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ELECTROPHORETIC STUDIES ON SEED PROTEINS OF SELECTED *Medicago* L SPECIES TO ASSESS THEIR RELATIVE PHYTOCHEMICAL AFFINITIES

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Key words : Electrophoresis, seed protein, variations, relative phytochemical affinities.

The genus *Medicago* L, popularly known as Medick, happens to be an important agronomic member of the Legume family Fabaceae. Many of the members are of great economic value, particularly as pasturage. However, during last 2-3 decades, importance of this legume genus has been widely realised as a protein rich fodder for all kinds of live stocks.

Electrophoretic studies for assessing seed protein variations carried out selectively on four different species, namely *M.polymorpha* L. (2n=2x=14), *M.intertexta* (L) Mill (2n=2x=16), *M.littoralis* Rhode (2n=2x=16) and *M.scutellata* (L) Mill (2n=4x=30) have revealed varying degrees of overlapping relationships.

The results have shown maximum percentage similarity between *M.intertexta* and *M.littoralis* (73%), the former species again showing next higher similarity with *M.polymorpha* (62%). However, *M.polymorpha* in turn has shown highest similarity to *M.scutellata* (60%). The similarity value between *M.polymorpha* and *M. littoralis* has been observed to be 46.10%.

INTRODUCTION

Over the years, the methods of detecting and assessing taxonomic as well as genetic diversity have extended from analysis of discrete morphological traits to biochemical traits. Seed proteins have extensively been studied from taxonomic point of view in a number of plants viz. Avena (Jain &Singh, 1979), Cowpea (Khan et al.,1980), Phlox (Levinand Schaal,1970), Vicia (Ladizinsky, 1975), Triticum and Aegilops (Caldwell and Kasarda, 1978). The study of protein band data, obtained with proteins extracted from various species of Solanum concluded that the protein profiles were specific for each species (Desborough and Piloquin,1967). Thus, the seed protein banding patterns as revealed by polyacrylamide gel electrophoresis in the presence of sodium dodecyle sulphate (SDS-PAGE) have provided a solid source of evidence for addressing relationships at the generic and specific levels and has been discussed at length by Crowford (1989), and others. This has also been employed in estimating the parental affinities of the interspecific hybrids.

Thus, in view of the facts mentioned above, the present electrophoretic investigations on four selected Medicago L. species have been undertaken here, the three diploids and a tetraploid one.

MATERIALS AND METHODS

The seeds of the selected taxa, namely *M.polymorpha* L (2n=2x=14), *M.intertexta* (L) Mill (2n=2x=16), *M.littoralis* Rhode (2n=2x=16) and *M.scutellata* (L) Mill (2n=4x=30) were pre-soaked overnight for the purpose of protein extraction and electrophoretic studies here.

ELECTROPHORETIC STUDIES

Electrophoresis involves the movement of dissolved or suspended material under the influence of an applied electric field. The procedure of Davis, (1964) was followed with a slight modification for separating gel.

I. **Ingredients for 10 % polyacrylamide**

Solution A- Stacking gel buffer	**pH-6.8**
Tris	6 gm
1 M HCl	48 ml
Distilled water	40 ml

The mixture was made to 100 ml with distilled water.

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Solution B- Separating gel buffer pH - 8.8

Acrylamide	36.3 gm.
1 M HCl	48 ml

The mixture was made to 100 ml with distilled water.

Solution C- Acrylamide stock solution

Acrylamide	30 gm
BIS (N ,N methylene bis acrylamide)	0.8 gm

Distilled water was added to make the final volume to 100 ml.

Solution D- SDS Solution (Prepared fresh)

SDS	1 gm
Distilled water	10 ml

Solution E- Ammonium per sulphate solution (Prepared fresh)

APS	0.1 g
Distilled water	1 ml

Stock solutions were kept separately at 80°C and mixed just before use.

Separating Gel:	Acrylamide stock (30 : 8)	-10 ml
	Separating gel buffer (Sol.B)	-7.5 ml
	10 % SDS solution	-0.3 ml
	Distilled water	-12.1 ml
	10 % APS	-150 µl
	TEMED	-10 µl

Stacking Gel:	Acrylamide stock (30 : 8)	-1.33 ml
	Stacking gel buffer(Sol. A)	-2.5 ml
	10 % SDS solution	-0.1 ml
	Distilled water	-6.0 ml
	10 % APS	-50 µl
	TEMED	- 5 µl

The separating gel was poured into gel caster having spacers of 1mm thickness and allowed to form gel. Stacking gel solution was then poured on top of separating gel and comb was put. Few drops of distilled water was placed on the top of the gel solution to avoid meniscus formation. The gel was allowed to polymerize for 30 minutes and subsequently used.

II. Running buffer : (pH 8.3)

Tris - 3.0 gm.

Glycine - 14.4 gm.

The final volm was made upto 1000ml by adding dist. water.

III. Tracking dye :

10 mg Bromophenol blue

10 ml distilled water

1 gm Sucrose (or Glycerol)

IV. Staining Solution :

Coomassie brilliant blue -0.1 gm

Methanol 50 ml

Glacial acetic acid 20 ml

Made to 100 ml with distilled water

V. **Destaining Solution :**

30 % methanol

10 % acetic acid

Made to 100 ml with distilled water .

Protein from overnight presoaked seeds were extracted in protein solubilization solution (62 m M Tris -HCl, pH 6.8, 10 % glycerol, 2% SDS, p-mercaptoethanol and traces of bromophenol blue) then transferred to Eppendorf tube and centrifuged for 30 seconds. The supernatant was transferred to a fresh tube and placed into a boiling water bath for 4 minutes. The extract was cooled and loaded on each gel tube. Electrophoresis was carried out at 20 mA current for 3-4 hours till the tracking dye reaches the bottom of the gel. After electrophoresis the gels were taken out of the tubes for the detection of protein bands.

Detection of Protein Bands in the gel

Staining and destaining :

Staining of the protein band was performed by dipping the gel into the plates containing coomassie brilliant blue for 24 hours. Destaining was carried in 10% acetic acid. The bands were examined in white light transilluminator and gels were photographed.

RESULTS AND DISCUSSION

The results showed marked variations in number and intensity of bands in the selected species. The Rf value of each band was calculated in terms of migration of each protein band as revealed by the movement of bromophenol marker dye. The Rf value was calculated as follows:

$$Rf = \frac{\text{Distance of the band from the top of the gel}}{\text{Distance travelled by tracking dye}}$$

The details of the observations made in each of the selected species in terms of protein bands and their corresponding Rf values are presented in the Tables 1 to 4:

TABLE-01

The Rf value of the bands that appeared on gel of *Medicago intertexta*

Band No.	Rf value
1	0.18
2	0.25
4	0.31
5	0.37
7	0.46
8	0.53
11	0.68
12	0.75
13	0.81

TABLE - 02

The Rf value of the bands that appeared on gel of *Medicago littoralis*

Band No.	Rf value
1	0.18
2	0.25
4	0.31
5	0.37
6	0.43
7	0.46
8	0.53
12	0.75
13	0.81
14	0.87

TABLE - 03

The Rf value of the bands that appeared on gel of *Medicago polymorpha*

Band No.	Rf value
1	0.18
2	0.25
5	0.37
7	0.46
9	0.56
10	0.62
11	0.68
12	0.75
13	0.81

TABLE - 04

The Rf value of the bands that appeared on gel of *Medicago scutellata*

Band No.	Rf value
1	0.18
2	0.25
3	0.28
4	0.31
7	0.46
10	0.62
11	0.68
12	0.75

The zymogram obtained after electrophoresis revealed various bands (Figs.1&2 Table -5).The Rf values amongst different species of Medicago ranged from 0.18 to 0.87 (Table - 6). This value depicts the mobility of proteins on Gel surface. Minimum Rf value of 0.18 has been seen in all the members of Medicago species studied here, while maximum Rf value 0.87 is specific in M. littoralis.

The comparative percentage similarity between the different pairs of species was calculated, based on the formula given below :

$$\text{Percentage similarity} = \frac{\text{No. of similar bands}}{\text{No. of different bands} + \text{No. of pairs of similar bands}} \times 100$$

Percentage value of similarity between *M. intertexta* and *M. littoralis* is 73 % , which is the maximum, while the same value for *M. littoralis* and *M. scutellata* is the minimum i.e. 38.70 % (Table - 7 and Fig. 3).

TABLE - 05

Number of bands revealed by experimented species of *Medicago*

Name of the Species	Bands
M. intertexta	9
M. littoralis	10
M. polymorpha	9
M. sativa	8

TABLE - 06

The Rf value of the bands that appeared on the gel of four *Medicago* spp.

S No.	Rf	*M. intertexta*	*M. littoralis*	*M. polymorpha*	*M. scutellata*
1	0.18	+	+	+	+
2	0.25	+	+	+	+
3	0.28	–	–	–	+
4	0.31	+	+	–	+
5	0.37	+	+	+	–
6	0.43	–	+	–	–
7	0.46	+	+	+	+
8	0.53	+	+	–	–
9	0.56	–	–	+	–
10	0.62	–	–	+	+
11	0.68	+	–	+	+
12	0.75	+	+	+	+
13	0.81	+	+	+	–
14	0.87	–	+	–	–

TABLE - 07

Percentage Similarity Index Between Four different pairs of ***Medicago*** **pp.**

S. No.	Species x Species	Percentage Similarity
1	*M. i* x *M. l*	73.00%
2	*M. i* x *M. p*	62.00%
3	*M. i* x *M. s*	54.50%
4	*M. l* x *M. p*	46.10%
5	*M. l* x *M. s*	38.7 0 %
6	*M. p* x *M. s*	60.00%

M. i = *M. intertexta*; M. l = *M. littoralis*

M. p = *M. polymorpha*; M. s = *M. scutellata*

The percentage similarity index based on electrophoretic studies of seed protein brought to light that *M. intertexta* (2n = 2x = 16) is very close to both *M. littoralis* (2n = 2x = 16) and *M. polymorpha* (2n = 2x = 14), but it is bit more closer to *M. littoralis* (73 %) than *M. polymorpha* (62 %). However, *M. polymorpha* in this respect stands quite away from *M. littoralis* (46.10%). The similarity index between *M. scutellata* and *M. littoralis* indicates that *M. littoralis* (2n=2x=16) has quite little affinity with *M.scutellata* (2n=4x=30), having a similarity value of just 38.70% .

Lastly, when similarity values amongst *M. scutellata* (2n = 4x = 30), *M.polymorpha* (2n=2x=14) and *M. intertexta* (2n = 2x = 16) is closely analysed, it indicates that the diploid *M. polymorpha* is more closer to the tetraploid *M.scutellata* (similarity 60%) as compared to the other diploid species, *M.intertexta* (similarity 54.50%) .

Thus, the diploid species of *M . polymorpha* and *M. intertexta* appear to show their near proximities with the tetraploid *M. scutellata* but the similiarity percentage results are not so encouraging. Therefore, the present investigation doesn't seem to provide any clear picture of the possible involvement of either of the diploids selected here in the origin of tetraploid *M. scutellata* (2n = 4x = 30). However, this will require a broader screening of other available Medicago L species for arriving at a meaningful interpretation.

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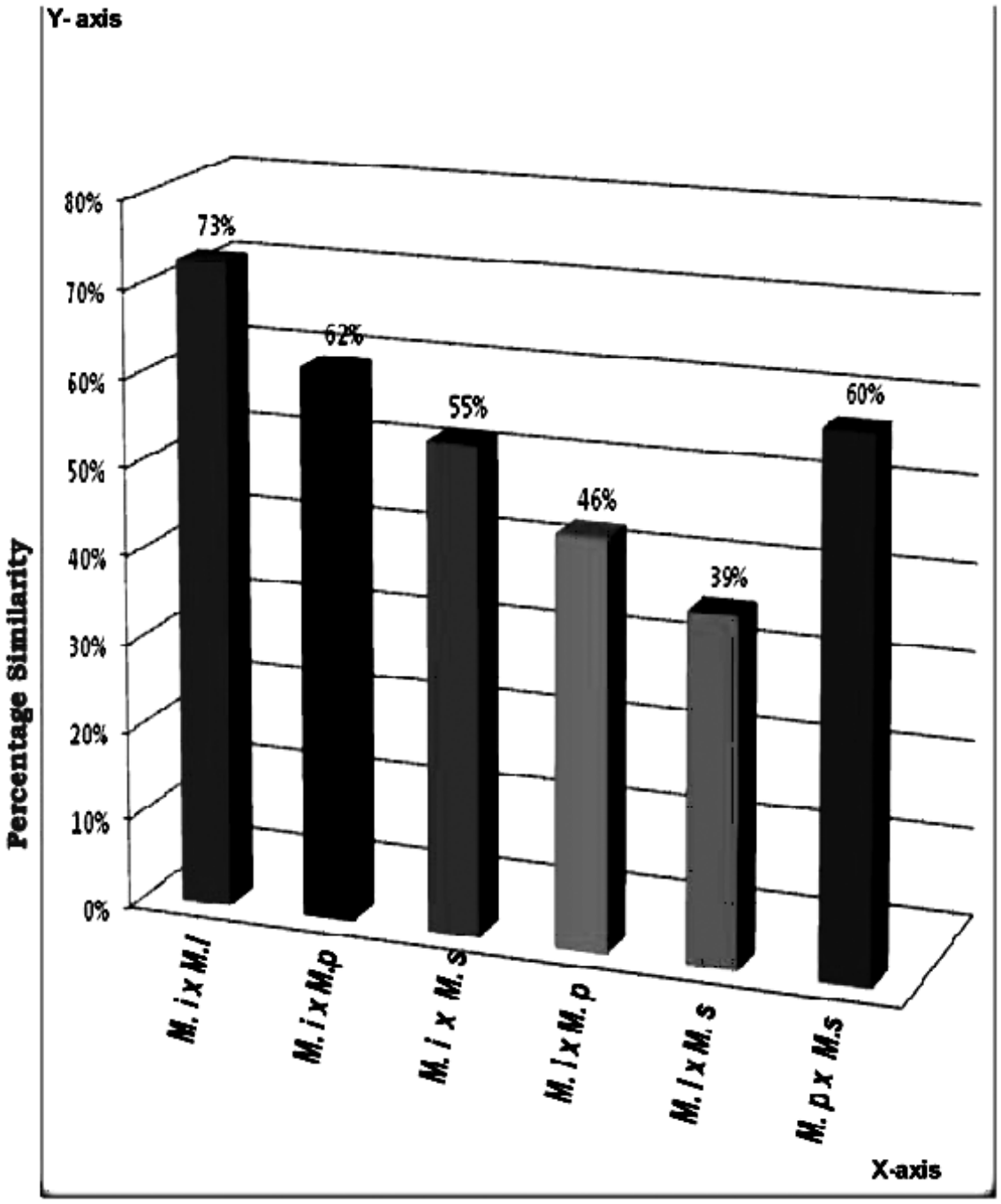

Fig. 3 **Showing % Similarity Index in the *Medicago* Spp.**

M. i x M. l - ***M. intertexta x M. littoralis***
M. i x M. p - ***M. intertexta x M. polymorpha***
M. i x M. s - ***M. intertexta x M. scutellata***
M. l x M. p - ***M. littoralis x M. polymorpha***
M. l x M. s - ***M. littoralis x M. scutellata***
M. p x M. s - ***M. polymorpha x M. scutellata***

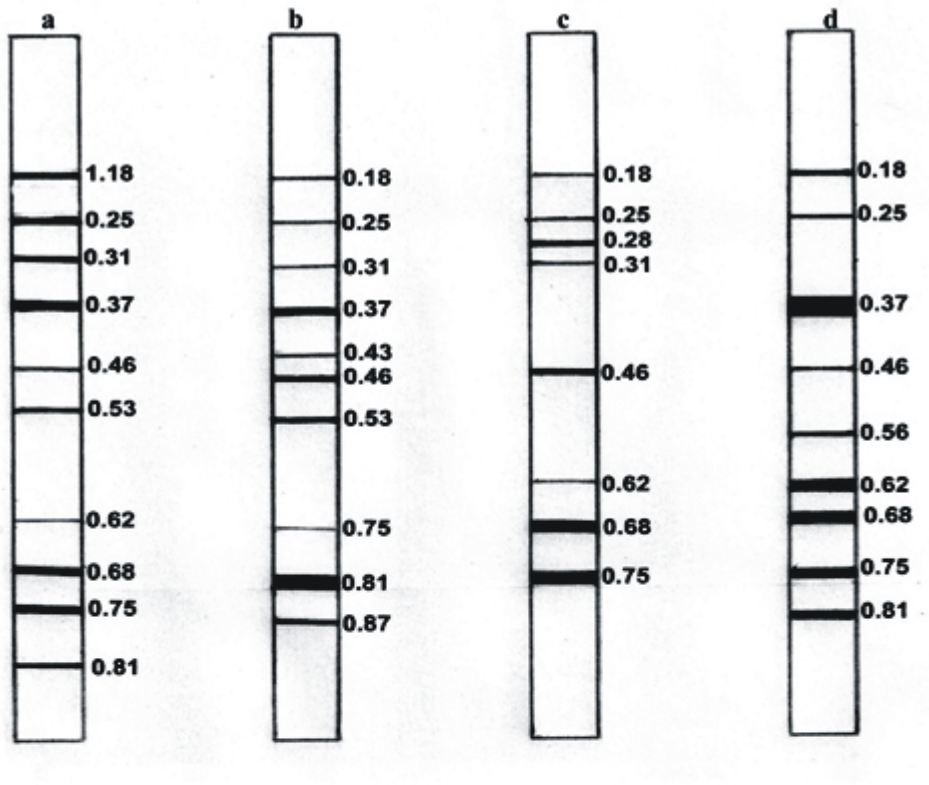

Fig. 1

Fig. 2

PLATE 1. Seed Storage Banding Patterns of *Medicago Spp*.
Fig 1.2 a. *M. intertexta*; b. *M. littoralis;*
c. *M. Scutellata*; *M. Polymorpha*