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EVALUATION OF GENETIC DIVERSITY OF SESAME BY THE STUDY OF SEED STORAGE PROTEIN THROUGH SDS-PAGE

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ABSTRACT

The sesame seed protein was electrophoretically separated through sodium dodecylesulphate polyacrylamide gel electrophoresis (SDS-PAGE). Electrophoretic banding homology among the genotypes was established not only through the banding pattern but also through estimation of dissimilarity percentage in protein profile. The advantage of SDS–PAGE is that the proteins are separated by their molecular weight and it has become one of the most widely used technique for storage protein separation. Freshly harvested seeds of 21 advance lines and their 8 parental lines were taken for the experiment of our study. The genetic diversity and the relationships among the 29 genotypes were evaluated using sodium dodecylesulphate polyacrylamide gel electrophoresis (SDS –PAGE) which shows difference in number of bands, band width and intensity for different genotypes. It was possible to identify as many as 21 different bands and these can be easily visualized. Dendogram of 21 advance lines and their 8 parents based on SDS – PAGE data using dissimilarity percentage was presented here. Mainly two clusters obtained from the dendrogram for all the 29 genotypes where cluster A forms 3 and cluster B forms 6 sub- clusters. Clustering pattern, size and constituents on the basis of electrophoretic banding pattern was independent of the same through the D^2 analysis on the basis of biochemical parameters and presence of distinct phylogenetic relationship could be recognized among the genotypes. The dissimilarity % in protein profile indicated that the B- 9 vs. adv. line 5, B-9 vs. adv. line 8, R – 9 vs. adv. line 8, adv. Line 14 vs. adv.18, HT-1 vs. adv. 11 would be useful in identifying parents for heterosis breeding.

Keywords: Sesame seed, storage protein, SDS-PAGE, genetic homology, dendrogram, genetic diversity.

Introduction

With the gradual increase of health awareness the people are much more concerned regarding the food security. The high level of under-nutrition in India is noticed due to lack of food security (Saxena, 2011). As the latest global nutrition report 2018 revels that India stands in top position with 46.6 million stunted children where in Pakistan 10.7 million, in Nigeria 13.9 million stunted children. In such situation people need nutritious food with high protein and Vitamin. In this aspect particularly in developing countries it has been shown that the average protein intake is less in people than the required amount. The plant protein is essential for proper growth and development of human beings (Sathe, 2002). Regarding this the food industry has given much attention for the production of plant protein to meet the demand of protein value in food market.

Sesame is one such important plant having seeds with good nutritive value and it can be exploited as a healthy food as it contains various nutritional components (Yokota *et. al*., 2007). As a nutritious oil seed crop sesame contains 40.4-59.8% oil and protein 19.4-26.3% (Yermanos *et al*., 1972). The sesame seeds mostly contain storage protein such as globulins (67.3%), albumins (8.6%), glutelins (6.9%) and prolamine (1.4%) (Achouri, A. 2012, Achouri, A 2013).

The demand of sesame as an edible oilseed is rising both from economic and nutritional aspects in global market. Sesame, known as the queen of oilseeds as it contains high quality oil (Johnson *et al.,* 1979) and in Asian countries same is used as healthy food (Miyake *et al.,* 2005). Actually it contains calcium, phosphorus, iron and the seeds are also rich in vitamin B, vitamin E and a small amount of trace elements. The sesame oil contains polyunsaturated fatty

acids, oleic acid and linoleic acid (Lyon 1972, Bedigian *et al*., 1985).

The use of sesame seeds increasing globally as the worldwide consumption of sesame seed is USD 6559.0 million in 2018 and it may be reach USD 7244.9 million by 2024, with a CAGR (compound annual growth rate) of 1.7% approximately (Morder Intelligence Home Page Nov 2019). The total annual consumption of sesame oil and sesame seed is about 65% and 35% (Morris, 2002). India produces 12.4% World's sesame followed by Tanzania (14.6%) and Myanmar where production around 12.78% (FAO STAT, 2020), but the average sesame yield in India is not good as compared to other sesame producing countries. So it is prime important to focus on the study on seed protein as well as genetic diversity to improve its productivity.

The charred sesame seeds were found in archaeological excavations in Harappa which are so ancient about 5000 years old (Bhat *et al.*, 1999). The knowledge of genetic diversity among the crops plays an crucial role regarding sustainable development and food security (Fukuda, 1985). Though the sesame as an oilseed crop it produces healthy oil because the oil contains high amounts of PUFA and high antioxidant content, its yield is very poor (Singh, 2003; Furat, 2010). It is reported that as an oilseed crop sesame could not compete with other oilseed crops such as sunflower, peanut, soybeans due to lack of improved varieties (Sharma *et al.,* 2014).

Information on genetic diversity and relationships among crop species and their wild relatives is essential for the efficient utilization of plant genetic resources (Chan and Sun, 1997; Helm *et al*., 1997).

The genetic variationin any crop is necessary for further improvement of the crop. Classical identification of genotypes and more so the germplasm diversity based on standard morphological markers has proved to be inadequate because of wide spectrum of phenotypic variation and their interaction with environment (Mannetji, 1984). In such instance, electrophoretic banding patterns illustrated through electro-phoregrams of storage proteins can be used effectively to decipher the similarities and differences between cultivars and genotypes (Hussain *et al.,* 1986). Population electrophoretic studies using non-enzymatic proteins of plants have been used and such studies are mainly carried out the seed storage proteins, although other storage protein such as tuber protein have been used. PAGE can be conducted with or without the inclusion of sodium dodecylsulphate (SDS).

The advantage of SDS–PAGE is that the proteins are separated by their molecular weight and it has become one of the most widely used techniques for seed storage protein separation. The technique of soluble seeds storage protein based marker assessed through sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) has been applied to evaluate the varieties in various plant species such as Mung (Ghaffar *et al*., 2002), Pea (Jha & Ohri, 2002), Einkorn wheat (Alvarez *et al*., 2006), Brassica (Khurshid & Rabbani, 2012). The molecular markers based study plays an important role in the evaluation of genetic diversity for high productivity and further improvement in crops (Sarwar *et al*., 2015; Woldesenbet *et al*., 2015; Bharathi *et al*., 2014). The electrophoresis study of protein is very essential for the study of genetic diversity as proteins beings stored in the seed are independent of environmental fluctuations (Alege *et al*., 2014; Eshsanpour *et al.*, 2010). Das *et al.* (2018) reported that the SDS-PAGE is less influenced by environmental fluctuation rather than phonological markers and it is more stable, more economic and it requires less time. According to Dudwadker *et al.* (2015) the diversity in protein profiles and seed storage proteins provides good knowledge for species delimitation and as marker for intra and interspecific hybridization. The main objective of our study is to separate the sesame seed protein and to evaluate the genetic diversity among the genotypes which is very essential for genetic improvement as well as high seed yield in sesame.

Materials and Methods

Seeds of eight parental genotypes and their 21 advance lines obtained after crossing were taken for the experiment. The parental genotypes were collected from different sources. The detail of the parental genotypes and advanced lines presented in Table 1(a) and Table 1(b)

The seeds of 8 parents and 21 advance lines were sown in Randomised Block Design (RBD) with 3 replications in five–rows plot for each genotypes with row to row spacing of 25 cm and plant to plant spacing of 12-15 cm. The irrigation and other agronomic practices including plant protection measures were done as usual and when it is required. The seeds were taken in brown paper envelops and labeled approximately and oven dried. Characterization of the different advance line, on the basis of sodium dodecylsulphate-polyacrylamide gel electrophoresis of the seed storage proteins was done from the harvested seeds.

SDS-PAGE analysis: Sodium dodecylsulphate polyacrylamide gel electrophoresis consists of the following three steps:

a) Extraction of soluble protein

b) Estimation of it and

c) Electrophoresis (gel casting, running and staining)

Extraction: 0.1 gm of dry powder of seeds was taken and macerated in 1.5 ml of Tris HCL buffer (pH 6.8). The homogenates were centrifuged at 10, 000 rpm for 30 minutes at 4'C. Supernatant was recovered and kept in a refrigerator for future use. Aliquot of the clear supernatant solution was taken for protein estimation.

Estimation: The seed protein of 8 parents and 21 advance lines of sesame was estimated by the method of Lowery *et al*. (1951) before the gel electrophoresis. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS- PAGE) was carried out according to the method proposed by Laemmli (1970). During this seed protein electrophoresis various chemical solutions and buffers were used. Ammonium persulphate (5%) and TEMED used as polymerizing agents. The pH of Electrode buffer maintained at 8.2, 3 – Mercaptoethanol and Bromophenol blue used in sample buffer. Coomassie brilliant blue R, methanol, acetic acid, water used in protein stain solution. Methanol, acetic acid and water used as distainer.

A large (17cm + 19cm) vertical gel system (Biotech) was used for carrying out the experiment. The glass plates and spacers were cleaned using ethanol and dried, then assembled properly. The assembly was held together with

full dog clip. The chamber was sealed using 2% agar (melted in a boiling water bath). 15% separating gel mixture was prepared by mixing the following one by one.

Stock acrylamide solution	20 ml
Tris HCl buffer (pH 8.8)	8ml
Water	11.4 ml
Ammonium Peroxidisulphate (APS)	0.2ml
10% SDS	0.4 ml
TEMED (N, N,N,NTetramethyl ethylene diamine)	60 µl

This mixture was poured inside the glass plates. After few minutes, the mixture inside the plates formed into gel. Then double distilled water was dropped above the separating gel. The water from top of the separating gel was then removed and stacking gel solution was poured.

Stacking gel 6% was prepared by mixing the following solutions

Stock acrylamide solution	6 ml
Tris Hcl (pH 6.8)	3ml
Water	20.5 ml
APS (5%)	180µl
10% SDS	0.3 µl
TEMED	30µl

Comb was then immediately placed in the stacking gel and was allowed to set for 30 minutes.

C) Electrophoresis : After polymerization, the comb was removed and the gel was installed carefully after removing the clips, agar etc. in the electrophoresis apparatus. The buffer tank of the apparatus was then filled with electrode buffer. The two electrodes were connected to the power pack to check briefly the electric circuit.

iii) Electrophoresis (gel casting, running and staining)

The protein concentration (quantity/volume) for each sample was adjusted with the extraction buffer. The samples were prepared by adding equal (1:1) volume of sample buffer (5X) to it. The total sample mixture was heated for 5 minutes in boiling water and then cooled. Same amount of protein (120ug) extract was loaded in each sample well. The standard marker protein (BSA) with sample buffer was also loaded in another well. 20 mA current was supplied for 15 minutes until the sample travel through the stacking gel. Then the run was continued at 30 mA until the bromophenol blue reached the bottom of the gel. The time required for the run was about 3 hours. After the run was completed the gel was separated carefully from the glass plates and immersed in staining solution overnight. Then in the next morning the gel was transferred to a large petridish containing 100 ml distaining solution. Initially destainer had to be changed frequently until the background of the gel become colourless. The gel was then photographed.

Relative mobility (Rm) o f the protein band was determined using the following formulae-

$$Rm = \frac{\text{Distance travelled by individual protein band (cm)}}{\text{Total distance run by protein samples (cm)}}$$

$$\text{Percentage dissimilarity} = \frac{\text{Number of pairs of dissimilar bands}}{\text{Number of dissimilar bands} + \text{Number of pairs of similar bands}} \times 100$$

Result & Discussion

Seed tuber storage protein gel electrophoresis was used to characterize and identify the different genotypes in a number of crop plants (Bushuk and Zillan, 1978; Cooke, 1984; Hussain *et al.*, 1986; Sehgal and Chandel, 1992; Babu, 1996). SDS-PAGE shows difference in number of bands, in band width and intensity for different genotypes (Fig.1 and Fig. 2 and Table no. 2).

It was possible to identify as many as 21 different bands. These can be very easily visualized from the zymogram presented here (Fig. 3 & Fig. 4).

None of the genotypes showed all the 21 bands in the SDS–PAGE (Fig. 1 & Fig. 2) at most 20 were found in only adv. line 1. Irrespective of genotypes band No. 8, 11, 19 and 21 showed wide width and intense intensity. Variation in the banding pattern among the genotypes was found only in the case of minor bands. The migration of bands was compared by Rm values which provided an approximation of their molecular weights. As lower the molecular weight of a protein, more would be in the migration in the charged gel. When Rm values of different bands were superimposed on the zymogram, 4 distinct groups of proteins could be identified:

1) Band number 1 to 7 (Rm values 0.050 to 0.225) – represented one group of protein, where molecular weights are likely to be above 66 KDa as their position stand close to the standard BSA (Bovine Serum Albumin) of 66 KDa
2) Band number 8 to 11 (Rm values 0.2562 to 0.365) appeared to be between 65 to 45 kDa;
3) Considering the relative position of bands numbering 12 to 17 (Rm values 0.462 to 0.5875), it is possible to draw same kind of approximation regarding their molecular weight. It is reasonable to suggest that they might have molecular weight in between 45 to 30 kDa;
4) Similarly, band number 18 to 21 (Rm values 0.6437 to 0.8062) representing another group of protein as per their Rm values and position on the zymogram.

It is further suggested that their molecular weight may fall within the range of 25 to 15 k Da. The band no. 21 was far away from others. This has appreciable lower molecular weight and it would likely to be around 15 kDa. However these are very tentative and unless standards of different molecular weight (kDa) are used, no accurate estimation would be possible. Nevertheless, such analysis would permit us to go into the details of seed protein characteristics.

The protein banding pattern showed in zymogram appeared to be very unique for each of the genotypes under study. A few studies have been reported earlier on SDS-PAGE of soluble proteins from sesame. Das *et al*. (1992) obtained protein profiles by repeated runs of the sesame samples of the buffer soluble protein fraction, reproduced in electro-phorograms, which showed clear differentiation in banding pattern among the varieties. The varieties differed not only in the number of protein bands but also in their nature of staining density, Differentiations in the banding

pattern revealed by PAGE-electrophoretic studies of native – seed proteins indicate genotypic variation at the varietal level. The banding pattern shows differences among the genotypes that provides the knowledge of genetic divergence among the sesame genotypes over evolutionary time which may be used for the crop improvement (Alege *et al.,* 2015).

Electrophoretic banding homology is considered to be a powerful approach in estimating genetic relatedness or differences between 2 or more species (Johnson and Thein, 1970) as well as in the identification and classification of genotypes (Cooke and Draper, 1983). The technique used in this study have shown the potential and usefulness in finding out the genotypic diversity. Following Whitney *et al*. (1968) the dissimilarity percentage of genotypes were worked out and presented in Table 3.

Among the genotypes it was indicated that 0 (adv.line 5vs adv.line 8) to 60% dissimilarity (B-9 vs adv. line 5, B-9 vs adv.line 8, R -9 vs adv.line 8 & 14 vs adv.line 18, HT-1 vs adv.line 11) in protein profiles indicating thereby a distinct deviation. Hence, from seed storageprotein profile standpoint the 29 genotypes appeared to be narrow divergent. Presence of major bands (major intense and broad) for all the genotypes (adv.line including parents) infer that the genotypes have same phylogenetic relationship. Dendogram of 21 advance lines and their 8 parents based on SDS-PAGE data using dissimilarity percentage is represented in Fig. 5.

Mainly two clusters may be assumed from the dendrogram for all the 29 genotypes where cluster A forms 3 and cluster B forms 6 sub-clusters. Thus the 9 sub-clusters, these are :-

The clustering pattern, size and constituents is somehow different from those of D^2 analysis derived based on biochemical parameters of sesame seeds (Roy *et al*., 2021).

Conclusion

The advantage of SDS-PAGE is that the proteins are separated by their molecular weight and it has become one of the most widely used technique for storage protein separation. Our study focused on genetic diversity of some advance lines of sesame revealed by SDS–PAGE of seed storage proteins. The dissimilarity percentage in protein profile indicated that the B- 9 vs.adv.line 5, B-9 vs.adv.line 8, R–9 vs. adv.line 8, adv. Line 14 vs. adv.18, HT-1 vs.adv. 11 would be useful in identifying parents for heterosis breeding, conservation and genetic improvement for seed yield in sesame. The protein banding pattern is helpful in divergence as well as phylogenetic study in sesame.

Table 1(a) : T The list of Parental genotypes

	Variety	Seed colour	Source of collection
1.	R-9	Small grayish white	U.S.S.R
2.	B-14	Black	Pulses & Oilseeds Research Station, Berhampur, W.B
3.	B-9	Brown	Pulses & Oilseeds Research Station, Berhampur, W.B
4.	B-67	Blackish brown	Pulses & Oilseeds Research Station, Berhampur, W.B
5.	T-12	White	Directorate of Oilseed Research , Hyderabad
6.	IDP-51	Blackish brown	Pulses & Oilseed Research Station, Berhampur
7.	IET-2	Light brown	Pulses & Oilseed Research Station, Berhampur
8.	HT-1	White	Pulses & Oilseed Research Station.

Table 1(b) : The parentage of advance lines

Adv. line.	Parentage	Adv. line.	Parentage	Adv. line	Parentage
1.	B-9 x T-12	8.	B-9 x IET-2	15.	HT-1 x B-14
2.	B-14 x HT-1	9.	B-9 x IET-2	16.	IET-2 x B-9
3.	HT-1 x B-14	10.	T-12 x R-9	17.	B-9 x HT-1
4.	IDP-51 x T-12	11.	T-12 x IDP-51	18.	IET-2 x R-9
5.	T-12 x B-9	12.	B-14 x IDP-51	19.	HT-1 x B-9
6.	IDP-51 x B-14	13.	R-9 x IET-2	20.	R-9 x T-12
7.	B-14 x B-9	14.	R-9 x HT-1	21.	IET-2 x R-7

Table 2 : Presence/absence of bands and their respective R_m values as revealed by SDS-PAGE of 8 parental and 21 advance lines of sesame

Band No. / Rm. Val / Sl. No. of Gent.	1 0.05	2 0.0625	3 0.0875	4 0.1125	5 0.1625	6 0.1875	7 0.225	8 0.2562	9 0.306	10 0.325	11 0.356	12 0.462	13 0.5125	14 0.525	15 0.55	16 0.562	17 0.5875	18 0.6437	19 0.6937	20 0.7437	21 0.806
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	×	✓	×	✓	✓	×	×	✓	✓	×	✓	×	✓	✓	×	✓	✓	✓	✓	✓	✓

1= IET-2, **2** = B-67, **3**= B-9, **4**= R-9, **5** = T-12, **6** = IDP-51, **7** = B-14, **8** =HT-1, **9** =Adv. L.-12, **10** = Adv. L.-13, **11** = Adv. L.-14, **12** = Adv. L.-2, **13** = Adv. L.-15, **14** = Adv. L.-1, **15** = Adv. L.-10, **16** = Adv. L.-6, **17** = Adv. L.-16, **18** = Adv. L.-4, **19** = Adv. L.-5, **20** = Adv. L.-8, **21** = Adv. L.-17, **22** = Adv. L.-9, **23** = Adv. L.-18, **24** = Adv. L.-19, **25**. Adv. L.-7, **26**. Adv. L.-20, **27** = Adv. L.-21, **28** = Adv. L.-11, **29** = Adv. L.-3.

Table 3 . Percentage dissimilarity between genotypes of 8 parental and 21 advance lines of sesame based on SDS-PAGE.

Genotypes	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
1	0	45.00	52.63	36.84	30.00	17.64	28.57	35.29	31.57	35.29	35.29	18.75	25.00	25.00	22.22	16.66	22.22	31.57	25.00	25.00	38.88	23.52	33.33	31.67	27.77	38.09	47.34	40.00	38.88
2		0	6.25	22.22	33.33	47.61	35.00	38.88	26.31	27.77	29.41	42.10	38.88	28.57	42.85	30.00	26.31	26.31	55.00	55.55	47.61	42.85	52.38	42.85	40.00	36.84	42.10	36.84	50.00
3			0	17.64	23.52	50.00	40.00	35.29	31.57	33.33	35.29	47.36	44.44	33.33	47.61	35.00	31.57	31.57	60.00	60.00	45.00	47.61	50.00	47.61	45.00	42.10	47.36	42.10	55.00
4				0	25.00	40.00	26.31	38.88	26.31	33.33	38.88	47.36	29.41	33.33	42.85	35.00	31.57	26.31	40.00	60.00	31.57	35.00	36.84	47.61	40.00	45.00	42.10	36.84	42.10
5					0	25.00	20.00	31.57	28.57	38.09	40.00	35.00	29.41	14.28	20.00	15.00	20.00	20.00	40.00	40.00	25.00	28.57	30.00	28.57	33.33	38.09	42.85	38.09	35.00
6						0	16.66	47.36	26.31	27.77	38.88	23.52	38.88	20.00	16.66	30.00	35.00	35.00	29.41	29.41	31.57	26.31	27.77	26.31	31.57	36.84	33.33	36.84	23.52
7							0	33.33	30.00	31.57	33.33	27.77	23.52	15.00	25.00	25.00	21.05	21.05	33.33	29.41	22.22	21.05	31.57	30.00	35.00	31.57	36.84	47.60	27.77
8								0	42.10	52.60	37.50	41.17	37.50	35.00	33.33	27.77	23.52	33.33	55.55	55.55	47.36	50.00	52.63	57.14	47.36	52.63	57.89	60.00	57.89
9									0	22.22	42.10	27.77	42.10	23.80	30.00	25.00	30.00	38.09	50.00	50.00	35.00	30.00	40.00	30.00	35.00	31.57	45.00	31.57	36.84
10										0	25.00	18.75	35.29	25.00	31.57	35.00	40.00	40.00	44.44	44.40	36.84	31.57	42.10	40.00	36.84	33.33	38.88	33.33	29.41
11											0	20.00	26.66	35.00	23.52	27.77	23.52	23.52	47.05	47.05	55.00	50.00	60.00	57.14	47.36	52.63	41.17	44.44	41.17
12												0	20.00	30.00	17.64	22.22	27.77	27.77	31.25	31.25	42.10	36.84	47.36	45.00	42.10	50.00	44.44	38.88	25.00
13													0	35.00	33.33	27.77	23.52	23.52	26.66	26.66	38.86	33.33	44.44	42.10	38.88	44.44	41.17	44.44	31.25
14														0	20.00	19.01	23.80	23.80	33.33	35.00	20.00	15.00	25.00	23.80	20.00	25.00	30.00	33.33	30.00
15															0	15.78	21.05	21.05	33.33	33.33	21.00	30.00	40.00	38.09	35.00	40.00	36.84	40.00	27.77
16																0	5.55	15.78	36.84	36.84	38.09	33.33	42.85	33.33	30.00	42.85	47.61	35.00	40.00
17																	0	11.11	42.10	42.10	42.85	38.09	47.61	38.09	35.00	47.61	45.00	40.00	45.00
18																		0	33.33	33.33	29.41	38.09	47.61	38.09	42.85	47.61	36.84	36.84	36.84
19																			0	0	29.41	23.52	25.00	23.52	29.41	35.29	31.25	35.29	20.00
20																				0	29.41	23.52	25.00	2352	29.41	35.29	31.25	35.29	20.00
21																					0	5.88	6.25	16.66	22.22	17.64	33.33	36.84	23.52
22																						0	11.76	11.11	16.66	11.76	27.77	31.57	17.64
23																							0	11.76	17.64	23.52	29.41	33.33	29.41
24																								0	16.66	22.22	27.77	23.52	27.77
25																									0	27.77	23.52	17.64	33.33
26																										0	38.88	42.10	29.41
27																											0	18.75	25.00
28																												0	29.41
29																													0

1= IET-2, **2** = B-67, **3**= B-9, **4**= R-9, **5** = T-12, **6** = IDP-51, **7** = B-14, **8** =HT-1, **9** =Adv. L.-12 **10** = Adv. L.-13, **11** = Adv. L.-14, **12** = Adv. L.-2, **13** = Adv. L.-15, **14** = Adv. L.-1, **15** = Adv. L.-10, **16** = Adv. L.-6, **17** = Adv. L.-16, **18** = Adv. L.-4, **19** = Adv. L.-5, **20** = Adv L.-8, **21** = Adv. L.-17, **22** = Adv. L.-9, **23** = Adv. L.-18, **24** = Adv. L.-19, **25**. Adv. L.-7, **26**. Adv. L.-20, **27** = Adv. L.-21, **28** = Adv. L.-11, **29** = Adv. L.-3.

Table 4: List of Clusters and Subclusters

Cluster-A	Sub-cluster I -consists of advance line 21, adv. line 11, adv.line7
	Sub-cluster II- consists of adv. line 20,adv.line 19, adv. line18, adv. line 9, adv. line 17
	Sub-cluster III- consists of adv. line 3, adv. line 8, adv. line 5
Cluster - B	Sub-cluster IV- consists of adv. line 13,adv line 12
	Sub-cluster V- consists of B-9, R-9, B-67.
	Sub-cluster VI- consists of adv. line 15,adv.line 14
	Sub-cluster VII- consists of HT -1
	Sub-cluster VIII- consists of adv. line 4, adv. Line 16, adv. Line 6, adv. line 1, T -12
	Sub-cluster IX- consists of B-14, IDP-51, adv. line 10, adv. line 2, IET -2

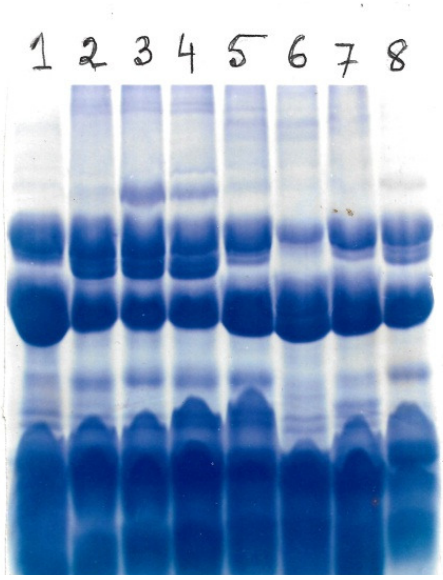
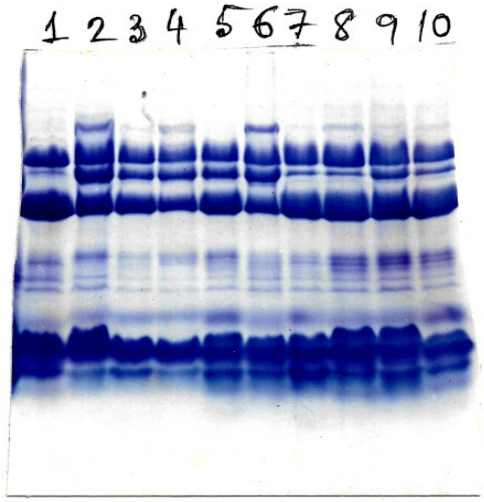


Fig. 1 : SDS Polyaccrylamide gel electrophoregram of 8 parental genotypes of sesame where lane (1) = IET-2, (2) = B-67, (3) B-9, (5) = T-12, (6) = IDP-51, (7) = B-14, (8) = HT-1



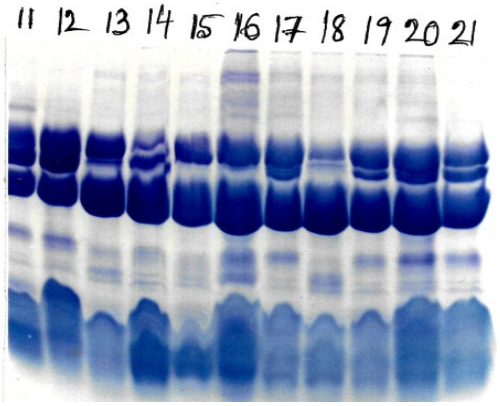


Fig. 2 SDS-polyacrylamide gel electrophoregram of 21 advance lines of sesame where lane **1** = ADV. L-12, **2** = Adv. L-13, **3** = Adv. L-14, **4** = Adv. L-2, **5** = Adv. L-15, **6** = Adv. L-1, **7** = Adv. L-10, **8** = Adv. L.-6, **9** = Adv. L.-16, **10** = Adv. L.-4, **11** = Adv. L.-5, **12** = Adv. L.-8, **13** = Adv. L.-17, **14** = Adv. L.-9, **15** = Adv. L.-18, **16** = Adv. L.-19, **17** = Adv. L.-7, **18** = Adv. L.-20, **19** = Adv. L.-21, **20** = Adv. L.-11, **21** = Adv. L.-3

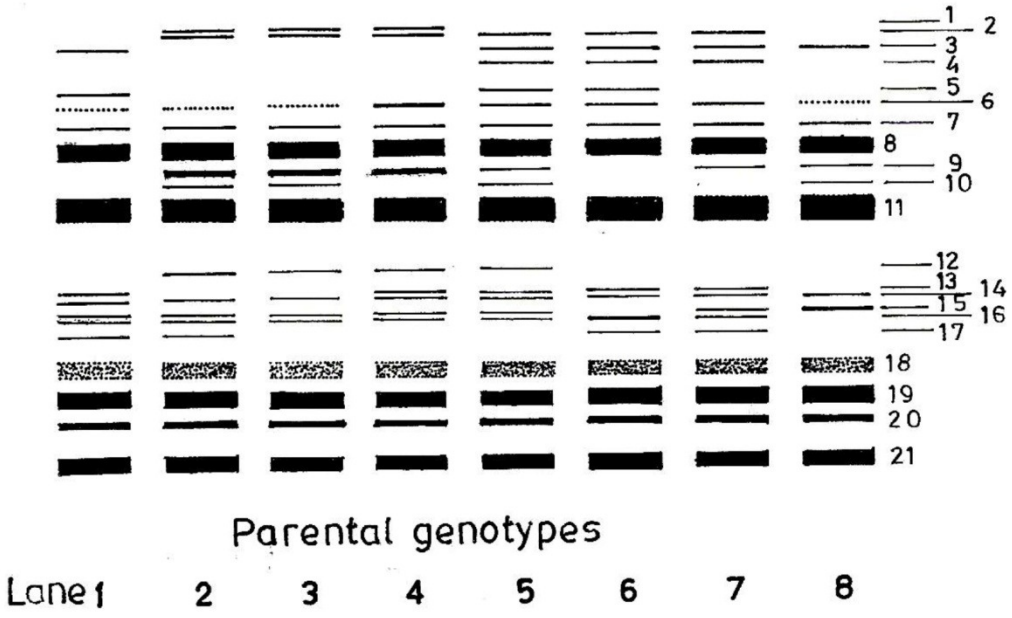


Fig. 3. Zymogram for SDS-PAGE of sesame and seed storage proteins from 8 parental genotypes. Where Lane 1= IET-2, 2 = B-67, 3= B-9, 4= R-9, 5 = T-12, 6 = IDP-51, 7 = B-14, 8 =HT-1

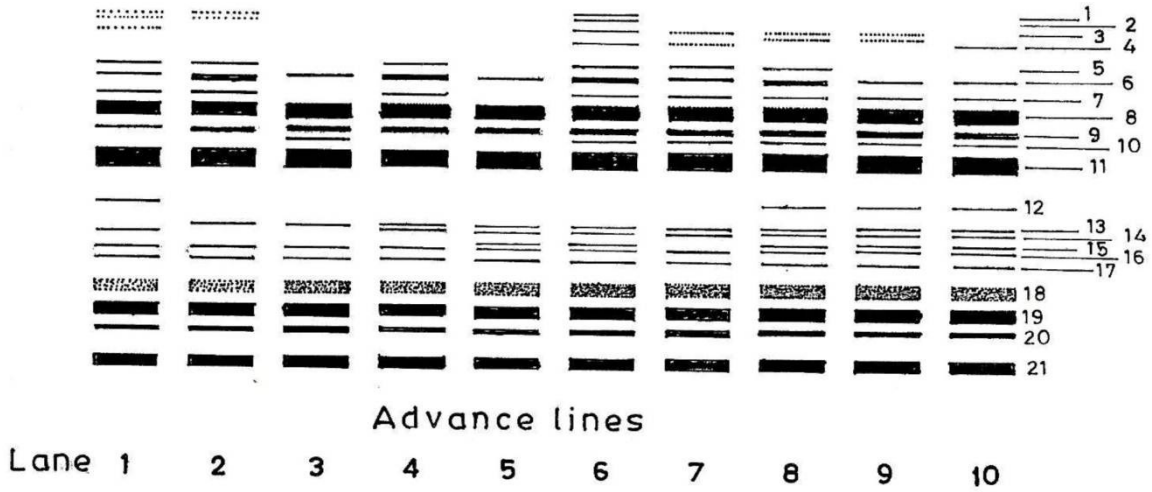


Fig. 4 Zymogram for SDS-PAGE of sesame seed storage proteins from 10 advance lines, Where Lane 1 =Adv. L.-12, 2 = Adv. L.-13, 3 = Adv. L.-14, 4 = Adv. L.-2, 5 = Adv. L.-15, 6 = Adv. L.-1, 7 = Adv. L.-10, 8 = Adv. L.-6, 9 = Adv. L.-16, 10 = Adv. L.-4,

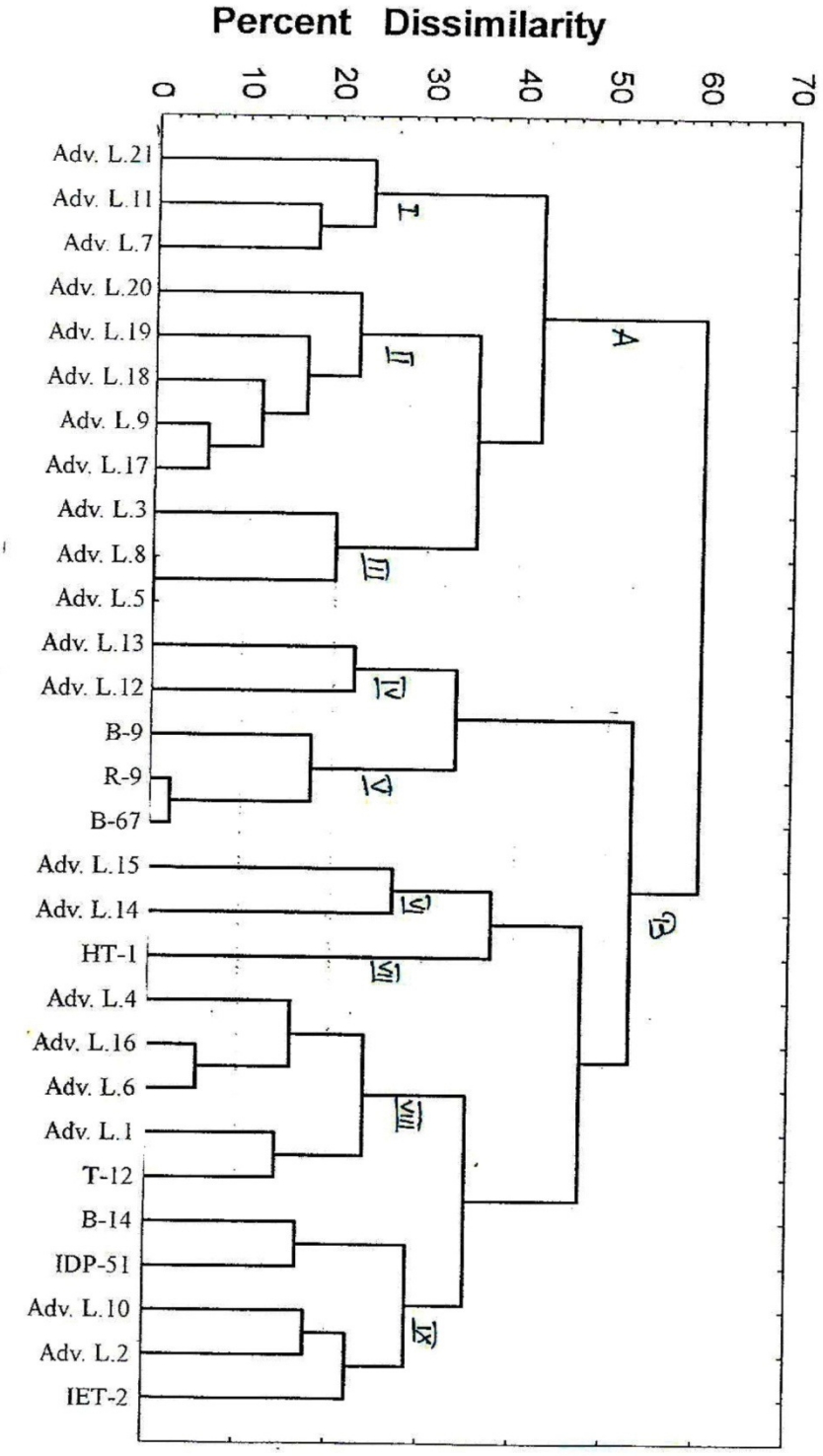


Fig. 5 Dendogram of 21 advance lines and their parents based on SDS-PAGE data using dissimilarity percentage.

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Author's contribution- B. Roy conducted the field and lab experiment and collected data, A.K. Basu designed the experiment, N. Mondal provided the support for manuscript preparation and A. Pal analyzed the data.

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