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Assessment of genetic diversity among different biotypes of *Physalis minima*

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ABSTARCT

Physalis minima is abundant weed species in India commonly found in non-cropped and crop areas during *Kharif* season. Fruits of this weed species has been reported for high nutritional and medicinal values. Despite, no study has been made, so far, for their molecular diversity analysis. In the present study, genetic diversity was assessed among a total of 17 biotypes of *P. minima* and 1 intermediate biotype (which exhibits floral characteristics of both *P. minima* and *P. peruviana*). Using 42 random amplified polymorphic DNA (RAPD) markers, a total of 224 bands were amplified among all studied biotypes. The band size of amplified fragments ranged from 100 - 2200 base pairs (bp). Out of 224 bands amplified, 52 were found to be monomorphic (23.2%) and remaining 172 were polymorphic (78.8%). Average number of bands per primer were 5.33 while average number of polymorphic bands per primer were 4.09. Cluster analysis grouped all biotypes into two groups. This is the first report on molecular diversity analysis among *Physalis minima* biotypes.

Key words: Genetic diversity, Biotype, *Physalis minima*, RAPD, Similarity, Weed

Physalis minima is a diploid (2n=24) plant and is one of the important weed species in Solanaceae family. It is locally known as *'Ban Tipariya'*, *'Panchkota'* or *'Chirpati'* in India. *Physalis* is a genus of 80 to 100 species of mostly neotropical herbs. The plant is an annual grow well in most soil types particularly sandy soil and commonly found as weed in *Kharif* crops in India. Almost all parts of this plant have been reported to be useful as treatment for ulcer, cancer and the decoction. The green-yellowish edible berry is encapsulated by the calyx and it is a popular seasonal fruit among tribal areas. Fruits of *P. minima* have been reported as rich source of flavonoids, terpenoids, vitamins, alkaloids and antibacterial substance which make it valuable as a medicinal plant (Nathiya and Dorcus 2012). It also has been reported for treating diseases like cancer, leukemia, malaria, asthma, hepatitis, dermatitis and rheumatism (Joshi and Joshi 2015). Utilization of the nutritional potential of *Physalis* may be helpful to alleviate malnutrition problems in India and elsewhere (Pagare *et al.* 2015). Recently, the demand for fruit of its cultivated species (*P. peruviana*) has increased due to its potential as antioxidant and anticancer (Pietro *et al.* 2000, Shariff *et al.* 2006).

Characterization of genetic diversity is required for further improvement. Morphological markers tend to fluctuate with environmental conditions. Molecular markers have been employed in characterization of genetic diversity among biotypes/ species. Random amplified polymorphic DNA (RAPD) is increasingly being employed in diversity analysis owing to its easy protocol and simplicity. To date, no report on molecular marker study is available regarding the genetic diversity of *P. minima* in India. Therefore, this study was conducted to investigate the genetic diversity among different biotypes of *P. minima* using RAPD markers.

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MATERIALS AND METHODS

A total of 18 biotypes of *P. minima* were collected from different locations in the year 2013-14 (Table 1). Out of these, 17 biotypes exhibited typical floral pattern of *P. minima* (designated as PM1, PM2,.....PM17), while one biotype exhibited mixed floral characteristics of *P. minima* and *P. peruviana* (designated as intermediate and abbreviated as PI). Plants were grown in the containment facility at ICAR-Directorate of Weed Research in *Kharif* season of 2015 using pot culture. Second leaf from top of 20 days old plants were used for DNA extraction.

Genomic DNA was extracted using DNeasy Plant Mini Kit (Qiagen) following supplier's instruction. Twenty days young leaves were sampled from a single plant of each population. After thorough washing in distilled water, samples were stored in liquid N_2 till further used. For DNA extraction, samples were crushed in liquid N_2 using pestle and mortar. Quality and quantity of extracted genomic

Table 1. Collection sites of different biotypes of *Physalis minima*

Biotypes	Collection sites	Latitude	Longitude
PM1	Jabalpur-random collection	23.1812° N	79.9866° E
PM2	Raipur (G.G.)	21.1797° N	81.7787° E
PM3	Anand (Gujarat)	22.5645° N	72.9289° E
PM4	Bengaluru (Karnataka)	12.9716° N	77.5946° E
PM5	Jorhat (Assam)	26.2006° N	92.9376° E
PM6	Jorhat (Assam)	26.2004° N	92.9378° E
PM7	DWR, Jabalpur (MP)	23.1815° N	79.9864° E
PM8	Jabalpur-random collection	23.1814° N	79.9865° E
PM9	Jabalpur-random collection	23.1818° N	79.9862° E
PM10	Jabalpur-random collection)	23.1816° N	79.9863° E
PM11	Jabalpur-random collection	23.1811° N	79.9860° E
PM12	Jabalpur-random collection	23.1812° N	79.9866° E
PM13	Jagdalpur (C.G.)	19.0741° N	82.0080° E
PM14	Jabalpur-random collection	23.1814° N	79.9868° E
PM15	Jabalpur-random collection	23.1810° N	79.9867° E
PM16	Jabalpur-random collection)	23.1811° N	79.9865° E
PM17	Jabalpur-random collection	23.1813° N	79.9868° E
PI	DWR, Jabalpur	23.1815° N	79.9864° E

DNA was assessed by UV-spectrophotometer (DS 11+, DeNovix) and submerged horizontal electrophoresis using agarose gel (0.8%).

PCR amplifications were performed in a programmable thermocycler (Takara). Each sample was amplified in a reaction mixture containing 50 ng genomic DNA, 1 unit of *Taq* polymerase (New England Biolabs), 10x PCR buffer with 2.5 mM $MgCl_2$ and 200 µM of each dNTP (New England Biolabs), 10 pmol of 10-mer RAPD primers (Operon Technologies, USA; Table 2). Cycling parameters for RAPD were adjusted to 5 min at 94 °C for pre-denaturation, 45 cycles each of 1 min at 94 °C for denaturation, 1 min for annealing at 37 °C, 2 min at 72 °C for extension and a final extension at 72 °C for 5 min. After cooling to 4 °C, amplified PCR products were stored at 4 °C till electrophoresis.

Amplified products were separated on 1.5% agarose gel in 1x TAE buffer with 1 Kb plus ladder (Fermentas) to determine the size of amplified DNA fragments. Gels were run for 2 h at 65 V, stained with ethidium bromide and documented with gel documentation system (Syngene, UK). Polymerase chain reactions (PCR) were repeated thrice to confirm reproducibility of primers. Reproducible bands were scored manually as '1' or '0' for presence or absence of the bands.

Evaluation of fragment patterns was carried out by similarity index. The final RAPD data generated was used to calculate pair-wise similarity co-efficient values (Jaccard 1908) using the similarity for qualitative data (SIMQUAL) format of NTSYS-pc version 2.1 (numerical taxonomy and multivariate analysis system) software package (Rohlf 2002).

Table 2. RAPD primers used for genetic diversity analysis

Primer	Sequence 5'-3'	GC (%)	TB	PB	PP
OPA-01	CAGGCCCTTC	70	4	3	75.0
OPA-02	TGCCGAGCTG	70	5	5	100
OPA-03	AGTCAGCCAC	60	4	4	100
OPA-04	AATCGGGCTG	60	5	1	20.0
OPA-05	AGGGGTCTTG	60	4	3	75.0
OPA-06	GGTCCCTGAC	70	7	3	42.8
OPA-08	GTGACGTAGG	60	6	4	66.7
OPA-09	GGGTAACGCC	70	5	4	80.0
OPA-10	GTGATCGCAG	60	6	6	100
OPA-11	CAATCGCCGT	60	7	7	100
OPA-12	TCGGCGATAG	60	6	5	83.3
OPA-15	TTCCGAACCC	60	7	5	71.4
OPA-17	GACCGCTTGT	60	5	3	60.0
OPA-18	AGGTGACCGT	60	6	2	33.3
OPA-19	CAAACGTCGG	60	5	3	60.0
OPA-20	GTTGCGATCC	60	7	6	85.7
OPE-01	CCCAAGGTCC	60	7	5	71.4
OPE-02	GGTGCGGGAA	70	5	4	80.0
OPE-04	GTGACATGCC	60	4	4	100
OPE-05	TCAGGGAGGT	60	7	5	71.4
OPE-06	AAGACCCCTC	60	4	4	100
OPE-07	AGATGCAGCC	60	6	6	100
OPE-08	TCACCACGGT	60	6	6	100
OPE-09	CTTCACCCGA	60	5	5	100
OPE-10	CACCAGGTGA	60	3	1	33.3
OPE-15	ACGCACAACC	60	6	5	83.3
OPE-16	GGTGACTGTG	60	5	1	20.0
OPE-19	ACGGCGTATG	60	6	5	83.3
OPE-20	AACGGTGACC	60	6	5	83.3
OPN-01	CTCACGTTGG	60	5	5	100
OPN-02	ACCAGGGGCA	70	5	5	100
OPN-03	GGTACTCCCC	70	4	4	100
OPN-04	GACCGACCCA	70	8	7	87.5
OPN-05	ACTGAACGCC	60	5	4	80.0
OPN-12	CACAGACACC	60	5	4	80.0
OPN-13	AGCGTCACTC	60	5	5	100.0
OPN-14	TCGTGCGGGT	70	5	4	80.0
OPN-15	CAGCGACTGT	60	5	5	100.0
OPN-16	AAGCGACCTG	60	4	3	75.0
OPN-17	CATTGGGGAG	60	4	3	75.0
OPN-20	GGTGCTCCGT	70	5	3	60.0
OPAB-07	GTAAACCGCC	60	6	3	50.0
Total			224	172	78.78
Average			5.33	4.09	-

Cluster analysis was performed on the basis of genetic similarity matrix, and the resulting similarity co-efficient values were used for constructing dendrogram using the unweighted pair group method with arithmetic average (UPGMA) with the SAHN module of NTSYS-pc (Sneath and Sokal 1973).

RESULTS AND DISCUSSION

In the present study, initially 60 RAPD primers were screened among 18 biotypess of *P. minima*. Forty two RAPD primers that produced consistently strong amplification products (Fig. 1) and polymorphic banding patterns were selected for further analysis. The polymerase chain reaction was carried out using a single decamer primer at a time.

The sequences of these primers are presented in Table 2. Selected 42 RAPD primers amplified a total of 224 loci. The band size of amplified fragments ranged from 100-2200 bp. Out of 224 bands amplified, 52 were found to be monomorphic (23.2%) and remaining 172 were polymorphic (78.8%). Average numbers of bands per primer was 5.33 while average number of polymorphic band per primer was 4.09. Maximum numbers of bands (8) were scored by primer OPN-04 while minimum numbers of bands (3) were scored by primer OPE-10 (Table 2). Banding patterns by primer OPE-05 and primer OPN-17 have been depicted in Fig. 1. Information on genetic diversity is essential in optimizing both conservation as well as utilization strategies for genetic resources. Molecular markers are preferred over conventional approaches to identify genetic variability among biotypes. Genetic diversity analysis through RAPD markers has been highlighted in a number of medicinally important plants including *Bacopa monnieri* (Tripathi *et al.* 2012) and *Coleus forskohlii* (Tripathi *et al.* 2013). DNA markers provide important data on diversity due to their capability to discover variation at the molecular level (Tripathi *et al.* 2013). The RAPD technique has been used very effectively in the discrimination of the individuals and can measure similarity present between a pair of biotypes.

The similarity coefficient values were obtained after multivariate analysis using Jaccard's value. These similarity coefficient values were then used to construct a dendrogram with the UPGMA method (Fig. 2). Cluster analysis revealed that biotypes of *P. minima* (PM) under study divided into two groups, minor group and major group. Minor group contained only three biotypes namely PM13, PM15 and intermediate PI. Major group comprised 15 biotypes and further divided into two subgroups. First subgroup contained 6 biotypes PM1, PM2, PM3, PM4, PM5 and PM6 while second subgroup contained 9 biotypes namely PM17, PM14, PM7, PM12, PM11, PM9, PM10, PM16 and PM8. Among all biotypes PM1, PM2, PM3, PM4 and PM6 showed higher similarity and grouped together while, PM7, PM18 and PM17 were grouped in a separate group. Among remaining biotypes in major group PM8, PM16 and PM10 were grouped separately. One *P. minima* biotype PM5 grouped distantly. Usaizan *et al.*

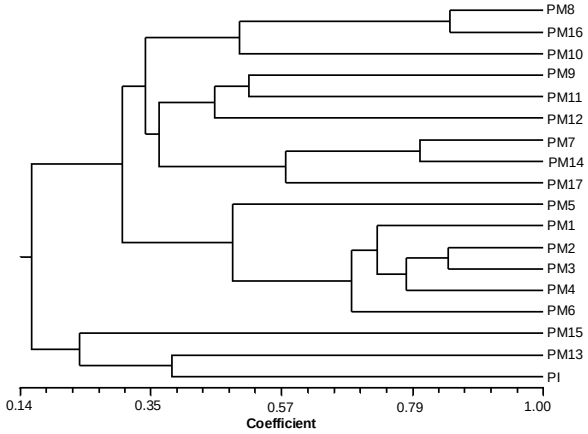

Fig. 2. UPGMA clustering among *Physalis minima* biotypes based on RAPD data

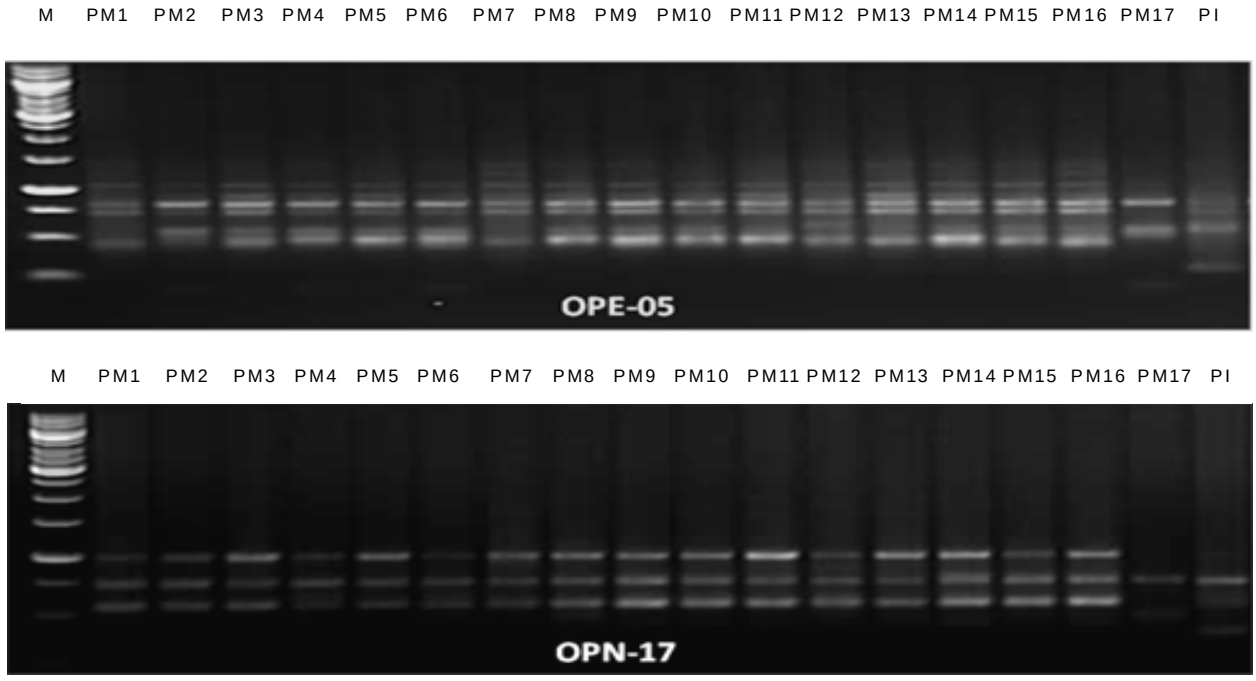

Fig. 1. Electrophoretic banding pattern of different biotypes using OPE-05 and OPN-17 RAPD markers

(2014) reported four clusters while analyzing genetic divergence among *P. minima* biotypes using ISSR markers. Results of this study further inferred that molecular diversity among different biotypes of *P. minima* may or may not be dependent on geographically locations. For example, biotypes from different places (Jabalpur, Jagdalpur and intermediate) fall in a single cluster (minor group). Similarly, first subgroup of major group has 6 bioypes namely PM1, PM2, PM3, PM4, PM5 and PM6. Despite of clustering in same subgroup, these belong to distant geographical locations [PM1 (Jabalpur), PM2 (Raipur, CG)), PM3 (Anand, Gujarat), PM4 (Bengaluru, Karnataka), and PM5, PM6 (Jorhat, Assam). On the other hand, biotypes from Jabalpur (except PM1 and PM15) formed a cluster (second subgroup) exhibiting high degree of genetic similarity. Our results showed the usefulness of RAPD markers for analysis of genetic diversity among biotypes even from same geographical locations or genetic similarity among biotypes from different geographical locations. Two-dimensional and three-dimensional scaling further elaborated the diversity analysis indicating that biotypes of minor groups (PM13, PM 15 and PI) placed at distant positions not only from the biotypes of major group by also within themselves (Fig. 3 and 4).

Component Analysis (PCA) conceded out using the similarity matrices for RAPD primers also produced trends similar to the UPGMA cluster analysis (Fig. 3). Further, in three-dimensional scaling, intermediate biotype showed higher diversity and grouped distantly from other biotypes (Fig. 4).

In conclusion, significant variations were observed among the *P. minima* biotypes after RAPD analysis can be used for genetic diversity analysis for characterization of germplasm at molecular level.

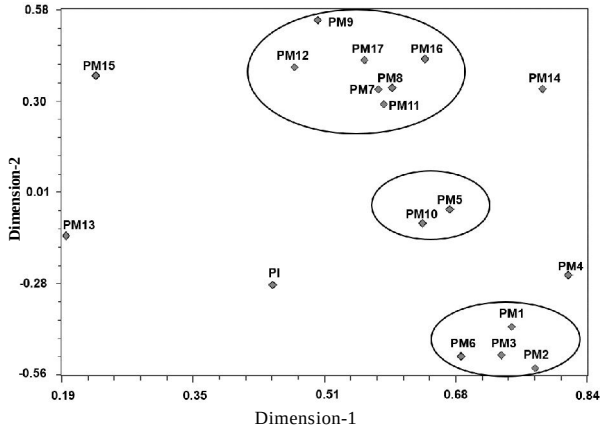

Fig. 3. Two-dimensional scaling among *P. minima* biotypes based on RAPD data

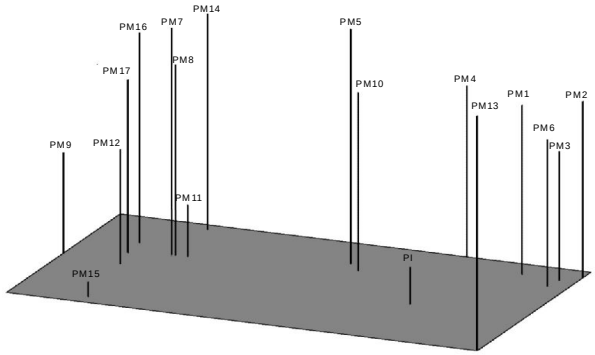

Fig. 4. Three-dimensional scaling among *P. minima* biotypes based on RAPD data

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