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Genetic variability among variety of sorghum [*Sorghum bicolor* (L.) Moench] based on protein profile of seed storage proteins

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Abstract
In India, the Government enacted the legislation on Protection of Plant Varieties and Farmers Rights (PVP and FR) Act in 2001. The act provides protection of new varieties including extant-notified and farmer's varieties. Novelty, Distinctness, Uniformity and Stability are the essential requirements for grant of protection to all the new varieties. Under the PVP and FR Act 2001 of India, it requires registration of varieties based on three principles know as DUS (Distinctness, Uniformity and Stability) criteria. The DUS testing principles are used for the protection of variety and award of Plant Breeder's Rights (PBR), a system of intellectual property protection which is available to breeders of all types of crops. On practical level, DUS assessment of agricultural crops generally involves growing field crops under appropriate ambient conditions, and recording various morphological characteristics of the seed and/or growing plants. Laboratory and green house tests can also be involved (Mauria, 2000) and the new (candidate) varieties are compared with existing varieties that are kept as reference collection. The present investigation comprised of 12 varieties of fodder sorghum (*Sorghum bicolor* (L.) Moench) was undertaken for genetic variability among varieties of sorghum based on protein profile of seed storage protein. The banding pattern of total soluble protein showed considerable variation among the twelve fodder sorghum varieties. Seven protein marker bands were present in all varieties but the intensity and thickness varies from variety to variety. The analysis of total soluble protein banding pattern in all the fodder sorghum varieties were carried out along with the standard protein molecular weight marker having 14.4 to 116.0 kDa. Unweighted paired group method with arithmetic mean (UPGMA) was used to construct dendrogram for all the twelve sorghum varieties. The dendrogram showed two major clusters at coefficient of 0.7774.

Keywords: sorghum bicolor, genetic variability, seed storage protein, protein profile

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) [6, 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) [5] 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]. Sharmila *et al.* (2013), in their studies they analyzed protein of six genotype (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. The phylogenetic relationships among six genotypes were investigated based on seed protein profile produce by SDS –PAGE. In their studies, they found 56 polypeptide bands were scored of which 30 were polymorphic and 26 were monomorphic. In the light of above facts, the present study on "Genetic variability among variety of sorghum [*Sorghum bicolor* (L.) Moench] based on protein profile of seed storage proteins."

was planned with the following main objectives to distinguish fodder sorghum varieties by Electrophoresis technique.

Material and Methods

Plant Material

Twelve varieties of Sorghum bicolor viz.., HC-136, HC-171, HC-308, HJ-513, HJ-541, Pant Chari-7, GFS-5, Pant Chari-3, PC-5, SSV-84, CSV-15, UP Chari-2 were used in present study. The seeds of different varieties were collected from Forage Section, Department of Genetics and Plant Breeding CCS Haryana Agricultural University, Hisar during 2013.

Electrophoresis of total soluble seed protein (SDS – PAGE)

The protein profile for fodder sorghum seed was obtained by electrophoresis technique (SDS-PAGE) following the procedure described by Dadlani & Varier (1993) [2] with some modification.

Preparation of protein samples

Five sound pure seed were randomly selected from each variety and ground into fine powder with the help of pestle and mortar. The powder transferred to test tube and 10 ml of defatting solvent mixture added. The test tube were covered with aluminum foil and allowed for defatting. After 3 h., solvent mixture decanted and the process was repeated three to four times so that the oil content could be removed completely. The seed powder was then dried by leaving the test tubes open at room temperature.

Protein extraction (Total tris soluble protein)

One hundred milligram of defatted seed powder was taken in eppendorf tube and 0.5 ml of 2x sample buffer was added to it. The content were thoroughly mixed and kept for overnight in refrigerator. The tubes were taken out and content were mixed properly and then it was subjected to centrifugation at 10,000 rpm for 10 minutes. The supernatants were taken into separate glass tubes. These samples were then boiled in water bath for 10 minute, cooled and finally it was used as protein source for electrophoresis.

Evaluation and documentation

The varieties were separated into different groups according to number of bands (presence or absence) and intensities of some specific bands. The relative mobility (Rm) value of each band was calculated as follows

$$\text{Relative mobility (Rm)} = \frac{\textit{Distance migrated by the protien band from the origin (cm)}}{\textit{Distance migrated by the tracking dye (cm)}}$$

Similarity index

Similarity index value was calculated based on proportion on common bands between two lines by using the following formula (Nei and Li, 1979).

$$F = \frac{2Mxy}{Mx + My}$$

Where F is the similarity index, Mx is the number of bands common in variety x, My is the number of bands in variety y and Mxy is the number of bands common to both x and y. F× 100 gives the percent similarity between two variety, thus F = 1.0 would mean that the patterns in the varieties are identical.

Dendrogram analysis

Analysis was done with the help of computer by using D-UPGMA software.

Result and Discussion

All 12 fodder sorghum varieties were subjected to SDS-PAGE analysis for total seed proteins (plate 1). A wide quantitative variation was noticed in terms of relative mobility (Rm) value, electrophoretic mobility of protein bands, intensity of bands and also in banding pattern of different sorghum varieties. Polymorphism in protein banding of all varieties were noticed and it was observed that they varied in intensity and thickness also.

The SDS- PAGE analyses of the total soluble proteins of twelve varieties revealed a maximum seventeen bands were recorded in total soluble seed protein electrophoresis profile. A wide quantitative variation (having different Rm values) was observed in pattern of protein bands, their electrophoretic mobility, position and intensity. The standard protein marker bands [2 (Rm 0.24), 6 (Rm 0.40), 9 (Rm 0.53), 11 (Rm 0.64), 13(Rm 0.68), 16 (Rm 0.88) and 17 (Rm 0.91)] were observed invariably common in almost all the varieties and which is presented in Table 1. The maximum number of bands were observed in varieties HC-136 (Dense-1, Medium-8, light-5 and weak-3), HJ-513 (Dense-3, medium-3, light-7 and weak 4), PC-5 (Dense-3, Medium-2, light-11, weak-1) and lowest were observed in variety PC-3 (Medium-2, light-6, weak-3). All the varieties were differentiated by using the presence/absence of specific combination of bands or single band. In this way, all the twelve varieties were individually identified. (Table 2)

The analysis was carried out along with the standard molecular weight marker having range 14.4 to 116.0 kDa. The bands between 45 to 66.2 kDa molecular weight were more thick and intense as compared to higher and lower molecular weight bands. This was a clear indication that these molecular size protein molecules constituted majority of total soluble seed protein in sorghum varieties. The respective protein band map summarized in table 3.

The similarity index was calculated based on the protein of all fodder sorghum varieties. Similarity index based on the relative mobility of protein band indicated the association among different varieties. The similarity index was calculated in all combination and their values obtained are present in Table 4. The similarity index value ranges from 0.143 to 1.000. The variety PC-3 showed less value with other varieties showing dissimilarity with other remaining varieties.

Unweighted paired group method with arithmetic mean (UPGMA) was used to construct dendrogram for all the twelve sorghum varieties. The dendrogram showed two major clusters at coefficient of 0.7774 as the varieties PC- 3 and PC 7 in first cluster and rest varieties in second cluster. The second cluster was further divided into two sub-clusters. The complete dendrogram is shown in Fig.1.

Table 1: Number of bands, their position and Rm values of seed protein in different varieties of fodder sorghum (*Sorghum bicolor* (L.) Moench).

Band number	Position (cm)	Rm value
1	1.89	0.18
2	2.52	0.24
3	2.94	0.28
4	3.26	0.31
5	3.36	0.32
6	4.20	0.40
7	4.73	0.45
8	5.04	0.48
9	5.57	0.53
10	5.99	0.57
11	6.72	0.64
12	6.93	0.66
13	7.14	0.68
14	7.56	0.72
15	8.51	0.81
16	9.24	0.88
17	9.56	0.91

Table 2: Seed Protein bands present or absent in different varieties of fodder sorghum (*Sorghum bicolor* (L.) Moench).

Band No.	HC 136	HC 171	HC 308	HJ 513	HJ 541	PC 3	PC 5	PC 7	UP Chari 2	SSV 84	CSV 15	GFS 5
1	+	+	++	+	+	----	+	-----	+	+	-----	+
2	++	+++	+++	+++	+++	+	+++	+++	++	+++	+++	+++
3	+	++	++	+	+	+	+++	++	++	+++	+	++
4	+++	++	+++	++	++	----	++	+	++	++	++	+
5	+++	++++	-----	+	++	+++	++++	+++	++++	++++	++++	++
6	+	++	++++	++++	++++	++	++	++	+++	+	-----	++
7	++++	++++	++	++	+++	++	++	++	+++	+++	+++	+++
8	+++	++++	++	++	++	+++	++++	++	+++	+++	+++	+
9	++	++	+++	++++	++++	----	++++	++	+++	+++	+++	+++
10	++	+++	++++	++++	++++	+	++	++	++	-----	------	+++
11	++	++	++	+++	++	-----	++	-----	+	++	+	-----
12	+++	+++	+++	+++	+++	++	++	++	+	-----	-----	+
13	+++	+++	++	++	-----	++	++	+	----	-----	-----	-----
14	+++	+++	+++	++	++	++	++	++	++	++	++	++
15	+++	+++	++	++	++	++	++	-----	++	++	++	++
16	+++	------	++	++	++	------	++	-----	++	++	++	++
17	++	++	++	+	++	------	++	++	-----	++	++	+

++++ Dense; +++ Medium; ++ Light; + weak; Band absen

Table 3: Band map based on seed protein analysis different varieties of fodder sorghum (*Sorghum bicolor* (L.) Moench).

Varieties / Protein marker Band	1	2	3	4	5	6	7
HC 136	1	1	1	1	1	1	1
HC 171	1	1	1	1	1	0	1
HC 308	1	1	1	1	1	1	1
HJ 513	1	1	1	1	1	1	1
HJ 541	1	1	1	1	0	1	1
PC 3	1	1	0	0	1	0	0
PC 5	1	1	1	1	1	1	1
PC 7	1	1	1	0	1	0	0
UP Chari 2	1	1	1	1	0	1	1
SSV 84	1	1	1	1	0	1	1
CSV 15	1	0	1	1	0	1	1
GFS 5	1	1	1	0	0	1	1

Table 4: Similarity index based on seed protein profile analysis in different varieties of fodder sorghum (*Sorghum bicolour* (L.) Moench).

S. No	Varieties	1	2	3	4	5	6	7	8	9	10	11	12
1.	HC 136	1	0.857	1.000	1.000	0.857	0.429	1.000	0.571	0.857	0.857	0.714	0.714
2.	HC 171		1	0.857	0.857	0.714	0.500	0.857	0.667	0.714	0.714	0.571	0.571
3.	HC 308			1	1.000	0.857	0.429	1.000	0.571	0.857	0.857	0.714	0.714
4.	HJ 513				1	0.857	0.429	1.000	0.571	0.857	0.857	0.714	0.714
5.	HJ 541					1	0.286	0.857	0.429	1.000	1.000	0.833	0.833
6.	PC 3						1	0.429	0.750	0.286	0.286	0.143	0.333
7.	PC 5							1	0.571	0.857	0.857	0.714	0.714

8.	PC 7								1	0.429	0.429	0.286	0.500
9.	UP Chari 2									1	1.000	0.833	0.833
10.	SSV 84										1	0.833	0.833
11.	CSV 15											1	0.667
12.	GFS 5												1

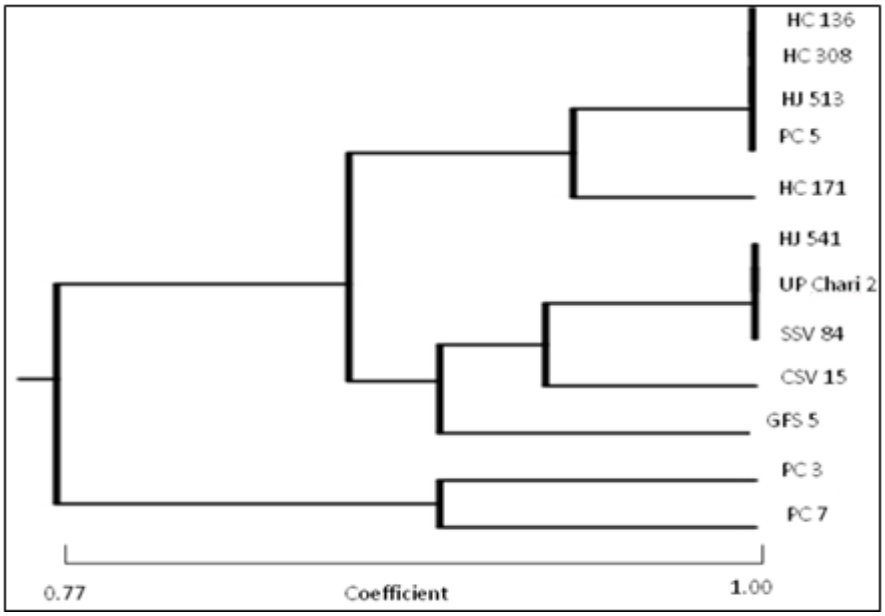

Fig 2: Dendrogram based on protein profile analysis of different varieties of fodder sorghum (*Sorghum biclour* (L.) Moench

Plate 1: Electrophoretic pattern of total soluble proteins in different varieties of fodder sorghum [*Sorghum bicolor* (L.) Moench].

{M -Marker, 1- HC 136, 2- HC 171, 3-HC 308, 4- HJ 513, 5-HJ541, 6-PC 3, 7-PC 5, 8-PC 7, 9-UP Chari 2, 10- SSV 84, 11-CSV 15, 12-GFS 5.}

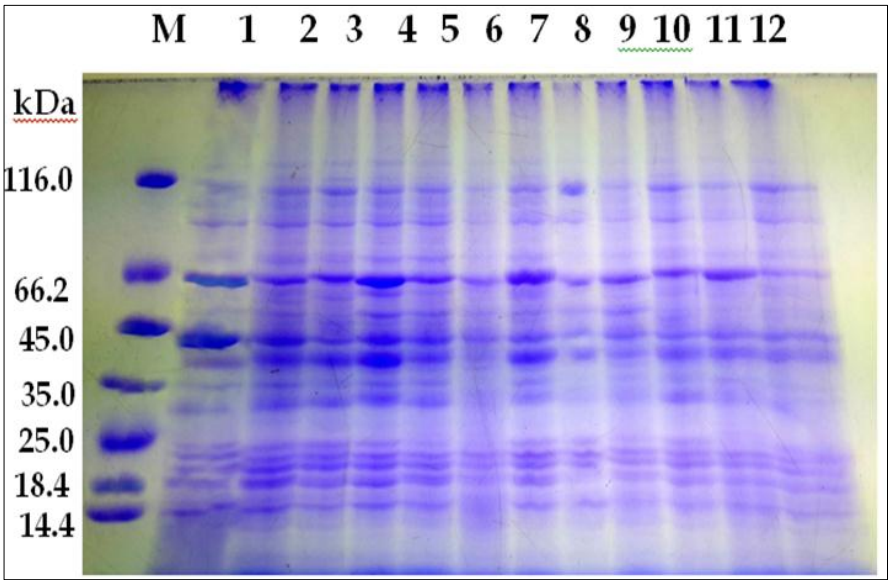

Fig 3

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