

## ESTIMATION OF GENETIC DIVERSITY AND SELECTION OF HIGH YIELDING GENOTYPES IN *PHASEOLUS VULGARIS* L. GERMPLASM

NAZIM HASSAN<sup>1</sup>, DELI WANG<sup>1</sup>, MUHAMMAD NISAR<sup>2\*</sup>, ZHIWEI ZHONG<sup>1</sup>, YU ZHU<sup>1</sup>, AJMAL IQBAL<sup>2</sup>, SAEED UR REHMAN KAKAR<sup>3</sup>, SYED FAZAL WADOOD<sup>2</sup> AND ABDUL GHAFOOR<sup>4</sup>

<sup>1</sup>Institute of Grassland Science and Key Laboratory of Vegetation Ecology, Northeast Normal University, Changchun, Jilin China

<sup>1,2</sup>Department of Botany University of Malakand Chakdara Dir (Lower) Pakistan

<sup>3</sup>Department of Botany University of Baluchistan Quetta, Pakistan

<sup>4</sup>Plant Genetic resources institute NARC, Islamabad, Pakistan

\*Corresponding author's email: mnshaalpk@yahoo.com

### Abstract

In present study 45 exotic *Phaseolus vulgaris* (L.) genotypes were assessed for assessment of genetic diversity through morphological & biochemical (SDS-PAGE) traits and selection of high yielding genotypes. One ways cluster dendrogram analysis of morphological traits segregated total genotypes to 2- lineages, lineages-1 and -2, separated at 80% similarity level. The lineages-1 further sub divided into 2 clusters i.e. cluster (C-I) and cluster (C-II), whereas lineage-2 consisted of one cluster (C-III). The high yielding genotypes were G3185 G (410g/plant) and G2785 (410g/plant) followed by G2613 (169g/plant) and G3185 (149g/plant). Biochemical characterization revealed that 11 reproducible polypeptide bands were observed in which 18.18% protein bands (band 2, 5) were monomorphic and 81.82% were polymorphic. Among polymorphic bands, band six showed outmost genetic diversity (17.7%) followed by band four and band eight showed 15.5% genetic diversity. Two way cluster dendrogram of biochemical characterization separated genotypes into two lineages L-1 and L-2. L-1 comprises of 20% genotypes with 50% genetic diversity, while L-2 comprised of 80% genotypes with 16.5% genetic diversity. Additional selection, multi locational trials for yield, and resistant to abiotic stress is significantly needed while, genotypes having similar banding pattern need additional characterization through morphological and 2-D electrophoresis in conjunctions with DNA based analysis.

**Key words:** Biochemical characterization, Morphological characterization, High yielding genotypes, *Phaseolus vulgaris* (L.).

### Introduction

*Phaseolus vulgaris* (L.) is an important legume crop which can be taken as source of protein grown for human consumption throughout the globe (Berber & Yasar, 2011). It can be grown from tropics to temperate regions with medium rain fall, however, in Pakistan it can be grown with average rain fed regions (Alghamadi, 2007). For fruit full grain yield the finest temperature for common bean is from 20°C to 25°C. High temperature effect seed quality and seed germination whereas low temperature stark growth (Alghamadi, 2004). The seed of common bean contains protein called phaseolin which is a rich source of vitamins, phenols, starch, and saccharides (Rodino *et al.*, 2001). Apart from food it is also an important medicinal plant which can be taken in oxidative stress, cardiovascular disorders, diabetes and cancer, (Camara *et al.*, 2013).

Genetic diversity play a vital role in crop improvement by widening the range of genes available to overcome yield challenges (Pervaiz *et al.*, 2010). The assessment of genetic diversity in germplasm encourages genetic variation by opening a breeding program (Rabbani *et al.*, 2010) and could be important in conservation (Zhou *et al.*, 2019). In crop plants genetic diversity is easily evaluated by using morphological characterization but morphological traits are adversely affected by environmental changes (Nisar *et al.*, 2009).

Using biochemical technique such as SDS PAGE for the estimation of genetic diversity is more precise and accurate because biochemical techniques are free of environmental fluctuations (Bretting and Widrlechner,

1995). It is trustworthy and widely used chemical technology to estimate genetic organization in germplasm (Sultana *et al.*, 2006) and among populations (Akbar *et al.*, 2019). Now a days this technology can be adopted for species identification (Sinha *et al.*, 2012; Sridharan *et al.*, 2013) and to study evolutionary and taxonomical relationships of numerous crop plants (Singh *et al.*, 2015). Because of rapid rise in population further 30% increase in common bean production is needed by 2050 (Palomino, 2012). To overcome this fall there is a dire need for the release of further high yielding common bean genotypes (Miles *et al.*, 2007).

Seed production in common bean is directly associated with agromorphic characters (Amanullah & Muhammad, 2011). In legume germplasm grain yield increased as pods and branches increased per plant, plant height, seeds per pod, dry matter, seed weight and harvest index (Ahmad *et al.*, 2000). The production of common bean is not enough in Pakistan to attain the needs, hence import it by paying a huge sum of money every year. Therefore the release of high yielding germplasm of common bean are highly needed for independence and to save imported exchange (Amanullah & Asim, 2011). Pakistan has limited lines/ genotypes of common bean, there is a dire need for selection of high yielding and potential genotypes of common bean to fulfill protein deficiency and support the life standard of farmers. Keeping in view the importance of common bean for country like Pakistan the current study was carried out, 1- To evaluate genetic diversity in exotic common bean germplasm, 2- To select high yielding genotypes in exotic common bean germplasm.

## Materials and Methods

**Plant materials:** The current research was conducted on 45 common bean genotypes received from PGRI (Plant genetic resources Institute National Agricultural Research Centre Islamabad Pakistan) to study genetic diversity through morphological and Biochemical (SDS-PAGE) characterization.

**Experiment:** Morphological traits were evaluated from 2013-2015, in the research area of Botanical Garden and Herbarium, Malakand University, Khyber Pakhtunkhwa situated at N 34° 40' 318" and E 72° 03' 753" with 726 meter elevation. The experimental design was randomized block design with three (03) replicates. Row to row distance was kept 50 cm while plant to plant as 10 inches. Weeds were controlled by hand during growth period and irrigations were made as per need (Ghamari & Ahmadvand, 2013). Plants were tagged/ selected randomly after seed germination from every line to score morphological traits. Total 39 morphological traits were evaluated, including 13 qualitative (Table 1) and 26 quantitative traits (Table 2).

**Table 1. Thirteen qualitative traits scored in 45 common bean genotypes.**

S. No.	Traits	S. No.	Traits
1.	Stem anthocyanin	8.	Pod color
2.	Leaf color	9.	Spots on tests
3.	Stipule color	10.	Cotyledon color
4.	Flower color	11.	Seed color
5.	Flower vein color	12.	Seed size
6.	Flower standard color	13.	Seed shape
7.	Keel color		

**Cluster analyses of morphological traits:** One way cluster analysis of 45 genotypes based on 39 morphological traits were achieved by using software PC-ORD (McCune and Grace, 2005). Morphological traits were examined to study similarity of traits through Pearson's correlation coefficient (Berber & Yasar, 2011).

**Genetic variation through SDS-PAGE:** For SDS-PAGE characterization dried seeds of every genotype were ground through pastel and mortal into fine powder. Protein was extracted through standard protocol following (Berber and Yasar, 2011; Laemmli, 1970). The electrophoretic technique was conducted using twelve percent (12%) polyacrylamide gel. A 20 µl of the supernatant was loaded in wells of polyacrylamide gel. The apparatus was linked with continuous electric source (120 V) till bromophenol blue (BPB) extended to bottom of gel plate (Laemmli, 1970). The gels were then stained and detained according to the protocol. To check the reproducibility of the score-able protein bands the experiment was carried out three times. The band presence was coded as 1, while the absence of bands as 0 following (Hassan *et al.*, 2016; Wadood *et al.*, 2016).

**Cluster analyses of molecular data:** Cluster analyses of the genotypes was determined by using Ward's method and two way cluster dendrogram was produced using the PC-ORD software (McCune & Grace, 2005).

## Results

**Morphological characterization:** A total of forty five (45) common bean genotypes were characterized for assessment of genetic diversity and selection of high yielding genotypes. During morphological characterization 13 qualitative and 26 quantitative characters were recorded.

**Cluster analysis of morphological traits:** One way cluster analysis of morphological traits was carried out through Ward's method (Fig. 1). Cluster separated entire genotypes into, lineages-1 and -2 parted at 80% similarity level. The lineages-1 was further sub-divided into 2 clusters i.e. cluster-1 (C-I) and cluster-2 (C-II), whereas lineage-2 consisted of only one cluster (C-III). In cluster-I, 7% were high yield producing genotypes while 93% were low yield producing genotypes. In cluster-II, 44% were high yield producing genotypes while 56% were low yield producing genotypes. Similarly, in cluster-III, 75% were high yield producing genotypes while 25% were low yield producing genotypes.

**Yield performance:** Total 45 common bean genotypes were evaluated for 39 different morphological traits during years 2013-15. The average grain yield of genotype G3185 and G2785 (410g/plant) was the highest followed by genotype G2613 (169g/plant) and G3185 (149g/plant).

**Correlation co-efficients:** Correlation co-efficients of twenty six (26) quantitative traits of forty five (45) genotypes were investigated (Table 2). Days to flowering showed high positive correlation with life span (0.93\*\*), grain yield (0.46\*\*), branches per plant (0.46\*\*), while high negative correlation with peduncle length (-0.44). Life span showed highly positive correlation with, grain yield (0.39). Seedling height showed high positive correlation standard width (0.41). Stipule length exhibited highly positive correlation with width (0.57) and seed width (0.42), Seed length exhibited highly positive correlation with seed width (0.51). Seed width exhibited high positive correlation with plant height (0.44). Nodes per plant showed highly positive correlation with height (0.52), grain yield (0.40). Stem diameter showed high positive correlation with number of pods (0.41), biomass (0.58), grain yield (0.55), and number of branches (0.47). Leaf length showed highly positive correlation with petiole length (0.48), leaf width (0.85). Leaf width showed high positive correlation with pod length (0.55), grain yield (0.40). Pod length exhibited highly positive correlation with the number of seed/pod (0.39). Number of pods showed high positive correlation with number of seed/pod (0.47), grain yield (0.55), plant height (0.40), biomass (0.57), total weight with pods (0.74) and number of branches (0.52). Plant height exhibited highly positive correlation with grain yield (0.41), number of branches (0.41). Biomass exhibited highly positive correlation with grain yield (0.86), number of branches (0.60). Grain yield showed high positive correlation with number of branches (0.62).

Table 2. Correlation co-efficient among 26 quantitative traits in common bean genotypes.

	DG	DF	LS	SH	SL	SW	Sd.L	Sd.W	N/P	IL	St.D	LL	PET.L	LW	PdL	Sdr.L	Sdr.W	PL	PW	P/P	S/P	PH	PB	GY	Hi
DF	-0.07																								
LS	-0.1	.93**																							
SH	-0.12	.30*	.32*																						
SL	0.07	-0.05	-0.04	0																					
SW	0.11	-0.15	-0.16	-0.01	.57**																				
Sd.L	0	0.11	0.19	0.2	.32*	0.26																			
Sd.W	-0.26	0.15	0.1	.41**	.42**	0.26	.51**																		
N/P	0.1	.35*	0.24	0.01	0.23	0.27	0.29	.35*																	
IL	-0.16	-0.11	-0.07	-0.17	0.28	0.06	0.07	0	-0.09																
St.D	-0.2	0.13	0.07	-0.07	0.19	0.22	0.1	0.26	0.28	0.02															
LL	0.07	0.05	-0.02	-0.18	-0.02	0.01	0.15	-0.09	0.11	0.24	0.18														
PET.L	-0.19	0.05	-0.01	-0.09	0	0	0.02	0.03	0.14	0.26	0.24	.48**													
LW	-0.03	-0.04	-0.06	-0.17	0.05	0.12	0.15	0.03	0.17	0.26	.31*	.85**	.37*												
PdL	0.02	-.44**	-.33*	-0.21	-0.09	-0.11	-0.24	-0.16	-0.06	0.09	-0.27	-0.19	0.19	-0.2											
Sdr.L	-0.02	0.19	0.07	-0.07	0.05	0.1	0.05	0.1	0.16	-0.07	0.1	0.11	-0.2	0.06	-.37*										
Sdr.W	0.05	0.08	0.02	0.12	0.04	0.22	0.01	0.23	0.09	-.32*	0.27	-0.28	-.30*	-0.21	-0.19	.321*									
PL	-0.18	0	0.05	-0.06	-0.17	-0.09	0.26	0.12	0.28	0.12	0.21	.35*	0.22	.55**	0.07	-0.14	-0.16								
PW	-0.06	-0.12	-0.09	-0.03	0.15	0.24	0.2	.31*	0.07	-0.14	.37*	0.16	0.05	.31*	-0.22	0.07	0.05	0.13							
P/P	-0.09	0.28	0.27	0.11	0.17	0.09	0.05	0.15	0.28	0.18	.41**	0.21	0.23	0.28	-0.11	-0.17	-0.29	0.11	0.17						
S/P	-0.14	.30*	0.29	0.03	0.13	0	0.18	0.12	0.29	0.28	0.17	0.29	.36*	0.25	-0.27	0.12	-0.02	0.28	0.27	.55**	0.18				
PH	-0.11	.32*	0.25	0.18	-0.01	0.09	0.17	.44**	.52**	0.09	.32*	0.13	0.17	0.28	-0.1	0.28	0.07	0.26	0.17	.40**	0.26				
PB	-0.12	.30*	0.23	0.17	0.1	0.16	0	0.2	0.26	-0.01	.58**	0.26	0.18	.40**	-.38*	0.13	0.06	0.17	.34*	.57**	0.18	.30*			
GY	-0.11	.46**	.39**	0.2	0.09	0.17	0.05	0.21	.40**	0.01	.55**	0.25	0.28	.35*	-.33*	0.05	-0.02	0.14	.37*	.74**	0.29	.41**	.86**		
Hi	-0.17	0.25	0.22	0.15	0.02	0.11	0.04	0.04	0.19	0.14	-0.09	-0.12	0.26	-.30*	0.01	-0.07	-0.22	-0.13	0.17	.32*	0.07	-0.17	0.16		
B/P	-0.12	.46**	.36*	0.14	-0.05	-0.09	-0.04	-0.06	.32*	0.08	.47**	0.26	0.15	.31*	-0.28	0.08	-0.03	.36*	-0.08	.52**	.33*	.414**	.60**	.62**	0

Note. DG=days to germination, LS= life span, SH=seedling height, DF= days to flowering, SW= seedling width, Sd.L= seed length, N/P= nodes/ plant, Sd.W =seed width, IL= internode length, LL= leaf length, St.D= stem diameter, PET.L= petiole length, PdL= peduncle length, LW= leaf width, SdL=standard length, SdW= standard width, PW= pod width, P/P= pods/ plant, PL= pod length, S/P= seed/pod, PB= plant biomass, PH= plant height, GY= grain yield, HI= harvest index, B/P= branches/plant

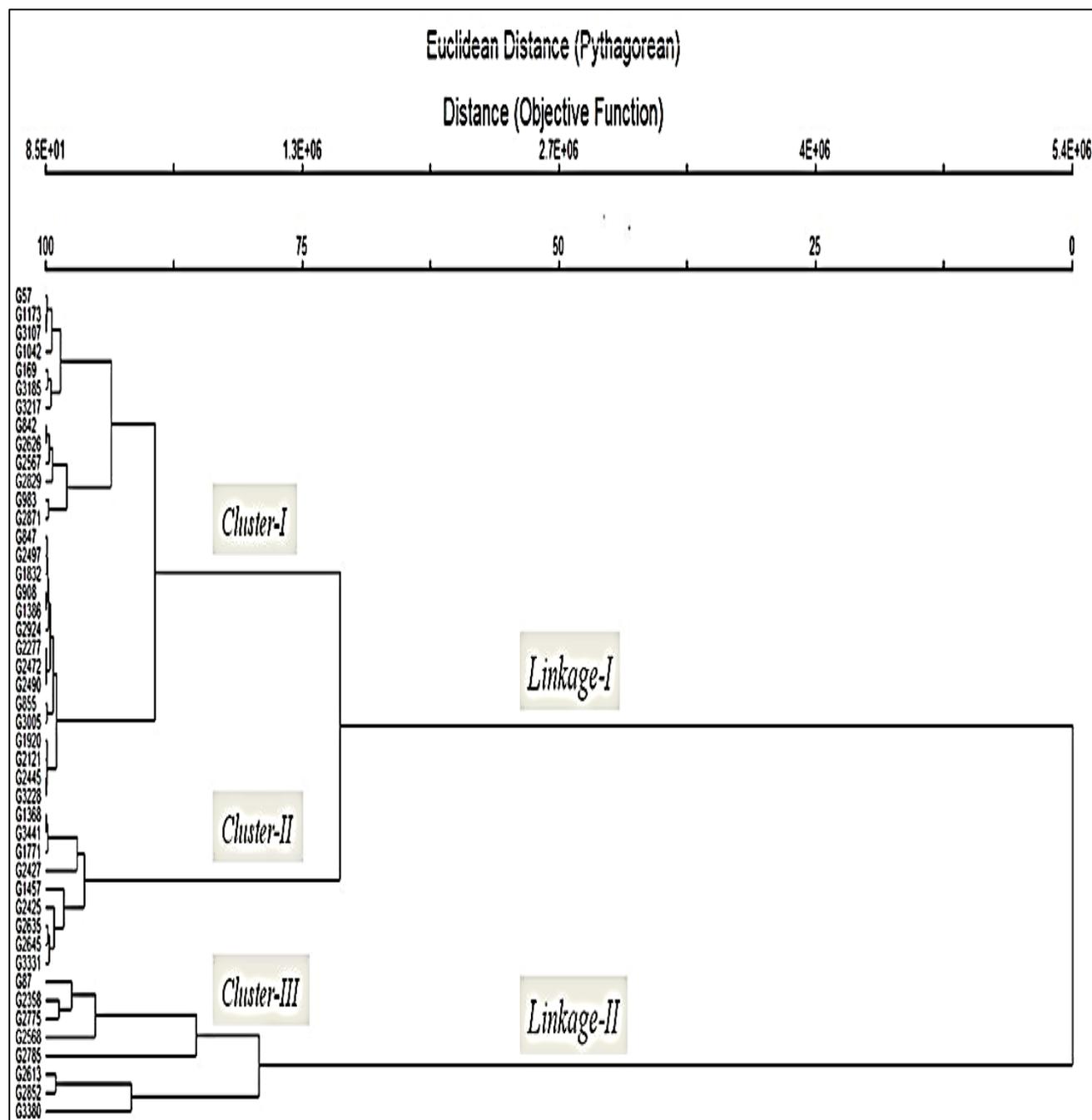


Fig. 1. One way cluster dendrogram of 45 common bean genotypes using PC-ORD software.

### Molecular characterization

**Genetic diversity within banding pattern:** The result of electropherogram showed that only two bands i.e. band-2 and band-5 were monomorphic and marked as specie specific bands while the remaining polypeptide bands were polymorphic (Fig. 2). Among polymorphic bands, band-1 showed 2.2% variation which is absent in genotype G2567, band-3 was absent in genotypes G2613, G3185 and G3441 with 6.6% genetic variation. The band-4 was absent in genotypes G1771, G1457, G1173, G2358, G57, G2497 and G1386 with 15.5% diversity. In genotypes G2852, G2775, G908, G2924, G3107, G2445, G2829 and G1386 the band-6 was absent with high level of genetic polymorphism i.e.17.7%. Band-7 displayed 6.6% genetic diversity and

band was absent in genotypes G3005, G169 and G1386. With 15.5% genetic polymorphism, the band-8 was missing in genotypes G2924, G855, G2635, G842, G847, G3185 and G3005. 11.1% variation was detected in band-9 due to missing in the genotypes G1771, G3228, G3107, G2445 and G2829. Band-10 was absent in genotypes G847, G1457, G2427, G3217 and G3441 while band-11 was found absent in G2829 and G1920 with 11.1% and 4.4% genetic diversity respectively.

**Intra genotypic genetic diversity:** The binary data was analyzed through construction of two way cluster dendrogram in order to find out intra genetic diversity present in common bean genotypes (Fig. 3). It was observed that all the genotypes were categorized in to two

lineages i.e. L-1 and L-2. 20% of bean population was separated by L-1, while a big portion of bean genotypes gathered in L-2. The genotypes G2852, G2775, G908, G2924, G1771, G3228, G3107, G2445, and G2829 were grouped in a single cluster of L-1 with 50% intra genotypic genetic diversity. The L-2 was further bifurcated into four clusters (C-II, C-III, C-IV and C-V) at 37.5% genetic diversity. The C-II clustered 50% genotypes of the L-II i.e. G2785, G1042, G2568, G2871, G2645, G3331, G87, G2425, G2121, G983, G3380, G2277, G1832, G2490, G2472, G2626, G2567 and G1920. Cluster C-III consisted the genotypes G855, G2635, G842, G847, G2613, G3185, G3005, G169, and G1386 with 25% of L-II bean genotypes. The cluster C-IV comprised of 11.11% genotypes consisting G1457, G2427, G327 and G3441 while C-V having the genotypes G1173, G2358, G57, G2497 and G1368 of the L-II genotypes. The genotypes belonging to similar cluster showed similarity at protein level and displayed low intra genotypic genetic diversity.

## Discussion

Common bean is used as a source of protein throughout the globe (Santalla *et al.*, 1999). In Pakistan countless peoples are suffering from malnutrition due to protein deficiency in their food. They only take pulses to full fill their proteins requirements. Its production decreases day by day as it is mostly cultivated in hilly areas. To overcome this problem there is a dire need for introduction of new exotic genotypes of common bean in Pakistan. In this connection 45 exotic common bean genotypes were characterized at research area of Botanical Garden University of Malakand Pakistan. Out of total only 16 genotypes were observed as high yielding. The outmost four high yielding and best fit genotypes were G3185, G2785 (410g/plant), followed by genotype G2613 (169g/plant) and G2568 (149g/plant).

Agromorphic characters play a vital role in dry matter production (Amanullah & Muhammad, 2011). In our study, number of pods/ plant exhibited highly positive and significant correlation with stem diameter and pod width exhibited significant and positive correlation with seed width, leaf width and stem diameter. Pod length showed significant and positive correlation with leaf length, and highly significant with leaf width. It might be due to genetic differences among germplasm. Same results were also observed in common bean by (Rodiño *et al.*, 2001) that there was positive and significant correlation between stem diameter, number of pods, pod length, number of branches per plant and leaf length.

Days to flowering exhibited positive correlation with plant height, seed number/ pod, grain yield, stem diameter, seed width, number of nodes and pod length. It might be due to genetic differences among germplasm and effects of ecological factors. The study is in connection with Awan *et al.*, (2014) who evaluated 13 local and exotic common bean genotypes and found positive correlation between stem diameter, pod height, pod length and, seed height, seed weight, seed length and seeds per pod.

Harvest index was observed varied significantly maximum (82.6%) for genotype G1920 and minimum (18.6%) for genotype G2775 with average harvest index (57.4%). Harvest index exhibited significant and negative correlation with width of leaf and significant positive correlation with seeds/ pod. It might be due to genetic differences among germplasm. Same was also observed by Shrivastava *et al.*, (2001) that harvest index in legumes significantly affect yield, similar results were also observed by Amanullah and Muhammad (2011) that, yield in cow peas germplasm is associated with harvest index, number of pods, seed weight, number of branches and plant height.

Branches number/ plant exhibited high positive and significant correlation with stem diameter, days to flowering while positive significant correlation with days to maturity, number of nodes and leaf width. Total seed weight exhibited high significant and positive correlation with leaf width and stem diameter. Same results in cow pea germplasm were also observed by Ahmad *et al.*, (2000), that Harvest index is positive correlated with seeds number/ pod and total yield exhibited positive correlation with branches number, dry matter, plant height, seeds per pod, and weight of seed. It might be due to genetic differences among germplasm. The results are in connection with Amanullah *et al.*, (2006) who observed positive correlation between yield, pod number and harvest index while branches/ plant exhibited positive correlation with plant height and yield.

Seeds number/ pod exhibited significant and positive correlation with petiole length and days to flowering whereas standard width exhibited negative and significant correlation with petiole and internode length. Seed width exhibited positive and high significant correlation with seed length, seedling height and stipule length. Seed length exhibited significant and positive correlation with length of stipule. It might be due to genetic differences among germplasm. The results are in close connection with Amanullah & Asim, (2011) who also observed significant variation in seeds per pod in common bean germplasm.

Plant total weight exhibited significant and positive correlation with days to flowering whereas highly positive and significant correlation with stem diameter, number of nodes, and leaf width. It might be due to genetic differences among germplasm with respect to seeds per pod plant height and vigor. Current results are in line with the results of Amanullah *et al.*, (2006) and Shrivastava *et al.*, (2001) who observed in common bean and soybean germplasm had significant difference in plant total weight with respect to number of nodes, seeds/pod and plant height.

Genetic diversity plays a vital role for survival and fitness of species in dissimilar agro- climatic zones (Lima *et al.*, 2012). The collection of improved traits depends on genetic variability percentage among cultivars (Amanullah *et al.*, 2006). Several studies have been conducted to evaluate genetic diversity within and among common bean genotypes on the basis of seed protein analysis and phenotypic traits (El-Awady & Hamed, 2016).

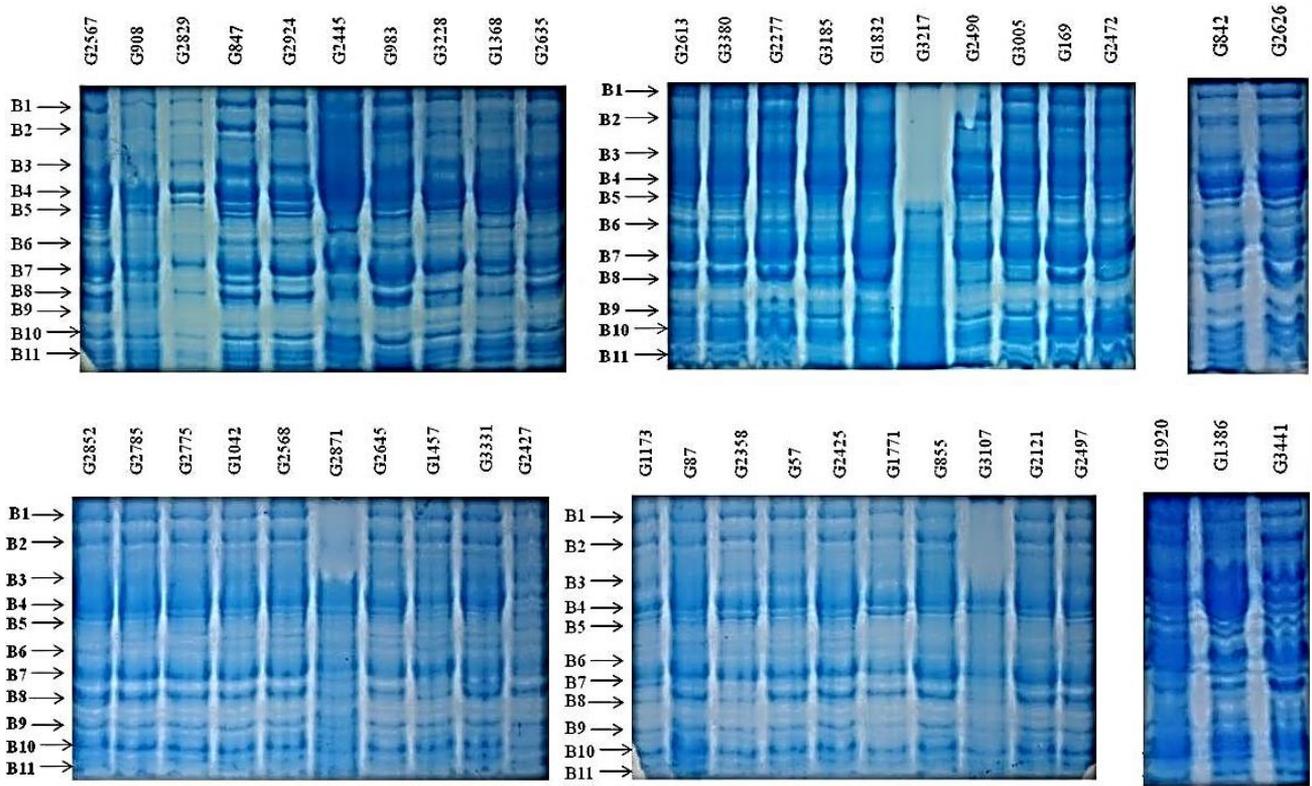


Fig. 2. Electrophorogram indicating banding pattern of 45 common bean genotypes.

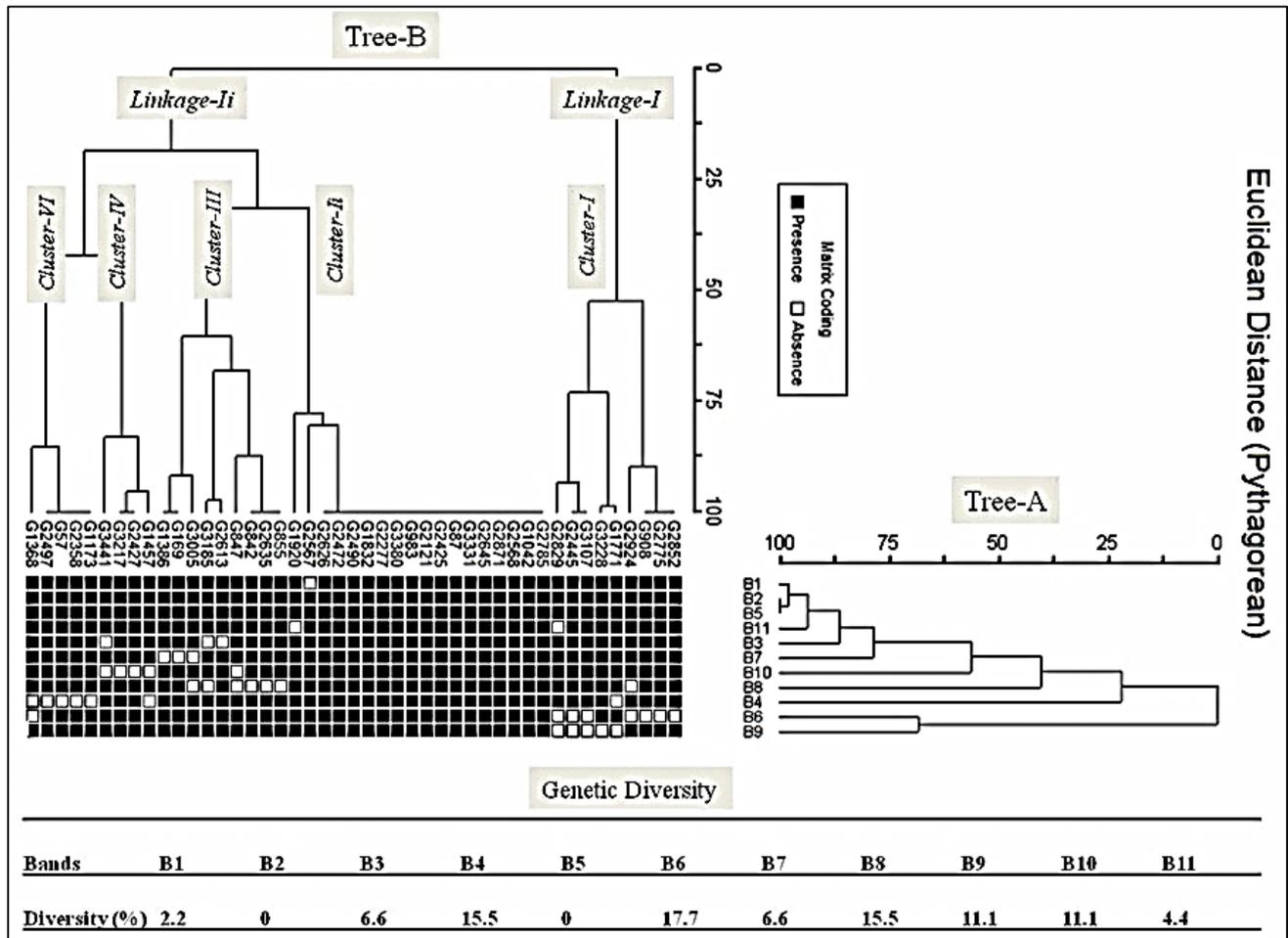


Fig. 3. Two way cluster analysis indicating intra genotypic genetic diversity in 45 exotic common bean genotypes.

For the study of genetic diversity and to distinguish cultivars SDS-PAGE has already been applied as a reliable and practical method hence, current research was carried out to evaluate and access intra specific genetic diversity of seed storage proteins in common bean through SDS PAGE. Out of total population 35.56% genotypes G2785, G1042, G2568, G2871, G2645, G3331, G87, G2425, G2121, G983, G3380, G2277, G1832, G2490, G2472 and G2626 indicating similarity in their banding pattern while the remaining 64.44% bean genotypes indicating genetic diversity in their protein banding pattern. Among all the reproducible protein bands, the B-II and B-V were specie specific bands; high level of genetic polymorphism was observed in B-VI with a percentage of 17.7%. Our results are in close connection with Nisar *et al.*, (2009) who studied genetic diversity in 97 genotypes of *Pisum sativum* L. and observed 34 bands among these, 26.7% bands were monomorphic, while 73.5% bands showed polymorphism and exhibited 70.6%, and 64.7% variation respectively, Similarly Bhargav *et al.*, (2016) while studying genetic diversity through SDS PAGE in 20, common bean genotypes, observed 22 bands in which 20 were polymorphic (91%) and 2 were monomorphic (9%) and (Alege *et al.*, 2014) observed maximum 0.93 genetic affinity in *Vigna subterranea* and *Arachis hypogaea* while minimum 0.32 in *Senna siamea* and *Albizia lebeck* using SDS-Protein electrophoreses. Same kind of results and process for evaluation of genetic diversity through SDS PAGE were also observed in Lima bean by Lioi *et al.*, (2005), common bean Scarano *et al.*, (2014), chickpea, Hajibarat *et al.*, (2015) *Pisum sativum*, Ghafoor and Ahmad, (2005), *Lens culinaris*, Sultana *et al.*, (2006) and in Guar germplasm (Jan *et al.*, 2019). Our study successfully determined high yielding genotypes, which could be best fit for cultivation in many localities of Pakistan especially in Khyber Pakhtunkhwa, Pakistan.

### Conclusion and Recommendations

High yielding genotypes i.e. G2785, G2613 and G2568 could be the best fit genotypes for common farming, particularly for improvement and good agronomic practices in different locations of Khyber Pakhtunkhwa, Pakistan. Apart from high production and best fit and effectiveness it will also increase the income of farmers. There is a dire need for classification and evaluation of these germplasm with respect to yield, quality and resistance to abiotic and biotic stress. Assessment of genetic diversity in crop germplasm through morphology and SDS-PAGE electrophoresis can be used economically. It is recommended that additional methods like random amplified polymorphic DNA (RAPD) must be used for fruitful results.

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