

# Morphological Diversity and Germplasm Conservation Strategies of *Phaseolus lunatus* L in East Java

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## 1 **Morphological Diversity and Germplasm Conservation Strategies of Phaseolus lunatus L in East Java**

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### Abstract

**Background/Objectives:** *Phaseolus lunatus L* has a high level of genetic diversity; however, there is a current issue of genetic erosion threat. This study was to characterize *P. lunatus L* (Lima bean) germplasm collections in East Java and evaluate phenotypic diversity to inform effective strategies for in situ and ex situ conservation and utilization of Lima bean breeding. **Methods:** This was a descriptive exploratory research aimed at revealing genetic variation and phylogenetic variants of *Phaseolus lunatus* in East Java based on morphological characters/phenotype. **Findings:** Exploration results of *P. lunatus L* in East Java obtained 15 genotype variants of *P. lunatus L* by morphological characters (qualitative-quantitative characters) that are different on each genotype. Variations between genotype are primarily on the shape, size, and color of the seed coat. Principle Component Analysis (PCA) classified the germplasm into four quadrants; Cluster analysis by Euclidean method drew similar genetic distance of *P. lunatus L* genotype grouping according to the morphometric characteristics. **Applications/Improvements:** Theoretically and practically, this current study resulted in the information data base of genetic variation of *P. lunatus L* in East Java based on morphological characters and the efforts of germplasm conservation strategies by in situ and ex situ and utilization in Lima bean breeding.

**Key words:** *Phaseolus lunatus L*, morphological diversity, Principal Component Analysis (PCA), Euclidean method, In situ/Ex situ conservation strategies

### Introduction

In Indonesia, *Phaseolus lunatus L.* is often named as lima bean, butter bean, koro glinding, kekara, kratok, or roway. It firstly found in Center of America (Mexico and Guatemala) with small seed morphology. In South America, particularly in Peru, *Phaseolus lunatus L.* has bigger seeds. Based on the characteristics derived from the region of origin, *Phaseolus lunatus L.* are classified into two types: Mesoamerican type (Mexico and Guatemala) and Andean type (South America). Andean-type spread limited only in the regions of Ecuador and Peru, while the Mesoamerican type spread almost all over the American continent. Further *Phaseolus lunatus L.* reach the Americas, Europe and then to Asia and was first discovered in the Philippines. It then grew extensively to Myanmar, from Myanmar and then to Java (PROSEA, 1989).

Based on data from IPGRI (2003), more than half of the primary gene pool of *Phaseolus lunatus L.* still has not been collected. It is essential for the deep survey in the centers of its primary diversity (in South America and Central America), also in its secondary centers in Africa, Malagasy, and Southeast Asia (including Indonesia). According to Rural Advancement Foundation International (RAFI), agricultural worldwide has lost three quarters of the genetic diversity in major food crops and this erosion continues at an annual rate of 1-2% (Mazhar 1997). Genetic erosion is the loss or reduction of genetic diversity between and within populations of the same species over time (Jarvis *et.al* 2000), and most of which result from agricultural, economic and social change (FAO, 1996). Genetic erosion is a significant issue in crop domestication areas since them: (a) concentrate the highest genetic diversity; (b) traditional producers conserve ancestral landrace, along with the knowledge and cultural practices that created this diversity; and (c) there exist inter-reproductive wild-weedy-domesticated complexes, favoring wild-domesticated gene flow (Bellon and Taylor 1993; brush 1991).

The conservation and development of genetic resources *Phaseolus lunatus* L. is very important because: 1) *Phaseolus lunatus* L. is a source of germplasm that need to be preserved as a source of biodiversity richness Indonesia. Therefore, conservation and development should be carried out to maintain biodiversity richness, 2) *Phaseolus lunatus* L. have a fairly high content of nutrients, 3) *Phaseolus lunatus* L. is ecologically tolerant of marginal habitat, dry habitat, deep root system, and has a high seed production potential, 4) the information of accessions diversity of *Phaseolus lunatus* L. in Indonesia, especially in East Java are extremely inadequate. The information of genetic diversity is required to support plant-breeding or conservations.

*Phaseolus lunatus* L. or frequently named lima bean or butter bean, was firstly found geographically in Central America with its small morphological seed; whereas *P. lunatus* L. existing in South America has larger seed. In accordance to its geographical origin, taxonomy experts classify this plant from its morphology, ecology, and types of protein content. Based on its origin characteristics, *P. lunatus* L. is divided into two types, Mesoamerican (Mexico and Guatemala) and Andean (South America). The geographical spread of Andean type is limited to several areas such as in Ecuador and Peru; meanwhile, Mesoamerican spreads throughout most of the regions in America continent. Afterwards, the plant was brought and cultivated in Europe and Asia, initiated from the Philippines, to Myanmar, then to Java island<sup>(24)</sup>.

The morphological characteristic is seen to be the most accurate way to determine plant's agronomical trait and its taxonomy (8). Morphological characteristic may be used to identify germplasm collections duplication, genetic diversity estimation study, and correlational study between morphology and other important agronomical traits (12; 12.,;15). One effort to conduct *P. lunatus* L management is conducting diversity identification through its characteristics and morphological level, in order to gain information about population diversity of *P. lunatus* L. Comprehensive identification and characterization of *P. lunatus* L is needed for biodiversity conservation and the development of plant varieties as well as for area conservation as the habitat of *P. lunatus* L. Plant characterization in its morphological level is used to identify its phenotype and change, related to the plant's ecotype (10).

The objective of this study was to characterize Lima bean germplasm collections in East Java and evaluate phenotypic diversity in order to determine strategies for effective *In situ* and *Ex situ* conservation and utilization in Lima bean breeding.

## Materials and Methods

**Plant sample and location observation.** In this study, samples were taken from several regions in East Java, based on the observation and plant survey conducted in Tulungagung, Probolinggo, and Madura on March 2015. The climate off East Java was: dominant type of red mediteran soil and litosol made from limestone and mediteran complex of grumosol, regosol, and litosol. Average rainfall was 1001 – 1500 mm/year, with average temperature of 21-34<sup>o</sup> C.

### 2.1. Research Stages

Morphological observation (quantitative and qualitative characters) was conducted in Biology Laboratory – University of Muhammadiyah Malang. The stages of the observation were as follows:

- a. Field study was done to collect field data, comprising the collection of ripen seeds of *P. lunatus* L from different regions in East Java (Madura, Probolinggo, and Tulungagung).
- b. Morphological observation is a form of qualitative observation (plant type, leaf form, flower petal color, seed pod form, seed form, and seed coat color) as well as quantitative characters (leaf length and width, pod length and width, seed length-width-thickness-weight/gram); it was done in Biology Laboratory of UMM.

- c. The collected seeds were planted in the experiment garden (arboretum) of UMM.

**2.2. Morphological traits:** This study selected plant materials from seed variants of *P.lunatus* L, taken from different places in East Java.

- a. Qualitative character observation: This observation was done by using a guide from “*Handbook on Evaluation of Germplasm*”: “*Characterization of Phaseolus Accesions*” sub-chapter (pg. 29-43) with the descriptors (Table 3.1) comprising: 1) plant type, 2) leaf form, 3) flower, 4) seed pod, 5) seed <sup>(25)</sup>.
- b. Quantitative character observation: The data were collected by conducting quantification/measurement of (repetitive parameter measurement by 10 times): 1) leaf: length, width; 2) seed pod: length, width; 3) seed: length, width, thickness: weight/g.

### 2.3. Data Analysis

Phylogenetic analysis of *P.lunatus* L based on its morphology (quantitative characters) was exercised through quantification of its morphometric characters. Morphometric analysis was performed by the utilization of *SPSS 16 software for windows*. Principal Component Analysis (PCA) was done by PAST program. To validate the result of PCA, the researcher conducted cluster analysis to classify the accessions of *P.lunatus* L based on its 8 distinguishing morphometry characters. Cluster analysis was regulated based on Euclidean distancing method.

## Results and Discussion

### 3.1. Phenotypic Characters of *Phaseolus lunatus* L in East Java

The exploration expedition of *Phaseolus lunatus* L in East Java resulted in the collection of *P. lunatus* variants, especially from Madura with its varied seed coat morphology, shape, and seed size. The exploration found 15 genotypes with different phenotype characters.

The result of variant analysis showed that there was a significant difference among groups/genotypes, observed from the 8 characters based on the measured parameters (weight/100 seeds, thickness, length, seed width, leaf length, leaf width, pod length, and pod width); the variant analysis showed that  $p < 0.01$  which means that there were significant differences in seed, seed pod, as well as leaf. These differences portray variations of genotype from different groups in accordance to their morphological characteristics in seed, pod, and leaf.

From the derived 15 genotypes, in line with Baudet <sup>(1)</sup> and Loi <sup>(9)</sup>, they were then classified into smaller cultigroups with characteristics: (1) Potato, small rounded seed (35.5 g/100 seeds, 9 mm length, 8mm width); (2) Potato-Sieva, (36.3 g/100 seeds, 11mm length, 8 mm width); (3) Sieva, kidney-shaped seed, medium size (30-45.3 g/100 seeds, 12 mm length, 9-10 mm width); (4) Sieva-Big *Lima*, 77.5g/100 seeds, 17 mm length, 11 mm width; and (5) Big *Lima*, big size and shape of seed, (100-110 g/100 seeds, 25 mm length, 14 mm width). Based on these quantitative characters, the subjects showed significantly different variability in potato, Sieve, and Big *lima* groups

For qualitative characters, most of the accessions (70%) have kidney-shaped seed, 30% oval, 6% seeds with cream seed coat color, 50% have dark brown coat color, 20% have blackish coat, 6% maroon, and 12 % with dots and dash ornaments. The shape of the seed pod beak showed 100% short size, 40% medium, with 40% white petals, 30% violet, 30% yellowish, and 100% are vines.



Figure 1. Genotype variations of *Phaseolus lunatus* L in East Java

### 3.2. Phenotypic Character Correlation among *Phaseolus lunatus* L. Seeds in East Java

Ordinal logistic regression analysis was performed to test factors affecting quantitative and/or qualitative characters. Quantitative character has eight tested parameters, while qualitative character goes with seven. Four out of seven characters were taken out since they could not match the given criteria due to high similarity of data, such as similar shape of leaf. Leaf shape of all successions was triangular; therefore, the ordinal logistic regression analysis with SPSS could not be performed. Some other excluded qualitative characters were plant type, color of young pod, and shape of pod beak.

Table 1: Result of Correlation Test of Each Quantitative Character

	Seed length	Seed width	Seed thickness	Seed weight	Leaf length	Leaf width	Pod length	Pod width
Seed Length	1							
Seed Width	0.79	1						
Seed Thickness	-0.03	-0.19	1					
Seed Weight	0.61	0.51	-0.02	1				
Leaf Length	0.3	0.04	0.28	0.23	1			
Leaf Width	0.72	0.56	0.3	0.57	0.3	1		
Pod Length	0.75	0.64	-0.23	0.44	0.25	0.7	1	
Pod Width	0.8	0.6	-0.21	0.44	0.42	0.68	0.9	1

Correlation test of qualitative-quantitative characters portrays: 1) the variables of pod width, leaf length, and pod length influenced the shape of *P. lunatus* L. seed and 2) the variables of seed length and pod length influenced the color of *P. lunatus* L's flower petals. The result of this ordinal logistic regression analysis described the testing of factors affecting the correlation of both qualitative and quantitative characters of *P. lunatus* L plant in East Java, thus, arriving into a prediction that there is a *genetic linkage or pleiotropy* among the groups being tested.

Sax <sup>(14)</sup> discovers the linkage between seed color and seed shape in the population segregation of *Phaseolus vulgaris*. Brittingham <sup>(2)</sup> reports the association between genes controlling the seed coat (qualitative) and its pod length (quantitative), as well as the linkage between the genes controlling the color and the shape of the seed in *Vigna unguilate*. In addition, Sounders <sup>(13)</sup> supports the assertion by finding the association between the color of seed coat (qualitative) and ripening duration (quantitative) within the same species. For this current study, the results suggest that there is a significant association between qualitative and quantitative characters for variables: pod width, leaf length and pod length that influence the seed shape of *P. lunatus* and some other variables such as the seed length and pod length that affect the color of the flower petals of *P. lunatus* L. This association/grouping can be classified into *genetic linkage or pleiotropy* occurrence. Further, a study conducted by Sounders <sup>(13)</sup> suggests the development of plant mapping to confirm and validate these findings. Therefore, seed morphotype is seen as the basic classification of *Lima bean*. Polymorphism of bean coat and the shape of pod beak were further observed as criteria to strengthen the classification. Association can prove that there is co-evolution of bean coat and bean beak shape (qualitative) for *P. lunatus* L./*Lima bean*. Those traits are urgently suggested for the characterization, conservation, and breeding of *Phaseolus* genus. Parental selection for breeding may be derived from this grouping and correlational information of the plant's phenotypic characters. Morphological classification is performed by measuring plant's qualitative and quantitative characters. The parameters are influenced by environment as well as inherited characters from its parent.

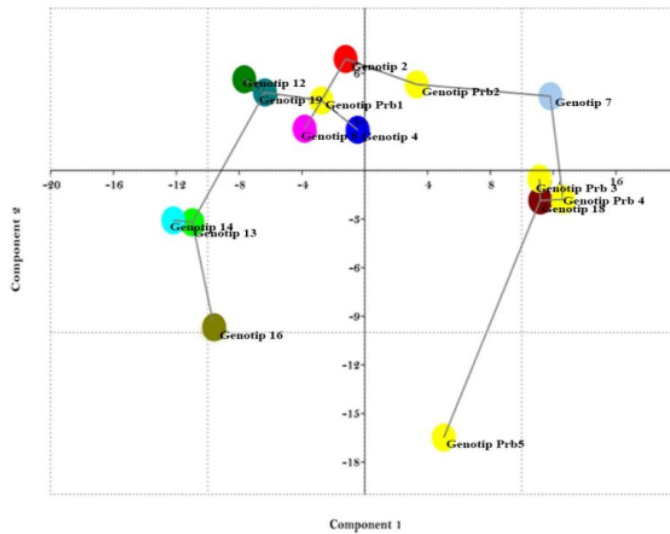
According to Greech and Reits <sup>(22)</sup>, phenotypic variations in one population are significant and used to determine genetic diversity, so that further selection to classify superior plant characters should be done. The more diverse the phenotypes for characters which are not influenced by environment factors, the higher the chance to get superior genotypes. Finger <sup>(5)</sup> shows that genetic resource of *Phaseolus* from wild species (not yet cultivated) could be a choice for variability source in phenotypic characterization. The bean coat color character and the bean shape are the examples of these phenotypic characters.

### 3.3. Cluster Analysis based on Quantitative Characters

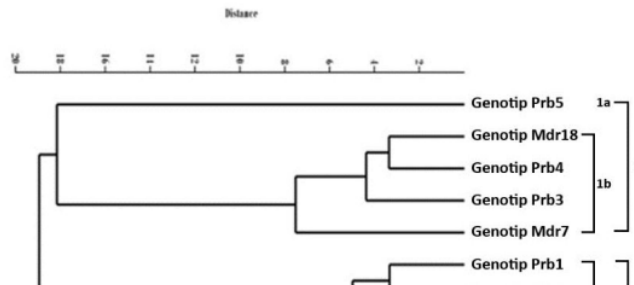
PCA (Principal Component Analysis), that is the grouping of genotypes into four quadrants, portrays the similar morphometrical characters of genotype within one quadrant. PCA results in holistic picture of genetic variation patterns for *P. lunatus* genotypes based on their morphometric characters. Eight scores are derived to identify genotypic classification for East Java area. PCA test results form four genotype classification quadrants, they are: Quadrant 1: Mdr7, Prb2 (bean size medium-big/sieva-*Big Lima*, ); Quadrant 2: Mdr18, Prb3, Prb4, Prb5 (bean size medium-big/sieva, sieve-*Big lima*, *Big lima*, average length, width, thickness are bigger than quadrant 1); Quadrant 3: Mdr13, Mdr14, Mdr16 (sieva-potato, sieva-*Big lima*, bean size is smaller than Quadrant 1 and 2); and Quadrant 4: Mdr2, Mdr4, Mdr8, Mdr12, Mdr19, Prb1 (potato-sieva, sieva-*Big lima*).

The classification of genotypes into four different quadrants is based on the plants' morphometrical variations in seed pod, seed and leaf, as well as seed's morphology. Pod, seed and leaf having similar morphological and morphometrical features are classified into the same quadrant. According to Koinange <sup>(7)</sup>, from the PCA test for *Phaseolus vulgaris*, it can be asserted that plant type and seed size parameters are logically significant to interpret character distribution among the genotypes of Phaseolus, Gepts and Debouck <sup>(21)</sup>, and Duran <sup>(4)</sup> emphasize that the diversification of genotypes into different quadrants has shown their different gene pools among Phaseolus species which have been reported in accordance to their leaf size, seed shape and size, and plant type. Further, morphological characters may also be recommended to observe differentiation in characters, conservation, and breeding processes. Material selection from parents or successors for breeding can be performed based on cluster diversity information and its phylogenetic information related to the plant's phenotypic characters.

Cluster analysis by Euclidean distance method results in 2 main clusters. For main cluster 1: two sub-clusters were formed, in D: 18, 1a sub-cluster consists of 1 Prb5 genotype, the second sub-cluster (1b) in D: 7 classifies the Mdr7, Mdr18, Prb3, Prb4 genotypes. Genotype 18 and genotype Prb4 are located in the genetic distance D: 2.5 from the main cluster, morphometrically the seeds are classified into **medium-large size cultigroup (Sieva-Big lima group)**. In the main cluster 2, the classification of D:13 comprises 2a cluster with: Mdr2, Mdr4, Mdr8, Mdr12, Mdr19, Prb2 genotype from the same group, then 2c cluster with Mdr13, Mdr14, Mdr16 genotypes. In the main cluster 2, the closest genetic distance was shown by Mdr13 and Mdr14 genotypes, (D: 1.5), then D: 3 by Mdr12 and Mdr19 genotypes, Mdr2 and Prb1 genotypes. Cluster 2 displays specific morphometric characters: small-medium sized seed, which, according to Baudet <sup>(1)</sup> is classified into Potato-sieve group. Cluster analysis based on Euclidean method portrays genetic distance similarity in the classification of *P. lunatus* L genotypes in accordance to their morphometric and morphology characters.



**Figure 2: PCA Analysis Result based on Eight Distinguishing Morphological Characters**



**Figure 3: Cluster Analysis Result by Euclidean Distance Method**

The high level of genetic diversity observed in the area of the study could be as the result of farmers' exchanging seeds to be more varied ones. On the other hand, according to CIAT <sup>(6)</sup>, Blair <sup>(2)</sup>, David and Sperling <sup>(7)</sup>, the high level of genetic diversity of *P. lunatus* L. is as the result of diverse planting seed types, and their tendency to breed varieties with superior seed coat quality for the purpose of local market. Therefore, genetic diversity is frequently found and conserved in tropical area. Mutation frequency and genetic recombination could possibly be other aspects of the species high diversity level.

Relevant to the mentioned theories, phenotypic plasticity affects the high level of phenotype diversity in Phaseolus. Phenotype diversity can be observed from one individual in a certain population group, between populations in one species or even among species Sofro <sup>(25)</sup>. The similarity and difference of phenotype accessions in one species can be used as predictor of their family relationship between accessions. Phenotypical characteristics that are genetically controlled will be inherited to the succeeding plant generation; however, environmental factors may affect the expression, although temporary.

Phenotypic plasticity is the ability of individual or genotype to produce more than one morphological alternative; it is a form of physiology or behavior to respond to environmental change. The plasticity reflects phenotypic sensitivity to the environment. Another definition of phenotypic plasticity is a variation of phenotypic expression of a genotype in response to certain environmental conditions and can improve plant's capability to survive and reproduce in the respective environmental conditions. Phenotypic plasticity shows the expression levels of different phenotypes in response to environmental variables. One aspect of phenotypic plasticity can produce different irritability scores when the organisms are in different environment Noor <sup>(19)</sup>.

Davis and Heywood <sup>(8)</sup> suggest that the term phenotypic plasticity can be applied to phenotypic variation in response to environmental changes. The possibility of the similarity of genotypes becomes a common environmental factor that forms different phenotypes. Plasticity extensively occurs on the outside only or not much going on genotypic variability. In fact, the difference between the characters is genetically determined and is less precise due to the environment, because both types are the result of genetically inherited and environmental controls. In other words, each genotype has a range of flexibility or plasticity which is genetically determined. Factors affecting the



phenotype plasticity are clearly of concern to the taxonomist. It is necessary to determine variations that are genetically derived or due to environmental factors that will shape the widespread modification of the same genotype. In fact the similarity in phenotype may be due to genetic modification. This situation is usually ignored by evolutionists as the possibility of non-genetic nature.

### Conclusion

Exploration results of *Phaseolus lunatus L* in East Java showed variants of *P. lunatus L* with the different morphological characters (qualitative-quantitative characters) on each genotype. Variations among genotypes are mainly shown in the shape, size, and color of the seed coat. Complete identification and characterization of *P. lunatus* is necessary for the conservation of germplasm, varieties development, and the spread of *P. lunatus* protection area. Characterization of the morphological level is required primarily for phenotype identification purposes and changes associated with their ecotypes.

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