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Seed storage protein profiling: A method to reveal genetic variation in local land races and wild forms of mungbean

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ABSTRACT

The SDS-PAGE of 81 land races, four improved varieties and four wild forms of mungbean revealed 11 scorable polypeptide bands each of albumin and globulin seed storage protein fraction with molecular weights ranging from 6.5 to 100.2kD and 18.2 to 85.9kD respectively. Barring two monomorphic bands at 45.0kD (albumin) and 53.7kD (globulin), rest of the polypeptide bands had shown polymorphism to the extent of 90.09% among the test genotypes. Sikri local and Sidheswar local could be identified owing to unique presence of globulin bands at mol. wt position 27.5kD and 18.8kD respectively. One albumin band at 48.2kD and two globulin bands at 85.9 and 18.8kD were visualized in TCR 20(wild accession) as well as in almost all local land races and cultivated check varieties, but such bands were specifically absent in all other viny wild forms. The clustering pattern revealed nine genetic clusters. Each of the viny wild forms (TCR 213, TCR 243 and TCR 192) were clearly isolated into three initial single clusters followed by two divergent phyllogenetic clusters of land races and a single cluster containing wild accession TCR 20. Thus, TCR 20 seems to have more proximity to cultivated forms than other wild accessions. Besides, a local land race, Balarampur Keonjhar local being morphologically distinct (vigorous growth, broad leaves, long pod, bold seeds) from other land races; was isolated to form single cluster. Further, it was revealed that genotypes in the same cluster based on seed protein profiling have some common agronomic performance and such clusters with unique morpho-economic traits could be identified for genetic improvement. TCR 20 and Balarampur Keonjhar local being highly divergent with high yield potential have been sorted out as valuable material for hybridization with local land races and ruling check varieties of mungbean.

Key words: Seed storage protein profiling, genetic variation, local land races, wild forms, mungbean(*Vigna radiata* L. Wilczek).

INTRODUCTION

Land races of crop plants are the gift of nature which constitutes the vital component of germplasm resources. Repeated use of selected genotypes in various breeding programs has resulted genetic erosion in gene pool. This becomes a major concern for increased vulnerability of crop plants to biotic and abiotic stresses (Smith *et al.*, 2004; Reif *et al.*, 2010). Climate change is inevitable. Hence, the wild gene pool and local land races; must be conserved and these should be allowed to co-evolve with

nature. India and Odisha in particular has a rich heritage of local land races of mungbean which have been evolved over several years and undergone a process of selection by farmers to suit local cultivation practices and needs. This resulted in a large number of land races adapted to a wide range of agro-climatic conditions (Panda, 2013). In general, genetic variability in available germplasm of mungbean is limited (Mohapatra, 2011). The existing varieties bred so far have low yield potential

Table 1. List of local land races of mungbean.

Sl. No.	Genotype	Seed coat colour	Sl. No	Genotype	Seed coat colour
1.	Banapur local A	Reddish brown	46.	Kendrapara local C	Brown
2.	Banapur local B	Green	47.	Kendrapara local D	Dull brown
3.	Jagatsinghpur local A	Reddish brown	48.	Phulbani local –A	Shiny greenish black
4.	Jagatsinghpur local B	Shiny greenish black	49.	Phulbani local –B	Shiny green
5.	Berhampur local	Reddish brown	50.	Phulbani local –D	Shiny brown
6.	Bhawanipatna local 2A	Reddish brown	51.	Hinjli local –A	Dull green
7.	Bhawanipatna local 2B	Dull black	52.	Hinjli local –B	Reddish brown
8.	Bawanipatna local 2C	Dull green	53.	Nayagarh local-A	Reddish brown
9.	Bhawanipatna local 1	Brown	54.	Nayagarh local-B	Dull green
10.	kopergaon local	Shiny yellowish green	55.	Nayagarh local-C	Brownish green
11.	Sashna ambagaon local A	Reddish brown	56.	Nayagarh local-D	Dull black
12.	Sashna Ambagaon local-B	Dull green	57.	kalamunga 1C	Dull black
13.	Paralakhmundi local	Shiny yellowish green	58.	Deogaon local	Glossy black
14.	Sidheswar local	Dull green	59.	Jyoti (Hyb 4-3)(check)	Green
15.	Sikri Local	Brown	60.	Nandika local	Dull green
16.	Makarjhola local –A	Shiny green	61.	Jharsuguda local-A	Dull green
17.	Sharagada local	Dull brown	62.	Jharsuguda local-B	Greenish black
18.	Purusattam local	Shiny green	63.	Jharsuguda local-C	Brown
19.	Anandapur local-A	Shiny black	64.	Jharsuguda local-D	Dull black
20.	Anandapur local-B	Dull green	65.	Dhenkanal local	Dull green
21.	Dayapalli local	Shiny brownish green	66.	Charapalli local	Brownish green
22.	Ratila local	Green	67.	Raipur local	Green
23.	Khadabhangalocal-A	Shiny green	68.	Kamakshya local A	Dull greenish brown
24.	Khadabhangalocal-B	Dull black	69.	Kamakshya local B	Glossy greenish black
25.	Samarjhola local	Shiny green	70.	kamakshya local C	Dull green
26.	Mayurbhanj local A	Black	71.	Ambagaon local A	Dull black
27.	Mayurbhanj local B	Green	72.	Ambagaon local B	Dull green
28.	Mayurbhanj local C	Dull green	73.	Mahimunda local	Black mosaic
29.	Banakhandi local A	Black	74.	Jhaimung Kharsel	Dull greenish brown
30.	Banakhandi local B	Green	75.	Jhaimung Kharsel sel.1	Dull greenish mosaic
31.	Sudhasarangilocal-A	Dull green	76.	Jhaimung Kharsel sel.2	Dull green
32.	Sudhasarangilocal-B	Dull green	77.	Jhaimung Kharsel Sel.3	Dull black
33.	Sudhasarangilocal-C	Dull black	78.	Jhaimung Kharsel Sel.4	Dull black
34.	Gope local-A	Brown	79.	Jhaimung Kharsel Sel.5	Brownish grey
3.5	Gope local-B	Green	80.	Jhaimung Kharsel sel.6	Glossy green
36.	Sheragarh local	Dull reddish brown	81.	Balarampur Kjr. local	Brownish green
37.	Athamallik local A	Glossy greenish mosaic	82.	Nipania munga	Dull green
38.	Athamallik local B	Glossy mosaic black	83.	TCR 20	Reddish brown
39.	Kalamunga -1A	Glossy black	84.	TCR 192	Brownish black
40.	Kalamunga -1B	Glossy mosaic green	85.	TCR 213	Brownish black
41.	Tigiria local B	Dull green	86.	TCR 243	Broownish black
42.	Tigiria local C	Blackish brown	87.	Pusa Vishal (check)	Brownish green
43.	Kodala local A	Shiny yellowish green	88.	Durga (best Check)	Green
44.	Kodala local B	Reddish brown	89.	TARM 1(check)	Green
45.	Kendrapara local B	Dull black	-	-	-

and are mostly vulnerable to cold stress and diseases. The paradox is that in order to enable to develop truly revolutionary new cultivars of tomorrow, plant breeders will need to have access to the wealth of genes which exist now only in exotic and /or local genetic backgrounds (Cole *et al.*, 2000) including wild related species. In this context, characterization of genetic resources is a vital step in generating new desirable plant types that help in increasing crop production as well as quality of the

produce. However, no systematic work has been done on characterization and evaluation of available local germplasm in this crop.

The scope of genetic improvement necessitates a broader range of genetic diversity comprising a set of genes carried by different genotypes of a species. The knowledge of genetic diversity is useful tool for efficient sampling and utilization of germplasm either by identifying and/or eliminating duplicates in the genetic

stock ultimately resulting in the development of core collection of genotypes (prebreeding). Morphological characterization of germplasm collections is an important step in this regard. Many often biochemical (Tripathy *et al.*, 2010a,b,c) and molecular markers (Lavanya *et al.*, 2008) are sought owing to paucity of specific morphological traits or descriptors for categorization of genotypes. Besides, assessment of genetic diversity based on morphological characters is often less efficient and biased owing to high environmental interaction. In the present investigation, a set of local land races including a few wild accessions of mungbean (*Vigna radiata* var. *sublobata*) was characterized for varietal identification and genetic diversity based on morphological traits and seed storage protein fingerprinting.

MATERIALS AND METHODS

A set of 89 popularly adapted *Vigna* local land races (Table 1) including four standard ruling varieties (Jyoti, Durga, Pusa Vishal and TARM 1) and four wild accessions of *Vigna radiata* var. *sublobata* (TCR 20, TCR 213, TCR 192 and TCR 243 received from NBPGR, New Delhi) were laid out in augmented design with five blocks and 17 test genotypes plus four promising standard checks in each block to assess their comparative performance. The crop was raised following recommended package of practices. Observations on all morpho-economic traits including seedling vigour (1-5 scale), yield and yield component characters, tolerance to cold stress and extent of powdery mildew and *Cercospora* leaf spot diseases (0-9 scale) were recorded in Rabi 2012-13. Similar field experiment comprising the same set of test genotypes was necessitated for proper assessment of drought and YMV (yellow mosaic virus disease) tolerance (0-9 scale) during Summer 2014. Actual values of agronomic data were adjusted as per the standard procedure of augmented design to eliminate block effects from actual data recorded.

Block effect r_j for each block = $B_j - M$ where, B_j = mean of all checks in the j th block, M = Grand mean of all checks, Adjusted data = Actual data - block effect.

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°C in hot water bath. Seed proteins were analysed through vertical slab gel (12.5% polyacrylamide gel) SDS-PAGE (Laemmli, 1970) 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, and 10% v/v glacial acetic acid for four hours with intermittent shaking followed by destaining overnight in 50% methanol and 10% glacial acetic acid; and finally, several washings with 5% methanol and 7% glacial 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 trypsinogen (24kD).

The binary data matrix of the presence (1)/absence (0) of polypeptide bands were analysed following NTSYS software programme to estimate Jaccard's similarity coefficient (Jaccard, 1908) values. The dendrogram was constructed using Unweighted Paired Group Method with Arithmetic Averages (UPGMA)-phenograms (Sokal and Michener, 1958) employing Sequential Agglomerative Hierarchic and Non-overlapping clustering (SAHN).

RESULTS AND DISCUSSION

The germplasm is a vital source of new plant types having desirable traits. It helps in increasing crop quality and production. Farmers and the end use consumers of the locality prefer to cultivate mungbean of their own choice. Breeders take such local preferences into account while developing improved varieties of mungbean. High yielding varieties are not always preferred by the farmers. Sometimes, local preferences like green, shiny, bold seeded genotypes dominate the market to fetch good prices.

Several biochemical and molecular marker loci are currently available for elucidation of genetic variation even between closely related germplasm stocks. However, no single method is adequate for assessing genetic variation as each of these differ in terms of sample variation at different level and difference in their power of genetic resolution, as well as in the quality of information content (Sultana and Ghafoor, 2008). SDS-PAGE of seed storage proteins is, however, considered as practical, cost effective and reliable method as it is largely independent of environmental fluctuations (Iqbal *et al.*, 2005; Dutta and Malik, 2012) as compared to soluble protein and isozyme analysis.

Total seed storage protein profiling through SDS-PAGE for differentiating mungbean genotypes is well established (Roy 2003, Gallab *et al.*, 2007 and Tripathy *et al.*, 2010a,b,c). The SDS-PAGE revealed 11 scorable polypeptide bands each of albumin and globulin seed storage protein fraction with molecular weights ranging from 6.5 to 100.2kD (AL1-AL11) and 18.2 to 85.9kD (GL1-GL11) respectively (data not shown). This envisaged that at least 22 multi-gene families are involved in seed storage protein expression in mungbean land races. Out

of these polypeptide bands, a thick albumin band with mol. wt. 45.0kD and a dense globulin band at 53.7kD mol. wt position were found to be monomorphic and rest of the bands had shown polymorphism to the extent of 90.09% among the test genotypes. As a whole, the resulting data matrix of the presence and absence of bands resolved a total of 620 albumin and 547 globulin polymorphic polypeptide bands out of total 709 albumin and 636 globulin bands over all the 89 test genotypes used in the study which reveals 86.76% polymorphism.

Genetic variation in relation to number of polypeptide bands in the electrophoregram ranged from 3 to 11 albumin and 2-11 globulin bands depending on the genotypes tested. Samarjhola local, Mayurbhanj local C and Kalamunga 1A had shown only three albumin bands each in the electrophoregram. Similarly, Sidheswar local, Sikri local and the wild accession TCR 243 revealed only two globulin bands; and Jagatsinghpur local A, Jagatsinghpur local B, Berhampur local and Kodala local A recorded only three globulin bands. In contrast, Jagatsinghpur local A, Kodala local A, Kodala local B and Kamakhya local A had shown all 11 albumin bands while, Kendrapara local D, Nayagarh local C, Jhaimung Kharsel Sel. 4 and Durga recorded all 11 globulin bands. These may be considered superior in protein quality provided the polypeptides revealed are not associated with anti-nutritional activity. A few of these land races e.g., Jagatsinghpur local A, Kodala local A, Kamakhya local A and Jhaimung Kharsel Sel. 4 are reported to be highly rich in protein content (Panda 2013). Hence, these may serve as excellent breeding material for recombination breeding for improvement protein yield and protein quality. Available literature does not reveal such a comparison.

In the present investigation, Sashna Ambagaon local A, Purusattam local, Dhenkanal local and Jhaimung Kharsel Sel.-5 can be identified by absence of band AL3(85.1kD), AL10(12.8kD), GL2(81.2kD) and GL11(18.8kD) respectively out of 11 bands each for albumin and globulin seed storage protein fractions revealed in the electrophoregram. Similarly, Sikri local and Sidheswar local could be identified owing to unique presence of globulin bands at mol. wt position 27.5kD (GL10) and 18.8kD (GL11) respectively. Tomooka *et al.* (1992) analysed 581 genotypes and of mungbean grouped them into eight protein types by combining albumin and globulin polypeptide bands. They could reveal one genotype-specific protein type. Asghar *et al.* (2003) observed 18 polypeptide bands and reported presence/ absence of specific bands in genotypes comprising different protein types. Thakare *et al.* (1988), however, could not able to recover any polymorphism in the vicilin seed protein derived from four cultivars of mungbean.

In contrast, wild Accession TCR--20 revealed band AL5 (48.2kD), GL1 (85.9kD) and GL11 (18.8kD) as well as in almost all local land races and check varieties, but

such bands were specifically absent in all other wild form. Such adaptational changes are hereditary and have direct bearing in total seed protein expression in the resulting cultivated test genotypes as well as TCR 20. Further, this signifies more proximity of TCR 20 with the cultivated land races than the viny wild forms of mungbean (TCR 213, TCR192 and TCR243). Sardar (2011) reported close resemblance of polypeptide banding pattern of TCR 20 with cultivated mungbean than a wild form TCR 213.

The genetic distance between genotypes suggests the relationship between the species and within the members of the same species. The crosses between parents with maximum genetic divergence are generally most responsive for genetic improvement as these can result better transgressive segregants through gene shuffling. However, to utilize such parental accessions with maximum genetic divergence, it is necessary to screen and characterize the available germplasm for the nature and extent of genetic similarity. In the present pursuit, overall similarity index (S.I., data not shown) values ranged from 0.15 (between V28 & V81 and V33 & V85.) to 1.00 (between V49 & V51, V61 & V62, V73 & V75, V73 & V76 and V75& V76). Among the test genotypes, V85 (TCR 213) exhibited least average genetic similarity (S.I. =0.33) followed by V86-TCR 243 (S.I. =0.38), while V45, V68, V67, V63, V42 and V55 revealed very high genetic homology (Av. S.I. =0.65-0.68) with rest of the test genotypes for seed storage protein expression. For instance, the viny wild accession V85 (TCR 213) had high genetic dissimilarity with rest of the land races with highest being with V33-Sudhasarangi local C (S.I. =0.15) to a minimum with V86-TCR 243 (S.I. =0.45). On an average, V85 (TCR 213) showed more than 80% genetic dissimilarity with V16, V40, V32, V24 and V81. Similarly, two wild forms e.g., V84 (TCR 192) and V83 (TCR 20) maintained wide genetic distance from other test genotypes in terms of polypeptide banding pattern. Besides, some of the few combinations e.g. V5 (Berhampur local) with V23 (Khadabhangha local A), V25 (Samarjhola local) and V36 (Sheragarh local); and V15 (Sikri local) with V36 (Sheragarh local) and V33 (Sudhasarangi local C) had high *inter se* genetic distance (S.I. ≤ 0.25). In contrast, V45 (Kendrapara local B) was shown to have high homology (S.I. =0.65 -0.90) with most of the entries except three viny wild forms e.g., V85 (TCR 213), V86 (TCR 243) and V84-TCR 192 (S.I. =0.33-0.46). Besides, some of the specific combinations e.g., V42 (Tigiria local C) with V45 (Kendrapara local B) and V68 (Kamakhya local A); V46 (Kendrapara local C) with V54 (Nayagarh local B) and V68 (Kamakhya local A); and V44 (Kodala local B) with V45 (Kendrapara local B) revealed high genetic similarity (S.I. =0.90) in seed storage polypeptide pattern.

Variation in polypeptide banding pattern have been reported by many workers, such as, Koenig *et al.* (1990) in French bean, Polignano *et al.* (1990) in *Vicia faba*

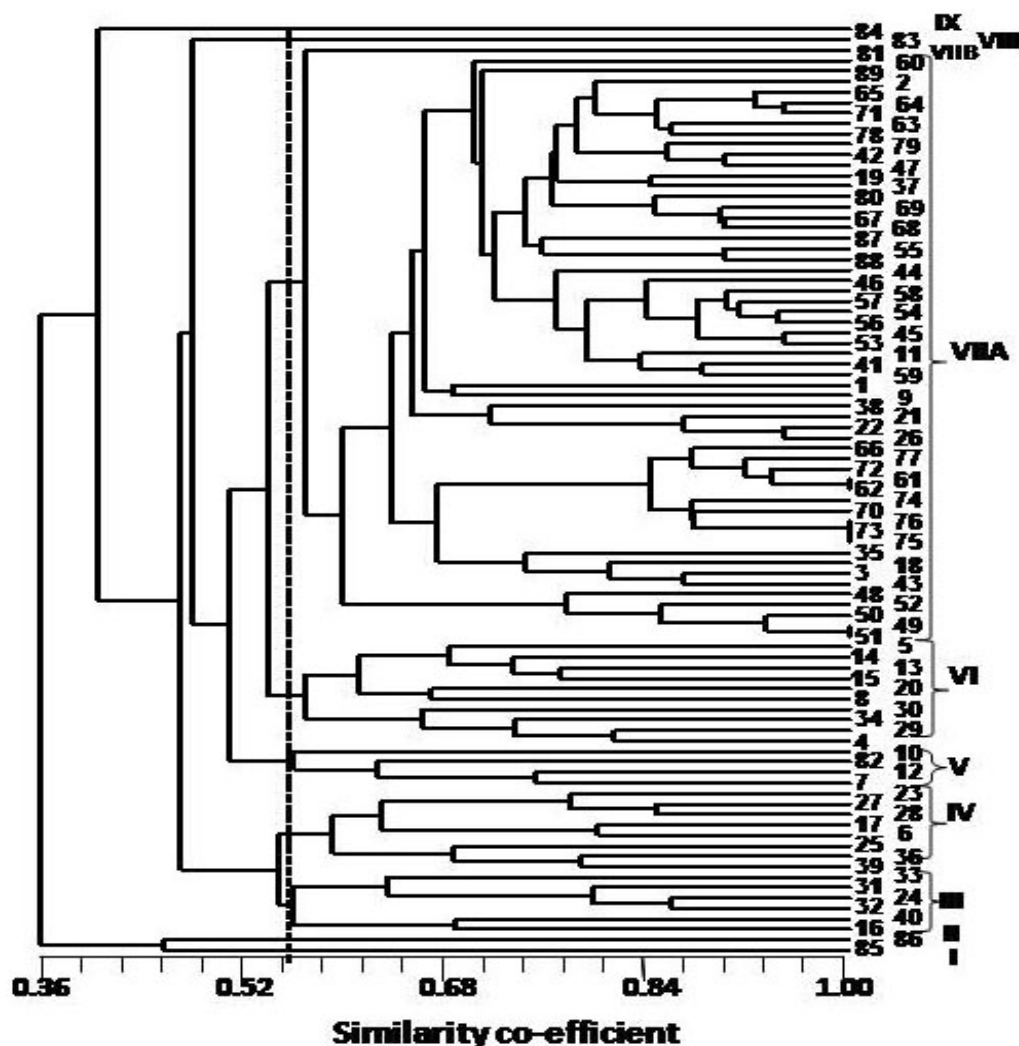


Figure 1. Dendrogram showing genetic diversity of local land races and wild forms of mungbean (marked with Sl. No. as in table 1) through seed storage protein (Albumin and globulin) polypeptide markers.

Ladizinsky and Adler (1975) in chickpea, Thakare *et al.* (1988) and Tripathy *et al.* (2010c) in *Vigna* spp. Thakare *et al.* (1988) reported varying degree of homology in polypeptide banding pattern in *Vigna radiata* var. *sublobata* accessions, *V. radiata* and *V. mungo*. However, isozymic variations have been also used by Sonnate *et al.* (1996) to reveal taxonomic relationship among *Vigna* species. Genetic variation in seed protein profiles has an important role in identification of varieties. The erstwhile mentioned genotype-specific banding pattern observed in the present investigation could serve the purpose.

Cluster analysis is *in vogue* used to study genetic relationship among a large number of accessions which enables planning for use of appropriate gene pool in crop improvement. This enables breeders to combine desirable genes in a single genotype through recombination breeding. Electrophoretic analysis of the seed storage proteins had direct relationship to the

genetic background of the proteins that reveal genetic diversity. Such analysis can be used to certify the genetic makeup of germplasm (Javid *et al.*, 2004; Iqbal *et al.*, 2005). The present investigation identifies the degree of genetic diversity based on seed storage protein profiles in mungbean from various localities. This might be the first attempt to study electrophoretic genetic variation of seed protein in large collection land races and wild forms of mungbean from various localities of India and Odisha in particular. The whole range of combined albumin and globulin based profiles of 89 test genotypes revealed nine clusters at even 56% phenon level (Figure. 1). Ghafoor *et al.* (2002) obtained four clusters in a set of genotypes of *Vigna mungo* and *V. radiata* resembling to *V. mungo* for seed characters. Ghallab *et al.* (2007) observed three different genetic clusters in 10 genotypes of mungbean. They had shown grouping of two genotypes L 3430 and L 2920 into a specific cluster owing to their similar polypeptide banding pattern. Similarly, Tripathy *et al.*

(2010a and 2010b) also reported clustering of few genotypes e.g., Pant M 5 and RCM 15 into a single cluster due to their characteristic polypeptide banding pattern. In the present pursuit, two wild forms of mungbean V85 (TCR 213) and V86 (TCR 243) were separated out and these constitute Cluster –I and Cluster-II. This follows sequential separation of another wild accession V84 (TCR 192) that formed Cluster IX. All these wild forms are morphologically distinct from rest of the test genotypes owing to their viny growth habit with basal branching, unique leaflet shape and solitary flower from nodes. Next to this, V16,V40,V32,V24,V31 &V33 formed Cluster III ; while V39,V36,V25,V6,V17,V28,V27 and V23 were grouped to form Cluster IV followed by isolation of the wild accession TCR 20 (V83) forming cluster VIII. This confirms that TCR 20 is more close to the cultivated forms than viny wild accessions as also reported by Sardar 2011 and Panda (2013). It is worth to note that stem, leaf, and pod characteristics of TCR 20 are alike to all cultivated forms of mungbean and the land races. Rest of the genotypes were sequentially grouped to form Cluster V with four genotypes(V7,V12,V82 and V10), Cluster VI with ten genotypes (V4,V29,V34,V30,V8,V20,V15,V13,V14 and V5), Cluster VIIB containing the land race Balarampur Keonjhar local and a large multivariety Cluster VIIA which contained rest 56 genotypes. The positioning of Balarampur Keonjhar local in the clustering pattern based on seed storage protein seems to be satisfactory as it is morphologically similar to wild accession TCR 20 (late maturity, broad leaves, vigorous growth and erect habit) as well as cultivated land races of mungbean. V49 (Phulbani local B) and V51 (Hinjlil local A); V61 (Jharsuguda local A) and V62 (Jharsuguda local B; and V73 (Mahimunda local), V75 (Jhaimung Kharsel Sel. 1) and V76 (Jhaimung Kharsel Sel. 2) within cluster VIIA could not be distinguished from each other even at 100% phenon level. Each of the above groups of similar genotypes may be designated as duplicates which the farmers were unaware about their origin and cultivate since long in different names.

The grouping of genotypes using three dimensional scaling based on PCA values (Figure.2) was found to be more or less consistent with that of UPGMA analysis. The three dimensional scaling with vectors represented clear grouping of test genotypes. Wild forms e.g.,V85 (TCR 213), V86 (TCR 243), V84 (TCR 192) and V83 (TCR 20) which were initially separated from rest of the test genotypes in case of UPGMA clustering (Figure.1), were also seen to be screened out to diverse extreme positions in PCA analysis. The above overall clustering pattern of wild forms and cultivated genotypes including land races indicates that the genetic variation in seed storage protein might have some relationship with morphological variation. Therefore, an effort was made to note agronomic performance of the test genotypes in

relation to their distribution in the dendrogram clusters using combined albumin and globulin profiles. The order of occurrence of the genotypes in the above seed protein based clusters was used as a reference for arranging performance values of the accessions for 12 agromorphological traits. The cluster mean values of these traits are given in Table 2. For better clarity, the significantly lower cluster mean values for days to 50% flowering and plant height; and for rest of the morpho-economic traits significantly higher cluster mean values compared to the experimental grand mean are marked astrick(*), as such plant types are of special consideration for mungbean genetic improvement. Genotype(s) with significant mean values were identified to assess relation (if any) of genotypic performance for yield and yield attributing traits with seed protein marker based clustering. The cluster-wise mean values allow a simultaneous comparison of several quantitative traits of the test genotypes with reference to the position of each genotype in the dendrogram. It is observed that the genotypes in the same cluster based on protein profiling have some common phenotypic performance and such clusters with unique phenotypic performance could be identified for genetic improvement.

Many workers attempted to integrate molecular data with morphological informations to derive genetic relationship among the genotypes (Betal *et al.*, 2004 and Lavanya *et al.*, 2008). Cluster analysis of both the data bases most often tend to overlap which guarantees successful assessment of inter and intra-specific genetic variation and interpretation for genetic relatedness /distance between the genotypes. Ghafoor *et al.* (2002), Ghafoor *et al.* (2005) in urdbean; and Hassan (2001) and Ghallab *et al.* (2007) in mungbean compared morphological data with seed protein polypeptide banding pattern to elucidate genetic diversity of genotypes. They reported typical agronomic performance of genotypes included in each of the clusters. Polypeptide banding pattern has been also effectively employed by Afiah and Rashed (2000) to isolate M₃ mutants for tolerance to calcareous soil in mungbean.

For Genetic improvement, dwarf plant types with early flowering habit are of special consideration. Cluster III revealed characteristically early flowering habit. Among 6 test genotypes comprising Cluster III; V24 (Khadabhanga local B) and V40 (Kalamunga 1B) came to 50% flowering as early as 30days after sowing. Similarly, cluster V included dwarf plant types with a cluster mean as low as 16.60cm. The local land race V82 (Nipania munga- a drought tolerant land race) and V12 (Sashna Ambagaon local B) included in cluster V had plant height even less than 15.0cm. Seed protein based clustering reflected differentiation of all four wild accessions(V85-TCR 213,V86- TCR 243, V84-TCR 192 and V83- TCR 20) as well as a local land race Balarampur Keonjhar local(V81) into separate single clusters(I, II, IX, VIII and

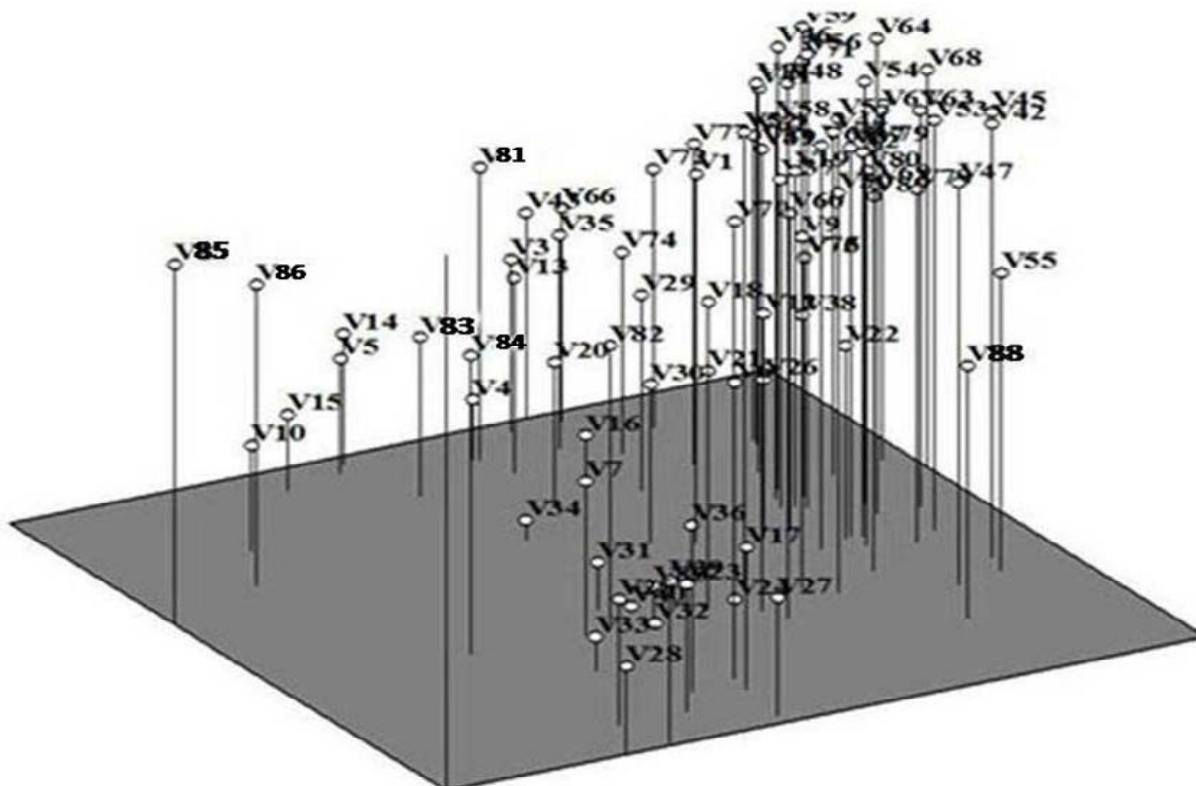


Figure 2. Three dimensional scaling of principal co-ordinates 1, 2 and 3 using seed storage protein markers. (For clarity; Sl. 81- Balarampur local, 83- TCR 20, 84- TCR 192, 85- TCR 213, 86-TCR 243 and 88-Durga (best standard check) are marked bold)

VII B). Each of these genotypes had distinctive morphological characteristics. TCR 213 -a viny wild form, recorded highest plant height (70.74cm.) and significantly late in 50% flowering (59.22days). Besides, it had shown appreciably high tolerance to powdery mildew and cold stress. On the other hand TCR 20 with erect habit and broad leaves was shown to have highest yield potential (7.67g/plant) along with high seedling vigour, resistance to drought and field tolerance to *Cercospora* leaf spot and yellow vein mosaic (YMV) disease. High productivity of TCR 20 could be attributed to late maturity, high pod number and more number of pods per cluster. TCR 192 and TCR 243 with trailing habit yielded significantly higher than grand mean (1.53g/plant). Besides, TCR 192 yielded (3.67g/plant) significantly higher than all local land races except Balarampur Keonjhar local (4.98g/plant) and it was at par (3.23g/plant) with the best standard check (Durga). Balarampur Keonjhar local had shown high yield potential (4.98g/plant) owing to its very long pod with exceptionally bold cowpea like seeds (100 seed wt. =8.86g). Besides, it had rapid growth rate with sturdy stem and large broad leaflets which accounts for high biomass production. Thus, it reveals that each of the seed protein based clusters had some sort of characteristic morphological and agronomic features. This could be due to the fact that some of the genotypes

may have been descended from common ancestry (Karuppanapandian *et al.*, 2006) and subjected to similar selection for agro-economic traits during breeding programme. Ghallab *et al.* (2007) correlated superiority of two mungbean genotypes (L 2520 and L 1720) in seed yield with absence of two bands at around 94.6kd and a presence of a polypeptide band with molecular weight 12.1kd under drought stress condition. Ghafoor *et al.* (2005) identified few QTLs for days to flowering, days to maturity, branches per plant, seeds per pod, 100-seed weight, biological yield and seed yield based on significant correlation of some polypeptide bands with the quantitative traits in urdbean. However, such an interpretation needs critical examination using DNA markers. In the present pursuit, none of the local land races except V81 (Balarampur Keonjhar local) could surpass the yield level of the standard check "Durga" (3.23g/plant). However, few local land races which recorded seed yield significantly more than grand mean (1.53g/plant) were listed as promising. These include V60 (Nandika local), V52 (Hinjli local B), V23 (Khadabhanga local A), V18 (Purusattam local), V28 (Mayurbhanj local C), V7 (Bhawanipatna local 2B), V10 (Kopergaon local) and V12 (Sashna Ambagaon local B) which can also be of immense value with regard to adaptability to local situations.

Table 2. Cluster composition based on AI + GL seed storage protein profiles and cluster mean of test genotypes for morpho-economic traits

Clusters	Genotype SI. No(s).	Seed -ling vigour	Days to 50% flowering	Plant height (cm)	Clust -ers/ plant	No. of pods/ cluster	No. of pods/ plant	Yield /PI. (g)	CLS tolera nce	Powdery mildew tolerance	YMV tole- rance	Drought tole- rance	Cold tole- rance
I (1)	85(TCR 213)	2.50	59.22	70.74	5.67*	1.34	25.52*	1.90	1.42	0.06	2.53	1.94	0.28
II (1)	86 (TCR 243)	2.84*	50.00	56.0	4.46*	3.04	13.80	2.66*	1.24	1.06	2.98	0.32	0.72
III (6)	16,40,32,24,31,33	1.46	32.20*	21.02	3.39*	5.42*	17.26	1.46	5.42	4.19	7.57	7.23	5.78
IV (8)	39,36,25,6,17,28,27,23	1.34	34.95	19.64	2.58	5.61*	15.37	1.68	6.54	3.25	7.59	6.28	5.15
V (4)	7,12,82,10	2.44	36.56	16.66*	3.08	5.46*	11.54	1.91	7.67	5.62	6.46	4.25	7.15
VI (10)	4,29,34,30,8,20,15,13,14,5	1.35	35.72	17.75	2.58	5.47*	13.95	1.33	7.92	2.39	7.78	6.39	8.08
VIIA (56)	Rest of the genotypes	1.64	34.94	19.86	2.81	4.88	13.37	1.28	5.93	3.90	7.20	6.49	6.36
VIIIB(1)	81 (Balarampur Kjr. Local)	5.02*	42.00	28.00	3.80*	4.02	15.2	4.98*	7.77	4.94	8.53	2.94	1.28
VIII(1)	83 (TCR 20)	4.52*	55.22	58.74	6.97*	5.54*	36.82*	7.67*	0.42	0.94	0.53	0.06	1.28
IX(1)	84 (TCR 192)	3.88*	50.00	64.0	5.23*	3.22	16.20	3.67*	0.98	1.02	3.02	0.42	1.28
Range of cluster means		1.34-5.02	34.94-59.22	70.74-16.66	2.58-6.97	1.34-5.61	11.54-36.82	1.28-7.67	0.42-7.92	0.06-5.62	0.53-8.53	0.06-7.23	0.28-8.08
Range of mean values of the test genotypes		5.02-0.5	59.22-30.22	70.74-12.31	6.97-1.52	6.29-1.34	36.82-7.92	7.67-0.24	9.42-0.58	8.94-0.81	9.53-0.03	9.44-0.06	9.28-0.28
Grand Mean		1.72	36.11	21.54	2.95	4.99	14.25	1.53	6.05	3.64	7.09	6.11	6.14
C.D.5%		1.02	2.61	4.73	0.39	0.32	4.10	0.52	1.32	1.74	1.32	1.02	1.41

CLS- Cercospora leaf spot, YMV-yellow vein mosaic virus disease, * - significant at P_{0.05}

Among the cultivated forms, Nipania munga (V82)-a land race had resistance to drought stress while, the best standard check variety Durga withstand cold stress appreciably. With regard to biotic stresses; TARM 1 and Pusa Vishal were noted to be YMV and *Cercospora* leaf spot tolerant respectively. Lower incidence of powdery mildew was noticed in Sashna Ambagaon local A(V11), Nayagarh local A(V53), Kendrapara local D(V47), Tigiria local-C(V42), Dhenkanal local(V65), Charapalli local(V66) and TARM -1 (V89) which scored < 0.19 (0-9 scale) and it is worth to note that all these were included in Cluster VIIA.

From above discussion, it is understood that each of the clusters based on seed protein

fingerprint has some characteristic features for performance of morpho-economic traits. This could be due to the fact that some of the genotypes may have been descended from common ancestry and subjected to similar selection for agro-economic traits during breeding programme.

Thus, the seed storage protein profiling emerge as a potent technique to generate wide array of polymorphism and as such, it could serve valuable information for varietal identification and extent of genetic diversity. Further, the perspective of such a biochemical approach could be more successful in conjunction with morpho-economic traits for genotype sorting and to support mungbean breeding programme.

CONCLUSION

Some of the valuable informations that emanate from the present pursuit are:

1. Wild accessions e.g. TCR 213, TCR 243, TCR 192 are genetically distant, but TCR 20 has close homology with cultivated mungbean and may be easily crossable.
2. TCR 20- a drought tolerant wild accession with resistance to YMV and *Cercospora* leaf spot; and Balarampur Keonjhar local with long and large size seeds maintained appreciable genetic distance with land races and these also have high yield potential along with important yield contributing characters. These may serve as useful materials for genetic augmentation of seed yield through hybridization.

3. Each of the seed protein based clusters had some sort of characteristic morphological and agronomic features indicating that seed protein profiling has relationship with genetic background of genotypes.
4. Seed storage protein analysis can be successfully applied for elucidation of genetic variation in wild and cultivated forms of mungbean.
5. The genotype-specific polypeptide fingerprints obtained in this study may be useful for varietal identification, and elimination of duplicates.

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