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EVALUATION OF TAXONOMIC STATUS OF MEDICINAL SPECIES OF THE GENUS *SOLANUM* AND *CAPSICUM* BASED ON POLY ACRYLAMIDE GEL ELECTROPHORESIS

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Abstract

Seed protein profiles of 54 accessions belonging to 11 species of 2 different genera (*Solanum* and *Capsicum*) of the family Solanaceae were analyzed by SDS-PAGE. Intra and inter specific relationship was estimated using Jaccard's similarity index. A dendrogram based on UPGMA revealed the generic status of *Solanum* and *Capsicum. S. surattense* with white flowers showed variation from the *S. surattense* with purple flowers not only morphologically but also based on protein profiles. However the high similarity index (82%) between them indicates that *S. surattense* (W) should be separated from *S. surattense* (P) as variety nova. *S. nigrum* and *S. americanum* are two distinct species, whereas *S. villosum* is the subspecies of *S. nigrum*. Similarity index of *S. villosum* and *S. americanum* was 53 % whereas it has similarity of 78% with *S. nigrum.* Similarity was 41% between *S. nigrum* and *S. americanum*. Based on the total seed protein profile, the genus *Solanum* can be divided into two sub genera. The distribution of species in these two subgenera is contrary to conventional classification. The present study provides useful information for the identification of the taxa, their relationship and the delimitation of their taxonomic status.

Introduction

Solanum and *Capsicum* are two medicinally important genera of the family Solanaceae and distributed in both tropical and temperate regions of the world. In Pakistan *Solanum* is represented by 15 species, of which 12 species have the medicinal properties (Nasir, 1985). *Capsicum* has two species, and only *C. frutescence* has therapeutic properties (Odeigah *et al*., 1999). Species of *Solanum* are useful in the headache, heart burning and heat of stomach (Edmonds & Chewya, 1997). Kirk (1927) and Ary & Gregory (1972) mentioned *S. nigrum* as a poisonous species. It is however being used as a cardiovascular stimulant and useful in lowering blood pressure and breaking down of cholesterol build-up. The warming properties of *Capsicum* are useful for people suffering from poor circulation in hands and feet, as it is a rich source of vitamin C and Capsaicine (Odeigah *et al*., 1999).

Most of the taxonomic information of these genera accumulated so far is based solely upon morphometry and has left many issues unresolved. *Solanum nigrum, S. americanum* and *S. villosum* are three medicinally important species of the genus *Solanum* (Jennifer & James, 1997). In the past their taxonomic status remained highly controversial. Clarke (1885) did not separate them and considered all of the three species as *S. nigrum*. Hawkes & Edmond (1972) gave the rank of subspecies to *S. villosum* and Baytop (1978) also found similar results. Nasir (1985) considered *Solanum nigrum* as species and *villosum* as the variety while Jennifer & James (1997) gave rank of species to all of these. Morphologically these species are very much similar. However they differ for one or two morphological traits. Similar kind of confusion related to morphology was observed in herbarium samples of *S. surattense* collected from the different areas of Pakistan.

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Table 1. Taxa of *Solanum* and *Capsicum* used in seed-protein electrophoresis analysis.

Species	Samples	Accessions	Localities
S. anguivi	1	Green house sample	Attock
S. incanum	3	78053, 11713, 29107	Rawalpindi, Attock, Choa Saidan Shah
S. torvum	1	3403	Bannu
S. melongena	5	56100, 90648, 106589, 73158, 46168	Kotli, Mianwali, Khyber, Quetta, Hyderabad
S. pseudo-capsicum	4	1465, 1263, 1975, 8363	Rawalpindi, Hazara, Shogran to Paras, Kashmir
S. cordatum	1	27868	Attock
S. erianthum	4	11666, 11811, 1570, 90704	Rawalpindi, Islamabad, Hazara, Muzaffarabad
S. surattense (P1)	3	11612, 68199, 29165	Tarnol, Hazara, Mangora
S. surattense (W)	3	79684, Freshly collected sample	Tarnol, Attock
S. surattense (P2)	3	43370, 44972, 112667	Lahore, Mianwali, Poonch
Solanum nigrum	6	11643, 56001, 47091, 46373, 88332, 47056,	Rawalpindi, Attock, Sialkot, Narowal, Sargodha, Mianwali
S. villosum	6	56090, 56055, 47080, 90697, 79696, 46182	Kotli, Mirpur, Muzaffarabad, Rawalpindi, Bannu
S. americanum	8	69940, 116328, 29113, 47001, 11710, 47083, 51333, 56102	Muzaffarabad, Leppa valley, Rawalpindi, Gujar Khan, Kala Chitta hills, Sahiwal, Sargodha, Chitral
C. frutescence	6	Green house, Freshly collected samples	Karachi, Mianwali, Rawalpindi, Attock

P= purple flowers, W= White flowers

Morphometery could not provide solution of complex taxonomic problems. Modern biological techniques are now available that can resolve these issues. In recent years SDS-PAGE of total seed proteins has found wide application in resolving systematic relationships and for inter and intra specific studies (Karihaloo *et al*., 2002). Khalifa *et al*. 1998 used SDS-PAGE to reassess the taxonomic relationships of 45 species belonging to 15 genera and 8 tribes of the Solanaceae. Based on the results they supported the conventional classification of this family. Two important species *C. annum* and *S. melongena* of the same family were also analyzed for seed protein (Karihaloo *et al*., 2002; Anu & Peter, 2003). SDS-PAGE was found effective for phylogenetic studies in these species. Edmonds & Glidewell (1977) confirmed the origin of *S. nigrum* from *S. americanum* and *S. villosum* by PAGE of seed proteins.

The aim of the present study was to find out the solution of existing taxonomic problems of species, which overlap in most of their morphological characters, and to elucidate relationship of the critical taxa by utilization of SDS-PAGE.

Materials and Methods

The taxa of *Solanum* and *Capsicum* used for electrophoretic analysis are presented in Table 1.

Fifty-four accessions belonging to 11 species of 2 different genera viz., *Solanum* and *Capsicum* from the family Solanaceae were used in this investigation. Total seed proteins were extracted from 0.01g of seed flour using 400µl of extraction buffer that contained 0.05M Tris-HCl pH 8.0, 0.2% SDS, 5M Urea, and 1% Mercaptoethnol. Seed flour was thoroughly mixed with buffer by vortexing. The extracted protein was separated by centrifuging the sample @ of 15000rpm for 10 mins. Electrophoresis was carried out in a

discontinuous SDS-PAGE system of Laemmli (1970) using 12.25% acrylamide gel. Electrophoresis was run at 100V. The gels were stained in the staining solution containing 44% methanol, 6% acetic acid, 500ml distilled water and 2.25g of coomassie brilliant blue for 45mins. Destaining was done in a solution containing 20% methanol, 5% acetic acid and 750ml of distilled water until the background color disappeared and protein bands were clearly visible.

Data analysis: Protein bands were scored depending on their presence (1) or absence (0). Jaccard's similarity indices were determined and hierarchical clustering was constructed by unweighted pair group method with arithmetic average (UPGMA). The computer software SPSS v ll.0 was used for this purpose.

Results

Allelic variation among the medicinally important species was studied on 12.25% acrylamide gel. Altogether 21 protein bands were observed on this concentration of gel.

The relative position of protein bands of different species of *Solanum* and *Capsicum* on 12.25% gel is presented in Fig. 1. The intensity of bands is represented by three different colors. Band 21 is present in all the species so it can be considered family specific band. Band 1 and 3 are characteristically present in one species each. Band 1 is recorded in *S. pseudo-capsicum* whereas band 3 is present in *S. americanum*. These bands can be identified as the species specific bands. The *S. surattense* (P1 and P2) are exactly similar. The intensity and number of bands are same in both specimens (Fig. 1). However *S. surattense* (P1 & P2) and *S. surattense* (W) differ from each other based on the protein profile. *S. surattense* (W) has 10 protein bands while *S. surattense* (P1 & P2) has 12 protein bands. Band 9 is present in *S. surattense* (W) but absent from other two samples. Similarly band 10, 12, and 13 are the part of protein profile of *S. surattense* (P1 &P2) and absent from *S. surattense* (W). The difference also lies in the intensities of the bands. Band 8 is of low intensity in *S. surattense* (W) but of intermediate in *S. surattense* (P1 & P2). The electropherogram of *S. nigrum* and *S. americanum* has 8 and 6 bands, respectively, whereas *S. villosum* has 8 bands of different intensities.

Jaccard's similarity indices were computed based on protein profile. The range of similarity found among the species is 0.004-0.82. Minimum similarity (0.004) is found between *S. americanum* and *C. frutescens.* The similarity indices for certain species is negative. The relationship of *S. anguivi* to *S. erianthum*, *S. incanum* to *S. cardatum* and *S. torvum* to *S. pseudo-capsicum* is also negative. *S. pseudo-capsicum* has negative relation with 7 species (Table 2). This species has only 2% similarity to *C. frutescence*. The highest similarity (12%) was exhibited by *S. pseudo-capsicum* with *S. cardatum. Solanum pseudo-capsicum* also made a separate cluster suggesting that it is highly distinct species. The maximum level of similarity indices is found among the *S. surattense* specimens. *Solanum surattense* having white flowers has 82% similarity with purple flower *S. surattense.* The interrelationship of *S. americanum*, *S. nigrum* and *S. villosum* is very important for deciding their taxonomic status. *S. villosum* is more closely related to *S. nigrum* rather than *S. americanum*. Similarity index of *S. nigrum* to *S. villosum* is 0.78 (Table 2) whereas the index for *S. americanum* to *S. villosum* is 0.53. The inter similarity of *S. nigrum* and *S. americanum* is 0.41.

Table 2. Jaccard's similarity indices of medicinally important species of the genus *Solanum* and *Capsicum* based on SDS-PAGE

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1													
2	0.62	1												
3	0.30	0.15	1											
4	0.44	0.30	0.41	1										
5	0.08	0.04	-0.17	-0.13	1									
6	0.22	-0.09	0.27	0.22	0.12	1								
7	-0.03	0.19	0.15	0.22	-0.36	-0.02	1							
8	0.28	0.23	0.48	0.48	-0.46	0.12	0.31	1						
9	0.38	0.23	0.48	0.48	-0.28	0.25	0.20	0.82	1					
10	0.28	0.23	0.48	0.48	-0.46	0.12	0.31	1.00	0.82	1				
11	0.56	0.30	0.30	0.44	-0.03	0.07	-0.03	0.48	0.58	0.48	1			
12	0.56	0.30	0.18	0.22	-0.03	0.07	0.09	0.48	0.58	0.48	0.78	1		
13	0.41	0.26	0.37	0.53	-0.06	0.27	0.15	0.48	0.48	0.48	0.41	0.53	1	
14	0.32	0.47	0.21	0.22	0.02	0.08	-0.06	0.20	0.29	0.20	0.12	0.12	0.004	1

1: *S. anguivi*, 2: *S. incanum*, 3: *S. torvum*, 4: *S. melongena*, 5: *S. pseudo-capsicum*, 6*: S. cardatum*,7: *S. erianthum*, 8: *S. surattense* (P1), 9: *S. surattense* (W) 10*: S. surattense* (P2), 11: *S. nigrum*, 12: *S. villosum*, 13: *S. americanum*, 14: *C. frutescens*

Dendrogram (Fig. 2) represents the division of species into two main groups (group 1 and 2). The group 1 is occupied by the *Capsicum frutescense* while *Solanum* species comprise group 2. All the species of *Solanum* are separated into two sub groups A and B. Sub group A is occupied by *S. pseudo-capsicum*, whereas sub group B consist of all other species of *Solanum*. *Solanum pseudo-capsicum* gets separated from all other species at the distance of 78. The genetic variability between *S. surattense (*P1) and *S. surattense* (P2) is only 5%.

Discussion

Seed protein profile obtained by electrophoresis has been successfully used to resolve the taxonomic problems of *Solanum* and *Capsicum* species. *Solanum americanum*, *S. nigrum* and *S. villosum* are phenotypically close to each other, and have controversial taxonomic status. From medicinal point of view they are being used for headache, heartburn, ear pains and eye inflammation (Jennifer & James 1997). Morphological similarities of characters contributed a lot toward the difficulty in identification of these species. There are very few morphological markers for distinguishing them. *Solanum americanum* can be distinguished from *S. nigrum* by its smaller seeds, umbellate inflorescence rather than the raciform as in *S. nigrum*, smaller anthers and shiny fruit (Edward, 1999). *S. nigrum* can be separated from *S. villosum* by yellow, orange or red colored fruit, its rotate corolla, white or purplish flowers. Whereas *S. americanum* can be differentiated from *S. villosum* because of purplish colored stem, subumbellate inflorescence, abaxially pubescent corolla, cup-shaped calyx, short filament and shiny black berry. Other important morphological marker is peduncle length in comparison of petiole length. *S. villosum* has peduncle and petiole of same length, while in *S. nigrum* and *S. americanum* peduncle is longer than petiole. However most of the morphological markers used for distinction are only prominent in fresh plant but lost in herbarium sample.

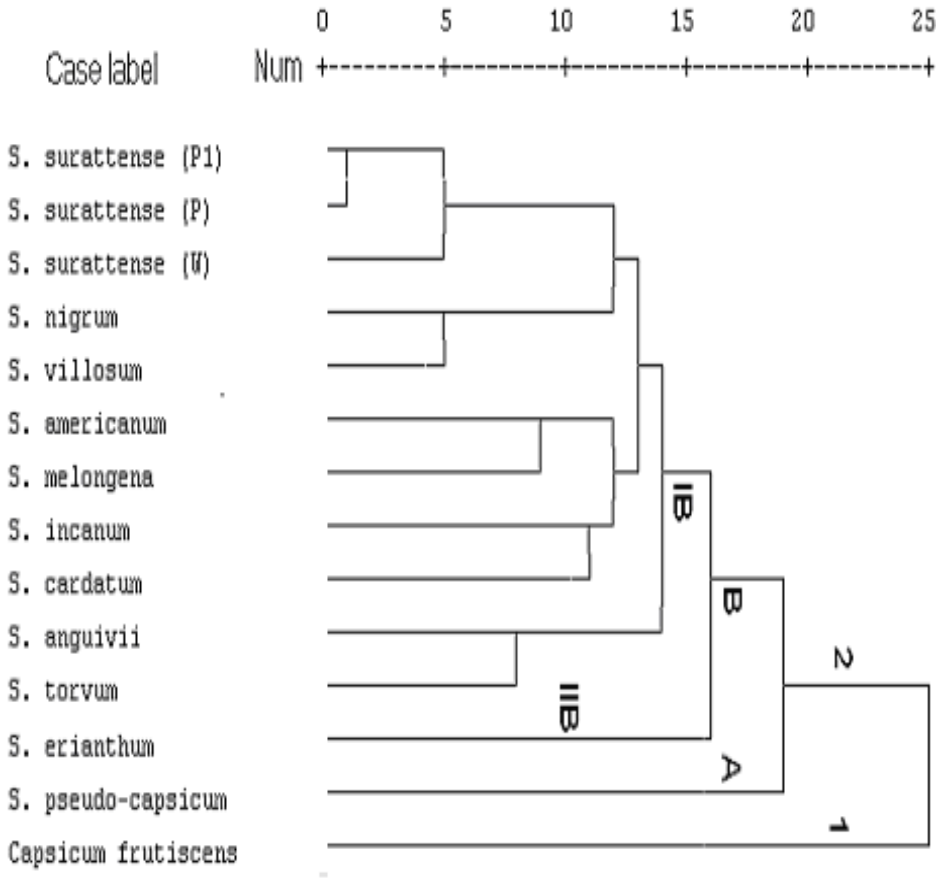

Fig. 2. A dendrogram of the medicinally important species of *Solanum* and *Capsicum*.

Protein profile shows clear differences between *S. nigrum*, *S. americanum* and *S. villosum* (Fig. 1). The major differentiating bands are 3, 7,9,13 and 20.It is obvious from the similarity indices that *S. villosum* is more closely related to *S. nigrum* as compared to *S. americanum*. Similarity index of *S. villosum* and *S. americanum* is 53% and for *S. nigrum* is 78% (Table 2). The inter similarity of *S. nigrum* and *S. villosum* is 41% (Table 2). These values of similarity indices favor the status of species for *S. nigrum* and *S. americanum*. However *S. villosum* is very close (78%) to *S. nigrum*. On dendrogram the *S. villosum* get separated from *S. nigrum* only at the 20% of segregating distance (Fig. 2). This distance revealed to consider *S. villosum* as the subspecies of *S. nigrum*. Our results are in accordance to Hawkes & Edmond (1972) and Baytop (1978) but contrary to Nasir (1985) and Jennifer & James (1997). Nasir considered it as variety of *S. nigrum* whereas Jennifer & James (1997) suggested status of species for *S. villosum*.

The status of critical taxa can be justified by using protein profile (Khan, 1992). *Solanum surattense* is another medicinally important species of the family Solanaceae. Different parts of this plant are being used as an expectorant, for sore throat and to relieve local pain (Hocking, 1958). Phenotypically this species is highly polymorphic.

Morphological characters such as point of origin of spines, arrangement of spines, presence of hairs on petiole and stem, flower colour, length of filaments and style showed polymorphism. Three specimens of *S. surattense* with different characters as mentioned above were selected for electrophoresis. The protein profile of *S. surattense* (P1) and *S. surattense* (P2) (vary from each other due to difference of arrangement of spines, length of filaments and style) was almost similar, however, *S. surattense* (W) showed variation. The similarity index of S. surattense (P1) and S. surattense (P2) is 100% whereas it was 82% in case of *S. surrattense* (W). Therefore specimens labelled as *S. surattense* (P1) and *S. surattense* (P2) could not be separated. It become obvious that point of origin of spines, arrangement of spines, length of filament and style are not enough markers for the differentiation of taxas. *S. surattense* (W) should be given a separate rank. As the similarity indices are very high therefore it can be treated as the variety of the *S. surattense*. The results of SDS-PAGE favor the importance of flower colour, the presence of hairs on stem and petiole for the differentiation of lower order taxa in case of *S. surattense*.

Solanum pseduo-capsicum contains the alkaloids solanine. The juice of the plant is diuretic and being used for the treatment of problems of urinary track (Nasir, 1985). This species has resemblance with *C. frutescens.* This resemblance is due to same life form, simple hairs on branches, short and obsolete Peduncle, white corolla and discoid seed. The dissimilarity between these two species is because of leaf shape (narrow oblong-lanceolate/ovate to lanceolate), inflorescence position (leaf opposite or solitary axillary/ axillary cymose), and dehiscence of anthers (by apical pore/longitudinal opening) and number of locules (2-5/2-3) present in ovary. These dissimilarity markers separated *S. pseudo-capsiucm* from *Capsicum* make it closer to *Solanum* species (Rechinger, 1958; Baytop, 1972). The protein profile of the *S. pseudo-capsicum* is different from the rest of the *Solanum* species therefore it segregated into separate group. The similarity indices justifies the presence of *S. pseudo-capsicum* in the *Solanum* genus because for *C. frutescens* its only 0.4% (Table 1). Based on the electrophoresis results it is suggested to divide the genus *Solanum* into two sub genera A and B. Sub genus B will consist of only *S. pseudo-capsicum* and all other species will be the part of sub genus A (Table 3). This lower order taxonomy of the *Solanum* genus is different from conventional classification. Schenobeck-Temesy (1972) divided the genus into two subgenera and six sections. He placed *S. pseudo-capsicum* and *S. nigrum* together into subgenus *Solanum*, whereas *S. melongena, S. incanum, S. cardatum* and *S. surattense* into subgenus *Leptostemonum.*

Table 3. Proposed classification of medicinally important species of the genus *Solanum*.

Genus	Subgenus	Species
Solanum	A	*S. nigrum,* subspp *S. villosum, S. americanum, S. anguivi, S. torvum, S. erianthum, S. melongena, S. incanum, S. cardatum, S. surattense, S. surattense Var. nova*
	B	*S. pseudo-capsicum*

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