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Revealing genetic diversity in land races and wild accessions of mungbean [*Vigna radiata* (L.) Wilczek] using SDS-PAGE of seed storage proteins

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ABSTRACT

Total seed storage protein profiles of 74 mungbean land races, three wild accessions and a popular variety 'Jyoti' of Odisha were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). 32 genotypes could be clearly identified based on genotype-specific seed protein fingerprints while rest of the test genotypes were categorized into eight protein types. Genotypes included in each protein type had 100% homology and some of these could be duplicates. In this pursuit, a few specific polypeptide markers have been detected for identification of the land races/ genotypes. Dendrogram based on electrophoretic data clustered the genotypes into seven groups at 70% phenon level. Paralakhemundi local, Samarjhola local and Phulbani local-D; and three wild accessions (TCR 20, TCR 213 and TCR 243) were comparatively divergent from other genotypes. Besides, Jyoti, Kalahandi local 2A, Sikri local, kodala local A and TCR 20 were identified to be protein rich with high seed yield. TCR 20 being morphologically similar to mungbean, moderately high protein content and high yielding as well as resistant to drought and bruchids; it may serve as a valuable source genotype in recombination breeding.

Key words: Cultivated *vigna*, Genetic diversity, Seed storage protein profile, Wild *vigna radiata.*

INTRODUCTION

Mungbean [*Vigna radiata* (L.) Wilczek] is the most important short duration pulse crop. India accounts for 45% of total world production in an area of 3.34m. ha. Among the mungbean growing states in India, Odisha ranks first in area and production. In Eastern India, mungbean is cultivated using local cultivars during Winter season mainly in rice fallow, under rain fed situation, in less fertile marginal and sub-marginal lands. In the process of such continuous minimal cultivation, the local land races sustained low productivity and the plant types have adapted to survival following natural selection against biotic and abiotic stresses (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 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 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.

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 diversity included in it. Characterization and cataloguing of germplasm are in vogue carried out on the basis of morpho-agronomic traits. However, the use of protein (Tripathy *et al.* 2010), isozyme (Sonnate *et al.* 1996), and molecular markers (Xu *et al.* 2000) in the past two decades has revealed tremendous genetic variation and genetic relatedness among mungbean genotypes. Each of these marker systems has its own limitation. In this context, SDS-PAGE of seed storage proteins being a simpler and cheaper technique which could be used to study the species relationship and genetic diversity of genotypes in crop plants (Dutta and Mallik 2012). An attempt was, therefore, was undertaken to characterize a set of local land races along with three wild mungbean accessions (*Vigna radiata* var. Sublobata) and a popular improved variety 'Jyoti' for genotypic identification and genetic diversity using total seed storage protein fingerprinting.

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MATERIALS AND METHODS

Seeds of 78 mungbean genotypes including 74 land races, three wild accessions (*Vigna radiata var*. Sublobata) and a popular variety 'Jyoti' of Odisha(Table 1) were grounded to fine powder and total seed storage protein was extracted with extraction buffer (0.5M Tris-HCl (pH 6.8), 2.5% SDS, 10% glycerol and 5% β-mercaptoethanol), denatured with a cracking buffer(0.125M Tris Hcl, pH 6.8, 4% SDS, 20% glycerol; 10% 2-Mercaptoethanol; 0.1% bromophenol blue) at 100°C for 20 sec in hot water bath. Total seed protein was then analysed through 12.5% polyacrylamide gel in a mini vertical electrophoresis apparatus (Tarsons Ltd., India) following Laemmli (1970) with minor modifications at 100v for four hours. Each set of genotypes were run twice simultaneously on two separate gels under similar electrophoretic conditions to check up reproducibility. After electrophoresis, the gels were stained with silver staining technique following Blum *et al.* (1987) with minor modification. The gels were placed on trans-illuminator for assessment of banding pattern and photographed with Digital Camera (Canon, 7.1 megapixel). The molecular weights of the dissociated polypeptides were determined by using molecular weight marker of protein standards which consisted four standard proteins of known molecular weight i.e., carbonic anhydrase (29kD), ovalbumin (43kD) and bovine serum albumin (66kD).

The genotypes were categorized according to polypeptide banding pattern of seed storage protein. The binary data matrix for presence(1) / absence(0) of bands were analysed to estimate Jaccard's similarity coefficient (Jaccard 1908) values and clustering of genotypes(dendrogram) was carried out using Unweighted Paired Group Method with Arithmetic means (UPGMA)-phenograms (Sokal and Michener, 1958) employing Sequential Agglomerative Hierarchic and Non-overlapping clustering (SAHN) (NTSYSpc2.02e). The entire research was carried out at Dept. of Plant Breeding and Genetics, College of Agriculture, OUAT, Bhubaneswar during the year 2012-13.

RESULTS AND DISCUSSION

Protein content: Seed is the edible portion of pulse crops and it contains high protein content which comprises about 70% globulins, 15% albumins and 15% glutelins among field crops (Aykroyed *et al.*1982). Variation in seed storage proteins either in terms of the amount of crude protein in seeds or its characteristic polypeptide banding pattern have become major concerns for pulse breeders and geneticists.

Each of the protein sample was quantified for the crude seed protein concentration in terms of µg/ml corresponding to the O.D. value (absorbance at 260/280nm) using UV-VIS Nanodrop-2000 spectrophotometer (Thermo Electron Scientific Instruments LLC, USA). Seed protein content (w/w in %) was estimated from the above estimated protein concentration (µg/ml) and the amount of extraction buffer (0.35ml) used for extraction of seed storage protein from fine grounded seed powder (0.05gm) of different land races and wild accessions of mungbean.

In the present investigation, there was wide variation in protein content that ranged from 18.42 to 29.96%. The highest value (29.96%) being observed in Jyoti -a released variety of Odisha followed by Kodala local A, Jagatsinghpur local A, Sudhasarangi local B and Anandapur local-A with protein content 28.84%, 27.45%, 26.73% and 26.67% respectively (Table 1). It is worth to note that mungbean as a pulse crop, harbour 22.87% protein (w/w) in seed and the local land races are in general poor in seed yield potential. However, V55 (Kamakshya local-A), V56 (Kamakshya local-B), V75 (Kalahandi local-2A), V31 (Anandapur local-B), V77 (TCR 243 -Wild accession),V21(Kopergaon local), V43 (Gope local-A), V26 (Sikrilocal)and V25 (Sidhaswara local) were found to harbour protein content more than 25% among the test genotypes (Fig 1a & 1b). Local land races in mungbean have not been well explored for their agronomic potential. These genotypes are worth studying for their seed protein content. Some of the protein rich genotypes identified above (Jyoti, Kalahandi local 2A, Sikri local, kodala local A and a wild accession of mungbean TCR 20) had also shown high seed yield. These test genotypes may be sorted out for inclusion in hybridization programme for genetic improvement of seed protein content in mungbean. Naik *et al.* (2000) identified protein rich genotypes e.g., MGG 330 and Nagpuri local (>29% seed protein); and BSN-1, TARM 18, MUS 288 and GDI 47-4 (>27% seed protein) in mungbean. Yan *et al.(*1991) reported negative association of seed glossiness with protein content in mungbean. In the present pursuit, a few of the protein rich local land races cited above e.g., Kodala local A(28.84%), Anandapur local(26.67%), Kamakhya local B(26.06%) and Kopergaon local (25.75%) had glossy seeds. Naik *et al*. (2000), however, revealed no association between seed lusture and protein content in mungbean.

Extent of polymorphism and polypeptide banding pattern: The polymorphism of SDS-PAGE 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. In the present investigation, SDS-PAGE of total seed storage protein revealed altogether 27 scorable polypeptide bands with molecular weights ranging from 12.2 to 110.2kD (Plate 1). Out of these, polypeptide bands at 53.40kD and 45.0kD were found to be monomorphic and rest of the bands had shown polymorphism to the extent of 92.6% among the test genotypes. For ease of detection of bands, five distinct zones of polypeptide migration were arbitrarily assigned in the

Table 1: Purity test of protein samples and estimation of protein content of mungbean genotypes.

Sl. No.	Genotype	Place of origin	Seed colour	Protein content (%)	No.of bands	Seed Yield (g/Pl)	Rem-ark
1.	Tigiria local-C	**Odisha**	Blackish brown	19.67	19	0.62	LC
2.	Kodola local-A	-do-	Bold glossy yellow green	28.84 **II**	20	**1.84**	LC
3.	Kodola local-B	-do-	Reddish brown	22.25	19	0.92	LC
4.	Kendrapada local-B	-do-	Dull black	21.23	16	0.89	LC
5.	Kendrapada local-C	-do-	Brown	21.14	19	0.77	LC
6.	Kendrapada local-D	-do-	Dull green	21.74	18	0.96	LC
7.	Phulbani local-A	-do-	Glossy greenish black	22.35	19	0.83	LC
8.	Phulbani local-B	-do-	Glossy green	21.61	19	0.45	LC
9.	Phulbani local-C	-do-	Dull green	19.77	17	0.88	LC
10.	Phulbani local-D	-do-	Glossy blackish green	20.98	13	1.37	LC
11.	Hinjili local-A	-do-	Dull green	20.16	20	1.15	LC
12.	Hinjili local-B	-do-	Reddish brown	20.69	20	2.24	LC
13.	Banapur local-A	-do-	Reddish brown	20.71	20	1.97	LC
14.	Jagatsingh pur local-A	-do-	Reddish brown	27.45**III**	19	1.24	LC
15.	Jagatsingh pur local-B	-do-	Glossy greenish black	19.88	19	1.45	LC
16.	Berhampur local	-do-	Reddish brown	20.82	12	1.03	LC
17.	Bhabanipatna local-2A	-do-	Reddish brown	23.27	20	1.90	LC
18.	Bhabanipatna local-2B	-do-	Dull black	21.02	19	2.05	LC
19.	Bhabanipatna local-2C	-do-	Dull green	22.36	19	1.64	LC
20.	Bhabanipatna local-1	-do-	Brown	24.99	18	1.77	LC
21.	Kopergaon local	**Maharashtra**	Bold glossy green	25.7 **XI**	20	2.05	LC
22.	Sashna Ambagaon local-A	**Odisha**	Reddish brown	23.69	19	0.58	LC
23.	Sashna Ambagaon local-B	-do-	Dull green	24.55	16	2.02	LC
24.	Paralakhemundi local	-do-	Glossy yellowish green	24.18	13	1.85	LC
25.	Sidheswara local	-do-	Dull green	25.1**XIII**	16	0.51	LC
26.	Sikri local	-do-	Brown	25.5**XII**	20	**1.93**	LC
27.	Makarjhola local-A	-do-	Glossy green	24.23	20	1.96	LC
28.	Saragada local	-do-	Dull brown	18.61	20	0.89	LC
29.	Purasattam local	-do-	Bold glossy green	19.90	20	2.05	LC
30.	Anandapur local-A	-do-	Glossy black	26.67**V**	20	1.24	LC
31.	Anandapur local-B	-do-	Dull green	25.8 **IX**	17	1.02	LC
32.	Dayapalli local	-do-	Glossy brownish green	22.52	20	1.90	LC
33.	Ratila local	-do-	Green	22.43	20	1.20	LC
34.	khadabhanga local-B	-do-	Bold glossy green	21.11	18	2.09	LC
35.	Samarjhola local	-do-	Glossy green	22.66	15	1.62	LC
36.	Mayurbhanj local-A	-do-	Black	23.15	19	1.84	LC
37.	Mayurbhanj local-B	-do-	Green	23.95	20	1.22	LC
38.	Banakhandi local-A	-do-	Black	23.77	19	1.92	LC
39.	Banakhandi local-B	-do-	Green	24.70	20	1.59	LC
40.	Sudhasarangi local-A	-do-	Dull green	22.64	16	0.75	LC
41.	Sudhasarangi local-B	-do-	Dull brown	26.7**IV**	19	1.05	LC
42.	Sudhasarangi local-C	-do-	Dull black	24.15	20	1.78	LC
43.	Gope local-A	**Odisha**	Brown	25.7**XI**	19	1.31	LC
44.	Gope local-B	-do-	Green	24.75	18	1.54	LC
45.	Sheragarh local	-do-	Dull reddish brown	24.98	20	1.11	LC
46.	Nayagarh local-A	-do-	Reddish brown	18.99	19	0.66	LC
47.	Nayagarh local-B	-do-	Dull green	22.63	19	0.72	LC
48.	Nayagarh local-C	-do-	Blackish green	20.87	17	1.68	LC
49.	Nayagarh local-D	-do-	Dull black	21.84	19	0.34	LC
50.	Jharsuguda local-A	-do-	Dull green	23.10	20	0.74	LC
51.	Jharsuguda local-C	-do-	Brown	24.80	20	0.86	LC
52.	Dhenkanal local	-do-	Small dull green	24.43	19	0.24	LC
53.	Charpalli local	-do-	Glossy brownish green	21.64	13	1.05	LC
54.	Raipur local	**Madhya Pradesh**	Green	22.86	18	1.08	LC

Contt...

55.	Kamakshya local-A	**Odisha**	Dull greenish brown	26.4**VI**	15	0.98	LC
56.	Kamakshya local-B	-do-	Glossy greenish black	26.0**VII**	14	0.88	LC
57.	Kamakshya local-C	-do-	Dirty green	23.67	18	0.69	LC
58.	Ambagaon local -A	-do-	Bold dull black	23.02	18	1.22	LC
59.	Ambagaon local- B	-do-	Faint green	19.58	19	1.03	LC
60.	Mahimunda local	-do-	Black mosaic	23.15	19	0.98	LC
61.	Jhaimunga Kharsel	-do-	Dull greenish grey	22.55	18	1.08	LC
62.	Jhaimunga Kharsel Sel-1	-do-	Dull greenish mosaic	20.68	19	1.59	LC
63.	Jhaimunga Kharsel Sel-2	-do-	Dull green	21.23	20	1.56	LC
64.	Jhaimunga Kharsel Sel.3	-do-	Dull black	21.35	18	1.88	LC
65.	Jhaimunga Kharsel Sel-4	-do-	Dull black bold	24.39	16	1.89	LC
66.	Jhaimunga Kharsel Sel-5	-do-	Bold glossy green	23.10	20	1.98	LC
67.	Jhaimunga Kharsel Sel-6	-do-	Glossy green	22.75	20	2.10	LC
68.	Athamallik local-A	-do-	Glossy green mosaic	23.86	20	1.28	LC
69.	Athamallik local-B	-do-	Glossy mosaic black	23.98	20	0.95	LC
70.	Kalahandi local-1A	-do-	Glossy black	21.25	16	1.85	LC
71.	Kalahandi local-1B	-do-	Glossy mosaic green	18.42	15	2.20	LC
72.	Kalahandi local-1C	-do-	Dull dirty black	23.48	17	1.96	LC
73.	Deogaon local	-do-	Glossy black	22.71	18	2.00	LC
74.	Jyoti	OUAT,BBSR	Green	29.96 **I**	13	**3.10**	RV
75.	Kalahandi local-2A	**Odisha**	Dull green	25.9**VIII**	18	**2.30**	LC
76.	TCR 20 (Wild accession)	NBPGR, New Delhi	Brown	24.9**XIV**	19	**7.67**	WA
77.	TCR 243 (Wild accession)	NBPGR, New Delhi	Small dull brown	25.72 **X**	19	1.00	WA
78.	TCR 213 (Wild accession)	NBPGR, New Delhi	Dirty blackish brown	22.80	20	1.90	WA

N.B. RV-Released variety, LC-Local collection, WA-Wild accession.

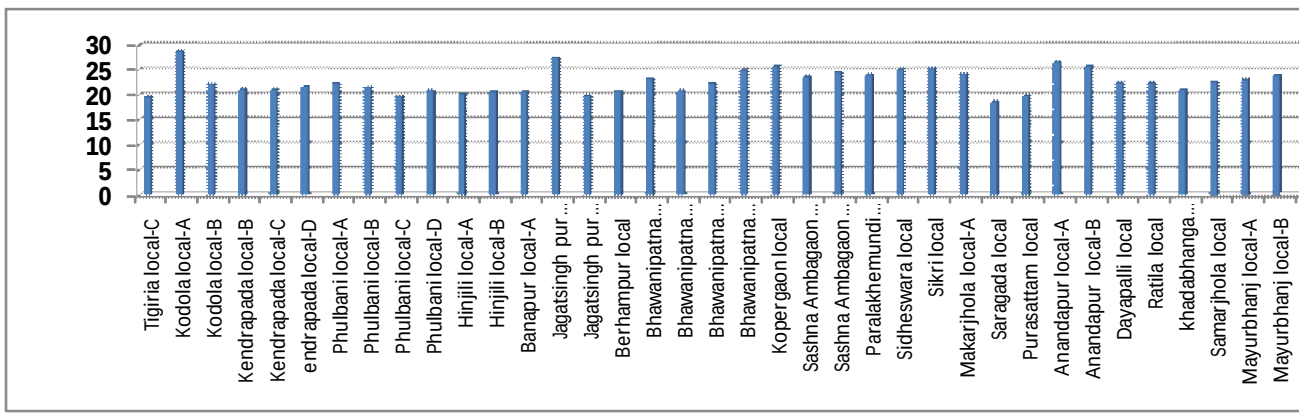

Fig 1a: Protein content(%) in seeds of different local land races of mungbean (genotype Sl. 1-39).

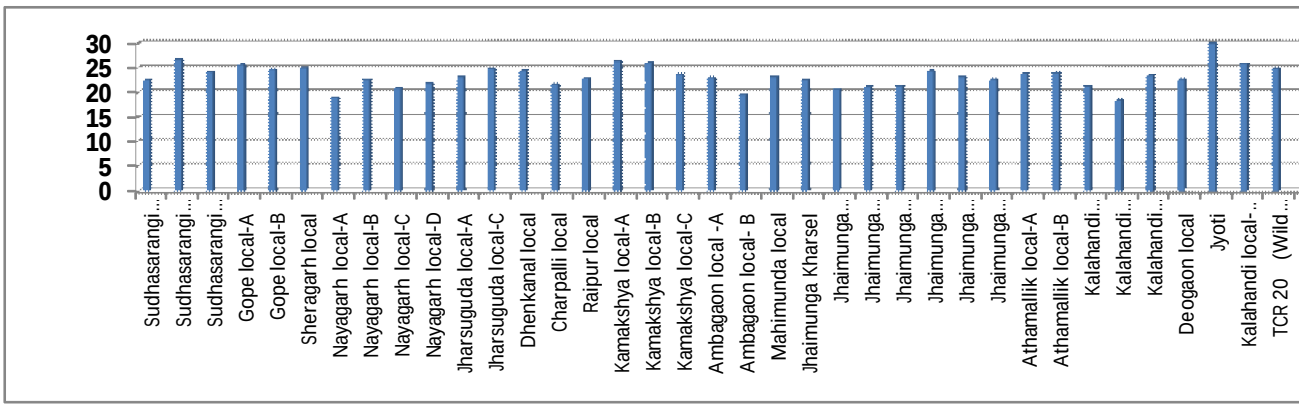

Fig 1b: Protein content(%) in seeds of different local land races of mungbean (genotype Sl. 40-78).

electrophoregrams i.e. A (89.4-110.2kD: 5 bands), B (37.4-85.9kD: 12 bands), C (34.3-36.2kD:3 bands), D (28.2-32.8kD: 5 bands) and E (12.2-16.4kD: 2 bands). Zone A showed five fine bands; Zone B had 12 bands with a characteristic monomorphic broad yellow dense band at 45kD. Zone C was designated by a cluster of three faint bands. Zone D constituted five bands characteristic to different genotypes and Zone E revealed two low molecular weight broad bands at 12.2 and 16.4kD.

As a whole, the resulting data matrix of the presence and absence of bands resolved a total of 1,266 polymorphic polypeptide bands out of total 1,422 bands over all the 78 test genotypes used in the study which reveals 89.02% polymorphism. A great array of polymorphism was revealed in terms of presence/absence, as well as intensity and colour of band following silver staining in different test genotypes. The intensity of bands reveal degree of quantitative variation in polypeptides dissociated following cleavage of storage protein fractions. Mungbean is characterized by a thick /broad yellow band of higher molecular weight (45kD) and two other bands approximately at 40 and 34.7kD. Thus, these polypeptide bands could be used as species-specific protein markers (Ghafoor *et al.* 2002). In the present investigation, however, the bands at 40 could not be electrophorically revealed, but five other clear dense bands at molecular weight positions 85.9, 32.8, 32.0, 29.0 and 28.2kD were visualized using silver staining technique.

Number of polypeptide bands in each genotype ranged from 12-20. 48 test genotypes recorded as high as 19-20 bands. These genotypes varied in polypeptide banding pattern and may be considered superior in protein quality provided the polypeptides revealed are not associated with antinutritional activity. Among the above 48 genotypes, a few land races e.g., Kodala local A, Kopergaon local, Sikri local and TCR 20 were highly rich in protein content with high seed yield. Hence, these may serve as excellent breeding material for recombination breeding for improvement of seed yield, protein yield and protein quality. Available literature does not reveal such a comparison.

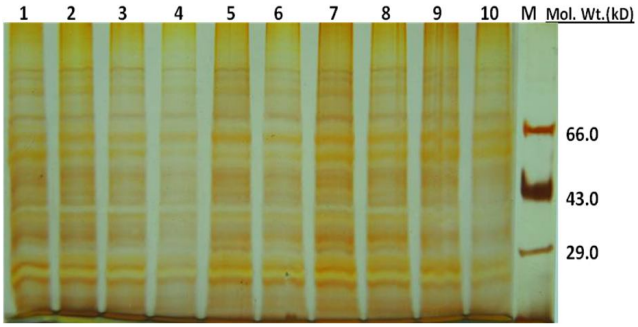

Plate 1: Total seed storage protein profile of different local varieties of mungbean. Lane 1-10: Tigiria local-C, Kodola local-A, Kodola local-B, Kendrapada local-B, Kendrapada local-C, Kendrapada local-D, Phulbani local-A, Phulbani local-B, Phulbani local-C, Phulbani local-D, M= molecular wt. marker.

In the present pursuit, forty protein types were detected for seed protein expression in the present set of test genotypes. Thirty two test genotypes revealed their characteristic genotype-specific polypeptide banding pattern, while rests of the land races are observed to reveal eight common protein types (data not shown). Three common protein types comprised 24, seven and five genotypes while rest five common protein types included two genotypes each. For instance, a common protein type comprising five land races namely, V15 (Jagatsinghpur local-B), V52 (Dhenkanal local), V59 (Ambagaon local- B), V62 (Jhaimunga Kharsel Sel-1) and V60 (Mahimunda local) exhibited the same 19 polypeptide bands imprinted at similar molecular weight positions. Such a high degree of homology in polypeptide banding pattern among some of the local land races might be due to the fact that these genotypes were domesticated in their respective ecological zones with narrow genetic base. Naik (1998) observed nine densely stained polypeptide bands (19.5kD to 62.4kD) and could differentiate 24 genotypes into fourteen protein types based on SDS-PAGE of crude proteins of mungbean seeds. Tomooka *et al.* (1992) analysed 581 genotypes of mungbean and grouped them into eight protein types by combining albumin and globulin polypeptide bands. They could reveal one genotype-specific protein type. Thakare *et al.* (1988), however, could not able to recover any polymorphism in the vicilin seed protein derived from four cultivars of mungbean.

A few polypeptide bands were observed to be specific to some test genotypes in terms of presence/absence of bands. Four polypeptide bands e.g., 110.2, 105.4, 85.9 and 28.2kD were expressed in all test genotypes except Charpalli local , Phulbani local D, TCR 243 and Anandapur local-B respectively. In contrast, 74.8kD and 70.0kD polypeptide bands were specific to the wild accessions TCR 243 and TCR 20 respectively. Similarly, 30.8kD was revealed in TCR 243 only. On the other hand, 66.8 and 34.3kD were specific to TCR 20 and TCR 213; while 37.4kD was unique to TCR 243 and TCR 213. Such genotype -specific protein markers could be reliably used for varietal certification.

Jhaimunga Kharsel Sel-5, Jhaimunga Kharsel Sel-6, Athamallik local-A and Athamallik local-B each with 20 bands, revealed additional bands at 100.2kD as compared to Dhenkanal local, Ambagaon local- B, Mahimunda local and Jhaimunga Kharsel Sel-1. Similarly, Nayagarh local-A and Nayagarh local-B expressed additional polypeptide bands at molecular position 100.2kD but absence of 12.2kD as compared to the above four later land races of mungbean. In this context, Sudhasarangi local B can be identified from that of Dhenkanal local, Ambagaon local- B, Mahimunda local and Jhaimunga Kharsel Sel-1 by absence of 89.4kD and presence of 100.2kD in the former. This evidences that the land races may have experienced minor spontaneous mutational changes in the genes of erstwhile mentioned

multigene families controlling seed storage protein expression (Panda 2013). On the other hand, Berhampur local had peculiar polypeptide banding pattern to that of other local land races. It had shown only 12 polypeptide bands and it revealed absence of many bands present in all other local cultivars. Such a genotype-specific banding pattern would serve reliable identification and maintenance of pure seeds in seed multiplication programme. Seed protein profile is usually species -specific. In the present investigation, each of the three wild accessions (TCR 20, TCR 213 and TCR 243) expressed considerably high number of bands (19-20 bands) with distinctly different *inter se* polypeptide profile as well as compared to local land races of mungbean under study. However, Sudhasarangi local-A maintained comparatively high homology (70%) with the wild accession TCR 20. Similarly, Jagatsinghpur local-A , Bhabanipatna local-2B, Nayagarh local-A and Nayagarh local-B had high congruence with TCR 243; and Gope local-B had shown high similarity with the wild accession TCR 213. Inter-specific variation in polypeptide banding pattern have been reported by many workers e.g., Koenig *et al.* (1990) in frenchbean, Polignanao *et al.* (1990) in *Vicia faba*, 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 sublobata* accessions, *V. radiata* and *V. mungo.* However, Tripathy *et al.* (2010c) reported resemblance of mungbean with *V. sublobata* except absence of a globulin band (seed storage protein) with molecular weight 30.2kD in a wild accession of *V. sublobata* Acc. TCR 213. Genetic variation in germplasm has an important role in identification of varieties. Polymorphism in electrophoretic banding pattern of seed storage proteins is associated with the genetic background of proteins and thus, this can be used to certify the genetic makeup. The erstwhile mentioned genotype-specific banding pattern observed in the present investigation could serve the purpose.

Genetic similarity/distance: Genetic distance is the inverse measure of genetic similarity. Similarity index values between paired genotypes ranged from 0.3 (between V53-Charpalli local and V10-Phulbani local-D) to 1.0. An attempt was taken to quest the most highly divergent combination(s) among the possible 3003 combinations comprising 78 genotypes. In this context, besides that between V53 (Charpalli local) and V10 (Phulbani local-D); V24 (Paralakhemundi local) was observed to have high genetic dissimilarity (SI=0.4) with V4 (Kendrapada local-B) and V10 (Phulbani local-D). Similarly, V77 (TCR 243) had shown high genetic distance with V16 (Berhampur local). Such genotypic combinations could be selected in hybridization programme.

Among the test genotypes, V10 (Phulbani local-D), V16 (Berhampur local), V24 (Paralakhemundi local) , V53 (Charpalli local) , V56 (Kamakshya local-B) , V74 (Jyoti) , V76 (TCR 20) , V77 (TCR 243) and V78 (TCR 213) had shown mean similarity index (SI) value of 0.6 while, V4 (Kendrapada local-B) , V23 (Sashna Ambagaon local-B), V25 (Sidhaswara local), V31 (Anandapur local-B) and V55 (Kamakshya local-A) maintained to have average SI of 0.7. These test genotypes may be sorted out for useful inclusion in the breeding programme. The rest of the test genotypes revealing Av. S.I. value beyond 0.7 may be discarded using the concept of pre-breeding. In fact, 1990 combinations out of possible 3003 paired genotypic combinations have similarity co-efficient value within the range of 0.8-1.0 indicating fair degree of homology among the test materials.

Clustering pattern: Genetic diversity is the diversity of the sets of genes carried by different genotypes of a species (Panda 2013). Information of genetic resources with broad genetic diversity is a pre-requisite for accelerated genetic improvement of crops.

The dendrogram showing genetic relationship among 78 test genotypes for total seed storage protein expression is presented in Fig 2. Initially, the genotypes were distributed into seven clusters e.g., Cluster I, Cluster II, Cluster III, Cluster IV, Cluster V, Cluster VI and Cluster VII within 70% phenon level. V24 initially separated out from rest of the test genotypes forming a single genotype cluster-I, while Cluster-II and Cluster III comprised the wild accessions of mungbean that included V78 (TCR 213) and V76 (TCR 20) ; and V77 (TCR 243) respectively. Cluster IV contained two genotypes (V35- Samarjhola local and V10- Phulbani local-D); Cluster V and Cluster VI grouped three (V53- Charpalli local, V55- Kamakshya local-A and V56- Kamakshya local-B) and four genotypes (V74- Jyoti, V71 Kalahandi local-1B, V70- Kalahandi local-1A and V16-

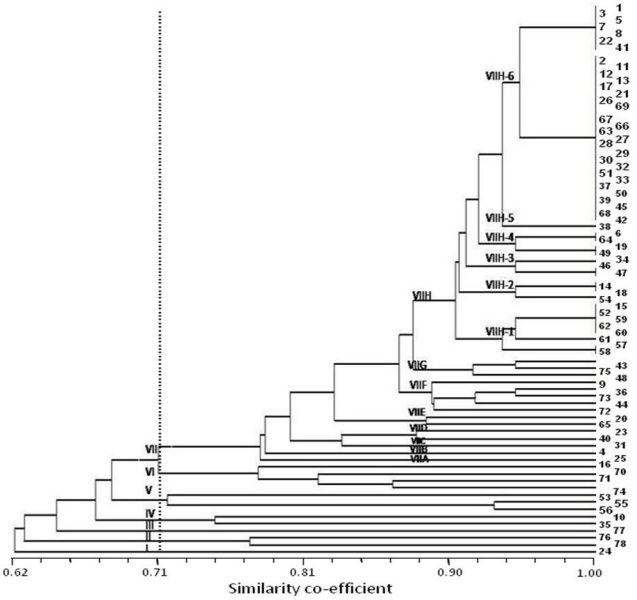

Fig 2: Dendrogram showing genetic diversity of genotypes based on total seed storage protein finger printing of mungbean land races.

Berhampur local) respectively. The rest of the test genotypes were clubbed into a large cluster-VII which comprised eight sub-clusters (Cluster VIIA to VIIH). These genotypes maintained high homology even upto 100% in seed protein expression. Such a high degree of homology could possibly be due to inclusion of duplicates which were otherwise collected from different places with different nomenclature. The SDS-PAGE in combination with 2-D electrophoresis is further suggested for documenting contrasting variations of isoforms of protein peptides.

In the present investigation, V14 (Jagatsinghpur local-A) and V18 (Bhabanipatna local-2B) could not be discriminated and suspected to be duplicate. Similarly, varietal identification for V57 (Kamakshya local-C) and V58 (Ambagaon local –A) could not be confirmed as these genotypes clubbed together at even 100% phenon level.

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. Asghar *et al*. (2003) classified 29 genotypes of chickpea into five clusters based on 18 polypeptide bands. They reported presence/ absence of specific bands in genotypes comprising different clusters. However, 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. 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.

The grouping of genotypes using three dimensional scaling based on PCA values (Fig.3) 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. V24 (Paralakhemundi local) , V77(TCR 243), V76(TCR 20) and V78(TCR 213) which were initially separated from rest of the test genotypes in case of UPGMA clustering (Fig.2), were also seen to be screened out to diverse extreme positions in PCA analysis.

Local land races and wild relatives harbour the wealth of valuable genes particularly for biotic and abiotic resistance. All the three wild accessions e.g., V76 (TCR 20), V77 (TCR 243) and V78 (TCR 213) proved to be most divergent

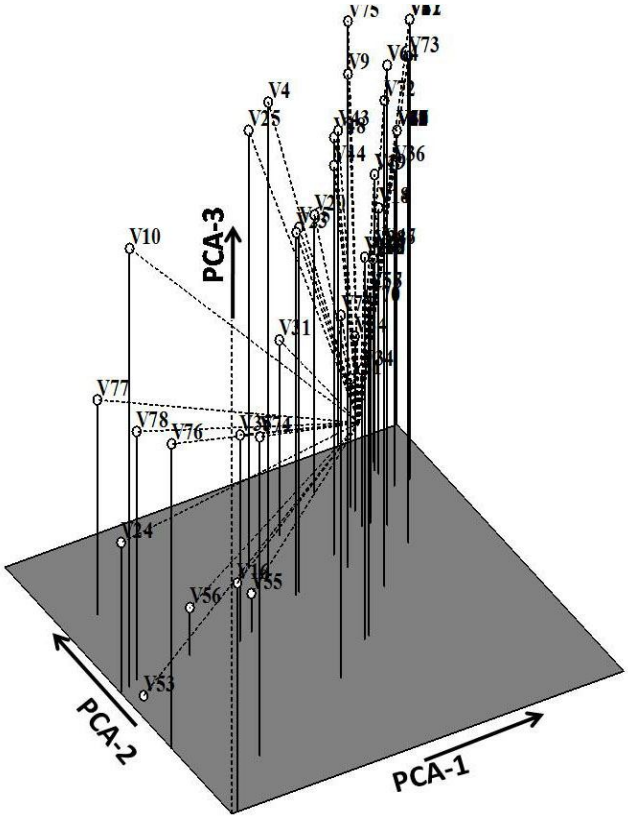

Fig 3: Three dimensional scaling of principal co-ordinates 1, 2 and 3 using seed storage protein markers with vectors

in the present pursuit. TCR 243 and TCR 213 bore small and thin pods and are viny. But, TCR 20(V76) is morphologically more similar to cultivated mungbean and it is reported to have high seed yield as well as drought and bruchid resistance (Sardar 2011). Among local land races e.g., V24 (Paralakhemundi local), V35 (Samarjhola local), V10 (Phulbani local-D), V56 (Kamakshya local-B), V55 (Kamakshya local-A), V53 (Charpalli local), V71 (Kalahandi local (Kalamunga)-1B), V70 (Kalahandi local-1A) and V16 (Berhampur local); and the popular high yielding variety Jyoti (high seed protein content) could be also sorted out as highly divergent. The high yielding and protein rich genotypes(Jyoti, TCR 20, Kamakhya local A, Kamakhya local B, Kalahandi local) identified in this pursuit could serve as a valuable source of genetic material for recombination breeding and other innovative approaches of crop improvement programme in mungbean.

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