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Assessment of genetic fidelity of micropropagated clones of strawberry genotypes using EST-SSR markers

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ABSTRACT

Micropropagated plants of two commercially important genotypes of strawberry (*Fragaria x ananassa* Duch.) viz. Festival and Sweet Charlie were subjected to evaluation of genetic uniformity using EST-SSR markers. Out of fifteen primers screened, twelve primers showed amplification and the PCR profiles obtained were found to be clear, reproducible and monomorphic. The regenerated plantlets were identical based on EST-SSR analysis and true-to-type character of the tissue cultured plants was established. The results concluded that the tissue culture raised plantlets of strawberry were genetically identical and clonally uniform. Thus, a comprehensive micropropagation protocol was developed for the two genotypes of strawberry.

KEYWORDS

Micropropagation;
Strawberry;
Clonal fidelity;
Molecular markers;
EST-SSR.

INTRODUCTION

Tissue culture of important fruit crops like strawberry has emerged as an important tool for modern plant improvement programs and can aid in developing suitable cultivars in minimum time, besides micropropagation. Several studies have attested that tissue cultured plants of strawberry are more advantageous than those of conventional propagation in terms of fruit yield[12], vigor, yield per plant[17]. These plants are of high quality, disease free and true to the parents. However, for use of tissue culture as continuous source of disease free planting material for commercial utilization, periodic monitoring of the degree of genetic stability among *in vitro* grown plantlets is of outmost importance. When plant tissue is passed through *in vitro* culture many of the regenerated plantlets appear to be no longer clonal copies of their donor genotype, probably due to somaclonal variations. Although somaclonal variation may be used as a source for variation to get superior clones, it could be also a very serious problem in the plant tissue culture industry resulting in the production of undesirable plant-off types i.e. non-true to type and plants practically not identical to the mother plant[6]. Thus, clonal fidelity is one of the major concerns in commercial propagation; true to type propagules and genetic stability are prerequisites for the application of strawberry propagation *in vitro*[3].

Molecular techniques are powerful and valuable tools used in analysis of genetic fidelity of *in vitro* propa-

gated plants. Strawberries have been extensively analyzed for clone identification, mapping and diversity studies using random amplified polymorphic DNA (RAPD) markers[4,7-9], amplified fragment-length polymorphisms (AFLPs)[4,16] and the simple sequence repeats (SSRs)[2,15]. There has been a growth of interest in the development of simple sequence repeats (SSRs), for *Fragaria* that can assist in the genetic studies of the cultivated strawberry[1,10,11,14]. The majority of these SSR markers have been developed from genomic DNA libraries. Moreover, costly genomic library screening - the source of the initial wave of *Fragaria* SSRs is being superseded by expressed sequence tag (EST) database mining as a means of discovering new SSR loci[10].

Thus, the objectives of the present study were to determine of clonal fidelity of micropropagated plants of two genotypes of strawberry using EST-SSR marker. This can be a useful tool for establishing a reliable micropropagation system for the production of genetically uniform plants before they are released for large scale cultivation or other commercial purposes.

MATERIAL AND METHODS

Plant material and micropropagation

Nodal stems of 0.5-10 cm along with internodes were used as explants. The MS medium[13] supplemented with BAP (1.5 mg/l) in combination with KN (0.5 mg/l) was used for induction of shoots as well as for further shoot multiplication and elongation. Rooting was performed on IBA (1.0 mg/l). The cultures were maintained thermal insulated tissue culture room with temperature of around 25±2^{0}C and relative humidity of 60-80%. A continuous light source of about 2 K lux was provided through compact fluorescent lamps. The rooted plantlets were transferred to 1:1 mixture of sterilized sand and FYM in plastic pots and acclimatized to greenhouse conditions.

DNA isolation and PCR amplification

Modified CTAB (Cetyl Trimethyl Ammonium Bromide) method based on Doyle and Doyle[5] was used for extraction of total DNA from both tissue cultured plants and mother plants of both genotypes. Fifteen EST-SSR primers (Eurofins mwg/operon) were used for assaying genetic uniformity. PCR amplifications were carried out in a reaction volume of 10 µl containing 2µl of genomic DNA (40 to 50 ng),1µl PCR buffer,1µl $MgCl_2$, 1.5µl dNTPs,1 µl each of Forward and Reverse primer,0.2 µl Taq DNA polymerase and 2.3 µl of Nuclease free water (Fermentas, USA). The PCR conditions were programmed for initial denaturation at 94°C for 3 min followed by 35 cycles of denaturation at 93°C for 40 sec, annealing for 40 sec (temperature specific to the primer) and an extension at 72°C for 1 min with a final extension at 72°C for 30 min. The amplified products were resolved on a 2% agarose gel in TBE (Tris-borate EDTA) buffer stained with Ethidium Bromide using 50 bp ladder as reference. The gel was visualized under gel documentation system from Bio-Rad®.

RESULT AND DISCUSSION

A generally accepted concept of plant tissue culture is that all of regenerated plants will be true to the type as tissue culture is considered to be method of clonal propagation. Therefore, at least theoretically, all the individuals produced should have the similar genetic constitution to that of the parents. A valid concern arises with respect to the genetic stability of tissue cultured plants.

In the present work, the genetic fidelity of the nuclear genome of the mother plant of two genotypes of strawberry and their tissue cultured clones was determined so that the true-to-type character of micropropagated plants could be established. Fifteen EST -SSR primers were used for initial screening of the eight micropropagated progenies of each genotype with their respective mother plants. However, only 12 primers generated clear, monomorphic and reproducible bands, ranging from 150 bp to 285 bp in size (TABLE 1). The banding profiles from micropropagated plants were monomorphic and similar to those of mother plant, which indicated that there was no genetic variation in the regenerated plantlet population (Figure 1).

Thus, our study reflects the suitability of use of axillary bud proliferation method for generation of clonally uniform plants of Festival and Sweet Charlie genotypes of strawberry and provides the first report on use of EST-SSR markers for assessment of clonal fidelity of tissue cultured plants of strawberry. It can ensure large scale

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TABLE 1 : Twelve EST–SSR forward (F) and reverse (R) primer pairs, GenBank Accession nos., motifs, annealing temperature and expected size of the PCR product in bps of two cultivars of *Fragaria x ananassa* Duch.

S. No.	Locus	Gene Bank Accession no.	Primer sequences	Motif	Ta (° C)	Amplified range size (bps)	
						Festival	Sweet Charlie
1	UFFa 01D03	CO816689	F-TTACTGAAATGGGTTTCAGAGC R-GACAGCACAGTCATGGAAGATG	$(TCT)_5$	53	275-285	275-285
2	UFFa 01E03	AJ870458	F-ACCCCATCTTCTTCAAATCTCA R-GACAAGGCCAGAGCTAGAGAAG	$(CAC)_{10}$	54	200-210	150-160
3	UFFa 02C07	CO816760	F-CTCTCCCCACAAAACCCTAAAC R-AAAGATCGGTAGGCACAGAGAG	$(CT)_{20}$	54	180-190	160-170
4	UFFa 02F07	CO816783	F-GAGGCTTACCGTTCCAATCTTC R-GTTGGGATCCTCTAACATCTGG	$(CT)_{15}$	54	200-210	180-185
5	UFFa 02H04	AJ870442	F-ATCAGTCATCCTGCTAGGCACT R-TACTCTGGAACACGCAAGAGAA	$(TCG)_6$	53	250-260	220-235
6	UFFa 03B05	AJ870443	F-GGAATCCAAGTTACAGGCTTCA R-AAGGAGCCTCTCCAATAGCTTC	$(AGC)_6$, $(CAG)_5(CAA)_4$	53	220-240	180-195
7	UFFa 03C04	CO816825	F-CGGTTCAGCAGGAGAATAAAAC R-GCCCCATACTACCATTATGACC	$(GGA)_5$	53	230-240	180-192
8	UFFa 04G04	AJ870445	F-ACGAGGCCTTGTCTTCTTTGTA R-GCTCCAGCTTTATTGTCTTGCT	$(TTC)_7$	52	150-160	160-170
9	UFFa 08C11	AJ870446	F-GGACGTCCCCTTCTTTATTTCT R-ACCCCACATTCCATACCACTAC	$(TGG)_6$	53	200-210	180-190
10	UFFa 08H09	CO817234	F-CTTCACCTAATCACTTGCCTGA R-GGTCTGTTCCTTTCCTTGTTTG	$(AT)_4$, $(TC)_4$, $(TA)_7(TA)_{10}$	52	180-190	-
11	UFFa 10H04	CO817377	F-AGATCATCAGGACAGCTACGACT R-CCTTCACAAGATAGTAACCACAGC	$(GA)_6$	54	160-170	-
12	UFFa 15H09	AJ870452	F-TTAGTAGTAGACCTGCCACAAGG R-CGGCTTATCTGTAGAGCTTCAA	$(CAGAG)_6$	53	210-220	275-285

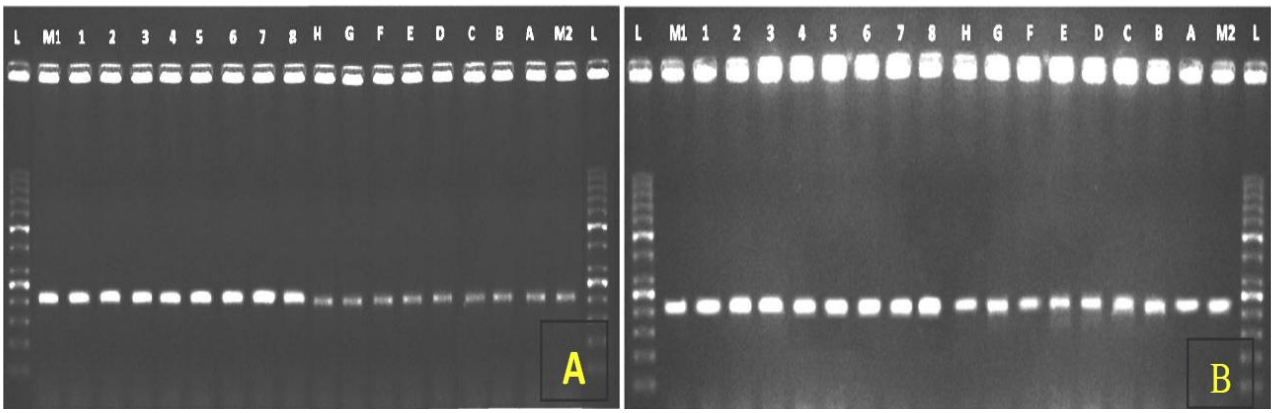

Figure 1 : DNA amplification products profile of two genotypes of strawberry (*Fragaria x ananassa* Duch.) using EST-SSR primer (A) UFFa 02C07 and (B)UFFa 02F07, lane L (50 bps)- ladder, lane M1- Mother plant of strawberry cv. Festival, (lane 1 -8) micropropagated plants of strawberry cv. Festival, lane M2- Mother plant of strawberry cv. Sweet Charlie,(lane A-H) micropropagated plants of strawberry cv. Sweet Charlie

propagation, as well as *ex situ* conservation of these commercially important cultivars of this horticultural crop and also aid in subsequent genetic manipulation studies.

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REFERENCES

[1] M.V.Ashley, J.A.Wilk, S.M.N.Styan, K.J.Craft, K.L.Jones, K.A.Feldheim, K.S.Lewers, T.L.Ashman; Theor.Appl.Genet., **107**, 1201-1207 **(2003)**.

[2] G.Cipriani, F.Pinosa, M.Bonoli, W.Faedi; J.Hortic. Sci.Biotechnol., **81**, 668-675 **(2006)**.
[3] S.C.Debnath, J.A.Teixeira da Silva; Fruit Veg.Cereal Sci.Biotechnol., **1**, 1-12 **(2007)**.
[4] C.Degani, L.J.Rowland, J.A.Saunders, S.C.Hokanson, E.L.Ogden, G.J.Galletta; Euphytica., **117**, 1-12 **(2001)**.
[5] J.J.Doyle, J.L.Doyle; Focus., **12**, 13-15 **(1990)**.
[6] R.M.Gaafar, M.M.Saker; World Journal of Agricultural Sciences, **2(1)**, 29-36 **(2006)**.
[7] M.G.García, M.Ontivero, J.C.Diaz Ricci, A.Castagnaro; Plant Breed., **121**, 76-80 **(2002)**.
[8] J.Graham, R.J.McNicol, J.W.McNicol; Theor.Appl. Genet., **93**, 402-406 **(1996)**.
[9] A.Kuras, M.Korbin, E.Zurawicz; Plant Cell Tiss. Org.Cult., **79**, 189-193 **(2004)**.
[10] K.S.Lewers, S.M.N.Styan, S.C.Hokanson, N.V.Bassil; J.Am.Soc.Hortic.Sci., **130**, 102-115 **(2005)**.
[11] A.Monfort, S.Vilanova, T.M.Davis; Mol.Ecol. Notes, **(2005)**.
[12] P.P.Moore, J.A.Robbins, T.M.Sjulin; HortScience., **26**, 192-194 **(1991)**.
[13] T.Murashige, F.Skoog; Physiologia Plantarum., **15**, 473-497 **(1962)**.
[14] D.J.Sargent, A.M.Hadonou, D.W.Simpson; Mol.Ecol.Notes., **3**, 550-552 **(2003)**.
[15] D.J.Sargent, J.Clarke, D.W.Simpson, K.R.Tobutt, A.Monfort, K.M.Folta; Theor.Appl.Genet., **112**, 1349-1359 **(2006)**.
[16] M.Tyrka, P.Dziadczyk, J.A.Hortynski; Euphytica., **125**, 273-280 **(2002)**.
[17] J.I.Zebrowska, J.Czernas, J.Gawronski, J.A.Hortynski; Food, Agriculture and Environment, **1**, 190-193 **(2003)**.