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Globulin seed storage protein based genotyping and Study of genetic diversity in core accessions of mungbean under drought stress

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Globulin seed storage protein profiles of 19 mungbean genotypes including two wild forms of *Vigna radiata var. sublobata* (TCR 20 and TCR 213) and two standard checks(T 2-1 and LGG 460) were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Thirteen genotypes could be clearly identified based on genotype-specific seed protein fingerprints. The combined dendrogram showed six genetic clusters within 68% phenon level. The clustering based on the combined clustering analysis revealed discrimination of all test genotypes even immediately beyond 88% phenon level, whereas individual clustering analysis based on protein and agro-morphological level failed to do so. Nipania munga, TCR 213, T 2-1, LGG 460, TCR 20 and Banapur local B were identified to be highly divergent genotypes. TCR 20 appears to have more genetic proximity to the mungbean genotypes than TCR 213. T 2-1, LGG 460 and TCR 20 are potentially high yielding. These may serve as valuable materials for recombination breeding in mungbean.

Key wards: Globulin storage protein profiling, genotyping, morphological traits, genetic diversity, mungbean

SDS-PAGE of seed storage proteins generates different profiles composed of several polypeptide sub-units which migrate in the gel according to their molecular weights. The number of such sub-units indicates the number of multi-gene families involved (de Lumen 1990). Mutation in these gene families or their regulator genes leads to deletion of some or all the genes or production of new alleles which forms the basis of variation in the polypeptide banding patterns involving presence or absence of bands. In addition, polymorphism in terms of intensity of bands could provide information in shutting of some members of the multi-gene family that could produce fewer copies in the genotype exhibiting faint bands. Electrophoregrams of a set of genotypes may also reveal distinct alleles 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. 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.

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, use of molecular marker in the past two decades has revealed tremendous genetic variation and genetic relatedness found in a population (Xu *et al*., 2000). However, polypeptide banding pattern based on SDS-PAGE of seed storage proteins does not require amplification as in case of RAPD or radio-isotope labeling as in case of restriction fragment length polymorphism(RFLP). In spite of the fact that, the degree of intra-specific variation exhibited by polypeptide banding pattern in SDS-PAGE is lower as compared to RAPD or RFLP, this simpler and cheaper technique could be used to study the species relationship and genetic diversity of genotypes in crop plants. In the present investigation, an attempt has been taken to explore genetic variation in globulin seed storage protein fraction and agro-morphological traits to compare and critically examine genetic diversity among a set of selected mungbean core germplasm lines.

MATERIALS AND METHODS

A set of 19 mungbean genotypes (Table 1) including two wild forms (*Vigna radiata* var. sublobata: TCR 20 and TCR 213) and two standard checks (LGG 460 and T 2-1) were selected as core germplasm lines from a large collection of 292 mungbean accessions using the concept of pre-breeding based on genetic variability of stable morphological descriptors and morpho-economic traits (Mohapatra 2011). These candidate genotypes were laid out in Randomized Block Design (RBD) with three replications to assess their comparative performance under drought stress.

Globulin seed storage protein fraction was extracted with 0.5M NaCl, 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 80oC in hot water bath. Seed proteins were analysed simultaneously by running two gels at a time in a vertical slab gel (12.5% polyacrylamide gel) at constant current of 60mA (2.5mA per lane for two gels run each time) for four hours following Laemmli (1970) with minor modifications. 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 tripsinogen (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 SDS-PAGE revealed altogether 15 scorable globulin polypeptide bands with molecular weights ranging from 10.0 to 102.2kd (Fig 1). This envisaged that at least 15 multi-gene families are involved in globulin seed storage protein expression in general. A minimum six polypeptide bands were observed in case of Nipania munga, TCR 213 and T2-1 while, it was as high as 13 in C. No. 35 and OUM 14-1. The latter two genotypes may be considered superior in protein quality provided the polypeptides revealed in these genotypes are not associated with anti nutritional activity. Available literature does not reveal such a comparison.

Out of 15 polypeptide bands scored in this investigation, two bands at molecular weight position 102.2kd and 53.7kd were found to be monomorphic and rest of the bands had shown polymorphism to the extent of 86.6% among the test genotypes. 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. Thakare *et al.* (1988), however, could not able to recover any polymorphism in the vicilin seed protein derived from four cultivars of mungbean. Similarly, Roy (2003) also revealed very restricted level of polymorphism among some Indian cultivars and wild accessions of mungbean.

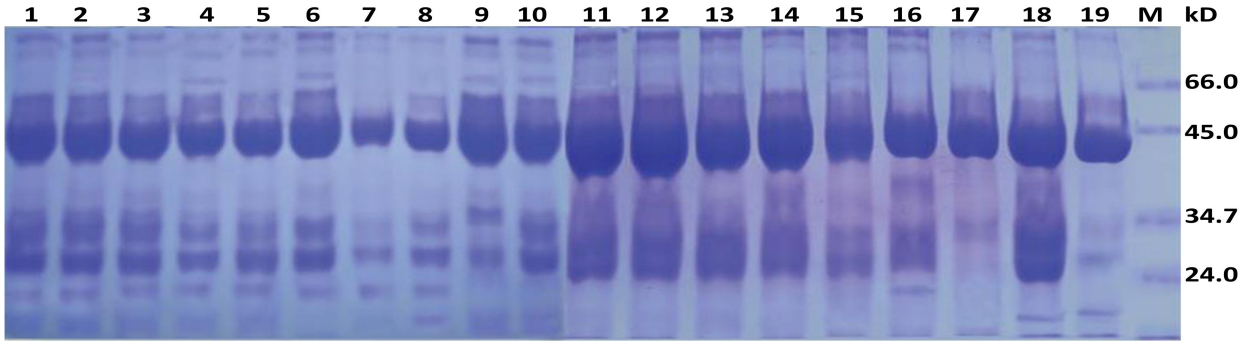

Fig 1. SDS-PAGE polypeptide profile of globulin seed storage protein fraction of 19 mungbean genotypes , Lane 1-8: C. No. 35, OUM 14-1, OUM 49-2, Pusa 9072, OM 99-3, Banapur local B, Nipania munga, Kalamunga 1-A; Lane 9-19: T 7-10 Sel . 3, OUM 13-2, OBGG 183, PMB 9, Pusa 2072, T 7-7 Sel.2, Nayagarh Local-C, TCR-20, TCR 213, LGG 460, T 2-1

A great array of polymorphism was revealed in terms of presence (1) / absence (0) of polypeptide bands as well as intensity of bands in different test genotypes. There exists a parallelism between staining intensity and amount of protein for each of the sub-units revealed as single bands. In the present pursuit, 53.7kd, 35.6kd and 29.0kd polypeptide bands were broad and densely stained in most of the genotypes. The test genotypes revealed their characteristic polypeptide banding pattern except in few which were categorized into common protein type(s).

It is worth to mention that band at 50.7kd and 45.8kd were specific to the wild accession TCR 20 while these were absent in all other genotypes except LGG 460. In contrast, another wild accession TCR 213 and a high yielding genotype T 2-1 marked the absence of 29.0kd polypeptide band. Tripathy *et al.* (2010c) reported unique absence of globulin bands at molecular positions 27.5kd and 30.2kd in Mayurbhanj local (a urdbean land race) and TCR 213 (a wild accession: *V. radiata* var. sublobata) respectively to serve as molecular marker(s) for species identification. Ghallab *et al*. (2007) reported one genotype-specific polypeptide band at molecular weight 39.66kd in a mungbean genotype L 1320. 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. However, Sahai and Rana (1977) reported almost similar polypeptide profiles in two genotypes (S 9 and Pusa Baisakhi) of mungbean. Similarly, among a set of 41 varieties, C. No.3 and C. No.36 were shown to have similar protein finger printing but yet differed in thickness of bands (Tripathy *et al.*, 2010c).

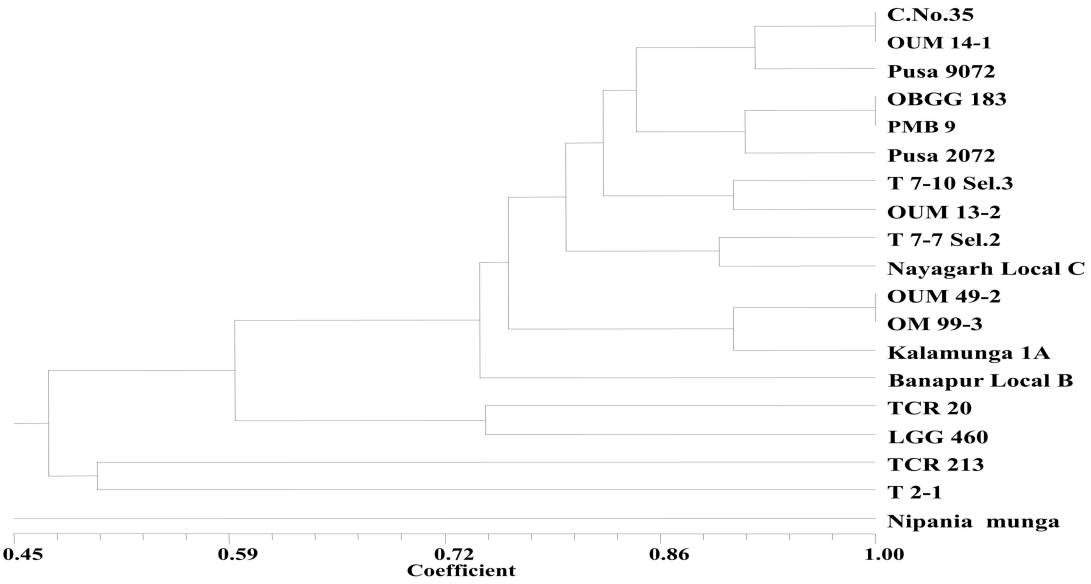

Fig 2. Dendrogram showing genetic diversity of genotypes based on globulin seed storage protein profiling.

Clustering based on SDS-PAGE of globulin seed proteins:

The dendrogram showing genetic relationship among 19 test genotypes for seed storage protein expression is presented in Fig 2. Initially, the genotypes were distributed into three clusters within 60% phenon level. Nipania munga formed a single variety cluster (Cluster-I). One of the improved mungbean genotype T 2-1 along with the wild accession TCR 213 constituted Cluster II. Rest of the genotypes had shown at least 75% inter se homology and formed a broad genetic group (Cluster III). At about 75% phenon level, Cluster III was subdivided into Sub-cluster IIIA and Sub-cluster IIIB. Cluster IIIA included TCR 20(wild accession) and LGG 460(best standard check) both of which had shown dissimilarity with rest of the 14 genotypes contained in Cluster IIIB. The genotypes in this large multivariety cluster dissociated progressively into small clusters.. Banapur local B exhibited high *inter se* genetic

distance among genotypes included in Cluster IIIB. In the present investigation, Nipania munga and TCR 20; and two high yielding genotypes T 2-1 and LGG 460 were shown to have very high genetic divergence based on globulin seed protein profile. Such germplasm lines may serve as valuable material for genetic improvement in mungbean.

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 munbean. 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.

Combined cluster analysis:

Morphological traits are *in vogue* used for study of genetic diversity in a set of genotypes. But, expression of these characters is highly influenced by environment in which the materials are tested. Many workers attempted to integrate molecular and biochemical data with morphological informations to derive genetic relationship among the genotypes. Cluster analysis of both the data bases 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 and Ahmad (2005) and 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. Betal *et al.* (2004) and Lavanya *et al.* (2008) adopted similar approach while comparing RAPD profile with agro-morphological traits in mungbean. Therefore, a combined approach for cluster analysis was attempted by incorporating all data relating to globulin seed protein banding pattern through SDS-PAGE and agro-morphological traits from field trial of a set of 19 selected test genotypes.

In case of agro-morphological traits, a value for any character (except drought tolerance score) exceeding significantly over the grand mean (Table 1) was scored as '1.0' and rest of the values for the character were marked '0'. In the present investigation, nine selected drought resistant genotypes (including wild accession TCR 20) which scored around zero(0-9 scale) were marked "1.0" and rest of the selected test genotypes were categorized with score value "0" to prepare the binary data. The selected genotypes widely differed in agro-economic traits and these were polymorphic for all eight visually expressed characters. The wild accession TCR 20 showed significantly high yield potential followed by cultivated forms e.g., T 2-1, T 7-7 Sel.2, T 7-10 sel.3, LGG 460 and OM 99 -3 (Table 1) under drought stress.

The combined analysis resolved unique characteristics of each of the selected test genotypes under study and each genotype was virtually separated into single variety clusters at higher phenon level (Fig 3). In contrast, the Globulin seed storage protein finger printing through SDS-PAGE failed to discriminate the paired genotypes e.g., OUM 49-2 and OM 99-3; C. No. 35 and OUM 14-1; and OBGG 183 and PMB 9 at even 100% phenon level (Fig. 2). The former two paired groups included drought resistant genotypes, while the last pair of genotypes was sensitive to drought stress.

Similarity coefficient values between paired genotypes indicated very high genetic

divergence of local land race 'Nipania munga' from high yielding varieties e.g., T 2-1, LGG 460, TCR 20(wild accession), C. No. 35, OM 99-3 and Pusa 9072. Among the test genotypes, Pusa 9072 and OM 99-3 maintained high *inter se* homology at 88 % phenon level (S.I. 0.88) and thereafter, both the genotypes were separated into single variety clusters. T 2-1, a high yielding improved mutant culture exhibited very high genetic distance from two drought resistant local land races e.g., Nipania munga and Banapur local. Both the wild accessions (TCR 20 and TCR 213) had shown high genetic divergence with rest of the selected mungbean genotypes. However, TCR 20 appears to have more genetic proximity to the mungbean genotypes than TCR 213 as revealed from similarity index values as well as comparatively similar morphological appearance with regard to growth habit, leaf and pod characteristics. 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, Tripathy *et al.* (2010c) reported resemblance of mungbean with *V. radiata* var. sublobata except absence of a globulin band with molecular weight 30.2kd in a wild accession of *V. radiata* var. sublobata (TCR 213).

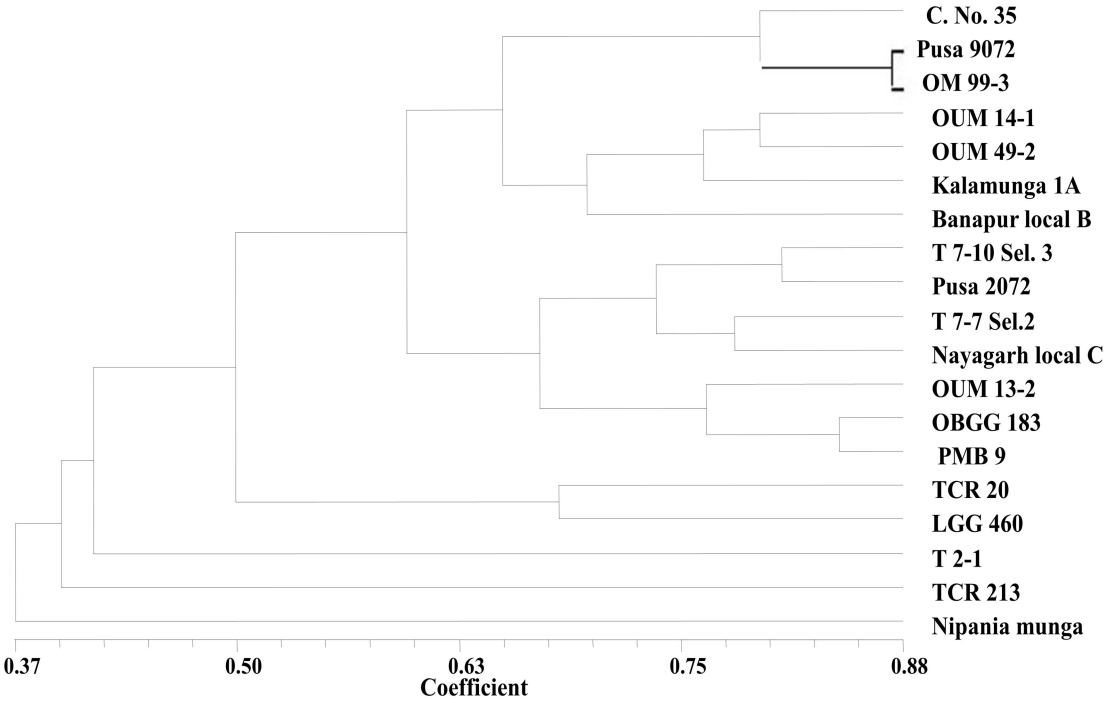

Fig 3. Dendrogram showing genetic diversity of genotypes based on combined analysis of globulin seed storage protein profile and agro-morphological traits.

The dendrogram showed six genetic clusters within 68% phenon level (Fig. 3). Cluster-I included the highly drought resistant local land race Nipania munga which is native to drought prone Kalahandi district of Odisha. It was initially separated from rest of the genotypes at even 37% phenon level and hence, considered as the most divergent genotype under drought stress condition. Cluster-II and Cluster III were also progressively separated as single genotype clusters containing the wild accession TCR 213 and T 2-1 respectively. Cluster IV comprised LGG 460 and TCR 20(wild accession), both being expressed high yield performance under drought stress.

These two test genotypes were separated from two broad genetic groups or clusters e.g., Cluster V and Cluster VI which constituted the erstwhile mentioned all seven drought sensitive (T 7-10 sel.3, OUM 13-2, OBGG 183, PMB 9, Pusa 2072, T 7-7 Sel.2 and Nayagarh Local-C) and all seven drought resistant genotypes (C. No. 35, OUM 14-1, OUM 49-2, Pusa 9072, OM 99 -3, Banapur Local-B, and Kalamunga -1A) respectively. This envisaged that drought stress resistant genotypes except Nipania munga and TCR 20 might have a common drought stress tolerance mechanism in form of seed storage protein expression as well as expression of morpho-economic characters in response to drought stress. In contrast, the drought sensitive test genotypes fail to response positively to combat water deficit and therefore, clubbed together to form Cluster V. Nipania munga being highly genetically divergent from the high yielding drought sensitive test genotypes e.g., T2-1, TCR 20(wild accession), T 7-10 Sel.3 and T7-7 Sel.2 (Table 1) , it could be used as a valuable source for transfer of drought tolerance. Besides, Nipania munga and TCR 20 being individually separated from the drought tolerance cluster(Cluster VI), are supposed to harbour different sets of drought responsive genes compared to the above seven drought resistant cultivars(Cluster VI). Harnessing such diverse sources of gene content through recombination breeding would pave the way for gene pyramiding for drought tolerance. Use of abiotic tolerant cultivars in breeding field crops has been also suggested by Horn (2001).

Table 1. Performance of 19 selected (resistant, susceptible and high yielding) genotypes under drought stress.

Sl. No.	Acces-sion No.	Genotype	Seed -ling vigour	Days to 50% flo-wering	Plant height	No. of clusters /plant	No. of pods/ cluster	No. of pods/ plant	Seed yield/ Plant(g)	Drought tole-rance
1.	**24**	C. No. 35	3.25*	29.22	26.59*	4.54*	6.44*	30.67*	2.95*	0.44
2.	**74**	OUM 14-1	2.25	33.47	15.41	2.59	6.19*	14.42	1.45	0.04
3.	**89**	OUM 49-2	2.25	33.47	19.61	2.79	5.39	13.42	2.59	0.24
4.	**157**	Pusa 9072	4.00*	35.47	27.61*	4.12*	5.13	21.67	2.96*	0.19
5.	**68**	OM 99 -3	5.25*	35.47	18.21	4.39*	5.45	21.42	3.09*	-0.06
6.	**240**	Banapur Local-B	1.25	34.72	13.71	1.72	5.70	9.92	1.22	0.26
7.	**268**	Nipania munga	1.50	37.22*	13.74	1.77	5.66	8.42	1.42	-0.06
8.	**267**	Kalamunga - 1A	0.50	37.22*	21.14	2.17	6.04*	11.42	1.98	-0.06
9.	**206**	T 7-10 sel.3	1.00	29.97	22.24	5.39*	5.79	34.17*	4.10*	7.44
10.	**72**	OUM 13-2	1.25	35.47	15.41	1.99	5.04	8.42	0.93	7.94
11.	**117**	OBGG 183	2.00	35.97	26.26*	3.07	3.29	19.17	1.72	6.69
12.	**147**	PMB 9	2.00	27.47*	21.81	2.92	4.55	13.67	2.24	9.19
13.	**165**	Pusa 2072	0.75	32.97	27.81*	4.22*	5.20	24.42*	3.05*	8.69
14.	**205**	T 7-7 Sel.2	4.00*	35.97	26.84*	4.39*	5.51	27.17*	4.28*	8.44
15.	**278**	Nayagarh Local-C	0.50	35.22	28.54*	3.97*	5.30	19.42	1.68	8.94
16.	**287**	TCR-20 (Wild form)	4.50*	55.22*	58.74*	6.97*	5.54	36.82*	7.67*	-0.06
17.	**288**	TCR 213 (Wild form)	2.50*	59.22*	70.74*	5.67*	1.34	25.52*	1.90	5.94
18.	**292**	LGG 460	4.67*	40.44*	21.64	3.98*	4.18	16.67	3.12*	5.22
19.	**200**	T 2-1	1.00	34.97	23.64	4.59*	5.91*	30.17*	5.31*	7.44
Overall Mean			1.87	34.02	21.38	3.14	5.55	17.44	1.99	6.98
C.D.			1.21	2.79	4.80	0.57	0.36	5.10	0.57	1.09

***-Significant at 5% level over the overall mean value.**

In the present investigation, the clustering based on the combined clustering analysis revealed discrimination of all test genotypes even immediately beyond 88% phenon level, whereas erstwhile mentioned clustering analysis based on protein level failed to do so. Unlike total soluble protein and isozymic variation at various plant growth stages, the seed storage proteins are relatively more stable and generate wide array of polymorphism. Thus, combined analysis could give an added advantage in discriminating genotypes as it involved study of entire variation due to polymorphism at genomic and proteomic level as well as phenotypic expression of agro-morphological traits. Available literature did not reveal such an approach for characterization and identification of elite germplasm lines for further utilization in mungbean.

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