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Regular article

Revealing contrasting genetic variation and study of genetic diversity in urdbean (*Vigna mungo* (L.)Hepper) using SDS-PAGE of seed storage proteins

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Total seed storage protein profiles of 20 urdbean genotypes including the popular variety T9 were analysed by Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). 14 genotypes could be clearly identified based on genotype-specific seed protein fingerprints while rest of the test genotypes were categorized into three protein types. Dendrogram based on electrophoretic data clustered the genotypes into seven groups at 78.5% phenon level. TU 95-1 with TU 12-25-4 revealed lowest similarity index value (0.33) followed by TU 95-1 with PU 30 and KU 96-3(SI=0.35). Clustering pattern revealed distinctly divergent group formed by TPU 95-1 and TPU 4. These may serve as a valuable source genotype in recombination breeding.

Key words: Seed storage protein profiling, SDS-PAGE, Genetic variation, urdbean.

Urdbean (*Vigna mungo* (L.)Hepper) is a self-pollinating crop and widely cultivated grain legume (Nag*et al*., 2006). The crop is resistant to adverse climatic conditions and improves the soil fertility by fixing atmospheric nitrogen in the soil. It is one of the rich source of vegetable protein and some

for selection of desirable traits (Nisar*et al*., 2008). Assessment of genetic diversity based on morphological characters is often less efficient and biased in assessment of genetic relationship owing to their high environmental interaction. Currently, several biochemical loci encoding seed storage proteins, soluble proteins and isozymes are available to measure genetic variation between closely related germplasm resources. Among these, the protocol of polypeptide banding pattern of seed storage proteins is simple, easy and cost effective. Besides, it can reveal a considerable degree of polymorphism and a simple genetic control

mental influence.

amplification or radio-isotope labeling for DNA profiling of genotypes. Germplasm being a vital source of valuable genes for which characterization of plant genetic resources is indispensable to improve production level and quality of the crop produce. The present study was, therefore, carried out to assess genetic variation and study of genetic diversity using

SDS-PAGE of total seed storage protein for identification of elite divergent urdbean genotypes for further use in hybridization programme.

Materials and Methods

Seeds of 20 urdbean genotypes including a popular variety 'T9'(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 three standard proteins of known molecular weight i.e., ovalbumin (43kD), bovine serum albumin (66kD) and phosphorylase-b (97.4kd).

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).

Results and Discussion

Seed storage protein profiling has been standardized in urdbean (Ghafoor and Ahmad, 2005), grasspea (Roy *et al.,* 2001; Emre, 2009), mungbean (Tripathy *et al*., 2010a; Tripathy *et al*., 2010c), lentil (Yuzbasioglu *et al*., 2008) and adzuki beans (Sai-Ut, 2009). In the present investigation, SDS-PAGE of total seed storage protein revealed altogether 18 scorable polypeptide bands with molecular weights ranging from 32.5 to 132kD (Fig 1, Table 1). This envisaged that at least 21 multi-gene families are involved in seed storage protein expression in pigeon pea. However, Gangwar and Bajpai (2006) observed 26 polypeptide bands both high as well as low molecular weight ranging from 11.0 to 98.0kD. Four polypeptide bands at molecular weight positions 86.5, 48.0, 45.5 and 32.5kD were found to be monomorphic among which 45.5kD band was characteristically broad and dense. Rest of the bands had shown polymorphism to the extent of 77.8% among the test genotypes. It indicated that the genes controlling the expression of the above four monomorphic protein bands appeared to behave in single blocks.

Genetic variation in seed storage protein expression:

Variation in polypeptide banding pattern in other legumes (frenchbean, *Vicia faba*, chick pea, urdbean and mungbean) have been reported by many workers (Koenig *et al*., 1990; Polignano *et al*., 1990; Ladizinsky and Adler, 1975; Thakare *et al*., 1988; Tripathy *et al*., 2010a), but report on urdbean is scanty. However, wide range of intra-specific variation in protein profiles was observed in the present pursuit. As a whole, the resulting data matrix of the presence and absence of

bands resolved 167 polymorphic polypeptide bands out of total 247 bands over all the 20 test genotypes used in the study which reveals 67.61% polymorphism.

The present study using silver staining technique revealed great array of polymorphism in terms of qualitative (presence/absence and colour of bands) and quantitative (colour intensity and thickness of bands) differences in polypeptides dissociated following cleavage of storage protein fractions (Fig 1). Roy *et al.* (2001) also noted differences in band mobility, width and intensity in legumes. The observed differences in protein band intensity could be utilized in identification of high protein rich genotypes. In the present pursuit, the 86.5kD band is comparatively thicker in all genotypes except TPU 95-1 and TPU 4. Such types of close relationships and/or minor variations were also observed in chickpea (Kharkwal, 1999).

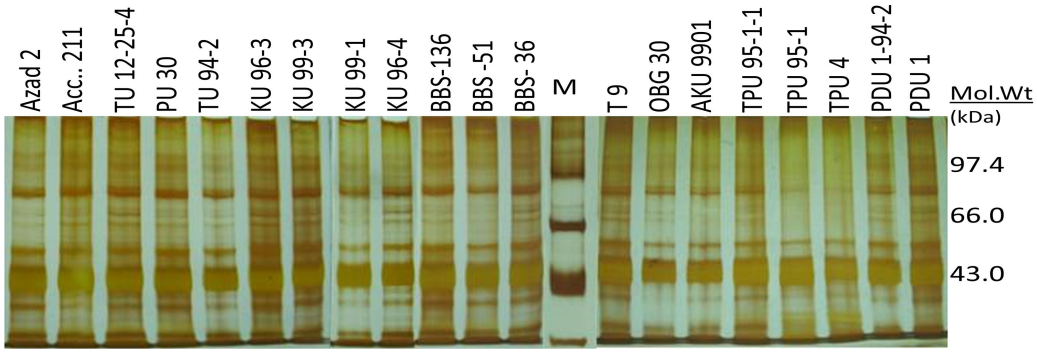

Fig 1. Seed storage protein profiles of 20 urdbean genotypes (lane 1-12 and lane 14-20), Lane 13: Molecular wt. marker (M).

In the present investigation, number of polypeptide bands in each genotype ranged from seven (TPU 95-1) to as high as 17 bands (TU 12-25-4) with an average of 12.35 bands/genotype. Besides, PU 30 and KU 96-3 recorded 16 polypeptide bandseach. These genotypes varied in polypeptide banding pattern and may be considered superior in protein quality provided the polypeptides revealed are not associated with anti-nutritional properties. Proteins being the direct gene products reflect the genomic composition of lines accurately to some extent and therefore, are ideal for study of genotypic distinctiveness. 17 protein types/profiles were detected for seed protein expression in the present set of test genotypes. Frequency of genotypes revealing each band was shown to be as minimum as 2 to maximum 20 indicating wide range of seed protein expression among the test genotypes. Fourteen test genotypes revealed their characteristic genotype-specific polypeptide banding pattern, while T 9 and TPU 95-1-1; BBS 51 and BBS 36; and both KU 99-3 and BBS 136 revealed common protein types. Such a high degree of homology in polypeptide banding pattern among some of the genotypes might be due to similar ancestry with narrow genetic base (Karup-panapandian *et al*., 2006) and subjected to similar selection for agro-economic traits during breeding programme. 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. Thakare*et al.* (1988), however, could not able to recover any polymorphism in the vicilin seed protein derived from four cultivars of mungbean.

Table 1. Electrophoretic (SDS-PAGE) polypeptide banding pattern of total seed storage protein in a set of 20 urdbean genotypes.

Bands	Mol. Wt (kDa)	Azad	Acc. 211	TU 12-25-4	PU 30	TU 94-2	KU 96-3	KU 99-3	KU 99-1	KU 96-4	BBS 136	BBS 51	BBS 36	T 9	OBG 30	AKU 9901	TPU 95-1-1	TPU 95-1	TPU 4	PDU 1-94-2	PDU 1	Total
		V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	
B1	**132.0**	0	1	1	0	0	1	1	0	0	1	1	1	0	0	0	0	0	0	0	0	7
B2	**123.3**	0	1	1	1	0	1	1	0	0	1	1	1	0	0	0	0	0	0	1	1	10
B3	**108.0**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	18
B4	**102.2**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	18
B5	**95.0**	0	0	1	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	6
B6	**86.5**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	20
B7	**75.8**	1	1	1	1	1	1	1	0	1	1	1	1	1	0	0	1	0	1	1	1	16
B8	**73.0**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0	1	1	17
B9	**680**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	19
B10	**59.2**	0	1	1	1	0	1	1	0	1	1	1	1	0	0	0	0	0	0	0	1	10
B11	**55.0**	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
B12	**48.0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	20
B13	**45.5**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	20
B14	**43.0**	0	0	1	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	1	8
B15	**38.2**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	19
B16	**35.5**	1	1	1	1	1	1	1	0	1	1	1	1	0	0	0	0	1	1	0	1	14
B17	**34.0**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	2
B18	**32.5**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**20**
Total		**11**	**15**	**17**	**16**	**13**	**16**	**15**	**11**	**13**	**15**	**14**	**14**	**10**	**8**	**8**	**10**	**7**	**9**	**11**	**14**	**247**

Table 2. Similarity coefficients between paired test genotypes for total seed storage protein banding pattern in a set of 20 urdbean genotypes.

Sl.No.	Genotypes	Azad	Acc. 211	TU 12-25-4	PU 30	TU 94-2	KU 96-3	KU 99-3	KU 99-1	KU 96-4	BBS 136	BBS 51	BBS 36	T 9	OBG 30	AKU 9901	TPU 95-1-1	TPU 95-1	TPU 4	PDU 1-94-2
		V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19
V2	Acc. 211	0.73																		
V3	TU 12-25-4	0.64	0.98																	
V4	PU 30	0.68	0.82	0.94																
V5	TU 94-2	0.84	0.64	0.76	0.81															
V6	KU 96-3	0.68	0.82	0.94	0.88	0.81														
V7	KU 99-3	0.73	0.87	0.88	0.82	0.75	0.93													
V8	KU 99-1	0.69	0.52	0.64	0.68	0.84	0.68	0.62												
V9	KU 96-4	0.84	0.75	0.76	0.81	0.85	0.81	0.75	0.71											
V10	BBS 136	0.73	0.87	0.88	0.82	0.75	0.93	1.00	0.62	0.75										
V11	BBS 51	0.88	0.93	0.82	0.76	0.68	0.87	0.93	0.56	0.80	0.93									
V12	BBS 36	0.78	0.93	0.82	0.76	0.68	0.87	0.93	0.56	0.80	0.93	1.00								
V13	T 9	0.90	0.66	0.58	0.62	0.76	0.62	0.66	0.75	0.76	0.66	0.71	0.71							
V14	OBG 30	0.72	0.53	0.47	0.50	0.61	0.50	0.53	0.72	0.61	0.53	0.57	0.57	0.80						
V15	AKU 9901	0.72	0.53	0.47	0.50	0.61	0.50	0.53	0.72	0.61	0.53	0.57	0.57	0.80	0.77					
V16	TPU 95-1-1	0.90	0.68	0.58	0.62	0.76	0.62	0.66	0.75	0.76	0.66	0.71	0.71	1.00	0.80	0.80				
V17	TPU 95-1	0.50	0.37	0.33	0.35	0.42	0.35	0.37	0.38	0.42	0.37	0.40	0.40	0.41	0.36	0.50	0.41			
V18	TPU 4	0.66	0.50	0.44	0.47	0.57	0.47	0.50	0.42	0.57	0.50	0.53	0.53	0.58	0.41	0.54	0.58	0.77		
V19	PDU 1-94-2	0.83	0.73	0.64	0.68	0.71	0.68	0.73	0.69	0.71	0.73	0.78	0.78	0.90	0.72	0.72	0.90	0.38	0.53	
V20	PDU 1	0.78	0.81	0.82	0.87	0.80	0.87	0.93	0.66	0.80	0.93	0.86	0.86	0.71	0.57	0.57	0.71	0.40	0.53	0.78

Genetic variation in a set of 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 (Javid, 2004 and Iqbal, 2005). TU 12-25-4 can be differentiated from PU 30 by absence of a high mol. wt polypeptide band at 132.5kD position. ACC 211, TU 12-25-4 and PU 30 revealed a unique 55kD band and this was absent in all other test genotypes. Similarly, 34.0kD band was unique to TPU 95-1 and TPU 4. In contrast, a 38.2kD polypeptide band was shown to be specifically present in all genotypes except OBG 30. Such genotype -specific protein markers could be reliably used for varietal certification and maintenance of pure seeds in seed multiplication programme.

Genetic similarity/distance: Genetic distance is the inverse measure of genetic similarity. Similarity index values between paired genotypes ranged from 0.33 to as high as 1.0 in between few combinations (Table 2). In fact, only 24 out of possible 190 paired genotypic combinations have similarity co-efficient value less than 0.50 indicating fair degree of homology among the test materials. TPU 95-1 followed by TPU 4 are the most divergent genotypes which revealed least average genetic similarity (0.41) with rest of the genotypes. Among all possible combinations; TU 95-1 (V17) with TU 12-25-4 (V3) revealed lowest similarity index value (0.33) followed by TU 95-1 with PU 30 and KU 96-3(SI=0.35). Such genotypic combinations could be selected in hybridization programme.

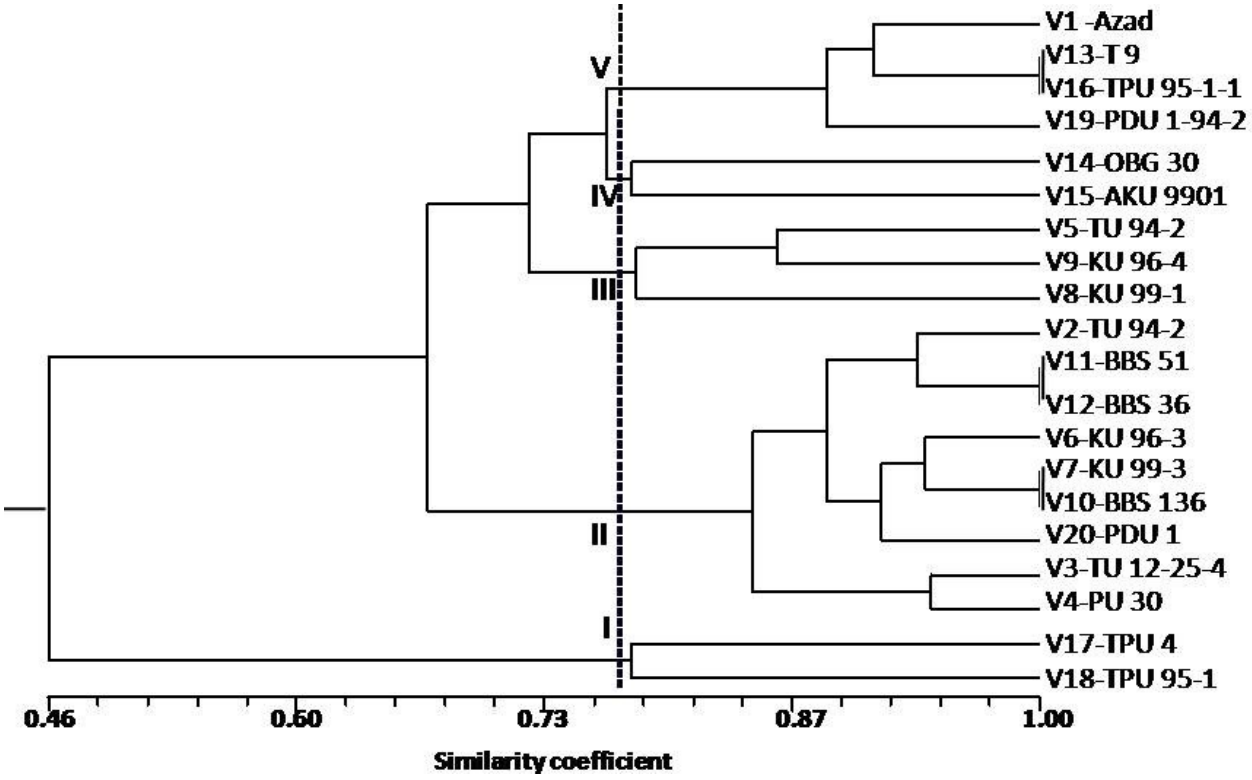

Fig 2. Dendrogram showing genetic diversity of urdbean genotypes based on total seed storage protein fingerprinting.

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 20 test genotypes for total seed storage protein expression is presented in Fig 2. Initially, the genotypes were distributed into five clusters e.g., Cluster I, Cluster II, Cluster III, Cluster IV and Cluster V within 78.5% phenon level. Initially, TPU 95-1 and TPU 4 separated out from rest of the test genotypes at 46% phenon level forming cluster I. Cluster-II was shown to be the next divergent group which included nine test genotypes. Cluster III Cluster-IV and Cluster V more or less maintained high inter-cluster homology. However, cluster VI was the largest cluster which included eighteen genotypes. Genotypes included under Cluster V find their position opposite extreme end of the dendrogram in comparison to Cluster I. In the present investigation, T 9 and TPU 95-1-1; BBS 51 and BBS 36; and both KU 99-3 and BBS 136 pair-wise clubbed together even at 100% phenon level as these exhibited exactly similar protein profiles. Therefore, SDS-PAGE in combination with 2-D electrophoresis is further suggested for documenting contrasting variation of isoforms of protein polypeptides.

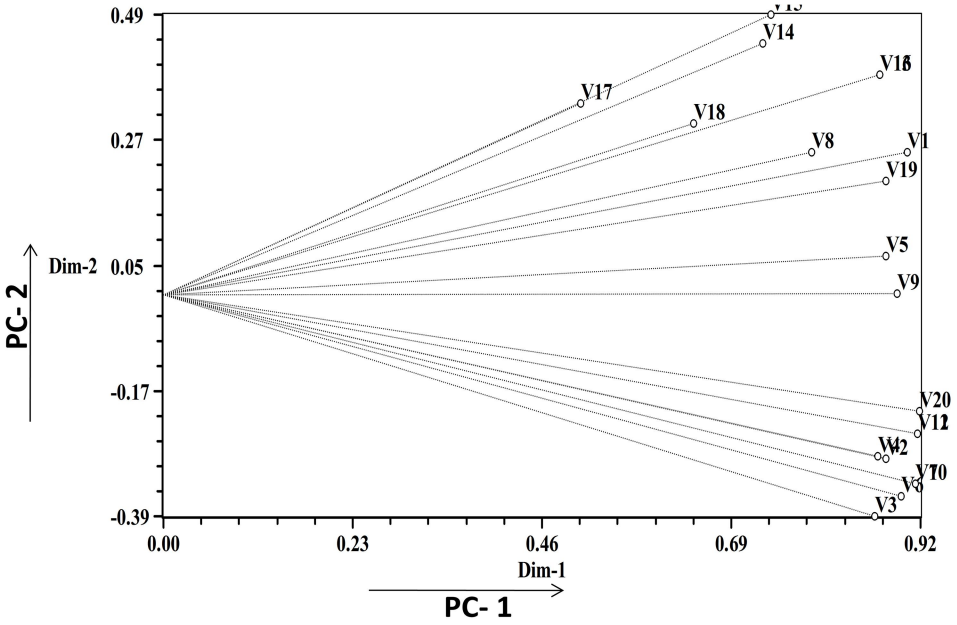

Fig 3. Two-dimensional scaling of principal co-ordinates(PC 1 and PC 2) using seed storage protein markers.

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, b) also reported clustering of few mungbean genotypes e.g., Pant M 5 and RCM 15 into a

single cluster due to their characteristic polypeptide banding pattern.

An effort was made to verify the protein profiles of the test genotypes in relation to their distribution in the dendrogram clusters., As expected, some of the genotypes with common protein type clubbed together in the dendrogram at 100% phenon level (Fig 2), whereas the rest of the genotypes isolated as single variety clusters were shown to have distinct unique protein types (Table 1). The positioning of unique protein type genotypes in the dendrogram was shown to have some relation with their mean genetic dissimilarity with rest of the test genotypes.

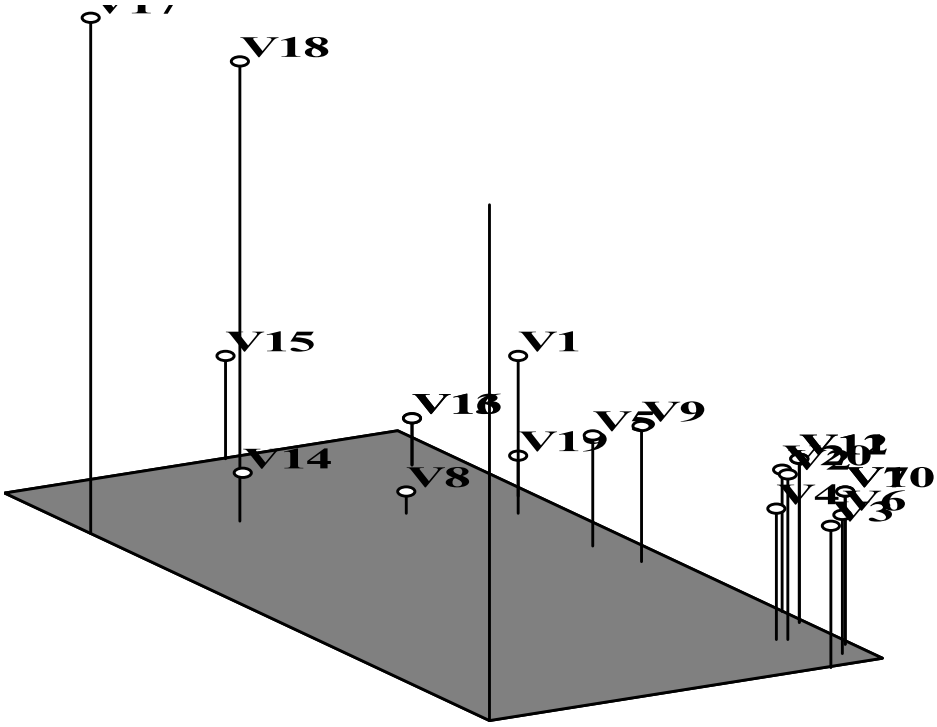

Fig 4. Three-dimensional scaling of principal co-ordinates (PC 1 ,PC 2 and PC 3) using seed storage protein markers.

Besides, the grouping of genotypes using two- and three dimensional scaling based on PCA values (Fig.3 &4) was found to be more or less consistent with that of UPGMA analysis. The three dimensional scaling represented clear grouping of test genotypes. TPU 95-1 and TPU 4 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. These genotypes could serve as a valuable source of genetic material for recombination breeding and other innovative approaches of crop improvement programme in urdbean.

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