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DNA fingerprinting of peach (*Prunus persica*) germplasm in accessing genetic variation using arbitrary oligonucleotide markers system

Parul Sharma and Rajnish Sharma*

Department of Biotechnology, Dr Y S Parmar University of Horticulture & Forestry, Nauni, Solan (HP) 173230, India

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Molecular characterization of 45 peach (*Prunus persica*) accessions was carried out using 48 RAPD and 46 ISSR molecular markers to assess the value and magnitude of genetic divergence. The RAPD primers revealed 84.20% polymorphism and ISSR markers generated 89.00% polymorphism. Pooled RAPD and ISSR along with UPGMA clustering based on Jaccard's coefficient were estimated with a view to assess efficiency of the marker system in *Prunus persica.* Polymorphic information conten (PIC) values varied from 0.13 to 0.50 in RAPD and 0.12 to 0.49 in ISSR with the mean values for all loci were 0.33 and 0.35, respectively. Jaccard's similarity coefficient among peach accessions with respect to RAPD and ISSR markers ranged from 0.37 to 0.95 and 0.43 to 0.95 which indicated a broad genetic base. Pooled analysis of both molecular markers concluded that genotypes 'Darli' and 'IC-2' are most distantly related to each other. The use of arbitrary oligonucleotide primers in the amplification reaction facilitated the study of uncharacterized genomes. In the present study, high level of polymorphism indicates their applicability in framing more extensive studies in development of superior progenies, quantitative trait loci (QTL) mapping, molecular breeding, investigation of population genetic diversity, comparative mapping, selection of the parents etc. among various peach crop improvement programmes.

Key words: Genetic diversity, peach, *Prunus persica*, RAPD, ISSR, molecular markers

Introduction

Peach (*Prunus persica*) belongs to family *Rosaceae*, is one of the important stone fruits and is also considered to be one of the most favourable crops for farmers worldwide. Peach is a self-fertile and naturally self-pollinating fruit species with very low genetic variability[1]. Peach breeding is usually time consuming, especially for fruit-specific characters and therefore, molecular markers linked to these traits are of great importance for the identification and selection of plant genotypes with the aspiration characters long before the traits are expressed. The International Peach Genome Initiative (IPGI) has released the early online access to the draft assembled and annotated peach genome sequence. Introduction of DNA-based markers has provided a large number of markers independent of environmental influences and are suitable for genetic typing at very early stages of development. RAPD and ISSR markers have a number of advantages for use in the detection of genetic variation such as technical simplicity, rapidity of assay, minimal DNA requirements, and low assay cost. In addition, no prior knowledge about the sequence under investigation is required[2]. Germplasm characterization is an important operation for a gene bank as it provides reference collection for genetic stability of genotypes, helps in authentication and identification of cultivars and varieties, for identification of potential donors with desirable traits, author's property rights protection, selection of parents suitable for creating of mapping population and for discriminating cultivated and wild genotypes. The role of RAPD and ISSR markers in determination of genetic diversity, thus, initiated our investigations to study genetic variation among different peach genotypes for future breeding. Considering these facts, our objective was to provide markers that can identify all prominent cultivars, test their efficiency in discriminating closely related cultivars, and evaluate future applications for maintenance of peach germplasm.

*Author for correspondence:
Tel: 01792-252310; Fax: 01792-252844
rajnish.sharma@yahoo.co.in

Materials and Methods

DNA Isolations

For molecular analysis, total 45 different accessions of peach germplasm (Table 1) were obtained from NBPGR Regional Station, Phagli, Shimla (H.P.).

Table 1 — List of peach germplasm used in molecular characterization studies

S.No.	Name of genotype	Origin
1	Darli	India (HP)
2	Ambri	India (HP)
3	Shan-e-Punjab	India (Punjab)
4	Nainital	India (UK)
5	LP/KBS/04-47 Nilgiris	India (TN)
6	SNS	India (HP)
7	IC1	India (HP)
8	IC2	India (UKD)
9	Independent	India (HP)
10	UKD	India (UKD)
11	RSSML17	India (HP)
12	Semi Wild Peach	India (Sikkim)
13	RSSML18	India (HP)
14	S-37	India (HP)
15	KP/8/44	Kullu (HP)
16	Silver King	India (HP)
17	Japan Peach	Japan
18	Nishiki	Japan
19	Nemaguard	USA
20	Fertilia	USA
21	Sun Red	USA
22	Floridason	USA
23	Summer Glo	USA
24	Sun Coast	USA
25	Burbank July Elberta	USA
26	Nunomwase	Korea
27	Yum-Yong	Korea
28	Sone Peach	Italy
29	Diaired	Bulgaria
30	Luna	Czechoslovakia
31	Red Gold	South Africa
32	Kanto-5	Japan
33	Candor	USA
34	Co-Smith	USA
35	Duke	USA
36	May Fire	USA
37	Snow Queen	USA
38	Flora Bella	USA
39	Early Red Fre	USA
40	Early Elberta	USA
41	Fire Prince	USA
42	Early Amber	USA
43	Okubu	Korea
44	Early Red Heaven	Italy
45	Alton Peach	USA

DNA was isolated and purified from young leaves of individual genotype using CTAB method[3] with some modifications. Quality and quantity of DNA preparations were checked by standard spectrophotometer and the samples were diluted to 50 ng DNA/µl concentration.

PCR Conditions

PCR was carried out in a 15 µl reaction volume containing *Taq* DNA polymerase (3 U/reaction), *Taq* DNA polymerase buffer (1X) with 1.5 mM $MgCl_2$, random decamer primers (10 pmol/reaction), deoxynucleotide triphosphate (dNTPs) (25 mM) of Genei, Bangalore, India and template DNA (50 ng/reaction). A total of 48 RAPD and 46 ISSR primers were employed to characterize 45 genotypes of *Prunus persica* at their respective annealing temperatures (Table 1 & 2) using a thermal cycler (Applied Biosystems, USA) programmed to initial cycle of 4 min at 95°C followed by 38 cycles of 1 min at 94°C, annealing temperature depending upon T_m value of primer for 1 min, elongation step of 2 min at 72°C, and a final extension step of 8 min at 72°C followed by a 4°C soak until recovery. Products were analysed by electrophoresis on different agarose (GeNei, Bangalore, India) concentrations i.e., 1.4% for RAPD and 2.0% for ISSR in 1X TAE buffer containing ethidium bromide (10 mg/ml) respectively and images were taken through gel documentation unit (Syngene, UK). The size of the amplified product was determined by co-electrophoresis 100 bp standard molecular weight markers (GeNei, Bangalore, India). Each of the reactions was carried out twice to establish the reproducibility of results. Only those primers which produced bands with all the samples were used to score for polymorphism.

Data Analysis

Two different softwares NTSYS-PC ver. 2.02i and DARwin5 ver. 5.0.158 were used to analyze the data after compiling the observations of bands on gel images of different primers and genotypes in molecular marker studies. The data on band position on agarose gel was recorded by assigning '0' for the absence of band and '1' for presence of band. The similarity matrix generated using Jaccard coefficient was used for unweighted pair group method on arithmetic-average (UPGMA) using software package NTSYS-PC ver.2.02i (Rohlf, 1998). The output data was graphically represented as dendrogram and neighbour-joining tree analysis using NTSYS-PC ver.2.02i[4] and DARwin software along with bootstrap values on the branches, respectively.

Polymorphic Information Content (PIC) Value

Marker index for respective molecular marker was calculated in order to characterize the capacity of each primer to detect polymorphic loci among the genotypes. It is the sum total of the polymorphism

Table 2 — Primer sequences, annealing temperature, size of amplicons, number of amplified bands, percent polymorphism and polymorphic information content values of RAPD markers studied in peach genotypes

S.No.	Primer	Sequence (5'-3')	Tm (°C)	Size of amplicons (bp)	No. of amplified bands	Polymorphism (%)	PIC
1	OPA-01	CAGGCCCTTC	35	250-1000	5	40	0.26
2	OPA-04	AATCGGGCTG	35	200-1300	7	42.85	0.22
3	OPA-05	AGGGGTCTTG	30	100-1000	8	75	0.37
4	OPA-08	GTGACGTAGG	35	200-1200	6	50	0.2
5	OPA-11	CAATCGCCGT	35	200-110	5	80	0.47
6	OPA-13	CAGCACCCAC	30	250-1100	5	50	0.36
7	OPA-18	AGGTGACCGT	30	300-1200	8	75	0.19
8	OPA-20	GTTGCGATCC	32	100-1100	7	71.42	0.39
9	OPB-01	GTTTCGCTCC	32	200-1200	9	55.55	0.38
10	OPB-02	TGATCCCTGG	32	100-1000	10	60	0.34
11	OPB-07	GGTGACGCAG	35	200-1150	8	62.5	0.49
12	OPB-12	CCTTGACGCA	35	150-1200	11	72.72	0.45
13	OPB-17	AGGGAACGAG	32	200-1200	8	62.5	0.47
14	OPB-18	CCACAGCAGT	30	150-1150	10	100	0.5
15	OPC-01	TTCGAGCCAG	32	250-1200	8	50	0.42
16	OPC-20	ACTTCGCCAC	30	200-900	9	33.33	0.22
17	OPC-08	TGGACCGGTG	32	200-1100	7	57.14	0.43
18	OPD-04	TCTGGTGAGG	32	100-1000	6	66.67	0.29
19	OPD-05	TGAGCGGACA	30	200-1000	4	50	0.27
20	OPD-12	CACCGTATCC	32	150-1100	8	87.5	0.47
21	OPE-03	CCAGATGCAC	32	200-1200	5	80	0.39
22	OPE-07	AGATGCAGCC	30	100-1200	5	60	0.34
23	OPE-14	TGCGGCTGAG	30	250-1000	6	50	0.33
24	OPE-15	ACGCACAACC	30	150-1100	5	40	0.19
25	OPF-04	GGTGATCAGG	32	250-1000	4	50	0.18
26	OPF-05	CCGAATTCCC	32	200-1200	5	80	0.13
27	OPG-01	CTACGGAGG	30	350-1350	5	100	0.45
28	OPG-04	AGCGTGTCTG	32	200-1200	11	100	0.43
29	OPH-01	GGTCGGAGAA	32	150-1200	8	50	0.32
30	OPH-17	CACTCTCCTC	32	100-1600	10	70	0.4
31	OPL-12	GGGCGGTACT	32	100-1000	9	44.44	0.31
32	OPL-18	ACCACCCACC	32	150-900	8	62.5	0.25
33	OPP-05	CCCCGGTAAC	32	200-1100	8	50	0.27
34	OPP-12	AAGGGCGAGT	30	250-1000	8	62.5	0.46
35	OPU-01	ACGGACGTCA	32	150-1200	7	57.14	0.27
36	OPY-07	AGAGCCGTCA	30	200-1100	6	50	0.3
37	OPY-16	GGGCCAATG	30	200-1200	9	66.66	0.38
		Mean			7.24	62.57	0.35

information content (PIC) values of all the markers produced by a particular primer. PIC value was calculated using the formula (Nie *et al*, 1983)[5];

$PIC = 1-\Sigma pi^2$,

Where, pi is the frequency of the i^{th} allele.

Results and Discussion

RAPD Studies

Of the 48 random RAPD primers used, only 37 were able to amplify the genomic DNA (Table 2). These 11 primers failed to amplify the genomic DNA uniformly and were not included into further analysis. All random primers were found polymorphic. For a total of 37 primers, the number of bands varied from 4 with OPF-05 & OPD-05 to 11 with primers OPG-04 & OPB-12 (Fig. 1), respectively with amplicon size ranging from 100-1600 bp (approx.) for all the informative primers. A total of 268 bands were amplified, of which 62.57% were polymorphic across all the subjected genotypes. On an average, total number of bands generated per primer was 7.24. The RAPD primer OPB10 showed the highest (84.20%) polymorphism, while the RAPD primer OPO-04 showed the lowest (11.10%) polymorphism among different peach cultivars (Nagaty *et al,* 2011)[6]. The average number of alleles in all loci ranged from 0.20 to 0.80 with a mean of 3.40 among the 12 peach genotypes (Bakht *et al,* 2013)[7].

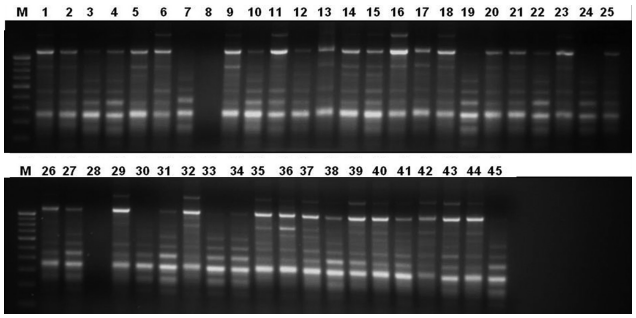

Fig. 1 — DNA banding profiles obtained using OPB-12 RAPD primer (1 - 45: Peach genotypes and M: 100 bp ladder).

The PIC value provides an estimate of the discriminatory power of a locus or loci, by taking into account not only the number of alleles that are expressed, but also relative frequencies of these alleles. Referring to PIC value recorded for all the informative RAPD primers, the PIC vary from a minimum of 0.13 for OPF-05 followed by 0.18 for OPF-04 and maximum of 0.50 for OPB-18 with an average of 0.35 (Table 2). The highest percent polymorphism was found to be 100% (OPB-18, OPG-01, OPG-04). Dendrogram grouped the subjected genotypes among three major clusters A, B and C with Jaccard coefficient ranged from 0.37 to 0.95. 'IC2' and 'Luna' genotypes were found to be highly diversified (0.42) with highest similarity coefficient for 'RSSML-17' and 'RSSML-18' (0.95). Therefore, RAPD fingerprinting confirmed certain molecular markers that might be associated with certain commercial characteristics. Our results holds good with the findings of Erturk *et al* (2009)[8] on the characterization of peach cultivars by using RAPD markers which allows interpreted information for future studies on the appropriate use of these cultivars in breeding programs, biodiversity assessment and better conservation of germplasm resources. The observed results are being subjected to SSR markers for their further characterization and respective interpretation with respect to these results is under progress. Many other workers reported the use of RAPD markers for molecular genetic evaluation in peach germplasm (Baranek *et al,* 2006; Bakht *et al,* 2012, 2013)[9-10].

ISSR Studies

Of the total 46 ISSR primers used, only 38 were able to amplify the genomic DNA (Table 3). Eight primers failed to amplify the genomic DNA uniformly and were not included into further analysis. All random primers were found polymorphic. For a total of 46 primers, the number of bands varied from 3 with UBC-816 to 10 with primer ISSR-826, respectively with amplicon size ranging from 100-1100 bp (approx.) for all the informative primers. A total of 249 bands were amplified, of which 60.53% were polymorphic across all the subjected genotypes. The highest percent polymorphism was found to be 100% using ISSR-857, ISSR-826 and ISSR-827 (Fig. 2), respectively. On an average, total number of bands generated per primer was 6.57. Similarily in *Prunus* genotypes, the number of bands per primer ranged from 5 to 17 with the average number of bands per primer being 9.8. Total 180 fragments were polymorphic, with an average of 89% polymorphism. The average number of polymorphic bands per primer was 9.0 (Yilmaz *et al*, 2009)[11].

Referring to PIC value recorded for all the informative ISSR primers, the PIC vary from a minimum of 0.12 for UBC-834 followed by 0.14 for ISSR-880 and maximum of 0.49 for ISSR-827 with an average of 0.33 (Table 3). Our results with respect to PIC values are consistent with the findings of Noormohammadi *et al* (2012)[12] in pomegranate with PIC values ranged from 0.27 for UBC-834 to 0.49 for UBC-811. The highest percent polymorphism was found to be 100% (ISSR-826, ISSR-827 and ISSR-857). Dendrogram grouped the subjected genotypes among three major clusters A, B and C with Jaccard coefficient ranged from 0.43 to 0.95. genotypes 'IC2', 'Luna' and 'Shan-e-Punjab' were found highly diversified (0.46) with highest similarity coefficient for 'Darli' and 'Independent' (0.95). Many other workers reported the use of ISSR markers for molecular genetic evaluation in peach germplasm (Li *et al* 2013, Fathi *et al,* 2013)[13-14].

Pooled Analysis of RAPD and ISSR Studies

For pooled RAPD and ISSR studies (Fig. 3), the similarity coefficient was as low as 0.41 to as high as 0.88 with a mean value of 0.64 indicated substantial diversity present in the germplasm. Maximum similarity coefficient 0.88 was observed between 'Darli' and 'Independent' while minimum 0.41 was observed in 'IC-2' and rest of the genotypes. While Khajuria *et al* (2012)[15] reported a pooled range of 0.10 to 0.80 with a mean value of 0.45 in apple genotypes. Further, the cluster tree analysis obtained after pooled RAPD and ISSR analysis showed that 45 *P. persica* genotypes were grouped into two major clusters viz; cluster A and B comprising of 40 and 4 genotypes, respectively (Fig. 3) while a

Table 3 — Primer sequences, annealing temperature, size of amplicons, number of amplified bands, percent polymorphism and polymorphic information content values of ISSR markers studied in peach genotypes

S.No.	Primer	Sequence (5'-3')	Repeat motifs	Tm (°C)	Size of amplicons (bp)	No. of amplified bands	Polymorphism (%)	PIC
1	ISSR-808	AGAGAGAGAGAGAGAGC	$(AG)_8C$	48	100-750	07	42.83	0.27
2	ISSR-810	GAGAGAGAGAGAGAGAT	$(GA)_8T$	46	175-900	10	60.00	0.30
3	ISSR-811	CACCACACACACACAAT	$(GA)_8C$	48	150-850	06	66.67	0.24
4	ISSR-814	CTCTCTCTCTCTCTCTTG	$(CT)_8TG$	52	100-800	06	33.33	0.19
5	ISSR-815	CTCTCTCTCTCTCTCTGT	$(CT)_8GT$	52	150-850	05	60.00	0.39
6	ISSR-818	CACACACACACACAC	$(CA)_8G$	50	200-900	07	57.14	0.40
7	ISSR-819	GTGTGTGTGTGTGTGT	$(GT)_8A$	48	150-750	05	80.00	0.33
8	ISSR-822	TCTCTCTCTCTCTCTAC	$(TC)_7TAC$	48	200-800	06	66.67	0.30
9	ISSR-823	TCTCTCTCTCTCTCTCC	$(TC)_8C$	50	200-1100	06	33.33	0.40
10	ISSR-824	TCTCTCTCTCTCTCTCG	$(TC)_8G$	50	150-800	08	50.00	0.32
11	ISSR-825	ACACACACACACACACT	$(AC)_8T$	48	100-750	07	37.50	0.36
12	ISSR-826	ACACACACACACACACC	$(AC)_8C$	50	100-900	10	100.00	0.47
13	ISSR-827	ACACACACACACACACGG	$(AC)_8GG$	52	150-850	09	100.00	0.49
14	ISSR-830	TGTGTGTGTGTGTGTGG	$(TG)_8G$	50	200-800	06	50.00	0.31
15	ISSR-840	GAGAGAGAGAGAGAGAGT	$(GA)_8GT$	52	175-750	07	71.42	0.44
16	ISSR-842	GAGAGAGAGAGACCCGGG	$(GA)_8GG$	58	150-800	07	85.71	0.46
17	ISSR-847	CACACACACACACACAGC	$(CA)_8GC$	52	150-900	08	75.00	0.37
18	ISSR-849	GTGTGTGTGTGTGTGAA	$(GT)_7GAA$	48	200-700	06	33.33	0.38
19	ISSR-851	GTGTGTGTGTGTGTGTCG	$(GT)_8CG$	52	250-750	04	50.00	0.26
20	ISSR-856	ACACACACACACACACG	$(AC)_8G$	50	100-800	06	66.67	0.40
21	ISSR-857	ACACACACACACACACGGTC	$(AC)_8GGTC$	56	175-700	05	100.00	0.36
22	ISSR-860	TGTGTGTGTGTGTGTGCA	$(TG)_8CA$	52	150-900	07	42.85	0.18
23	ISSR-864	ATGATGATGATGATGTG	$(ATG)_5TG$	45	150-800	05	60.00	0.36
24	ISSR-873	GACAGACAGACAGACA	$(GACA)_4$	48	100-750	06	50.00	0.42
25	ISSR-880	GGAGAGGAGAGGAGAGT	$(GGAGA)_3T$	52	225-650	07	42.85	0.14
26	UBC-807	AGAGAGAGAGAGAGAGT	$(AG)_8T$	50	200-800	07	71.42	0.38
27	UBC-809	AGAGAGAGAGAGAGAGG	$(AG)_8G$	52	150-600	05	60.00	0.41
28	UBC-812	GAGAGAGAGAGAGAGAA	$(GA)_8A$	50	150-750	06	50.00	0.30
29	UBC-816	CACACACACACACACAT	$(CA)_8T$	52	300-700	03	33.33	0.32
30	UBC-817	CACACACACACACACAA	$(CA)_8A$	50	175-850	07	71.42	0.44
31	UBC-834	AGAGAGAGAGAGAGAGTT	$(AG)_8TT$	50	200-750	05	40.00	0.12
32	UBC-835	AGAGAGAGAGAGAGAGCC	$(AG)_8CC$	54	150-900	08	75.00	0.42
33	UBC-836	AGAGAGAGAGAGAGAGTA	$(AG)_8YA$	50	175-850	07	57.14	0.30
34	UBC-841	GAGAGAGAGAGAGAGACC	$(GA)_8CC$	54	150-900	06	66.67	0.23
35	UBC-844	CTCTCTCTCTCTCTCTCGC	$(CT)_8RC$	56	200-950	05	80.00	0.25
36	UBC-855	CTCTCTCTCTCTCTCTGG	$(CT)_8GG$	54	150-850	07	85.71	0.36
37	UBC-868	GAAGAAGAAGAAGAAGAA	$(GAA)_6$	52	175-850	09	44.44	0.35
38	UBC-874	CCCTCCCTCCCTCCCT	$(CCCT)_4$	56	100-800	08	50.00	0.23
Mean						6.57	60.53	0.33

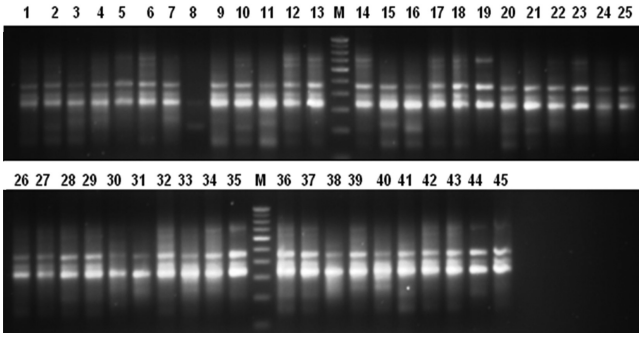

Fig. 2 — DNA banding profiles obtained using ISSR-827 primer (1-45: Peach genotypes and M: 100 bp ladder).

single genotype 'IC-2' was remained ungrouped. In cluster A, 'Darli' and 'Florabella' were found more distantly related whereas 'Darli' and 'Independent' were more closely related. In cluster B, 'IC1' and 'Luna' were more distantly related whereas 'Sun Coast' and 'Candor' were more closely related to each other. Our results further strengthened previous findings of Bhattacharya *et al* (2010)[16] on *Cymbopogon winterianus*, and Parveen *et al* (2013)[17] Karnal bunt of on wheat pathogen with the similarity coefficients ranging from 0.83 - 0.98 and 0.61 - 0.96, respectively. This DNA based data can

reliably be used for studying phylogenetic relationship among various accessions of a species based on geographic origin. It is concluded from the pooled analysis of both molecular markers that genotypes 'Darli' and 'IC-2' are most distantly related to each other. Similar kind of studies has already been taken into consideration in our laboratory for characterizing *Capsicum* germplasm, (Rana *et al* 2014)[18]. Hence, it is recommended that these two genotypes should be crossed to create a segregating population with maximum genetic diversity. Further, it is suggested that the subjected molecular markers taken in the present study are valid tags for the assessment of genetic diversity in *P. persica* germplasm.

The neighbor-joining cluster analysis with boot strap support values of 45 genotypes of peach obtained after pooled molecular marker analysis revealed high diversity among each fruit crop (Fig. 4). These genotypes were grouped into three major groups while, a single genotype 'IC-2' was remained ungrouped. Similarly, same genotype was found to be ungrouped during cluster analysis. Individuals grouped under major cluster B obtained using NTSYS software consisting of 'IC-1', 'Luna', 'Sun Coast' and 'Candor' were also found in same group obtained during NJ cluster analysis. This clearly revealed the precise genetic analysis of genotypes taken into consideration in the present study. Similar results were reported by Coart *et al* (2003)[19] in which bootstrap support value clustered the apple genotypes in five major groups with value ranging from 77% to 100%, separating ornamental and edible cultivars. Further NJ tree generated using UPGMA by Bhatt *et al* (2013)[20] clustered pear genotypes into two main groups I and II having 5 and 6 genotypes, respectively. Our result also holds well with Bao *et al* (2007)[21] and Bassil *et al* (2009)[22] in apple and pear, respectively. Hence, the obtained results confirmed the potential of RAPD and ISSR technology as a reliable, rapid and inexpensive screening method to discriminate subjected genotypes of peach fruit crop in the present study.

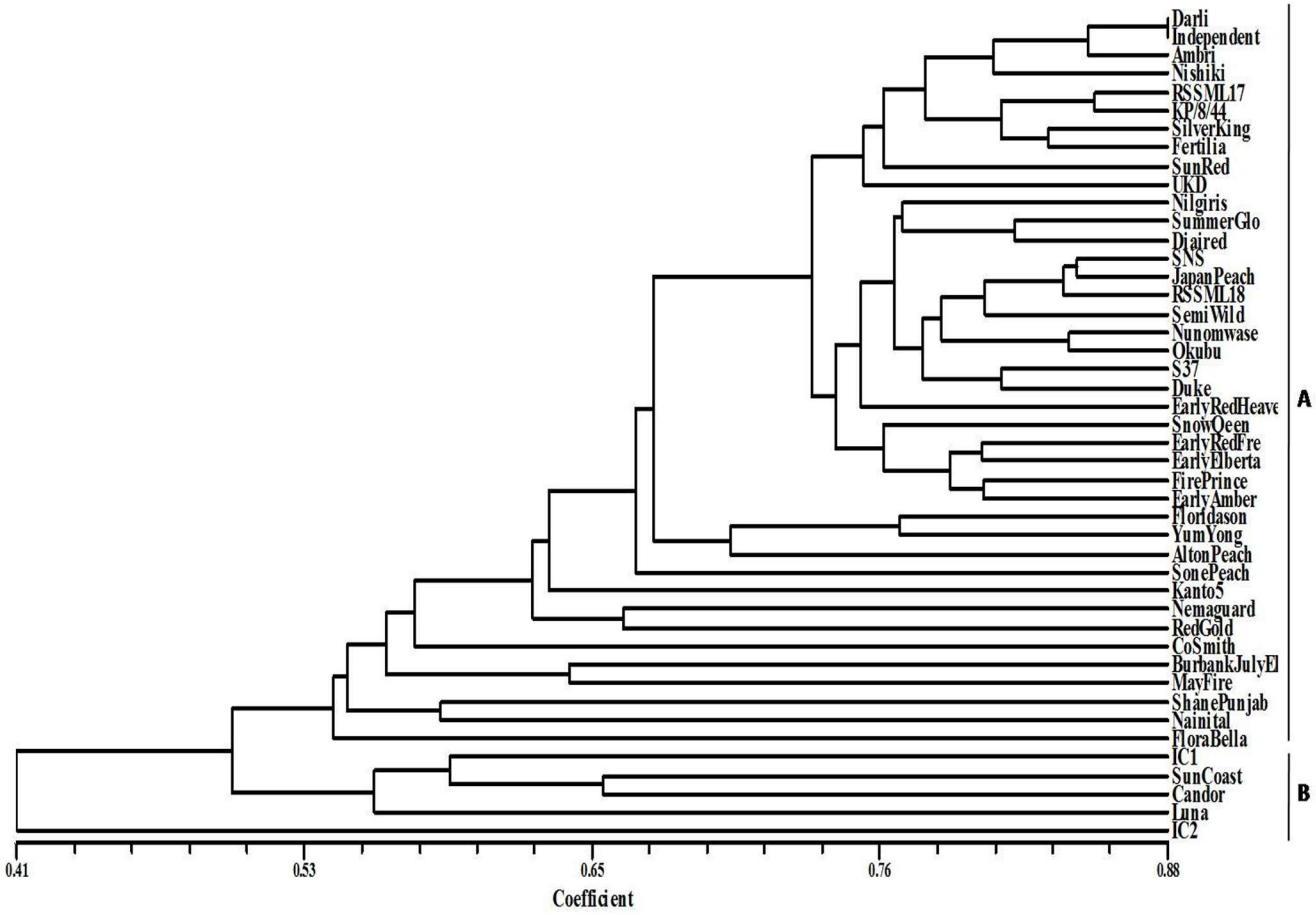

Fig. 3 — Dendrogram obtained after pooled RAPD and ISSR analysis in peach germplasm.

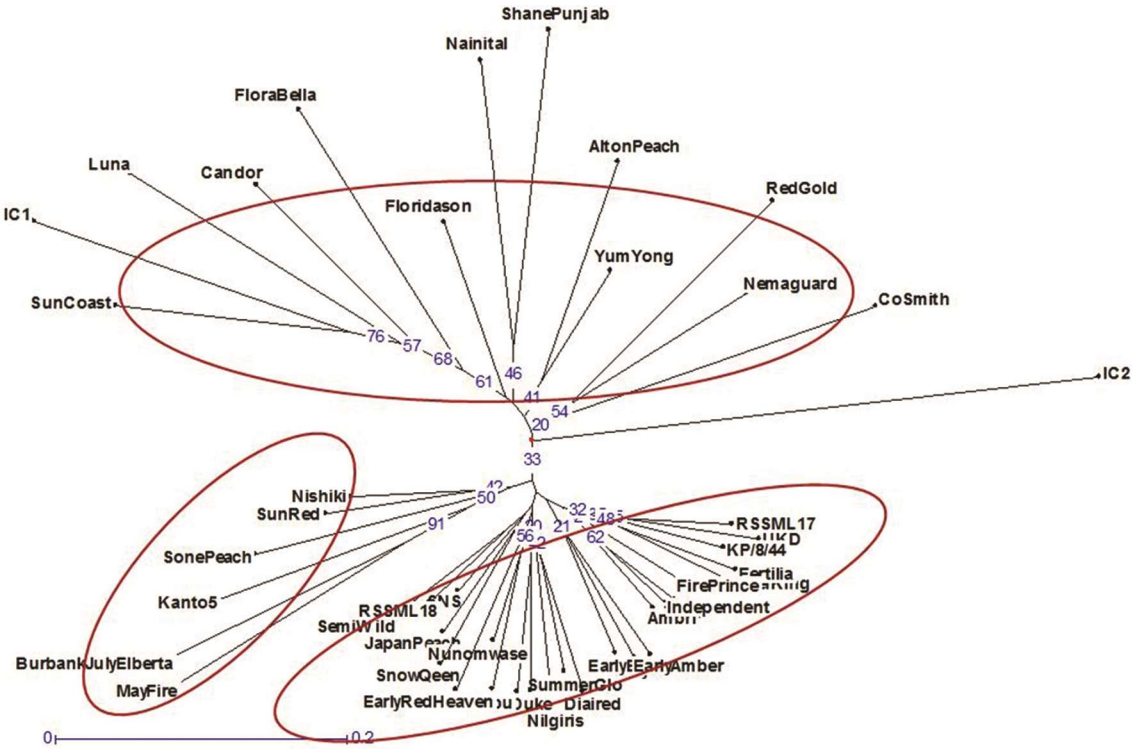

Fig. 4 — Neighbour-Joining tree obtained after pooled RAPD and ISSR marker analysis (number on branches are bootstrap values).

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