ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Phylogenetic diversity and relationships of some medicinally important species of *Solanum* as revealed by seed morphometric and biochemical analysis

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Manuscript Info

Manuscript History:

Received: 15 May 2015
Final Accepted: 22 June 2015
Published Online: July 2015

Key words:

Dendrogram, polygraph, seed morphology, seed protein, *Solanum*, sub genera.

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Abstract

Morphometric parameters like seed length and width; dry seed weight; biochemical data like total soluble seed protein content per gm of tissue as well as protein content per grain of seed were analyzed in the eight selected species of *Solanum* (*S. nigrum*, *S. americanum*, *S. villosum*, *S. torvum*, *S. xanthocarpum*, *S. sisymbriifolium*, *S. macranthum* and *S. indicum*). It was revealed that *S. macranthum* and *S. americanum* produce heaviest and lightest seeds respectively. *S. nigrum* and *S. macranthum* contain maximum and minimum soluble seed protein per gm of tissue, while protein contents per seed were highest and lowest in *S. macranthum* and *S. americanum* respectively. Phylogenetic relationship among the selected species has been established by constructing dendrograms based on morphological and biochemical data separately which show striking similarity. From the dendrograms it was revealed that the selected species are clearly divided in to two major clusters- upper cluster (UC) and lower cluster (LC). UC is sub-divided into two sub-clusters, viz. UC1 and UC2. *S. nigrum* complex and *S. torvum* are placed in UC1 and UC2 respectively. LC is also divided in to two sub-clusters- LC1 and LC2. *S. sisymbriifolium*, *S. macranthum* and *S. indicum* are closely associated and placed in LC1, while LC2 is represented by *S. xanthocarpum*. The relationship and diversity among these species were confirmed through polygraph analysis. Based on overall seed morphometric and biochemical characters, the genus *Solanum* can be divided into two sub genera and the distribution of species in these two sub genera is in contrary to conventional classification.

INTRODUCTION

Solanum is one of the largest economically as well as medicinally valuable genera of Solanaceae with approximately 2700 species (Olmstead and Bohs, 2006). The great abundance of the species of *Solanum* represents nearly 1% of total angiosperm flora in the world (Whalen and Caruso, 1983). 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 presence of different kinds of secondary metabolites.

Although species of *Solanum* exhibited much uniformity in external appearance, some contradictory external diversified morphology has also been detected (Roe, 1972; Lester and Hasan, 1991). Thus, there have always been debates concerning the taxonomic complexity of the species of *Solanum* (Stebbins and Paddock, 1949; Symon, 1970). Therefore, only morphological markers that have been used in past are proved to be insufficient for their correct identification and proper phylogenetic classification.

To investigate probable phylogenetic relationships among the species of *Solanum*, studies on morphometric characters along with gene diversity study may be considered as a powerful technique, since morphological diversity is the external manifestation of differential expression of genes. Genetic diversity refers to variation in the nucleotide sequence, chromosomes or genome of a particular organism. Proteins are relatively direct gene products, because a particular protein is synthesized via translation of a specific messenger RNA, which is the transcript product of a particular gene. Therefore, if any variations of total amount of seed proteins are observed, it could be due to alteration of the genetic configuration of that organism (Crawford, 1990).

The aim of present study is to determine interspecific relationship and diversity among eight selected species of morphologically diversified, medicinally important, non-tuberous wild *Solanum*, based on some quantitative seed macro morphological features along with dominant biochemical characters such as amount of soluble seed proteins. The results of this study may provide reliable information to the plant breeders and taxonomists in establishing 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 regions of Assam and West Bengal including Santiniketan. The mature seeds of collected species are sun dried, stored in a desiccator for their morphological analysis and extraction of seed proteins.

Measurement of seed morphometric data:

Regarding the analysis of seed morphometric characters; seed length, seed width and dry seed weight of selected species are considered. For obtaining accurate data of each character of the selected species, at least 20 seeds of each species have been taken into consideration.

Estimation of seed proteins:

Total seed protein of each species was extracted from 0.01g of seed flour using 400µl of extraction buffer that contained 0.5M Tris-HCl, 0.01 M $MgCl_2$, 18% (w/v) sucrose and 40 mM β-mercaptoethanol having pH- 6.8. Crushed seed samples were thoroughly mixed with buffer by vortexing, transferred to 1.5 ml eppendorf tubes and the extracted proteins were separated by centrifuging at 10000 rpm for 15 min and the supernatant was collected and stored at 4^0C as a protein stock for analysis. Total soluble seed protein was estimated following the method of Bradford (1976).

Data Analysis:

For determination of phylogenetic relationship among the selected species of *Solanum*, hierarchical clustering or dendrograms were constructed. Initially, two separate dendrograms were constructed based on the morphological and biochemical characters of seeds respectively. Regarding morphological characters, such as seed length, width and dry weight of seed are taken together; similarly within biochemical features total amount of soluble seed protein present per gram of seed tissue and the seed protein contents of each seed are considered together and were subjected to hierarchical clustering respectively. Finally all these morphological and biochemical features of seeds were taken together to construct a single hierarchical cluster for establishment of more accurate relationship among the investigated species of *Solanum*. For construction of these dendrograms SPSS vs 16 was used. Polygraph analysis, based on seed morphological and biochemical data, was done for determination of relationship and diversity among these species.

Results

Present investigation revealed that all the eight selected species of *Solanum* produce in general pale yellow to blackish yellow colored seeds (Table 1, Fig. 1). Great variation was observed among them in respect of their seed morphometric as well as biochemical characteristics (Table 1). With regard to the seed morphology, *S. macranthum* possesses highest seed length and seed width; and *S. americanum* has lowest seed length and seed width. Thus *S. macranthum* and *S. americanum* possess heaviest and lightest seeds respectively among the selected species of *Solanum* in respect of dry seed weight. Considering the biochemical aspects, the soluble seed protein content / gm of seed tissue was found highest in *S. nigrum* and least in *S. macranthum*. However, in contrast to that, when total amount of soluble seed protein per seed is concerned, it is clearly evident that *S. macranthum* and *S. americanum* possess highest and least amount respectively (Table 1).

From Table 1, it is indicated that variation of seed weights among the selected species of *Solanum* is more or less proportional to the variation of total amount of soluble seed protein content per grain of seed. It clearly indicates that concentration of soluble seed protein is more or less symmetrical with the dry weight of seeds of selected species of *Solanum*.

Based on seed morphometric values (seed length, width and dry weight), a hierarchical clustering or dendrogram (Fig. 2A) shows interspecific relationships among the selected species of *Solanum*. This dendrogram reveals two main clustered groups- upper cluster (UC) and lower cluster (LC). *S. nigrum*, *S. villosum*, *S. americanum* along with S. *torvum* and *S. xanthocarpum* are placed in UC, while LC consists of three species- *S. sisymbriifolium*, *S. indicum* and *S. macranthum*. UC is sub-divided in to two sub-clusters - UC1 and UC2. From Fig. 2A it is clearly evident that *S. nigrum* is more closely related to *S. villosum* than *S. americanum*, while *S. torvum* is quite distantly related to the *S. nigrum* complex, which includes *S. nigrum*, *S. villosum*, and *S. americanum*; though these four species are placed in sub- cluster UC1. The seed morphology of *S. xanthocarpum* is quite dissimilar to the other species, so this species is placed in different sub-cluster UC2. Similarly LC is sub-divided in to two sub-clusters- LC1 and LC2. *S. sisymbriifolium* is more closely related to *S. indicum* than *S. macranthum* in respect of seed morphological parameters; therefore *S. sisymbriifolium* and *S. indicum* are placed in sub-cluster LC1 and *S. macranthum* is placed in LC2 sub-cluster. Polygraph analysis (Fig. 3) also revealed more or less similar trends.

Based on biochemical parameters of seeds (total amount of soluble seed protein content per gm of tissue and per grain of seed), another hierarchical clustering or dendrogram (Fig. 2B) has been constructed, which shows striking similarity with the previous results (Fig. 2A). In this case selected plants are also divided in to two major clusters- upper cluster (UC) and lower cluster (LC). UC is sub-divided in to two sub-clusters - UC1 and UC2. *S. nigrum* complex is placed in UC1 and *S. torvum* is placed in UC2 sub-cluster. Similarly LC is sub-divided in to two sub-clusters - LC1 and LC2. Here concerning LC, result is slightly altered from previous hierarchical cluster (Fig. 2A). In this dendrogram (Fig. 2B) *S. sisymbriifolium* shows close similarity with *S. macranthum* which are placed in the LC1 sub-cluster, and *S. xanthocarpum* is less closely related to *S. indicum*, though they are placed in same sub-cluster LC2. Polygraph analysis (Fig. 3) also revealed more or less identical results.

Finally another dendrogram has been constructed considering all these morphological and biochemical seed characters of selected species (Table 1, Fig. 2C). Results are quite similar to the earlier two dendrograms (Fig. 2A and 2B). This combined dendrogram also exhibited two major clustered groups- upper cluster (UC) and lower cluster (LC). UC of this dendrogram (Fig. 2C) is exactly similar to the UC of previously drawn dendrogram (Fig. 2B) which indicated that *S. nigrum* complex is closely related to *S. torvum* in respect of combined morphological and biochemical seed characters. LC of this dendrogram (Fig. 2C) is sub-divided in to two sub-clusters - LC1 and LC2. *S. sisymbriifolium* is more closely related to *S. macranthum* compared to *S. indicum*, and they all are placed in LC1, while seeds of *S. xanthocarpum* are quite different from other species and thus placed in separate sub-cluster LC2. Polygraph analysis (Fig. 3) also supports this conclusion.

Discussion

In the present study, seed morphological and biochemical data (Table 1) are considered for construction of three separate dendrograms (Fig. 2A, 2B and 2C). Regarding phylogenetic diversity and relationships among these selected species of *Solanum*, dendrograms (Fig. 2) exhibited more or less similar result, where the selected species are clearly divided in to two major clusters. The first group is *S. nigrum* complex, which includes *S. nigrum*, *S. villosum* and *S. americanum* and these are always closely associated in UC with *S. torvum*. This relationship confirmed and supported the observations of Yousaf et al. (2006, 2010). On the other hand, considering all concerned aspects, *S. sisymbriifolium*, *S. macranthum* and *S. indicum* exhibited close resemblance and form the LC. However, proper placement of *S. xanthocarpum* in these hierarchical clusters is quite paradoxical. Thus from these cluster analysis, taxonomic complexity amongst the species of *Solanum* becomes 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 (*S. nigrum*, *S. villosum* and *S. americanum*) as *Solanum nigrum*. According to our analysis, members of *S. nigrum* complex are always placed in same cluster UC1 in every dendrogram (Fig. 2) and *S. nigrum* and *S. villosum* exhibited a high degree of similarity in respect of seed morphometric and biochemical features, than *S. americanum*, which is already established by polygraph analysis (Fig. 3). Therefore clustering of these species indicated a close evolutionary relationship and suggests the common origin of the two (*S. nigrum* and *S. villosum*) taxa, which was already confirmed and supported by Heiser et al. (1965) and Schilling and Heiser (1976). From these dendrograms it is also clearly evident that *S. villosum* gets separated from *S. nigrum* only at the 4% of segregating distance (Fig. 2). This distance may be revealed to consider *S. villosum* as the sub-species of *S. nigrum*. Thus, our results are in accordance to Hawkes and Edmonds (1972),

Baytop (1978) and Yousaf et al. (2006, 2008, 2010) but contrary to Nasir (1985), Edmonds and Glidewell (1977) and Edmonds and Chweya (1997).

S. xanthocarpum or yellow berried nightshade exhibited a distinct seed morphological and biochemical features than rest of the selected species of *Solanum*. Phenotypically this species is highly polymorphic (Yousaf et al., 2010). Therefore, the taxonomic position of *S. xanthocarpum* is still a matter of confusion. In the first dendrogram (Fig. 2A), which is based on seed morphometric characters, *S. xanthocarpum* is more closely associated with *S. torvum* and placed in UC, though in a separate sub-cluster UC2, which was supported by Yousaf et al. (2006, 2010). Based on biochemical characters (Fig. 2B), this species is placed in LC and more closely associated with *S. indicum* in the sub-cluster LC2. Finally similar result was revealed in the last dendrogram (Fig. 2C), which is based on all concerned morphometric and biochemical characters taken together, where *S. xanthocarpum* is placed in a separate sub-cluster LC2 alone. Polygraphs (Fig. 3) also supported these conclusions.

Whalen (1984) treated *S. sisymbriifolium* as one of the "unusual species" since it could not be accommodated in any of the groups. However, our result is totally contrasted with his view. Here based on hierarchical clustering study, *S. sisymbriifolium* gets associated 88% with *S. macranthum* and 80% with *S. indicum* in respect of segregating distance scale (Fig. 2C). The taxonomical similarity between *S. sisymbriifolium* with *S. torvum* is again a matter of controversy. D'Arcy (1972) placed them on separate clusters in UPGMA dendrogram based on seed protein analysis. Our results corroborate with that but contrary to Karihaloo et al. (2002).

Finally, from the hierarchical cluster and polygraph analyses based on overall seed morphological and biochemical features among these selected species of *Solanum*, it may be suggested that these species can be divided into two sub genera A and B. The sub-genus A comprises of *S. nigrum* complex and *S. torvum* and sub-genus B comprises of rest of the investigated species of *Solanum* (Table 2). This lower order taxonomy of *Solanum* genus is different from conventional standard classification.

Conclusion

Solanum, in general has a paradoxical and confusing taxonomy. Inter-specific taxonomic relationship within the genus *Solanum* is a matter of debate, due to the presence of high degree of morphological divergence along with genetic and biochemical polymorphism. Biochemical features, such as proteins are the resultant gene products of living organisms, which are ultimately reflected in external phenotypic characters. This hierarchical clustering study based on seed morphometric characters along with biochemical features is one of the potential tools to study proper and accurate phylogenetic status of species from taxonomic point of view.

Table 1: morphological and biochemical characters of some selected species of *Solanum*

Sl. No.	Name of the species	Color of the seeds	Morphometric characters			Biochemical characters	
			Length of seed (mm)	Width of seed (mm)	Dry seed weight (mg)	Total amount of soluble seed proteins (mg/gm tissue)	Total amount of soluble seed proteins (µg/grain of seed)
1.	*Solanum nigrum*	Yellow	1.82±0.18	1.2±0.06	0.65±0.03	8.67±0.06	5.63±0.03
2.	*Solanum americanum*	Bright yellow	1.45±0.14	1.01±0.08	0.25±0.02	8.5±0.09	2.12±0.01
3.	*Solanum villosum*	Bright yellow	1.96±0.05	1.42±0.07	0.58±0.02	8.13±0.02	4.71±0.02
4.	*Solanum torvum*	Blackish pale yellow	2.16±0.18	1.86±0.12	0.81±0.02	5.04±0.08	4.08±0.02
5.	*Solanum xanthocarpum*	Blackish yellow	2.0±0.15	1.86±0.05	1.46±0.03	6.71±0.09	9.80±0.07
6.	*Solanum sisymbriifolium*	Pale yellow	2.18±0.15	2.08±0.04	2.32±0.11	5.3±0.13	12.30±0.09
7.	*Solanum macranthum*	Blackish pale yellow	2.96±0.1	2.54±0.17	3.42±0.21	3.78±0.12	12.93±0.11
8.	*Solanum indicum*	Pale golden yellow	2.94±0.12	2.08±0.07	2.42±0.12	3.97±0.11	9.61±0.06

Table 2: Proposed classification of selected species of the genus *Solanum*, based on seed morphometric and biochemical characters

Genus	Sub genus	Species
Solanum	**A**	*S. nigrum*, subspp. *S. villosum*, *S. americanum*, *S. torvum*
	B	*S. sisymbriifolium*, *S. macranthum*, *S. indicum*, *S. xanthocarpum*

Fig. 1: Seeds of selected species of *Solanum*. (a-h); a: *S. nigrum*, b: *S. americanum*, c: *S. villosum*, d: *S. torvum*, e: *S. xanthocarpum*, f: *S. sisymbriifolium*, g: *S. macranthum* and h: *S. indicum*.

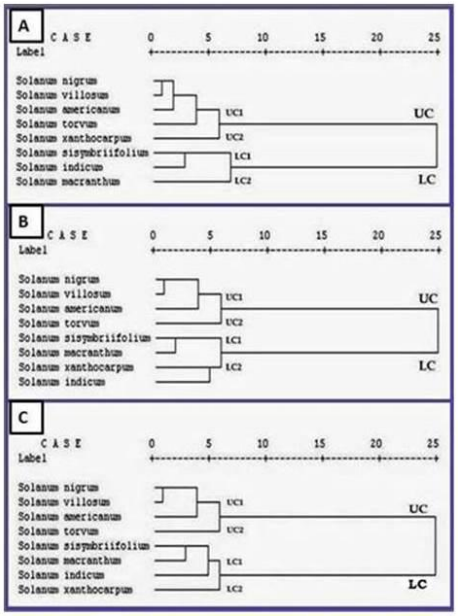

Fig. 2: Dendrogram indicating the phylogenetic relationships among the eight selected species of *Solanum* based on: (A) morphological characters, (B) biochemical characters and (C) both morphological and biochemical characters together.

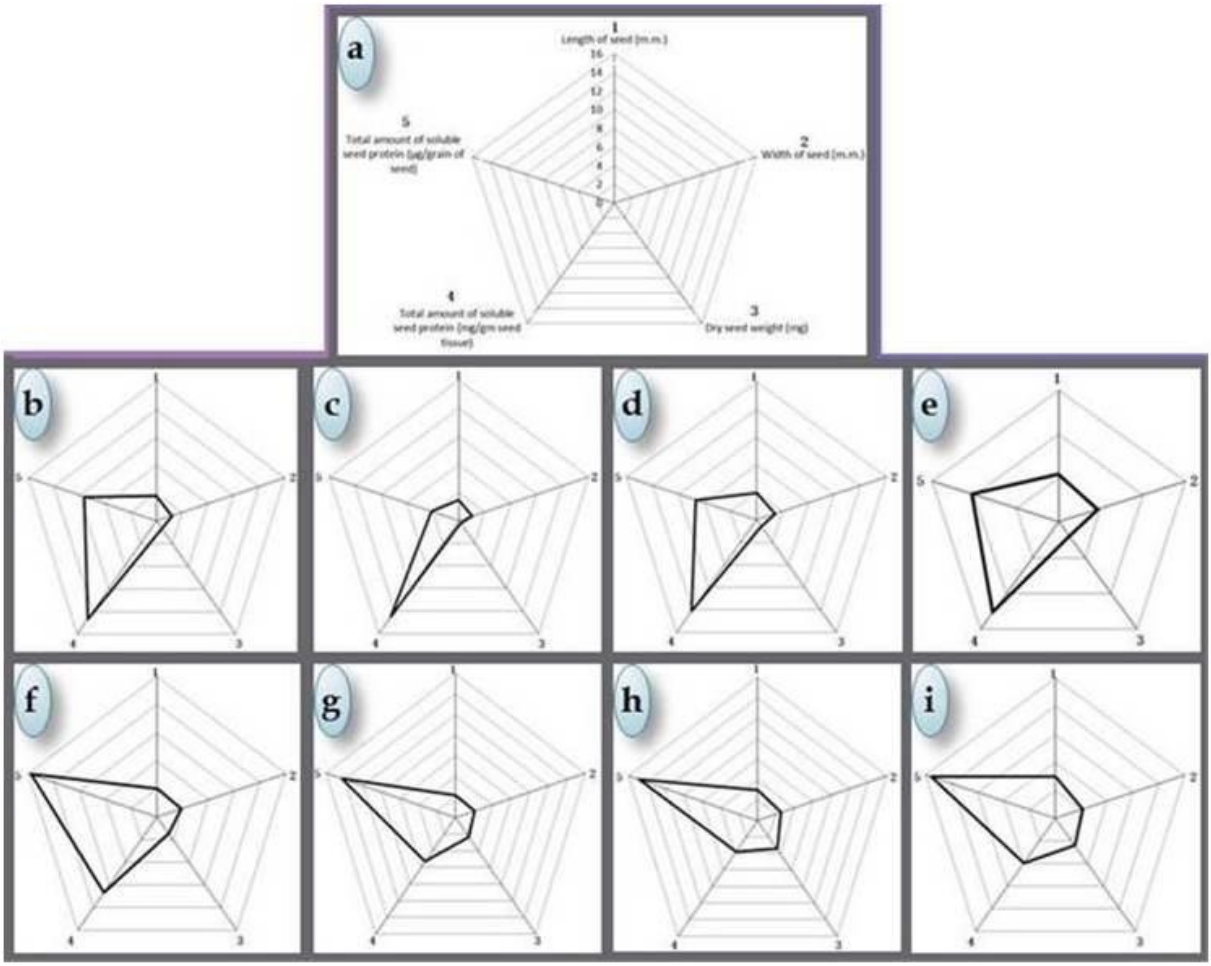

Fig. 3: Polygraph showing similarities and differences among the selected species of *Solanum* based on different concerned seed morphological and biochemical characters. (a-i); a: a generalized format of polygraph includes concerned parameters and relative measurement scales, b: *S. nigrum*, c: *S. americanum*, d: *S. villosum*, e: *S. torvum*, f: *S. xanthocarpum*, g: *S. sisymbriifolium*, h: *S. macranthum* and i: *S. indicum*.

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