Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; SP1: 636-641

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(Special Issue- 1)
2nd International Conference
"Food Security, Nutrition and Sustainable Agriculture - Emerging Technologies"
(February 14-16, 2019)

Paternal contribution in the Intra-specific hybrid of *Grewia optiva* as reveled by paternity analysis using molecular markers

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Abstract
Superior genotype of *Grewia optiva*, SO-2 (Nauni, Solan) as female and BI-4 (Khutira, Bilaspur) as male genotype were selected on the basis of their morphological and genetic variability, the plants were hybridized to produce an intra-specific hybrid (F_1). The F_1 obtained is morphologically similar to its paternal parent to confirm its parentage, fourteen RAPD and eleven ISSR markers were used in the study. The primers used were successful in confirming hybridity. The hybrid was highly similar to their male parents. Similarity of hybrid between the combined banding of genotypes for individual primers varied from 17 to 100 % (RAPD) and 33 to 80 % (ISSR). Whereas, overall similarity of hybrid was 71 % for RAPD and 68 % in ISSR. In the present study, RAPD and ISSR profile generated by individual primers exhibited up to 100 % and 80% polymorphism respectively. From the present investigation, we can conclude that the intra-specific hybrid (F_1) produced is a cross between SO-2 and BI-4 and it is evident from both morphological character and molecular phylogeny using RAPD and ISSR profiling the F_1 is tends to be more similar to its paternal parent (BI-4) than that of maternal (SO-2). So, RAPD and ISSR profiling can be successfully used for genetic fingerprinting including verification of intra-specific hybrid in *G. optiva.*

Keywords: *Grewia optiva*, intra-specific, RAPD, ISSR, paterntage

Introduction
India is an agriculture dominated country having largest livestock population in the world. Apart from this livestock rearing is one of the major occupations found in India that provides multiuse products and forms important source of food and cash income to millions of households across various parts of the country. Closer scrutiny of the sector, however, reveals that the contribution to the GDP by livestock sector is far too low from such a large size of livestock population. This low productivity of the sector is as much attributable to underfeeding of the livestock. An idea of the meager fodder availability can be had from the fact that about 50% of the cattle population, depending largely upon free rangeland grazing in forests, pastures, village commons, agroforestry filed and they like, end up getting only about 1.5 kg of dry fodder/ day/ ACU (Adult Cattle Unit) as against the healthy fodder requirement norm of 3% body weight. In developing country like India, the production of fodder has not been gained much emphasis and it is estimated to be only about four per cent of total cultivable area of the country under this category (Anonymous 2014, Verma, 2012) [2, 15]. The reason for the shortage of fodder are mainly because it is handicapped by the difficulty in setting apart sufficient cultivated area for fodder production, lack of irrigation facilities, low economic returns from fodder crops as compared to cash crops, *etc.*, trees which can be grown either in combination with agricultural crops or on separate land usually offer opportunity of producing green nutritious fodder for the livestock (Singh and Kanstra 1981) [13]. Moreover, Fodder production is very essential component of hilly people for their ecological and economy security. By 2020, the country is about to face an estimated shortage of 728 million tons of green fodder and 157 million tones of dry fodder (Anonymous 2011) [3]. The available fodder is not only scarce but also poor in nutritive value and seasonal. With the increase in

demand for fodder, there is a need to develop new hybrids of green leaf fodder species. The major sources of fodder are the trees growing on forests and common lands. However, with high timber value plantations coming up on scrub forests; this resource is getting depleted from near habitations. Alternative source of tree leaf fodder can be obtained from the trees growing on farm lands. One of the important fodder species that aid in improving the nutrition of livestock in the hills is *Grewia optiva* Drummond.

Grewia optiva Drummond, locally called as 'Beul' or 'Bhimal', belongs to family Tiliaceae which is highly a cross pollinated species with some per cent of self pollination characters. It is a very important agroforestry tree species of the low and mid-hills regions in the western and central Himalaya's (Watson and Dallwitz 1992) [17]. It is an important fodder tree during the winters for villagers when no other fodder is available. Apart from fodder, species is highly valued for its fibre, fuel and small timber for making agricultural implements. *G. optiva* is suited to different agro-climatic zones with improved yield. Therefore, it is essential to understand the genetic architecture of *G. optiva*, which provides useful guidelines to determine the source population and from which it is possible to derive appropriate genotypes with desired characters. The selection of parent, developing the hybrid and assessing their purity is an important aspect of tree breeder. Conventional breeding programmes reach this goal by generating an F_1 hybrid and F_2 segregating population and then screening, which is followed by a process of repeated backcrossing, selfing and testing, which is time consuming and difficult to achieve with classical methods. To optimize time and costs, it is necessary to verify the hybridity of new seedlings at an early stage. Use of molecular markers facilitate these breeding processes, since it can provide means of detecting and resolving complications and accelerate the breeding programme. Molecular markers not only help in studying molecular phylogeny but also allow the easy and reliable identification of F_1 hybrids which contain DNA from both parents, identification of male and female parent-specific markers will allow differentiation of true hybrids. In this regard, in recent years molecular techniques are providing a useful tool for the correct identification of plant species, included hybrid taxa. In particular, DNA markers have been often used for hybrid characterization in tree species. Among these, RAPDs and ISSRs have been used for finger printing tree species (Dumolin *et al*. 1995) [7]. As *G. optiva* is an important fodder and agroforestry species of hilly regions of Himalayas, there is a wide scope exist for hybridization process for the overall improvement of yield of this species and there is no evidence regarding attempt to improve the quality characters and testing its purity was found so this was an attempted to find the molecular phylogeny and parentage analysis in intra-specific hybrid of *G. optiva* using RAPD and ISSR markers.

Materials and Methods

Plant material

G. optiva genotypes from different districts of Himachal Pradesh are maintained in the seed orchard of Department of Tree Improvement and Genetic Resources, Dr Y. S. Parmar University of Horticulture and Forestry Nauni, Solan (H.P.) India. For carrying out present study, two superior genotype, SO-2 (Nauni) of Solan (30°86'N and 77°16'E) as female and BI-4 (Khutira) of Bilaspur (31° 56'N and 76°48'E) as male genotype were selected on the basis of their morphological character (Table 1) and genetic variability, the plant material were hybridized to produce the F_1 (Table 2 & 3). The morphological characters such as petiole shape, number of teeth per centimeter on the broadest part of leaf blade, number of lateral vein was counted by using magnifying lens, fully grown leaves were collected and leaf area (cm^2) was measured with the help of leaf area meter. Fresh and dry weight (g) of 10 matured leaves weighed unbiasedly using the electronic balance. However, length of petiole (cm), width of apex (cm) and length of apex (cm) was measured by measuring scale and petiole (mm) was measured by digital Vernier calliper. For fodder quality parameter we followed standard described by AOAC (1995) [4]. The above mentioned characters were also observed in F_1 which showed superiority of male or female for some characters and for some they exhibited intermediate response against their parents (Table 1). The above mentioned observation was recorded in fully matured leaves of parents as well as in F_1. The morphological and fodder quality characters that the F_1 obtained has compared with its parents and found that it is having more resemblance to the BI-4 (male) genotype.

So, to prove its genetic worth fresh and disease free leaves were collected in brown paper bags collected from the parents and hybrid for molecular phylogeny and parentage analysis and kept in deep freezer (- 20°C) for future DNA extraction.

Table 1: Leaf and fodder quality characters of SO-2, BI-4 genotype and their F_1.

Leaf characters	SO-2 (Nauni)	BI-4 (Khutira)	F_1 (SO-2 X BI-4)
Petiole shape	Ovate	Rhomb	Rhomb
Number of teeth per cm on the broadest part of leaf blade	4	5	5
Length of Petiole (cm)	0.82	0.98	0.92
Diameter of petiole (mm)	<1.2 mm	1.2-1.5 mm	>1.5 mm
Number of Lateral Vein	14.3	12.2	12.3
Lateral veins of 2nd and 3rd order on abaxial surface	Not raised	Raised	Intermediate
Width of Apex (cm)	0.82	0.85	0.75
Length of Apex (cm)	1.43	1.25	1.45
Leaf Area (cm^2)	27.00	34.02	32.51
Fresh weight of 10 leaf (g)	1.48	1.90	1.97
Dry weight of 10 leaf (g)	0.64	0.86	0.86
Fodder quality			
Crude protein (%)	16.1	17.54	19.69
Crude fat (ether extract %)	5.07	5.83	5.44
Crude fibre (%)	18.95	19.65	19.60
Total ash (%)	9.00	9.67	9.82
Nitrogen free extract (%)	47.44	45.68	46.46

Table 2: RAPD analysis showing details on amplification product produced, polymorphism and similarity between parents and hybrids

No.	Primers	Sequence	Number of amplification products					Number of bands gained/ lost by F_1	Polymorphism between SO-2 & BI-4 (%)	Similarity between the combined banding pattern & the hybrid banding pattern (%)
			SO-2 Genotype (Female)	BI-4 Genotype (Male)	Combined banding pattern of SO-2 & BI-4	Polymorphic banding pattern of SO-2 & BI-4	F_1 (SO-2 X BI-4)			
1	OPA 01	AAGCCTCGTC	5 (1)*	4 (0) *	5 (1,0) *	4	3 (0,0) *	-1	80.00	60.00
2	OPA 04	GGGAATTCGG	4 (0) *	6 (2) *	6 (0,2) *	4	6 (0,2) *	+2	66.67	100.00
3	OPA 05	GGGATATCGG	4 (1) *	5 (2) *	6 (1,2) *	3	5 (0,2) *	+2	50.00	83.33
4	OPA 07	ACGGTACCAG	5 (0) **	6 (1) *	6 (0,1) *	5	5 (0,0) *	+0	83.33	83.33
5	OPAC 10	CAGGCCCTTC	4 (2) *	2(0) *	4 (2,0) *	2	2 (0,0) *	+0	50.00	50.00
6	OPAC 15	AATCGGGCTG	3 (0) *	6 (3) *	6 (0,3) *	3	5 (0,2) *	+2	50.00	83.33
7	OPAE 02	AGGGGTCTTG	8 (3) *	5 (0) *	8 (3,0) *	5	5 (0,0) *	+0	62.50	62.50
8	OPAE 10	ACACACGCTG	3 (0) *	5 (2) *	5 (0,2) *	3	3 (0,0) *	+0	60.00	60.00
9	OPAE 15	CTCAAGCGCA	5 (2) *	4 (1) *	6 (2,1) *	3	1 (0,0) *	-2	50.00	16.67
10	OPC 13	TGCCGTGAGA	4 (2) *	3 (1) *	5 (2,1) *	2	6 (2,1,1#)*	+3+1#	40.00	83.33
11	OPF 06	CTCTCGGCGA	4 (1) *	3 (0) *	4 (1,0) *	3	2 (0,0) *	-1	75.00	50.00
12	OPF 08	CACGAACCTC	8 (1) *	7 (0) *	8 (1,0) *	7	7 (0,0) *	+0	87.50	87.50
13	OPF 12	GTGCCCGATC	7 (0) *	8 (1) *	8 (0,1) *	7	8 (0,1) *	+1	87.50	100.00
14	OPO 20	AGTCCGCCTG	6 (1) *	6 (1) *	8 (1,1) *	5	6 (0,1) *	+1	75.00	75.00
Total/ average			70(14) *	70(14) *	85(14, 14) *	57	59 (2,9,1#)*	+12-4 = +6	65.54	71.07

* Value in the parentheses represents the unique band(s) produced by RAPD marker in parent genotype and their contribution in F_1

Unique band produced only in the F_1

Table 3: ISSR analysis showing details on amplification product produced polymorphism and similarity between parents and hybrids.

No.	Primers	Sequence	Number of amplification products					Number of bands gained/ lost by F_1	Polymorphism between SO-2 & BI-4 (%)	Similarity between the combined banding pattern & the hybrid banding pattern (%)
			SO-2 Genotype (Male)	BI-4 Genotype (Female)	Combined banding pattern of SO-2 & BI-4	Polymorphic banding pattern of SO-2 & BI-4	F_1 (SO-2 X BI-4)			
1	809	AGAGAGAGAGAGAGAGG	3 (1)*	4 (2) *	5 (1,2) *	2	4 (0,2) *	+2	40.00	80.00
2	810	GAGAGAGAGAGAGAGAT	5 (0) *	6 (1) *	6 (0,1) *	5	4 (0,0) *	-1	83.33	66.67
3	811	GAGAGAGAGAGAGAGAC	7 (1) *	7 (1) *	8 (1,1) *	6	6 (1,1) *	+2	75.00	75.00
4	825	ACACACACACACACACC	4 (0) *	7 (3) *	7 (0,3) *	4	4 (0,0) *	+0	57.14	57.14
5	834	AGAGAGAGAGAGAGAGYT	4 (1) *	3 (0) *	4 (1,0) *	3	3 (0,0) *	+0	75.00	75.00
6	835	AGAGAGAGAGAGAGAGYC	5 (1) *	7 (3) *	8 (1,3) *	4	3 (0,0) *	-1	50.00	37.50
7	864	ATGATGATGATGATGATG	5 (1) *	4 (0) *	5 (1,0) *	4	4 (0,0) *	+0	80.00	80.00
8	881	GGGGTGGGGTGGGGTC	3 (2) *	1 (0) *	3 (2,0) *	1	1 (0,0) *	+0	33.33	33.33
9	UBC-807	AGAGAGAGAGAGAGAGT	5 (1) *	4 (0) *	5 (1,0) *	4	4 (0,0) *	+0	80.00	80.00
10	UBC-826	ACACACACACACACACAC	5 (1) *	4 (0) *	5 (1,0) *	4	4 (0,0) *	+0	80.00	80.00
11	UBC-841	GAGAGAGAGAGAGAGAYC	6 (1) *	5 (0) *	7 (1,0) *	6	7 (0,0,1#)*	+1#	85.71	80.00
Total/Average			53 (10) *	53 (10) *	63 (10, 10) *	43	44 (1,3,1#)*	+5-2=3	67.23	67.69

* Value in the parentheses represents the unique band(s) produced by ISSR marker in parent genotype and their contribution in F_1

Unique band produced only in the F_1

DNA extraction

Fresh leaf tissue (~ 0.5 g) was crushed in 7 ml extraction buffer (10 per cent (w/v) SDS (sodium dodecylsulfate), 0.5M EDTA (pH 8.0), 5M NaCl, 1M Tris pH 8.0 and 0.75 g PVP (polyvinylpyrrolidone) was added to remove polyphenols. The powder was either stored at -40^{0}C or used for DNA isolation immediately. Total genomic DNA was isolated using the Dellaporta *et al*. (1983) [6] method with slight modification made in buffer concentrations.

RAPD amplification

In earlier studies, the parent plants were subjected to RAPD analysis (Verma, 2012) [15] and among them fourteen decamer primers which are highly polymorphic and which exhibited unique bands in the parents were used for the current study to know the contribution of particular parents to its F_1 (Table 2). DNA was amplified by PCR amplification reaction. The 25µl of reaction mixture contained 20ng of DNA, 0.75 units of Taq DNA polymerase 2.5µl of 10X Taq buffer (50mM $MgCl_2$, 10mM Tris-Cl), 1.25µl of pooled dNTP's (2.5mM each) and 10ng of primer. PCR conditions used for RAPD amplification included initial denaturation for 3 min at 94°C followed by 45 cycles of amplification (denaturation at 92°C for 45 seconds, annealing of primer at 36°C for 1min and primer amplification at 72°C for 2 min) and final extension at 72°C for 10 min.

ISSR amplification

Verma (2012) [15] has subjected the parent plants to ISSR analysis and among them eleven primers which are highly polymorphic and exhibited unique bands in the parents were used for the current study to know the contribution to its F_1 (Table 3). DNA was amplified by PCR amplification reaction. The 25µl of reaction mixture contained 20ng of DNA, 0.75 units of Taq DNA polymerase, 2.5µl of 10X Taq buffer (50mM MgCl2, 10mM Tris-Cl), 1.25µl of pooled dNTP's (2.5mM each) and 10ng of primer. PCR conditions used for ISSR amplification included initial denaturation for 3 min at 94°C followed by 45 cycles of amplification (denaturation at 92°C for 45 seconds, annealing of primer at 55°C for 1min and primer amplification at 72°C for 2 min) and final extension at 72°C for 10 min.

Amplification products stained in ethidium bromide were separated on 2 per cent agarose gel using 1X TBE buffer (Tris HCI pH 8.0, boric Acid, Ethylene diamine-tetra acetic acid) on horizontal gel electrophoresis apparatus (Banglore Genei, India Limited) and photographed in Alpha Imager gel documentation system. 1kb and 100bp DNA mass ladder were used as molecular weight markers in first and last well of respective gel.

Combined banding Pattern of parents

According to Parani *et al.* (1997) [10] total number of polymorphic and monomorphic bands produced in parent genotype during DNA amplification was considered as combined banding pattern of the parents.

Per cent polymorphism (%) between the parents

To calculate the per cent polymorphism between the parent genotype, the scoring of bands was carried out and total number of bands produced in both parent and F_1 were recorded and calculation done according to formula suggested by Parani *et al*. (1997) [10].

Per cent polymorphism (%)

$$= \frac{\text{Number of bands polymorphic between the genotype}}{\text{Number of bands in combined banding pattern}} \times 100$$

Per cent similarity of F_1 with the parents.

Per cent similarity was calculated as the number of bands in hybrid out of number of bands in the combined banding pattern and expressed in percentage (Parani *et al*. 1997) [10].

Per cent similarity (%)

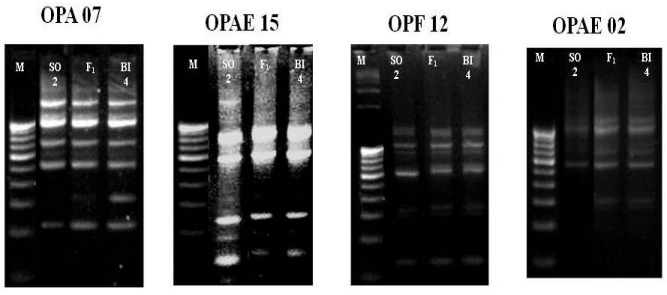
$$= \frac{\text{Number of bands in the hybrid}}{\text{Number of bands in combined banding pattern}} \times 100$$

In case of non-parental bands in F_1 the number of bands in the hybrid was the denominator to calculate the per cent similarity (%).

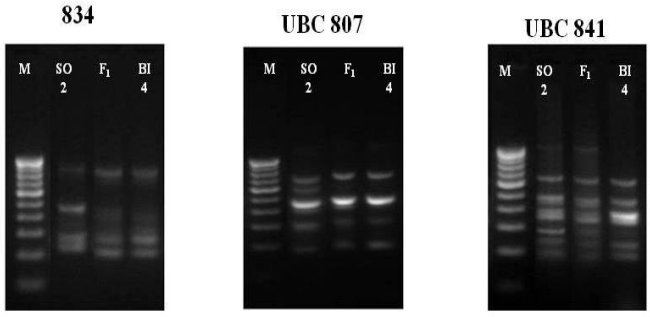
Results and Discussion

Parentage analysis

The hybrid obtained after the completion of hybridization programme was tested for their paternity using RAPD and ISSR markers. A total of fourteen initially screened RAPD markers and eleven ISSR markers were used to confirm the hybrids on the basis of banding pattern. A close appraisal of the RAPD and ISSR banding pattern (Fig.1) obtained after the amplification of genomic DNA of both the parents and their hybrid revealed that all the hybrids were true to type. The RAPD and ISSR fragments obtained after PCR amplification was subjected to analysis for obtaining their polymorphism between their parent, similarity between the combined banding pattern of parents and the hybrid banding pattern (%). The scoring of bands for presence as 1 (band present) and 0 (band absent) for each hybrid combination was done separately by using Alpha Imager software.



(A) RAPD Primers



(B) ISSR Primers

Fig 1: Parentage analysis of SO-2 X BI-4 using

DNA markers are very useful for confirming hybridity of artificial sexual hybrids or somatic fusion hybrids. Molecular markers are especially useful when hybridity is questioned by morphological reasons or for early screening of large putative hybrid populations (Rajendra 2009) [11]. RAPD and ISSR markers have been successfully used for genetic fingerprinting including verification of controlled crosses (hybrids) in tree species (White *et al*. 2007) [18]. RAPD and ISSR markers based on the presence or absence of polymorphism among the individuals was employed for hybrid verification in the selected genotypes of *G. optiva*.

RAPD analysis

PCR amplification of genomic DNA for 14 primers produced 70 (14), 70 (14), 59 (2, 9, 1#) amplification products in SO-2, BI-4 and the F_1, respectively (Table 2.). Polymorphism (proportion of polymorphic bands to the total number of bands) between the genotype for individual primers varied from 40 % (OPC 13) to 87.50 % (OPF 08 and OPF 12) with an average of 65.54 %. RAPD analysis for SO-2 (female) parent having 14 genotype specific bands produced by 9 decamer primers, among these primers OPAE 02 produced 3 bands, OPAC 10, OPAE 15 and OPC 13 showed 2 bands each, whereas, OPA 01, OPA 05, OPF 06, OPF 08 and OPO 20 exhibited one band each. In BI-4 (male) parent genotype also had 14 genotype specific bands produced by 10 decamer primers, among them primers OPAC 15 produced 3 bands, OPA 04, OPA 05 and OPAE 10 produced 2 bands each, However, OPA 07, OPAE 15, OPC 13, OPF 12 and OPO 20 exhibited one band each. The banding pattern of 14 primers, which was observed in the F_1 was present either in SO-2 or in BI-4 or in both, but OPC 13 primer produced an non-parental band in F_1. Total 57 polymorphic bands produced in parents. The banding pattern of the two genotypes were combined to simulate the expected F_1 banding pattern and compared with that of the actual hybrid in order to calculate percentage of similarity. The similarity for individual primers varied from 16.67 (OPAE 15) to 100 % (OPA 04 and OPF 12) with an average of 71.07 %. The F_1 is the result of crossing over between two parents, during this process there may be restriction or free movement in the transfer or expression of gene which may be in recessive form in their parents. The RAPD analysis reveled that the F_1 has a total of 59 amplified bands among these bands two band which is genotypic specific to SO-2 (female) and nine from BI-4 (male) and a non- parental band was found in F_1. Among the additional 12 new bands, OPC 13 primer produced a non-parental band, in primer, OPA 01, OPF 06 (one band each) and OPAE 15 (two) bands found missing in the F_1. The result showed that the F_1 obtained has having more genetically resemblance to the BI-4 (male) genotype (Table 2.).

ISSR analysis

The genomic DNA with eleven ISSR primers followed by its polymerized chain reaction (PCR) showed 100% polymorphism in between two parent genotype (SO-2 & BI-4) and it's F_1. The amplification product of genomic DNA for 11 ISSR primers produced 53 (10), 53(10), 44 (1, 3, 1#) amplification products in SO-2, BI-4 and the F_1, respectively. Polymorphism between the genotype for individual primers varied from 33.33 % (881) to 85.71 % (UBC-841) with an average of 67.23 %. SO-2 (female) parent showed 10 genotype specific bands produced by 9 primers. Primer 809, 811, 834, 835, 864, UBC-807, UBC-826 and UBC-841 produced one band each, 881 exhibited 2 bands. For BI-4 (male) which had 10 genotype specific bands produced by 5 ISSR primers, among them primers 810 and 811 produced 1 band, 809 produced 2 bands, However, 825 and 835 exhibited three bands each (Table 3.).

The banding produced in F_1 by 11 primers was either of the parents SO-2 or BI-4 or of both. UBC-841 primer produced a non-parental band in F_1. Total 43 polymorphic bands were showed in the parents. The combined banding pattern of parents which can be expected in F_1 was estimated and compared with that of the actual hybrid to know the percentage of similarity. The similarity for individual primers varied from 33.33 % (881) to 80 % (809, 864, UBC-807, UBC-826 and UBC-841) with an average of 67.69 %. The hereditary transformation or expression of gene to their F_1 from the parents can be determined by estimating banding pattern of F_1 with the parents. The analysis reveled that the F_1 has a 5 new bands which are carry forward to their half-spring by the parents. The F_1 obtained has produced 44 amplified bands among these bands one genotypic specific band of SO-2 (female) and three from male (BI-4) genotypic specific band. Moreover UBC-841 primer produced a non-parental band. In primer, 810 and 835 one band found missing in the F_1. The ISSR amplification also reveled that the genetic contribution of male is dominating in the F_1 tested. These are evident from the morphological and fodder quality characters in which the majority of the observation resembles the male (BI-4) genotype than the female (SO-2) genotype (Table 3.).

Fourteen RAPD primers *viz.*, OPA 01, OPA 04, OPA 05, OPA 07, OPAC 10, OPAC 15, OPAE 02, OPAE 10, OPAE 15, OPC 13, OPF 06, OPF 08, OPF 12 and OPO-20, whereas, eleven ISSR primers *viz.*, 809, 810, 811, 825, 834, 835, 864, 881, UBC-807, UBC-826 and UBC-841 were successful in confirming hybridity of intra-specific hybrid of *G. optiva.* The investigation disclose that hybrid were highly similar to their male parents. Similarity of hybrid between the combined banding of genotypes for individual primers varied from 17 to 100 % (RAPD) and 33 to 80 %. Whereas overall similarity of hybrid was 71 % for RAPD and 68 % in ISSR. In the present study, RAPD and ISSR profile generated by individual primers exhibited up to 100 % and 80% polymorphism respectively. Since it is not possible to take exactly the same parental genotypes to the hybrid, because missing bands and non-parental bands may appear due variations within the genotypes involved in the hybridization. Moreover, missing bands and non-parental bands were also observed in RAPD analysis of hybrids of known parental genotypes and were not readily explainable (Wang *et al*. 1994, Marshall and Marchand 1994) [16, 9]. Considering these facts, a deviation of 3.5 % from expected similarity is taken as insignificant, and SO-2 and BI-4 are proposed as parental genotypes of the hybrid.

The present findings are consisted with the results of Thawaro and Te-Chato (2009) [14] on application of molecular markers in the hybrid verification in oil palm. Amalendu and Kamble (2009) [1] obtained polymorphism of genomic DNAs of nine parents of *Morus* and their seven hybrids from two wild and four cultivated species. Twelve arbitrary primers of Random Amplified Polymorphic DNA (RAPD) unraveled that in F_1 hybrids, the banding pattern indicates similar to their respective parents. However, a few hybrids showed unique bands, which are different from respective parents. The RAPD technique demonstrated that hereditary variability occurred in between wild and cultivated *Morus* species at

inter and intra-specific levels. Choudhary (2011) [5] tested paternity of *salix* hybrids using four RAPD markers. Results disclosed that six out of nine hybrids were highly similar to their male parents, whereas, three hybrids showed more similarity towards female parents. Similarly study was carried by Samal *et al*. (2012) [12] in 65 mango (*Mangifera indica* L.) genotypes of India including 20 commercial cultivars, 18 hybrids, 25 local genotypes and two exotic cultivars based on qualitative and quantitative fruit characters as well as RAPD profiles. Fifteen RAPD primers yielded 27 monomorphic and 129 polymorphic bands with percent polymorphism averaging 82.7% with its parents. Similar paternity analysis was done by Gao *et al*. (2006) [6] using the Inter-Simple Sequence Repeat (ISSR) for genetic fingerprinting and identification of 28 important *Populus L.* (poplar) cultivars (varieties/clones), and determination of the genetic relationships among these cultivars. Out of 27 ISSR primers tested, eight primers generated clear multiplex profiles. The best three primers produced 154 easily detectable fragments, 129 (84%) of which were polymorphic among the cultivars. Each of these 3 primers produced fingerprint profiles unique to each of the accessions studied, and thus could be solely used for their identification. Samal *et al*. (2012) [12] in mango (*Mangifera indica* L.) genotypes based on qualitative and quantitative fruit characters as well as ISSR profiles. Of a total 70 ISSR bands generated from eight ISSR primers, 60 bands (85.71%) were found to be polymorphic. Cumulative band data from these two methods precisely arranged accessions into eight clusters which correspond well with their pedigree relationship.

A close appraisal of the data (Table 2 & 3) revealed that banding pattern of parent and hybrid generated by fourteen different RAPD and eleven ISSR primers matched with each other. All the hybrids showed the DNA pattern between the two parents involved. From the present investigation, we can conclude that the intra-specific hybrid (F_1) produced is a cross between SO-2 and BI-4 and it is evident from both morphological character and molecular phylogeny using RAPD and ISSR profiling the F_1 is tends to be more similar to its paternal parent (BI-4) than that of maternal (SO-2). So, RAPD and ISSR profiling can be successfully used for genetic fingerprinting including verification of intra-specific hybrid of *G. optiva.*

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