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1 **Exploring genetic diversity of yellow-berried night shade (*Solanum virginianum* L.) using**
2 **genetic divergence and molecular markers**

3 **Abstract**

4 The rationale of this research was to explore the genetic divergence of 54 kantakari
5 accessions (*Solanum virginianum* L. syn. *S. surattense* Burm. f.), to identify their significant traits,
6 and unravel their genetic variations. Kantakari is a widely used medicinal plant in traditional
7 medicine worldwide and is also known for its allopathic properties. The morphological descriptors
8 and molecular markers (Random Amplified Polymorphic DNA) used to characterize these samples
9 revealed significant within-accession heterogeneity, indicating the possibility of future selection.
10 Twenty-three morphological and biochemical features were examined to assess taxonomic traits.
11 Accessions were classified into six clusters based on Mahalanobis D^2 statistics of 23 quantitative
12 traits. Clusters V and VI had the largest intercluster distance (1183.81), whereas Cluster IV had
13 the largest intracluster distance (D^2 = 275.67) (Cluster V and VI). Random Amplified Polymorphic
14 DNA marker amplification using 10 polymorphic primers revealed 79 alleles with an overall mean
15 of 7.90 alleles per marker, with Polymorphism Information Content values varying from 0.11 to
16 0.37 (Mean=0.30). Cluster assessment produced three distinct groups. In conclusion, the
17 investigation underscores that substantial diversity was observed among the surveyed kantakari
18 accessions. Using molecular markers in tandem with morphological characterization, presents a
19 robust approach for elucidating the intricacies of species-specific genetic variation with greater
20 accuracy and depth.

21 **Keywords:** *Solanum surattense*, Molecular characterization, Genetic diversity, D^2 analysis,
22 AMOVA, RAPD primers, Kantakari

23 **1. Introduction**

24 Traditional medicine uses a wide variety of plants for the treatment of common diseases
25 and the promotion of good health. According to the WHO, 80% of the global population uses
26 ethnomedicine (Sen and Chakraborty, 2017). India has an extensive history of traditional medicine
27 dating back to ancient times, which is still practised today. Bioactive phytochemicals are a major
28 source of novel pharmaceuticals. Globally, there has been a growing recognition of the use of
29 herbal remedies.

30 Species belonging to the genus Solanum (Solanaceae) are widely used in traditional
31 medicine and are known reservoirs of bioactive compounds, including steroidal saponins (Kunwar
32 et al., 2021). Due to the extensive spectrum of biological activities, including antibacterial,
33 antirheumatic, anticonvulsant, analgesic, antioxidant, and anticancer properties (Kumar et al.,
34 2010), Solanum alkaloids (Solasodine) have long drawn attention in pharmacological and
35 therapeutic investigations. Alkaloids are vital to the pharmaceutical industry as fundamental
36 constituents or precursors for various steroidal medications employed in treating inflammation,
37 menopause, and arteriosclerosis (Jayakumar and Murugan, 2016).
38 *S. virginianum* L. is frequently referred to as Indian nightshade in India, kantakari in
39 Sanskrit, or yellow-berried nightshade in English. It is a bright green, spreading perennial plant
40 with a prickly appearance. The younger branches are densely covered in stellate tomentum, with
41 straight, glabrous, shiny yellow prickles often exceeding 1.3 cm in length. The leaves are ovate to
42 elliptic, measuring 5-10 × 2.5–5.7 cm, and feature stellate hairs on both sides, particularly
43 prominent on the underside, although the leaves become non-pubescent on maturation. Petioles
44 vary in length from 1.3 to 2 cm. Flowers have bluish-violet petals with attractive yellow stamens
45 and are born in axillary clusters. The berries of *S. virginianum* L. measure 1.3–2.0 cm in diameter
46 and are characterized by yellow stripes with green stripes, enclosed within an enlarged calyx. The
47 seeds are approximately 0.25 cm in diameter, smooth (glabrous), sub-reniform in shape, and
48 yellowish-brown in colour. (Nithya et al., 2018; Parmar et al., 2017). This species is diploid, with
49 a base number of x=12. According to Sun et al., (2020), the presence of heterochromatin segments
50 along the chromosome arms helps these plants to tolerate high temperatures. Hence, kantakari are
51 primarily distributed in warm, dry regions, especially in southeastern Asia and tropical Australia.
52 In India, kantakari is commonly found along roadsides and in barren lands, particularly in regions
53 such as Tamil Nadu, Rajasthan, Gujarat, Haryana, Madhya Pradesh, and Uttar Pradesh (Parmar et
54 al., 2010).
55 Owing to its well-known ethnobotanical properties, kantakari has attracted researchers from
56 all over the world. The fruits of *S. virginianum* L. are edible, and in Manipur, India, locals use
57 them as traditional medicines to treat various ailments. The unripe, cooked fruits of S. *virginianum*
58 L. have traditionally been consumed as a vegetable by the Irular tribes residing in the Hasanur
59 Hills of Tamil Nadu. The Kattunaikka, Paniya, and Kurumba tribal people of Kerala's Wayanad
60 District consume both fruit and seeds, as documented by Revathi and Parimelazhagan (2010) and

Narayanan et al. (2011). Within the Ayurvedic system of medicine, kantakari is stated to be pungent, bitter, assimilative, and an alternative astringent. Its fruits, flowers, and stems are bitter and carminative. In accordance with Kumar et al. (2012); Singh and Singh (2010) the root infusion of *S. virginianum* L. is used as a febrifuge, expectorant, and strong diuretic. In addition, the plant extract is used for conditions such as bronchial asthma, hemorrhoid, tympanitis, dysuria, mis-peristalsis and rejuvenation, as reported by Thippeswamy et al. (2024). Although the entire plant possesses medicinal properties, dried berries are primarily used because of their rich solasodine content. Currently, there are no available cultivars of this plant; therefore, it is predominantly harvested from natural wild populations.

Owing to its pharmaceutical potential, a comprehensive understanding of the molecular diversity of kantakari is imperative for the advancement of novel, high-yielding varieties abundant in solasodine. This understanding is crucial not only for enhancing therapeutic applications and effective conservation efforts. Solasodine is a nitrogen analogue of sapogenins and an aglycone component of glycoalkaloids in most solanaceous plants. Its C27 cholestane structure renders it easier for the break down into 16-dehydropregnenolone, which is a crucial precursor for the synthesis of steroidal drugs like cortisone and progesterone (Pawar et al.,2008). In addition, it has various pharmaceutical effects such as anticancer, diuretic, antifungal, immunomodulatory, antiandrogenic,antipyretic, cardiotonic and CNS (Patel et al., 2013). In order to build linkages within species and to discover more about genetic affinities, it is essential to look into genetic variation (Munda et al., 2021). In addition, genetic heterogeneity research facilitates genotypic comparisons among various geographic regions (Kumaret al., 2013). Because the molecular markers are neutral, economical, highly informative, and free of the growing stage, tissue, or environmental conditions, these offer advantages over phenotypic markers while assessing the plant's genetic makeup (Kumar et al., 2013, Munda et al., 2021). PCR-based RAPD markers represent a common, cost-effective method for identifying patterns of genetic variation in plants due to their simplicity, minimal DNA requirements, insensitivity to environmental factors, and codominant nature (Yadav et al., 2013). Specifically, RAPD markers offer distinct advantages for investigating previously unstudied species. Not requiring DNA sequence information, RAPDs enable the identification and discrimination of accessions, along with providing a means to assess phenotypic expression and investigate phylogenetic associations in the accessions under study.

2. Materials and methods

2.1. Experimental materials

Explorations were conducted across India to obtain kantakari (*Solanum virginianum* L.) genetic material. In total, germplasm was collected from 54 sites across six Indian states (Fig. 1). The species identity was verified with the assistance of the Botanical Survey of India, TNAU, Coimbatore. The seeds of the collected species were deposited in the National Bureau of Plant Genetic Resources (NBPGR) Gene bank for obtaining the Indigenous Collection (IC) Number, and the seeds were available in the Ramiah gene bank, TNAU, Coimbatore. Seeds were treated with 500 ppm GA_3 for 24 h to promote germination as per the trail conducted by Boomiga et al., (2021a). Treated seeds were sown in a seed pan with coir pith and vermicompost (1:1) as the potting medium. To assess and select the most appropriate genotypes, experimental fields were established at the Department of Medicinal and Aromatic Plants, TNAU Botanical Garden, Coimbatore (11°00′50.8″N 76°55′52.7″E, 411 MSL). Ten plants per accession (45-day-old seedlings) were planted in a randomized complete block design (RCBD) having three replications on October 16, 2021. The total experimental area was 0.27 acre containing 11 blocks with 100 m^2. Each block consist of 15 ridges of 10 m length with 15 plants planted at the spacing of 60 × 60 cm. The genotypes were cultivated as per the cultural practises proposed and standardized by NMPB (2014).

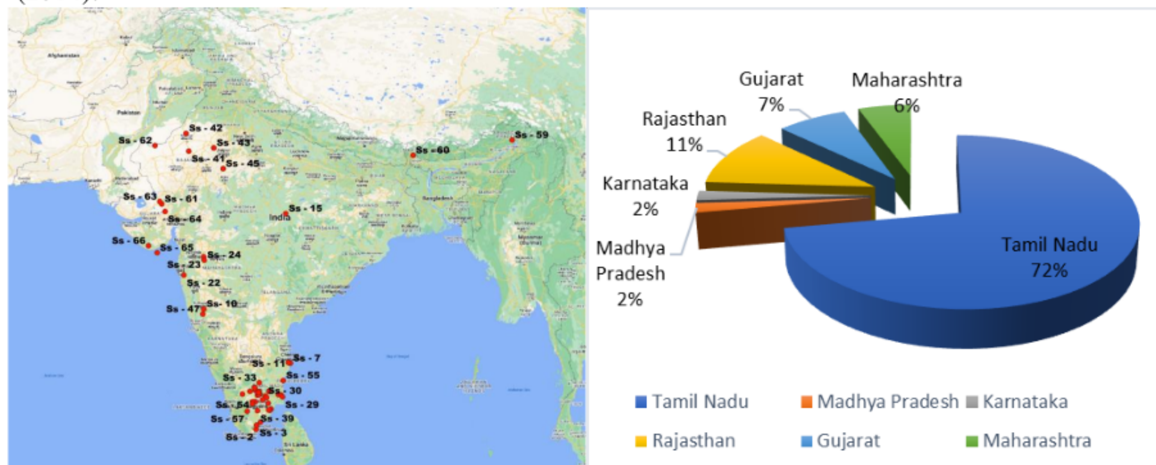

Fig. 1. Map showing the locations of the kantakari accessions

111 Phenotypic observations were recorded from five randomly selected plants from each
112 accession across all replications based on the National Bureau of Plant Genetic Resources minimal
113 descriptors for Agri-horticultural crops (Part IV) (Singh et al., 2003). Morphological characters:
114 North– South spread of the plant (cm), East– West spread of the plant (cm), number of branches
115 plant^{-1}, diameter of the stem (mm), leaf length and width (cm), length of the petiole (cm), length
116 of the internodes (cm), and number of thorns on the upperand lower surface of leaf were recorded
117 upon completion of the vegetative stage of the plant. Flowering traits: days to first flowering,
118 number of flower clusters plant^{-1} and days taken for 50% flowering were observed at the full bloom
119 stage. Yield characteristics: total number of berries plant^{-1}, diameter of a berry (mm), fresh and
120 dry weight of single berry (g/plant), fresh and dry berry yield plant^{-1} (g) were recorded at fruit
121 maturity. Biochemical analyses were conducted as suggested by Rakesh et al.(2021) to assess the
122 total phenol content (mg GAE/g) by the Folin-Ciocalteu method; total protein content (mg/g) by
123 Lowry's method, total flavonoid content (mg QE/g) by aluminium trichloride method; and total
124 antioxidant content using DPPH assay (mg ASAE/DW). All these observations were analyzed
125 using Mahalanobis's (1936) D^2 statistics for assessing genetic divergence between the kantakari
126 accessions.

Variations in leaf (upper and lower side), spine, flower, and berry colour were documented using the Royal Horticultural Society (RHS) Color Chart (**Fig. 2**)

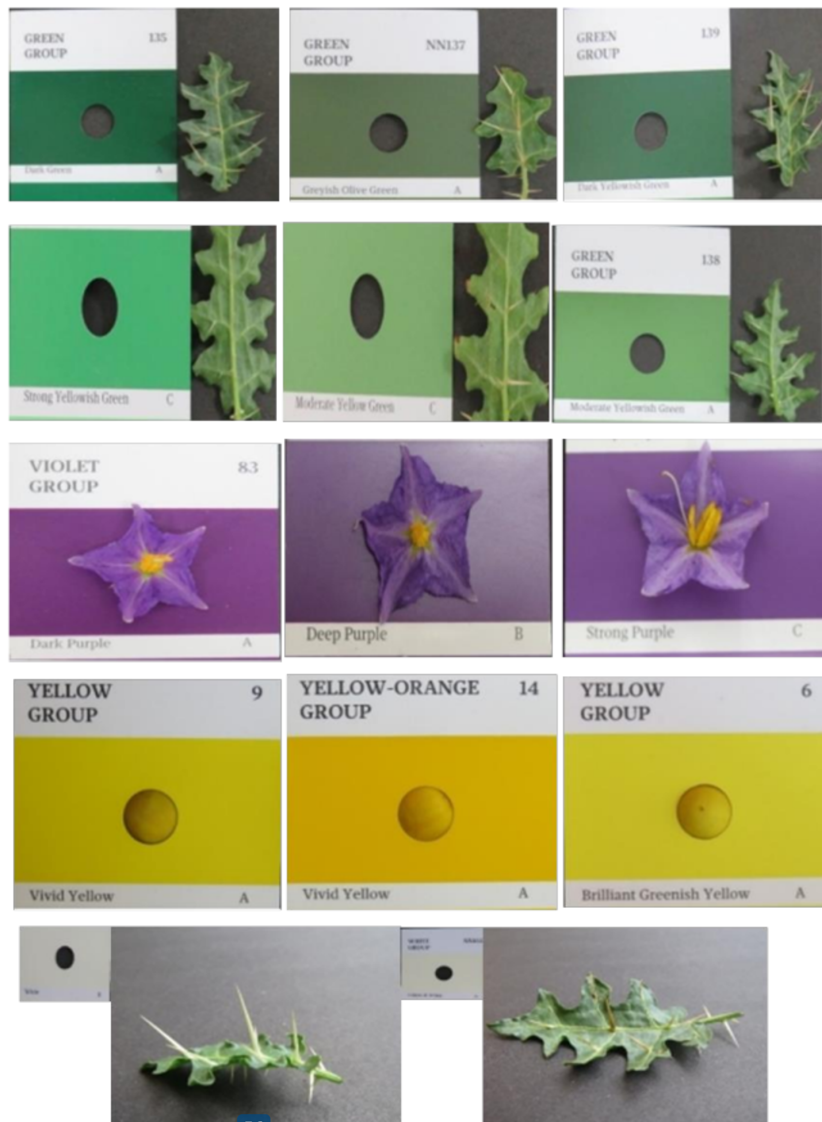

Fig. 2. Lane 1: Variation in leaf colour (upper side); Lane 2: Leaf colour (lower side); Lane 3:Flower colour; Lane 4: Dry berry colour; Lane 5: Thorn colour

2.2. Extraction of genomic DNA

Genomic DNA analysis was performed according to the protocol outlined by Doyle and Doyle (1990). On the day of extraction, fresh young leaves of *S. virginianum* L. were collected from each

accession, washed with tap water, and wiped with 70% (aq) ethanol. The cleaned leaf blades were then cut into small pieces and macerated in 1000 μL of slightly modified CTAB buffer with 10 μL ofmercaptoethanol. Ground samples were incubated in a water bath at 60°C for 45 min. Subsequently, 600 μL of a phenol, chloroform, and isoamyl alcohol solution (25:24:1) was added, and the mixture was centrifuged in a refrigerated centrifuge at 12,000 rpm for 10 min. The supernatant was collected, to which 600 μL of chloroform and isoamyl alcohol mixture (24:1) was added and centrifuged again. Because of the high phenol concentration of *S. virginianum* L. leaves, this procedure was performed twice to remove the phenols. Finally, 50 μL of sodium acetate (7.5 M) was added to enhance nucleic acid precipitation, and an equal amount of frigid isopropanol was added. The samples were kept overnight at 4ºC. The DNA pellets precipitated were subsequently rinsed with 70% ethanol on the following day after the samples had been centrifuged at 10,000 rpm for 8 min. The pellets were dried until the alcohol Odor disappeared. The pellets were then reconstituted in TE buffer and kept at -20 ºC for storage. A nanodrop spectrophotometer (Jenway™ Genova) was used to evaluate the quantity and purity of DNA samples at 260 and 280 nm. Using 0.8% (w/v) agarose gel electrophoresis and 6 X DNA loading dye (Thermo Fisher Sci, Cat. No. R06111), the DNA was examined for quality.

2.3. RAPD amplification

Ten decamer oligonucleotides derived from Operon series (OP) RAPD primers (Eurofins Advinus Agrosciences Services Pvt. Ltd., Bengaluru) (Table 1) were used to evaluate genetic polymorphisms in *S. virginianum* L. Amplification was performed in a 10 μL reaction volume, with 2 μL of 100 ng DNA, 2 μL of primer and 6 μL of 2X mastermix (smartPRIME). In a Proflex Thermal Cycler, PCR reactions were performed with an initial denaturation at 94°C for 5 minutes, 45 cycles at 94°C for 1 minute, 37°C for 1 minute, and 72°C for 1 minute, and a final extension at 72°C for 5 min. The amplified products were separated using a 1.5% agarose gel in 0.5X TBE buffer and then electrophoresed at 90 V for 3 h. The Gel Documentation System was used to visualize the results using EtBr staining. Using GeneDirex Cat. No. DM0150 as a molecular standard, the 1-kb marker RTU was used to quantifythe amplified product size.

Table 1. List of primers used to screen the genotypes of *Solanum virginianum* L.

Primers	Sequence 5'→3'	Molecular Weight (bp)	Annealing Temperature (°C)
OPA-02	TGCCGAGCTG	200-2500	47
OPA-20	GTTGCGATCC	400-2500	44
OPD 02	GGACCCAACC	300-2000	46

OPS-20	TCTGGACGGA	400-1000	46
OPT-15	GGATGCCACT	300-3000	44
OPT-18	GATGCCAGAC	300-2000	44
OPT 20	GACCAATGCC	400-2000	44
OPT 17	CCAACGTCGT	200-2500	44
OPP 09	GTGGTCCGCA	300-2500	45
OPR 02	CACAGCTGCC	200-2000	40

162

163 After gel electrophoresis, the amplified fragments were scored in a binary fashion, and
164 a phylogenetic tree was created using the neighbour joining approach with 100 bootstraps in the
165 DARWIN computer program (Perrier and Jacquemoud-Collet, 2006). The PIC values were
166 calculated using the formula,

167 $$PIC = 1 - \sum fi^2$$

168 where, "f" - represents the "ith" allele frequency (Botstein et al. 1980).
169 The formula for calculating resolving power was also as follows:

170 $$Marker\ Index\ (MI) = PIC\ \times EMR$$

171 where, EMR is the multiple of number of polymorphic loci and fraction of polymorphic loci
172 (Milbourne et al. 1997)

173 $$Rp = \sum Ib\ (1 - (2 \times |0.5 - p|)$$

174 where, Ib (band informativeness) and 'p' is the number of individuals containing the band (Prevost
175 and Wilkinson, 1999). Using AMOVA, which is a feature of the GenAlEx 6.5 Excel software
176 suite, molecular variation within and across populations was analyzed (Peakall and Smouse, 2012).

177 **3. Results and Discussion**

178 **3.1. Variation in phenotypic traits**

179 Table 2 represents the assessment of variance results unequivocally demonstrated
180 significant accession-level differences for each of the twenty-four characteristics.

181 **Table 2.** Study of variance (mean sum of squares) for morphological parameters of *Solanum*
182 *virginianum* L. accessions

S.No.	Character	Source of Variance		
		Replication	**Accession**	**Error**
	Degrees of freedom	2	53	106
1	Plant spread (N-S) (cm)	18.92	906.96**	99.42
2	Plant spread (E-W) (cm)	276.18	589.14**	139.94
3	Number of branches	0.50*	2.14**	0.18

4	Number of flower clusters per plant	7.14*	37.04**	1.10
5	Days to the first flowering	7.79	218.29**	10.72
6	Days to 50% flowering	2.34	216.61**	3.08
7	Leaf length (cm)	1.71*	8.80**	0.49
8	Leaf width (cm)	0.84	8.56**	0.51
9	Stem diameter (mm)	1.13	5.34**	0.49
10	Petiole length (cm)	0.19	5.17**	0.29
11	Total no. of thorns on the upper leaf surface	0.20	3.92**	0.79
12	Internode length (cm)	6.47	396.99**	138.82
13	Total no. of thorns on the lower leaf surface	0.71	4.41**	0.37
14	Number of berries	1.36	2271.25**	35.35
15	Berry diameter (mm)	0.17	8.07**	0.57
16	Fresh single berry weight (g)	0.004	0.97**	0.01
17	Fresh berry yield per plant (g)	2864.94*	15433.38**	1113.82
18	Dry single berry weight (g)	0.02*	0.03**	0.01
19	Dry berry yield per plant (g)	6.04*	964.60**	31.97
20	Phenolic content (mg GAE/g)	0.002	8.16**	0.03
21	Protein content (mg/g)	0.03	26.40**	0.13
22	Flavonoid content (mg QE/g)	14.67	2641.67**	12.27
23	Total antioxidant activity (mg ASAE/DW)	3.17	148.55**	8.40

** Significant at p =0.01; * Significant at p =0.05

The observation of qualitative traits also revealed variation within the accessions. Three leaf-colour classes were observed: 16 accessions showed dark green and strong yellowish green leaf colour whereas 43 showed dark yellowish green and moderate yellowish green colour and only one accession was categorized in the greyish olive green and moderate yellowish green group (Figure 2; Lanes 1 and 2). In case of thorn colour, there were two types (Figure 2; Lane 5). Three groups were observed in flower as well as berry colour (Figure 2; Lanes 3 and 4).

3.2. Genetic divergence

Genetic diversity is one of the most significant features of plant breeding. Measures of genetic divergence can be used to optimise accession selection for breeding programmes by providing information on both the type and amount of diversity present within plant populations (Smith et al., 2015). The significance of parental accessions selected based on a set of critical qualities may outweigh those selected based on a single characteristic. In order to develop novel variability and suitable recombinants with improved yield and quality, genetically diverse parents must be included. Therefore, multivariate analysis using Mahalanobis D^2 statistics has been

employed to quantitatively measure phenotypic divergence (Kovacic, 1994). Table 3 revealed that these 54 kantakari accessions clustered into six groups with appreciable diversity. Fresh single berry weight, days to 50% flowering, berries plant^{-1}, flower clusters plant^{-1} and dry berry yield plant^{-1} were the most significant contributors to overall differentiation (**Fig 3**).

Table 3. Clustering of kantakari accessions using Mahalanobis D^2 statistics for 23 quantitative traits

Cluster	No. of accessions	Name of the accessions
I	16	*Ss*49, *Ss*42, *Ss*45, *Ss*26, *Ss*3, *Ss*25, *Ss*2, *Ss*34, *Ss*17, *Ss*35, *Ss*31, *Ss*62, *Ss*24, *Ss*14, *Ss*63, *Ss*18
II	17	*Ss*53, *Ss*54, *Ss*55, *Ss*41, *Ss*10, *Ss*6, *Ss*33, *Ss*4, *Ss*7, *Ss*11, *Ss*13, *Ss*36, *Ss*2, *Ss*1, *Ss*19, *Ss*6, *Ss*39
III	08	*Ss*22, *Ss*27, *Ss*21, *Ss*38, *Ss*58, *Ss*23, *Ss*50, *Ss*9
IV	10	*Ss*51, *Ss*56, *Ss*57, *Ss*32, *Ss*64, *Ss*15, *Ss*44, *Ss*48, *Ss*52, *Ss*43
V	2	*Ss*12, *Ss*65
VI	1	*Ss*20

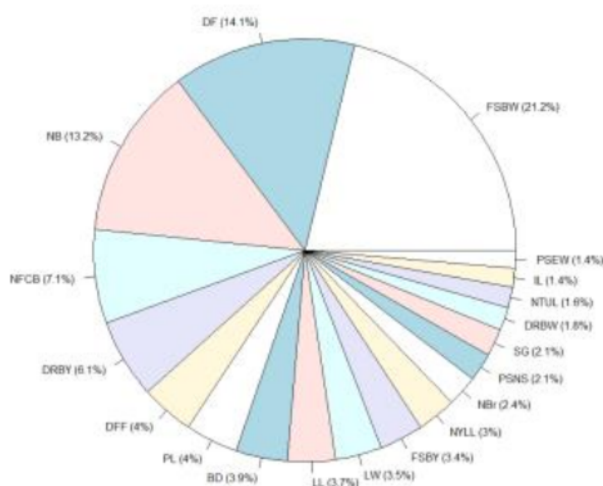

Fig 3. Percent contribution of characters towards genetic divergence in kantakari

* FSBW- Fresh weight of a berry, PSEW -Plant spread (E-W), IL -Internode length, NTUL-Number of thorns on upper leaf surface, DRBW- Dry weight of single berry, SG- Stem girth, PSNS- Plant spread (N-S),NBr- Branches per plant, NYLL- Number of thorns on lower leaf surface, FSBY- Berry yield (fresh), LW- Leaf width, LL- Length of the leaf, BD-Diameter of the berry PL- Petiole length, DFF- First flowering (days),DRBY- Yield of berry (Dry), NFCB- Flower cluster per branch, NB- number of berries and DF- days to 50% flowering.

214
215
216
217 **Table 4.** Cluster distances of yellow berried nightshade accessions (Intra & Inter)
218

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	**139.96**	338.29	225.51	438.70	647.57	335.17
Cluster II		**252.07**	394.79	484.85	361.14	638.26
Cluster III			**237.52**	674.46	783.72	417.50
Cluster IV				**275.67**	590.80	683.64
Cluster V					**135.27**	1183.81
Cluster VI						**0.00**

219 Bold values on the diagonals indicate the intra-cluster distance

220 The inter-cluster values of D^2 analysis ranged from 1183.81 (between Cluster-V and VI) down to
221 674.46 (between Cluster-III and IV) whereas, the highest intra- cluster distance was observed in
222 Cluster-IV (D^2= 275.67) with nine accessions (Table 4). Considering that the greatest phenotypic
223 distance between clusters VI and V, the accessions selected from these clusters for hybridization
224 should yield broad variation in segregating generations, perhaps including transgressive
225 segregates. Similarly, Boomiga et al. (2021b) classified *Solanum surattense* accessions into six
226 groups based on quantitative traits. These findings are consistent with studies on other Solanaceous
227 crops, including tomato (Meena and Bahadur, 2015), eggplant (Begum et al., 2013), and chili
228 (Rahevar et al., 2021).

229 **3.3. Quantitative trait cluster mean performance**

230 In represent to Table 5 plant spread (N-S) showed the widest plants in cluster II (mean =
231 106.10 cm) whereas cluster VI (at 105.86 cm) recorded the widest plants for plant spread (E-W).
232 In addition, cluster VI produced the most branches per plant (6.14). The highest number of flower
233 clusters per branch was observed in clusters I and VI (15.20). The fewest thorns on the upper and
234 lower sides of the leaf were also noted in cluster VI (11.90 and 12.20 respectively). Cluster II
235 recorded the highest mean number of berries (85.52). The yield attributes: fresh (3.26 g) and dry
236 (0.75 g) single berry weight, and fresh (238.09 g) and dry (74.07 g) berry yield per plant was
237 highest in cluster V. Hence, the selection of parental lines for hybridization from diverse clusters
238 results in greater variability. Dutta et al. (2009) have similarly observed similar findings in
239 aubergine. Hybrids with desired segregants could potentially result from accessions originating

240 from the most divergent clusters. These results are in line with the kantakari divergence studies of
241 Boomiga et al. (2021) and research demonstrated substantial variation in the yield of dried
242 berries/plant among the 34 traits analyzed. and that 49 accessions could be grouped into six groups
243 using cluster analysis.
244 **Table 5.** Mean performance of kantakari accessions for 23 quantitative traits
245

Characters	I	II	III	IV	V	VI
Plant spread (N-S) (cm)	102.18	106.10	95.46	97.41	96.18	103.47
Plant spread (E-W) (cm)	101.75	98.58	91.12	97.63	87.75	105.86
Branches/ plant	5.41	5.27	5.21	6.00	5.54	6.14
Flower clusters/plant	15.20	15.06	13.73	14.90	14.82	15.20
Length of the leaf (cm)	8.35	9.76	9.03	9.84	8.71	8.59
Leaf width (cm)	6.44	6.99	7.88	6.46	5.71	4.51
Diameter of the stem (mm)	7.90	8.97	8.51	7.89	8.93	8.22
Leaf Petiole length (cm)	4.27	5.45	4.75	5.31	4.99	3.44
Internodal distance (cm)	7.97	8.58	7.47	8.14	6.62	8.15
Number of thorns on upper leaf surface	13.01	13.10	13.18	12.59	12.74	11.90
Number of thorns on lower side of the leaf	13.52	12.85	13.37	12.81	12.35	12.20
Days to first flowering	39.09	37.92	40.42	43.93	28.78	34.67
Days to 50% flowering	52.94	55.28	52.38	56.79	47.57	45.67
Number of berries	66.75	85.52	62.52	61.98	78.91	83.46
Berry diameter (mm)	16.59	16.55	16.53	16.83	16.57	16.00
Fresh single berry weight (g)	2.49	2.42	2.47	2.72	3.26	1.73
Fresh berry yield (g/plant)	168.75	193.18	144.71	167.86	238.09	133.78
Dry single berry weight (g)	0.63	0.61	0.61	0.69	0.75	0.62
Dry berry yield (g/plant)	53.10	60.59	47.18	51.66	74.07	43.56
Total phenol content (mg GAE/g)	6.68	6.64	7.50	7.72	10.09	7.96

Total flavonoid content (mg QE/g)	127.57	127.16	123.24	140.67	210.37	148.89
Total protein content (mg/g)	12.76	12.72	12.32	14.07	21.04	14.89
Total antioxidant activity (mg ASAE/DW)	133.16	130.38	132.32	125.57	143.76	129.50

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247 **3.4. AMOVA**

248 **Table 6.** Analysis of molecular variance within and among populations of kantakari

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Source	df	SS	Variance component	Variation (in per cent)
Among Pops	1	12.218	0.310	3%
Within Pops	52	463.542	8.914	97%
Total	53	475.759	9.224	

250

251 Data from Table 6, revealed that just 3% of variation was identified among populations,
252 whereas 97% of the total geneticdifference was found within populations. This significant level of
253 within-population variation may be indicative of elevated gene flow or the presence of allelic
254 variation that was not resolved in the observed banding patterns. High levels of within-population
255 variation allow populations to better adapt to changing environments (Danusevičius et al., 2024).
256 Future studies should investigate these RAPD banding patterns to determine whether single bands
257 are truly mono-allelic, and, if so, whether those alleles are identical by descent. The findings of
258 this study echo earlier research conducted by Akaffou et al. (2020), wherein molecular variance
259 analysis revealed 14% of molecular variance among groups, with the remaining 86% attributed to
260 within-group diversity in brinjal. Additionally, Shimira et al. (2021) reported that within-
261 population variation in scarlet eggplant accounted for 81%, while among-population variation
262 constituted 19%. In the case of potato, analysis of molecular variance demonstrated 3% variation
263 among locations, with 97% stemming from diversity within locations, and further among the
264 elevations, 2% variability coupled with 98% attributable to diversity within elevation (Anoumaa
265 et al., 2017).

266 **3.5. Molecular characterization by RAPD primers**

267 **Table 7. Discriminatory power of used primers**

268

Primers	NB	NPB	PP	PIC	MI	R_P
OPA-02	9	5	55.5	0.28	2.15	4.57
OPA-20	8	3	37.5	0.22	2.30	3.81

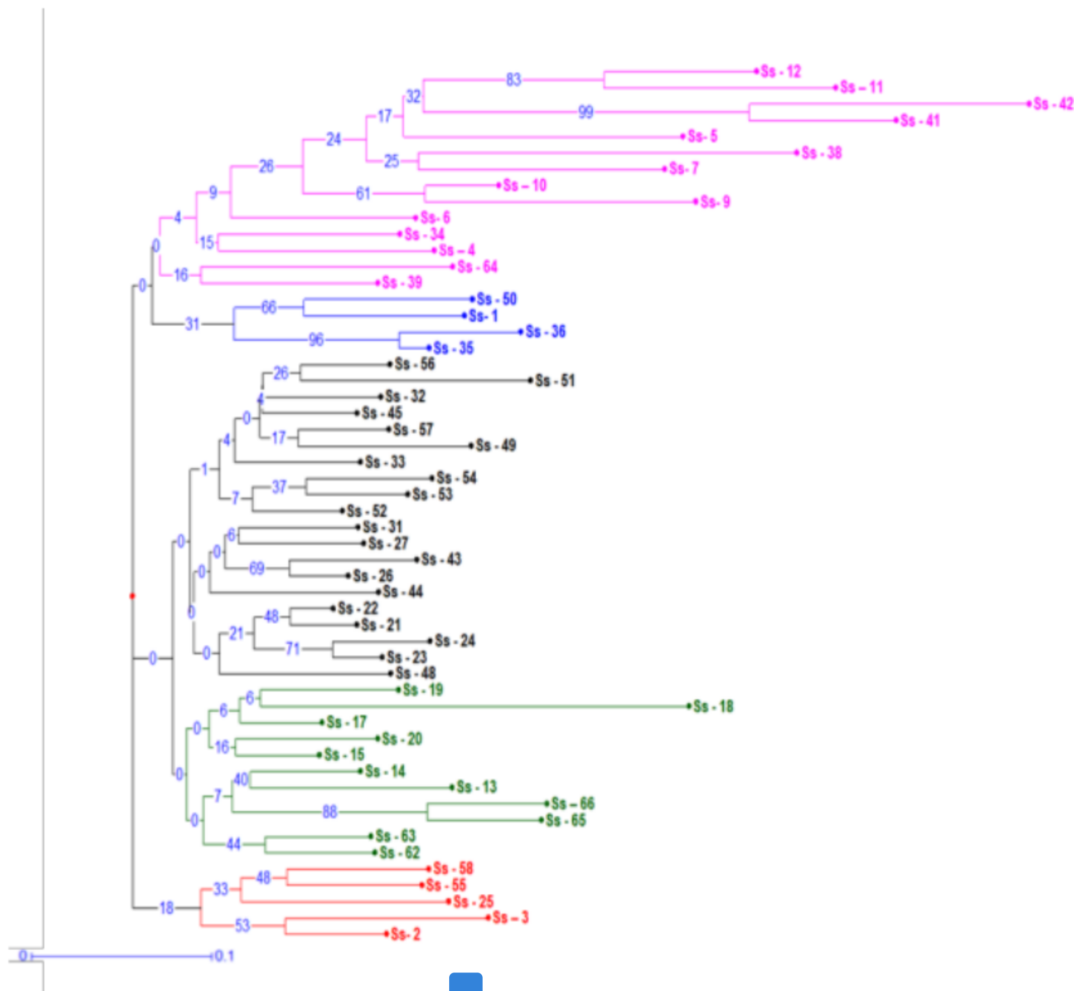
OPD 02	11	9	81.8	0.34	2.55	4.81
OPS-20	8	2	25.0	0.11	2.03	3.56
OPT-15	10	4	40.0	0.32	2.51	4.39
OPT-18	11	9	81.8	0.33	2.40	4.52
OPT 20	11	10	91.0	0.36	2.42	4.67
OPT 17	13	12	92.3	0.35	2.96	7.74
OPP 09	11	11	100	0.33	2.55	5.33
OPR 02	14	14	100	0.37	2.73	4.22
Mean	**10.6**	**7.9**	**70.46**	**0.30**	**2.46**	**4.76**

269 *NB- Number of bands, NPB – Number of polymorphic bands, PP- Percentage of polymorphism, PIC- Polymorphism
270 Information Content, MI- Marker Index, Rp- Resolving power
271

272 Ten RAPD primers (Table 7), producing repeatable, polymorphic banding patterns (Fig.
273 4), were used to characterize the 54 kantakari accessions. Out of total 106 scorable bands, 79 were
274 polymorphic, average of 7.9 polymorphic bands per primer. Among the ten primers, six showed
275 polymorphism levels above 80 %, whereas primers OPP 09 and OPR 02 were fully polymorphic.
276 The marker index was determined for all markers, as it quantifies their discriminatory strength. A
277 marker index value of 2.46 was determined for this set of RAPD primers, a value resulting from a
278 higher multiplex ratio component (7.49), results consistent with those of previous authors (Ansari
279 and Singh, 2013; Tiwari et al., 2009; Verma et al., 2012,).

280 PIC of a primer is a crucial component that shows how well it can distinguish between
281 different species. An average PIC of 0.30 was observed for the ten RAPD primers, whereas the
282 highest PIC was observed for primer OPR 02 (0.37) (Table 6). In addition, the resolving power of
283 each marker was assessed, indicating the primer's ability to effectively differentiate between
284 genotypes or individuals. Across the primers, the mean resolving power was 4.76 with the highest
285 value (7.74) detected for primer OPT 17. A neighbour-joining algorithm was used to generate a
286 cluster analysis for the RAPD markers (Fig. 4). Larger differences in bootstrap values, greater
287 divergence in genotypes and greater stability within clusters. The tree produced three major
288 groupings (but with very low bootstrap values 18 accessions form Cluster I with two sub-clusters
289 (shown in pink and blue); 31 accessions form Cluster II also with two sub-clusters (in black and
290 green), and 5 accessions form Cluster III (in red). In an earlier study involving 24 accessions of *S.*
291 *surattense* from Yadav et al. (2013), accessions were divided into 4 groups using UPGMA
292 dendrogram with a 40% resemblance for RAPD and ISSR markers. In this study, it is noteworthy
293 that the dendrogram did not unveil a discernible pattern of geographic grouping. Similar agro-

294 climatic conditions and widespread gene flow may be the cause of these associations between
295 accessions from various locales (Saengprajak and Saensouk, 2012). Parallel research outcomes



296 were documented by prior investigations conducted by Mastan et al. (2012) for Jatropa and Sarwat
297 et al. (2011) for *Terminalia*. Semagn (2002) explained that the low relationship between molecular
298 markers and phenotypical traits is indeed relevant to our study. Molecular markers, such as RAPD,
299 encompass a broader portion of the genome, spanning both coding and non-coding regions, unlike
300 morphology. Furthermore, molecular markers are not subject to artificial selection as morphology
301 is. Consequently, phenotypic and molecular data approaches might not always be tightly linked.
302 Enhanced association between them might be achieved with the inclusion of additional
303 morphological markers in the analysis or by utilizing a wider array of RAPD primer combinations.

Fig 4. Dendrogram using neighbour-joining method, utilizing kantakari populations screened with RAPD markers. (The number following accession names denote individual genotypes with boot-trap values, expressed as a percentage of 100 replicates, indicated at the nodes.)

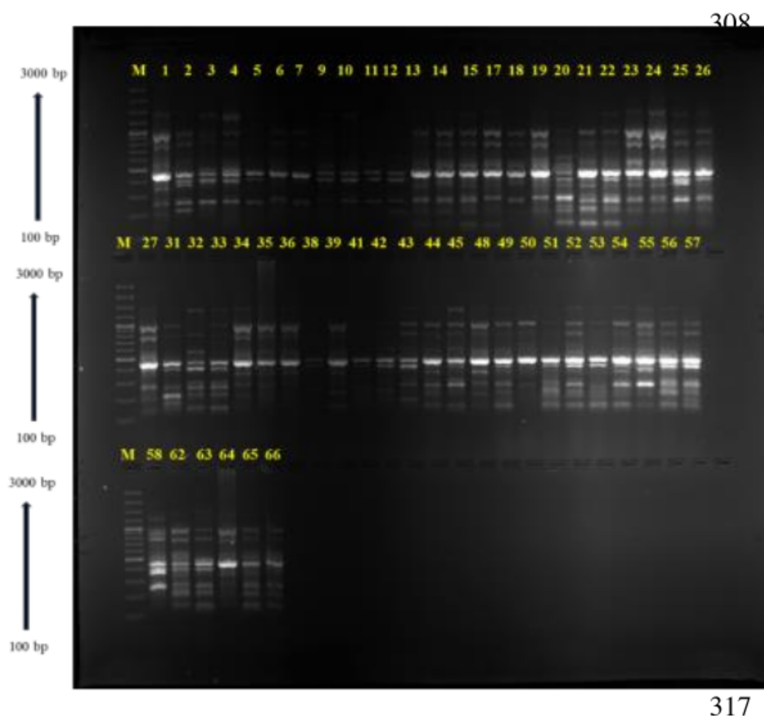


Fig 5. Gel image of OPT -17 RAPD primer showing amplified bands of 54 kantakari accessions

4. Conclusions

This is the first report of an evaluation of the genetic and phenotypic diversity of kantakari populations gathered from diverse regions of India. Investigating diversity and group accessions may be done using any of the examined approaches; yet, no two approaches may be used in the same way. The selection of a genetic diversity estimation method will largely depend on the resources available to the researcher and how these integrate with the breeding strategy. Thus, it is impossible to reliably distinguish between closely related genotypes and analyse the genetic similarities between them using phenotypic evaluations. Among the 54 assessed accessions, Ss-13, Ss-14, Ss-34, and Ss-66 exhibited superior performance concerning yield and associated traits,

328 while Ss-44 displayed a reduced number of thorns. These accessions could be recommended for
329 commercial cultivation of kantakari. Additionally, Ss-48 demonstrated elevated solasodine
330 content, and its methanolic extract showed potential antibacterial properties.

331 It is crucial to implement proactive conservation and breeding strategies for the economic
332 cultivation of kantakari. Exploring genetic diversity of *Solanum virginianum* through molecular
333 work will provide a strong foundation in developing new varieties with superior yield and quality
334 traits useful for various phytomedicine industries. To prevent genetic diversity loss and for further
335 crop development work, it is imperative to cultivate and preserve high-solasodine lines and other
336 genetically diverse accessions for future exploitation.

337

338 **Author Contributions: Dheebisha Chandirasekaran**: Writing- Original Draft; Methodology,
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340 Analysis, Software **Suresh Jesuraj**: Conceptualization, Investigation, Funding Acquisition,
341 Review &Editing. **Arumugam Thangaiah**: Supervision andResources; Validation.
342 **ManikandaBoopathi Narayanan**: Instrumentation Facilities, Resources and Project
343 Administration. **NalinaLakshmanan**: Conceptualization; Supervision, Research Administration.
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355 **Declarations**

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