Doi: HTTPS://DOI.ORG/10.23910/2/2021.0396

Genetic Divergence Study in *Grewia* Optiva through Quantitative and Molecular Markers

Shikha Bhagta[1*], Poonam Thakur[2] and Dushyant Sharma[1]

[1]Dept. of Tree Improvement and Genetic Resources, [2]Dept. of Biotechnology, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, HP (173 230), India

Corresponding Author

Shikha Bhagta
e-mail: bhagtashikha@gmail.com

Article History

Article ID: IJEP0396
Received in 25th November, 2020
Received in revised form 13th February, 2021
Accepted in final form 27th February, 2021

Abstract

Diversity analysis amongst 10 different families of *Grewia optiva* was carried out by using RAPD (random amplified polymorphic DNA) and ISSR (inter simple sequence repeats) marker. *Grewia optiva* families were raised by seed collected from various districts of Himachal Pradesh (India) and selected based on morphological parameters. Using 15 RAPD and 20 ISSR primers and 9 RAPD and 12 ISSR primers show amplification respectively. Nine RAPD primers showed 68.96% polymorphism and 12 ISSR primers showed 71.25% polymorphism. Similarity matrices and Dendrograms were generated using SAHN module of NTSYSpc ver.2.02h. Jaccard's similarity matrix revealed maximum similarity coefficient 0.88 between 'SO-7' and 'SO-3' with RAPD primers. For ISSRs, coefficient values ranged from 0.52 to 0.80. Dendrograms also revealed to larger extent similar results and maximum similarity found among the 10 families of *Grewia optiva* collections was 88% between 'SO-7' and 'SO-3'with RAPD primers and 80% between 'SH-7' and 'SO-4' with ISSRs. RAPD and ISSR were found effective in revealing polymorphisms among 10 different genotypes of *Grewia optiva*. UPGMA based dendrograms of both RAPD and ISSR confirmed the placement of different genotypes into different clusters and sub clusters as per geographic distribution and genetic constitution. Family SH-7 came as outliner as revealed by both RAPD and ISSR study.

Keywords: Fodder tree, genetic diversity, *Grewia optiva*, ISSR, RAPD

1. Introduction

The genus *Grewia* consists of some 150 species in world, out of which 42 species are found in Indian subcontinent whereas five species of genus Grewia are found in Himachal Pradesh. *Grewia optiva* (local name: Beul; 2n=18) belonging to family Tiliaceae is one of the most important fodder tree species found in Himachal Pradesh. *G. optiva* is very popular agroforestry tree of low and mid-hills regions in the western and central Himalaya on account of its utility as fodder, fuel and fiber. It is naturally distributed in India, Bhutan, Nepal and Pakistan. In India, it is distributed in areas of Himachal Pradesh, Jammu and Kashmir, Punjab, Sikkim and Uttar Pradesh (Hooker, 1875). It occurs at elevations from 500-2100 meters, where the temperature ranges from 2° to 38°C and rainfall from 1,200 to 2,500 mm in the year (Joshie and Narain, 1992).

With the increase in demand for fodder, there is a need to select superior genotypes for inclusion in breeding program develop clones of genetically superior trees. Therefore, it is essential to understand the genetic architecture of *Grewia optiva*, which provides useful guidelines to determine the source population and from which it is possible to derive appropriate genotypes with desired characters. Use of molecular markers facilitate breeding processes, since it can provide means of detecting and resolving complications and accelerate the generation of new varieties and allow association of phenotypic traits with genomic loci. Molecular markers not only help in studying genetic diversity but also allow the easy and reliable identification of breeding lines, hybrids and cultivars. Because F_1 hybrids 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 (Dumolin et al., 1995). Among these, RAPDs and ISSRS have been the preferred markers for finger printing tree species. RAPDs are fragments of genomic DNA amplified through PCR using a decamer primer of random sequence, where polymorphism depends upon the presence or absence of an amplification product. The use of RAPDs in different organisms began in the late 80's (Williams et al., 1990), and due to their simplicity and speed they have become a very valuable tool for cultivar identification and genetic similarity studies in plants. On the

other hand, ISSR markers share most of the advantageous features of RAPD markers with the addition of a potentially co-dominant pattern of inheritance (Zietckiewicz et al., 1994) and have been considered a promising source of a large number of reliable, highly-polymorphic markers (Salimath et al., 1995).

2. Materials and Methods

In the present study, top ten best performing families (Table 1) out of forty families of *G. optiva* evaluated for morphometric and fodder quality parameters were evaluated for molecular diversity through molecular markers. Fresh and disease free leaves were collected from the selected ten trees in seedling seed orchard of *Grewia optiva.* Fresh, green leaves were separately excised from different plants. Before plucking, the leaves were wiped off the soil with tissue paper and then wrapped in aluminum foil and brought to the laboratory in icebox and stored in deep freezer at -80°C till further use.

Table 1: Top ten selected families based on morphomrteic and fodder quality parameters for molecular studies

Sr. No.	District	Family	Code
1.	Sirmour	Madhobag	SI-15
2.	Solan	Dharja	SO-3
3.	Hamirpur	Patta Balakhar	HA-2
4.	Hamirpur	Bassi	HA-3
5.	Hamirpur	Hamirpur Kanal	HA-4
6.	Solan	Oyali	SO-7
7.	Shimla	Taradevi	SH-7
8.	Solan	Deog	SO-4
9.	Sirmour	Nainatikker	SI-6
10.	Sirmour	Saraha Chakli	SI-14

Genomic DNA from the collected leaves of top ten families separately isolated using CTAB method of Doyle and Doyle (1987) with some modifications wherever required.

2.1. RAPD amplification

DNA amplification was carried out for RAPD analysis using fifteen decamer random primers. DNA was amplified by PCR amplification reaction. The 25 µl of reaction mixture contained 4 µl of DNA (5 ng μl^{-1}), 0.25µl of Taq DNA Polymerase (3U μl^{-1}), 2.5 µl of Taq buffer (10X), 1.25 µl of dNTPs (2.5 mM), 2.0µl of Primer (10 ng) and 15 µl of sterile distilled water. PCR condition for RAPD amplification included initial denaturation for 3min at 94°C followed by 45 cycles of amplification (denaturation at 92°C for 45 seconds, annealing of primer at 36°C for 1 min and primer amplification at 72°C for 2 min) and final extension at 72°C for 10 min.

2.2. ISSR amplification

Fifteen ISSR primers synthesized by M/S Banglore Genei, India Limited were used in the current study. DNA was amplified by PCR amplification reaction. The 24 µl of reaction mixture contained 4µl of DNA (5ng μl^{-1}), 0.25 µl of Taq DNA polymerase (3U μl^{-1}), 1.0 µl of primer (10 ng), 1.25 µl of dNTPs (2.5 mM), 2.5µl of Taq buffer (10X) and 15µl of Sterile distilled water. PCR condition 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. The amplified DNA was mixed thoroughly with 6X loading dye and then electrophoresed in 2% agarose gel in 1X TAE buffer. The gel was run at constant voltage at the rate of 5V cm^{-1} under submerged conditions for about two hours. Ethidium bromide at the rate of 0.5 µg ml^{-1} was incorporated in the gel. Stock solution of ethidium bromide @ 10 mg ml^{-1} was kept ready. DNA profiles were visualized on UV Transilluminator and photographed on Gel Documentation System (Syngene, Cambridge, UK).

2.3. Scoring of bands and data analysis

The scored bands were analyzed in the form of binary system to prepare the similarity index. The bands with same molecular weight and mobility were treated as identical fragments. Data matrices were prepared in which the presence of a band was coded as one whereas the absence as zero. The data matrices were analyzed by the SIMQUAL Program of NTSYS-PC (Version 2.2) and similarities between Families were estimated using Jaccard similarity coefficient, calculated as J = A ÷ (N-D), where A is the number of positive matches (*i.e.* presence of band in both samples), D is the number of negative matches (*i.e.* absence of band in both samples) and N is the total sample size including both the number of matched and unmatched. Dendrogram was produced from the resultant similarity matrices using the UPGMA method.

3. Results and Discussion

3.1. RAPD (Random amplified polymorphic DNA) studies

After initial screening of 15 RAPD, nine RAPD primers producing intense banding pattern and showing polymorphism were used for further study. Total of 29 amplified bands were scored with 9 primers in 10 families of *Grewia optiva*. Six polymorphic and 4 monomorphic bands were observed. Amplification pattern of the primers maximum number of amplified bands i.e. five were produced by primers 'P1'and 'P4' whereas minimum number of bands i.e. one was produced by primer P9. Out of the total 29 scorable bands, 20 showed polymorphism and 9 bands exhibited monomorphism resulting in 68.96% polymorphism among ten families. Five of nine primers exhibited 100% polymorphism.

3.1.1. RAPD data analysis

The data matrix so obtained was analyzed with NTSYS-2.2 software to obtain the Jaccard's similarity correlation coefficient. The mean coefficient value of any families or accession gave an idea about its overall relatedness with

all other families or accession in the study. The coefficient values ranged from 0.413 (among the family HA-2 and SI-14) to 0.875 (among the family SO-7 and SO-3). This indicated a fair range of variability in the similarity coefficient values suggesting a broad genetic base of ten accessions included in the experiment. The above finding are in agreement with Qi et al. (2003), who reported screening of twenty-five primers from 119 random primers in jute, and a total of 329 DNA fragments were amplified ranging from 0.3-3.0 kb, 253 (87.78%), which were polymorphic. Similar results were also revealed by Vaishali et al. (2008) who reported that out of total 145 fragments generated by random decamer primers, 126 (86%) were polymorphic with an average of 10 polymorphic products primer^{-1}. The number of products amplified by the polymorphic primers varied from 8-17. Similarly, Wang et al. (2011) screened 16 decamer primers that showed polymorphisms within five populations of *Dalbergia sissoo* used, and that generated 101 bands ranging in molecular size 200 to 1700 bp (Table 2).

Table 2: Total number of amplified and polymorphic fragments generated by PCR using RAPD primers

Sl. No.	Primer name	Base Sequences (5'-3')	Total no. of scorable bands (y)	Total no. of polymorphic bands(x)	Total no. of monomorphic bands	Polymorphism (%) (x/y)×100
p1	OPC-08	TGG ACC GGT G	5	0	5	0
p2	OPC-11	AAA GCT GCG G	3	3	0	100
p3	OPC-13	AAG CCT CGT C	3	3	0	100
p4	OPF-08	GGG ATA TCG G	5	5	0	100
p5	OPF-11	TTG GTA CCC C	4	3	1	75
p6	OPA-01	CAG GCC CTT C	3	3	0	100
p7	OPA-04	AAT CGG GCT G	3	3	0	100
p8	OPO-17	GGC TTA TGC C	1	0	1	0
p9	OPO-18	CTC GTA TCC	2	0	2	0
		TOTAL	29	20	9	68.96

In the dendrogram (Figure 1), the 10 families separated into two main clusters, 'I' and 'II', at 59% similarity. This revealed less similarity between cluster I and cluster 'II'. Cluster 'II' was further subdivided into two clusters i.e. IIa and IIb at similarity value of 70%. It was concluded that '*S0-3*' and '*SO-7*' were closely related as they showed 88% similarity.

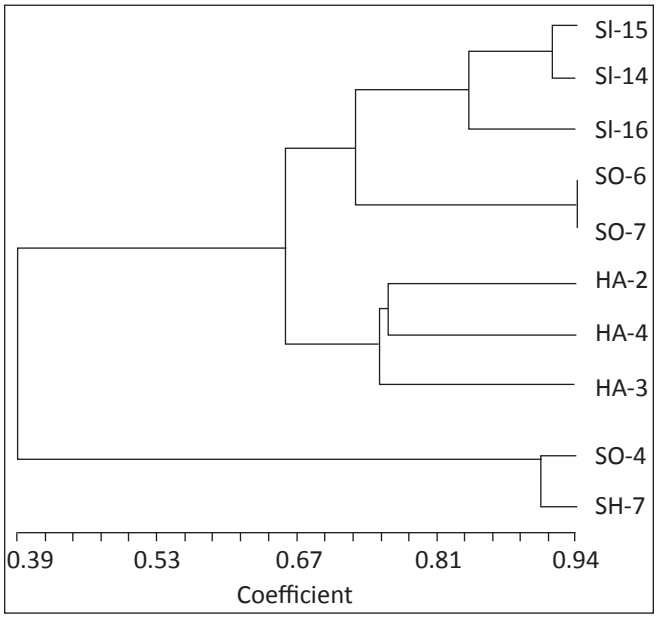

Figure 1: Dendrogram based on UPGMA analysis among 10 families of *Grewia optiva* using RAPD markers

3.2. Inter simple sequence repeat (ISSR) studies

Initial screening of 20 ISSR primers 12 primers produced ISSR profiles with intense banding pattern, which showed polymorphism between 10 accessions used in the study. ISSR analysis revealed high levels of genetic diversity within the reference set of *G. optiva* families. Out of the total 74 scorable bands, 57 showed polymorphism and 17 bands exhibited monomorphism. Total number of amplified and polymorphic fragments generated per ISSR primer revealed 71.25% polymorphism among families (Table 3).

3.2.1. ISSR data analysis

The similarity coefficient values ranged from 0.52 to 0.80. This indicated a fair range of variability suggesting a broad genetic base of thirty accessions included in the experiment. The highest value (0.80) was found between SH-7 and SO-4. The lowest value of 0.52 was exhibited between SI-15 and SO-7, SI-15 and HA-2 depicting that the families were more diverse respectively.

In the dendrogram (Figure 2), the 10 families separated into two main clusters, 'I' and 'II', at 60% similarity. Cluster 'I' contained only one family i.e. SH-7 'and Cluster'II' accommodated rest nine families. This revealed less similarity between cluster I and cluster 'II'. Cluster 'II' was further subdivided into two clusters i.e. IIa and IIb at similarity value of 63%. Families 'SO-3' and 'SO-7' were closely related as they showed 80% similarity.

Table 3: Total number of amplified and polymorphic fragments generated by PCR using ISSR primers in *Grewia optiva*

Sl. No.	Primer name	Base Sequences (5'-3')	Total no. of scorable bands (y)	Total no. of polymorphic bands (x)	Total no. of monomorphic bands	Polymorphism (%) (x/y)×100
1.	809	AGAGAGAGAGAGAGAGG	7	7	0	100.0
2.	810	GAGAGAGAGAGAGAGAT	8	8	0	100.0
3.	811	GAGAGAGAGAGAGAGAC	5	2	3	40.0
4.	812	GAGAGAGAGAGAGAGAA	5	4	1	80.0
5.	830	TGTGTGTGTGTGTGTGG	6	6	0	100.0
6.	834	AGAGAGAGAGAGAGAGYT	6	6	0	100.0
7.	850	GTGTGTGTGTGTGTGTYA	6	6	0	100.0
8.	861	ACCACCACCACCACCACC	6	4	2	66.7
9.	862	AGCAGCAGCAGCAGCAGC	9	3	6	33.3
10.	UBC-807	AGAGAGAGAGAGAGAGT	5	5	0	100.0
11.	UBC-826	ACACACACACACACACAC	4	2	2	50.0
12.	UBC-841	GAGAGAGAGAGAGAGAYC	7	4	3	57.1
		TOTAL	74	57	17	71.25

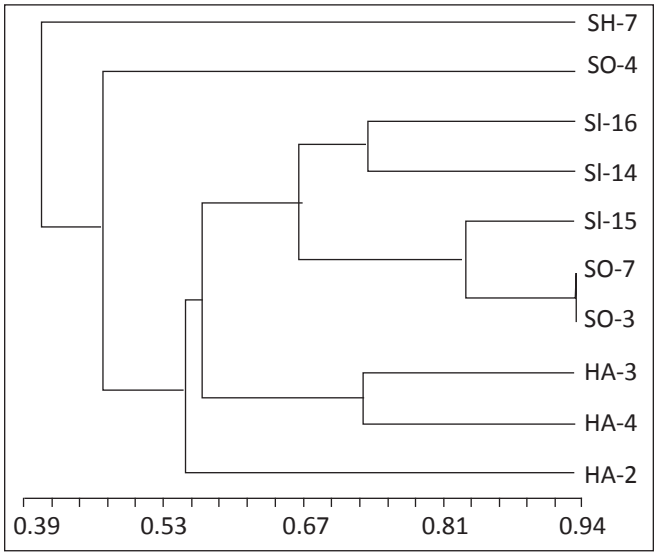

Figure 2: Dendrogram based on UPGMA analysis among 10 families of *Grewia optiva* using ISSR markers

ISSR markers have been successfully used for varietal identification and assessment of genetic relationships in many plant species (Ajibade et al., 2000). In a similar study by Chatterjee et al. (2004) in *Morus alba*, ten ISSR primers generated a total of 58 bands, out of which 43 were polymorphic, thus generating 74.13% polymorphism. In contrast, Verma (2012) in the study of *Grewia optiva* showed that RAPD primers revealed more DNA polymorphism (96.31%) among the genotypes than ISSR primers (91.72%).

4. Conclusion

RAPD and ISSR were found effective in revealing polymorphisms among 10 different families of *Grewia optiva*. UPGMA based dendrograms of both RAPD and ISSR confirmed the placement of different genotypes into different clusters and sub clusters as per both RAPD and ISSR study.

5. References

Ajibade, S.R., Weeden, N.F., Chite, S.M., 2000. Inter simple sequence repeat analysis of geneticrelationships in the genus Vigna. Euphytica 111, 47–55.

Chatterjee, S.N., Nagaraja, G.M., Srivastava, P.P. Naik, G., 2004. Morphological and molecular variation of *Morus laevigata* in India. Genetica 121, 133–143.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissues. Phytochemistry Bulletin 19, 11–15.

Dumolin, S., Demesure, B., Petit, R.J., 1995. Inheritance of chloroplast and mitochondrial genomes in pedunculate oak investigated with an efficient PCR-based method. Theoretical and Applied Genetics 91, 1253–1256.

Hooker, J.D., 1875. Flora of British India. L. Revve & Co., London.

Joshie, P., Narain, P., 1992. Bhimal: A multipurpose tree for agroforestry. Indian Farming 41, 14–15.

Qi, J.M., Zhou, D.X., Wu, W.R., Lin, L.H., Fang, P.P., Wu, J.M., 2003. The application of RAPD technology in genetic diversity detection of Jute. Yi Chuan XueBao 30, 926–932.

Salimath, S.S., De Oliveria, A.C., Godwin, I.D., Bennetzen, J.L., 1995. Assessment of genome origins and genetic diversity in the genus Eleusine with DNA markers. Genome 38, 757–763.

Vaishali, Khan, S., Sharma, V., 2008. RAPD based assessment of genetic diversity of *Butea monosperma* from different agro-ecological regions of India. Indian Journal of Biotechnology 7, 320–327.

Verma, A., 2012. Estimation of genetic diversity and cross ability pattern in *Grewia optiva* Drummond. Thesis, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, 171.

Wang, B.Y., Shi, L., Ruan, Z.Y., Deng, J., 2011. Genetic diversity and differentiation in *Dalbergia sissoo* (Fabaceae) as revealed by RAPD. Genetics and Molecular Rresearch 10**,** 114−120.

Williams, J.G.K., Kubelik, A., Livak, K.J., Rafalski, J.A., Tingey, S.V., 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18**,** 6531−6535.

Zietckiewicz, E., Rafalski, A., Labuda, D., 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20, l77−l83.