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Identification of Mesta Varieties through Gel electrophoresis of Tris-soluble Seed Proteins

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ABSTRACT

A study was conducted with an objective of varietal identification by using gel electrophoresis of tris-soluble seed proteins. Nine genotypes of Mesta *(Hibiscus cannabinus* and *H. sabdariffa*) were characterized by utilizing soluble proteins from extracts of single seeds by SDS-PAGE. The specific banding patterns of total soluble seed proteins by SDS-PAGE was quite different for each of these genotypes and therefore, it was possible to identify each genotype individually indicating the usefulness of electrophoretic variation in soluble seed protein for varietal characterization.

Key words : Mesta, Rm, Storage protein,SDS-PAGE.

Mesta is an important fibre crop in India. The development of new cultivars has caused a large increase in crop yields in the last decades (Association of Official Seed Analyst, AOSA). However the release of closely related mesta cultivars *(Hibiscus cannabinus* and *H. sabdariffa*) with similar phenotypes is causing difficulties to analysts regarding varietal differentiation. Traditionally, cultivar identification techniques are based mainly on morphological characteristics, mainly those expressed under field conditions. Their number is limited and their expression is frequently influenced by environmental and management conditions. To assure, the genetic purity of seed lots, besides controlling eventual contamination, it is necessary to employ an identification test which is not affected by environmental effects. Therefore, the conventional method has been supplemented by bio-chemical tests. Among these, seed protein electrophoresis techniques are now being used for the characterization and identification of varieties (Mc Donald and Drake) and it has been reported that SDS-PAGE profiles are polymorphic and environmental influence on their pattern is limited (Gepts *et al*. 1986 and Sadia *et al*. 2009) for assessing genetic relationships among species, all of which are most important for seed technologists, geneticists and plant breeders.

MATERIAL AND METHODS

The present study comprising genetically pure seed of nine varieties of mesta *(Hibiscus cannabinus* and *H. sabdariffa*) obtained from the respective breeders of the research stations of ANGRAU and the experiment was conducted during 2008-09 at Seed Research and Technology Centre, Hyderabad.

Electrophoretic technique of total soluble seed proteins:

SDS–PAGE of total soluble seed proteins was carried out by using 15 per cent resolving gels according to the methods prescribed by Laemmli (1970) with slight modifications.

Protein extraction:

Matured and well filled seeds were selected and ground in a prechilled pestle and mortar. Powdered samples were defatted using defatting solution. (Chloroform, methanol and acetone in 2:1:1 ratio), which was performed for 24 hours. 400µl of sample buffer (0.5 m Tris HCl, pH 6.8 : 5.0 ml, distilled water :43 ml, Glycerol 1.0 ml and absolute alcohol :1.0 ml) was added to 100 mg of seed powder and kept overnight at room temperature. The samples were centrifuged at 15000 rpm for 20 minutes and supernatant was collected, 20 µl of (SDS) Sodium dodecyl

sulphate + Mercapto ethanol mixture, 10 µl of bromophenol blue and two pellets of sucrose were added to the 50 µl of supernatant. The samples were boiled for five minutes and cooled and were used immediately for electrophoresis.

Electrophoresis:

Electrophoresis was performed with a model of A E - 6290 Resolmex Slab gel unit (ATTO Corporation, Japan) with 16 x 16 cm glass plates separated by 0.75 mm spacers. The resolving gel (15%) was de-aerated and poured to a height of 11.5 cm and over layered with distilled water. After polymerization, the water was decanted and the stacking gel solution was poured. A 12 well comb was inserted into a depth of 1.5 cm in stacking gel. An aliquot of 15 ìl of sample was loaded into each well. Electrophoresis was performed with a constant current of 30 µA (at fixed voltage) at 20 º C maintained with circulating cooling water. The run is terminated when the dye marker has migrated about 10 cm. Bovine Serum Albumin (BSA) of 66.2 kDa was added as molecular weight marker. The gels were stained with a staining solution with 0.25% (w/v) Coomassie brilliant blue R 250 in 50% (v/v) methanol and 10% (v/v) acetic acid. Gels were stained over night followed by two rinses with distilled water and were destained in 25% (v/v) methanol, 7 % (v/v) acetic acid destaining solution, until the bands were clear. The gels were preserved in preserving solution (10 % (v/v) glycerol in destaining solution. Three electrophoretic runs were performed for checking the repeatability of the protein profiles.

The electrophoresis can be measured by observing gels over a transilluminator measuring the distance of each band from the point of loading. Relative mobility (R_m) of each band was calculated as

$$R_m = \frac{\text{Distance traveled by protein sample (cm)}}{\text{Distance traveled by tracking dye (cm)}}$$

Scoring of bands:

Protein bands were traced on to the graph by visual observation of the gels and mean Rm value calculated for each genotype and the banding pattern were measured as qualitative and quantitative variation (Wouters and Booy, 2000).

Qualitative variation: When a particular band as designated by its Rm value was present in the electrophoregram of one cultivar but absent in that of another, the variation was referred as qualitative variation.

Quantitative variation:

When a particular band was observed in the electrophoregram of two or more different cultivars, but differs in band size or staining intensity.

RESULTS AND DISCUSSION

Seed storage protein profiling was studied as it is a useful technique for the characterization of crop varieties at molecular level. The consistency of the protein profile suggests that each species has a reproducibly stable profile as a consequence of its specific gene arrangement (Ladizinsky, 1975). Though no unique band was observed specific for a variety, all the varieties studied exhibited unique banding patterns (Fig.1).The differences were either in the total number of bands present, location of bands or intensity of bands or it can even be the presence or absence of four categories of bands namely dense, medium, light and very light.

Nine varieties of mesta showed wide variability for various morphological traits exhibited. The total of protein bands present in different genotypes ranged from 16 to 23. The lowest number of bands was observed in AMV-108 (16) and maximum number of bands i.e 23 was observed in AMV-3, HS 7910 and AMV-2. The number of bands present was 19 in AMV-1 and AMV-4, 21 in K12(Y) and HC 583 and 22 in HC 4288 respectively.

Even though some of the genotypes produced same number of bands, certain differences were found with respect to Rm (Relative mobility) of the bands and also the intensity and size of the bands.

The varieties AMV-1 and AMV-4 showed 19 bands, but the differences were observed in Rm values and intensity of staining. The major difference is presence of medium band at Rm value 4.0 in AMV-1 and it is dark in AMV-4. Whereas at Rm 5.8 it is dark in AMV-1 and medium in AMV-4. The bands present at Rm 0.9 and 8.4 in AMV-1 and absent in AMV-4, whereas bands at Rm 3.6

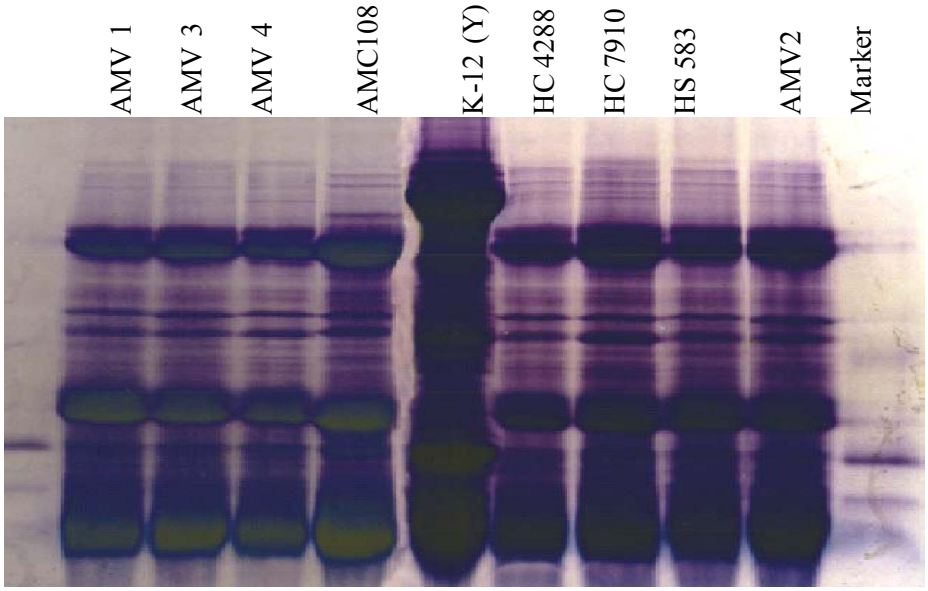


Figure 1. Electrophoretic (SDS-PAGE) pattern of tris soluble seed proteins in mesta varieties.

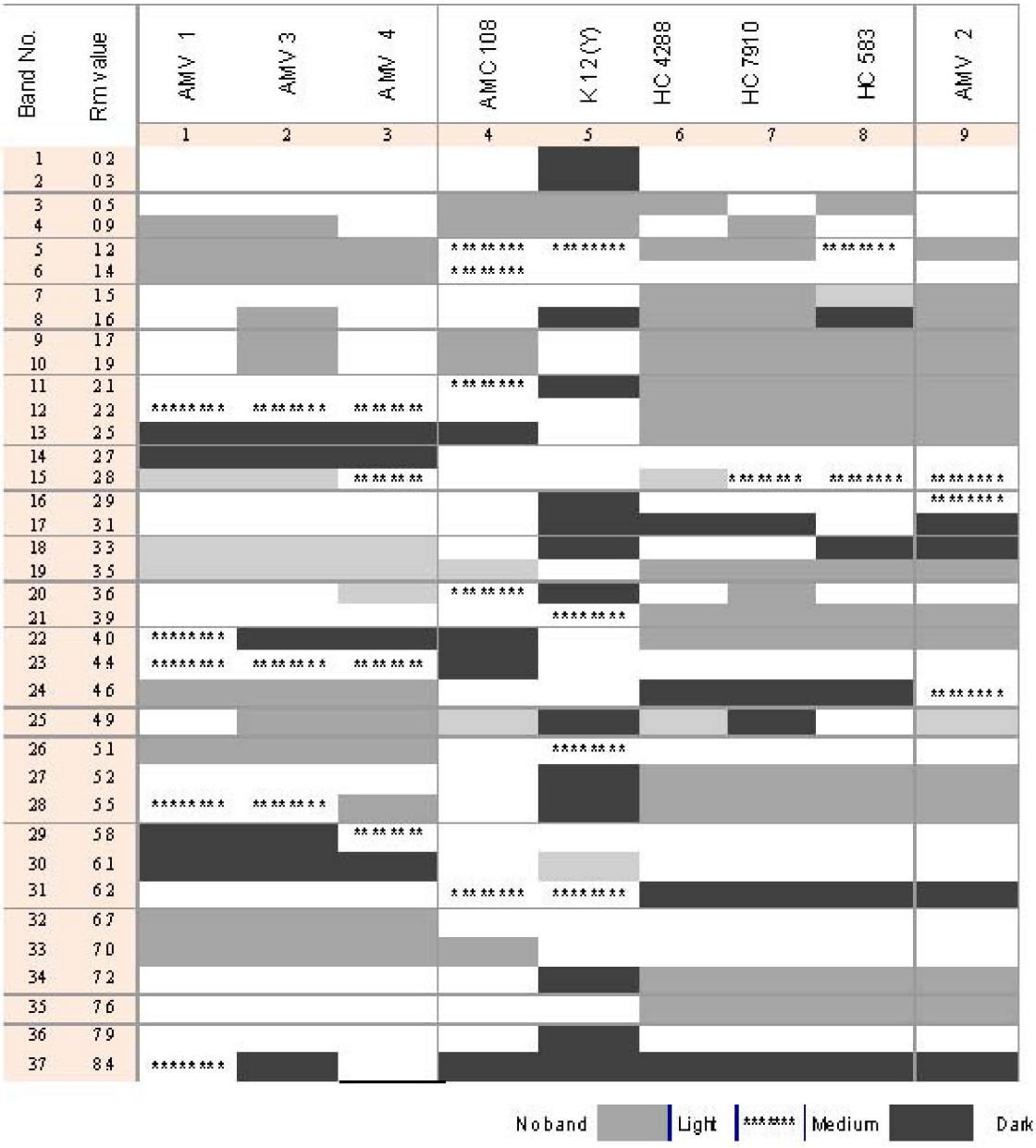
and 4.9 were absent in AMV-1 and were present in AMV-4.

The varieties K 12(Y) and HC 583 have same number of bands (21), but the distinct variation was observed in presence of 14 bands in K 12 (Y) and only 5 dark bands in HC 583. The genotype K 12 (Y) can be differed from HC 583 by observing the presence of dark bands at Rm values 0.2, 0.3, 2.9,3.1,3.6,4.9 and 7.9, where as there were no bands at these Rm values in HC 583.

The varieties AMV-3, HS 7910 and AMV-2 have equal number of bands (23), but the variation was observed in the intensity and location of bands. The genotype AMV-3 can be differentiated from other two genotypes by observing presence of dark band at Rm 2.7, 5.8 and 6.1, The genotype HS 7910 can be differentiated from other two genotypes by observing presence of dark band at Rm 5.1 and is light in other two, where as light band is present at Rm 3.6 and is absent in other two. The genotype AMV-2 can be differentiated from other two genotypes by observing presence of medium band at Rm 4.6 and is absent in other two genotypes.

The overall differential banding pattern of seed proteins revealed qualitative and quantitative variations among the different genotypes, which enable us to identify a particular genotype with the presence or absence of specific position of band and also the intensity of band which could be used as a powerful technique for ascertaining genetic homology at the molecular level and for resolving taxonomic and phylogenetic problems (Ladizinsky and Hymowitz, 1979). These specific protein bands migrating to the same distance gave some evidence of homology in molecular structure and function. The analysis of protein composition as a means of plant variety identification is now well established and has been thoroughly reviewed (Cooke, 1988 and Cooke, 1984). The success of electrophoretic procedures depends on the wide ranging polymorphism of seed and seedling proteins and the fact that these proteins represent primary gene products. Their expression and detection are thus largely unaffected by environmental interactions and they can be conveniently used as gene markers (Cooke, 1984). Electrophoresis method is widely used because of simplicity, relatively low cost and short time required to provide reliable results even under different environmental conditions.

Figure 2. Electrophoregram of Tris-soluble seed proteins in mesta varieties.



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