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ISSR, anthocyanin content and antioxidant activity analyses to characterize strawberry genotypes

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Abstract

Data on molecular markers, anthocyanin contents and antioxidant activities are increasingly used in breeding programs of many horticultural crops. Inter simple sequence repeat (ISSR) analysis, anthocyanin contents and antioxidant activities were used to characterize 10 strawberry (*Fragaria* x *ananassa* Duch.) cultivars and nine breeding lines. Fifteen primers generated 240 polymorphic ISSR-PCR bands. Cluster analysis by the unweighted pair-group method with arithmetic averages (UPGMA) revealed a substantial degree of genetic similarity among the genotypes ranging from 45% to 73% that were in agreement with the principal coordinate (PCO) analysis. Wide genetic diversity was observed among the strawberry genotypes for anthocyanin contents and antioxidant activities. The ISSR analysis together with data for antioxidant activities and anthocyanin contents in strawberries could be used for germplasm management and more efficient choices of parents in current strawberry breeding programs.

Key words: Anthocyanin content, antioxidant activity, *Fragaria* x *ananassa* Duch., ISSR markers, strawberry

Introduction

The cultivated strawberry (*Fragaria* x *ananassa* Duch.), an octoploid (2n = 56) hybrid between the Scarlet or Virginia strawberry (*F. virginiana* Duch.) and the pistillate South American *F. chiloensis* (L.) Duch., is a dicotyledonous, perennial low-growing herb grown in most arable regions of the world and is enjoyed by millions of people in all kinds of climates including temperate, Mediterranean, subtropical and taiga zones (Hancock *et al.,* 1991). Strawberries are good sources of natural antioxidants including carotenoids, vitamins, phenols, flavonoids, dietary glutathionine and endogenous metabolites and exhibit a high level of antioxidant capacity against free radical species: superoxide radicals, hydrogen peroxide, hydroxyl radicals and singlet oxygen (Wang and Jiao, 2000). The benefits of these high antioxidant activity fruit include reduction of carcinogens in humans (Chung *et al.,* 2002), protection against tumor development (Kresty *et al.,* 2001) and reversal of age-related effects on memory (Bickford *et al.,* 2000). Anthocyanins are typically present at high levels in strawberries and are thought to significantly contribute to the total antioxidative activity of this fruit (Wang *et al.,* 1997). Total antioxidant activity, as well as total anthocyanin content, can vary among cultivars, and these may affect overall protective benefits of human health and are worth further investigation.

There is a pressing need to develop reliable methods for identifying strawberry cultivars and for assessing genetic diversity/relatedness in strawberry genotypes for practical breeding purposes and proprietary-rights protection. Molecular markers are increasingly used in breeding programs of many horticultural crops. The introduction of molecular biology techniques, such as DNA-based markers, allows direct comparison of different genetic material independent of environmental influences (Weising *et al.,* 1995). The degree of similarity between the banding patterns can provide information about genetic similarity, and relationships between the samples studied.

Strawberries have been extensively analyzed for clone identification, mapping and diversity studies using randomly amplified polymorphic DNA (RAPD) markers (Graham *et al.,* 1996; Degani *et al.,* 2001; García *et al.,* 2002; Kuras *et al.,* 2004), amplified fragment-length polymorphisms (AFLPs) (Degani *et al.,* 2001; Tyrka *et al.,* 2002) and the simple sequence repeats (SSRs) (Cipriani *et al.,* 2006; Sargent *et al.,* 2006). Gil-Ariza *et al.* (2006) described expressed sequence tags (ESTs)-derived microsatellites from cultivated strawberry and their potential use for varietal identification and diversity study. Inter simple sequence repeat markers (ISSRs) (Zietkiewicz *et al.,* 1994) have been used successfully in a number of horticultural crops including blueberry (Debnath, 2009), lingonberry (Debnath, 2007) and strawberry (Arnau *et al.,* 2002; Debnath *et al.,* 2008). The ISSR primers target microsatellites that are abundant throughout the plant genome (Wang *et al.,* 1994). These markers have proven to be more reproducible than RAPD markers and generally reveal higher levels of polymorphism because of the higher annealing temperature and longer primer sequences (Qian *et al.,* 2001). They cost less and are easier to use than AFLPs and do not require prior knowledge of flanking sequences like SSRs (Reddy *et al.,* 2002).

The purpose of this study was to examine the level of genetic variation among 10 strawberry cultivars and nine advanced selections for antioxidant activity and anthocyanin content, to study genetic diversity using ISSR- PCR fingerprints and to compare the genotypic classification based on molecular marker with those of chemical marker. Although the scientific literature does include reports of ISSR analysis for strawberry (Arnau *et al.,* 2002; Kuras *et al.,* 2004; Debnath *et al.,* 2008), anthocyanin

content and antioxidant activity in relation to molecular analysis have not been documented in a given set of strawberries. The goal of the study was to combine data on anthocyanin contents and antioxidant activities with DNA analysis to better identify genotypes used in breeding programs.

Materials and methods

Plant material: For this study, the strawberry cultivars and advances selections (Table 1) developed by Agriculture and Agri-Food Canada Research Centres in St-Jean-sur-Richelieu, Quebec (QC); Kentville, Nova Scotia (NS); and in Agassiz, British Columbia (BC) in Canada were grown in 10.5 cm^2 plastic pots containing ProMix BX (Premier Horticulture Limited, Rivière-du-Loup, QC, Canada) potting medium, in the greenhouse under natural light conditions at a maximum photosynthetic photon flux of 90 µmol m^{-2} s^{-1} at 20 ± 2°C, 85% RH. Irrigation and artificial fertilization were applied when necessary.

DNA extraction: Leaf tissues used for DNA extraction were collected from actively grown shoots, immediately placed on ice and then frozen at -80°C until extraction. DNA was extracted by the GenElute Plant Genomic DNA Miniprep extraction kit (Sigma Chemical Co., Oakville, ON, Canada) following the manufacturer's instructions. The concentration of DNA was estimated spectrophotometrically (Ultrospec 2000, Pharmacia Biotech, Cambridge, UK) at 260 nm. DNA purity was measured by the ratio of the absorbance at 260 nm over 280 nm. Template DNA with an A_{260}/A_{280} ratio of 1.8: 2.1 in a dilution of 10 ng μL^{-1} was used for PCR.

ISSR primers, PCR amplification and electrophoresis: A set of 100 primers (UBC set 9) was procured from the Biotechnology Laboratory, University of British Columbia, Vancouver, Canada. Out of these, 15 (Table 2) that gave clear banding patterns were used in the final study. Different concentrations of template DNA and *Taq* polymerase were tested for optimal amplification. The optimized amplification reaction mixture (25 µL) contained 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], 30 pmol primer, 200 µM of each dNTP, 1.25 U of *Taq* DNA polymerase (Sigma) and PCR grade dH_2O (Sigma). DNA Reaction mixtures were amplified in a PTC-100 Programmable Thermal Controller (MJ Research Inc., Watertown, MA, USA) using an initial hot start of 94°C for 10 min, followed by 45 cycles of 1 min at 94°C, 1 min at 45°C and 2 min at 72°C. The reaction was terminated with a final extension at 72°C for 10 min before holding the sample at 4°C for analysis. Amplified fragments, along with a 1kb DNA ladder (Invitrogen, Burlington, ON, Canada) used as a molecular weight standard, were resolved by 1.6% agarose gel electrophoresis in tris-borate-EDTA (TBE). After ethidium bromide staining for 35 min (0.5 µg L^{-1} of TBE) and a distilled water wash of 20 min, DNA was visualized using a GeneGenius gel documentation system (Syngene**,** Beacon House, Cambridge, UK). DNA amplification with each primer was repeated at least twice and congruence between replicates was verified. Gels were scored for polymorphic and monomorphic bands. Non-replicated bands were eliminated from analysis. Bands of similar molecular weight and migration distance across individuals were assumed to be homologous (Adams and Rieseberg, 1998).

Fruit extraction for chemical analysis: Mature ripe fruit, with a well-developed red colour, were harvested from three replicates per genotype and frozen immediately at -20°C until analysis. Four g of berries from each genotype were homogenised overnight at 4°C in 2.5 mL ethanol : 1.5 M HCl (85:15 v/v) to extract anthocyanins. The extracts were filtered through 0.2 µM syringe filters before analysis.

Total anthocyanin content: The total anthocyanin content was measured in triplicate by the pH differential method as described

Table 1. Parentage, anthocyanin contents and antioxidant activities of strawberry genotypes used in inter simple sequence repeat (ISSR) analysis

Genotype	Code	Parentage	Origin in Canada[a]	Anthocyanins (mg $100g^{-1}$ fr wt)	DPPH (ED_{50})[b]
Cultivar					
Bounty	BO	(Jerseybelle × Senga Sengana)	Kentville, NS	27.3 ± 5.5	27.5 ± 7.4
Cavendish	CA	(Glooscap × Annapolis)	Kentville, NS	31.3 ± 4.3	9.4 ± 0.42
Clé des champs	CL	(SJ89244-6E × SJ8518-11)	Saint-Jean-sur-Richelieu, QC	16.1 ± 4.3	23.1 ± 4.0
Kent	KE	[(Redgauntlet × Tioga) × Raritan]	Kentville, NS	25.4 ± 3.1	23.2 ± 7.3
Micmac	MC	(Tioga × Guardsman S1)	Kentville, NS	27.0 ± 4.7	10.7 ± 0.13
Mira	MR	(Scott × Honeoye)	Kentville, NS	21.0 ± 14.7	39.5 ± 1.6
Rosalyne	RO	[Fern × (SJ9616-1 × Pink Panda)]	Saint-Jean-sur-Richelieu, QC	23.6 ± 2.9	19.0 ± 11.0
Saint-Pierre	SP	(Chandler × Jewel)	Saint-Jean-sur-Richelieu, QC	14.1 ± 2.3	11.6 ± 0.10
Stolo	SL	(Puget Reliance × Whonnock)	Agassiz, BC	16.3 ± 2.5	16.7 ± 1.3
Wendy	WE	[{Sable x (Cavendish x Selkirk)} H Evangeline]	Kentville, NS	35.1 ± 6.1	20.3 ± 4.0
Advanced selections					
APF9313-126	AP	Unreleased breeding line	Saint-Jean-sur-Richelieu, QC	22.6 ± 1.0	19.0 ± 0.32
BC92-20-85	B1	(Cavendish × Nanaimo)	Agassiz, BC	43.7 ± 5.3	20.2 ± 2.7
BC96-1-7	B2	(Marmolada × Nanaimo)	Agassiz, BC	15.4 ± 2.5	27.6 ± 7.5
FIN005-7	F1	Unreleased breeding line	Saint-Jean-sur-Richelieu, QC	23.7 ± 5.0	20.63 ± 6.5
FIN005-55	F2	Unreleased breeding line	Saint-Jean-sur-Richelieu, QC	22.2 ± 5.0	21.0 ± 1.9
FIN0016-115	F3	Unreleased breeding line	Saint-Jean-sur-Richelieu, QC	32.5 ± 1.3	23.4 ± 4.7
KRS-10	KR	(K94-15 × K95-24)	Kentville, NS	10.5 ± 4.4	18.6 ± 1.5
SJO001-99	S1	Unreleased breeding line	Saint-Jean-sur-Richelieu, QC	6.3 ± 1.2	19.4 ± 3.2
SJO9611-23	S2	Unreleased breeding line	Saint-Jean-sur-Richelieu, QC	41.7 ± 4.8	18.7 ± 4.4

[a]BC = British Columbia, NS = Nova Scotia, QC = Quebec. [b]ED50 value is used to express the concentration of an antioxidant required to quench 50% of the initial 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Each value is expressed as mean ± standard deviation (n = 3).

by Foley and Debnath (2007). Absorbance was measured in a spectrophotometer (Ultrospec 2000; Pharmacia Biotech, Cambridge, UK) at 510 nm and 700 nm in buffers at pH 1.0 and pH 4.5. Absorbance readings were converted to total milligrams of cyanidin 3-glucoside per 100 g fresh weight of strawberry using the molar extinction coefficient of 26 900 and absorbance of $A = [(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}]$ (Meyers *et al.,* 2003).

Anti-oxidant activity analysis: The anti-oxidant activity in each fruit extract was measured, in triplicate, using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH, from Sigma-Aldrich Co., St. Louis, MO, USA) method as previously described (Anttonen *et al.,* 2006; Foley and Debnath, 2007). Using a calibration curve with different amounts of DPPH, the ED_{50} was calculated. The ED_{50} is the concentration of an antioxidant that is required to quench 50% of the initial DPPH radicals under the given experimental conditions.

Data analysis: The ability of the most informative ISSR primers to differentiate between genotypes was assessed by calculating their resolving power (Rp) (Gilbert *et al.,* 1999):

$Rp = \sum Ib$, where, $Ib = 1 - (2 \times |0.5 - p|)$,
where, p is the proportion of the 19 genotypes containing the band. The value of Ib was calculated for 15 informative ISSR primers.

Presence or absence of each ISSR fragment was coded as 1 and 0, where 1 = the presence of a specific allele, and 0 = its absence. Since ISSR markers are typically dominant, it was assumed that each band represented the phenotype at a single bi-allelic locus. The presence of a band was interpreted as either a heterozygote or a dominant homozygote, and the absence of a band as a recessive homozygote. The basic data structure finally consisted of a binomial (0/1) matrix, representing the scored ISSR markers. The similarities were calculated using the following coefficients, described by Sneath and Sokal (1973): *Simple Matching coefficient*: $S_{ij} = (a+d)/(a+b+c+d)$; *Dice's coefficient*: $S_{ij} = 2a/(2a+b+c)$, equivalent to the coefficient of Nei and Li (1979); and *Jaccard's coefficient*: $S_{ij} = a/(a+b+c)$, where S_{ij} is the similarity between two individuals, i and j; a = number of bands shared by both individuals, b = number of bands presented by i, but not by j; c = number of bands presented by individual j but not by i; and d = number of bands absent in both. Jaccard's and Nei and Li's (1979) coefficients calculate similarities based on the shared presence, but not the absence, of DNA bands. The similarity matrix was used as input data for cluster analysis by applying unweighted pair-group method with arithmetic averages (UPGMA), and to compute a principal coordinate (PCO) analysis (Gower, 1966) using NTSYS-pc (Version 2.1) software (Rohlf, 1998). The SAHN option was used to cluster the data according to the UPGMA method. Co-phenetic matrices were created from the dendrogram and compared with the similarity matrix using the Mantel matrix comparison function in NTSYS to test whether clusters in the dendrogram agreed with information from the similarity matrix. The cophenetic correlation coefficient (CCC) value obtained gives a goodness of fit for the clusters (Rohlf ,1998).

Data for anthocyanin contents and antioxidant activities were subjected to analysis of variance (ANOVA) using the SAS statistical software package (Release 8.2; SAS Institute, Inc., Cary, NC, USA). Statistical F-tests were evaluated at $P \leq 0.05$. Differences among treatments were further analysed using Duncan's multiple range test. For cluster analysis, variables were standardized using STAND module, similarity matrices (in SIMINT) were generated using Euclidean distance, and the dendrogram was constructed using the UPGMA with NTSYS-pc (Version 2.1) software (Rohlf, 1998).

Results

ISSR amplification: Of the 15 selected primers, 12 (seven 3′ mono-anchored and five 3′ di-anchored) were designed to anneal to di-nucleotide repeats, two to tetra-nucleotide repeats and one to mixed nucleotide repeats. The primers revealed the presence of polymorphisms in the amplified DNA fragments in a range from 300 to 4100 bp. The number of polymorphic bands produced ranged from 13 for the 811 and 879 primers to 20 for the 876 primer, with an average of 16 bands per primer (Table 2). The ability of the 15 most-informative primers to differentiate strawberry genotypes was assessed on the basis of Rp. The Rp values varied from 5.5 for primers 811 and 879 to 11.5 for 876. Primers with higher Rp values were generally able to distinguish more genotypes and showed higher polymorphic bands (Table 2).

Genetic relationships among strawberry genotypes: The Dice, Jaccard and the Simple Matching coefficients were used to generate similarity matrices for ISSR cluster analysis. The CCC relates the level of distoration between the similarity matrix and cluster analysis, a higher CCC indicates a better fit. The CCCs of the Dice, Jaccard and Simple Matching-based cophenetic matrices were 0.83, 0.83 and 0.81, respectively. Despite the similar discriminating power of both Dice-and Jaccard-based co-phenetic matrices, genetic similarity values were lower for Jaccard than for Dice (data not shown). This result is expected because the Dice index differs from the Jaccard index by the higher weight that it gives to coincidences of the presence of a band in relation to non-coincidences. The UPGMA clustering algorithm based

Table 2. Inter simple sequence repeat (ISSR) primers: their sequence, anchored end, repeat motif and the data on DNA profile and polymorphism generated in 10 strawberry cultivars and nine advanced selections using 15 ISSR primers

Primer Code[a]	Sequence[b]	Polymorphic bands		Resolving power
		Number	Size range (bp)	
807	$(AG)_8$ T	15	400-2200	7.4
808	$(AG)_8$ C	18	500-2400	8.4
810	$(GA)_8$ T	18	500-3100	9.3
811	$(GA)_8$ C	13	500-2700	5.5
817	$(CA)_8$ A	18	400-3200	10.4
823	$(TC)_8$ C	14	500-3300	6.8
827	$(AC)_8$ G	18	400-3700	7.9
834	$(AG)_8$ YT	17	300-2300	7.5
835	$(AG)_8$ YC	15	400-2400	6.8
836	$(AG)_8$ YA	16	500-2100	10.4
840	$(GA)_8$ YT	15	400-2500	8.8
841	$(GA)_8$ YC	14	400-2200	6.8
876	$(GATA)_2$ $(GACA)_2$	20	400-4100	11.5
879	$(CTTCA)_3$	13	700-3300	5.5
895	AGA GTT GGT AGC TCT TGA TC	16	500-2800	7.3

[a] Primer numbers follow those in UBC set 9 (no. 801 - 900).
[b] Y: C,T.

on Dice's and Jaccard's similarity matrix gave similar results (data not shown). Results on Dice-based matrix clustering are described hereafter. UPGMA clustering identified three clusters at a similarity index of approximately 0.54 leaving APF9313-126 as an outlier at 0.45 similarity index. Within Cluster I, two sub-clusters were resolved at approximately 57% similarity value: the first one with seven cultivars and three breeding lines, and the second sub-clusters with two cultivars and two breeding lines. In the first sub-cluster of Cluster I, Bounty, FIN005-7 and FIN0016-115 were grouped together with about 60% similarity and were separated from six cultivars and one advanced line that formed a sub-sub-cluster, at a similarity index of approximately 0.59. Cavendish and Kent had the maximum similarity (73%), and both were separated from BC96-1-7 at a similarity value of about 0.64. Mira, Saint-Pierre, Wendy and Stolo formed a group in the first sub-cluster of Cluster I at approximately 0.65 similarity index. In the second sub-cluster of Cluster I, Micmac was separated from KRS-10, Rosalyne and FIN005-55 at a similarity value of approximately 0.57. The later two were grouped together with 63% similarity and both were separated from KRS-10 at about 0.60 similarity index. Clé des champs and BC92-20-85 formed Cluster II with 56% similarity, and SJO001-99 and SJO9611-23 formed Cluster III with approximately 58% similarity (Fig. 1).

PCO analysis on the frequencies of occurrence of the polymorphic ISSR markers among the genotypes showed that the plotting of the first three components, representing 11.0, 9.2 and 8.2% of the total variation for the first, second and the third component, respectively, indicating existence of significant variation among the genotypes. Most of the genotypes are separated by the first or second or third PCO (Fig. 2) which demonstrates distinct groups of genotypes corresponding to cluster analysis (Fig. 1). The genotypes Kent, Stolo, Wendy, Saint-Pierre, Mira and BC96-1-7 were grouped together by the third axis (Fig. 2) as was also observed by cluster analysis where they were grouped together at a similarity value of 0.63 within the first sub-cluster of Cluster I (Fig. 1). Similarly, Rosalyne, FIN005-55 and KRS-10 were in a group corresponding to cluster analysis where they were grouped together within second sub-cluster of Cluster I at a similarity value of about 0.60. However, FIN005-55 and FIN0016-115 were tightly clustered in PCO analysis although they were well separated in cluster analysis (Fig. 1). The third axis revealed another level of separation for BC92-20-85 and Clé des champs (Fig. 2) and these were not apparent from the dendrogram.

Antioxidant activities and anthocyanin contents: Significant differences ($P \leq 0.05$) were observed among genotypes tested for anthocyanin contents and for antioxidant activities (data not shown). Among the 19 genotypes tested for anthocyanin contents, BC92-20-85 fruits had the highest total anthocyanin content and it was followed by SJO9611-23. The unreleased advanced line SJO001-99 had the lowest anthocyanin content (Table 1).

The strawberry genotypes differed in their activity to react and quench DPPH radicals (Table 1). The ED_{50} value is used to express the concentration of an antioxidant required to quench 50% of the initial DPPH radicals under the experimental conditions given. A smaller ED_{50} value corresponds to a greater DPPH radical scavenging activity. While Cavendish was the best for antioxidant activity, Mira was the worst (Table 1). The correlation between total anthocyanin content and antioxidant activity was positive but insignificant (r = 0.06).

The relationships between the 19 genotypes revealed by UPGMA

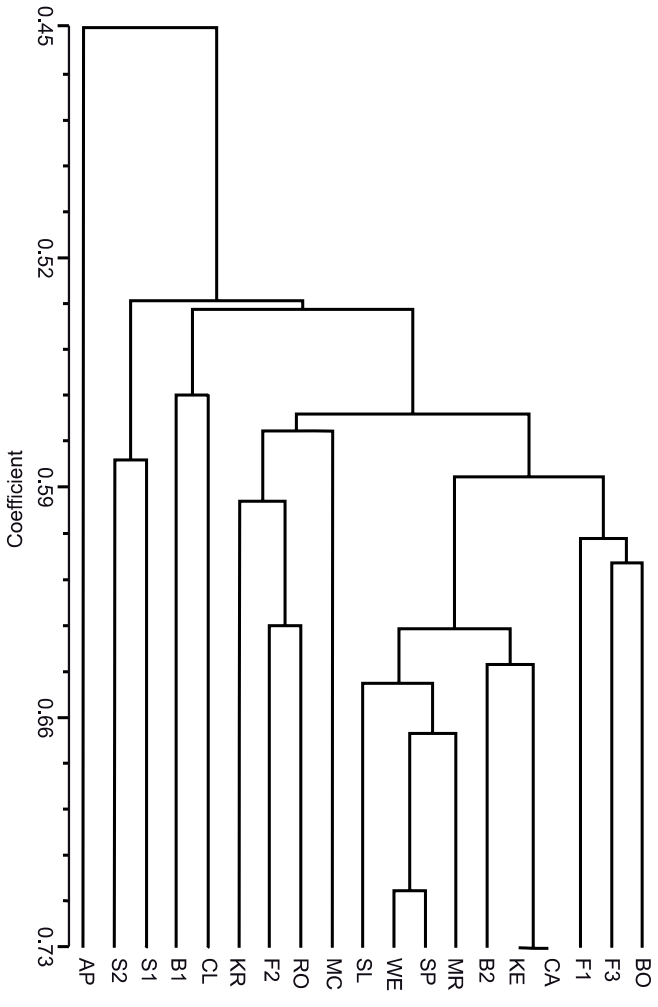

Fig. 1. Unweighted pair-group method with arithmetic averages (UPGMA) dendrogram estimating the genetic distance among 19 strawberry genotypes designated by codes given in Table 1, based on inter simple sequence repeat (ISSR) coefficient- derived Dice matrix.

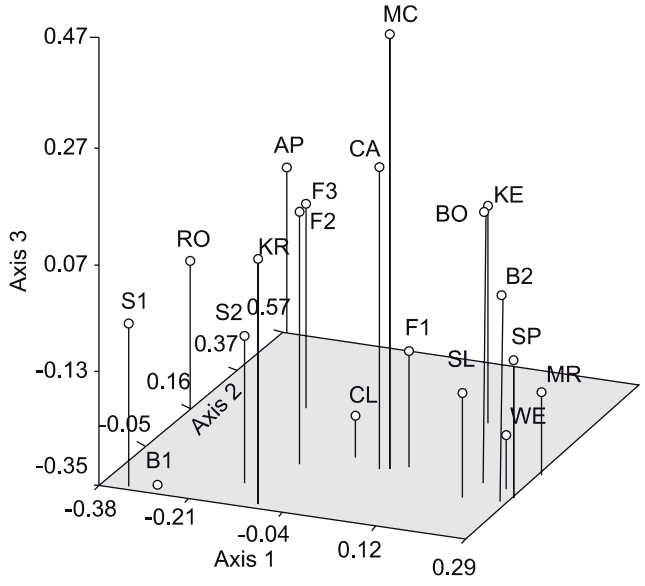

Fig. 2. Three-dimensional plot of the principal coordinate (PCO) analysis of distance among 19 strawberry genotypes designated by codes given in Table 1, on inter simple sequence repeat (ISSR) markers.

cluster analysis based on anthocyanin content and antioxidant activity data and Euclidean distance are presented in Fig. 3. Two major clusters were resolved leaving FIN005-7 as an outlier. While all cultivars and six advanced selections formed Cluster I, two advanced lines BC92-20-85 and SJO9611-23 were grouped in Cluster II. As was observed in the ISSR analysis (Figs. 1 and 2), the cultivars were intermixed with selections in both anthocyanin content and antioxidant activity-based dendrogram. Within Cluster I, two sub-clusters were evident. Within the first sub-cluster of Cluster I, Bounty, Micmac and Kent formed a group and were separated from the other group consisting of Mira, Rosalyne, APF9313-126 and FIN005-55 at a coefficient value of approximately 0.42. These two groups were again separated from a sub-sub-cluster of Cluster I consisting of Cavendish, FIN0016-115 and Wendy, at approximately 0.86 coefficient value. Within the second sub-cluster of Cluster I, Clé des champs and Stolo possessed the minimum distance (0.02 coefficient value) with each other and both cultivars grouped with BC96-1-7 and Saint-Pierre at a coefficient value of approximately 0.19 forming a sub-sub-cluster within the second sub-cluster of Cluster I. Two advanced selections, KRS-10 and SJO001-99 formed another sub-sub-cluster within the second sub-cluster of Cluster I (Fig. 3).

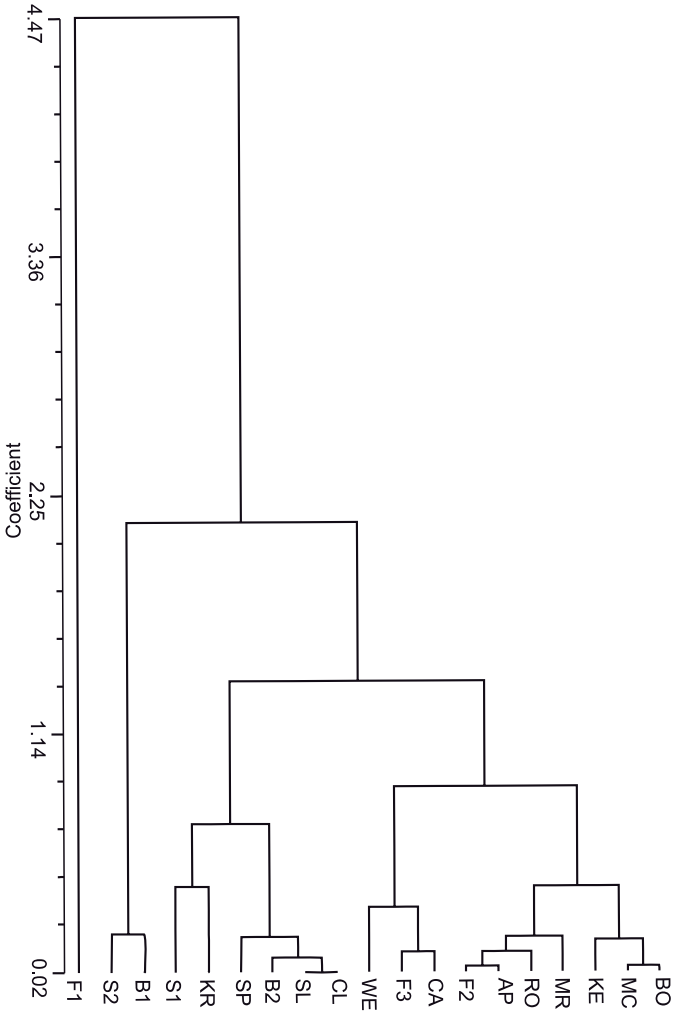

Fig. 3. Unweighted pair-group method with arithmetic averages (UPGMA) dendrogram estimating the genetic distance among 19 strawberry genotypes designated by codes given in Table 1, based on anthocyanin content and antioxidant activity data.

Discussion

The ISSR and chemical markers were used in this study to evaluate the levels of genetic relatedness among 10 strawberry cultivars and nine advanced selections. Fifteen primers detected enough genetic variation and relatedness among the genotypes to allow for complete differentiation as was revealed from PCR-derived gel analysis. The ISSR primers varied in their ability to distinguish strawberry genotypes, and primers with high Rp values were generally more effective in distinguishing between genotypes (data not shown). This is in agreement with the findings in lupin (Gilbert *et al.,* 1999) and lingonberry (Debnath, 2007). The results clearly demonstrate that ISSR markers can be used in a genetic diversity study as well as in genotypic identification of strawberries, as noted by Kuras *et al.* (2004) and Arnau *et al.* (2002) or in helping breeders to select diverse parents with similar phenotypes for breeding.

Two different methods of multivariate molecular analysis, PCO and cluster analysis, were used to group the individuals in this study, as each method is designed to sort the data on a different basis and therefore can be used comparatively to elucidate relationships. The UPGMA clustering algorithm assumes a constant evolutionary rate among accessions or clones. The results of the present study showed a significant level of genetic relatedness among strawberry cultivars and lines. The strawberry cultivars and advanced selections showed 45 to 73% genetic similarity. Graham *et al.* (1996) found a level of similarity for strawberry cultivars ranging from 62 to 89% indicating their closely related nature even though they resulted from four independent breeding programs (Scotland, England, USA and The Netherlands). Similar results have also been reported by Tyrka *et al.* (2002) confirming small gene pool variation in strawberry cultivars. In the present study, out of 19 strawberry cultivars and lines, 14 were grouped in one cluster with about 54% similarity. The five cultivars originating in NS: Bounty, Cavendish, Kent, Mira and Wendy, have 59% similarity. Degani *et al.* (2001) reported 73 and 86% similarity by RAPD and AFLP analysis respectively, between two NS cultivars, Annapolis and Kent. Although BC92-20-85 did not cluster with its parent Cavendish, they had a relatively high similarity value of 54%. Cavendish and Kent, although developed from two different crosses, were grouped together with 73% similarity (Fig. 1). This is unexpected as these cultivars have very different genetic backgrounds. Similar results were also reported by Degani *et al.* (2001) where a UPGMA-derived dendrogram based on genetic similarities from banding data did not reflect known pedigree information. These might be due to incomplete coverage of the genome (Degani *et al.,* 2001).

Strawberries are a good source of dietary antioxidants. The present data provide evidence of a genetic similarity and divergence for anthocyanin contents and for antioxidant activities among the tested genotypes. Previous studies reported variation in anthocyanin content and antioxidant activity in strawberry genotypes (Meyers *et al.,* 2003; Anttonen *et al.,* 2006). The observed poor correlation between anthocyanin content and antioxidant activity indicated that this fruit antioxidant did not play a major role in increasing antioxidant activity in the present material. Similar results have also been reported in strawberry (Hansawasdi *et al.,* 2006).

Out of 19 strawberry genotypes, 14 were grouped together in one cluster by ISSR and 16 by chemical UPGMA analysis (Figs. 1 and 3). However, clustering based on ISSR data was different from that based on the chemical data. ISSR markers are distributed throughout the genome and in the majority of cases most regions of the genome are not expressed at the phenotypic level. The non-coding regions (un-expressed) of genome are not accessible to phenotypic expression and might have resulted in disagreement between the chemical and molecular diversity. The weak correspondence between genetic distances from chemical and ISSR data most probably implies that these markers differ in their degree of genomic coverage. García *et al.* (2002) failed to correlate morphological and RAPD characterization in strawberry.

In conclusion, this study should facilitate the use of chemical analysis of fruits and of ISSR fingerprints for marker-assisted applications in strawberry breeding. The study identified promising genotypes with anthocyanin contents and high antioxidant activities. Fifteen ISSR primers that generated substantial polymorphism among the strawberry genotypes have also been identified. As ISSRs have higher reproducibility and reveal higher levels of polymorphism than RAPDs (Qian *et al.,* 2001), cost less and are easier to use than AFLPs, and as they do not require prior knowledge of flanking sequences like SSRs (Reddy *et al.,* 2002), similar genetic analysis might be applicable to other strawberry genotypes. Given the resource limitations on identifying genotypes at the molecular level, this study demonstrated the use of ISSR markers combined with chemical markers as potential quality-assurance tools to identify and maintain diverse genotypes and helps assess the genetic diversity of strawberry genotypes. The resultant molecular markers can also be used for pre-selection in seedling populations, to discard unfavourable genotypes at an early stage and to identify successful crosses between different strawberry genotypes.

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