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Phylogenetic relationship among selected wild and cultivated Fabaceae species

Noor Muhammad[1,2*], Muhammad Khalil Ullah Khan[1], Nisar Uddin[2], Niaz Ali[2] and Muhammad Romman[3]

[1]College of Horticultural, Hebei Agricultural University, Baoding Hebei, **China**
[2] Department of Botany Hazara University Mansehra, KP, **Pakistan**
[3]Department of Botany, University of Chitral, KP**, Pakistan**

***Correspondence:** noorpk_1990@yahoo.com **Received 11-10-2020, Revised: 12-11-2020, Accepted: 20-11-2020 e-Published: 21-11-2020**

The phylogenetic and comparative analysis of the Fabaceae family is an important and powerful tool for legume plant breeders since it identifies close relatives as focal points for legume crop improvement and also provides information on wild relatives of legumes crop species. The present study was designed to examine the phylogenetic relationship amongst the selected species of family Fabaceae. For the estimation of phylogenetic relationship among 160 genotypes of family Fabaceae belonging to 8 different species. Both morphological and biochemical characterizations were explored. Data were analyzed using computer software SPSS and PC-ord5. In SDS-PAGE analyses, nine loci were recorded; out of the nine loci, B-9 was missing in *V. unguiculata* and *V. radiata R. rothii and A. platycarpa*, B-1and B-9 were absent *V. radiata* var. *sublobata* while B-7 & 9 were absent in *R. capitata*. The absence or missing band could be helpful in the identification of these species. The interspecies locus contribution was 88.888%. Locus/band 6 (B-6) was present in all collected genotypes of the current study. The current data reveal ample intra and inter-species genetic diversity within these eight species, each species maintaining species-specific individuality in the area irrespective of environmental fluctuation. The presence of Locus B-6 in all the collected genotypes was considered as a family-specific locus suggest their close genetic similarity and common heritage, and was considered as a family-specific locus for the selected species.

Keywords: Fabaceae, Morphology, SDS-PAGE, Phylogeny, Cluster analysis

INTRODUCTION

The leguminous family is an important food legume family generally known as Fabaceae consists of approximately 20,000 species, considering the third largest family after the Orchidaceae and Asteraceae (Noor et al. 2018). It is found all over the world. The most important earliest legumes crops of mankind were soybean and *V. radiata* in East Asia; faba bean, lentil, chickpea and pea in the Fertile Crescent of the Near East; and common bean or lupin in Central and South America. Legumes usually associate with nitrogen-fixing bacteria provides additional value to agriculture and thereby play a vital role in natural environments. Furthermore, the legume species pea was the fundamental experimental entity for Mendel's pioneering work in founding the fundamental basis of heredity (Sm'ykal et al. 2014).

All the legumes are valuable sources of nutrients (proteins, minerals, vitamins) and play a crucial role in the human diet. The grain legumes have a unique place in world agriculture due to their high protein content (Muhammad et al.

2018). The word pulse in Pakistan is applied for edible legumes, and Dal is used for decuticled split legumes. Legumes are the primary sources of amino acids, principally lysine and leucine, in addition to some vitamins and β carotene. Legumes take second place after cereals as a source of calories and protein in human nutrition. Several nutritionists have proposed partial replacement of animal food with legumes to enhance the overall nutritional dietary status. Pulses are rich in proteins. e.g., garden pea, broad bean, soya bean, black gram, green gram, Cajanuscajan, etc. as well as it is a source of vegetables like garden pea, mungphali or groundnut. Oil can be extracted from soya bean and groundnut.

Numerous attempts have been made for identifying genetic variations and improvement in protein quality and quantity. The use of available genetic diversity among selected germplasms in a breeding program plays a crucial role in crop improvement (Ghafoor et al., 2002; Muhammad et al., 2018). Usually, the classification of several subgenera, species, and subspecies is based mainly on morphological features (Ghafoor et al., 2002; Muhammad et al., 2018; Muhammad et al., 2019). Nonetheless, these traits may not be significantly distinct and usually require growing plants to maturity before identification. Furthermore, morphological features may be unstable due to environmentally induced phenotypes (Ghafoor et al., 2002; Muhammad et al., 2018). Over the decades, the approaches for perceiving and assessing genetic diversity have extended from the study of distinct morphological characters to biochemical and molecular characters. Among biochemical practices, (SDS-PAGE) is one of the most widely used techniques due to its robustness and easiness for revealing crop plants genetic organization. SDS-PAGE is thought to be a consistent technique because seed storage proteins are mainly free of ecological variations (Gepts, 1989; Muhammad et al., 2019). The seed protein patterns acquired by electrophoresis have been successfully used to resolve the taxonomic and evolutionary problems of several crop plants (Ladizinsky, 1979; Muhammad et al., 2019a). This technique can also be applied for distinguishing cultivars of particular crop species (Moller and Spoor 1993; Muhammad et al., 2019b). The seed storage proteins have been used as genetic markers in four major areas; analyses of genetic diversity within and between accession, plant domestication concerning genetic resources conservation and breeding, genome relationship, and as a tool in crop improvement (Ghafoor et al., 2002; Muhammad et al., 2019b). The genetic variation of seed proteins of some *Vigna* species grown in China evaluated by SDS-PAGE and reported that the seed proteins profiles of typical species of *Vigna* such as yardlong bean, rice bean, and small bean to be more similar than mung bean and black gram. The seed storage profiling demonstrated to be a powerful tool for discriminating *Vigna radiata* and *Vigna mungo* (Ghafoor et al., 2002). The electronic conductivity is proportional to both the density and the drift mobility of the charge carrier (Bhad and Sangawar, 2012; Muhammad et al., 2019b).

Rebuilding the phylogenetic relationship of the Fabaceae is vital to the understanding of the origin and diversification of this family. Phylogenetic studies of Fabaceae initiated by the plastid *rbcL* gene (Kass, 1996) tracked by investigation, including the more variable *matK* gene (Lewis et al., 2005). Both are now recognized as universal barcoding regions for plants (Charmaine, 1998). Still, the representation is far from being complete, however, as many species have not yet been sequenced or are characterized by just one or two germplasms. With each day passing on, more sophisticated techniques are being developed, and many of these tools have been applied for *Cicer aretinum*, *P. vulgaris*, cowpea, and soybean, as well as for the model legumes *Medicago truncatula* Gaertn. and, *Lotus japonica* (Regel) K. Larson. The monophyly of the family has been continually validated through molecular systematic (Doyle, 1995). Seed protein is of specific importance in legumes and holds additional benefits. Using the electrophoretic pattern of seed storage protein is cost-effective, easy handling and thus suits developing countries where research grants are scarce (Singh and Ntare, 1985).

Previously, a lot of work was carried out in genetic diversity of Pakistani legumes. However no phylogenetic systematic attempt has been carried out to comprehend the degree of genetic variation in Pakistani legumes involving wild and cultivated species. The current study aimed to understand the interspecies variation and phylogenetic relationship among Fabaceae species growing in the Malakand Division, KP, Pakistan.

MATERIALS AND METHODS

Plant materials

In this current project, several investigative trips were arranged to different agro-ecological zones of Malakand Division, KP, Pakistan in 2017 – 2018. During the expedition, different zones were visited which are presented in table 1. A total of 160 genotypes of 8 species of family Fabaceae were collected from the below Zones to assess the interspecies phylogenetic relationship and genetic diversity in seed storage protein profile.

Morphological characterization

Morphologically both the (qualitative and quantitative) characters were scored. Quantitative traits which were measured with the help of Verneircalipers are: petiole length, leaf length, leaf width, seed length, seed width, seed thickness, and seed weight, pod length, No. of seed per pod, No. of pod per plant, inflorescence length, inflorescence width, 100 seed weight, No. of branches per plant, plant height, stipule length, Biomass. Characters mean was found out after measuring 3 different samples (small, medium, large) of each quantitative character.

The observed qualitative characters are leaf color, leaf shape, seed texture, Hilum color, seed coat color, seed shape, leaf pubescent, leaf stipule, flower color, spots on the seed.

The data of both quantitative and qualitative characters of 160genotypes (total of 27 characters) was noted, and the binary matrix data was subjected to computer software the PC-ord shown in (figure 1). The result of the cluster analysis was presented as a phylogenetic tree (Dendrogram) based on the linkage distance (figure 1).

Protein Profiling

To estimate the level of phylogenetic relationship, the total seed protein profile was carried out using SDS-PAGE. For this purpose, a single seed of each landrace was crushed into powder. 400µl of Protein Extraction Buffer using the protocol of Laemmli, 1970, modified by Noor et al. (2018). Relationship catalogs of 160 genotypes were designed for all possible pairs of protein sorts and used to create a dendrogram by computer software PC-ord. v 5 McCune (McCune, 1997) in Window 8. The data were noted from the destined gel based on the presence and absences of protein bands, i.e., '1' for the presence and '0' for the absence.

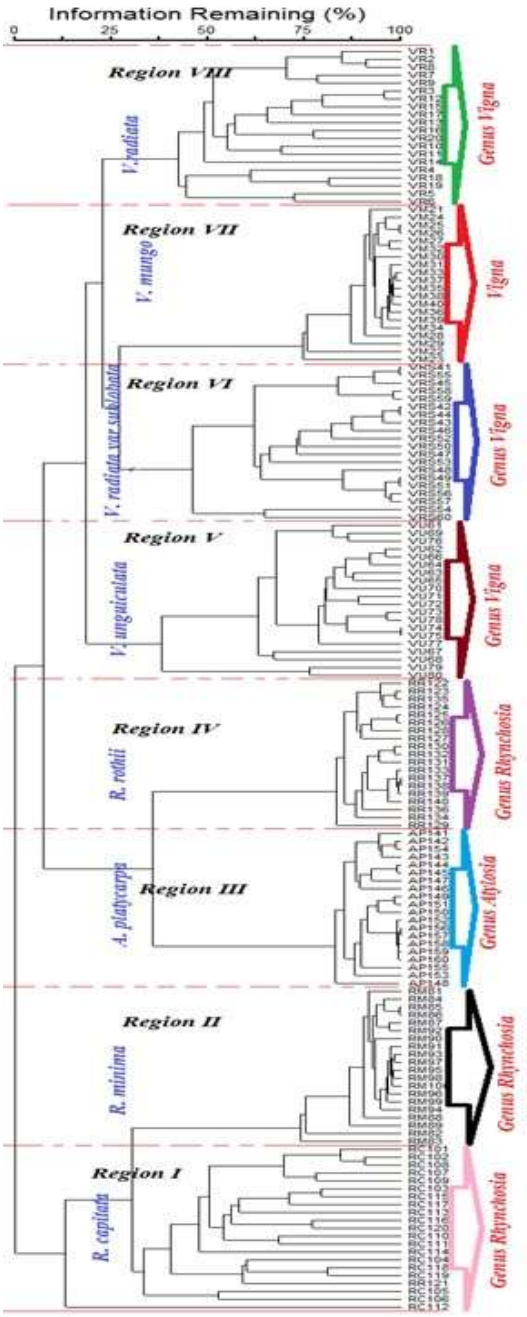

Figure 1: Interspecies phylogenetic relationship identified through morphological traits analysis in 160 different genotypes of *various* species of Fabaceae collected from Malakand Division, Khyber Pakhtunkhwa, Pakistan. VU indicate genotypes of *V. unguiculalata,* VRS indicate genotypes of *V. radiata varsublobata*, VM indicates genotypes of *V. mungo* and VR represents *V. radiata*

Table 1: One hundred and sixty genotypes collected from different geographical regions of Malakand Division, KP, Pakistan

Genotypes	Collection Sites	Genotypes	Collection Sites	Genotypes	Collection Sites	Genotypes	Collection Sites
Vigna radiata		*V. radiata* var. *sublobata*		*R. minima*		*R.rothii*	
VR1	Khwazakhela	VRS41	Soray	RM81	Ashreet	RR121	Khwazakhela
VR2	Mungultan	VRS42	Tangai Chena	RM82	Domail	RR122	Mungultan
VR3	Derai	VRS43	Qalagay	RM83	Qashqar	RR123	Derai
VR4	Kanju	VRS44	Sarkhanai	RM84	GaramChashma	RR124	Kanju
VR5	Kandak	VRS45	Yakhtangay	RM85	Beriel	RR125	Kandak
VR6	Cheno Baba	VRS46	Dokat	RM86	Boni	RR126	Cheno Baba
VR7	Nasrat	VRS47	Sarkhazano	RM87	Chakeser	RR127	Nasrat
VR8	Dughalgo	VRS48	Sarbala	RM88	Qandeel	RR128	Dughalgo
VR9	Tooth Banrai	VRS49	Qambo	RM89	Madiyan	RR129	Tooth Banrai
VR10	Besham Mayera	VRS50	Landai Shah	RM90	Bahrain	RR130	Besham
VR11	Bezobanr (Swat)	VRS51	Kasai	RM91	Parai	RR131	Mayera
VR12	Jawaro	VRS52	Banjo Banda	RM92	Chagharzo	RR132	Bezobanr
VR13	Jangir	VRS53	Tangai	RM93	Gadi	RR133	Jawaro
VR14	NawagaiSar	VRS54	Biakot	RM94	Swegalai	RR134	Jangir
VR15	Hazara	VRS55	Sarsinaai	RM95	Ziarat	RR135	NawagaiSar
VR16	GulJaba	VRS56		RM96	Rangila	RR136	Hazara
VR17	Kalakaly	VRS57	Drag	RM97	Gharai	RR137	GulJaba
VR18	Sarsinai	VRS58	Taranr	RM98	Dadahara	RR138	Kalakaly
VR19	Mahak	VRS59	Landay	RM99	Jawand	RR139	Sarsinai
VR20	Akhun Kalay	VRS60	Kandao	RM100	Melagah	RR140	Akhun Kalay
V. mungo		*V. unguiculata*		*R.rothii*		*Atylosia platycarpa*	
VM21	Ziarat	VU61	Batal	RC101	Jawand	AP141	Tangai
VM22	Swegalai	VU62	Khwago-Obo	RC102	Shamra	AP142	Biakot
VM23	Dadahara	VU63	Bekari	RC103	Mula Hassan Baba	AP143	Sarsinaai
VM24	Kohay	VU64	Patrok	RC104	Malak Abad	AP144	Drag
VM25	Gadi	VU65	Shaoor	RC105	Kalakaly	AP145	Taranr
VM26	Sharif Abad	VU66	Barikot	RC106	Kandao	AP146	Landay
VM27	Zarakhela	VU67	Jandrai	RC107	Jawaro	AP147	Kandao
VM28	Jalala	VU68	Islam-Gat	RC108	Jangir	AP148	Sati
VM29	Gatkoto	VU69	Jelar	RC109	NawagaiSar	AP149	Shamra
VM30	Gora Gat	VU70	Haji Shai	RC110	Hazara	AP150	Mula Hassan Baba
VM31	Chongai	VU71	Kharkani	RC111	GulJaba	AP151	Malak Abad
VM32	Qabar Shah	VU72	Thal	RC112	Kalakaly	AP152	Kandao
VM33	Landakay	VU73	Kalakot	RC113	Sarsinai	AP153	LoyeNao
VM34	Aboha	VU74	Lamotai	RC114	Mahak	AP154	Jawaro
VM35	Thana	VU75	Jagram	RC115	Akhun Kalay	AP155	Jangir
VM36	Terang	VU76	Bandagai	RC116	Dadahara	AP156	NawagaiSar
VM37	Dool	VU77	Asbnar	RC117	Hazara	AP157	NawagaiSar
VM38	Rangila	VU78	Laspoor	RC118	Ramora	AP158	Hazara
VM39	ChargoTangay	VU79	Mastooj	RC119	Faqir Abad	AP159	GulJaba
VM40	AmlookGarai	VU80	Drosh	RC120	Goragat	AP160	Ziarat

Note: V= *Vigna*, VR= *V. radiata*, VM= *V. mungo*, VRS= *V. radiata* var. *sublobata*, VU= *V. unguiculata*, R= *Rhynchosia*, RM= *R. minima*, RC= *R. capitata*, RR= *R. rothii*, A= *Atylosia*, AP= *A. platycarpa*

RESULTS

Morphological characterization

The morphological data of 160 genotypes was studied for the construction of phylogenetic tree to describe the similarity of these species, and the eight species were examined for similarities and the phylogenetic tree was made (Fig. 1). The phylogenetic tree divided all the eight species into eight Regions (Region I, Region II, Region III, Region IV, Region V, Region VI, Region VII and Region VIII). The Region I enclosed the 20 genotypes of *R. capitata* adjacent to this Region the dendrogram clustered the genotypes of *R. minima* in Region II which shows closed affinities to one another. Similarly the Region III enclosed the genotypes *A. platycarpa* whereas the phylogenetic tree placed the 20 genotypes *R. rothii* near *A. platycarpa* genotypes in in Region IV show closest similarity with another based on morphology. After Region IV the phylogenetic tree clustered the species of the genus *Vigna.* The Region V was consisted of 20 genotypes *V. unguiculata* after that the Region VI enclosed 20 genotypes *V. radiate varsublobata*. The Region VII has the genotypes of *V.mungo* and genotypes *V. radiata* was clustered in Region VIII (Fig: 1) and further the cluster analysis was confirmed by scattered plot detected through Principal Components analysis (Fig: 2)

The significant correlation coefficient naked a significant positive and a negative association is shown by the Pearson correlation coefficient (p = 0.05 and 0.01) among the studied traits of eight species (Tables 2, 3, 4and 5). Several features revealed strong interrelationships within phenotype categories, particularly leaf traits with yield donating characters and a few traits correlating with other groups, such as inherently linked growth and phenology-related characters (Tables 2,3, 4and 5).

The similarity indexes were performed based on qualitative and quantitative traits for all eight species' genotype, for qualitative characteristics; the similarity was 70% for *V. radiata* var. *sublobata* and *V. mungo*, Whereas *R. minma* and *R. capitata* were 80% similar morphologically. While *R. rothii* and *A. platycarpa* revealed 100% similarity. Similarly, *V. radiata* and *V. radiata* var *sublobata* expressed 100% relatedness. *R. rothii* and *A. platycarpa* were 100% similar morphologically, *V. radiata* and *V. mungo* were 80% similar whereas *V. radiata and R. capitata* were 80% similar. *V. radiata* and *R. rothii* were 80% similar. *V. radiata* and *A. platycarpa* had 80% similarity. Whereas *V. mungo* and *V. radiata* var *sublobata* were 80% similar morphologically, similarly *V. mungo* and *V. unguiculata* were 90% similar. *V. mungo* and *R. minima* were 80% similar. *V. mungo* and *R. capitata* were 80% similar. Similarly *V. mungo* and *R. rothii* were also 80% similar, based on qualitative traits. *V. mungo* and *A. platycarpa* have 90% affinities. *V. radiata* var *sublobata* and *V. unguiculata* were 80% similar morphologically. *V. radiata* var *sublobata* was 80% similar with *R. minima, R. capitata, R. rothii* and 90% with *A. platycarpa*. Whereas V. unguiculata was 70% similar with *R. minima*, *R. capitata*, *R. rothii* and *A. platycarpa*. *R. minima* was 80% similar with *R. capitata* and *R. rothii* and *A. platycarpa,* similarly the *R. capitata* was 90% identical with *R. rothii* and *A. platycarpa* while *R. rothii* was 90% similar with *A. platycarpa* (Table 6).

The similarity indexes based on the quantitative traits for all genotypes were 18% for *V. radiata* and *V. mungo. V. radiata* var.*sublobata and V. unguiculata* were 9.4% similar. No similar traits were found among *R. rothii* and *A. platycarpa*. *V. radiata* and *V. radiata* var *sublobata* was 12% similar morphologically. *V. radiata* and *V. unguiculata* was 18% similar. The traits similarity between V. radiata var sublobata and R. rothii was 0%. The *V. radiata* was 6% similar with *R. capitata*, 0% similarity was observed between *R. rothii* and *A. platycarpa*. *V. mungo* was 6% similar with *V. radiata* var *sublobata*, 12% *V. unguiculata*, 6% with *R. minima, R. capitata, R. rothii* and *A. platycarpa. V. radiata* var *sublobata* was 6% similar with *V. unguiculata, R. rothii,A. platycarpa* and 0% similar with *R. capitata*, and *R. minima*. The *V. unguiculata* and *A. platycarpa* had no similarity. The *R. minima* was11.8% similar with *R. capitata*, 6% with *R. rothii* and *A. platycarpa*. No resemblance was found between *R. capitata* and *R. rothii. R. capitata* and *A. platycarpa* was 6% similar. *R. rothii* and *A. platycarpa* was 24% similar morphologically (Table 7).

SDS-PAGE Investigation

Nine bands were detected in the *V. mungo*, eight protein bands were noticed in *V. radiata* and *V. radiata* var. *sublobata, V. unguiculata, R. minima and R. capitata* whereas six and seven bands were noted in *R. rothii* and *A. platycarpa* with molecular weight ranging from 180 to 10kDa in 8 species of family Fabaceae.

The data of 160 genotypes based on SDS-PAGE was scrutinized for the construction of phylogenetic tree Fig 3. It represents the similarity of various genotypes, and the 160 genotypes of 8 species of familyFabaceae (20 genotypes of each) were considered for similarities, and the dendrogram was constructed (Fig 3). This tree divided all the 160 genotypes into ten regions (R-I-R-X). Region I was composed of 20 genotypes of *V. mungo* with 8.97% genetic diversity and its genotypes were 12.5% similarity with the genotypes of Region II. Whereas Region II (R-II) has 25% genetic similarity with Region III (R-III). It was composed of the genotypes of *A. platycarpa.* Region III (R-III) was consisted of the genotypes of *R. rothii* and was 50% genetically similar to R-IV. The genotypes of Region IV have 62% genetic similarity with the genotypes of Regions (R-V). The R-V has the genotypes of *R. capitata* Fig: 4. the genotypes of R-V and R-VI have 75% genetic similarity. The R-V consisted of *R. minima.* The R-VI has genotypes of *V. unguiculata.* These genotypes were clustered near the genotypes of *R. minima* based on 75% genetic similarity. The genotypes of R-VIII and R-IX have 81.50% similarity. The R-VIII was consisted of the genotypes of *V. radiata.* The Region IX and Region X was 96% similar genetically. The R-IX and R-X has the genotypes of *V. radiate* var.*sublobata* and *V. radiata* respectively.

Locus variation

SDS-PAGE has exposed the ability to understand the genetic relationships in angiosperms at both generic as well as at the specific levels and is consistent method for measuring polymorphisms in crops. Remarkably, Table 8 shows interspecific variation among 160 genotypes of eight species belongs to family Fabaceae. Among all nine loci (B1-B9), Locus 6 (L6) was monomorphic and was marked as family specific which was used to classify the species of various genera of family Fabaceae. The loci B-1, 2, 4, 8 and 9 marked as polymorphic with 60, 45.62, 28.75, 62.5, 11.87, 37.5, 25 and 87.5 percent genetic diversity, respectively. The inter species genetic disagreement *was* 88.88% of 160 genotypes of the 8 species (Table: 8).

Intra-specific locus genetic diversity among 20 genotypes of *V. radiate* is shown in Table 9, among nine loci/ bands, L-9 band was disappeared in this specie hence this locus can be useful to distinguish this specie. Especially, B-4, 5, 6, 7, 8 was monomorphic in *V. radiata*. B-1, 2, 3 shows70, 50, 25 percent diversity, and genetic disagreement of *V. radiata* was 33.33%.

Whereas, the intra-specific variation among the 20 genotypes of *V. mungo* had high intra-specific locus variation was found. Among nine loci, B-1, 2, 3, 4, 5, 6 7, 8, and 9 were monomorphic; B-3 represents a 70 percent variation. The genetic disagreement *of V. mungo was* 11.11% (Table 9).

Whereas in *V. radiata* var. *sublobata* among nine loci, out of which B-2, 3, 5, 6 7 and 8 were monomorphic. The B-1 and B-9 were absent in 20 *V. mungo* genotypes. Hence, these missing bands in this specie can be supportive to isolate this specie. The locus contribution toward genetic disagreement of *V. radiata* var. *sublobata* was 0.00% (Table 9).

While intra-specific locus difference among 20 genotypes of *V. unguiculata* is represented in Table 9 and nine loci/ bands, L-9 band was missing in this species. Hence, this locus can help identify this species. Notably, B-1, B-3, B-5, B-6,B-7 and B-8 were monomorphic in *V. unguiculata*. B-2, B-4 was polymorphic and represents 40 and 50 percent variation respectively and genetic disagreement of *V. radiata* was 22.22%, as shown in table 9.

Table 9, whereby the intra-specific variation among the 20 genotypes of *R. minima* out of nine loci, B-5, 6, 7 and 8 were monomorphic while B-1, 2 and 3 were polymorphic with, 95, 65 and 35 percent diversity. The B-9 was missing in 20 *R. minima* genotypes. Hence, this missing band in this species can be helpful to identify this specie. The genetic disagreement of *R. minima* was 33.33% (Table 9).

Intra-specific locus difference among 20 genotypes of *R. capitata* is represented in Table 9 and nine loci/ bands, B-7 and B-9 bands were missing in this species hence this locus can be cooperative to classify this specie. Mainly, B-3, 4, 5, 67, 8 were monomorphic in *R. capitata*. B-1, 2, was polymorphic and shows 55 and 10 percent variation, and the genetic disagreement of *R. capitata was* 22.222% Table 9.

Intra-specific locus dissimilarity among 20 genotypes of *R. rothii* is represented in Table 9 and nine loci/ bands, B-7, 8 and 9 bands were missing in this species; hence these bands can be useful to categorize this species. Remarkably, B-4, 5, 6, 7, 8 was monomorphic in *R. rothii*. B-1, 5 shows 60 and 40 percent difference and the genetic disagreement of *R. rothii was* 22.22%.

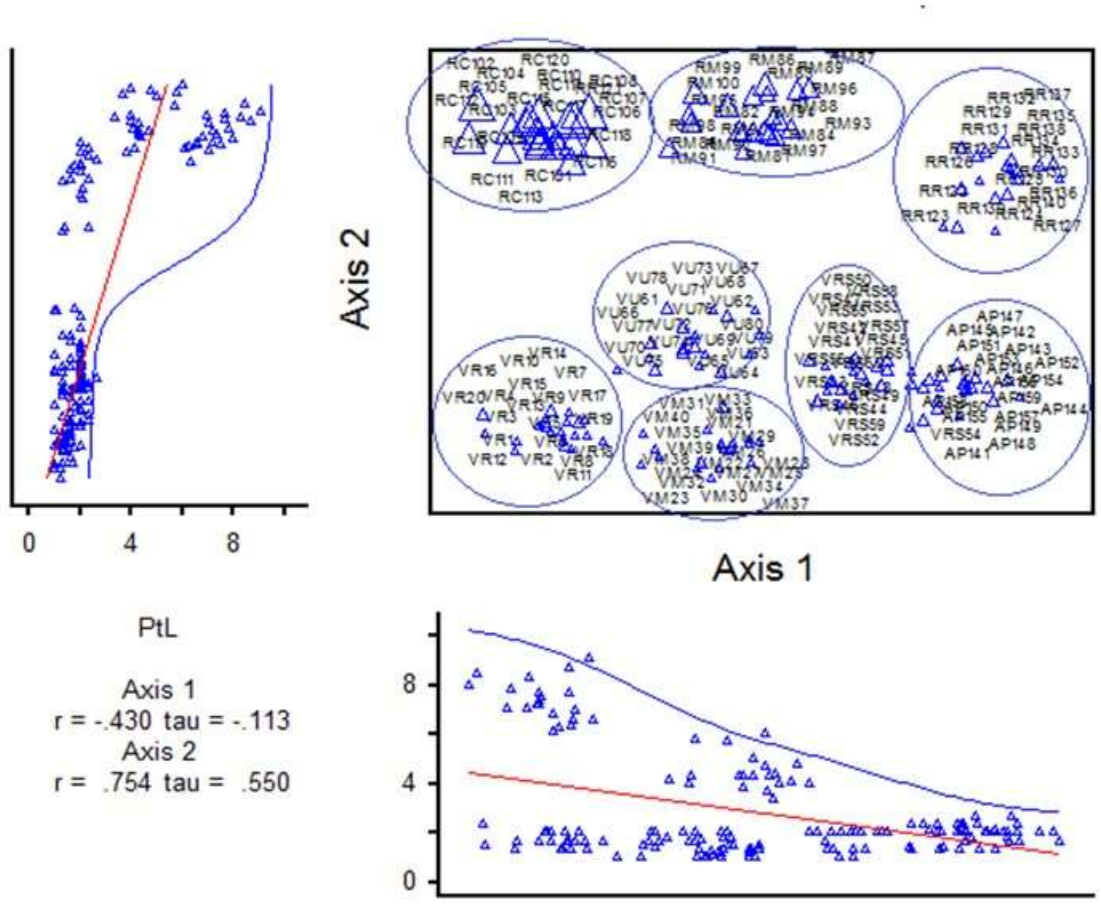

Figure 2: Confirmation of cluster analysis by scattered plot detected through Principal Components based on the morphology of 8 species of different genera belonging to Fabaceae in 160 genotypes collected from Malakand Division Pakistan.

Table 2: Correlation coefficient among seventeen quantitative traits of *V. mungo* (italic numbers) and *V. radiata*

	PtL	LL	LW	STL	IL	IW	SL	SW	ST	Pod L	S/Pod	Npod/P	100 SWt	NB/P	PH	BM	Y/P
PtL	1.00	*-0.04*	*-0.07*	*0.15*	*-0.07*	*-0.20*	*0.41*	*-0.09*	*0.31*	*0.07*	*-0.27*	*0.32*	*0.04*	*-0.15*	*0.27*	*0.11*	*0.19*
LL	0.17	1.00	*-0.18*	*-0.07*	*0.09*	*0.06*	*0.02*	*-0.04*	*-0.13*	*0.31*	*-0.17*	*-0.02*	*0.17*	*0.01*	*0.00*	*0.19*	*0.26*
LW	0.08	-0.16	1.00	*0.4*	*0.2*	*0.1*	*0.0*	*0.0*	*-0.3*	*0.0*	*-0.2*	*0.0*	*0.0*	*-0.2*	*-0.1*	*0.0*	*0.1*
STL	0.14	-0.05	.796**	1.00	*.489**	*0.4*	*-0.1*	*-0.1*	*-0.1*	*-0.4*	*-0.4*	*-0.4*	*0.0*	*-0.4*	*0.2*	*0.3*	*0.0*
IL	0.19	0.24	-0.38	-0.34	1.00	*.873***	*-0.1*	*-0.1*	*-0.1*	*-0.1*	*-0.1*	*-0.2*	*0.0*	*0.0*	*-0.1*	*0.4*	*0.4*
IW	0.19	0.17	-0.21	-0.17	.874**	1.00	*-0.3*	*-0.3*	*-0.1*	*0.0*	*-0.1*	*-0.2*	*0.1*	*0.2*	*-0.2*	*0.2*	*0.3*
SL	.505*	0.30	0.17	0.16	0.06	-0.07	1.00	*.527**	*.480**	*0.1*	*0.0*	*0.4*	*0.0*	*-0.2*	*-0.1*	*-0.2*	*0.3*
SW	.450*	0.11	0.08	0.07	-0.15	-0.27	.563**	1.00	*.581***	*-0.1*	*0.3*	*0.1*	*-0.2*	*0.1*	*-0.2*	*-0.1*	*-0.1*
ST	0.22	-0.25	0.17	0.28	-0.42	-.525*	0.33	.543*	1.00	*0.2*	*0.4*	*0.2*	*-0.3*	*0.3*	*-0.1*	*0.1*	*-0.1*
Pod L	.481*	-0.03	-0.13	0.00	-0.03	-0.10	0.18	.496*	0.36	1.00	*0.0*	*0.4*	*-0.3*	*.487**	*-0.3*	*-0.2*	*0.1*
S/Pod	-0.04	0.01	-0.41	-0.07	-0.18	-0.21	0.18	0.14	0.21	0.23	1.00	*0.2*	*-0.2*	*.451**	*-0.4*	*0.2*	*-0.4*
Npod/P	0.02	-0.14	0.13	-0.07	-0.19	-0.15	-0.09	-0.08	-0.09	0.28	-0.21	1.00	*-0.1*	*0.0*	*0.2*	*-0.3*	*0.2*
100SWt	0.21	0.39	.599**	561*	0.27	0.11	0.07	0.03	-0.31	0.00	0.01	0.23	1.00	*-0.2*	*.547**	*-0.2*	*0.0*
NB/P	-0.13	-0.32	0.40	0.13	-0.31	0.06	-0.34	-0.35	-0.44	0.25	-0.30	0.40	-0.12	1.00	*.500**	*0.1*	*-0.3*
PH	-0.13	0.26	-0.06	0.22	-0.20	-0.19	-0.03	0.12	0.19	0.24	0.28	-0.44	-0.05	-0.22	1.00	*-0.1*	*0.0*
BM	0.41	0.11	-0.21	508*	0.18	0.13	0.20	0.29	0.04	0.29	-0.29	0.20	0.26	-0.02	.548*	1.00	*0.1*
Y/P	-0.07	.581**	0.12	0.12	.609**	-.546*	0.01	0.19	.605**	0.11	0.37	0.07	-0.44	0.05	-0.06	0.11	1.00
*. Correlation is significant at the 0.05 level (2-tailed).																	
**. Correlation is significant at the 0.01 level (2-tailed).																	

PtL= Petiole length, LL= Leaf length, LW=Leaf Width, STL= Stipule length, IL=Inflorescence length, IW= Infloresence width, SL=Seed length, SW= Seed Width, ST= Seed thickness, PodL= Pod length, S/Pod= No. of Seed/Pod, Npod/P= No. of Pod/ Plant, 100SWt= 100 seed weight, NB/P= No. of Branches/ Plant, PH= Plant Height, BM= Biomass, Y/P= Yield/ Plant

Table 3: Correlation coefficient among seventeen quantitative traits of *R. capitata* (italic numbers) and *R.minima*

	PtL	LL	LW	STL	IL	IW	SL	SW	ST	Pod L	S/Pod	Npod/P	100 SWt	NB/P	PH	BM	Y/P
PtL	1.00	*-0.04*	*-0.18*	*-0.07*	*0.09*	*0.06*	*0.17*	*0.02*	*-0.04*	*-0.13*	*0.31*	*-0.17*	*-0.02*	*0.01*	*0.00*	*0.19*	*0.26*
LL	0.10	1.00	*-0.07*	*0.15*	*-0.07*	*-0.20*	*0.04*	*0.41*	*-0.09*	*0.31*	*-0.07*	*-0.27*	*0.32*	*-0.15*	*0.27*	*0.11*	*0.19*
LW	0.38	0.12	1.00	*0.44*	*0.21*	*0.12*	*-0.04*	*0.00*	*0.05*	*-0.25*	*-0.04*	*-0.24*	*-0.01*	*-0.24*	*-0.15*	*0.01*	*0.10*
STL	0.35	0.20	.908**	1.00	*.489**	*0.44*	*0.00*	*-0.09*	*-0.08*	*-0.06*	*-0.36*	*-0.41*	*-0.42*	*-0.38*	*0.19*	*0.32*	*0.01*
IL	-0.18	0.24	-0.10	-0.08	1.00	*.873***	*0.00*	*-0.13*	*-0.13*	*-0.14*	*-0.09*	*-0.14*	*-0.18*	*0.02*	*-0.14*	*0.42*	*0.43*
IW	0.03	0.25	0.21	0.08	.701**	1.00	*0.13*	*-0.31*	*-0.33*	*-0.14*	*0.04*	*-0.05*	*-0.24*	*0.16*	*-0.15*	*0.23*	*0.27*
SL	-0.07	-0.08	-0.36	-0.38	-0.27	-0.06	1.00	*-0.02*	*-0.20*	*-0.32*	*-0.26*	*-0.19*	*-0.06*	*-0.23*	*.547**	*-0.15*	*-0.03*
SW	-0.25	0.25	-0.24	-0.15	-0.37	-.475*	0.31	1.00	*.527**	*.480**	*0.10*	*-0.02*	*0.43*	*-0.19*	*-0.08*	*-0.15*	*0.33*
ST	-0.23	0.13	-0.02	0.11	-0.23	-0.43	-0.12	.802**	1.00	*.581***	*-0.08*	*0.35*	*0.12*	*0.14*	*-0.17*	*-0.07*	*-0.14*
Pod L	0.30	-0.04	-0.35	-0.41	-0.11	-0.15	0.15	-0.05	-0.18	1.00	*0.15*	*0.36*	*0.17*	*0.27*	*-0.15*	*0.05*	*-0.05*
S/Pod	-0.20	0.02	-0.24	-0.22	0.29	-0.04	-.454*	0.02	0.24	0.17	1.00	*0.00*	*0.37*	*.487**	*-0.27*	*-0.19*	*0.09*
Npod/P	0.11	-0.08	-0.27	-0.09	-0.36	-0.30	0.32	0.08	0.08	0.44	0.03	1.00	*0.20*	*.451**	*-0.38*	*0.17*	*-0.43*
100SWt	0.19	0.39	0.38	0.25	0.19	.611**	-0.02	-0.34	-0.44	-0.30	-0.32	-.487*	1.00	*0.03*	*0.15*	*-0.34*	*0.17*
NB/P	-0.15	-0.40	0.08	-0.10	.446*	0.44	-0.19	-0.33	-0.19	-0.09	0.28	-0.43	0.08	1.00	*-.500**	*0.06*	*-0.26*
PH	0.41	-0.22	0.14	0.13	0.21	0.19	-0.19	-.480*	-0.31	0.24	0.36	0.26	-0.15	0.03	1.00	*-0.12*	*0.01*
BM	-0.35	.573**	0.13	0.14	0.38	0.18	-.495*	0.01	0.08	-0.27	0.18	-.477*	0.27	0.01	-0.27	1.00	*0.11*
Y/P	-0.40	0.11	0.18	0.12	0.02	-0.07	-0.20	0.19	0.24	-0.36	-0.04	-0.29	0.10	0.23	-.514*	0.35	1.00
**. Correlation is significant at the 0.01 level (2-tailed).																	
*. Correlation is significant at the 0.05 level (2-tailed).																	

PtL= Petiole length, LL= Leaf length, LW=Leaf Width, STL= Stipule length, IL=Inflorescence length, IW= Infloresence width, SL=Seed length, SW= Seed Width, ST= Seed thickness, PodL= Pod length, S/Pod= No. of Seed/Pod, Npod/P= No. of Pod/ Plant, 100SWt= 100 seed weight, NB/P= No. of Branches/ Plant, PH= Plant Height, BM= Biomass, Y/P= Yield/ Plan

Table 4: Correlation coefficient among seventeen quantitative traits of *V. radiate* var. *sublobata* (italic numbers) and *V. unguiculata*

	PtL	LL	LW	STL	IL	IW	SL	SW	ST	Pod L	S/Pod	Npod/P	100 SWt	NB/P	PH	BM	Y/P
PtL	1.00	*0.0*	*-0.1*	*0.2*	*-0.1*	*-0.2*	*0.4*	*-0.1*	*0.3*	*-0.1*	*-0.3*	*0.3*	*0.0*	*-0.1*	*0.3*	*0.1*	*0.2*
LL	0.08	1.00	*-0.2*	*-0.1*	*0.1*	*0.1*	*0.0*	*0.0*	*-0.1*	*0.3*	*-0.2*	*0.0*	*0.2*	*0.0*	*0.0*	*0.2*	*0.3*
LW	0.41	0.10	1.00	*0.4*	*0.2*	*0.1*	*0.0*	*0.0*	*-0.3*	*0.0*	*-0.2*	*0.0*	*0.0*	*-0.2*	*-0.1*	*0.0*	*0.1*
STL	0.08	-0.19	.542*	1.00	*.489**	*0.4*	*-0.1*	*-0.1*	*-0.1*	*-0.4*	*-0.4*	*-0.4*	*0.0*	*-0.4*	*0.2*	*0.3*	*0.0*
IL	-0.16	0.25	-0.19	0.36	1.00	*.873***	*-0.1*	*-0.1*	*-0.1*	*-0.1*	*-0.1*	*-0.2*	*0.0*	*0.0*	*-0.1*	*0.4*	*0.4*
IW	-0.16	0.31	-0.23	0.19	.942**	1.00	*-0.3*	*-0.3*	*-0.1*	*0.0*	*-0.1*	*-0.2*	*0.1*	*0.2*	*-0.2*	*0.2*	*0.3*
SL	0.01	0.35	-0.19	-0.22	-0.12	-0.04	1.00	*.527**	*.480**	*0.1*	*0.0*	*0.4*	*0.0*	*-0.2*	*-0.1*	*-0.2*	*0.3*
SW	-0.18	0.04	0.05	0.04	-0.17	-0.27	-0.18	1.00	*.581***	*-0.1*	*0.3*	*0.1*	*-0.2*	*0.1*	*-0.2*	*-0.1*	*-0.1*
ST	0.01	-0.05	0.03	0.06	0.10	0.01	-0.28	.805**	1.00	*0.2*	*0.4*	*0.2*	*-0.3*	*0.3*	*-0.1*	*0.1*	*-0.1*
Pod L	-.687**	0.14	-0.17	0.06	0.18	0.04	0.09	0.35	0.02	1.00	*0.0*	*0.4*	*-0.3*	*.487**	*-0.3*	*-0.2*	*0.1*
S/Pod	-0.43	0.03	-0.24	-0.11	-0.01	0.01	0.18	0.41	0.24	0.41	1.00	*0.2*	*-0.2*	*.451**	*-0.4*	*0.2*	*-0.4*
Npod/P	-0.26	0.32	0.18	0.10	-0.04	-0.04	0.01	.449*	0.25	0.33	0.11	1.00	*-0.1*	*0.0*	*0.2*	*-0.3*	*0.2*
100SWt	-0.09	-0.15	0.21	0.03	-0.11	-0.22	0.16	-0.19	-0.16	0.20	-0.16	0.13	1.00	*-0.2*	*.547**	*-0.2*	*0.0*
NB/P	-0.11	0.05	-0.21	-0.42	-0.23	-0.21	-0.44	0.41	0.42	0.02	0.12	0.24	-0.23	1.00	*-.500**	*0.1*	*-0.3*
PH	0.00	0.24	0.04	-0.34	0.08	0.04	-0.27	-0.22	-0.05	-0.03	-0.17	-0.01	0.37	0.28	1.00	*-0.1*	*0.0*
BM	-0.14	0.26	-0.26	-0.30	0.40	0.44	0.07	-0.30	-0.15	-0.08	-0.04	-0.20	0.23	-0.14	.457*	1.00	*0.1*
Y/P	-0.11	0.10	0.06	-0.06	-0.31	-0.38	0.03	0.15	-0.11	0.33	0.12	0.18	0.24	0.37	0.12	-0.34	1.00
****. Correlation is significant at the 0.01 level (2-tailed).**																	
***. Correlation is significant at the 0.05 level (2-tailed).**																	

PtL= Petiole length, LL= Leaf length, LW=Leaf Width, STL= Stipule length, IL=Inflorescence length, IW= Infloresence width, SL=Seed length, SW= Seed Width, ST= Seed thickness, PodL= Pod length, S/Pod= No. of Seed/Pod, Npod/P= No. of Pod/ Plant, 100SWt= 100 seed weight, NB/P= No. of Branches/ Plant, PH= Plant Height, BM= Biomass, Y/P= Yield/ Plant

Table 5: Correlation coefficient among seventeen quantitative traits of *A. platycarpa* (italic numbers) and *R. rothii*

	PtL	LL	LW	STL	IL	IW	SL	SW	ST	Pod L	S/Pod	Npod/P	100 SWt	NB/P	PH	BM	Y/P
PtL	1.00	*-0.20*	*.952***	*.944***	*-0.01*	*0.13*	*-.852***	*-.481**	*-0.05*	*-0.01*	*.860***	*.921***	*.556**	*.927***	*.870***	*.922***	*.649***
LL	0.17	1.00	*-0.18*	*-0.20*	*-0.16*	*-0.17*	*0.11*	*0.09*	*-0.17*	*-0.11*	*-0.38*	*-0.19*	*-0.31*	*-0.25*	*-0.20*	*-0.12*	*-0.24*
LW	0.08	-0.23	1.00	*.992***	*0.05*	*0.20*	*-.866***	*-.563***	*-0.14*	*-0.05*	*.865***	*.977***	*.549**	*.913***	*.929***	*.916***	*.567***
StL	-0.04	0.12	.682**	1.00	*0.11*	*0.24*	*-.857***	*-.560**	*-0.14*	*-0.05*	*.875***	*.967***	*.554**	*.887***	*.933***	*.901***	*.545**
IL	0.24	0.19	-.465*	-0.36	1.00	*.938***	*-0.22*	*-0.23*	*-0.28*	*-0.19*	*0.24*	*0.15*	*-0.03*	*-0.02*	*0.17*	*-0.20*	*-0.30*
IW	0.17	0.19	-0.31	-0.19	.874**	1.00	*-0.40*	*-0.33*	*-0.37*	*-0.23*	*0.32*	*0.30*	*0.01*	*0.14*	*0.35*	*-0.07*	*-0.30*
SL	0.39	0.21	-0.39	-.556*	0.27	0.11	1.00	*0.44*	*0.11*	*0.00*	*-.818***	*-.888***	*-0.42*	*-.852***	*-.923***	*-.696***	*-0.29*
SW	0.28	.564**	0.16	0.15	0.11	-0.03	0.06	1.00	*.804***	*.767***	*-.445**	*-.639***	*-0.07*	*-0.40*	*-.513**	*-0.41*	*-0.22*
ST	0.11	.450*	0.01	0.06	-0.15	-0.27	0.03	.599**	1.00	*.924***	*0.01*	*-0.23*	*0.20*	*0.00*	*-0.11*	*-0.04*	*0.12*
Pod L	-0.20	0.09	0.39	0.33	-.578**	-.641**	-0.32	0.40	.490*	1.00	*0.07*	*-0.15*	*0.09*	*0.10*	*0.02*	*0.02*	*0.09*
S/Pod	-0.03	.481*	-0.17	-0.02	-0.03	-0.10	0.00	0.22	.496*	0.27	1.00	*.868***	*.537**	*.812***	*.872***	*.722***	*.452**
Npod/P	0.01	-0.04	0.03	-0.06	-0.18	-0.21	0.01	0.17	0.14	0.25	0.23	1.00	*.561***	*.908***	*.923***	*.842***	*.504**
100 SWt	-0.14	0.02	-0.03	-0.06	-0.19	-0.15	0.23	-0.11	-0.08	-0.06	-0.28	-0.21	1.00	*.588***	*0.41*	*.526**	*.458**
NB/P	-0.32	-0.13	0.27	0.16	-0.31	0.06	-0.12	-0.40	-0.35	-0.31	-0.25	-0.30	0.40	1.00	*.859***	*.856***	*.624***
PH	0.26	-0.13	0.29	0.24	-0.20	-0.19	-0.05	-0.06	0.12	0.29	0.24	0.28	-0.44	-0.22	1.00	*.755***	*0.32*
BM	0.11	0.41	-.444*	-.522*	0.18	0.13	0.26	0.24	0.29	-0.07	0.29	-0.29	0.20	-0.02	-.548*	1.00	*.705***
Y/P	-.581**	-0.07	0.03	0.10	-.609**	-.546*	-0.44	0.03	0.19	.567**	0.11	0.37	0.07	0.05	-0.06	0.11	1.00
**. Correlation is significant at the 0.01 level (2-tailed).																	
*. Correlation is significant at the 0.05 level (2-tailed).																	

PtL= Petiole length, LL= Leaf length, LW=Leaf Width, STL= Stipule length, IL=Inflorescence length, IW= Infloresence width, SL=Seed length, SW= Seed Width, ST= Seed thickness, PodL= Pod length, S/Pod= No. of Seed/Pod, Npod/P= No. of Pod/ Plant, 100SWt= 100 seed weight, NB/P= No. of Branches/ Plant, PH= Plant Height, BM= Biomass, Y/P= Yield/ Plant

Table 6: Traits Similarity among *V. radiata*, *V. mungo*, *V. unguiculata*, *V. radiata* var. *sublobata*, *R. capitata*, *R. minima*, *R. rothii* and *A. platycarpa* based on qualitative traits.

Species	LS	LC	LP	Ls	FC	St	HC	SCc	SS	SpT	TSI
VRS & VU	NA	Green	Present	Present	NA	Smooth/Rough	yellow	white/ Yellow	NA	Present	70
RM & RC	NA	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	NA	Present	80
RR & AP	Ovate	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	Flat	Present	100
VR & VRS	Lanceolate	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	Oblong/Cylindrical	Present	100
VR & VU	NA	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	NA	Present	80
VR & RM	NA	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	NA	Present	80
VR & RC	NA	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	NA	Present	80
VR & RR	NA	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	NA	Present	80
VR & AP	NA	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	NA	Present	80
VM & VRS	Ovate-Rhomboid	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	Oblong/Cylindrical	Present	100
VM &VU	Lanceolate	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	NA	Present	90
VM & RM	NA	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	NA	Present	80
VM & RC	NA	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	NA	Present	80
VM & RR	NA	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	NA	Present	80
VM & AP	Lanceolate	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	NA	Present	90
VRS & VU	Lanceolate	Green	Present	Present	NA	Smooth/Rough	yellow	white/ Yellow	NA	Present	80
VRS & RM	NA	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	NA	Present	80
VRS & RC	NA	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	NA	Present	80
VRS & RR	NA	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	NA	Present	80
VRS & AP	Lanceolate	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	NA	Present	90
VU & RM	NA	Green	Present	Present	NA	Smooth/Rough	yellow	white/ Yellow	NA	Present	70
VU & RC	NA	Green	Present	Present	NA	Smooth/Rough	yellow	white/ Yellow	NA	Present	70
VU & RR	NA	Green	Present	Present	NA	Smooth/Rough	yellow	white/ Yellow	NA	Present	70
VU & AP	NA	Green	Present	Present	NA	Smooth/Rough	yellow	white/ Yellow	NA	Present	70
RM & RC	NA	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	NA	Present	80
RM & RR	NA	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	NA	Present	80
RC & RR	NA	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	Flat	Present	90
RC & AP	NA	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	Flat	Present	90
RM & AP	NA	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	NA	Present	80
RR & AP	NA	Green	Present	Present	Yellow	Smooth/Rough	yellow	white/ Yellow	Flat	Present	90

TSI= homologus traits/ Total traits*100, VR= *V. radiata*, VM= *V. mungo*, V. unguiculata, VRS= *V. radiaita var. sublobata*, R. minima, RC= R. capitata, RR= R. rothii and AP= *Atylosiaplatycarpa*, LS= leaf Shape, LC= leaf color, LP= leaf pubescent,St= Seed texture, FC= flower color, HC=Hilum color, SCc= Seed coat color, SS= Seed Shape, SpT= Spot on Seed

Table 7: Traits Similarity among *V. radiata*, *V. mungo*, *V. unguiculata*, *V. radiata* var. *sublobata*, *R. capitata*, *R. minima*, *R. rothii* and *A. platycarpa* based on quantitative traits

Traits	VR & VM	VRS & VU	RM & RC	RR & AP	VR & VRS	VR & VU	VR & RM	VR & RC	VR & RR	VR & AP	VM & VRS	VM & VU	VM & RM	VM & RC	VM & RR	VM & AP	VRS & VU	VRS & RM	VRS & RC	VRS & RR	VRS & AP	VU & RM	VU & RC	VU & RR	VU & AP	RM & RC	RM & RR	RC & RR	RC & AP	RM & AP	RR & AP
PtL	**1.6**	**1.6**	-	-	**1.6**	**1.59**	-	-	-	-	**1.6**	**1.6**	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LL	-	-	**1.6**	-	-	-	-	-	-	-	**4.3**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.58	1.58	-	-	-	-
LW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
StL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	23	-	-	-	-	-	-	-	-	-	-	-
IL	-	**3.3**	-	-	-	-	-	5	-	-	-	-	4	-	-	-	-	-	-	-	-	-	3.3	-	-	-	-	-	-	-	3.29
IW	**3.3**	**2.8**	-	-	-	3.29	3.3	-	-	-	2.8	2.8	3.3	-	-	-	3	-	-	-	-	3.3	2.8	-	-	3.29	-	-	-	-	2.75
SL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11	-	-	-	-	-	-	-	-
SW	-	-	**5.5**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.3	-	-	-	-	-	-	-	-	-	-
ST	**2.5**	-	-	-	2.5	2.54	-	-	-	-	2.5	-	-	-	2.5	3	-	-	2.5	-	2.5	-	-	4	-	-	-	-	-	-	-
Pod L	-	-	-	-	-	-	-	-	-	-	58	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-
S/Pod	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Npod/P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SWt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NB/P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	150
BM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	93
Y/P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TSI=	18	9.4	9.4	0	12	18	0	6	0	0	6	12	6	6	6	6	6	0	0	6	6	6	18	6	0	11.8	6	0	6	6	24

*.Traits Similarity index (TSI) = homologous traits/Total traits*100, VR= *V. radiata*, VM= *V. mungo*, VU=*V. unguiculata*, VRS= V. radiaita var. *sublobata*, RM=*R. minima*, RC= *R. capitata*, RR= *R. rothii* and AP= *Atylosia platycarpa,* PtL= Petiole length, LL= Leaf length, LW=Leaf Width, STL= Stipule length, IL=Inflorescence length, IW= Infloresence width, SL=Seed length, SW= Seed Width, ST= Seed thickness, PodL= Pod length, S/Pod= No. of Seed/Pod, Npod/P= No. of Pod/ Plant, 100SWt= 100 seed weight, NB/P= No. of Branches/ Plant, PH= Plant Height, BM= Biomass, Y/P= Yield/ Plant

Table 9: Intra Specific diversity among the genotypes of *V. radiata, V. radiata* var. *sublobata, V. mungo, V. unguiculata, R. minima, R. Capitata, R. rothii and A. platycarpa*

	Loci	Present (%)	Absent (%)	Variation (%)	Status			Locus	Present (%)	Absent (%)	Variation	Status
V. radiata	B-1	6(30)	14(70)	70	Poly		*V. mungo*	B-1	20(100)	0.00	Nil	mono
	B-2	10(50)	10(50)	50	Poly			B-2	20(100)	0.00	Nil	mono
	B-3	15(75)	5(25)	25	Poly			B-3	6(30)	14(70)	70	poly
	B-4	20(100)	0.00	Nil	Mono			B-4	20(100)	0.00	Nil	mono
	B-5	20(100)	0.00	Nil	Mono			B-5	20(100)	0.00	Nil	mono
	B-6	20(100)	0.00	Nil	Mono			B-6*	20(100)	0.00	Nil	mono
	B-7	20(100)	0.00	Nil	Mono			B-7	20(100)	0.00	Nil	mono
	B-8	20(100)	0.00	Nil	Mono			B-8	20(100)	0.00	Nil	mono
	B-9	0.00	20(100)	Nil	Mono			B-9	20(100)	0.00	Nil	mono
	GD= 33.33% Poly loci/Total loci*100)							**GD= 11.11% GD= (Poly/ Total loci*100)**				
	Locus	**Present (%)**	**Absent (%)**	**Variation**	**Status**			**Locus**	**Present (%)**	**Absent (%)**	**Variation**	**Status**
V. radiata var. sublobata	B-1	0.00	20(100)	Nil	mono		*V. unguiculata*	B-1	20(100)	0.00	Nil	mono
	B-2	20(100)	0.00	Nil	mono			B-2	12(60)	8(40)	40	Poly
	B-3	20(100)	0.00	Nil	mono			B-3	20(100)	0.00	Nil	mono
	B-4	20(100)	0.00	Nil	mono			B-4	10(50)	10(50)	50	Poly
	B-5	20(100)	0.00	Nil	mono			B-5	20(100)	0.00	Nil	mono
	B-6	20(100)	0.00	Nil	mono			B-6*	20(100)	0.00	Nil	mono
	B-7	20(100)	0.00	Nil	mono			B-7	20(100)	0.00	Nil	mono
	B-8	20(100)	0.00	Nil	mono			B-8	20(100)	0.00	Nil	mono
	B-9	0.00	20(100)	Nil	mono			B-9	0.00	20(100)	Nil	mono
	GD=0.00 GD (Poly loci/ Total loci*100)							**GD=22.22% (GD= Poly loci/ Total loci*100)**				
	Locus	**Present (%)**	**Absent (%)**	**Variation**	**Status**			**Locus**	**Present (%)**	**Absent (%)**	**Variation**	**Status**
R. minima	B-1	1(5)	19(95)	95	Poly		*R. capitata*	B-1	9(45)	11(55)	55	poly
	B-2	7(35)	13(65)	65	Poly			B-2	18(90)	2(10)	10	poly
	B-3	13(65)	7(35)	35	Poly			B-3	20(100)	0.00	Nil	mono
	B-4	20(100)	0.00	Nil	mono			B-4	20(100)	0.00	Nil	mono
	B-5	20(100)	0.00	Nil	mono			B-5	20(100)	0.00	Nil	mono
	B-6*	20(100)	0.00	Nil	mono			B-6*	20(100)	0.00	Nil	mono
	B-7	20(100)	0.00	Nil	mono			B-7	0.00	20(100)	Nil	mono
	B-8	20(100)	0.00	Nil	mono			B-8	20(100)	0.00	Nil	mono
	B-9	0.00	20(100)	Nil	mono			B-9	0.00	20(100)	Nil	mono
	GD=33.33% (GD= poly loci/ Total loci*100)							**GD=22.22% (GD= poly loci/Total loci*100)**				
	Locus	**Present (%)**	**Absent (%)**	**Variation**	**Status**			**Locus**	**Present (%)**	**Absent (%)**	**Variation**	**Status**
R. rothii	B-1	8(40)	12(60)	60	Poly		*A. platycarpa*	B-1	19(95)	1(5)	5	Poly
	B-2	20(100)	0.00	Nil	mono			B-2	9(45)	11(55)	55	Poly
	B-3	20(100)	0.00	Nil	mono			B-3	13(65)	7(35)	35	Poly
	B-4	20(100)	0.00	Nil	mono			B-4	17(85)	3(15)	15	Poly
	B-5	12(60)	8(40)	40	poly			B-5	17(85)	3(15)	16	Poly
	B-6	20(100)	0.00	Nil	mono			B-6	20(100)	0.00	Nil	mono
	B-7	0.00	20(100)	Nil	mono			B-7	12(60)	8(40)	Nil	Poly
	B-8	0.00	20(100)	Nil	mono			B-8	0.00	20(100)	Nil	mono
	B-9	0.00	20(100)	Nil	mono			B-9	0.00	20(100)	Nil	mono
	GD= 22.22 (GD= Poly loci/total loci*100	)						**GD=66.66**	**(Poly loci/ Total loci*100)**			

Table 8: Inter-locus variations among VR, VM, VU, VRS, RM, RC, RR and AP

Locus	Present (%)	Absent (%)	Variation (%)	Status	GD
B-1	64(40)	36(60)	60	Poly	0.4
B-2	87(54.37)	73(45.62)	45.62	Poly	0.5437
B-3	114(71.25)	46(28.75)	28.75	Poly	0.7125
B-4	150(93.75)	10(62.5)	62.5	Poly	0.9375
B-5	141(88.125)	19(11.87)	11.87	Poly	0.882
B-6* family specific	160(100)	0.00	Nil	Mono	1.00
B-7	100(62.5)	60(37.5)	37.5	Poly	0.625
B-8	120(75)	40(25)	25	Poly	0.75
B-9	20(12.5)	140(87.5)	87.5	Poly	0.125
GD= 88.888% (GD= poly loci/Total loci*100)					

B= Protein Band, VR= *V. radiata*, VM= *V. mungo*, *V. unguiculata*, VRS= *V. radiaita* var. *sublobata minima*, RC= *R. capitata*, RR= *R. rothii* and AP= *Atylosiaplatycarpa*

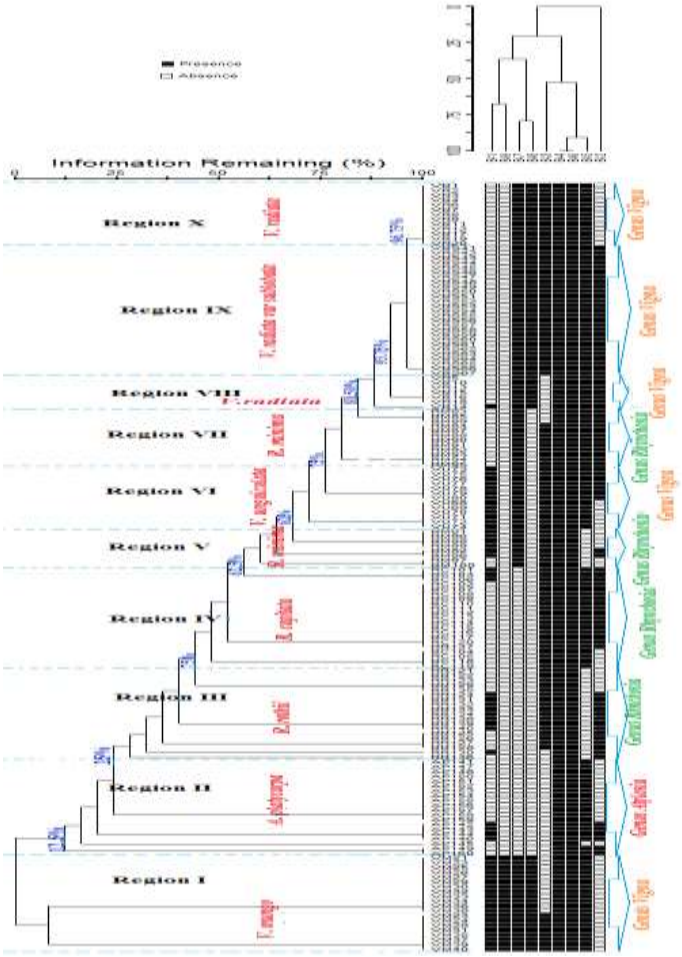

Figure 4: Inter -species phylogenetic relationship identified through Seed storage protein analysis in 160 different genotypes of various species of Fabaceae collected from Malakand Division, Khyber Pakhtunkhwa, Pakistan. VU indicate genotypes of *V. unguiculalata,* VRS indicate genotypes of *V. radiata varsublobata*, VM indicates genotypes of *V. mungo* and VR represents *V. radiata,* RM= *R. minima,* RC= *R. capitata,* RR= *R. rothii,* AP= *A. platycarpa.*

And Intra-specific locus dissimilarity among 20 genotypes of *A. platycarpa* is represented in Table 8 and nine loci/ bands, B-9 bands were missing in this specie hence this locus can be useful to isolate this specie. Remarkably, B-2, 3, 4, 5, 6, 7, 8 was polymorphic in *A. platycarpa* and shows 55 and 10 percent variation. B-6 was monomorphic and the genetic disagreement (GD) of *A. platycarpa was* 66.66% Table 9.

DISCUSSION

Phylogenetic relationship and genetic diversity has a critical role in germplasms identification and crop improvement. Genetic bottlenecks pose potential threats to breeding for adaptation to biotic stresses, like diseases, and abiotic stresses, such as drought or salt tolerance. Moreover, it is necessary to investigate the genetic diversity in legumes germplasm in order to broaden the genetic variation in future breeding (Muhammad et al., 2018).

But our study indicated extensive diversity among all important agro morphological trails analyzed and suggest the importance of conservation of the vital germplasm resources present as landraces in the remote areas of the country. Morphological characterization is the first step to investigate genetic diversity however such traits are adversely affected by environmental fluctuations (Noor et al., 2018; Muhammad et al., 2019).

One hundred and sixty genotypes of eight species of Family Fabaceae were studied for inter and intra-species variability and seed protein profiling. Genetic diversity delivers a vital understanding of genetic diversity and selective breeding investigations (Kouam et al., 2012). Narrow genetic diversity stances a risk to the existence of species as it limit (Muhammad et al., 2019c). Generally, the classification of several subgenera, species, and subspecies is based mainly on morphological features. However, these qualities may not be significantly distinct and usually require growing plants to maturity earlier to documentation. Furthermore, morphological characters may be unstable due to environmental effects. Among biochemical practices, SDS PAGE is most widely applied due to its validity and easiness for describing the genetic structure of crop genotypes. SDS PAGE is considered a consistent process because seed Storage proteins are largely autonomous of environmental fluctuations (Noor et al. 2018).

Information on genetic distance and diversity at the molecular level among genotypes is essential for description and documentation of gene flow among populations (Muhammad et al., 2019b). In contrast, several studies have assessed the molecular diversity of common bean (Zargar et al., 2014). Similarly, in our current work, a dendrogram based on seed storage protein analyses of selected species showed that the 8 species had close similarity to one another. The result showed that the *R. capitata* was clustered adjacent to *R. minima* and was relatively close to one another. *R. rothii* was clustered near to *A. platycarpa* has exposed kinship to one another. The *V. unguiculata* and *V. radiate var sublobata* was found adjacent to one another similarly *V. radiata* and *V. mungo* was placed nearest to one another.

After SDS-PAGE electrophoresis, the result disclosed that the technique provided an influential apparatus for consistent germplasms judgment based on genetic differences in seed storage protein compared to selected germplasms of *Vigna*. Thus, the present study explores the existing polymorphism of total proteins through SDS PAGE to facilitate the characterization of selected germplasms of *Vigna.*

Seed storage protein profiling is a suitable tool for assessing genetic diversity in legumes species, such as the work carried out in some *Vigna* species cultivated in China (Chen et al. 2006). The SDS- PAGE is shown to be a dominant tool for judgment of *Vigna radiata* and *Vigna mungo* (Gafoor et al. 2002); SDS-PAGE has been applied as a practical and reliable scheme for species phylogenetic relationship and identification. Therefore, the current research was led that has shown promising results with low intra-specific and high inter-specific diversity that has able us to differentiate all the species through SDS-PAGE.

The eight plant species under the three genera belong to the family Fabaceae study exposed that no plants have similar protein banding patterns which show the presence of genetic diversity among these species. The presence of a common band/locus (L-6) among these eight species suggests their close genetic resemblance and common ancestry (Muhammad et al., 2019a). This locus coded for by a gene that has become fixed in different species under these genera over evolutionary time (Azeez, and Morkinyo, 2004; Muhammad et al. 2018) that the existence of common bands in *Lycopersicum* and *Trichosanthes* species designates their common evolutionary origin. Also, Alkinwusi and llloh et al. (1995) documented the occurrence of a common

band in all individuals in a population to the fact that the gene coding for the enzyme or protein does not vary.

Due to High inter-species locus, genetic diversity, SDS-PAGE could be a dependable procedure for documentation of these eight species. In contrast, intra-specie locus contribution toward genetic disagreement was 33.33% in *V. radiata,* 11.11% *in V. mungo,* 33.33%, 0.00 in *V. radiata* var. *sublobata*, 22.22% in *V. unguiculata,* 33.33% in *R. minima,* 22.22% in *R. capitata* and *R. rothii* whereas 66.66% in *A. platycarpa*. In the same way, inter species locus/band contribution toward genetic diversity was 88.88%.

In our current work, phylogenetic tree based on seed storage protein analyses of selected species showed that the 8 species had close similarity to one another. The result showed that the *V. mungo* was clustered adjacent to *A. platycarpa* and was relatively close to one another; this in contrast to dendrogram based on morphometric analyses, this may be due to morphometric traits are under the influence environmental fluctuations (Muhammad et al., 2018) *R. rothii* was clustered near *R. capitata.* Similarly *V. unguiculata* and *R. minima* placed closed to one another. *V. radiata* var. *sublobata* had clustered near to *V. radiata* has revealed relatedness to *V. radiata*. The results obtained after SDS-PAGE electrophoresis disclosed that the technique delivered a powerful tool for dependable genotypes determination based on genetic variation in seed storage protein. Thus, the current project discovers the existing polymorphism of total proteins through SDS PAGE to facilitate classification of selected germplasm. Similar study was carried out in 10 different species of family Fabaceae in which all species have shown genetic affinity to each other (Alege et al. 2014).

CONCLUSION

In the present investigation, we have attempted to assess the genetic polymorphism and phylogenetic relationship amongst selected 8 Fabaceae species; this may prove important in improving the economically important legume crops by manipulating their wild relatives. The genetic disagreement within the specie was 33.33% in *V. radiata,* 11.11% in *V. mungo,* 0.00 in *V. radiata* var. *sublobata*, 22.22% in *V. unguiculata,* 33.33% in *R. minima,* 22.22% in *R. capitata* and *R. rothii* whereas, 66.66% in *A. platycarpa*. Further, inter species locus/band contribution toward genetic diversity was 88.88. Presence of common Locus/band 6 (B-6) in all collected genotypes of the current study suggest their close genetic affinity and common ancestry.

Significant Statement:

The genetic polymorphism and phylogenetic relationship among the selected legume species, could demonstrate an important in improving the economically important legume crops by manipulating their wild relatives. The species chosen for the analysis by morphometric and SDS-PAGE bared a considerable genetic variations in the study of total genotypes. Hence, the results obtained by this study could be of a broader range. Today there is still a need to evaluate phylogenetic relationship and genetic variability and conserve genetic resources, particularly wild species, and pulses, for prospective plant breeding benefits. There is a general understanding that growth of the genetic base is a real need if genetic vulnerability is to be reduced and further advancement to be made.

CONFLICT OF INTEREST

The authors declared that the present study was performed in the absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

NM collected plants materials, carried out experimental work, and wrote the manuscript, NA conceived the overall project; NU and MKK helped in interpretation of the results, and MR critically reviewed the manuscript. The authors have read and approved the final manuscript.

with these terms.

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