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Protein electrophoresis for identification of hybrids and their parents in *Sorghum bicolor* (L.) Moench

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The applicability of protein markers was studied for identification of sorghum hybrid and its parents and to test the genetic purity of commercial samples. The experimental materials consisted of four sorghum hybrids Viz. CHS-14, CSH-9, CSH-19R and CSH-15R and their respective parental lines. The separation of protein on the basis of their molecular mass was performed by denaturing proteins in presence of SDS and then subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis. The hybrid CSH-14 and its parents produced distinguishing protein banding pattern by SDS-PAGE analysis of soluble proteins which was found to be useful in identifying hybrid CSH-14 from its parents and also from the other genotypes under study. No polymorphism in protein profile of hybrid CSH-19R and male parent AKR 354 was observed in the present investigation indicating limitation of SDS-PAGE analysis in CSH-19R for identification and determination of genetic purity and further study to confirm the same. However some of protein markers obtained in hybrid CSH-9 and CSH-15R showed the possibility of application of this technique to some extent for genetic purity analysis in these hybrids.

Key Words: Electrophoresis, Protein profile, SDS-PAGE, Sorghum, identification and genetic purity.

INTRODUCTION

Sorghum is fifth in worldwide economic importance among cereal crops. It is a staple food crop of millions of the poor in semi-arid tropics of Africa and Asia. In India it is third most important cereal after wheat and rice. In India, the area under high yielding cultivars of sorghum has increased from 0.7 million hectare in the early 1970's to 6.5 million hectares in the late 1990's (Anonymous 2005). Most of the area under high yielding cultivars is planted with about 70 private sector's hybrids. The green revolution was based mainly on the use of high yielding varieties and hybrids of crop plants. The success of hybrid technology depends upon the timely production and adequate supply of genetically pure hybrid seeds to the farmers. In order to determine the genetic purity, grow out tests or field test is most commonly used technique. In most of the laboratory and field tests of 'purity' and 'characterisation', it is difficult to identify the hybrid and the parental lines. It is mainly due to lack of availability of an adequate number of ideal morphological markers in several cultivars. Morphological markers are simple, quick and requires inexpensive techniques, however the number of different markers per cross is less than 10. Whereas protein markers detected as electrophoretic variants of proteins scores up to 30 different markers per cross (Singh, 2005). Proteins (Gene products) markers could be very successfully used to verify the Identity of a variety. A major advantage of using protein markers lies in their being least or not influenced by the growing environment and the fact that if a suitable protocol is standardized, it can be used with equal reproducibility at any time and any test center.

India is a signatory to the GATT (General Agreement on Tariff and Trade) agreement. With the introduction of IPR (IBR) at the global level, it has become imperative to register, characterize and prepare documentation of hybrids /varieties in seed production chain. For registration of variety/hybrid, the Government of India has enacted its 'sui generic' system called Protection of Plant Varieties and Farmers Rights Act 2001. In view of the above act the identification of cultivar has gained more importance and moreover the ability to distinguish and identify the crop varieties and hybrids is a fundamental operation in seed trade. Therefore, the present investigation was undertaken to study the possibility of applying an alternative or supportive method to determine the genetic purity and to distinguish the hybrids and parents.

MATERIALS AND METHODS

The present investigation was carried out at Biotechnology

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Centre, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola and on the farm of Sorghum Research Unit, Dr. PDKV, Akola during 2002-2006. The experimental material of present investigation2006. The experimental material of present investigation comprised of four sorghum hybrids and their parents namely CSH-9 (MS 296 A x CS 3541), CSH-14 (AKMS 14 A x AKR 150), CSH-15R (MS 104 A x RS 585) and CSH-19R (MS 104 A x AKR 354), which are now in seed production chain.

Protein extraction from single sorghum seed was carried out by using 0.1 M Tris – HCl buffer (pH 7.5). Soluble seed protein estimation in different varietal seeds was carried out by Lowry's method (Lowry *et al.*, 1951). Sodium dodecyl sulphate polyacrylamide gel electrophoresis method was followed for the separation of total soluble seed proteins as described by Anuradha Varier and Malvika Dadlani (1992).

For analysis of protein gel the image was captured in Gel Documentation System (Bio- Rad). Apparent molecular weight of protein bands was determined by comparison with the molecular weight protein marker from Bio-Rad. The Rf value, intensity and molecular weight of protein bands were obtained by using Gel Doc EQ 4.5.0 software.

RESULTS AND DISSCUSSION

In the presence of an electric field and while passing through a semi porous gel medium, the electrophoretic variants cause dissimilar forms of a protein. The resulting variability was used as biochemical marker for the present study.

Eighteen types of polypeptide bands were resolved in four sorghum hybrids and their parents studied for seed protein profile as observed in Plate 1. The molecular weight for these polypeptides was ranged between 255 kDa to 37 kDa. The polypeptides in the same range were obtained by Maiti *et. al.*, (1994) while studying protein profile of some glossy and non glossy sorghum lines.

The qualitative differences in the protein banding pattern of four sorghum hybrids and their parents were scored in terms of presence or absence of the specific bands and were categorised into seven types of markers as given in Table 1. The marker category, which includes bands common in hybrid and its parents, are good markers to confirm that the hybrid is from their parents. This includes bands of marker type I, III and IV. The bands of marker type II, IV and VI markers are good markers to identify off types from the hybrid as these markers includes bands of parents which are not shared with hybrid. The bands of type VII markers are useful markers for identifying the specific hybrid as this type includes the bands which are expressed only in hybrid.When the protein profile of hybrid CSH-14 and its parents was compared, 80% bands were found monomorphic of type I marker and one band i.e. band no. 3 of 157 kDa size was expressed as male specific band of type IV marker. Thus, presence of male specific band in hybrid may help to identify true hybrid as it rules out the possibility of self-pollination. Band no. 5 of 122 kDa size expressed only in female (Type V marker) and band no. 14 of 50 kDa size expressed only in male (Type VI marker) were important markers to identify the male and female plants in the hybrid seed lot. This will help to decide the percentage of off types in the sample. The banding pattern of CSH-14 differed from the banding pattern of remaining genotypes. Thus band no. 3, 5, and 14 may serve as useful markers in deciding the genetic purity of seed lot of hybrid CSH-14.

In comparison of protein profile of hybrid CSH-19Rand its parents, only one band i.e. band no. 1 of 255 kDa size was found to be polymorphic as male specific

Table 1: Seven types of protein markers observed in the four sorghum hybrids and their parents

Type of Marker	Nature of band	Property of marker			Bands amplified							
					CSH-14		CSH-19R		CSH-9		CSH-15R	
		Female	Hy.	Male	No.	%	No.	%	No.	%	No.	%
Type I	Monomorphic	+	+	+	12	80.00	17	94.44	2	16.67	11	64.70
Type II	Monomorphic for parents	+	-	+	-	-	-	-	1	8.33	1	5.88
Type III	Female specific	+	+	-	-	-	-	-	2	16.67	1	5.88
Type IV	Male specific	-	+	+	1	6.67	1	5.56	-	-	-	-
Type V	Female band	+	-	-	1	6.67	-	-	-	-	4	23.53
Type VI	Male band	-	-	+	1	6.67	-	-	7	58.33	-	-
Type VII	Hybrid specific	-	+	-	-	-	-	-	-	-	-	-
Total					15	-	18	-	12	-	17	-

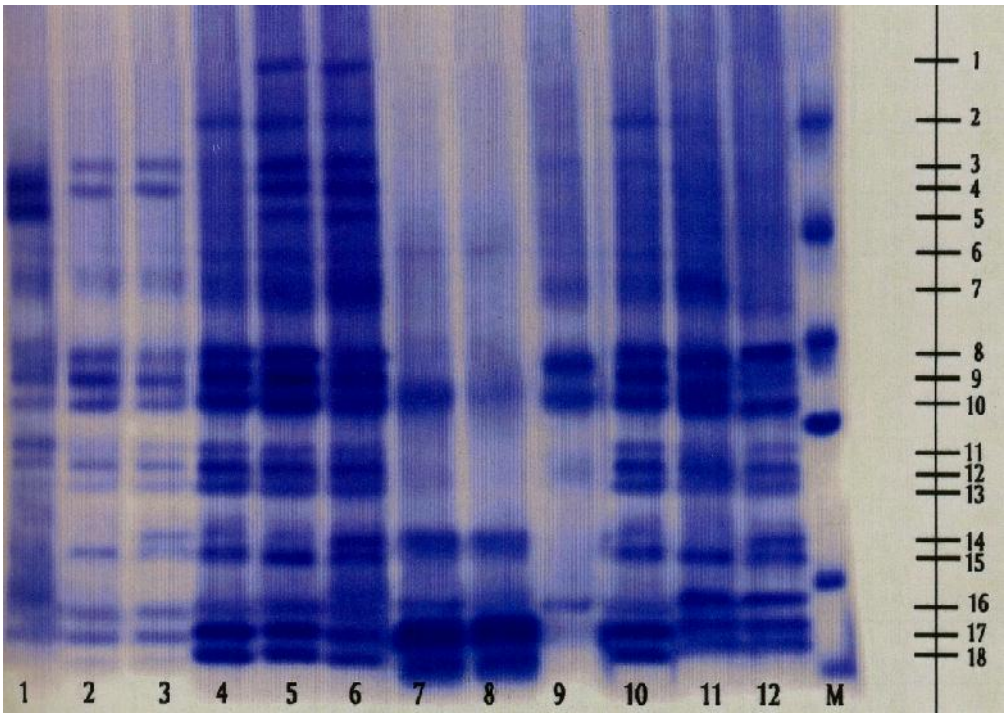

Plate 1: Protein banding pattern of four sorghum hybrids and their parents.

band of type IV marker. This band is likely to serve as useful marker to ascertain the hybridity eliminating the possibility of self-pollination. All other protein bands were found to be monomorphic and hence of no use in deciding the genetic purity of hybrid seed lot of hybrid CSH-19R 16.67% bands were monomorphic of type I marker and 16.67% bands were female specific bands of type III marker when the hybrid CSH-9 was compared with its parents. Identification of self plants and male plants could be possible as band no. 16 of 42 kDa of type II marker was expressed in both parents, but was absent in hybrid. Thus, presence of this marker specifies the sample may be of male, female or other variety but not of the hybrid. The pollen shedder male plants can more easily be identified due to presence of seven male bands of marker

The comparative study of protein profile of hybridCSH-15 R and its parents showed 64.70% monomorphic bands of type I marker. One band was

Lane No.	Genotype
1	AKMS 14 A
2	CSH-14
3	AKR 150
4	MS 104 A
5	CSH-19R
6	AKR 35
7	MS 296 A,
8	CSH-9
9	CS 3541
10	MS 104 A
11	CSH-15R
12	RS 585
M	Marker

Band No.	Band size (kDa)
1	253-255
2	192-196
3	156-157
4	139-143
5	120-122
6	110-111
7	104-106
8	85-91
9	79-81
10	71-74
11	61-62
12	59
13	56
14	50
15	47-48
16	41-43
17	39-40
18	37

expressed as female specific band of type III marker. The band no. 14 of 50 kDa size of type II marker was expressed in both the parents. The self, male and other variety plants can be roughed out on basis of such band of type II marker. The four bands found in female and not shared with hybrids were useful for identifying the self plants from hybrid. These were band no. 2 (176 kDa), 3 (157 kDa), 4 (142 kDa) and 6 (111 kDa).

The electrophoretic banding patterns of four sorghum hybrids and its parents revealed that the hybrid CSH-14 and its parents had comparatively more clear distinguishing protein banding pattern by SDS PAGE analysis of soluble proteins than other hybrid and its parental combination studied in present investment. Thus SDS PAGE analysis of sorghum was found to be useful in identifying hybrid CSH-14 from its parents and also from the other genotypes under study. Abdel Tawab et al. (1993) also identified sorghum cultivars on the basis of SDS PAGE analysis of seed proteins. Similarly Anuradha Varier et al. (1990), Nagaraja et al., (2000) and Chauhan et al. (2002) also found SDS PAGE of soluble protein as a useful technique in varietal identification in some other crops.

100% similarity in banding patterns of CSH-19 R and its male parents AKR 354 unable us to distinguish between them. This limits the usefulness of SDS-PAGE analysis for hybrid identification and genetic purity determination in hybrid CSH-19R which needs further study to confirm the same. The similar results were obtained by Chauhan et al. *(2002) for forage varieties HC-171 and HC-308, which had equal number of bands and could not be differentiated on the basis of presence/absence of specific bands.*

In hybrid CSH-9 and CSH-15R, the identification of self plant, pollen shedder plant was possible on the basis of the bands of type II and type V and type VI markers. Due to lack of male specific bands of type IV markers, the true hybrids can not be confirmed as either it was pollinated by same male parent or not. The limited number of marker types obtained in CSH-9 and CSH-15R could not produce clear spectrum for identification and genetic purity analysis of CSH-9 and CSH-15R and needs further study. Findings of Jaiswal and Agarwal (1990) also reflect similar results that banding patterns of proteins extracted from seed could not present a clear spectrum in some of the paddy varieties for the verification of genetic purity.

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