MORPHOLOGICAL CHARACTERIZATION OF BRINJAL (*Solanum melongena* L.) CULTIVARS IN SRI LANKA AND PRELIMINARY MOLECULAR EVALUATION FOR BACTERIAL WILT RESISTANCE

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Abstract

Brinjal (*Solanum melongena* L.) is the second most economically important solanaceous crop in the world. Brinjals are low in calories but rich nutritional factors, antioxidants, vitamins and minerals. Wild as well as genetically modified brinjal cultivars are available in Sri Lanka. Information on available local and imported brinjal cultivars can aid in brinjal breeding programmes and help farmers select suitable cultivars for planting. The study assessed morphological diversity in selected brinjal cultivars in Sri Lanka using plant growth and leaf characteristics of 1- month and 6-month-old seedlings. Seeds of nine brinjal cultivars (*Padagoda, Raveena 135, Lucky green, Plastic batu, Lena iri batu, Deshiya Ela batu, Thalana batu, Thith batu and Hybrid bride*) were obtained from the Horticultural Crops Research Development Institute (HORDI), Department of Agriculture, Sri Lanka and characterization was carried out in a plant house at the Open University of Sri Lanka, Nawala. A total of 25 plants from each cultivar (arranged in Randomized Complete Block Design [RCBD]) was subjected to characterization following the minimum descriptors (plant and fruit) for eggplant published by the International Board for Plant Genetic Resources. The data was analyzed using descriptive and inferential statistical methods including ANOVA, Cluster Analysis and a PCA using SPSS ver.20.0.

The study reveals a wide range of variation in morphological characteristics among brinjal cultivars in Sri Lanka. ANOVA results showed statistically significant differences ($p \leq 0.05$) in plant height, petiole length, leaf number, growth habit, leaf blade length and width, leaf blade lobing, and leaf prickles. According to the dendrogram obtained from tree cluster analysis, nine brinjal cultivars were grouped into eight clusters, indicating significant variation in plant height and leaf morphologies. Leaf blade width, leaf blade color, leaf blade length, and tip angle can be used to classify these cultivars as the tree cluster diagram indicates. Further, correlation matrix of Principle component analysis indicated negative correlation between growth habit and leaf blade characters.

A preliminary molecular evaluation for bacterial wilt resistance using five specific primers was carried out for five different brinjal cultivars which showed varying banding patterns among the cultivars. Further analyses with more primers are required to confirm the extent of bacterial wilt resistant among brinjal cultivars.

Keywords: Brinjal, *Solanum melongena* L., morphological characterization, bacterial wilt

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Introduction

Brinjal (*Solanum melongena* L.) is an agronomically important, non-tuberous crop of Solanaceae family which is native to Southern India, and widely grown in America, Europe and Asia. It is the second most economically important solanaceous fruit crop after tomato, with a total annual production of approximately 51 Mt (Aubriot *et al*., 2018). Simplicity in cultivation, year-round availability, considerably high yield and utilization in many ways as a vegetable, salad, chutney, pickles etc., has made brinjal the king of vegetables among Asians (Priyanka *et al*., 2018). It is also one of the most important and highly marketable vegetable crops grown in Sri Lanka (DOA, 2022).

Brinjal has a very low caloric value and is considered among the healthiest vegetables for its high content of vitamins, minerals and bioactive compounds for human health (Plazas *et al*., 2014; Docimo *et al*., 2016). It is an important source of fibre, potassium, manganese as well as vitamins C, K and B6. The bioactive properties of brinjal are mostly associated with a high content in phenolic compounds (Plazas *et al*., 2014) and serves as one of the most powerful free radical scavengers found in plants and is an antimicrobial, antiviral and anti-carcinogenic agent. Successful cultivation of brinjal crop has been hindered due to infestation of many insect pests and diseases. Bacterial wilt disease is the most devastating disease throughout the tropical, sub-tropical and temperate regions of the world (Hayward, 1991; Gopalakrishnan, 2014). Bacterial wilt in brinjal is caused by *Ralstonia solanacearum* and it is the most important limiting factor for brinjal cultivation in Sri Lanka. Bacterial wilt is an important disease of many plant species, especially, in Solanaceae (Liu *et al*., 2016) crops causing enormous economic losses, which limits eggplant production from 4.24% to 86.14% (Sabita *et al*., 2000). *R. solanacearum* perpetuates in the soil, enters the plant through the roots, progressively invades the stem vascular tissues and blocks the vessels, and finally leads to partial or complete wilting of the plant.

Farmers need improved brinjal cultivars for sustainable production and adaptation to climate change challenges. In general, brinjal breeding programmes aim to develop high-yielding varieties; mostly, F1 hybrids, with higher fruit quality, longer shelf-life and resistance to major disease and insect pests and broader adaptation to environmental stresses (Daunay and Hazra, 2012).

The Department of Agriculture (DOA) in Sri Lanka has recommended several brinjal cultivars including selections and hybrids. In addition, seeds of genetically improved exotic hybrids are also imported and available for farmers and are grown in considerable amounts. However, the agro-morphological traits, especially of the imported cultivars, are not systematically studied and no comparative studies of them with DOA-recommended cultivars have been undertaken in Sri Lanka. Accordingly, farmers do not have sufficient information in selecting suitable cultivars to be planted. In addition to agro-morphological traits, comparative genetic information of the locally available varieties and the exotic varieties is also deficient. Though extensive studies with respect to agro-morphological traits are carried out in the cultivars recommended by DOA prior to recommendation, such information is

critically lacking with respect to imported cultivars. If available, such information would provide useful information for farming community in the selection of suitable cultivars for planting and in plant breeding programmes for parental selection. As importation of exotic planting material of brinjal is undertaken by the private sector, there is an urgent need of the above information in decision making within a short period of time on the varieties for importation. In addition, the farmers need such information on decision making in selecting cultivars for planting and utilizing in plant breeding programmes.

Accordingly, the present study was carried out to evaluate the morphological diversity on plant growth and leaf characteristics of brinjal cultivars in Sri Lanka. Further, a preliminary molecular evaluation was carried out to identify brinjal cultivars with bacterial wilt resistance.

Materials and methods

The seeds of nine (09) brinjal cultivars (*Padagoda*, *Raveena 135*, *Lucky green*, *Plastic batu*, *Lena iri batu*, *Deshiya Ela batu*, *Thalana batu*, *Thith batu* and *Hybrid bride*) were evaluated in the study.

Morphological characterization was carried out in a plant house at the OUSL, Nawala. A total of 25 plants from each cultivar (arranged in Randomized Complete Block Design [RCBD]) at one month and six-month-old brinjal plants were subjected to characterization following the minimum descriptors (plant and fruit) for eggplant published by the IBPGR in 1990 (Table 1).

Table 1: Morphological characters (IBPGR, 1990) used to characterize brinjal cultivars

Character code	Character	Levels of character (if any)
1	Plant height	Measured in cm
2	Petiole length	Measured in cm
3	Petiole color	1-green, 3-greenish violet, 5-violet, 7-dark violet,9-dark brown
4	Leaf blade color	1-light green, 3-green, 5-dark green, 7-greenish violet,9-violet
5	Leaf blade length	Measured in cm
6	Number of leaves	counted
7	Plant growth habit	3-upright,5-intermediate, 7-prostrate
8	Leaf blade width	Measured in cm
9	Leaf blade lobing	1-very weak, 3-weak, 5-intermediate,7-strong, 9-very strong
10	Leaf blade tip angle	1-very acute,3-acute,5-intermediate,7-obtuse,9-very obtuse
11	Leaf prickles	0-none, 1-very few, 3-few, 5-intermediate, 7-many, 9-very many
12	Leaf hairs	1-very few, 3-few, 5-intermediate, 7-many, 9-very many

Statistical analyses were carried out using SPSS.ver.20.0. Analysis of variance (ANOVA) and a tree cluster analysis was performed.

A preliminary molecular evaluation was carried out to test the bacterial wilt resistance of five (05) selected brinjal varieties, *Thithbatu*, *Lena iri*, *Plastic batu*, *Padagoda* and *Raveena 135* against bacterial wilt caused by *Ralstonia solanacearum*. DNA was extracted using CTAB method and Polymerase chain reaction was carried out using five specific SSR primers for bacterial wilt resistance: 3F-KIM, AF, 1F, IR-KIM and 4r (Prohens *et al*, 2005). Then, gel electrophoresis was performed to analyze and elucidate the banding patterns.

Results

Descriptive analysis

The data were first subjected to descriptive analysis where means for each character were compared (data not shown) and graphed (graphs not shown) to observe the variation between the studied cultivars.

Thith batu showed the highest value for the mean height of one-month old seedlings whereas the lowest values were with *Raveena 135* and *Plastic batu*. The trait leaf blade colour was comparatively different in *Thith batu,* compared to rest of the cultivars. Mean petiole length was also the highest in *Thith batu* and the least was found in *Thalana balu*. The highest number of leaves were found in *Thith batu* and the lowest was found in *Plastic Batu*. The highest mean leaf blade length and width were recorded in *Thith batu* and the lowest was recorded in *Plastic Batu*. *Thith batu* and *Deshiya Ela batu* showed a prominent leaf lobing than other cultivars. Descriptives in 6 months (not shown), plant height was highest in *Thith batu* and lowest in *Lena iri.* The highest mean leaf blade length was recorded in *Thith batu.* The Leaf blade colour, number of leaves and growth habit showed no difference among cultivars.

Inferential statistical analysis

One way ANOVA of the morphological characters and cultivars was carried out to determine if there were any significant differences ($p <= 0.05$) in morphological characters among cultivars (Table 2). According to the ANOVA results, plant height, petiole length, number of leaves, growth habit, leaf blade length, leaf blade width, leaf lobing and leaf prickles were significantly different among the studied brinjal cultivars.

Table 2: ANOVA of the morphological characters in one month old brinjal cultivars

Character	Sums of squares	df	Mean squares	F	Sig.
Plant height * Cultivar	130.229	8	16.279	17.948	.000
Petiole length * Cultivar	5.561	8	.695	2.701	.007
No. of Leaves * Cultivar	57.636	8	7.204	11.077	.000
Growth Habit * Cultivar	1.636	8	.204	2.464	.014
Leaf blade length * Cultivar	33.522	8	4.190	13.295	.000
Leaf blade width * Cultivar	19.283	8	2.410	12.614	.000
Leaf Blade Lobing * Cultivar	106.809	8	13.351	22.700	.000
Leaf prickles * Cultivar	76.169	8	9.521	8.768	.000

A tree-cluster analysis was also performed (Figure 1), and it exhibited that leaf blade width, leaf blade colour, leaf blade length and tip angle can be used to classify these brinjal cultivars.

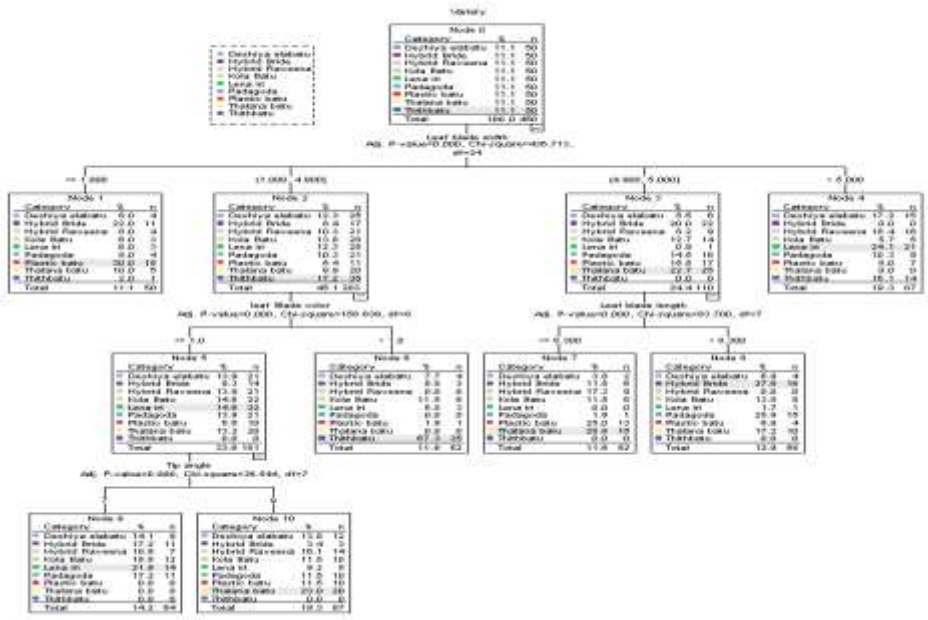

Figure 1: Tree cluster diagram

A dendogram was constructed (Figure 2), and based on the morphological characters, nine (09) cultivars were grouped into eight (08) at 20% phenon level.

Table 3: Corrolation matrix of Principle component analysis

Correlation Matrix

		Leaf prickels	Leaf Blade Lobing	Leaf blade length	Leaf blade width	No. of Leaves	Growth Habit	leaf Blade color	Petiole length	Plant Height	Petiole length
Correlation	Leaf prickels	1.000	.227	.309	.303	.220	-.097	.294	.	.286	.277
	Leaf Blade Lobing	.227	1.000	.615	.607	.428	-.019	.616	.	.596	.552
	Leaf blade length	.309	.615	1.000	.982	.701	-.096	.910	.	.969	.908
	Leaf blade width	.303	.607	.982	1.000	.687	-.088	.901	.	.948	.906
	No. of Leaves	.220	.428	.701	.687	1.000	-.004	.658	.	.700	.627
	Growth Habit	-.097	-.019	-.096	-.088	-.004	1.000	.009	.	-.102	-.096

	leaf Blade color	.294	.616	.910	.901	.658	.009	1.000	.	.893	.825
	Petiole length	.	.	.	.	.	.	.	1.000	.	.
	Plant Height	.286	.596	.969	.948	.700	-.102	.893	.	1.000	.883
	Petiole length	.277	.552	.908	.906	.627	-.096	.825	.	.883	1.000

Molecular evaluation for bacterial wilt resistance

Agarose gel electrophoresis results showed different cultivars showing varied banding patterns. The cultivar *Raveena-135* showed banding patterns with four primers out of five, whereas, the cultivar *Padagoda* did not show any banding pattern. The cultivars *Thith batu*, *Lena Iri* and *Plastic Batu* showed band patters with either one or two primers.

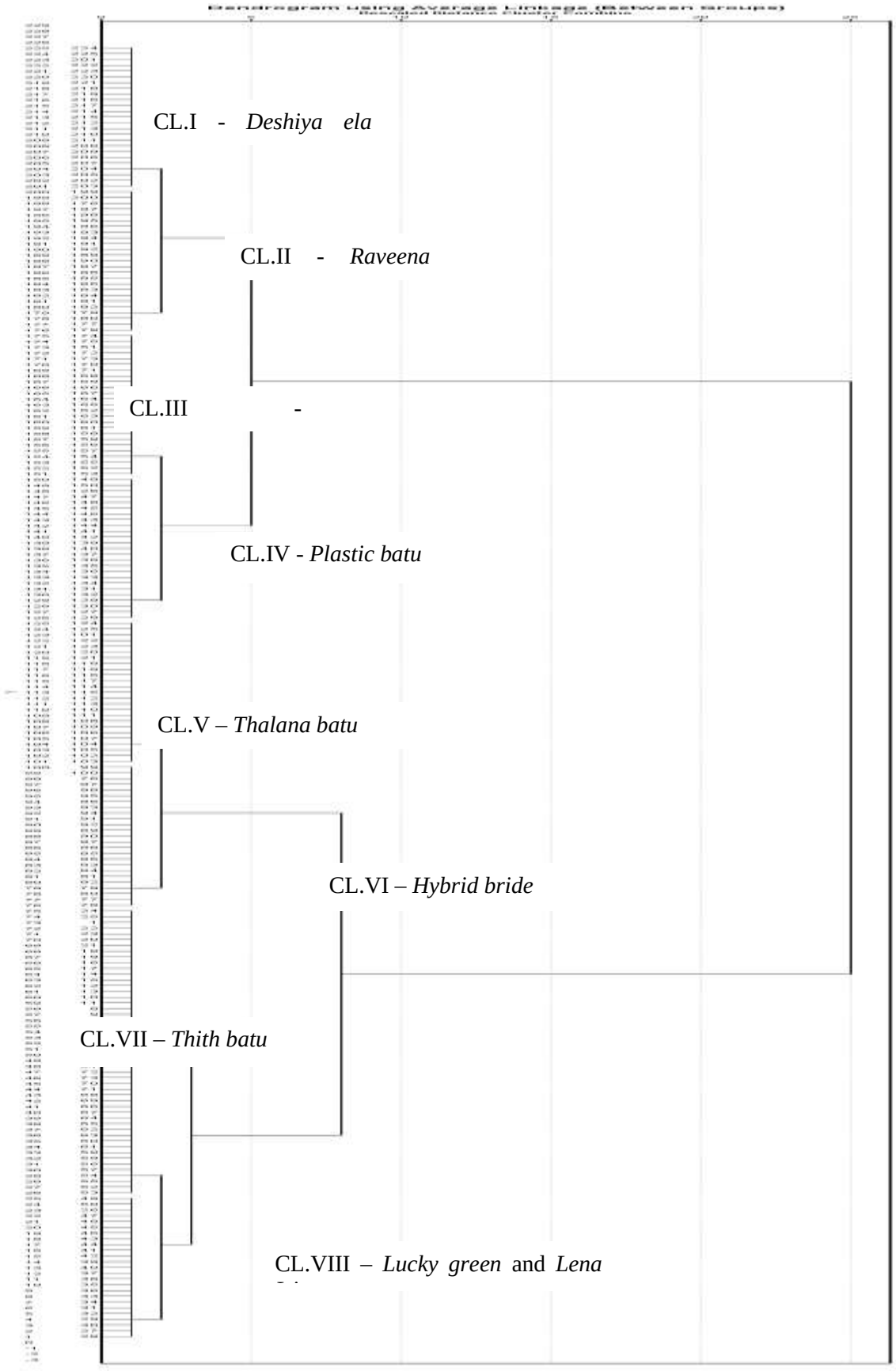

Figure 2: Dendrogram of the hierarchical classification of brinjal cultivars

Discussion

ANOVA revealed that there is a significant difference between the brinjal cultivars based on eight (08) morphological characters: Plant height, petiole length, number of leaves, growth habit, leaf blade length and width, leaf lobing and leaf prickles. Descriptive statistics showed that *Thith batu* cultivar had more vigorous morphological characters such as higher plant height, longer leaf blades and more prickles on leaves than other cultivars. A dendrogram was constructed based on tree cluster analysis and the nine (09) brinjal cultivars were grouped into eight (08) clusters; CL.I- *Deshiya Ela batu*, CL.II – *Raveena 135*, CL.III - *Padagoda*, CL.IV - *Plastic batu*, CL.V – *Thalana batu*, CL.VI – *Hybrid bride*, CL.VII – *Tith batu* and CL.VIII – *Lucky green* and *Lena Iri batu*. Tree-cluster analysis further proved that leaf blade width, leaf blade colour, leaf blade length and tip angle can be used to classify these brinjal cultivars. The correlation matrix of the Principle component Analysis (table 3) negative corrolations between plant growth habit and petiole length, leaf prickles, plant height, leaf blade length and leaf blade width. Results obtained in this study also proved previous studies of Dash *et al*., 2019 and Kumar *et al*.,2008.

Molecular evaluation with five specific primers for bacterial wilt resistance showed different brinjal cultivars showing a varied banding pattern.

Conclusion

Brinjal cultivars in Sri Lanka display considerable variation in plant height and leaf morphologies. Coupled with yield assessments, the findings will be useful for farmers in selecting varieties for cultivation among the locally improved and imported brinjal cultivars. In addition to morphological data, yield parameters and molecular studies are important in characterization of brinjal cultivars and confirmation of present results. Molecular evaluation with five specific primers for bacterial wilt showed different brinjal cultivars showing a varied banding pattern. Further analyses with more primers are required to confirm the extent of bacterial wilt resistant among brinjal cultivars.

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