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Analysis of seed storage protein pattern: a method for studying genetic variation and diversity among *vigna* genotypes

S. K. Tripathy, Suchinnata S. Sardar and P. K. Mishra

S.K. Sinha Molecular Lab., Plant Breeding and Genetics, College of Agriculture, OUAT, Bhubaneswar 751 003

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Abstract

A method based in protein gel electrophoresis was employed in order to improve the analysis of genetic relationships among populations of *Vigna.* It has been used to estimate genetic divergence among 34 improved varieties, five local land races, one wild progenitor of mungbean (*Vigna sublobata*) and a land race of urdbean.In total, 20 polypeptide bands were resolved for seed storage proteins (albumin and globulin) by SDS-PAGE. The electrophoregrams revealed 11 and nine polypeptide bands for albumin and globulin respectively that have exhibited an array of polymorphism both in quality and quantity of bands. The varieties, C. No. 3 and C. No. 36 had similar protein type for albumin and globulin seed protein fraction, but differed in thickness of bands. Mayurbhanj local (an urdbean local cultivar) and TCR 213 (a wild progenitor of mungbean) had absence of a globulin band GL9 (27.5kd) and GL8 (30.2kd) respectively, but such bands were present in all other accessions leading to serve as molecular marker(s) for their identification. Mutants of mungbean differed in polypeptide banding pattern as compared to their parents indicating the mutation of genes in multigene families for seed storage protein expression. Cluster analysis revealed high genetic diversity of Keonjhar local, Pant M-5, OUM 75-1 and Mayurbhanj local(urdbean) from rest of the genotypes. The results obtained support the idea that seed storage protein analysis can be successfully applied to phylogenetic analysis of *Vigna* genotypes.

Key words: Genetic variation, seed storage protein, SDS-PAGE, protein finger printing, mungbean

Introduction

Mungbean is a major pulse crop of India grown particularly in Orissa and serves as an important source of protein in human diet. It has high vit.B content and possesses high degree of digestibility among legumes. Protein content of mungbean ranges from 17.2 to 29.9% (w/w) in seeds with an average of 22.8%. The majority of proteins in seeds are present in the form of stable storage reserves of amino acids that provides energy and nutrients for germination and seedling growth. Seed storage proteins in mungbean mainly consists of globulin (70%), albumin (15-20%) and traces of prolamin [1]. Hence, mungbean could serve as an ideal test crop for study of genetic variation and characterization of genotypes based on SDS-PAGE of seed storage proteins based on globulin and albumin. The nature and extent of inter and intra-specific variation in protein profile provides a clue for improvement of protein quality. However, no significant breakthrough has been achieved for amelioration of protein quality in mungbean. Thus, there is an urgent need to breed suitable genotypes with desirable traits for increasing crop production as well as quality of produce. In this pursuit, a set of mungbean germplasm lines were tested in the field for yield performance and analyzed for seed proteins (albumin and globulin) through SDS-PAGE to assess the status of the genotypes for protein quality and molecular characterization.

Materials and methods

The experimental materials comprised of 41 genotypes including 34 improved varieties, five local land races of Orissa, one wild progenitor of mungbean (*Vigna sublobata*) and a land race of urdbean. Albumin and globulin seed storage proteins were extracted with pre-chilled distilled water and 0.5M NaCl respectively, denatured with an equal volume of cracking buffer (0.125M Tris HCl pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.1% bromophenol blue) at 80^{o}C in hot water bath. Seed proteins were analyzed through

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vertical slab gel (12.5% polyacrylamide gel) SDS-PAGE [2] with minor modifications running at constant current of 60mA (2.5mA per lane for two gels run each time) for four hours. Reproducibility was confirmed by minimum of two repeats of each run of gel electrophoresis under similar electrophoretic conditions. After electrophoresis, gels were stained with 0.125% w/v Coomassie brilliant blue R 250, 50% v/v methanol, 10% v/v glacial acetic acid for four hours with intermittent shaking followed by distaining overnight in 50% methanol and 10% Acetic acid; and finally several washings with 5% methanol and 7% acetic acid. The molecular weights of the dissociated polypeptides were determined by using molecular weight marker with known molecular weights i.e, Bovine plasma albumin (66kd), egg albumin (45kd), glyceraldehydes-3-phosphate dehydrogenase (34.7kd) and bovine pancreas tripsinogen (24kd).

Results and discussion

SDS-PAGE of seed proteins generate different profiles composed of several polypeptide subunits which migrates in the gel according to their molecular weights. The number of such subunits indicates the number of multigene families involved [3]. Mutation in these gene families or their regulatory regions leads to deletion of some or all the genes or production of new alleles which form the basis of variation in the polypeptide banding patterns involving presence or absence of bands. In addition, intensity of polymorphism could provide information in shutting of some members of the multigene family that could produce fewer copies in the genotype exhibiting faint bands. Electrophoregrams of a fairly large number of genotypes may also reveal distinct allelism for gene families comprising two molecular variants of a polypeptide subunit under monogenic control. Such polymorphic polypeptides with varying molecular weights are considered as polypeptide markers. Electrophoretic patterns of storage seed protein could be used for verification of varietal identity [4].

Seed storage proteins contain various protein fractions including globulin, albumin, glutelin, prolamin, arcelin and lectin which differ in their solubility.. Since,albumin is water soluble and globulin is salt (sodium chloride) soluble, polypeptide banding pattern has been generated for these protein fractions singly or in combination. The polymorphism of polypeptide markers are *in vogue* used for characterization and categorization of genotypes in addition to its use in hybrid selection, marker assisted selection, elucidation of genetic control of protein expression, linkage of polypeptide bands, stability of polypeptide banding patterns, genome homology, centre of genetic diversity and evolutionary pathways.

Electrophoresis results for albumin and globulin (accounting to 85-90% of seed storage proteins in mungbean) protein fractions revealed 11 and nine polypeptide bands respectively with different molecular weights (Fig. 1). The albumin profile included bands, AL1 to AL11 with molecular weights 94.4, 85.1, 74.1, 67.2, 60.2, 53.2, 50.2, 38.9, 32.0, 28.5, 18.8kd. Globulin profile is a bit smaller which included bands, GL1 to GL9 with molecular weights 85.9, 81.2, 60.5, 53.7, 50.7, 38.2, 35.1, 30.2, 27.5kd, respectively.

Polymorphism of polypeptide bands were revealed in terms of presence (+)/ absence (–) of bands as well as thickness of bands at appropriate molecular weight positions in the electrophoregrams. The thickness of bands was given due importance to reveal degree of quantitative variation in polypeptides. Four distinct major bands each of albumin (AL3: 74.1kd, AL6: 53.2kd, AL9: 32.0kd, and AL11: 18.8kd) and globulin (GL4: 53.7kd, GL7: 35.1kd, GL8: 30.2kd and GL9:27.5kd) were observed in the electrophoregrams. The present set of genotypes did not reveal any polymorphism for polypeptide band AL9 (32.0kd) and GL4 (53.7kd) as these major bands were present in all accessions. However, thickness of these bands varied in six genotypes. The polypeptide band GL4 (53.7kd) was thick in all genotypes except var. Sujata and Banakhandi local indicating low concentration of polypeptide in the protein band. Similarly, var. MGG 489, TM 98-55, Khadabhanga local-A, Mayurbhanj local (urdbean) exhibited faint band for AL9 (32.0kd) compared to rest of the genotypes which revealed a thick molecular print for such polypeptide band. Tomooka *et al*. [5] recognized four albumin and two globulin polymorphic bands in mungbean. However, 48 polymorphic subunit bands could be discriminated from seed protein electrophoretic profiles of five species of *Vigna* and seven species in its related genera [6].

Comparing polypeptide banding patterns of albumin between var. C. No.3 and C. No. 36, between Dhauli and Hum-3 and between OUM 75-1 and T2-1 revealed 100% homology and hence, could be characterized by a common specific protein type. A characteristic polypeptide banding pattern of globulin storage protein fraction was observed in the genotypes e.g., C.No.3, C. No. 36, Nayagarh local-A and ML 729 which were categorized into one protein type. Similarly, COGG 912 and Dhauli; OGG 57 and T 32-2; RCM 6

and Khadabhanga local; and Nandika local and Pant M-4 exhibited same banding pattern or protein type. Naik [7] categorized 37 genotypes of mungbean into four protein types but no genotype-specific polypeptide finger print was recognized in any of the test genotypes. Varietal categorization based on electrophoretic banding pattern of seed storage proteins has been also reported in several other legumes e.g., frenchbean [8], urdbean [9], cowpea [10], Chickpea [11] and Azuki bean (*Vigna angularis*) [12].

Considering polypeptide banding pattern pooled over albumin and globulin, Var. C.No.3 and C.No.36 had similar protein type, but differed in intensity of globulin bands. Further, Dhauli and Hum-3 were of same protein type for albumin, but differed in globulin polypeptide banding pattern. The potential power of electrophoresis techniques for determining genetic variation in crop germplasm have been demonstrated for seed storage proteins and isozymes [13, 14].

Genotype OUM 75-1 and Mayurbhanj local (urdbean) revealed absence of globulin band GL9 (27.5kd), but it was present in all other accessions. Similarly, globulin band GL8 (30.2kd) was present in all genotypes except in the wild accession TCR 213. Absence of these bands could serve as molecular marker(s) for identification of urdbean and wild accession of mungbean. Specific polypeptide bands have been reported to discriminate *Vigna radiata* from *Vigna mungo* [15]. Absence of few polypeptide bands have been observed in wild genotypes of *Phaseolus* and *Vigna* species [16] and *Cicer* [17] compared to some cultivated varieties. This could be due to the fact that the cultivated varieties could have acquired new protein components either in the course of domestication (evolution) or through extensive crosses in the process of genetic improvement in different breeding programmes.

Presence of 17 polypeptide bands (out of 20 bands scored) in variety Pant M-4 and Nandika local were observed. Similarly, genotype TM 98-55 and T 32-2-3 revealed altogether 16 bands. Hence, such accessions may be considered as superior in seed protein quality provided the polypeptides revealed are not associated with antinutritional activity. These genotypes differed in protein type and may serve as excellent breeding material for further crop improvement in protein quality and quantity *per se.*

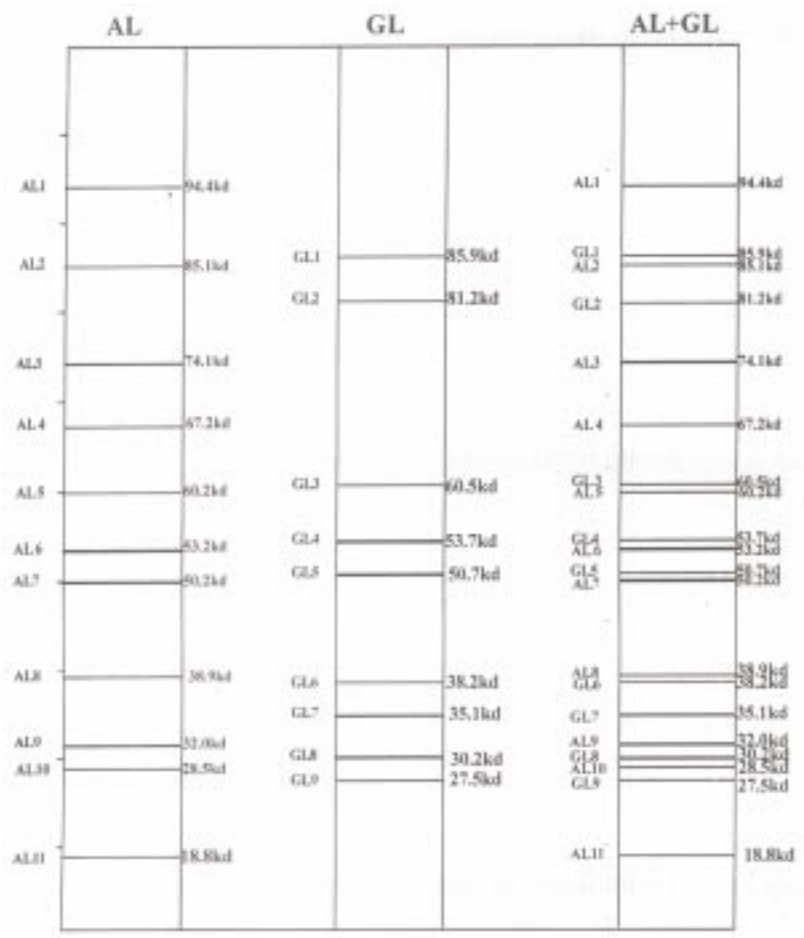

Fig. 1. Seed storage protein fraction profile (AL : albumin, GL : globulin and AL+GL : combined albumin and globulin

Polypeptide banding pattern of mutants e.g. T 2-1(derived through gamma irradiation), OUM-7, OUM 11-5, OUM 52-3, OUM 75-1 (all derived through EMS treatment) differed from their parent variety “Dhauli” indicating mutation of genes in multigene families for seed storage protein expression. The mutants of Khurda local e.g. T 32-2 and T 32-2-3 (sister lines derived through combined gamma ray and EMS treatment) were similar in plant height (31 cm), days to 50% flowering (34 days) and yield performance (3.2g per plant), but the former differed morphologically by having lobed leaf; and at molecular level by absence of bands AL1 (94.4kd) and AL8 (38.9kd). Thus, such bands may serve as molecular marker for identification of genotypes derived from the above ancestry.

Genetic variation in germplasm has an important role in identification of desirable varieties. Polymorphism in electrophoretic banding pattern of seed storage proteins is associated with the genetic background of proteins and thus, could be used to certify the genetic make up. Further, the genetic variation studied in the present set of material could serve as the source of genetic diversity. The erstwhile mentioned superior local land race Nandika local revealed absence of only 3 bands e.g. AL1 (94.4kd), AL2(85.9kd) and AL7 (50.2kd) out of 20 bands scored, but these bands were conspicuous in genotype T2-1. Combining these genotypes through hybridization would lead to possibility of isolation of qualitatively superior segregants showing a protein profile with presence of all the 20 polypeptide bands. A choice of few other genotype combinations can also be made basing on complementary banding pattern for further genetic improvement of protein quality. Besides, the protein finger printing developed in the present investigation would serve as a molecular tool for identification and characterization of promising genotypes.

The present set of *Vigna* genotypes were distributed over seven divergent clusters at comparatively lower phenon level (Fig. 2). At finer level (SI=1.00), the genetic variation was virtually dissociated into single variety clusters except only one cluster which

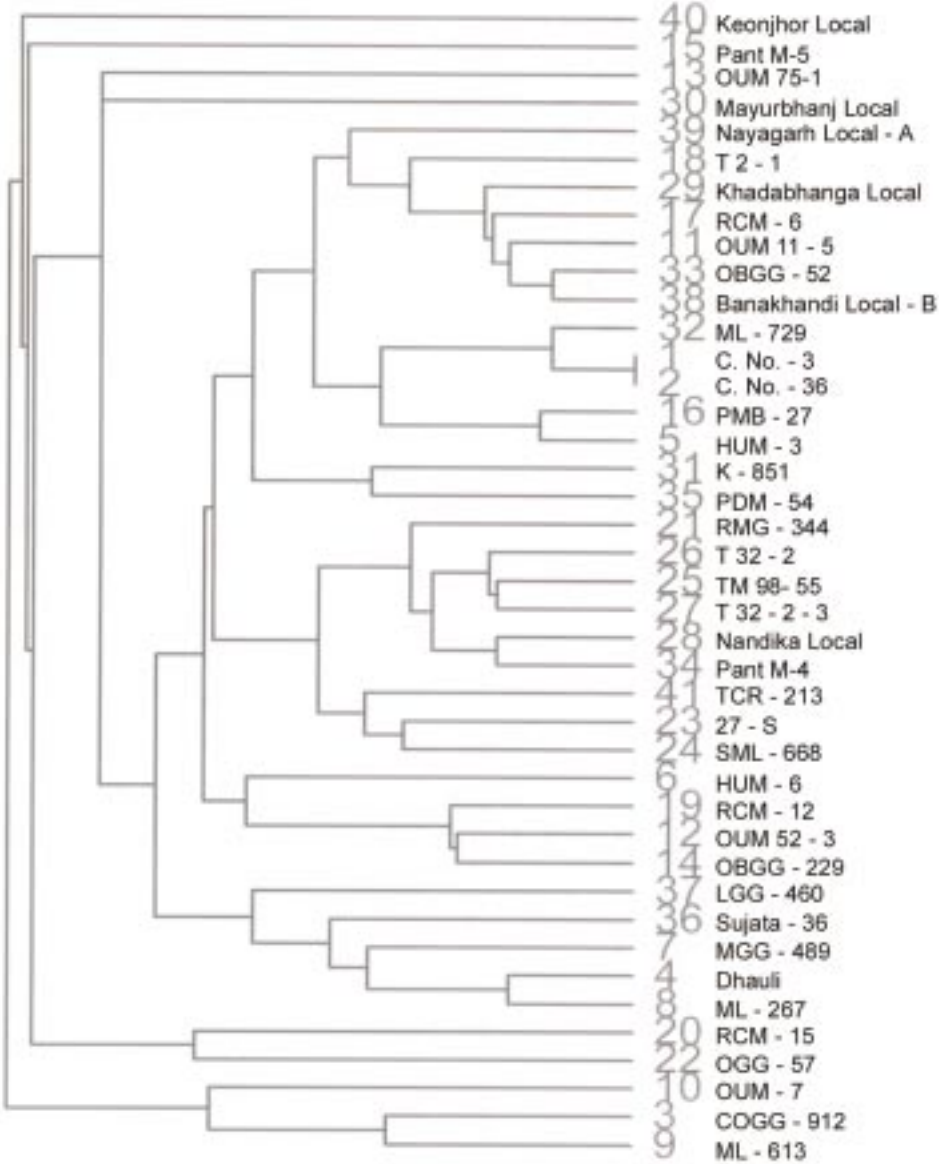

Fig. 2. Dendrogram showing genetic diversity of genotypes based on combined albumin and globulin polypeptide banding pattern

contained two genotypes e.g., C. No. 3 and C. No. 36. This envisaged large genetic diversity and genotype-specific protein types among the test genotypes. Gallab *et al*. [18] delineated the genetic relationship among a set of mungbean genotypes from a dendrogram which showed three different genetic clusters. Electrophoretic assessment of 581 local strains of mungbean collected from different regions of Asia, revealed eight protein types on the basis of combination of four albumin and two globulin bands[5]. However, Roy [19] observed very restricted level of polymorphism among some Indian cultivars and wild accessions of mungbean.

In this pursuit, few genotypes e.g., ML 613, COGG 912 and OUM 7 were clubbed in a separate cluster at lower phenon level. Besides, each of the genotypes e.g, keonjhar local, Pant M-5, OUM 75-1, Mayurbhanj local (urdbean), RCM 15, OGG 57 had also maintained their genotypic identity. The wild accession being placed in a separate small cluster at moderate phenon level, exhibited appreciable genetic difference in polypeptide banding pattern with other genotypes. However, more polymorphic bands needed to be scored to reveal its distinctiveness at lower phenon level. Thus, all these above genotypes are considered to be quite divergent from rest of the genotypes and could serve as valuable breeding material for improvement in protein quality and quantity *per se.*

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