J. Indian bot. Soc. e-ISSN:2455-7218, ISSN:0019 - 4468 Vol. 99 (3&4) 2020:00-00

DELINEATION OF TWO MORPHO-TYPES OF *SOLANUM MELONGENA* VAR. *INSANUM* (L.) PRAIN. USING MORPHOLOGICAL, ANATOMICAL AND MOLECULAR TECHNIQUES

R. THULASI AND K. KRISHNAKUMAR[1]

Department of Botany, Sree Neelakanta Government Sanskrit College, Pattambi, Palakkad – 679306, Kerala, India. [1]Dept. of Botany, Maharaja's College (Government Autonomous), Ernakulam, Kerala, India.
Email: kkrishnakumar.alp@gmail.com
Date of online publication: 30th June , 2020
DOI:10.5958/2455-7218.2020.00016.9

Many of the Solanaceae members, especially the genus *Solanum*, are with taxonomical controversies. There were two morpho-types of *Solanum melongena* var. *insanum,* botanically considered as same. One of it is used in Ayurvedic preparations, locally known as 'Cheruvazhuthina', and the other locally known as 'Punyahachunda', was not. The morphological, macro-morphological, micro-morphological and anatomical features showed similarities and little differences. Micro-morphological characters are unchangeable within a species or variety. Differences in micro-morphological features showed that these two plants may belong to two different varieties. So for confirmation, the advanced molecular techniques are used. DNA barcoding using two genes, ITS and matK, was carried out as preliminary molecular assay. The results showed similarities for both the morpho-types. Success of these two genes, matK and ITS, in Solanaceae family is doubtful. However a detailed molecular profiling is warranted to confirm the recently obtained results to solve the taxonomic position of the two morpho-types of *S. melongena* var. *insanum.*

Key words:*Solanum melongena* var. *insanum*, DNA barcoding, ITS, *matK*

From the time of origin, man began to classify the plants based on the usefulness as poisonous and non-poisonous plants. This system of classification got modified time to time and the recent system of classification is APG system of classification. Taxonomic classification of plant species basically depends on the morphological and anatomical characters, these features are changeable and sometimes difficult to observe, so it is necessary to be supported by molecular techniques, in which molecular markers are used to detect the genetic variability. Molecular markers are biochemical constituents viz. secondary metabolites and macromolecules like proteins and deoxyribo nucleic acids (DNA) that play a very important role in plant taxonomy, physiology, embryology, plant breeding, ecology, genetic engineering, etc.(Joshi *et al*. 1999). So today taxonomical classification is not merely based on the morphological characters, it uses other biological techniques like spectroscopic studies and the emerging techniques based on molecular biology for classifying plants (Rosselló-Mora *et al*. 2001), and also for solving the taxonomic ambiguity among complex groups like the genus *Solanum.*

The Solanaceae family consists of approximately 98 genera and some 2,700 species, with a great diversity of habitats, morphology and ecology (Kumari *et al*. 2017.) Plants belonging to this family contain different secondary metabolites coming under alkaloids, terpenoids, phenolics, etc. Tropane alkaloids, such as hyoscyamine and scopolamine, constitute one of the most distinctive group of secondary metabolites of the Solanaceae (Griffin and Lin 2000) and many plants containing them have long been utilised for their medicinal, hallucinogenic and poisonous properties. Withanolides represent a group of steroidal lactones with strong insecticidal properties which appear to be restricted to the Solanaceae (Harborne and Baxter 1993).

Solanum is a largest, cosmopolitan genus with around 1700 species. The genus *Solanum,* however, has certain difficulty in identifying natural species groups. Poor definition of the generic limits, the occurrence of suites of

Received on April 03, 2020 www.indianbotsoc.org Accepted on April 07, 2020

attributes in varying combinations throughout the genus, the phenotypic plasticity and genetic variation in many of the species, and the continual reclassification of parts of genus has ended in nomenclatural confusion (Kumar and Murugan, 2013).

The present investigation focuses on two morpho-types of *Solanum* to identify its uniqueness if any in Ayurveda. There are two morpho-types available for the genus *Solanum* and are locally known as 'Cheruvazhuthina' and 'Punyahachunda', considered as separate plants in Ayurveda. The root of 'Cheruvazhuthina' is included in 'Dasamool'. But 'Punyahachunda' is not used in Ayurvedic preparations. In certain places roots of 'Punyahachunda' is used as substitute for roots of 'Cheruvazhuthina'. But taxonomically these plant specimens are treated as ecotypes of a single plant variety – *Solanum melongena* var. *insanum*(L.) Prain. (Gamble 2016). Both of these plants show many differences in morphology, but considered as same plant variety of a species. Hence the aim of the present investigation is to assess its difference if any. In the present study the two plants are considered as two morpho-types of *S. melongena* var. *insanum*. For convenience 'Cheruvazhuthina' is denoted as morpho-type 1 and 'Punyahachunda' as morpho-type 2 throughout this work.

MATERIALS AND METHODS

The samples for the present investigation were collected from different localities in Kerala such as Nhangattiri, Thirumittacode and Koottanad of Palakkad district, Guruvayur of Thrissur district and Evoor in Alappuzha district of Kerala and voucher specimens were prepared and deposited in the Herbarium of Department of Botany, Sree Neelakanta Govt. Sanskrit College, Pattambi. (Fig.1 a and b)

Morphological Studies

Macro morphological study: Macromorphological characters (trichome and seed coat ornamentation) were studied by close observation using handlens and stereomicroscope (Leica). The morphological characters were analysed using the work of Beentje (2010). Freshly collected leaf and mature dried seed samples were used for investigation. The trichome morphology was analysed according to Payne (1978).

The mature dried seeds of each sample was stained with safranin and observed under compound microscope in 10x magnification. The ornamentation pattern was analysed using the standard work on seed coat ornamentation by Barthlott (1981).

Anatomical Studies: For anatomical studies thin hand sections of stem, leaf and petiole of each sample were observed under compound light microscope after stained with safranin. Stem samples of the third internode of four plants with almost same maturity were selected for the study. For DNA barcoding DNA isolates from both the plant specimens were submitted for barcoding in collaboration with Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram.

Plant genomic DNA extraction was done as per the method described by Sambrook *et al*. (1989). DNA barcoding was carried out for two genes – *mat K* and ITS. These genes showed sequence polymorphism between the species and constant within a species. (Patwardhan *et al*. 2014)

RESULTS AND DISCUSSION

Morphological Studies

Micro morphological study (Table. 1): The micro morphological peculiarities of the two morpho-types were studied using the collected samples. The results obtained are summarised in the Table 2.

The two morpho-types were analysed for macro morphological as well as micro morphological characters. Macro-

Table 1. The macro morphological characters analysed for the two morpho-types (Bold words denote the characters in difference)

Characters	'Cheruvazhuthina' (Morpho-type 1)	'Punyahachunda' (Morpho-type 2)
Habit	**Highly branching shrubs**, ~1m height	Shrub, **less branched**, ~1 m height
Habitat	Terrestrial, slightly wet	Terrestrial, slightly wet
Stem	**Highly branching; dense, long purple coloured spines; young parts dark purple coloured, less tomentose**	**Less branching; sparsely distributed, short, yellow coloured spines; young parts dark purple coloured, densely tomentose**
Leaves	Alternate, simple, **undulate, ~8 spines on adaxial surface, 3-6 on abaxial surface**	Alternate, simple, **sinuate, ~2 spines on adaxial surface, 0-3 spines on abaxial surface**
Inflorescence	**Racemose, 2-3 flowers from single point**, in sub-axillary position	**Solitary cyme**, sub-axillary position
Flower	Bisexual, regular, hypogynous, complete	Bisexual, regular, hypogynous, complete
Calyx	5 sepals, gamosepalous, persistant, **spiny**	5 sepals, gamosepalous, persistant, **not very spiny**
Corolla	5 petals, gamopetalous, **corolla lobes prominent**	5 petals, gamopetalous, **corolla lobes not prominent, umbrella shaped**
Androecium	5 stamens, epipetalous, porous dehiscence, alternipetalous	5 stamens, epipetalous, porous dehiscence, alternipetalous
Gynoecium	Superior ovary, bicelled, axile placentation on swollen placenta, capitate stigma	Superior ovary, bicelled, axile placentation on swollen placenta, capitate stigma
Leaf Characters		
Leaf length (Mean ± SD)	13 ± 0.6 cm	7.5 ± 0.37 cm
Leaf width (Mean ± SD)	11 ± 1.5 cm	6 ± 1.75 cm
Intermodal length (Mean ± SD)	8 ± 0.5 cm	4 ± 0.5 cm
Flower and Inflorescence Characters		
Petiole length (Mean ± SD)	4.5 ± 0.6 cm	2.25 ± 0.58 cm
Pedicel length (Mean ± SD)	2.25 ± 0.49 cm	1.5 ± 0.5 cm
Diameter of flower (Mean ± SD)	3.25 ± 0.5 cm	1.5 ± 0.52 cm
Stamen length (Mean ± SD)	0.85 ± 0.3 cm	0.45 ± 0.12 cm
Style length (Mean ± SD)	1.3 ± 0.32 cm	0.75 ± 0.11 cm
Corolla colour	Dark violet	Dark violet **with pale striations in the petal junctions**
Spine characters		
Spine length (Mean ± SD)	0.6 ± 0.1 cm	0.25 ± 0.1 cm
Spine distribution	Upper – 6- 8 Lower – 3-6	Upper – 1-3 Lower – 0-3
Spine morphology	**Dimorphic spines**	**Simple spines**

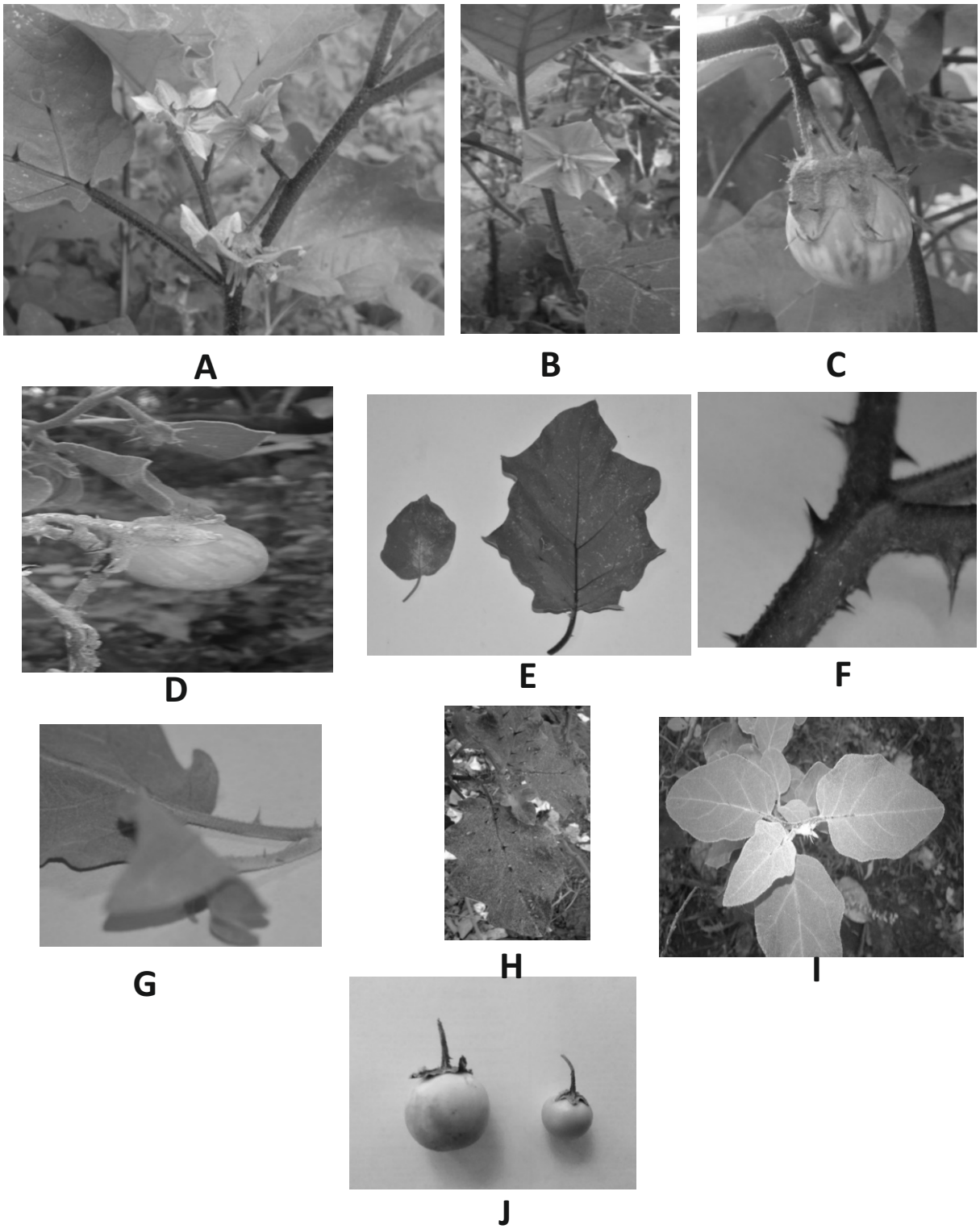

Figure. 1(A-J): Macro morphological characters of two morpho-types of *Solanum melongena*var. *insanum.* 1**A.** Inflorescence of morpho-type1, **B**. Inflorescence of morpho-type 2, **C**. Fruit of morpho-type 1, **D**. Fruit of morpho-type 2, **E**. Leaf of morpho-type 2 and 1, **F.** Dimorphic spines of morpho-type 1, **G.** Simple spines of morpho-type 2 **H.** Leaf with numerous spines in morpho-type 1, **I**. Leaf with less spines in morpho-type 2, **J**. Ripe fruit of morpho-type 1 and 2 respectively.

Table 2: The micro morphological peculiarities of the two morpho-types

characters	'Cheruvazhuthina' (Morphetype 1)	'Punyahachunda' (Morphetype 2)
	Trichome characters (Fig. 3a and b)	
Morphology	Stellate trichome	Stellate trichome
Number of arms	**6 - 7 arms**	**8 arms**
Number of trichomes in adaxial surface	**2 – 3**	**Numerous**
	Seed characters (Fig3. c and d)	
Size	2 – 3 mm	2 – 3 mm
Seed coat ornamentation	**Thick muri with wide lumen**	**Thick and compact muri**

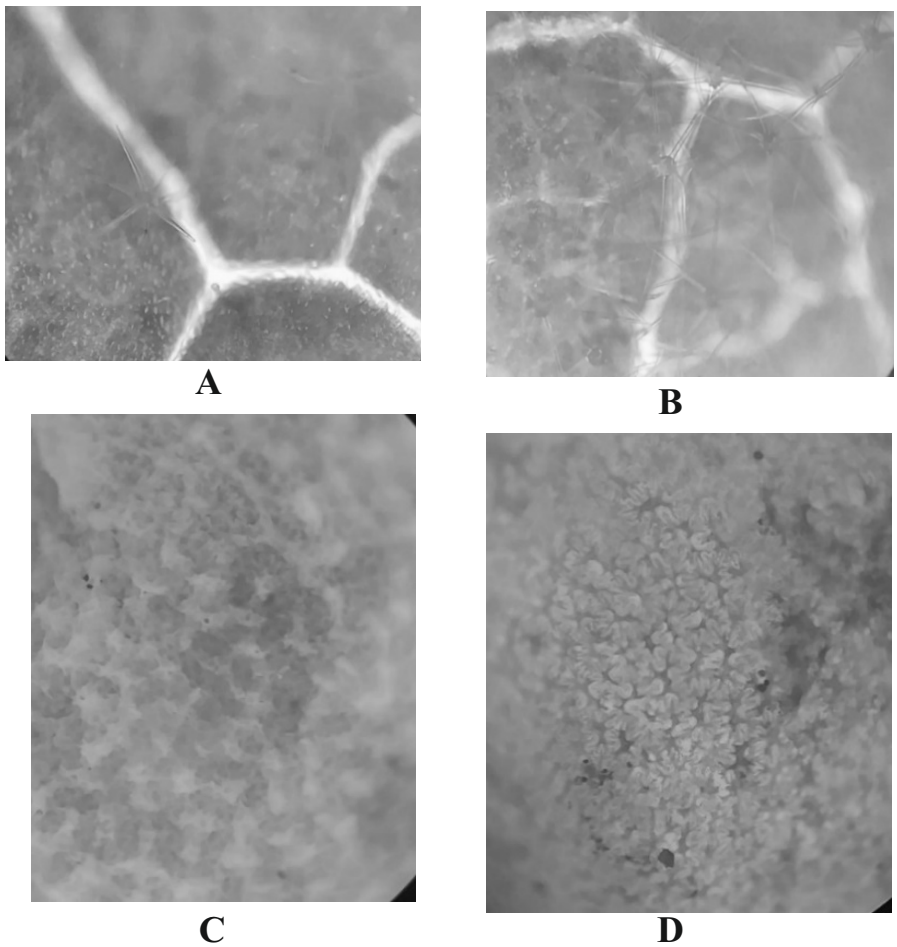

Figure 2 (A-D). Micro morphological characters of two morpho-types of *Solanum melongena* var. insanum **A**.Trichomes ofmorpho-type 1**B.** Trichomes of morpho-type 2 **C**. Seed coat ornamentation of morpho-type 1 **D**. Seed coat ornamentation of morpho-type 2

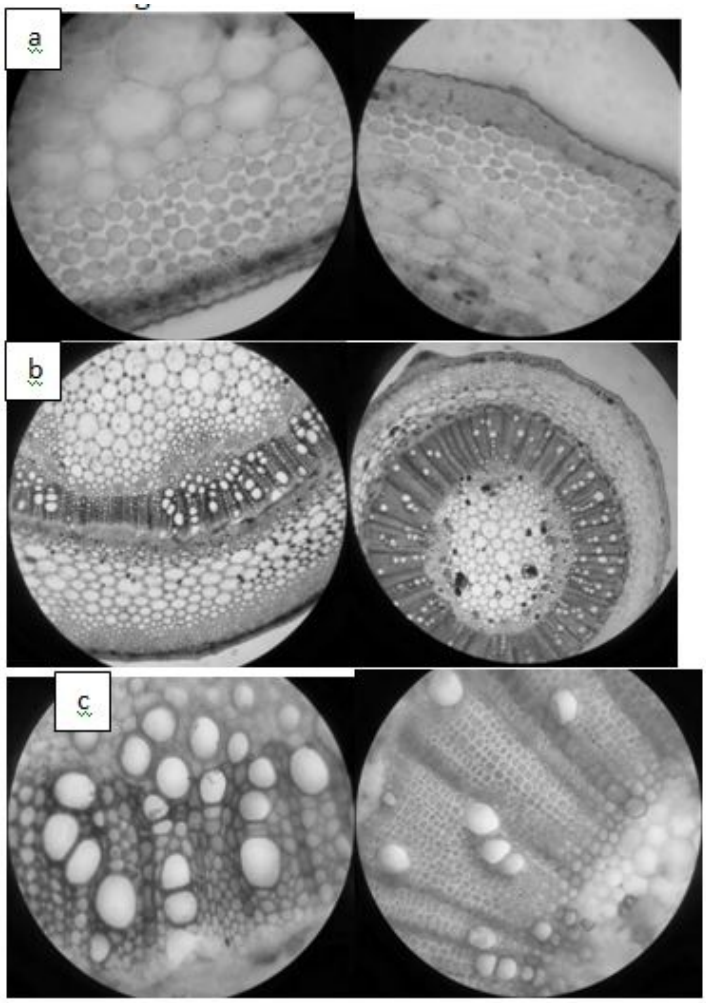

Figure 3 (a-c): a. The epidermal and cortical regions morpho-typ e 1 and 2 respectively, **b.** Stem T.S. of morpho-type 1 and 2 respectively, after secondary thickening, **c.** The secondary xylem of morpho-type 1 and 2 respectively.

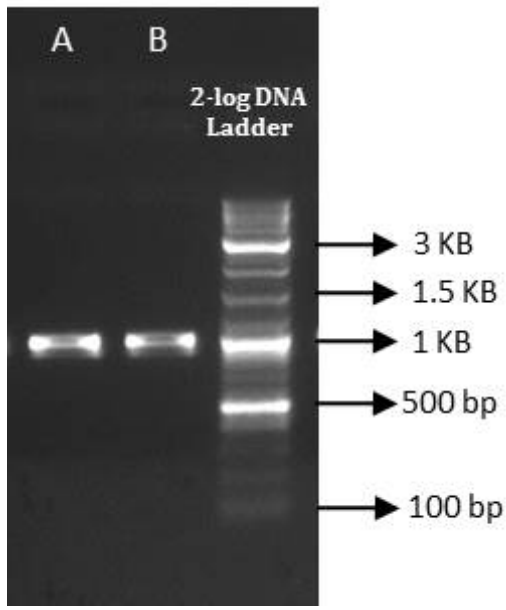

Figure. 4: Isolated DNA from two morpho-types after gel electrophoresis – A: morpho-type 1 and B: morpho-type 2.

```
SR593-A-MATK       1 CTTTTTTAGAGGACAATTTGTCACATCTAAATTATGTATTAGATATACTA 50
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
SR593-B-MATK       1 CTTTTTTAGAGGACAATTTGTCACATCTAAATTATGTATTAGATATACTA 50

SR593-A-MATK      51 ATACCCTACCCCGTTCATCTGGAAATCTTGGTTCAAACTCTTCGTTATTG 100
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
SR593-B-MATK      51 ATACCCTACCCCGTTCATCTGGAAATCTTGGTTCAAACTCTTCGTTATTG 100

SR593-A-MATK     101 GGTAAAAGATGCCTCTTCTTTACATTTATTACGATTCTTTCTCCACGAAT 150
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
SR593-B-MATK     101 GGTAAAAGATGCCTCTTCTTTACATTTATTACGATTCTTTCTCCACGAAT 150

SR593-A-MATK     151 ATTGTAATTTGAATAGTCTTATTACTTCAAAGAAGCCCGGTTACTCTTTT 200
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
SR593-B-MATK     151 ATTGTAATTTGAATAGTCTTATTACTTCAAAGAAGCCCGGTTACTCTTTT 200

SR593-A-MATK     201 TCAAAAAAAAAATCCAAAATTCTTCTTCTTCTTATATAATTCTTATGTATA 250
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
SR593-B-MATK     201 TCAAAAAAAAAATCCAAAATTCTTCTTCTTCTTATATAATTCTTATGTATA 250

SR593-A-MATK     251 TGAATGCGAATCCACTTTCGTCTTTCTACGGAAACAATCTTTTCATTTAC 300
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
SR593-B-MATK     251 TGAATGCGAATCCACTTTCGTCTTTCTACGGAAACAATCTTTTCATTTAC 300

SR593-A-MATK     301 GATCAACATCTTTTGGAGCCCTTCTTGAACGAATATATTTCTATGGAAAA 350
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
SR593-B-MATK     301 GATCAACATCTTTTGGAGCCCTTCTTGAACGAATATATTTCTATGGAAAA 350

SR593-A-MATK     351 ATAGAACGTCTTGTAAAAGTCTTTGCTAAGGATTTTCAGGTTACCCTATG 400
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
SR593-B-MATK     351 ATAGAACGTCTTGTAAAAGTCTTTGCTAAGGATTTTCAGGTTACCCTATG 400

SR593-A-MATK     401 GTTATTCAAGGATCCTTTGATGCATTATGTTAGGTATGAAGGAAAATCAA 450
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
SR593-B-MATK     401 GTTATTCAAGGATCCTTTGATGCATTATGTTAGGTATGAAGGAAAATCAA 450

SR593-A-MATK     451 TTCTGGCTTCAAAAGGGACGTTTCTTTTGATGAATAAATGGAAATTTTAC 500
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
SR593-B-MATK     451 TTCTGGCTTCAAAAGGGACGTTTCTTTTGATGAATAAATGGAAATTTTAC 500

SR593-A-MATK     501 CTTGTCAATTTTTGGCAATGTCATTTTTCTATGTACTTTCACACAGGAAG 550
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
SR593-B-MATK     501 CTTGTCAATTTTTGGCAATGTCATTTTTCTATGTACTTTCACACAGGAAG 550

SR593-A-MATK     551 GATCCATATAAACCAATTATCCAACCATTCCCGTGACTTTATGGGCTATC 600
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
SR593-B-MATK     551 GATCCATATAAACCAATTATCCAACCATTCCCGTGACTTTATGGGCTATC 600

SR593-A-MATK     601 GTTCAAGTGTGCGACTAAATCATTCAATGGTACGTAGTCAAATGTTCGAA 650
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
SR593-B-MATK     601 GTTCAAGTGTGCGACTAAATCATTCAATGGTACGTAGTCAAATGTTCGAA 650

SR593-A-MATK     651 AATTCATTTCTAATCAATAATCCAATTAAGAAATTCGATACCCTTGTTCC 700
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
SR593-B-MATK     651 AATTCATTTCTAATCAATAATCCAATTAAGAAATTCGATACCCTTGTTCC 700

SR593-A-MATK     701 AATTATTCCTTTGATTGGATCATTAGCTAAAGCACACTTTTGTACCGTAT 750
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
SR593-B-MATK     701 AATTATTCCTTTGATTGGATCATTAGCTAAAGCACACTTTTGTACCGTAT 750

SR593-A-MATK     751 TAGGGCATCCCATTAGTAAACCGGTTTGGTCCGATTTATCAGATTCTGAT 800
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
SR593-B-MATK     751 TAGGGCATCCCATTAGTAAACCGGTTTGGTCCGATTTATCAGATTCTGAT 800

SR593-A-MATK     801 ATTATTGACCGATTTGGGCGTATATGCAGAAATCTTTTTCATTATTATAG 850
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
SR593-B-MATK     801 ATTATTGACCGATTTGGGCGTATATGCAGAAATCTTTTTCATTATTATAG 850

SR593-A-MATK     851 CGGATCTTCCAAAAAAAAGAC 871
                     |||||||||||||||||||||
SR593-B-MATK     851 CGGATCTTCCAAAAAAAAGAC 871
```

Figure 5: The pair-wise sequence alignment of *matK* using BLAST

```
8
A ITS 1   TTTCCGTAGGTGAACCTGCGGAAGGATCATTGTCGAAACCTGCACAGCAGAACGACCCGC 60
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
B ITS 1   TTTCCGTAGGTGAACCTGCGGAAGGATCATTGTCGAAACCTGCACAGCAGAACGACCCGC 60

A ITS 61  GAACACGTTCAAACACCGGGGGAGCCGCGCGGCGTGGGCCGCTCCGGCGCCGCCCCGCGC 120
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
B ITS 61  GAACACGTTCAAACACCGGGGGAGCCGCGCGGCGTGGGCCGCTCCGGCGCCGCCCCGCGC 120

A ITS 121 GTCTGCCCCTCGCCCCCTCTTCGGGGGGGCCAAACGAACCCCGGCGCGGAAAGCGCCAAG 180
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
B ITS 121 GTCTGCCCCTCGCCCCCTCTTCGGGGGGGCCAAACGAACCCCGGCGCGGAAAGCGCCAAG 180

A ITS 181 GAATACTCAAACGAGAGCCCTCCGCCCGTGCCCCGTCCGCGGGGCGTGCGGGCGGATGCG 240
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
B ITS 181 GAATACTCAAACGAGAGCCCTCCGCCCGTGCCCCGTCCGCGGGGCGTGCGGGCGGATGCG 240

A ITS 241 TGCTTCTTTCGAAACCAAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATG 300
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
B ITS 241 TGCTTCTTTCGAAACCAAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATG 300

A ITS 301 AAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTC 360
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
B ITS 301 AAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTC 360

A ITS 361 TTTGAACGCAAGTTGCGCCCGAAGCCGTCAGGCCGAGGGCACGTCTGCCTGGGCGTCACG 420
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
B ITS 361 TTTGAACGCAAGTTGCGCCCGAAGCCGTCAGGCCGAGGGCACGTCTGCCTGGGCGTCACG 420

A ITS 421 CATCGCGTCGCCCCCCCGCACGCCGCTCGGCGTCGCGGGGGCGGATACTGGCCTCCCGTGC 480
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
B ITS 421 CATCGCGTCGCCCCCCCGCACGCCGCTCGGCGTCGCGGGGGCGGATACTGGCCTCCCGTGC 480

A ITS 481 GCCTCGCGCCCGCGGCCGGCCTAAATGCGAGTCCACGTCGACGGACGTCGCGGCAAGTGG 540
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
B ITS 481 GCCTCGCGCCCGCGGCCGGCCTAAATGCGAGTCCACGTCGACGGACGTCGCGGCAAGTGG 540

A ITS 541 TGGTTGTAGCTCAACTCTCTTGGTGCCGCGGCCACAGCCCGTCGCGCGTGCGCGCTCCAC 600
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
B ITS 541 TGGTTGTAGCTCAACTCTCTTGGTGCCGCGGCCACAGCCCGTCGCGCGTGCGCGCTCCAC 600

A ITS 601 GGCCCCTCCGGCGCTAGCGCGCTCCGACCGCGACCCCAGGTCAGGCGGGATTACCC 656
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||
B ITS 601 GGCCCCTCCGGCGCTAGCGCGCTCCGACCGCGACCCCAGGTCAGGCGGGATTACCC 656
```

Figure 6: The pair-wise sequence alignment of ITS using BLAST

morphological characters include qualitative and quantitative characters. The qualitative macro morphological characters including habit, habitat, characters of stem, leaf, inflorescence and flower were investigated. About 10 quantitative characters viz., leaf length and width, inter-nodal length, petiole length, pedicel length, diameter of flower, stamen, style, length and number of spines were examined. The qualitative as well as quantitative characters show some differences. Among the characters investigated the two morpho-types showed variations in leaf characters, flower morphology, spine characters, seed and fruit morphology (Fig. 1). In leaf morphology the leaf shape of the morpho-type 1 was undulate and that of morpho-type 2 was sinuate (Fig. 1 E). The length and width of the leaf of morpho-type 1 (13 ± 0.6 cm) was higher than that of morpho-type 2 (7.5 ± 0.37 cm) (Table 1). Morpho-type 1 is spinier than morpho-type 2 and morpho-type 1 has dimorphic spines, but morpho-type 2 possess only one type of spine (Fig. 5 f and g).

Colour of spine was also showing considerable difference ie. morpho-type 1 has dark purple coloured spines whereas morpho-type 2 has small yellow coloured spines (Fig. 1 F and G). The seed coat ornamentation also showed some differences (Fig. 1 C and D). Morpho-type 1 has thick muri with wide lumen seed coat ornamentation, whereas the morpho-type 2 has thick, compact muri seed coat ornamentation. Morpho-type 1 has racemose inflorescence, whereas morpho-type 2 has solitary cyme inflorescence. Trichome distributions on leaf also showed variations. Morpho-type 2 has densely distributed stellate trichomes on the upper surface of the leaf and that of morpho-type 1 has scarcely distributed stellate trichomes on the upper leaf surface (Fig. 2A and B). The collected samples showed distinguishable differences (Table 1 and 2). Using these characters the collected samples can be distinguished as morpho-type 1 and morpho-type 2. So it might be pointed out that the characters showing differences were conserved in each morpho-type. The significant variations in the micro morphological characters such as trichome characters (Fig. 2 A and B) and seed coat ornamentation (Fig. 2 C and D) were conserved in each morpho-types. Krishnakumar *et al.* (2018) reported that majority of the accessions of *Abelmoschus* e*sculentus* could be easily distinguishable using trichome characters even in the early vegetative stage of the plants. This variability in the micro morphological characters were supported by RAPD studies of *Abelmoschus esculentus* (L.) Moench. (Christeena, 2014). Zhigila et al. (2015) also used trichome characters to distinguish five varieties of *Capsicum annuum* L. Fareedkhan *et al*. (2012) conducted a study on the morphological characterisation of the wild varieties of *Solanum tuberosum* L. and the variations found were confirmed by RAPD.

Anatomical Studies: The cross section of the leaf and petiole of two morpho-types had no significant differences. However cross section of stem showed some differences among the morpho-types studied. The anatomy of the two morpho-types were almost same; a single epidermal layer followed by 1 – 2 layers of chlorenchyma, then by 4 layers of collenchyma in morpho-type 2 and 8 layers of collenchyma in morpho-type 1. Then 4 layers of paranchyma followed by the stelar region (Fig. 3 a). The stelar region contain single layered endodermis and stone cells above the secondary phloem in discontinuous pattern in morpho-type 2. The stone cells were absent or poorly developed in morpho-type 1. Morpho-type 1 contain more xylem vessels than morpho-type 2. Morpho-type 2 started its secondary thickening earlier than morpho-type 1. Both morpho-types contain intra-xylary phloem in pith region and cell inclusions like crystals in pith cells (Fig. 3 b and c).

Anatomical studies of the stem, leaf and petiole showed no significant differences. The secondary growth in morpho-type 2 is started earlier than that of morpho-type 1(Fig. 3 b). One notable difference observed was the number of xylem vessels in stem. Morph-type 1 possessed more xylem vessels than that of morpho-type 2. It may be due to the increased girth of the stem of morpho-type 1. Further detailed anatomical studies are warranted to confirm the anatomical difference. Earlier stuies revealed that anatomical differences were observed in different varieties of *Artemisia absinthium* (Konowalik and Kreitschitz, 2012).

DNA Barcoding of the two Morpho-types: The isolated DNA from the two morphotypes were subjected for polyacrylamide gel electrophoresis. The results showed that the isolated DNA from two morpho-types were of similar equal size ie.1 kb (Fig.4).

Two genes- the chloroplast gene *mat K* and the nuclear gene ITS – were sequenced. Both sequence were submitted to NCBI GenBank using BankIt submission tool. ITS sequence got accession number MG572181 and *mat K* sequence got accession number MG543677.

The sequence of *mat K* and ITS of two morpho-types were then pair-wise aligned using NCBI

BLAST (Fig. 5 and 6). The pair-wise sequence alignment results obtained from BLAST showed that the sequences of both *mat K* and ITS genes were 100% similar in both morpho-types.

In the present investigation the morphological as well as anatomical analysis showed many differences between the two morpho-types. In another study these two morpho-types showed a characteristic difference in phytochemical constituents present in root and fruit (Thulasi and Krishnakumar, 2018). In such cases of taxonomical controversies molecular level analysis, like DNA barcoding is relied for further clarification. Hence in the present study DNA barcoding of the two morpho-types were performed. The sequence of the two genes *mat K* and ITS were compared. These two genes are frequently used for DNA barcoding studies. *Mat K*, the chloroplast gene, coded maturase kinase and ITS, the nuclear gene, is the internal transcribed spacer region which present between the small rRNA subunit gene and large rRNA subunit gene. These genes were selected because these two genes showed sequence polymorphism between species and constant within a species (Patwardhan *et al*., 2014). Pair wise sequence alignment using the NCBI tool BLAST was carried out. But this analysis showed 100% similarity. Based on the DNA barcoding results, the two morpho-types were with same taxonomic status. The difference seen in the morphology, anatomy and phytochemical constituents might be due to the environmental differences. However there were reports that the use of DNA barcoding for differentiating two verities is a failure in the case of complex groups like wild potatoes (Spooner, 2009) where whole chloroplast genome sequencing was used. So for further confirmation the complete chloroplast genome sequencing is to be performed. We could suggest that one of the morpho-type (morpho-type1) might be the wild alley of the other morpho-type.

In conclusion, morphologically the two morpho-types were different in many macro morphological characters like spine morphology, leaf shape, inflorescence type and fruit morphology, as well as micro morphological characters like seed coat ornamentation and trichome characters. Micro morphological characters are less changed within a species or variety as the influence of ecological factors. So micro morphologically the two morpho-types are different varieties of *Solanum melongena*. Anatomically two morpho-types does not showed much variations. Except in the number of xylem vessels and early starting of secondary thickening in morpho-type 2 than in morpho-type 1. Finally the DNA barcoding studies showed that the two morpho-types are same. The success of the two genes – *mat K* and ITS – used in barcoding is doubtful. So a further confirmation is needed, by whole chloroplast genome sequencing, to confirm the taxonomical position of the two morpho-types.

We express our sincere gratitude to Dr. Biju P. (Government College, Kasaragod, Kerala), Dr. Maya C. Nair (Govt. Victoria College, Palakkad, Kerala), Dr. Mallika V and Mr. Bijesh George (RGCB, Thiruvananthapuram, Kerala) for their valuable help and suggestions during this work.

REFERENCES

Barthlott W 1981 Epidermal and seed surface characters of plants: Systematic applicability and some evolutionary aspects. *Nord J Bot* **1:** 345-355.

BeentjeHenk 2010 *The Kew Plant Glossary*. Kew Publishing, Kew

Christeena T G 2014. Evaluation of morphological and molecular diversity in Okra (*Abelmoschusesculentus* (L.) Moench). Diss. University of Calicut.

Fareedkhan M, Najma Tabassum, Anila Latif, Abdul Khalid and Mansoor Malic 2012

Morphological characterisation of potato (*Solanum tuberosum* L.) germplasm under rain fed environment. *Afr J Biotechnol* **12:** 3214-3223.

Gamble J S 2016 *Flora of the Presidency of Madras*. Adlard& Son Limited (reprinted).

Griffin W J and Lin G D 2000. Chemotaxonomy and Geographical distribution of Tropane Alkaloids. *Phytochemistry* **53:** 623-637.

Harborne J B and Baxter H 1993. *Phytochemical Dictionary- A handbook of Bioactive compounds from Pants*. Taylor and Francis, London.

Joshi S P, Ranjekar P K and Gupta V S 1999. Molecular markers in plant genome analysis. *Current Science*, 230-240.

Konowalik, K. and Kreitschitz, A. 2012. Morphological and anatomical characterisation of *Artemisia absinthium* var. *absinthium*and its Polish endemic variety *A. absinthium* var. *caicigena*. *Plant SystEvol* **298:** 1325-1330.

Krishnakumar K, Rajeena P and Veena V 2018. Study on diversity of foliar trichomes in thirty accessions of okra (*Abelmoschusesculentus*(L.) Moench.). *Ind. J. Agr. Res*. **52(4)**: 439-443.

Kumar A V S and Murugan K 2013. Electron microscopic and IR spectral studies of spiny *Solanum – Solanum melongena* var. *insanum* L. *IJAB* **2:** 29-35.

Kumari S, Anokhe A and Kumar R 2017. Nutritional Quality and Health Benefits of Solanaceous Vegetables. *Progressive research* **12:** 1942 – 1945.

Patwardhan A, Ray S and Roy A 2014. Molecular markers in phylogenetic studies-a review. *Journal of Phylogenetics& Evolutionary Biology* **2:** 1-9.

Payne W W 1978 A glossary of Plant hair terminology. *Brittonia***30:** 239-255.

Rosselló-Mora R and Amann R 2001. The species concept for prokaryotes. *FEMS microbiology reviews*, **25**: 39-67.

Sambrook J, Fritsch E F and Maniatis T. 1989. *Molecular cloning* (14-19). Cold spring harbor laboratory press, New York.

Spooner D M 2009. DNA barcoding will frequently fail in Complicated Groups: An example in Wild Potatoes. *Am J Bot* **96:** 1177-1189.

Thulasi R and Krishnakumar K 2018 Phytochemical and Anti-bacterial analysis of two morpho-types of *Solanum melongena var. insanum* (L.) Prain. An aaaaayurvedic herb. *Indian J. Pharm. Biol. Res.***6**: 39 – 47.

Zhigila D A, Sawa B J, Aluko T A, Oladele, F A and Abdul Rahman A A 2015 Leaf epidermal anatomy in five varieties of *Capsicum annuum* L. Solanaceae. *AJEA***5:** 392-399.