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Molecular Analysis of genetic diversity of field bean genotypes showing varied resistance to pod borer, *Sphenarches caffer* using Isozyme analysis and RAPD

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ABSTRACT: *Isozyme and RAPD analysis of selected eight field bean genotypes detected a high level of genetic variation. The isozyme analysis of the genotypes for peroxidase showed slight variations with a total of 3 bands. More intense bands were observed at Rm value of 0.166 in all the genotypes except PLS-22016 and FB-2. Bands observed in the genotypes TNAU Purple Pod, GA-102 and EC-7467 at Rm value of 0.7916 with high and medium intensity respectively and the rest showed low intensity. In RAPD studies, a high degree of polymorphism in general was obtained with most of the primers especially with OPB-11 and OPB-12 (6 bands) and OPB-10 (2 bands) indicating a wide range of variability among the genotypes at DNA level. The similarity index values ranged from 0.50 to 0.951 indicating a wide range of genetic diversity. Based on RAPD marker analysis, the most diverse pair was found to be FB-2 and Devangundappa where as maximum closeness was observed between EC-7467 and Devangundappa. In general, the genotype PLS-22016 exhibited wide variation with the other genotypes and was genetically more distinct and diverse. TNAU purple pod and GA-102 belonging to the same group (Group A) possessing the desirable attributes of resistance with the similarity index of 79.6 per cent expressed resistance to pod borer,* S. caffer *could be exploited and utilized in the genetic enhancement studies.*

Keywords: *Field bean genotypes, peroxidase isozyme, RAPD, plume moth, Sphenarches caffer, resistance.*

INTRODUCTION

Field bean is a multipurpose crop grown for pulse, vegetable and forage. Both pods and grains are nutritious, serving as a good source of proteins, several minerals and vitamins and form an essential daily diet of humans. The host plant resistance / tolerance is one of the most viable components of integrated pest management and utilisation of resistant or tolerant cultivars reduces the use of insecticides and involves no extra cost to the farmers and forms an ideal way of effective, economical and eco-friendly management strategy against insect pests.

Polymorphism due to different molecular forms of the enzyme with conserved activity was detected by differential migration of isozymes within the gel; any consistent banding variation between varietal zymograms is a result of genotypic differences between varieties under comparison [Hussain *et al*. 1].

RAPD can be used in studying genetic diversity, varietal identification etc. The information on polymorphism for genetic resistance using RAPD in a set of genotypes is useful in tagging genes of interest and genetic mapping in long run to facilitate marker assisted selection. With this in view, present study was taken up on eight genotypes of field bean that were selected among thirty three accessions based on their reaction to pod borer, *Sphenarches caffer* and were subjected to Isozyme and RAPD analysis for molecular characterization.

I. Electrophoretic analysis of peroxidase isozyme

Identification of molecular diversity among the various genotypes was done by isozymes analysis and polymorphism for the peroxidase isozyme, adopting standard protocols [Shaw and Prasad, 2].

Evaluation and Documentation/ Zymogram preparation

Gels were photographed over diffused U.V light immediately after appearance of bands. The positions of bands were drawn. The Relative electrophoretic

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mobility (Rm) values for the bands were calculated and zymograms were constructed from Rm values. Rm values of peroxidase were calculated as the ratio of the movement of the band to that of the tracking dye. The Rm value was calculated as given below:

$$R_m = \frac{Distance\ traveled\ by\ the\ isozyme\ band\ (cm)}{Distance\ traveled\ by\ the\ tracking\ dye\ (cm)}$$

Bands were numbered on the basis of increasing R_m values, the relative intensities of protein bands and presence or absence of specific bands or combination of different bands.

II. Studies on the genetic diversity of selected genotypes

II. RAPD Analysis

Detection of molecular polymorphism among the selected genotypes (resistant and susceptible) from field screening of field bean was performed by RAPD analysis.

Extraction of plant DNA

DNA was extracted as per modified CTAB (Cetyl Trimethtyl Ammonium Bromide) method [Murray and Thompson, 3].

STATISTICAL ANALYSIS

Data on genetic diversity studies were entered using a matrix in which all observed bands or characters were listed. The RAPD pattern of each genotype was evaluated; assigning character state '1' to all the bands that could be reproducible and detected in the gel and '0' for the absence of band.

The data matrix thus generated was used to calculate Jaccard's similarity co-efficient for each pair-wise comparison. The coefficients were calculated *insilico* following Jaccard (1908), using the following formula.

Similarity coefficient = a/n

Where, a = Number of matching bands for each pair of comparisons

n = Total number of bands observed in two samples

The similarity co-efficients were subjected to Unweighted Pair-Group Method of Arithmetical averages (UPGMA) cluster analysis to group the genotypes based on their overall similarities. Statistical Package for Social Sciences (SPSS) package was used for the cluster analysis and subsequent dendrogram preparation.

RESULTS AND DISCUSSION

Electrophoretic analysis of peroxidase isozyme

Occurrence of multiple forms popularly called isozymes has been used as a biochemical marker to estimate genetic diversity and trueness to type [Smith and Smith, 4]. In the present study, peroxidase isozyme system was used to estimate the genetic diversity among different clusters of field bean genotypes.

The similarities and dis-similarities in the zymograms showed the extent of genetic diversity existing among different clusters. The isozyme profile of peroxidase was presented in Fig.1 and Plate 1. The electrophoretic analysis of field bean genotypes for peroxidase showed slight variations with a total of 3 bands. More intense bands were observed at Rm value of 0.166 in all the genotypes except PLS-22016 and FB-2. Bands observed in the genotypes TNAU Purple Pod, GA-102 and EC-7467 at Rm value of 0.7916 with high and medium intensity respectively and the rest showed low intensity.

Peroxidase isozyme banding pattern of different Rm values indicated the different mobility patterns for 3 bands suggesting the presence of polymorphism for peroxidase isozyme. Mobility values ranged from 0.166 to 0.7916 indicating a wide range of variability in the molecular weights for peroxidase bands. The genotypes of cluster II, with TNAU purple pod and GA-102, exhibited a genetic similarity due to presence of similar type of banding pattern with high intensity with Rm value of 0.7916 and EC-7467 had medium intensity with Rm value of 0.7916. The high intensity was as consequence of higher peroxidase activity resulting in resistance nature among the entries. The susceptible entries could be recognized by less intense bands. The field and lab results with respect of TNAU purple pod and GA-102 exhibited resistance reaction to pod borers of field bean with special reference to *S. caffer*. Relevance of peroxidise assays are in accordance with Karban and Myers [5] and increased activity of defensive enzymes like peroxidases, polyphenol oxidases and phenyl ammonia lyase are related to resistance inducement in plant. Peroxidase is related to lignin and suberin synthesis, which increase the hardness of tissues, and to the production of quinones and active oxygen, which possess antibiotic properties [Goodman *et al*. 6, Bowler 7, Stout *et al*. 8]. Quinones, complex with proteins, thus decreasing the nutritional quality of food, making protein digestion difficult. [Felton and Duffy 9, Felton *et al*. 10, Mohammed and Kazani 11].

Studies on the genetic diversity of selected genotypes

Eight genotypes of field bean were selected among 33 genotypes based on their reaction to *S. caffer* from field and laboratory assays. They were subjected to RAPD technique to study the level of diversity and to establish genetic similarities among them.

Primers survey and selection

The DNA samples from eight selected genotypes produced clear, sharp and high molecular weight band is 0.8 per cent agarose gel.

Thirteen random primers *viz*., OPB-2, 5, 6, 7, 10, 11, 12, 14, 15, 17, 18 and 20 are used for amplification of field bean Genotypes. Out of thirteen primers, OPB-2 and OPB–20 resulted in non-distinct amplification products and were discarded; remaining eleven primers were used for PCR amplification which gave reproducible bands with high percentage of polymorphism. PCR amplification with these primers was done twice before scoring for presence or absence of bands.

Banding Pattern

A high degree of polymorphism in general was obtained with most of the primers especially with OPB-11 and OPB-12 (6 bands) and OPB-10 (2 bands) indicating a wide range of variability among the Genotypes at DNA level (Plate 2).

Banding profiles obtained with 11 primers for 8 Genotypes of field bean were analyzed on the basis of presence or absence of bands. Jaccard's similarity co-efficients of eight selected genotypes were calculated to establish the genetic relationships and presented in Table 1.

The similarity index values ranged from 0.50 to 0.951 indicating the presence of genetic diversity at DNA level among eight genotypes of field bean evaluated. The most diverse pair was found to be FB-2 and Devangundappa where as maximum closeness was observed between EC-7467 and Devangundappa.

In general, the genotype PLS-22016 exhibited wide variation with the other genotypes and was genetically more distinct and diverse.

Cluster Analysis

Relationships were evaluated by cluster analysis of data and based on similarity matrix, the dendrogram was generated by Unweighted Pair Group Method with Arithmetic mean (UPGMA) using paired matrix values, all the entries were grouped into 3 close knit clusters and are presented in Fig. 2.

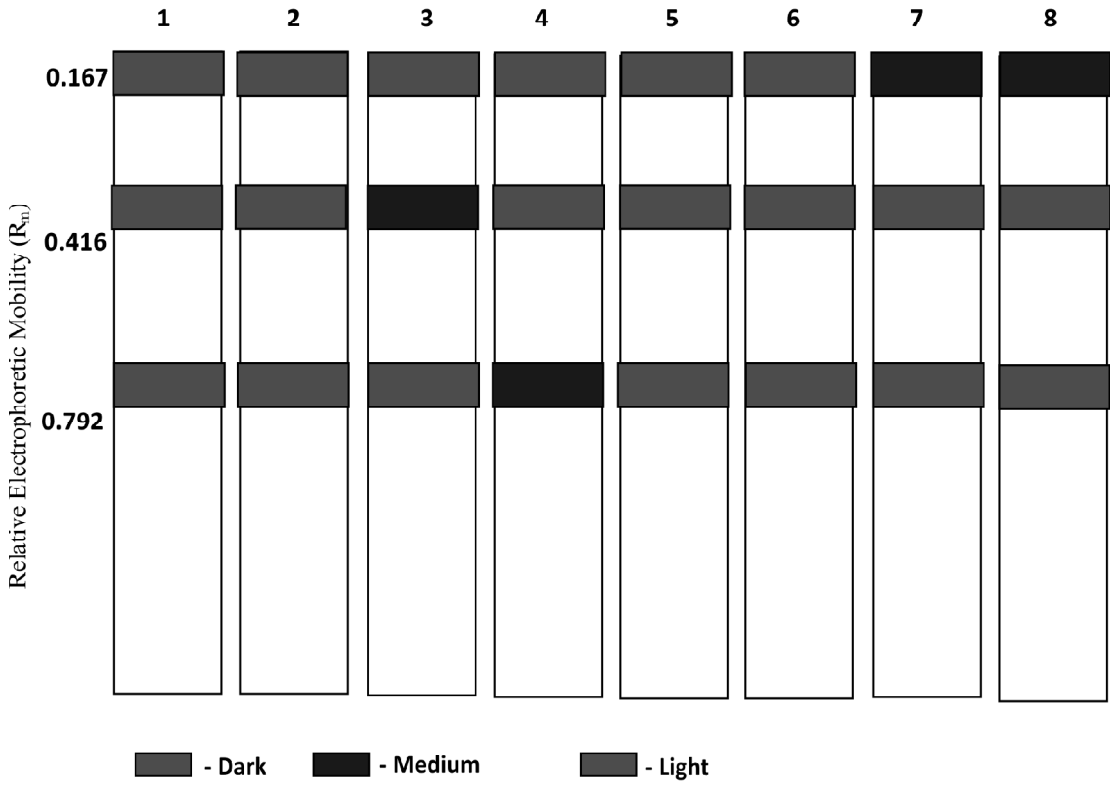
Molecular characterization of genotypes is considered to be reliable method to find out genotypic variability and polymorphism at genetic level among the entries.

Extensive variations for morphological and physiological characteristics were reported in field bean by Gnanesh [12]. Out of 11 primers used, 10 primers produced polymorphism for 2 to 6 bands with fragment size varying from 1.6 Kbp to 4.0 Kbp. A total of 68 bands were obtained with the above process, among which 41 bands were polymorphic (60.29%) in nature. It was in accordance with the findings of Gnanesh [12], Kamakshi [13] and Sujithra [14] where 42, 69 and 65.8 per cent polymorphism was reported in field bean genotypes, respectively.

Jaccard's similarity co-efficients among 8 genotypes were calculated to establish the genetic relationships. The similarity index values ranged from 0.50 to 0.951 indicating the presence of wide range of genetic diversity at molecular level among 8 genotypes. Hence the genetic diversity was apparent with field bean according to Liu [15] and Renu and Mishra [16] who reported wide range of genetic diversity by RAPD techniques at molecular level in germplasm lines of peas.

Table 1
Jaccard's similarity co-efficient of 8 genotypes of field bean based on Polymorphism obtained with 11 primers

Genotype	*AVT FB-SD 13-1-8*	*TNAU Purple Pod*	*GA-102*	*EC-7467*	*PL-3196*	*Devangu-ndappa*	*PLS-22016*	*FB-2*
AVT FB- SD 13-1-8	1.00							
TNAU Purple Pod	.786	1.00						
GA-102	.783	.796	1.00					
EC-7467	.825	.694	.750	1.00				
PL-3196	.738	.684	.746	.850	1.00			
Devangundappa	.810	.733	.790	.951	.864	1.00		
PLS-22016	.508	.580	.564	.541	.604	.550	1.00	
FB-2	.818	.642	.721	.923	.785	.879	.500	1.00



Genotypes:

1. AVT -FB (SD) 13-1-8
2. TNAU Purple pod
3. GA-102
4. EC-7467
5. PL-3196
6. Devangundappa
7. PLS-22016
8. FB-2

Figure 1: Zymogram of Electrophoretic banding patterns for peroxidase isozyme of selected field bean genotypes

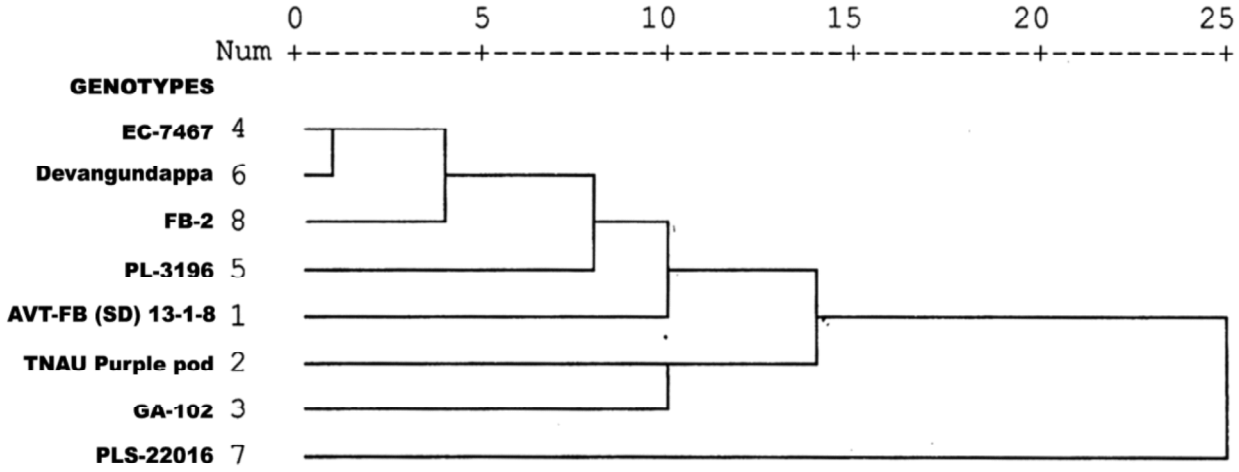


Figure 2: Dendrogram depicting variation among selected genotypes of field bean based on RAPD

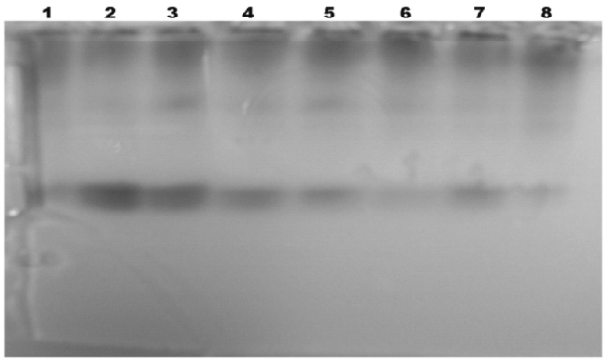


Plate 1: Electrophoretic banding pattern for peroxidise isozyme of selected field bean genotypes

Genotypes

1. **AVT-FB (SD) 13-1-8**
2. **TNAU Purple pod**
3. **GA-102**
4. **EC-7467**
5. **PL-3196**
6. **Devangundappa**
7. **PLS-22016**
8. **FB-2**

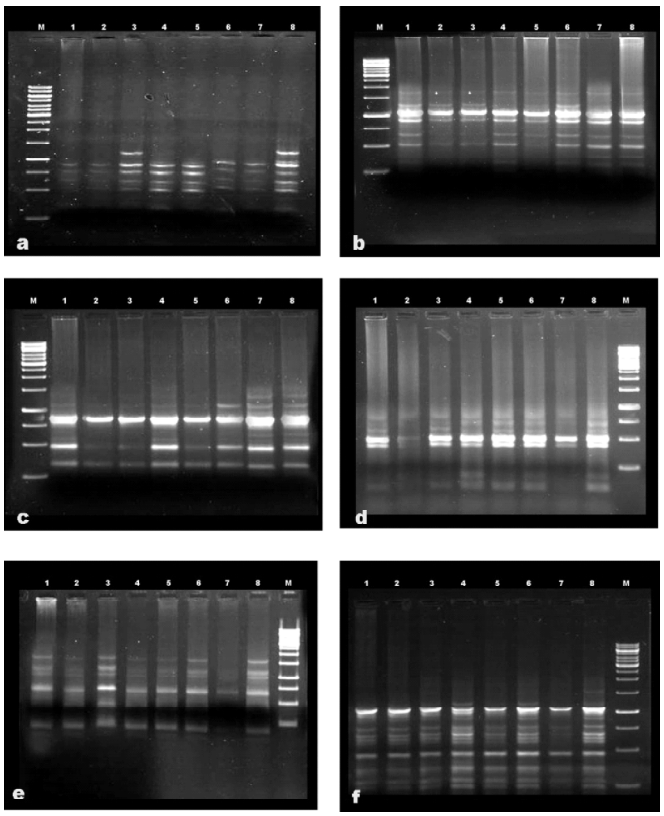


Plate 2: RAPD gel profile of 8 field bean genotypes using various primers a. OPB-5; b. OPB-11; c. OPB-12; d. OPB-14; e. OPB-15 and f. OPB-18

Genotypes

1. **AVT-FB (SD) 13-1-8**
2. **TNAU Purple pod**
3. **GA-102**
4. **EC-7467**
5. **PL-3196**
6. **Devangundappa**
7. **PLS-22016**
8. **FB-2**

The clustering pattern using RAPD resulted in 3 major clusters. The dendrogram of similarity coefficients of cluster I indicated that the genotypes Devangundappa, FB-2, PL-3196, AVT-FB(SD) 13-1-8, which were susceptible to pod borer, *S. caffer* (Group B) and EC-7467 resistant to pod borer were genetically similar.

In the cluster II two genotypes i.e. TNAU purple pod and GA-102 belonging to the same group (Group A) with the similarity index of 79.6 per cent expressed resistance to pod borer, *S. caffer*. But in cluster III, only one genotype PLS-22016 which is susceptible to the pod borer had 50.8% similarity with AVT-FB (SD) 13-1-8 belonging to the same group (Group B).

However, information on diversity for pest resistance is lacking. Thus from current investigation, genotype TNAU purple pod and GA-102, possessing the desirable attributes of resistance to plume moth, *S. caffer* could be exploited and utilized in the genetic enhancement studies in view of its diverse base variation in DNA profile.

CONCLUSION

RAPD assay revealed the presence of wide range of genetic diversity using molecular method among 8 genotypes. The results from the field screening, pertaining to TNAU purple pod to express resistance reaction to plume moth, *S. caffer* were supported by the laboratory evaluations, which were further confirmed by the peroxidase enzymatic patterns and molecular characterization of DNA. Further, polymorphic studies involving more number of RAPD primers and techniques may reveal better similarity index in phenotypic and genotypic resistance.

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