Evaluation of Phylogenetic Relationships of Some Medicinally Important Species of *Solanum* Based on Seed Protein Profile of SDS-PAGE

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Abstract Total amount of soluble seed protein along with its protein profile of nine species of *Solanum* was investigated through SDS-PAGE. *S. nigrum* and *S. macranthum* contain maximum and minimum amount of total soluble seed protein per gm of tissue respectively. A dendrogram based on Jaccard's similarity index and also on the basis of presence and absence of peptide bands revealed two major clusters- upper cluster (UC) and lower cluster (LC). Both the clusters are again sub-divided in two sub-clusters like UC1, UC2 and LC1, LC2. *S. nigrum* being evolutionary more closely (91%) related to *S. villosum* than *S. americanum*, has been placed in UC1 while *S. americanum* along with *S. sisymbriifolium*, *S. macranthum* and *S. torvum* are placed within UC2. *S. indicum* and *S. erianthum* showed close resemblance and are placed in LC1 while LC2 contains only *S. xanthocarpum*, which shows least similarity with other studied species of *Solanum* and thus occupies a distinct place on the dendrogram. Based on these results, the genus *Solanum* can be divided into two sub genera and the distribution pattern of these species in the two sub genera does not corroborate with the conventional classification. The present study thus provides useful information for the identification of the taxa, their relationship and delimitation of their taxonomic status. So, this omega taxonomical approach may be very much beneficial for future proteomics study.

Keywords Dendrogram, SDS-PAGE, Seed Protein,

Solanum, Sub Genera

1. Introduction

Solanum is one of the most economically valuable genera among the 98 genera of 'nightshade' family Solanaceae; which contains approximately 2700 species [1]. Members of this genus are distributed throughout the world, especially in tropical and warm temperate regions, among which largest sub-genus *Leptostemonum* or 'spinous *Solanum*' are predominant in India widely [2]. The genus is not only important for its food value but also equally valuable for its pharmaceutical demands. Several major cultivated crops of *Solanum* such as *S. tuberosum* L. (potato), *S. melongena* L. (eggplant), *S. lycopersicum* (tomato), *S. muricatum* Aiton (pepino) etc. are cultivated throughout the world and they provide lots of food security in most of the countries of developing world [2].

More than hundreds of pharmaceutically important alkaloids are found in different wild and cultivated species of *Solanum* such as solasodine, solasonine, solamargine, solanidine etc. Beside alkaloids sterols, saponins, flavonoids, fatty acids, amino acids, glycosides etc. are also present among the members of *Solanum* [3]. That is why most of the species are used as analgesic, antinarcotics,

emollient, diuretic, tonic, laxative, anticancer, antiulcer and also in different types of neuro-vegetative disorders [2-10].

This genus has very rich species diversity among angiosperms [11,12]. However, *Solanum* has a paradoxical and confusing taxonomy; though it shows much more uniformity in external appearance, it exhibits some contradictory external diversified morphology [2,9,10,13,14]. The exact calculations of divergence times of the genus *Solanum* is problematic, due to lack of fossil evidence. Many earlier workers failed to construct exact phylogenetic tree of the species of *Solanum* based on different morphological parameters associated with the taxon, which has led too much of the taxonomic confusion among the species [2,9,10].

To investigate probable phylogenetic relationship among the species of *Solanum*, genetic diversity study may be a powerful tool which has a consistent role during the course of natural evolution. Genetic diversity refers to any variation in the nucleotides, genes, chromosomes or genomes of the organism. Proteins are relatively direct gene products, because a particular protein is synthesized via translation of a specific messenger RNA, which was the transcript product of a particular gene. So, if any variation in expression of protein profile is observed, it may be stated that genetic configuration of that organism has been altered [2,9,15]. These proteins are the characteristic raw materials for the anatomical and physiological development of an organism.

The aim of present study was to determine interspecific genetic diversity among nine selected species of wild non-tuberous *Solanum* that are medicinally important as well as morphologically diversified; using total soluble seed protein profile as a dominant biochemical marker through SDS-PAGE analysis. The results of this study may provide valuable information not only to plant breeders but also to taxonomists in establishing phylogenetic relationships among wild non-tuberous *Solanum* species.

2. Materials and Methods

The selected species of *Solanum* are *Solanum nigrum* L., *Solanum americanum* Mill., *Solanum villosum* Mill., *Solanum torvum* Sw., *Solanum xanthocarpum* Schard. and Wendl., *Solanum sisymbriifolium* Lam., *Solanum macranthum* Dunal, *Solanum indicum* L. and *Solanum erianthum* D. Don. These species are collected from in and around Santiniketan, Hazaribag and Assam regions. The mature seeds of collected species are sun dried, stored in a desiccator for extraction of seed proteins.

2.1. Isolation 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 MgCl₂, 18% (w/v)

sucrose and 40 mM β-Mercaptoethanol having pH6.8. 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°C as a protein stock for protein analysis. Total soluble seed protein was estimated following the method of Bradford [16].

2.2. SDS-PAGE Analysis

Electrophoresis was carried out in a discontinuous sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) system of Laemmli [17] using 12% acrylamide gel. A total volume of 10 µl protein extract solution was loaded into each well and electrophoresis was carried out at 100 volts until the tracking dye bromophenol blue reached at the bottom of the gel. The gel was stained in the staining solution containing 44% methanol, 6% acetic acid, 500 ml double distilled water and 2.25 g of Coomassie Brilliant Blue R-250 for 45 minutes, followed by destaining done in a solution containing 20% methanol, 5% acetic acid and 750 ml of double distilled water until the background colour of gel disappeared and protein bands became clearly visible. Banding profile was photographed in gel documentation system manufactured by Perkin Elmer.

2.3. Data Analysis

Banding patterns of seed proteins of SDS-PAGE were analyzed and designated into specific types depending on the mobility of the bands. Interspecific comparisons were made by computing the Jaccard's similarity index (S.I.) values between any two selected species based on the presence (1) and absence (0) of polypeptide bands, using the formula, S.I. = Number of similar bands / Number of similar bands + Number of dissimilar bands. Hierarchical clustering or dendrogram was constructed based on the similarity index table using Word's method. The computer software SPSS vs 16 was used for this purpose. Relative mobility (R_f) value of each polypeptide band was determined by using the formula, R_f = distance migrated by proteins / distance migrated by tracking dye. The molecular weights of the dissociated polypeptides were calibrated through standard curve by using a mixture of standard broad range PMW-B protein markers from Genei, which include myosin (205 KDa), phosphorylase b (97.4 KDa), bovine serum albumin (66 KDa), ovalbumin (43 KDa), carbonic anhydrase (29 KDa), soyabean trypsin inhibitor (20.1 KDa), lysozyme (14.3 KDa), aprotinin (6.5 KDa) and insulin (3 KDa).

3. Results

Great variation was observed among the nine selected

species of *Solanum* in respect of total amount of soluble seed protein content per gram of seed. The amount of soluble seed protein ranges from 3.78 ± 0.12 to 8.67 ± 0.06 mg/g tissue and *Solanum nigrum* possesses highest and *Solanum macranthum* contains lowest amount of soluble seed protein (Table 1).

Figure 1 shows the SDS-PAGE polypeptide band patterns of soluble seed proteins of selected non-tuberous species of *Solanum*. A total of 40 major polypeptide bands of different sizes were resolved in nine selected species after SDS-PAGE of total soluble seed proteins. The number of polypeptide bands varies from 3 to 6 among the selected species of *Solanum*. *S. xanthocarpum* and *S. villosum* exhibited a greater number of bands (6); and least number of bands (3) were observed in *S. sisymbriifolium* and *S. indicum*.

Table 1. Amount of total soluble seed proteins of selected species of *Solanum*

Sl. No.	Name of the species of <i>Solanum</i>	Total amount of soluble seed proteins (mg/g tissue)
1.	<i>S. nigrum</i>	8.67 ± 0.06
2.	<i>S. americanum</i>	8.5 ± 0.09
3.	<i>S. villosum</i>	8.13 ± 0.02
4.	<i>S. torvum</i>	5.04 ± 0.08
5.	<i>S. xanthocarpum</i>	6.71 ± 0.09
6.	<i>S. sisymbriifolium</i>	5.3 ± 0.13
7.	<i>S. macranthum</i>	3.78 ± 0.12
8.	<i>S. indicum</i>	3.97 ± 0.11
9.	<i>S. erianthum</i>	6.45 ± 0.12

Figure 1. Seed protein profile of SDS-PAGE of nine selected species of *Solanum*, where S.n.=*S. nigrum*, S.a.=*S. americanum*, S.v.=*S. villosum*, S.t.=*S. torvum*, S.x.=*S. xanthocarpum*, S.s.=*S. sisymbriifolium*, S.m.=*S. macranthum*, S.i.=*S. indicum* and S.e.=*S. erianthum*; M= Molecular marker

Molecular weight of each polypeptide band was calculated using standard curve. A total of 19 polypeptide bands of different molecular weight (Table 2) was observed, which ranged from 3.2 KDa to 76.5 KDa. Polypeptide (76.5 KDa) band of highest molecular weight was observed in four species viz. *S. americanum*, *S.*

sisymbriifolium, *S. macranthum* and *S. indicum*. However, *S. xanthocarpum* shows lowest molecular weight polypeptide (3.2 KDa) band. A specific polypeptide band of 15 KDa was constantly present in six species viz. *S. nigrum*, *S. americanum*, *S. villosum*, *S. torvum*, *S. sisymbriifolium* and *S. macranthum*. Similarly, 33.3 KDa polypeptide band is also common in five species- *S. nigrum*, *S. americanum*, *S. villosum*, *S. torvum* and *S. sisymbriifolium*. This indicates that these two polypeptide bands (33.3 and 15 KDa) are dominating and they are the major bands among these five species except *S. macranthum*.

Similarity indices (Table 3) were computed based on SDS-PAGE protein profile of selected species of *Solanum*. Maximum similarity (91%) is found between *S. nigrum* and *S. villosum*. Based on SDS-PAGE seed protein profile it is observed that *S. americanum*, *S. torvum*, *S. sisymbriifolium* and *S. macranthum* do not shown any similarity with *S. erianthum*. Similarly, *S. nigrum*, *S. sisymbriifolium*, *S. macranthum*, *S. indicum* and *S. erianthum* exhibited no similarity with *S. xanthocarpum*. Any similarity is also not found between *S. indicum* and *S. torvum*. Based on similarity indices it may be clearly stated that *S. xanthocarpum* is a species which exhibited least similarity with rest of the species of *Solanum*. From the similarity indices table (Table 3) it is also clearly evident that *S. sisymbriifolium* and *S. americanum* exhibited 75% similarity, indicating that those are evolutionary closely related species. Similarly, it is also commented that *S. macranthum* is more closely related to *S. sisymbriifolium* (0.57 S.I.) than *S. torvum* (0.25 S.I.). The interrelationship of *S. americanum*, *S. nigrum* and *S. villosum* is very important for deciding their proper taxonomic status. Based on the present study, it is also observed that, similarity indices between *S. nigrum* and *S. villosum*; *S. americanum* and *S. villosum*; *S. nigrum* and *S. americanum* are 0.91, 0.73 and 0.60 respectively. Thus, it can be clearly concluded that *S. nigrum* is very closely related to *S. villosum* than *S. americanum* and it is also evident that *S. americanum* is more distantly related to *S. nigrum* than *S. villosum* particularly from evolutionary point of view.

Based on the similarity indices values, a hierarchical clustering or dendrogram (Figure 2) showing interspecific relationships was obtained using square Euclidean distance interval. This dendrogram shows two main clustered groups- Upper cluster (UC) and Lower cluster (LC). UC comprises of six species (*S. nigrum*, *S. americanum*, *S. villosum*, *S. sisymbriifolium*, *S. macranthum* and *S. torvum*). UC is further sub-divided into two sub-clusters- UC1 and UC2. *S. nigrum* and *S. villosum* are closely related and placed at UC1 cluster. But *S. americanum* is distantly related to both the species. Therefore, *S. americanum* is placed in the UC2 cluster along with *S. sisymbriifolium*, *S. macranthum* and *S. torvum*. From the dendrogram it may be predicted that within the cluster UC2, *S. americanum* is more closely related to *S. sisymbriifolium* than *S.*

macranthum and obviously from *S. torvum*. LC comprises of three species (*S. indicum*, *S. erianthum* and *S. xanthocarpum*). LC is also sub-divided into two sub-clusters- LC1 and LC2. *S. indicum* and *S. erianthum*

show 28% similarity and placed in LC1 cluster; and LC2 is represented only by *S. xanthocarpum*, which shows least or no similarity with other species of *Solanum* and thus occupies a distinct place as revealed in the dendrogram.

Table 2. Molecular weight, Rf value of total soluble seed protein profile of SDS-PAGE of selected species of *Solanum*; where *S.n.*=*S. nigrum*, *S.a.*=*S. americanum*, *S.v.*=*S. villosum*, *S.t.*=*S. torvum*, *S.x.*=*S. xanthocarpum*, *S.s.*= *S. sisymbriifolium*, *S.m.*=*S. macranthum*, *S.i.*=*S. indicum* and *S.e.*=*S. erianthum*; M= Molecular weight (KDa) of protein band and Rf = Relative mobility value of protein band

Sl. No.	<i>S.n.</i>		<i>S.a.</i>		<i>S.v.</i>		<i>S.t.</i>		<i>S.x.</i>		<i>S.s.</i>		<i>S.m.</i>		<i>S.i.</i>		<i>S.e.</i>	
	M	Rf	M	Rf	M	Rf	M	Rf	M	Rf	M	Rf	M	Rf	M	Rf	M	Rf
1	-	-	76.5	0.23	-	-	-	-	-	-	76.5	0.23	76.5	0.23	76.5	0.23	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	66	0.28
3	52.35	0.37	52.35	0.37	52.35	0.37	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	49.05	0.39	-	-	-	-
5	-	-	42.9	0.43	42.9	0.43	-	-	42.9	0.43	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	38.4	0.48	-	-
7	-	-	-	-	-	-	35.85	0.51	-	-	-	-	-	-	-	-	-	-
8	33.3	0.54	33.3	0.54	33.3	0.54	33.3	0.54	-	-	33.3	0.54	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-	32.4	0.55	-	-	-	-
10	30.3	0.58	-	-	30.3	0.58	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	25.05	0.65	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	23.1	0.69
13	-	-	-	-	-	-	20.7	0.72	20.7	0.72	-	-	-	-	-	-	-	-
14	17.25	0.75	-	-	17.25	0.75	-	-	-	-	-	-	-	-	17.25	0.75	17.25	0.75
15	15	0.79	15	0.79	15	0.79	15	0.79	-	-	15	0.79	15	0.79	-	-	-	-
16	-	-	-	-	-	-	-	-	12	0.84	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	5.25	0.89	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.3	0.94
19	-	-	-	-	-	-	-	-	3.2	0.95	-	-	-	-	-	-	-	-

Table 3. Jaccard's Similarity index of selected species of *Solanum* based on seed protein profile study, where *S.n.*=*S. nigrum*, *S.a.*=*S. americanum*, *S.v.*=*S. villosum*, *S.t.*=*S. torvum*, *S.x.*=*S. xanthocarpum*, *S.s.*= *S. sisymbriifolium*, *S.m.*=*S. macranthum*, *S.i.*=*S. indicum* and *S.e.*=*S. erianthum*

Species name	<i>S.n.</i>	<i>S.a.</i>	<i>S.v.</i>	<i>S.t.</i>	<i>S.x.</i>	<i>S.s.</i>	<i>S.m.</i>	<i>S.i.</i>	<i>S.e.</i>
<i>S.n.</i>	1.00								
<i>S.a.</i>	0.60	1.00							
<i>S.v.</i>	0.91	0.73	1.00						
<i>S.t.</i>	0.44	0.44	0.40	1.00					
<i>S.x.</i>	0.00	0.18	0.17	0.20	1.00				
<i>S.s.</i>	0.50	0.75	0.44	0.57	0.00	1.00			
<i>S.m.</i>	0.22	0.44	0.20	0.25	0.00	0.57	1.00		
<i>S.i.</i>	0.25	0.25	0.22	0.00	0.00	0.33	0.28	1.00	
<i>S.e.</i>	0.22	0.00	0.20	0.00	0.00	0.00	0.00	0.28	1.00

4. Discussion

Solanum is a taxonomical paradox, exhibiting both uniformity and extreme diversity in its morphology [13]. Hence, morphological markers as used in the past are insufficient for their correct and proper identification and placement in taxonomical group [2,9,10]. Various classical, experimental and numerical studies have demonstrated that, this complexity can be attributed to a number of causes such as phenotypic plasticity and genetic variation [18,19]. A high level of genetic polymorphism has been detected among the non-tuberous members of *Solanum*. Despite their morphological similarity, diversity was exhibited by the extensive polymorphism at the DNA and protein levels. Proteins are the direct gene products, i.e. they are generated from the transcriptionally active parts of DNA, called genes. Thus, protein profile is in general regarded as the active and direct reflection of genomic configuration. The advantage behind the protein marker based phylogenetic study is that, the non coding portions of the DNA are not involved in this process. It is already known that the percentage of non-genic portion is quite significant in comparison to gene bearing region within DNA [20]. So, interference of non-coding portion of DNA is totally nullified during the analysis of phylogenetic relation of *Solanum* plants based on poly peptides or protein through SDS-PAGE. Thus, this study has close resemblance with DNA marker based phylogenetic study to some extent.

Seed protein profile obtained by SDS-PAGE has been successfully employed to resolve the taxonomic disputes of different medicinally important non-tuberous species of *Solanum*. Variation in the major polypeptide profile indicates substantial differences in amino acid composition and represents genetic differences among the species [21]. In the past, taxonomic position of *S. nigrum*, *S. villosum* and *S. americanum* remained highly paradoxical and controversial. Clarke [22] did not separate them and considered all of the three species as *Solanum nigrum*. Hawkes and Edmonds [23] gave the rank of subspecies to *S. villosum*; and *S. americanum* was totally overlooked by him. While Edmonds and Chweya [7] gave separate rank to all of these three species. Morphologically these three species exhibit marked similarity.

Figure 2. Dendrogram showing genetic relationship among nine selected species of *Solanum* based on SDS-PAGE seed protein profiling

The status of taxa has been justified by protein profile

analysis [24]. In the present investigation, clear differentiation among *S. nigrum*, *S. americanum* and *S. villosum* has been revealed in the SDS-PAGE seed protein profiles (Figure 1). It is detected from the similarity indices (Table 3) that *S. villosum* is more closely (91%) related to *S. nigrum* as compared to *S. americanum*. Thus, inter-similarity between *S. americanum* with *S. nigrum* is 60% and that of *S. americanum* and *S. villosum* is 73%. These values of similarity indices based on seed protein profile favour the status of species for *S. nigrum* and *S. americanum*. These findings are directly reflected also in the dendrogram, where *S. nigrum* and *S. villosum* exhibit a high similarity and thus placed in the same cluster UC1 (Figure 2). The clustering of these species indicates a close evolutionary relationship and suggests the common origin of the two taxa. This assumption of common origin is already confirmed and supported by several workers [25,26], using different numerical analysis. From the dendrogram it is also clearly evident that *S. villosum* gets separated from *S. nigrum* only at the 3.6% of segregating distance (Figure 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 [23], Baytop [27] and Yousaf *et al.* [28] but contrary to Edmonds and Chweya [7], Edmonds and Glidewell [29] and Nasir [30].

Based on seed protein analysis D'Arcy [31] placed *S. sisymbriifolium* and *S. torvum* in sections *Cryptocarpum* and *Torva* respectively and were located on separate branches of the UPGMA dendrogram. Our results also corroborate with Karihaloo *et al.* [32], where similarity between *S. sisymbriifolium* and *S. torvum* is 57% and occupy same cluster UC2 (Figure 2) and get separated from *S. torvum* at the 29% of segregating distance on dendrogram. Whalen [33] 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. Though in the present study *S. sisymbriifolium* shows no similarity with *S. erianthum* and *S. xanthocarpum* but maintain moderate similarity (33%-75%) with rest of the selected species and showing highest similarity (75%) with *S. americanum* based on seed protein profile study. Although there may be lots of morphological distinctions present between *S. sisymbriifolium* and *S. americanum*; they become very close to each other based on this molecular marker and get separated only by 11% segregating distance on dendrogram (Figure 2). Therefore, sometimes molecular marker exhibits more pronounced effects than conventional morphological characters.

S. macranthum, the giant potato tree or giant star potato is a tropical, perennial, large, woody, bushy shrub that can grow into a medium-sized tree, which bears beautifully stunning flowers throughout the year. This plant exhibits distinct morphological similarity with *S. torvum*, which is also a perennial, woody shrub of comparatively small

height (2-3 m). These two species show 25% similarity (Table 3) in their seed protein profiles and have been placed within same cluster UC2 and get separated 40% from each other on dendrogram (Figure 2).

S. erianthum is medicinally important plant, contains steroidal saponins and free genins as well as steroidal alkaloids of the spirosolane group [34]. This species has quite morphological resemblance with *S. indicum*. The resemblance is due to same life form, simple, ovate-elliptical, slightly wavy, densely wooly hairy, acute leaves, globose berry etc. This morphological similarity is reflected in our results, which shows 28% similarity (Table 3) based on seed protein profile and placed within same cluster LC1 (Figure 2).

S. xanthocarpum or yellow berried nightshade is a road side, medicinally important, perennial, prostrate herb with scattered stellate hairs and zigzag branched with prickly stem, showing morphologically distinctive features than rest of the selected species of *Solanum*. In the past the nomenclature of *S. xanthocarpum* remained controversial. Burmanii [35] described this plant first and gave the name of the plant as *S. surattense*. Later various names have been given by several taxonomist of different era. Schard and Wendelbo gave the most accepted and commonly used synonym of *S. surattense* as *S. xanthocarpum* [36]. Phenotypically this species is highly polymorphic [2,9,10,36]. Therefore, a great deal of taxonomic confusion exists in this species. In our study, most remarkable observation is that, the SDS-PAGE protein profile of this particular species exhibited 2-3 polypeptide bands of comparatively lower molecular weight out of 6 bands found in the other studied species of *Solanum* (Table 2; Figure 1). From this protein profile study of *S. xanthocarpum* it could be assumed that, this species may possess many genes of short nucleotide length, which supports its uniqueness and shows no similarity with majority of the species of *Solanum*. Thus, on dendrogram only this species remains isolated and placed in LC2 cluster (Figure 2).

Based on the electrophoresis results, it may be suggested that the selected species of *Solanum* can be divided into two sub-genera A and B. Sub-genus B comprises of *S. indicum*, *S. erianthum* and *S. xanthocarpum* and sub-genus A comprises of rest of the selected species of *Solanum* (Table 4). Therefore, this lower order taxonomy of *Solanum* genus is different from conventional classification.

Table 4. Proposed classification of medicinally important selected species of the genus *Solanum*, based on seed protein profile

Genus	Sub genus	Species
<i>Solanum</i>	A	<i>S. nigrum</i> , subsp. <i>S. villosum</i> , <i>S. americanum</i> , <i>S. sisymbriifolium</i> , <i>S. macranthum</i> , <i>S. torvum</i>
	B	<i>S. indicum</i> , <i>S. erianthum</i> , <i>S. xanthocarpum</i>

5. Conclusions

The taxonomy of *Solanum* is addressed by momentous changes, which can be attributed to the advent of molecular genetic techniques together with the combination of various biochemical and morphometric parameters. The evolving view about this diverse genus will lead to significant changes in phylogenetics. Thus, the concept of species complex (various morpho and cytotypes existing in the populations of the same species) described in *Solanum nigrum* for quite a long time has now been changed and presently three species have been described based on their diversified characteristics (*S. nigrum*, *S. villosum* and *S. americanum*). Therefore, understanding the relationship in this genus, which is such a paradox of uniformity and hyper diversity, will have repercussions for other genera. The SDS-PAGE study based on seed protein profiles is a powerful tool to achieve a clear understanding of the molecular systematics as well as the correct identification of genotypes. This investigation has provided new information and reinforced suggestions made in previous phylogenetic studies, which were not the last word regarding evolutionary relationships within *Solanum*.

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