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ORIGINAL ARTICLE

Studies on Genetic diversity in some Selected Medicinal plants of Solanaceae family using Reproductive characters and protein profiling

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ABSTRACT
Reproductive characters and protein profiles of five medicinal plants (Solanum nigrum, Solanum xanthocarphum, Withania somnifera, Capsicum annum and Datura stramonium) of family Solanaceae were estimated through polyacrylamide gel electrophoresis and pollen viability. The present investigation revealed that the highest percentage pollen viability was recorded in Datura stramonium (84%) which revealed that this genus has highest reproductive power as well as power of division and lowest percentage pollen viability was obtained in Withania somnifera (67%), which means this genus become scarce and endangered. Total seed storage protein profile was examined using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). A total of 51 bands were scored, out of them 50 were polymorphic with a total of 98.03% polymorphism and only 1 band was monomorphic with 1.96 % monomorphism. The electrophoresis of the total seed proteins revealed protein bands in the range of 125 kD to less than 13 kD molecular weight. The similarity index calculated on the basis of presence and absence of bands ranged from 0.029 to 0.333. A dendrogram constructed based on UPGMA (unweighted pair group method using arithmetic averages) shows distinct separation of the collected species into three clusters. This study reveals high genetic variability among the selected members and suggests possibilities for improvement through hybridization programs.
Key words: *Genetic diversity, Solanaceae, Pollen viability, SDS-PAGE, Dendrogram and UPGMA.*

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INTRODUCTION

Solanaceae is a family of flowering plants that contains a large number of important agricultural plants as well as toxic plants. The name of the family comes from the Latin word Solanum "the nightshade plant" which refers to sedative effects associated with many of the species due to the presence of alkaloids. The Solanaceae is a large varied family of herbs, shrubs and trees including 98 genera and 3000-4000 species [16]. The family has a universal distribution, being present in all continents except Antarctica. The greatest diversity in species is found in South America and Central America. Some species of the family are used as food eg. *Solanum tuberosum* L. (potato), *Lycopersicon esculentum* L. (tomato), *Solanum melongena* L. (egg plant) and drugs (eg. *Solanum nigrum, Withania somnifera*, and *Atropa belladonna*).
Genetic diversity refers to any difference in the nucleotides, genes, chromosomes or genomes of the organism. Thus each gene comprises a heritable section of DNA that occupies a specific place of the chromosome and controls a particular characteristic of an organism. Assessment of genetic variation in any species is an important component of crop improvement programs. The complete understanding of relationship between inbred lines and pure lines can be useful in planning crosses [8]. Genetic diversity studies can classify alleles that might influence the ability of the organism to survive in its existing habitat, or might enable it to survive in more diverse habitats. Therefore, this knowledge is valuable for germplasm conservation, individual, population, variety or breed identification [6].

Morphological characters can be used for analyzing genetic diversity but are often influenced by the environment. The use of biochemical and molecular markers for the estimation of genetic diversity has received much attention in recent years. The establishment of biochemical techniques has made possible and a more accurate evaluation of genetic variation, bringing greater precision to measure of genetic diversity. Among the biochemical techniques, SDS-PAGE is an economical, simple and considerably used biochemical technique for describing the seed protein diversity of crop germplasm [2, 3, 7, 13]. This technique has provided a promising tool to distinguish cultivars of a particular crop species [15]. Seed storage proteins have also been used as genetic markers in four major areas [1] analysis of genetic variation within and between species (2) plant domestication in relation to genetic resource management and breeding (3) creating genome relationships and [4] as a implement in crop improvements [9-12, 14].
In order to improve the yield of medicinal plants, their genetic analysis is the most important and urgent tasks and need is greatest in the country particularly in Madhya Pradesh. Where genetic diversity is great and the existence of many species are threatened. Genetic resources play important role for any breeding and improvement programs [4]. Although the cultivation of medicinal plants has been known for countries, but their germplasm collection and exploitation in breeding has been very limited in this area [17-22, 24]. Hence, the present study has been planned to evaluate genetic diversity in selected medicinal plants of Solanaceae family by using reproductive characters and protein profiling. This work will help in further hybridization programme and development of new varieties of selected medicinal plants of Solanaceae family having evolved characters.

MATERIAL AND METHODS

Pollen viability

Flower buds or flowers were collected at different locations of Chitrakoot and fixed in Carnoy's solution in the ratio of 3:1 ethanol: glacial acetic acid for 24 hours at room temperature and stored in 70% alcohol in a freezer at 3°C. Selected five plant species belonging to Solanaceae family were tested for pollen viability status by using the acetocarmine staining method. To determine the pollen viability, dark stained pollen grains were considered as fertile and viable, and unstained or very lightly stained pollen grains were recorded as sterile or non viable.

Formula used

$$\%\ \textbf{Pollen viability} = \frac{\textbf{Number of viable pollen grains}}{\textbf{Total number of pollen grains analysed}} \times 100$$

SDS-PAGE

Extraction of total seed protein

Total seed proteins were extracted from 100 mg seed flour using 500 micro liters (µl) of extraction buffer that contained 500 mM Tris Hcl, pH 7.5, 2% Beta-mercaptoethanol, 0.7 M Sucrose, and 0.5 M Sodium Chloride and protease inhibitor cocktail. Equal volume of cold Tris-saturated phenol (pH 7.5) was added. This mixture was shaken vigorously for 30 min. at 4°C. Ammonium Acetate 0.1 M was added five times the volume of phenol phase, mix well and kept for precipitation over night at 20°C. Next day, the mixture was centrifuge at 5000 rpm for 30 min. at 4°C. The supernatant was discarded and precipitates were washed twice and thrice in ice-cold Acetone, these precipitates were dissolved in 1 ml of modified lysis.

Protein estimation

Protein concentration of extract was measured immediately and directly from the supernatant by dye binding assay as described by Bradford. A standard curve of absorbance at 595 nm versus 10-80 µg of BSA was also drawn and from this curve, the amount of protein in sample was calculated and finally expressed as mg per g of seed.

Electrophoresis

SDS-PAGE gels were run on Bio-red protein vertical gel electrophoresis apparatus. A 10% resolving gel (1.5 M Tris, pH 8.8, 10% SDS and 5% stacking gel (1M Tris, pH 6.8, 10% SDS) were prepared and polymerized chemically by addition of 0.008ml of tetra methylene diamine (TEMED) and 10% Ammonium Per Sulphate. Then Electrode buffer (25mM Tris base, 250 mM glycine, 10% SDS, pH 8.3) was added to the top pool of the apparatus. 10µl of the extracted protein were loaded with the micropipate into the wells of the gel. The apparatus was connected with constant electric supply and electric current of 70 V was applied. The gels were run till the tracking dye "Brilliant Blue, R250" reached the bottom of the gel. Gels were stained in staining solution for 30 minutes and destained in destaining solution until clear background was obtained. After destaining the gels were photographed.

RESULTS AND DISCUSSION

Significant variations were obtained in reproductive characters and biochemical characters (protein profiling) among some selected medicinal plants of Solanaceae family. To find out the genetic diversity among selected medicinal plants of family Solanaceae, reproductive (viz; pollen viability) and biochemical analysis (viz; SDS-PAGE) was done during present investigation. The pollen viability results obtained using an Acetocarmine are described in table 1. The percentage of pollen viability was 84% for *Datura stramonium*, 67% for *Withania somnifera*, *Solanum nigrum* had 71%, *Solanum xanthocarpum* had 81%, while *Capsicum annum* had 77%. The cytological study reveals that *Datura stramonium* has highest value of pollen viability i.e. 84% which revealed that this genus has highest reproductive power as well as power of division among experimental species and lowest pollen viability in *Withania somnifera* i.e. 67% indicated that this genus has lowest power of division. Present investigation on SDS-PAGE revealed that protein profiling is one of the basic and reliable methods to analyze inter varietal genetic diversity and study phylogenetic relationship among the five selected medicinal plants of family Solanaceae. Seed protein pattern can also be used as a promising tool for differentiating cultivars of particular crop species [15]. The protein band for highest molecular weight (i.e. 125 kD) was present in *Withania somnifera* and lowest molecular weight (i.e.13 kD) was generated in *Solanum xanthocarpum*. When bands of all selected medicinal plants were compared, a total of 51 bands were analyzed. Out of them 50 bands were polymorphic with a total of 98.03% polymorphism and only 1 band was monomorphic with 1.96 % monomorphism. The bands observed in different members were seventeen in *Withania somnifera*, sixteen in *Solanum xanthocarpum* and *Capsicum annum*, thirteen in *Datura stramonium* and 20 in *Solanum nigrum*. The near polymorphism percentage i.e. 100% was found in different five species of Solanaceae family [23].The similarity index calculated on the basis of presence and absence of bands ranged from 0.029 to 0.333. Dendrogram was constructed based on unweighted pair group method using arithmetic averages (UPGMA). Clustering based on seed storage protein profile provides information about the phylogenetic relationships of genotypes as all the genotypes have at least one or more unique seed storage protein marker that can separate them from one another. Cluster analysis of data placed five selected medicinal plants of family Solanaceae into three clusters. Among the three clusters, cluster I contain only 1 species *Withania somnifera,* cluster II contain 3 species in grouped namely *Solanum xanthocarpum, capsicum annum*, and *Datura stramonium*, in which *Solanum xanthocarpum* and *Capsicum annum* were more close to each other. Cluster III contain only one species namely *Solanum nigrum*, which occupies a distinct place as revealed in the dendrogram.

Table 1. Showed percentage pollen viability of selected members of Solanaceae family

S. No.	Common name	Botanical name	No. of pollen grains analyzed	No. of viable pollen grains	No. of non - viable pollen grains	% of pollen viability
1	Datura	*Datura stramonium*	100	84	16	84%
2	Bhatkateli	*Solanum xanthocarpum*	100	81	19	81%
3	Chilli	*Capsicum annum*	100	77	23	77%
4	Makoi	*Solanum nigrum*	100	71	29	71%
5	Ashwagandha	*Withania somnifera*	100	67	33	67%

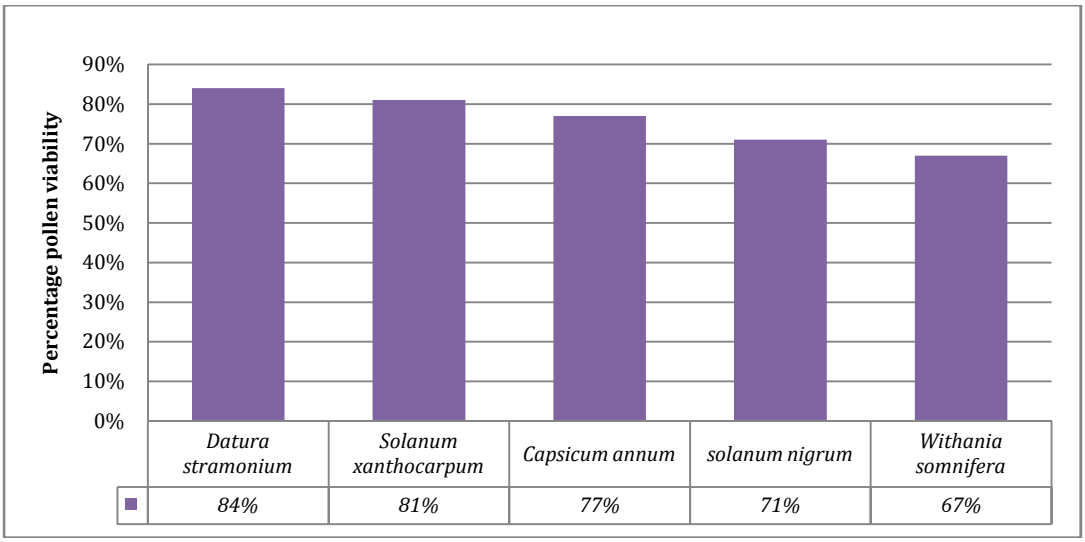
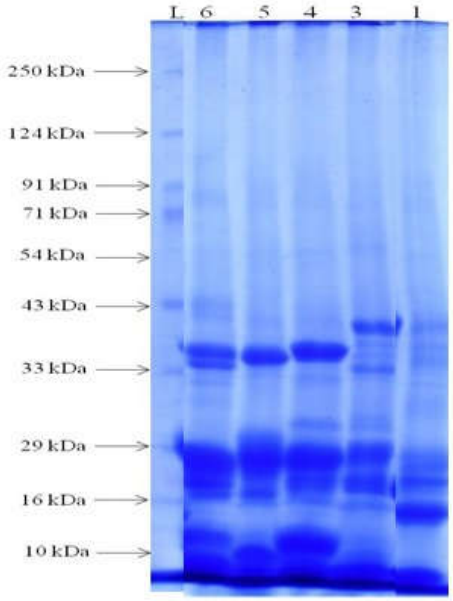


Figure 1. Showed percentage pollen viability in selected medicinal plants of Solanaceae family



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

Figure 2. Protein profiles of five medicinal plants of Solanaceae family

Table 2. Showing presence and absence of bands in SDS-PAGE profile

No. of band	Band mass in Kda	*Withania somnifera*	*Solanum xanthocarpum*	*Capsicum annum*	*Datura stramonium*	*Solanum nigrum*
1	125	+	-	-	-	-
2	114	-	-	-	-	+
3	108	+	-	-	-	-
4	102	-	-	-	-	+
5	99	-	-	+	-	-
6	93	-	-	-	-	+
7	83	-	-	-	-	+
8	81	-	-	-	+	-
9	76	-	+	+	-	-
10	75	+	-	-	-	+
11	66	-	-	-	-	+
12	59	+	-	-	-	-
13	58	-	-	-	-	+
14	56	-	-	-	+	-

15	**54**	**-**	**+**	**-**	**-**	**-**
16	**53**	**+**	**-**	**-**	**-**	**-**
17	**52**	**-**	**-**	**+**	**-**	**-**
18	**51**	**-**	**-**	**-**	**-**	**+**
19	**49**	**-**	**-**	**-**	**+**	**+**
20	**48**	**+**	**-**	**-**	**-**	**-**
21	**47**	**-**	**-**	**+**	**-**	**-**
22	**46**	**-**	**-**	**-**	**+**	**-**
23	**45**	**+**	**-**	**+**	**-**	**+**
24	**44**	**-**	**+**	**-**	**-**	**-**
25	**43**	**-**	**-**	**+**	**+**	**-**
26	**42**	**+**	**-**	**-**	**-**	**-**
27	**41**	**-**	**+**	**+**	**-**	**-**
28	**40**	**+**	**+**	**+**	**+**	**+**
29	**37**	**+**	**-**	**-**	**-**	**-**
30	**36**	**-**	**+**	**-**	**-**	**-**
31	**35**	**-**	**-**	**-**	**-**	**+**
32	**34**	**+**	**-**	**-**	**-**	**-**
33	**33**	**-**	**-**	**-**	**+**	**+**
34	**32**	**-**	**-**	**-**	**+**	**-**
35	**31**	**-**	**+**	**+**	**-**	**-**
36	**30**	**-**	**-**	**-**	**-**	**+**
37	**27**	**-**	**+**	**-**	**+**	**-**
38	**26**	**-**	**-**	**+**	**-**	**+**
39	**25**	**+**	**+**	**-**	**+**	**-**
40	**24**	**-**	**-**	**-**	**-**	**+**
41	**23**	**+**	**+**	**+**	**-**	**-**
42	**22**	**-**	**-**	**-**	**-**	**+**
43	**21**	**+**	**+**	**-**	**+**	**-**
44	**20**	**-**	**+**	**+**	**-**	**-**
45	**19**	**-**	**+**	**+**	**-**	**-**
46	**18**	**-**	**-**	**-**	**-**	**+**
47	**17**	**+**	**-**	**+**	**+**	**+**
48	**16**	**+**	**+**	**-**	**-**	**-**
49	**15**	**+**	**-**	**+**	**-**	**+**
50	**14**	**-**	**+**	**+**	**+**	**-**
51	**13**	**-**	**+**	**-**	**-**	**-**

Table 3. Showing the similarity index of selected medicinal plants of family Solanaceae

	Withania somnifera	***Solanum xanthocarpum***	***Capsicum annum***	***Datura stramonium***	***Solanum nigrum***
Withania somnifera	1.000	0.179	0.179	0.154	0.156
Solanum xanthocarpum		1.000	0.333	0.208	0.029
Capsicum Annum			1.000	0.160	0.161
Datura Stramonium				1.000	0.138
Solanum Nigrum					1.000

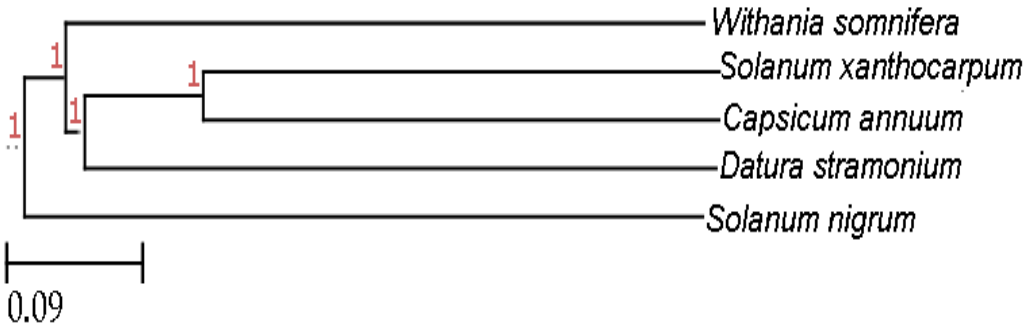


Figure 3. Dendrogram showing the genetic relationship among the selected five medicinal plants of family Solanaceae

CONCLUSION

The presence of diversity is important for improving any plant species. An understanding of the magnitude and patterns of genetic diversity in any plant species has important suggestions in breeding programs and for conservation of genetic resources. Seed protein profiling through SDS-PAGE is the most important tool for estimation of genetic diversity. Seed storage protein profiles helps in cultivars identification by avoiding the external environmental influences. During present investigation three medicinal plants of Solanaceae family (i.e. *Solanum xanthocarpum* with *Capsicum annum* and *Solanum xanthocarpum* with *Datura stramonium*) show highest value of similarity index (i.e. 0.333 and 0.208) respectively which revealed that these plants are phylogenetically very close to each other while as *Solanum xanthocarpum* and *Solanum nigrum* having lowest similarity index (i.e. 0.029) which means that these plants are distantly related from each other. The plants which are distantly related either at protein or gene level should be used for plant breeding programs in future. On the basis of present study it is concluded that SDS-PAGE revealed considerable amount of variation in five selected medicinal plants of Solanaceae family.

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