Molecular markers based genetic relatedness studies in tissue culture propagated Japanese plum cultivars Santa Rosa and Frontier

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Abstract

Santa Rosa and Frontier are the major Japanese plum cultivars grown throughout the world. The present investigation was performed to understand the genetic relatedness among *in vitro* propagated plum cultivars Santa Rosa and Frontier using PCR based molecular markers. For the study, three arbitrary markers viz. RAPD (Random amplified Polymorphic DNA), ISSR (Inter-Simple Sequence Repeats) and SCoT (Start Codon Targeted) were used. In RAPD analysis, 18 primers out of 28 amplified and generated 33 scorable bands. The allelic variations when analysed, revealed 84 percent similarity between these two cultivars with highest polymorphic information content of 0.78. Similarly, 15 ISSR primers produced 73 amplicons with an average of 4.86 amplicon per primer and similarity coefficient ranging from 62 to 67 percent. Seven SCoT primers out of 26 resulted in a total of twenty- six scorable bands with 24 polymorphic bands. Cluster analysis from all the three markers used broadly divided plum cultivars Santa Rosa and Frontier into two major clusters containing *in vitro* shoots, their progenies and mother trees of respective genotypes. The study concluded that these three marker systems were found to be effective in revealing genetic relationship of these two commercially important plum cultivars.

Introduction

Plums are widely grown in most of the temperate regions of the world. Plum belongs to family Rosaceae, genus *Prunus* and subgenus *Prunophora*. Most of the commercial varieties of plum are divided into European (*Prunus domestica*; hexaploid) and Japanese (*Prunus salicina*; diploid) type (Okie and Weinberger, 1996). Japanese plum is a dominant species with broad adaptation from temperate to subtropical regions of the world. Though it is indigenous to China but was refined in Japan and United States (Okie and Hancock, 2008). All the cultivars of plum planted today were developed by Luther Burbank during 19th century by crossing *P. salicina* with *P. simonii*, *P. cerasifera*, *P. americana* and many American species of plum (Howard, 1945). The great variability among plum cultivars is due to poly-specific breeding and natural outcrossing. Plums are self-compatible hence it is difficult to elucidate if cultivars are pure species or hybrids. Santa Rosa and Frontier are two prime cultivars of Japanese plum planted commercially in India (Thakur et al., 2020) whose genetic relationship is not known.

Awareness of genetic diversity and relatedness is the key in designing methods for conservation of species for the use in breeding programmes. Molecular markers have proved to be a productive tool in deciphering genetic relatedness in plants (Agarwal et al., 2008). All the data obtained from these markers provide details on population structure, relation and inbreeding (Dobson et al. 1998; Surridge et al. 1999). Recently, we revealed the genetic fidelity of tissue culture propagated plum cvs. Santa Rosa and Frontier using RAPD, ISSR & SCoT markers (Thakur et al., 2018; Thakur et al., 2020; Thakur et al., 2021). The outcome of these studies was used to elucidate genetic relatedness between these two commercial plum cultivars for their future use in breeding for the development of new cultivar.

Materials And Methods

Fresh leaves were collected randomly from axenic shoot cultures of Santa Rosa and Frontier, two years old hardened plants and their mother trees maintained in the Department of Biotechnology and in the fields of Horticultural Research and Training Station & Krishi Vigyan Kendra, Kandaghat, Solan (HP) India for DNA extraction. Leaves were macerated in 1.2 ml extraction buffer (65°C) and DNA was isolated following Doyle and Doyle (1987) protocol. Purification of DNA was carried out by successive RNase treatment followed by treatment of phenol: chloroform: Isoamyl alcohol (25:24:1) and centrifugation at 12,000 rpm for 10 minutes. The DNA pellet formed was mixed in TE buffer for storage till further use. Quantification of DNA was performed using nanodrop spectrophotometer (BioPhotometer) at 260 nm. Each sample was diluted to 12.5ng/μl in 1X TE buffer and stored at 4°C.

PCR amplifications were executed in 10 μl PCR reaction containing 1X PCR buffer, 1.5 mM MgCl₂, 200 μM dNTPs, 0.3 U Taq DNA polymerase, 1.0 μM primer and 50ng DNA. DNA was amplified in MJ Mini Personal Thermal cycler (BioRAD, Singapore). PCR amplification included initial denaturation at 94 °C for 3–4 minutes followed by 35 cycles of initial denaturation at 94 °C, for 1 min, annealing depending upon T_m of the primer for 1 min, extension at 72 °C for 2 min and final extension at 72 °C for 5–7 min. The amplified products were mixed with 6X loading dye and then electrophoresed at 1.5% agarose gel and stained with ethidium bromide using 1X TAE buffer. Each reaction was repeated twice. Electrophoretic separations were conducted at 80 mA (70V) for 1–2 hrs and gels were photographed using gel documentation system (Alpha Imager, CA).

Reproducible and well-defined amplicons were scored for their presence (1) or absence (0) through visual observations. All the diversity parameters i.e. number of effective alleles (Ne), Shannon's information index (I), total genetic diversity (Ht) and Nei's expected heterozygosity (h) were determined using POPGENE version 1.31 (Yeh et al. 1999). Dendrograms were constructed by Jaccard's genetic similarity matrix to display cultivars relationship using the unweighted pair group method with arithmetic mean (UPGMA) from data generated using NTSYSpc 2.2 software.

Results And Discussion

There is very limited genetic information on Plum species (Esmenjaud and Dirlewanger 2007) required for plum breeding which happens to be slow due to high degree of heterozygosity, self-incompatibility and long juvenile phase (Guerra and Rodrigo 2015). In this study resolving power of RAPD, ISSR and SCoT was used to confirm the genetic relatedness in Plum cvs. Santa Rosa and Frontier. The relationship within and between two cultivars was drawn by cluster analysis from binary data generated through these marker systems. For RAPD analysis eighteen RAPD primers out of 28 resulted in 33 scorable bands ranging from minimum of 1 in primers OPA 08, OPB 01, OPB 02, OPB 06, OPB 07, OPB 08, OPB 10, OPB 14, OPE 04 and OPE 14 to a maximum of 5 in OPC 02. All the amplified products ranged from 100–1500 bp. Genetic similarity between the two cultivars ranged from 0.74 to 0.94 having an average of 0.84 (Fig. 1a) indicating very low level of polymorphism, similarly the Nei's gene diversity ranged from 0 to 0.37 depicting very high similarity between the two cultivars. The highest PIC value of RAPD primers was 0.78, indicating the efficiency of these primers in genetic diversity studies. Shimada et al (1999) reported a polymorphism of 24% while studying the genetic variations in plum commercial genotypes through RAPD markers which is nearly similar to our studies.

During ISSR analysis all of 15 ISSR primers used for the study amplified the genomic DNA generating 73 detectable bands which ranged from 3 to 8. Minimum of 3 bands were amplified by ISSR 825, ISSR 841, ISSR 845 and ISSR 873 whereas, maximum of 8 bands was produced through ISSR 807, having an average of 4.86 bands per primer ranging from 100- 3000bp size. The similarity coefficient between the two cultivars ranged from 0.62 to 0.67 (Fig. 1b) and Shannon's information index was between 0 to 0.67 depicting low genetic diversity. Previously, ISSR markers were utilized for studying the genetic variability of many plum varieties of Southern China through by Wu et al. (2019). In SCoT analysis seven out of 26 primers resulted in amplification of genomic DNA ranging from 200 to 2000bp having genetic similarity of 0.67 to 0.89 (Fig. 1c) and Nei's gene diversity index ranging from 0 to 0.25.

All the three markers were effective in distinguishing the two genotypes as majority of the amplicons generated were polymorphic (0.75%) amongst both the genotypes. However, the dendrogram pertaining to the above data broadly divided these two cultivars into two major clusters containing *in vitro* shoots, their progenies and mother trees of respective cultivars which depicted a genetic similarity of 0.65 to 0.84 between plum cvs. Santa Rosa and Frontier. Amongst all the three markers used for the study SCoT were found to be more efficient having highest PIC (0.88) and Rp value (15.09) followed by ISSR having highest PIC value of 0.86. Many RAPD primers had the lowest PIC and Rp values. On the contrary low polymorphism has been reported by SCoT markers in date palm (Qurainy et al., 2015)

Plums are self-incompatible, therefore it becomes tough to decide if cultivars are pure species. Many of the varieties cultivated today are hybrids with unknown parents. Previously a close genetic relationship between Plum cvs Golden Japan and Santa Rosa was derived by Hend et al., (2009) with the aid of RAPD markers the information regarding which can be utilized for the improvement of this crop. Santa Rosa is a complex hybrid developed by Luther Burbank (Athanasiadis et al., 2013) and is a parent to many other varieties of plum including Frontier (Jonathan, 1993) that is justified by our studies showing a molecular relatedness between them indicating a common lineage.

Conclusion

The present investigation concluded that Japanese plum cultivars Santa Rosa and Frontier are related to each other in their genetic makeup which was revealed using arbitrary PCR based molecular markers. Hence, reporting that both of these plum cultivars have a common origin.

Declarations

Authors declare no conflict of interest

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Tables

Table: List of RAPD, ISSR and SCoT primers with number of amplicons produced in Plum cvs Santa Rosa and Frontier

Primer	Primer code	Primer Sequence	Tm (°C)	No. of amplicons	No. of polymorphic bands	Fragment size range (bp)	PIC	Rp	E	MI	ne	h
RAPD	OPA 03	AGTCAGCCAC	28.90	2	2	100-400	0.50	1.57	0.043	0.015	1.00	0.00
	OPA 08	GTGACGTAGG	28.90	1	0	600	0.00	0.00	0.000	0.000	1.00	0.00
	OPA 13	CAGCACCCAC	33.00	2	2	400-600	0.50	3.13	0.043	0.015	1.00	0.00
	OPA 14	TCTGTGCTGG	28.90	3	3	300-1000	0.67	3.00	0.065	0.023	1.00	0.00
	OPA 15	TTCCGAACCC	28.90	3	3	600-1500	0.67	5.74	0.065	0.023	1.00	0.00
	OPA 18	AGGTGACCGT	28.90	2	2	200-300	0.50	3.83	0.043	0.015	1.00	0.00
	OPB 01	GTTTCGCTCC	28.90	1	1	100	0.00	1.00	0.022	0.008	1.00	0.00
	OPB 03	CATCCCCCTG	33.00	2	0	100-300	0.50	0.00	0.000	0.000	1.00	0.00
	OPB 06	TGCTCTGCCC	33.00	1	0	400	0.00	0.00	0.000	0.000	1.00	0.00
	OPB 07	GGTGACGCAG	33.00	1	0	500	0.00	0.00	0.000	0.000	1.00	0.00
	OPB 08	GTCCACACGG	33.00	1	0	300	0.00	0.00	0.000	0.000	1.00	0.00
	OPB 10	CTGCTGGGAC	33.00	1	1	300	0.00	1.00	0.022	0.008	1.00	0.00
	OPB 14	TCCGCTCTGG	33.00	2	2	200	0.50	1.91	0.043	0.015	1.09	0.08
	OPC 02	GTGAGGCGTC	33.00	5	5	200-1000	0.78	6.57	0.109	0.039	1.59	0.37
ISSR	OPE 01	CCCAAGGTCC	33.00	2	2	300-700	0.50	3.91	0.043	0.015	1.00	0.00
	OPE 04	GTGACATGCC	28.90	1	0	100	0.00	0.00	0.000	0.000	1.00	0.00
	OPE 06	AAGACCCCTC	28.90	1	0	100	0.00	0.00	0.000	0.000	1.00	0.00
	OPE 14	TGCGGCTGAG	33.00	2	2	200-1000	0.47	2.61	0.043	0.015	1.44	0.31
	ISSR 807	AGAGAGAGAGAGAGAGT	50.36	8	3	100-800	0.86	3.00	0.065	0.049	1.44	0.30
	ISSR 812	GAGAGAGAGAGAGAGAA	50.36	7	4	100-800	0.84	4.96	0.087	0.066	1.51	0.34
	ISSR 817	CACACACACACACAA	50.36	6	1	200-900	0.83	1.00	0.022	0.016	1.18	0.15
	ISSR 818	CACACACACACACAG	52.77	6	2	100-800	0.83	3.83	0.043	0.033	1.03	0.03
	ISSR 825	ACACACACACACACT	50.36	3	3	900-3000	0.64	4.74	0.065	0.049	1.45	0.31
	ISSR 827	ACACACACACACACG	52.77	4	0	200-1000	0.75	0.00	0.000	0.000	1.00	0.00
	ISSR 835	AGAGAGAGAGAGAGAGCTC	56.67	7	5	200-1000	0.84	5.91	0.109	0.082	1.70	0.41
	ISSR 840	GAGAGAGAGAGAGAGACTT	54.51	4	4	300-700	0.72	5.83	0.087	0.066	1.61	0.38
	ISSR 841	GAGAGAGAGAGAGAGACTC	56.67	3	0	300-700	0.67	0.00	0.000	0.000	1.00	0.00
	ISSR 845	CTCTCTCTCTCTCTAGG	56.67	3	1	300-700	0.64	1.00	0.022	0.016	1.38	0.28

	ISSR 847	CACACACACACACACAAGC	56.67	4	3	200-500	0.72	2.87	0.065	0.049	1.91	0.48
	ISSR 868	GAAGAAGAAGAAGAAGAA	46.85	5	4	300-700	0.78	4.00	0.087	0.066	1.92	0.48
	ISSR 873	GACAGACAGACAGACA	58.39	3	3	300-1000	0.67	5.87	0.065	0.049	1.00	0.00
	ISSR 880	GGAGAGGAGAGGAGA	50.57	6	2	200-700	0.82	2.00	0.043	0.033	1.38	0.28
	ISSR 888	CGTAGTCGTCACACACACACA	62.43	4	4	300-800	0.73	6.83	0.087	0.066	1.29	0.22
SCoT	S1	CAACAATGGCTACCACCA	60.6	2	2	800-1200	0.50	3.30	0.043	0.025	1.00	0.00
	S3	CAACAATGGCTACCACCG	62.7	9	9	200-2000	0.88	15.09	0.196	0.111	1.33	0.25
	S4	CAACAATGGCTACCACCT	58.5	5	3	200-1200	0.79	4.65	0.065	0.037	1.30	0.23
	S7	CAACAATGGCTACCACGG	62.7	2	2	500-1200	0.50	3.30	0.043	0.025	1.00	0.00
	S11	AAGCAATGGCTACCACCA	61.8	2	2	600-2000	0.50	3.39	0.043	0.025	1.00	0.00
	S12	ACGACATGGCGACCAACG	63.5	5	5	300-1200	0.80	8.48	0.109	0.062	1.15	0.13
	S35	CATGGCTACCACCGGCC	71.0	1	1	500	0.00	1.78	0.022	0.012	1.00	0.00
	Mean			3.30	2.20	310.00	0.52	3.15	0.048	0.028	1.19	0.13

PIC= polymorphic information content; *Rp*: resolving power; *E*: effective multiplex ratio; *MI*: marker index; *h* = Nei's gene diversity; *I* = Shannon's information index; *ne* = effective number of alleles; *Ht* = variability within treatments

Figures

Figure 1

Fruits, in vitro multiplying shoots and hardened plants of Plum cvs. Santa Rosa (a-c) and Frontier (d-f)

Figure 2
Dendrograms of Plum cvs. Santa Rosa and Frontier obtained through RAPD (a) ISSR (b) and SCoT (c) analysis