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Assessment of Genetic Variation in Selected Members of Family Apocynaceae using SDS-PAGE

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ABSTRACT

Genetic variation of any plant species is very interesting in reducing genetic vulnerability as well as stabilizing production. In this regard, a study was undertaken to analyze the genetic variation among selected members of family Apocynaceae by using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). In this study, a total of 53 protein bands were analyzed, out of them all bands were polymorphic with a total of 100% polymorphism. The electrophoresis of the proteins revealed protein bands in the range of 151 kD to less than 11 kD molecular weight. The similarity index calculated on the basis of presence and absence of protein bands ranged from 0.04 to 0.200. A dendrogram was constructed based on UPGMA (unweighted pair group method using arithmetic averages) clustering method revealed three clusters. Cluster I contained three species namely *Thevetia peruviana*, *Catharanthus roseus* and *Nerium indicum*, in which *Thevetia peruviana* and *Catharanthus roseus* were more close than *Nerium indicum*, while cluster II included only one species namely *Rauvolfia serpentina*. *Carissa carandus* emerged as the most primitive species forming an out group (cluster III). Thus, this study revealed that the SDS-PAGE method plays a key role in the study of protein based variation among selected plant species.

Figures : 02 References : 31 Tables : 02
KEY WORDS : Apocynaceae, Dendrogram, Genetic variation, SDS-PAGE, UPGMA.

Introduction

The Apocynaceae is a family of flowering plants that includes herbs, shrubs, trees and climbers. Many species are tall trees which occur in tropical rain forests, and most are from the tropics and sub tropics but some found in tropical dry and xeric environments. Many species of the family have milky sap and many are toxic if ingested[5]. Today in Apocynaceae, 5100 species are distributed among 357 genera [6,21,26]. This family is one of the ten largest families of angiosperms and also one of the most popular due to the traditional wide spread use of some of its species as medicinal plants. The family carries considerable importance in the field of herbal medicine because of its species are used in the treatment of malaria, diarrhea, diabetes and skin diseases.

The evaluation of genetic diversity is a suitable precursor to improve any plant species because it provides baseline information to guide selection of parental lines and design of a breeding system. Genetic variation within and among populations is a major attraction of plant breeders and geneticists because it provides the efficient sampling and exploitation of germplasm resources. The information on genetic variation and relationships between populations is important to understand the available genetic variability and its potential use in breeding programs. For the efficient evaluation, conservation and utilization of germplasm, the understanding of genetic basis of any population is very essential. Several markers have been utilized for studying the genetic variation and relationships among different species such as morphological, biochemical and molecular markers. Morphological markers are not quite sufficient to analyze the genetic diversity because they are influenced by the environment[20]. Molecular markers are useful tools for assessing the genetic variations because they are not influenced by environment and do not require the prior knowledge of DNA sequence.

Among biochemical techniques, seed storage protein analysis represents a valid alternative for varietal identification[18]. SDS-PAGE is most economical, simple

TABLE-1 : Showing the presence and absence of bands in SDS-PAGE profile

No. of band	Band mass in kDa	RF Value	*Thevetia peruviana*	*Catharanthus roseus*	*Nerium indicum*	*Rauvolfia serpentina*	*Carissa carandus*
1	151	0.56	-	-	-	-	+
2	132	0.215	-	+	-	-	-
3	130	0.221	+	-	-	+	-
4	127	0.226	-	-	-	-	+
5	122	0.242	-	+	-	-	-
6	116	0.250	-	-	-	+	-
7	114	0.265	-	+	-	-	-
8	108	0.285	-	-	-	-	+
9	104	0.297	+	-	-	-	-
10	102	0.303	-	+	-	-	-
11	100	0.310	-	-	+	-	-
12	98	0.316	+	-	-	-	-
13	96	0.320	-	-	-	+	-
14	90	0.347	-	-	+	-	-
15	80	0.388	+	-	-	-	-
16	78	0.396	-	-	+	+	+
17	76	0.404	-	+	-	-	-
18	74	0.418	-	-	+	-	-
19	69	0.442	-	+	-	-	-
20	66	0.452	-	-	-	+	-
21	63	0.467	+	-	-	-	-
22	60	0.484	-	+	-	-	+

23	57	0.503	-	-	+	-	-
24	55	0.514	+	+	-	+	-
25	52	0.535	-	-	+	-	-
26	49	0.556	-	+	+	-	-
27	46	0.578	+	-	-	-	-
28	45	0.584	-	-	-	-	+
29	44	0.594	-	-	-	+	-
30	43	0.598	-	-	+	-	-
31	42	0.608	-	-	+	-	-
32	41	0.614	-	+	-	-	-
33	40	0.626	-	-	+	-	-
34	39	0.630	-	-	-	-	+
35	38	0.634	+	+	-	-	-
36	35	0.658	-	+	+	-	-
37	32	0.705	-	+	+	+	-
38	30	0.724	-	+	+	-	-
39	28	0.745	-	-	+	-	-
40	27	0.762	+	+	-	-	-
41	26	0.777	-	+	-	-	-
42	25	0.786	-	-	+	-	-
43	24	0.796	-	-	-	+	-
44	23	0.818	-	-	-	-	+
45	22	0.831	+	+	-	-	-
46	20	0.865	-	+	+	-	-

47	19	0.883	+	-	+	+	-
48	18	0.900	-	+	+	-	+
49	17	0.918	+	-	+	-	-
50	16	0.941	+	+	-	-	+
51	15	0.965	+	+	+	-	-
52	14	0.982	-	+	-	-	+
53	11	0.998	-	-	-	+	+

and effectively used biochemical technique for estimating the genetic variation of crop germplasm[3, 4,7,13]. Seed storage protein analysis helps in protein identification and description of diversity in crop varieties and their wild relatives. Seed storage protein markers are highly polymorphic and independent of environmental factors[9,28]. It can also be utilized for various purposes such as species identification, characterization of germplasm[13,15], biosystematics analysis and establishment of phylogenetic relationships among different species[10,14,29]. Therefore the present study was undertaken to analyze the genetic variation in selected members of Apocynaceae family for further improvement in breeding programs.

Material and Methods

A. 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 systematically for 30 min. at 4°C. Ammonium Acetate 0.1 M was added five times the volume of phenol phase, mixed well and kept for precipitation overnight at 20°C. Next day, the mixture was centrifuged 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.

B. Protein Estimation

Protein concentration of extracts was measured immediately and directly from the supernatant by dye binding assay. A standard curve of absorbance at 595 nm for the standards *versus* protein concentration was also drawn and from this curve, the amount of protein in sample was calculated and finally expressed as mg per g of seed.

C. Electrophoresis (SDS-PAGE)

The electrophoretic procedure was carried out using slab type SDS-PAGE with 10% polyacrylamide gel. A 10% resolving gel (1.5 M Tris, pH 8.8, 10% SDS) and 5% stacking gel (1M Tris, pH 6.8, 10% SDS) was prepared and polymerized chemically by addition of 0.008ml of N,N,N,N tetramethylenediamine (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 micropipette 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 using gel documentation systems.

D. Data Analysis

The data obtained from SDS-PAGE were scored as presence (+) or absence (-) of protein polypeptide bands. Bands were identified and RF value was calculated in the images using Gel Analyzer 19.1. Phylogenetic tree was constructed by using software "Dendro UPGMA a dendrogram construction utility" and then analyzed with Jaccord index (Tanimoto) with 100 Boostrap Replicates and clustering method of UPGMA.

Results and Discussion

Significant variations were observed in SDS-PAGE analysis among five selected members belonging to family Apocynaceae. To find out genetic variation among five selected members, seed storage protein profile was done during present investigation. Germplasm characterization based on morphological traits is not upto the mark and

TABLE-2 : Showing the similarity index of selected members of family Apocynaceae

	Thevetia peruviana	*Catharanthus roseus*	*Nerium indicum*	*Rauvolfia serpentina*	*Carissa carandus*
Thevetia peruviana	1.000	**0.200**	0.097	0.136	**0.04**
Catharanthus roseus		1.000	**0.200**	0.065	0.133
Nerium indicum			1.000	0.107	0.067
Rauvolfia serpentina				1.000	0.095
Carissa carandus					1.000

requires confirmation at molecular or at least at protein level. Electrophoresis of proteins is a powerful tool for detection of the genetic diversity and the SDS-PAGE of seed storage protein is particularly considered as a reliable technology because seed storage proteins are highly independent of environment fluctuations. The present study revealed that seed storage protein profiling is one of the basic and reliable methods to study inter

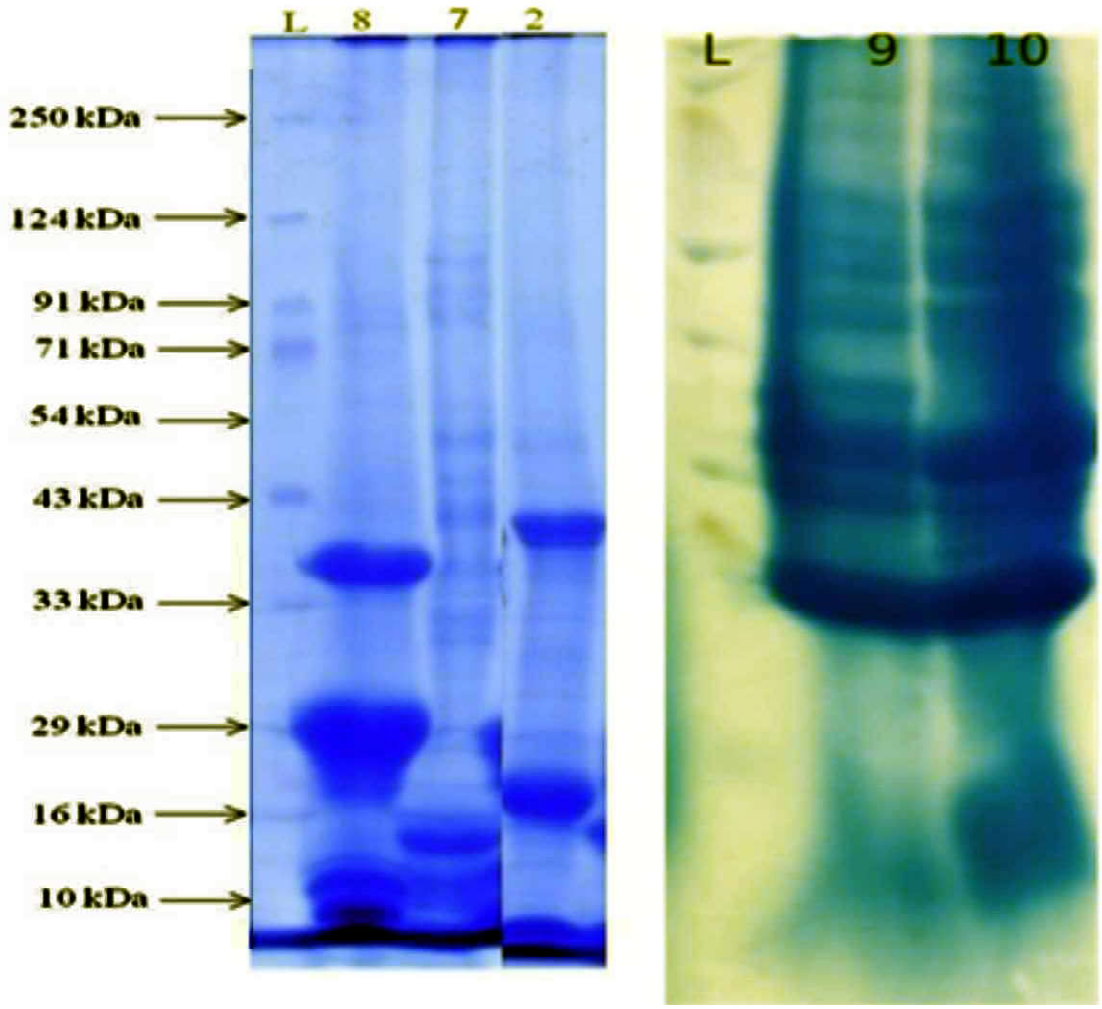

Fig. 1 : Protein profiles of selected members of Apocynaceae family (Where 8 is *Thevetia peruviana*, 7 is *Catharanthus roseus*, 2 is *Nerium indicum*, 9 is *Rauvolfia serpentina*, 10 is *Carissa carandus*)

varietal genetic variation and phylogenetic relations among the five selected members of family Apocynaceae. In the present study, a total of 53 bands were analyzed, in which all bands were polymorphic with a total of 100% polymorphism. This level of observed bands agree with the findings of workers[2] who also reported 100% polymorphism in different species of Solanaceae family by using seed protein profiling. Similarly workers[8] used SDS-PAGE technique in *Oryza sativa* and found 100% polymorphism in case of seed proteins. The protein size based polymorphism revealed the range of protein bands on their molecular weights which ranged from 151 kDa to 11 kDa. The protein band for highest molecular weight (*i.e.* 151 kDa) was present in *Carissa carandus* and lowest molecular weight (*i.e.* 11 kDa) was generated in *Rauvolfia serpentina* and *Carissa carandus*. These findings are in agreement with the results of workers[23] who also found the molecular weight in the range of 150 kDa to 14 kDa. The selected medicinal plants showed considerable variation in band number of proteins in the present study which ranged from 11 to 22. These results agreed with the reports of some workers[31], they also obtained the total number of bands varied from 10 to 22 in different varieties of wheat by using SDS-PAGE. More number of Protein bands (*i.e.* 22) was produced in *Catharanthus roseus* and less number of bands (*i.e.* 11) was observed in *Rauvolfia serpentina*.

The presence or absence of bands was used to generate binary data for each of the selected medicinal plant. Using this binary data and Gel Analyser 19.1 software, the Jaccard's similarity coefficient was prepared. The Jaccard's similarity coefficient based on presence and absence of observed bands ranged between 0.04-0.200. The similarity index computed with Jaccord's coefficient revealed the maximum similarity between *Thevetia peruviana* with *Catharanthus roseus* and *Catharanthus roseus* with *Nerium indicum i.e.* 0.200 respectively, while distantly related members were *Thevetia peruviana* and *Carissa carandus* with similarity coefficient 0.04 indicating high level of genetic diversity.

The dendrogram, which represents the genetic relations among the selected members of family Apocynaceae is presented in figure II. The dendrogram constructed based on UPGMA (unweighted pair group method using arithmetic averages) clustering method revealed three clusters. Cluster I contains three species in group namely *Thevetia peruviana*, *Catharanthus roseus* and *Nerium indicum*, in which *Thevetia peruviana* and *Catharanthus roseus* were more closed than *Nerium indicum*, while cluster II included only one species (*Rauvolfia serpentina*). *Carissa carandus* emerged as the most primitive species forming an out group (cluster III). Therefore, the dendrogram as a whole revealed high genetic diversity because most of species are distantly related to each other.

Conclusion

Quantification and characterization of genetic diversity has always been a primary concern in population and evolutionary genetic studies because genetic variability provides the material basis for evolutionary changes. Seed protein electrophoresis could be proved to be a successful technique in certain cases to distinguish morphologically very similar genotypes. Assessment based on protein and selection of desirable genotype is of immense importance for crop breeders. The seed storage protein profiling based on SDS-PAGE is an effective method to analyze genetic diversity. Based on the present study, the species *Thevetia peruviana* was most distantly related to *Carissa carandus*. Hence, it was

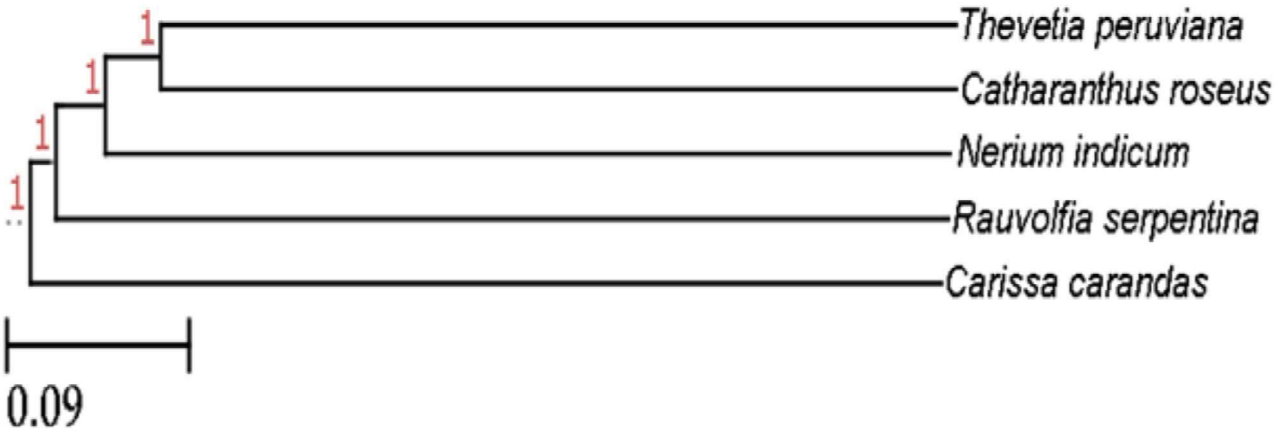

Fig. 2: Dendrogram showing the genetic relationship among the selected five members of family Apocynaceae

concluded that these two species (*Thevetia peruviana* and *Carissa carandus*) could be utilized for crossing programme to create more genetic diversity or segregants of desired characteristics through breeding programme.

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