



Genetic diversity analysis among male and female *Jobba* genotypes employing gene targeted molecular markers, start codon targeted (SCoT) polymorphism and CAAT box-derived polymorphism (CBDP) markers



Monika Heikrujam, Jatin Kumar, Veena Agrawal

Department of Botany, University of Delhi, Delhi 110007, India

ARTICLE INFO

Article history:

Received 28 January 2015

Revised 3 June 2015

Accepted 3 June 2015

Available online 16 June 2015

Keywords:

Jobba

Genetic diversity

PIC

Start codon targeted (SCoT) polymorphism

CAAT box-derived (CBDP) polymorphism

ABSTRACT

To detect genetic variations among different *Simmondsia chinensis* genotypes, two gene targeted markers, start codon targeted (SCoT) polymorphism and CAAT box-derived polymorphism (CBDP) were employed in terms of their informativeness and efficiency in analyzing genetic relationships among different genotypes. A total of 15 SCoT and 17 CBDP primers detected genetic polymorphism among 39 *Jobba* genotypes (22 females and 17 males). Comparatively, CBDP markers proved to be more effective than SCoT markers in terms of percentage polymorphism as the former detecting an average of 53.4% and the latter as 49.4%. The Polymorphic information content (PIC) value and marker index (MI) of CBDP were 0.43 and 1.10, respectively which were higher than those of SCoT where the respective values of PIC and MI were 0.38 and 1.09. While comparing male and female genotype populations, the former showed higher variation in respect of polymorphic percentage and PIC, MI and Rp values over female populations. Nei's diversity (h) and Shannon index (I) were calculated for each genotype and found that the genotype "MS F" (in both markers) was highly diverse and genotypes "Q104 F" (SCoT) and "82-18 F" (CBDP) were least diverse among the female genotype populations. Among male genotypes, "32 M" (CBDP) and "MS M" (SCoT) revealed highest h and I values while "58-5 M" (both markers) was the least diverse. Jaccard's similarity co-efficient of SCoT markers ranged from 0.733 to 0.922 in female genotypes and 0.941 to 0.746 in male genotype population. Likewise, CBDP data analysis also revealed similarity ranging from 0.751 to 0.958 within female genotypes and 0.754 to 0.976 within male genotype populations thereby, indicating genetically diverse *Jobba* population. Employing the NTSYS (Numerical taxonomy and multivariate analysis system) Version 2.1 software, both the markers generated dendrograms which revealed that all the *Jobba* genotypes were clustered into two major groups, one group consisting of all female genotypes and another group comprising of all male genotypes. During the present investigation, CBDP markers proved more informative in studying genetic diversity among *Jobba*. Such genetically diverse genotypes would thus be of great significance for breeding, management and conservation of elite (high yielding) *Jobba* germplasm.

© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Simmondsia chinensis (Link) Schneider is a dioecious desert shrub, native to South-western USA and Mexico, cultivated mainly for its seed oil. Its seeds contain a light yellow, odorless wax that makes up to 45–55% of the seed weight (Gentry, 1958). *Jobba* oil's physical and chemical properties are similar to Sperm whale oil thus it has numerous applications in various industries such as lubricants, cosmetics, pharmaceuticals, paints, varnishes, waxes, detergents, resins, plastics, computer industries, transformer oil, leather industry and biodiesel (Benzioni and Forti, 1989; Wisniak, 1994). Because of its valuable seeds, female *Jobba* plants are preferred than the male plants but being dioecious in nature

its plantation through seeds is uneconomical due to its high ratio of males compared to females (5 males:1 female). Moreover, sex of this plant can only be ascertained when it starts flowering i.e. after 3–4 years of planting. Large scale cultivations of this crop are thus mainly via vegetative propagation from a limited number of elite lines/clones which are subsequently used in the production of many more cultivars. The large-scale cultivation of such genetically uniform cultivars has resulted in an increasingly narrow genetic base for the crops, leading to genetic vulnerability (Rao and Hodgkin, 2002).

For sustainable production of a crop species, understanding the extent and organization of genetic variations and its relationship in any population is extremely necessary. In dioecious plant species, male and female populations display different levels of genetic variations (Hilfiker et al., 2004; Vandepitte et al., 2010). These differences in the levels of genetic diversity between the populations of dioecious plants

E-mail address: drveena_du@yahoo.co.in (V. Agrawal).

might be because of the distribution patterns of individuals across the populations, sex ratio variation and stochastic events (De Jong and Klinkhamer, 2005; Engen et al., 2003; Vandepitte et al., 2010). The amount of genetic variation can have important biological consequences on population fitness and persistence (Reusch et al., 2005). An assessment of the genetic diversity is crucial for breeders in many ways: to better understand the evolutionary and genetic relationships among different genotypes, to select germplasm in a more systemic and effective fashion and to develop strategies to incorporate useful diversity in their breeding programs (Li and Nelson, 2001; Paterson et al., 1991).

The advent of different molecular techniques led breeders to estimate genetic diversity among the species, genotypes, landraces, varieties, cultivars etc., on the basis of data generated by the markers. In recent years, many molecular markers which generate polymorphism from gene regions of the genome have been developed such as sequence related amplified polymorphism (SRAP; Li and Quiros, 2001), target region amplification polymorphism (TRAP; Hu and Vick, 2003), conserved region amplification polymorphism (CoRAP; Wang et al., 2009), start codon targeted polymorphism (SCoT; Collard and Mackill, 2009) and CAAT box derived polymorphism (CBDP; Singh et al., 2014). The start codon targeted (SCoT) polymorphism is a simple and reliable gene targeted marker technique based on the conserved region surrounding the translation codon ATG (Collard and Mackill, 2009). They are dominant like RAPDs and could be used for genetic analysis, quantitative trait loci (QTL), mapping and bulk segregation analysis (Collard and Mackill, 2009). The SCoT molecular markers have been successfully used in a diverse set of plant species for genetic diversity analysis: potato, mango, *Dendrobium nobile*, tomato, Cicer and peanut (Amirmoradi et al., 2012; Bhattacharyya et al., 2013; Gorji et al., 2011; Luo et al., 2011; Shahlaei et al., 2014; Xiong et al., 2011). Another gene targeted marker, CAAT box-derived polymorphism (CBDP) exploits CAAT box region of promoters in plant genes (Singh et al., 2014). CAAT box has a distinct pattern of nucleotides with a consensus sequence GGCCAATCT located ~80 bp upstream of the start codon of eukaryote genes and plays an important role during transcription (Benoist et al., 1980). This marker has been validated through studies on jute cultivars, cotton (*Gossypium* species) and linseed (*Linum usitatissimum*) cultivars (Singh et al., 2014).

In the past few years, several publications have appeared pertaining to sex linked marker in Jojoba (Agarwal et al., 2011; Agrawal et al., 2007; Heikrujam et al., 2014a, 2014b; Hosseini et al., 2011; Ince et al., 2010, 2011 and Sharma et al., 2008) however, there are scant information regarding molecular diversity among different genotypes (Bhardwaj et al., 2010; Sharma et al., 2009). The present investigation has been carried out to evaluate the genetic diversity among different Jojoba genotypes using two gene targeted molecular markers (SCoT and CBDP) for the first time in these genotypes.

Materials and methods

Plant materials and DNA extraction

The experimental materials used in the present study consisted of 39 Jojoba genotypes (22 females and 17 males), individually collected from the Association of Rajasthan Jojoba Plantation and Research Project (AJORP) (Table 1). Total genomic DNA was isolated from the leaves of each genotype separately using the modified CTAB method (Saghai-Maroo et al., 1984). DNA concentrations were estimated by both spectrophotometry (260/280) and gel electrophoresis (0.8% agarose gel). The final concentration of DNA for PCR analysis was made to 50 ng/ μ L.

SCoT-PCR amplification

A total of 15 SCoT primers (Integrated DNA Technologies, Inc., USA) were used for genetic diversity analysis. PCR amplification was carried

Table 1
Jojoba genotypes procured from AJORP (Jaipur, Rajasthan).

S. no.	Genotypes collected	S. no.	Genotypes collected	S. no.	Genotypes collected	S. no.	Genotypes collected
1	Q106 F	11	96 F	21	CSMCRI F	31	92 M
2	MS F	12	82–18 F	22	CAZRI F	32	Forti M
3	C-64 F	13	58–5 F	23	Q106 M	33	96 M
4	879–154 F	14	85 F	24	MS M	34	82–18 M
5	17–21 F	15	K-11 F	25	C-64 M	35	58–5 M
6	Local F	16	48–25 F	26	879–154 M	36	85 M
7	Q104 F	17	16–10 F	27	17–21 M	37	K-11
8	32 F	18	C ₈ R ₂₃ F	28	Local M	38	48–25 M
9	92 F	19	12–18 F	29	Q104 M	39	Male cutting
10	Forti F	20	24–8 F	30	32 M		

F = female; M = male.

out in 20 μ L volumes containing 50 ng of template DNA, 2.5 μ L of 10 \times PCR buffer, 0.8 μ M SCoT primers, 200 mM dNTPs and 1 U Taq DNA polymerase (Bangalore Genei, India). DNA amplifications were carried out in a 2720 thermal cycler (Applied Biosystems, USA). SCoT-PCR amplifications were carried out with a preliminary cycle of 2 min at 94 $^{\circ}$ C, followed by 35 cycles of 1 min at 94 $^{\circ}$ C, 1 min at 50 $^{\circ}$ C, 2 min at 72 $^{\circ}$ C and a final extension of 5 min at 72 $^{\circ}$ C. The amplification products were resolved on 1.2% agarose gels in 0.5 \times Tris–borate EDTA buffer (45 mM Tris–borate and 1 mM EDTA) and stained with ethidium bromide (0.5 μ g/mL).

CBDP-PCR analysis

A total of 20 CBDP primers (Integrated DNA Technology, Inc., USA) were used for the study. PCR amplifications were carried out in 20 μ L volumes containing 50 ng of template DNA, 2.5 μ L 10 \times PCR buffer, 0.8 μ M CBDP primers, 200 mM dNTPs and 1 U Taq DNA polymerase (Bangalore Genei, India). DNA amplifications were carried out in a 2720 thermal cycler (Applied Biosystems, USA). CBDP-PCR amplifications were carried out with a preliminary cycle of initial DNA denaturation at 95 $^{\circ}$ C for 4 min, followed by 5 cycles of 1 min denaturation at 94 $^{\circ}$ C, 1 min annealing at 36 $^{\circ}$ C and 2 min of extension at 72 $^{\circ}$ C. In the following 30 cycles, the annealing temperature was increased to 50 $^{\circ}$ C with a final extension of 72 $^{\circ}$ C for 7 min. The amplification products were resolved on 1.2% agarose gels in 0.5 \times Tris–borate EDTA buffer (45 mM Tris–borate and 1 mM EDTA) and stained with ethidium bromide (0.5 μ g/mL).

Data scoring and statistical analysis

PCR products of SCoT and CBDP primers were scored visually. Only clearly distinguishable bands in both cases were considered for final scoring and data analysis. The presence of a band was recorded as “1” and the absence of a band as “0”. All amplifications were repeated at least thrice and only reproducible bands were considered for analysis. A binary matrix of the presence/absence was obtained from gels for each marker. Separate binary matrices for male genotype population, female genotype population and combined male and female genotype populations were generated.

Discriminatory power of the primers was evaluated by means of three parameters; Polymorphic information content (PIC), marker index (MI) and resolving power (Rp). PIC value was calculated following Roldan-Ruiz et al. (2000) as: $PIC = 2fi(1 - fi)$ where fi is the frequency of the amplified allele (band present) and $(1 - fi)$ is the frequency of the null allele (band absent) of marker i . MI, a measure of overall efficient of a molecular marker technique, was obtained by multiplying the average PIC with the Effective multiplex ratio (Powell et al., 1996). Effective multiplex ratio (EMR) is the product of the number of polymorphic loci per primer (n) and the fraction of polymorphic fragments (β). Rp of each primer was calculated according to Prevost and Wilkinson (1999): $Rp = \sum bi$ where bi = band informativeness. The BI index (Band informativeness) was calculated for each primer using

the formula $BI = 1 - [2(0.5 - p)]$ where p is the proportion of occurrence of bands in the genotypes out of the total number of genotypes. R_p is based on the distribution of alleles within the genotypes. R_p of primers is a very useful parameter for molecular diagnosis of any species from a mixed population. The basic parameters for genetic diversity were calculated in the POPGENE application (Yeh et al., 1999). The data for the number of observed alleles (na), the mean number of effective alleles (ne), the mean Nei's gene diversity index (h) and the Shannon index (I) were determined.

NTSYS (Numerical taxonomy and multivariate analysis system) Version 2.1 software (Rohlf, 2000) was used to perform distance matrix and cluster analysis of the dataset. Genetic association among the Jojoba genotypes was measured by Jaccard's similarity coefficient (Jaccard, 1908) with SIMQUAL (Similarity for qualitative data program in NTSYS) module. A dendrogram was generated using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) with Sequential agglomerative hierarchical and nested clustering method program in NTSYS (SAHN) to show a phenetic representation of genetic relationships as revealed by similarity coefficient.

Results

SCoT diversity analysis

Fifteen SCoT primers were screened to study genetic diversity among Jojoba genotypes; all the primers produced reproducible polymorphic bands in all the 39 Jojoba genotypes as shown in the Table 2.

Females SCoT analysis

A total of 151 amplification products were generated, out of which 75 bands were polymorphic across 22 female Jojoba genotypes. The size of the fragments ranged from 200 to 2500 bp. The highest and lowest number of polymorphic bands was 10 with SCoT 13 and 2 with SCoT 11 and SCoT 36, on an average of 5 polymorphic bands per primer. The percentage of polymorphism varied from 20% (SCoT 36) to 76.9% (SCoT 13). PIC of the 15 primers ranged from 0.22 (SCoT 36) to 0.48 (SCoT 28) with an average of 0.40 per primer. MI of the primers ranged from maximum 3.63 (SCoT 13) to minimum 0.08 (SCoT 36). R_p of the SCoT primers ranged from 10 (SCoT 16) to 20.1 (SCoT 15) with an average of 14.3 per primer (Table 2). The primers with high R_p values were considered to be more informative in distinguishing the genotypes but none of the polymorphic primers could discriminate all the female genotypes independently.

The genetic parameters were calculated for all the female genotypes amplified with SCoT primers. Nei's gene diversity (h) ranged from 0.386 (Q104 F) to 0.488 (MS F), with a mean of 0.440. A similar pattern was observed for the Shannon's information index (I), with the highest value of 0.680 observed in genotype MS F population and the lowest value of 0.551 observed in population Q104 F, with a mean of 0.625. The observed number of alleles (na) ranged from 1.866 to 2.000. The effective number of alleles (ne) ranged from 1.955 (MS F) to 1.706 (Q104 F) which shows similar pattern for values of h and I (Table 3).

The Jaccard's genetic similarity values of the female genotypes based on SCoT markers varied from 0.733 (between CSMCRI F and MS F) to 0.922 (between 879–154 F and Clone 64 F). Such a range in genetic distances suggested genetically diverse female genotype population. UPGMA cluster analysis of the SCoT dataset grouped female Jojoba genotypes into four distinct clusters in the dendrogram. Cluster I consisted of genotypes Q104 F, Q106 F, MS F, Clone-64 F, 879–154 F, Local F, 32 F, 17–21 F, 82–18 F and 58–5 F; Cluster II consisting of 92 F, 48–25 F, C8R23 F, 16–10 F, CAZRI F, 28–8 F, Forti F, K-11 F and 85 F. Cluster III consisted of only one genotype (96 F). The genotype CSMCRI F forms an outgroup from the other genotypes forming the cluster IV.

Table 2
Sequence information and amplification efficiency of the 22 female and 17 male genotypes using SCoT primers.

S. no.	SCoT primer	Sequence (5'-3')	Range of amplified fragments (bp)	Female genotypes				Male genotypes							
				Total number of bands	Number of polymorphic bands	Polymorphism %	PIC	MI = EMR * PIC	Rp	Total number of bands	Number of polymorphic bands	Polymorphism %	PIC	MI = EMR * PIC	Rp
1	SCoT 11	AAGCAATGGCTACACCA	300–1815	8	2	25	0.40	0.20	11.7	8	3	37.5	0.49	0.55	11.8
2	SCoT 13	ACGACATGGCGACCATCG	200–2500	13	10	76.9	0.47	3.63	16.2	12	9	75	0.43	2.88	16.6
3	SCoT 15	ACGACATGGCGACCGGA	300–2500	13	9	69.2	0.36	2.24	20.1	12	8	66.7	0.39	2.10	17.5
4	SCoT 16	ACCATGGCTACCCGAC	200–2000	8	5	62.5	0.46	1.46	10	8	5	62.5	0.46	1.43	10.4
5	SCoT 18	ACCATGGCTACCCGCC	300–1815	9	4	44.4	0.38	0.67	13.5	9	4	44.4	0.27	0.49	15.1
6	SCoT 19	ACCATGGCTACCCGGC	300–2000	11	4	36.3	0.41	0.60	15.6	11	6	62.5	0.30	0.97	14.5
7	SCoT 23	CACATGGCTACACACAG	300–1185	9	4	44.4	0.47	0.84	11.2	8	5	62.5	0.30	0.91	12.8
8	SCoT 24	CACATGGCTACACACAT	600–1815	9	3	33.3	0.40	0.40	13.1	9	3	33.3	0.41	0.42	21.2
9	SCoT 28	CCATGGCTACCCGCCA	200–3000	10	7	70	0.48	2.34	12.1	8	4	50	0.26	0.52	18.8
10	SCoT 31	CCATGGCTACCCGCCCT	400–2000	9	4	44.4	0.43	0.77	12.3	10	5	50	0.37	0.92	15.2
11	SCoT 34	ACCATGGCTACCCGCCA	400–2500	8	4	50	0.43	0.85	11.1	8	2	25	0.32	0.16	13.2
12	SCoT 35	CATGGCTACCCGCCCC	300–2000	12	6	50	0.41	1.23	17.1	12	8	66.7	0.27	1.11	12.7
13	SCoT 36	GCAACAATGGCTACCC	400–1500	10	2	20	0.22	0.08	17.5	10	2	20	0.27	0.11	16.8
14	SCoT 38	AAGCAATGGCTACACCG	300–2000	12	5	41.7	0.33	0.70	18.9	12	5	41.7	0.34	0.70	13.5
15	SCoT 39	ACGACATGGCGACACGGC	200–1185	10	6	60	0.40	1.41	14.6	11	7	63.6	0.45	2.0	18
Average				10.06	5	48.54	0.40	1.16	14.3	9.9	5.1	50.2	0.35	1.02	15.2

Table 3
Genetic diversity within populations of male and female Jojoba genotypes revealed by SCoT markers.

Genotypes	na	ne	h	I
Q104 F	1.867	1.706	0.386	0.551
Q106 F	2.000	1.831	0.451	0.643
17–21 F	2.000	1.888	0.467	0.659
Clone-64 F	1.933	1.862	0.445	0.625
82–18 F	2.000	1.892	0.469	0.662
58–5 F	2.000	1.907	0.474	0.667
879–154 F	2.000	1.919	0.477	0.670
MS F	2.000	1.955	0.488	0.680
Local F	1.933	1.862	0.447	0.627
32 F	2.000	1.917	0.477	0.670
92 F	1.866	1.825	0.422	0.589
Forti F	1.933	1.851	0.444	0.623
96 F	1.866	1.721	0.390	0.556
K-11 F	1.933	1.789	0.424	0.603
85 F	1.933	1.801	0.425	0.603
48–25 F	1.933	1.841	0.441	0.620
16–10 F	1.933	1.797	0.428	0.607
C8R23 F	1.933	1.884	0.453	0.634
12–18 F	1.933	1.830	0.437	0.616
CSMCRI F	1.933	1.838	0.438	0.617
CAZRI F	1.933	1.825	0.434	0.613
24–8 F	1.933	1.844	0.442	0.622
Q104 M	2.000	1.873	0.461	0.653
Q106 M	1.933	1.858	0.446	0.626
MS M	2.000	1.947	0.485	0.678
Clone 64 M	2.000	1.933	0.481	0.674
82–18 M	2.000	1.916	0.476	0.669
58–5 M	1.800	1.745	0.384	0.539
879–154 M	1.866	1.807	0.417	0.584
17–21 M	1.933	1.849	0.443	0.623
Local M	1.933	1.840	0.441	0.621
32 M	2.000	1.894	0.470	0.663
92 M	2.000	1.906	0.474	0.666
Forti M	1.933	1.8770	0.452	0.632
96 M	2.000	1.9230	0.479	0.672
K-11 M	2.000	1.8834	0.467	0.660
85 M	2.000	1.8645	0.461	0.653
48–25 M	2.000	1.8711	0.463	0.655
Male cutting	1.8667	1.7751	0.408	0.575

Males SCoT analysis

A total of 148 bands were produced by 15 SCoT primers among 17 genotypes. Out of the bands, 76 were found to be polymorphic. The extent of polymorphism per primer ranged from 75% (SCoT 13) to 20% (SCoT 36) with a mean of 50.2% (Table 2). The average PIC value was 0.35 per primer; maximum being 0.49 with SCoT 11 and minimum being 0.26 with SCoT 28. MI with maximum value was 2.88 (SCoT 13) and minimum was 0.11 (SCoT 36). Primer SCoT 24 revealed the highest Rp (21.2) value while primer SCoT 16 revealed the lowest Rp (10.4) (Table 2). None of the polymorphic primers could discriminate all the male genotypes independently.

Nei's gene diversity (h) ranged from 0.485 (MS M) to 0.384 (58–5 M), with a mean of 0.45. A similar pattern was observed for the Shannon's information index (I), with the highest value observed in genotype MS M (0.678) and the lowest value observed in genotype 58–5 M (0.539). The observed number of alleles (na) ranged from 1.800 to 2.000. The effective number of alleles (ne) ranged from 1.947 (MS M) to 1.745 (58–5 M), with a mean of 1.868 which also shows similar pattern observed for h and I (Table 3).

Pair wise comparison of the 17 male genotypes based on Jaccard similarity indicated relative genetic similarity between the male genotypes ranging from maximum of 0.941 (32 M and Local M) to a minimum of 0.746 (K-11 M and 85 M). The similarity co-efficient generated from the SCoT data were used to construct UPGMA dendrogram. The dendrogram grouped the 17 male genotypes into four clusters; Cluster I consisted of eleven genotypes (Q104 M, Q106 M, MS M, 879–154 M, 17–21 M, Clone-64 M, 82–18 M, Local M, 32 M, 96 M, K-11

M); Cluster II consisted of genotypes 92 M and Forti M; Cluster III consisted of three genotypes (85 M, 48–25 M and Male cutting) and Cluster IV was distinct as it consisted of only one genotype '58–25 M'.

CBDP diversity analysis

Out of 20 CBDP primers tried, 17 primers produced polymorphic bands in all the 39 genotypes while the remaining 3 primers produced monomorphic bands and were not included in the further study.

Females CBDP analysis

The selected 17 primers amplified 126 bands with 66 bands being polymorphic (Table 4). The maximum and minimum numbers of polymorphic amplified bands were 7 (CBDP 6; CBDP 11 and CBDP 18) and 1 (CBDP 25), respectively. The values of polymorphism were between 87.5% (CBDP 6) and 28.6% (CBDP 4) with an average of 3.9 bands per primer. The maximum and minimum PIC values were 0.50 (CBDP 10) and 0.32 (CBDP 25), respectively with an average of 0.42. The range of MI was from 3.00 (CBDP 6) to 0.11 (CBDP 25) with an average of 1.03. The resolving power of CBDP ranged from 15.50 to 4.60. None of the polymorphic primers could discriminate all the female genotypes independently.

The Nei's gene diversity measure for each genotype was highest in genotypes 92 F and MS F ($h = 0.46$) while the genotype 82–18 F had the least diversity ($h = 0.39$) with an average value of 0.419. The Shannon's information index ranged from 0.65 (92 F and MS F) to 0.55 (82–18 F) with an average value of 0.62. The value of observed number of alleles (na) ranged from 2.000 to 1.88. The effective number of alleles (ne) ranged from 1.88 (92 F) to 1.71 (82–18 F) (Table 5).

Jaccard similarity index from the CBDP marker data ranged from 0.751 (between CSMCRI F and 82–18 F) to 0.958 (between 92 F and Local F). The UPGMA clustering algorithm from CBDP marker analysis grouped the female genotypes into four clusters. Cluster I consisted of fifteen genotypes (Q104 F, Clone-64 F, 58–5 F, 879–154 F, 17–21 F, Q106 F, MS F, Local F, 92 F, 32 F, 96 F, Forti F, 48–25 F, 16–10 F, C₈R₂₃ F, 12–18 F). Cluster II consisted of genotypes CSMCRI F and CAZRI F; Cluster III consisted of three genotypes (K-11 F, 85 F and 24–8 F). Cluster IV consisted of only one genotype "82–18 F" which was quite divergent from the remaining genotypes.

Males CBDP analysis

A total of 133 amplification products were scored, out of which 72 were polymorphic, exhibiting an average of 54.94% polymorphism. The polymorphic amplification products using 17 primers ranged from 83.3% (CBDP 8) to 18.2% (CBDP 17) (Table 4). The PIC value for the CBDP primers ranged from 0.50 (CBDP 9 to 0.33 (CBDP 17) with an average of 0.44. The maximum MI value was 3.313 (CBDP 18) and minimum was 0.12 (CBDP 17) with an average of 1.16. The Rp value of the CBDP primers ranged from 22.6 (CBDP 17) to 7.88 (CBDP 8) with an average of 12.9. None of the polymorphic primers could discriminate all the male genotypes independently.

The Nei's gene diversity ranged from 0.48 (32 M) to 0.36 (58–5 M, Local M and 17–21 M) with an average value of 0.40. The Shannon's information index ranged from 0.67 (32 M) to 0.51 (58–5 M and 17–21 M) with an average value of 0.575. The highest na and ne were 2.00 and 1.93, respectively (Table 5).

The genetic distance recorded using Jaccard's coefficients of similarity ranged from 0.754 (32 M and K-11 M) to 0.976 (17–21 M and Local M). The genetic similarity matrix was applied for cluster analysis through the UPGMA method and generated a dendrogram which grouped the 17 male genotypes into four clusters. Cluster I formed a major group in which thirteen genotypes were clustered together; Cluster II consisted of only one genotype 96 M; Cluster III consisted of two genotypes 48–25 M and Male cutting while Cluster IV consisted of only one genotype 'K-11 M' distant from the other genotypes.

Table 4
List of CBDP primers used in the present study and the extent of polymorphism among the 17 male and 22 female genotypes.

S. no.	Primer code	Primer sequence (5'–3')	Range of amplified fragments (bp)	Females genotypes					Male genotypes						
				Total no. of amplified fragments	Polymorphic fragments (%)	PIC	MI = PIC × EMR	Rp	Total no. of amplified fragments	Polymorphic fragments (%)	PIC	MI = PIC × MR	RP		
1	CBDP 4	TGAGCAGATCCAATAG	1185–300	7	2	28.6	0.38	0.22	10.73	7	4	57.14	0.39	0.90	10.24
2	CBDP 5	TGAGCAGATCCAATCTA	900–300	5	3	60	0.44	0.80	6.73	5	3	60.0	0.38	0.68	9.65
3	CBDP 6	TGAGCAGATCCAATCAG	1600–400	8	7	87.5	0.49	3.00	9.18	8	6	75.0	0.42	1.89	12.0
4	CBDP 7	TGAGCAGATCCAATCGA	1185–250	7	3	42.9	0.39	0.50	10.36	6	2	33.3	0.46	0.31	10.0
5	CBDP 8	TGAGCAGATCCAATCGG	1815–300	6	4	66.7	0.48	1.28	4.60	6	5	83.3	0.45	1.88	7.88
6	CBDP 9	TGAGCAGATCCAATGAT	1500–450	7	3	42.9	0.41	0.53	10.45	7	4	57.1	0.50	1.14	9.06
7	CBDP 10	TGAGCAGATCCAATGTT	1200–400	9	6	66.7	0.50	2.00	9.45	9	5	55.6	0.47	1.31	14.6
8	CBDP 11	TGAGCAGATCCAATGCG	1500–400	11	7	63.6	0.47	2.09	13.64	11	4	36.7	0.46	0.67	18.1
9	CBDP 13	TGAGCAGATCCAATGAG	1185–400	4	3	75	0.42	0.95	6.45	6	4	66.7	0.47	1.25	9.65
10	CBDP 14	TGAGCAGATCCAATCGC	800–300	6	2	33.3	0.45	0.30	10.00	7	3	42.9	0.48	0.62	10.9
11	CBDP 17	TGAGCAGATCCAATTTG	1185–250	9	4	44.4	0.43	0.76	15.50	11	2	18.2	0.33	0.12	22.6
12	CBDP 18	CTGAGCAGATCCAATAG	1400–250	9	7	77.7	0.45	2.45	13.00	11	9	81.8	0.45	3.31	19.0
13	CBDP 19	CTGAGCAGATCCAATAC	1815–400	8	3	37.5	0.39	0.44	14.70	9	6	66.7	0.46	1.84	15.0
14	CBDP 21	CTGAGCAGATCCAATCA	1500–300	9	3	33.3	0.39	0.39	14.54	10	5	50.0	0.48	1.2	15.6
15	CBDP 23	CTGAGCAGATCCAATGG	1185–250	9	3	33.3	0.45	0.45	15.18	7	2	28.6	0.41	0.23	13.9
16	CBDP 24	CTGAGCAGATCCAATGA	2500–300	9	5	55.5	0.43	1.19	13.45	7	5	71.4	0.48	1.7	11.0
17	CBDP 25	CTGAGCAGATCCAATGT	600–300	3	1	33.3	0.32	0.11	5.63	6	3	50.0	0.45	0.68	10.3
Average				7.4	3.9	51.9	0.42	1.03	10.80	7.82	4.24	54.94	0.44	1.16	12.9

Comparison between the male and female genotypes

The male and female genotypes showed high polymorphism with both the markers (SCoT and CBDP) but comparatively, male genotypes showed slightly higher polymorphism than female genotypes. In SCoT analysis, male genotypes showed higher polymorphism (50.2%) than the female genotypes (48.54%). The average values of Rp (15.2) were also higher in the case of males than the Rp (14.3) of females whereas the average PIC value and MI was higher in females (0.4 and 1.16, respectively) than in males (0.35 and 1.02, respectively). Similarly, CBDP markers also generated high polymorphism in male genotypes than female genotypes. In terms of average percentage polymorphism between the male and female genotypes, there were no major differences but male genotypes showed slightly higher polymorphism (54.94%) than the female genotypes (51.9%). The average values of PIC, MI and Rp of male genotypes were 0.44, 1.16 and 12.9, respectively which were also higher than PIC (0.42), MI (1.03) and Rp (10.80) values of female genotypes. Among females, SCoT markers detected highest *h* and *I* values in genotype “MS F” while genotype MS F and 92 F had highest *h* and *I* values in CBDP analysis. Genotype Q104 F has the lowest *h* and *I* values in SCoT analysis and 82–18 F in CBDP analysis. Among males, MS M (SCoT analysis) and 32 M (CBDP analysis) had highest *h* and *I* values while 58–5 M had lowest *h* and *I* values in both the marker analysis.

UPGMA clustering algorithm based on Jaccard's similarity matrix of the female and male genotype data, independently generated dendrograms. Female genotypes were grouped in 4 clusters in both the SCoT and CBDP data generated dendrograms. The two markers generated a similar pattern of clustering with slight differences. One similarity

Table 5
Genetic diversity parameters of Jojoba genotypes revealed by CBDP marker.

Genotypes	<i>na</i>	<i>ne</i>	<i>h</i>	<i>I</i>
Q104 F	1.94	1.80	0.43	0.61
Q106 F	1.94	1.81	0.43	0.61
879–154 F	2.00	1.84	0.45	0.64
Clone-64 F	2.00	1.86	0.45	0.64
82–18 F	1.88	1.71	0.39	0.55
58–5 F	2.00	1.80	0.43	0.62
MSF	2.00	1.87	0.46	0.65
17–21 F	2.00	1.83	0.44	0.64
Local F	2.00	1.85	0.45	0.64
32 F	2.00	1.84	0.45	0.64
92 F	2.00	1.88	0.46	0.65
Forti F	2.00	1.77	0.41	0.61
96 F	1.94	1.77	0.41	0.59
K-11 F	1.94	1.75	0.41	0.58
85 F	2.00	1.76	0.41	0.60
48–25 F	2.00	1.83	0.44	0.63
16–10 F	2.00	1.81	0.43	0.62
C8R23 F	2.00	1.85	0.45	0.64
12–18 F	1.94	1.74	0.40	0.58
CSMCRI F	2.00	1.75	0.41	0.60
CAZRI F	2.00	1.77	0.42	0.60
24–8 F	1.94	1.79	0.42	0.60
Q104 M	1.88	1.79	0.41	0.58
Q106 M	1.88	1.76	0.39	0.56
MS M	1.88	1.77	0.40	0.57
Clone 64 M	1.94	1.82	0.43	0.61
82–18 M	1.82	1.73	0.38	0.54
32 M	2.00	1.93	0.48	0.67
879–154 M	1.94	1.82	0.43	0.61
17–21 M	1.76	1.69	0.36	0.51
Local M	1.82	1.68	0.36	0.52
58–5 M	1.82	1.66	0.36	0.51
92 M	1.82	1.70	0.37	0.53
Forti M	1.94	1.80	0.43	0.61
96 M	1.88	1.76	0.40	0.57
K-11 M	1.94	1.80	0.42	0.61
85 M	1.94	1.78	0.42	0.60
48–25 M	1.94	1.82	0.43	0.61
Male cutting	1.88	1.74	0.40	0.57

pattern was observed in both the dendrograms, i.e. genotypes Local F and 32 F were always grouped together, similarly genotypes K-11 F and 85 F were also grouped together in both the marker analysis. In SCoT analysis, genotype CSMCRI F forms an out-group to other groups while in CBDP analysis 82–18 F was out-group to other groups.

Male genotypes were clustered into four clusters in both the marker analysis. The grouping of the genotypes varied in both the markers but two genotypes 48–25 M and Male-cutting were found to be always grouped together.

Comparison between the two marker systems (SCoT and CBDP markers)

Both the markers were found to be effective in studying genetic diversity as evident from the high polymorphic percentage, PIC, MI and Rp values. When compared, CBDP marker generated higher values in all the genetic parameters studied as compared to the SCoT marker. The combined average percent polymorphism, PIC and MI values were 53.4%, 0.43, and 1.10 respectively for CBDP while % polymorphism, PIC and MI values were 49.4, 0.38 and 1.09, respectively for SCoT analysis.

Both the markers generated dendrograms (combined male and female genotype data) in which all the Jojoba genotypes were clustered into two major groups distinguishing the genotypes on the basis of gender: one group consisting of all the female genotypes and another group consisting of all the male genotypes. (Figs. 1, 2). In SCoT marker analysis, though majority of the genotypes were clusters in their respective sex groups but two genotypes, Q104 M and 96 F were not grouped in

their respective sex groups as compared to CBDP markers. The reason might be the incompetency of SCoT marker to separate the genotypes on the basis of sex or may be these genotypes were genetically more distant from the rest of the genotypes.

Discussion

Jojoba is an important oil yielding crop having immense industrial applications. The reduction of genetic variation in Jojoba through domestication and breeding has resulted in the need of conservation, characterization and utilization of genetic resources. During present investigation, two gene targeted molecular markers i.e. SCoT and CBDP were adopted to study the genetic diversity among different male and female Jojoba genotypes. A comparative account of the two markers in detecting genetic variations was also studied. Application of gene targeted markers has advantages over the use of random type of markers (such as RAPD, ISSR) as they measure genetic diversity from the genic regions, i.e. functional diversity present in any species (Paliwal et al., 2013). The existing variations in the nature of genotype or group of genotypes can be identified using a specific statistical method or combination of methods (Kubik et al., 2009). PIC, EMR, MI values of a primer help in determining its effectiveness in genetic diversity analysis. Sivaprakash et al. (2004) suggested that the ability of a marker system to resolve genetic variation may be more directly related to the degree of polymorphism. In our study, both the markers (SCoT and CBDP) revealed independent results and were found to be effective in

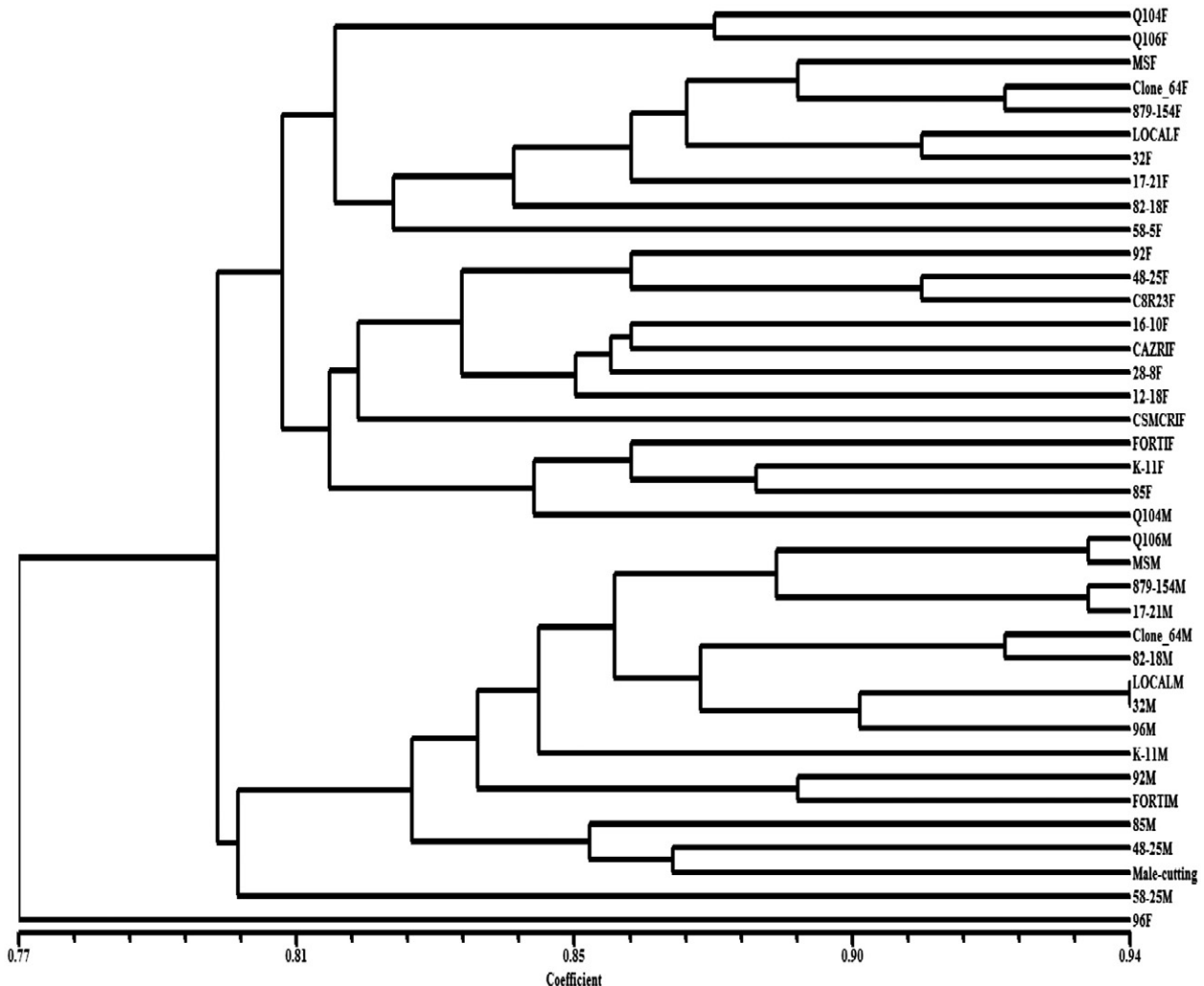


Fig. 1. Dendrogram generated using UPGMA analysis of the SCoT dataset of 39 genotypes (22 females and 17 males) showing genetic relationships among Jojoba genotypes.

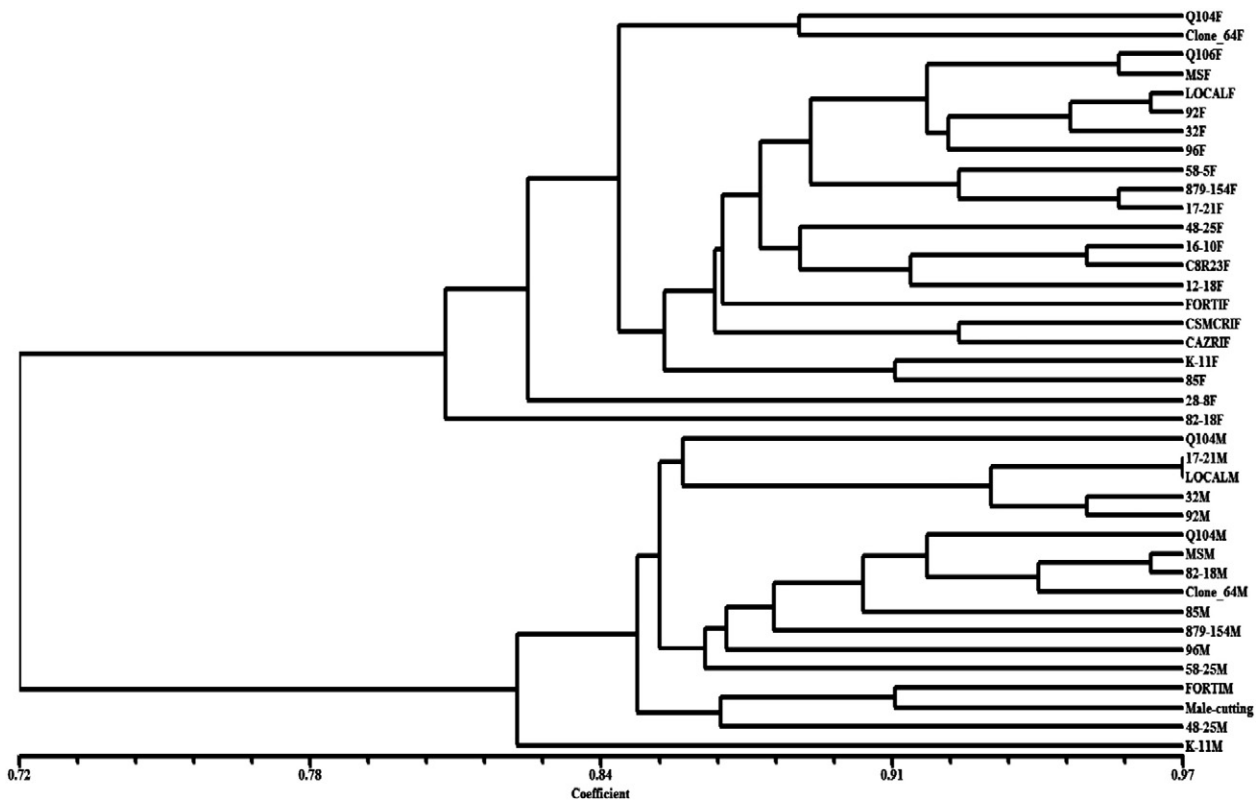


Fig. 2. Dendrogram generated using UPGMA analysis of the CBDP dataset of the 39 genotypes (17 males and 22 females) showing genetic relationships across the Jojoba genotypes.

the estimation of genetic diversity in different genotypes of Jojoba. Their efficiency was evident from high values of polymorphism percent, PIC, Multiplex ratio and average number of polymorphic bands per primer. A diverse level of polymorphism in Jojoba had been reported earlier by Sharma et al. (2009) and Bhardwaj et al. (2010). They compared ISSR and RAPD markers for its efficiency in detecting genetic variability among the genotypes. High genetic variation within the Jojoba population is expected because dioecious plant species in nature tends to have high genetic diversity within populations and low genetic differentiation among populations due to obligate outcrossing (Hamrick and Godt, 1996).

In our study, CBDP markers were found to be more effective than SCoT markers with regard to average percentage polymorphism which was higher (53.4%) compared to 49.4% in SCoT markers. The average PIC value of CBDP marker (0.43) was also higher than the SCoT marker (0.38). Average MI of CBDP marker (1.10) was also higher than the SCoT marker (1.09). However, the average Rp of SCoT markers (14.8) was more than that of CBDP markers (11.85). The two marker techniques were found to resolve differently, which could be due to differences in the resolution of targeted different regions of the genome as suggested by Souframanien and Gopalakrishna (2004) and Gajera et al. (2010), reinforcing the importance of the number of loci and their coverage of the whole genome for obtaining estimates of genetic relationship among cultivars (Souframanien and Gopalakrishna, 2004).

CBDP marker utility has been reported only in Jute, cotton and linseed (Singh et al., 2014) while SCoT has been proved effective in analysis genetic diversity in diverse plant species (Amirmoradi et al., 2012; Bhattacharyya et al., 2013; Gorji et al., 2011; Guo et al., 2012; Luo et al., 2011; Paliwal et al., 2013; Que et al., 2014; Shahlaei et al., 2014; Xiong et al., 2011).

The genetic diversity index (h) reflects diversity and differentiation among the germplasm collections while Shannon's index (I) reflects genetic diversity within and between the populations (Que et al., 2014). The higher the indices, the greater the genetic diversity. The extent of variability among na , ne , h and I indices in both the SCoT and CBDP

markers indicated a high level of genetic diversity among the 22 female and 17 male Jojoba genotypes. Genotype "MS F" had the highest genetic diversity parameters among female genotypes in both analyses while Genotype "Q104 F" in SCoT and "82–18 F" in CBDP analysis had the least values. Among male genotypes, MSM genotype in SCoT and 32 M in CBDP analysis showed maximum genetic diversity while 58–5 M genotype and 17–21 M showed least genetic values. Though the genetic diversity parameters for the two markers revealed different values, the overall values for all the genotypes are almost same or have nearby values. Dje et al. (2000) reported that higher the genetic distance between parents, the higher the heterosis in the developed progenies. So, most diverse set of parents can be considered for further breeding programs. Thus, in Jojoba the genotypes showing diverse values can be used as parental combinations for breeding purposes. The more diverse the parents, the more chances of getting new genetic combination.

Comparison of the genetic polymorphism between male genotype population and female genotype population by SCoT and CBDP markers revealed that the male genotype populations in both cases yielded higher polymorphism and PIC, MI, Rp values than the female populations. This is in agreement with the reports on *Pistacia atlantica* (Nosrati et al., 2012) and *S. chinensis* (Sharma et al., 2009). Nosrati et al. (2012) concluded from their study that higher levels of genetic variation in male populations resulted due to the higher number of male plants and even distribution of males than females. Similar interpretation could be drawn for Jojoba because of its male biased population (5:1; male:female ratio) in nature. Sex ratio in dioecious populations in general deviates from 1:1 ratio because of the more resource allocation of the female plants to reproduction leading them to be vulnerable (Stehlik and Barrett, 2005). Vandepitte et al. (2010) also suggested that in most cases male populations have higher individual numbers as compared to female populations.

It is expected that the identified variations would form a pattern of genetic relationship useable in grouping of genotypes. The recorded variations are primarily because of the differences in the nature of genetic materials. On the basis of these data, dendrograms were created

and both the markers showed different clustering patterns revealing genetic variation pattern among the Jojoba genotypes. The differences in the pattern of dendrograms when two markers were used had also been reported in Shisham (Arif et al., 2009) and potato (Gorji et al., 2011). The dendrograms were mainly clustered into two major groups, one representing all the female genotypes and another showing all the male genotypes, indicating an apparent gender-based distinction among the Jojoba genotypes. Similar clustering of the males and females separately in a dendrogram had also been reported earlier in dioecious taxa such as *Actinidia deliciosa* (Shirkot et al., 2002); *Piper betle* (Verma et al., 2004), *S. chinensis* (Sharma et al., 2009), *Pandanus tectorius* (Panda et al., 2010) and *P. atlantica* (Nosrati et al., 2012). Based on the aforesaid findings, it is concluded that Jojoba genotypes collected from AJORP are genetically diverse and the breeders can choose the most diverse parents for breeding programs and for its germplasm collection and management.

Acknowledgments

The authors are grateful to the Science and Engineering Research Board, Government of India, New Delhi for the sanction of a Major research project (SERB/SR/SO/PS/05/2012) to Veena Agrawal. Monika Heikrujam is indebted to the Council of Scientific and Industrial Research for the award of CSIR JRF and SRF.

References

- Agarwal, M., Shrivastava, N., Padh, H., 2011. Development of sex-linked AFLP markers in *Simmondsia chinensis*. *Plant Breed.* 130, 114–116.
- Agrawal, V., Sharma, K., Gupta, S., Prasad, M., 2007. Identification of sex in *Simmondsia chinensis* (Jojoba) using RAPD markers. *Plant Biotechnol. Rep.* 1, 207–210.
- Amirmoradi, B., Talebi, R., Karami, E., 2012. Comparative of genetic variation and differentiation among annual Cicer species using start codon targeted (SCoT) polymorphism, DAMD-PCR, and ISSR markers. *Plant Syst. Evol.* 298, 1679–1689.
- Arif, M., Zaidi, N.W., Singh, Y.P., 2009. A comparative analysis of ISSR and RAPD markers for study of genetic diversity in Shisham (*Dalbergia sissoo*). *Plant Mol. Biol. Rep.* 27, 488–495.
- Benoist, C., O'hare, K., Breathnach, R., Chambon, P., 1980. The ovalbumin gene – sequence of putative control regions. *Nucleic Acids Res.* 8, 127–142.
- Benzioni, A., Forti, M., 1989. Jojoba. In: Roebelen, G., Downey, R.K., Ashri, A. (Eds.), *Oil Crops of the World; Their Breeding and Utilization*. McGraw-Hill, New York, pp. 448–461.
- Bhardwaj, M., Uppal, S., Jain, S., Kharb, P., Dhillon, R., Jain, R.K., 2010. Comparative assessment of ISSR and RAPD marker assays for genetic diversity in Jojoba [*Simmondsia chinensis* (Link) Schneider]. *J. Plant Biochem. Biotechnol.* 19, 255–258.
- Bhattacharyya, P., Kumaria, S., Kumar, S., Tandon, P., 2013. Start Codon Targeted (SCoT) marker reveals genetic diversity of *Dendrobium nobile* Lindl. An endangered medicinal orchid species. *Gene* 529, 21–26.
- Collard, B.C.Y., Mackill, D.J., 2009. Start Codon Targeted (SCoT) polymorphism: a simple novel DNA marker technique for generating gene-targeted markers in plants. *Plant Mol. Biol.* 27, 86–93.
- De Jong, T.J., Klinkhamer, P.G.L., 2005. *Evolutionary Ecology of Plant Reproductive Strategies*. Cambridge University Press, Cambridge, UK.
- Dje, Y., Hevretz, M., Letebure, C., Vekemans, X., 2000. Assessment of genetic diversity within and among germplasm accessions in cultivated sorghum using microsatellite markers. *Theor. Appl. Genet.* 100, 918–925.
- Engen, S., Lande, R., Saether, B.E., 2003. Demographic stochasticity and Allee effects in populations' with two sexes. *Ecology* 84, 2378–2386.
- Gajera, B.B., Kumar, N., Singh, A.S., Punvar, B.S., Ravikiran, R., Subhash, N., Jadedja, G.C., 2010. Assessment of genetic diversity in castor (*Ricinus communis* L.) using RAPD and ISSR markers. *Ind. Crop. Prod.* 32, 491–498.
- Gentry, H.S., 1958. The natural history of Jojoba (*Simmondsia chinensis*) and its cultural aspects. *Econ. Bot.* 12, 261–295.
- Gorji, A.M., Pocza, P., Polgar, Z., Toller, J., 2011. Efficiency of arbitrarily amplified dominant markers (SCoT, ISSR and RAPD) for diagnostic fingerprinting in tetraploid potato. *Am. J. Potato Res.* 88, 226–237.
- Guo, Da-Long, Zhang, J.Y., Liu, C.H., 2012. Genetic diversity in some grape varieties revealed by SCoT Analyses. *Mol. Biol. Rep.* 39, 5307–5313.
- Hamrick, J.L., Godt, M.J., 1996. Effects of life history traits on genetic diversity in plant species. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 351, 1291–1298.
- Heikrujam, M., Sharma, K., Kumar, J., Agrawal, V., 2014a. Validation of male sex-specific UBC-8071₂₀₀ ISSR marker and its conversion into sequence tagged sites marker in Jojoba: a high precision oil yielding dioecious shrub. *Plant Breed.* 133, 666–671.
- Heikrujam, M., Sharma, K., Kumar, J., Agrawal, V., 2014b. Generation and validation of unique male sex-specific sequence tagged sites (STS) marker from diverse genotypes of dioecious Jojoba—*Simmondsia chinensis* (Link) Schneider. *Euphytica* 199, 363–372.
- Hilfiker, K., Gugerli, F., Schutz, J.P., Rotach, P.A., Holderegger, R., 2004. Low RAPD variation and female-biased seed ratio indicate genetic drift in small populations of the dioecious conifer *Taxus baccata* in Switzerland. *Conserv. Genet.* 5, 357–365.
- Hosseini, F.S., Hassani, H.S., Arrin, M.J., Baghizadeh, A., Mohammadi-Nejab, G., 2011. Sex determination of Jojoba (*Simmondsia chinensis* cv. Arizona) by random amplified polymorphic DNA (RAPD) molecular marker. *Afr. J. Biotechnol.* 10, 470–474.
- Hu, J., Vick, B., 2003. Target region amplification polymorphism: a novel marker technique for plant genotyping. *Plant Mol. Biol. Report.* 21, 289–294.
- Ince, A.G., Karaka, M., 2011. Early determination of sex in Jojoba plant by CAPS assay. *J. Agric. Sci.* 149, 327–336.
- Ince, A.G., Karaca, M., Onus, A.N., 2010. A reliable gender diagnostic PCR assay for Jojoba [*Simmondsia chinensis* (Link) Schneider]. *Genet. Resour. Crop. Evol.* 57, 773–779.
- Jaccard, P., 1908. Nouvelles recherches sur la distribution florale. *Bull. Soc. Vaud. Sci. Nat.* 44, 223–270.
- Kubik, C., Honig, J., Meyer, W.A., Stacy, A.B., 2009. Genetic diversity of creeping bentgrass cultivars using SSR markers. *Intern. Turfgrass Soc. Res. J.* 11.
- Li, Z.L., Nelson, R.L., 2001. Genetic diversity among soybean accessions from three countries measured by RAPDs. *Crop Sci.* 41, 1337–1347.
- Li, G., Quiros, C.F., 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. *Theor. Appl. Genet.* 103, 455–461.
- Luo, C.H., Chen, X.H., Ou Shi-jin, H., Mei-ping, G., Brown, S.J., Tondo, C.T., Schnell, R.J., 2011. Genetic diversity of mango cultivars estimated using SCoT and ISSR markers. *Biochem. Syst. Ecol.* 39, 676–684.
- Nosrati, H., Husainpourfeizi, M.A., Khorasani, M., Razban-Haghighi, A., Nikniazi, M., 2012. Sex ratio and genetic diversity in the dioecious *Pistacia atlantica* (Anacardiaceae). *J. Agri Biol.* 29, 41–46.
- Paliwal, R., Singh, R., Singh, A.K., Kumar, S., Kumar, A., Majumdar, R.S., 2013. Molecular characterization of Giloe (*Tinospora cordifolia* Willd. Miers ex Hook. F. and Thoms.) accessions using Start Codon Targeted (SCoT) markers. *Int. J. Med. Aromat. Plants* 3, 413–422.
- Panda, K.K., Sahoo, B., Dasb, A.B., Pandaa, B.B., 2010. Use of RAPD markers to detect sex differences in *Pandanus tectorius* Parkinson, an important bioresource plant in Orissa, India. *Int. J. Biodivers. Sci. Ecosyst. Serv. Manag.* 6, 28–34.
- Paterson, A.H., Tanksley, S.D., Sorrells, M.E., 1991. DNA markers in plant improvement. *Adv. Agron.* 46, 39–90.
- Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S., Rafalski, A., 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol. Breed.* 2, 225–238.
- Prevost, A., Wilkinson, M.J., 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theor. Appl. Genet.* 98, 107–112.
- Que, Y., Pan, Y., Lu, Y., Yang, C., Yang, Y., Huang, N., Xu, L., 2014. Genetic Analysis of Diversity Within a Chinese Local Sugarcane Germplasm Based on Start Codon Targeted Polymorphism. <http://dx.doi.org/10.1155/2014/468375>.
- Rao, V.R., Hodgkin, T., 2002. Genetic diversity and conservation and utilization of plant genetic resources. *Plant Cell Tissue Organ Cult.* 68, 1–19.
- Reusch, T.B.H., Ehlers, A., Hammerli, A., Worm, B., 2005. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proc. Natl. Acad. Sci. U. S. A.* 102, 2826–2831.
- Rohlf, F.J., 2000. *NTSYSpc: Numerical Taxonomy and Multivariate Analysis System*. Version 2.11x. Exeter Software, Setauket, New York.
- Roldan-Ruiz, I., Gilliland, T.J., Calsyn, E., Gilliland, T.J., Coll, R., Van Eijk, M.J.T., De Loose, M., 2000. Estimating genetic conformity between related ryegrass (*Lolium*) varieties. II. AFLP characterization. *Mol. Breed.* 6, 593–602.
- Saghai-Marouf, M.A., Soliman, K.M., Jorgensen, R.A., Allard, R.W., 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proc. Natl. Acad. Sci. U. S. A.* 81, 8014–8018.
- Shahlaei, A., Torabi, S., Khosroshahi, M., 2014. Efficacy of SCoT and ISSR markers in assessment of tomato (*Lycopersicon esculentum* Mill.) genetic diversity. *Int. J. Biol. Sci.* 5, 14–22.
- Sharma, K., Agrawal, V., Prasad, M., Gupta, S., Kumar, R., Prasad, M., 2008. ISSR marker-assisted selection of male and female plants in a promising dioecious crop, jojoba (*Simmondsia chinensis*). *Plant Biotechnol. Rep.* 2, 239–243.
- Sharma, R., Chowdhury, V.K., Jain, S., Jain, R., 2009. A comparative study of genetic relationships among and within male and female genotypes of dioecious Jojoba (*Simmondsia chinensis* L. Schneider) using RAPD and ISSR markers. *Asian J. Hortic.* 4, 184–193.
- Shirkot, P., Sharma, D.R., Mohapatra, T., 2002. Molecular identification of sex in *Actinidia deliciosa* var *deliciosa* by RAPD markers. *Sci. Hortic.* 94, 33–39.
- Singh, A.K., Rana, M.K., Singh, S., Kumar, S., Kumar, R., Singh, R., 2014. CAAT box-derived polymorphism (CBDP): a novel promoter-targeted molecular marker for plants. *J. Plant Biochem. Biotechnol.* 23, 175–183.
- Sivaprakash, K.R., Prasanth, S.R., Mohanty, B.P., Parida, A., 2004. Genetic diversity of black gram landraces as evaluated by AFLP markers. *Curr. Sci.* 86, 1411–1415.
- Soufframanien, J., Gopalakrishna, T., 2004. A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. *Theor. Appl. Genet.* 109, 1687–1693.
- Stehlik, I., Barrett, S.C.H., 2005. Mechanism governing sex-ratio variation in dioecious *Rumex nivialis*. *Evolution* 59, 814–825.
- Vandepitte, K., Honnay, O., De Meyer, T., Jacquemyn, H., Roldan-Ruiz, I., 2010. Patterns of sex ratio variation and genetic diversity in the dioecious forest perennial *Mercurialis perennis*. *Plant Ecol.* 206, 105–114.
- Verma, A., Kumar, N., Ranade, S.A., 2004. Genetic diversity amongst landraces of a dioecious vegetatively propagated plant, betelvine (*Piper betle* L.). *J. Biosci.* 29, 319–328.
- Wang, Q.H., Zhang, B.L., Lu, Q.S., 2009. Conserved region amplification polymorphism (CoRAP), a novel marker technique for plant genotyping in *Salvia miltiorrhiza*. *Plant Mol. Biol. Report.* 27, 139–143.
- Wisniak, J., 1994. Potential uses of Jojoba oil and meal—a review. *Ind. Crop. Prod.* 3, 43–68.
- Xiong, F., Zhong, R., Han, Z., Jiang, J., He, L., Zhuang, W., Tang, R., 2011. Start codon targeted polymorphism for evaluation of functional genetic variation and relationships in cultivated peanut (*Arachis hypogaea* L.) genotypes. *Mol. Biol. Rep.* 38, 3487–3494.
- Yeh, F.C., Yang, R.C., Boyle, T., 1999. POPGENE 32-version 1.31. Population genetics software. <http://www.ualberta.ca/~fyeh/fyeh/>.