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Evaluation of snake bean (*Vigna sesquipedalis*) through agro-morphological and SDS-PsAGE base markers

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Abstract

The present panel of work was conducted to investigate intra-specific genetic variability among Snake bean landraces. Genetic variation is considered as a basic feature to plants breeding and makes it viable to choose plants with appropriate traits. The methods to study genetic variation are morphological traits and SDS-PAGE. For this purpose, seeds of 40 landraces were collected. The morphometric parameters include both qualitative and quantitative traits. In 4 qualitative parameters significant level of variation was observed in seed shape, seed color and pod pigmentation except legume colour. For 11 quantitative traits descriptive statistics analysis done in which 100 seed weight show maximum coefficient of variance CV (0.37%) followed by pod length (0.32%), While minimum coefficient of variance showed by internode length (0.10%) followed by plant height 0.16% and seed length (0.18%). In comparative correlation, the plant height show highly positive correlation with plant biomass (.705**), seed per pod (.464**) and pod length (.362*), while highly negative correlation obtained with leaf length (-.335*).The protein profile of 40 snake bean landraces tested through SDS-PAGE shown 15 reproducible bands. High level of variation was observed in band B14 (0.93%), followed by B11 (0.80%), B15 (0.78%) B12 (0.75%), B13 (0.75%), B1 (0.65%), B5 (0.50%) and B8 (0.50%). Similarly low level of variation was noted in B3 (0.15%) followed by B4 (0.33%), B10 (0.38%), B6 (0.40%), B2 (0.45%), B7 (0.48%) and B9 (0.50%). The entire bands show polymorphism. All the traits show outmost level of divergence which is advantageous for further improvement of best variety.

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Introduction

Snake bean (*Vigna unguiculata* sub.sp. *sesquipedalis*) is a dicotyledon plant belong to leguminosae family, usually considered as a sub species of *Vigna unguiculata* (cowpea) of genus *Vigna* (Verdcourt, 1970). The chromosomes number of all domesticated *Vigna* are same which is 2n=2x=22 except creole bean. Snake bean is phenotypically different from cowpea and other species of *Vigna*. Its physiological and morphological traits make them differ from their wild ancestors. (Hammer, 1984). According to Newman 2006 snake bean was originated from Yunnan province of southern china. But majority of scientist belief that ancestors of domesticated snake bean was come from central Africa (Hansen, 2013).The annual production of this crop exceed to 13,450kg/ha and the area covered by it is approximately 300,000 ha (Timko *et al.,* 2007; Huque *et al.,* 2012). *Sesquipedalis* mean one-and-a-half-foot long.

The name was given because of its very long pod. In different countries it is known by various name such as yard long bean, asparagus bean, barboti, bora, string bean, podded bean, pole sitao, kusasagemae, chori, dawgauk (Newman, 2006). Growth habit notified in snake bean is dwarf, bush and climbing, pole. (Ofori and Klogo, 2005; Rambabu *et al;* 2016).Various colour of flower are found in snake bean plant such as light purple, dark purple, white and pink. Pod shows light green and dark green colour while some show purple with green tip and light green with purple tip. Seed are kidney shaped and from red colour to deep red, off white, red with white tip, brown, buff and black colour. The seed eyed pattern varied from small black ring, dark red ring, large mottled brown ring, brown holstienand red holstien pattern. (Rambabu *et al.,* 2016). Mature seeds of snake bean is composed of (2.8g) of protein, (8.35 g) of carbohydrate, (62 µg) folate, (0.41g) niacin, (0.11mg) riboflavin, (0.06mg) pantothenic acid. Vitamins A and C present in (865 IU) and (18.8mg) respectively. Other minerals such as iron 0.47mg, phosphorus 59mg, magnesium (44mg), sodium (4mg), calcium (50mg), zinc (0.37mg), copper (0.05mg), selenium (5 µg) are also found in their seeds (Benchasri *et al.,* 2017).

Genetic assortment show significant role in the population for survival of genotype. That's why important because the change in environment is continuously occurring and a genotype can adopt such ability to facing the challenges of surrounding environment (Andrew 2002). Furthermore, genetic diversity provides basic necessities to obtained high yielding variety in plant breeding program (Mansur and Rahman, 2009). According to (Nagalakshmi *et al* 2010), the genotypes collection and evaluation provide greater scope for genetic manipulation. The domestication of snake bean [*Vigna unguiculata* (L.) *Walp.ssp. unguiculata cv.-gr. sesquipedalis*] is of certain importance because the genome of snake bean (*Vigna sesquipedalis*) has experienced differing domestication from cowpea (*Vigna unguiculata*).

The genetics of domesticated crops is much interesting for plant breeders, their intentions was to identify useful genes, genome region or allele that have never seen in the wild ancestor of cultivated harvests and have a great impression in the enhancement of crops (Fuller, 2009; Izawa *et al.,* 2009 and Purugganan 2009). The determination of genetic diversity among different crop population was done by using different techniques such as SDS_PAGE, DNA marker analysis or morphological characterization (Ghafoor *et al.*, 2008; Nisar *et al.*, 2008). In the above three method the most prevalent and less expensive method are Electrophoresis and DNA marker analysis which needs less material and consume less time. By protein Electrophoresis the genetic diversity detects in functional genes. In protein electrophoresis SDS-PAGE are used Due to its effortlessness and independence of environmental features it is one of most commonly used biochemical methods to scrutinize the genetic construction of harvest plants (Edwards and Karp 1997, Ghafoor *et al.,* 2005). The purpose of the present work are to explore the genetic diversity for better improvement of crop.

Materials and methods

A total 40 genotypes of snake bean (*Vigna sesquipedalis*) were collecting from different area of district Dir and swat. The research work comprises of two phases conducted in field and laboratory for (SDS_PAGE) in the Department of Botany University of Malakand Khayber Pakhtunkhwa Pakistan during 2017-2018.

Morphological traits

In the present investigation 11 quantitative traits include plant biomass, plant height, seed width, seed length, leaf length, leaf diameter, pod length, pod diameter, seed per pod, 100 seed weight and internode length and 4 qualitative traits which is seed colour, seed shape. Mature dry Pod colour and pod pigmentation were observed to evaluate allelic variation in the 40 genotypes.

Biochemical characterization

For protein extraction the seeds of 40 genotypes were grind to fine powder with the help of pestle and mortle. From each samples 0.01g were taken in 1.5 eppendrof tube. Add 2ml of water, 2ml of 0.5ml NaCl or 1ml of water followed by subsequent grinding of 1m NaCl respectively. And use for SDS, 1ml transferred into new tube and centrifuge for 20 minute on 14000 rpm. Then 0.5 ml of supernatant transfer into new tube and add 0.5ml protein extraction buffer. After this kept in incubator for 2 hour on 70C^{o,} then40 micro liters added to each wells of 12.5% polyacrylamide gels containing separation gel (0.4% SDS, 3M Tris-HCL pH 9) and stacking gel (0.4% SDS, 0.4M Tris-HCL pH 7). The gel were then kept in the electrode buffer solution which contain 0.125% SDS, 129M Glycine, 0.025 M tris, and run it on 120V. The gel were then transfer to staining solution (440ml Methanol, Acetic acid 60ml, 2.25g coosmassie briliant blue CBB, 500ml Distilled water) and run the shaker for 20 minutes to stained the protein bands. The gel was then kept in de-staining solution (750ml distilled water, Methanol200ml, Acetic acid50ml) overnight until the bands become clear and visible. The protein data (0 1) was recorded in M.S Excel 2013. The presence of bands was represent by 1 while the absence of bands showed by 0 (M.S Excel 2013 sheet) for all genotypes.

These match coefficients were used to find out the relationship among study genotype by cluster analysis using Un-weighted Pair Group Matrix Average strategies (UPGMA) and dendogram was constructed by software PECORD

Results and discussion

Qualitative characters

Seed colour: Total 9 colour of seed were observed. On the basis of frequency and percentage distribution 2.5% seeds had black colour, 7.5% showed grey colour, brown colour were showed by 7.5% seeds. Some bean showed 15% dark brown colour with pointed head while 20% seeds had light brown colour with pointed head. Red colour seeds were also found in the beans which is light red (7.5%) and dark red (25%). Some seeds about 12.5% had pointed red colour while 2.5% seeds had white colour (Table 1).

Table 1. Frequency distribution of 4 qualitative traits of 40 snake bean.

Character	Categories	Frequency	Percentage
Seed shape	Kidney	16	40%
	Ovoid	4	10%
	Rhomboid	20	50%
Seed color	Black	1	2.5%
	Grey	3	7.5%
	Brown	3	7.5%
	Light Red	3	7.5%
	White	1	2.5%

	Reddish	10	20%
	Light brown pointed	8	20%
	Dark brown pointed	6	15%
	Reddish pointed	5	12.5%
Pigmentation	Highly pigmented	29	72.5%
	Rare	5	12.5%
	No pigment	6	15%
Pod color	Creamy white	40	100%

Seed shape: Three types of seed shapes were observed that is kidney shape, ovoid and rhomboid. Total 50% seeds had Rhomboid shape and 40% had kidney shape while the remaining 10% showed ovoid shape seeds (Table 1). Pod colour: In total 40 landraces of Snake bean there was no variation found in mature dry pod colour, all pod of bean show creamy white colour (Table 1).

Table 2. Descriptive statistics for 11 quantitative traits of 40 snake bean.

Parameters	Mean	Standard Error	Standard Deviation	Sample Variance	Range		CV%
					Minimum	Maximum	
PH	216.55	5.47	34.62	1198.46	153.00	321.00	0.16
PB	177.10	5.89	37.23	1386.04	88.00	238.00	0.21
LL	9.55	0.33	2.07	4.28	4.05	13.50	0.22
LD	5.99	0.16	1.02	1.04	4.00	7.50	0.17
SL	1.08	0.03	0.19	0.04	0.70	1.50	0.18
S\w	18.44	1.09	6.89	47.53	1.36	30.69	0.37
SW	0.58	0.02	0.12	0.01	0.40	0.90	0.20
S\P	14.85	0.64	4.07	16.59	6.00	22.00	0.27
PW	0.93	0.03	0.19	0.03	0.50	1.40	0.20
PL	28.61	1.45	9.16	83.96	7.90	47.20	0.32
IL	15.16	0.24	1.49	2.22	12.10	18.10	0.10

Note: PH plant height, PB plant biomass, LL leaf length, LD leaf diameter, SL seed length, S/W seed weight, SW seed width, S/P seed per pod, PW pod width, PL pod length, IL internode length.

Pod pigmentation: The pigmentation in pod was noticed in high level. 72.5% pod showed pigmentation in total of 40 snake bean. 12.5% had rare pigmentation while the remaining 15% showed no pigmentation (Table 1).

Quantitative traits

Descriptive Statistical analysis: Plant height of snake bean was divided into three categories (low, intermediate and high) the minimum plant height range from 153.0 cm while maximum range from 321.0 cm with mean 216.55,sample variance 1198.46 and standard deviation 34.62. In case of plant biomass categories into three categories (low, intermediate and high) the maximum plant biomass range from 238.00g while minimum range 88.00 g with mean 177.10 sample variance 1386.04 and standard deviation 37.23.leaf length of the samples divided into three categories (Low, intermediate and high) minimum range from 4.05 cm while maximum was 13.50 cm with mean 9.55, sample variance was 4.28 and standard deviation was 2.07.leaf diameter also divided into three categories (low, intermediate and high). Maximum range from 7.50 cm while minimum was 4.00 cm with mean 7.99, sample variance 1.04 and standard deviation 1.02.

Seed length categories into three categories (low, intermediate and high) the minimum range for seed length was from 0.70 cm while maximum range was 1.50 cm, mean was 1.08, sample variance was 0.04, standard variation was 0.19. for 100 seed weight the sample were divided into three categories (low, intermediate and high) minimum range was 1.36 g while maximum range was 30.69 g with mean value 18.44, sample variance 47.53 and standard deviation 6.89. Seed diameter of samples was divided into three categories (low, intermediate and high) the minimum range for seed diameter was range from 0.40 cm while maximum range was 0.90 cm with mean 0.58, sample variance 0.01 and standard deviation 0.12. Seed per pod categories into three categories (low, intermediate and high**)** maximum range for seed per pod was 22.00 while minimum range was 6.00 with mean 14.58 sample variance 16.59 and standard deviation 4.07. Pod diameter of snake bean divided into three categories (low, intermediate and high) maximum range from 1.40 cm while maximum was 0.50 cm with mean 0.93 sample variance 0.03 and standard deviation 0.19. In case of Pod length the sample were categories into three categories (low, intermediate and high) maximum range for pod length was 47.20 cm while minimum was 7.90 cm with mean 28.61, sample variance 83.96 and standard deviation 9.16.

Internode length of snake bean plant divided into three categories (low, intermediate and high) the minimum value was 12.10, the maximum was 18.10, the mean was 15.16, the sample variance was 2.22 and the standard deviation was 1.49 (Table 2).

Table 3. Correlation among the 11quantitative traits of 40 genotype**.**

	PH	PB	LF	LD	SL	S/W	SW	S/P	PW	PL	IN/L
PH	1.00										
PB	.705**	1.00									
LF	-.335*	-0.25	1.00								
LD	-0.13	0.06	.374*	1.00							
SL	-0.07	0.09	0.03	0.02	1.00						
S/W	0.07	0.17	0.02	0.10	.385*	1.00					
SW	-0.14	-0.04	-0.09	0.00	.705**	0.27	1.00				
S/P	.464**	.631**	-0.22	-0.04	0.11	0.04	0.07	1.00			
PW	0.02	0.06	0.10	-0.12	0.29	0.23	0.11	0.15	1.00		
PL	.362*	.594**	-0.26	-0.07	.363*	0.18	0.22	.728**	0.27	1.00	
IN/L	0.17	0.18	0.05	-0.11	-0.14	-0.17	0.10	0.04	-0.24	-0.17	1.00

** Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Correlation analysis of quantitative traits: Total 55 correlation coefficient value was observed in which 38 was positively correlated while 17 was negatively correlated. Among the 38 coefficient value 28 value were significantly positively correlated while 10 were strongly significant correlated. In negative correlation 1 value observed significantly strongly negative while the remaining 16 was negatively correlated. Highly significant positive correlation was noted for plant height with plant biomass (.705**), seed per pod (.464**) and pod length (.362*).

Plant biomass showed highly positive correlation with seed per pod (.631**) and pod length (.594**).

The highly positive correlation of leaf length was observed only with leaf diameter (.374*). Seed length showed highly positive correlation with seed weight (.385*), seed diameter (.705**) and pod length (.363*). Seed per pod showed highly positive correlation with pod length (.728**) (Table 3).

Table 4. Total genetic diversity present in 15 bands of 40 *Snake bean* landraces.

Bands	F	P%	A%	TGD%
B1	14	35	65	0.65%
B2	22	55	45	0.45%
B3	34	85	15	0.15%
B4	27	67.5	32.5	0.33%
B5	20	50	50	0.50%
B6	24	60	40	0.40%
B7	21	52.5	47.5	0.48%
B8	18	45	55	0.55%
B9	20	50	50	0.50%
B10	25	62.5	37.5	0.38%
B11	8	20	80	0.80%
B12	10	25	75	0.75%
B13	10	25	75	0.75%
B14	3	7.5	92.5	0.93%
B15	9	22.5	77.5	0.78%

Seed storage protein: The protein profile of 40 snake bean (*Vigna sesquipedalis*) landraces tasted through SDS-PAGE shown 15 reproducible bands. High level of variation was observed in band B14 (0.93%), followed by B11 (0.80%), B15 (0.78%) B12 (0.75%), B13 (0.75%), B1 (0.65%), B5 (0.50%) and B8 (0.50%).

Similarly low level of variation was noted in B3 (0.15%) followed by B4 (0.33%), B10 (0.38%), B6 (0.40%), B2 (0.45%), B7 (0.48%) and B9 (0.50%). The entire bands show polymorphism (Table 4 and Fig.5).

Cluster analysis of seed storage protein: During present work it was detected that dendogram separated genotypes into two linkages i.e. Linkage-1 and Linkage-2 which was further comprise of seven (7) clusters.

There were five clusters present in linkage-1 i.e. cluster 1, 2 and cluster 3, cluster (4), cluster 5 while lineage-2 consist of two cluster 6 and clusters 7.

Cluster-1 of linkage-1 consist of total 9 varieties of *Vigna sesquipedalis* V001, V004, V003, V005, V006, V008, V009, V007, V010. Cluster-2 consist of 7 lines i.e. V002, V013, V027, V022, V036, V023, V037. Cluster-3 has only two genotypes V014 and V028. Cluster-4 of the same linkage contain V021, V035 and V040 while cluster-5 of linkage-1 contain 4 genotypes V024, V038, V025 and V039.

Cluster-6 of linkage-2 consist of 9 genotypes of *Vigna sesquipedalis* V011, V012, V026, V018, V032, V019, V033, V020, V034 while cluster-7 of same linkage contain 6 lines V015, V029, V016, V030, V017, V031 (Fig.1 and2).

Cluster analysis of morphological data: In order to find out genetic diversity based on 11 different morphological traits.

The data of 40 landraces of snake bean were subjected to PC-ORD software. From the analysis of 40 genotype of snake bean a dendogram tree was obtained which delineated landraces into two linkage linkage-1 and linkage-2.

Which was further divided into 6 cluster. Linkage-1 consist of cluster-1 cluster-2, cluster-3 and cluster-4 while linkage-2 contain cluster-5 and cluster-6. Cluster-1 of linkage-1 contain 7 genotypes which

include V001, V016, V015, V035, V036, V026 and V029. Cluster-2 of linkage-1 comprises of 8 genotypes which include V002, V014, V037, V021, V027, V034, V024 and V031. Cluster-3 of linkage-1 consist of 6 genotypes included V007, V022, V013, V008, V009 and V018. Cluster-4 of linkage-4 comprises of 6 genotypes include V010, V012, V011, V038, V023 and V020.

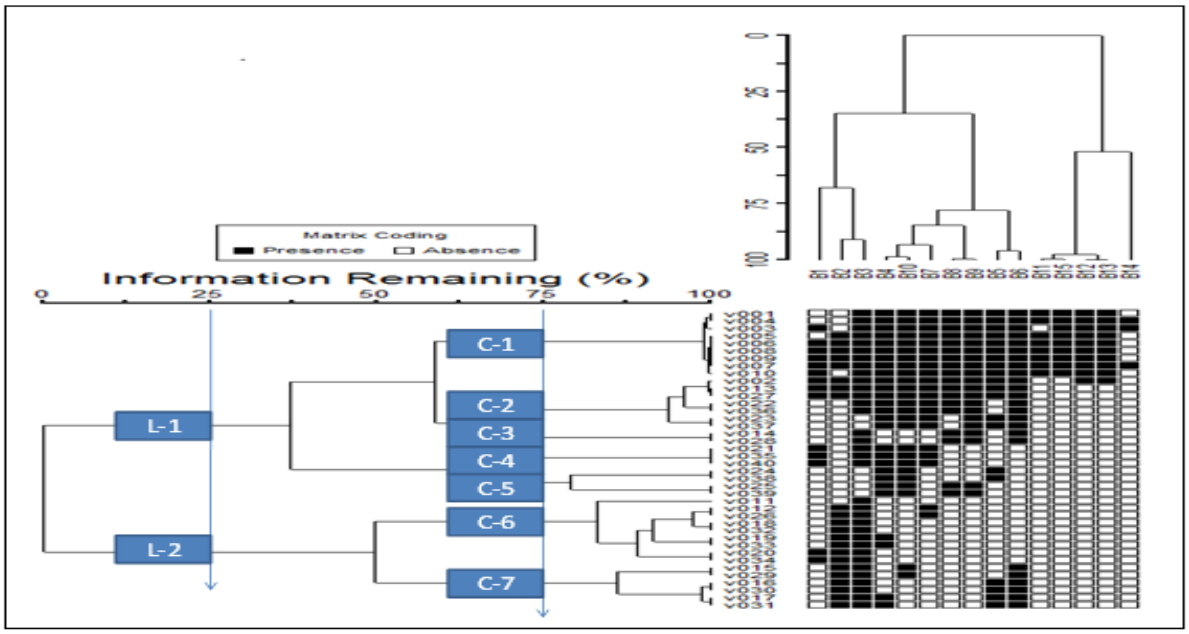

Fig. 1. Two-way Clusters Analysis based on seed storage proteins profile in 40 *Snake bean* landraces.

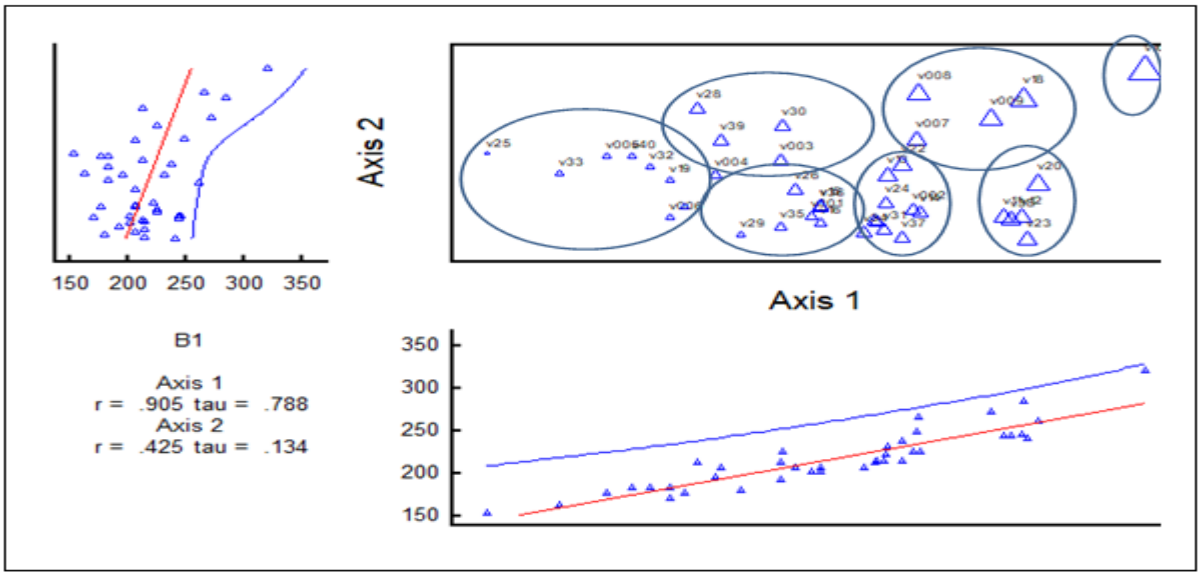

Fig. 2. PCA for 40 genotype of snake bean on the base of seed storage protein.

Cluster-5 of linkage-2 consist of 10 genotypes which include V003, V004, V030, V039, V028, V040, V006, V017, V019 and V032. Cluster-6 of linkage-2 comprises of only 3 genotypes included V005, V033 and V025 (Fig.3 and4).

Plant morphology

A significant variation found in qualitative traits on the basis of frequency distribution except mature dry pod colour all were creamy white and show no variation. In the present research, seed colour show high variability which is reddish, brownish, white,

black, light brown, light red, brown colour. Similarly to (kishtij Khatri *et al;* 2015*)* who's also reported that seed colour from brown to black and some other mottled type. (Karistsapol and Quanchi2015) worked on seven varieties of snake bean they reported four colour of seed that is white, black, brown and two colour's white-brown. In case of seed shape the present study observe three types of shape which is rhomboid ovoid and kidney similar to the research work of (kishtij Khatri *et al;*2015*)*. Rao *et al.,* 2006 also reported seed shape in his work from globular to kidney shaped.

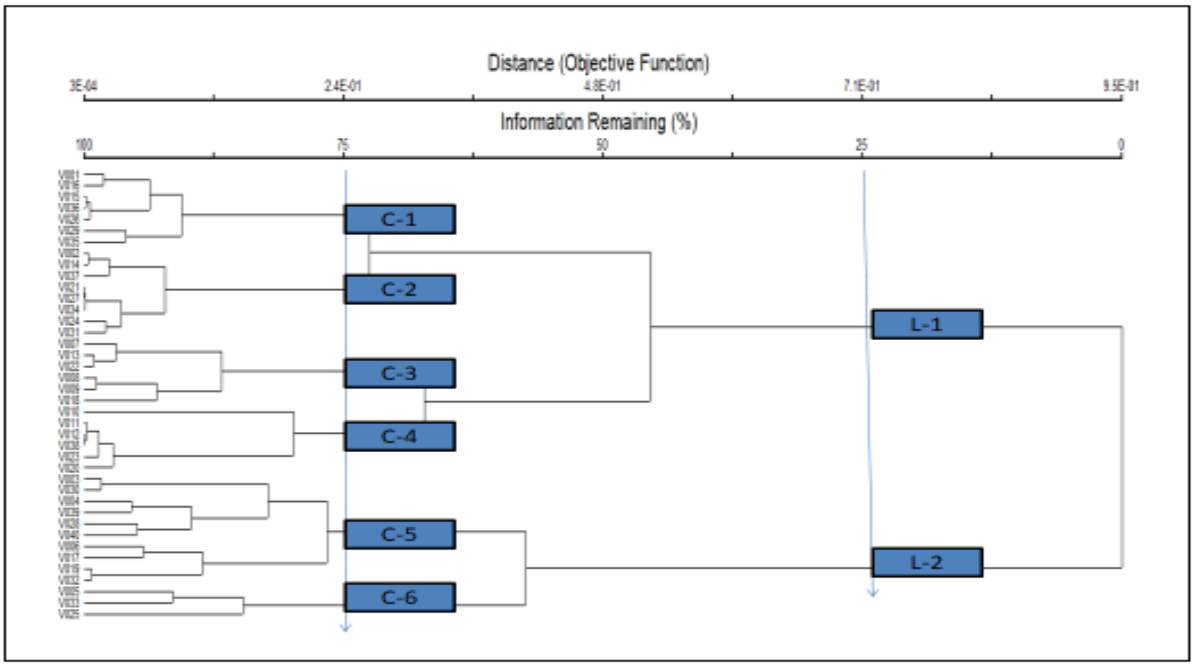

Fig. 3. One way cluster analysis on the base of 11 morphological traits.

The presence of Pigment is a dominant character over non pigmentation (Mustapha and Singh 2008). During the present study remarkable variability observe among different accession of snake bean. significant variation were observed in plant height from 150 to 321, plant biomass observe 80 to 238, Leaf length from 4.05 to 13.5, leaf diameter 4 to 7.5, internodes length 12.1 to 18.1, Seed length 0.7 to 1.5, seed width 0.4 to 0.9. During the present research work of snake bean the Pod length, pod width, seed per pod and 100 grain weight were similar to observation of (Littyverghase *et al,* 2015*)*. the pod length ranged was (7.9 to 47.2cm), highest pod length was (85.07), pod width observe from (0.5 to 1.4), snake bean pod width reported (-634.), while in the present research seed per pod reported from (6 to 22), ranged of maximum number of seeds in pod was 21.57, observation 100 seed weight was 23.65g similar as the present study the 100 seed weight ranged reported from (1.363 to 30.69).

(Rachie, 1985) reported in his statement the pod length 30-90cm, while another scientist Teerawat Sarutayophat *et al;* 2007 also reported about pod length ranged from 34.0 to 48.7 cm. Branca and La Malfag 2008) investigated the length of pod from 20-30cm.

Biochemical assessment

Seed storage protein profile is used as to resolve the taxonomic and evolutionary complications of various crops (Ladizinshy and Hymowitz 1979).

According to this when the genetic diversity high in a species then higher the chances of improvement in that species. During the present research work 40 genotypes of snake bean were analyzed through SDS-PAGE in which total 15 polymorphic bands was observed. Within banding pattern of snake bean genotypes high level of Genetic diversity observed, which an improvement is for researchers in future

breeding programs. Similarly (Asaf *et al.*, 2016) reported the same result for genetic diversity of *Pisum sativum* (L.). Our work is greatly support by (Vittal *et al* 2011) who analyzes 23 genotypes and observed deviation in the banding pattern of prolamine profiles. PC-ORD software was used to draw a Scatter Plot and dendogram tree for morphological data and also for seed storage protein, which showed significant deviation among the snake bean landraces. Scatter plot or PCA was created for the confirmation of genetic divergence which divided genotypes into 7 clusters on the basis of similarities and difference present among the genotype. As the genotypes in these clusters is from different areas of Malakand division.

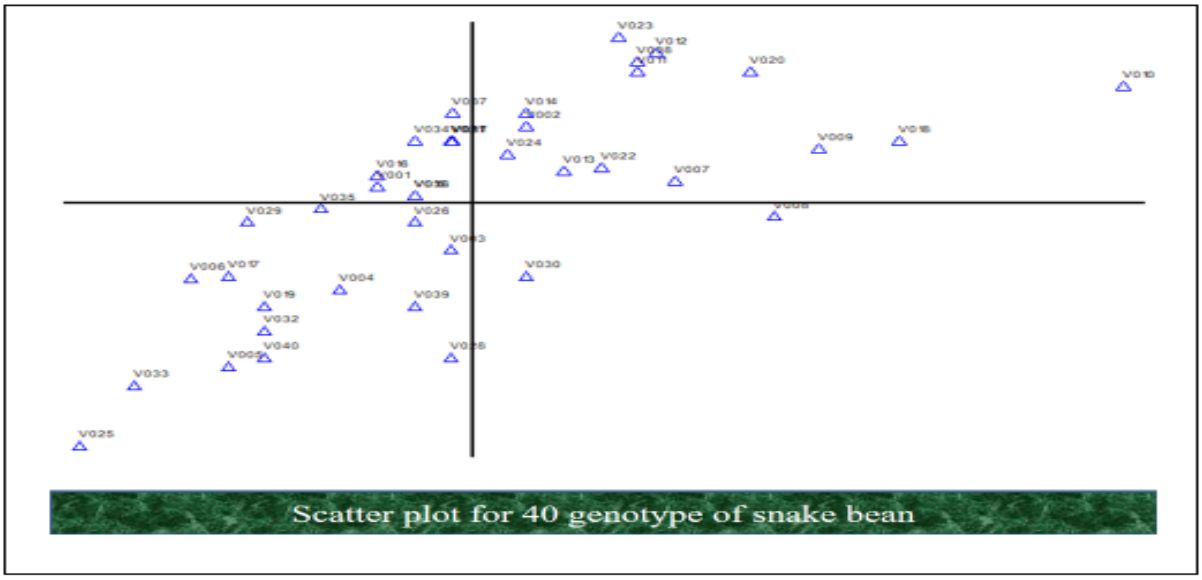

Fig. 4. Scatter plot for 40 genotypes of snake bean on the basis of 11 morphological traits.

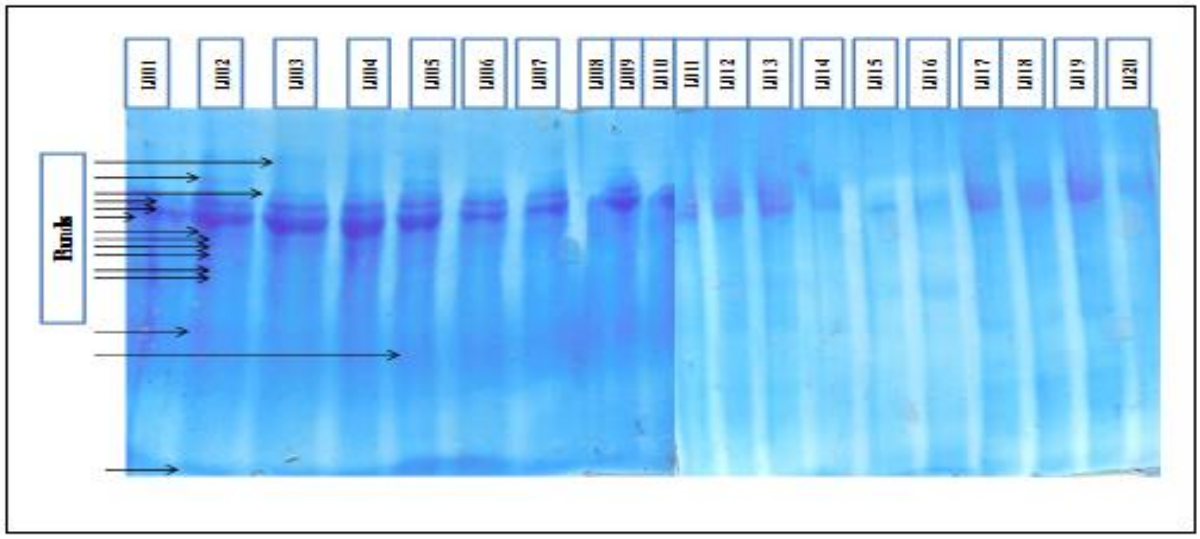

Fig. 5. Gel picture of 20 landraces of snake bean.

Dendogram tree separated snake bean accessions into two linkages i.e. Linkage-1 and Linkage-2 which is further comprises of seven (7) clusters. There were five clusters present in Linkage-1 i.e. cluster-1, cluster-2 and cluster-3, cluster-4, cluster-5 while lineage-2 consist of cluster-6 and clusters-7.As to construct a dimensional scatter plot and to complement the cluster analysis information of the

genotypes a PCA is used. It is a multivariate approach which is considered more explanatory concerning distance between the landraces (Hassan *et al* 2016).

Conclusion

The 40 snake bean landraces revealed highly significant variation for all the quantitative and qualitative characters. The variety V23, V11 and V9 obtained higher values for characters such as plant biomass, seed per pod, plant height and internode length, therefore these variety are recommended for general cultivation, while V33 and V25 show lowest results for plant height plant biomass and seed per plant, it may improve further through breeding program.

In protein profiling total 15 reproducible bands was observed in which High level of variation was observed in band B14 (0.93%), followed by B11 (0.80%), B15 (0.78%) and B12 (0.75%).These landraces shown some distinctiveness and interestingly. It makes them important for further investigation.

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