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Characterization of Castor Bean Genotypes Using SDS-Page of Total Soluble Seed Proteins

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ABSTRACT

Six varieties, nine hybrids of castor and their parents were characterized on the basis of electrophoresis of total soluble seed proteins. The SDS-PAGE profile of the Tris-soluble proteins from seeds could distinguish the hybrids from their female parent. A key was prepared for using the above parameters to identify the castor varieties, hybrids and their parents.

Key words: *Rm value, Seed proteins, SDS-PAGE, Seed keys.*

INTRODUCTION

Castor bean (*Ricinus communis* L.) is an indeterminate, non-edible oil seed crop grown in low rainfall regions of semi-arid tropics and sub-tropics. A number of hybrids of castor having a significantly higher yield potential than open pollinated varieties are being cultivated, particularly under rain-fed conditions. For an effective seed quality control programme it is very important to describe, differentiate and characterize these hybrids and their parental lines. Many of the descriptors that are conventionally used, based on plant morphology are unable to distinguish these genotypes. Also the expression of some of these characters is effected by the environment[4]. Hence there is a need to develop alternative descriptors which can supplement the morphological data. The use of electrophoresis of seed proteins and isozymes for variety characterization in various crops has been reviewed by Smith and Smith[5] and Cooke[1]. In the recent decades considerable interest has been focused on the use of biochemical methods for plant variety discrimination and identification. Seed protein banding patterns are considered to be particularly reliable, as seed storage proteins are largely independent of environmental factors[3]. The objective of the present study is to characterise castor bean varieties, hybrids and inbreds on the basis of electrophoresis of seed proteins.

Based on protein profiles an identification key was developed for identification and characterization of castor hybrids and their parents. These markers (proteins) together have been used successfully for distinguishing varieties, hybrids and their parents.

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MATERIAL AND METHODS

Plant materials: Plant materials consisted of 27 genotypes of castor bean, which are released since 1987 and the old ones still active in seed production chain (Table 1).

Table 1: Varieties and hybrids along with parental lines of castor released after 1987

Variety	Hybrid	Female parent	Male parent
AKC 1	DCH 32	LRES 17	DCS 5
Aruna	DCH 177	DPC 9	DCS 9
GC 2	GAUCH 1	VP 1	VI 9
Kranthi	GCH 2	VP 1	JI 35
	GCH 4	VP 1	48-1
	GCH 5	Geetha	SH 72
	GCH 6	JP 65	JI 96
	PCH 1	VP 1	PCS 136
	TMVCH 1	LRES 17	TMV 5

Protein extraction: For the extraction of proteins, whole individual grains of castor bean were taken, removed seed coat, crushed and ground to fine powder in a mortar and pestle. The seed samples were defatted with defatting solution (Chloroform, Methanol and Acetone in 2:1:1 ratio) for 24 hours with 3 times solvent changes. Castor grain powder (0.1g) was transferred into 1.5 ml eppendorf tube to which 400 µl of sample buffer (5 ml 0.5 mM Tris HCl ph 6.8, 1 ml Glycerol, 1 ml Absolute Alcohol and 43 ml Distilled water) was added and kept overnight at room temperature. The samples were centrifuged at 15,000 rpm for 20 minutes. 50µl of supernatant was taken in 1.5 ml eppendorf tube to which 10µl of bromophenol blue dye and 20µl of SDS+ mercapto ethanol mixture was added Two pellets of sucrose was added and the sample was boiled for 5 minutes and cooled. Then the sample was ready for loading on to the gel.

Electrophoresis: SDS-PAGE of total seed protein was carried out in polyacrylamide slab gels in a discontinuous buffer system according to the modified method of Laemmli[2]. Vertical gel slabs were prepared in a glass sandwich which was tightened by a set of plastic clips lined with a band of foamed silicon rubber. Separation gel was put into the space between a set of glass plates (left up to 2 cm from the top). Small amount of distilled water (120 µl) was added on separation gel gently to prevent gel surface from air and promote fixation. The set up was left for 30 minutes so that gel was fixed. The separating gel (15%) was prepared by mixing of 9 ml of 30% Acrylamide stock solution (29.2g Acrylamide and 0.8 g of bis Acrylamide dissolved in distilled water and made upto 100ml), 4.5 ml Tris HCl (pH 8.8) {18.2g Tris Hydroxy Methyl Amino Methane ,2.0 ml HCl dissolved in distilled water and made upto 100ml}, 1.5 ml Glycerol, 3 ml distilled water 80 of 10% Ammonium per sulphate and 10 µl of TEMED. TEMED was added and the mixture was swirled just before pouring the gel mixture. After polymerisation of separating gel stacking gel (4.5%) was prepared by mixing 0.9 ml of 30%Acytomide stock solution, 1.5 ml Tris HCl (pH6.8) {18.2g Tris Hydroxy Methyl Amino Methane ,8.4 ml HCl dissolved in distilled water and made upto 100ml} 3.6 ml distilled water, 20 µl 10% APS and 10 of TEMED.

When separation gel was fixed, distilled water was removed from its top and stacking gel solution poured on it. Combs were fixed into the stacking gel. Combs were put with special care and it was confirmed that there was no air bubble at the bottom of the combs. The set up was left for 15 minutes so that the stacking solution became gel. Combs, clips and gaskets were removed from glass plates carefully and confirmed that there was no any air bubble at this stage. The anode

buffer is made with 17.3 g Glycine (192 mM) and 3.6 g Tris HCl (25mm) was mixed and made up to 1200ml with distilled water. The cathode buffer is made with 0.3 g SDS (0.1%) dissolved is 300 ml of anode buffer (300ml taken from 1200ml anode buffer). The upper and lower reservoirs of electrophoretic unit were filled with cathode and anode buffers, respectively. The gel having twelve loaded wells was placed in the electrophoretic unit and fixed. Twenty-five µl of protein supernatant were applied into the stacking gel sample wells with a micro syringe, followed by 20 µl of reservoir buffer containing bromophenol blue which served as the tracking dye. Then electrophoresis was performed the specific time till the tracking dye reached the lower gel front. The unit was switched off and the gel was taken out and placed in staining solution. The staining solution was prepared by mixing 0.31 g coommassie brilliant blue, 62 ml methanol and 50ml distilled water. The gel was kept for 24 hours and transferred to destaining solution. The destining solution was changed 3-4 times till the banding pattern is clearly visible.

Evaluation and documentation

Electrophoregrams or zymograms were prepared by observing gels over a trans-illuminator and measuring the distance traveled by each band. Relative mobility (Rm) of each band was calculated using the following formula.

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

Scoring of the bands

By visual screening of the gels, protein bands were traced on the graph and mean "Rm" value calculated for each genotype and the banding patterns were drawn. The banding patterns thus obtained were observed to detect the differences among the genotypes in terms of presence or absence of a particular band. If the same band was present in two or more genotypes the intensity of that band was considered for the genotype identification (Fig. 1).

RESULTS AND DISCUSSION

The electrophoretic profiles of soluble proteins from castor bean revealed an overall qualitative variation among all the genotypes (Fig. 1). A total of 29 bands were observed with a fair degree of polymorphism among the genotypes (Fig. 2). The bands were identified by their Rm values. The total number of bands per genotype was ranged from 11 to 24. The lowest number of bands was observed in LRES-17 genotype and higher 24 were observed in GCH-6. Only bands with high intensity were considered while scoring gels. The genotypes could be differentiated based on the presence or absence of the other bands.

The total genotypes (27) were categorized into 12 groups based on number of bands. The 6 groups were constituted by single genotype only i.e., LRES 17(11), DCH 177 (12), DCS-(15), DCH-32(20), JP 65(23) and GCH 6(24) rest of genotypes could be grouped into 6 groups. Most of the hybrids were similar to the male percent profiles. The GCH-6 is more resemblance to the female percent. The 14 No of bands group (VP-1, TMVCH-1 and DCS-5), 16 No of bands group (GAUCH-1, TMV-5 and DPC-9), 17 No of bands (PCS-136, Geetha and AKC-1), 18 No of bands group V1-9, PCH -1, GC-2 SH -72 and Aruna), 19 No of bands group (GCH-2, GCH-4 and Kranthi) and 21 No of bands group (JI-35,48-1, GCH-5 and J2-96) showed distant variation in the position of bands i.e. Rm values and thickness of bands. where as in the 14 No bands group the genotypes TMVCH -1 and DCS -5 shown the similarity is no of light medium and dark bands, but the variation was observed is the position of bands i.e Rm values.

The data entry was done into a binary data matrix as discrete variables. Jaccard's co-efficient of similarity was measured and dendrogram (Fig. 3) based on similarity co-efficient was generated by using Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The computer package RAP Distance version 1.55 was used for cluster analysis. Based on protein profiles an identification key was developed for identification and characterization of castor hybrids and their parents. These protein markers could be used successfully for distinguishing varieties, hybrids and their parents.

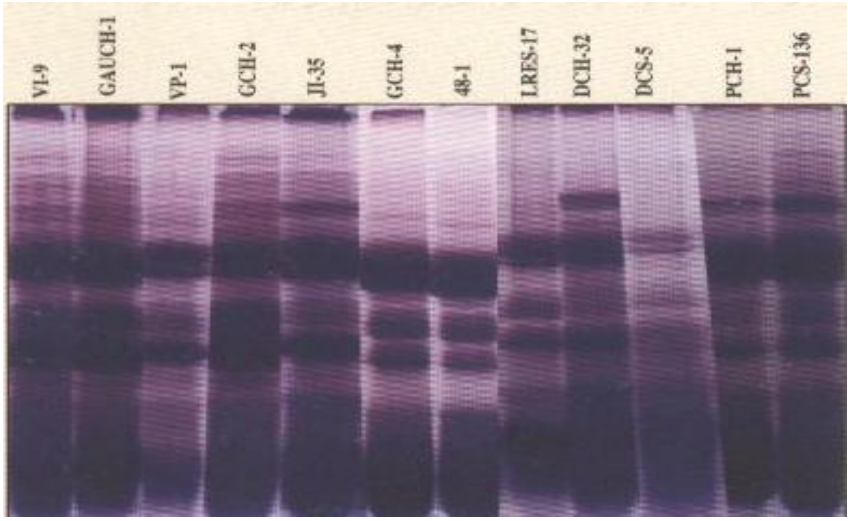

Fig. 1: SDS-PAGE profile of soluble seed proteins in castor genotypes

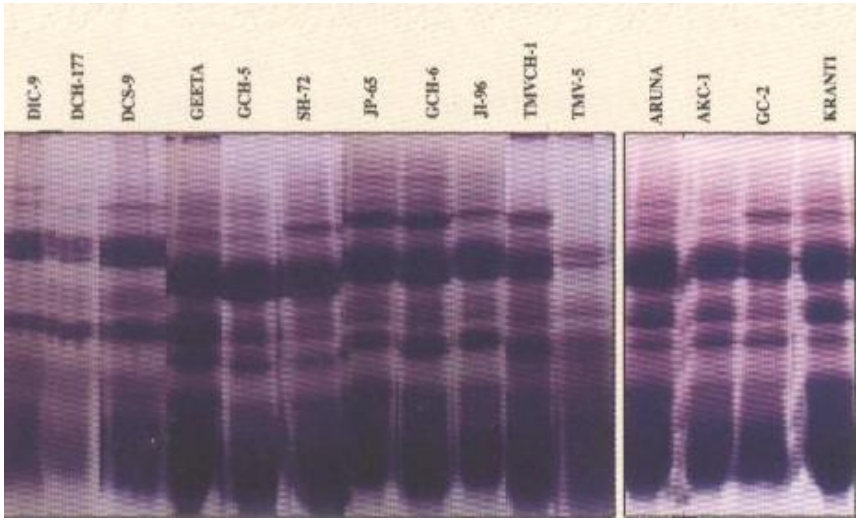

Fig. 2: Electrophorograms of total soluble seed protein of castor bean genotype

S. No.	Band No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
	Genotype *Rm*	0.13	0.16	0.19	0.20	0.21	0.22	0.23	0.24	0.26	0.28	0.30	0.32	0.36	0.38	0.40	0.42	0.45	0.47	0.49	0.50	0.51	0.53	0.55	0.57	0.59	0.63	0.65	0.68	0.80
1	VP 1																													
2	VI 9																													
3	GAUCH 1																													
4	JI 35																													
5	GCH 2																													
6	48 1																													
7	GCH 4																													
8	PCS 136																													
9	PCH 1																													
10	LRES 17																													
11	TMV 5																													
12	TMVCH 1																													
13	DCS 5																													
14	DCH 32																													
15	Geeta																													
16	SH 72																													
17	GCH 5																													
18	DPC 9																													
19	DCS 9																													
20	DCH 177																													
21	JP 65																													
22	JI 96																													
23	GCH 6																													
24	Aruna																													
25	AKC 1																													
26	GC 2																													
27	Kranti																													

Dark | Medium | Light | Absent

Fig. 2: Electrophorograms of total soluble seed protein of castor bean genotypes

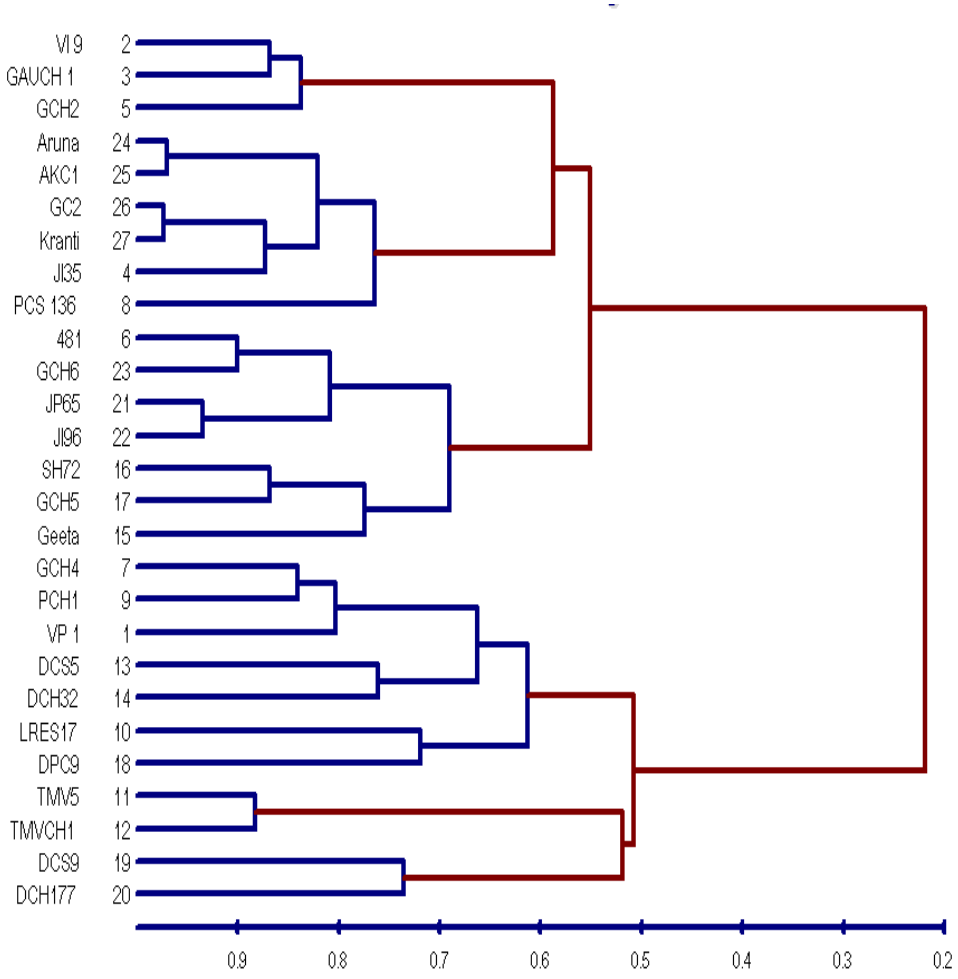

Fig. 3: Dendrogram of castor genotypes using soluble seed proteins

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