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Research Article

CHARACTERIZATION OF GENETIC DIVERSITY OF *VIOLA ODORATA* FROM DIFFERENT REGIONS OF JAMMU AND KASHMIR USING RAPD PRIMERS

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ABSTRACT

Viola odorata has been recognized as an important medicinal plant due to its role in the treatment of respiratory disorders and secondary cancer tumors. Genetic diversity was studied in ten *Viola odorata* samples collected from 10 different regions of Jammu and Kashmir. A total of 118 bands were amplified in ten *V. odorata* samples using 9 RAPD primers. Out of these 107 bands were polymorphic and 11 were monomorphic. Maximum numbers of bands were generated from the primer OPA19 giving 25 bands out of which 24 were polymorphic. A 100% polymorphism was obtained with two primers OPA-04 and by OPH-08 followed by primer OPA-19 showing a polymorphism of 96%. The genetic dissimilarity index calculated varied from 0.33 to 0.94 for ten *Viola* samples. The generated dendrogram based on the dissimilarity matrix using the neighbor- joining approach showed three distinct clusters.

Keywords: *Viola odorata*, characterization, genetic diversity, molecular markers, RAPDs

INTRODUCTION

Viola odorata belonging to family Violaceae, includes 200 species. It is widely distributed in the temperate and tropical regions of the world that includes Europe, Northern Asia and North America. In India, *Viola odorata* in Jammu and Kashmir is mostly found in regions like Kud, Patnitop, Reasi, Kishtwar, Mahore and Panchari. *Viola odorata* is generally used in the treatment of various diseases especially in the treatment of cancer and whooping cough (Ghani et al., 1997). It contains salicylic acid, which is used to make aspirin and thus is effective in the treatment of headaches, migraine and insomnia. The whole plant plays role in anti-inflammatory, antidyslipidemic and antihypertensive activity (Siddiqi et al., 2012). It is taken internally in the treatment of bronchitis, respiratory problems, coughs, asthma, and cancer of the breast, lungs or digestive tract. Externally, it is used to treat mouth and throat infections.

Genetic variation is essential for long term survival of species and it is a critical feature in conservation (Godt and Hamrick 1998). For efficient conservation, the genetic composition and genetic structure of the species in different geographical areas must be known. Molecular markers like Random amplified polymorphic DNA (RAPDs), is a powerful tool which involves the use of primers of arbitrary sequence to discriminate and identify genetically diverse genotypes in many plant systems (Williams et al., 1990; Sachaal and Leverich 2001). The technique diversity has been successfully used in a number of plant species at both species and cultivar level. Till date, there is no report available on applications of molecular markers in assessing the genetic diversity in *viola odoarata.*

The objective of the present study was to assess the genetic diversity in ten *Viola Odorata* samples collected from different regions of Jammu and Kashmir using RAPD primers with the aim to study the genetic variation so to manage these genetic resources in breeding programs.

MATERIAL AND METHODS

Leaf samples of ten *Viola odorata* samples were collected from different regions of Jammu and Kashmir (Table-1).

S.No.	Location	Code
1	Banihal(Ramban)	J-1
2	Basantgarh(Udampur)	J-2
3	Bharakh(Reasi)	J-3
4	Kashmir	J-4
5	Pouni (Reasi)	J-5
6	Mahore(Reasi)	J-6
7	Kud(Udampur)	J-7
8	Panchari(Udampur)	J-8
9	Patnitop(Udampur)	J-9
10	Reasi	J-10

Table1: *Viola odorata* samples and their codes

DNA Isolation:

Young and tender leaves were collected and surface sterilized. The genomic DNA was isolated from sample using the CTAB based method by Saghai-Maroof *et al.* 1984.The RNA was eliminated from the isolated DNA by adding 5U of RNase and the vials were incubated at 37^{o}C for 50-60 minutes. The DNA was finally suspended in 1X TE (10mm Tris HCl and 1mM EDTA, pH 8.0) and quantified by UV spectrophotometer and also on 0.8% (w/v) agarose gel and were stored in -20^{o}C till further use.

In vitro amplification of DNA using polymerase chain reaction (PCR) (Mullis et al. 1985) was performed in 0.2ml PCR tubes using 1ul of genomic DNA of each sample in a final volume of 25µl reaction mixture. A total of nine different RAPD primers procured from Operon Technologies were used for analysis (Table 2). The PCR reaction mixture contained 5.0 ul template DNA, 12.2 µL ddH_2O, 2.5 µl 10X PCR buffer, 3.5 µl of 100 mM dNTPs, 1.5 µl of 5 µM primer and 0.3 µL Taq polymerase (5 U/µl). Amplification of DNA was done with thermal cycler. Each reaction was performed using initial denaturation of Template DNA at 94°C for 4 min followed by 45 cycles of PCR amplification following: 1 min of denaturation at 94°C, 1 min of primer annealing at 37°C and 2 min of primer extension at 72°C. Final incubation was done at 72°C for 7 min so as to complete primer extension. The amplified products were electrophoretically resolved on a 1.0% agarose gel in 0.5X Tris-acetate-EDTA (TAE) and visualized under UV light after staining with 0.1% ethidium bromide.

Data collection and diversity analysis:

All the gels were scored manually for the bands. The dissimilarity matrix used for clustering was based on the unweighted neighbor-joining method and the analysis was performed using DARWIN 5.0 (http://darwin.cirad.fr). Confidence limits of different clades were tested by bootstrapping 500 times to

assess the repetitiveness of genotype clustering (Felsenstein 1985).

RESULTS AND DISCUSSION

Marker Analysis:

Nine different RAPD primers (OPA19, OPHO8, OPA04, OPBB08, OPBA03, OPBD17, OPU20, OPBB10 and OPY06) were used to evaluate the level of genetic diversity amongst the different samples of *Viola odorata*. The amplified product was scored on the basis of presence and absence of bands. The scoring of bands was done independently and only the distinct well separated bands were used to generate the input 1, 0 matrix that was used for all further computations. A total of 118 bands were amplified in ten *Viola odorata* samples using 9 RAPD primers. Out of these 118 bands, 107 bands were polymorphic and 11 bands were monomorphic (Table 2). Maximum numbers of bands were generated from the primer OPA-19 giving 25 bands out of which 24 were polymorphic, followed by primer OPBD17 generating 16 bands with 14 polymorphic bands. A 100% polymorphism was obtained with the primers OPA-04 and primer OPH-08 followed by primer OPU-20 (92.85%). Slomka et al. (2011) used ISSR primers to study diversity in *Viola tricolor*. The plants collected from polluted sites showed 84% of genetic diversity. Similarly, Cennamo et al. (2011) used nuclear and chloroplast DNA in *Viola subsect* to separate tracking of maternal lineages and detect dubious herbarium specimens. Their findings confirmed a correlation between chloroplast sequences at species or subspecies level.

S. No.	Primer	Total Bands	Polymorphic Bands	Polymorphic percentage
1	OPA-19	25	24	96%
2	OPA-04	8	8	100%
3	OPU-20	14	13	92.85%
4	OPBD-17	16	14	87.5%
5	OPH-08	15	15	100%
6	OPY-06	7	5	71.4%
7	OPBA-03	11	10	90.9%
8	OPBB-08	12	10	83.3%
9	OPBB-10	10	8	80%
Total		118	107	--
Mean		11.8	10.7	90.6%

Table2: Level of polymorphism obtained in *Viola odorata* samples

Analysis of genetic dissimilarity:

The clear bands were scored from the gel and "0" and "1" were standardized as the least and maximum of dissimilarity respectively. The dissimilarity coefficients used for cluster analysis was based on the unweighted neighbor- joining method and a dendrogram was generated to study the relationship among *Viola odorata* samples collected from different regions of Jammu and Kashmir. The genetic dissimilarity index calculated varied from 0.33 to 0.94 for all the ten *Viola odorata* samples (Table 3). The maximum genetic diversity i.e. 0.94 was calculated for samples collected from Kashmir (J-4) and Reasi (J-10). This shows that the *Viola odorata* samples collected from these two places are highly genetically diverse. The sample J-3 and J-5 collected from Bharakh and Pouni respectively do not show much diversity (0.33). This clearly indicated that these two samples may have same genetic makeup with no or very little genetic difference and these may have spread to different areas because of human intervention.

	J-1	**J-2**	**J-3**	**J-4**	**J-5**	**J-6**	**J-7**	**J-8**	**J-9**
J-2	0.48								
J-3	0.55	0.39							
J-4	0.62	0.65	0.73						
J-5	0.53	0.37	0.33	0.70					
J-6	0.60	0.43	0.44	0.77	0.42				
J-7	0.50	0.42	0.50	0.67	0.47	0.54			
J-8	0.56	0.48	0.56	0.73	0.54	0.60	0.34		
J-9	0.67	0.59	0.67	0.84	0.65	0.71	0.45	0.40	
J-10	0.77	0.60	0.61	0.94	0.59	0.54	0.71	0.77	0.88

Table3: Diversity matrix between ten *Viola odorata* samples as revealed by RAPD primers

The generated dendrogram based on neighbourhood joining method approach of the UPGMA method showed three distinct clusters (Fig 1):

Cluster I has samples (J-9) collected from Patnitop and sample J-8 collected from Panchari are closely related with each other and also with sample 7 collected from Kud. Sample J-4 collected from Kashmir and sample J-1 collected from Banihal are also group together in cluster I as they are closely related to each other.

Cluster II has sample J- 5 collected from Pooni, sample J- 3 collected from Bharakh, sample J-10

collected from Reasi and sample J-6 collected from Mahore. Samples sample J-5 and J-3 show close relationship whereas samples J-10 and J-6 are genetically more closely related.

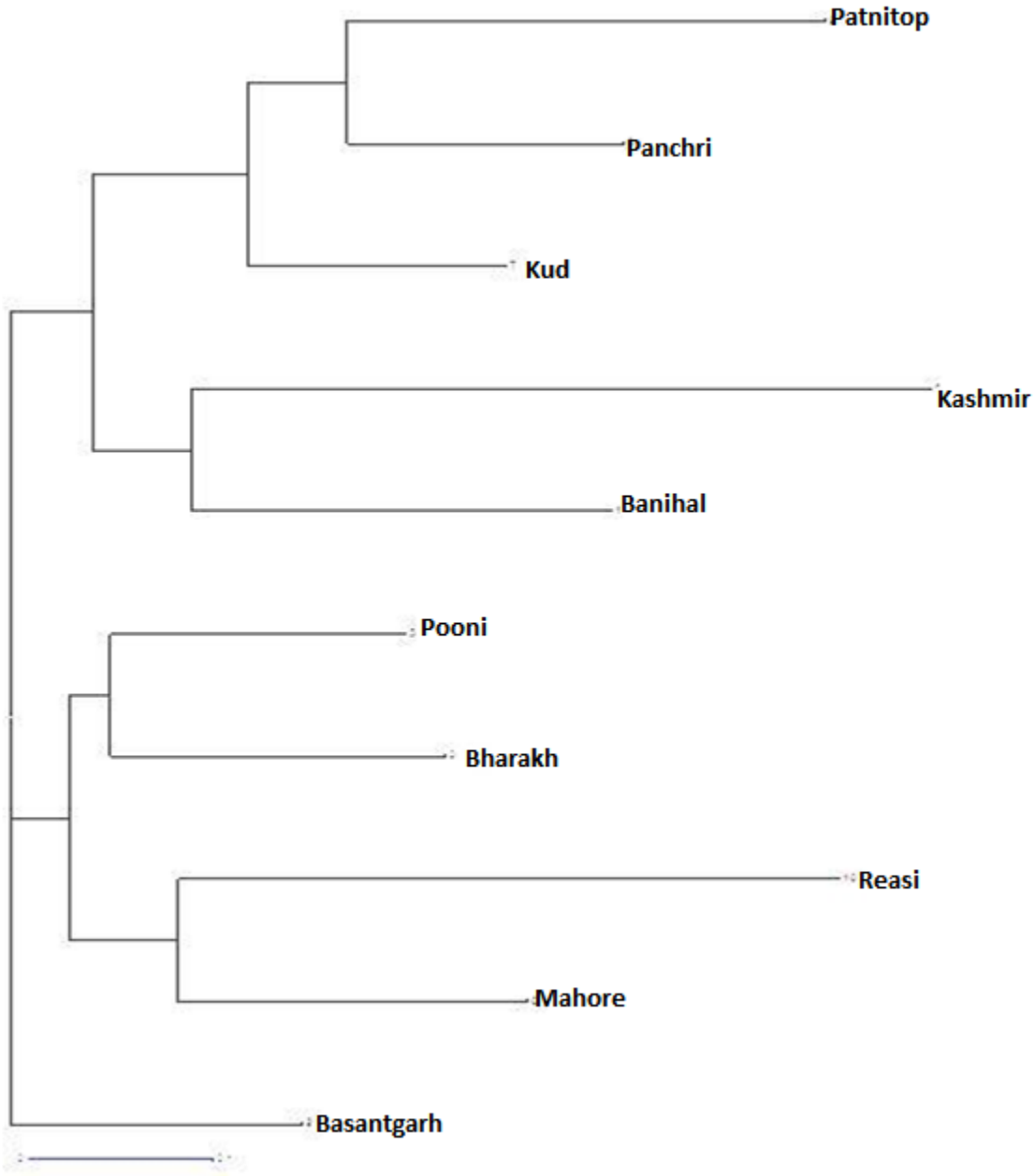

Figure 1: UPGMA based dendrogram of ten *Viola odorata* samples

Cluster III has sample J-2 collected from Basantgarh is genetically diverse from all the *V. odorata* samples and do not show relationship with any other collected samples in the dendrogram.

CONCLUSIONS

The UPGMA tree generated clearly indicates a strong genetic differentiation among different accessions of *Viola odorata* collected from different locations of J&K and RAPD being an efficient marker can distinguish the differences at molecular level. The germplasm diversity in *Viola odorata* plants from Jammu and Kashmir can be exploited in the future for breeding programs so as to develop new and better *Viola odorata* varieties.

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