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# GENETIC VARIABILITY AMONG GENOTYPES OF SORGHUM BASED ON PROTEIN PROFILE OF SEED STORAGE PROTEINS

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## ABSTRACT

Sorghum (*Sorghum bicolor (L.)Moench)* is an important cereal crop, a significant dietary food for one-third of world population and principle source of energy, protein, vitamins and minerals. The current study aims at protein analysis of six genotypes (HC 260, M 35-1, CSV-15, CSV-17, CSV-20, CSV-22) of Sorghum by using SDS-PAGE, protein estimation and Phylogenetic relationship among them. Phylogenetic relationships among six genotypes were investigated based on seed protein profiles produced by SDS-PAGE. A total of 56 polypeptide bands were scored of which 30 were polymorphic and 26 were monomorphic. The molecular weights of polypeptides ranged between 25KDa to 70KDa. A distance matrix was generated from the similarity matrix which was computed by using Jaccard's coefficient. The similarity coefficients among six genotypes ranged from 0.500 to 0.900 and the genotypes were categorized into two main clusters. The UPGMA dendrogram depicted that CSV 15, CSV 20 and CSV 22 are closely related.

**Key words:** *Sorghum bicolor*, SDS-PAGE, Jaccard's coefficient, seed storage proteins, Phylogenetic tree,

## INTRODUCTION:

Seed storage proteins of cereals contribute major source for nutrition of Mankind. Cereal seed proteins are of importance for human and animal nutrition, plant breeding and cultivar identification (Skylas and Wrigley 2004)[19]. Sorghum is the fifth major cereal crop in the world after wheat, rice, corn and barley (Awika and Rooney, 2004)[1]. Inspite of it, scanty information is available concerning the genetic variability among the sorghum genotypes (N.P.Eswara Reddy, M.Jacobs 2002)[7]. The unique property of its adaptation to semi-arid environmental conditions attains special interest when compared to other cereals. In recent years, considerable focus has been shown in breeding cereal grain with high protein content provided good nutritional quantity can be maintained. Sorghum grain proteins play an important role in the utilization of sorghum for its nutritional and functional properties in respect to dietary requirements and in our present study SDS – PAGE has been a tool used to study sorghum proteins. Sorghum protein content of grain sorghum is approximately 13% (Beta et al. 1995)[2]

with storage proteins comprises of 70-90% of total protein (Lokhart et al 2000)[14].The advent of the electrophoretic as an analytical tool provide an indirect methods for genome probing by exposing structural variations in enzymes or other protein genomes (Cooke RJ 1984, Gilliland TJ 1989)[6, 9]. Electrophoresis is an effective method for testing seed purity and has been widely used for the identification of cereal varieties (International seed testing association 1999)[10]. PAGE is one of the most reliable methods available for the separation of proteins in complex mixtures for assessing protein purity and for determination of protein relative molecular mass ($M_r$) (Richard J Simpson 2002)[16]. SDS-PAGE is a relatively low cost and high through put method for analyzing proteins of cereals (Lookhart, G.L., Bean, S.R. 2000)[14] and a simple and powerful way to dissociate proteins into individual chains and separate them according to their molecular weights (Shapiro et al 1967)[18]. For efficient conservation and breeding practices characterization and evaluation of genetic diversity with a species is important (Bretting PK and Widrlechner MP 1995; Gracia E et al., 1998)[5, 8]. Knowledge about degree of genetic diversity among and within natural population in and outside centre of origin is required to gain the first idea about where to find potentially valuable genetic material (Varsha khurana kaul et al., 2012)[21]. The objective of this research was to study genetic variability among six genotypes of sorghum using extraction of seed storage proteins and analysis by using SDS-PAGE, their estimation using spectrophotometer to find out genotype with higher protein content.

## MATERIALS AND METHODS:

### Plant Material:

Six cultivars of *Sorghum bicolor* HC 260, M 35-1, CSV-15, CSV-17, CSV-20, and CSV-22 were used to assess the total protein and genetic variability. The seeds were provided by Directorate of Sorghum Research (NRCS), Rajendranagar, Hyderabad.

### Protein Extraction:

Seeds of six different varieties of sorghum were grinded to fine powder with the help of mortar and pestle. Sample buffer (Naushad et al., 2010)[15] of 400 µl was added to a 0.02 g of fine seed flour as an extraction liquid and bromophenol blue (BPB) to follow the movement of protein in the gel. The active ingredients used for the extraction of protein buffer contained 0.5 M Tris-HCl (pH 8.0), 0.2% SDS, 5 M urea and 1% 2-mercaptoethanol. When all these chemicals are tightly put together then the solution needs to be purified and homogenate, so the samples were thoroughly mixed by vortexing and centrifugation at 15,000 rpm for 5 min at room temperature. After centrifuging samples, the crude proteins were recovered as clear supernatant on the top of the tube. Then the supernatant were transferred into new 1.5 ml eppendorf tubes and were stored at - 20°C until gel electrophoresis.

### Protein estimation:

Protein content of each sample was determined using Bradford's reagent (Bradford, M.M. 1976)[4] using BSA as standard. The extractions of proteins for estimation were carried out by applying the method followed by Naushad et al (2010)[15].

### SDS-PAGE Analysis:

SDS PAGE analysis was carried out applying the method of Sambrook et al., (1989)[17]. Gel is prepared in concentration of 10% resolving gel and 4.4% stacking gel. Electrophoresis was carried out according to Laemmli (1970)[13] after adding sample loading buffer. Bands were visualized by silver staining method which was described by Blum et al (1987)[13] and UNSW Biological mars spectrophotometry and protein analysis laboratory. Molecular weights of dissociated polypeptides were determined by using Molecular size marker in the range between 29KDa to 205KDa (Merck).

**Data analysis:**

**Jaccard similarity co-efficient:**

The Jaccard similarity (Jaccard 1902, Jaccard 1912)[11, 12] is a common index for binary variables. It is defined as the quotient between the intersection and the union of the pair wise compared variables among two objects.

$d^{JAS}(i,j) = \frac{J11}{J01 + J10 + J11}$. In the equation $d^{JAD}$ is the Jaccard distance between the objects *i* and *j*. For two data records with *n* binary variables *y* the variable index *k* ranges from 0 to *n*-1. Four different combinations between $y_{i,k}$ and $y_{j,k}$ can be distinguished when comparing binary variables. These combinations are (0/0), (0/1), (1/0) and (1/1). The sums of these combinations can be grouped by:

- $J_{01}$: the total number of variables being 0 in $y_i$ and 1 in $y_j$.
- $J_{10}$: the total number of variables being 1 in $y_i$ and 0 in $y_j$.
- $J_{11}$: the total number of variables being 1 in both $y_i$ and $y_j$.
- $J_{00}$: the total number of variables being 0 in both $y_i$ and $y_j$.

As each paired variable belongs to one of these groups it can be easily seen that: $J_{00} + J_{01} + J_{10} + J_{11} = n$ As the Jaccard similarity is based on joint presence, $J_{00}$ is discarded. The Jaccard dissimilarity is defined as $d^{JAD} = 1 - d^{JAS}$. In some cases the Jaccard similarity is computed as $d^{JAS} = 2d^{BCD} / (1 + d^{BCD})$, where $d^{BCD}$ is the Bray–Curtis dissimilarity. This equation does not reduce values to binary states. Thus, results are different when using on the one hand a presence/absence matrix and on the other hand a count matrix. The results are the same, when the count matrix is converted to a binary matrix beforehand.

**Drawing Phylogenetic tree:**

Electrophoregrams for each variety were scored by the presence (1) or absence (0) of each band noted. Presence and absence of bands were entered in a binary data matrix. Based on electrophoresis band spectra, Jaccard's similarity index (JSI) was calculated by the formula (Sneath and Sokal, 1973). S= W/ (A+B-W) Where 'W' is the number of bands of commonmobility, 'A' the number of bands in type 'A' and 'B' is the number of bands in type'B'. The similarity matrix generated was converted to a dissimilarity matrix (dissimilarity = 1-similarity) and used to construct a dendrogram by the unweighed pair group method with arithmetic means (Sneath and Sokal, 1973). All analysis was carried out using a statistical package NTSYS-pc, version 1.8 (Rohlf, 1993) and STATISTICA for window 98. Closely observing the gel banding pattern, the binary matrix for presence (1) or absence (0) of a band was denoted.

**RESULTS:**

**Protein estimation:**

Protein estimation studies reveal that HC-260 possesses high protein content followed by M35-1.

| Protein isolate | Amount of protein in mg/gm of seed. |
|---|---|
| HC 260 | 5.00 |
| M-35-1 | 4.03 |
| CSV-15 | 1.85 |
| CSV- 17 | 2.00 |
| CSV-20 | 2.55 |
| CSV-22 | 3.00 |

Table 1: Protein Estimation of six genotypes of sorghum.

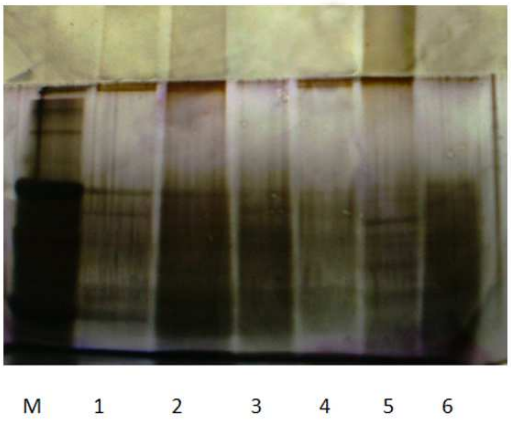
**SDS-PAGE analysis:**

Protein profile of the six genotypes produced a total number of 56 bands out of which 30 were Polymorphic and 26 were monomorphic. Molecular weight of the proteins ranged from 25KDa to 70KDa. Highest number of bands was observed in the genotype CSV 20 that scored 12 bands. Genotype HC 260 scored 8 bands; M-35-1 scored 11 bands; CSV-15 scored 9 bands; CSV-17 scored 6 bands; CSV-22 scored 10 bands. The Molecular weight range of six genotypes was depicted below.

| Genotype | Molecular weight range |
|---|---|
| HC 260 | 29KDa-67KDa |
| M-35-1 | 29KDa-70KDa |
| CSV-15 | 29KDa-67KDa |
| CSV- 17 | 29KDa-67KDa |
| CSV-20 | 25KDa-70KDa |
| CSV-22 | 25KDa-67KDa |

**Table 2:** Molecular weight range of six genotypes

(a)




(b)

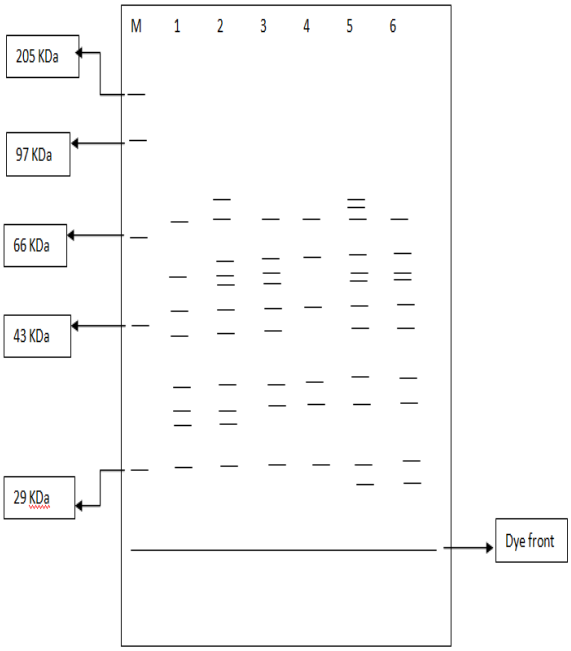



Fig 1(a-b): Electrophoretic protein profile of six genotypes of Sorghum using SDS-PAGE.
(1-HC260; 2-M-35-1; 3-CSV-15; 4-CSV-17; 5-CSV-20; 6-CSV-22; M-Marker)

## Cluster analysis:

The Jaccard's similarity coefficient of six genotypes ranged from 0.500 to 0.833. A Phylogenetic tree was constructed using UPGMA method. Cluster analysis showed that genetic relatedness of 6 genotypes ranged from 0.100 to 0.500. The sorghum genotypes were clustered into two main clusters. Cluster 1 denoted one genotype CSV-17, Cluster II is divided into two accessions one is HC 260 and Second accession is further divided into two sub-groups one is M-35-1 and in another sub group further divided into two sub groups. Genotypes CSV15, CSV22 grouped in single branch and CSV20 placed in another group, revealing that CSV-15 and CSV-22 are very closely related i.e., of same origin. CSV-20 is closely related to CSV-15 and CSV-22. CSV-17 is distant in Phylogenetic relation with all other genotypes.

**Similarity matrix:**

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| **2** | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| **3** | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 |
| **4** | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 |
| **5** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 |
| **6** | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 |

**Similarity matrix with Jaccard coefficient:**

| | HC260 | M351 | CSV15 | CSV17 | CSV20 | CSV22 |
|---|---|---|---|---|---|---|
| HC260 | 1 | 0.727 | 0.700 | 0.556 | 0.538 | 0.636 |
| M351 | | 1 | 0.818 | 0.545 | 0.769 | 0.750 |
| CSV15 | | | 1 | 0.667 | 0.750 | 0.900 |
| CSV17 | | | | 1 | 0.500 | 0.600 |
| CSV20 | | | | | 1 | 0.833 |
| CSV22 | | | | | | 1 |

**Distance matrix using Jaccard coefficient:**

| | HC260 | M351 | CSV15 | CSV17 | CSV20 | CSV22 |
|---|---|---|---|---|---|---|
| HC260 | 0 | 0.273 | 0.300 | 0.444 | 0.462 | 0.364 |
| M351 | | 0 | 0.182 | 0.455 | 0.231 | 0.250 |
| CSV15 | | | 0 | 0.333 | 0.250 | 0.100 |
| CSV17 | | | | 0 | 0.500 | 0.400 |
| CSV20 | | | | | 0 | 0.167 |
| CSV22 | | | | | | 0 |

**Phylogenetic Tree:**

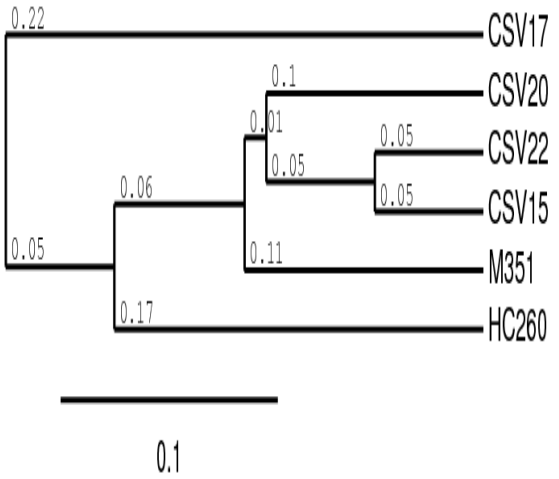



Fig 2: Phylogenetic tree UPGMA method relationship between genotypes of sorghum.

## CONCLUSION:

Electrophoresis has been widely used for separating proteins from all cereals. Sorghum proteins play an important role in the utilization of sorghum and its nutritional properties and SDS-PAGE has been a major tool used to study sorghum proteins. The present work is the first report on SDS-PAGE Protein profile based genetic variability study on selected genotypes of Sorghum and the method proved to be effective. Protein estimation studies also provide data regarding protein content for these genotypes.

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