BIOCHEMICAL CHARACTERIZATION OF PIGEON PEA (*Cajanus c*ajan)

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Abstract

Pigeon pea (Cajanus cajan (L) Millsp) is an important legume and belongs to family Leguminoseae. Pigeon pea contains 21% protein. Pigeon pea is highly proteinous plays an important role in food security, balanced diet and alleviation of poverty because it can be used in diverse ways as a source of food, feed, fodder (Rao et al., 2002). The aim of present study was to characterize cultivars eight of pigeon pea differentiating in Fusarium wilt resistance and susceptible cultivars based on SDS -PAGE.

The estimation of protein was done by Follin and Lowry's method, the protein content in all eight genotypes found in range between 21% to 25%. Production of pigeon pea of high-protein genotype should be encouraged. For SDS-PAGE analysis, prestained protein molecular weight marker of range 14 kDa to 94.4 kDa was used. Seed protein profile for majority of the genotypes showed less polymorphism. A unique band of molecular weight 60 kDa was found in only BWR-87 line and the band of molecular weight 16 kDa was found in only genotype khadaki. The diversity revealed by molecular and biochemical characterization of pigeon pea cultivars can be used for further crop improvement of pigeon pea through plant breeding programme.

Keywords- *Pigeon pea, protein content, eight genotypes, crop improvement.*

Introduction

Pigeon pea (*Cajanus cajan* (L) Millsp) is a important legume and belongs to family Leguminoseae. The term Cajanus is derived from Malay word '*katschang'* or '*katjang'* meaning pod or bean. The genus *Cajanus* comprises 32 species, most of which are found in India and Australia, although one species is native to West Africa. The origin of pigeon pea is Nile river and coastal region in Africa. Pigeonpea is the only cultivated food crop of the *Cajaninae* sub-tribe and has a diploid genome comprising 11 pairs of chromosomes (2n = 22) with a physical size estimated at about 853 Mbp (Greilhuber and Obermayer, 1988).

Electrophoresis (SDS-PAGE) is used due to its validity and simplicity for describing genetic structure of crop germplasm. Seed storage proteins have been used as genetic markers obtained by electrophoresis to resolve the taxonomic and evolutionary problems of several crop plants (Ladizinsky & Hymowitz, 1980; Das & Mukarjee, 1995). Researchers can use genetic similarity information to make decisions regarding the choice for selecting superior genotypes for improvement or to be used as parents for the development of future cultivars through hybridization. The present study was initiated to study biochemical diversity on the basis of seed protein profile and its relationship with agronomic traits in pigeon pea.

Characterization of germplasm using biochemical fingerprinting has got special attention. The protein profiling of germplasm and use of genetic markers have been widely and effectively used to determine the taxonomic and evolutionary aspects of several crops (Murphy *et al*., 1990; Khan, 1990; Das & Mukarjee, 1995; Ghafoor *et al*., 2002).

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is most economical simple and extensively used biochemical technique for analysis of genetic structure of germplasm. Seed storage proteins have been used as genetic markers in four major areas: 1) Analysis of genetic diversity within and between species, 2) Plant domestication in relation to genetic resources conservation and breeding, 3) Genome relationship and 4) A tool in crop improvement (Ghafoor *et al*., 2002). As seed storage proteins are largely independent of environmental fluctuations, their profiling using SDS-PAGE technology is particularly considered as a consistent tool for economic characterization of germplasm (Javaid *et al*., 2004; Iqbal *et al*., 2005).

Materials and Methods

The present study regarding biochemical analysis of pigeon pea cultivars (*Cajanus cajan*) was carried out at the Department of Post Harvest and Food Biotechnology, College of Agricultural Biotechnology, Latur, constituent college of Marathwada Krishi Vidyapeeth, Parbhani.

Experimental materials

In the present study, the experimental material was comprised of 8 cultivars of pigeon pea were obtained from Agriculture Research Station, Badnapur, District Jalna, (Maharashtra).

Table No.1 List of genotypes.

Sr. no.	Genotypes	Response to Fusarium Wilt as per data provided by Agriculture Research Station, Badnapur.
1	BSMR- 736	Resistance
2	BSMR – 853	Resistance
3	BWR 134	Resistance
4	BWR 87	Resistance
5	BDN-708	Moderately resistance
6	BDN-2	Susceptible
7	ICPL-2376	Susceptible
8	Khadaki	Susceptible

Inoculation of *Fusarium udum* on pigeon pea genotypes

Confirmation of wilt susceptible and resistant cultivars was done by inoculating the *Fusarium udum* inoculum on the different cultivars as per method followed by (Joshi, 2001).

Material:

PDB (Potato Dextrose Broth) – 100ml
Culture of *Fusarium udum*

Procedure

Single conidium culture of *Fusarium udum* was multiplied on 100ml of potato dextrose broth in 250ml of flask.

The flasks were placed on rotary shaker for 10 days at 25-30^{0}C.

After 10 days, the entire culture was diluted with sterile distilled water and final volume was made to 100 ml containing 2.5 ml inoculum.

7-10 days old, pigeon pea seedlings were immersed in the spore suspension of *Fusarium udum* for 30min.

The seedlings were transplanted in sterilized sand and soil (1:1) mixture in plastic pots.

The inoculated plants were kept at greenhouse for 20 days. The disease incidence and observation were taken on 21 days after transplanting.

The use of biochemical markers for characterization of pigeon pea has been promising**.** However, limited studies on seed storage proteins have been conducted in this section. In this study, SDS-PAGE was used to analyse pigeon pea biochemical diversity of protein.

Isolation of protein from seeds

Dry seeds of pigeon pea were powdered using mortar and pistal. The powder was defatted with several washing with acetone and hexane. The solvent was removed by centrifugation at 6000 rpm for 15 min.

1 ml of sample buffer- I (0.2 M Tris-HCl, pH: 6.8 and 2% SDS) was added to 200 mg of seed powder.

The tubes containing protein sample with sample buffer-I were incubated at 4^0 C overnight with intermittent shaking.

After overnight incubation, the samples were centrifuged at 10,000 rpm for 20 minutes at 4^0 C.

The supernatant was collected and sample buffer-II (0.25M Tris-HCl, pH: 6.8, 10% SDS, 10% Sucrose, 0.1 % Mercaptoethanol) was added in 1:1 ratio. By this addition the protein samples were prepared.

This supernatant-buffer-II mixture was the protein isolated from seeds of pigeon pea.

For SDS-PAGE, the protein sample was denatured at 90^0C for 3 mins.

The gel slab was prepared according to Laemmlis (1970) protocol for further analysis of protein

Quantification of protein

Protein quantification was performed by Follin and Lowry's method.

Result and Discsussion

Protein Estimation-

Protein was isolated from dry seeds of eight genotypes of pigeon pea cultivars which appears as pale yellow in colour. The quantification of this protein sample was done by Follin and Lowry's method (Lowry, *et al.,* 1951) The protein percentage of the unknown sample is determined by comparing its absorbance with the absorption of standard sample by plotting calibration curve (Fig. 1). The protein percent ranges from 21% to 25%. The lowest protein percentage is found in the genotype BSMR-853 and the highest protein percent was found in genotype ICPL-2376.

Protein percentage of 8 genotypes

Sr. no.	Lines	Percent protein
1	BSMR-736	22%
2	BSMR-853	21%
3	BWR-134	21.5%
4	BWR-87	22.5%
5	BDN-708	24%
6	BDN-2	23%
7	ICPL-2376	25%
8	Khadki	23.5%

SDS-PAGE Analysis

Seed storage protein profile of 8 pigeon pea genotypes were analysed by SDS-PAGE (Fig, 2). Total crude protein was resolved on 15% SDS polyacrylamide gel. For SDS-PAGE analysis, prestained protein molecular weight marker range 97.4 kDa to 14 kDa was used.

The protein bands of molecular weight 97.4 kDa were found in all the 8 genotype of pigeon pea namely BSMR-736, BSMR-853, BWR-134, BWR-87, BDN-708, BDN-2, ICPL-2376, Khadaki. The protein bands of molecular weight 66.0 kDa were also common in all the 8 genotype.

The unique band of molecular weight approximately 60 kDa was found in only BWR-87 line. The bands 43 kDa were found in all pigeon pea line namely BSMR-736, BSMR-853, BWR-134, BWR-87, BDN-708, BDN-2, ICPL-2376 and Khadaki.

The band of molecular weight 27 kDa was found in all the 8 genotypes of pigeon pea cultivars. The bands of molecular weight 25 kDa were found in lines BSMR-853, BWR-134, BWR-87, BDN-708, BDN-2, ICPL-2376, Khadaki but absent in BSMR-736. The bands of molecular weight 20.1 kDa were found in all genotype of pigeon pea. The unique band of molecular weight 16 kDa was present in only Khadaki line. The bands of molecular weight 14 kDa were present in 5 genotype of pigeon pea BSMR-853, BWR-134, BWR-87, BDN-708, and BDN-2 and absent in BSMR-736, ICPL-2376 and Khadaki. This study reveals some polymorphism at the protein level of the different cultivars of pigeon pea.

Estimation of protein was carried out by the Follin and Lowry's method. Lowry's assay for total protein is one of the most common colorimetric assays performed by biochemists. The seed protein content in the pigeon pea cultivar is found slightly variable. The protein percent ranges from 21% to 25%. The highest protein content was found in line ICPL-2376 which is wilt susceptible and lowest protein was found in the line BSMR-853 which is wilt resistance. The varieties susceptible to pests or diseases were found to possess higher amount of proteins (Chakravarthy and Sahni, 1972). This correlation can be studied in further experiments.

Electrophoresis of protein is a powerful tool for detection of genetic diversity and SDS-PAGE of seed protein is particularly considered as a reliable technology because seed storage proteins are highly independent of environmental fluctuation (Iqbal *et al*, 2005 and Javid *et al*, 2004). Proteins being the direct gene products reflect the genomic composition of lines accurately to some extent and therefore, are ideal for genotypic distinctness.

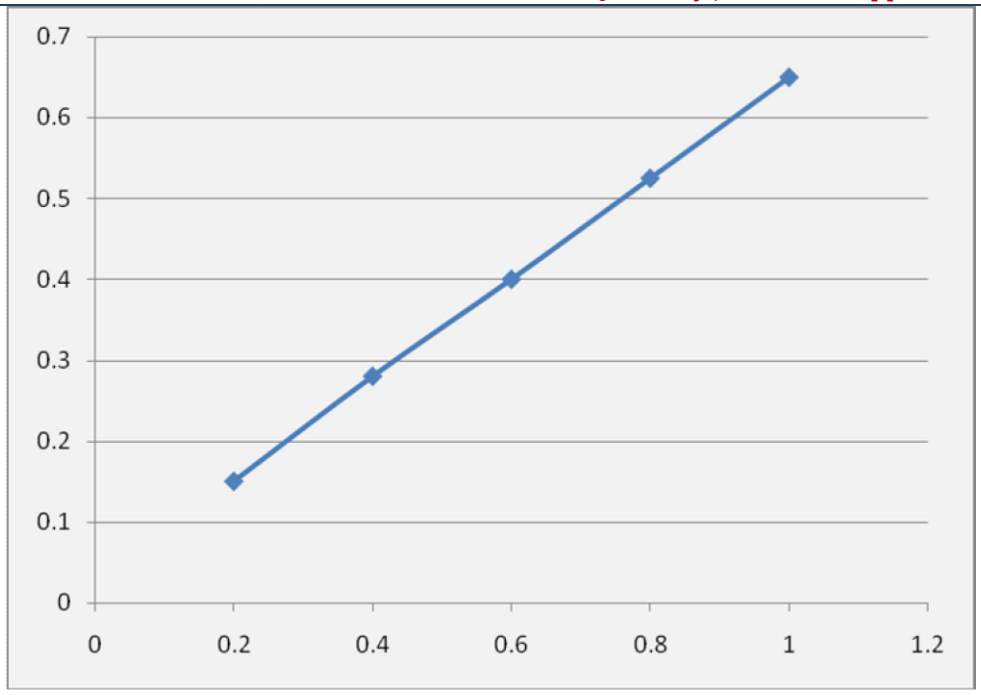
It is important to note that a low level of intra-specific variation has been reported in various legumes, i.e. chickpea (Ghafoor *et al*., 2003*b*), lentil (Piergiovanni & Taranto, 2003; Sultana & Ghafoor, 2007), groundnut (Javaid *et al*., 2004), pigeon pea (Jha & Ohri, 1996) and black gram (Ghafoor *et al*., 2003*a*) but in the case of pea, a considerable amount of variation was observed based on SDS-PAGE that indicated the valid utilization of seed protein markers for germplasm classification in pea. Although SDS-PAGE showed diversity in the storage protein banding patterns of the present material, its magnitude was low.

Gangwar *et al*. (2006) studied storage seed proteins of plant A134, UPAS 120, ICPL 84023 and of *Cajanus scarabaeoides* wild species and their hybrids were analyzed by SDS PAGE. In all 26 protein bands were observed ranging from 11.0 kDa to 98.0 kDa.

In the present study, the polymorphism at protein level is found to be low level. The band of molecular weight 60 kDa was found in only the line BWR-87 which is the wilt resistance line. The unique band of molecular weight 16 kDa was found in only the wilt susceptible line Khadaki.

The bands of molecular weight 14 kDa were present in 5 genotype of pigeon pea out of which 4 lines namely BSMR-853, BWR-134, BWR-87, BDN-708 are wilt resistance and one genotype BDN-2 is the wilt susceptible. No bands of 29.0 kDa were not found in any genotype of pigeon pea. The bands of molecular weight 94.7 kDa, 66 kDa, 43 kDa, 27 kDa were present in all the 8 genotypes.

This polymorphism at molecular and biochemical level can be utilized with present sequence database of pigeon pea genome and their character.

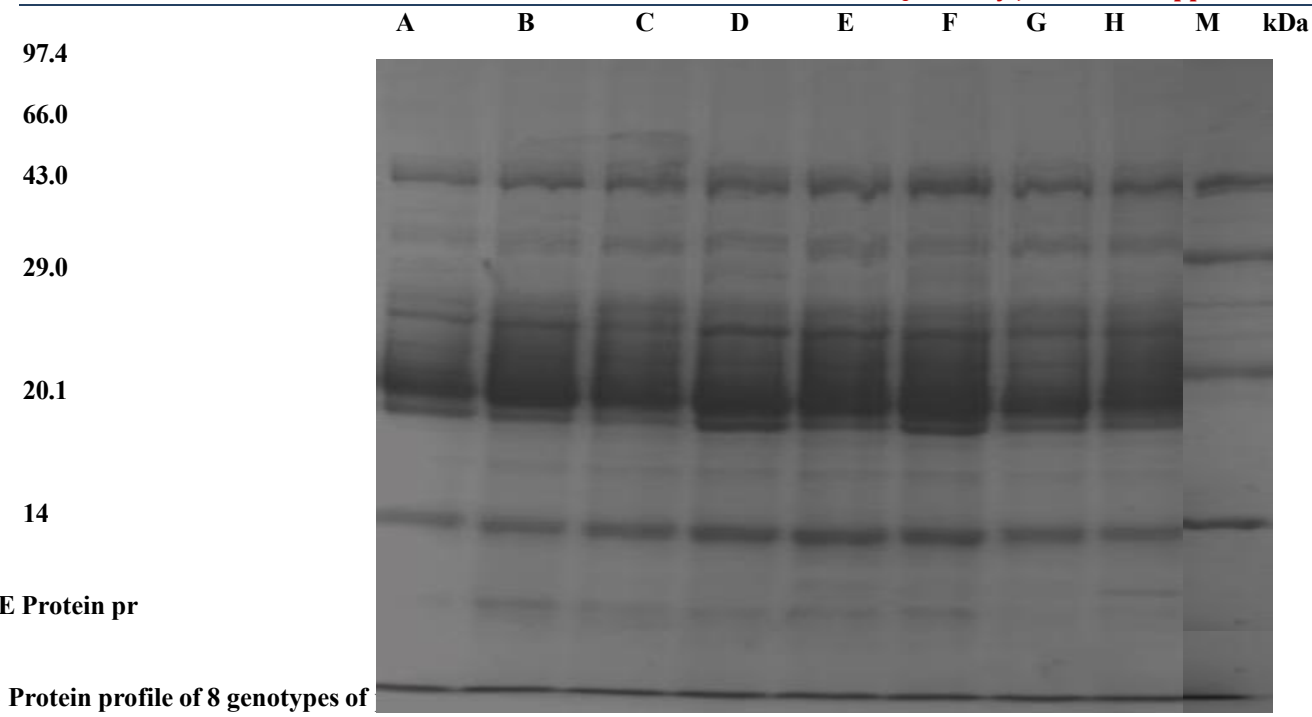


X axis – protein concentration Mg/ml
Y axis - absorbance

Fig. 1 Standard calibration curve

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Protein profile of 8 genotypes of