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Analysis of Genetic Diversity using Cytological Characters and Protein Profiling in Some Medicinal Plants of Solanaceae Family

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Abstract

Genetic variation of plants/species is very interesting in terms of minimizing genetic vulnerability as well as stabilizing production. In this regard, a study was conducted to assess the genetic diversity among five selected medicinal plants (i.e. *Withania somnifera*, *Solanum xanthocarpum*, *Capsicum annum*, *Datura stramonium* and *Solanum nigrum*) of family Solanaceae by using cytological characters viz. mitotic index and protein profiling. Present investigation revealed that *Datura stramonium* had highest mitotic index i.e. 45.46% meaning that species has highest power of division while *Withania somnifera* had the lowest 22.50%. Total seed storage protein profiles were also estimated through SDS-PAGE. At protein profile level, total 51 protein bands were recorded, 50 bands show polymorphism (98.04%) and only one band show monomorphism (1.96%). The similarity coefficient calculated based on obtained bands ranged from 0.03 to 0.33. The greatest similarity (0.33) was observed between *Solanum xanthocarpum* and *Capsicum annum*, while lowest similarity (0.03) was obtained between *Solanum xanthocarpum* and *Solanum nigrum*. Upon UPGMA analysis the dendrogram grouped the five selected medicinal plants into two clusters. Cluster I am comprising of only one species *Withania somnifera* and cluster II comprising of three species i.e., *Datura stramonium*, *Solanum xanthocarpum* and *Capsicum annum*. While the species *Solanum nigrum* occupies a distinct group as revealed in the dendrogram. It is evident from the dendrogram that the species *Solanum xanthocarpum* and *Solanum nigrum* are genetically diverse, hence it is recommended that these species could be utilized for future breeding programs to create higher amount of genetic variability in selected medicinal plants.

Keywords
Genetic Diversity, Mitotic index, SDS-PAGE, Solanaceae, UPGMA

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INTRODUCTION

Solanaceae is the largest and economically most important families of angiosperms that include a number of important agricultural plants, vegetables, ornamentals and many medicinal plants [1, 2]. The Solanaceae family consists of 3000-4000 species which are divided into 90 genera. The family is highly diverse, includes perennial trees as well as herbaceous annual species and occupies a wide range of terrestrial habitats from deserts to tropical rainforests [3]. The species of the family are mostly occurred in tropical and temperate regions with centers of diversity in Southern hemisphere, particularly in South America [5]. Several plants/species of the family attained importance in human civilization as food sources (Tomato, Potato, Eggplant, and Pepper), ornamentals (Datura, Petunia, and some Solanum species) and drugs (Atropa, Tobacco, Ashwagandha, Hyoscyamus) [4].

Genetic markers such as morphological and biochemical characteristics (protein profiles) are powerful tools for the analysis of genetic diversity

and relatedness among genotypes, species, and large plant communities. Although morphological traits can be used for assessing genetic diversity, but morphological data may not provide an accurate indication of genetic diversity because of environmental influences upon the expression of observed traits [6]. In view of these difficulties, the introduction of biochemical techniques has made possible and a more accurate evaluation of genetic variations; bringing greater precision to measures of genetic diversity [7]. The use of biochemical markers to estimate the genetic diversity has received much attention in recent years. A significant number of species can be characterized for biochemical markers in a short period of time. Moreover, the data more accurately reflects genetic variations because biochemical markers are direct products of genes and their expression is not affected by environmental situations [8, 9, 10].

Among the biochemical techniques, SDS-PAGE is an inexpensive, simple method that provides the best resolution in the identification of germplasm by protein patterns and is one of the most commonly used method for describing the seed protein diversity of crop germplasm [11, 12, 13,]. Nowadays, this method is considered as a low cost, high reproducible and rapid approach, because of that it became accepted valuable tool [15]. Seed storage protein profiling aids in the identification and characterization of diversity in plant species as well as phylogenetic relationship of the species, generating potential information to supplement evaluation and passport data [16]. In view of above consideration, the present investigation was initiated to analyze the genetic diversity within selected species of family Solanaceae based on cytological characters and seed protein profile by using SDS-PAGE [17].

MATERIAL AND METHODS

Plant sample

The selected Solanaceae species *Withania somnifera*, *Solanum xanthocarpum*, *Capsicum annum*, *Datura stramonium* and *Solanum nigrum* were identified and collected from various locations of Chitrakoot regions. The collected samples were kept in deep freezer for further use.

Mitotic Index

Mitotic index was analyzed from the root tips of selected medicinal plants of Solanaceae family. The root tips were harvested between 5 AM to 7 AM in the morning and transferred them in fixative i.e. acetoalcohol (1:3 glacial acetic acid: alcohol). After 12 hours of fixation, the root tips were preserved in 70% alcohol. Mitotic index of the root tips observed is calculated by using the given formula-

$$\textbf{Mitotic index} = \frac{\textbf{Number of dividing cells}}{\textbf{Total number of cells studied}} \textbf{X100}$$

Protein profiling (SDS-PAGE)

Isolation of protein

The seed samples of selected species were ground using mortar and pestle. 100 mg seed flour was transferred to each eppendrof tube with addition of 500 µl extraction buffers (500 mM Tris HCl, pH 7.5, 9M UREA, 2% Beta Mercaptoethanol, 0.7 M Sucrose and 0.5 M Sodium Chloride and Protease Inhibitor Cocktail). The homogenate sample was shaken vigorously for 25 minutes at 4ºC and then centrifuged at 5000 rpm for 30 minutes at 4ºC. The extracted proteins were recovered as supernatant and stored in the refrigerator for further use.

Protein estimation

`The protein estimation was done by Bradford method. Measured the absorbance of the sample, calibration standards and reference at 595 nm. A calibration plot was prepared by graphing the net A_{595} values for standards versus protein concentration and determines the protein concentration for the sample by interpolation from the plot.

Electrophoresis

The electrophoretic procedure was carried out in the discontinuous buffer system in a vertical electrophoresis apparatus using 10% polyacrylamide gel. A 10% separating gel (1.5 M Tris HCl, pH 8.8, 10% SDS) and 5% stacking gel (1M Tris HCl, pH 6.8, 10% SDS) was prepared and polymerized by addition of TEMED and 10% Ammonium Per Sulphate. The electrode buffer solution (250 mM Glycine, 25 mM tris base, 10% SDS and pH adjusted to 8.3) was poured to the top pool of the apparatus. 10 microliter of the extracted protein was loaded with the help of micropipette into each well of the gel. Electrophoresis was conducted at a constant current 70 V until the tracking dye reached to the bottom of the gel. The gel was stained in Coomassie Brilliant Blue G250 and destained in methanol and Acetic Acid for 30 minutes until the background was clear enough for band scoring.

Data analysis

The molecular weights of the dissociated proteins were determined by using standard curve. The gels were scored for the presence (+) or absence (-) of every protein band. These data were used to

calculate similarity coefficient using gel analyzer 19.1. Depending upon the presence or absence of protein bands between selected species, similarity index was calculated using following formula-

$$\textbf{Similarity index} = \frac{A}{X+Y} X100$$

Where, A= number of common bands between two samples, and X+Y = total number of bands shared in two samples.

Dendrogram was constructed to elucidate the variations among the selected plants studied. It was constructed by using software "Dendro UPGMA- A dendrogram construction utility" and analyzed with clustering method of UPGMA. Mobility of protein in SDS-PAGE was expressed as relative mobility (RF) with respect to the tracking dye. RF value of each band was calculated as follows-

RF= Distance migrated by the protein band from origin/ Distance migrated by tracking dye.

RESULTS AND DISCUSSION

Significant variations were observed in cytological characters (viz. mitotic index) and protein profiling (viz. SDS-PAGE) among five selected medicinal plants belonging to family Solanaceae. Cytological study was done to find out mitotic index in different selected medicinal plants of Solanaceae family. The highest percentage of mitotic index was found 45.46% in *Datura stramonium*, followed by 36.00% in *Solanum nigrum*, 35.71% in *Solanum xanthocarpum* and 34.48% in *Capsicum annum*. While the lowest percentage of mitotic index was found 22.50% in *Withania somnifera*. The cytological investigation revealed that *Datura stramonium* had highest value of mitotic index i.e. 45.46% which revealed that this species had highest power of division among the present experimental species as compared to other species viz. *Withania somnifera*, *Solanum nigrum*, *Solanum xanthocarpum* and *Capsicum annum*. Different workers from time to time analyzed cytological characters among different plants/species and revealed different rates of genetic variations [18, 19, 20, 21, 22, 23].

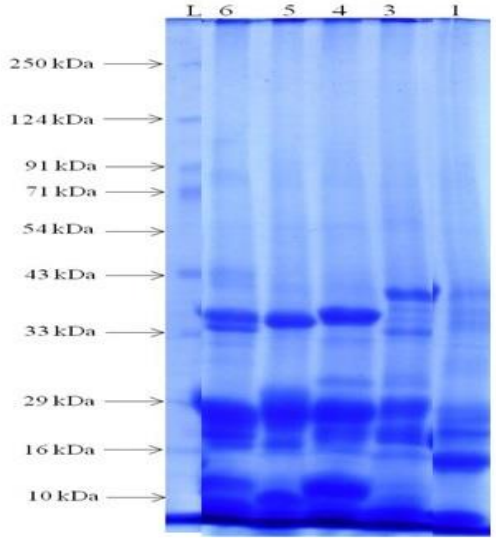

Figure 1. [Where 6= *Withania somnifera*, 5= *Solanum xanthocarpum*, 4= *Capsicum annum*, 3= *Datura stramonium* and 1= *Solanum nigrum*]

Total seed storage protein profiles were examined using SDS-PAGE. Protein electrophoresis (seed protein profiling) is a better tool for the identification of genetic diversity and tracing evolutionary processes in plants than morphological markers [24, 25]. It is a suitable choice of studying genetic variability of crops in a short period of time [26]. Seed protein profiling can be used as a promising tool for distinguishing cultivars of particular species [27, 28]. It can also be used for different purposes like germplasm characterization, varietal identification, determination of phylogenetic relationship between different species and their biosystematics analysis [29]. In our study, the total seed proteins in selected species were ranged between 7.29 - 17.09 mg/ml by Bradford method. The size of polypeptide bands produced by SDS-PAGE ranged from 125 kDa to 13 kDa. The highest molecular weight (i.e. 125 kDa) was present in *Withania somnifera* and lowest molecular weight (i.e.13 kDa) was generated in *Solanum xanthocarpum*. In total 51 protein bands were distinguished among the selected five species. Out of

51 protein bands calculated, only 1 band was monomorphic (1.96% monomorphism) and rests of 50 bands were polymorphic with 98.04% polymorphism. This level of observed bands agrees with the findings [18] who reported 100% polymorphism in different species of family Solanaceae by using SDS-PAGE. The number of bands present in five medicinal plants ranged from 13 to 20 with Rm value 0.236 to 0.990. In first lane, *Withania somnifera* showed 17 protein bands in the range of 125 and 15 kDa (Rm value 0.236-0.965). In lane second, *Solanum xanthocarpum* showed 16 bands in the range of 76 and 13 kDa (Rm value 0.404-0.990). It was also observed that in lane third of *Capsicum annum* was reported with 16 protein bands in the range of 99 and 14 kDa (Rm value 0.313-0.982). In the fourth lane of *Datura stramonium* 13 protein bands were observed between 81 and 14 kDa (Rm value 0.381-0.982). Moreover, *Solanum nigrum* was observed in lane fifth and obtained 20 bands in the range of 114 and 15 kDa (Rm value 0.265-0.965).

Table 1. The mitotic index in different medicinal plants of Solanaceae family

S. No.	Local Name	Botanical name of the species	Percentage of Mitotic index
1	Ashwagandha	*Withania somnifera*	22.50
2	Bhatkateli	*Solanum xanthocarpum*	35.71
3	Mirch	*Capsicum annum*	34.48
4	Datura	*Datura stramonium*	45.46
5	Makoy	*Solanum nigrum*	36.00

Table 2. Molecular weights and RF values of different protein bands in selected medicinal plants of Solanaceae family

S. No.	*Withania somnifera*		*Solanum xanthocarpum*		*Capsicum annum*		*Datura stramonium*		*Solanum nigrum*	
	Mol. Wt.	RF Value	Mol. Wt.	RF Value	Mol. Wt.	RF Value	Mol. Wt.	RF Value	Mol. Wt.	RF Value
1	125	0.236	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	114	0.265
3	108	0.285	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	102	0.303
5	-	-	-	-	99	0.313	-	-	-	-
6	-	-	-	-	-	-	-	-	93	0.337
7	-	-	-	-	-	-	-	-	83	0.376
8	-	-	-	-	-	-	81	0.381	-	-
9	-	-	76	0.404	76	0.404	-	-	-	-
10	75	0.408	-	-	-	-	-	-	75	0.408
11	-	-	-	-	-	-	-	-	66	0.452
12	59	0.493	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	58	0.498
14	-	-	-	-	-	-	56	0.510	-	-
15	-	-	54	0.522	-	-	-	-	-	-
16	53	0.528	-	-	-	-	-	-	-	-
17	-	-	-	-	52	0.535	-	-	-	-
18	-	-	-	-	-	-	-	-	51	0.545
19	-	-	-	-	-	-	49	0.556	49	0.556
20	48	0.561	-	-	-	-	-	-	-	-
21	-	-	-	-	47	0.569	-	-	-	-
22	-	-	-	-	-	-	46	0.578	-	-
23	45	0.584	-	-	45	0.584	-	-	45	0.584
24	-	-	44	0.594	-	-	-	-	-	-
25	-	-	-	-	43	0.598	43	0.598	-	-
26	42	0.608	-	-	-	-	-	-	-	-
27	-	-	41	0.614	41	0.614	-	-	-	-
28	40	0.626	40	0.626	40	0.626	40	0.626	40	0.626
29	37	0.643	-	-	-	-	-	-	-	-

30	-	-	36	0.649	-	-	-	-	-	-
31	-	-	-	-	-	-	-	-	35	0.658
32	34	0.673	-	-	-	-	-	-	-	-
33	-	-	-	-	-	-	33	0.680	33	0.680
34	-	-	-	-	-	-	32	0.705	-	-
35	-	-	31	0.709	31	0.709	-	-	-	-
36	-	-	-	-	-	-	-	-	30	0.724
37	-	-	27	0.762	-	-	27	0.762	-	-
38	-	-	-	-	26	0.777	-	-	26	0.777
39	25	0.786	25	0.786	-	-	25	0.786	-	-
40	-	-	-	-	-	-	-	-	24	0.796
41	23	0.818	23	0.818	23	0.818	-	-	-	-
42	-	-	-	-	-	-	-	-	22	0.831
43	21	0.845	21	0.845	-	-	21	0.845	-	-
44	-	-	20	0.865	20	0.865	-	-	-	-
45	-	-	19	0.883	19	0.883	-	-	-	-
46	-	-	-	-	-	-	-	-	18	0.900
47	17	0.918	-	-	17	0.918	17	0.918	17	0.918
48	16	0.941	16	0.941	-	-	-	-	-	-
49	15	0.965	-	-	15	0.965	-	-	15	0.965
50	-	-	14	0.982	14	0.982	14	0.982	-	-
51	-	-	13	0.990	-	-	-	-	-	-
Total bands	**17**		**16**		**16**		**13**		**20**	

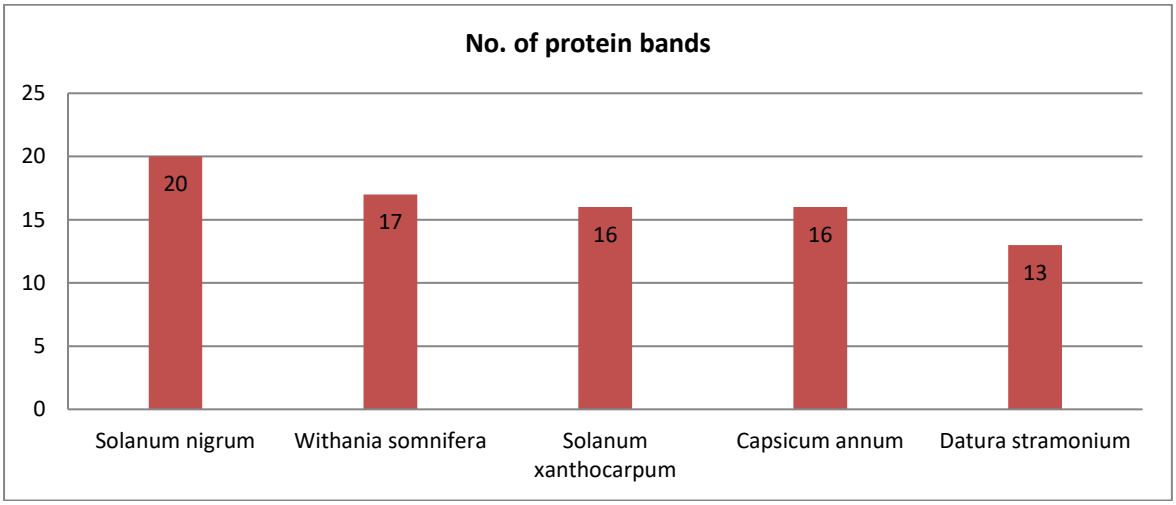

Figure 2. Showing total number of protein bands generated in five different medicinal plants of Solanaceae family

Based on the similarity index among the studied medicinal plants, the similarity coefficient values were ranged from 0.03 to 0.33. (Table3). The highest similarity 0.33 was recorded between *Capsicum annum* and *Solanum xanthocarpum* while lowest similarity 0.03 was found between *Solanum nigrum* and *Solanum xanthocarpum*. The dendrogram which represents the genetic relations among the selected species of family Solanaceae is presented in figure 3. The dendrogram was constructed based on the Jaccard's similarity coefficient, which classified the selected species into two main clusters I and II. Cluster I consisted of only one species *Withania somnifera*. While cluster II comprised of three species namely *Datura stramonium*, *Solanum xanthocarpum* and *Capsicum annum*, in which *Solanum xanthocarpum* and *Capsicum annum* were closed to each other with 0.33 similarities. The species *Solanum nigrum* occupies a distinct place as revealed in the dendrogram.

Table 3. Similarity coefficient matrix of selected medicinal plants of Solanaceae family

	Withania somnifera	*Solanum xanthocarpum*	*Capsicum annum*	*Datura stramonium*	*Solanum nigrum*
Withania somnifera	1.00				
Solanum xanthocarpum	0.18	1.00			
Capsicum annum	0.18	**0.33**	1.00		
Datura stramonium	0.15	0.21	0.16	1.00	
Solanum nigrum	0.16	**0.03**	0.16	0.14	1.00

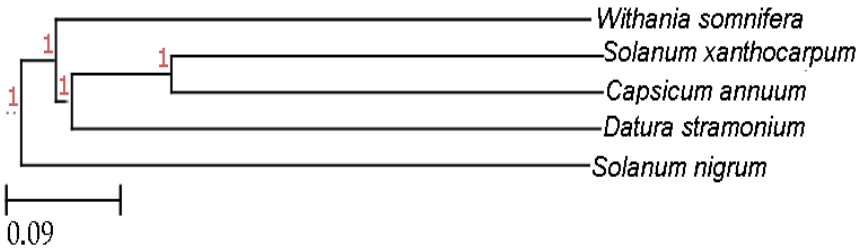

Figure 3. Dendrogram showing the genetic relationship among the selected five medicinal plants of Solanaceae family

CONCLUSION

Measurement and Characterization of genetic diversity has always been a primary concern in population and evolutionary genetic studies because genetic variation provides the material basis for evolutionary changes. It provides opportunities for plant breeders to develop new and improved varieties/cultivars with desirable characters. Considering all the data of present study, it is concluded that SDS-PAGE is a most powerful tool to discriminate between closely related species. During present study two species i.e. *Capsicum annum* and *Solanum xanthocarpum* showed highest value of similarity index (0.33) which revealed that these two species are phylogenetically close to each other while as *Solanum xanthocarpum* and *Solanum nigrum* showed 0.03 similarity index which means that these two species are distantly related to each other. It is recommended that genetically distinct species observed among selected medicinal plants should be used in future breeding programs for selected plant species.

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