.96% with combination of three markers), which confirmed the previous studies with lowbush blueberry using ISSR marker (73%, Debnath, 2009), lingonberry using ISSR (90%, Debnath, 2007b), and RAPD marker (89.2%, Persson and Gustavsson, 2001); *V. uliginosum* (96%, Albert et al., 2005) and *V. myrtillus* using RAPD marker (86%, Albert et al., 2004), and with cranberry using RAPD marker (90%, Debnath, 2007a). The differences among these studies might be due to the materials, account the distribution of genotypes within and among provinces, types of DNA markers, and the number of primers used.

This study of genetic variation helps the collection of cranberry germplasm. The sufficient variation among the wild clones and cultivars provided potential genetic resource from the wild to complement the limitation of cranberry crops (Debnath, 2007a).

5. Summary

The cranberry (*Vaccinium macrocarpon* Ait.) is a evergreen, perennial, woody vine plant native to North America. Because of its commercial value and its great potential for medical and health benefits, cranberry breeding is increasingly becoming a high concern. Thus, knowledge on the taxonomy, phylogeny, and geographical distribution of the wild germplasm is required for their best use in a breeding program. Genetic diversity provides us with the sustained ability to develop new plant cultivars that can resist diseases, pests, and environmental stresses. In the present study, genetic variation was studied among 102 wild cranberry clones collected from four Canadian provinces and five cranberry cultivars using ISSR, EST-PCR, and EST-SSR markers to generate sufficient information on their genetic diversity. The informativeness of three DNA markers were compared. All of three DNA markers showed a high efficiency on generating polymorphism among the 107 genotypes. ISSR was most informative marker with highest number of polymorphic bands and highest values of PIC (0.97), expected heterozygosity (0.97), and marker index (1.20). ISSR was followed by EST-PCR with PIC of 0.56, expected heterozygosity of 0.60, and marker index of 0.56. The EST-SSR was less informative than ISSR with 0.74. 0.77, and 0.77 for PIC, expected heterozygosity, and marker index.

UPGMA analysis, PCo analysis and AMOVA analysis were applied for each marker, and for the combination of the three markers. Genotypes trended to form groups in the similar way, in UPGMA analyses and in PCo analyses, although differences were observed for some genotypes. The outputs for UPGMA and PCo analyses were confirmed by AMOVA, detecting a higher amount of variation among genotypes within communities than among communities within provinces for each marker and for the combined markers.

In conclusion, all three types of DNA markers employed in this study detected a high level of diversity among 102 wild cranberry clones and five cultivars. The study identified ISSR, EST-PCR, and EST-SSR primers that are suitable for cranberry fingerprinting. These primers assessed the level of genetic variations among wild cranberry clones and cultivars and the level of genetic polymorphism as well as the relative importance among these three genetic markers in molecular diversity analysis. Although it is difficult to differentiate cranberry clones and cultivars by phenotypic characteristics, the present results obviously provided the extend of genetic variation among the cranberry clones and cultivars studies. The sufficient genetic variation studied provided genetic source for crossing experiments among the wild clones next step, which can improve the breeding of cranberry by combining the advantages of parent plants to one offspring plant.

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Appendix I: Gel pictures of the rest samples with ISSR, EST-PCR, and EST-SSR markers.

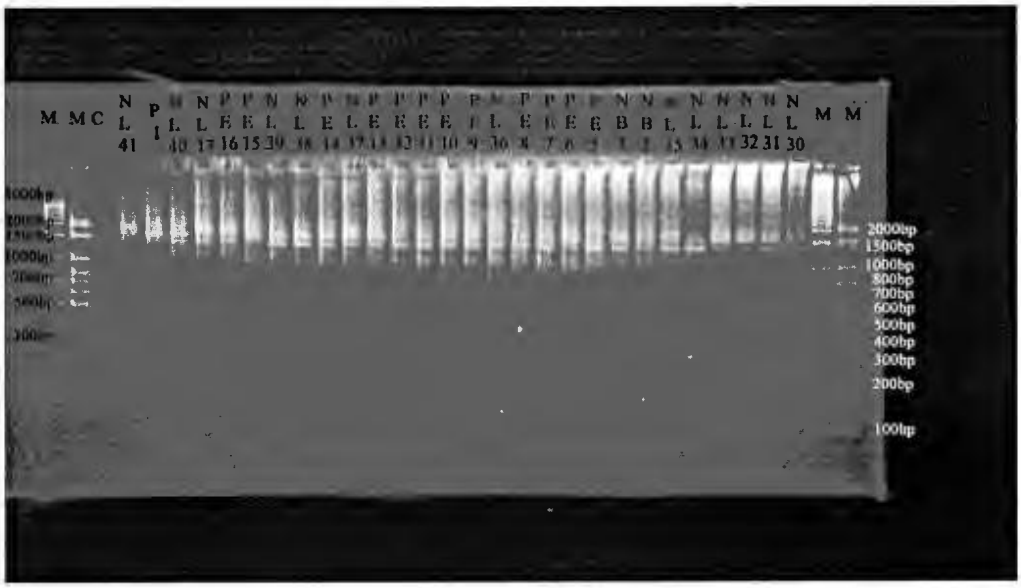

Figure 17: Inter simple sequence repeat (ISSR) banding patterns of 11 Newfoundland and Labrador (NL30-41), two New Brunswick (NB2-3), and 12 Prince Edward Island wild cranberry clones (PE5-16), and cultivars, 'Pilgrim' (PI) generated by primer UBC 801. M-standard molecular sizes: 100 bp ladder (right) and 1kb (left).

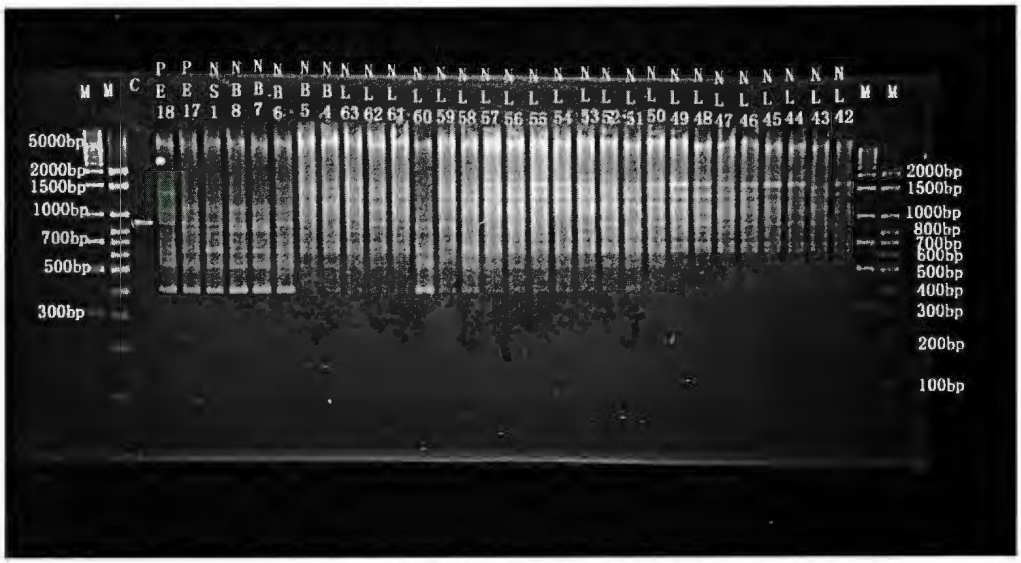

Figure 18: Inter simple sequence repeat (ISSR) banding patterns of 22 Newfoundland and Labrador (NL42-63), five New Brunswick (NB4-8), one Nova Scotia (NS1), and two Prince Edward Island wild cranberry clones (PE17-18) generated by primer UBC 801. M-standard molecular sizes: 100 bp ladder (right) and 1kb (left).

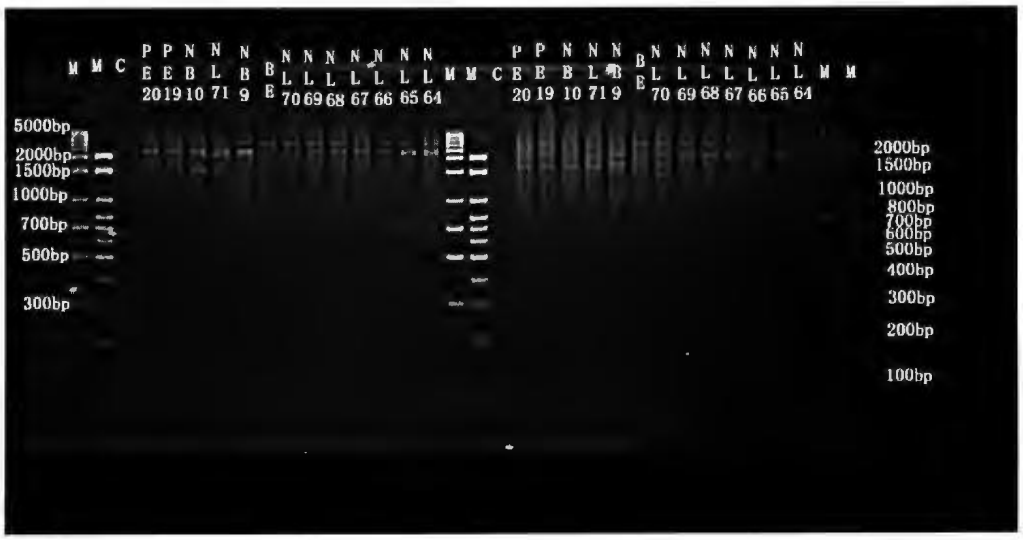

Figure 19: Inter simple sequence repeat (ISSR) banding patterns of eight Newfoundland and Labrador (NL64-71), two New Brunswick (NB9-10), and two Prince Edward Island wild cranberry clones (PE19-20) generated by primer UBC 801(right) and primer UBC808 (left). M-standard molecular sizes: 100 bp ladder (right) and 1kb (left).

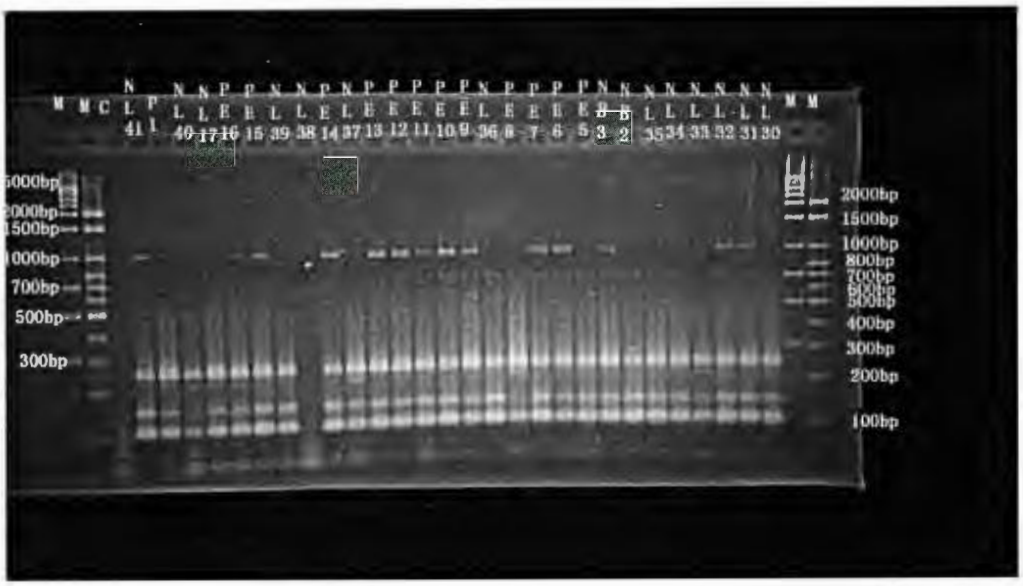

Figure 20: Expressed sequence tag- polymerase chain reaction (EST-PCR) banding patterns of 11 Newfoundland and Labrador (NL30-41), two New Brunswick (NB2-3), and 12 Prince Edward Island wild cranberry clones (PE5-16), and cultivars, 'Pilgrim' (PI) generated by primer CA 231. M-standard molecular sizes: 100 bp ladder (right) and 1kb (left).

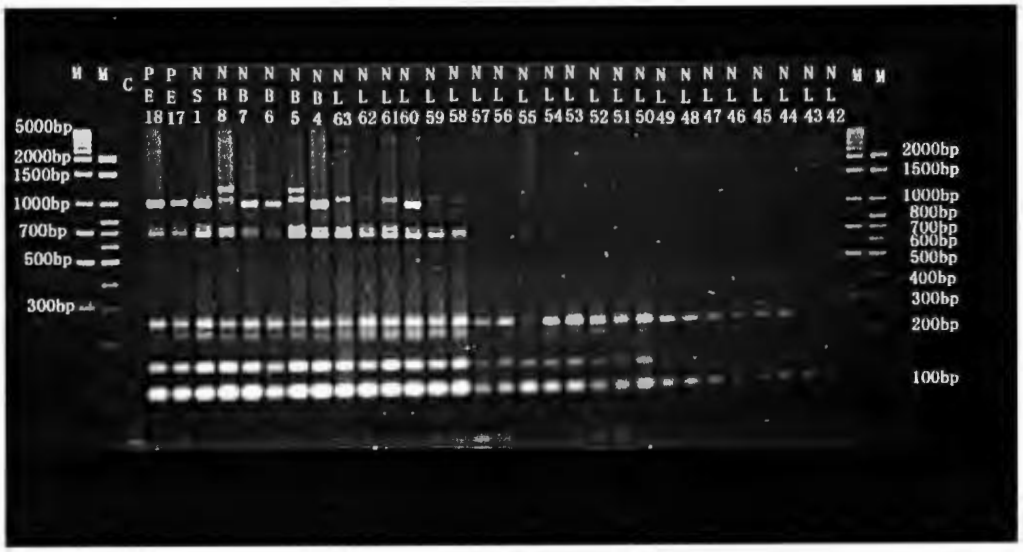

Figure 21: Expressed sequence tag- polymerase chain reaction (EST-PCR) banding patterns of 22 Newfoundland and Labrador (NL42-63), five New Brunswick (NB4-8), one Nova Scotia (NS1), and two Prince Edward Island wild cranberry clones (PE17-18) generated by primer CA 231. M-standard molecular sizes: 100 bp ladder (right) and 1kb (left).

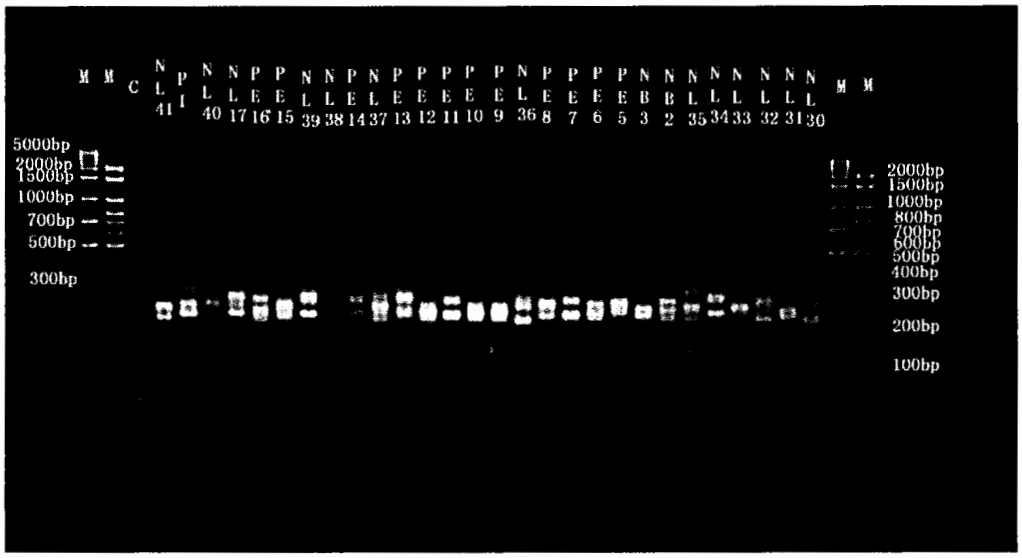

Figure 22: Expressed sequence tag- simple sequence repeat (EST-SSR) banding patterns of 11 Newfoundland and Labrador (NL30-41), two New Brunswick (NB2-3), and 12 Prince Edward Island wild cranberry clones (PE5-16), and cultivars, 'Pilgrim' (PI) generated by primer NA 800. M-standard molecular sizes: 100 bp ladder (right) and 1kb (left).

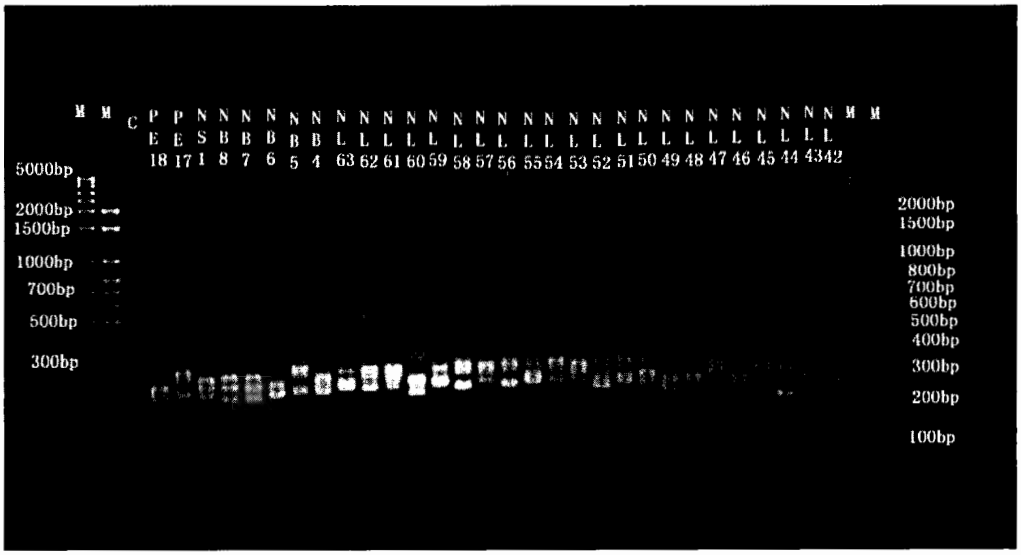

Figure 23: Expressed sequence tag- simple sequence repeat (EST-SSR) banding patterns of 22 Newfoundland and Labrador (NL42-63), five New Brunswick (NB4-8), one Nova Scotia (NS1), and two Prince Edward Island wild cranberry clones (PE17-18) generated by primer NA 800. M-standard molecular sizes: 1 bp ladder (right) and 1kb (left).

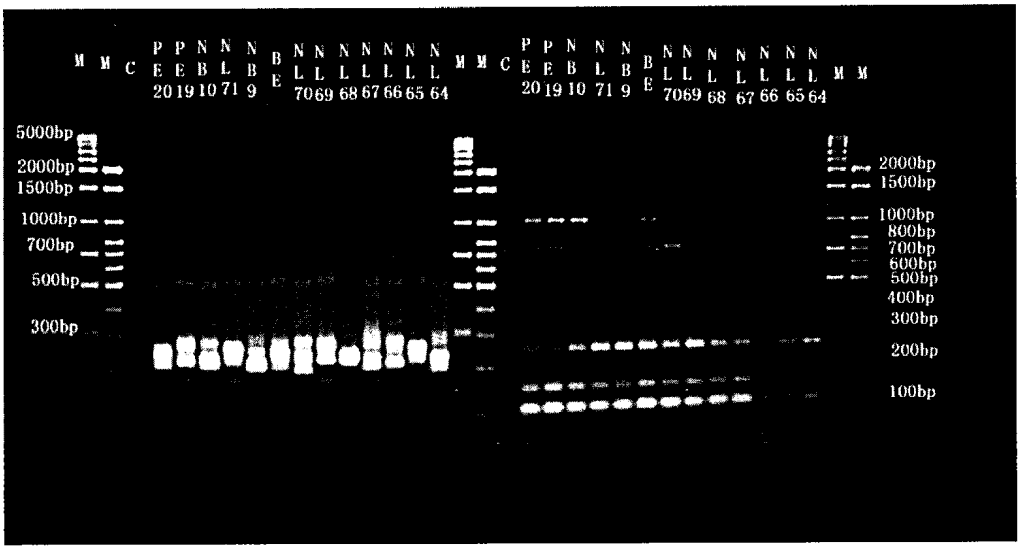

Figure 24: Expressed sequence tag- polymerase chain reaction (EST-PCR) and expressed sequence tag- simple sequence repeat (EST-SSR) banding patterns of eight Newfoundland and Labrador (NL64-71), two New Brunswick (NB9-10), and two Prince Edward Island wild cranberry clones (PE19-20) generated by primer CA231 (right) and primer NA800 (left). M-standard molecular sizes: 100 bp ladder (right) and 1kb (left).

Appendix II: Tabes of pairwise differentiation between genotypes among zones in Newfoundland and Labrador, New Brunswick and Prince Edward Island, and among cultivars.

Table 18: Pairwise differentiations in AMOVA analysis for ISSR marker within clones of Bauline Line/ Portugal Cove, Cape Spear, Logy Bay, Ferryland and Soldiers Pond in Newfoundland and Labrador

	NL2	NL7	NL29	NL33	NL37	NL40	NL42	NL47	NL55
NL2	0								
NL7	40	0							
NL29	52	32	0						
NL33	67	63	73	0					
NL37	73	69	63	46	0				
NL40	77	69	69	48	44	0			
NL42	58	64	72	63	73	69	0		
NL47	59	63	67	64	74	72	27	0	
NL55	76	72	68	63	67	57	44	37	0

Table 19: Pairwise differentiations in AMOVA analysis for ISSR marker within the clones of Lamline, Lords Cove/ Pump Cove, Freshmans Cove, Corbin, Pork Lirwarm, Freshwater and Mobile in Newfoundland and Labrador.

	NL4	NL12	NL21	NL31	NL41	NL44	NL49	NL50
NL4	0							
NL12	49	0						
NL21	50	43	0					
NL31	66	57	66	0				
NL41	62	59	54	50	0			
NL44	74	71	64	66	68	0		
NL49	68	73	64	62	66	34	0	
NL50	64	65	56	66	66	38	26	0

Table 20: Pairwise differentiations in AMOVA analysis for ISSR marker within clones of Bell Island East in Newfoundland and Labrador

	NL1	NL5	NL6	NL10	NL14	NL20	NL22	NL23	NL24	NL25	NL30	NL32	NL34	NL35	NL39	NL53	NL54	NL57	NL58	NL65	NL66	NL69	NL70	NL71
NL1	0																							
NL5	27	0																						
NL6	38	23	0																					
NL10	42	41	38	0																				
NL14	46	45	42	26	0																			
NL20	43	36	39	35	41	0																		
NL22	44	35	32	40	38	21	0																	
NL23	42	33	38	34	40	17	22	0																
NL24	40	31	36	40	42	21	20	8	0															
NL25	40	29	38	38	40	21	26	20	18	0														
NL30	60	61	58	66	66	63	64	62	60	66	0													
NL32	55	62	65	67	71	68	73	65	65	67	23	0												
NL34	63	62	59	59	71	60	65	59	65	63	33	28	0											
NL35	60	63	60	64	66	65	72	62	62	66	26	21	21	0										
NL39	61	64	63	67	67	64	69	65	61	67	47	42	48	47	0									
NL53	67	72	71	77	69	66	73	71	69	65	65	70	70	71	64	0								
NL54	71	70	67	73	63	60	65	61	63	65	61	70	68	69	58	24	0							
NL57	61	60	59	71	69	60	57	59	57	59	65	72	72	73	62	34	28	0						
NL58	68	69	72	70	66	65	68	66	68	66	68	69	67	74	67	35	35	33	0					

NL65	58	65	70	74	74	69	68	70	68	62	64	55	63	64	59	55	65	65	62	0				
NL66	70	69	70	72	72	77	72	74	74	72	56	55	63	60	63	63	71	65	64	30	0			
NL69	63	70	69	71	69	68	65	71	69	69	59	58	68	67	60	48	62	62	61	19	33	0		
NL70	63	66	65	65	61	66	63	73	73	63	65	66	68	69	62	52	62	62	63	23	35	14	0	
NL71	62	67	74	70	70	67	64	68	70	66	56	57	63	64	61	55	61	61	64	28	30	19	23	0

Table 21: Pairwise differentiations in AMOVA analysis for ISSR marker within the clones of Bell Island West in Newfoundland and Labrador.

	NL3	NL8	NL9	NL11	NL15	NL16	NL17	NL26	NL27	NL51	NL52	NL56	NL59	NL60	NL61	NL62	NL63	NL67	Nl68
NL3	0																		
NL8	40	0																	
NL9	52	28	0																
NL11	50	38	30	0															
NL15	47	31	31	35	0														
NL16	42	48	40	30	25	0													
NL17	59	49	49	41	34	37	0												
NL26	39	39	43	41	42	33	52	0											
NL27	35	47	43	45	44	35	54	22	0										
NL51	63	71	63	71	58	57	62	66	62	0									
NL52	67	69	65	71	64	67	60	64	64	22	0								
NL56	71	77	69	63	72	69	62	68	70	28	26	0							
NL59	69	71	71	73	62	67	62	64	62	38	38	34	0						
NL60	65	71	73	69	66	71	58	62	62	38	34	34	32	0					
NL61	66	70	76	64	63	66	55	65	59	43	47	41	29	27	0				
NL62	68	70	76	64	67	68	55	71	73	41	37	31	31	21	22	0			
NL63	66	72	68	62	67	68	59	63	67	39	39	27	31	31	34	28	0		

NL67	73	65	73	67	72	67	58	66	72	66	64	70	82	66	63	61	67	0	
NL68	64	68	78	72	73	64	61	61	63	65	61	67	79	73	66	64	66	15	0

Table 22: Pairwise differentiations in AMOVA analysis for ISSR marker within the other clones of Newfoundland and Labrador

	NL13	NL18	NL19	NL28	NL36	NL38	NL43	NL45	NL46	NL48	NL64
NL13	0										
NL18	35	0									
NL19	40	27	0								
NL28	40	43	40	0							
NL36	67	72	73	69	0						
NL38	72	79	80	74	49	0					
NL43	70	69	66	62	71	68	0				
NL45	77	70	69	73	80	71	29	0			
NL46	78	71	70	74	75	66	30	23	0		
NL48	74	65	64	72	69	72	34	25	26	0	
NL64	70	73	68	76	65	62	72	61	64	64	0

Table 23: Pairwise differentiations in AMOVA analysis for ISSR marker within the clones of Prince Edward Island

	PE1	PE2	PE3	PE4	PE5	PE6	PE7	PE8	PE9	PE10	PE11	PE12	PE13	PE14	PE15	PE16	PE17	PE18	PE19	PE20
PE1	0																			
PE2	32	0																		
PE3	38	24	0																	
PE4	47	39	33	0																
PE5	69	69	65	78	0															
PE6	71	73	73	74	24	0														
PE7	57	59	61	68	30	36	0													
PE8	69	75	71	76	34	36	36	0												
PE9	66	66	68	75	31	31	27	31	0											
PE10	64	64	62	73	31	35	27	35	18	0										
PE11	66	64	64	61	41	37	37	43	38	32	0									
PE12	67	71	73	72	42	40	46	46	35	33	39	0								
PE13	63	71	69	76	44	42	42	44	43	43	35	28	0							
PE14	67	75	75	76	40	32	50	48	37	41	45	28	34	0						
PE15	69	75	75	82	48	40	56	54	47	51	53	42	46	38	0					
PE16	71	77	73	74	48	44	56	50	53	47	45	44	38	34	40	0				
PE17	77	73	63	64	68	70	72	60	65	67	67	68	70	64	62	68	0			
PE18	68	68	56	63	63	59	63	57	66	66	64	67	63	65	71	67	33	0		

PE19	66	66	70	73	59	57	61	63	62	68	62	59	61	61	65	67	65	60	0	
PE20	71	67	69	64	64	58	70	68	69	67	59	56	54	60	64	62	60	55	21	0

Table 24: Pairwise differentiations in AMOVA analysis for ISSR markers within the clones of New Brunswick

	NB1	NB2	NB3	NB4	NB5	NB6	NB7	NB8	NB9	NB10
NB1	0									
NB2	61	0								
NB3	58	27	0							
NB4	67	64	63	0						
NB5	67	68	67	32	0					
NB6	68	67	72	25	29	0				
NB7	68	75	74	31	33	30	0			
NB8	73	74	79	32	32	23	23	0		
NB9	66	67	58	59	55	58	62	63	0	
NB10	66	63	60	55	63	58	64	61	24	0

Table 25: Pairwise differentiations in AMOVA analysis for ISSR marker within the cultivars.

	Franklin	Stevens	Wilcox	Pilgrim	Ben Lear
Franklin	0				
Stevens	38	0			
Wilcox	53	39	0		
Pilgrim	75	73	68	0	
Ben Lear	80	76	77	59	0

Table 26: Pairwise differentiations in AMOVA analysis for EST-PCR marker within the clones of Bauline Line/ Portugal Cove, Cape Spear, Logy Bay, Ferryland and Soldiers Pond in Newfoundland and Labrador

	NL2	NL7	NL29	NL33	NL37	NL40	NL42	NL47	NL55
NL2	0								
NL7	8	0							
NL29	12	6	0						
NL33	8	12	10	0					
NL37	7	9	11	11	0				
NL40	10	16	14	10	11	0			
NL42	14	10	8	10	13	14	0		
NL47	15	13	11	9	12	9	11	0	
NL55	12	10	10	8	9	10	10	5	0

Table 27: Pairwise differentiations in AMOVA analysis for EST-PCR marker within the clones of Lamline, Lords Cove/ Pump Cove, Freshmans Cove, Corbin, Pork Lirwarm, Freshwater and Mobile in Newfoundland and Labrador.

	NL4	NL12	NL21	NL31	NL41	NL44	NL49	NL50
NL4	0							
NL12	8	0						
NL21	16	8	0					
NL31	19	15	11	0				
NL41	18	12	12	9	0			
NL44	16	12	8	9	10	0		
NL49	13	13	13	10	11	7	0	
NL50	11	9	9	10	9	7	10	0

Table 28: Pairwise differentiations in AMOVA analysis for EST-PCR marker within the clones of Bell Island East in Newfoundland and Labrador

	NL1	NL5	NL6	NL10	NL14	NL20	NL22	NL23	NL24	NL25	NL30	NL32	NL34	NL35	NL39	NL53	NL54	NL57	NL58	NL65	NL66	NL69	NL70	NL71
NL1	0																							
NL5	4	0																						
NL6	9	7	0																					
NL10	8	6	9	0																				
NL14	9	7	8	7	0																			
NL20	9	7	10	5	6	0																		
NL22	8	4	11	8	9	5	0																	
NL23	10	10	9	8	9	7	6	0																
NL24	10	6	11	8	11	7	2	6	0															
NL25	6	4	9	6	7	5	4	6	4	0														
NL30	12	10	11	10	9	11	12	12	10	10	0													
NL32	18	14	11	14	9	15	16	16	14	14	6	0												
NL34	9	7	10	9	8	10	9	9	9	7	5	9	0											
NL35	11	9	10	11	10	12	9	7	9	9	7	9	4	0										
NL39	13	15	14	17	16	16	15	13	13	13	9	11	12	8	0									
NL53	12	12	7	10	11	11	10	8	8	10	8	10	9	7	9	0								
NL54	13	11	10	11	10	12	11	11	9	11	3	7	8	6	8	5	0							
NL57	11	11	10	9	8	8	9	9	9	11	5	11	10	10	10	7	6	0						

NL58	12	10	11	10	7	11	10	10	8	10	4	8	9	9	11	8	3	5	0					
NL65	10	14	11	12	9	11	14	12	16	12	14	14	13	13	17	12	13	13	14	0				
NL66	8	8	9	14	11	11	8	12	8	8	10	14	9	13	15	10	11	11	10	8	0			
NL69	10	12	11	10	11	9	10	8	8	8	8	12	13	13	11	8	9	7	8	10	8	0		
NL70	10	10	9	8	9	7	8	6	8	6	10	14	11	13	15	8	11	9	10	10	8	4	0	
NL71	13	13	8	9	10	10	11	7	9	11	7	11	12	12	12	5	6	4	5	13	11	5	5	0

Table 29: Pairwise differentiations in AMOVA analysis for EST-PCR marker within the clones of Bell Island West in Newfoundland and Labrador

	NL3	NL8	NL9	NL11	NL15	NL16	NL17	NL26	NL27	NL51	NL52	NL56	NL59	NL60	NL61	NL62	NL63	NL67	NL68
NL3	0																		
NL8	7	0																	
NL9	8	5	0																
NL11	7	4	5	0															
NL15	7	6	7	4	0														
NL16	10	7	6	3	7	0													
NL17	7	2	3	2	4	5	0												
NL26	10	9	6	9	9	8	7	0											
NL27	9	8	7	8	8	7	6	7	0										
NL51	15	12	9	12	16	9	12	7	12	0									
NL52	15	12	9	10	14	7	10	7	10	2	0								
NL56	11	8	7	6	8	7	6	3	8	8	6	0							
NL59	13	8	7	8	10	5	8	7	10	6	8	8	0						
NL60	13	8	7	6	8	3	6	5	6	8	6	4	4	0					
NL61	12	7	6	5	7	4	5	8	9	9	7	5	3	3	0				
NL62	12	7	6	5	7	4	5	8	9	9	7	5	3	3	0	0			
NL63	13	8	7	6	8	3	6	7	8	8	6	6	2	2	1	1	0		

NL67	16	9	12	9	11	8	9	10	9	11	11	9	9	7	10	10	9	0	
NL68	14	9	10	9	11	8	9	8	9	9	11	9	7	7	10	10	9	2	0

Table 30: Pairwise differentiations in AMOVA analysis for EST-PCR marker within the other clones of Newfoundland and Labrador

	NL13	NL18	NL19	NL28	NL36	NL38	NL43	NL45	NL46	NL48	NL64
NL13	0										
NL18	8	0									
NL19	4	8	0								
NL28	5	5	7	0							
NL36	7	9	5	8	0						
NL38	21	19	21	18	24	0					
NL43	7	9	9	6	6	18	0				
NL45	12	10	10	11	9	19	7	0			
NL46	13	13	11	14	10	20	10	5	0		
NL48	14	12	12	13	7	17	7	8	7	0	
NL64	10	10	6	13	9	19	15	10	9	12	0

Table 31: Pairwise differentiations in AMOVA analysis for EST-PCR marker within the clones of Prince Edward Island

	PE1	PE2	PE3	PE4	PE5	PE6	PE7	PE8	PE9	PE10	PE11	PE12	PE13	PE14	PE15	PE16	PE17	PE18	PE19	PE20
PE1	0																			
PE2	10	0																		
PE3	7	3	0																	
PE4	9	11	12	0																
PE5	12	14	15	7	0															
PE6	12	10	11	11	6	0														
PE7	11	15	14	12	7	7	0													
PE8	11	11	12	12	9	9	12	0												
PE9	11	13	14	12	9	11	10	8	0											
PE10	10	12	11	13	10	8	9	9	7	0										
PE11	12	10	11	13	8	6	7	9	7	6	0									
PE12	7	11	12	6	7	7	10	10	8	7	7	0								
PE13	10	10	11	11	8	8	11	9	7	6	8	5	0							
PE14	15	15	16	14	9	11	10	14	10	11	7	12	13	0						
PE15	12	14	13	11	6	8	7	9	9	10	8	11	12	7	0					
PE16	13	15	12	14	11	13	12	10	12	13	11	14	13	8	5	0				
PE17	12	14	15	9	8	10	7	17	9	12	8	7	10	9	10	15	0			
PE18	11	13	14	8	7	9	6	16	8	11	7	6	9	8	9	14	1	0		

PE19	9	9	10	8	9	9	8	12	10	11	7	6	9	12	13	18	5	4	0	
PE20	10	10	11	7	6	8	7	13	11	14	8	7	10	11	10	15	4	3	3	0

Table 32: Pairwise differentiations in AMOVA analysis for ISSR marker within the clones of New Brunswick

	NB1	NB2	NB3	NB4	NB5	NB6	NB7	NB8	NB9	NB10
NB1	0									
NB2	12	0								
NB3	14	10	0							
NB4	11	5	9	0						
NB5	9	9	13	4	0					
NB6	14	8	10	3	5	0				
NB7	13	7	11	2	4	3	0			
NB8	13	7	11	2	4	1	2	0		
NB9	10	10	16	7	5	8	7	7	0	
NB10	11	9	15	6	6	7	6	6	1	0

Table 33: Pairwise differentiations in AMOVA analysis for EST-PCR marker within the cultivars

	Franklin	Stevens	Wilcox	Pilgrim	Ben Lear
Franklin	0				
Stevens	5	0			
Wilcox	7	8	0		
Pilgrim	16	15	19	0	
Ben Lear	9	12	8	19	0

Table 34: Pairwise differentiations in AMOVA analysis for EST-SSR marker within the clones in Bauline Line/ Portugal Cove, Cape Spear, Logy Bay, Ferryland and Soldiers Pond of Newfoundland and Labrador

	NL2	NL7	NL29	NL33	NL37	NL40	NL42	NL47	NL55
NL2	0								
NL7	11	0							
NL29	10	9	0						
NL33	7	8	5	0					
NL37	15	8	13	12	0				
NL40	11	12	11	10	8	0			
NL42	8	11	8	5	11	9	0		
NL47	8	11	6	5	11	9	4	0	
NL55	11	12	9	8	10	8	7	3	0

Table 35: Pairwise differentiations used for AMOVA analysis for EST-SSR marker within the clones of Lamline, Lords Cove/ Pump Cove, Freshmans Cove, Corbin, Pork Lirwarm, Freshwater and Mobile in Newfoundland and Labrador

	NL4	NL12	NL21	NL31	NL41	NL44	NL49	NL50
NL4	0							
NL12	9	0						
NL21	9	6	0					
NL31	5	8	8	0				
NL41	9	12	8	10	0			
NL44	6	9	11	9	11	0		
NL49	6	9	9	7	13	6	0	
NL50	7	10	10	8	14	5	3	0

Table 36: Pairwise differentiations in AMOVA with EST-SSR within the clones of Bell Island East in Newfoundland and Labrador

	NL1	NL5	NL6	NL10	NL14	NL20	NL22	NL23	NL24	NL25	NL30	NL32	NL34	NL35	NL39	NL53	NL54	NL57	NL58	NL65	NL66	NL69	NL70	NL71
NL1	0																							
NL5	6	0																						
NL6	8	4	0																					
NL10	10	8	8	0																				
NL14	9	7	7	5	0																			
NL20	9	7	7	9	6	0																		
NL22	9	7	7	7	4	6	0																	
NL23	12	10	8	8	7	9	7	0																
NL24	10	8	6	4	3	5	3	4	0															
NL25	10	8	6	6	3	3	3	6	2	0														
NL30	10	8	4	6	7	9	5	8	6	6	0													
NL32	10	6	10	8	9	9	7	10	8	8	8	0												
NL34	11	9	5	9	10	8	10	7	7	7	7	7	0											
NL35	7	5	5	3	4	8	6	7	5	5	3	7	8	0										
NL39	11	9	9	9	10	12	12	7	11	9	11	9	8	8	0									
NL53	10	8	10	12	7	7	7	10	8	6	10	6	9	9	11	0								
NL54	11	11	9	13	8	8	10	11	9	7	9	9	8	10	12	3	0							

NL57	9	7	9	9	4	6	8	9	7	5	9	5	8	6	8	3	4	0						
NL58	8	8	10	10	5	5	9	10	8	6	10	6	9	7	9	4	5	1	0					
NL65	7	3	5	11	10	8	8	9	9	9	9	7	6	8	8	7	10	8	9	0				
NL66	4	6	10	10	9	7	9	12	10	10	12	10	11	9	11	10	13	9	8	5	0			
NL69	4	6	8	8	7	5	9	12	8	8	10	10	11	7	11	10	11	7	6	7	2	0		
NL70	4	4	4	6	7	9	9	10	8	8	6	8	7	3	7	10	11	7	8	5	6	4	0	
NL71	5	7	7	7	4	6	6	9	5	5	9	9	10	6	8	9	10	6	5	8	5	3	5	0

Table 37: Pairwise differentiations in AMOVA analysis for EST-SSR marker within the clones in Bell Island West of Newfoundland and Labrador

	NL3	NL8	NL9	NL11	NL15	NL16	NL17	NL26	NL27	NL51	NL52	NL56	NL59	NL60	NL61	NL62	NL63	NL67	NL68
NL3	0																		
NL8	23	0																	
NL9	26	19	0																
NL11	19	14	14	0															
NL15	19	22	20	16	0														
NL16	19	24	22	18	16	0													
NL17	22	23	25	17	19	11	0												
NL26	15	20	20	20	14	16	21	0											
NL27	14	19	21	21	17	17	18	9	0										
NL51	23	24	26	28	26	28	27	26	23	0									
NL52	18	25	23	21	23	21	24	23	20	17	0								
NL56	26	31	29	31	29	29	30	27	26	17	16	0							
NL59	24	29	29	29	27	25	26	27	28	15	18	18	0						
NL60	24	29	27	33	27	29	32	23	28	17	24	18	12	0					
NL61	24	27	30	27	27	27	28	21	24	13	20	20	12	10	0				
NL62	25	28	28	30	26	28	29	26	29	14	21	19	15	13	7	0			
NL63	26	27	25	27	27	25	26	23	26	21	26	24	16	16	12	13	0		

NL67	23	24	22	26	24	28	27	22	25	26	27	33	27	25	25	26	29	0	
NL68	18	21	23	23	17	21	20	21	18	23	22	30	28	26	26	27	26	17	0

Table 38: Pairwise differentiations in AMOVA analysis for EST-SSR marker within the other clones of Newfoundland and Labrador.

	NL13	NL18	NL19	NL28	NL36	NL38	NL43	NL45	NL46	NL48	NL64
NL13	0										
NL18	5	0									
NL19	9	4	0								
NL28	3	4	8	0							
NL36	6	11	13	7	0						
NL38	12	11	9	11	14	0					
NL43	11	10	6	8	9	11	0				
NL45	9	12	10	10	9	13	4	0			
NL46	12	11	9	9	10	12	3	3	0		
NL48	10	13	9	11	8	12	3	1	4	0	
NL64	10	13	9	11	8	12	7	5	8	4	0

Table 39: Pairwise differentiations in AMOVA analysis for EST-SSR marker within the clones of Prince Edward Island.

	PE1	PE2	PE3	PE4	PE5	PE6	PE7	PE8	PE9	PE10	PE11	PE12	PE13	PE14	PE15	PE16	PE17	PE18	PE19	PE20
PE1	0																			
PE2	5	0																		
PE3	6	5	0																	
PE4	9	12	11	0																
PE5	7	6	5	10	0															
PE6	4	5	2	11	3	0														
PE7	6	5	2	9	5	2	0													
PE8	7	6	5	14	4	3	5	0												
PE9	7	6	5	12	2	3	5	4	0											
PE10	8	7	6	11	1	4	6	5	3	0										
PE11	9	10	5	12	6	5	5	6	8	7	0									
PE12	8	5	4	11	1	4	6	5	3	2	7	0								
PE13	7	6	1	10	6	3	3	6	6	7	6	5	0							
PE14	11	6	5	8	8	7	5	8	8	9	10	7	4	0						
PE15	7	4	5	10	6	5	5	6	6	7	10	5	6	4	0					
PE16	9	6	7	16	8	7	9	6	8	7	10	7	6	8	8	0				
PE17	8	7	10	5	9	10	8	11	11	10	11	10	9	7	7	11	0			
PE18	4	5	6	9	5	4	6	5	5	6	9	6	7	7	3	9	6	0		

PE19	7	6	7	8	6	5	5	8	8	7	8	7	8	8	8	10	7	9	0	
PE20	9	6	9	10	6	7	7	8	8	7	8	7	10	6	6	10	7	7	4	0

Table 40: Pairwise differentiations in AMOVA analysis for EST-SSR marker within the clones of New Brunswick

	NB1	NB2	NB3	NB4	NB5	NB6	NB7	NB8	NB9	NB10
NB1	0									
NB2	10	0								
NB3	8	4	0							
NB4	7	11	9	0						
NB5	4	8	8	7	0					
NB6	6	8	8	7	4	0				
NB7	5	7	7	8	3	1	0			
NB8	5	7	7	8	3	1	0	0		
NB9	6	6	4	5	6	6	5	5	0	
NB10	10	6	6	9	10	8	7	7	6	0

Table 41: Pairwise differentiations in AMOVA analysis for EST-SSR marker within the cultivars

	Franklin	Stevens	Wilcox	Pilgrim	Ben Lear
Franklin	0				
Stevens	7	0			
Wilcox	10	9	0		
Pilgrim	4	5	8	0	
Ben Lear	7	6	9	7	0

Table 42: Pairwise differentiations in AMOVA analysis for the combination of ISSR, EST-PCR, and EST-SSR markers within the clones of Bauline Line/ Portugal Cove, Cape Spear, Logy Bay, Ferryland and Soldiers Pond in Newfoundland and Labrador

	NL2	NL7	NL29	NL33	NL37	NL40	NL42	NL47	NL55
NL2	0								
NL7	59	0							
NL29	74	47	0						
NL33	82	83	88	0					
NL37	95	86	87	69	0				
NL40	98	97	94	68	63	0			
NL42	80	85	88	78	97	92	0		
NL47	82	87	84	78	97	90	42	0	
NL55	99	94	87	79	86	75	61	45	0

Table 43: Pairwise differentiations in AMOVA analysis for the combination of ISSR, EST-PCR, and EST-SSR markers within the clones of Lamline, Lords Cove/ Pump Cove, Freshmans Cove, Corbin, Pork Lirwarm, Freshwater and Mobile in Newfoundland and Labrador

	NL4	NL12	NL21	NL31	NL41	NL44	NL49	NL50
NL4	0							
NL12	66	0						
NL21	75	57	0					
NL31	90	80	85	0				
NL41	89	83	74	69	0			
NL44	96	92	83	84	89	0		
NL49	87	95	86	79	90	47	0	
NL50	82	84	75	84	89	50	39	0

Table 44: Pairwise differentiations in AMOVA analysis for the combination of ISSR, EST-PCR, and EST-SSR markers within the clones of Bell Island East in Newfoundland and Labrador

	NL1	NL5	NL6	NL10	NL14	NL20	NL22	NL23	NL24	NL25	NL30	NL32	NL34	NL35	NL39	NL53	NL54	NL57	NL58	NL65	NL66	NL69	NL70	NL71
NL1	0																							
NL5	37	0																						
NL6	55	34	0																					
NL10	60	55	55	0																				
NL14	64	59	57	38	0																			
NL20	61	50	56	49	53	0																		
NL22	61	46	50	55	51	32	0																	
NL23	64	53	55	50	56	33	35	0																
NL24	60	45	53	52	56	33	25	18	0															
NL25	56	41	53	50	50	29	33	32	24	0														
NL30	82	79	73	82	82	83	81	82	76	82	0													
NL32	83	82	86	89	89	92	96	91	87	89	37	0												
NL34	83	78	74	77	89	78	84	75	81	77	45	44	0											
NL35	78	77	75	78	80	85	87	76	76	80	36	37	33	0										
NL39	85	88	86	93	93	92	96	85	85	89	67	62	68	63	0									
NL53	89	92	88	99	87	84	90	89	85	81	83	86	88	87	84	0								
NL54	95	92	86	97	81	80	86	83	81	83	73	86	84	85	78	32	0							

NL57	81	78	78	89	81	74	74	77	73	75	79	88	90	89	80	44	38	0						
NL58	88	87	93	90	78	81	87	86	84	82	82	83	85	90	87	47	43	39	0					
NL65	75	82	86	97	93	88	90	91	93	83	87	76	82	85	84	74	88	86	85	0				
NL66	82	83	89	96	92	95	89	98	92	90	78	79	83	82	89	83	95	85	82	43	0			
NL69	77	88	88	89	87	82	84	91	85	85	77	80	92	87	82	66	82	76	75	36	43	0		
NL70	77	80	78	79	77	82	80	89	89	77	81	88	86	85	84	70	84	78	81	38	49	22	0	
NL71	80	87	89	86	84	83	81	84	84	82	72	77	85	82	81	69	77	71	74	49	46	27	33	0

Table 45: The pairwise differentiations in AMOVA analysis for the combination of ISSR, EST-PCR, and EST-SSR markers within the clones of Bell Island West in Newfoundland and Labrador

	NL3	NL8	NL9	NL11	NL15	NL16	NL17	NL26	NL27	NL51	NL52	NL56	NL59	NL60	NL61	NL62	NL63	NL67	NL68
NL3	0																		
NL8	53	0																	
NL9	67	38	0																
NL11	64	45	39	0															
NL15	61	44	44	45	0														
NL16	58	59	53	38	37	0													
NL17	73	52	58	45	44	45	0												
NL26	57	56	58	57	56	49	66	0											
NL27	52	59	57	56	57	48	63	33	0										
NL51	84	93	83	92	83	74	83	79	80	0									
NL52	87	88	84	87	86	79	76	78	79	27	0								
NL56	92	97	87	80	89	86	79	79	86	40	37	0							
NL59	90	87	87	92	81	80	79	79	80	48	51	46	0						
NL60	88	87	89	86	85	84	73	79	78	54	47	44	40	0					
NL61	86	85	91	78	79	76	67	81	76	56	57	50	34	34	0				
NL62	89	86	92	79	82	79	68	86	89	53	48	39	35	29	23	0			

NL63	88	89	85	78	83	78	73	77	82	50	49	36	34	38	36	29	0		
NL67	94	83	91	84	89	84	77	85	92	88	85	92	102	84	84	83	88	0	
NL68	84	83	91	86	91	80	77	79	78	82	81	86	94	90	86	83	84	26	0

Table 46: Pairwise differentiations in AMOVA analysis for the combination of ISSR, EST-PCR, and EST-SSR markers within the other clones of Newfoundland and Labrador

	NL13	NL18	NL19	NL28	NL36	NL38	NL43	NL45	NL46	NL48	NL64
NL13	0										
NL18	48	0									
NL19	53	39	0								
NL28	48	52	55	0							
NL36	80	92	91	84	0						
NL38	105	109	110	103	87	0					
NL43	88	88	81	76	86	97	0				
NL45	98	92	89	94	98	103	40	0			
NL46	103	95	90	97	95	98	43	31	0		
NL48	98	90	85	96	84	101	44	34	37	0	
NL64	90	96	83	100	82	93	94	76	81	80	0

Table 47: The pairwise differentiations used for AMOVA with the combination of ISSR, EST-PCR, and EST-SSR markers within the genotypes of clones in Prince Edward Island

	PE1	PE2	PE3	PE4	PE5	PE6	PE7	PE8	PE9	PE10	PE11	PE12	PE13	PE14	PE15	PE16	PE17	PE18	PE19	PE20
PE1	0																			
PE2	47	0																		
PE3	51	32	0																	
PE4	65	62	56	0																
PE5	88	89	85	95	0															
PE6	87	88	86	96	33	0														
PE7	74	79	77	89	42	45	0													
PE8	87	92	88	102	47	48	53	0												
PE9	84	85	87	99	42	45	42	43	0											
PE10	82	83	79	97	42	47	42	49	28	0										
PE11	87	84	80	86	55	48	49	58	53	45	0									
PE12	82	87	89	89	50	51	62	61	46	42	53	0								
PE13	80	87	81	97	58	53	56	59	56	56	49	38	0							
PE14	93	96	96	98	57	50	65	70	55	61	62	47	51	0						
PE15	88	93	93	103	60	53	68	69	62	68	71	58	64	49	0					
PE16	93	98	92	104	67	64	77	66	73	67	66	65	57	50	53	0				
PE17	97	94	88	78	85	90	87	88	85	89	86	85	89	80	79	94	0			

PE18	83	86	76	80	75	72	75	78	79	83	80	79	79	80	83	90	40	0		
PE19	82	81	87	89	74	71	74	83	80	86	77	72	78	81	86	95	77	73	0	
PE20	90	83	89	81	76	73	84	89	88	88	75	70	74	77	80	87	71	65	28	0

Table 48: Pairwise differentiations in AMOVA analysis for the the combination of ISSR, EST-PCR, and EST-SSR markers within the clones of New Brunswick

	NB1	NB2	NB3	NB4	NB5	NB6	NB7	NB8	NB9	NB10
NB1	0									
NB2	83	0								
NB3	80	41	0							
NB4	85	80	81	0						
NB5	76	85	88	43	0					
NB6	88	83	90	35	38	0				
NB7	86	89	92	41	40	34	0			
NB8	91	88	97	42	39	25	25	0		
NB9	82	83	78	71	66	72	74	75	0	
NB10	87	78	81	70	79	73	77	74	31	0

Table 49: Pairwise differentiations in AMOVA analysis for the combination of ISSR, EST-PCR, and EST-SSR markers within the cultivars

	Franklin	Stevens	Wilcox	Pilgrim	Ben Lear
Franklin	0				
Stevens	50	0			
Wilcox	70	56	0		
Pilgrim	95	93	95	0	
Ben Lear	96	94	94	85	0