MJSIR
Mountain Journal of Science and Interdisciplinary Research
PRINT ISSN: 2619-7855
ONLINE ISSN: 2651-7744
July-December 2022 • 82 (2) : 132-151

Diversity of Ricebean (*Vigna umbellata* L.) Germplasm in the Northern Philippine Highlands Based on Agro-morphological Characteristics and Simple Sequence Repeat Markers

Belinda A. Tad-awan* and Hilda V. Palaes
College of Agriculture, Benguet State University
**Corresponding author e-mail address: belle_tadawan@yahoo.com.ph*

ARTICLE INFO
Date Received: 03-11-2022
Date Last Revised: 06-20-2022
Date Accepted: 06-24-2022

KEYWORDS
agro-morphological characters
genetic diversity
genetic similarities
ricebean germplasm
simple sequence repeats

Abstract

Rice bean (*Vigna umbellata* L.) is one of the underutilized food legumes in the Philippines but is integral in the cropping system of highland local farmers. To get more understanding on the germplasm diversity of this legume in the area for conservation and breeding purposes, morpho-agronomic and genetic characterization using simple sequence repeats (SSR) markers were carried out on 28 ricebean germplasm. Morpho-agronomic characters exhibited mid to high diversity (0.42 to 0.71). Characters such as climbing habit, primary leaflet width, seed width, terminal leaf width, and pod width had the highest diversity indices. Ward's cluster analysis grouped the ricebean germplasm into five clusters. In SSR analysis using a set of 10 SSR primer pairs, the genetic similarities among selected ricebean germplasm ranged from 0.39 to 0.86 showing mid to high similarities. A total of 89 alleles were detected with an average of 8.9 alleles for each locus, and the polymorphism information content value for SSR loci varied from 0.45 for CEDG021 to 0.94 for primer CEDG003 with an average of 0.78. Cluster diagram based on the similarity matrix generated six clusters at 0.86 similarity index. Fruit setting ability, pod width, seed width, and 100-seed weight are important characters for seed yield improvement in ricebean germplasm. Further, the grouping of ricebean germplasm with high seed weight and yield, many pods per cluster, and many seeds per pod suggest a potential gene pool of ricebean in the northern Philippine highlands, which can be utilized for future breeding improvement programs.

Introduction

Crop landraces have played important role in diversifying the production systems in the Philippines highlands (Tad-awan & Sagalla, 2015). These landraces are maintained by local farmers for subsistence. Among these crops is ricebean (*Vigna umbellata*) which is a traditional legume crop grown across South and Southeast Asia. Ricebean and other legume crop species are traditional part of the diet of the Cordilleran in the Philippine highlands. Landraces are adapted to specific environments particularly in marginal areas.

The local adaptations of crop species to certain growing environments result in genetic diversity that could be shown in their morphological traits

(Carović-Stanko et al., 2017). However, landraces are at risk of genetic erosion at allelic level due to the low profitability of farming lands and the introduction of modern varieties (Belshaw, 1947; Upadhyaya et al., 2008). Thus, the conservation of local landraces could provide important sources of genetic resistance for breeding improvements. The locally adapted landraces or lines of species possess potential genes for crop breeding improvements that could be used to develop new and improved varieties that are needed in the changing agro-environments. Yet, few studies were conducted to characterize the genetic resources of underutilized crops.

Researches on underutilized crop species like ricebean could be of great potential scientifically due to their useful traits that can be used in breeding improvements. These crops are not as subjected to genomic studies unlike other legumes such as peas, mungbean, and soybean. An important part of conservation is to use genetic markers in assessing the genetic variability within germplasm and the cultivar identity in various crop species. Additionally, this can be coupled with morphological markers that are the traditional technique for studying diversity. Several studies have assessed the genetic diversity of germplasm collections of crops using morphological traits (Carović-Stanko et al., 2017; Malik et al., 2010; Gaad et al., 2018) and molecular markers (Gangadhar & Mishra, 2016; Rajkovic et al., 2015; Moiana et al., 2015; Choudhury et al., 2008; Smykal et al., 2008; Khazaei et al., 2016). Example of morphological traits used as markers are growth characters and yield components (Malik et al., 2014; Mafakheri et al., 2017; Marconato et al., 2016), flower character, number of pods per plant, and fruit set (Arif et al., 2016). However, morphological traits have limitations in determining genetic diversity among different crop species due to strong environmental influence which could be augmented by molecular markers (Kameswara, 2004; Valladares, 2007; Kumar et al., 2020; Bhalodiya et al., 2019; Wang et al., 2018). Fortunately, genebanks, where germplasm are conserved with large numbers of accessions showing genetic polymorphism of agronomically important genes, are available to molecular approaches for rapid assessment of genetic diversity.

The use of SSR markers could help in this regard, having been proven to be an efficient tool for genetic diversity analysis. Also, significant progress had already been made on its application to various genomic researches in crop species such in legume (Chen et al., 2017; Ramya et al., 2014; Hamwieh et al., 2009). SSR markers are also efficient for the selection of desirable traits in legumes and other economic crops (Rajkovic et al., 2015; Moiana et al., 2015; Kaila et al., 2011; Keneni et al., 2012; Rana et al., 2015). Taking into account these advantages, the study used agro-morphological and SSR markers to investigate the level of genetic diversity in *V. umbellata*. The results could be used in breeding improvements and in selecting genotypes of high yielding landraces.

Methodology

Ricebean Germplasm

A total of 28 ricebean germplasm were collected from the Northern Philippine Highlands (12°16' to 120°12"N and 120°26' to 121°40"E; 290 to 1,524 m asl). Figure 1 shows the distribution of the ricebean germplasm used in the study. Thirteen ricebean germplasm were collected in the four municipalities of Benguet (Bakun, Kapangan, Sablan) and 15 were from Mt. Province (Bauko, Bontoc, Natonin, Paracelis, Sabangan, Sadanga, Sagada and Tadian)

Study Site and Experimental Treatments

The field experiment was carried out at the experimental station of Benguet State University, La Trinidad, Benguet, Philippines (1, 332 m asl; 16°27 latitude, 16°27 '19.2"N 120°35'33.9"E) on November 2018 to May 2019. The ricebean germplasm were laid out in a randomized complete block design with three replications. Each replication was further subdivided into 32 plots each measuring $3m^2$. Three seeds of each ricebean germplasm were planted per hill at a depth of 2.0-2.5cm in a double row with a distance of 25.0cm x 40.0cm between rows and hills. After three weeks, seedlings were thinned to maintain two plants per hill (Joshi et al., 2008). Compost was applied at a rate of 1.2kg $3m^{-1}$ during land preparation. Locally available organic pesticides such as wood vinegar were applied at the rate of 16.0ml: 16 liters of water during the vegetative to flowering stages of the crop.

Agro-meteorological Data

During the growing period of the crop, temperatures varied from 10.7°C in November to 20.8°C in April with a mean of 17.8°C. At the vegetative stage, the mean temperature was 18.5°C, while at flowering pod development to maturity stages were 17.5°C and 20.25°C. Relative humidity ranged from 79.0% (November) to 88.0% (May). The amount of rainfall ranged from 0.08mm to 13.5.0mm. The amount of rainfall recorded was relatively low in some months except in May, whereas sunshine duration ranged from 221 min to 490.5 min.

Morphological Characterization

A standard characterization for the data observation of morphological characters such as the vegetative, inflorescence and pod, and seed characters was done using the descriptors list for *V. mungo* and *V. radiata* (IBPGR, 1985). Tables 1 and 2 present the list of qualitative and quantitative characters used to evaluate the plants and the seeds, respectively.

SSR Genotyping

Of the 28 ricebean germplasm, 11 selected genotypes were assessed for SSR genotyping. Samples were brought at the Philippine Genome Center, Diliman, Quezon City for DNA extraction and SSR analysis. Four seeds from each genotype were pre-germinated in Petri plates and transferred in pots in a greenhouse for leaf sample collection. Fresh young leaves of a 15-day old seedlings were collected for DNA extraction (Wamalwa et al., 2016). DNA extraction was carried out according to the prescribed protocol of the NucleoSpin® Plant II kit that was designed for genomic DNA from plant species (Macherey-Nagel, 2014). Genomic DNA of each sample (2ul) was checked on a 1.2% gel and run at 80V for 100 minutes and 1kb plus ladder (Invitrogen) in an ENDURO™ Gel XL Electrophoresis System, or 90 minutes in a Biometra Compact M (Standard Power Pack P25 T and it was quantified on spectrometer NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, Delaware USA). In the PCR assay, 20 primer pairs from previous studies were initially tested, and a set of ten primer pairs that showed amplification

Figure 1

Geographical Location of 28 Ricebean Germplasm in the Northern Philippine Highlands

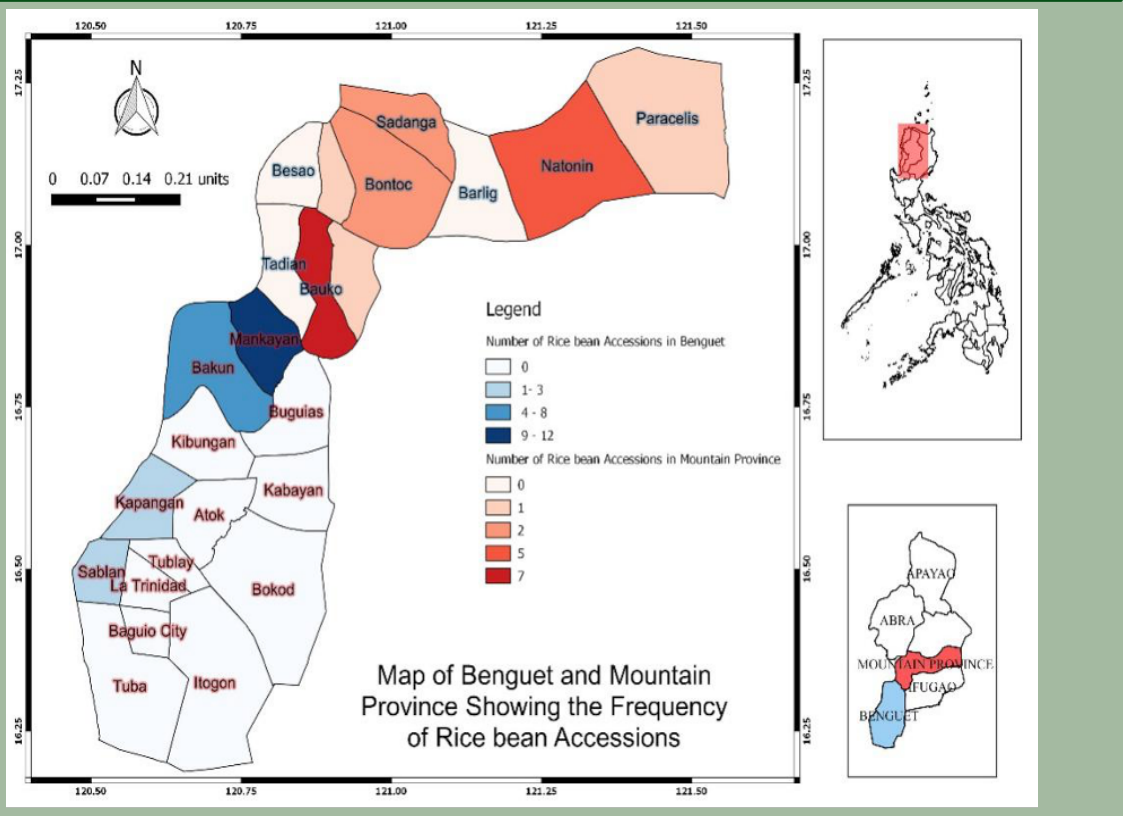


Table 1

Qualitative Traits (19) Used in the Characterization of Ricebean Germplasm Evaluated in the La Trinidad, Benguet, Philippines

Character	Description
Petiole color	1-green with purple spots, 3-greenish-purple, 4-purple, 5-dark purple, and 6-others
Leaf Color	3-light green, 5-intermediate, and 7-dark-green
Terminal leaf shape	1-deltate, 2-ovate, 3-ovate-laceolate, 4-lanceolate, 5-rhombic, 6-obvate, 7-others
Primary leaflet shape	1-ovate-lanceolate, 2-lanceolate, 3-other
Leafiness	3-spars (main stem easily visible), 5-intermediate green, and 7-dark green (very sparse)
Leaf senescence	0-no visible senescence, 3-slightly visible senescence, 5-moderate senescence, 7-conspicuous concurrent senescence
Climbing habit	3-viny, 4-slightly, 5- intermediate, 6-slighlty non-viny, 7-non-viny
Twining tendency	0-none, 3-slight, 5-intermediate, and 7-pronounce
Branching pattern	1-basal, 2-center, 3-top, and 4-all over
Corolla color	1-yellow, 2-greenish-yellow, 3-yellowish green, 4-green purple yellow, and 5-others
Calyx color	1-green, 2-purplish-green, 3-greensih purple, and 4-others
Raceme position	1-mostly above canopy, 2-intermediate, and 3-no pods visible above canopy
Pod color	1-straw, 2-tan, 3-brown, 4-brown and black, 5-black, and 6-others
Pod shattering	0-absent, +-present
Pod cross-section	1-semi-flat, 2-round
Constriction of pod between seeds	0-absent, +-present
Seed Color	0-light gree, 1-dark-green, 2-green yellow, 3-yellow, 4-brown, 5-mottled, and 6-mottled
Luster on the seed surface	0(dull) and +(shiny)
Seed shape	1-globose, 2-ovoid, 3-drum-shaped, and 4-others

were used in the MultiNA DNA sizing (Table 3). The SSR primers were acquired from IDT® (Integrated DNA Technologies, Singapore) for the amplification of each sample. Samples showed genomic DNA were amplified. The polymerase chain reaction mixture used for amplification contained the following reagents: 5X Green GoTaq® Flexi Buffer (Cat. #M891A) or 5X Colorless GoTaq® Flexi Buffer (Cat. #M890A), GoTaq® Flexi DNA polymerase (Cat. #M829B), Promega dNTP mix (Cat. #U151V), and Vivantis 50 mM MgCl2 (Cat. #RB0204-1).

The PCR components of 2.725 µl reaction were as follows: 1.0 µl 5x GoTaq buffer, 0.25 ul dNTP, 0.25 ul 50 mM MgCl2, 0.25 µl forward and 0.25 µl reverse primer, 0.025 µl Taq polymerase and 0.50 ul genomic samples. The approximate genomic size range of 117 to 235 bp of the PCR products for each sample was based on the study conducted on ricebean (Thakur et al., 2017) to optimize the PCR condition. The PCR cycling conditions were as follows: initial denaturation of 94°C for 2 min; followed by 35 cycles at 94°C for 30 s, annealing of 58°C for 1 min. and 72°C for 1 minute; final extension at 72°C for 5 min. and incubation at 4°C. Amplicon products were sized using DNA-500 assay with 25bp as the marker. In the MultiNA

Table 2

Quantitative Traits (18) Used in the Characterization of Ricebean Germplasm Evaluated in La Trinidad, Benguet, Philippines

Character	Description
Petiole Length	Average petiole length (cm) in 10 plants randomly selected plants per plot
Primary leaflet length	Average leaflets length (cm) in 5 plants randomly selected plants per plot
Primary leaflet width	Average leaflets width (cm) in 5 plants randomly selected plants per plot
Terminal leaflet length	Average leaflets length (cm) in 5 plants randomly selected plants per plot
Terminal leaflet width	Average leaflets width (cm) in 5 plants randomly selected plants per plot
Days to flowering	Days from planting to 50% of plants have flowered
Days to pod maturity	Days from planting to 75% of plants have matured
Fruit-setting ability	Percentage of flowers that set pods when 50% of pods are mature
Flowers per cluster	Average number of flowers per peduncle in 10 randomly selected plants per plot
Pods per cluster	Average number of pods per peduncle in 10 randomly selected plants per plot
Peduncle length	Average peduncle length (cm) in 10 plants randomly selected plants per plot
Pod length	Average pod length (cm) in 10 pods in 10 randomly selected plants per plot
Pod width	Average pod width (cm) in 10 pods in 10 randomly selected plants per plot
Seed length	Average seed length (cm) in 10 seeds in 10 randomly selected plants per plot
Seed width	Average seed width (cm) in 10 seeds in 10 randomly selected plants per plot
Seeds per pod	Average number of seeds per pod in 10 randomly selected plant per plot
1000-seed wt	1000 seeds weight (g) three groups of 1000 seeds
Seed yield	Total weight (g) of seed per plot ($3m^2$)

Table 3

SSR Prime Pairs for the Reaction of Amplication of the DNA Fragments Through PCR Within Ricebean Germplasm

SSR Marker	Primer Sequence 5'-3' (F/R)	Reference
CEDC016	ACTCTTGTCAATTGTCCAGG/ TAACTTGTCACTGGAAAGGC	
CEDG003	CCACTTTCTCTTGACTTTGC/GACCAAAGTGAAGCCAAGAG	[35]
CEDG021	GCAGAATTTTAGCCACCGAG/ AAAGGATGCGAGAGTGTAGC	
CEDG015	CCCGATGAACGCTAATGCTG/ CGCCAAAGGAAACGCAGAAC	
CEDG043	AGGATTGTGGTTGGTGCATG/ ACTATTTCCAACCTGCTGGG	
CEDG073	CCCCGAAATTCCCCTACAC/ AACACCCGCCTCTTTCTCC	
CEDAAG002	GCAAAACTTTTCACCGGTACGACC/GCAGCAACGCACAGTTTCATGG	[36]
CEDG021	AAAGGATGCGAGAGTGTAGC/ GCAGAATTTTAGCCACCGAG	
CEDG008	AGGCGAGGTTTCGTTTCAAG/ GCCCATATTTTTACGCCCAC	
CEDG011	CCCAACCAAAGCGTTTTG/ CTTCTAGACTCTGAGCACTG	

assay 3uL of amplicon was loaded for each well. A master mix of separation buffer (included in the DNA-500 kit) and SYBR gold nucleic acid stain were prepared for each run while marker used was 25bp. Samples were loaded into MultiNA and run using MultiNA Control Software and data was generated using MultiNA Viewer Software (MultiNA Control Software and Data Analysis Software Viewer, Dongil Shimadzu). Peak sizes on electropherogram were automatically detected and recorded. The genomic polymorphism of ricebean germplasm was analyzed using 10 selected SSR markers. The SSR marker peaks were scored as 1 (presence of peak) or 0 (absence of peak) for each ricebean germplasm (Lim et al., 2017). While the diversity of SSR markers were obtained by counting the frequency of allele generated based on polymorphism information content (PIC) using the equation:

$$PIC = 1 - J = 1 - \sum_{J=1}^{n} P^2 ij$$

Statistical Analysis

All quantitative data were statistically analyzed using analysis of variance using PROC GLM of SAS® PC for Windows Version 9.1.3 (SAS Institute Inc., Cary, NC) statistical software. Means between treatments were compared with the DMRT ($P \leq 0.05$). Variability for the morphological characters was computed using the Shannon-Weaver diversity Index (Ortiz-Burgos, 2016). Cluster analysis was performed on the software Statistical Product and Service Solutions (SPSS Inc., Chicago, IL, USA) version 9.1.3 to generate a dendrogram of 28 germplasm into a distinctive grouping based on the morphological characters measured. A measure of similarity was computed using the squared Euclidian (Minkowski distance metric) formula. The dendrogram was constructed using Ward's method. The genetic similarity coefficient was calculated among all possible pairs with the SIMQUAL option and organized in a similarity matrix using the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc), version 2.02 package (Rohlf, 1993). Cluster analysis of the samples based on similarity values was done using an unweighted pair group method arithmetic method (UPGMA) in sequential, agglomerative, hierarchical, and nested (SAHN) Programme to construct a dendrogram (Thakur et al., 2017).

Results and Discussion

Morpho-agronomic Characters of Ricebean Germplasm

Significant variations were observed in the petiole length and leaf characters among ricebean germplasm (Table 4). The longest petiole was exhibited by germplasm RB PAR-01 and RB MAN-01. As to the leaf characters, a broad terminal leaflet was observed in RB MAN-12, RB MAN-01, RB KBAK-01, and RB TAD-01 while RB TAD-01 produced the longest primary leaves but comparable with RB BAU-05 and RB KBAK-01. RB KBAK-01 and RB TAD-01 had the broadest leaves among the germplasm.

Significant variations were noted in other quantitative characters among ricebean germplasm (Table 5). The days from planting to 50% flowering and pod maturity differed significantly among the ricebean germplasm. RB-BAU-01, RB-BAU-03, RB-BAU-04, RB-BAU-05RB-PAR-01, RB-SABA-01, RB-SAD-01, and RB-SAG-01 were the earliest to flower after 67 days after planting (DAP) while most of the germplasm flowered after 89 to 107 DAP. Pod maturity among the ricebean germplasm ranged from 107 to 178 DAP. RB PAR-01, RB SAD-01, RB SAG-01, and RB BON-02 had the highest percentage of fruit-setting but did not vary with RB BAU-01 and RB BAU-03. Flowers and pods per cluster were found to vary among the germplasm, RB NAT-04, RB NAT-05, RB BON-01, RB MAN-01, RB MAN-12, and RB KBAK-02 produced the most flowers per cluster, while RB MAN-12, RB PAR-01, and RB NAT-04 had significant pods per cluster. Flowers per cluster varied from 22.9 to 5.6 while pods per cluster ranged from 5.7 to 2.3. Consequently, germplasm having fewer flowers and pods per cluster had the highest fruit-setting ability. It was observed that most germplasm from Benguet are observed to have low fruit-setting ability. Further, more flowers and pods per cluster from germplasm had longer peduncles. Peduncle length, pod length and seeds per pod significantly varied among the ricebean germplasm. Peduncle length and pod length among the ricebean germplasm ranged from 29 to 13.8cm and 8.7 to 13.4cm, respectively. RB KBAK-01 and PBAK-01 significantly had the longest peduncles. Long peduncles were exhibited by germplasm from Benguet. RB PBAK-01 and RB PBAK-02 had the longest pods. RB MAN-06, RB MAN-11, and RB

Table 4

Quantitative Leaf Character of the 28 Ricebean Germplasm Evaluated in La Trinidad, Benguet, Philippines

Ricebean germplasm	Petiole length (cm)	Primary leaflet length (cm)	Primary leaflet width (cm)	Terminal leaflet length (cm)	Terminal leaflet width (cm)
RB KBAK-01	13.5 d-i	8.0 a-c	6.4 a	9.4 a-c	5.4 ab
RB KBAK-02	12.0 g-j	7.2 b-f	3.7 c-e	9.1 a-d	4.2 d-g
RB KBAK-03	13.3 e-i	7.9 a-d	3.8 cd	9.5 ab	5.0 a-d
RB PBAK-01	12.8 f-j	7.3 b-f	3.7 c-e	9.1 a-d	4.5 c-f
RB PBAK-02	14.2 b-h	7.4 a-f	3.9 cd	9.0 a-e	5.0 a-d
RB PBAK-04	10.7 i-k	6.2 e-g	3.3 de	7.7 e-i	3.9 fg
RB KAP-01	10.4 jk	6.6 c-g	3.3 de	7.2 hi	4.0 e-g
RB KAP-02	8.8 k	5.6 g	2.9 e	6.8 i	3.6 g
RB MAN-01	16.8 ab	7.0 b-g	4.3 bc	7.9 d-i	5.4 ab
RB MAN-06	16.4 a-c	7.5 a-f	3.9 cd	8.5 a-h	4.7 b-f
RB MAN-11	13.0 f-j	6.1 fg	3.9 cd	7.4 g-i	4.8 a-e
RB MAN-12	13.6 c-i	7.2 b-f	4.1 b-d	8.2 b-h	5.7 a
RB SABL-01	13.2 f-j	6.8 b-g	3.7 c-e	8.1 c-i	4.4 c-f
RB BAU-01	14.3 b-h	7.2 b-f	3.7 c-e	8.2 b-h	4.6 b-f
RB BAU-03	16.2 a-e	6.6 d-g	4.3 bc	9.0 a-e	4.7 b-f
RB BAU-04	14.8 a-g	7.4 a-f	4.1 b-d	8.9 a-e	4.5 c-f
RB BAU-05	15.4 a-f	8.1 ab	4.3 bc	8.7 a-f	5.0 a-d
RB BON-01	12.7 f-j	7.8 a-d	4.1 b-d	8.6 a-g	4.8 b-e
RB BON-02	16.3 a-d	7.8 a-d	3.9 cd	8.3 b-h	4.7 b-f
RB NAT-01	11.7 h-k	6.7 c-g	3.6 c-e	8.0 d-i	4.6 b-f
RB NAT-02	14.3 b-h	6.7 c-g	3.8 c-e	7.5 f-i	4.3 d-g
RB NAT-04	15.3 a-f	6.8 b-g	4.2 bc	8.5 a-h	4.7 b-f
RB NAT-05	13.0 f-j	7.7 a-d	4.1 b-d	8.6 a-g	4.9 a-d
RB PAR-01	17.5 a	7.5 a-f	3.9 cd	8.5 a-h	4.7 b-f
RB SABA-01	13.6 c-h	7.6 a-e	3.9 cd	8.7 a-g	4.8 d-e
RB SAD-01	14.1 b-h	7.4 a-f	4.0 b-d	8.3 b-h	4.7 b-f
RB SAG-01	14.3 b-h	7.1 b-f	3.7 c-e	9.7 a	4.5 c-f
RB TAD-01	15.4 a-f	8.7 a	4.9 b	9.7 a	5.2 a-c
CV (%)	**12.8**	**11.9**	**14.0**	**9.4**	**11.1**

MAN-12 significantly produced the highest average seeds per pod. Ricebean germplasm produced an average of 8.4-11.0 seeds per pod.

Significant differences were observed on the seed length, 1000-seed weight, and seed yield. Longer seeds were obtained from RB PBAK-01, RB BON-02, RB BAU-05, and RB BAU-03. These germplasm from Mt. Province had bigger seeds. Seed length and seed width varied from 8.4-6.5cm and 4.9-3.5cm, respectively. RB PBAK-01 produced the heaviest 1000 seeds of 286.7g followed by RB BAU-04 (240.0g), RB BAU-05 (239.7g), RB BON-02 (233.7g), PBAK-04 (233.3g) and RB BAU-03 (233.3g). Low seed weights were noted from RB BON-01, RB NAT-02, and RB NAT-05

Table 5

Plant Maturity, Yield, and Yield Component and Other Characters in 28 Ricebean Germplasm Evaluated in La Trinidad, Benguet, Philippines

Ricebean germplasm	Days ro flowering	Days matu-rity	Flowers per cluster	Pods per cluster	Fruit setting ability (%)	Peduncle length (cm)	Pod Length (cm)	Pod width (cm)	Seeds per pod	Seed length (cm)	1000-Seed weight (g)	Seed yeild (g/3m2)
RB KBAK-01	89.0 ^{b}	143.0 ^{g}	19.3 ^{ab}	4.5 $^{a-f}$	22.$^{8\ f}$	29.0 ^{ab}	11.9 $^{e-f}$	5.6 ^{kl}	10.1 $^{a-g}$	7.7 $^{a-f}$	166.7 ^{jk}	194.5 $^{b-i}$
RB KBAK-02	89.0 ^{b}	138.0 ^{f}	12.7 $^{d-f}$	2.9 $^{c-f}$	23.8 ^{f}	27.0 ^{bc}	12.0 $^{c-f}$	6.1 ^{ef}	9.7 $^{d-j}$	7.8 $^{a-f}$	190.0 $^{g-i}$	112.2 $^{f-i}$
RB KBAK-03	89.0 ^{b}	165.0 ^{d}	11.7 $^{e-g}$	2.7 ^{f}	23.3 ^{f}	25.8 $^{b-d}$	11.0 $^{g-i}$	5.2 ^{m}	9. 5 $^{e-k}$	8.0 $^{a-d}$	163.3 ^{k}	22.0 ^{hi}
RB PBAK-01	89.0 ^{b}	178.0 ^{l}	13.2 $^{c-f}$	2.3 ^{f}	17.9 ^{f}	31.6 ^{a}	13.1 ^{ab}	6.3 ^{bc}	10.3 $^{a-e}$	8.4 ^{a}	286.7 ^{a}	178.6 $^{c-i}$
RB PBAK-02	89.0 ^{b}	138.0 ^{f}	9.8 $^{e-h}$	2.5 ^{ef}	27.3 $^{d-f}$	27.0 ^{bc}	13.4 ^{a}	6.2 ^{cd}	10.1 $^{a-g}$	7.8 $^{a-f}$	196.7 $^{f-g}$	341.3 $^{a-e}$
RB PBAK-04	89.0 ^{b}	124.0 ^{e}	12.3 ^{ef}	3.5 $^{a-f}$	31.7 $^{b-f}$	18.5 ^{fg}	12.4 $^{b-f}$	5.5 ^{l}	9.1 $^{h-l}$	8.0 $^{a-d}$	233.3 ^{bc}	60.0 $^{g-i}$
RB KAP-01	91.0 ^{c}	169.0 ^{j}	9.3 $^{e-h}$	2.9 $^{c-f}$	31.5 $^{b-f}$	26.5 ^{bc}	12.8 $^{a-c}$	6.2 ^{cd}	10.5 $^{a-d}$	7.9 $^{a-e}$	196.7 $^{f-g}$	489.1 ^{a}
RB KAP-02	89.0 ^{b}	169.0 ^{j}	13.3 $^{c-f}$	2.4 ^{f}	15.6 ^{f}	26.4 ^{bc}	11.8 $^{d-g}$	5.6 ^{kl}	10.3 $^{a-e}$	7.3 $^{b-g}$	186.7 ^{hi}	122.3 $^{e-i}$
RB MAN-01	107.0 ^{f}	178.0 ^{l}	22.7 ^{a}	4.3 $^{a-f}$	20.4 ^{f}	24.3 $^{c-e}$	9.5 ^{kl}	5.0 ^{n}	10.3 $^{a-e}$	7.0 $^{d-g}$	143.3 ^{l}	10.0 ^{i}
RB MAN-06	107.0 ^{f}	178.0 ^{l}	14.3 $^{b-e}$	4.3 $^{a-f}$	30.6 $^{c-f}$	25.0 $^{b-e}$	10.6 $^{h-j}$	5.2 ^{m}	11.0 ^{a}	6.9 ^{fg}	176.7 $^{i-k}$	16.7 ^{hi}
RB MAN-11	102.0 ^{e}	178.0 ^{l}	18.2 $^{a-c}$	4.2 $^{a-f}$	22.1 ^{f}	22.2 $^{d-f}$	12.4 $^{b-e}$	5.7 ^{i}	10.7 $^{a-c}$	8.0 $^{a-d}$	183.3 $^{h-j}$	178.5 $^{c-i}$
RB MAN-12	107.0 ^{f}	165.0 ^{i}	21.3 ^{a}	5.7 ^{a}	26.7 $^{d-f}$	26.6 ^{bc}	11.7 $^{e-g}$	5.6 ^{j}	10.9 ^{ab}	7.5 $^{a-f}$	176.7 $^{i-k}$	258.5 $^{b-g}$
RB SABL-01	114.0 ^{e}	178.0 ^{l}	18.0 $^{a-d}$	3.7 $^{a-f}$	20.9 ^{f}	15.8 ^{gh}	11.4 $^{f-h}$	5.9 ^{h}	10.0 $^{b-h}$	7.6 $^{a-f}$	126.7 ^{lm}	13.3 ^{hi}
RB BAU-01	67.0 ^{a}	107.0 ^{a}	5.6 ^{h}	2.7 ^{f}	51.6 ^{ab}	19.0 ^{g}	12.5 $^{a-e}$	6.2 ^{de}	9.3 $^{g-l}$	7.7 $_{a-f}$	219.3 $^{c-e}$	350.7 $^{a-d}$
RB BAU-03	67.0 ^{a}	115.0 ^{b}	5.7 ^{h}	3.0 $^{c-f}$	53.0 ^{ab}	19.0 ^{g}	11.7 $^{e-g}$	5.8 ^{h}	8.4 ^{l}	8.2 ^{ab}	233.3 $^{b-d}$	308.1 $^{s-f}$
RB BAU-04	67.0 ^{a}	121.0 ^{d}	6.8 ^{gh}	3.1 $^{b-f}$	48.6 $^{a-c}$	16.8 ^{g}	12.2 $^{b-f}$	6.4 ^{a}	8.7 $^{j-l}$	8.1 ^{ab}	240.0 ^{b}	311.4 $^{a-f}$
RB BAU-05	67.0 ^{a}	107.0 ^{a}	6.2 ^{gh}	2.8 ^{cf}	45.7 $^{a-e}$	18.7 ^{fg}	12.6 $^{a-e}$	6.4 ^{a}	9.0 $^{i-l}$	8.3 ^{a}	239.7 ^{b}	316.8 $^{a-f}$
RB BON-01	107.0 ^{f}	164.0 ^{h}	22.3 ^{a}	4.7 $^{a-e}$	20.7 ^{f}	21.7 ^{ef}	8.7 ^{l}	4.5 ^{q}	8.8 $^{j-l}$	6.5 ^{g}	103.3 ^{o}	140.5 $^{h-j}$
RB BON-02	95.0 ^{d}	119.0 ^{c}	5.9 ^{h}	3.3 $^{b-f}$	55.7 ^{a}	16.3 ^{gh}	11.7 $^{e-g}$	6.0 ^{g}	8.6 ^{kl}	8.4 ^{a}	233.7 ^{bc}	236.1 $^{b-h}$
RB NAT-01	107.0 ^{f}	174.0 ^{k}	22.7 ^{a}	4.7 $^{a-e}$	21.3 ^{f}	21.9 $^{d-f}$	10.5 ^{ij}	6.0 ^{g}	8.9 $^{i-l}$	7.7 $^{a-f}$	132.3 ^{lm}	99.8 $^{f-i}$
RB NAT-02	107.0 ^{f}	164.0 ^{h}	18.2 $^{a-c}$	4.9 $^{a-d}$	27.4 $^{d-f}$	23.6 $^{c-e}$	9.8 ^{jk}	4.9 ^{o}	9.1 $^{h-l}$	7.5 $^{a-f}$	106.7 ^{no}	33.3 $^{g-i}$
RB NAT-04	119.0 ^{g}	174.0 ^{k}	22.8 ^{a}	5.3 ^{ab}	23.3 ^{f}	25.4 $^{b-e}$	12.2 $^{c-f}$	4.6 ^{p}	10.2 $^{a-f}$	7.0 $^{e-g}$	180.0 $^{h-k}$	195.5 $^{b-i}$
RB NAT-05	107.0 ^{c}	174.0 ^{k}	20.0 ^{a}	5.1 $^{a-c}$	25.2 ^{ef}	18.2 ^{fg}	11.9 $^{c-g}$	5.2 ^{m}	10.3 $^{a-e}$	7.1 $^{c-g}$	123.3 ^{nm}	78.3 $^{g-j}$
RB PAR-01	67.0 ^{a}	115.0 ^{b}	8.6 $^{f-h}$	5.6 ^{a}	65.9 ^{a}	15.0 ^{gh}	12.5 $^{b-e}$	4.3 ^{r}	9.3 $^{f-l}$	6.5 ^{g}	144.9 $^{i-k}$	417.6 ^{ab}
RB SABA-01	67.0 ^{a}	115.0 ^{b}	6.6 ^{gh}	3.0 $^{c-f}$	46.3 $^{a-c}$	21.9 $^{d-f}$	12.2 $^{c-f}$	6.4 ^{ab}	8.8 $^{j-l}$	8.1 $^{a-c}$	167.5 $^{d-f}$	380.7 $^{a-c}$
RB SAD-01	67.0 ^{a}	107.0 ^{a}	5.8 ^{h}	3.2 $^{b-f}$	56.6 ^{a}	13.8 ^{h}	12.2 $^{b-f}$	6.1 ^{f}	9.8 $^{c-i}$	7.9 $^{a-f}$	178.7 $^{b-e}$	345.6 $^{a-e}$
RB SAG-01	67.0 ^{a}	143.0 ^{g}	6.5 ^{gh}	3.7 $^{a-f}$	56.5 ^{a}	16.9 ^{h}	12.7 $^{a-d}$	5.7 ^{i}	9.6 $^{e-k}$	7.6 $^{a-f}$	159.9 $^{e-g}$	494.8 ^{a}
RB TAD-01	89.0 ^{b}	138.0 ^{f}	9.3 $^{e-h}$	4.5 $^{a-f}$	48.8 $^{a-c}$	17.2 ^{h}	12.1 $^{c-f}$	5.6 ^{jk}	9. 7 $^{d-j}$	7.8 $^{a-f}$	151.8 $^{f-g}$	399.1 $^{a-c}$
CV (%)	0.0	0.0	23.4	9.9	31.5	10.0	4.7	0.9	6.1	8.1	5.5	33.2

having 103.3g, 106.7g and 123.3g, respectively, this could be due to short and narrow seeds. RB SAG-01 (494.8g) and RB KAP-01 (489.1g) significantly produced the highest seed yield but were comparable with RB PAR-01, which yielded 417.6g per plot (g/3m^2). Most of these germplasm are from Mt. Province and characterized as early maturing and big seeded.

Diversity of Ricebean Germplasm Using Morpho-agronomic Traits

Table 6 shows the diversity indices for the 37 morphological characters of ricebean germplasm. Low diversity index was observed in all the qualitative characters among the 28 ricebean germplasm (0.27). The low diversity indices of plant characters observed resulted in low variability implying that most of the qualitative characters of germplasm are relatively uniform. Corolla color had the lowest diversity index among the qualitative characters measured with only two colors of corolla flower observed from the different ricebean germplasm. The low variability could be due to the predominant characters observed. On the quantitative characters, the mean diversity index is moderate (0.56). Most of the characters showed moderate diversity except for pod maturity, flowering, seed weight, and seed yield. Moderate diversity indicates variability among the ricebean germplasm. Significant variations were observed for qualitative and quantitative morphological characters in the ricebean germplasm. For the qualitative traits, substantial variations in the growth habit, leaflet color, and shape, inflorescence, pod and seed characters were noted. Similarly, Stoilova and Perira (2013) found variability on qualitative characteristics such as flower color, growth habit, raceme position, testa texture, seed shape, and seed coat color in cowpea. In vigna species, found variation of some qualitative traits in the different strains between Vigna mungo and radiata. Such variations of traits are useful and effective in distinguishing different lines in a germplasm.

On quantitative traits, leaf variability was observed similar to that in ricebean genotypes reported by Pandey et al. (2015) and other legume species such as cowpea (Gerrano et al., 2015). The variations in the maturity among the ricebean germplasm are largely attributed to the genotypes. Ricebean germplasm are characterized as short and late maturing with flowering days ranging from 45-110 days and pod maturity from 65-148. Previous studies on ricebean have similar results. Joshi et al. (2008) reported high variation in the flowering days (61-104) and maturity (99-134) among ricebean germplasm. Further, early maturing genotypes were reported among different ricebean accessions (Sachan, 2017; Pratap et al., 2018; Gupta et al., 2009), black gram (Pyngrope et al., 2015), and lentil (Mohammed et al., 2019). Variability in the plant maturity was also observed in the different cowpea genotypes (Stoilova & Perira, 2013). Early maturity is a good agronomic trait for the uniformity of yield and developing dwarf varieties with high yield (Musango et al., 2016) and a good trait suitable during the dry season (Doumbia et al., 2013). Variations in the number of flowers and pods per cluster among ricebean germplasm were found by Sharma et al. (2015) and Ahamed et al. (2014) in cowpea genotypes. Peduncle length among the genotypes varied among the cowpea genotypes (Ahamed et al., 2014). High-yielding genotypes were reported in ricebean genotypes by Gupta et al. (2009). The mean seed yield and 100-seed weight values of ricebean genotypes ranged from 44.0 to 274.27g and 3.77g to 11.0g, respectively. Pandey et al. (2015) reported a mean seed yield per plant ranging from 6.34 to 12.36g. Significant variations in the seed yield per plot (2.40m^2) and 100-seed weight were likewise reported by Katoch (2011). Further, significant genetic variations in the yield components in cowpea, mungbean, soybean, limabean, and other legume crop species were reported (Al-Saady et al., 2018; Razvi et al., 2018; Hamawaki et al., 2017; Silva et al., 2019). Gul et al. (2013) reported significant genetic variability for seed yield and yield components in chickpea genotypes. Different studies reported that high seed yield is associated with yield components. These characters are pods per plant and seeds per pod which are important yield components in determining seed yield (Painkra et al., 2019; Tajoddin et al., 2012; Punia et al., 2014). These findings indicate that there is a great potential for making genetic enhancement as traits vary (Joshi et al., 2008). Low to moderate diversity of morphological characters among ricebean germplasm was found in the study, however, climbing habit and primary leaflet shape, pod per cluster, primary leaflet width, seed width, terminal leaf width, and pod width were found to have high diversity index. Arif et al. (2016) reported low to moderate diversity of quantitative characters in bambara groundnut.

Table 6

Diversity Indices for Morphological Characters in 28 Ricebean Germplasm

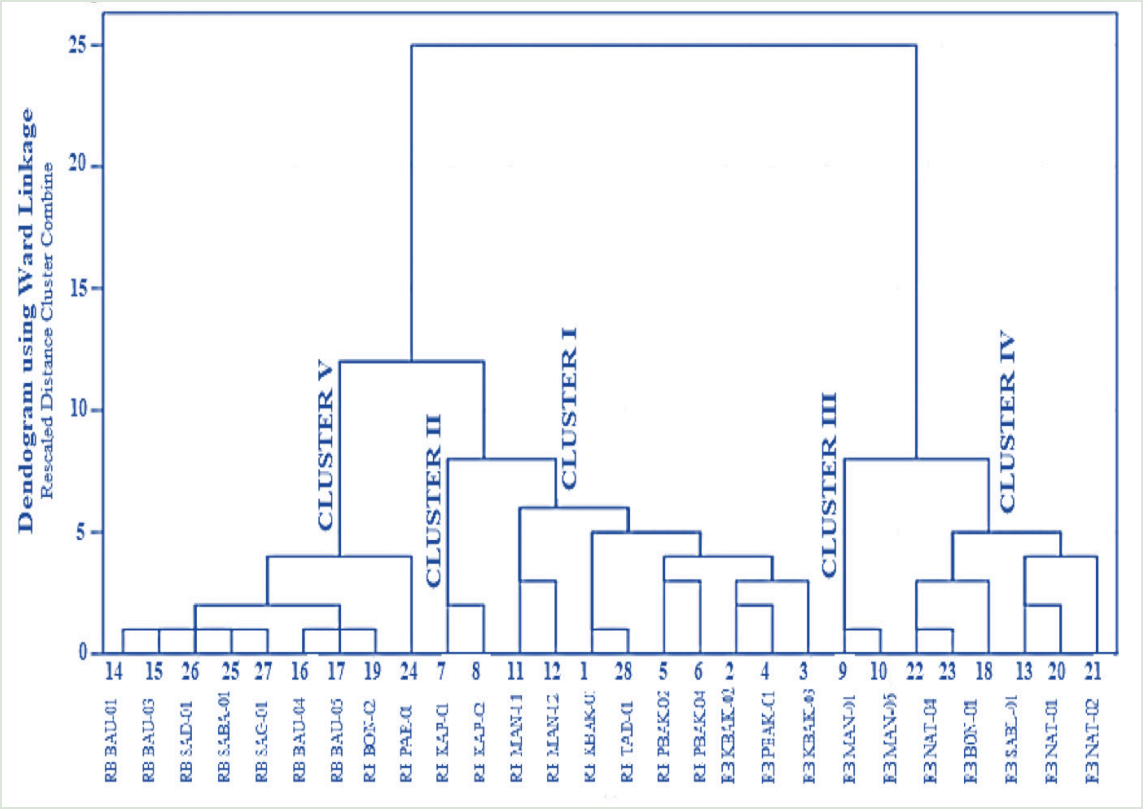
Qualitative	Index Value	Quantitative	Index Value
Petiole color	0. 29	Petiole length (cm)	0.56
Leaf color	0.22	Terminal length (cm)	0.61
Terminal leaf shape	0.39	Primary length (cm)	0.63
Primary leaflet shape	0.41	Primary width (cm)	0.70
Leafiness	0.31	Terminal width (cm)	0.68
Leaf senescence	0.30	Days to pod maturity	0.40
Climbing habit	0.42	Days to flowering	0.42
Twinning tendency	0.36	Peduncle length	0.52
Branching pattern	0.32	Pods per cluster	0.71
Corolla color	0.12	Flowers per cluster	0.55
Calyx	0.27	Fruit capacity (%)	0.47
Raceme position	0.30	Pod length (cm)	0.57
Pod color	0.37	Pod width (mm)	0.66
Pod shattering	0.21	Seeds per pod	0.59
Pod cross section	0.20	Seed length (mm)	0.62
Constriction of pod between seeds	0.20	Seed width (mm)	0.69
Seed color	0.24	100-sed wt. (g)	0.42
Luster on seed surface	0.18	Seed yield (g/3m^2)	0.35
Seed shape	0.14		
Mean	**0.27**		**0.56**

Cluster Analysis of the Ricebean Germplasm Based on Morphological Traits Using Ward's Linkage

Twenty-eight ricebean germplasm from Benguet and Mt. Province were classified into five main clusters based on morphological traits (Figure 2). Among the groups, Clusters I and V are the largest composed of nine (9) germplasm each and further subdivided into sub-clusters. Most of the ricebean germplasm in the Cluster I are collections from Benguet. These germplasm are characterized as viny plants, with pronounced twinning, having greenish-purple calyx, with racemes found above the canopy, fully distributed branches, short petioles, and long peduncles, low-fruit setting capacity, late maturing, have high number of seeds per pod, and with heavy seeds. All germplasm in Cluster V are from Mt. Province and characterized as viny, slightly-pronounced twinning, high fruit-setting ability, and early maturing. These germplasm are also characterized by purplish-green calyx, branches that are found mainly in the middle portion of the plant, short petioles and peduncles, round pod cross-section, green-yellow seeds, few seeds per pod, and heavy seeds. Cluster IV consists of six germplasm and further subdivided into four sub-groups. Most germplasm in this cluster are collections from Mt. Province characterized as slightly non-viny plants and slightly twinning. These are also observed to have green-purplish yellow corolla with purple to green calyx, long petioles and peduncles, and branches that are mainly from the basal portion of plant. Other characters include no pod shattering, with constriction of pod between seeds, and semi-flat pods. Further, these germplasm have low fruit-setting ability and late maturing. Seeds are drum-shaped, few, and low weight. In the subgroups, RB NAT-02, RB NAT-01, and RB-SABL-01 are more associated with each other while RB BON-01 are closely associated with RB NAT-04 and RB NAT-05. The smallest groups

Figure 2

Dendogram of the Ricebean Germplasm Based on the Morphological Traits Using Ward's Linkage



are found in Clusters II and III consisting only two (2) germplasm. Cluster II contains RB KAP-01 and RB KAP-02 which are characterized as intermediate to pronounced twinning, early maturing, low-fruit setting ability, having mottled green seeds, with many seeds per pod, and heavy 1000 seeds. Cluster III consists of RB MAN-01 and RB MAN-06. These germplasm are characterized as slightly non-viny and slightly twinning plants. Germplasm are also distinguished by having dark green leaves, yellow corolla with green calyx, branches that are found mainly in the middle portion of the plant, long petioles, and peduncles, and constricted and semi-flat pods. Low-fruit setting ability and late maturing, drum-shaped seeds, many seeds per pod, and low weight of 1000 seeds are also the characters of the germplasm in this Cluster. The clustering of germplasm based on the different morphological markers has been reported in different legume species such as soybean, bambara groundnut, and limabean (Teixeira et al., 2017; Malek et al., 2014; Kumar et al., 2015; Gonne et al., 2013; Martínez-Castillo et al., 2006). Gixhari et al. (2014) reported different clusters in garden pea and lentils (Roy et al., 2013). In the present study, it was observed that high-yielding germplasm were grouped into one cluster (Cluster V). Similar observation was reported by Leila et al. (2016) in field pea. Twelve genotypes were separated into two clusters, first group of genotypes was characterized by having high pod yield while the second group comprises the less productive genotypes. Stoilova and Perira, (2013) reported in cowpea seven clusters generated from the 48 accessions, and one of which consists only one accession.

Polymorphism and Efficiency of SSR Marker

All the 10 SSR markers produced reproducible and polymorphic bands and yielded a total of 89 fragments of which 85 amplicons (96.0%) were polymorphic (Table 7). A total of three monomorphic bands were observed among the SSR markers which were exhibited by CEDG0214, CED021, and CEDG003. Polymorphic bands ranged from 4 to 13 with an average of 8.5. CEDG073 (13), CEDG015 (11), and CEDG002 (10) produced the highest total bands while CEDG021 had the lowest total bands amplified. All the SSR markers are found to be highly polymorphic which shows high genetic diversity among the ricebean germplasm. The peak of fragments among the eleven ricebean germplasm ranged from 50 bp to 460 bp. The PIC value varied from 0.45 to 0.94 with an average of 0.78. In the present study, about 80-100% polymorphism was observed with a mean of 78%. Of the 10 SSR primers tested, six had 100% polymorphism (CEDG043, CEDG015, CEDC016, CEDG073, CEDG008, and CEDG011) which is revealed by having high PIC. All the markers showed high PIC except CEDG021, which had a PIC value lower than 0.50. CEDG003 had the highest PIC of 0.94 followed by CEDG073 with 0.88, while CEDG021 had the lowest but had 80% polymorphism. All germplasm showed a relatively detectable band across all primers except CEG008 and CEDG011 in RB KAP-02. The gel image of SSR profile of the 10 primers is shown in Figure 3. On the assessment of rice bean germplasm diversity based on SSR DNA markers, 10 SSR markers were found to be polymorphic. High PIC value of SSR markers was previously reported in ricebean germplasm (Chen et al., 2016). Tian et al. (2013) also reported a high diversity of 13 SSR markers in wild ricebean landraces. Iangrai et al. (2017) reported a high gene diversity (mean 0.534) in ricebean germplasm having a total of 179 amplified alleles with an average of 6.393 alleles per locus. In the present study, the PIC values ranged from 0.221 to 0.682 with an average of 0.438, relatively lower than the values recorded by Pyngrope et al. (2015) in blackgram but almost close to the values found by Isemura et al. (2010) in adzuki bean. The high PIC from the 48 SSR markers ranged from 0.37 to 0.91 (Sefera et al., 2011). High PIC values of 10 SSR loci markers were also observed in pea varieties with an average of 0.89 (Smykal et al., 2008). Mathivathana et al. (2018) also revealed that out of 10 SSR primer pairs, only two primer markers exhibited polymorphism among the six mungbean genotypes. Information about diversity and genetic relationships is of fundamental importance for breeding improvement and germplasm management. The molecular markers such as SSR, ISSR and RAPD showed that SSR markers were found to be the most efficient in molecular characterization among genotypes or lines of four Vigna species. The pooled allelic diversity separated the 35 genotypes into 4 major clusters according to the species (Singh et al., 2014). SSR markers successfully assessed genetic variability in pigeon pea (Babasaheb et al., 2013), garden pea (Sarikamiş et al., 2010), chickpea (Gul et al., 2013), green bean (Madakbaş et al., 2016), and strawberry (Lim et al., 2017) germplasm. Previous findings showed that different SSR markers in determining genetic polymorphism result in several clusters generated as reported by previous authors (Tian et al., 2013; Wang et al., 2015; Sathees et al., 2021). For instance, seven out of 44 SSR markers were found to be polymorphic which grouped the 40 ricebean genotypes into two clusters (Thakur et al., 2017). The UPGMA dendrogram defined by 15 polymorphic SSR markers revealed genetic relatedness of the pea accessions (Ahmad et al., 2012). The efficiency of molecular markers in determining genetic diversity was reported by Zaccardelli et al. (2012) in 14 lentil germplasm grouped into clusters and sub-clusters by 16 primer pairs of SSR markers. Tantasawat et al. (2011) found genetic diversity and relatedness among soybean genotypes based on based on SSR markers. Choudhary et al. (2012) revealed the efficiency of SSR markers in chickpea. Further, molecular markers such as RAPD were also successful in genetic characterization among the ricebean varieties and their crosses (Shafiqul et al., 2017).

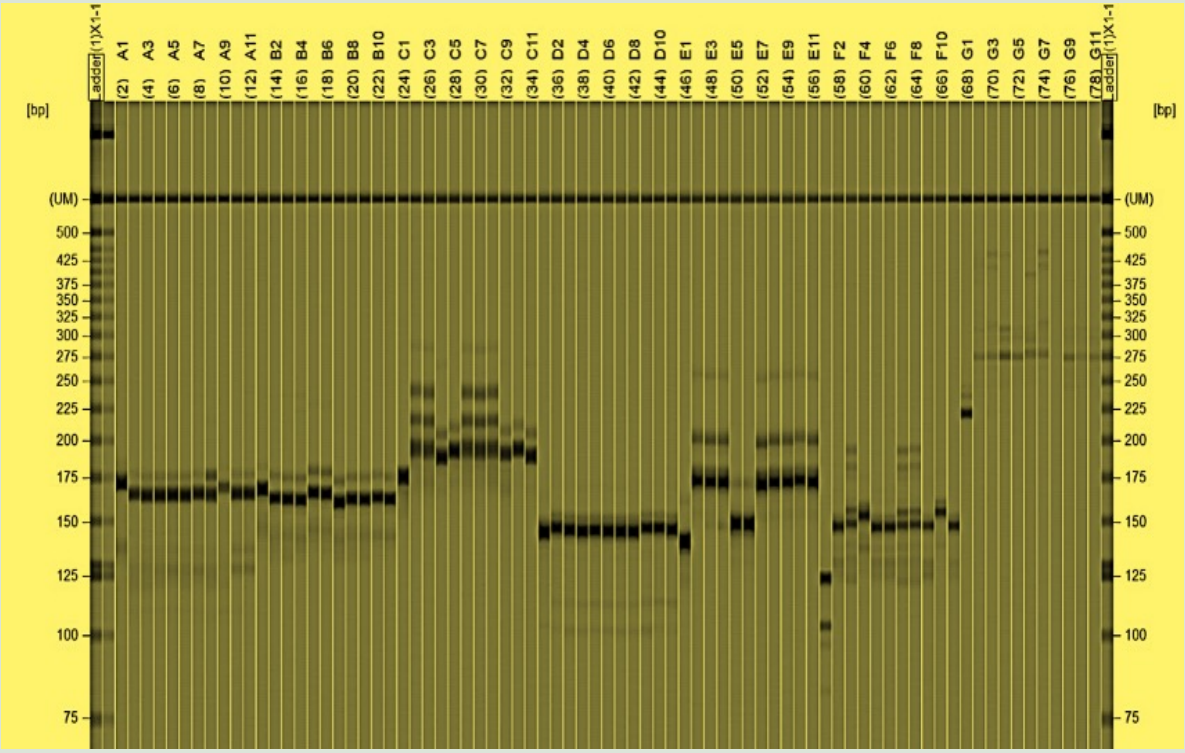
Table 7

Summary of Amplication and Polymorphic Rates of 10 SSR Markers in the Ricebean Germplasm

SSR PRIMER	No. of bands	Monomorphic bands	Polymorphic Bands	Polymorphism (%)	Peak ranges (bp)	PIC Value
CED214	8	1	7	87.5	100-165	0.81
CEDG043	8	0	8	100.0	100-170	0.86
CEDG015	11	0	11	100.0	155-280	0.86
CEDG021	5	1	4	80.0	115-250	0.45
CEDG002	10	0	9	90.0	50-140	0.74
CEDC016	9	0	9	100.0	50-460	0.81
CEDG003	9	1	8	88.9	95-135	0.94
CEDG073	13	0	13	100.0	50-270	0.88
CEDG008	9	0	9	100.0	85-130	0.64
CEDG011	7	0	7	100.0	50-220	0.80
Total	89	3	8.5	946.4		7.79
Average	8.9	0.30	8.50	94.64		0.78

Figure 3

Gel Image Showing Polymorphins of SSR Markers Within the 11 Ricebean Germplasm



Similarity Index and Cluster Membership of the Ricebean Germplasm Based on SSR Markers

The similarity coefficient among the clusters ranged from 0.39 to 0.86 indicating diversity among the ricebean germplasm (Table 8). RB PBAK-04 and RB MAN-12 had high similarity index (0.86) indicating close relationship, while RB KAP-02 shows close relatedness with RB MAN-11, RB SABA-01, and RB SAG-01 as shown by their low similarity indices. RB SAG-01 showed a far relatedness with RB KAP-02 and RB PBAK-04. The polymorphism of SSR markers grouped the ricebean germplasm into six clusters (Figure 4). A dendogram generated based on UPGMA revealed six clusters. Cluster I consists of four germplasm which grouped RB PBK-04, RB MAN-12, RB MAN-11, and RB KAP-01. These germplasm were from Benguet. Cluster II contains two germplasm which include RB NAT-01 and RB BON-01. Cluster V consists of germplasm RB SAG-01 and RB KBAK-02, and other clusters consisting only one accession each. RB KAP-02, KBAK-01 and RB SABA-01 segregate separately. RB KBAK-01 showed relatedness with RB SABA-01, RB SAG-01, and RB KBAK-02. RB KAP-02 was placed distantly among other groups showing clear genetic divergence from other germplasm.

Table 8

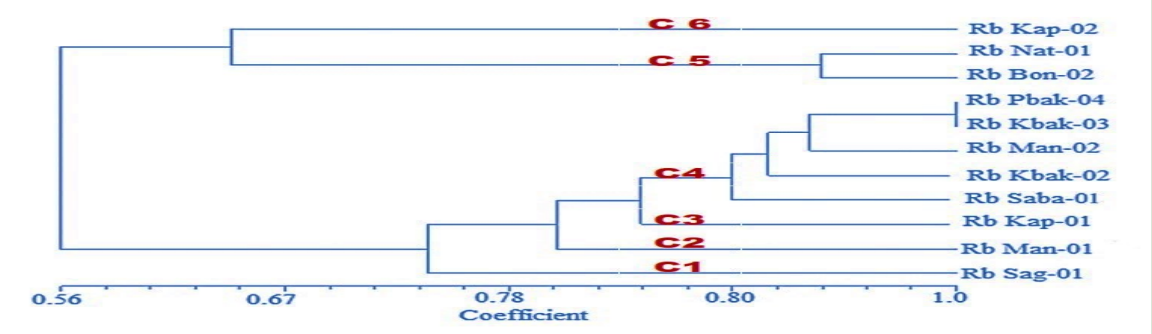
Jaccard's Similarity Coefficient of the 11 Ricebean Germplasm

CODE	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	A_9	A_{10}	A_{11}
A_1	1.00										
A_2	0.57	1.00									
A_3	0.51	0.86	1.00								
A_4	0.48	0.70	0.73	1.00							
A_5	0.60	0.77	0.83	0.68	1.00						
A_6	0.54	0.74	0.73	0.72	0.84	1.00					
A_7	0.49	0.75	0.80	0.66	0.76	0.64	1.00				
A_8	0.52	0.80	0.82	0.63	0.73	0.63	0.82	1.00			
A_9	0.39	0.65	0.66	0.70	0.58	0.63	0.60	0.67	1.00		
A_{10}	0.47	0.73	0.72	0.71	0.70	0.58	0.67	0.68	0.66	1.00	
A_{11}	0.52	0.76	0.77	0.72	0.73	0.65	0.68	0.70	0.76	0.77	1.00

Legend: A_1-RB KAP-02, A_2-RB-PBAK-04, A_3-RB MAN-12, A_4-RB KBAK-01, A_5-RB NAT-01, A_6-RB BON-01, A_7-RB MAN-11, A_8-RB KAP-01, A_9-RB SABA-01, A_{10}-RB SAG-01, A_{11}-RB KBAK-02

Figure 4

Dendrogram Constructed Using UPGMA Based on Jaccard's Similarity Matrix Data Generated by SSR Analysis



Conclusions

Ricebean germplasm exhibit significant variation among parameters on days to flowering, pod maturity, fruit-setting ability, seed yield and weight of 1000 seeds. Fruit setting ability are high in RB PAR-01, RB SAD-01, RB SAG-01, RB BON-02, RB BAU-01 and RB BAU-03. RB BAU-01, RB BAU-05 and RB SAD-01 are the earliest in terms of plant maturity. The highest yielders are RB SAG-01, RB KAP-01, RB PAR-01, RB BAU-01, RB PBAK-02, RB BAU-05, and RB BAU-04. Morpho-agronomic traits reveal that ricebean germplasm have low to moderate diversity. SSR markers on the other hand reveal that ricebean germplasm have moderate to high diversity. The combination of morpho-agronomic traits and SSR markers complemented in determining relationships and distinguishing ricebean germplasm. Fruit setting ability, pod width, seed width, and 100-seed weight are important characters for seed yield improvement in ricebean germplasm. The grouping of ricebean germplasm with high fruit setting ability, long and broad pods, large seeds, many pods per cluster and many seeds per pod, high seed weight and yield suggest a potential gene pool of ricebean, which can be utilized for future breeding improvement programs. Further, results suggest that genetic markers are efficient for measuring genetic diversity and relatedness as well as identifying ricebean germplasm. Genetic diversity and relationship assessments among ricebean germplasm could provide useful information for efficient utilization of these materials especially for genetic improvement of a new variety.

Acknowledgement

The authors gratefully acknowledge the Commission on Higher Education (CHED) for the funding; Benguet State University-Higher Education Regional Research Center (BSU-HERRC) for the assistance in the implementation; Benguet State University for the counterpart and support in the implementation of the project; and the Philippine Genome Center for the laboratory use and technical assistance.

References

Ahmad, S., Singh, M., Lamb-Palmer, N.D., Lefsrud, M., & Singh, J. (2012). Assessment of genetic diversity in 35 *Pisum sativum* using microsatellite markers. *Canadian Journal of Plant Science*, 92(6): 1075-1081.

Ahamed, K.U., Akhter, B., Islam, M.R., Humaun, M.R., & Alam, M.J. (2014). Morphological characterization and genetic diversity in lentil (*Lens culinaris* medikus ssp. Culinaris) germplasm. *International Journal of Agricultural Research, Innovation and Technology*, 4(1): 70-76.

Al-Saady, N. A., Nadaf, S. K., Al-Lawati, A. H., & Al-Hinai, S. A. (2018). Germplasm collection and seed diversity of cowpea (*Vigna unguiculata* (L.) Walp.). *Asian Journal of Agriculture and Food Sciences*, 6(4).

Arif, A., Kendarini, N., & Kuswanto, K. (2016). *Evaluation of Genetik Purity on 20 Genotypes of Bambara Groundnut (Vigna subterranea L. Verdcourt) Selected From Single Seed Descent Morphological Characters*. Doctoral dissertation. Brawijaya University.

Babasaheb, W., Parihar, A., Chaurasia, P., Pachchigar, K., & Chauhan, R.M. (2013). Genetic analysis of wild and cultivated germplasm of pigeonpea using random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR) markers. *African Journal of Biotechnology,* 12(40): 5823-5832.

Belshaw, H. (1947). Races, Lands and Foods. P*acific Affairs*, 20(1), 71-80.

Bhalodiya, D., Dhaduk, H.K.L., Kumar, S., Gediya, L.N., & Patel, H.P. (2019). Line× tester analysis for seed yield, protein and oil content and SSR based diversity in sesame (*Sesamum indicum* L.). *Ecological Genetics and Genomics*, 13, 100048.

Carović-Stanko, K., Liber, Z., Vidak, M., Barešić, A., Grdiša, M., Lazarević, B., & Šatović, Z. (2017). Genetic diversity of Croatian common bean landraces. *Frontiers in Plant Science*, 8, 604.

Chen, H., Chen, X., Tian, J., Yang, Y., Liu, Z., Hao, X., & Cheng, X. (2016). Development of gene-based

SSR markers in rice bean (*Vigna umbellata* L.) based on transcriptome data. *PloS one*, 11(3), e0151040.

Chen, H., Chen, H., Hu, L., Wang, L., Wang, S., Wang, M.L., & Cheng, X. (2017). Genetic diversity and a population structure analysis ofaccessions in the Chinese cowpea [*Vigna unguiculata* (L.) Walp.] germplasm collection. *The Crop Journal,* 5(5): 363-372.

Choudhary, P., Khanna, S.M., & Jain, P.K. (2012). Genetic structure and diversity analysis of the primary gene pool of chickpea using SSR markers. *Genetics of Molecular Research*, 11(2): 891-905.

Choudhury, P., Singh, I.P., George, B., Verma, A.K., & Singh, N.P. (2008). Assessment of genetic diversity of pigeonpea cultivars using RAPD analysis. *Biologia Plantarum*, 52(4): 648-653.

Doumbia, I.Z., Akromah, R., & Asibuo, J.Y. (2013). Comparative study of cowpea germplasms diversity from Ghana and Mali using morphological characteristics. *Journal of Plant Breeding and Genetics*, 1(3): 139-147.

Gaad, D., Laouar, M., Abdelguerfi, A., & Gaboun, F. (2018). Collection and agro-morphological characterization of Algerian accessions of lentil (Lens culinaris). *Biodiversitas Journal of Biological Diversity,* 19(1): 183-193.

Gangadhar B.H.M., & Mishra, R.K. (2016). Comparative study of EST-SSR, SSR, RAPD, and ISSR and their transferability analysis in pea, chickpea and mungbean. *European Academic Research,* 4(2): 1346- 1352.

Gerrano, A. S., Adebola, P. O., Jansen van Rensburg, W.S., & Laurie, S. M. (2015). Genetic variability in cowpea (*Vigna unguiculata* (L.) Walp.) genotypes. *South African Journal of Plant and Soil*, 32(3): 165-174.

Gixhari, B., Vrapi, H., & Hobdari, V. (2014). Morphological characterization of pea (*Pisum sativum* L.) genotypes stored in Albanian genebank. *Albanian Journal of Agricultural Sciences*, 169.

Gonné, S., Félix-Alain, W., & Benoît, K.B. (2013). Assessment of twenty bambara groundnut (*Vigna subterranea* (L.) Verdcourt) landraces using quantitative morphological traits. *International Journal of Plant Research*, 3(3): 39-45.

Gul, R., Khan, H., Bibi, M., & Imran, B. (2013). Genetic attributing traits in chickpea (*Cicer arietinum* L.). *Journal of Animal and Plant Sciences*, 23(2), 521-526.

Gupta, S., Kozak, M., Sahay, G., Durrai, A. A., Mitra, J., Verma, M. R. & Das, A. (2009). Genetic parameters of selection and stability and identification of divergent parents for hybridization in rice bean (*Vigna umbellata* Thunb. (Ohwi and Ohashi)) in India. *The Journal of Agricultural Science,* 147(5), 581-588.

Hamawaki, O.T., Rezende, G.F., Jorge, G.L., Silveira, I.C., Medeiros, L.A., & Hamawaki, R.L. (2017). Phenotypic and genotypic correlations between soybean agronomic traits and path analysis. *Genet. Mol. Res.,* 16 2–11.

Hamwieh, A., Udupa, S.M., Sarker, A., Jung, C., & Baum, M. (2009). Development of new microsatellite markers and their application in the analysis of genetic diversity in lentils. *Breeding Science*, 59(1), 77-86.

Iangrai, B., Pattanayak, A., Khongwir, D.E.A., Pale, G., Gatphoh, E.M., Das, A., & Chrungoo, N.K. (2017). Development and characterization of a new set of genomic microsatellite markers in rice bean (*Vigna umbellata* (Thunb.) Ohwi and Ohashi) and their utilization in genetic diversity analysis of collections from North East India. *PloS one*, 12(7), e0179801.

IBPGR. (1985). *Descriptors for Vigna mungo and Vigna radiata (Revised)*. International Board Plant Genetic Resources, Rome.

Isemura, T., Kaga, A., Tomooka, N., Shimizu, T., & Vaughan, D.A. (2010). The genetics of domestication of rice bean, *Vigna umbellata*. Annals of Botany, 106(6): 927-944.

Joshi, K.D., Bhanduri, B., Gautam, R., Bajracharya, J., & Hollington, P.B. (2008). *Rice bean: a multi-purpose underutilized legume*. In: Smart, J. Haq, N. *New crops and uses: their role in a rapidly changing world*. CUC, UK, 234-248.

Kameswara, R.N. (2004). Plant genetic resources: Advancing conservation and use through biotechnology. *African Journal of Biotechnology,* 3(2): 136-145.

Kalia, R.K., Rai, M.K., Kalia, S., Singh, R., & Dhawan, A.K. (2011). Microsatellite markers: an overview of the recent progress in plants. *Euphytica*, 177(3): 309-334.

Katoch, R. (2011). Morpho-physiological and nutritional characterization of rice bean (*Vigna umbellata*). *Acta Agron. Hungarica*, 59, 109–20.

Keneni, G., Bekele, E., Imtiaz, M., Dagne, K., Getu, E., & Assefa, F. (2012). Genetic diversity and population structure of Ethiopian chickpea (*Cicer arietinum* L.) germplasm accessions from different geographical origins as revealed by microsatellite markers. *Plant Molecular Biology Reporter*, 30(3), 654-665.

Khazaei, H., Caron, C.T., Fedoruk, M., Diapari, M., Vandenberg, A., Coyne, C.J., McGee, R., & Bett, K.E. (2016). Genetic diversity of cultivated lentil (*Lens culinaris* Medik.) and its relation to the world's agro-ecological zones. *Frontiers in Plant Science*, 7, 1093.

Kumar, A., Pandey, A., Aochen, C., & Pattanayak, A. (2015). Evaluation of genetic diversity and interrelationships of agro-morphological characters in soybean (Glycine max) genotypes. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 85(2), 397-405.

Kumar, S., Hash, C.T., Singh, G., Basava, R.K., & Srivastava, R.K. (2020). Identification of polymorphic SSR markers in elite genotypes of pearl millet and diversity analysis. *Ecological Genetics and Genomics*, 14, 100051.

Lim, S., Lee, J., Lee, H.J., Park, K.H., Kim, D.S., Min, S.R., Jang, W.S., Kim, T., & Kim, H. (2017). The genetic diversity among strawberry breeding resources based on SSRs. *Scientia Agricola*, 74(3): 226-234.

Leila, O., Alane, F., Rahal-Bouziane, H., & Abdelguerfi, A. (2016). Agro-morphological diversity within field pea (*Pisum sativum L.*) genotypes. *African Journal of Agricultural Research,* 11(40): 4039-4047.

Macherey-Nagel. (2014). Genomic DNA from plant: User manual, NucleoSpin 96 Plant II, NucleoSpin 96 Plant II Core Kit.

Madakbaş, S.Y., Sarıkamış, G., Başak, H., Karadavut, U., Özmen, C.Y., Daşçı, M.G., & Çayan, S. (2016). Genetic characterization of greenbean (*Phaseolus vulgaris* L.) accessions from Turkey with SCAR and SSR markers. *Biochemicalgenetics*, 54(4): 495-505.

Mafakheri, K., Bihamta, M.R., & Abbasi, A.R. (2017). Assessment of genetic diversity in cowpea (*Vigna unguiculata* L.) germplasm using morphological and molecular characterisation. *Cogent Food & Agriculture*, 3(1), 1327092.

Malik, S. R., Shabbir, G., Zubir, M., Iqbal, S.M., & Ali, A. (2014). Genetic diversity analysis of morpho-genetic traits in desi chickpea (*Cicer arietinum*). *International Journal of Agriculture and Biology*, 16(5).

Malik, S.R., Bakhsh, A., Asif, M.A., Iqbal, U., & Iqbal, S.M. (2010). Assessment of genetic variability and interrelationship among some agronomic traits in chickpea. *Int. J. Agric. Biol.,* 12: 81–85.

Malek, M.A., Rafii, M.Y., Afroz, S.S., Nath, U.K., & Mondal, M. (2014). Morphological characterization and assessment of genetic variability, character association, and divergence in soybean mutants. *The Scientific World Journal*. https://doi.org/10.1155/2014/968796

Marconato, M.B., Pereira, E.M., Silva, F.M., Bizari, E.H., Pinheiro, J.B., Mauro, A.O., & Unêda-Trevisoli, S.H. (2016). Genetic divergence in a soybean (Glycine max) diversity panel based on agro-morphological traits. *Genetics and molecular research*, 15, 1-10.

Martínez-Castillo, J., Zizumbo-Villarreal, D., Gepts, P., Delgado-Valerio, P., & Colunga-García Marín, P. (2006). Structure and genetic diversity of wild populations of Lima bean (*Phaseolus lunatus* L.) from the Yucatan Peninsula, Mexico. *Crop Science*, 46(3): 1071-1080.

Mathivathana, M.K., Jagadeeshselvam, N., Madhumitha, B., Karthikeyan, A., Pandiyan,M., Karthikeyan, G., Vanniarajan, C., Raveendran, M., Senthil, N., & Sudha M. (2018). Screening and Identification of SSR Markers for Genetic

Diversity for Mungbean [*Vigna radiata* (L.) Wilczek]. *Int. J. Curr. Microbiol. Appl. Sci.,* 7, 789–93.

Mohammed, N.A., Refay, Y.A., Migdadi, H.M., Alsamin, B., Muharram, A.A., Al-Selwey, W., Abdela, K.A., Alghamdi, S.S. & Farooq, M. (2019). Agro-Morphological Characterization of Lentil Genotypes in Dry Environments. *International Journal of Agriculture and Biology*, 22(6): 1320-1330.

Moiana, L.D., Filho, P.S.V., Gonçalves-Vidigal, M.C., & de Carvalho, L.P. (2015).Genetic diversity and population structure of upland cotton Brazilian cultivars (*Gossypium hirsutum* L. race'latifolium'H.)using SSR markers. *Australian Journal of Crop Science,* 9(2): 143-152.

Musango, R., Kudzai, K., Mhungu, S., & Tibugar, H. (2016). Phenotypic characterization of common bean (*Phaseolus vulgaris* L.) accessions conserved at the genetic resources and biotechnology institute. *Journal of Biodiversity and Environmental Sciences,* 8(6): 26-36.

Ortiz-Burgos, S. (2016). Shannon-Weaver diversity index. U: Kennish MJ (ur.) *Encyclopedia of estuaries.* Encyclopaedia of Earth sciences series.

Painkra, P., Shrivastava, R. & Nag, S.K. (2019). Agro-morphological characterization of soybean germplasm. *Indian Soc. Oilseeds Res.,* 85. 85–8.

Pandey, G., Prasad, R., Prasad, B., & Chauhan, P. (2015). Co-efficient of variation, heritability, genetic advance and variability for ricebean (*Vigna umbellata* (Thunb.) genotypes under mid hill conditions of Uttarakhand. *Journal of Applied and Natural Science*, 7(2): 794-798.

Pratap, A., Prajapati, U., Singh, C.M., Gupta, S., Rathore, M., Malviya, N., & Singh, N.P. (2018). Potential, constraints and applications of in vitro methods in improving grain legumes. *Plant Breeding*, 137(3): 235-249.

Punia, S.S., Gautam, N.K., Ram, B., Verma, P., Dheer, M., Jain, N.K., Koli, N.R., Mahavar, R., & Jat, V.S. (2014). Genetic variability and correlation studies in urdbean (Vigna mungo L.). *Legume Research-An International Journal*, 37(6): 580-584.

Pyngrope, A.H., Noren, S.K., Wricha, T., Sen, D., Khanna, V.K., & Pattanayak, A. (2015). Genetic diversity analysis of blackgram [*Vigna mungo* (L.) Hepper] using morphological and molecular markers. *Int. J. Appl. Pure Sci. Agric,* 1(8): 104-113.

Rajković, D., Taški-Ajduković, K., Nagl, N., Isakov, M., Milić, D., Karagić, Đ., & Mikić, A. (2015). Potential use of SSR and ISSR markers in estimation of DNA polymorphism within genus Vicia. *Ratarstvo i povrtarstvo*, 52(2): 85-89.

Ramya, B., Nallathambi, G., & Ram, S.G. (2014). Genetic variability, heritability and genetic advance in induced mutagenesis blackgram (*Vigna mungo* (L.)Hepper). *Plant Archives*, 14(1): 139-141.

Rana, J.C., Chahota, R.K., Sharma, V., Rana, M., Verma, N., Verma, B., & Sharma, T.R. (2015). Genetic diversity and structure of Pyrus accessions of Indian Himalayan region based on morphological and SSR markers. *Tree genetics & genomes*, 11(1): 1-14.

Razvi, S.M., Khan, M.N., Bhat, M.A., Ahmad, M., Ganaie, S.A., Sheikh, F.A., & Parry, F.A. (2018). Morphological variability and phylogenetic analysis in Common bean (*Phaseolus vulgaris* L.). *Legume Research-An International Journal*, 41(2): 208-2012.

Rohlf, J.F. (1993). *Numerical Taxonomy and Multivariate Analysis System*, version 2.1. Exeter Software, New York.

Roy, S., Islam, M.A., Sarker, A., Malek, M.A., Rafii, M.Y., & Ismail, M.R. (2013). Determination of genetic diversity in lentil germplasm based on quantitative traits. *Australian Journal of Crop Science*, 7(1): 14-21.

Sachan, M.S., (2017). Genetics of yield and its components in rice bean under mid altitude areas of Mon district Nagaland India. *Journal of Hill Agriculture*, 8(4), 366-371.

Sarikamis, G., Yanmaz, R., Ermis, S., Bakir, M.E.L.İ.K.E., & Yuksel, C. (2010). Genetic characterization of pea (*Pisum sativum*) germplasm from Turkey using morphological and SSR markers.*Genetics and Molecular Research*, 9(1).

Sathees, N., Shoba, D., Saravanan, S., Kumari, S.M.P., & Pillai, M.A. (2021). Assessment of the genetic diversity of black gram [*Vigna mungo* (L.) hepper]

collections for yellow mosaic virus resistance using simple sequence repeat markers. *Legume Research: An International Journal*, 44(7).

Sefera, T., Abebie, B., Gaur, P.M., Assefa, K., & Varshney, R.K. (2011). Characterisation and genetic diversity analysis of selected chickpea cultivars of nine countries using simple sequence repeat (SSR)markers. *Crop and Pasture Science*, 62(2): 177-187.

Shafiqul I., Dutta M., Shachi S., Kumar P., & Bhatt, K.V. (2017). Assessment of Genetic Diversity of Ricebean [(*Vigna umbellata*) (Thunb.) Ohwi & Ohashi)] Varieties and their Narrow Leaf Cross Derivatives using RAPD Markers. *Int. J. Agric. Environ. Biotechnol.,* 10, 415.

Sharma, L.A., Singh, M.R.K., & Singh, N. (2015). Heterosis and combining ability for grain yield and its components in rice bean (*Vigna umbellata* (thunb) Ohwi and Ohashi). *Indian Journal of Hill Farming*, 11, 27-33.

Silva, R.N.O., Lopes, A.C.A., Gomes, R.L.F., Pádua, J.G., & Burle, M.L. (2019). High diversity of cultivated lima beans (*Phaseolus lunatus*) in Brazil consisting of one Andean and two Mesoamerican groups with strong introgression between the gene pools. *Genet. Mol. Res.,* 18(4): GMR18441. https://doi.org/10.4238/gmr18441

Singh, A., Dikshit, H.K., Jain, N., Singh, D., & Yadav, R.N. (2014). Efficiency of SSR, ISSR and RAPD markers in molecular characterization of mungbean and other Vigna species. *Indian Journal of Biotechnology,* 13, 81-88.

Smýkal, P., Hýbl, M., Corander, J., Jarkovský, J., Flavell, A.J., & Griga, M. (2008). Genetic diversity and population structure of pea (*Pisum sativum* L.) varieties derived from combined retrotransposon, microsatellite and morphological marker analysis. *Theoretical and Applied Genetics*, 117(3): 413-424.

Stoilova, T., & Pereira, G. (2013). Assessment of the genetic diversity in a germplasm collection of cowpea (*Vigna unguiculata* (L.) Walp.) using morphological traits. *African J. Agric. Res.,* 8, 208–15.

Tad-awan B.A. & Sagalla E.J.D. (2015). Cultivation and Conservation of Traditional Food Crops and Perceptions of Farmers on Biodiversity Loss in the Cordillera Administrative Region. *Mt. J. Sci. Interdiscip. Res. (formerly Benguet State Univ. Res. Journal),* 74: 62–81.

Tajoddin, M., Manohar, S., & Lalitha, J. (2012). Agronomic Characteristics and Seed Yield of Mung Bean Cultivars Differing in Seed Color using Organic and Conventional Farming in Indian Subtropical Region. *The Asian and Australasian Journal of Plant Science and Biotechnology,* 6, 38-40.

Tantasawat, P., Trongchuen, J., Prajongjai, T., Jenweerawat, S., & Chaowiset, W. (2011). SSR Analysis of Soybean ('Glycine max' (L.) Merr.) Genetic Relationship and Variety Identification in Thailand.*Australian Journal of Crop Science*, 5(3): 283-290.

Teixeira, F.G., Hamawaki, O.T., Nogueira, A.P.O., Hamawaki, R.L., Jorge, G.L., Hamawaki, C.L. & Santana, A.J.O. (2017). Genetic parameters and selection of soybean lines based on selection indexes. *Genetics and Molecular Research*, 16(3).

Thakur, S., Bhardwaj, N., & Chahota, R.K. (2017). Evaluation of genetic diversity in ricebean [*Vigna umbellata* (Thunb.) Ohwi and Ohashi] germplasm using SSR markers. *Electronic Journal of Plant Breeding,* 8(2): 674-679.

Tian, J., Isemura, T., Kaga, A., Vaughan, D.A., & Tomooka, N. (2013). Genetic diversity of the rice bean (Vigna umbellata) genepool as assessed by SSR markers. *Genome,* 56(12), 717-727.

Upadhyaya, H.D., Reddy, K.N., Vetriventhan, M., Ahmed, M.I., Krishna, G.M., Reddy, M.T., & Singh, S.K. (2017). Sorghum germplasm from West and Central Africa maintained in the ICRISAT genebank: Status, gaps, and diversity. *The Crop Journal*, 5(6), 518-532.

Valladares, F., Gianoli, E., & Gómez, J.M. (2007). Ecological limits to plant phenotypic plasticity. *New phytologist*, 176(4): 749-763.

Wamalwa, E.N., Muoma, J., & Wekesa, C. (2016). Genetic diversity of cowpea (*Vigna unguiculata* (L.) Walp.) accession in Kenya gene bank based on simple sequence repeat markers. *International journal of genomics*. https://doi.org/10.1155/2016/8956412

Wang, L., Bai, P., Yuan, X., Chen, H., Wang, S., Chen, X., & Cheng, X. (2018). Genetic diversity assessment of a set of introduced mung bean accessions (*Vigna radiata* L.). *The Crop Journal*, 6(2), 207-213.

Wang, L.X., Elbaidouri, M., Abernathy, B., Chen, H.L., Wang, S.H., Lee, S.H. & Cheng, X.Z. (2015). Distribution and analysis of SSR in mung bean (*Vigna radiata* L.) genome based on an SSR-enriched library. *Molecular breeding*, 35(1), 1-10.

Zaccardelli, M., Lupo, F., Piergiovanni, A.R., Laghetti, G., Sonnante, G., Daminati, M.G., Sparvoli, F., & Lioi, L. (2012). Characterization of Italian lentil (Lens culinaris Medik.) germplasm by agronomic traits, biochemical and molecular markers. *Genetic Resources and Crop Evolution,* 59, 727–738. https://doi.org/10.1007/s10722-011-9714-5