

## Diversity Analysis of Selected *Solanum* Species in Sri Lanka using Molecular and Morphological Descriptors

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**ABSTRACT:** The family Solanaceae is composed of a broad list of species, which includes both commercial and wild accessions with enormous medicinal importance. The published phylogenies on Sri Lankan wild and naturalized *Solanum* spp. confers that *S. hispidum*, and *S. torvum* are sister species. However, this has not been proved using multiple barcoding markers. Moreover, *S. torvum*, an underutilized crop is expected to contain multiple varieties. However, varietal descriptions using morphological and DNA markers have not been done so far. Therefore, our study was aimed at accurate identification of four *Solanum* spp. found in Sri Lanka (*S. torvum* 'Bindu' and landraces *S. hispidum* and *S. pubescens*) using chloroplast marker based DNA barcoding combined with a morphological description. We used 29 quantitative and 22 qualitative parameters, comprised of vegetative and reproductive traits along with eight DNA barcoding markers. We sequenced *rbcL* and *trnL-F* to check the DNA polymorphisms. According to the combined morphological analysis of reproductive and vegetative parameters, the four species clustered separately. There were no separation observed between *S. torvum* and *S. hispidum*. *Solanum pubescens* diverged out and cladded with old world nightshades in molecular analysis. These results are in line with published literature and validates that *trnL-trnF* could be used as a potential marker to carry out a combined DNA barcoding analysis with *matK*. However, *trnL-trnF* could not be used to discriminate between *S. torvum* and *S. hispidum* species.

**Keywords:** DNA barcoding, solanum morphological and molecular assessments, conservation, wild relatives

## INTRODUCTION

The genus *Solanum* belongs to the tribe Solaneae, subfamily Solanoideae, and family Solanaceae which is comprised of species with substantial economic importance worldwide (Mace *et al.*, 1999; Seguí-Simarro, 2016). The members of Solanaceae species found in the tropics are generally considered as annual herbs while those in the temperate regions are annuals. They grow as a bush up to a height of 0.5-2.5 m. The fruits produced may vary from a small number to several (Rubatzky and Yamaguchi, 1997).

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The members of the family Solanaceae are well known for the remarkable medicinal properties found in leaves, fruits and roots (Jeyakumar *et al.*, 2016). The fruits of *Solanum torvum* Swartz are used as a cure for cough, liver and spleen disorders (Siemonsma and Piluek, 1994). It has further been discovered to have tranquilizing and diuretic properties and a broad array of antibacterial and antifungal effects on human and animal pathogens (Gousset *et al.*, 2005; Chah *et al.*, 2000; Abas *et al.*, 2006). The phenolic compound present in the fruits of *S. torvum* is used as an anti-diabetagenic and antioxidant (Gandhi *et al.*, 2011; Loganayaki *et al.*, 2010). The leaves are utilized as a stabilizing agent of blood flow. Tonics and blood cell regeneration agents are prepared from mature fruits and they are also employed for the curing pains and skin diseases (Kala, 2005). *Solanum hispidum* has been evaluated for the anti-fungal activity due to the presence of a novel spirostanol and several other saponins (González *et al.*, 2004; Chakravarty *et al.*, 1982).

The species of Solanaceae grown in Sri Lanka include. *S. melongina*, *S. torvum*-Bindu ('Thibbatu'), *S. torvum*-Landrace ('Thibbatu'), *S. hispidum* ('Gonabatu') and *S. pubescens* ('Walthibbatu'). Majority of the research conducted in Sri Lanka are focused on 'wambatu' or *S. melongena* eggplant which thrives well in the rain-fed and minimum irrigation facilities due to great stress resilience of the plant (DOA, 2018). *Solanum melongena* is cultivated as a commercial crop in Sri Lanka. However, the ability of the varieties of eggplant to resist biotic stress such as bacterial and fungal and other nematode and insect attacks is poor. Thus, the development of resistance by identification of resistant genes from the wild relatives is highly recommended (Collonnier *et al.*, 2001). Certain wild relatives of *S. melongena* such as *S. torvum* harbors a consortium of resistant genes which can be readily employed in breeding programmes. *S. torvum* has been identified as resistant to wilt diseases and certain other pests such as nematodes. The resistance has been transferred to the commercial varieties of *Solanum* spp. through various breeding programs (Jarl *et al.*, 1999). Interspecific hybridization has been carried out between commercial eggplant cultivars and *S. torvum* (Collonnier *et al.*, 2003; Clain *et al.*, 2004; Gousset *et al.*, 2005). However, the precise identification of species/varieties play a crucial role in subsequent breeding protocols as most of the wild relatives possess undesirable traits which can be easily transferred to the genomes of improved cultivars when subjected to breeding (Prohens *et al.*, 2017).

DNA barcoding can be identified as a key and robust strategy in successful species identification (Group *et al.*, 2009). Several research studies have been carried worldwide for successful identification of the *Solanum* spp. especially to detect the adulterations when used as medicinal plants (Techen *et al.*, 2014). *Solanum* species have also been used in DNA barcoding projects carried out for conservation of local biodiversity in certain other regions of the world (Boessenkool *et al.*, 2014). However, studies have not yet been carried out for the varieties/species of the genus *Solanum* found in Sri Lanka especially for the wild relatives such as *S. torvum*. Thus, the present study was conducted to accurately identify the varieties of *Solanum* spp. found in Sri Lanka for the future use of the species in wide hybridization protocols and conservation of the wild germplasm of *Solanum* spp.

## METHODOLOGY

### Collection of the plant material

The study was carried out with four species of wild relatives of brinjal (*Solanum melongena*) (*S. torvum* 1: 'Thibbatu' variety Bindu recommended by the Department of Agriculture

(DOA), Sri Lanka; *S. torvum* 2: landrace of 'Thibbatu'; *S. hispidum*: 'Gonabatu'; *S. pubescens*: 'Walthibbatu'). Breeder seeds of *S. torvum* 1 were sourced from the Regional Agriculture Research and Development Center, Aralaganwela, Sri Lanka and *S. torvum* 2 from a farmer in *Girandurukotte*. *Solanum hispidum* was collected from roadsides at Haggala, Nuwara Eliya, while *S. pubescens* were collected from roadsides at Baragama, Hambanthota.

### Establishment of plant material

The nursery bed was prepared by using soil and coir dust (1:1), treated with Topspin and was kept for 24 hours for disinfection. Seeds were soaked in pure water and was allowed to germinate. Pits of 0.09 cm<sup>2</sup> dimensions were prepared on 10 m<sup>2</sup> plots with a space of 1 m between rows and 2 m between plants. Plots were maintained at the open fields of the Regional Agriculture Research and Development Centre, Bandarawela (IU3a-average annual rainfall of > 1900 mm, maximum average temperature of 27 °C, minimum average temperature of 14 °C), Welimada, and farmer fields at Masnawatta and Mirahawatta, Welimada (U3d-Average annual rainfall of > 1300 mm, maximum average temperature of 28 °C, minimum average temperature of 15 °C) (Reddish yellow Podsolc soil in both locations). The pits were filled with a mixture of decomposed cattle manure and topsoil in 1:1 ratio and the basal fertilizer was added according to the recommendation of the Department of Agriculture, Sri Lanka. Four weeks old seedlings were transplanted according to an incomplete block design (IBD) with four genotypes, and five blocks in Bandarawela and three blocks in Welimada sites.

Three individuals per each genotype in each block were assessed for 29 quantitative and 22 qualitative (vegetative and reproductive) parameters. Vegetative parameters including cotyledon length (CL), cotyledon width (CW), cotyledon length / cotyledon width ratio (CL/CW), leaf blade length (LBL), leaf blade width (LBW), leaf petiole length (LPL), leaf blade tip angle (LBTA), number of leaf prickles (NLP), plant height (PIH), mean canopy width (MCW), number of branches per plant (NB) were measured to describe the morphological variability of leaves and stems. The reproductive traits at flowering stage; number of flowers per inflorescence (NFPI), flower diameter (FD), petal length (PL), petal width (PW), length of stigma exerted (LSE), length of anther (AL), width of anther (AW), days to flowering (DF) were measured. Similarly, at the harvesting stage, the fruit parameters including fruit length (FL), fruit breadth (FB), fruit length/fruit breadth ratio (FL/FB), length of stalk (LS), thickness of stalk (TS), individual fruit weight (IFW), 100 fruit weight (HFW), seed diameter (SD), and 100 seed weight (HSW) were recorded.

Furthermore, 22 qualitative parameters on cotyledon, plant, leaf, stem, flower, seed, fruit, were descriptively assessed for three and five replicates from Bandarawela and Welimada sites respectively. The color of the cotyledon and the plant growth habit were recorded to distinguish among the species/varieties according to the RHS Color Chart (Royal Horticultural Society, 2001). The parameters recorded to assess the leaf variability were leaf blade color, blade lobbing and the leaf blade tip angle. The stem characteristics recorded in this study were the color of the stem, and presence of hairs and prickles on the stem. The floral parameters included the type of the inflorescence, the color of corolla, position of the stigma and the presence of prickles in the pedicel and color of the seeds. The shape of the fruit apex, fruit flesh density, relative fruit calyx length, color of the petiole, the position of the fruit, the color of fruits, the nature of fruit curvature, cross section and locules, and the number of prickles in calyx were recorded as fruit characteristics. These parameters were used to assess the variability among the species/varieties.

## DNA extraction and PCR

Immature, fresh leaf samples were used in the DNA extraction process. DNA was extracted using modified CTAB (hexadecyl trimethyl ammonium bromide) protocol (Porebski *et al.*, 1997). PCR amplification was performed in TP600: Takara (Otsu Shiga, Japan) thermal cycler using eight DNA barcoding primer pairs (Table 1) in a 15  $\mu$ l the PCR reaction mixture with Go Taq Green Master Mix (7.5  $\mu$ l), 10  $\mu$ M forward and reverse primers (0.5  $\mu$ l each) and 10  $\mu$ M Spermidine (3.5  $\mu$ l). The PCR profiles used were as given in Table 1 (Levin *et al.*, 2003; Shaw *et al.*, 2005). The volume of template DNA for each marker was optimized considering each marker profile. The standard PCR protocol was modified for certain markers for an optimal amplification (Table 1).

## DNA sequencing

The viable PCR products of two selected DNA markers, which are commonly used for more profound phylogenetic relationship analysis in plants were purified using Qiagen Qiaquick PCR purification kit (Catalog number: 28104, Qiagen, Hilden, Germany) and sequenced using ABI 3500 automated sequencer (Catalog number 622-0010).

## Data analysis

All the quantitative morphometric data were subjected to normality testing in Minitab 17 (Minitab Inc. USA), and General Linear Model (GLM) and LS means/pdiff mean separation procedures using statistical package SAS 9.4 (SAS Institute, NC, Cary, USA). Following the data analysis, a dendrogram was constructed for the vegetative and reproductive data using the algorithms of Complete Linkage and Pearson Distance method in Minitab 17 (Minitab Inc. USA). Principal component analysis (PCA) was conducted using reproductive, vegetative and combined data in Minitab 17 (Minitab Inc. USA). The principal component 1 (PC 1) and principal component 2 (PC 2) were employed in drawing scatter plots. All the PCs were used to draw dendrogram using Euclidean distances.

## Phylogenetic analysis

The raw sequencing data generated for two markers *rbcL* and *trnL-trnF* were first visualized in MEGA 7 (Kumar *et al.*, 2016) in order to define the initial and end noise. Then, the trimmed datasets were subjected separately to a BLAST search and homology sequences with high E values (threshold E value =  $10^{-5}$ ) were retrieved (Table 2). The selected reference sequences were then aligned with the sequences by ClustalW algorithm (Thompson *et al.*, 1994) in MEGA 7 (Kumar *et al.*, 2016) with manual editing. The evolutionary relationship of *S. torvum*, *S. pubescens* and *S. hispidum* was inferred based on the phylogenetic tree reconstructed based on *rbcL* and *trnL-trnF*. A tree search was conducted in Maximum Likelihood (ML) framework in RAxML (Stamatakis, 2006) through a rapid bootstrap algorithm (Stamatakis, 2008) for 1000 iterations and GTRGAMMA (Rodriguez *et al.*, 1990) as the evolutionary model to compensate the dataset. All the bootstrap bipartitions were used to get a single tree topology by constraining the bootstrap values to ML consensus tree. Further modification of the final majority rule consensus tree was carried out using Figtree 1.4.3 (Rambaut, 2014). Furthermore, to support the analysis, a tree was built in the Bayesian framework and interpretations on the best nucleotide substitutions were made by carrying out a model selection in Akaike Information Criteria (AIC) (Akaike, 1974) and corrected Akaike Information Criteria (AICc) (Cavanaugh, 1997) in J model test (Posada, 2008).

Table 1. DNA Barcoding markers and PCR conditions.

A marker	Sequence (5'-3')	Initial denaturation		Denaturation		Annealing		Extension		Final extension		Reference
		T (°C)	Time	T (°C)	Time	T (°C)	Time	T (°C)	Time	T (°C)	Time	
<i>trnH-psbA</i>	F- CGCGCATGGTGGATTCACAATCC R- GTTATGCATGAACGTAATGCTC	98	45 sec	98	10 sec	64	30 sec	72	40 sec	72	10 mins	Tate and Simpson (2003) Sang <i>et al.</i> (1997)
<i>rbcL</i>	F-ATGTCACCACAAACAGAGACTAAAGC R- GTAAAATCAAGTCCACCRCG	98	45 sec	98	10 sec	55	30 sec	72	40 sec	72	10 mins	Levin <i>et al.</i> (2003) Kress <i>et al.</i> (2009)
<i>trnL-trnF spacer</i>	F- CGA AAT CGG TAG ACG CTA CG R- ATT TGA ACT GGT GAC ACG AG	80	5 mins	94	1 min	50	1 min	72	2 mins	94	4 mins	Taberlet <i>et al.</i> (1991)
<i>trnS<sup>GCU</sup>-trnG<sup>UUC</sup></i>	F- AGA TAG GGA TTC GAA CCC TCG GT R- GTA GCG GGA ATC GAA CCC GCA TC	80	5 mins	95	1 min	66	4 min	66	4 mins	66	10 mins	Shaw <i>et al.</i> (2005)
<i>atpB-rbcL spacer</i>	F-GAAGTAGTAGGATTGATTCTC R-TACAGTTGTCCATGTACCAG	94	4 mins	94	30 sec	45	30 sec	72	2 mins	72	5 mins	Hoot and Taylor (2001)
<i>atpB gene</i>	F-TATGAGAATCAATCCTACTACTTCT R-TCAGTACACAAAGATTTAAGGTCAT	92	3 mins	92	1 min	55	1 min	72	3 mins	72	7 mins	Hoot <i>et al.</i> (1995)
<i>matK-trnT spacer</i>	F-GCATAAAATATAYTCCYGAAARATAAGTGG R-TGGGTTGCTAACTCAATGG	95	90 sec	95	30 sec	48	1 min	68	2 mins	68	20 mins	Wicke and Quandt (2009)
<i>trnL(tRNA-leu gene)</i>	F-CGAAATCGGTAGACGCTACG R-GGGGATAGAGGGACTTGAAC	95	10 mins	95	30 sec	50	30 sec	72	2 mins	94	4 mins	Taberlet <i>et al.</i> (2007)

Then, the criteria for the best model were used to construct the tree in the Bayesian framework using MrBays (Huelsenbeck and Ronquist, 2001). Four Markov Chain Monte Carlo (MCMC) were implemented for 50 million cycles to generate precise posterior probability distributions. Burn-in point of the analysis was set to 5000 generations and 10% of the trees generated initially were omitted as burn-in. The 50% majority rule consensus tree out of the remaining trees was drawn. The posterior probability values from each branch were subsequently inferred. The model selection and all the tree constructions were carried out in CIPRES super computer (Miller *et al.*, 2010). Finally, the resemblance of Bayesian and ML trees was modelled, and the posterior probability values were incorporated to the nodes of the Bayesian tree.

**Table 2. The GenBank sequences used in the present study.**

<b>Accession No.</b>	<b>Species name</b>	<b>Reference</b>
AY266235	<i>Solanum sisymbriifolium</i>	Bohs 2004
AY266246	<i>S. torvum</i>	Bohs 2004
DQ180394	<i>S. aethiopicum</i>	Weese and Bohs 2007
DQ392958	<i>S. capense</i>	NCBI
DQ392959	<i>S. cyaneo-purpureum</i>	NCBI
DQ392961	<i>S. tomentosum</i>	NCBI
DQ812103	<i>S. anguivi</i>	Bohs <i>et al.</i> , 2007
EU176156	<i>S. richardii</i>	NCBI
EU176158	<i>S. supinum</i>	NCBI
GQ163556	<i>S. rigescentoides</i>	NCBI
GU590997	<i>S. albidum</i>	Stern <i>et al.</i> , 2011
GU590999	<i>S. asperolanatum</i>	Stern <i>et al.</i> , 2011
GU591003	<i>S. bolivianum</i>	Stern <i>et al.</i> , 2011
GU591007	<i>S. chrysotrichum</i>	Stern <i>et al.</i> , 2011
GU591013	<i>S. donianum</i>	Stern <i>et al.</i> , 2011
GU591016	<i>S. glutinosum</i>	Stern <i>et al.</i> , 2011
GU591023	<i>S. lanceolatum</i>	Stern <i>et al.</i> , 2011
GU591035	<i>S. pluviale</i>	Stern <i>et al.</i> , 2011
GU591044	<i>S. stellatovelutinum</i>	Stern <i>et al.</i> , 2011
HQ457406	<i>S. metrobotryon</i>	Stern <i>et al.</i> , 2011
HQ721897	<i>S. aethiopicum</i>	NCBI
HQ721899	<i>S. anguivi</i>	NCBI
HQ721909	<i>S. catombelense</i>	NCBI
HQ721927	<i>S. mauense</i>	NCBI
HQ721941	<i>S. ruvu</i>	NCBI
HQ721958	<i>S. zanzibareense</i>	NCBI
KC539171	<i>S. euacanthum</i>	Wahlert <i>et al.</i> , 2014
KC539172	<i>S. hasslerianum</i>	Wahlert <i>et al.</i> , 2014

Accession No.	Species name	Reference
KC539180	<i>S. sisymbriifolium</i>	Wahlert <i>et al.</i> , 2014
KU719749	<i>S. trilobatum</i>	Aubriot <i>et al.</i> , 2016
KU719755	<i>S. dammerianum</i>	Aubriot <i>et al.</i> , 2016
KU719760	<i>S. poka</i>	Aubriot <i>et al.</i> , 2016
KU719781	<i>S. pseudosaponaceum</i>	Aubriot <i>et al.</i> , 2016
KU719784	<i>S. torvum</i>	Aubriot <i>et al.</i> , 2016
KU719786	<i>S. trilobatum</i>	Aubriot <i>et al.</i> , 2016
KU719790	<i>S. torvum</i>	Aubriot <i>et al.</i> , 2016
AB586589	<i>Solanum sp.</i>	Tobe <i>et al.</i> , 2010
JN407385	<i>S. torvum</i>	NCBI
JN407386	<i>S. torvum</i>	NCBI
KF270202	<i>S. environmental</i>	NCBI
KT178124	<i>S. triflorum</i>	Aust <i>et al.</i> , 2015
KY293568	<i>S. nigrum</i>	NCBI
MH283707	<i>S. trilobatum</i>	Aubriot <i>et al.</i> , 2018
MH283708	<i>S. melongena</i>	Aubriot <i>et al.</i> , 2018
MH283709	<i>S. glabratum</i>	Aubriot <i>et al.</i> , 2018
MH283710	<i>S. umtuma</i>	Aubriot <i>et al.</i> , 2018
MH283711	<i>S. melongena</i>	Aubriot <i>et al.</i> , 2018
MH283712	<i>S. violaceum</i>	Aubriot <i>et al.</i> , 2018
MH283713	<i>S. incanum</i>	Aubriot <i>et al.</i> , 2018
MH283715	<i>S. polhillii</i>	Aubriot <i>et al.</i> , 2018
MH283717	<i>S. aethiopicum</i>	Aubriot <i>et al.</i> , 2018
MH283718	<i>S. campylacanthum</i>	Aubriot <i>et al.</i> , 2018
MH283719	<i>S. usambarensense</i>	Aubriot <i>et al.</i> , 2018
MH283720	<i>S. agnewiorum</i>	Aubriot <i>et al.</i> , 2018
MH283721	<i>S. incanum</i>	Aubriot <i>et al.</i> , 2018
MH283722	<i>S. richardii</i>	Aubriot <i>et al.</i> , 2018
MH283724	<i>S. anguivi</i>	Aubriot <i>et al.</i> , 2018
MH360739	<i>S. nigrum</i>	NCBI

## RESULTS AND DISCUSSION

### Variation of the quantitative morphological parameters

Mean separation of a total of 29 quantitative parameters including 11 vegetative and 18 reproductive parameters for three *Solanum* species are given in the Table 3. The individual effects of site (*Bandarawela* and *Welimada*), species/variety (*S. torvum* 1, *S. torvum* 2, *S.*

*pubescens* and *S. hispidum*) and the integration effect of site  $\times$  species/variety interaction were assessed on all the parameters.

The mean values of CL, CW, LBL, LBW, NLP, PIH, MCW, and NB were found to be significantly variable among the species/varieties, and so as the site species/variety interaction. A significantly highest mean CL was recorded for *S. hispidum* (2.7 cm), while it was the lowest in *S. pubescens* (1.46 cm) (Table 3,  $P < 0.05$ ). When considering the site  $\times$  species/variety interaction, the highest CL was recorded for *S. hispidum* in both the sites (2.7 cm). Similarly, CW was found to be the highest for *S. hispidum* (8.58 cm) whereas *S. pubescens* was the lowest (4.93 cm). However, no significant difference of CL/CW ratio could be recorded among the three species in two sites (Table 3,  $P < 0.05$ ), thus suggesting that CL/CW could not be employed in the delimitation of studied *Solanum* species.

The morphological variation of leaf parameters also could be employed to differentiate species. The mean values of LBL, LBW, and LPL were the highest for *S. hispidum* (37.19 cm, 34.83 cm and 12.59 cm respectively). On the contrary, *S. pubescens* were found to be the lowest for each of the three parameters mentioned above (6.15 cm, 6.20 cm, and 2.69 cm, respectively) (Table 3,  $P < 0.05$ ). Moreover, the highest mean values of LBL, LBW, and LPL were recorded by *S. pubescens* in Bandarawela site than Welimada site. The mean values of LBTA were significantly variable depending on site, species/variety and site  $\times$  species/variety interaction. Accordingly, LBTA was found to be very high in Welimada area. Regarding the effect of the species/variety, *S. torvum* 2 and *S. pubescens* varieties were shown to have the highest LBTA values (67.25 and 69.17 cm, respectively). The variation of both the micro-climatic conditions and the genetic components may result in the observed differences in leaf morphology.

Furthermore, NLP was significantly high in *S. hispidum* (21.42 cm) and was highly variable concerning the site  $\times$  species/variety interaction. It was also noticed that the mean values of PIH for *S. pubescens* in both Bandarawela and Welimada areas were markedly low (98.18 cm and 160.84 cm, respectively) compared to other site  $\times$  species/variety interactions (Table 3,  $P < 0.05$ ). The significant highest MCW was recorded for *S. torvum* 2 (322.72 cm). The species *S. pubescens*, in contrast, recorded the lowest MCW (79.46 cm). NB was markedly high in *S. torvum* species (*S. torvum* 1- 20, *S. torvum* 2- 19) while it was the lowest in *S. pubescens* (5.77). However, all the mean values of MCW and NB recorded were highly variable depending on site  $\times$  species/variety interaction (Table 3,  $P < 0.05$ ). Therefore, the variation in all the vegetative characters evaluated in this study except CL/CW, indicate their potential applicability in delimiting of the species. Similar levels of variations in plant, canopy and leaf characters of *Solanum* spp. have been reported in Jeyakumar *et al.* (2016) and Bello *et al.* (2013) showing higher genetic determination in the considered traits.

### Variation of the reproductive traits

Out of the 18 reproductive parameters, NFPI, FD, PL, PW, AW, FB, LS, TS, yield, SD, and HSW were not significantly different between two sites. However, NFPI was the highest in *S. torvum* 1 and *S. torvum* 2 varieties (41.31 and 41.00, respectively) and the lowest in *S. pubescens* (12). In contrast, NFPI was highly variable regarding the site  $\times$  species/variety interaction (Table 4,  $P < 0.05$ ). However, other floral traits; FD, PL, PW, LSE, AL, AW, LS are the highest in *S. pubescens* (5.06 cm, 1.66 cm, 0.91 cm, 13.80 cm, 12.02 cm, 3.01 cm, 2.06 cm respectively) and some of the seed traits; SD, and HSW were the highest in *S. hispidum* (0.31 cm, and 0.29 g respectively). Moreover, in both the sites, *S. pubescens* recorded high mean values for FD (Bandarawela-4.98 cm, Welimada-5.14 cm), PL (Bandarawela-1.65 cm, Welimada-1.68 cm), PW (Bandarawela-0.9 cm, Welimada-0.91 cm), AL



(*Banadarawela*-12.62 cm, *Welimada*-11.45 cm), LS (*Banadarawela*-2.07 cm, *Welimada*-2.10 cm), SD (*Banadarawela*-0.31 cm, *Welimada*-0.30 cm), and HSW (*Banadarawela*-0.27 g, *Welimada*-0.30 g) (Table 4,  $P < 0.05$ ).

Floral characters, in contrast were not significantly different with respect to the site  $\times$  species/variety interaction. Moreover, the mean values of LSE, AL and DF were significantly high in trees grown in *Banadarawela* site than those in *Welimada* site (9.83 cm, 8.29 cm and 124.74 respectively). *S. torvum* 1 recorded the highest DF (133.09) while *S. pubescens* recorded the lowest (122.78) (Table 4,  $P < 0.05$ ). Also, the mean values of FL and FL/FB ratio were significantly high in *Welimada* site (1.38 cm, 1.5 respectively). However, *S. pubescens* recorded comparatively a less mean FL (1.06 cm) and less mean FB value (1.04 cm) whereas, other species were found to have fruits with similar length and breadth measurements (Table 4,  $P < 0.05$ ). The lowest TS, HFW, and yield were recorded by *S. pubescens* (0.34 cm, 68.81 g, and 14.01 g) and the yield was significantly high in *S. torvum* 2 (242.23 g). Despite the differences in overall yield, IFW was significantly different among the species/varieties, thus suggesting the fact that, there was a correlation between IFW and yield traits. However, all the reproductive traits except FL/FB were useful to distinguish among the species quantitatively (Table 4,  $P < 0.05$ ). Similar levels of variability in floral, seed and fruit morphology have been reported in Jeyakumar *et al.*, (2016).

#### Analysis of qualitative parameters

Qualitative characters provided an indispensable source for the differentiation of species. The variability of the qualitative morphometric parameters was descriptively assessed and reported in Table 5 and Table 6. Morphological variability of cotyledon, plant, leaf, stem, flower, seed and fruit of *Solanum* species/varieties were descriptively evaluated at vegetative, flowering and harvesting stages.

The cotyledon color at the seedling stage was variable among the species. *S. torvum* were found to have green cotyledons, while those of the *S. pubescens* and *S. hispidum* were light violet. The growth habits of *Solanum* spp. were different, as the plants of *S. torvum* and *S. pubescens* species developed an upright growth habit and the stems of *S. hispidum* plants showed an intermediate growth pattern (Table 5). Three parameters of leaf were assessed to detect the variability among the species. Accordingly, the color of the leaf blade of *S. torvum* sp. was moderately olive green, whereas *S. pubescens* and *S. hispidum* possessed leaves with greyish green. Leaf blade lobbing was slightly variable among the species. The blade lobbing of *S. torvum* and *S. hispidum* were strong, while that of the *S. pubescens* was intermediate. However, it was noticed that there was no any difference in the leaf blade tip among the species, such that all the leaves had an acute tip (Table 5) (Plate 1 A and B).

**Table 3. The means of the parameters explaining vegetative morphology.**

Variable		CL (cm)	CW (cm)	CL/CW	LBL (cm)	LBW (cm)	LPL (cm)	LBTA	NLP	PIH (cm)	MCW (cm)	NB
Site	<i>Bandarawela</i>	2.04 <sup>a</sup>	6.40 <sup>a</sup>	3.17 <sup>a</sup>	22.81 <sup>a</sup>	21.08 <sup>a</sup>	5.60 <sup>b</sup>	60.29 <sup>b</sup>	6.48 <sup>a</sup>	215.81 <sup>a</sup>	267.31 <sup>a</sup>	15.35 <sup>a</sup>
	<i>Welimada</i>	2.03 <sup>a</sup>	6.09 <sup>a</sup>	3.32 <sup>a</sup>	21.79 <sup>a</sup>	20.59 <sup>a</sup>	6.26 <sup>a</sup>	64.81 <sup>a</sup>	5.89 <sup>a</sup>	226.78 <sup>a</sup>	269.06 <sup>a</sup>	16.25 <sup>a</sup>
Species / Variety	<i>S.torvum</i> 1	1.97 <sup>b</sup>	5.96 <sup>b</sup>	3.32 <sup>a</sup>	23.80 <sup>b</sup>	21.67 <sup>b</sup>	6.24 <sup>b</sup>	63.86 <sup>b</sup>	4.95 <sup>b</sup>	236.96 <sup>a</sup>	311.49 <sup>b</sup>	19.55 <sup>a</sup>
	<i>S.torvum</i> 2	2.01 <sup>b</sup>	6.03 <sup>b</sup>	3.36 <sup>a</sup>	23.83 <sup>b</sup>	21.97 <sup>b</sup>	5.72 <sup>b</sup>	67.25 <sup>a</sup>	3.10 <sup>c</sup>	239.03 <sup>a</sup>	322.72 <sup>a</sup>	18.72 <sup>a</sup>
	<i>S. hispidum</i>	2.70 <sup>a</sup>	8.58 <sup>a</sup>	3.17 <sup>a</sup>	37.10 <sup>a</sup>	34.83 <sup>a</sup>	12.59 <sup>a</sup>	44.32 <sup>c</sup>	21.42 <sup>a</sup>	254.87 <sup>a</sup>	283.21 <sup>b</sup>	15.27 <sup>b</sup>
Site × Species/ Variety	<i>S. pubescens</i>	1.46 <sup>c</sup>	4.93 <sup>c</sup>	3.12 <sup>a</sup>	6.15 <sup>c</sup>	6.20 <sup>c</sup>	2.69 <sup>c</sup>	69.17 <sup>a</sup>	4.44 <sup>b</sup>	133.25 <sup>a</sup>	79.46 <sup>c</sup>	5.77 <sup>c</sup>
	<i>S.torvum</i> 1 <sup>#</sup>	1.99 <sup>b</sup>	5.69 <sup>c</sup>	3.55 <sup>a</sup>	24.67 <sup>c</sup>	22.33 <sup>c</sup>	5.57 <sup>b</sup>	59.19 <sup>b</sup>	4.92 <sup>b</sup>	236.41 <sup>a</sup>	317.80 <sup>a</sup>	18.78 <sup>a</sup>
	<i>S.torvum</i> 2 <sup>#</sup>	2.00 <sup>b</sup>	6.10 <sup>c</sup>	3.25 <sup>a</sup>	22.91 <sup>c</sup>	20.32 <sup>c</sup>	5.26 <sup>b</sup>	66.44 <sup>a</sup>	4.05 <sup>b</sup>	234.78 <sup>a</sup>	334.59 <sup>a</sup>	18.04 <sup>a</sup>
	<i>S. hispidum</i> <sup>#</sup>	2.70 <sup>a</sup>	9.44 <sup>a</sup>	2.87 <sup>a</sup>	40.69 <sup>a</sup>	37.54 <sup>a</sup>	12.65 <sup>a</sup>	42.18 <sup>c</sup>	21.64 <sup>a</sup>	256.20 <sup>a</sup>	262.03 <sup>a</sup>	15.19 <sup>b</sup>
	<i>S. pubescens</i> <sup>#</sup>	1.47 <sup>c</sup>	5.13 <sup>d</sup>	3.02 <sup>a</sup>	5.28 <sup>d</sup>	5.85 <sup>d</sup>	2.65 <sup>c</sup>	66.99 <sup>a</sup>	4.08 <sup>b</sup>	98.18 <sup>b</sup>	64.95 <sup>b</sup>	5.82 <sup>c</sup>
	<i>S.torvum</i> 1 <sup>#</sup>	1.95 <sup>b</sup>	6.25 <sup>c</sup>	3.09 <sup>a</sup>	22.95 <sup>c</sup>	21.01 <sup>c</sup>	7.00 <sup>b</sup>	67.93 <sup>a</sup>	4.97 <sup>b</sup>	237.52 <sup>a</sup>	305.06 <sup>a</sup>	20.29 <sup>a</sup>
	<i>S.torvum</i> 2 <sup>#</sup>	2.02 <sup>b</sup>	5.96 <sup>c</sup>	3.47 <sup>a</sup>	24.76 <sup>c</sup>	23.63 <sup>c</sup>	6.22 <sup>b</sup>	68.04 <sup>a</sup>	2.37 <sup>c</sup>	243.21 <sup>a</sup>	310.39 <sup>a</sup>	19.38 <sup>a</sup>
	<i>S. hispidum</i> <sup>#</sup>	2.70 <sup>a</sup>	7.80 <sup>b</sup>	3.47 <sup>a</sup>	33.66 <sup>b</sup>	32.21 <sup>b</sup>	12.89 <sup>a</sup>	46.26 <sup>a</sup>	21.20 <sup>a</sup>	253.53 <sup>a</sup>	302.90 <sup>a</sup>	15.36 <sup>b</sup>
	<i>S. pubescens</i> <sup>#</sup>	1.45 <sup>c</sup>	4.73 <sup>d</sup>	3.22 <sup>a</sup>	7.01 <sup>d</sup>	6.55 <sup>d</sup>	2.74 <sup>c</sup>	71.21 <sup>a</sup>	4.82 <sup>b</sup>	160.84 <sup>b</sup>	97.71 <sup>b</sup>	5.73 <sup>c</sup>

Means denoted by the same letters within the column are not significantly different at  $P < 0.05$ . CL=Cotyledon length, CW= Cotyledon width, CL/CW= Cotyledon length / cotyledon width ratio, LBL= Leaf blade length, LBW= Leaf blade width, LPL= Leaf petiole length, LBTA= Leaf blade tip angle, NLP= Number of leaf prickles, PIH= Plant height, MCW= Mean canopy width, NB= Number of branches per plant. <sup>\*\*\*</sup>= *Bandarawela*, <sup>#</sup>=*Welimada*

**Table 4. The variation of the reproductive morphology.**

Variable		NFPI	FD (cm)	PL (cm)	PW (cm)	LSE (cm)	AL (cm)	AW (cm)	DF	FL (cm)	FB (cm)	FL/FB	LS (cm)	TS (cm)	IFW (g)	HFW (g)	Yield (g)	SD (cm)	HSW (g)
Site	<i>Bandarawela</i>	28.14 <sup>a</sup>	3.54 <sup>a</sup>	1.27 <sup>a</sup>	0.77 <sup>a</sup>	9.83 <sup>a</sup>	8.29 <sup>a</sup>	2.44 <sup>a</sup>	124.74 <sup>a</sup>	0.93 <sup>b</sup>	1.22 <sup>a</sup>	0.45 <sup>b</sup>	1.61 <sup>a</sup>	0.42 <sup>a</sup>	1.32 <sup>a</sup>	107.85 <sup>a</sup>	60.35 <sup>c</sup>	0.23 <sup>a</sup>	0.16 <sup>a</sup>
	<i>Welimada</i>	25.97 <sup>a</sup>	3.52 <sup>a</sup>	1.29 <sup>a</sup>	0.73 <sup>a</sup>	8.50 <sup>b</sup>	7.63 <sup>b</sup>	2.49 <sup>a</sup>	119.70 <sup>b</sup>	1.38 <sup>a</sup>	1.22 <sup>a</sup>	1.50 <sup>b</sup>	1.60 <sup>a</sup>	0.43 <sup>a</sup>	1.21 <sup>a</sup>	110.15 <sup>a</sup>	74.73 <sup>c</sup>	0.23 <sup>a</sup>	0.16 <sup>a</sup>
Species/Variety	<i>S. torvum</i> 1	41.31 <sup>a</sup>	2.71 <sup>c</sup>	1.12 <sup>c</sup>	0.71 <sup>b</sup>	6.65 <sup>c</sup>	5.85 <sup>c</sup>	2.18 <sup>b</sup>	133.09 <sup>a</sup>	1.22 <sup>a</sup>	1.29 <sup>a</sup>	0.85 <sup>a</sup>	1.43 <sup>b</sup>	0.44 <sup>a</sup>	1.24 <sup>a</sup>	115.16 <sup>a</sup>	119.75 <sup>b</sup>	0.19 <sup>c</sup>	0.11 <sup>c</sup>
	<i>S. torvum</i> 2	41.00 <sup>a</sup>	2.68 <sup>c</sup>	1.10 <sup>c</sup>	0.69 <sup>b</sup>	6.70 <sup>c</sup>	5.79 <sup>c</sup>	2.33 <sup>b</sup>	125.10 <sup>b</sup>	1.20 <sup>a</sup>	1.27 <sup>a</sup>	0.82 <sup>a</sup>	1.46 <sup>b</sup>	0.47 <sup>a</sup>	1.22 <sup>a</sup>	120.34 <sup>a</sup>	242.23 <sup>a</sup>	0.19 <sup>c</sup>	0.11 <sup>c</sup>
	<i>S. hispidum</i>	25.50 <sup>b</sup>	4.24 <sup>b</sup>	1.31 <sup>b</sup>	0.72 <sup>b</sup>	11.36 <sup>b</sup>	9.83 <sup>b</sup>	2.43 <sup>b</sup>	108.51 <sup>d</sup>	1.28 <sup>a</sup>	1.25 <sup>a</sup>	0.96 <sup>a</sup>	1.54 <sup>b</sup>	0.46 <sup>a</sup>	1.36 <sup>a</sup>	122.69 <sup>a</sup>	50.05 <sup>c</sup>	0.31 <sup>a</sup>	0.29 <sup>a</sup>
	<i>S. pubescens</i>	12.37 <sup>c</sup>	5.06 <sup>a</sup>	1.66 <sup>a</sup>	0.91 <sup>a</sup>	13.80 <sup>a</sup>	12.02 <sup>a</sup>	3.01 <sup>a</sup>	122.78 <sup>c</sup>	1.06 <sup>b</sup>	1.04 <sup>b</sup>	0.97 <sup>a</sup>	2.06 <sup>a</sup>	0.34 <sup>b</sup>	0.69 <sup>b</sup>	68.81 <sup>b</sup>	14.01 <sup>d</sup>	0.22 <sup>b</sup>	0.17 <sup>b</sup>
	<i>S. torvum</i> 1 <sup>#</sup>	39.37 <sup>a</sup>	2.75 <sup>c</sup>	1.13 <sup>c</sup>	0.75 <sup>b</sup>	7.13 <sup>c</sup>	6.11 <sup>c</sup>	2.17 <sup>a</sup>	141.53 <sup>a</sup>	0.90 <sup>d</sup>	1.27 <sup>a</sup>	0.40 <sup>b</sup>	1.43 <sup>b</sup>	0.42 <sup>a</sup>	1.18 <sup>a</sup>	112.91 <sup>a</sup>	82.63 <sup>c</sup>	0.19 <sup>c</sup>	0.11 <sup>c</sup>
Site × species/variety	<i>S. torvum</i> 2 <sup>#</sup>	36.18 <sup>a</sup>	2.73 <sup>c</sup>	1.10 <sup>c</sup>	0.75 <sup>b</sup>	7.35 <sup>c</sup>	6.08 <sup>c</sup>	2.17 <sup>a</sup>	126.51 <sup>b</sup>	0.84 <sup>d</sup>	1.27 <sup>a</sup>	0.35 <sup>b</sup>	1.44 <sup>b</sup>	0.48 <sup>a</sup>	1.21 <sup>a</sup>	117.65 <sup>a</sup>	223.86 <sup>a</sup>	0.19 <sup>c</sup>	0.12 <sup>c</sup>
	<i>S. hispidum</i> <sup>#</sup>	27.38 <sup>b</sup>	4.23 <sup>b</sup>	1.29 <sup>b</sup>	0.71 <sup>b</sup>	12.35 <sup>a</sup>	10.10 <sup>b</sup>	2.41 <sup>a</sup>	109.19 <sup>c</sup>	1.08 <sup>c</sup>	1.27 <sup>a</sup>	0.51 <sup>b</sup>	1.57 <sup>b</sup>	0.45 <sup>a</sup>	1.36 <sup>a</sup>	117.73 <sup>a</sup>	49.31 <sup>c</sup>	0.31 <sup>a</sup>	0.27 <sup>a</sup>
	<i>S. pubescens</i> <sup>#</sup>	16.08 <sup>c</sup>	4.98 <sup>a</sup>	1.65 <sup>a</sup>	0.90 <sup>a</sup>	14.44 <sup>a</sup>	12.62 <sup>a</sup>	3.14 <sup>a</sup>	122.79 <sup>b</sup>	0.86 <sup>d</sup>	1.04 <sup>b</sup>	0.58 <sup>b</sup>	2.07 <sup>a</sup>	0.34 <sup>b</sup>	0.72 <sup>b</sup>	77.95 <sup>b</sup>	14.54 <sup>d</sup>	0.23 <sup>b</sup>	0.17 <sup>b</sup>
	<i>S. torvum</i> 1 <sup>#</sup>	43.35 <sup>a</sup>	2.67 <sup>c</sup>	1.12 <sup>c</sup>	0.67 <sup>b</sup>	6.20 <sup>d</sup>	5.61 <sup>c</sup>	2.18 <sup>a</sup>	124.92 <sup>b</sup>	1.43 <sup>a</sup>	1.27 <sup>a</sup>	1.47 <sup>a</sup>	1.44 <sup>b</sup>	0.45 <sup>a</sup>	1.19 <sup>a</sup>	117.37 <sup>a</sup>	173.54 <sup>a</sup>	0.19 <sup>c</sup>	0.11 <sup>c</sup>
	<i>S. torvum</i> 2 <sup>#</sup>	46.46 <sup>a</sup>	2.64 <sup>c</sup>	1.10 <sup>c</sup>	0.64 <sup>b</sup>	6.11 <sup>d</sup>	5.51 <sup>c</sup>	2.50 <sup>a</sup>	123.69 <sup>b</sup>	1.42 <sup>a</sup>	1.27 <sup>a</sup>	1.49 <sup>a</sup>	1.48 <sup>b</sup>	0.47 <sup>a</sup>	1.23 <sup>a</sup>	122.97 <sup>a</sup>	262.11 <sup>a</sup>	0.19 <sup>c</sup>	0.11 <sup>c</sup>
<i>S. hispidum</i> <sup>#</sup>	23.75 <sup>b</sup>	4.25 <sup>b</sup>	1.33 <sup>b</sup>	0.73 <sup>b</sup>	10.46 <sup>b</sup>	9.56 <sup>b</sup>	2.44 <sup>a</sup>	107.83 <sup>c</sup>	1.43 <sup>a</sup>	1.27 <sup>a</sup>	1.56 <sup>a</sup>	1.50 <sup>b</sup>	0.47 <sup>a</sup>	1.38 <sup>a</sup>	127.45 <sup>a</sup>	50.79 <sup>c</sup>	0.30 <sup>a</sup>	0.30 <sup>a</sup>	
<i>S. pubescens</i> <sup>#</sup>	9.51 <sup>d</sup>	5.14 <sup>a</sup>	1.68 <sup>a</sup>	0.91 <sup>a</sup>	13.18 <sup>a</sup>	11.45 <sup>a</sup>	2.89 <sup>a</sup>	122.78 <sup>b</sup>	1.02 <sup>b</sup>	1.03 <sup>b</sup>	1.46 <sup>a</sup>	2.10 <sup>a</sup>	0.35 <sup>b</sup>	0.64 <sup>b</sup>	58.26 <sup>b</sup>	13.50 <sup>d</sup>	0.22 <sup>b</sup>	0.17 <sup>b</sup>	

Means denoted by the same letters within the column are not significantly different at  $P < 0.05$ . NFPI= Number of flowers per inflorescence, FD= Flower diameter, PL= Petal length, PW= Petal width, LSE= Length of stigma exerted, AL= Length of anther, AW= Width of anther, DF= Days to flowering, FL= Fruit length, FB= Fruit breadth (Fruit diameter), FL/FB= Fruit length/fruit breadth ratio, LS= Length of stalk, TS= Thickness of stalk, IFW= Individual fruit weight, HFW= 100 fruit weight, SD= Seed diameter, HSW= 100 seed weight. <sup>\*\*\*</sup>= *Bandarawela*, <sup>#</sup>=*Welimada*

**Table 5. Variability of the qualitative vegetative parameters.**

Species/ Variety	Cotyledon	Plant	Leaves			Stem	
	Colour	Growth habit	Blade colour	Blade tip Lobe pattern	Leaf blade	Presence of hairs and prickles	Colour
<i>S. torvum 1</i>	Green	Upright	Moderate olive green	Strong	Acute	Hairs with prickles	Yellowish grey
<i>S. torvum 2</i>	Green	Upright	Moderate olive green	Strong	Acute	Hairs with prickles	Yellowish grey
<i>S. hispidum</i>	Light violet	Intermediate	Greyish olive green	Strong	Acute	Hairs with prickles	Light olive grey
<i>S. pubescens</i>	Light violet	Upright	Greyish olive green	Intermediate	Acute	Prickles only	Moderately olive green

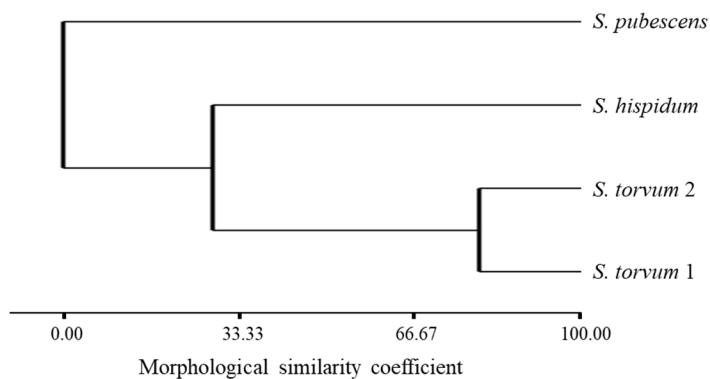
**Table 6. Variability of floral, seed and fruit characteristics.**

Species/variety	Flower				Seed				Fruit						
	Type of inflorescence	Corolla colour	Stigma position	Prickles in pedicel	Colour	Apex shape	Fruit flesh density	Relative fruit calyx length	Petiole colour	Position	Colour	Curvature	Cross section	Locules	Prickles
<i>S. torvum 1</i>	Compound dichasium	Greyish white	Included /exerted	None	Pale yellow	Rounded	Average dense	Short	Moderate yellow green	Semi erect	Strong yellow green	Straight	Circular	No grooves	None
<i>S. torvum 2</i>	Compound dichasium	Greyish white	Included /exerted	None	Moderate yellow	Rounded	Average dense	Short	Moderate yellow green	Semi erect	Strong yellow green	Straight	Circular	No grooves	None
<i>S. hispidum</i>	Compound dichasium	White	exerted	None	Moderate yellow	Protruded	Dense	Short	Strong yellow green	Semi erect	Strong yellow green	Straight	Circular	No grooves	None
<i>S. pubescens</i>	Helicoid cyme	Strong purple greyish white	exerted	None	Light yellow	Rounded	Average dense	Long	Strong yellow green	Semi erect	Strong yellow green	Straight	Circular	No grooves	None

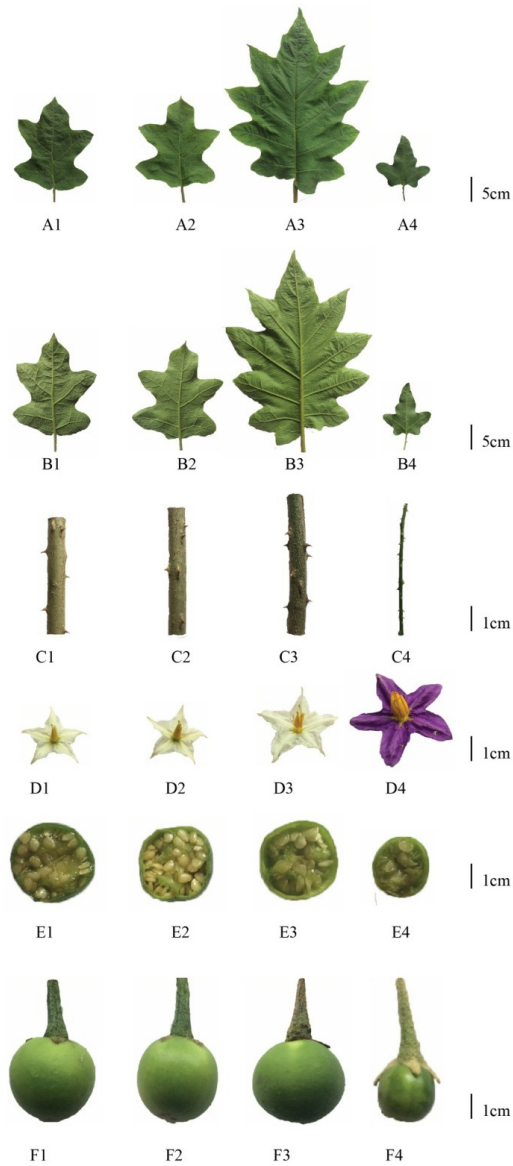
The stem color at the maturity stage of *S. torvum* was yellowish green, and that of the *S. pubescens* and *S. hispidum* were light olive grey and moderate olive grey respectively. Stems of the trees were moreover assessed for the presence of hairs and prickles (Plate 1 C). Accordingly, *S. torvum* and *S. hispidum* were found to have hairs and prickles on the stem while the stems of *S. pubescens* only possessed the prickles (Table 5). All the species possessed flowers in clusters. *S. torvum* and *S. hispidum* possessed inflorescence with compound dichasium whereas, the type of inflorescence of *S. hispidum* was helicoid cyme. The color of the corolla was also variable among the species. *S. torvum* had flowers with greyish white whereas those of the *S. hispidum* and *S. pubescens* were white and strong purple respectively. The position of the stigma in *S. torvum* was either included/exserted while those of the *S. pubescens* and *S. hispidum* were found to be exserted. However, none of the flowers possessed prickles in their pedicels (Plate 1 D). Moreover, the color of the seeds was found to be of variable yellows among the species (Table 6). It was also noted that most of the fruit characters were not variable among the three species revealing their less importance in discriminating of species. All the *Solanum* species possessed strong yellow-green, semi-erect fruits with a straight curvature, circular cross-section, no grooved locules and no prickles in fruit calyx. Nevertheless, the fruits of *S. torvum* and *S. pubescens* possessed a round apex and average dense fruit flesh while those of the *S. hispidum* possessed a protruded apex and dense fruit flesh. The relative length of the fruit calyx of *S. torvum* and *S. hispidum* was short whereas that of the *S. pubescens* was comparatively long. Furthermore, the color of the petiole at the maturity stage in *S. torvum* species was moderately yellowish green while those of the *S. pubescens* and *S. hispidum* were found to have a strong yellow-green (Table 6) (Plate 1 E and F). Qualitative traits however, played a key role in differentiating the three species thus providing a better way to develop easily utilizable morphometric markers for species identification within the same genera. Although a variation was detected, it is relatively difficult to distinguish the species based on the quantitative traits, as compared to qualitative morphological traits. Our results are in conformity with the previous findings in Yousaf *et al.*, (2010).

### Cluster analysis of quantitative parameters

The dendrogram constructed considering both vegetative and reproductive characteristics showed a clear separation between three different *Solanum* spp. *S. torvum* 1 and *S. torvum* 2 which belong to *S. torvum* were clustered together at 79.78 % similarity level. *S. pubescens* was highly diverse from the other varieties based on the morphological characteristics (Figure 1).

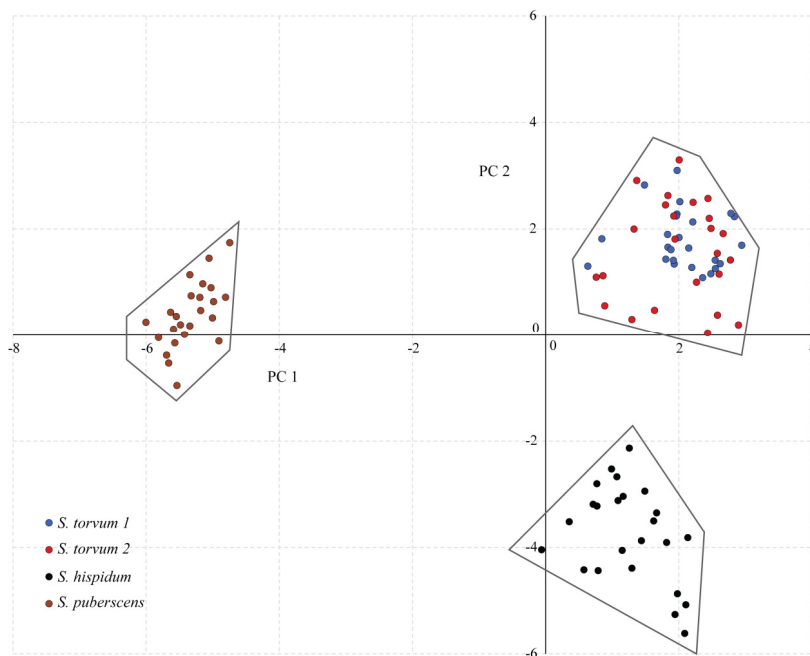


**Figure 1. Dendrogram obtained for *Solanum* spp. based on both vegetative and reproductive characters. The X-axis shows the level of similarity.**



**Plate 1. Variability of vegetative (A, B, C) and reproductive traits (D, E, F) among three species of *Solanum*. (1: *S. torvum* var. *Bindu*; 2: *S. torvum* strain Landrace; 3: *S. hispidum*; 4: *S. pubescens*) The characteristics A and B represent abaxial and adaxial surface of leaf lamina, and C, D, E, F represent stem cuttings, flowers, cross sections of fruits and whole fruits, respectively.**

The PCA yielded 29 components corresponding to 29 morphometric parameters including 11 vegetative and 18 reproductive traits (Figure 2). A total variance of 98.2% was obtained collectively for the first two components of the PCA performed where PC1 and PC2 accounted for 67.8% and 30.4% respectively. The third component produced a variance of 1.9% which did not improve the outcomes of the study and all the other components were uninformative. Therefore, third and all the other components were not considered. In the PCA scatter plot, the four species/varieties were divided into three distinct groups as *S. hispidum* (black), *S. pubescens* (brown) and *S. torvum* (red and blue) where the *S. torvum*; Bindu and *S. torvum*; Landrace were overlapped with no distinct separation. The extensive overlap of the two *S. torvum* varieties indicates that the morphological data cannot provide discrimination between the two varieties.

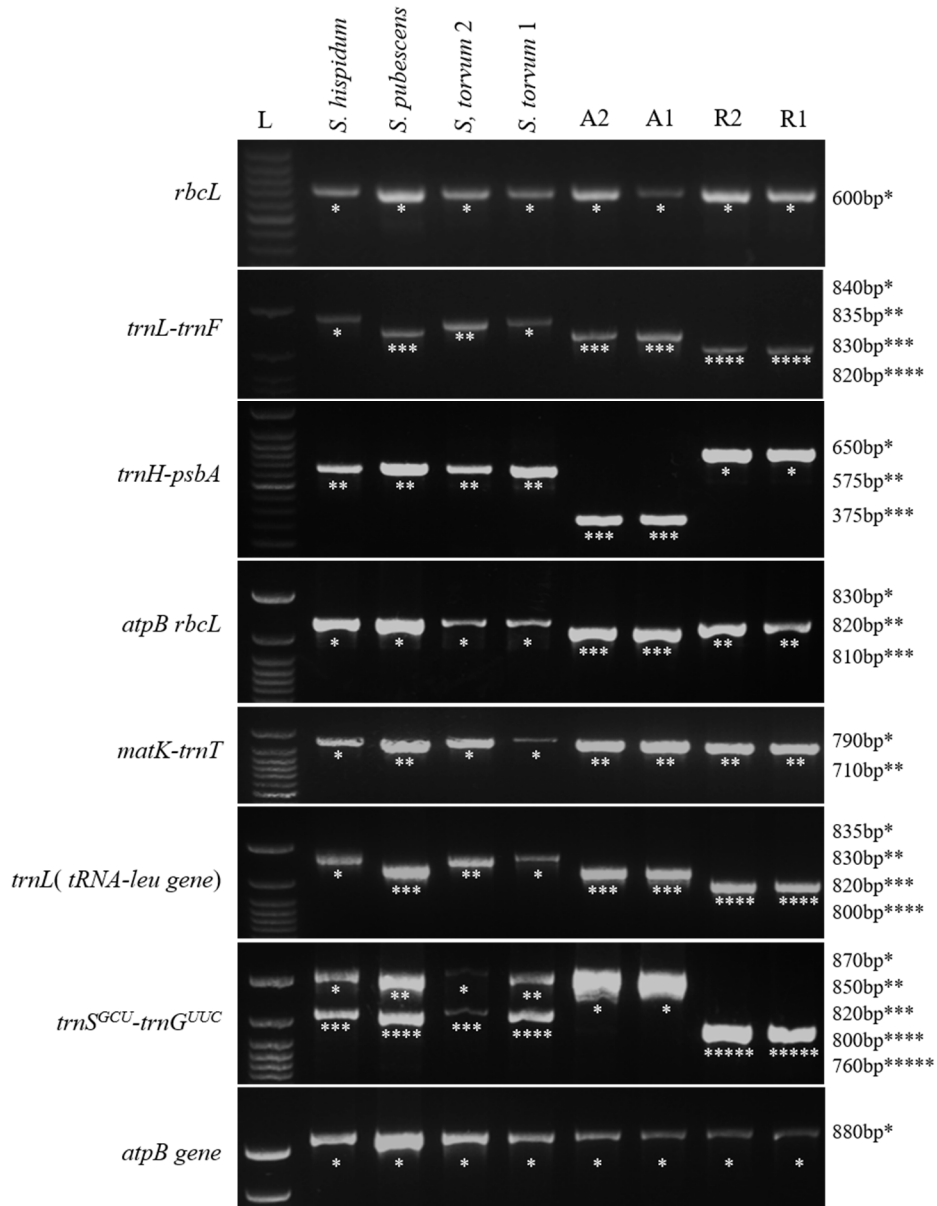


**Figure 2.** Scatter plot for *Solanum* species/varieties based on both reproductive and vegetative parameters using Minitab 17 (Minitab Inc. USA). Three distinct groups were obtained for *S. pubescens*, *S. hispidum* and *S. torvum* where the two varieties of *S. torvum* were overlapped revealing that the morphological parameters employed in the present study were not utilizable for a better discrimination between *S. torvum* varieties.

### Assessing the DNA length polymorphism

The DNA bands resulted a polymorphic banding pattern for four markers namely, *trnL*, *trnS<sup>GCU</sup>-trnG<sup>UUC</sup>*, *matK-trnT* and *trnL-trnF*. Considering the aforementioned loci, *S. pubescens* indicated a banding pattern which is clearly distinguishable from the other varieties indicating the genetic variability of *S. pubescens*. All the species produced monomorphic bands for *atp*

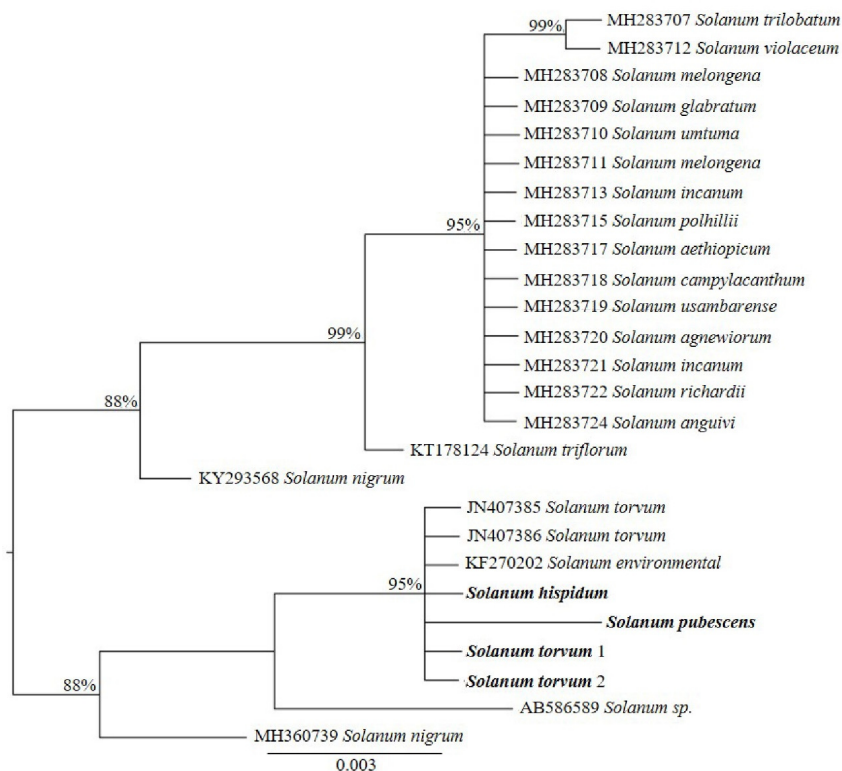
*B* gene, *atpB-rbcL*, *trnH-psbA* and *rbcL* (Plate 2). A total of 16 alleles were detected for eight loci. Thereby an average of two alleles per loci was identified.



**Plate 2.** The polymorphic bands detected for the DNA barcoding loci (*Solanum torvum*; Bindu, *S. torvum*; Landrace, *S. hispidum*, *S. pubescens*), PCR amplified with eight markers; *rbcL*, *trnL-trnF*, *trnH-psbA*, *atpB-rbcL*, *matK-trnT*, *trnL (tRNA-leu gene)*, *trnS<sup>GCU</sup>-trnG<sup>UUC</sup>* and *atpB gene* obtained by 2.5% agarose gel electrophoresis carried out alongside 50bp ladder (L). The relative sizes of each PCR product is denoted by \* mark. The increasing order of the band size is designated by increasing the number of \* marks.

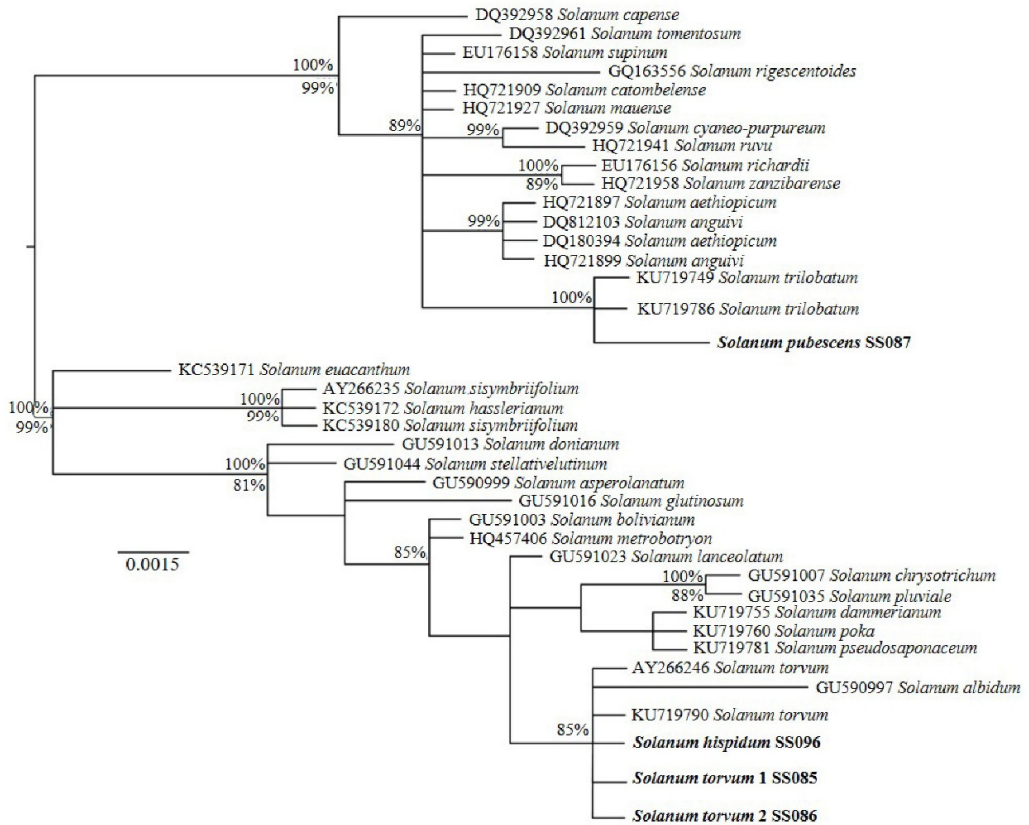
### Assessing the DNA sequence polymorphism

Out of the two (*rbcL* and *trnL-trnF*) DNA barcoding markers employed in this study, there were only one SNP detected in *trnL-trnF* marker for all four species/varieties. Among the individuals of nightshades, a less polymorphism was observed in *rbcL* gene region, thus a shallow separation was obtained in the phylogeny. The 50% majority rule consensus tree drawn in the Bayesian framework had low divergence between each sequences. The ML tree constructed for *rbcL* marker had many polytomies and low support value (Figure 3) thus the employment of *rbcL* marker would not be suitable for species/varietal discrimination. However, both the phylogenies (ML consensus tree and the Bayesian 50% majority rule consensus tree) constructed for *trnL-trnF* marker had congruence in the branching pattern with a higher node support (Figure 4). The branching pattern in the *trnL-trnF* phylogeny is mostly similar to previously published phylogenies where the *S. torvum* and *S. pubescens* were claded as sister groups (Ranaweera *et al.*, 2018; Särkinen *et al.*, 2013). Thus the *trnL-trnF* could be used as candidate marker for phylogenetic studies. It is essential to combine the *trnL-trnF* sequence data with other potential markers such as *trnH-psbA* and *ITS* to obtain a robust phylogeny. The generated sequences were submitted to GenBank under the Accession numbers MK122636-MK122643.



**Figure 3. The mid-point rooted 50% majority rule consensus tree drawn in the Bayesian criteria for *rbcL* marker. The nodes having posterior probabilities (pp) > 85 given above the node and the bootstrap values (bs) are given below the nodes. The sequences obtained in this study are given in bold letters. The scale bar represents the number of substitution per site.**





**Figure 4.** The 50% majority rule consensus tree drawn in the Bayesian criteria for *trnL-trnF* marker. The nodes having posterior probabilities (Figpp) > 85 given above the node and the bootstrap values (bs) are given below the nodes. The sequence obtained in this study are given in bold letters. The scale bar represents the number of substitution per site.

## CONCLUSIONS

The analyses of morphological diversity of *Solanum* spp. revealed that the species identification can be successfully accomplished by considering both the vegetative and reproductive morphological parameters together. There was a less sequence polymorphism observed in *rbcL* coding region, thus could not be used as a marker for characterization of *Solanum* spp. The *trnL-trnF* marker provides the required separation for genus *Solanum* and it separates *S. pubescens* from *S. hispidum* and *S. torvum*.

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