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Research Article

CHARACTERIZATION OF CULTIVARS BASED ON ELECTROPHORETIC ANALYSIS OF SEED PROTEINS AND ISOZYMES IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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ABSTRACT
The identification of sunflower hybrids KBSH-1, TCSH-1, PKVSH-27, APSH-11 and DSH-1, their parental lines and the varieties, Morden, Surya, AKSF-9, CO-2, CO-3, CO-4, GAUSF-15, NDSH-15 and SS-56 was possible from the genotype specific intensity of dark, light and medium bands as well as their relative position in the seed protein and isozymes profiles analysed by the polyacrylamide gel electrophoresis. Their characterizations were difficult through the total number of bands and as several of them were common in more than one genotype.

Key Words: *Electrophoresis, Gel, Genotypes, Identification, Polyacrylamide, Isozymes, Protein, Seed, Sunflower*

INTRODUCTION
The traditional field plot techniques to identify crop varieties with narrow morphological deviations are tedious and time consuming. The resent approach to analyze the seed proteins and isozymes by polyacrylamide gel electrophoresis is the rapid, accurate and dependable technique. It has been successfully adapted to identify the varieties of several crops like cotton (Dadlani and Varier, 1994; Ankaiah, 2002), jute (Pathak and Chattopadhyay, 1989), soybean (Blogg and Imrie, 1982; Goyal and Sharma, 1998) and tomatoes (Chakrabarty *et al.*, 1992). The technique may be useful to ensure the genetic purity of sunflower genotypes and their parental lines in commercial seed production of hybrids predominantly cultivated in India (Larence *et al.*, 1999 and Krishnasamy and Gayathri Devi, 2002) analysed the seed and seedlings for proteins and isozymes composition by polyacrylamide gel electrophoresis to identify lines, varieties and hybrids of sunflower. Keeping this in view the present study was carried out to characterize 27 sunflower genotypes as a quick aid to identify these genotypes for their genetic purity.

MATERIALS AND METHODS
Seed Proteins
SDS-PAGE of total soluble seed proteins was carried out by using 15 per cent gels according to the methods prescribed by Laemmli (1970) with slight modifications. Mature and well filled seeds of each genotype were dehulled and crushed. The powder was defatted in three changes of a mixture of chloroform, methanol and acetic acid (2:1:1). Defatted seed material was dried. Soluble seed protein was extracted by adding 0.2 ml of Tris HCl extraction buffer (0.125 M, pH6.8). The suspension was centrifuged at 10000 rpm for 15 minutes. The extract was dissolved in equal amount of working buffer and kept in boiling water for 5 minutes for denaturation of proteins. Then centrifuged at 10000 rpm for 15 minutes and the supernatant were used for loading in to the gel. A current of 1.5 mA per well with a voltage of 80 volts was applied until the tracking dye crossed the stacking gel. Later the current was increased to 2 mA per well and voltage up to 120 volts. The electrophoresis was stopped when the tracking dye reached the bottom of the resolving gel. Then the gel was stained using coomassie blue solution overnight and destained using a mixture of 227 ml of methanol, 46 ml of acetic acid and 227 ml of distilled water until the bands were clearly visible.

Isozymes

Polyacrylamide gel electrophoresis was carried out by using 8 per cent gel according to methods prescribed by Dadlani and Varier (1993) with slight modifications. Three day old seedling in each genotype was ground in 250 µl of extraction buffer (0.1M Tris-HCl, pH 7.5) and centrifuged at 10000 rpm for 15 minutes and the supernatant was used for loading. The gels were stained for isozymes Malate dehydrogenase using malic acid 15 ml, 15 ml Tris buffer, 50 mg NAD, 50 mg NBT, 25 mg PMS, 75 ml distilled water and for isozymes alcohol dehydrogenase using 7.5 ml ethanol, 15 ml Tris buffer, 50 mg NAD, 50 mg NBT, 25 mg PMS and 75 ml distilled water under dark.

RESULTS AND DISCUSSION

Electrophoretic separation of seed proteins has been utilized for resolving taxonomic and evolutionary divergence as well as species and cultivar identification in sunflower. In the current study a total of 31 bands were observed (Figure 1, 2, and 3) and varied from 7 (CMS-2B) to 17 (Surya). Nine bands were noticed in genotypes CMS-2A, AK-1R and APSH-11, ten bands in PKVSH-27, eleven bands in CO-2, CO-3, CO-4, Morden and RHA-857, twelve in CMS-234B, thirteen bands in genotypes CMS-234A, 6 D-1, TCSH-1 and CMS-7-1A. Genotypes KBSH-1, AKSF-9, CMS-7-1B, DSF-15B and DSF-15A showed fourteen bands. Fifteen bands were noticed in genotypes RHA-272, Surya, DSH-KBSH-1, and GAUSUF-15, sixteen bands in NDSH-15 and RHA-271 (Table 1).

Band number 2 (Rm: 0.125), 7 (Rm: 0.200) and 14 (Rm: 0.395) were useful to distinguish KBSH-1 hybrids from its parent's viz., CMS-234A, CMS-234B and 6D-1. Band number 3 (Rm: 0.150) and 6 (Rm: 0.190) may be used to distinguish hybrid DSH-1 from its parent's viz., DSF-15A, DSF-15B and RHA-857. In hybrid APSH-11 band number 8 (Rm: 0.215), 12 (Rm: 0.310) and 19 (Rm: 0.475) can be used to differentiate with its parents. Band number 3 (Rm: 0.146), 13 (Rm: 0.339), 14 (Rm: 0.360), 20 (Rm: 0.530) and 21 (Rm: 0.550) were used to characterize the hybrid PKVSH-27 from its parent's viz., CMS-2A, CMS-2B and AK-1R. Band number 13 (Rm: 0.339), 14 (Rm: 0.360) and 25 (Rm: 0.680) were used to distinguish hybrid TCSH-1 with its parents CMS-234A, CMS-234B and RHA-272. Band number 15 (Rm: 0.382), 17 (Rm: 0.445) and 21 (Rm: 0.550) were common in varieties CO-2, CO-3, CO-4 and CMS-7-1A with high intensity. Band number 25 (Rm: 0.680) in CO-2, 8 (Rm: 0.225) in CO-3, 28 (Rm: 0.750) in CO-4 and 20 (Rm: 0.530) in Morden were differed from each other. Band number 9 (Rm: 0.382), 11 (Rm: 0.445), 13 (Rm: 0.480), 16 (Rm: 0.550), 18 (Rm: 0.625) and 22 (Rm: 0.850) were common in genotypes NDSH-15, SS-56, Surya, AKSF-9 and GAUSUF-15 with high intensity. Band 14 (Rm: 0.510) was differed from these varieties with medium intensity similar type of results were obtained by Theertha Prasad and Channakrishnaiah (1990), Lawrence *et al.* (1999) and Prakash Chandrakanth (1999) in sunflower genotypes.

The electrophoretic banding patterns of isozymes of seedlings for Malate dehydrogenase, a total of three bands were observed (Figure 4 and 5). Band number 1 (Rm: 0.483) distinguished PKVSH-27, 6D-1 and SS-56. Band number 2 (Rm: 0.540) was absent in RHA-272, Morden and GAUSUF-15. Band number 3 (Rm: 0.610) was present in KBSH-1, APSH-11, DSH-1, CMS-2A, 6D-1, AK-1R, RHA-271, RHA-857, CO-2, CO-3, CO-4, Morden, AKSF-9 and it was absent in TCSH-1, PKVSH-27, CMS-234A, CMS-2A, DSF-15A, RHA-272, NDSH-15, SS-56, Surya and GAUSUF-15. The intensity of banding pattern differed among all genotypes for all three bands. Krishnasamy and Gayathri (2002) observed similar results. Isozymes alcohol dehydrogenase could not be used for the characterization, as it was expressed only one band with same relative mobility and intensity in the present study.

Thus the present study suggested that any number of sunflower genotypes could be identified based on the total soluble seed proteins and isozymes as the banding pattern and intensity were distinct for each genotype.

Table 1: Region wise classification of genotypes based on number and intensity of electrophoretic protein bands in sunflower genotypes

S. No.	Genotypes	Region A			Region B			Region C			Region D			Region E		
		*L**	M	D	L	M	D	L	M	D	L	M	D	L	M	D
1.	KBSH-1	2	2	0	0	1	0	1	0	3	1	0	2	0	0	2
2.	CMS-234 A	2	2	0	1	1	0	1	0	2	0	1	1	0	0	2
3.	CMS-234 B	2	2	0	2	0	0	0	1	1	0	1	2	0	0	1
4.	6 D-1	3	1	0	1	0	0	0	2	0	2	0	2	0	0	2
5.	TCSH-1	0	1	0	1	2	0	2	0	1	0	1	3	0	0	2
6.	RHA-272	0	0	0	2	1	0	2	1	1	2	0	3	1	0	2
7.	CO-2	0	0	0	1	0	0	0	1	1	1	1	3	2	0	1
8.	CO-3	0	0	0	2	1	0	0	1	1	1	1	2	1	1	0
9.	CO-4	0	0	0	1	0	0	1	0	1	1	2	1	1	1	2
10.	MORDEN	0	0	0	0	1	0	0	1	1	0	2	3	1	1	1
11.	NDSH-15	0	1	0	1	2	1	1	1	1	0	3	2	2	0	3
12.	SS-56	0	1	0	2	2	1	2	0	1	0	2	3	1	0	2
13.	PKVSH-27	0	1	0	0	1	0	1	0	2	2	0	1	2	0	2
14.	CMS-2A	0	0	0	0	1	0	2	0	1	0	1	1	1	0	2
15.	CMS-2B	0	0	0	0	0	0	0	1	0	0	2	1	1	0	2
16.	AK-1-R	0	0	0	0	0	0	1	0	0	3	1	0	3	1	0
17.	SURYA	0	0	0	0	3	1	2	0	1	0	2	3	0	0	3
18.	AKSF-9	0	0	0	1	2	1	0	2	2	0	2	3	0	0	2
19.	APSH-11	0	1	0	0	0	0	1	1	0	2	1	1	0	1	1
20.	CMS-7-1 A	2	1	0	1	0	0	1	1	1	0	2	2	0	0	2
21.	CMS-7-1 B	2	2	0	1	0	0	1	1	1	1	1	2	0	0	2
22.	RHA-271	4	1	0	1	0	0	1	1	3	1	1	1	0	1	1
23.	DSH-1	4	0	0	1	0	0	1	0	2	1	1	3	0	0	3
24.	DSF-15 B	3	0	0	2	1	0	1	1	1	0	2	1	0	0	3
25.	DSF-15 A	2	1	0	2	1	0	1	0	2	0	1	2	0	0	3
26.	RHA-857	3	0	0	1	0	0	0	1	1	1	1	1	0	0	3
27.	GAUSUF-15	0	0	0	0	3	0	1	1	2	0	1	3	0	0	4

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