Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jls.htm
2015 Vol. 5 (3) July-September, pp.51-56/Paul and Banerjee
Research Article

PHYLOGENETIC RELATIONSHIP OF SOME SPECIES OF *SOLANUM* BASED ON MORPHOLOGICAL, BIOCHEMICAL AND CYTOLOGICAL PARAMETERS

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ABSTRACT
Solanum has some contradictory diversified external morphology. Only morphological markers are insufficient for their correct identification and proper phylogenetic position. In the present investigation, phylogenetic relationship among the eight selected species of *Solanum* (*S. nigrum, S. americanum, S. villosum, S. torvum, S. xanthocarpum, S. sisymbriifolium, S. macranthum and S. indicum*) has been established through dendrogram analysis, based on morphological, biochemical and cytological data. Of the morphological parameters; seed length, seed width, dry seed weight, leaf area and fresh leaf weight are taken into consideration. Regarding biochemical parameters, total amount of soluble seed and leaf proteins and total amount of leaf chlorophylls of these plants have been studied. In the cytomorphometric study, chromosome number, total genome size and mean chromosome length have been recorded. Dendrogram analysis exhibited two hierarchical clusters- upper cluster (UC) and lower cluster (LC). *S. macranthum* is only placed in LC while the rest of all seven species are placed in UC. UC has been again sub-divided into two sub clusters- UC1 and UC2. S. *torvum*, *S. indicum* and *S. sisymbriifolium* are included in UC1 while *S. nigrum* complex along with *S. xanthocarpum* are placed in UC2. Thus the present study provided useful information for the identification of the taxa, their relationship and delimitation of their taxonomic status.

Keywords: *Biochemical Aspects, Cytomorphometric Study, Dendrogram, Morphological Study, Solanum*

INTRODUCTION
Solanum is the largest economically as well as medicinally valuable genus among the 98 genera of Solanaceae with approximately 2700 species (Olmstead and Bohs, 2006). The genus is wildly distributed throughout the world especially in tropical and worm temperate regions, such as America, Australia, Africa and India (Bukenya and Carasco, 1995). This genus is extremely useful for its food value and pharmaceutical demands due to the presence of different types of secondary metabolites.
The genus *Solanum* has a paradoxical and confusing taxonomy. Although it shows much more uniformity in external appearance, it also exhibits some external diversified morphology (Roe, 1972; Lester and Hasan, 1991). Therefore, debates have always been retained regarding identification, phylogenetic relationship and proper taxonomic position among the species of *Solanum* based on only morphological aspects (Stebbins and Paddock, 1949; Symon, 1970). So, mere morphological markers used in the past are insufficient for their correct identification and proper phylogenetic classification.
In the present time, data from different branches of biological sciences, such as biochemistry, molecular biology, anatomy, cytology etc. along with traditional morphology are extremely useful in studying more accurate phylogenetic relationship among the species of *Solanum*.
Regarding biochemical parameters, variation in chlorophyll contents, protein diversity study etc. are important parameters for cladistics study. In the era of modern biological techniques, study of chromosome profile is still a valuable tool for taxonomy, phylogeny and diversity studies. The cytological features like chromosome number, their morphology, genome size etc. may have provided valuable information regarding interspecific relationships and delimitation of the genus *Solanum*.
The aim of the present study is to determine interspecific relationship among the eight selected species of medicinally important, morphologically diversified, non-tuberous wild *Solanum*, based on some morphological characters, along with dominant biochemical and cyto-morphometric characters. The

results of this study may provide useful information to plant breeders and taxonomists in establishing proper phylogenetic relationships among wild non-tuberous *Solanum* relatives.

MATERIALS AND METHODS

The selected species of *Solanum* are *Solanum nigrum* Linn., *Solanum americanum* Mill., *Solanum villosum* Mill., *Solanum torvum* Swartz, *Solanum xanthocarpum* Schard and Wendl (Syn. *Solanum surattense* Burm. f.), *Solanum sisymbriifolium* Lam, *Solanum macranthum* Dunal (Syn. *Solanum crinitum* Lam, *Solanum wrightii* Benth), and *Solanum indicum* Linn. These species are identified and collected from different regions of Assam and West Bengal including Santiniketan.

For phylogenetic analysis, following morphological, biochemical and cytological parameters were investigated in the experimental plants-

A. Morphological Characters

(i) Seed length and (ii) seed width: measured by standard scale of measurement; (iii) dry seed weight and (iv) fresh leaf weight: measured by electronic fine balance; (v) area of leaf lamina: measured by graphical scale.

B. Biochemical Characters

(i) Total amount of soluble seed protein was estimated by Bradford (1976) method, (ii) total amount of soluble leaf protein was also estimated by Bradford (1976) method and (iii) total content of leaf chlorophyll was assayed by Arnon (1949) method.

C. Cytological Characters

(i) Chromosome number, (ii) total genome length and (iii) mean chromosome length were measured with an ocular micrometer. For this purpose good metaphase plates with well spread chromosomes were prepared by the method of Sharma and Sharma (1980).

Data Analysis

Hierarchical cluster analysis was performed based on above mentioned morphological, biochemical and cytological parameters using SPSS computer software.

RESULTS AND DISCUSSION

Results

Present investigation revealed that selected species of *Solanum* exhibited highly diversified features in each of the concerned morphological, biochemical and cytological parameters (Table 1; Figure 1 and 2). Regarding seed and leaf morphological aspect, *S. macranthum* exhibited maximum seed length, seed width, dry seed weight, leaf area and fresh leaf weight. On the other hand, *S. americanum* exhibited minimum seed length, seed width, dry seed weight and total amount of soluble leaf protein present /gm tissue (Figure 1). *S. nigrum* exhibited largest genome size (Table 1; Figure 2) and highest amount of soluble seed protein/gm tissue and shown lowest fresh leaf weight (Figure 1). *S. villosum* exhibited least leaf area and contain minimum amount of leaf chlorophyll/ gm leaf tissue (Figure 1). In respect of cyto-morphometric characters *S. xanthocarpum* exhibited smallest genome size as well as mean chromosome length. Longest chromosome is found in *S. sisymbriifolium* (Table 1; Figure 2). Regarding the biochemical parameters, *S. indicum* exhibited lowest amount of total soluble leaf protein/ gm leaf as well as total content of leaf chlorophyll/ gm tissue. *S. macranthum* contained minimum amount of soluble seed protein in each gm of seed (Figure 1).

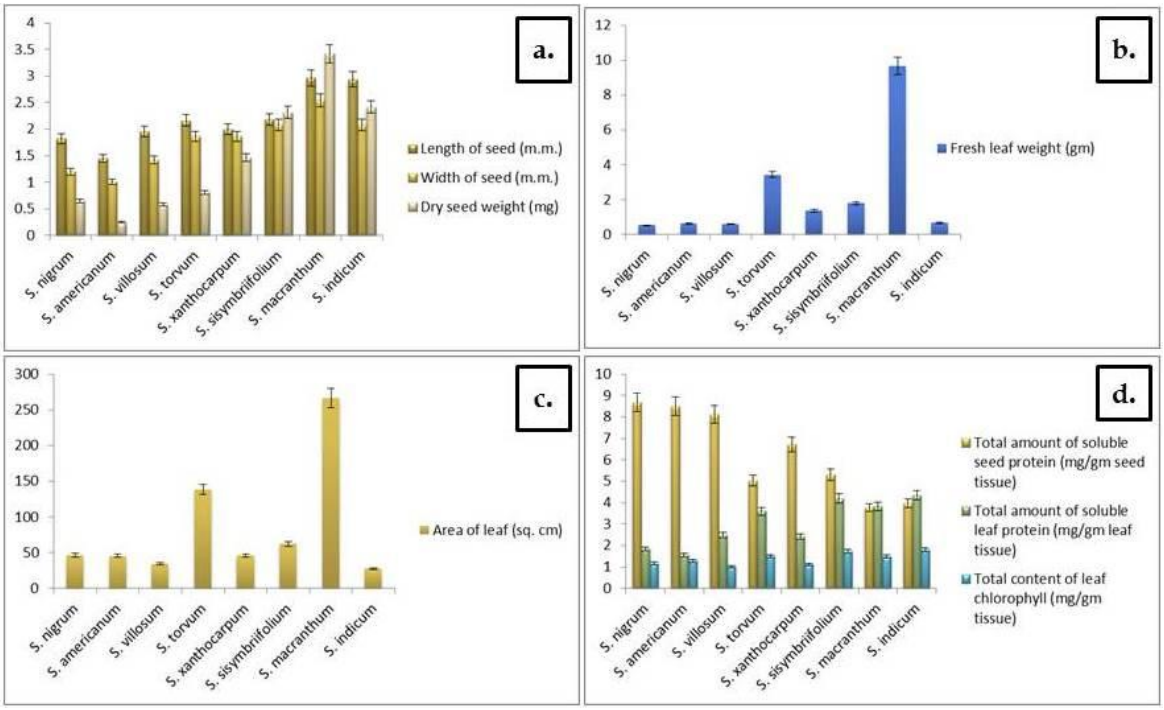

Figure 1: Variation in different morphological and biochemical parameters of selected species of *Solanum* (a-d); a: Morphological variation of seed, b: Variation of fresh leaf weight, c: Variation of leaf area and d: Variation based on selected biochemical characters

A total of 11 characters were taken together to construct hierarchical cluster (Figure 3) that exhibited inter-specific phylogenetic relationship of *Solanum*. The results are shown diagrammatically in the form of dendrogram (Figure 3). Two major hierarchical clusters are formed among the selected species of *Solanum*- upper cluster (UC) and lower cluster (LC). *S. macranthum* is the only representative in LC while the rest of all seven species represent the UC. UC is again sub-divided into two sub clusters- UC1 and UC2. *S. torvum*, *S. indicum* and *S. sisymbriifolium* form the UC1. *S. nigrum* complex, which includes *S. nigrum*, *S. villosum* and *S. americanum* are placed in UC2 along with *S. xanthocarpum*. From Figure 3 it is clearly evident that *S. americanum* is more closely related to *S. villosum* than *S. nigrum*. All these clusters are formed on the basis of the similarity and dissimilarity of different morphological, biochemical and cytological parameters of the studied species.

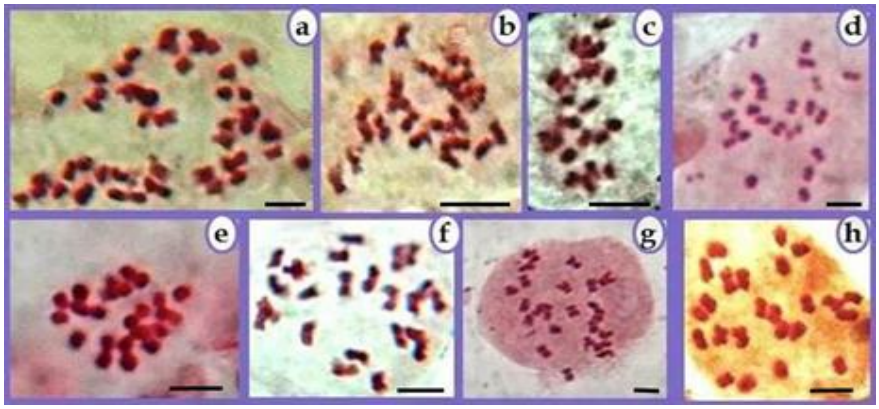

Figure 2: Mitotic chromosome plates of selected species of *Solanum* (a-h); a: *S. nigrum* (2n=48), b: *S. americanum* (2n=24), c: *S. villosum* (2n=24), d: *S. torvum* (2n=24), e: *S. xanthocarpum* (2n=24), f: *S. sisymbriifolium* (2n=24), g: *S. macranthum* (2n=24) and h: *S. indicum* (2n=24). (Bar = 5 µm)

Table 1: Cytological characters of some selected species of *Solanum*

Cyto-morphometric data	Selected plant species of *Solanum*							
	S.n.	***S.a.***	***S.v.***	***S.t.***	***S.x.***	***S.s.***	***S.m.***	***S.i.***
Total number of Chromosome /cell	48	24	24	24	24	24	24	24
Total genome length (µm)	54.61±0.13	24.7±0.18	22.41±0.01	27.89±0.06	21.58±0.09	37.14±0.11	25.18±0.04	33.75±0.04
Mean chromosome length (µm)	2.27	2.06	1.87	2.32	1.8	3.09	2.1	2.81

Abbreviations: *S.n.: Solanum nigrum; S.a.: Solanum americanum; S.v.: Solanum villosum; S.t.: Solanum torvum; S.x.: Solanum xanthocarpum; S.s.: Solanum sisymbriifolium; S.m.: Solanum macranthum and S.i.: Solanum indicum*

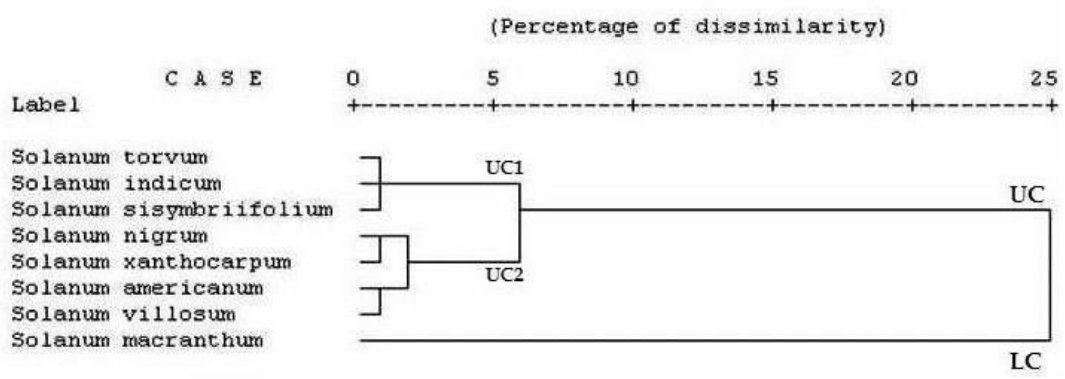

Figure 3: Dendrogram showing the phylogenetic relationship among the eight selected species of *Solanum* based on morphological, biochemical and cytological characters together

Discussion

The biochemical and cytological data were considered as additional powerful tools along with the general morphological characters to study the interspecific relationship among the species of *Solanum*. These additional data might be helpful to construct more accurate dendrogram. Generally morphological characters seem to be of more or less similar nature in a particular genus and thus it sometimes becomes difficult to differentiate them for their identification at the species level. With the help of cluster analysis, some taxonomic complexity among the species of *Solanum* remains clear.

In the past, taxonomic position of *S. nigrum* complex remained highly paradoxical and controversial. Clarke (1885) did not separate them and considered all the three species as *Solanum nigrum*. According to the present investigation, members of *S. nigrum* complex are always placed in the same cluster UC2 (Figure 3), and *S. americanum* and *S. villosum* exhibit a high degree of similarity in respect to all concerned characters, than *S. nigrum*. From this dendrogram it is also evident that *S. villosum* gets separated from *S. americanum* only at the 4% of segregating distance and *S. nigrum* gets separated from *S. americanum* at the 8% of segregating distance (Figure 3). Therefore, *S. nigrum* exhibits high range of diversified characters in all concerned aspects. It has 2n = 4X = 48 chromosomes; i.e. tetraploid (4n) and its genome size is more than twice that of *S. americanum* and *S. villosum*. Thus our results are in accordance with Edmonds and Chweya (1997) and Khan (1992) but contrary to Hawkes and Edmonds (1972), Baytop (1978) and Yousaf *et al.,* (2006, 2008 and 2010).

S. xanthocarpum is phenotypically highly polymorphic (Yousaf *et al*., 2010). Therefore, the taxonomic position of *S. xanthocarpum* is still a matter of confusion. According to our investigation this species is

closely associated with *S. nigrum* complex (Figure 3) in respect of all concerned morphological, biochemical and cytological features. Our result is contrasted with the view of Yousaf *et al.*, (2006 and 2010). Whalen (1984) treated *S. sisymbriifolium* as one of the "unusual species" since it could not be accommodated in any of the groups. But our result is totally contrasted with his view. Based on dendrogram study *S. sisymbriifolium* is closely associated with *S. torvum* and *S. indicum* (Figure 3). Our results are in accordance with Karihaloo *et al.*, (2002) but contrary to D'Arcy (1972).

S. macranthum or the giant potato tree exhibits a high range of diversified characters in all concerned aspects. Mainly seed and leaf morphological features of this species, such as seed length and width, dry seed weight, leaf area and fresh leaf weight exhibit greater percentage of dissimilarity than other species. Therefore *S. macranthum* is placed in a separate cluster in the dendrodram (Figure 3).

Inter-specific taxonomic relationships in the genus *Solanum* is a matter of debate, due to the presence of high degree of morphological divergence along with the biochemical and cytological polymorphism. This investigation has provided new information about the taxonomical affinities among the selected species of *Solanum* which might be helpful in proper taxonomic characterization.

ACKNOWLEDGEMENT

The first author gratefully acknowledges the UGC-SAP (DRS) and DST-FIST supported Department of Botany, Visva-Bharati for providing laboratory space and instrument facilities.

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