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Ex situ conservation of agro-biodiversity of major food legumes in the Philippines

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Abstract. The increase in crop productivity brought about by the Green Revolution has inspired the continuous development and release of more profitable varieties in most crops. The adoption of these varieties however, displaced many traditional cultivars and landraces in production areas. On-farm agro-biodiversity have been threatened and/or lost for decades. To address this, the National Plant Genetics Resources Laboratory (NPGR) collected and conserved a considerable amount of agricultural diversity across the country. Food legumes which include mungbean, peanut, and soybean are among the most important crops conserved at NPGR. The study aims to: i) elucidate the importance of *ex situ* conservation in relation to breeding, selection, and crop production; and ii) characterize and assess the agro-biodiversity of three major food legumes conserved in the National repository in one season. Food legumes were characterized at the Institute of Plant Breeding (IPB) experimental area for one season. Morphological diversity of the accessions was assessed using Shannon-Weaver's Diversity Index (H') for both qualitative and quantitative traits. Based on diversity analyses, mungbean (43 descriptors), has an index (H') ranging from 0 to 0.98 with seed surface luster as the highest. In peanut (42 descriptors), H' ranged from 0 to 0.90 with peg color as the highest. For soybean (41 descriptors), H' ranged from 0 to 0.98 with flower standard color as the highest. The high diversity found in the food legumes accessions indicates their potentials for future use in the legumes breeding programs.

1. Introduction

With the increase in crop productivity through intensive agriculture, monoculture, and introduction of high-yielding crop varieties brought about by the Green revolution, most farmers have replaced or have already abandoned their old cultivars. As a result, agro-biodiversity in the Philippines especially in mainstream agricultural areas have been endangered and/or lost for decades. On-farm conservation which is supposed to be a sustainable and an complementary approach was not developed and recognized on time and is considered as one of the root causes for the loss [1]. The establishment of plant genetic repositories (*ex situ* conservation) in the 20th century has mitigated the threat of biodiversity loss. Globally, over 6 million accessions have been accumulated with more than 90% of these are stored as seeds [2]. The National Plant Genetic Resources Laboratory (NPGR) has



collected and conserved a considerable amount of agricultural diversity across the country through field collections and other forms of germplasm acquisition.

Food legumes, belonging to family Fabaceae, are among the crops conserved in the seed genebank. As of 2014, three of the most important food legumes conserved are mungbean (*Vigna radiata* (L.) R. Wilczek), peanut (*Arachis hypogaea* L.), and soybean (*Glycine max* (L.) Merr.) with total number of accessions of 6684, 1195, and 387, respectively. Various types of food legumes or pulses accounted for 80.3 million hectares of crop area producing 72.3 million metric tons of grain globally in 2011-2013[3]. In 2015, the total area planted with mungbean in the Philippines is 41,400 hectares, producing 33,600 metric tons and valued at 1,827.8 million pesos in 2015. For peanut, the total area planted is 24,600 ha, with 29,200 metric tons produced and valued at 1,176.6 million pesos [4]. In the country, mungbean is used as raw ingredient in major processes such as sprout production, 'sotanghon' (glass noodles) manufacturing, 'hopia' (bean-filled pastry) processing, soup dishes, porridge, snacks, bread, noodles and ice cream. Another important food legume is peanut. This crop also have multiple uses such as snack food, ingredients in recipes, components of solvents and oils, medicines, textile materials, processed into peanut butter and other confections, and as soil fertility amelioration. The third is soybean, which is used as a major protein ingredient in the formulation of livestock and poultry feeds. Locally, it is a raw material of processed foods and food additives like soy sauce, tofu, soymilk, and 'tokwa'. While in other countries, it is processed into soy paste (miso) and soy milk. This study aims to: i) elucidate the importance of *ex situ* conservation in relation to breeding, selection, and crop production; and ii) characterize and assess the agro-biodiversity of three major food legumes conserved in the National repository in one season.

2. Materials and Methods

2.1. Regeneration of accessions

While seed viability in storage is obviously not infinite, seeds from old collections (20-30 years in storage) needs to be rejuvenated. In 2013, a total of 467 accessions from the base collection of food legumes composed of 250 mungbean, 190 peanuts, and 27 soybeans were prepared and sown at the experimental field of the Institute of Plant Breeding (IPB). Two 5-meter rows were allotted for every accession with planting distance of 1m between rows and 0.25m within row. Cultural management practices (e.g. irrigation, tillage, weed control, and disease and pest management) were implemented to maintain the field and ensure sufficient quantity and quality of seeds to be harvested for storage.

2.2 Morphological characterisation

Morphological characterization was done to all accessions of the three species using modified descriptors list based on existing descriptor lists by Bioversity International, International Union for the Protection of New Varieties of Plants (UPOV), and Plant Variety Protection Office (PVPO). Agronomic traits were gathered from plant emergence to maturity. Morphometric traits were recorded from ten sample plants per accession.

2.3 Diversity analyses

Diversity of qualitative and quantitative traits of each food legume species was assessed using the standardized Shannon-Weaver diversity index (H'). Each quantitative trait was categorized into 10 classes based on the mean observation using the Pecetti method of descriptive statistics. H' for each character was computed using:

$$H' = -\sum_{i=1}^n P_i * \log_2(P_i) / \log_2 n \quad (1)$$

where: n = number of classes for a character

p_i = proportion of entries in the i^{th} class

2.4 Harvesting and seed processing

Accessions at maturity were harvested in net bags and labelled accordingly. Pods were initially air-dried, threshed manually, and seeds cleaned using seed blowers. Clean seeds were further dried inside desiccators (air-tight drum containers with Silica gels) to achieve appropriate moisture contents for long term storage (5-7% for peanut and 3-7% for mungbean and soybean).

2.5 Seed Moisture Testing and Storage

Seed moisture determination was done directly by Low constant temperature oven method (17 ± 1 hrs at 103°C), following ISTA rules (2 replicates, each with 4g seeds). After attaining the proper %MC for storage, seeds were contained in hermetically-sealed containers (metalized film packs) and placed in freezers (0°C) for medium to long term storage (base collection) and Convicon chamber (air-conditioned room) for short term storage (active collection).

3. Results and Discussion

A total of 467 accessions were regenerated, characterized, and stored in 2013WS. Shannon-Weaver's diversity analyses for each trait of the three species revealed considerable intra-specific diversity.

3.1. Mungbean

The qualitative and quantitative traits of mungbean (43 descriptors) are illustrated in Figures 1 and 2. For qualitative traits, 13 out of 29 traits (descriptors) have $H' \geq 0.50$ (Figure 1); while 8 out of 14 quantitative traits have $H' \geq 0.50$ (Figure 2). These plant traits are hence considered as moderately to highly diverse.

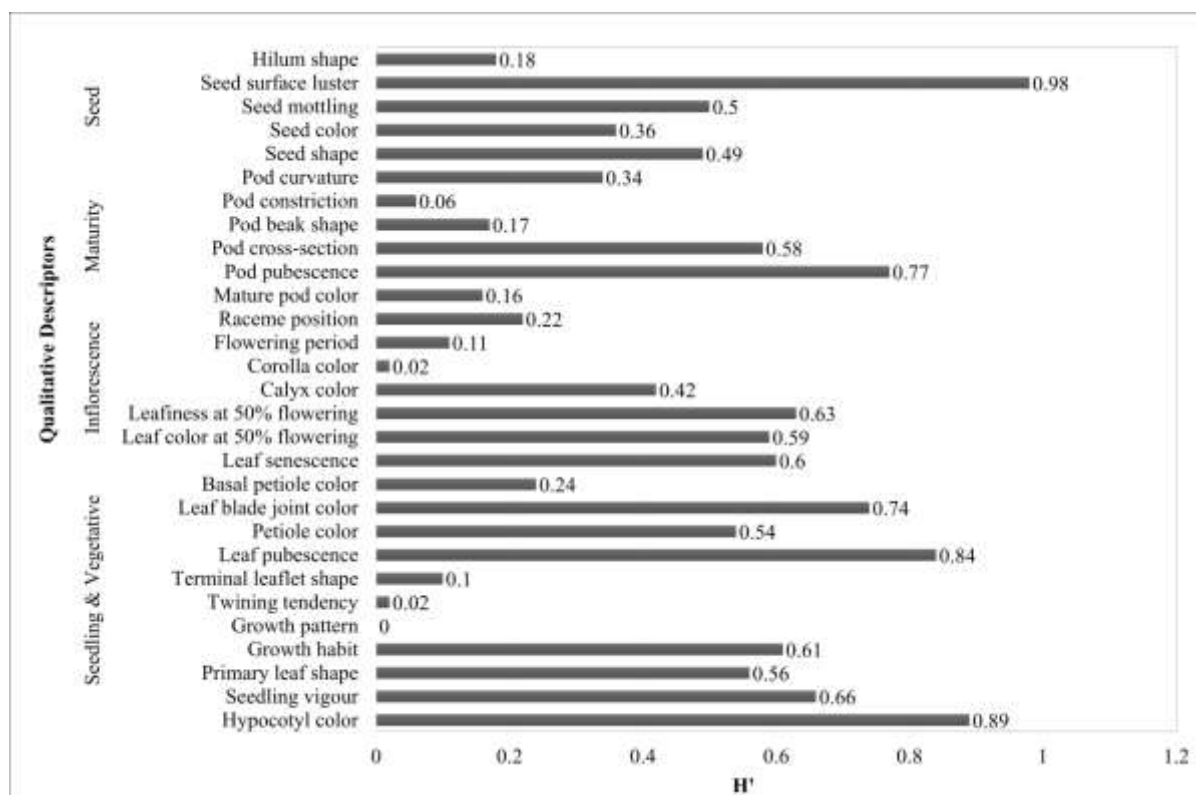


Figure 1. Shannon-Weaver's diversity index (H') for qualitative traits of mungbean.

Among all traits observed for mungbean, seed surface luster has the highest index ($H'=0.98$). High diversity index in seed surface luster is attributed to almost equal number of dull and shiny seed coat

among the accessions (data not shown). The trait is concomitant to the coefficient of static friction of the grain which is the degree of interaction between two surfaces. This coefficient plays its role in transport and packaging of mungbean grains [5]. The coefficient of static friction also showed a positive linear relationship with moisture content [6]. Since seed moisture is critical in seed storage, seed longevity between the stored accessions may significantly vary.

All quantitative traits observed at maturity showed high diversity. The diversity indicates the availability of genetic materials with specific traits preferred by farmers, food processors, and consumers. Pod length ($H'=0.88$) for example is positively correlated to seed yield [7]; suggesting that mungbean accessions with relatively high seed yield is available for use. Days to first mature pods also showed a high degree of variation ($H'=0.63$) which implies the wide range of maturity between accessions. Plant breeders work on developing early maturing varieties to avoid yield-limiting factors like drought, diseases like mungbean yellow mosaic virus (MTMV) and *Cercospora* leaf spot (CLS) [8]. However, ideal large seeds and pods are positively correlated with late maturity [9].

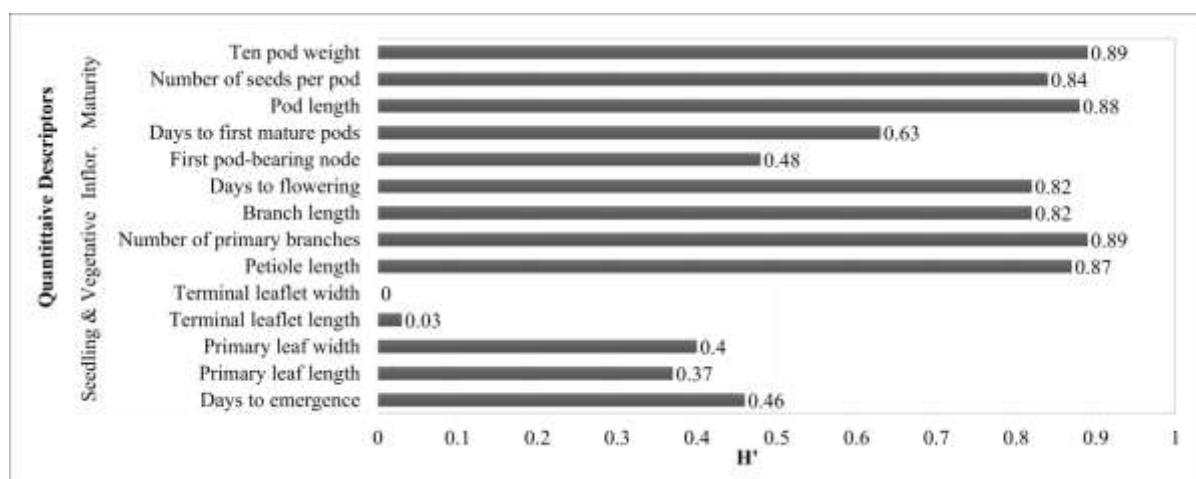


Figure 2. Shannon-Weaver's diversity index (H') for quantitative traits of mungbean.

3.2. Peanut

Diversity analysis for peanut (42 descriptors) is shown in Figures 3 and 4. For qualitative traits, 13 out of 26 have $H' \geq 0.50$ (Figure3); while for quantitative traits, 11 out of 16 have $H' \geq 0.50$ (Figure4). The highest index for peanut is observed in peg color ($H'=0.90$). The peg of peanut is an intercalary meristem extending from the base of the flower, penetrating the soil with embryo at their tips. Peg coloration is due to anthocyanin pigmentation which is cultivar dependent and very much influenced by the environment [10]. Other qualitative traits with high index are standard petal markings ($H'=0.89$), kernel shape ($H'=0.75$), and stem hairiness ($H'=0.73$).

Certain traits are useful in preventing or minimizing the damages from plant pests. The presence of long trichomes for example, makes the plant resistant to serious insect pests such as the leaf hopper and aphids. This trait is reported to be controlled by one or more recessive genes with additive effects [11]. The conserved accessions, therefore, which indicated the presence of trichomes can be used for developing peanut varieties with improved morphological mechanism to insect resistance; it may also be deployed directly in areas with mild to moderate insect infestation. Moreover, peanut varieties with upright growth habits show less disease than those with more leaves in contact with the soil [12]. Plant growth habit is not as diverse as with other traits ($H'=0.24$), however, this is because the number of accessions with erect or semi-erect growth habit outnumbered those with prostrate (data not shown).

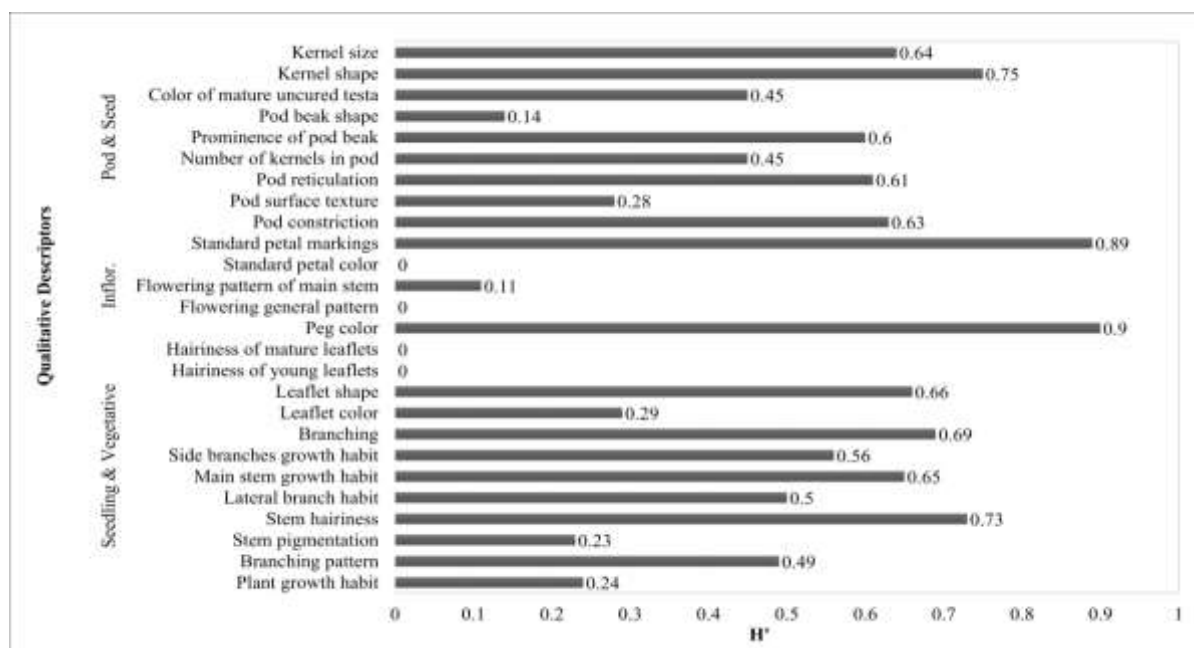


Figure 3. Shannon-Weaver's diversity index (H') for qualitative traits of peanut.

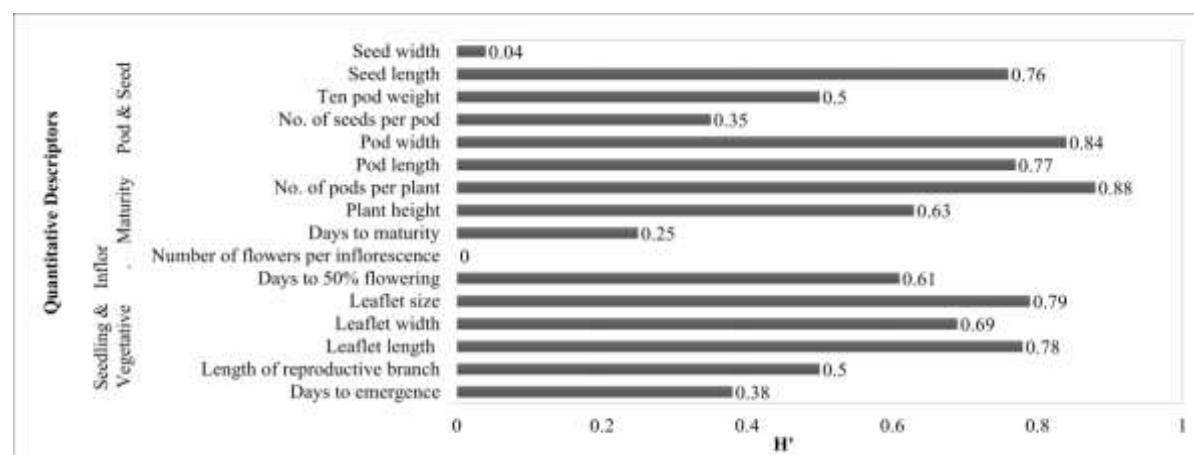


Figure 4. Shannon-Weaver's diversity index (H') for quantitative traits of peanut.

Majority of quantitative traits were highly diverse. The yield of peanut depends on number of mature pods and 100 kernel weights [10]. The Number of pods per plant was computed as highest ($H'=0.88$); while no data was obtained for 100 kernel weight. Other highly diverse traits are pod width ($H'=0.84$), leaflet size ($H'=0.79$) and length ($H'=0.78$), pod length ($H'=0.77$), and seed length ($H'=0.76$). These observations represent the wide range of values for each numeric trait. Most of these traits are economically important.

3.3. Soybean

In soybean, thirteen qualitative traits have $H' \geq 0.50$ out of 28 (Figure 5); and 11 in 13 quantitative traits have $H' \geq 0.50$ (Figure 6). For qualitative traits, the highest index is found in flower standard color with $H'=0.98$. High diversity can be observed from traits in various stages; however, the economically important traits involve seed hilum color ($H'=0.59$), seed size (no data), and seed coat color ($H'=0.28$). As an example, food processors prefer big seeds, yellow or cream seed coat, and

yellow or white hilum [13]. The plant is also a favorite host of aphids. Similar to peanut, morphological mechanism of resistance (i.e. presence of trichomes) will render the plant resistant to the pest. The characterized accessions were observed to have normal to dense pubescence (data not shown), with $H'=0.54$.

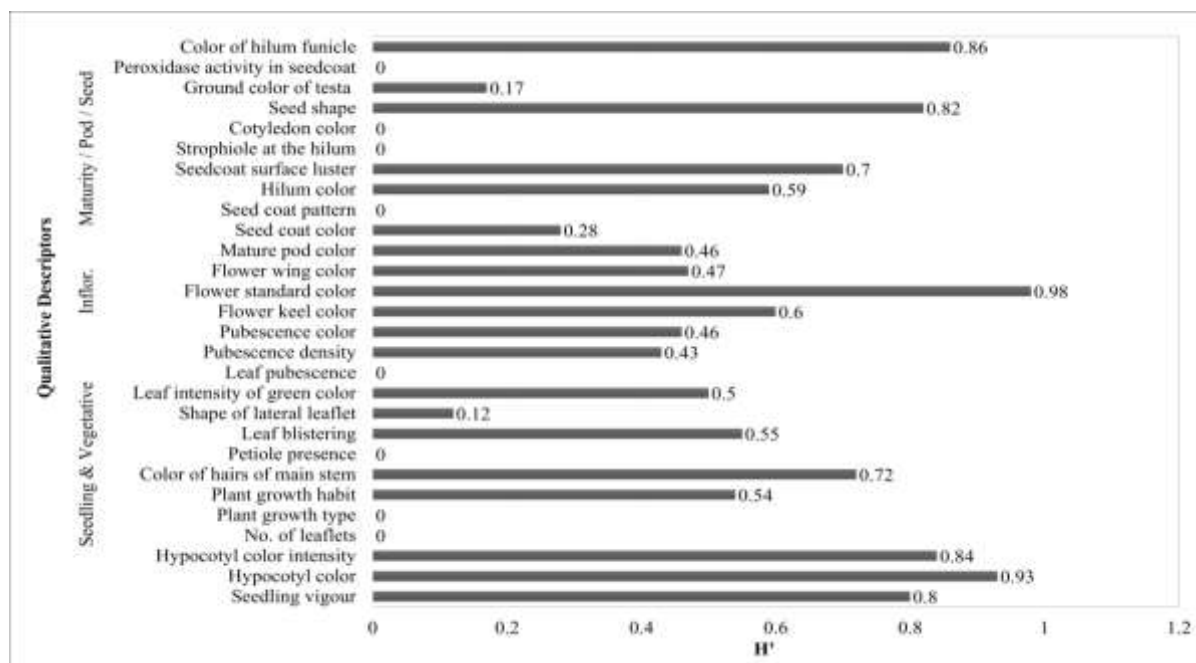


Figure 5. Shannon-Weaver's diversity index (H') for qualitative traits of soybean.

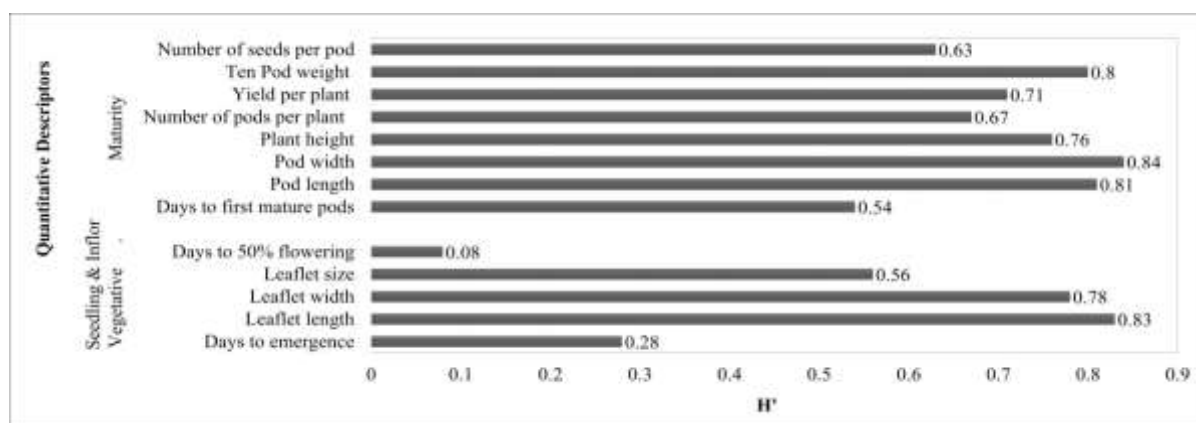


Figure 6. Shannon-Weaver's diversity index (H') for quantitative traits of soybean.

High diversity of numeric traits of soybean was observed particularly at maturity. In many literatures, attributes such as yield, resistance to lodging, and maturity are primarily considered in breeding and selecting the best varieties [14]. As far as yield is concerned, traits like pod length ($H'=0.81$) and width ($H'=0.84$), number of pods per plant ($H'=0.67$), yield per plant ($H'=0.71$), and number of seed per pod ($H'=0.63$) are indispensable. Resistance to lodging may be associated with plant height ($H'=0.76$) and plant maturity on days to first mature pods ($H'=0.54$).

4. Conclusion

Ex-situ conservation of diverse germplasm plays an important role in broadening the genetic background of breeding legume varieties. As well, the conserved accessions may be directly utilized

for production; and accessions with different traits may be tapped for diverse purposes by different end-users. The results indicate a substantial biodiversity of food legumes conserved in the national repository. Furthermore, conserving such agro-biodiversity ensures the availability of legume genetic resources in the future which integrates with the concept of sustainable crop production.

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