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Phylogenetic Relationship of Some *Ipomoea* Seed Proteins by SDS-PAGE

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Abstract

To investigate the phylogenetic relationship of nine Ipomoea species, seed proteins were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Based on the analysis, total 50 bands were identified. The number of bands varies from 8 bands in Ipomoea obscura to 4 in Ipomoea mauritiana. Phylogenetic tree was constructed based on the presence or absence of protein bands using Freetree and Treeview software programme.

Keywords: *Ipomoea*, protein, phylogenetic tree, bands, SDS-PAGE

Introduction

The convolvulaceae (Morning Glory Family) is a beautiful family which is widely cultivated as ornamentals. About 55 genera and 1930 species of the convolvulaceae are widely distributed throughout temperate and tropical regions and abundant in tropical America and tropical Asia[1]. One of the major genus is *Ipomoea*, represented by 600 species[2].

There are number of sections in the genus *Ipomoea*[3]. The morphological traits and biochemical analysis they go hand in hand[4]. Phylogenetic analysis of 40 species representing the three subgenera and nine sections within the *Ipomoea* using sequence data from the ITS region and waxy sequences revealed a close relationship between species of section Pharbitis subgenus *Ipomoea* and species of subgenus *Quamoclit*[5] The cladistic analysis of the tribe *Ipomoeae* based on 45 morphological and palynological characters, and suggested that the *Ipomoeae* is a monophyletic tribe[6]. The phylogenetic relation of the genus *Ipomoea* with other genera from the tribe *Ipomoeae* based on morphology and phylogeny and found that *Ipomoea* is paraphyletic[7]. The objective of our study is to clarify the relationship among the nine *Ipomoea* species using the molecular technique of sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of seed proteins.

Material and Methods

Seeds of *Ipomoea* species were collected from different parts of Karnataka. These *Ipomoea* species were used to study uni-dimensional SDS-PAGE (12.5% resolving gel and 4% stacking gel) in a vertical gel system[8] (Bio-Rad).

Samples were prepared as follows. 0.2gm of seeds grounded in 2ml of 50mM phosphate buffer (pH 7.4) in cold conditions to get 10% of homogenate. Then it is centrifuged in micro-centrifuge machine for 10 minutes at 10,000 rpm. The supernatant was separated and used as protein sample. The protein concentration in the supernatant was then determined for gel electrophoresis by the method of Bradford[9], with bovine serum albumin (BSA) as the standard and using spectrophotometer at 595 nm. The protein sample along with the gel loading buffer containing bromophenol blue were denatured in boiling water (1 minute), cooled and 100 µg of each extract loaded in lanes with micropipette. A protein molecular weight marker (Aristogene, Bangalore, Cat No. BCL-039) was also incorporated into the gel (as marker lane) as reference to detect molecular weights of the bands. The gel was ran initially at 50mA for half an hour later on at 100mA for 2 hours.

Following electrophoresis, the gel was stained with a solution containing Coomassie brilliant blue (CBB-R-250), destained with double distilled water. All chemicals were purchased from Sigma and stock solutions were prepared before making a working solution. The gel was scanned using Alpha Innotech 1.2 version.

The presence or absence of each band was treated as binary character in a data matrix i.e coded 1 and 0 respectively. Data were analyzed using Freetree software programme[10] with Jaccard method[11] and UPGMA (Unweight Pair-Groups Method using Arithmetic Average) by tree construction method using Treeview software[12].

Results and Discussion

The seed protein banding profile among nine species of *Ipomoea* is compared in figure 1. The zymogram of the same is presented in figure 2. Electrophoretic analysis of proteins exposed a total of 50 protein bands in the seeds of the 9 species of *Ipomoea.* The analysis of the results reveals that some bands are characteristic and constant markers for species. Other bands are shared by more than one species. The number of bands and intensity of bands varies from one species to another. The common band which is shared by all the plant species is at 55.5KDa (Rf value 0.655).

The Rf values, molecular weight, intensity and position of protein bands of *Ipomoea* species is given in table 1. The data

matrix of *Ipomoea* species showing the seed protein characters (0= band absent, 1= band present) is given in table 2. The phylogenetic tree obtained through Freetree and Treeview software figure 3, which indicates a very clear picture of the species inter-relationship.

The phylogram shows that the two groups of plant species seperated very early from each other and thus originated in seperate ways. Eight plant species are in one group, only one species in another group. Further these 8 plant species forms two separate groups of one plant species and seven plant species. On the other hand, the 8 plant species, *I. mauritiana, I. triloba, I. carnea, I. cairica, I. campanulata, I. obscura, I. hederifolia, I. muricata,* originated from same ancestor shared by *I. alba* but seperated further into 2 groups represented by *I. mauritiana* in one group and *I. triloba, I. carnea, I. cairica, I. campanulata, I. obscura, I. hederifolia, I. muricata,* in the other group during the process of evolution.

According to Biju sectional classification of the genus *Ipomoea* are Mina (*I. hederifolia*), Calonyction (*I. muricata* and *I. alba*), Erpipomoea (*I. cairica* and *I. obscura*), Eriospermum (*I. carnea*, *I. mauritiana* and *I. campanulata*) and Batatas (*I. triloba*) based on the morphological characters.The phylogenetic tree figure 3 constructed for 9 species of *Ipomoea* exhibit unrelatedness between the sections. For example *I. cairica* and *I. obscura* are placed distantly, similarly *I. alba* and *I. muricata* exhibit far and wide relationship. The *I. triloba* sandwiched between Eriospermum. The section Mina and Calonyction go hand in hand, but *I. alba* is distinct. There is no correlation between SDS-PAGE profile and Biju's morphological characters. The only possibility is to approach the species holistically.

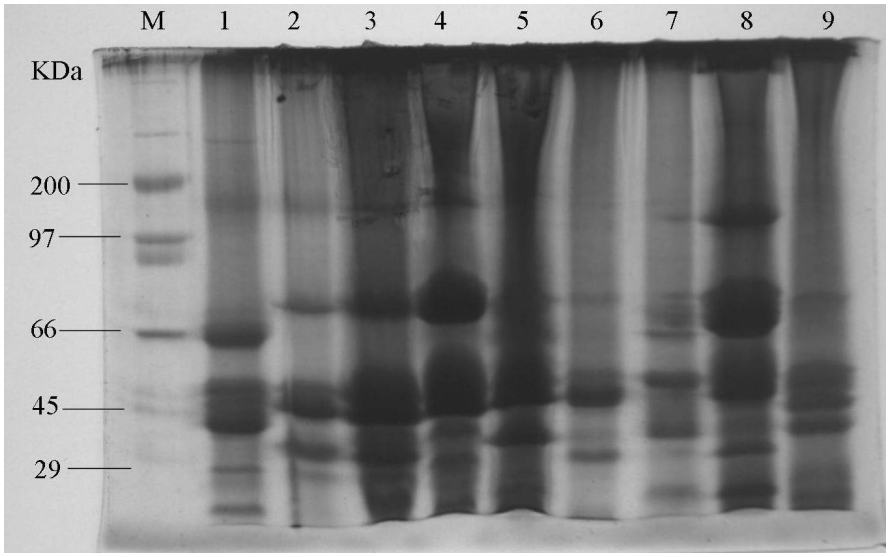

Figure-1

Electrophoretic patterns of the seeds of Ipomoea Species: M- Marker (Wide range molecular weight standard from top to bottom 200, 97, 66, 45, 29 KDa.). 1- *I. alba*, 2- *I. cairica,* 3- *I. campanulata*, 4- *I. carnea*, 5- *I. hederifolia*, 6- *I. mauritiana*, 7- *I. muricata*, 8- *I. obscura,* 9- *I. triloba*.

Table-1

Rf values, molecular weight, intensity and position of protein bands of *Ipomoea* species using SDS-PAGE

Band	Rf value	Mol.wt. in KDa.	1	2	3	4	5	6	7	8	9
1	0.310	155.8	+	+	++	++	-	-	-	-	-
2	0.345	119	-	-	-	-	-	-	-	+++	-
3	0.500	72.6	-	++	+++	++++	+++	+	+	++++	+
4	0.569	66	+++	-	-	-	++++	-	+	++++	-
5	0.655	55.5	+++	++	++++	++++	++++	++	++	++++	+++
6	0.724	45	+++	+++	++++	++++	++++	+++	-	+++	+++
7	0.776	38.3	-	+++	++++	++++	++++	-	++	++	+++
8	0.862	29	+	-	-	++	-	++	-	++	-
9	0.914	14.5	+	-	+	-	-	-	+	++	++

1- *I. alba*, 2- *I. cairica,* 3- *I. campanulata*, 4- *I. carnea*, 5- *I. hederifolia*, 6- *I. mauritiana*, 7- *I. muricata*, 8- *I. obscura,* 9- *I. triloba*.
(+: Low intensity, ++: Medium intensity, +++: High intensity, ++++: Very high intensity)

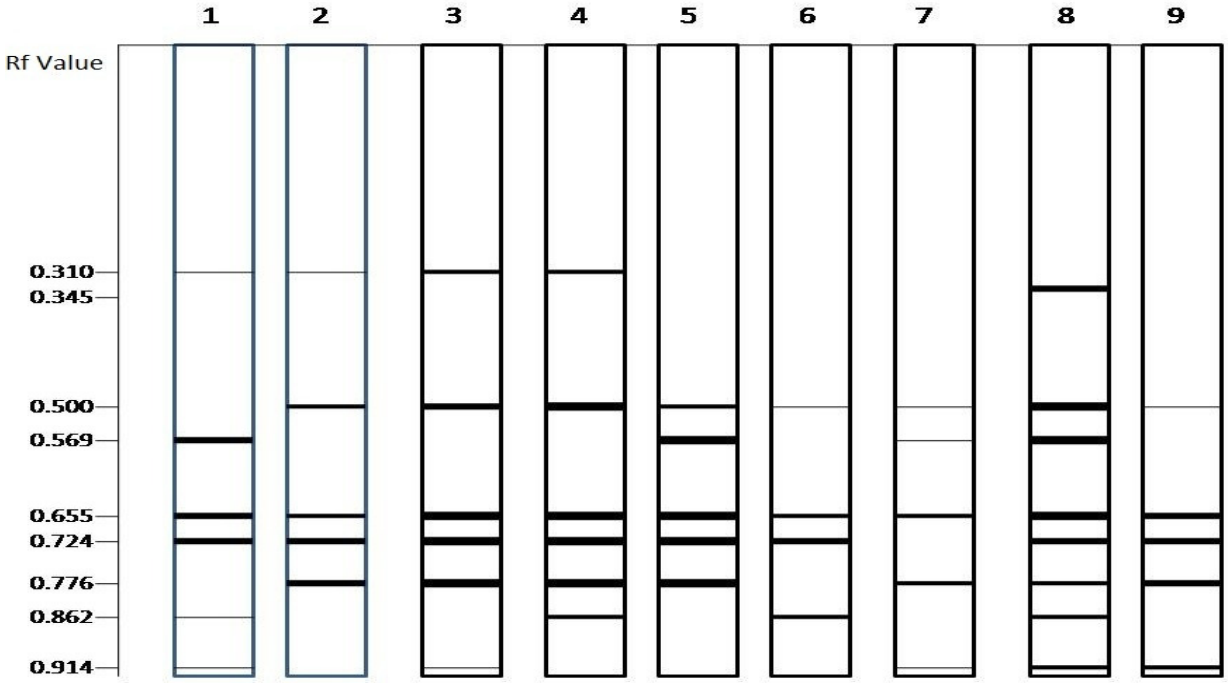

Figure-2
Zymogram of total soluble seed protein of *Ipomoea* species through SDS-PAGE

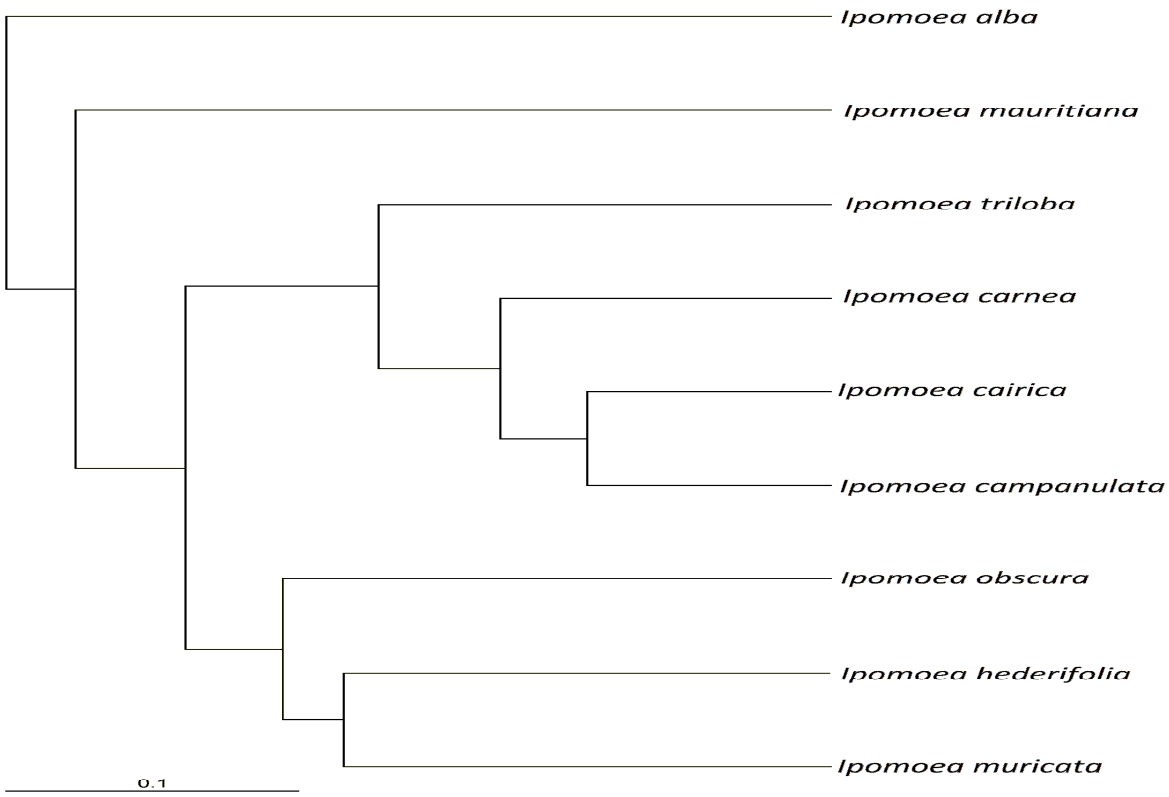

Figure-3
Phylogenetic tree obtained through banding comparisons among nine species of *Ipomoea*

Table-2
Data matrix of *Ipomoea* species showing the characters (seed protein pattern) which is represented by fifty band, 0 = band absent 1 = band present

Species	Characters states
Ipomoea alba	100111011
Ipomoea cairica	101011100
Ipomoea campanulata	101011101
Ipomoea carnea	101011110
Ipomoea hederifolia	001111100
Ipomoea mauritiana	001011010
Ipomoea muricata	001110101
Ipomoea obscura	011111111
Ipomoea triloba	001011101

Conclusion

Electrophoresis of seed proteins showed total of 8 bands in *Ipomoea obscura*, 6 bands in *Ipomoea alba, Ipomoea campanulata, Ipomoea carnea,* 5 bands in *Ipomoea cairica, Ipomoea hederifolia, Ipomoea muricata, Ipomoea triloba,* 4 bands in *Ipomoea mauritiana*. The intensity of the band also varied among all the plant species. Hence the present study obviously indicated the use of SDS-PAGE profile to draw inter-relatedness between the species of *Ipomoea*.

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