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Characterization of *Solanum nigrum* L. Genotypes by Morphological and RAPD Markers

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ABSTRACT

Randomly amplified polymorphic DNA markers were used to assess genetic diversity among 12 cultivated genotypes of *Solanum nigrum* L. obtained from diverse locations of India and were compared with their morphological data obtained for vegetative and reproductive characters. DNA was isolated using CTAB method and 113 amplified fragments were obtained from 15 random 10-mer primers. Intense, clear and repeatable bands ranging from 250 bp to 6 Kb were used for RAPD analysis. Dendrograms were constructed for both morphological and RAPD data using Wards method, which grouped the genotypes into two clusters 'A' and 'B' at 5.56 and 4.0 linkage distances, respectively. Cluster 'A' consisted of eight genotypes, which were tall plants and required less time to flower. Cluster 'B' consisted of four genotypes showing short and widely spread plants with high pollen viability and had more number of seeds per berry. The genetic dissimilarity matrix calculated for the RAPD markers was based on Squared Euclidean Distance, showed a variation from 16 to 53% among the cultivars. Thus, RAPD markers combined with morphological analysis proved to be a quick, simple and significant testing method to assess genetic diversity among *S. nigrum* L. genotypes.

Keywords: cluster analysis, genetic variability, Indian genotypes, Makoy, medicinal plant, STATISTICA

INTRODUCTION

Makoy (*Solanum nigrum* L.) is a newly emerging medicinal plant belonging to the family Solanaceae. It is considered as a widely spread weed and a native of Eurasia (Schilling 1981). It is an annual herbaceous plant, which grows to 30-80 cm in height with an erect and divaricately branched stem. The leaves are numerous, alternate, ovate or lanceolate, sub-acute or acuminate and entire-toothed tapering into the petiole. The flowers are white, small, produced in an extra-axillary sub-umbell, with 3 to 8 cymose inflorescences. It grows on a well-drained, fairly light fertile loamy soil with good moisture holding capacity. It is found growing throughout dry parts in India up to an elevation of 2100 m above sea level. It also grows well under different climatic conditions of the country, but prefers a moderate climate. The plant is sensitive to frost and shade, and highly tolerant to dry conditions. It is commonly found in Sri Lanka, China, India, Madagascar, South Africa, Zimbabwe and some European countries (Sreeramu *et al*. 2005).

All parts of plants are economically and medicinally important. It is used for the treatment of scrofulous virulent gonorrhoea, wounds, sores and malaria (Nadkarni 1995; Chatterjee 2000). The plant extract is effective in the treatment of liver cirrhosis and serves as an antidote against opium poisoning (Dhar *et al*. 1968). The berries are considered to possess tonic, diuretic and cathartic properties and are effective against heart diseases (Raju *et al*. 2003; Son *et al*. 2003). They are the domestic remedy for liver pains, leucoderma, piles, diarrhoea, ulcers, vomiting, asthma, bronchitis and eye troubles (Krithikar and Basu 1975). The leaves are found to suppress carcinogenesis and can act as a protective agent against cancer (Aruna and Sivaramakrishan 1990). The alcoholic extract of leaves is active against *Escherichia coli* and *Staphylococcus aureus* (George *et al*. 1947). The crude extract from berries can inhibit the growth of *Bacillus subtilis*, *S. aureus*, *Pseudomonas aurginosa*, *Aspergillus niger* and *E. coli* (Khan *et al*. 1993). The alcoholic extract of berries inhibits edema and acts as an anti-inflammatory agent. The plant is also used as an antiseptic, emollient, diuretic, laxative and narcotic (Dhar *et al*. 1968).

S. nigrum contains a number of alkaloids of economical importance. The alkaloids α-solamargine and α-solasonine are obtained from green unripe fruits (Ridout *et al*. 1989). The leaves and berries contain steroidal glyco-alkaloids such as α-solanigrine, β-solanigrine, solamargine and solasonine. The total alkaloid content of the fruit and leaves ranges from 0.101 and 0.131%, respectively. The anti-inflammatory activity of the plant has been attributed to the presence of steroidal glyco-alkaloids present in the leaves and berries (Nadeem and Hussain 1996). An SNL glycoprotein (150 KDa) isolated from the plant is an effective anticancer agent (Sreeramu *et al*. 2005).

S. nigrum exhibits considerable genetic variation, both florally and vegetatively, corresponding to the ploidy levels. It is a hexaploid (2n=6x=72), probably derived from a cross between a tetraploid *S. luteum* Mill (2n=2x=24) and a diploid Solanaceae member. It is also believed to have been developed from a sterile triploid which had spontaneously doubled the number of chromosome to become a stable and fertile hexaploid (Edmonds 1979). The morphological characters of the hexaploid individuals obtained from different geographical locations in India showed distinct characteristic features and growth morphology. The leaf margin varied from entire to sinuate-dentate and was characterized by different fruit colour ranging from green to orange and red. Owing to almost complete autogamous nature in *S. nigrum* all the progenies are very similar to the plant that produces the seeds (Stankiewicz *et al*. 2001).

Intrageneric variability was first described morphologically, to indicate the separation of different cultivars due to fixed traits. Random Amplified Polymorphic DNA (RAPD) marker is described as fast, simple, easy and inexpensive for detecting polymorphisms, based on the amplification of

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random DNA segments with single primers of arbitrary nucleotide sequence (Williams *et al*. 1990). Typically a PCR-based method requires only small quantities of template DNA. Moreover, the amplified fragments provide a large number of potentially polymorphic loci, making them very useful for detection of types within a species or with low genetic variability. Unlike most PCR-based methods, no DNA sequence information is required prior to investigation of previously unstudied individuals (Dawson *et al*. 1995). The present investigation is attempted to genetically characterize the morphologically distinct hexaploid *S. nigrum* L. genotypes using morphological and RAPD markers.

MATERIALS AND METHODS

Plant materials

The plant materials used for the study comprised of 12 morphologically and geographically distinct genotypes of *S. nigrum* L. collected from Andhra Pradesh (SN_1), Assam (SN_2), Gujarat (SN_3), Haryana (SN_4), Himachal Pradesh (SN_5), Karnataka (SN_6), Kashmir (SN_7), Kerala (SN_8), Maharastra (SN_9), Orissa (SN_{10}), Rajasthan (SN_{11}) and Tamilnadu (SN_{12}). The seeds of the genotypes were obtained from the University of Agricultural Sciences, Bangalore and cultivated in separate plots at R.B.S. College, Dr. B. R. Ambedkar University, Agra. Fifty g of young and healthy leaves that were free from pest and disease damage were harvested from the 12 genotypes individually in an ice box from the field, wiped with 70% ethanol, air-dried and stored at 4°C in sealed polythene covers for the isolation of DNA.

Morphological characteristics

The morphological data was recorded for the characters such as plant height (cm), plant spread (cm), secondary branches (number), leaf area (cm^2), flowering duration (months), flowers per inflorescence, pollen viability (%), colour of berry, fresh weight of berry (g), number of seeds per berry and size of seeds (µm) for two successive cycles. The mean average of 20 individuals of each genotype was selected for the determination of morphological characteristics (**Table 1**). For numerical classificatory analysis, the general similarity coefficient (S_G) was used as a measure of resemblance between the different genotypes and was calculated according to Sneath and Sokal (1973). Based on the presence or absence of character, a dendrogram was constructed by Wards method of clustering (Wards 1963). Analysis, correlation and interpretation were employed by using RCBD for field studies as suggested by Fischer and Yates (1963).

DNA isolation

DNA was extracted according to a modified Cetyl trimethyl ammonium bromide (CTAB) method (Shashidhara *et al*. 2003). Two g of leaf sample was powdered in liquid nitrogen to extract DNA. The powder was mixed with 10 ml extraction buffer, preheated to 65°C, and containing 100 mM Tris-HCl, pH 8.0, 20 mM EDTA, 1.4 M NaCl, 3% (w/v) CTAB, 2% polyvinyl pyrrolidone and 1% β-mercaptoethanol, then incubated at 65°C for 1 h. The mixture was cooled to room temperature, 6 ml cold 24:1 (v/v) chloroform: isoamylalcohol was added, and the contents were mixed well. After centrifugation at 6500 × *g* for 8 min at 4°C, the supernatant was transferred to a fresh tube and the chloroform:isoamylalcohol step was repeated until a clear supernatant was obtained. 5 M NaCl was added to the supernatant (0.5 v/v) and mixed gently followed by the addition of 1 volume of cold isopropanol to precipitate the DNA. The mixture was incubated overnight at 4°C, and then centrifuged at 7500 × *g* for 15 min. The resulting pellet was washed with 70% (v/v) ethanol, air dried, and dissolved in TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). Two µg RNase (Bovine pancreatic ribonuclease, Bangalore Genei, Bangalore, India) was added to each sample which was incubated for 3 h at 37°C, mixed with an equal volume of phenol and centrifuged at 7500 × *g* for 10 min at room temperature. This step was followed by a washing with an equal volume of 1:1 (v/v) phenol:chloroform then with chloroform alone. DNA was precipitated overnight at 4°C with 0.5 vol of 5 M NaCl and 1 vol cold isopropanol, and the resulting pellet obtained after centrifugation was dissolved in TE buffer, analysed on an agarose gel and quantified using a spectrophotometer (NanoDrop Technologies, Wilmington, USA).

PCR amplifications

PCR amplification followed was the protocol of Williams *et al*. (1990) with minor modifications. Of the 36 primers screened using the pool DNA, 15 primers showing clear and distinguishable bands during screening were selected for RAPD-PCR analysis (**Table 2**). Reproducibility of the primers was tested by repeating the PCR amplification three times under similar conditions. PCR reactions were carried out in a volume of 25 µl containing 30 ηg template DNA, 150 µM each dNTP, 1.5 mM $MgCl_2$, 1 unit *Taq* DNA polymerase (Sigma Aldrich Chemicals, Bangalore, India), 5 pmol primer (OPA, OPB, OPC, OPD, OPE, OPF, OPG, OPH, OPI, OPJ and OPK series, Operon Technologies, Alameda, CA, US) in PCR buffer (50 mM KCl, 10 mM Tris-HCl pH 9.0, 0.05% (v/v) NP40 and 0.05% (v/v) Triton X-100). Amplifications were performed in a MJ Research PTC-100 Thermocycler (Bio-Rad Laboratories, Bangalore, India), programmed for an initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 35°C for 1 min, primer extension at 72°C for 2 min, and a final extension at 72°C for 10 min. PCR products were resolved in a 1.2% (w/v) agarose gel, visualized and documented using an Alpha Digidoc system (Alpha Innotech, San Leandro, CA, USA).

Table 1 Morphological characters of 12 genotypes of *Solanum nigrum* L.

Genotypes	Plant characters										
	Plant height (cm)	Plant spread (cm)	Secondary branches (№)	Leaf area (cm^2)	Flowering duration (Months)	Flowers per inflorescence (№)	Pollen viability (%)	Berry		Seed	
								Colour	Weight (g)	№ per berry	Size (µm)
SN_1	63±1.527	23.4±1.965	8±0.88	20.44±1.366	3.0±0.424	4.8±0.374	33.8±5.678	Black	0.267±0.151	28.6±4.045	1.58±0.014
SN_2	72±0.864	39.0±2.550	3±0.31	19.79±2.384	3.5±0.586	4.2±0.374	38.8±4.341	Black	0.414±0.005	35.0±2.280	1.56±0.009
SN_3	51±1.255	39.8±2.354	6±0.70	30.99±4.475	5.0±0.242	4.4±0.748	20.2±5.267	Black	0.120±0.053	28.8±2.691	1.76±0.009
SN_4	53±2.129	29.8±5.286	5±0.70	33.20±2.093	5.0±0.246	6.0±0.447	46.8±2.596	Black	0.110±0.007	33.8±3.513	1.58±0.031
SN_5	42±1.453	38.6±1.327	3±0.44	34.93±3.592	5.5±0.318	5.8±0.583	45.2±1.530	Black	0.411±0.069	28.6±3.655	1.85±0.021
SN_6	62±1.192	29.6±3.326	5±0.70	24.47±2.377	4.0±0.209	5.6±1.249	38.8±4.341	Black	0.121±0.053	38.0±2.000	1.84±0.137
SN_7	83±1.269	27.0±2.490	5±0.94	15.86±0.771	3.0±0.402	5.0±0.316	22.6±2.015	Orange	0.114±0.006	27.6±1.965	1.84±0.020
SN_8	82±1.084	46.0±9.649	5±1.03	40.53±3.788	3.0±0.310	4.8±0.200	14.2±0.735	Black	0.112±0.010	26.0±5.891	1.83±0.025
SN_9	72±0.934	41.4±6.337	8±0.78	53.31±3.184	3.0±0.331	4.0±0.632	16.6±1.400	Black	0.135±0.006	21.0±2.864	1.82±0.006
SN_{10}	85±0.673	30.6±3.187	7±1.00	22.21±4.270	4.0±0.392	4.6±0.510	32.6±2.874	Black	0.534±0.158	22.0±3.194	1.53±0.016
SN_{11}	86±0.723	32.0±6.208	5±1.04	10.17±0.302	4.0±0.480	5.0±0.548	19.6±4.438	Black	0.532±0.157	27.4±2.542	1.52±0.014
SN_{12}	28±0.657	24.8±5.490	3±0.54	14.10±0.885	3.5±0.302	4.6±0.245	43.4±5.183	Black	0.088±0.012	21.0±4.069	1.85±0.009
Mean	64.9	33.5	5.25	26.67	3.88	4.9	31.1	-	0.247	28.15	1.71
SEm±	1.152	4.776	0.77	3.22	0.370	0.591	3.716	-	0.076	3.508	0.042
CD	3.295	13.659	2.22	9.52	1.057	1.628	10.628	-	0.217	10.033	5.568

Table 2 RAPD-PCR primers, their sequence and level of polymorphism.

Primer	Sequence	Total № of bands	№ of polymorphic shared bands	№ of polymorphic unique bands	№ of monomorphic bands
OPA 16	AGCCAGCGAA	7	6	0	1
OPB 08	GTCCACACGG	7	4	1	2
OPB 18	CCACAGCAGT	9	8	0	1
OPB 20	GGACCCTTAC	7	5	0	2
OPC 07	GTCCCGACGA	7	7	0	0
OPD 11	AGCGCCATTC	7	7	0	0
OPD 13	GGGGTGACGA	7	5	0	2
OPF 01	ACGGATCCTG	7	6	0	1
OPF 03	CCTGATCACC	7	4	0	3
OPF 07	CCGATATCCC	10	7	1	2
OPF 10	GGAAGCTTGG	5	1	1	3
OPF 11	TTGGTACCCC	11	10	0	1
OPG 02	GGCACTGAGG	7	5	0	2
OPK 07	AGCGAGCAAG	8	8	0	0
OPK 19	CACAGGCGGA	7	5	0	2

RAPD profile analysis

Each reproducible band was visually scored as '1' for presence and '0' for absence, and the binary data were used for statistical analysis. The band sizes were determined by comparing with 500 bp DNA ladder, which was run along with the amplified products. The Dissimilarity Matrix was developed using Squared Euclidean Distance which estimated all pairwise (Sneath and Sokal 1973) and the dendrogram was computed based on Ward's method of clustering using a minimum variance (Wards 1963).

RESULTS AND DISCUSSION

Information on genetic diversity in medicinal plant species is important for efficient utilization of plant genetic resources. Geographical isolation of a population may cause its genome to drift away from other populations of the same species (Biron *et al*. 2002). Hence, authentic identification of taxa is necessary both for breeders to ensure protection of intellectual property rights, and also for propagators and consumers. The traditional method of identifying species by morphological characters is now been accompanied by DNA profiling that is more reliable or proteins largely because of several limitations of their morphological data. Therefore, organisms with a high tendency for morphological differentiation, studies considering both molecular and morphological characters are highly relevant (Bartish *et al*. 2000).

Young and recently matured leaves were used for the extraction of DNA since matured leaves were rich in phenols and polysaccharides that hinder extraction of highly pure DNA (Prakash *et al*. 2002). The leaves collected were washed with distilled water to remove dust particles, wiped with 70% ethanol to remove microbial contaminations (Shashidhara *et al*. 2003). The extraction buffer containing 3% CTAB was found optimal to release the nucleic acid from the cell. Centrifugation of the buffer containing DNA with chloroform: isoamylalcohol at 6500 × *g* for 8 minutes twice was found optimal to remove all protein contaminations. The pellet obtained after precipitation was transparent, whitish and easily dissolved in TE buffer. The purification steps effectively removed RNA and proteins rendering the DNA suitable for PCR amplification. The average spectrophotometer readings for the DNA samples at 260/280 ηm showed 1.82 OD value which was found optimal for PCR amplifications. The concentration of DNA obtained at 260 nm varied from 0.7 to 1.2 μg g^{-1} of leaf tissue. An intact good quality DNA was obtained when analysed using 0.7% agarose gel electrophoresis.

The PCR amplifications were followed according to the standard protocol (Williams *et al*. 1990) with minor modifications produced good amplifications having intense and clear banding pattern. The screening of primers using pool DNA facilitated the selection of primers that can amplify and produce more reproducible fragments pattern. Such screenings are essential to save time, cost and to reject primers which are not informative for the analysis (Prakash *et al*. 2002). Thirty six primers were screened of which fifteen producing intense, clear and reproducible banding patterns were selected for the analysis. About 113 clear, readable and reproducible RAPD markers were produced from the selected 15 primers. The number of bands obtained per primer varied from 5 to 11 with an average of 7.5 bands per primer from 250 bp was obtained. Out of the 113 bands, 88 (77.9%) were polymorphic and shared among at least two individuals, 22 (19.5%) were monomorphic common to all the individuals and 3 (2.6%) were polymorphic and unique to one genotype (**Table 2**). The polymorphic gel profiles of primer OPF-01 and OPF-11 are shown in **Figs. 1** and **2**.

The pair-wise genetic dissimilarity matrix (**Table 3**) was calculated using Squared Euclidean Distance. The highest genetic dissimilarity of 53% was noticed between the genotypes 'SN_2' and 'SN_{11}' having contrasting morphological characters such as leaf area, seed size, number of secondary

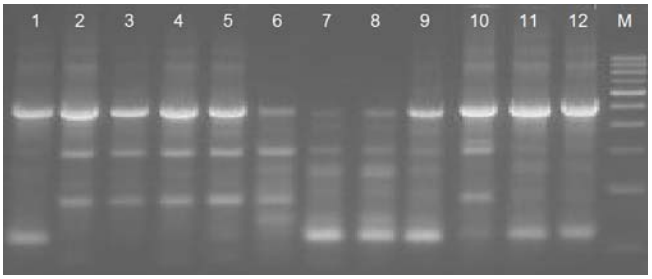

Fig. 1 Gel profile of *S. nigrum* using RAPD-PCR primer OPF–01. Genotypes (Lanes 1- 12): 1. 'SN_1', 2. 'SN_2', 3. 'SN_3', 4. 'SN_4', 5. 'SN_5', 6. 'SN_6', 7. 'SN_7', 8. 'SN_8', 9. 'SN_9', 10. 'SN_{10}', 11. 'SN_{11}' and 12. 'SN_{12}'. Lane M: 500 bp DNA ladder.

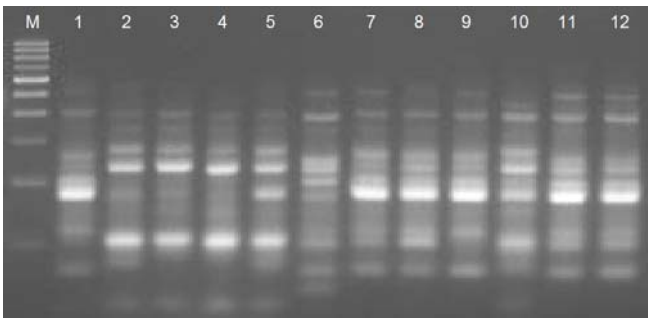

Fig. 2 Gel profile of *S. nigrum* using RAPD-PCR primer OPF–11. Genotypes (Lanes 1-12): 1. 'SN_1', 2. 'SN_2', 3. 'SN_3', 4. 'SN_4', 5. 'SN_5', 6. 'SN_6', 7. 'SN_7', 8. 'SN_8', 9. 'SN_9', 10. 'SN_{10}', 11. 'SN_{11}' and 12. 'SN_{12}'. Lane M: 500 bp DNA ladder.

Table 3 Genetic dissimilarity matrix of 12 genotypes of *Solanum nigrum* L. based on polymorphism of RAPD markers.

SN_1	0	0	0	0	0	0	0	0	0	0	0	0
SN_2	48	0	0	0	0	0	0	0	0	0	0	0
SN_3	45	23	0	0	0	0	0	0	0	0	0	0
SN_4	41	27	16	0	0	0	0	0	0	0	0	0
SN_5	36	34	25	19	0	0	0	0	0	0	0	0
SN_6	41	43	36	36	33	0	0	0	0	0	0	0
SN_7	43	49	48	42	35	24	0	0	0	0	0	0
SN_8	41	45	44	44	41	30	20	0	0	0	0	0
SN_9	38	46	43	41	38	37	25	27	0	0	0	0
SN_{10}	41	47	38	40	31	38	34	34	35	0	0	0
SN_{11}	37	53	40	40	35	30	22	30	23	30	0	0
SN_{12}	28	46	41	43	38	31	33	29	20	33	23	0

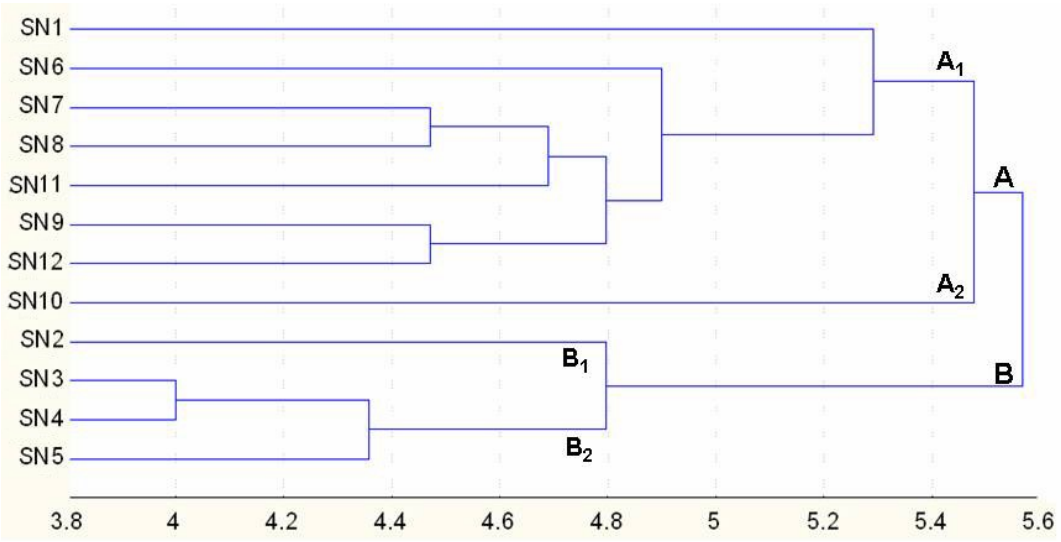

Fig. 3 Dendrogram showing RAPD-marker-based genetic relationships among 12 *Solanum nigrum* L. genotypes and grouping them into two clusters 'A' and 'B' with two sub-clusters each.

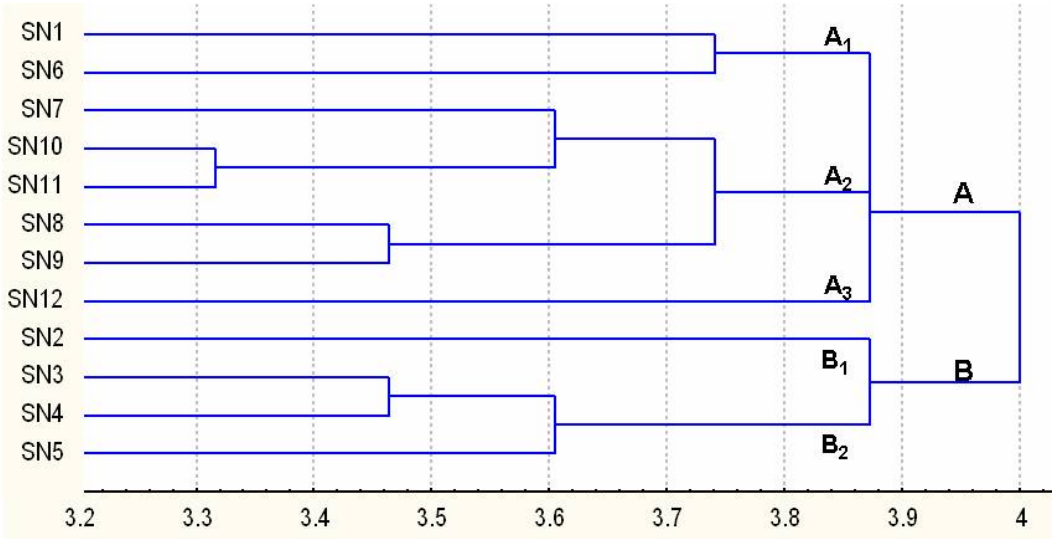

Fig. 4 Dendrogram showing morphological-marker-based genetic relationships among 12 *Solanum nigrum* L. genotypes and grouping them into two clusters 'A' and 'B' with three and two sub-clusters, respectively.

branches and pollen viability. The lowest genetic diversity of 16% was noticed between the genotypes 'SN_3' and 'SN_4' which was similar in most of the morphological characters. The dendrograms (**Figs. 3, 4**) divided the 12 accessions clearly into two major clusters *viz*., 'A' and 'B' at 5.56 linkage distance for the RAPD analysis and 4.0 for the morphological data. Both the dendrograms segregated the genotypes into two clusters: 'A' consisting of eight genotypes ('SN_1', 'SN_6', 'SN_7', 'SN_8', 'SN_9', 'SN_{10}', 'SN_{11}' and 'SN_{12}') and 'B' with four genotypes ('SN_2', 'SN_3', 'SN_4' and 'SN_5').

Cluster 'A' based on morphological data clustered the accessions into three sub-clusters. The sub-cluster 'A_1' consisted of two genotypes 'SN_1' and 'SN_6' linked at 3.74 distances. Both the genotypes shared similar morphological characters but differed by the number of flowers per inflorescence, and the number and size of seeds. The sub-cluster 'A_2' consisted of five genotypes linked at 3.74 distances and was dispersed into two groups. The genotypes 'SN_{10}' and 'SN_{11}' were linked at 3.32 distances which were alike in most of the characters except for leaf area and the percentage of pollen viability, and were closely related to genotype 'SN_7' which had smaller fruits of orange colour and large seed size. The genotypes 'SN_8' and 'SN_9' were linked at 3.46 distance, and contrasted in their leaf area and number of secondary branches i.e., five and eight, respectively. Sub-cluster 'A_3' consisted of one genotype 'SN_{12}', characterized as dwarf plants with few secondary branches (three) and high pollen viability (43.4%).

The dendrogram constructed from RAPD analysis

grouped cluster 'A' into two sub-clusters ('A_1' and 'A_2') clustered at 5.48 linkage distance. Sub-cluster 'A_1' consisted of seven genotypes, segregated into two groups at 5.28 linkage distance. Group one with only one genotype 'SN_1' was characterized by a medium-sized plant (63 cm) with the highest number of secondary branches (eight). Group two with six genotypes was divided into two sub-groups '2a' and '2b'. Genotype 'SN_6' stood as a single entity in group '2a' sharing similar characters of plant height, number of secondary branches and duration for flowering with the accessions of group '1b'. However, genotype 'SN_6' was distinctly characterized with plants producing the highest number of seeds per berry (38).

The genotypes 'SN_7' and 'SN_8' in group '2b' were linked at 4.48 units and shared similar morphological characters but differed in their berry colour: orange and black, respectively. The genotype 'SN_{11}' was closely linked to 'SN_7' and 'SN_8' at 4.68 distances with similar morphological features except its fruit weight (0.532 g) and small sized seeds (1.52 µm). The genotype 'SN_9' was characterized by its large sized leaves (53.31 cm^2) and the highest number of secondary branches (eight) and was linked to 'SN_{12}' at 4.48 linkage distance. The genotype 'SN_{12}' showed a dwarf character (28 cm) and the smallest leaf size (14.1 cm^2). The sub-cluster 'A_2' consisted of one accession 'SN_{10}' characterized by tall plants (85 cm) producing many secondary branches (seven) with large-sized berries (0.534 g) and fewer seeds (22). Cluster 'A' was characterized by a RAPD marker of 369 bp produced by primer OPF-11 (**Fig. 2**) specific to all eight genotypes. A comparison of morphological characters of group 'A' consists predominantly of tall plants with more secondary branches and requires less time to flower.

Cluster 'B' of the RAPD analysis and the morphological data separated the four genotypes in similar fashion into two sub-clusters 'B_1' and 'B_2' with 1 and 3 accessions, respectively. The genotype 'SN_2' in sub-cluster 'B_1' was characterized as tall plants (72 cm) with small leaf size (19.79 cm^2), requiring lower duration for flowering (3.5 months) and high berry weight (0.414 g). The genotypes 'SN_3' and 'SN_4' were closely related but differed in their pollen viability and number of flowers per inflorescence. The genotype 'SN_5' shared similar morphological features with 'SN_3' and 'SN_4' but differed in its plant height (42 cm), number of secondary branches (3), fresh weight of berry (0.411 g) and the greatest seed size (1.85 µm). Cluster 'B' was characterized by three RAPD markers of 356, 1440 and 1621 bp produced by primers OPD-11, OPA-16 and OPF-07, respectively. The comparison of morphological features of cluster 'B' showed that the plants were predominantly short with higher plant spread requiring more time to flower, flowers with high pollen viability and berries with high number of seeds. The results indicate clearly that the association between 'SN_2', 'SN_3', 'SN_4' and 'SN_5' were similar in both dendrograms.

All the individuals in the present studies were collected from different geographical locations. *S. nigrum* is a self-pollinated plant and shows a low degree of cross pollination (Gadamski *et al*. 1996) hence showed relatively low to moderate polymorphism, regardless of the locations from where they were obtained signifying their common origin. The cluster analysis helped in grouping genotypes with similar morphological features. The genotypes grouped under cluster 'A' were early flowering with high fruit yield, which could be cultivated for the extraction of alkaloids from berries and seeds. The types grouped under cluster 'B' recorded extensive vegetative growth and larger leaf size characters. Similar association within the 'B' cluster have depicted the precise methodology of morphological and RAPD analysis. Because of the high differentiation of polymorphism in the genetic structure of *S. nigrum*, maintenance of a limited number of populations will not be sufficient to preserve most of its genetic diversity at the species level. Hence, priority should at least be placed on maintaining representatives from both of the identified groups. *S. nigrum* is an important medicinal plant primarily cultivated for its valuable alkaloids. Similar studies on potential medicinal plants have been conducted in *Chaenomeles* (Bartish *et al*. 2000), *Changium smyrnioides* (Chengxin *et al*. 2003), *Anisodus tanguticus* (Zheng *et al*. 2008), *Hypericum perforatum* (Jana and Jan 2003) and *Phyllanthus amarus* (Neeraj *et al*. 2003). This study effectively revealed the use of RAPD and morphological markers in estimating genetic diversity in *S. nigrum*, which could be the first step towards efficient germplasm studies among herbaceous medicinal plants.

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