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STUDY OF TOTAL SEED PROTEINS PATTERN OF SESAME (*SESAMUM INDICUM* L.) LANDRACES VIA SODIUM DODECYL SULFATE POLYACRYLAMIDE GEL ELECROPHORESIS (SDS-PAGE)

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Abstract

The sesame (*Sesamum indicum* L.) germplasm, comprising of 105 accessions was characterized for total seed storage proteins using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The germplasm was collected from diverse agro-ecological regions of Pakistan. To our information, no studies have yet been carried out in Pakistan on the genetic evaluation of sesame genotypes based on total seed protein. Total seed proteins were electrophoretically separated on 12% polyacrylamide gels by standard protocols. A total of 20 polypeptide bands were observed, of which 14 (70%) were polymorphic and 6 (30%) were monomorphic, with molecular weight ranging from 13.5 to 100 kDa. Six bands i.e., 7, 11, 12, 15, 16 and 18 were common in all genotypes. Similarity coefficients varied from 0.50 to 1.00. The dendrogram based on dissimilarity matrix using unweighted pair group method with arithmetic averages (UPGMA) separated all sesame accessions into three main groups i.e., A, B, C, comprising 89, 14 and 2 genotypes, respectively. Overall a low to medium level of genetic variability was observed for SDS-PAGE (single dimension). As SDS-PAGE alone did not reveal high level of genetic variability, hence 2-D gel electrophoresis along with other advanced type DNA markers and more number of sesame accessions from all over the country are recommended for the future genetic evaluation. Our investigation will significantly support the classification, development, genetic evaluation and conservation of sesame germplasm in Pakistan.

Introduction

Sesame (*Sesamum indicum* L.) is considered one of the most important and oldest oil crops that belong to the Pedaliaceae family (Noorka *et al*., 2011). The genus Sesamum contains more than 30 species of which *S. indicum* is the commonly cultivated (Kobayashi *et al*., 1990; Nayar & Mehra, 1970). Sesame is considered to be the oldest of oilseed plants and is under cultivation in Asia for more than 5000 years. The exact natural origin of the sesame is mysterious, even though many wild species found in Africa and a few members in India. Bedigian (1981) suggested that it is appropriate to believe Africa as the primary centre of origin, due to broad genetic diversity, while India may be considered as secondary centre due to its huge production of sesame seeds. Ashri (1998) felt that settling the dispute on the origin of sesame will involve more comprehensive cytogenetic and decent DNA assessments.

India positions first in production and one third of the world production and almost 30% of the sesame acreage in the world are from India only. Honduras and Egypt are leading countries producing yield of 1267 and 1063 kg/ha, respectively. In Pakistan it is locally known as Til in Urdu and Punjabi, Tir in Sindhi, Konzola in Pashto and Kunjit in Balochi (Hatam & Abbasi, 1994). Sesame is grown in all provinces of Pakistan and is well scattered in irrigated as well as rainfed zones. Sesame seeds are used in confectioneries like cakes, cookies and similar many other backing. The occurrence of antioxidants such as sesamolin sesamin and sesamol makes the sesame oil extremely preservable as a consequence of which it does not rotten (Ahuja *et al*., 1971). Small uses of sesame oil consist of pharmaceutical and skin care products and are synergic for insecticides (Hatam & Abbasi, 1994). Mucilaginous leaves or leaf of sesame sap are used to treat fever, as a remedy for cough and sore eyes and to kill head lice; the sap is taken to facilitate childbirth, to treat dysentery and gonorrhea and is used in dressings after circumcision. In eastern and southern Africa the leaves play a role in the treatment of snakebites. Ash from burned stems is used as a medicinal salt.

In spite of being the first oilseed crop known to man and its extended history, sesame is a neglected crop. It is not studied by any of the international agricultural research centers (Ashri, 1998). Sesame has been mentioned as an 'orphan crop' because it is not commanded to any of the CGIAR institutes (Ashri, 1995). Pakistan is rich of sesame genetic diversity, more than172 sesame accessions are there in different regions of the country (Masood *et al*., 2003). The local varieties can be employed as a raw material for agricultural development (Ali *et al*., 2009). Genetic enhancement of local sesame has the potential to overcome the low production limitations. Although Pakistani sesame germplasm has ample genetic variability, but so for this important natural asset has not been evaluated for the development of high quality varieties. According to Arriel *et al*., (2000) the classification of sesame is still in the premature period in Pakistan. The use of biochemical markers for the assessment of genetic diversity has received a great deal of attention in current years. Among the biochemical techniques, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is widely used due to its easiness and effectiveness for telling the genetic construction of crop germplasm (Ghafoor & Ahmed, 2005; Siddiqui *et al*., 2010; Jatoi *et al*., 2011; Nisar *et al*., 2011). SDS-PAGE is considered to be a practical and dependable method because seed storage proteins are extremely sovereign of environmental fluctuations (Gepts, 1989; Murphy *et al*., 1990; Javaid *et*

al., 2004; Iqbal *et al*., 2005). Seed protein patterns obtained by electrophoresis have been effectively used to solve the taxonomic and evolutionary relationships amongst crops and their wild relatives, and study the various classes of storage proteins (Rao *et al*., 1992; Das & Mukarjee, 1995; Khan *et al*., 2010). Genetic diversity assessment based on SDS-PAGE for sesame is not reported in Pakistan. This study was made to investigate genetic diversity of 105 sesame accessions collected from different agro-ecological localities of Pakistan.

Materials and Methods

Plant material: Plant material consisted of 105 accessions of Pakistani sesame (*Sesamum indicum* L.). Details are given in Table 1. The germplasm accessions were obtained from PGRP Gene-bank, Institute of Agri-Biotechnology & Genetic Resources (IABGR), NARC, Islamabad. This material was collected from diverse ecologies of Pakistan.

Table 1. List of sesame accessions used in present study.

No.	Accession	Collection area	No.	Accession	Collection area	No.	Accession	Collection area
1.	19531	Lahore	36.	19700	Rawalpindi	71.	22094	Quetta
2.	19533	Lahore	37.	21271	Jhang	72.	22175	Hyderabad
3.	19534	Lahore	38.	21302	Jhang	73.	22179	Khairpur
4.	19542	Okara	39.	21334	Jhang	74.	22180	Ghotki
5.	19545	Sahiwal	40.	21354	Layyah	75.	22189	Sanghar
6.	19547	Sahiwal	41.	21372	Layyah	76.	22191	Sukkur
7.	19551	Sahiwal	42.	21376	Muzaffargarh	77.	22192	R.Y. Khan
8.	19552	Sahiwal	43.	21402	Attock	78.	22193	Nawabshah
9.	19556	Khanewal	44.	21403	Attock	79.	22194	Larkana
10.	19559	Khanewal	45.	21404	Attock	80.	22196	Jacobabad
11.	19560	Khanewal	46.	21406	Attock	81.	22199	Nawabshah
12.	19562	Khanewal	47.	21478	T.T. Singh	82.	22200	Peshawar
13.	19564	Khanewal	48.	21523	D.G.Khan	83.	22209	D.I. Khan
14.	19567	Khanewal	49.	21530	D.G.Khan	84.	22216	D.I. Khan
15.	19570	Khanewal	50.	21558	D.G.Khan	85.	22217	D.I. Khan
16.	19571	Khanewal	51.	21568	T.T. Singh	86.	22238	Bhakkar
17.	19573	Khanewal	52.	21608	T.T. Singh	87.	22241	Bhakkar
18.	19576	Khanewal	53.	21611	T.T. Singh	88.	22243	Bhakkar
19.	19580	Multan	54.	21660	Bahawalnagar	89.	22247	Mianwali
20.	19583	Multan	55.	21662	Faisalabad	90.	22249	Mianwali
21.	19590	Bahawalpur	56.	21663	Pakpattan	91.	22251	Bahawalpur
22.	19591	Bahawalpur	57.	21664	Pakpattan	92.	22253	Bahawalpur
23.	19592	Bahawalpur	58.	21666	Bahawalpur	93.	22256	Bahawalpur
24.	19593	Bahawalpur	59.	21674	Narowal	94.	22272	Layyah
25.	19600	Bahawalpur	60.	21688	Bahawalnagar	95.	22318	Rawalpindi
26.	19602	Bahawalpur	61.	21820	Quetta	96.	SG8	Islamabad
27.	19608	Vihari	62.	21832	Quetta	97.	SG62	Islamabad
28.	19612	Faisalabad	63.	21880	Faisalabad	98.	SG119	Islamabad
29.	19615	Faisalabad	64.	21885	Faisalabad	99.	21258	Jhang
30.	19628	Faisalabad	65.	21888	Sargodha	100.	21661	Sialkot
31.	19630	Sheikhupura	66.	21898	Sargodha	101.	21885	Faisalabad
32.	19631	Sheikhupura	67.	21905	Sargodha	102.	22248	Mianwali
33.	19635	Gujranwala	68.	21907	Sargodha	103.	21127	Faisalabad
34.	19636	Gujranwala	69.	21921	Lahore	104.	26805	Islamabad
35.	19696	Karachi	70.	21943	Karachi	105.	TS	Faisalabad

Protein extraction: For the extraction of proteins, whole seeds were crushed and ground to fine powder with mortar and pestle. Around 0.1 gram seed flour was put into 1.5ml micro-tube. To extract proteins from flour, protein extraction buffer (400µl) was added to flour as an extraction liquid and mixed methodically in eppendorf tube with a small glass rod. The extraction buffer contained 0.5M Tris-HCl (pH 8.0), 0.2%SDS, 5M Urea, and 1% 2-mercaptoethanol. Bromophenol blue was added to extraction buffer as a dye to show the movement of protein in the gel. To clean the extraction, the homogenate samples were varied thoroughly by vortexing and centrifugation at 15,000 rpm for 10 minutes at room temperature (RT), and stored at -4^{o}C until gel electrophoresis.

Preparation of electrophoretic gel: SDS-PAGE of total seed protein was carried out in 20% polyacrylamide slab gels in discontinuous buffer system according to method of Laemmli (1970). Vertical slab gel was organized in a glass sandwich. The separating gel contained 20% by weight acrylamide and 0.135% by weight N.N-methylene-acrylamide in 0.5M Tris-HCl buffer (pH 8.8) with 0.27% SDS. The gel was polymerized chemically by adding 15 microlitters TEMED (Tetramethylene-diamine) and 10% APS (Ammonium per sulphate). The staking gel consisted of 30% acryamide and 0.8% N.N-methylene-bis-acrylamide in 0.25M Tris-HCL buffer (pH 6.8) containing 0.2 SDS. Polymerization of the staking gel was done chemically in the same technique as for the separation gels. The electrode buffer enclosed Tris-glycine (9.0g Tris-HCl and 43.2g glycine per 3 liters buffer solution at pH 8.9) with 3.0g SDS (0.1%). Ten microliters of sample was applied into the separation stacking gel sample wells.

Electrophoresis: Electrophoresis was carried out at 75V for around 3 hours until bromophenol blue marker achieved bottom of the gel. The molecular weights of separated polypeptides were dogged by co-electrophoresis of molecular weight protein standards, #SM0431 (Fermentas Life Sciences). After electrophoresis, the gels were stained with 2% commassie blue solution for one an hour. Gels were then destained by washing with a solution containing 5% (v/v) acetic acid, 20% (v/v) methanol and distilled water in the ratio of 5:20:75 (v/v) for about 2 hour.

Data analysis: Depending upon the presence or absence of polypeptide bands, similarity index was designed for all potential pairs of protein types. The score was 1 for the presence and 0 for absence of bands. Based on outcome of electrophoretic band spectra, similarity index (s) was deliberated for all possible pairs of protein type electrophoregrams by using the following formula (Sneath & Sokal, 1973):

$$S = w / (a + b – w)$$

where, S = similarity index, w = number of bands of common mobility, a = number of bands of protein a, b = number of bands in protein type b. The similarity matrix thus generated was converted in to a dissimilarity matrix (dissimilarity = 1 – similarity) and used to build dendogram by unweighted pair-group method with arithmetic averages (Sneath & Sokal, 1973). All the analyses were carried out using statistical package NTSYS-pc, version 2.1 (Applied Biostatistics Inc., USA).

Results

A total of twenty bands were scored among the 105 sesame accessions evaluated. Of these 20 bands, 14 (70%) were polymorphic and 6 (30%) were monomorphic. Size of the protein bands generated by SDS-PAGE (measured by Unstained Protein Molecular Weight Marker ranging from 14.4 to 116 kDa) ranged from 13.5 to 100 kDa. The bands 11, 12, 15, 16 and 18 were present in all the accessions, whereas band 10 was present in 13 out of 105 sesame accessions and band 17 was missing in 5 accessions only. All the accessions in which band 17 was missing were from Punjab i.e., from Multan (19583), Bahawalpur (19590), Vehari (19608), Gujranwala (19636) and Faisalabad (21127). It was examined that protein profile of most sesame accessions differed from one another in their minor bands (Fig. 1). Variability in intensity was viewed in many protein bands that showed the amount of protein peptides mounting up at a specific molecular weight.

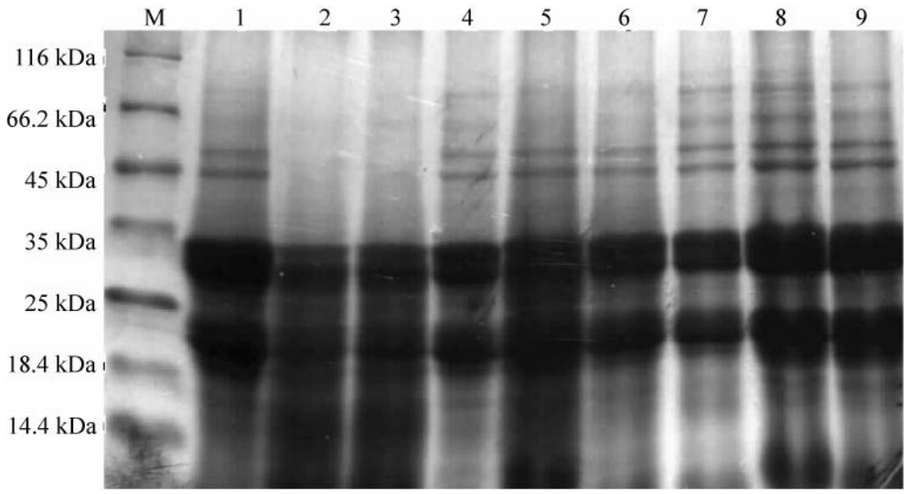

Fig. 1. Electrophoretic banding pattern generated by SDS-PAGE of seed storage proteins of some of sesame accessions. M = Protein ladder, 1 = 21403, 2 = 21404, 3 = 21406, 4 = 21478, 6 = 21530, 7 = 21558, 8 = 21568 and 9 = 22238.

The cluster diagram revealed 3 major groups i.e., A, B and C (Fig. 2). Group A consisted of four sub-groups or sub-clusters A1, A2, A3 and A4. Cluster A1 consisted of 14 accessions, A2 consisted of 66 accessions, A3 consisted of 7 accessions and A4 comprised of only 2 accessions. Group B consisted of two sub-groups B1 and B2. B1 contained 3 accessions and B2 contained 11 accessions. Group C was the smallest group of only 2 accessions (Table 2). Similarity coefficients ranged from 0.50 (19600- Bahawalpur and 19531-Lahore) to 1.00.

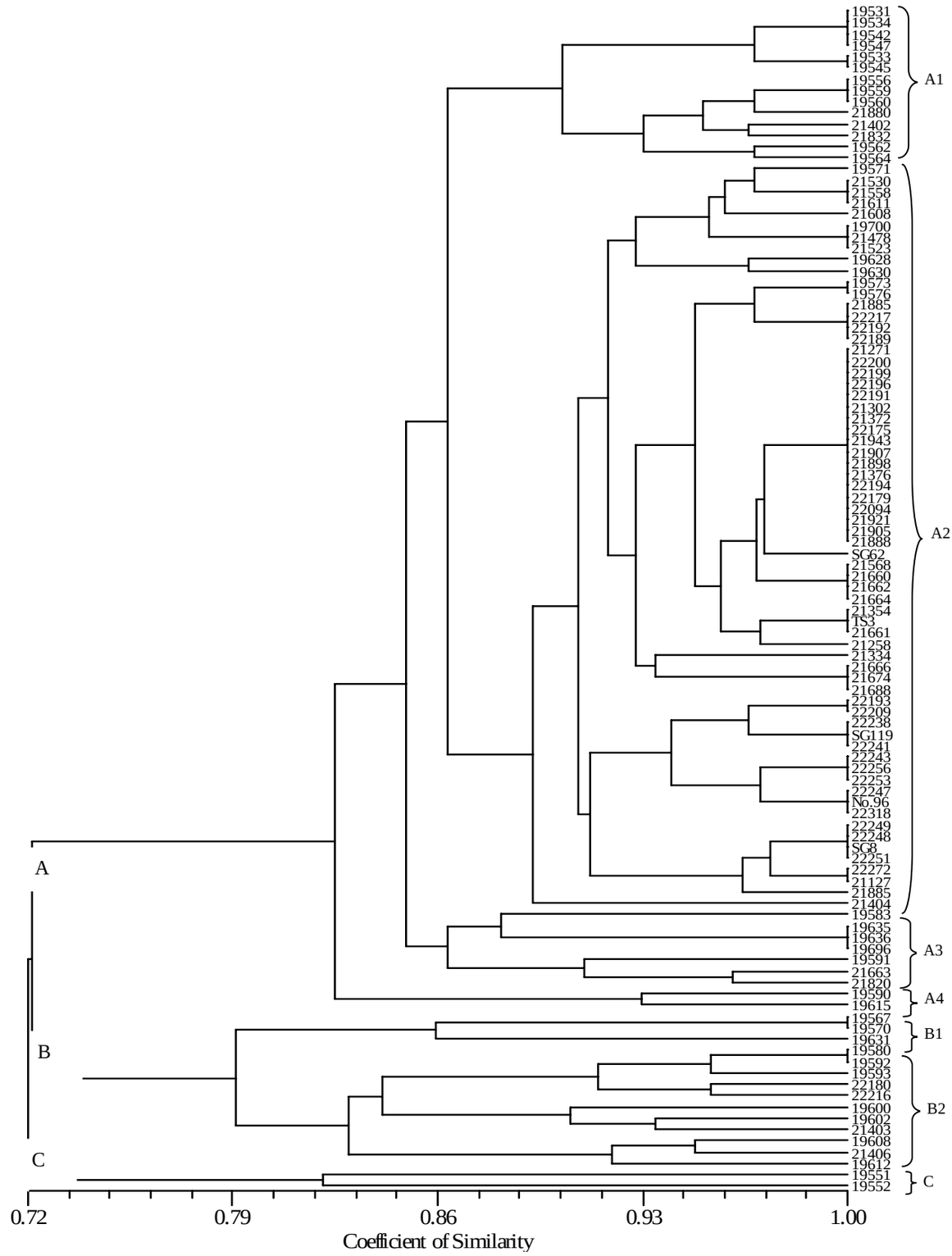

Fig. 2. Dendrogram showing the relationship among Pakistani sesame genotypes based on SDS-PAGE of total seed proteins.

Table 2. Grouping of 105 sesame genotypes based on cluster analysis using SDS-PAGE analysis.

Groups	Clusters	No. of genotypes	Genotypes
A	A1	14	19531, 19534, 19542, 19547, 19533, 19545, 19556, 19559, 19560, 21880, 21402, 21832, 19562 and 19564
	A2	66	19571, 21530, 21558, 216111, 21608, 19700, 21478, 21523, 19628, 19630, 19573, 19576, 21885, 22217, 22192, 22189, 21271, 22200, 22199, 22196, 22191, 21302, 21372, 22175, 21943, 21907, 21898, 21376, 22194, 22179, 22094, 21921, 21905, 21888, SG62, 21568, 21660, 21662, 21664, 21354, TS3, 21661, 21258, 21334, 21666, 21674, 21688, 22193, 22209, 22238, SG119, 22241, 22242, 22256, 22253, 22247, No.96, 222318, 22249, 22248, SG8, 22251, 22272, 21127, 21885 and 21404
	A3	7	19533, 19535, 19536, 19596, 19591, 21663 and 21820
	A4	2	19590 and 19615
B	B1	3	19567, 19631 and 19570.
	B2	11	19580, 19592, 19593, 22180, 22216, 19600, 19602, 21403, 19608, 21406 and 19612
C	-	2	19551 and 19552

Discussion

The SDS-PAGE technique is mostly thought as a reliable means for the reason that total seed proteins are mainly free of environmental variations (Iqbal *et al*., 2005; Javaid *et al*., 2004). Genetic diversity can be easily evaluated through biological markers (Rabbani *et al*., 2001; Akhtar, 2001). Protein types and their diversity varied among a variety of crop species, which may assist us for the early detection of species at seed level and to acquire the information on clarity of genetic assets (Rehman & Hirata. 2004). Our results exposed that a limited level intra-specific diversity was present in the evaluated sesame germplasm. Variations based on major bands were present in a few accessions such as 21404, and 21402 (Attock, Punjab), but diversity based on minor bands were available in most of the sesame accessions. The equality in major bands among a variety of accessions specifies that the genes coding these proteins are preserved (Ali *et al*., 2007). Our results were supported by Ghafoor *et al*., (2003) and Mehrani (2002) who reported a limited level intra–specific variation for seed protein among in pea and chickpea. Our results did not support the findings of Nisar *et al*., (2007), who reported a high level of intra-specific variation for seed protein among local and exotic chickpea germplasm. The disagreement is in fact due to use of different gene pools both from the exotic and local resources.

Results of SDS-PAGE revealed that this technique provided a means for steady genotypes discrimination based on genetic variation in seed protein comparison in sesame accessions, but no other relationship among the sesame germplasm observed. Sesame germplasm showed the same banding pattern may be duplicated; it should be verified through the use of advanced molecular markers. Over all a low to medium level of genetic diversity was observed. In the present investigation intra-specific difference was narrow and it was noticed that SDS-PAGE technique only did not show high level of intra-specific dissimilarity; so, different genotypes based on SDS-PAGE are suggested to be obtained from a variety of sources, to make a wide based gene pool with maximum diversity. Present evaluation offered first details to document sesame genotypes in Pakistan based on total seed storage protein markers. Our research work will be supportive to set up a gene bank of genetic resources of different sesame genotypes found Pakistan.

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