

Morphological Characterization and Assessment of Selected Biochemical Properties of *Solanum Insanum* L.: Screening of Local Plant Genetic Resources for Crop Improvement

S.M.P.H. Samarakoon¹, R.H.G. Ranil^{1*}, R.M. Fonseka¹, T. Sivarnanthawerl¹, H. Fonseka², S.M.A.C. Senarathna³, K.H. Sarananda³, D.M.B.G.A.I.K. Dissanayaka², H.M.V.T. Welegama² and H.M.L. Niran⁴

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ABSTRACT

Purpose : *Solanum insanum* L. is remaining as an underutilized crop due to little evidence from scientific studies on its medicinal and nutritional values. Lack of such basic information on the plant obscured the in-depth biochemical or molecular studies as well as its uses in plant breeding. The study was aimed to identify the morphological variations of *S. insanum* and its selected biochemical properties.

Research Method : Characterization of 71 accessions collected from different agro-ecological regions was carried out following the descriptor developed by the IBPGR and antioxidant activity, total phenolic content and browning of the fruits were also measured at the level of 5% probability.

Findings : The results revealed that there were two distinct groups of *S. insanum* accessions, mainly based on prickliness of the plants. Non prickly plants showed $20.30 \pm 0.52\%$ radical scavenging activity (DPPH), 51.98 ± 0.16 mg/100 g fruit weigh (FW) of total phenolic content while prickly plants with $7.10 \pm 0.59\%$ radical scavenging activity (DPPH) and 50.62 ± 0.09 mg /100 g FW of total phenolic content in their fruits at the edible maturity. In terms of browning, time taken for color development up to “Grayed orange group 165 b” was 380 ± 10 seconds in fruits of non-prickly plants while 450 ± 17.32 seconds for fruits of prickly plants.

Research limitations : Due to the out-crossing nature of the plant, it is difficult to find typical *S. insanum* plant either from wild or cultivated lands.

Originality/Value : Characterization and evaluation of different accessions of *S. insanum* via morphological characteristics is a prime need before carrying out comprehensive biochemical or molecular studies. It provides fundamental basis for the classification based on vegetative, inflorescence and fruiting characteristics. Accordingly, the results will provide baseline information on *S. insanum* paving way to select superior germplasm for the taxonomists, breeders and those who are engaged in studies on *S. insanum*.

Keywords: *Solanum insanum*, characterization, prickled plants, antioxidant activity, total phenolic content.

INTRODUCTION

Solanum insanum L. (subgenus *Leptostemonum* Bitter, Solanaceae), is the neglected wild progenitor of cultivated eggplant (*Solanum*

^{1*}Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Sri Lanka. rhgranil@gmail.com

²Horticultural Crop Research and Development Institute, Gannoruwa, Peradeniya, Sri Lanka

³Food Research Unit, Department of Agriculture, Gannoruwa, Peradeniya, Sri Lanka.

⁴Agriculture Research Station, Girandurukotte, Sri Lanka.

melongena L.) and widespread in tropical Asia from Madagascar to the Philippines (Syfert *et al.*, 2016). Though *S. insanum* has a high potential for use in eggplant breeding, it still remains as an underutilized crop and not properly studied (Ranil *et al.*, 2016). However, few researches conducted in India revealed that, *S. insanum* presents significant morphological and genetic variation, particularly in regions of Central and Southern India (Deb, 1979; Karihaloo & Rai, 1995; Davidar *et al.*, 2015, Mutegi *et al.*, 2015). The most updated taxonomic information are given by Vorontsova and Knapp (2016) for *S. insanum* which were mainly confined to the Madagascar, but not for *S. insanum* in Asia. The most comprehensive account on genetic diversity and population structure of *S. insanum* in South India was given by Mutegi *et al.*, (2015) whereas Aubriot *et al.*, (2016) provides useful information on the evolutionary history of the spiny solanums including *S. insanum*, native to tropical Asia, the region encompassing Western India to New Guinea. Ranil *et al.*, (2016) has compiled all information of *S. insanum* while highlighting its potential for eggplant breeding.

Even though *S. insanum* is considered as the wild progenitor of cultivated eggplant (*S. melongena*), its important and potential characters have not adequately used in eggplant breeding. Two recent studies conducted in Southern India reveal the potential of *S. insanum* for hybridization with *S. melongena* and highlight the genetic variation of *S. insanum* and their implications for conservation, respectively (Mutegi *et al.*, 2015; Davidar *et al.*, 2015). Such studies will provide baseline information to select superior germplasm to improve and popularize eggplant among farmers. Moreover, *S. insanum* L. is a drought tolerant species and resistant to most of the insect-pest and pathogens (personal communication with traditional farmers). Wild relatives of eggplant grow in diverse climates viz; desert areas and in environments with a wide range of temperatures, waterlogged and swampy areas (Daunay & Hazra, 2012). Such characteristics are important in developing

new varieties adapted to the changing climate. Furthermore, crop wild relatives play an important role with respect to the food and nutritional security especially in the developing countries (Vincent *et al.*, 2013; Syfert *et al.*, 2016). Hence, there is a high potential lying with this plant for usage in culinary preparations apart from its reputation for curing diseases like diarrhea, coughs, rheumatism and lung ailments (Marasinghe *et al.*, 2016). *Solanum insanum* can be found either as a home-garden crop or in wild in many climatic regions of Sri Lanka comprising variable characters due to its high cross pollinating nature. Hence, the vulnerability of dwindling the true *S. insanum* plant population from its natural habitats is apparent. Therefore, it is vital to study on its genetic variation to screen the characteristics which can be utilized in future crop improvement programs. The lack of studies done on chemical and pharmacognostical properties have also marginalized the plant's usage and commercialization and therefore, the crop still remains as underutilized and one of the poor man's favorites. Studies on morphological and genetic diversity are essential to promote its utilization in eggplant breeding due to its potential compatibility to successfully hybridize with cultivated eggplant.

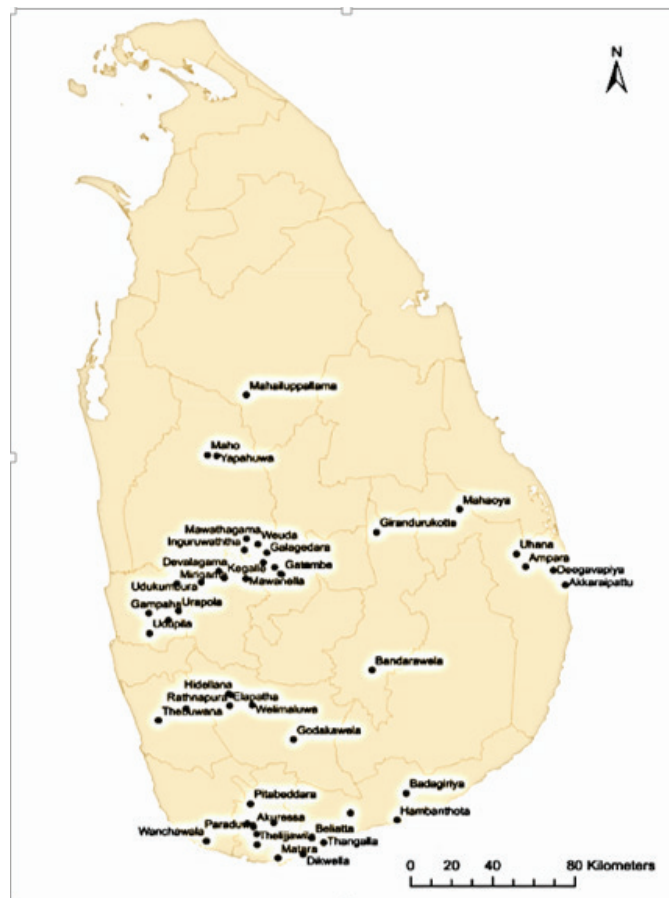
MATERIALS AND METHODS

Field assessment

Location: The experiment was carried out in selected agro-ecological regions which represent dry, intermediate and wet zones of Sri Lanka. Selected agro-ecological regions and sampling locations are given in Table 01 and Map 01, respectively. Samples were collected following standard sampling techniques. The Number of samples collected varied from 2-6 from each agro-ecological region based on the information gathered from farmers and agriculture officers. Approximately, a 10 Kilometers distance was maintained between two sampling points.

Table 01: Sampling locations of three climatic zones of Sri Lanka

Climatic zone	Agro-ecological regions	No. of samples
Wet zone	WM2b	05
	WM3b	02
	WL1a	04
	WL1b	05
	WL2a	06
	WL2b	04
	WL3	03
Intermediate zone	IU3c	03
	IU3a	03
	IM3a	03
	IL1a	03
	IL1b	03
	IL3	05
Dry Zone	DL1b	04
	DL1c	04
	DL2a	03
	DL2b	04
	DL5	04



Map 01: Distribution of sampling locations

Morphological characterization:

Morphological characteristics were evaluated according to the descriptor developed for eggplant by the International Board for Plant Genetic Resources in 1990. The following characteristics were recorded in plants which represent each selected agro-ecological region.

Vegetative characteristics:

Plant growth habit, plant height, canopy width, branching. Petiole; length and colour. Leaf blade; length and width, lobbing, tip angle and colour. Leaf prickliness, and prickles colour.

Flower and fruit characteristics:

Flower; number of flowers per inflorescence, corolla colour. Fruit; length, breadth, length/breadth ratio, curvature, cross section, shape, apex shape, colour at commercial ripeness, colour distribution at commercial ripeness, colour at physiological maturity, flesh density, calyx length, calyx prickles, position, pedicel length, pedicel thickness, pedicel prickles, number of locules per fruit, number of fruits per inflorescence and number of seeds per fruits.

Seed characters:

Seed color and 100 seed weight (g).

Laboratory analysis

The laboratory analysis was conducted to measure antioxidant activity, total phenolic content and time taken for browning of fruits at the Food Research Unit, Department of Agriculture Sri Lanka.

Antioxidant activity:

Fruits at table maturity stage were used to assess the antioxidant activity following the DPPH assay method. Five samples were evaluated from each cluster. DPPH radical scavenging activity of the samples was determined using the following equation. Higher DPPH radical scavenging activity reveals higher amount of antioxidant properties (Razak, 2012).

$$\text{DPPH Radical Scavenging Activity (\%)} = [(A_0 - A_1)/A_0] \times 100$$

Where, A_0 is the absorbency of control and A_1 is the absorbency of samples (Kandoliya *et al.*, 2015).

Assessment of total phenolic content:

Five samples were evaluated from each cluster. It was done according to the procedure described by Velioglu *et al.*, (1998) with a slight modification. Total phenolic content was examined using fruits at edible maturity stage.

Assessment of browning:

Fruits at table maturity were used to evaluate browning characteristics of fruits. Time taken for color development up to “Grayed orange group 165 b” of colour chart of Royal Horticulture Society (RHS) was measured. The anthocyanin content was also visually observed and compared with colour range in RHS colour chart.

Data analysis

Non parametric data were analyzed using hierarchical cluster analysis whereas Least Significant Difference (LSD) test was used for mean separation in parametric data using SAS software (version 9.1).

RESULTS AND DISCUSSION

The results revealed that 71 accessions were clearly grouped into two main clusters based on the presence or absence of prickles. As appeared in the Figure 01, cluster one contained fifty one accessions while cluster two ended up with twenty accessions. Furthermore, they were clustered into specific groups according to plant growth habit, leaf, flower and fruits' characteristics.

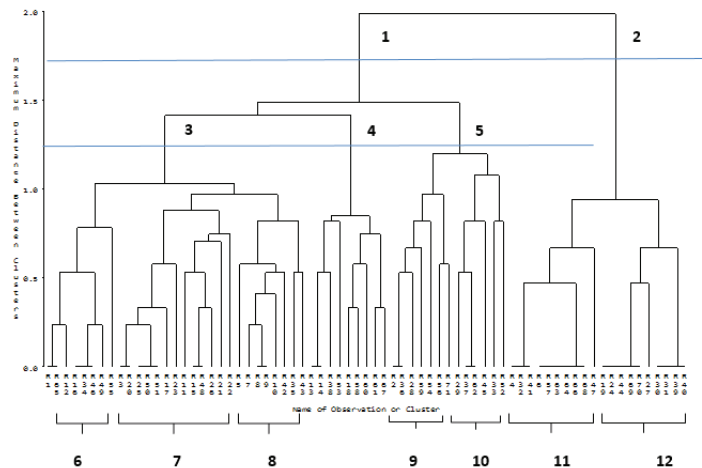


Figure 01: Dendrogram obtained from cluster analysis

There was a significant difference found between cluster 1 and cluster 2 as depicted by the results of MANOVA ($P \leq 0.05$). Accessions were differentiated into two distinct clusters mainly based on prickliness of the plants. Cluster 1 consists of plants with prickles on their vegetative parts while cluster 2 consists of plants that had no prickles in their vegetative parts and contained high fruit flesh density than that of the fruits of cluster 1. Cluster 1 was further grouped into several sub clusters based on plant habit. Cluster 4 and 9 showed intermediate growth habit while cluster 8 and 10 depicted prostrate growth habit. All other clusters represented an erect growth habit. It was also observed that with the fruits in accessions clustered under the cluster 8 had higher numbers of prickles (4-5) than in fruits of others and those prickles were observed especially in areas of calyx and pedicel of the fruits. It indicates that accessions of sub cluster 8 are taxonomically more close to typical *S. insanum*.

Non-prickled plants (cluster 2) depicted high intensity of purple color than prickled plants. Cluster 2 was further divided into two groups, based on the purple color pigments present in their vegetative parts. Higher purple color intensity was visually observed from cluster 11 than cluster 12. Purple colour exhibits higher antioxidant activity which attributed to the higher phenolic and anthocyanin contents (Somawathi *et al.*, 2014). According to the Nakabayashi *et al.*, (2014) purple pigmentation exhibits the

accumulation of anthocyanin in petiole, leaf blade and veins. It is one of the flavonoid sub groups having consistent beneficial effects on humans and shows allelopathic reactions which possess either positive or negative effects on the growth of organisms such as fungi, insects and plants. And also anthocyanin accumulation directly associates with drought tolerance (Bahler *et al.*, 1991). Drought stress induced the accumulation of reactive oxygen radicals (eg; hydroxyl radical, superoxide radicals, and hydrogen peroxide) and anthocyanin and that over accumulation of such radicals would cause to reduce the accumulation of hydrogen peroxide and thereby prevent oxidative damage while making the plant drought tolerance (Bahler *et al.*, 1991; Jiang and Zhang, 2002). This will help to elicit long term stress by tolerating biotic and abiotic stress conditions. In this study, purple colour pigments were observed visually. Further study is required to measure the Anthocyanin concentration using standard laboratory procedure.

There was no significant difference between prickly plants and non-prickly plants in the total phenolic content at ($P \leq 0.05$). Non prickly plants had higher amount of total phenolic content than prickly plants. Table 02 and Figure 2A show the difference between the total phenolic contents in fruits of prickled and non-prickly plants. Several researches have reported that there is a correlation between the total phenolic content of plant extracts and antioxidant activity

(Dudonne *et al.*, 2009; Velioglu, 1998). In general, higher phenolic content was associated with higher antioxidant capacity (Kandoliya *et al.*, 2015). The findings of the present study also revealed that, non-prickly plants had the highest total phenolic content with higher antioxidant activity (Table 2). Moreover, table 2 and Figure 2B show the difference of radical scavenging activity in fruits between prickly and non-prickly plants. Further, the table 2B gives the difference among groups as depicted by results of two sampled t test. In general, higher DPPH scavenging activity in plant parts reveals higher quantity of antioxidant

properties (Nisha, 2009). It is a well known fact that antioxidant properties are important in reducing risk of human disease such as cancer, arthritis, diabetes, and other aging related non-communicable diseases. Antioxidants come up frequently in discussions about good health and preventing diseases. These powerful substances mostly come from fresh fruits and vegetables. This specifies that, findings of this study would benefit in promotion of human health as consumption of antioxidant rich foods would help in preventing or minimizing the risk of such diseases.

Table 02: Selected chemical properties of fruits of prickly and non-prickly plants

Group	Total Phenolic content (mg/100g)	DPPH Radical Scavenging Activity %	Time taken for browning (sec.)
Group 1(Prickled plants)	50.32 ± 0.13	7.10 ± 0.59	450 ± 17.32
Group 2 (Non-prickled plants)	51.45 ± 0.12	20.30 ± 0.52	380 ± 10

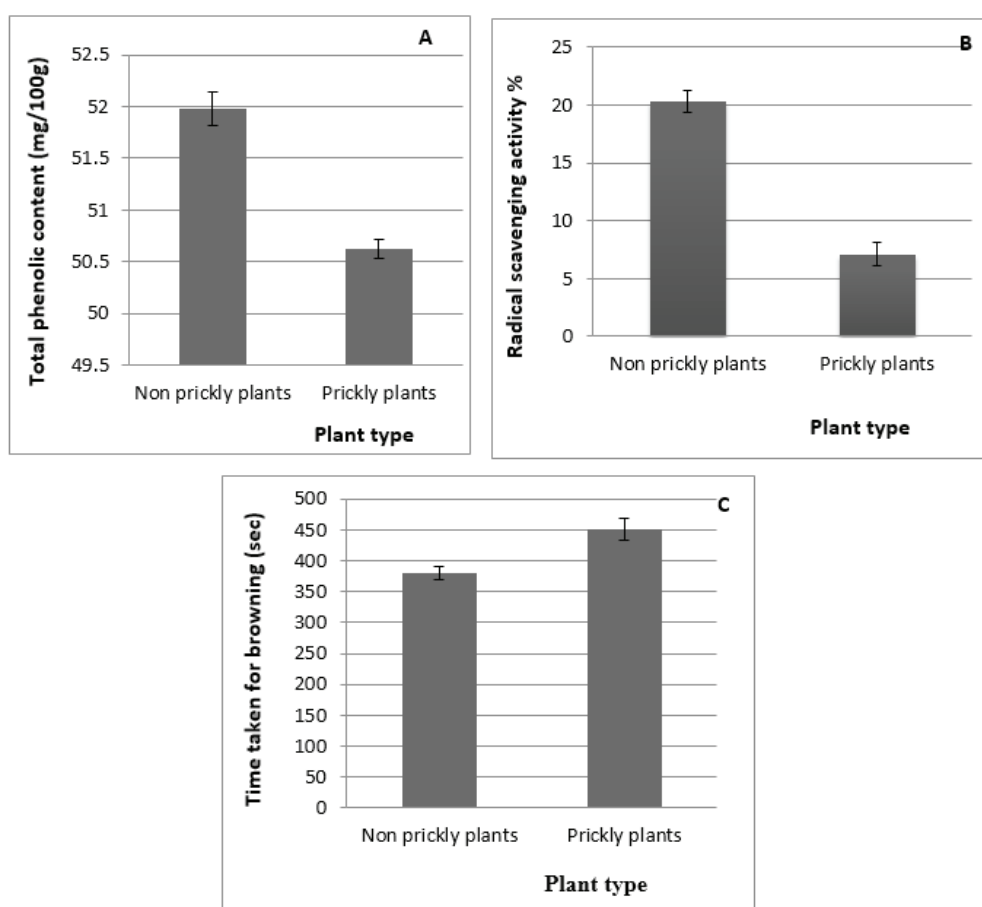


Figure 02: Selected chemical properties of fruits of prickly and non-prickly plants. A: Total phenolic content mg/100g in two types of *S. insanum* fruits at their edible stage. B: Percent radical scavenging activity (DPPH) value in two types of *S. insanum* fruits at their table maturity level. C: Time taken for browning (sec) of *S. insanum* fruits at consumable stage.

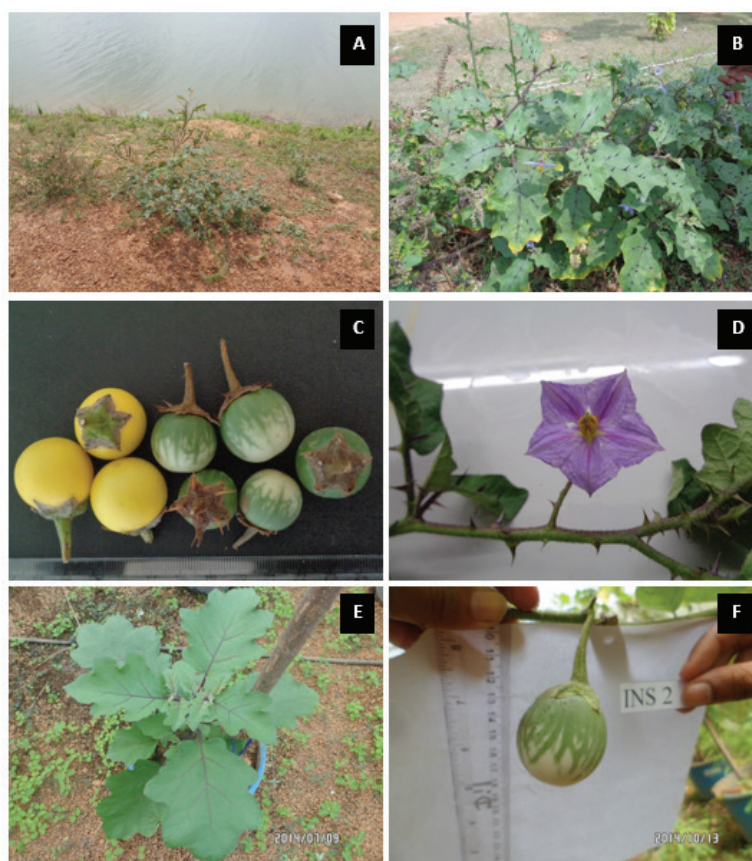


Figure 03: Morphology of *Solanum insanum* plant photographed from bank of water reservoir in Kilinochchiya district of North Central province of Sri Lanka (A-D) and accession (INS 2) cultivated in plant house at the Horticultural Crop Research and Development Institute, Gannoruwa, Sri Lanka (E-F). A: Natural habitat of *S. insanum*, B: luxury grown plant, C: ripen and unripen fruits, D: flower. E: prickliness form of *S. insanum*, F: fruit of prickliness form.

There was a significant difference between prickly plants and non-prickly plants in time taken for browning ($P \leq 0.05$). Non prickly-plants have shown significantly low time for colour development up to “Grayed orange group 165 b” of colour chart than prickly-plants. Table 2 and Figure 2C show the difference in time taken to browning in fruits between prickly and non-prickly plants. The table 2 also gives difference among groups as depicted by results of two sampled t test. Time taken for browning is given at 5% probability.

Non prickliness could have occurred due to farmer selection or due to the natural selection. Perhaps those may be results of natural hybridization with typical prickly *S. insanum*. Non-prickliness would definitely be the farmer’s choice due to easiness of cultural practice, harvesting and handling practices while it would become the preferred type of house wives

and consumers due to low degree of browning. The results of this study will provide useful information for plant breeders to select superior germplasm for the breeding programs aiming for development of new varieties adapted to future changing climate. It is also a well known fact that human involvement and some of natural incidents largely contribute to the erosion of plant genetic resources from farmlands, natural and semi-natural habitats. Therefore, collection, evaluation, conservation and utilization of existing crop genetic resources are important, as such germplasm can be effectively utilized to develop climate smart varieties to ensure future food and nutritional security.

CONCLUSIONS

According to the study, seventy one accessions were categorized into two main groups based

on ten significant morphological characteristics. Fifty one accessions that had prickles in plant parts were grouped into one cluster while twenty accessions without prickles in their plant parts were grouped into the other cluster. Fruits belong to non-prickly plants had a significantly higher amount of antioxidant activity than fruits belong to prickly plants which took lesser time to browning. Undoubtedly, non-prickliness could be the farmer's choice or natural selection. It can further be suspected that it may be a result of natural hybridization

of typical prickly *S. insanum* and cultivated *S. melongena*. However, the results clearly show that both prickled and non-prickled plants play a vital role in the endeavor of future crop improvement. Furthermore, these results will provide a platform to select superior germplasm for breeders to develop new varieties aiming for changing climate. Consequently, collection, evaluation and conservation of variable forms of *S. insanum* found in both natural habitats and farmland are vital for the better utilization of this hidden treasure.

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