Research Article

Evaluation of Genetic Diversity in Wild *Rhynchosia capitata* through Morphomatric and Biochemical Character Collected from District Dir Lower

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Abstract | The present research work was carried out on 36 genotypes based on morphological and biochemical characterization collected from different regions of District Dir Lower. A total of 12 morphological parameters were recorded, of which 3 of them were qualitative characters: leaf colors, seed coat color, and seed shape. For quantitative trait, the maximum coefficient of variance (0.95%) was found in petiole length, biomass per plant (0.49%), and pod per plant (0.41%), whereas the minimum coefficient of variance showed by leaf width (0.25%), followed by leaf length (0.27%) and internode length (0.29%). Coefficient correlation analysis was computed for all the quantitative traits, where a positive correlation was recorded for plant height (0.37), leaf length (0.18), and seed per plant (0.127). Biomass per plant shows a positive correlation with leaf length (0.118), leaf width (0.27), and seed per plant (0.22) whereas a negative correlation was found in petiole length (-0.044), plant height (-0.002), pod length (-0.59) and pod per plant (-0.99). Principal component analysis (PCA) based on 9 quantitative traits showed significant divergence among the 36 genotypes of *R. capitata*. It was determined that the 4principal component with an Eigenvalue of above 0.99 accounted for 61.4% of the total variation, where the 1st PC shows a total variation of 19%, the 2nd PC showed 34.87%, 3rd PC had 49.38% while the 4th PC showing a total variation of 61.4%. Based on cluster analysis all the genotypes were divided into 2 main lineages and further subdivided into 6 clusters where the genotypes A1 and A9 were found the most diverse and were found at the extreme of the Dendrogram. For total seed, storage proteins all the genotypes were subjected to SDS-PAGE analysis using 12.5% acrylamide gel, where a total of 14 polymorphic bands were observed. In Band 1 the highest degree of variation (0.83%), followed by Band 2 and Band 3 with a value of 0.75% variation, whereas the lowest (0.17%) was found in Band 12, followed by Band 14 with a value of 0.25% respectively. Based on two-way cluster analysis all the genotypes were divided into 2 main groups and further subdivided into 6 groups where the genotypes RC01 and RC16 were found the most variant genotypes and were placed at the extreme of the cluster Dendogram. The entire bands loci show polymorphism, report addressing genetic variability in *Rhynchosia capitata*.

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Introduction

The *Rhynchosia capitata* (Roth) DC, belong to Fabaceae which is widely distributed in tropical areas, In Pakistan, *Rhynchosia capitata*, is a perennial summer plant, It is indigenous to Sri Lanka, India and Pakistan Southern Punjab, is appropriate a more serious pest of agricultural (Dogra *et al*., 2009; ILDIS, 2010; Ali *et al*., 2013). After watering, the plant emerges from the seed in the field, it is an annual plant with numerous branches that surround and root at each node. A nearly month-old plant starts to bloom and produces oval-shaped pods with two seeds in each pod. Typically, the spherical seeds are dark in hue. The plant starts to dry out after three months, and the seeds mature. From May to October, when the typical low and high temperatures are 29/21 ˚C and 39/29 ˚C, respectively, the season for growth is in effect (Noor *et al*., 2018).Despite the fact that they are influenced by environmental changes and conflict with the study of genetic variability, morphological traits are important for the estimation of genetic diversity in plants (Nisar *et al*., 2016). On the other hand, there are several advantages to using DNA-based techniques and molecular methodologies for genetic diversity estimation as opposed to traditional morphology (Ndiaye *et al*., 2012). Comparatively to biochemical analysis at the protein level, investigating DNA characterization at the molecular level is too expensive (Win *et al*., 2011). The protein isolation technique is a straightforward, trustworthy, affordable, and ecologically consistent method for biochemical measurements (Wadood *et al*., 2016). SDS-PAGE method that is increasingly being used to determine the genetic makeup of agricultural species (Hameed *et al*., 2009). SDS-PAGE has received a lot of attention over the last 20 years for evaluating genetic variation, making reliable decisions, and cataloguing plant species. Cultivated variants of a number of crop plant species have been successfully identified and taxonomic relationships determined using seed storage protein characterization; Beans Chickpea (Ghafoor *et al*., 2003; Nisar *et al*., 2007), *Pisum sativa* (Nisar *et al*., 2009), Lens culin (Win *et al*., 2011). The latent uses of the wild relatives as genetic tools for crop breeding and enhancement are the focus of this assessment, and we concise the different genetic/genomic slants postulated for these drives. For tree improvement, programs can be developed on the results of the proper study using morphological and biochemical characterization. The aim of the present research is to find out the genetic diversity in food legumes. To evaluate the genetic diversity in *Rhynchosia capitata* through morphological characterization. To score out total seed storage proteins using SDS-PAGE methods also to score out the most promising genotypes future breeding program.

Materials and Methods

The present study was carried out in 2023 at the University of Malakand Department of Botany. A total of 36 genotypes of *Rhynchosia capitata* were used for agro morphological and biochemical characteristics. A total of 12 morphological traits in which nine quantitative traits: Petiole length, leaf length, leaf width, seed length, seed width, and seed weight, pod length, number of seed per pod, number of pods per plant, number of branches per plant, and plant height, and three qualitative traits were used in the current study.

SDS-PAGE analyses were performed on all the studied genotypes to determine total seed storage proteins. Five healthy and mature seeds were finely ground with a mortar and pestle, about 0.02g of the ground material was weighed and transferred into 1.5 ml centrifuge tubes with 400 protein extraction buffer. The material was than homogenized for 1 minute in a vortex before being centrifuged at 12,000 rpm for 10 minutes at room temperature. For total seed protein, 12.5% polyacrylamide gels were used, as per Laemmli's 1970 procedure.

Data analysis

To determine genetic diversity, five plants were randomly selected and the mean value of each genotype was used for data analysis. Microsoft Excel 2016 was used to compute basic statistics (Mean, Maximum, Minimum, and Coefficient of variation). For cluster analysis and principal component analysis PC ORD software version 6, and correlation analysis SPSS version 22 were used. Similarly, for total seed storage protein the binary matrix data was calculated as the presence and absence of bands. Two way cluster Dendrograms were generated with the help of PC ORD version 6 using the unweighted pair group method with arithmetic average (UPGMA) technique.

Results and Discussion

Genetic diversity based on agro morphological characterization

The current research investigated at both qualitative

(3) and quantitative (9) traits in *Rhynchosia capitata* genotypes from Dir (L). During the investigation 3 qualitative characters of *Rhynchosia capitata* were studied which are leaf colors, seed coat color, and seed shape. A single allele was found to be in charge of regulating leaf color; each was determined to be yellow-green. Every seed was circular and had a head on top. For all of the genotypes, the colour of the seeds was found to be brown 42%, grey 54%, and pale grey 4%. There were two types of seed texture: Rough 55% and smooth 45%.

Quantitative characters

During the present study, 9 quantitative traits were studied in *Rhynchosia capitata* collected from 2 Districts, and there was found a large number of variations for most of the traits. The maximum value for internodes length was recorded 4.1 cm while minimum was 1.7 cm with the mean value of 2.58 cm. Minimum value for biomass per plant of 2 gm and maximum value of 8 gm with the mean value of 4.14 gm, standard deviation 0.328, sample variance 3.773. .Minimum value 0.2 cm and maximum of 2 cm for the Petiole length with the mean of 1.01 cm, with a mean value of 18.9 cm, a standard deviation of 6.568, and a sample variance of 43.14 percent, the highest range for plant height was 35 cm, while the minimum was 10 cm. Leaf length had a mean value of 3.24 cm, a standard variation of 0.877, a sample variance of 0.770, and a range of 2 cm to 6 cm, respectively. The mean value for leaf width was 2.36 cm, standard deviation of 0.597, sample variance of 0.356, with the minimum value of 1 cm and maximum value of 3.2 cm. Similarly pod length has maximum range from 18 cm while minimum range from 1.1 cm with the mean value of 2.87 cm, standard deviation 2.738 and sample variance 7.495%. Furthermore, seed per pod with the mean value of 2.86, standard deviation 0.912, sample variance 0.832%, minimum value of 2 and maximum value of 4. The mean value for pod per plant was 14.2 standard deviation 5.759, sample variance 33.165, minimum value 8 and maximum value of 40 pods per plant. Similarly the association coefficients among 9 quantitative traits are, Internodes length (2.56), Branch per plant (3.96), petiole length (0.94). Total plant height (31.42), leaf length (3.28), leaf width (2.35), pod length (3.05), seed per pod (2.79) and pod per plant (14.54) (Table 1, Figure 1).

Correlation analysis

For nine morphological characteristics, correlation was calculated using MS Excel 2016. Internode length showed a negative correlation with biomass per plant (-0.07), petiole length (-0.15), pod length (-0.14), and pod per plant (-0.13), while a positive correlation was seen with plant height (0.03), leaf length (0.18), leaf width (0.10), and seed per plant (0.12). Biomass per plant show positive correlation with leaf length (0.11), leaf width (0.02) and seed per plant (0.22) whereas negative correlated with petiole length (-0.04), plant height (-0.02), pod length (-0.05) and pod per plant (-0.09). Petiole length show negative correlation to plant height (-0.15), leaf length (-0.08), leaf width (-0.27) and seed per plant (-0.03) while positive correlated to pod length (0.08) and pod per plant (0.04). Plant height show positive correlation to leaf length (0.16), leaf width (0.15), pod length (0.13), seed per pod (0.10) and pod per plant (0.18). Leaf length show positive correlation to all traits except seed per pod (-0.32). Leaf width show positive correlation to pod length (0.12) and seed per pod (0.24) while negative to pod per plant (-0.31).

Table 1: *Descriptive statistics of Rhynchosia capitata collected from Dir Lower.*

Traits	Mean	Standard error	Standard deviation	Sample variance	Minimum	Maximum	CV %
IN	2.58	0.125	0.739	0.546	1.7	4.1	0.29
B/P	4.14	0.328	1.942	3.773	2	8	0.47
PtL	1.01	0.084	0.495	0.245	0.2	2	0.49
PH	18.9	1.110	6.568	43.14	10	35	0.35
LL	3.24	0.148	0.877	0.770	2	6	0.27
LW	2.36	0.101	0.597	0.356	1	3.2	0.25
PL	2.87	0.463	2.738	7.495	1.1	18	0.95
S/P	2.86	0.154	0.912	0.832	2	4	0.32
P/P	14.2	0.973	5.759	33.165	8	40	0.41

IN, Internodes length; B/P, branch per plant; PtL, petiole length; PH, total plant height; LL, leaf length; LW, leaf width; PL, pod length; S/P, seed per pod; P/P, Pod per plant.

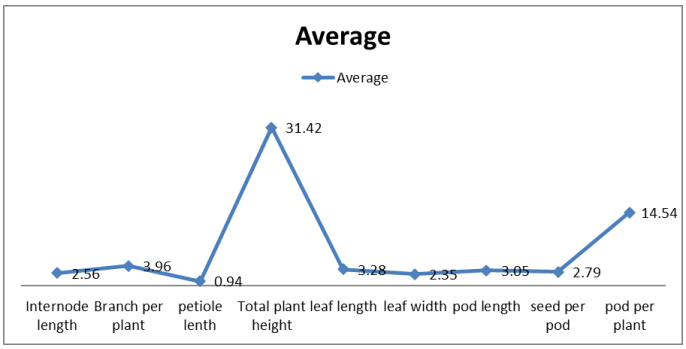

Figure 1: *Average frequency distribution of 9 quantitative traits of genotype.*

Pod length show negative correlation with seed per pod (-0.05) while positive to pod per plant (0.16). Seed per plant show negative correlation with pod per plant (-0.11) (Table 2).

Table 2: *Correlation analysis in 36 R. capitata collected from Dir Lower.*

Traits	IN	B/P	PtL	PH	LL	LW	PL	S/P	P/P
IN	1								
B/P	-0.07	1							
PtL	-0.15	-0.04	1						
PH	0.03	-0.02	-0.15	1					
LL	0.18	0.11	-0.08	0.16	1				
LW	0.10	0.02	-0.27	0.15	0.01	1			
PL	-0.14	-0.05	0.08	0.13	0.00	0.13	1		
S/P	0.12	0.22	-0.03	0.10	-0.32	0.24	-0.06	1	
P/P	-0.13	-0.09	0.04	0.18	0.01	-0.31	0.16	-0.11	1

IN, Internodes length; B/P, branch per plant; PtL, petiole length; PH, total plant height; LL, leaf length; LW, leaf width; PL, pod length; S/P, seed per pod; P/P, Pod per plant.

Table 3: *Principal component analysis (PCA) based on 9 morphological traits.*

AXIS	PC1	PC2	PC3	PC4
% of Variance	19	15.85	14.51	12.02
Cum.% of Var.	19	34.87	49.38	61.4
Eigenvalue	2.83	1.829	1.329	0.996
Traits	**Eigenvector**			
IL	0.33	-0.17	0.37	0.235
B/P	0.22	0.119	-0.02	-0.85
PL	-0.4	0.317	-0.05	-0.08
PH	0.15	-0.51	-0.38	-0.12
LL	0.01	-0.57	0.395	-0.31
LW	0.54	-0.11	-0.22	0.227
PDL	-0.1	-0.25	-0.53	0.127
S/P	0.43	0.35	-0.37	-0.14
P/P	-0.4	-0.28	-0.3	-0.12

Principal component analysis (PCA)

Principal component analysis (PCA) based on 9 quantitative traits shown significant divergence among the 36 genotypes of *R. capitata*. It was determined that 4principal component with an Eigen value of above (0.99) accounted for 61.4% of the total variation (Table 3, Figure 2). In PC1 the total variation was 19% and the variation found with the highest values are associated with internode length (0.33), branches per plant (0.22), leaf width (0.54) were found positively weighted. The entire variance in PC2 was 34.87%. Plant height (-0.51) and leaf width (-0.57) were found to be negatively weighted, while the variation associated with pod length (0.31) and seed per pod (0.35) was found to be favorably weighted. Similarly, internode length (0.37) and leaf length (0.39) were favorably correlated with weight in PC3, while plant height (-0.38), seeds per pod (-0.37), and pod length (-0.53) were negatively correlated. The total variation in PC3 was 49.38%. Internode length (0.23) and leaf width (0.22) were found to have positive weight contributions in PC4, whereas branches per plant (-0.85), leaf length (-0.31), and seeds per pod (-0.14) were found to have negative weight contributions. The overall variation in PC4 was 61.4% (Table 3).

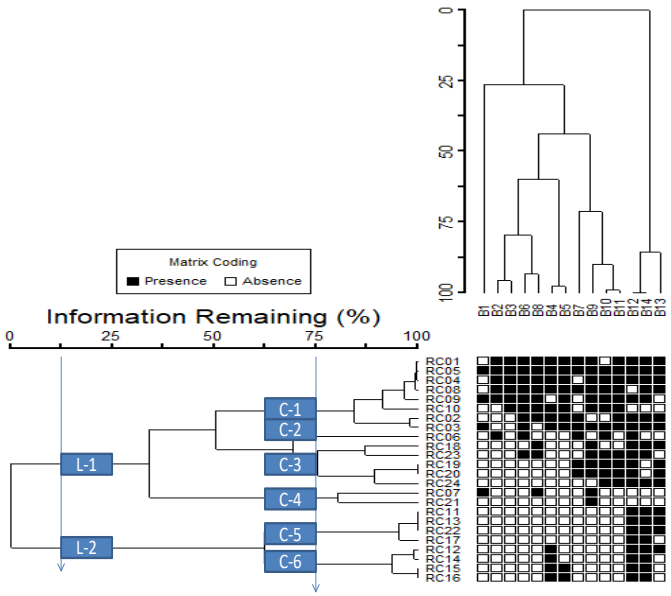

Figure 2: *Two-way clusters analysis based of seed storage proteins profile in genotype. Dendogram tee indicates genetic relationship of 36 genotype. Genotype based on bands.*

SDS-PAGE characterization and genetic association within genotypes

To determine the genetic variation in seed storage proteins, all *R. capitata* genotypes were examined using total seed storage proteins. In the current research, 12.5% acrylamide gel was employed. There were a total of 14 polymorphic bands observed; the highest degree of variation was observed in B1 (0.96%) and B2 (0.94%), B4 (0.85%), B6 (0.75%), and B7 (0.73%), in that order. Similar to B12 (0.2%), B14 (31%), B13 (0.34%), B11 (0.41%), B9 (0.55%), and B8 (0.65%) all showed modest levels. cluster dendogram was construct to show the level of genetic variation among the collected genotypes (Figure 3 & 4), and all the genotypes were divided into 2 main linkage and further divided in sub cluster. Linkage 1

consist of 4 cluster such as C-1 (RC01, RCO5, RC04, RC08, RC09, RC10, RC02, RC03) C-2 (RC06) C-3 (RC18, RC23, RC19, CR20, RC24) C-4 (RC07, RC21) and linkage 2 consist of 2 sub cluster, C-5 (RC11, RC13, RC22, RC17) C-6 (RC12, RC14, RC15, RC16).

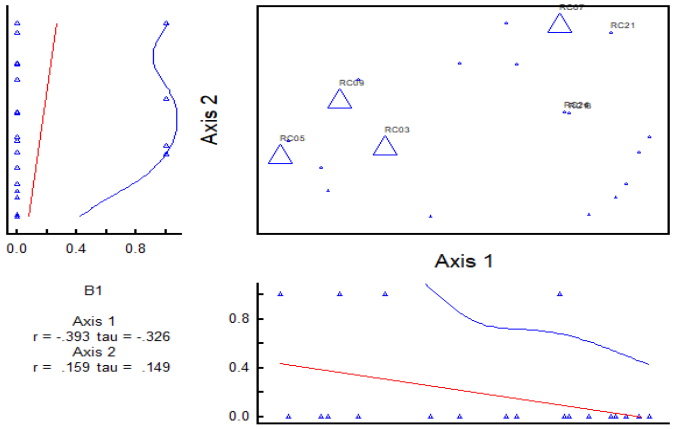

Figure 3: *Scatter plot diagram of principal component analysis using different quantitative traits of rhynchocia capitata genotype 2023.*

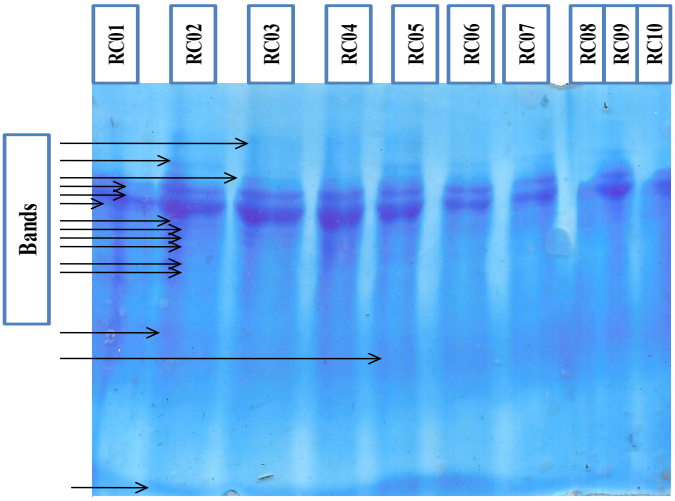

Figure 4: *The gel picture shows polymorphic subunits of different R. capitata genotypes of the present study.*

The validation of group analysis by clustering by scatter plot by Principal Components based on SDS-PAGE in 20 *R. capitata* genotypes. The entire population, which has been divided into 9 groups, exhibits a high degree of genomic divergence rather than genetic connection. Each group contains a striping pattern that denotes a particular landrace (Figure 2).

The importance of genetic diversity within genotypes for crop growth programmes cannot be overstated (Win *et al.*, 2011; Simon *et al.*, 2007). The sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of seed storage protein is a common method for demonstrating genetic diversity and relationships between different taxa (Nisar *et al.*, 2007). Researchers have studied genetic variation in different plant species using electrophoretic representations of total seed proteins (Sundin *et al.*, 2004; Ayten *et al.*, 2009; Ghafoor and Arshad, 2008). SDS-PAGE has been used in numerous Leguminosae studies (Hussain *et al.*, 2005; Hussein and George, 2002). Although in Pakistan, the current research represents the first known attempt to use SDS-PAGE to identify intra-specific genetic polymorphism in *R. capitata* genotypes. In the current study, phenotypic characterization of the seeds and SDS-PAGE descriptions of 36 genotypes of *R. capitata* showed a high degree of intra-genotypic diversity. One allele was found to be responsible for regulating the specific foliage color. For all genotypes, the colour of the seeds was discovered to be one of brown, grey, or pale grey. There were two kinds of seed texture: Rough and smooth. Roughness was 55%, while smoothness was 45%.

Because environmental changes have no impact on storage proteins, SDS-PAGE protein profiling is regarded as a reliable technique for economically describing germplasm (Javid *et al.*, 2004; Iqbal *et al.*, 2005). The protein profiles of 20 cowpea landraces were examined using 10% slab gel electrophoresis, and the findings demonstrate how similar the genotypes are. To eliminate duplicates and create a core collection of *R. capitata*, improved gene bank management requires an accurate and thorough knowledge of agricultural and biochemical data (protein and DNA). Variations in SDS-PAGE can be used to understand the degree of genetic variation and the connections among Pakistani *R. capitata*.

Conclusions and Recommendations

A significant genetic diversity was found in qualitative characters on the basis of frequency distribution shown variation. On behalf of these traits descriptive statistics, correlation and cluster analysis was made the quantitative traits was observed with significant variation for all the traits. Their genetic diversity studied through the biochemical analysis by SDS-PAGE. There was significant diversity among the genotypes. A total 14 bands were found, all the protein bands were polymorphic, there were no monomorphic bands found.

Novelty Statement

This is the first study of evaluating the Genetic Diversity in wild *Rhynchosia capitate* in Dir Lower (KP), Pakistan.

Author's Contribution

Shahid Iqbal: Helped in collection of data fromthe field.
Arshad Khan: Result collection and field work,Objective and title configuration.
Mohammad Ihsan: Resultcalibration with software's.
Ali Hazrat: Discussion calibrationwith result.
Gul Rahim: Help in experimental work.
Maryam Bibi: References designing according to the journal standard.
Umar Zad Gul: Review of literature.
Khadija Bibi and Muhammad Mukhtiar: Overall compilation of the paper.

Conflict of interest
The authors have declared no conflict of interest.

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