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ISSR MARKERS FOR COMPARATIVE ANALYSIS OF GENETIC VARIABILITY IN *SOLANUM* L. SPECIES OF SOUTHERN WESTERN GHATS OF KERALA, SOUTH INDIA

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ABSTRACT

Aims: To analyze the genetic variability among 17 *Solanum* L. taxa from the Southern Western Ghats of Kerala along with common cultivars *S. melongena* Neelima and *S. tuberosum* by employing inter simple sequence repeats. **Study design:** using15 ISSR primers, the purified DNA from the plant samples were tested for their variability. **Methodology:** The DNA samples were isolated and purified from various plant materials. PCR amplification was done. ISSR primers were used for hybridization and the scorable bands were analyzed. This was compared for the presence of shared and unique bands and finally the percentage of genetic distance calculated and ISSR dendrogram was constructed. The grouping of the clusters denotes their genetic relatedness. **Results:** 14 ISSR primers showed polymorphism and the highest number of polynmorphic amplification fragments was 22, obtained with the primer number 02. The least number of bands obtained with the primers 05 and 03. **Conclusion:** The genetic variability existing among various *Solanum* species could be effectively demonstrated using ISSR analysis.

KEYWORDS: Dendrogram, Inter simple Sequence Repeats, Polymerase Chain Reaction, *Solanum.*

INTRODUCTION

Solanum L. is the largest genus of Solanaceae with about 1500 species having cosmopolitan distribution.[1] It is recognised to be one of the largest angiosperm group. Nearly 48 species of *Solanum* have been reported from India.[2] In Kerala, about 34 *Solanum* species are found among which, many are having uses as vegetables and herbal drugs Fruits of *S. melongena* L. and *S. torvum* Sw. as well as the tubers of *S. tuberosum* are consumed as vegetables, while that of *S. nigrum* L., *S. mauritianum* Scop., *S. indicum* L., *S. aculeatissimum* Jacq. are being eaten by many tribal people. *Solanum torvum, S. nigrum, S. erianthum* D.Don., *S. violaceum* Ortega., *S. melongena, S. xanthocarpum* Schrad and wendle. etc. are medicinally important and there are several studies validating its pharmacological significance.[3,6] Precise identification is a pre requisite for quality assurance of the medicinal raw materials. Hence, it is inevitable to ensure reproducible quality of herbal drugs which further contribute to its efficacy and safety. Several strategies including morphological, anatomical, biochemical and molecular characteristics have been employed in pharmacognostic standardization of plant material. Even though the genus *Solanum* shows rich species diversity, ambiguity still exists related to morphology. This often warrants the use of molecular markers as an aid in assigning the taxonomic status at species level accurately. The phylogeny and classification of *Solanum* species is a complex issue, a widely accepted consensus. The high degree of interspecific hybridization exhibited by the species poses great dilemma in discriminating intra- and interspecific variations. Conventional systematic recognition of various species relies largely on morphological markers. However, in the case of *Solanum*, high variability coupled with the prevalence of hybrids makes it difficult to delineate the different taxa purely based on morphological traits.

Inter Simple Sequence Repeats (ISSR) is a reliable and efficient technique for the analysis of genetic variability at the molecular level. The DNA-based ISSR 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.[7,9] To the best of our knowledge, no report has been published on the genetic diversity of *Solanum* spp. in Kerala based on ISSR markers. Hence, the study was attempted to analyze relationship among 17 *Solanum* species located in the Southern Western Ghats of Kerala along with the common cultivars *S. melongena* Neelima and *S. tuberosum* which can be further utilized in assigning taxonomic status at species, subspecies and varietal levels to a larger extent.

MATERIALS

Nineteen *Solanum* species collected including the locality (Table 1).

Table 1: List of *Solanum* species.

Acc. No	*Solanum* species (Herbarium voucher Number)	Collection locality (latitude & longitude)
1	*S. giganteum* Jacq. (TBGT 24067)	Mannavanchola, Idukki; kundala dam, Idukki (9° 51′ 0″ N, 76° 56′ 24″ E)
2	*S. seaforthianum* Andr. (TBGT 24070)	Kanthallur, Idukki (9° 51′ 0″ N, 76° 56′ 24″ E); Ponmudi, Thiruvananthapuram (8° 30′ 0″ N, 76° 55′ 12″ E)
3	*S. torvum* Sw. (TBGT 24076)	Thiruvananthapuram (8° 30′ 0″ N, 76° 55′ 12″ E); Palakkad (10° 46′ 30″ N, 76° 39′ 3.6″ E); Wayanad (11° 36′ 18″ N, 76° 4′ 58.8″ E)
4	*S. violaceum* (Ortega) subsp. *multiflorum* (Clarke) Matthew (TBGT 24075)	Munnar, Idukki; Kanthallur, Idukki (9° 51′ 0″ N, 76° 56′ 24″ E)
5	*S. pseudocapsicum* L. (TBGT 24068)	Marayur, Idukki; Rajamala, Idukki (9° 51′ 0″ N, 76° 56′ 24″ E)
6	*S. americanum* Mill. (TBGT 24074)	Thiruvananthapuram (8° 30′ 0″ N, 76° 55′ 12″ E), Wayanad (11° 36′ 18″ N, 76° 4′ 58.8″ E), Palakkad (10° 46′ 30″ N, 76° 39′ 3.6″ E)
7	*S. violaceum* (Ortega) subsp. *violaceum* (TBGT 24077)	Thiruvananthapuram (8° 30′ 0″ N, 76° 55′ 12″ E), Wayanad (11° 36′ 18″ N, 76° 4′ 58.8″ E), Palakkad (10° 46′ 30″ N, 76° 39′ 3.6″ E), Kollam (8° 48′ 0″ N, 76° 36′ 0″ E)
8	*S. mammosum* L. (TBGT 014)	Thiruvananthapuram (8° 30′ 0″ N, 76° 55′ 12″ E)
9	*S. mauritianum* Scop. (TBGT 24073)	Mannavanchola, Idukki (9° 51′ 0″ N, 76° 56′ 24″ E)
10	*S. melongena* (L.) var *insanum* (L.) Praine. (TBGT 24066)	Thiruvananthapuram (8° 30′ 0″ N, 76° 55′ 12″ E)
11	*S. macrocarpon* L. (TBGT 015)	Wayanad (11° 36′ 18″ N, 76° 4′ 58.8″ E); Thiruvananthapuram (8° 30′ 0″ N, 76° 55′ 12″ E); Kollam (8° 48′ 0″ N, 76° 36′ 0″ E)
12	*S. wendlandii* Hook (TBGT 24071)	Idukki, Kambilikkandam; Marayur (9° 51′ 0″ N, 76° 56′ 24″ E)
13	*S. aculeatissimum* Jacq. (TBGT 017)	Idukki (9° 51′ 0″ N, 76° 56′ 24″ E); Thiruvananthapuram (8° 30′ 0″ N, 76° 55′ 12″ E)
14	*S. trilobatum* L. (TBGT 013)	Thiruvananthapuram (8° 30′ 0″ N, 76° 55′ 12″ E)
15	*S. capsicoides* All. (TBGT 24069)	Thiruvananthapuram (8° 30′ 0″ N, 76° 55′ 12″ E); Wayanad (11° 36′ 18″ N, 76° 4′ 58.8″ E); Idukki (9° 51′ 0″ N, 76° 56′ 24″ E)
16	*S. exarmatum* Anil *et al*. (TBGT 016)	Dhoni, Palakkad (10° 46′ 30″ N, 76° 39′ 3.6″ E); Thiruvananthapuram (8° 30′ 0″ N, 76° 55′ 12″ E)
17	*S. erianthum* D. Don (TBGT 24072)	Marayur, Idukki (9° 51′ 0″ N, 76° 56′ 24″ E)
18	*S. tuberosum* L. (TBGT 4133)	Wayanad (11° 36′ 18″ N, 76° 4′ 58.8″ E); Idukki (9° 51′ 0″ N, 76° 56′ 24″ E)
19	*S. melongena* L. Neelima (TBGT 4134)	Thiruvananthapuram (8° 30′ 0″ N, 76° 55′ 12″ E), Wayanad (11° 36′ 18″ N, 76° 4′ 58.8″ E), Palakkad (10° 46′ 30″ N, 76° 39′ 3.6″ E), Kollam (8° 48′ 0″ N, 76° 36′ 0″ E)

RESULTS AND DISCUSSION

Of 15 ISSR primers, 14 exhibited polymorphism among the *Solanum* taxa (Table 2). The highest number of polymorphic amplification fragments is obtained with the primers 02 (22), followed by 01 (18) and 10 (18), whereas the primers 05 (1) and 03 (3) resulted with the lowest number of bands. Polymorphisms are observed among different species and also among the two accessions within the species. Total number of bands in the taxa also showed diversity i.e., primer 01 produced the highest number of bands (98) followed by primer 02 (90) and similarly, primer 08 yielded the lowest number of bands (09). Among the species *S. tuberosum* produced 68 bands followed by *S. giganteum* Jacq. (66) and *S. violaceum* Ortega subsp *multiflorum* (Clarke) Matthew (65). Minimum bands are seen with *S. aculeatissimum* (26). Primers 01, 11 and 13 yielded bands for all the species. Primer 01 produced an unique band of 1500 bp for *S. trilobatum* L., primer 03 with bp 100 for *S. pseudocapsicum* L., primer 04 yielded 1500 and 1200 bp bands with *S. erianthum*, primer 05 with 200 bp and primer 06 with 650 bp for *S. mammosum* L., primer 06 with 900 bp for *S. americanum* Mill., primer 08 with 900 bp for *S. erianthum*, 600, 500, 400 bp for *S. peseudocapsicum*, primer 10 with 250 bp for *S. violaceum* subsp. *multiflorum*, primer 11 with 1500 bp for *S. pseudocapsicum*, primer 12 with 1200 bp for *S. wendlandii* Hook.f., 800 bp *S. exarmatum* Anil et al., 100 bp for *S. seaforthianum* Andr., primer 13 with 1200 bp for *S. seaforthianum*, 900 bp *S. aculeatissimum*, 750 bp for *S. capsicoides* All. 200 bp for *S.melongena* Neelima, primer 14 with 1200 and 100 bp for *S. giganteum*, primer 15 with 900 bp for *S. giganteum*, 400 bp for *S. capsicoides.* Primers 1, 7 and 14 produced the maximum number of shared bands compared to other primers among the analyzed *Solanum* species i.e., 10 and 11 respectively with *S. violaceum* subsp. *violaceum* and *S. giganteum.*

Table 2: ISSR shared and polymorphic bands in *Solanum* species with 15 primers.

ISSR primers	Total bands	Shared bands	Polymorphic bands
1	98	79	18
2	90	68	22
3	51	47	03
4	73	59	12
5	64	62	01
6	60	52	06
7	82	72	10
8	09	05	00
9	81	66	15
10	86	67	18
11	77	63	13
12	73	53	17
13	72	54	14
14	82	72	08
15	31	20	08
Total	**1029**	**839**	**165**

Number of unique bands with the primers also showed variations i.e., primers 08 and 13 yielded 4 bands (Table 3). The ISSR profile showed low polymorphism among the *Solanum* taxa compared to RAPD. The primers yielded 1029 highly reproducible ISSR bands ranging from 100 to 1500 bp in size (Figure 1-5). From the summary in Table 2 and 4, 16.03% (165/1029) of the bands are polymorphic, indicating low profile of genetic diversity among the analyzed species. This is unexpected, since the ISSR technique amplifies microsatellite regions that are potentially polymorphic.[10]

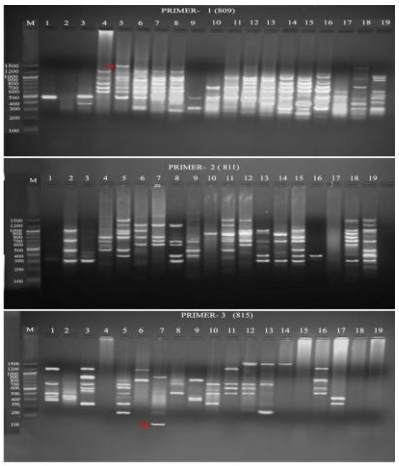

Figure 1.

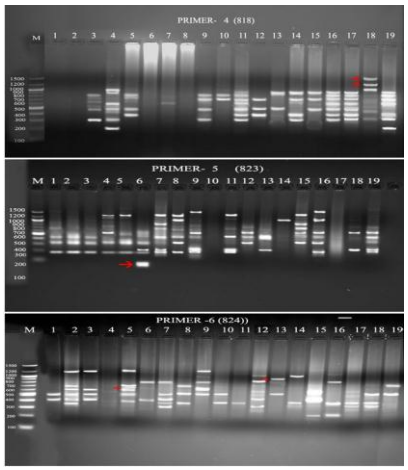

Figure 2.

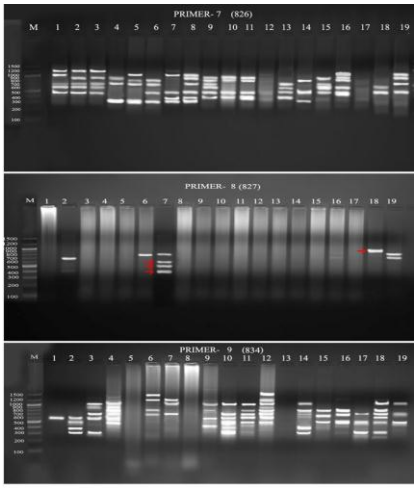

Figure 3.

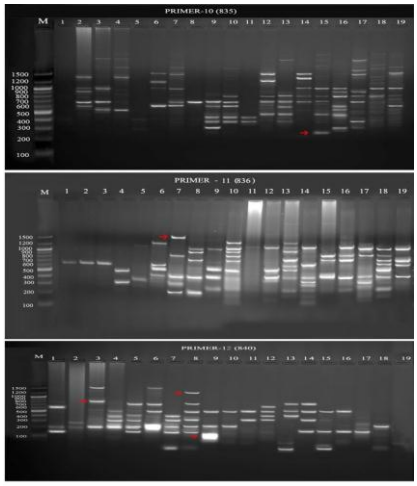

Figure 4.

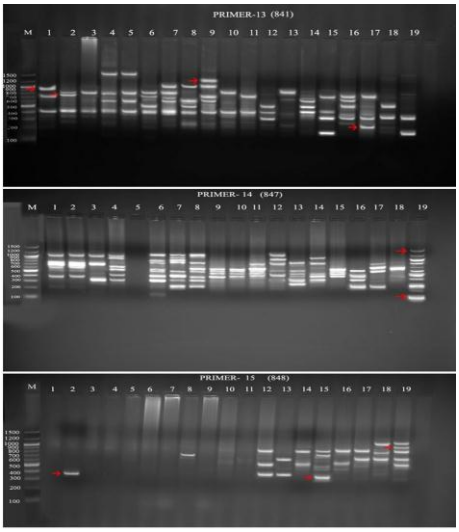

Figure 5.
Figures 1-5: ISSR profile of *Solanum* species.

(1- *Solanum aculeatissimum* Jacq., 2- *Solanum capsicoides*-All. 3- *Solanum exarmatum* Anil *et al*.4- *Solanum mauritianum* Scop., 5- *Solanum trilobatum* L., 6- *Solanum mammosum* L., 7- *Solanum pseudocapsicum* L., 8- *Solanum wendlandii* Hook., 9- *Solanum seaforthianum* Andr., 10- *Solanum violaceum* Ortega subsp. *violaceum*, 11- *Solanum melongena* L. var *insanum*(L.) Prain, 12- *Solanum torvum* Sw., 13- *Solanum americanum* Mill., 14- *Solanum macrocarpon* L., 15- *Solanum violaceum* Ortega subsp. *multiflorum* (Clarke) Matthew, 16- *Solanum tuberosum*, 17- *Solanum melongena* L. var 'Neelima', 18- *Solanum erianthum* D. Don, 19- *Solanum giganteum* Jacq.).

Zukauskiene et al.[11] reported genetic polymorphism in the natural *Lilium martagon* group and were 84%. But in the present study, polymorphism was considerably lower. Mean genetic similarity was 0.49. The expected level of heterozygosity if all the subpopulations were pooled together (Ht) is 0.34 ± 0.009 and the expected level of heterozygosity in a subpopulation (Hs) is 0.29 ± 0.005. FST is 0.147, indicating a reduction of genetic diversity 14.7%. Gene flow is Nm = 2.69. Genetic diversity within and among populations is measured as the percentage of polymorphic bands with Nei's gene diversity (h) 0.342 ± 0.09. The mean diversity (DST) is 0.05. The number of alleles per locus (Na), effective number of alleles per locus (Ne) and the Shannon information index (I) at the group level were 2.00 ± 0.09, 1.55 ± 0.22, and 0.52 ± 0.11, respectively. The measure of gene diversity, Shannon's information index (I) are much higher in the *Solanum* taxa (0.52 ± 0.11) compared to other plant species analyzed. For example, in wild lily the value was 0.244 ± 0.18. The genetic differentiation among species was (GST) 0.16. Furthermore pair-wise Nm at the species level is 2.69. Nm predicts the relative importance of gene flow and genetic drift based on the observed pattern of genetic differentiation in a population.

Table 3: ISSR unique bands in *Solanum* species with 15 primers.

ISSR primers	Total bands	Unique bands
1	98	01
2	90	00
3	51	01
4	73	02
5	64	01
6	60	02
7	82	00
8	09	04
9	81	00
10	86	01
11	77	01
12	73	03
13	72	04
14	82	02
15	31	03
Total	**1029**	**25**

Population fixation index (Fst) represents the genetic differentiation among species, ranging from 0 to 1. The larger the Fst value, the higher the degree of differentiation among populations. Gene flow (Nm) values grouped them into 3 categories: high (> 1.0), intermediate and low (< 0.249).[12] In this study, low Fst and significantly high Nm, indicating the lack of isolation among the studied taxa. This suggests further that the genetic difference is minimum within the species.

The Principal coordinate analysis is performed with ISSR data in order to establish the relationship among species and to compare with dendrogram. Distribution pattern of species in this aspect is dissimilar to the result extracted from dendrogram UPGMA analysis. The first three principal axes accounted for 38.12, 20.93 and 15.60% of the total variation, respectively, indicating the complex multidimensional nature of ISSR variation. The Cumulative % was 74.65.

According to the NJ dendrogram (Figure 6), *Solanum* taxa are separated into two main clusters, one large and a minor (A1 & A2).

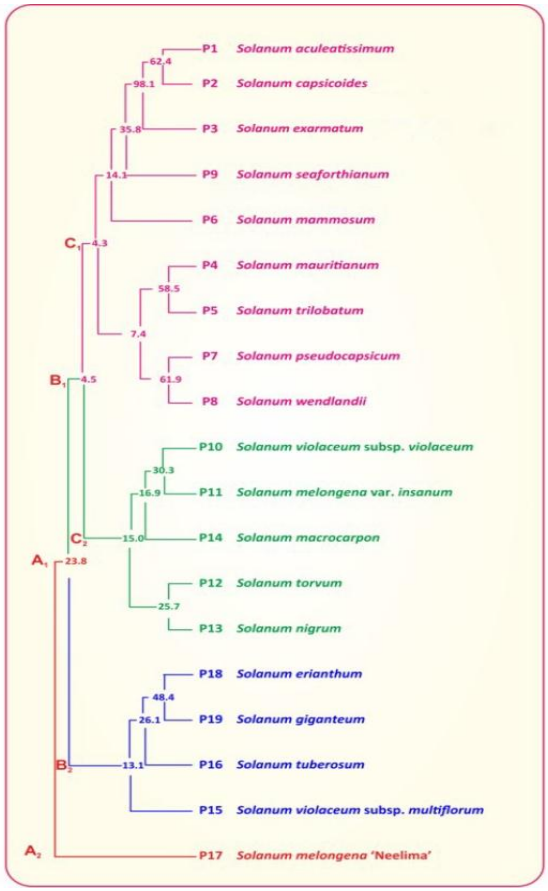

Figure 6: ISSR- UPGMA Dendrogram cluster analysis of *Solanum* species.

The larger cluster is divided into two subclusters. Cluster B1 includes *S. erianthum, S. giganteum, S. tuberosum* and *S. violaceum* subsp. *multiflorum.* The second cluster bifurcated into C1 and C2 cluters. C1 subcluster contained taxa like *S. violaceum* subsp. *violaceum, S. melongena* var. *insanum, S. macrocarpon, S. torvum* and *S. americanum.* The cluster C2 have *S. aculeatissimum, S. capsicoides, S. exarmatum, S. seaforthianum, S. mammosum, S. mauritianum, S. trilobatum, S. pseudocapsicum* and *S. wendlandii*. Interestingly, *S. melongena* var. *insanum* and *S.melongena* Neelima are seated in far away branches of the dendrogram. The former is an offshoot of *S. violaceum* subsp. *violaceum* and the latter *S.melongena* Neelima seated as a separate clade of the first major bifurcated branch (A2).

Table 4: ISSR unique bands in *Solanum* species with 15 primers.

Sl. No.	*Solanum species*	Total number of bands scored in ISSR
P1	*Solanum aculeatissimum* Jacq. (TBGT 017)	26
P2	*Solanum capsicoides*-All. *spiny accession* (TBGT 24069)	45
P3	*Solanum exarmatum* (TBGT016)	47
P4	*Solanum mauritianum* Scop. (TBGT 24073)	53
P5	*Solanum trilobatum* L. (TBGT 013)	45
P6	*Solanum mammosum* L. (TBGT 014)	54
P7	*Solanum pseudocapsicum* L. (TBGT 24068)	64
P8	*Solanum wendlandii* Hook. (TBGT 24071)	56
P9	*Solanum seaforthianum* Andr. (TBGT 24070)	57
P10	*Solanum violaceum* Ortega subsp. *Violaceum* (TBGT 24077)	47
P11	*Solanum melongena* L. var *insanum* (L.) Prain (TBGT 24066)	50
P12	*Solanum torvum* Sw. (TBGT 24076)	62
P13	*Solanum americanum* Mill. (TBGT 24074)	57
P14	*Solanum macrocarpon* L. (TBGT 015)	58
P15	*Solanum violaceum* Ortega subsp. *multiflorum* (Clarke) Matthew (TBGT 24075)	65
P16	*Solanum tuberosum* (TBGT 4133)	68
P17	*Solanum melongena* L. var 'Neelima' (TBGT 4134)	50
P18	*Solanum erianthum* D. Don (TBGT 24072)	59
P19	*Solanum giganteum* Jacq. (TBGT 24067)	66
	Total number of bands	**1029**

ISSR markers have been employed for the molecular identification of egg plant cultivars[13], among brinjal varieties[14], potato genotypes[15] as well as some non tuberous wild *Solanum* species from North India. Thus, the study provides significant insight into genetic similarity and diversity of *Solanum* taxa. The information and data on genetic variability obtained from this study might be a potential source for *Solanum* data base. The results also confirm that the ISSR marker is feasible tool for the assessment of genetic diversity in plant species. The suitability of the methodology in analyzing the genetic variability has been demonstrated in several other taxa.[16,18]

The genetic distance values based on ISSR analysis reveals the highest value of 0.74 is between *S. aculeatissimum* and *S. capsicoides* followed by *S. capsicoides* and *S. exarmatum* and the lowest value is 0.21 between *S. aculeatissimum* and *S.melongena* Neelima. The distance matrix based on ISSR data sets is used to construct a dendrogram, to further determine phylogenetic relationships among the *Solanum* taxa. UPGMA clustering is carried out using Nei's unbiased genetic distance matrix. The dendrogram (Figure 6) clearly showed differentiation among *Solanum* taxa which is further substantiated by the co ordinate plot (Figure 7).

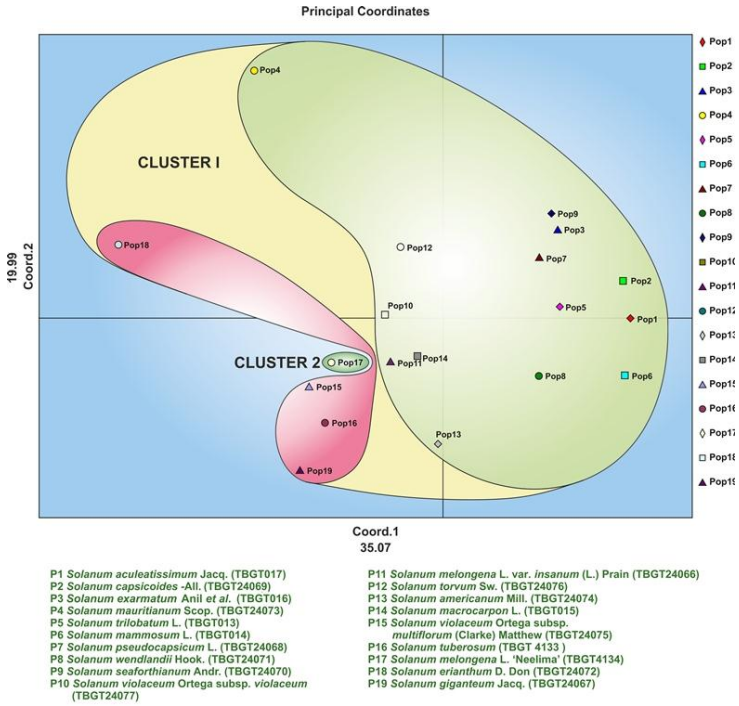

Figure 7: ISSR co-ordinate plot of *Solanum* species.

Although there are published reports on the use of other molecular marker techniques such as RAPD, AFLP and others to analyze various species in the past, this is the first report of the use of ISSR markers in analyzing *Solanum* taxa. *Solanum* species along with others of the Solanaceae display diversity in habitat, ethno botanical use and morphology. These findings suggest that the maternal origins of the individuals in bigger group are closely related. Therefore, UPGMA analysis is useful to show genetic structure in different groups.

Similarily, this study offered the first detailed analysis of the genetic diversity and its structure of different *Solanum* species and varieties reported from Kerala based on ISSR molecular markers that have been proven to be valuable for the determination of genetic diversity. The ISSR-PCR analysis in the present study identified relatively low genetic diversity, considerable genetic differentiation among species (GST) and the gene flow (Nm) at the species level.

The level and distribution of genetic variability among and within groups can be explained with many potential factors including pollination, seed dispersal, geographic distribution range, adult density, mating system, colonization history and natural selection. Additional phylogenetic studies using chloroplast or mitochondrial gene sequences or appropriate nuclear gene sequences can help to evaluate the systematic positions of *Solanum* species.

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