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Confirmation of sunflower F_1 hybrids using SDS-PAGE analysis

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Among bio-chemical techniques, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) is most widely used due to its validity and simplicity for describing genetic structures of group of plants. In the present study, SDS-PAGE was used to confirm sunflower hybrids. Four male sterile lines, four restorer lines and their sixteen F_1 hybrids were analyzed. Based on results of electrophoretic band spectra, Jaccard's similarity index (S) was calculated for all possible pairs. The similarity matrix, thus, generated was converted to a dissimilarity matrix and used to construct dendrogram, using unweighed pair-group method with arithmetic means. Euclidean distance of 1.0 was observed among 5 comparisons, whereas maximum distance (3.87) was observed for 1 comparison only. In most of the cases, female parents, along with their respective crosses, were found in one cluster, indicating that SDS-PAGE can be used reliably for the identification of hybrids in sunflower.

Key words: Sunflower, sodium dodecyle sulphate polyacrylamide gel electrophoresis, F_1 hybrids, heterosis**.**

INTRODUCTION

Like many other crop species of commercial importance, sunflower hybrids are proven to be more vigorous, uniform, self fertile, high yielding and resistant to foliar diseases (Seetharam et al., 1975). Hybrid sunflower yields about 50% more than the better open pollinated varieties (Miller, 1987; 1998). In recent years, sunflower hybrids are being planted in all parts of the world where sunflower is grown commercially (USDA, 1995). Major producers of sunflower are Eastern and Western Europe, Russia, South America, Australia, South Africa, Turkey, China and India. Total area under sunflower cultivation in the world is approximately 16.5 million hectares, out of which 11.5 million hectares are planted with hybrid sunflower genotypes (FAO, 2006). In Pakistan, sunflower is planted as an oilseed crop over an area of 1250000 acres with an annual production of 625000 tons giving an average yield of 500 kg/acre. Because of the huge gap in production and demand, Pakistan spends 22 billion US Dollar annually to import edible oil (MINFAL, 2009). To bridge this gap, availability of quality hybrid sunflower seed needs to be ensured. This requires development of indigenous heterosis breeding program of sunflower.

Biochemical assays have extensively been used to study genome structure in various crops of agronomic importance like wheat, maize, soybean, sunflower, etc (Weber et al., 2005, Zhu et al., 2005). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is most widely used due to its validity and simplicity for describing genetic structures of group of plants. Present study was undertaken to analyze seed storage protein variants and their utilization in confirmation of parental lines and F_1 populations of sunflower. The findings will be utilized in designing better strategies for future breeding programs aimed at exploitation of heterosis in local breeding programs of sunflower.

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Abbreviations: SDS-PAGE, Sodium dodecyl sulphate-polyacrylamide gel electrophoresis; **S,** Jaccard's similarity index; **CMS,** cytoplasmic male sterile.

Table 1. Parental lines and their origin of sunflower used during present study.

S/N	Inbred lines	Parentage	Origin	Salient feature
1	TS-17	S-2 x F-6201 x DM-6	USA	Maximum number of leaves and large head size.
2	TS-18	S-4 x F-11 x F-15	USA	Early maturing, short stature, maximum stem curvature and maximum harvest index.
3	TS-228	TS-7 x TF-6201 x DM-7	Pakistan	Minimum stem curvature.
4	TS-335	TS-17 x TF-11 x TF-7	Pakistan	Late maturing and having large sterile zone diameter.
5	291RGI	RHA-365 x R-8	USA	Maximum height and low harvest index.
6	R-25	R-8 x R-III	Pakistan	Medium stem curvature type with minimum number of leaves.
7	TR-9	TRL-13 x RHA-857	Pakistan	Small heads
8	TR-6023	TRL-13 x R-5	Pakistan	Small heads

MATERIALS AND METHODS

Four cytoplasmic male sterile (CMS) lines (TS-17, TS-18, TS-228 and TS-335) and four restorer lines (291RGI, R-25, TR-9 and TR-6023), having sufficient morphological variation, were used during present research work (Table 1).

The parental genotypes were sown in sixteen blocks having four rows of five meter length during growing season, 2003 - 2004. Each cytoplasmic male sterile line was crossed with four restorer lines. The cytoplasmic male sterile lines were bagged during the last week of October 2003. Crosses were made manually in the morning from 8:00 am to 10:00 am. After pollination, the heads were rebagged to avoid contamination and to ensure genetic purity.

Total seed storage proteins were extracted from single seed using protocols described by Hajduch et al. (2005). Seed proteins were analysed using 7.5% polyacrylamide gel. Electrophoresis was carried out in discontinuous buffer system (Laemmli, 1970) at 100 V until the Bromophenol blue reached the bottom of the gel. Molecular weights of dissociated polypeptides were determined by using molecular weights protein standards "MW-SDS-70 Kit" (Sigma Chemical Company, USA). Gels were stained with 0.2% (w/v) Commassie brilliant blue R-250 dissolved in 10% (v/v) acetic acid, 40% (v/v) methanol and water in the ratio of 10: 40: 60 (v/v) and destained in destaining solution containing 5% (v/v) acetic acid, 20% (v/v) methanol and water in the ratio of 5: 20: 75 (v/v). In order to check the reproducibility of the results, two separate gels for each sample were run under similar electrophoretic conditions.

Each protein band was considered as a single allele/locus. Presence/absence of the alleles was entered in a binary data matrix ("1" for presence and "0" for absence). Jaccard's similarity index (S) was calculated for all possible pairs. The similarity matrix thus generated was converted to a dissimilarity matrix (dissimilarity = 1-similarity) and used to construct dendrogram by the unweighted pair-group method with arithmetic means (Sneath and Sokal, 1973; Nei and Li, 1979). All the analyses were carried out using a statistical package NTSYS-pc, version 1.8 (Rohlf, 1973) and "STATISTIA" for Windows 95.

RESULTS AND DISCUSSION

The electrophoretic seed protein profiles for sixteen hybrids along with eight parents used during present study are presented in Figures 1 - 2. A total of twenty-seven alleles (protein bands) were scored. Many other proteins sub units of lower molecular weight observed during present study were not included in the analysis because of inconsistency in reproducibility.

The Euclidean distance matrixes for sixteen F1 hybrids along with eight parents are presented in Table 2. It is evident that (1.0) Euclidean distance was observed between 5 comparisons, whereas maximum distance (3.87) was observed between 1 comparisons only (TS18 X 291RGI vs. TS335 X TR9).

The cluster diagram using Ward's method revealed two major lineage groups at linkage distance 10 (Figure 3). Lineage 'A' at linkage distance 4 was further divided into three clusters (I, II and III). Among the lineage 'A', cluster I consists of 5 genotypes including four F_1 hybrids (TS-18 x TR-9, TS-18 x TR-6023, TS-18 x 291RGI and TS-18 x R-25) and female parent, TS-18. Cluster II consists of four F_1 hybrids viz; TS-17 x TR-9, TS-17 x R-25, TS-17 x 291RGI and TS-17 x TR-6023 and one female parent, TS-17. Cluster III comprises three male parents viz; 291RGI, TR-6023 and TR-9, while lineage group 'B' comprises two clusters (IV and V). Cluster IV comprises four F_1 hybrids viz; TS-335 x R-25, TS-335 x 291RGI, TS-335 x TR-9 and TS-335 x TR-6023 and one female parent, TS-335. Cluster V comprises four F_1 hybrids viz; TS-228 x TR-9, TS-228 x 291RGI, TS-228 x TR-6023 and TS-228 x R-5, one male parent, R-25 and one female parent, TS-228. It is evident from the dendogram that in most cases, the female parents along with their respective crosses were found in one cluster, showing that they share same protein banding profile, which revealed that in F_1 population, SDS-PAGE can reliably be used for identification of hybrids. Present findings support previous reports of Hadjuch et al. (2005) and Sultana et al. (2005).

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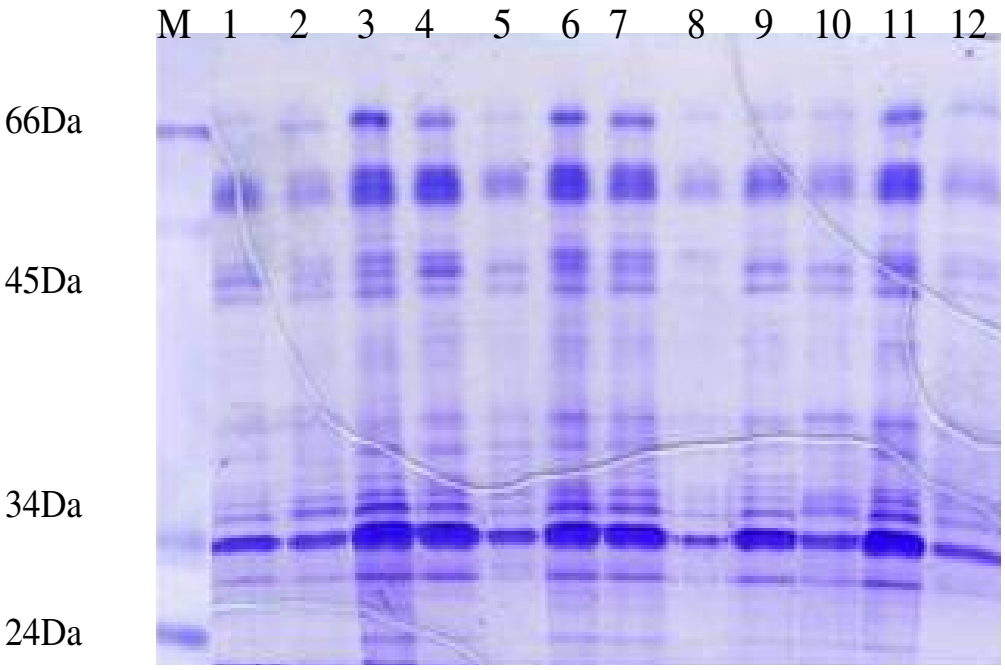

Figure 1. SDS-PAGE profile of seed storage protein for parents and F_1 hybrids of sunflower used during the present study. M = Molecular weight marker, 1 = 291RGI, 2 = TS335X29RGI, 3 = TS336XR25, 4 = TS228XR25, 5 = R25, 6 = TR9, 7 = TS17, 8 = TS18XTS9, 9 = TS228, 10 = TS335XTR9, 11 = TS18XTR6023 and 12 = TS228XTR9.

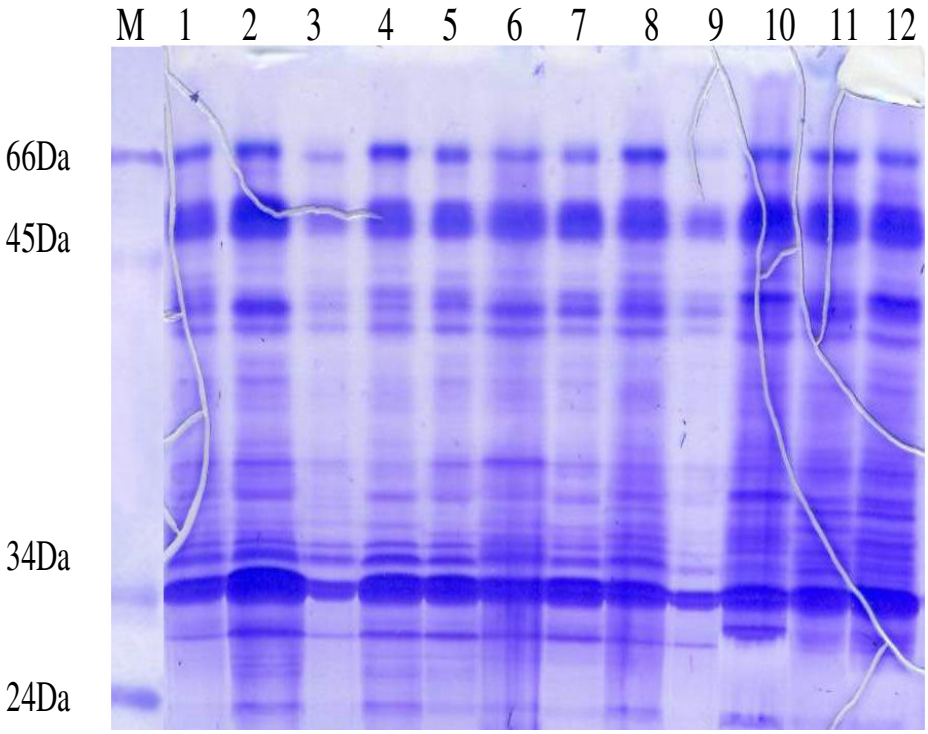

Figure 2. SDS-PAGE profile of seed storage protein for parents and F_1 hybrids of sunflower used during the present study. M = Molecular weight marker, 1 = TR6023, 2 = TS228X291RGI, 3 = TS335XTR6023, 4 = TS228XTR6023, 5 = TS18, 6 = TS17XTS6023, 7 = TS18X291RGI, 8 = TS17XR25, 9 = TS335, 10 = TS17XTS9, 11 = TS17X291RG and 12 = TS18XR25.

Table 2. Euclidean distances for 16 F_1 hybrids along with 8 parents of sunflower using data obtained from SDS-PAGE.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
TS-228 x TR-9	2.00	2.83	1.41	2.24	2.00	3.16	2.00	2.45	2.83	3.00	3.16	3.16	3.00	3.46	3.32	3.61	3.46	3.61	2.45	2.45	2.45	2.45	2.83
TR-9		3.16	2.00	2.24	2.45	3.16	2.45	2.83	2.83	3.00	3.16	2.83	2.65	3.46	3.32	3.61	3.46	3.61	3.16	2.83	3.16	2.83	3.46
TS-228 x R-25			2.45	3.00	2.83	2.45	2.83	2.45	2.83	3.00	3.16	2.83	3.32	3.16	3.00	3.00	2.83	3.00	3.16	3.46	2.83	3.46	3.16
R-25				2.24	1.41	2.83	1.41	2.83	2.45	2.65	2.83	3.16	3.00	3.46	3.00	3.32	3.16	3.32	2.83	2.83	2.83	2.83	2.83
TS-228 x TR-6023					2.24	3.00	2.65	3.00	2.65	2.83	3.00	2.65	2.45	3.00	2.83	3.16	3.00	3.16	2.65	2.65	3.00	3.00	2.65
TR-6023						2.83	2.00	3.16	2.45	2.65	2.83	3.16	3.00	3.16	2.65	3.00	2.83	3.00	2.45	2.83	2.83	2.83	2.45
TS-228 x 291 RGI							2.83	2.83	2.45	2.65	2.45	2.83	3.32	2.00	2.24	2.24	2.00	1.73	3.16	3.74	3.16	3.74	3.16
291 RGI								3.16	2.83	3.00	2.83	3.46	3.32	3.46	3.00	3.32	3.16	3.32	2.45	2.45	2.45	2.45	2.45
TS-18									3.16	3.00	3.46	3.16	3.32	2.83	2.65	3.00	2.83	3.00	3.16	3.46	3.16	3.16	3.46
TS-18 x R-25										1.00	1.41	2.00	2.24	3.16	3.00	3.00	2.83	3.00	3.16	3.74	3.46	3.74	3.16
TS-18 x 291 RGI											1.73	2.24	2.45	3.00	2.83	2.83	2.65	2.83	3.32	3.87	3.61	3.61	3.32
TS-18 x TR-6023												2.45	2.65	3.16	3.32	3.32	3.16	3.00	3.16	3.74	3.46	3.74	3.16
TS-18 x TR-9													1.73	3.16	3.32	3.32	3.16	3.32	3.16	3.46	3.16	3.46	3.46
TS-17														3.61	3.46	3.74	3.61	3.74	3.00	3.32	3.61	3.32	3.32
TS-17 x 291-RGI															1.73	1.73	1.41	1.00	3.16	3.46	3.16	3.46	3.16
TS-17 x TR-9																1.41	1.00	1.41	3.00	3.32	3.32	3.32	2.65
TS-17 x R-25																	1.00	1.41	3.32	3.61	3.32	3.61	3.00
TS-17 x TR-6023																		1.00	3.16	3.46	3.16	3.46	2.83
TS-335																			3.32	3.61	3.32	3.61	3.00
TS-335 x TR-9																				2.00	2.00	2.00	1.41
TS-335 x R-25																					2.00	1.41	2.00
TS-335 x 291RGI																						2.00	2.45
TS-335 x TR-6023																							2.45

1 = TS228XTR9, 2 = TR9, 3 = TS228XTR9, 4 = R25, 5 = TS228XTR6023, 6 = TR6023, 7 = TS228X29IGRI, 8 = 29IGRI, 9 = TS18, 10 = TS18XR25, 11 = TS18X29IGRI, 12 = TS18XTR6023, 13 = TS18XTR9, 14 = TS17, 15 = TS17X29IGRI, 16 = TS17XTR, 17 = TS17XR25, 18 = TR17XTR6023, 19 = TS335, 20 = TS335XTR9, 21 = TS335XR25, 22 = TS335X29RG and 23 = TS335XTR6032.

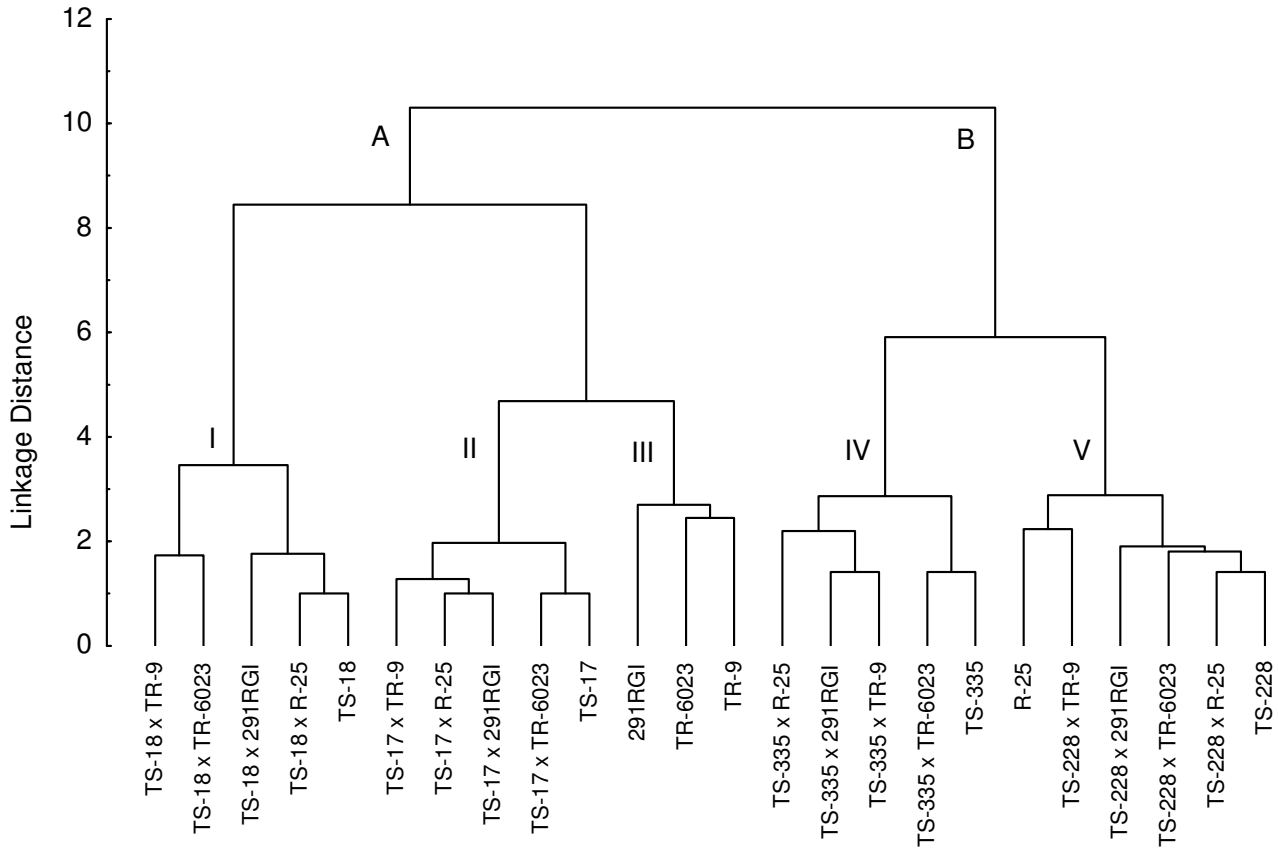

Figure 3. Dendogram constructed for twenty-four sunflower genotypes based on SDS-PAGE.

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