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Standardization and validation of EST-SSR markers on peach (*Prunus persica* L)

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ABSTRACT

In present study among different concentrations of PCR reaction mixture components used, 1X buffer A (1.5 mM $MgCl_2$), 0.7 mM $MgCl_2$, 1 mM dNTP, I µM primer (each Forward and Reverse), 1 U taq DNA polymerase and 30 ng DNA gave best results and were further used to validate 43 EST-SSRs among six genotypes of peach of which 38 primers produced amplification consisting of 18 monomorphic and 20 polymorphic primers. Total number of amplified bands was 75 out of which 51 were polymorphic representing 68.00 per cent polymorphism. High level of polymorphism revealed using EST-SSRs confirms their promising use in molecular studies.

Keywords: EST-SSRs; peach; standardization; polymorphism

INTRODUCTION

Peach is the model fruit crop for molecular biology work as it is genetically most characterized fruit crop and has small genome size (4230 Mb) and also due to availability of genomic resources. In today's scenario molecular markers hold a very important position because of their use in widespread areas like assessment of genetic diversity, hybridity testing, gene tagging/QTL identification, DNA fingerprinting, marker assisted selection etc. Among various molecular markers used simple sequence repeats (SSRs) are of immense importance due to their hypervariability, polymorphism rate, co-dominance nature and cross-transferability (Kaur et al 2015, Vaidya et al 2015). But in the genus *Prunus* there is lack of a common standardized set of SSRs that can be used by various research groups for different studies. Thus it is necessary to develop standardized protocols for the efficient use of EST-SSRs in various studies.

MATERIAL and METHODS

Experimental material

DNA isolation was done from leaf samples of six peach genotypes procured from Department of Fruit Science, Dr YS

Parmar University of Horticulture and Forestry, Nauni, Solan (Table 1). Leaves were stored at -80°C until further use.

DNA isolation and PCR standardization

To carry out DNA isolation method given by Doyle and Doyle (1987) was used. Quality of DNA was assessed by running DNA on 0.8 per cent agarose gel supplemented with ethidium bromide and quantity was accessed by running it on 0.8 per cent agarose gel alongwith λ (lambda) DNA marker of known concentration.

Concentrations of taq buffer A, MgCl$_{2,}$ dNTP and taq DNA polymerase were kept constant viz 1X, 2.2 mM, 1 mM and IU respectively. Primer concentrations (Forward and Reverse) from 0.1-1 µM and DNA of 30 and 40 nanomoles were tried (Table 2). PCR profile followed was initial denaturation at 95°C for 5 minutes, denaturation at 94°C for 1 minute, annealing at Tm of primer for 1 minute, extension at 72°C for 2 minutes followed by 40 cycles repeat and final extension at 72°C for 5 minutes. After standardization the best combination was chosen and used to validate 43 EST-SSRs on six genotypes of peach.

RESULTS

PCR standardization

Out of ten sets tried set 3 gave best results producing more number of bands without smears and dimmers (Fig 1). This set consisted of 1X buffer A (1.5 mM MgCl$_2$), 0.7 mM MgCl$_2$, 1 mM dNTP, I µM primer (each Forward and Reverse), 1 U taq DNA polymerase, 30 ng DNA and autoclaved distilled water to make up final

Table 1. List of peach genotypes used in the study

Genotype	Parentage	Genotype	Parentage
July Elberta	Unknown origin	Suncrest	Cross of Alamar x Gold Dust
Early Red haven	Bud mutant	Florida Grande	Unknown origin
Early Elberta	Unknown origin	Pratap	Unknown origin

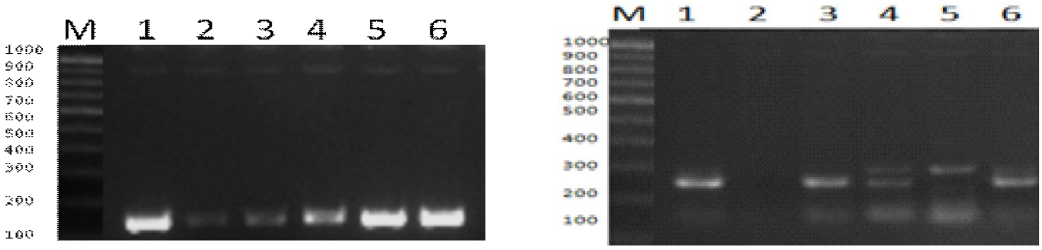

Fig 1. **Validation of EST-SSR markers on six genotypes of peach using gi|209180357| and gi|209180377| primers, (1-6 numbers have been decoded in Table 1), M= 100 bp marker**

Table 2. Different concentrations of PCR components used for standardization

Component		Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7	Set 8	Set 9	Set 10
10 X buffer A	µl	2	2	2	2	2	2	2	2	2	2
$MgCl_2$ (0.7 mm)	µl	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56
dNTP (1 Mm)	µl	2	2	2	2	2	2	2	2	2	2
Primer-Forward	µl	0.1	0.1	0.2	0.2	0.4	0.4	0.6	0.6	1	1
	µM	0.5	0.5	1	1	2	2	3	3	5	5
Primer-Reverse	µl	0.1	0.1	0.2	0.2	0.4	0.4	0.6	0.6	1	1
	µM	0.5	0.5	1	1	2	2	3	3	5	5
taq DNA polymerase (1 U)	µl	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
DNA (30-40 ng)	µl	3	4	3	4	3	4	3	4	3	4
Autoclave distiller water	µl	11.94	10.94	11.74	10.74	11.34	10.34	10.94	9.94	10.14	9.14

volume of 20 µl (Table 2). The bands produced by this set were of high resolution. This was because of required optimum concentration of each PCR reaction mixture component which is of utmost importance.

Validation and polymorphism study

Of 43 EST-SSR primers 38 produced amplification consisting of 18 monomorphic and 20 polymorphic primers (Table 3). Total number of amplified bands was 75 out of which 51 were polymorphic representing 68.00 per cent polymorphism and remaining 24 were monomorphic. Average number of polymorphic bands per primer was recorded to be 2.55. Average number of amplified fragments per accession was 43.33 and per informative primer was 6.84.

DISCUSSION

EST-SSR markers are laborious to work with. Hence standardization is must to obtain good results. In present study all possible combinations of different PCR reaction mixture components were tried and from these the best was used to validate EST-SSR primers among peach germplasm. It was found that primer and DNA concentration hold the most important position. When the concentrations of these two components varied keeping other components constant, variation in results was observed. High level of polymorphism (68%) was revealed when the EST-SSR primers were validated on peach

Table 3. List of polymorphic EST-SSR primers used for validation among peach genotypes

Sequence ID	Primer sequence (5”!3’)	Tm (^{o}C)	GC (%)	Sequence ID	Primer sequence (5”!3’)	Tm (^{o}C)	GC (%)
gi|209180377|	F: AGATGGCTGAAGAAGCCAAA	59.96	45.00	gi|209180320|	F: GACGCCCATAGTTCTTCACC	59.56	55.00
	R: CTCACAACATGCTGAGTACACG	59.43	45.00		R: GGGCTCGGATCTTTGTTGTA	60.07	50.00
gi|209180174|	F: GAGTGGTGATGATGGCTTTG	59.09	50.00	gi|282845644|	F: CGCCTTCGACTTTCTTCTTC	59.19	50.00
	R: TTTATACGAGTGGCGGTGCT	60.66	50.00		R: ACTAGGCATTTGGCCATCAG	60.10	50.00
gi|209179911|	F: CGACACAATTCAGCAAGCAT	59.87	45.00	gi|209180112|	F: CGAGAGGGCCATTCAGATTA	60.17	50.00
	R: GACGGGGGAGGAAAAAGTAG	59.93	55.00		R: ATCAAATTTCACCCCTGCTG	59.93	45.00
gi|209179307|	F: ATCGGTGGTGAGATTGAAGG	59.93	50.00	gi|282845600|	F: ACGGGGTTCTAGCAGCACTA	59.90	55.00
	R: CATGCCAAAAACTGAAAGCA	59.85	40.00		R: AAATTTGCTGGGTCAGTTGG	59.97	45.00
gi|209180051|	F: CTTCCCCTTCTCGTTTCCTC	60.18	55.00	gi|209179890|	F: ACTCGTGCTCACAACTCACG	60.10	55.00
	R: GAGAAATGGGTGTGCTTGGT	59.97	50.00		R: GTAGGGGTTGGAGTCCTCGT	60.37	60.00
gi |209179708|	F: TGCTCTTTGGTCGTCTCCTT	59.99	50.00	gi|282844913|	F: GAACCAAGGTGAGGACTGGA	60.09	55.00
	R: CCTCCTGGTCATGGAAGAAA	60.04	50.00		R: TTCGACGCTCCAGTTTCTTT	59.99	45.00
gi |209180380|	F: CCAAGCTGATGGAGGAAGAC	59.80	55.00	gi|209179588|	F: ACTCTTCCTCTTCCCCCAAA	60.04	50.00
	R: GTTACCCATCGTGGCTGACT	60.00	55.00		R: CCAGCATCAACTGCAAAAGA	59.99	45.00
gi |209180366|	F: GAACTTGCTCTGGCATTTCC	59.82	50.00	gi|209179588|	F: GGCTTGCAGATCCTGAGTTC	59.96	55.00
	R: TGGATGAGCTTCGTCTTGTG	59.98	50.00		R: GCCATTGACTCAGCCAAACT	60.26	50.00
gi |282845836|	F: CGAACAGGGTATGCAAAAGG	60.49	50.00	gi|209179472|	F: CAACCCATGGAATTTGCTTT	59.80	40.00
	R: TTGTCAGGTGGAGGAGGAAC	60.09	55.00		R: GCCCTGTTCTTTTCCTCTCC	60.19	55.00
gi |282845819|	F: TCAAACAGGGTATGCAAAAGG	59.98	42.86	gi|209179290|	F: TGTTTCTGGGAGGAGGAGAA	59.77	50.00
	R: TTGTCAGGTGGAGGAGGAAC	60.09	55.00		R: TTCCCACCCTTTATCCACAA	60.16	45.00

germplasm indicating high variability among the germplasm studied. Mnejja et al (2010) and Wunsch (2009) also obtained high polymorphism level of 76.4 and 72.2 per cent in *Prunus*. Some other findings also supported the results of higher level of polymorphism such as 70 per cent in wheat (Li et al 2008), 63.7 per cent in rose (Park et al 2010) and 92 per cent in strawberry (Zorrilla-Fontanesi et al 2010) using EST-SSRs and proving that ESR-SSRs are valuable markers in transcribed regions of the genome. These markers can further be used to carry out QTL/gene tagging, DNA fingerprinting and marker assisted selection studies.

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