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Diversity of Local Indonesian Mungbean Germplasm Based on Morphological Quantitative and Qualitative Traits

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Abstract. Mungbean (Vigna radiata L.) is one of an important cash crop legume in Indonesia, especially in dry regions. Identification and evaluation diversity of germplasm collections play important role for mungbean breeding program. The aim of this study was to determine the relationship among mungbean accessions based on quantitative and qualitative traits. A total of 122 local mungbean accessions from Indonesian was obtained from ILETRI germplams collections which have been cultivated in Jambegede Research Station Thirteen variables quantitative and five variables qualitative were observed. Data were analysis using principal component analysis (PCA) and cluster analysis. The results showed that five principal components (PC) contributed 76% of total variation. The most important characters for PC 1 was number of branches, number of fertile nodes per branches, number of pod cluster, and number of filled pod, PC 2 was days 50% to flowering and maturing days, PC 3 was percentage plant affected to root rot diseases, PC 4 was seed weight per plant and plant height, and PC 5 was 100- seed weight. The dendogram clustered 122 accessions into four groups. Based on clusters analysis there were four clusters with similarity distance 72.29%. There was no parallelism between geographical distributions in each cluster.

Keywords: multivariate analysis, mungbean breeding, plant genetic resources, root rot

1. Introduction

Mungbean is one of important cash crop legume in Indonesia, its position in third rank as the most important food crop legumes, after soybean and groundnut. Mungbean has been long cultivated in Indonesia, planting center of mungbean located in Central Java, East Java, West Java, South Sulawesi, West and East Nusa Tenggara [1]. Mungbean has special characteristics, due to its short life (early maturity) and well adapted in drought condition compare to other legume crops. Mostly mungbean utilized as food such as porridge, flour products, beverage products, cakes, noodles, sprouts and a small portion of fodder. Traditional foods made from mungbean are bakpia, onde-onde and tauge.

Mungbean grown in Indonesia probably originated from India, introduced at the beginning of the seventh century coincided with trade and religious relations between India and Indonesia. The development of mungbean planting area is very slow compared to soybean and groundnut. In 1960, mungbean have not been put into agricultural statistical data. In the beginning of 1970s along with improved early-maturing varieties, high yielding, and can be harvested simultaneously, the mungbean total area planted increased rapidly [2].

In the past few decades, rapid economic development in agriculture may have resulted in possible losses of genetic resources. The loss of genetic resources, which sustain the people now widely
recognized to be critical. Endangered or extinction of some species occurred caused by several factors, land used, modern agriculture, availability of instantly foods in market or people consumption pattern and natural disasters. Based on Statistic Indonesia [3], over the last ten years (2003-2013) mungbean harvested area decline by 30.85%, this led the mungbean production decreased 24.18%.

Evaluation of germplasm is useful not only in selection of core collection but it is also important for breeding programs [4]. Future progress in mungbean breeding requires urgent action to identify accessions with favourable agronomic traits for mungbean improvement [5]. The main targets of mungbean breeding in Indonesia are high yield, early maturing, tolerant to main pest and disease, and suitable for food industry (large or small seed size). Broad genetic diversity needed to achieve mungbean breeding purposes. Sarkar and Kundagrami [6] stated that mungbean genotypes exhibited a wide range of variation for most of the investigated traits. Lestari et al. [7] reported that the collection of diverse local cultivar and their sub-sequent genotyping would enhance germplasm diversity and provide information, both of which are beneficial for developing collection strategies and breeding purposes with desirable agromorphological characteristics.

According to Jusup [8], there were three analyses that can be used for classification or grouping germplasm based on the characters or genetics, i.e. cluster analysis, principal component analysis and discriminant analysis. The multivariate techniques, such as cluster analysis and principal component analysis are widely used to estimate various quantitative traits genetic variation. Jeberson et al. [9] stated that cluster analysis techniques were helped to identify the superior genotypes. Lavanya et al [10] agrees that the multivariate techniques, may be an efficient tool in the quantitative estimation of genetic variation to select germplasm in a more systemic and effective way and to develop strategies to incorporate useful diversity in their breeding programs. Musalamah et al. [11] successfully classify 75 genotypes mungbean into 11 major groups. The same thing has been done by Yimram et al. [12] who classify 344 accessions into 5 major and 1 minor cluster. Zubair et al. [13] reported that 40 genotypes can classify into four main groups based on high yield, days to flowering and days to maturity.

The aims of this study was to determine the genetic relationship of 122 mungbean accessions based on quantitative characters and to find out group of accessions using cluster analysis and principal component analysis.

2. Materials and methods

2.1 Plant materials and cultivation

The experiment was conducted at Jambegede Research Station in Malang (335 m above sea level). A total of 122 accessions of mungbean germplasm from different regions in Indonesia were used in this study (table 1). Each accession was planted in a row along 4 m with plant spacing of 50 x 10 cm, and two plants were maintained in every hole. Land preparation was zero-tillage due to the land used was previously cultivated with wetland rice crop. Before planting, the planting plots were irrigated to maintain optimum soil moisture. Weed control was performed at two and four weeks after planting. Water irrigation and weeding was managed based on soil condition. Fertilization was done by adding 50 kg Urea, 75 kg SP36, dan 75 kg KCl/ha at planting time. Insecticides were applied by periodically. Harvesting was undertaken when 80% of the filled pods have already matured.

| Origins            | No of accessions |
|--------------------|------------------|
| Bali               | 16               |
| Central Java       | 16               |
| East Java          | 69               |
| West Nusa Tenggara| 9                |
| South Sulawesi     | 8                |
| Yogyakarta         | 4                |

Table 1. Origins of 122 mungbean accessions used in this study
2.2 Trait measurement
Thirteen variables quantitative (plant height, number of branches per plant, number of fertile nodes per plant, number of fertile nodes branches, number of cluster, filled pods number, days to flowering (50%), days to maturity, 100-seed weight, seed yield per plant, pod length, number of seeds per pod, percentage affected plants to wilt disease) and 5 qualitative characters (hypocotyl colour, mature pod colour, seed colour, seed luster, pod attachment to peduncle) were observed. The data was collected from five randomly samples plants for each characters.

2.3 Data analysis
Principal component analysis (PCA) was done after data were standardized. Cluster and principal component analysis was done by using average linkage method and euclidean distance. The data were analyzed by Minitab ver. 16 programs [14].

3. Results

3.1 Distribution frequency and descriptive statistic
Based on distribution frequency for qualitative traits (table 2), it is known local Indonesian mungbean dominated by purple hypocotyls, black mature pod color, pedant pod attachment to peduncle, mix seed color and luster. Descriptive statistics of 122 mungbean for quantitative traits are summarized in (table 3). High variability is shown for the characters plant affected to root rot disease with a standard deviation 16.98. Moderate variability is shown for the characters plant height, days to flowering, days to maturity, and filled pods number per plant. Less variability can be seen in the characters of seed yield per plant, 100-seed weight, number of branches, number of fertile nodes per plant, number of fertile nodes branches, number of cluster, pod length, and number of seed per pod.

| Table 2. Distribution frequency for qualitative traits |
|-----------------------------------------------|
| Traits                  | Frequency (%) |
|--------------------------|---------------|
| Hypocotyl color          |               |
| Green                    | 12 (9.83)     |
| Purple                   | 110 (90.16)   |
| Mature pod color         |               |
| Black                    | 105 (86.06)   |
| Black brownish           | 9 (7.38)      |
| Brown                    | 7 (5.74)      |
| Straw                    | 1 (0.82)      |
| Seed colour              |               |
| Green                    | 61 (50)       |
| Brown                    | 4 (3.28)      |
| Mix                      | 57 (46.72)    |
| Seed luster              |               |
| Shiny                    | 9 (7.38)      |
| Dull                     | 47 (38.52)    |
| Mix                      | 66 (54.09)    |
| Pod attachment to peduncle|             |
| Pendant                  | 86 (70.49)    |
| Erect                    | 36 (29.51)    |

3.2 Principal component analysis
Five principal components together contributed 76% of total phenotypic variability in this study (table 4). PC-1 with eigenvalue 3.393 contributed 26.1% of total variations, PC-2 with eigenvalue 2.558 contributed 45.8% of total variations, PC-3 with eigenvalue 1.680 contributed 58.7% of total
variations, PC-4 with eigenvalue of 1.237 contributed 68.2% of total variations and PC-5 with eigenvalue 1.013 contributed 76% of total variation. The character which contributed more positively to PC-1 were number of branches per plant, number of fertile branches node per plant, number of cluster and number of seeds per pod. The variance extracted by PC-2 largely resulted from showed days to flowering (50%) and days to maturity. Percentage affected plants to root rot diseases was contributed maximum genetic variation in PC-3. The variance accounted for PC-4 originated from seed yield per plant and plant height. The variance of PC-5 was characterized positively by 100-seed weight.

Table 3. Descriptive statistics for quantitative traits of 122 mungbean accessions

| Characters                        | Minimum | Maximum | Average | StDev |
|----------------------------------|---------|---------|---------|-------|
| Plant height (cm)                | 36.30   | 84.2    | 57.61   | 8.65  |
| Number of cluster                | 3.20    | 17.40   | 5.78    | 1.97  |
| Number of branches per plant     | 0.00    | 3.20    | 1.32    | 0.74  |
| Number of fertile nodes per plant| 1.00    | 6.60    | 2.82    | 0.99  |
| Number of fertile nodes branches | 0.00    | 5.40    | 1.30    | 1.11  |
| Filled pods number per plant     | 5.60    | 30.60   | 14.15   | 4.71  |
| Days to flowering                | 40      | 56      | 37.63   | 2.05  |
| Days to maturity                 | 58      | 78      | 70.52   | 5.52  |
| 100-seed weight (g)              | 2.19    | 7.36    | 4.35    | 0.88  |
| Seed yield per plant (g)         | 1.04    | 6.82    | 2.86    | 1.22  |
| Number of seeds per plant        | 7       | 14.4    | 11.28   | 1.29  |
| Pod length                       | 5.70    | 12.90   | 7.79    | 1.06  |
| % plant affected to wilt disease | 2.63    | 97.14   | 38.52   | 16.98 |

Table 4. Principal components analysis of 122 mungbean accessions

| Quantitative characters | PC1  | PC2  | PC3  | PC4  | PC5  |
|-------------------------|------|------|------|------|------|
| Plant height (cm)       | 0.023| 0.321| -0.315| 0.491| 0.177|
| Number of cluster       | 0.474| 0.018| -0.165| 0.034| 0.015|
| Number of branches per plant | 0.429| 0.072| -0.033| -0.365| 0.052|
| Number of fertile nodes per plant | 0.251| 0.097| 0.196| 0.378| 0.403|
| Number of fertile nodes branches | 0.446| 0.093| 0.055| -0.190| 0.200|
| Filled pods number per plant | 0.460| 0.035| 0.045| 0.106| -0.122|
| Days to flowering       | -0.090| 0.561| -0.017| -0.144| 0.136|
| Days to maturity        | -0.089| 0.543| -0.003| -0.144| 0.202|
| 100-seed weight (g)     | -0.207| -0.265| -0.267| -0.136| 0.647|
| Seed yield per plant (g) | 0.146| -0.262| -0.230| 0.493| 0.012|
| Number of seeds per pod | 0.460| 0.035| 0.045| 0.106| -0.122|
| Pod length              | 0.030| -0.146| -0.622| -0.257| 0.190|
| % plant affected to root rot diseases | 0.176| -0.293| 0.206| -0.243| 0.124|
| Eigenvalue              | 3.3927| 2.5582| 1.6796| 1.2365| 1.0134|
| Proportion              | 0.261| 0.197| 0.129| 0.095| 0.078|
| Cumulative              | 0.261| 0.458| 0.587| 0.682| 0.760|

3.3 Cluster analysis
Based on cluster analysis there were four clusters with similarity distance 72.29% (figure 1). Cluster I was the largest group which consisted of 60 accessions. Cluster II consisted of 45 accessions. Cluster III consisted of 10 accessions and Cluster IV consisted of seven accessions. Among them, cluster III was the most interesting as its member consist of accessions which moderately resistant to root rot diseases.
4. Discussion

Agronomic traits (qualitative and quantitative) are a low level technique but powerful taxonomic tool for the preliminary grouping of germplasm before using marker technologies. Identification of qualitative traits is useful in order to meet consumer preferences. Trustinah et al. [15] reported that each region in Indonesia has a typical preference for utilizing mungbean. Some production centers such as Central Java, East Java, West Nusa Tenggara and South Sulawesi, the dominant consumer characteristic interest are seed color (dull or glossy) and seed size (small or large). In the regions close to the bakery and food and beverage industry, farmers grow large seed mungbean. On the contrary, in the regions near mungbean sprouts industry, farmers grow small seed and green hypocotyl. Some accessions in table 2 consisted of mix seed colour and luster. Local collection of mungbean germplasm obtained from the market or farmers. In several regions such as Nusa Tenggara, some community is still doing local wisdom by planting several mungbean various in one hole. Composition of the mixture from the past original exploration area is still maintained until today. In addition, the position of the pods erect to peduncle is also preferable because it easier for farmers to harvest.

Beside qualitative characters, quantitative characters are also important for breeding purposes. Mungbean breeding purpose in Indonesia is still high seed yield, early maturing, tolerant to main pest and disease and suitable for food industry. According to Hakim [16] the characters of number of pods per plant, pod length, number of seeds per pod and 100-seeds weight have positive phenotypic correlation with seed yield per plant. Gul et al. [17] reported the seed yield was positively correlated with number of pods per plant, yield/ha, and harvest index. Hapsari [18] reported that 100-seeds weight and pod length have high heritability and positively correlated with seed yield/plot. Yimram et al. [12] reported that 100-seed weight, seed weight per plant, plant height and number of pods per plant expressed high genetic variability with moderate to high heritability and expected genetic advance. From these references, we can collect information that number of pods per plant, pod length, and 100-seeds weight characters can be considered to choose superior accession for high yield mungbean breeding purpose. In this study, none of the accessions have these characters simultaneously. However some accessions have an advantage such as MLGV 0444 had long length pod characters (12.90 cm), MLGV 0951 and MLGV 1064 had big seed size (>7g/100 seed), and high number of seed per pod (MLGV 0967, MLGV 0897, and MLGV 0990).

Figure 1. Dendogram of cluster analysis of 122 mungbean accessions
A large number of accessions may lead difficulties to pick up the potential genotypes which representatively it’s phenotypic and originated. According to Gaspersz [19] principal component analysis is useful to data reduction which removes interrelationship among components. Iqbal et al [20] also reported that multivariate analysis techniques, which simultaneously analyze multiple measurements on each individual, are widely used in analysis of genetic diversity irrespective of data set. Based on Table 4, eigenvalue under one is not used to calculate the principal component. The first five principal component with eigenvalue >1 contributed 76% of the total variability among accessions. Several researchers reported that mungbean total variation ranging from 71-85% were assessed as eligible for determining significance of a principal component [11,13,21]. The characters which contributed more positively for the first five principal components respectively are number of branches per plant, number of fertile branches node per plant, number of cluster, number of seeds per pod, days to flowering (50%), days to maturity, percentage plants affected to root rot diseases, seed yield per plant, plant height and 100-seed weight (table 4).

Based on cluster analysis, 122 mungbean accessions were classified into four clusters (figure 1). Each cluster content accessions member with spesific trait. Cluster I was the largest group, entirely constituted by accessions with inferior traits in number of cluster per plant, number of filled pod, and pod length. Cluster II was consisted accessions with short duration types, shorter plant length, lower number seeds per pod, high seed weight per plant. Whereas cluster III was characterized by moderately resistant to root rot accessions, taller plant type and high number of seed per pod. Contrary with cluster III, the members of cluster IV showed susceptible to root rot diseases, early maturing, and high number of filled pod. Cluster III and IV were interesting as its member consist of accessions which had correlation to root rot. Root rot are common in mungbean cultivation, especially in ILETRI research experimental station and the dominant ones are caused by fungal infections of Phytophthora sp., Rhizoctonia sp., Sclerotium rolfsii and Collerotrichum sp. Root rot caused by Rhizoctonia diseases is one of important diseases in mungbean which could infect since seedling and cause plants wilt and die. The study to evaluated of 460 accessions to wilt disease have been conducted in ILETRI since 2004, the result showed that there was no accessions resistant to Rhizoctonia root rot disease up to reproductive stage [22]. Similar result reported by Inayati et al. [23] that evaluation of 91 mungbean accessions were classified as susceptible to Rhizoctonia root rot disease. Resistance to root rot disease is an important character in the improvement of mungbean genotype in the future.

If associated with origin of accessions, not all accessions come from the same area in one group. For example, in cluster I consist of accessions from East Java, Bali, Central Java, South Sulawesi, Yogyakarta and West Nusa Tenggara. The same thing also happened in other clusters, whereas one group consists of various origin of accessions. Arunachalam in Saini et al. [24] reported that mungbean genotypes originating from the same area do not always have in a grouped. It is also reported Bisht et al. [25] and Afzal et al. [26] that there is no association between variations in geographical areas with genetic diversity of mungbean. The result is in agreement with Lestari et al. [7] who reported that the genetic cluster did not reflect the geographical origin of the accessions.

5. Conclusion
The cluster analysis based on 72.29% similarity successfully split 122 accessions into four clusters. Genetic improvement of mungbean can be done by using the gene source of MLGV 0965 and MLGV 0925 (for high seed yield), MLGV 1064 and MLGV 0951 (for large seed size), and MLGV 0886, MLGV 0890, MLGV 0929, MLGV 0661, MLGV 0967, MLGV 0879, and MLGV 0972 (for moderately resistant to root rot disease).

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