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Molecular Identification and Genetic Relationships of Six Strawberry Varieties using ISSR Markers

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ABSTRACT

The objectives of the present investigation were to molecularly identify and fingerprint six strawberry varieties. The genetic relationships among the six strawberry varieties were achieved using the Inter Simple Sequence Repeats (ISSRs) technique with nine primers. Primers generated 102 total amplified fragments, of which 86 (84.3%) polymorphic fragments discriminated the varieties under the present investigation. A dendrogram-tree generated across the analysis demonstrated that six strawberry varieties are grouped into two clusters. The first cluster contained the Capitola variety only, while Tamar, Chandler, Sweet Charl, Rosa and Diamond comprised the second one. The Sweet Charl variety showed the same high genetic similarity index (83%) to Diamond and Chandler. On the contrary, the most genetically distant varieties were Tamar and Capitola with 45% similarity index. It is concluded that the ISSR technique along with the nine primers in this study were useful tools for the identification of the six strawberry varieties.

Key Words: Strawberry; Molecular identification; ISSR

INTRODUCTION

Strawberry (*Fragaria ananassa*) is a vegetatively propagated and one of the important export vegetable crops in Egypt. Fruits are exported as fresh, frozen and/or manufactured in different forms. The genus *Fragaria* belongs to the family *Rosaceae* and was produced from the hybridization between *F. chiloensis* and *F. virginiana*. The fruit is rich in vitamins such as vitamin C or ascorbic acid (60 mg 100 g^{-1}), niacin (0.6 mg 100 g^{-1}) and contains a large amount of carbohydrates (8 g 100 g^{-1}) on fresh weight basis.

The application of DNA technology in agricultural research is progressed rapidly over the last two decades, especially in the area of variety identification. More recently, molecular marker systems based on the Polymerase Chain reaction (PCR) technique have become increasingly popular for fingerprinting and variety identification. The Inter-Simple Sequence Repeat marker (ISSR, anchored microsatellite) use simple sequence repeats anchored at the 5' or 3' end by a short arbitrary sequence as PCR primers (Zietkiewicz *et al*., 1994). ISSRs are ideal markers for genetic mapping and population studies due to their abundance and the high degree of polymorphism between individuals with a population of closely related genotypes (Cregan *et al*., 1994; Jarret & Bowen, 1994; Hokanson *et al*., 1998; Lanham & Brennan, 1998). The ISSR technique has been reported as a good alternative to AFLP when tested on poplar, roses, pea and hortensien; being cheaper, more rapid and more reproducible (Arnau *et al.,* 2000).

The ISSR technique is a potentially useful tool for the identification of strawberry varieties as it is simple, cost-effective, fast and highly discriminant and reliable (Arnau *et al*., 2003). The objectives of the present study were to detect genetic relationships and identify molecular fingerprints among six strawberry varieties using ISSR markers.

MATERIALS AND METHODS

Source of plant materials. Six strawberry varieties (*Fragaria ananassa*) namely Capitola, Chandler, Diamond, Rosa, Sweet Charl and Tamar were kindly provided from Strawberry and Non-Traditional Improvement Center, Faculty of Agriculture, Ain Shams University for preserving in NGBGR.

DNA extraction. Five to six healthy *in vitro* tissue cultured plantlets of each variety were collected and used for DNA extraction. Their genomic DNA was extracted and purified using the DNeasy Plant Mini Kit (QIAGEN, Chatsworth, CA). The concentration of DNA was determined at a wavelength of 260 nm (Biophotometer, Eppendorf) and the quality verified by electrophoresis on a 0.8% agarose gel. PCR reactions were conducted using nine ISSR primers, as detailed in Table I.

The amplification reactions were carried out in 25 µL reaction volume containing 1 x PCR buffer, 1.2 mM $MgCl_2$, 0.2 mM dNTPs, 50 pmol primer, one unit of Taq DNA polymerase (ABgene) and 50 ng template DNA. Amplification was performed in a thermal cycler (MJ Research PTC-200) for total of 35 cycles after an initial

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denaturation of the template DNA at 94ºC for 4 min. This was followed by 10 cycles of 94ºC for 45 sec, touch-down one-degree decrement for annealing temperature started with 5ºC above Tm for each primer for 30 sec and 72ºC for 2 min. Then, followed by 25 cycles of 94ºC for 45 sec, last annealing temperature for 30 sec (Table I) and 72ºC for 2 min and final extension of 72ºC for 5 min. The amplification products were visualized in an ultraviolet transilluminator, following horizontal electrophoresis in 2.2% agarose gel and photographed by gel documentation system (Alpha Innotech).

Analysis of molecular data. ISSR bands were scored as present (1) or absent (0) for all strawberry varieties and were examined to estimate the relationships among the investigated varieties. The similarity matrix was estimated by pairwise comparisons of the varieties based on the percentage of common fragments. Each band was assumed to represent a unique genetic locus. Molecular results were analyzed visually and with Phoretix 1D Pro software from non-linear dynamics. A dendrogram was generated using the Un-weighted Pair-Group Method using Arithmetic Averages (UPGMA) reported by Sokal and Michener (1985).

RESULTS AND DISCUSSION

In the present study, nine ISSR primers exhibited polymorphism with the six strawberry varieties (Fig. 1) and summarized in (Table I). Nine ISSR primers detected a total of 102 amplification fragments, varying from 7 (IS-7) to 16 (IS-10) fragments per primer and ranged from 166 to 1750 base pair in size (Table II). All tested primers revealed polymorphisms among six varieties ranging from 61.5% for primer (IS-8) to 100% for primer (IS-7). The overall polymorphism for the nine primers across all six varieties was 84.3% (Table II). The estimates of the genetic similarity ranged from 45% for the most distant varieties (Tamar & Capitola) to 83% between Sweet Charl variety and with each of Diamond and Chandler varieties (Table III).

Varieties specific markers. The specific markers for strawberry varieties under the present investigation across ISSR analysis are listed in Table (IV). Forty six out of 102 ISSR fragment were found to be variety-specific markers. They represented 45% of the total fragments. These markers were scored whether they were present or absent as a unique band for a given variety. The highest number of variety-specific markers was recorded for Capitola (18 markers) and the lowest number of markers was recorded for the strawberry Rosa variety (3 markers).

Rest of the strawberry varieties showed intermediate numbers of variety specific markers. The present results indicated that ISSR technique gave adequate distinctions among all strawberry varieties under investigation. The Dice ISSR-based coefficients of the genetic similarities among the six strawberry varieties resulted in a dendrogram comprised of two clusters (Fig. 2). The first cluster included

Table I. Names, Sequences, melting temperatures (Tm) and annealing temperatures (Ta) of different primers used

Primers Name	Primer code	Sequences	Tm	Ta
17898B	IS-1	$(CA)_6GT$	43.4	49-40
17899B	IS-2	$(CA)_6GG$	46.3	51-42
17898A	IS-3	$(CA)_6AC$	43.4	49-40
814	IS-5	$(CT)_8TG$	53.7	58-49
17899A	IS-6	$(CA)_6AG$	43.4	49-40
844A	IS-7	$(CT)_8AC$	57.6	62-53
HB-8	IS-8	$(GA)_6GG$	52.61	57-48
HB12	IS-10	$(CAC)_3GC$	49.2	54-45
HB13	IS-11	$(GAG)_3GC$	49.2	54-45

Fig. 1. PCR products of genomic DNA from six strawberry varieties with nine ISSR primers. M = Marker 100 bp, 1 = Tamar, 2 = Diamond, 3 = Chandler, 4 = Sweet Charl, 5 = Capitola, 6 = Rosa

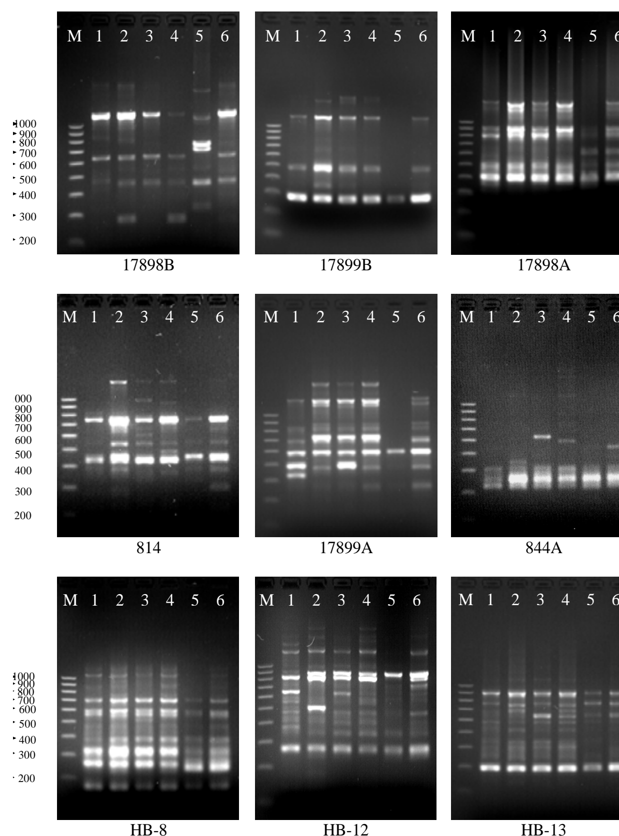

Fig. 2. Dendrogram demonstrating the relationships among the six strawberry varieties based on ISSRs

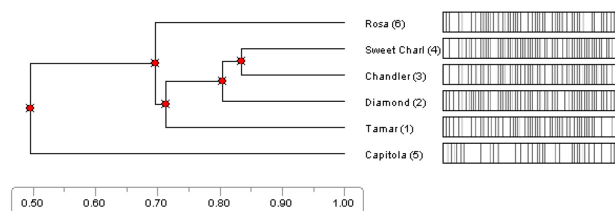

Table II. Number of amplified fragments and specific markers of six strawberry varieties based on ISSR analysis using nine primers

Primers code	TAF	PB	PB (%)	Tamar		Diamond		Chandler		Sweet Charl		Capitola		Rosa		TSM
				AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	
IS-1	12	11	91.7	5	0	6	0	3	0	4	0	7	8	4	0	8
IS-2	8	7	87.5	3	0	6	2	5	2	4	0	1	2	3	0	6
IS-3	10	8	80.0	7	3	6	0	6	0	7	0	4	2	8	0	5
IS-5	13	12	92.0	2	0	7	0	7	2	7	3	3	0	6	0	5
IS-6	12	11	91.7	6	2	10	0	8	0	9	0	2	3	8	0	5
IS-7	7	7	100	3	1	3	1	3	1	3	1	1	0	2	1	5
IS-8	13	8	61.5	10	0	9	0	9	1	11	1	6	0	7	0	2
IS-10	16	14	87.5	11	3	10	2	10	0	10	0	4	2	9	0	7
IS-11	11	8	72.7	8	0	10	0	10	0	11	0	6	1	5	2	3
Total	102	86	84.3	55	9	67	5	61	6	66	5	34	18	59	3	46

TAF= Total Amplified Fragment, PB= Polymorphic Bands, %= Percentage of polymorphism, AF = Amplified Fragment, SM = Specific Marker (including either the presence or absence of a band in specific variety), TSM = Total no of specific markers across varieties

Table III. Genetic similarity indices between each pairs of the six strawberry varieties (*Fragaria ananassa*) based on ISSR fragments analysis

Varieties	Tamar	Diamond	Chandler	Sweet Charl	Capitola
Diamond	0.69				
Chandler	0.72	0.78			
Sweet Charl	0.73	0.83	0.83		
Capitola	0.45	0.48	0.48	0.46	
Rosa	0.69	0.69	0.71	0.69	0.60

Table IV. Specific markers for six strawberry varieties using ISSR-analysis

No.	Varieties name	Specific markers	
		Present	**Absent**
1	Tamar	IS-3-809, IS-3-574, IS-6426, IS-7-327, IS-10-1343, IS-10-365	IS-3-848, IS-6-363, IS-10-335
2	Diamond	IS-2-641, IS-2-338, IS-7-327, IS-10-1567, IS-10-487	
3	Chandler	IS-2-1482, IS-2-356, IS-5-1000, IS-5-607, IS-7-627, IS-8-505	
4	Sweet Charl	IS-5-932, IS-5-367, IS-7-587, IS-8-1130	
5	Capitola	IS-1-1550, IS-1-1113, IS-1-754, IS-1-700, IS-1-542, IS-1-333,	IS-1-1155, IS-1-638, IS-2-1159, IS-2-453, IS-3-1342, IS-3-930, IS-6-1189, IS-6-822, IS-6-485, IS-10-1262, IS-10-785, IS-11-347
6	Rosa	IS-7-542	IS-11-584, IS-11-435

only Capitola variety, while the second was divided into two sub-clusters. The first one combined Rosa variety, whereas the second was subdivided into two groups. The first one comprised of varieties Sweet Charl, Chandler and Diamond, while the second included the Tamar variety.

PCR-based molecular markers are playing an increasingly important role in the analysis of genetic diversity of horticulture and field-crop species (Torres *et al*., 1993; Wolf *et al*., 1995; Debener *et al*., 1996; Swoboda & Bhalla 1997). The similarity values based on ISSR data were reported to be higher than those based on RAPD. In this regard, parallel study using RAPD and ISSR techniques showed that RAPD required the testing of six times more primers than ISSR (Korbin *et al*., 2002). It is reported that ISSR profiling is a powerful method for the identification and molecular classification of *Leucadendron* varieties (Pharmawati *et al*., 2005) and proved to be a potentially useful tool for the identification of strawberry varieties, because it is simple, fast, cost-effective, highly discriminant and reliable (Arnau *et al*., 2003). In addition, the study of the genetic relationships among 24 strawberry varieties using RAPD and ISSR showed similar results for both techniques, although similarity values based on ISSR data were higher than those based on RAPD (Anita *et al*., 2004). The present investigation clearly demonstrated that the six strawberry varieties could be distinguished by these ISSR primers. The dendrogram tree indicated that Rosa, Sweet Charl, Chandler, Diamond and Tamar varieties might have common ancestor which is different from that of the Capitola variety.

Fingerprint of the varieties. The present study revealed different pattern for each variety across the nine primers used. The all package of information gained from the primers used for each variety considered as fingerprint for it.

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