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EVALUATION OF GENETIC VARIABILITY USING RANDOM AMPLIFIED POLYMORPHIC DNA MARKERS IN SELECTED SPECIES OF *Solanum* L. - A POTENTIAL MEDICINAL GENUS

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ABSTRACT

Molecular techniques have been found to be more useful and accurate for the determination of both interspecific and intraspecific genetic variation among plants. Randomly amplified polymorphic DNA (RAPD) markers, in particular, have been successfully employed for determination of intraspecific genetic diversity among several plant species. *Solanum*, the genus selected for the present study is the largest and complex genus of the *Solanaceae*. It comprises more than 1,500 species with geographical origin in Asia and Africa. The centers of diversity are in South America, Australia and Africa, while relatively less diverse species are found in Europe and Asia with a promise as primarily in medicinal followed by vegetable crop. The present investigation was carried out to assess the genetic diversity of 16 selected species including one variant accession based on RAPD mark ers. Most of the selected species are having potential medicinal properties and used in modern system of medicine as well as in traditional system by the ethnic societies of Kerala against various ailments. The 234 polymorphic bands were scored using 15 random primers employed suggesting 100% polymorphism across the genotypes. UPGMA dendrogram cluster analysis indicated three distinct clusters, one comprising accessions of *S. mauritianum*, *S. capsicoides* (spiny accession) and *S. trilobatum*, while second included four species viz., *S. pseudocapsicum, S. mammosum, S. capsicoides* (lax spiny accession) and *S. giganteum*. *S. seaforthianum, S. erianthum, S. nigrum, S. melongena* var. *insanum, S. torvum,*

S. violaceum ssp. *multiflorum, S. macrocarpon, S. aculeatissimum, S. violaceum* ssp. *violaceum and S. wendlandii* formed third larger cluster indicating its genetic relatedness. The overall grouping pattern of clustering corresponds well with principal component analysis confirming patterns of genetic diversity observed among the species. The result provides valid guidelines for collection, conservation and characterization of *Solanum* genetic resources in particular, the medicinal species.

Keywords: Dendrogram, Genetic diversity, Polymorphic bands, Random primers, *Solanum* spp.

INTRODUCTION

Solanum L. is the most representative of the Solanaceae with about 1500 species with cosmopolitan distribution[1]. It is considered to be one of the largest group among the angiosperms[2]. Several species of *Solanum* viz. *S. torvum*, *S. nigrum, S. melongena, S. tuberosum, S. macrocarpon* are found in India. In Kerala, the genus is represented by about 30 species and many of them are being used as vegetables and some are medicinally important. *Solanum schizanthus pinnatus, S. grahami*, and *S. retuses* are cultivated as garden ornamentals. Fruits of *S. melongena* and *S. torvum* and tubers of *S. tuberosum* are of vegetable value, while that of *S. nigrum, S. mauritianum, S. indicum, S. aculeatissimum* are edible. *Solanum indicum* fruits are used in colic and roots in tooth aches, cough and catarrhal affections. *Solanum torvum, S. nigrum, S. erianthum, S. violaceum, S. melongena, S. xanthocarpum* etc. are medicinally important and there are several studies validating its pharmacological significance[3,4,5,6]. Correct identification and quality assurance of the medicinal raw materials is an essential pre-requisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. Morphological, anatomical, biochemical and molecular characteristics have been used in pharmacognostic standardization of plant material. Though the genus *Solanum* has rich species diversity, there is still ambiguity regarding morphology, therefore, molecular markers can be an aid in assigning accurately the taxonomic status at species level. The classification and phylogeny of the species belonging to *Solanum* is a complex issue, a widely accepted consensus. These species diverged recently, are still closely related and, in some cases, are still even capable of interspecific hybridization, thereby blurring the difference between intra- and interspecific variation. Most of the taxonomic information of the genus relies on morphological markers. When the plants are highly variable and contain large number of hybrids, identification based on morphological characters is quite difficult.

The Random Amplified Polymorphic DNA (RAPD) analysis is a reliable and efficient technique for the analysis of genetic variability at the molecular level. The DNA-based RAPD markers have been widely used in plant species for a variety of purposes such as accession identification, diversity studies, percentage determination, developing breeding programs and conservation strategies [7]. To our knowledge, no report has been published on the genetic diversity of *Solanum* spp. in Kerala based on molecular markers. In this study, we applied RAPD markers to analyze relationship among 16 *Solanum* species located in the geographical region of Kerala which can be further utilized in assigning taxonomic status at species, subspecies and varietal levels to a larger extent.

MATERIALS AND METHODS

Plant material

Solanum species selected for the present study were collected during spring season from its natural habitat along different parts of Kerala (Table 1). The Plants were planted in garden pots filled with mixed peat moss and perlite (1:1) in the green house of Department of Botany, University College, Thiruvananthapuram, Kerala, India. The herbarium voucher specimens were deposited in the herbarium depository at Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Thiruvananthapuam, Kerala, India. Fresh leaves were collected for DNA analysis.

Table 1: Details of plant materials in the study

Acc.No.	*Solanum* species	Collection locality (Kerala state, South India)
1	*S. giganteum* Jacq.	Mannavanchola, Idukki; kundla dam, Idukki
2	*S. seaforthianum* Andr.	Kanthallur, Idukki; Ponmudi, Thiruvananthapuram
3	*S. torvum* Sw.	Thiruvananthapuram, Palakkad, Wayanad
4	*S. violaceum* Ortega ssp *multiflorum*(Clarke)	Munnar, Idukki; Kanthallur, Idukki
5	*S. pseudocapsicum* L.	Marayur, Idukki, Rajamala, Idukki
6	*S. nigrum* sensu Gamble	Thiruvananthapuram, Wayanad, Palakkad
7	*S. violaceum* Ortega ssp *violaceum*	Thiruvananthapuram, Wayanad, Palakkad, Kollam
8	*S. mammosum* L.	Thiruvananthapuram
9	*S. mauritianum* Scop.	Mannavanchola, Idukki
10	*S. melongena* var *insanum* L.	Thiruvananthapuram
11	*S. macrocarpon* L.	Wayanadu, Trivandrum, Kollam

12	*S. wendlandii* Hook	Idukki, Kambilikkandam; Marayur
13	*S. aculeatissimum* Jacq.	Idukki, Thiruvananthapuram
14	*S. trilobatum* L.	Thiruvananthapuram
15	*S. capsicoides* All.(spiny accession)	Thiruvananthapuram, Wayanad, Idukki
16	*Solanum capsicoides* All.(lax spiny accession)	Dhoni, Palakkad; Thiruvananthapuram
17	*S. erianthum* D.Don	Marayur, Idukki

DNA extraction

Total genomic DNA was extracted following the procedure as described by Doyle and Doyle [8], with minor modifications.

PCR amplification

PCR reaction was performed as described by Williams et al. [9] with 10-mer oligonucleotides (Operon technologies).

Data analysis

The matrix of similarity (Jaccard) and similarity of coefficients [10] were calculated and the dendrogram was constructed by clustering according to the Unweighted Pair-Group Method with Arithmetic averages (UPGMA) using SPSS, Version (11.0) software.

RESULTS AND DISCUSSION

A total of 15 RAPD Operon primers were screened for 16 species of *Solanum* collected from different localities of Kerala. RAPD was performed in triplicate using genomic DNA with 15 arbitrary decamers to test the reproducibility. The number of RAPD markers required for distinguishing the accessions varies with the test material. Forty-nine primers have been used for the RAPD analysis of Indian *Musa* germplasm and found that 12 primers were sufficient to distinguish all the 57 accessions included in the study [11]. The use of the most polymorphic bands may reduce the time and cost of RAPD analysis [12]. In the present study, using 15 selected RAPD primers, a total of 234 bands were generated (an average of 15.6 bands per primer) of which 100% bands were polymorphic (Table 2 & 3). The number of bands varied from 6 for primer OPR-08 to 16 for primer OPR-04. The size of the amplified fragments varied from 100 to 2500 bp. The average number of polymorphic bands for each primer was estimated at 15.6. In total 234 polymorphic bands were generated and the maximum number of bands belonged to primer OPR 08 with 26 bands followed by OPR 07 with 20 bands and the least belonged to OPR 06 with 8 bands followed by OPR 01 with 9 bands. All the species

exhibited polymorphic bands and they were unique for 14 species and the variant accession of *Solanum capsicoides* except in *S. mauritianum* and *S. mammosum.*

Table 2: Total number of bands exhibited in each *Solanum* species

Sample number	*Solanum* species	Total no. of bands
1	*S. violaceum* ssp. *violaceum*	10
2	*S. aculeatissimum*	10
3	*S. macrocarpon*	12
4	S. *capsicoides* (lax spiny accession)	4
5	S. *capsicoides* (spiny accession)	12
6	*S. trilobatum*	12
7	*S. mauritianum*	9
8	*S. pseudocapsicum*	6
9	*S. giganteum*	8
10	*S, mammosum*	6
11	*S. wendlandii*	11
12	*S. torvum*	20
13	*S. melongena* var. *insanum*	23
14	*S. nigrum*	20
15	*S. violaceum* ssp. *multiflorum*	15
16	*S. erianthum*	23
17	*S. seaforthianum*	23

Table 3: Number of polymorphic bands in *Solanum* species against the selected 15 primers

Sl. No.	Primers	Primer sequence (5'→3')	No. of polymorphic bands
1	OPR 01	AACCGACGGG	9
2	OPR 02	GGGGGTCGTT	15
3	OPR 03	TGCCCTGCCT	15
4	OPR 04	CCAGACCCTG	11
5	OPR 05	AAGCTCCCCG	19
6	OPR 06	TACCACCCCG	8
7	OPR 07	GGCGGACTGT	20
8	OPR 08	GTCACTCCCC	26
9	OPR 09	ACCGCGAAGG	19
10	OPR 10	GGACCCAACC	19
11	OPR 11	GTCGCCGTCA	10

12	OPR 12	TCTGGTGAGG	17
13	OPR 13	TGAGCGGACA	15
14	OPR 14	ACCTGAACGG	18
15	OPR 15	TTGGCACGGG	13
Total			234

The results presented are more commendable than recent findings by Gracelin et al. [13]. A total number of 234 amplified fragments were obtained with all primers, which agreed well with the work of Welsh and McClelland [14], who found that simple and reproducible fingerprints of complex genomes can be generated using single primers and PCR. Fourteen species and the variant accession of *Solanum* showed the following specific fragments such as *S. violaceum* ssp. *violaceum*: 5 specific fragments (OPR 03 with 850 bp, OPR 05 with 700 bp, OPR 11 with 550 bp, OPR 13 with 380 bp and OPR 14 with 2200 bp), *S. aculeatissimum*: 1 specific marker (OPR 13 with 400 bp), *S. macrocarpon* displayed two fragments (OPR 05 with 290 bp and OPR 07 with 880 bp), *S. capsicoides* (lax spiny accession) one fragment (OPR 04 with 250 bp), and the other accession (spiny accession) with three fragments (OPR 4 with 120 bp, OPR 5 with 680 bp, OPR 07 with 800 bp), *S. trilobatum*: 7 fragments (OPR 04 with 200 bp, OPR 09 with 800 and 350 bp, OPR 12 with 580 bp, OPR 13 with 550 bp, OPR 14 with 500 and 700 bps, *S. pseudocapsicum* with one fragment (OPR 02 with 300bp), *S. giganteum* with 4 fragments (OPR 06 with 1800 bp, OPR 13 20000bp, OPR 14 2000 and OPR 15 2000bp), *S. wendlandii* with 4 fragments (OPR 02 with 2500 bp, OPR 05 450 bp, OPR 06 2500 bp, OPR 08 2500bp), *S. torvum* with two fragments (OPR 03 200bp, OPR 07 270 bp), *S. melongena* var. *insanum* with one specific fragment with OPR 04 (350bp), *S. nigrum* with two fragments (OPR 01 1500, OPR 03 250bp), *S. violaceum* ssp. *multiflorum* with two fragments (OPR 02 1100 bp, OPR 08 1500bp), *S. erianthum* showed six fragments (OPR 01 2000, OPR 02 150bp, OPR 03 400bp, OPR 05 10000 bp, OPR 07 600bp and OPR 10 350bp) and *S. seaforthianum* with three fragments (OPR 07 300bp, OPR 14 900bp and OPR 15 390bp) while *S. mauritianum* and *S. mammosum* did not have any specific bands (Fig.1a, b and c).

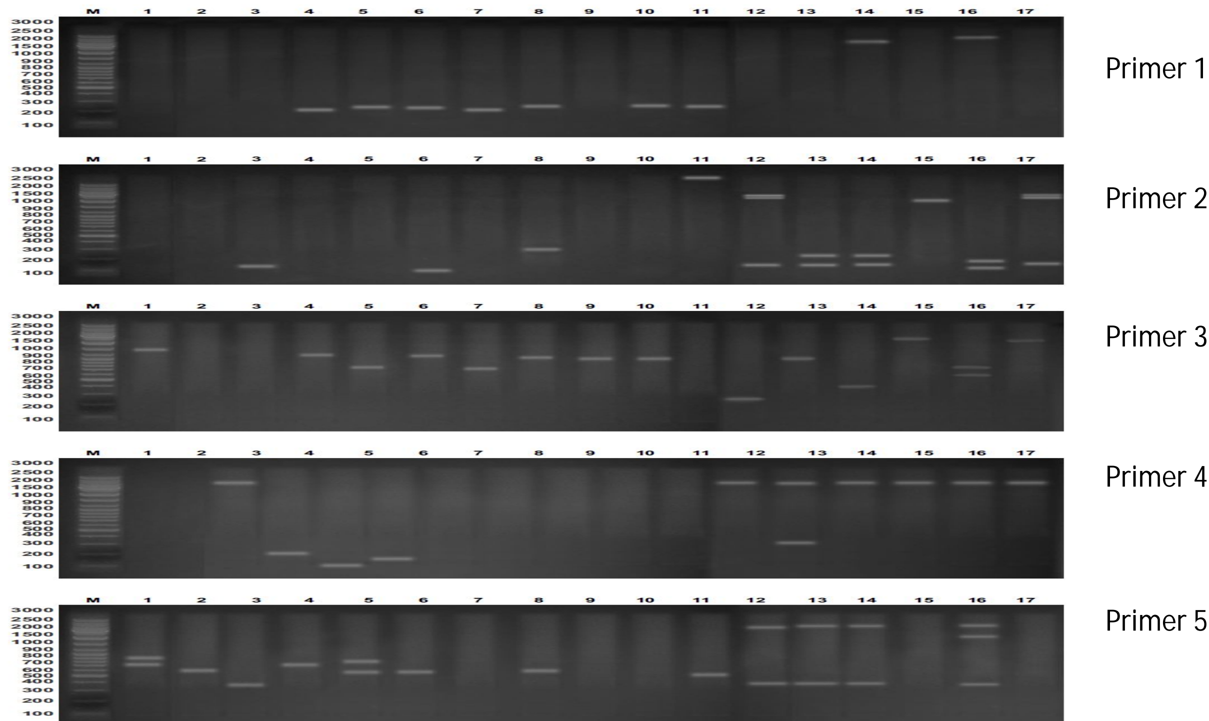


Fig 1a: RAPD banding patterns in *Solanum* species

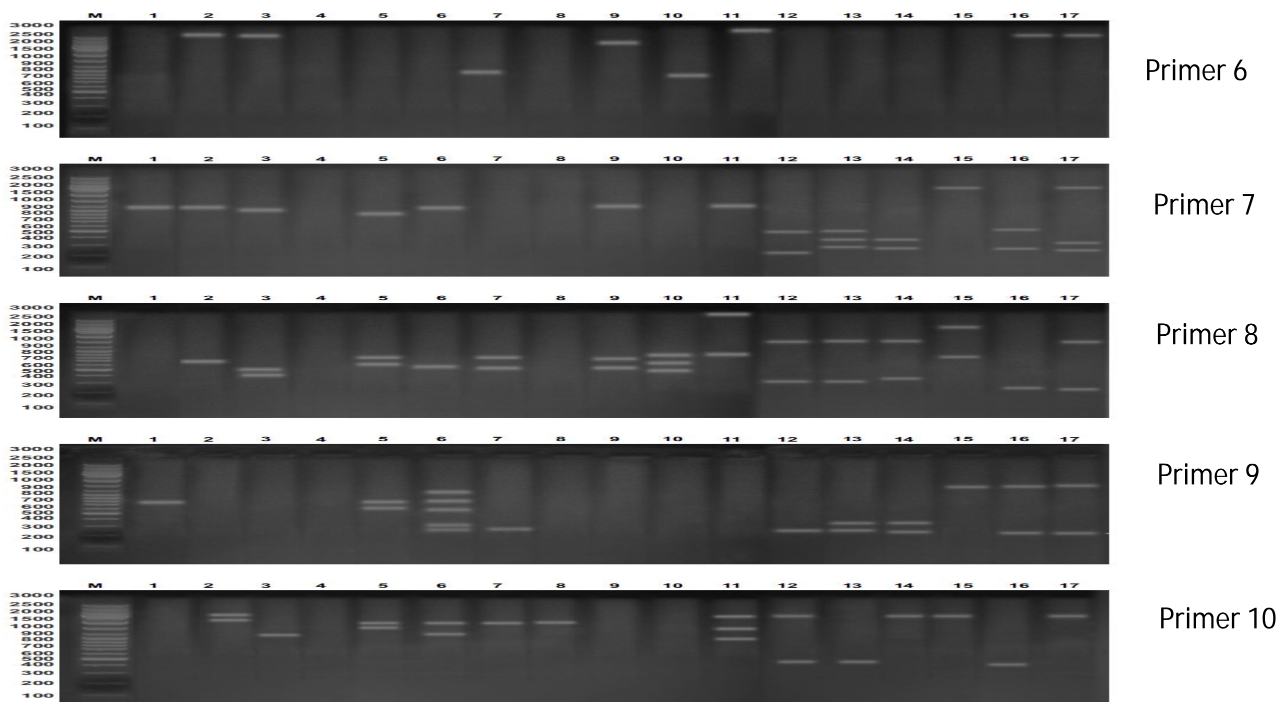


Fig.1b: RAPD banding patterns in *Solanum* species

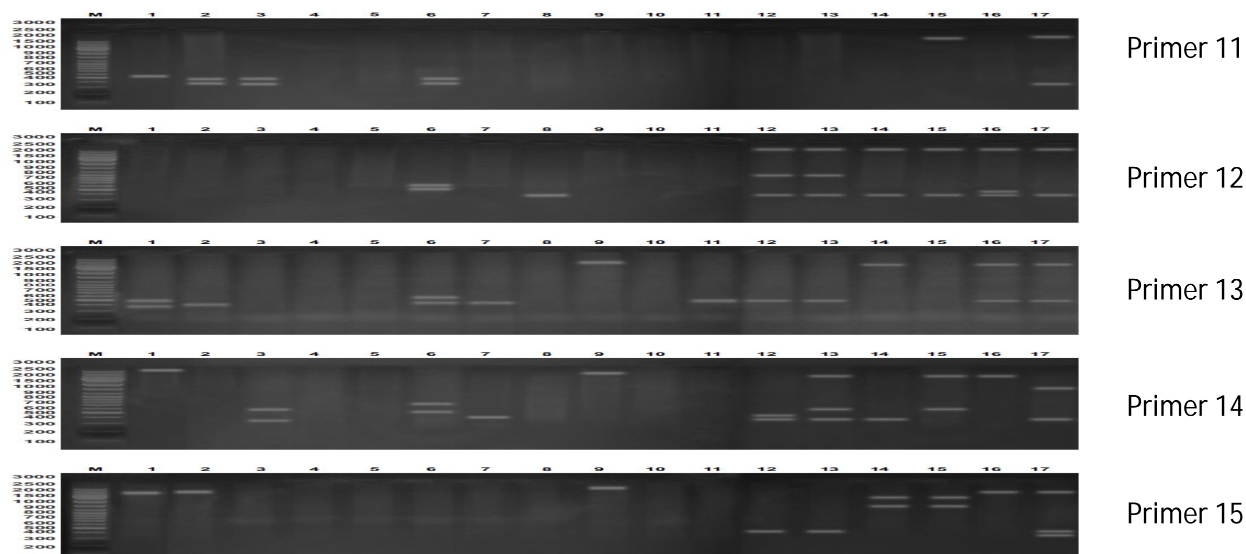


Fig.1c: RAPD banding patterns in *Solanum* species

These bands could be considered as potential species-specific markers after checking that every individual from that species shows that specific marker in question [15]. The results of *Solanum* species in the present study confirmed the utility of RAPD analysis to characterize each species based on specific markers that produce bands which distinguishes them. Similar findings were obtained with molecular studies in Zygophyllaceae [16,17].

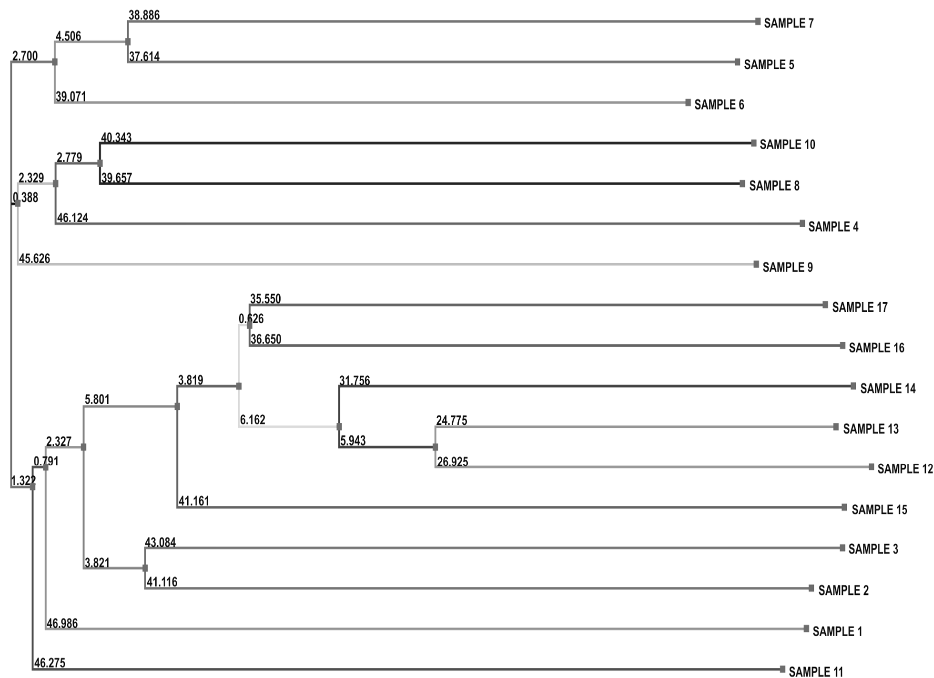
Molecular techniques have been found to be more useful and accurate for determination of both interspecies and intraspecies genetic variation in plants. Both molecular and morphological data has been utilized for characterization among *Iris* species and *I. spuria* was segregated from the others [18]. RAPD was used to determine genetic relationships in the genus *Crataegus* and the species were rearranged based on DNA polymorphism data [19]. There were several instances of correlated frequency change overs of single RAPD markers and morphological characters along the macrogeographic gradient and a few cases of markedly parallel patterns between RAPD markers [20]. Al-Rawashdeh [21] also used RAPD technique to study the molecular taxonomy and genetic relationship between two *Mentha* species namely, *Mentha spicata* and *Mentha longifolia*, and *Ziziphora tenuior*. Tripathi and Goswami [22] analyzed generic relationships among twenty-four species belonging to genus *Cassia* L., *Senna* Mill. and *Chamaecrista* Moench using RAPD markers. Total 80 primers were initially screened, 514 amplification products obtained with 38 informative primers, of which 514 were polymorphic. A very high degree of polymorphism (100%) was observed among them. The genus *Terminalia* is considered as complex group having many problems associated with taxonomic identification. Five taxonomically critical *Terminalia* L. species

were analysed by Deshmukh et al. [23] with 31 random primers to evaluate genetic diversity and species relationships. From the total 31 primers screened, 26 primers amplified across all species scoring 336 bands of which 305 were polymorphic. Similarly, Lina Zybartaite et al. [24] employed randomly amplified polymorphic DNA effectively in *Impatiens glandulifera* across geography or habitats using 8 primers. At the species level, all DNA bands (188) were polymorphic. Among populations of *I. glandulifera* genetic parameters ranged in the following intervals: 40–56% of polymorphic DNA bands, 0.115–0.165 for Nei's gene diversity, 0.179–0.255 for Shannon's information index.

The percentage of genetic distance between *Solanum* species showed variations from 100 to 51.7%. Majority of the species exhibited shared bands except *S. capsicoides* accessions i.e., lax spiny and spiny. Interestingly, these two accessions also displayed sound morphological variations in the size and distribution of spines. In the spiny accession spines are profusely distributed along the entire plant body including the calyx while, in the lax spiny accession spines are sparse and completely absent in calyx. The fruit colour also showed variation from red to orange red. Similarly, no shared bands are exhibited between *S. violaceum* ssp. *violaceum* and *S. violaceum* ssp. *multiflorum*. This was supported by sound morphological differences and was treated taxonomically into two different subspecies. In this context *S. capsicoides* accessions requires revision i.e., may be treated into separate subspecies or varieties.

Dendrogram based on Nei's [25] genetic distance using UPGMA indicated segregation of the 17 species of *Solanum* into three main clusters (Fig. 2): *S. mauritianum*, *S. capsicoides* (spiny accession) and *S. trilobatum* grouped into cluster 1; *S. mammosum*, *S. pseudocapsicum*, *S. capsicoides* (lax spiny accession) and *S. giganteum* grouped in cluster 2 and the third larger cluster included *S. seaforthianum*, *S. erianthum*, *S. nigrum*, *S. melongena* var. *insanum*, *S. torvum*, *S. violaceum* ssp. *multiflorum*, *S. macrocarpon*, *S. aculeatissimum*, *S. violaceum* ssp. *violaceum* and *S. wendlandii*. In cluster 1, there are two subclusters: *S. mauritianum* and *S. capsicoides* (spiny accession) formed sub cluster 1 while, *S. trilobatum* was in sub cluster 2. Among the genotypes of cluster 2, *S. mammosum*, *S. pseudocapsicum* formed sub cluster 1 and *S. capsicoides* (lax spiny accession) belonged to sub cluster 2, though, *S. giganteum* formed another single sub cluster. Further, the cluster 3 was divided into five groups, *S. seaforthianum* and *S. erianthum* belonged to sub group 1. *S. nigrum* another sub group, *S. melongena* var. *insanum* and *S. torvum* formed another sub cluster, *S. violaceum* ssp.

multiflorum produced a separate sub group, *S. macrocarpon* and *S. aculeatissimum* another sub cluster, *S. violaceum* ssp. *violaceum* yet another sub cluster and *S. wendlandii* into a single sub group. The grouping of *S. capsicoides* accessions in the dendrogram strongly supported the genetic variations in them (Fig. 2).



Sample 1	*S. violaceum* ssp. *violaceum*	Sample 10	*S. mammosum*
Sample 2	*S. aculeatissimum*	Sample 11	*S. wendlandii*
Sample 3	*S. macrocarpon*	Sample 12	*S. torvum*
Sample 4	*S. capsicoides* (lax spiny accession)	Sample 13	*S. melongena* var. *insanum*
Sample 5	*S. capsicoides* (spiny accession)	Sample 14	*S. nigrum*
Sample 6	*S. trilobatum*	Sample 15	*S. violaceum* ssp. *multiflorum*
Sample 7	*S. seaforthianum*	Sample 16	*S. erianthum*
Sample 8	*S. pseudocapsicum*	Sample 17	*S. seaforthianum*
Sample 9	*S. giganteum*		

Fig. 2: Dendrogram of *Solanum* species

Evaluation of important qualitative characters among different species showed morphological characters viz. leaves, flower, leaf cross section and flower scent as very valuable traits in terms of economic viability in *Solanum*. It can be seen that although these traits can differentiate species based on morphometrics, the expression of phenotype of each plant is due to the interaction of environmental and genetic elements. Molecular markers can be used as an alternative way to identify species with very small genetic variation such as mutants.

Both molecular and morphological characteristics (quantitative and qualitative) among the seventeen *Solanum* species under study discriminated one species from the other. It is not expected that all morphological data corroborate molecular results, but using simultaneous markers is more useful for effective grouping. The results are completely compatible with each other.

CONCLUSION

Thus it can be concluded that all of these investigated species have high molecular variation and therefore, RAPD markers can be used for managing the genetic resources of *Solanum*. The findings also provide useful information about applying these genetic materials for transferring useful genes to generate new species for breeders. Similarly, it will also be a basis for future investigation aimed at defining intra- and inter-specific levels of genetic diversity, distinguishing hybrids among species and to preserve the diversity of this medicinally potential genus.

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REFERENCES

1. Bohs L. Major clades in *Solanum* based on ndhF sequences. In: Keating RC, Hollowell VC (eds.), Croat monographs in Systematic Botany from the Missouri Botanical Garden, A festschrift for William G. D'Arcy: the legacy of a taxonomist, St. Louis: Missouri Bot. Garden Press, 2005;104, pp. 27-49.
2. D'Arcy WG. The Solanaceae since 1976, with a review of its biogeography. In JG Hawkes, RN Lester, M Nee N. Estrada (eds.), Solanaceae III: Taxonomy, Chemistry, Evolution, Royal Botanic Garden, Kew: 1991; pp. 75-138.
3. Kirtikar KR, Basu BD. Indian Medicinal Plants, Bishen Singh Mahendra Pal Singh, Dehradun: 1994, p.26.
4. Jain R, Sharma A, Gupta S, Sarethy IP, Gabrani R. *Solanum nigrum*: Current perspectives on therapeutic properties. Alternaive Medicine Review, 2011; 16(1): 78-85.

5. Roshy Joseph C, Ilanchezhian R, Patgiri BJ. Therapeutic potentials of Kantakari (*Solanum xanthocarpum* Schrad. & Wendl). Ayurpharm Int J Ayur Alli Sci, 2012; 1(2): 46 – 53
6. Ranka D, Aswar M, Aswar U, Bodhankar S. Diuretic potential of aqueous etract of roots of *solanum xanthocarpum* Schrad & Wendl, a preliminary study. Ind J Exp Biol, 2013; 51: 833-839.
7. Ongusoa JM, Kahangi EM, Ndiritu DW, Mizutani F. Genetic characterization of cultivated bananas and plantains in Kenya by RAPD markers. Sci Hortic, 2004; 99: 9-20.
8. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytol Bull, 1987; 19: 11-15.
9. Williams JGK, Kubelik AR, Livak KJ, Rafalski SA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res, 1990; 18: 653-665.
10. Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences of the USA, 1979; 76: 5269-5273.
11. Bhat KV, Jarret RL. Random amplified polymorphic DNA and genetic diversity in Indian *Musa* germplasm. Genet Resour Crop Evol, 1995; 42: 107-118.
12. Fanizza G, Colonna G, Resta P, Ferrara G. The effect of the number of RAPD markers on the evaluation of genotypic distances in *Vitis vinifera*. Euphytica, 1999; 107: 45–50.
13. Gracelin DHS, John De Britto A, Raj LS, Rathna Kumar PBJ. Assessment of genetic relationships among five species of *Solanum* as revealed by RAPD markers. Life Sci Leaflets, 2011; 19: 809-814.
14. Welsh J, McClelland M. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res, 1990; 18: 7213-7218.
15. Roman B, Alfaro C, Torres AM, Moreno MT, Pujadas A, Rubiales D. Genetic Relationships among *Orabanche* Species as revealed by RAPD Analysis. Ann Bot, 2003; 91: 637-642.
16. Sheahan MC, Mark WC. Phylogenetic relationships within Zygophyllaceae based on DNA sequences of three plastid regions, with special emphasis on Zygophylloideae. Syst Bot, 2011; 25: 371-384.
17. Hammad SH Qari. Genetic diversity among *Zygophyllum* (Zygophyllaceae) populations based on RAPD analysis. Gene Mol Res, 2010; 9: 2412-2420.

18. Azimi MH, Sadeghian SY, Razavi Ahari V, Khazaei F, Fathi Hafashjani A. Genetic variation of Iranian *Iris* species using morphological characteristics and RAPD markers. Int J Agri Sci, 2012, 2: 875-889.
19. Beigmohamadi M, Rahmani F. Genetic variation in hawthorn (*Crataegus* spp.) using RAPD markers. Afr J Biotechnol, 2011; 10: 7131-7135.
20. Gonza´ Lez-Rodri´Guez A, Arias DM, Valencia S, Oyama K. Morphological and RAPD analysis of hybridization between *Quercus affinis* and *Q. laurina* (Fagaceae) two mexican red oaks. Am J Bot, 2004; 91: 401-409.
21. Al-Rawashdeh IM. Molecular Taxonomy among *Mentha spicata, Mentha longifolia* and *Ziziphora tenuior* Populations using the RAPD Technique. Jordan J Biol Sci, 2011; 4: 63-70.
22. Tripathi V, Goswami S. Generic relationship among *Cassia* L., Senna Mill. and *Chamaecrista* Moench using RAPD markers. Int J Biodiversity Cons, 2011; 3: 92-100.
23. Deshmukh VP, Prashant V, Thakare S, Uddhav Chaudhari A, Prashant Gawande, Vinod Undal S. Assessment of Genetic Diversity among Terminalia Species Using RAPD Markers. Global J Biotechnol Biochem, 2009; 4: 70-74.
24. Lina Zybartaite, Judita Zukauskiene, Milda Jodinskiene, Steven Janssens B, Algimantas Paulauskas, Eugenija Kupcinskiene. RAPD analysis of genetic diversity among Lithuanian populations of *Impatiens glandulifera.* Zemdirbystė Agriculture, 2011; 98: 391-398.
25. Nei M. Genetic distance between populations. Am Nat, 1972; 106: 283-292.