Research Article

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ASSESSMENT OF GENETIC DIVERSITY BASED ON POLYPEPTIDE BANDING PATTERN AMONG DIFFERENT ISOLATES OF *ASPERGILLUS FLAVUS* USING SDS-PAGE

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Abstract: A quantitative categorization of total storage proteins profile of 6 isolates of *Aspergillus flavus* was performed by sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE). This technique was used to explore the level of genetic discrepancy in *A. flavus* isolates. Total soluble proteins were resolved on 10% resolving gel. A total of 27 polypeptide bands were obtained among which 20 bands were present in all isolates but other 7 bands of molecular weight (127.38, 110.14, 109.74, 97.62, 93.83, 74.98 and 59.03 KDa) showed variation. Dendrogram based on electrophoretic data clustered the varieties mainly into two groups. In conclusion, electrophoretic bands (SDS-PAGE) of storage proteins can provide a potent tool to estimate genetic variation and relation among isolates. The specific bands of seed storage protein profiles may be used as markers for identification of the varieties.

Keywords: *Aspergillus flavus*, Genetic diversity, SDS-PAGE, Polypeptide bands

INTRODUCTION

Aspergillus flavus is a haploid filamantous fungus. It is a saprophyte which obtains its nutrient from decaying material. It belongs to the phylum ascomycota. Members of this phylum reproduce sexually by forming ascospores. Like other molds, it also grows by producing hyphae. The network of hyphae or mycelia is responsible for secreting catabolic enzymes. The enzyme secreted is used to down complex food services. The complex food sources are broken down into small molecules which then absorbed by the mycelium to produce asexual spores called conidia[1]. Conidia are globes, smooth and small with size of 2-2.5 mm in diameter.

Markers such as morphological traits, biochemical characteristics (isozyme, protein profiles) and DNA-based molecular markers are powerful tools for the analysis of genetic diversity and relatedness among different genotypes/isolates of fungus. Though morphological trait can be used for assessing genetic diversity, but it is often influenced by the environmental factors[2]. Protein polymorphism serves as genetic marker as they are direct products of active genes and are quite polymorphic and generally heritable[3,4]. The polymorphism observed in the protein profiles reflects the changes in the active part of the genome. Though protein polymorphism can be analysed through a variety of techniques, polyacrylamide gel electrophoresis is generally favoured technique for rapid analysis[5,6,7,8] due to its validity and simplicity for describing genetic variations[9].

The present study was undertaken to assess genetic diversity based on storage proteins among 6 isolates of *A. flavus*.

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MATERIALS AND METHODS

Fungal Culture:

For the present investigation, fungal isolates of *Aspergillus flavus* were obtained from school of life sciences, Jaipur National University, Jaipur.

Extraction of Proteins:

In this investigation, Crude protein samples were prepared from the culture supernatant. Proteins were extracted using protein extraction buffer. The resulting supernatant was used as protein sample.

Determination of Protein Concentration:

The concentrations of proteins extracted by the above method were determined spectro-photometrically using the Bradford method[10].

Sample Application and Gel Electrophoresis:

Protein sample was mixed with 4× gel loading dye to make its final concentration of 1× in mixture and was heated at 95°C in water bath for 10 min prior to loading. Protein sample (100µg) was loaded in each lane. Protein molecular weight marker (Bangalore Genei, India) was used as reference. Protein samples were electrophoresed at 8 V/cm for about 4h at constant current.

Staining of Gel:

Preparative gels were visualised by staining with Coomassie Brilliant Blue R-250.

Data Analysis:

Gels were placed on a white light Transilluminator and photographed. Gels were subjected to careful manual scoring. The presence or absence of bands was scored as '1' and '0', respectively. Only prominent

bands were scored. Molecular weight of the polypeptides was determined on the basis of their migration distance. Migration distance of the marker polypeptides measured and their Rf values were calculated.

A graph was plotted by the Rf values of the marker polypeptides against the logarithm of their molecular weight. Rf values of the sample proteins were calculated and their corresponding log10 molecular weights were interpolated from the calibration plot.

RESULTS AND DISCUSSION

SDS-PAGE of fungal proteins was performed in order to investigate genetic diversity among *A. flavus* isolates. The six isolates used in the present study showed various banding pattern using SDS-PAGE technique. Figure showed the polypeptide banding pattern of different *A. flavus* genotypes. A total of 27 polypeptide bands were obtained among which 20 bands were present in all isolates but other 7 bands of molecular weight (127.38, 110.14, 109.74, 97.62, 93.83, 74.98 and 59.03 KDa) showed variation (Figure.1).

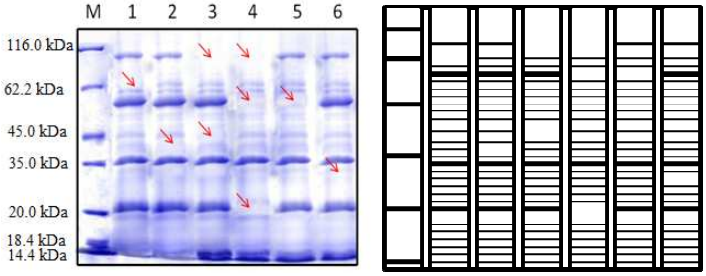

Figure.1: Comparative evaluation of SDS-PAGE protein profile of six isolates of *A. flavus*. M=molecular weight marker

The genetic similarity coefficient matrix of six *A. flavus* isolates based on SDS-PAGE using UPGMA method was used to construct a dendrogram using a computer program NTSYS-PC, version 2.02i, to find the diversity among these isolates. The results of the cluster analysis are given in a dendrogram (Figure 2) on the basis of similarity coefficient. The dendrogram revealed that the two main groups A and B; the group A is further divided into two sub clusters A1 and A2. Sub cluster A1 contains A and F isolates. Another sub cluster A2 contains B and C isolates. The group B contains D and E isolates.

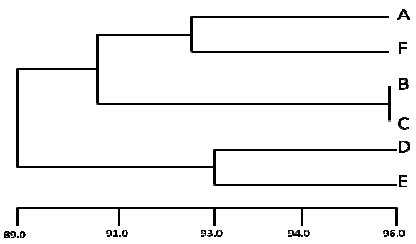

Figure.2: Dendrogram obtained from polypeptide bands analysis using UPGMA demonstrating relationship among six isolates of *A. flavus*.

Table.1: Comparative evaluation of protein profiles among different isolates of *A. flavus*

S. No.	Molecular Weight	1	2	3	4	5	6
1	127.38	+	+	-	-	+	+
2	110.14	-	+	+	+	+	+
3	109.74	+	+	+	-	-	+
4	107.42	+	+	+	+	+	+
5	106.94	+	+	+	+	+	+
6	106.16	+	+	+	+	+	+
7	102.34	+	+	+	+	+	+
8	99.32	+	+	+	+	+	+
9	97.62	+	+	-	+	+	+
10	93.83	+	-	+	+	+	+
11	91.14	+	+	+	+	+	+
12	85.17	+	+	+	+	+	+
13	82.53	+	+	+	+	+	+
14	79.47	+	+	+	+	+	+
15	76.21	+	+	+	+	+	+
16	74.98	+	+	+	+	+	-
17	71.32	+	+	+	+	+	+
18	68.17	+	+	+	+	+	+
19	62.17	+	+	+	+	+	+
20	59.03	+	+	+	-	+	+
21	53.54	+	+	+	+	+	+
22	50.23	+	+	+	+	+	+
23	49.21	+	+	+	+	+	+
24	48.17	+	+	+	+	+	+
25	45.52	+	+	+	+	+	+
26	43.25	+	+	+	+	+	+
27	42.17	+	+	+	+	+	+

Jaccard's similarity coefficient value ranged from 0.89 to 0.96. The similarity matrix was subjected to UPGMA clustering to generate dendrogram. Lowest Jaccard's similarity value represents maximum diversity. The variation in protein banding pattern was revealed by SDS-PAGE. From the results of the SDS-PAGE, the overall blueprint of storage-proteins showed the very low degree of heterogeneity. A low level of genetic diversity may be attributed to narrow genetic base.

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