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Genetic polymorphism within the wild population of *Rhynchosia himalensis* Benth. ex Baker

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Abstract

The importance of plant genetic diversity is now being recognized as a specific area since exploding population with urbanization and decreasing cultivable lands are the critical factors contributing to food insecurity in developing world and are also helpful in conservation planning strategies of threatened plant species. For this purpose thirty genotypes of *Rhynchosia himalensis* were collected from the different areas of Swat and were assessed for morphometric and for SDS-PAGE and this study revealed that genetic assortment were present at both morphological and molecular levels. The intraspecific variations were present among the genotypes. The examined morphological data (which includes quantitative and qualitative data) exposed that variation was present among the genotypes of above specie. Likewise it was found that two alleles were responsible in controlling leaf color, leaf upper surface with Emerald green 70% and leaf lower surface with 30% yellow green. Other leaf color was 70% Moss green and 30% white respectively. 100% seeds were flat shape. The seed color was found to be brown for all of the genotypes, Hillum color was found to be yellow. Seed texture was of two types Rough and Smooth. The rough was 60% and Smooth was of 40%.The seed storage protein profiles of twenty *R. himalensis* genotypes were studied for SDS-PAGE and total seed storage proteins were best resoluted on 12% polyacrylamide Gel. A total of 9 reproducible bands with molecular weight ranging from 10 to 180kDa were detected. The genotypes were distributed into two Regions R-I, R-II and R-III. Based on the intraspecific locus variation among 30 genotypes of *R. himalensis* L-3, L-6 and L-8 were monomorphic and the locus contribution toward genetic disagreement *(LCTGD)* of *Rhynchosia himalensis was* 33.333%. Further investigation is required for genetic diversity in the *R. himalensis*.

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Introduction

The genus *Rhynchosia* belongs to Fabaceae family is extensively dispersed in the mountainous regions of the tropics. *Rhynchosia himalensis* is an emerging annual summer weed in Pakistan (Ali *et al.* 2013). It is native to India (Dogra *et al.*, 2009), Pakistan and Sri Lanka (Ildis, 2010; Lopez, 2012). It has invaded the agro-ecosystems of Southern Punjab, Pakistan and is increasingly becoming a problematic weed in farming systems (Ali *et al.* 2013).

Rhynchosia himalensis is a climbing plant with twining stems that scramble over the ground and climb into the surrounding vegetation for support (Lopez, 2012). The plant is harvested from the wild for local use as a food and a medicine. The plant has remarkable potential to be used in forage, pharmaceutical and other agricultural products, and more importantly, there are no known major threats to this species (Lopez, 2012); however, the plant has negligible uses worldwide, including in Pakistan (Muhammad *et al.* 2018). *Rhynchosia himalensis* has a wide distribution range and is present in protected areas. However, further research and surveys are recommended to confirm the distribution range of the species. The plant is classified as 'Least Concern' in the IUCN Red List of Threatened Species (2013). Seeds are spherical and usually brown in color. Seeds mature within 3 months when the plant also starts to dry (Sharma *et al.* 1978). The growing season is from May to October. Aerial parts of *Rhynchosia himalensis* Benth. ex Bakershows significant analgesic activity comparable to that of standard drug Aspirin.

A widespread genetic base is serious to adaptation and will define the future severity of climate change effects (Muhammad *et al.* 2018).

Recent attitudes to biodiversity conservation are mainly based on geographic areas, ecosystems, ecological communities and species, with little attention on genetic diversity and the evolutionary continuum from populations to species. Conservation management generally rests on discrete categories, such as identified species, and, for threated taxa, intraspecific units. Species, in particular, provide a common measure of biodiversity yet in theory and nature, speciation is typically a protracted process progressing from connected populations to unambiguous species with variable rates of phenotypic, ecological and genetic divergence. Thus, most recognized species are not genetically uniform and are sometimes highly structured into historically isolated populations worthy of consideration as intraspecific units that represent unique genetic diversity for conservation.

The morphometric traits have vital role in the analysis of genetic diversity in plants but affected by environmental changes severely and there by contradictory the study of genetic divergence (Muhammad *et al.* 2018). On the other side genetic diversity estimation through molecular methods such as biochemical evaluation at protein level and DNA based practices have a number of benefits over the classical morphology (Ndiaye *et al.,* 2012) but compared to biochemical evaluation at protein level, molecular study of DNA markers are too costly (Muhammad *et al.* 2018). Among biochemical measures, Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) method is a simple, reliable, cheap and free of environmental changes (Muhammad *et al.* 2018).SDS-PAGE is now commonly used biochemical way to designate the genetic structure of crop species (Muhammad *et al.* 2018a). Massive attention has been focused on the use of SDS-PAGE over the last two decades for evaluation of genetic polymorphism, consistent judgment and documentation of plant species. Seed storage protein markers have been effectively used to determine taxonomic interactions and characterize cultivated varieties in a number of crop plant species; Lima bean (Lioi*et al.*, 1999), *Phaseolus vulgaris* (Ferreira *et al.*, 2000), Chickpea (Ghafoor *et al.* 2003) *Lens culinaris* (Sultan *et al.* 2006) *V. unguiculata* (Win *et al.*, 20011). Proteins are being the end products of gene expression; SDS-PAGE can be employed to identify varities, describe polygenetic relationship in various plants, biosystematics analysis

and assess the passport data (Sammour, 1991).

Pakistan is in the vicinity of the center of diversity of *Rhynchosia himalensis* Benth. ex Baker but yet there is no report about the genetic diversity of *Rhynchosia himalensis* Benth. ex Baker based on biochemical characterization. In this article, we report on SDS-PAGE characterization of 30 Pakistani *Rhynchosia himalensis* Benth. ex Baker germplasm, which hold important local adaptation and are of widespread throughout the entire country.

The objective of the study is to estimate the level of genetic diversity in Pakistani *Rhynchosia himalensis* Benth. ex Baker genotypes based on morphological assessment and SDS-PAGE characterization.

Material and methods

Plant materials

To explore the genetic diversity both morphological and SDS-PAGE was carried out. Samples from 30 genotypes of *Rhynchosia himalensis* Benth. ex Baker, were collected from different loculities of, District Swat (Malam, Jaba, serai, Banda, Raniyal, Derai, Qandeel, Lalko, AlamGanj, Nawagai, Swegalai, Shagai, Kalabat, Dokat, Bela, shagai, Kokarai, Sara Shah, Kukrai, AmlookDara, Naji Gram ,Mangaltan, Shalping, AmlookDara, Naj Gram, BaghDeraiDadahara, Dam, Gadi, Kotlai, Dagay, Zawra), KPK, Pakistan. Mature seed of 30 samples were collected from each genotype and the branches along with seeds were preserved. These seeds were then subjected to SDS-PAGE to extract seed storage protein.

Morphological characterization

Both qualitative and quantitative descriptions were carried out. Qualitative traits were recorded on the general visualization (phenotypic observations). Seven qualitative traits i.e. Leaf upper and lower surface color, flower color, seed color, seed shape testa texture, Hilum color) were studied in the present investigation. Similarly quantitative traits which were measured with the help of Vernier calipers are: petiole length (PL), leaf length (LL), leaf width (LW), seed length (SL), seed width (SW), seed thickness (ST), and seed weight (SWt), pod length (PodL), No. of seed per pod (SP), No. of pod per plant (PP), inflorescence length (IL), inflorescence width (IW), 100 seed weight, No. of branches per plant (B/P), plant height (PH), stipule length (StL), Biomass(BM). Characters mean was found out after measuring of 3 different samples (small, medium, large) of each quantitative character.

Protein profiling

To guesstimate the level of genetic diversity poly acryl amid gel electrophoresis SDS-PAGE was carried out. For seed storage protein profile, single seed of each genotype was crushed into a fine powder. About 400µl of Protein Extraction Buffer (PEB) with a composition of 0.5M Tris-HCL, 0.2%SDS, 5M Urea, 1%B-mercaptoethanol under 8-pH was added to 0.01g of seed fine powder. The E-tube containing PEB and seed fine powder (PEB-FP) was Vortexed thoroughly to homogenize the mixture.

The Comassive Brilliant Blue (CBB) was added to the E-tube as tracking dye to see the movement of PEB-FP on the separation PAG. The homogenated samples were centrifuged at 13,000 rpm for 10 minutes under room temperature.

The electrophoretic process was carried out using 12% polyacrylamide gel (composition of resolution gel: 3.0M Tris-HCl pH9.0, 0.4% SDS and staking gel 0.4M Tris-HCl pH 7.0, 0.4% SDS).

The electrode buffer containing 0.025M Tris, 129M Glycine and 0.125% SDS was poured in the Electrophoresis tank. Similarly, 15µl PEB-FP was loaded in each well of 10% PAG. The electrophoresis was run at 100V until the blue line passed through the bottom of gel plates. The PAG were than stained and destained for data scoring of seed storage protein profile.

Data analysis

Morphological data was analyzed through cluster plotting using software's SPSS and PC-ORD. Principal

Component Analysis (PCA) was conducted by using PC-ORD.

Results

Qualitative characters

The results obtained for the assessment of genetic diversity in 7 qualitative characters of *Rhynchosia himalensis* presented in Table 1. It was found that two alleles were responsible in controlling leaf color, leaf upper surface with Emarld green 70% and leaf lower surface with 70% yellow green. Other leaf color was 30% Moss green and whites each respectively. 100% seeds were flat shape.

The seed color was found to be brown for all of the genotypes, Hillum color was found to be yellow. Seed texture was of two types Rough and Smooth. The rough was 60% and Smooth was of 40%.

Table 1. Qualitative traits noted on 30 genotypes of *R. himalensis.*

Traits	Color	%age
Leaf Upper Color Surface	Emerald green	70%
	Moss green	30%
Leaf lower Color Surface	Yellow green	70%
	White	30%
Flower color	Yellow	100%
Seed color	Brown	100%
Seed Shape	flat	100%
Hilum color	Yellow	100%
Testa texture	Rough	60%
	Smooth	40%

Quantitative characters

Data were recorded on 17 quantitative traits of 30 genotypes *Rhynchosia himalensis.* By using the Pearson correlation coefficient the result for the association coefficient among the various traits for the *R. himalensis* was accomplished (Table 2).

Table 2. Correlation coefficient among seventeen quantitative traits of *R. himalensis*

	PL	LL	LW	StL	IL	IW	SL	SW	ST	PodL	S/P	P/P	SWt	B/P	PH	BM	Y/P
PL	1.00																
LL	-0.05	1.00															
LW	-0.10	.918**	1.00														
StL	0.29	-0.02	0.14	1.00													
IL	0.37	-0.37	-0.36	0.19	1.00												
IW	0.25	-0.25	-0.19	0.19	.874**	1.00											
SL	0.26	0.25	0.17	.664**	0.11	-0.03	1.00										
SW	0.18	0.20	0.06	.480*	-0.15	-0.27	.599**	1.00									
ST	-0.26	0.44	0.33	0.09	-.568**	-.641**	0.40	.490*	1.00								
PodL	0.08	-0.01	-0.02	.481*	-0.06	-0.10	0.23	0.496*	0.27	1.00							
S/P	-0.05	-0.06	-0.05	-0.06	-0.20	-0.24	0.15	0.13	0.26	0.21	1.00						
P/P	-0.04	-0.14	-0.11	0.12	-0.07	-0.07	-0.10	-0.02	-0.12	-0.19	-0.25	1.00					
SWt	0.36	-.646**	-.556*	0.21	0.27	0.11	0.05	0.03	-0.32	0.00	0.01	0.21	1.00				
B/P	-.447*	0.05	0.16	-0.13	-0.31	0.06	-0.40	-0.35	-0.31	-0.26	-0.28	0.27	-0.12	1.00			
PH	0.16	0.24	0.24	-0.13	-0.20	-0.19	-0.06	0.12	0.29	0.24	0.29	-.483*	-0.05	-0.22	1.00		
BM	0.21	-0.42	-.522*	0.41	0.18	0.13	0.24	0.29	-0.07	0.29	-0.30	0.28	0.26	-0.02	-.548*	1.00	
Y/P	-.584**	0.21	0.14	-0.15	-.690**	-.601**	0.03	0.14	.608**	0.05	0.40	0.04	-0.44	0.13	-0.01	0.04	1.00

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

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

PL= petiole length, LL= leaf length, LW= leaf width, StL= stipule length, IL= inflorescence length, IW= inflorescence width, SL= seed length, Sw= seed width, ST= stipule length,PodL= Pod length, No. of the seed/pod, S/P= seed/ pod, P/P= per plant, Swt=100 seed weigth, PH= plant height, BM= Biomass,Y/P=Yield Plant

In correlation study, the petiole length in the *R. himalensis* was negatively correlated with leaf length whereas the stipule length was significantly positively correlated with inflorescence length. Seed length was significantly positively correlated with seed width. No. of pod/ plant was significantly positively correlated with plant height. The yield /plant (Y/P) was significantly positively correlated with Biomass (BM).

Table 3. Intra locus polymorphism in *Rhynchosia himalensis* genotypes.

Locus	Present%	Absent%	Variation%	Status	GD
L-1	40	60	60	poly	0.4
L-2	70	30	30	poly	0.7
L-3	100	0.00	40	mono	1.00
L-4	30	70	70	poly	0.3
L-5	30	70	70	poly	0.3
L-6	100	0.00	Nil	mono	1.00
L-7	90	10	10	poly	0.9
L-8	100	0.00	Nil	mono	1.00
L-9	75	25	25	poly	0.75

GD=33.33% Poly loci/Total loci*100

GD= Genetic disagreement.

The double data matrix of 30 genotypes based on morphology was evaluated for the construction of phylogenetic tree. It denotes the similarity of various genotypes and the 30 genotypes of the *R. himalensis* were studied for similarities and the phylogenetic tree was made (Fig. 1).

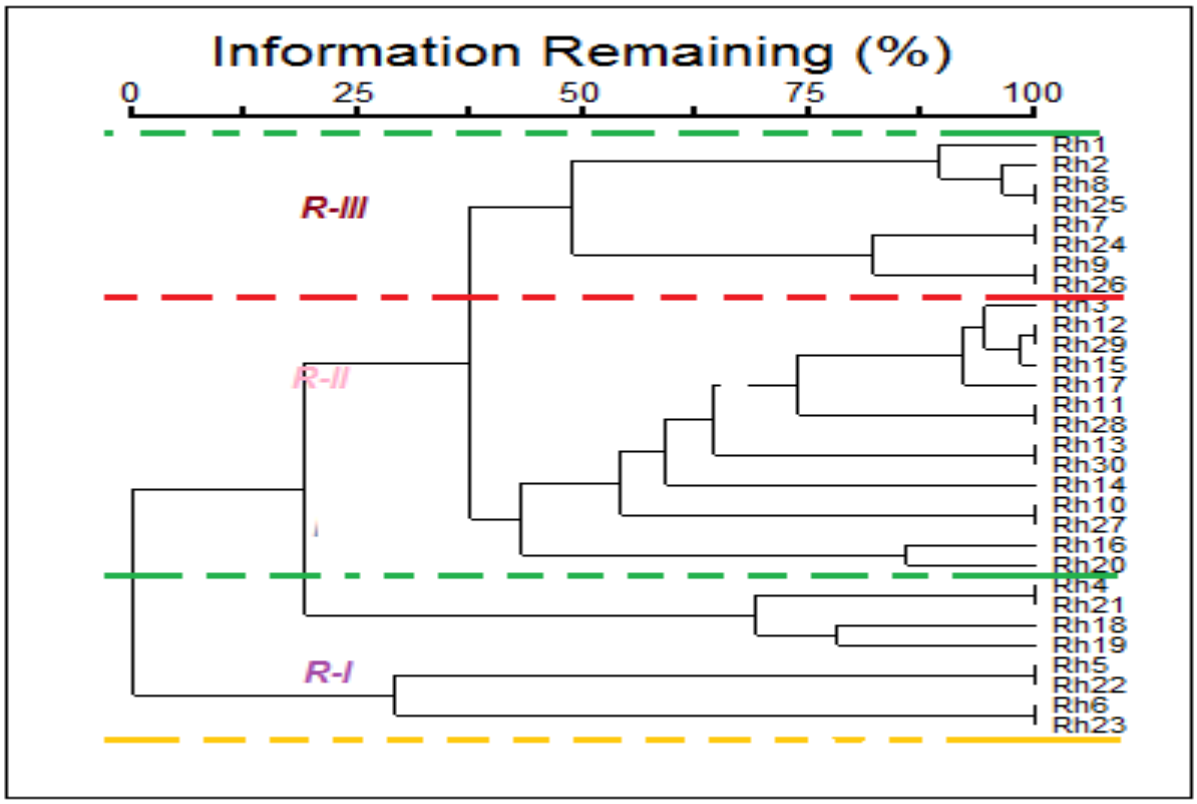

Fig. 1. Intra -specific genetic diversity identified through morphological traits analysis in 30 different genotypes of *Rhynchosia himalensis* Benth. ex Baker collected from Swat. Khyber Pakhtunkhwa, Pakistan. Rh represents *Rhynchosia himalensis* Benth. ex Baker.

The phylogenetic tree divided all the 30 genotypes of *R. himalensis* into three Region I and R- II and R-III. R-I and R-III were composed of 8, 8 genotypes each and R-II were composed of 14 genotypes.

SDS-PAGE characterization

A total of nine reproducible bands were observed in 30 genotypes of *R. himalensis*. (Fig. 2).

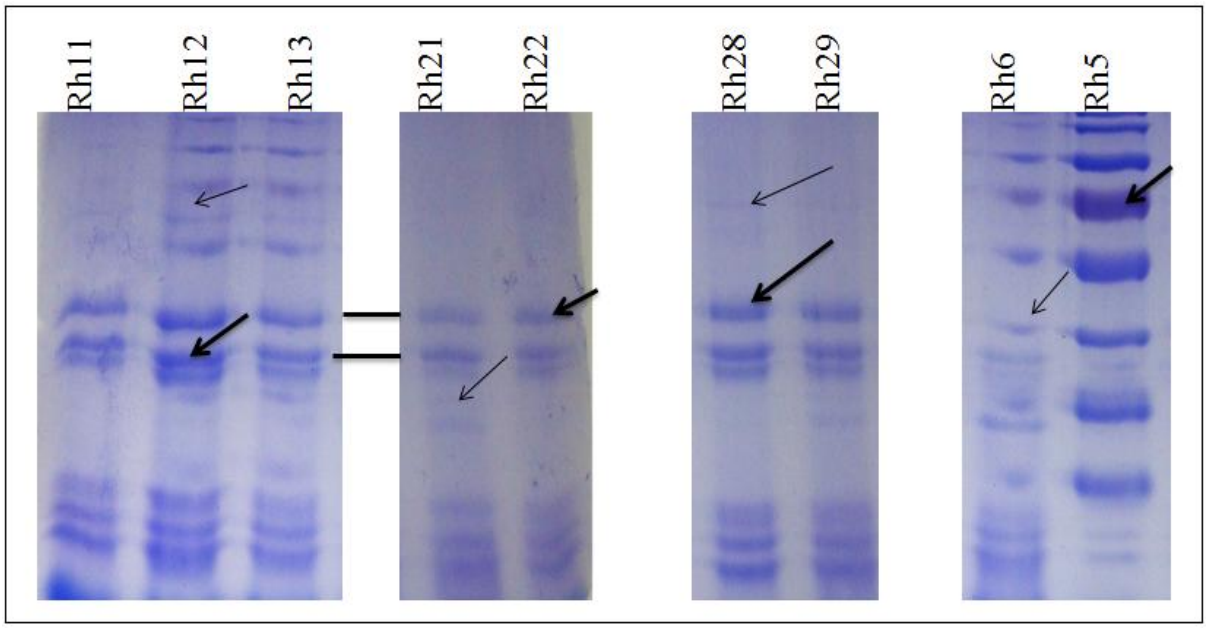

Fig. 2. Electrophoregram represents Seed storage protein profile of different 9 genotypes *Rhynchosia himalensis* Benth. ex Baker. Arrow indicates the location of protein bands in the electropherogram. Rh= *Rhynchosia himalensis* Benth. ex Baker.

Genetic association within genotypes

A total of nine reproducible bands were observed in 30 genotypes of *R. himalensis*. (Fig 3). The R-I had 11 genotypes (Rh9 Kokarai, Rh11Malam, Rh12Jaba, Rh13serai, Rh15Banda, Rh20Derai, Rh22 Raniyal, Rh23 Lalko, Rh27 Qandeel, Rh9Nawagai, Rh11AlamGanj, Rh29 Nawagai while R-II had 13 genotypes (Rh2Shagai, Rh3Swegalai, Rh6Kalabat, Rh7Dokat, Rh8Bela, Rh10Sara Shah, Rh14Kukrai, Rh18Sara Shah, Rh19Mangaltan, Rh24Shalping, 28AmlookDara, Rh30 Naj Gram, Rh21Bagh Derai Whereas R-III Rh1Dadahara, Rh4Dam, Rh5 Gadi, Rh16 Kotlai, Rh17 Dagay and Rh26Zawra. The data was confirmed of cluster analysis by scattered plot detected through Principal Components based on SDS-PAGE.

Genetic diversity identified through SDS-PAGE

Intraspecific locus variation among 30 genotypes of *R. himalensis* is represented in Table 3 Notably, L-3, L-6 and L-8 were monomorphic in *R. himalensis* variation respectively and the locus contribution toward genetic disagreement *(LCTGD)* of *Rhynchosia himalensis was* 33.333% table 3. A total of 9 reproducible bands with molecular weight ranging from 10 to 180kDa were detected in 30 genotypes of *R. himalensis* (Table 3).

Discussion

In the present investigation, 30 genotypes of *Rhynchosia himalensis* Benth. ex Baker revealed a considerable level of Intra-genotypic genetic diversity tested through seeds phenotypic characterization and SDS-PAGE description. It was found that two alleles were responsible in controlling leaf color, leaf upper surface with Emarld green 65% and leaf lower surface with 65% yellow green. Other leaf color was 35% Moss green and white respectively. 100% seeds were flat shape. The seed color was found to be brown for all of the genotypes, Hillum color was found to be yellow. Seed texture was of two types Rough and Smooth. The rough was 55% and Smooth was of 45%.

Protein profiling by SDS-PAGE is measured as reliable tool for economic description of germplasm because environmental fluctuations does not affect storage proteins (Javid *et al.* 2004; Iqbal *et al.* 2005).

The protein profiling of 30 *Rhynchosia himalensis* Benth.ex Baker was tested through 10% slab gel electrophoresis.

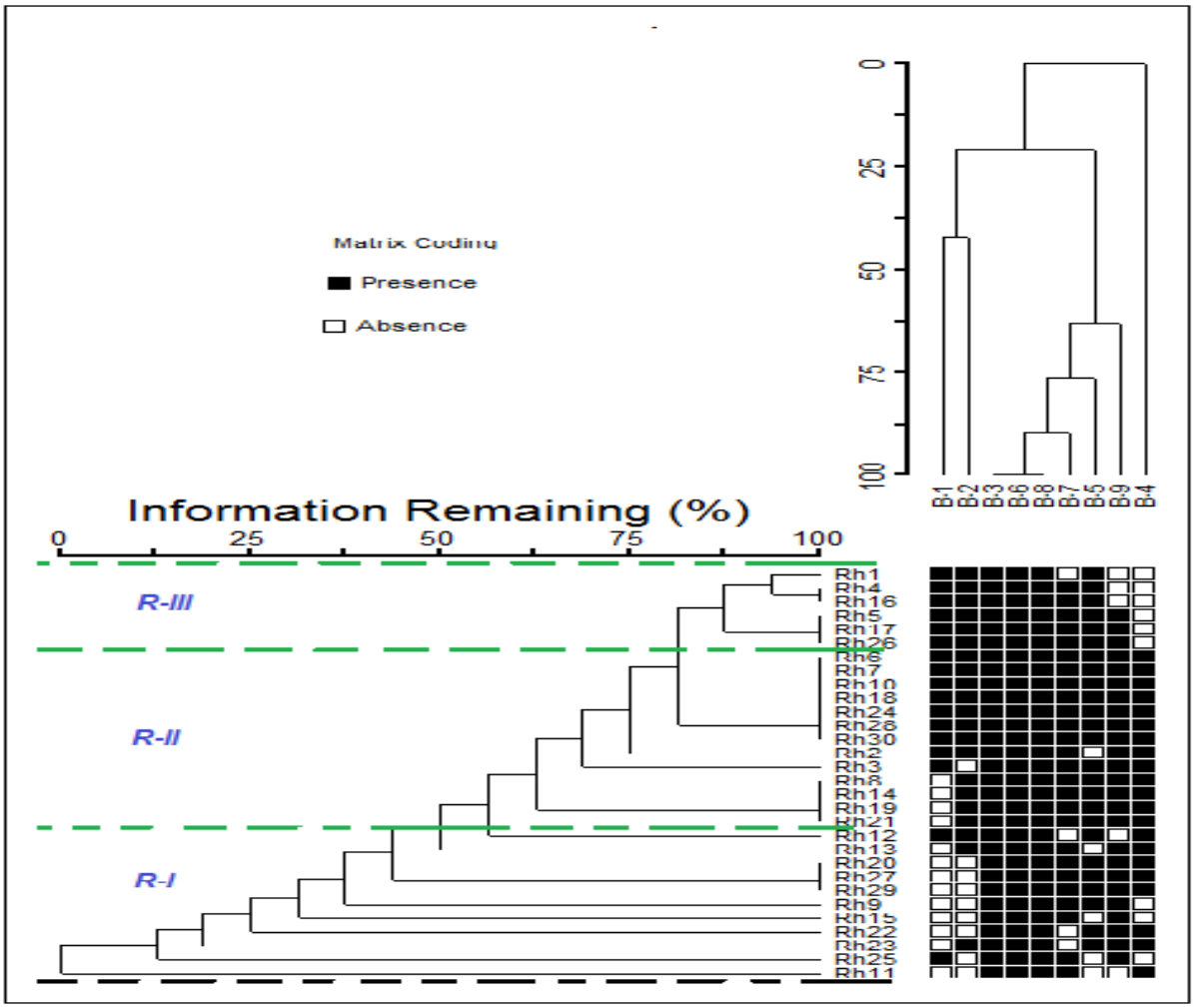

Fig. 3. Two-way Cluster analysis Tree represents the banding profile, showing genetic relationship of 30 *R. himalensis* based on protein bands showing genetic diversity in 9 bands based on bands presence/absence.

It denotes the similarity of various genotypes and the 30 genotypes of the *Rhynchosia himalensis* Benth. ex Bakerwere studied for similarities and the phylogenetic tree was built. The phylogenetic tree divided all the 30 genotypes of *R. himalensis* into two Region I and R- II. R-I has 11% genotypes and R-II has 9 genotypes. The R- I and R-II was 62. 5%.

Intraspecific locus variation among 30 genotypes of *Rhynchosia himalensis* Benth. ex Baker Notably, L-3, L-6 and L-8 were monomorphic in *R. himalensis* variation respectively and the locus contribution toward genetic disagreement (LCTGD) of *Rhynchosia himalensis* was33.333%.A total of 9 reproducible bands with molecular weight ranging from 10 to 180kDa were detected in 30 genotypes of *R. himalensis*.

Description of genetic diversity within the genotypes is of principal significance to crop development programs (Simon *et al.*, 2007; Win *et al.*, 2011). Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of seed storage protein is a common approach of revealing genetic assortment and relationship among different taxa (Muhammad *et al.* 2018). Genetic polymorphism in

different plant species have been carried out by using electrophoretic designs of total seed proteins as exposed by SDS-PAGE of seed storage protein (Ladizinsky & Hymowitz, 1979; Potokina*et al.*, 2000; Ghafoor & Arshad, 2008; Ayten*et al.* 2009). In Leguminosae many studies have been performed based on the SDS-PAGE (Hussein and George, 2002; Hussein *et al.*, 2005). Other plants have been particularized through seed storage protein using SDS-PAGE (Win *et al.* 2011; Sundin*et al.* 2004; Oppong-konadu *et al.* 2005).

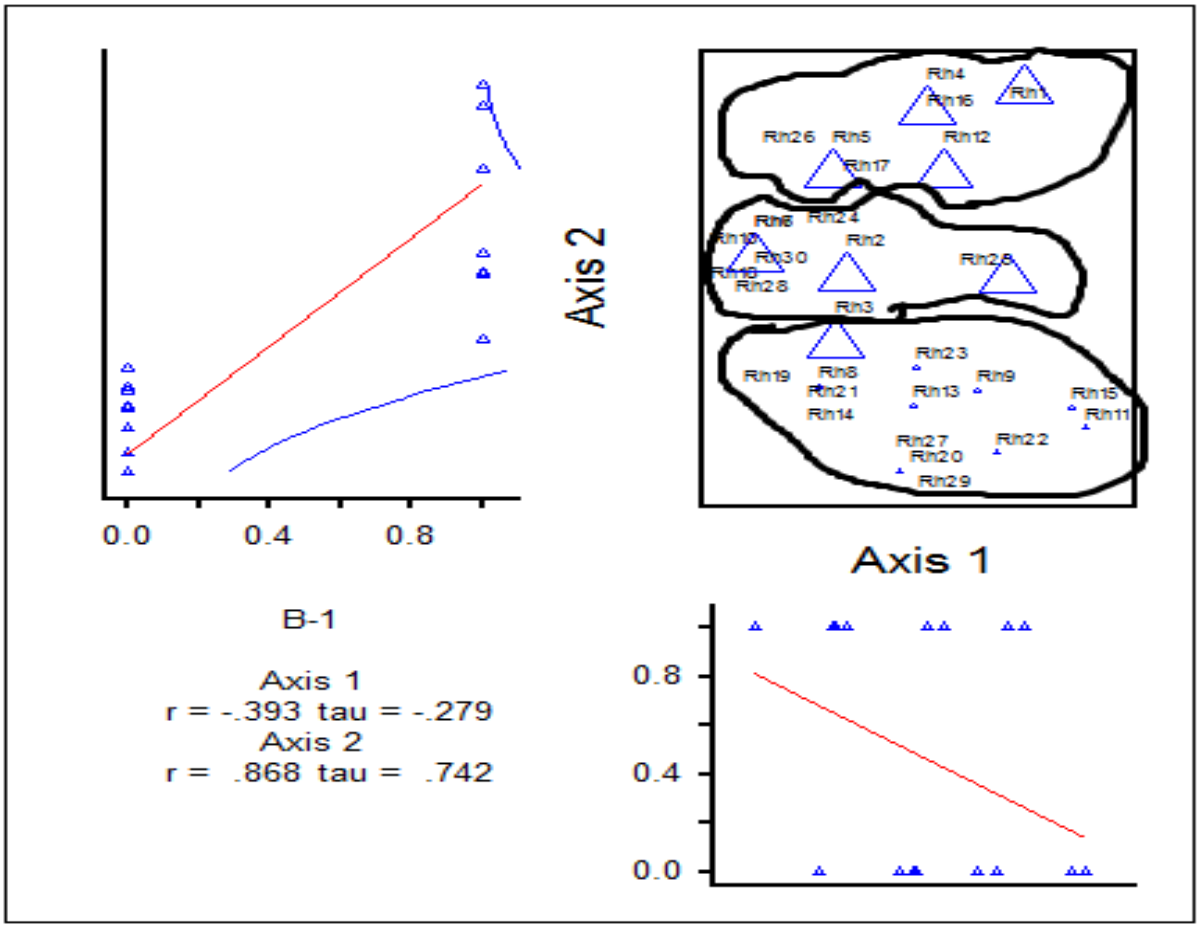

Fig. 4. Confirmation of cluster analysis by scattered plot detected through Principal Components based on SDS-PAGE collected from Swat.

Conclusion

For better management of the gene bank, precise and comprehensive knowledge of agricultural and biochemical data (protein and DNA) is essential so that duplicates can be eliminated; this will help in compiling a core collection of *R. himalensis*. Variations in SDS-PAGE can be exploited to understand the extent of genetic diversity and the relationship among Pakistani *R. himalensis*.

Genotypes with similar banding patterns have been suggested to be further studied for detailed agronomic and biochemical analyses, including 2-D electrophoresis and DNA markers, for better management of the gene bank.

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Conflict of interest

The authors declare to have no conflict of interest.

Author's contribution

MKK and NM collected plants and carried out written

work, IK, NA, RU and NU helped in interpretation of the results, and wrote and critically reviewed the manuscript. All authors have read and approved the final manuscript.

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