

Original Research Article

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Analysis of Leaf and Seed Protein of Pigeonpea Genotype (*Cajanus cajan* L. Millspaugh) Including One Wild Species Revealed by Gel Electrophoresis

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ABSTRACT

Pigeonpea is the major source of dietary protein for vegetarian population in India and other developing countries. The following study was taken to understand the genetic relationship between genotypes on the basis of protein profiling of leaves and seed protein. Ten genotypes of Pigeon pea [*Cajanus cajan* (L.) Millspaugh] including one wild species was sown in the field at Student Instructional Farm, ANDUA & T Kumarganj Ayodhya. Total protein was extracted using 0.1 M Sodium phosphate buffer (pH 7.2) containing NaH_2PO_4 and Na_2HPO_4 . Protein was electrophoresed on 12% SDS- PAGE along with standard protein marker and detected by commassive brilliant blue staining. Pigeonpea leaves and seeds protein showed variability in banding pattern of polypeptide on gel. RM values of different pigeonpea genotype were measured. The results showed that both leaves and seeds protein migrated between a range of < 10 to 250 kDa. NDA-3, MAL-6 and MAL-13 had minimum protein bands (8 bands) while maximum protein bands was observed in NDA-14-6 (12 bands) in leaves. In protein profile of seeds it has been seen that *Cajanus scarabaeoides* showed maximum bands (20 bands) while NDA-2 showed minimum protein band (11). All the accessions commonly showed at least six major bands in leaves while 11 major bands in seeds. In this context we can say that seeds of wild genotype contain more protein than cultivated pigeonpea genotype. The main objective of this study is to reveal intra-specific similarities and genetic diversity in protein content amongst ten genotypes of pigeon pea.

Keywords

Pigeonpea, *Cajanus scarabaeoides*, SDS-PAGE

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Introduction

The pigeon pea [*Cajanus cajan* (L.) Millspaugh] is an often cross-pollinated diploid ($2n = 2x = 22$) crop with genome size 833.07 Mb belongs to the family Fabaceae. It is an important grain legume crop of rain fed agriculture in the tropical and sub-tropical regions of the world. It is the first seed legume which has been complete genome sequenced (Singh *et al.*, 2012; Varshney *et al.*, 2012). Pigeon peas production is

estimated at 4.49 million tons in world (FAOSTAT 2018). In India Pigeonpea production is 4.25 Million tons in a total area of 4.43 Mha at productivity level of 960 kg/ha (ever highest yield) during 2017-18 (DES 2018).

Pigeon pea seeds are a rich source of protein and are widely consumed by people in developing countries to meet their caloric and protein needs (Salunkhe *et al.*, 1986; Singh *et al.*, 1990). Due to high protein content (18–

30%) and its ease of digestibility (68%), it is a major source of proteins particularly for a large section of vegetarian population of India (Reddy *et al.*, 1979; Chitra *et al.*, 1996; Sharma *et al.*, 2011). Cellular proteins from different tissues often vary in their properties with respect to their localization, hydrophobicity, ligand interactions, proteolysis, size and charge (Isaacson *et al.*, 2006). It is important to understand the genetic diversity available in this crop and its wild relatives for a planned and better utilization of germplasm in pigeonpea breeding.

There are some methods that are available to study the genetic diversity in any crop species which includes protein analysis by electrophoresis (Singh *et al.*, 1991), isozyme profiles (Ahmad *et al.*, 1992) and eDNA-based methods (Miller and Tanksley, 1990; Devos and Gale, 1992; Hongtrakul *et al.*, 1997; Wang *et al.*, 1998). Protein and isozyme analyses (Ladizinsky and Hamel, 1980; Krishna and Reddy, 1982) and molecular methods like RFLPs, RAPDs (Nadimpalli *et al.*, 1993 and Ratnaparkhe *et al.*, 1995) SSRs, AFLPs and ISSRs have successfully elucidated the phylogenetic relationship of pigeonpea and its wild relatives. Therefore to determine tissue-specific protein constituents of pigeonpea protein was extracted from mature leaf, immature pods and dry seeds collected from field-grown pigeonpea. The results demonstrated the efficacy of different protein dependent on the metabolic constituents of the tissues. In this study tissue-specific protein extracted for their quantitative and qualitative properties are discussed.

Materials and Methods

Plant material

Ten Pigeonpea genotype including one wild

variety was used in the present study. Leaf and pod samples were collected from the field maintained at Student Instructional Farm ANDUA&T, Kumarganj Ayodhya. Seeds were taken from fully mature plant.

Extraction of protein

Total soluble proteins were extracted separately from leaves and seeds of ten pigeonpea genotypes; the extraction was carried out at 4⁰C. 1g of seeds was soaked into phosphate buffer for overnight before extraction and then grind using mortar pestle. 1g of freshly collected leaves were cut into small pieces and macerated into phosphate buffer. The ratio of plant material and buffer was 1:4 (w/v). After that samples were centrifuged at 5000 rpm for 20 minutes. Discard pellet and collect supernatant that was crude protein.

Protein quantification

Concentration of protein were measured by protein assay reagent (Bio-Rad) based on Bradford method (Bradford 1976). Protein estimation involved the addition of assay reagent to protein samples, incubated for 5 min and measurement of absorbance at 595 nm. Bovine serum albumin served as standard. Quality of protein samples was examined on denaturing polyacrylamide gel.

Gel electrophoresis

Electrophoresis of protein samples was carried out in a Mini-PROTEAN®3 vertical gel electrophoresis system (Bio-Rad USA) by following the standard protocol of Laemmli, (1970). Protein samples were mixed with equal volume of 2x gel loading buffer, denatured by heating (100⁰C, 5 min). The gel was electrophoresed at a constant current (20mA) and stained with coomassie brilliant blue a triphenylmethane textile dye. After

staining the gel was de-stained with 45% methanol containing 10% glacial acetic acid until the protein bands become visible with negligible background.

Results and Discussion

Proteins from leaf and seeds of ten pigeonpea genotype were extracted and analysed separately on polyacrylamide gel respectively. Protein bands were compared with respect to their Rf values and molecular weight, and the range of M.W. of proteins has been shown in Table 1 and 2.

It is evident from the results 11 leaf proteins and 17 seed proteins have been observed in genotype NDA-1. 10 leaf proteins and 11 seed proteins have been observed in genotype NDA-2.

It has been observed that genotype BAHAR showed 11 leaf proteins and 12 seed proteins. The leaves of NDA-14-6 showed 12 protein bands, while seeds have 17 bands. Genotype NDA-13-6 showed 9 protein bands in leaves and 17 protein bands in seeds. In case of genotype NDA-3, 8 leaf protein and 16 seed protein have been observed. 10 leaf protein and 16 seed protein have been observed on the polyacrylamide gel in case of UPAS-120. Genotype MAL-6 showed 8 protein bands in leaves and 14 bands in seeds. The leaves of MAL-13 showed 8 protein bands while seeds have 17 bands. *Cajanus scarabaeoideis* (wild genotype) showed 10 protein bands in leaf and 20 protein bands in seeds. It is valuable to emphasize the difference between electrophoretic mobility of protein fractions obtain from two sources have greater import for taxonomic purpose than the similarities of mobility. The possibility of two dissimilar proteins having identical electrophoretic mobility is known (Hayward *et al.*, 1970), yet the assumption is made that bands derive from two different

accessions that migrate the same distance in polyacrylamide gel are considered to be produced by gene(s) common to both accessions.

In leaf at molecular range low than 10 kDa single band was observed in all genotype except in MAL-6 and wild species and in the seed a maximum of 6 bands observed in NDA-13-6, MAL-13 and wild species followed by five bands observed in NDA-1, NDA-14-6, NDA-3 and UPAS-120. Three bands observed in the genotype NDA-3 followed by BAHAR and MAL-6 (four bands).

At molecular range 10 to 20 kDa in leaf single bands have been observed in NDA-2, BAHAR, NDA-3, UPAS-120 and In MAL-6. Rest of all genotype showed 2 bands while wild species contain one more protein which is completely absent in the cultivated genotype. In the seed a NDA-1 have three protein bands while rest of all genotype showed two polypeptide bands. Wild species have one more bands (total 4 bands) that was absent in cultivated genotypes.

Single protein were observed in leaf of all pigeonpea genotype at molecular range between 20 to 25 kDa and between 25 to 50 kDa. While in seed 2 bands observed in all genotype except NDA-2 and BAHAR showed single protein bands between 20 -25 kDa. At 25 to 50 kDa range single bands observed in NDA-2, BAHAR and in MAL-6. Only NDA-14-6 and wild genotype have been showed three protein bands while rest genotype showed 2 bands on polypeptide gel.

In leaf, all pigeonpea genotype showed two protein bands on gel except NDA-1, MAL-6 and MAL-13 showed single protein bands at molecular weight 50-75 kDa while in seed protein gel, all the genotype have commonly presence of single protein bands falling in this

range.

At molecular range between 75 to 100 kDa two polypeptide bands observed in genotype NDA-1, NDA-2 BAHAR, NDA-14-6 and wild genotype while rest genotype have only single protein bands in leaves.

However seeds contained only single protein bands in all genotype, only wild species contain one additional band falling in this range. The leaves of NDA-1, BAHAR, NDA-

14-6, UPAS-120 and MAL-6 showed two protein bands and NDA-2 have single band on polyacrylamide gel while rest genotypes have no protein bands with molecular weight between 100 to 150 kDa. In seed single bands observed in all genotypes which are absent in wild species. At molecular weight between 150 to 250 single polypeptides appear to be common in the leaves as well as seeds (only absent in wild genotype in seed).

Table.1 Number of protein bands in leaf sample with their corresponding gel in different molecular weight ranges

Protein range (KDa)	No. of bands present in leaf sample									
	NDA-1	NDA-2	BAHAR	NDA-14-6	NDA-13-6	NDA-3	UPAS-120	MAL-6	MAL-13	Wild
<10	1	1	1	1	1	1	1	-	1	-
10< 20	2	1	1	2	2	1	1	1	2	3
20<25	1	1	1	1	1	1	1	1	1	1
25<50	1	1	1	1	1	1	1	1	1	1
50<75	1	2	2	2	2	2	2	1	1	2
75<100	2	2	2	2	1	1	1	1	1	2
100<150	2	1	2	2	-	-	2	2	-	-
150<250	1	1	1	1	1	1	1	1	1	1
>250	-	-	-	-	-	-	-	-	-	-
Total bands	11	10	11	12	9	8	10	8	8	10

Table.2 Number of protein bands in seed sample with their corresponding gel in different molecular weight ranges

Protein range (KDa)	No. of bands present in seed sample									
	NDA-1	NDA-2	BAHAR	NDA-14-6	NDA-13-6	NDA-3	UPAS-120	MAL-6	MAL-13	Wild
<10	5	3	4	5	6	5	5	4	6	6
10< 20	3	2	2	2	2	2	2	2	2	4
20<25	2	1	1	2	2	2	2	2	2	2
25<50	2	1	1	3	2	2	2	1	2	3
50<75	1	1	1	1	1	1	1	1	1	1
75<100	1	1	1	1	1	1	1	1	1	2
100<150	2	1	1	2	2	2	2	2	2	2
150<250	1	1	1	1	1	1	1	1	1	-
>250	-	-	-	-	-	-	-	-	-	-
Total bands	17	11	12	17	17	16	16	14	17	20

It has been predicted that globulins represent about 61% of pigeonpea total seed proteins (Krishnan *et al.*, 1977). Protease Inhibitors are generally present at high concentration in seeds. It is also accumulated in vegetative tissue in response to the attack of insects and pathogens (Ryan *et al.*, 1990).

The result of the electrophoretic banding patterns from the leaves and seeds of pigeonpea genotypes reveals some characteristics that could be used for recognizing the genotypes having stress related proteins. Similarities and differences observed in this work agreed with the studies of Odeny (2007); Flower and Ludlow (1987) and Agbolade *et al.*, (2013) who employed comparative electrophoretic protein banding pattern of different species and accession in establishing relation among various taxa.

Pigeonpea is well known for its high nutritive value and uses by a large number of vegetarian population that depends on it. From the protein profile of pigeonpea genotype it is clear that most of the proteins are common in all genotype we have studied and some proteins are unique to a particular genotype. These proteins found in leaves and seeds of pigeonpea require more investigation to exploit the improvement of this crop from major diseases and insect pests as well as improving nutritive value. Certain proteins found in wild species may be useful for improving of cultivars against disease and major insect pests. This data can be used for understanding the close relationship between the cultivated and wild genotype and better understanding of future research.

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