

Variation in morphological traits of a selection of Indonesian winged bean accessions (*Psophocarpus tetragonolobus*) and its analysis to assess genetic diversity among accessions

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Abstract. Yulianah I, Waluyo B, Ashari S, Kuswanto. 2020. Variation in morphological traits of a selection of Indonesian winged bean accessions (*Psophocarpus tetragonolobus*) and its analysis to assess genetic diversity among accessions. *Biodiversitas* 21: 2991-3000. In Indonesia, winged bean (*Psophocarpus tetragonolobus* (L.) DC.) is a traditional vegetable crop grown mainly for its edible green pods. Plant breeding programs aim to produce cultivars with high production and good nutritional qualities. The objective of this present study was to assess genetic diversity among 21 selected Indonesian winged bean lines based on observation of morphological characters. This was the first step in determining an appropriate breeding program for the development of improved vegetable cultivars. Twelve qualitative characters and eight quantitative variables were assessed for each of the 21 lines. Categorical differences among lines were observed in characters such as leaflet, pod and seed shape, pod surface texture, anthocyanin pigmentation of stem, flowers and pods. Several of these characters are useful as genetic markers, and cluster analysis of the 21 lines on the basis of qualitative characters enabled two distinct groupings to be identified. Quantitative variation across line means was also high for several of the quantitative variables (a coefficient of variation > 25% for pod length, number of pods per plant, and total pod weight per plant). Principal component analysis applied to the eight variables accounted for 86% of the total variation in just three components with eigenvalues > 1. On Component 1, the characters number of days to first open flower, pod length and pod weight were closely aligned with total weight of pods per plant. Number of pods per plant was not closely aligned with weight of pods per plant. This study has enabled broad differences between groups of lines to be categorized and has identified particular lines with characteristics that recommend them for inclusion as parents in inheritance studies designed to elucidate the contribution that individual characters make to overall productivity, attractiveness, and nutrition of this useful, high protein, vegetable species.

Keywords: genetic diversity, Indonesian, morphological characters, plant breeding, winged bean

INTRODUCTION

Indonesia is recognized as one of the genetic diversity centers for winged bean. However, the area planted to winged bean in Indonesia appears not to have expanded beyond what it was when Thompson and Haryono (1980) wrote their optimistic report. Cultivation still uses traditional low input methods, and currently, winged bean is best considered as a backyard plant, its utilization being limited to household consumption (Handayani 2013).

For the most part, winged bean landraces in Indonesia are facultative perennials. They can be planted both in rainy and dry seasons, with a capacity to produce vegetable pods in low to moderate quantities over several seasons if allowed. Winged beans planted in the rainy season produce more leaf but fewer flowers and pods (Kuswanto et al. 2016). The abundance of leaves during the vegetative phase appears to inhibit the transition to the generative phase, resulting in a long, drawn out harvest time. The time from planting to first harvestable pod in winged bean is often almost twice as long as for other popular leguminous vegetable species; i.e. 13-14 weeks for winged bean, compared to just 8-9 weeks for crops like common bean

(*Phaseolus vulgaris* L.) and yardlong bean (*Vigna unguiculata* L. Walp. subsp. *sesquipedalis* (Eagleton 2019)). Several approaches have been carried out with the aim of increasing winged bean pod and seed production. Throughout the traditional growing areas in tropical Asia and Melanesia, winged beans are indeterminate climbers in growth habit, so one avenue of research has been to attempt to manipulate biomass production by such things as adjustments to trellising support and vegetative pruning with the aim of stimulating flower and pod production. Using two-metre high trellising, ratooning, and multiple harvesting over an extended time, period Wong (1980) and (Motior et al (1998b) have reported obtaining very high green pod yields and seed yields of 35 t/ha and 6.26 t/ha respectively, but the cost of the trellising is high. Another approach has been to use plant breeding methods to generate varieties with modified plant architecture, particularly branching pattern, with a view to maximizing pod set (Tanzi et al. 2019). Approaches using plant breeding have included classification of genetic diversity across the species (Khan 1976; Eagleton et al. 1980; Mohamadali and Madalageri 2015; Handayani et al. 2015); expansion of genetic diversity through the induction of

genetic mutations (Klu et al. 1997); baseline inheritance studies (Erskine and Khan 1977; Erskine 1980; Erskine 1981); hybridisation followed by screening and selection in the segregating generations of crosses (Erskine and Kesavan 1982; Eagleton 1983; Mohamadali and Madalageri, 2004; Kuswanto et al. 2016; identification of disease resistant genotypes (Thompson and Haryono 1979); and genomic sequencing studies (Mukhopadhyay 2000; Sharma et al. 1996).

Plant breeding research on winged bean has been ongoing since the 1970-80's era, but it has to be admitted that there have been few developments that have resulted in significant improvements in winged bean productivity, and the way it is grown and utilized for human consumption. The research task in Indonesia today is for plant breeders to develop superior cultivars of winged bean that combine high productivity of vegetable pods with high nutritional value under relatively low input conditions; the kind of variety that would assist traditional horticulturists produce winged bean not only for their own needs but also enable them to benefit from expanding urban market opportunities.

Genetic diversity within a population is a prerequisite for a successful plant breeding program (Borojevic 1990). Adequate genetic diversity provides opportunities for adaptive genotypic responses to environmental change. The results of research on clover (*Trifolium subterraneum* L.) show that there was a correlation between several agromorphological characters with environmental variables. These can be considered highly adaptive to environmental pressures (Abdi et al. 2020). Furthermore, it provides opportunity for plant breeders to manipulate the gene pool to generate new genotypes expressing desired combinations of traits. Germplasm evaluation studies over many years have recorded morphological variations in a wide range of characters including in leaves, flowers, pods, seeds, stems and tubers (Khan 1975; Erskine and Kesavan 1982; Mohamadali and Madalageri 2004; Fatihah et al. 2012; Mohanty et al. 2013; Handayani et al. 2015).

Principal component analysis, as applied to data obtained from the measurement of a number of observable characteristics on a large collection of plant accessions, is a technique used for objectively reducing the dimensionality of the dataset to a limited number of uncorrelated, new variables. It can be used to identify those particular characteristics that contribute most to the total observed variation within the collection of evaluated accessions. The output from cluster analysis consists of coefficients of similarity or dissimilarity used to measure overall distance between accessions and between identified groups of accessions. Genetic distance information derived from the analysis can be used in decision-making about which accessions to include as parent lines in on-going plant breeding programs. Accessions with a wide distance between them, if included in a breeding program, expand the selection options available for developing new cultivars.

There have been few studies on the genetic diversity of Indonesian winged bean, and none that have attempted to estimate the genetic distance between accessions in germplasm collections.. The aim of this study is to glean

information on genetic variation and distance between the accessions as a preliminary step in the selection of parents for the eventual development of new productive winged bean cultivars.

MATERIALS AND METHOD

Study area

The experiment was carried out between November 2017 and June 2018 in Dadaprejo Village, Junrejo District, Batu City, East Java Province. The field site was located at an altitude of 600 m asl., at longitude 112.572947E and latitude -7.908635S and. The daily temperature at the site ranged between 17°C and 30.8°C. The average humidity ranged from 62% to 94% (BMKG 2018).

Materials

The 21 winged beans (*Psophocarpus tetragonolobus*) lines evaluated in the study (Table 1) derived from a collection of accessions assembled from five provinces of Indonesia (Bengkulu; Central Java; East Java; West Nusa Tenggara; and Southeast Sulawesi). Nineteen of the winged bean accessions originated from farmers and/or communities who plant winged bean in small amounts as a backyard garden crop or in fringing hedges around fields or household compounds. These accessions underwent preliminary screening and selection to identify lines with interesting, potentially useful characteristics indicative of possible underlying genotypic differences (Table 1). Seed from these was multiplied by line-breeding to form 19 of the lines evaluated in this study. The other two lines (UB1.1 and UB 1.2) included in this study came from a collection held in the Faculty of Agriculture at Universitas Brawijaya. Thus the 21 lines evaluated in the study were a sampling of Indonesian winged bean germplasm with wide potential for genotypic variation.

Method

The research method used a randomized block design to evaluate the 21 winged bean lines in three replicate blocks. Each block was 3.6 m x 27 m. Individual plots consisted of beds 1 m wide by 3.6 m in length with an intended plant spacing within the beds of 0.6 m x 1 m. Each plant was provided by thick bamboo poles that are separated from other plants. Bamboo poles height of about 2 m, consisting of three bamboos and tied at the top. To foster germination, seeds were scarified using sandpaper to abrade the back of the seeds and then soaked in warm water for 24 hours. The 21 seed batches were then sown into trays containing a 1: 1 mixture of soil and manure, and grown in the trays for two weeks before planting out into the field at the two trifoliolate leaf stage. The field planting was carried out in the afternoon. Each bed plot consisted of six planting holes with each planting hole filled by a single winged bean seedling. Re-planting has carried out for two weeks to gap-fill any missing planting points. Fertilization was conducted on three occasions: (i) one week after planting (7 DAP), (ii) during flowering, and (iii) during pod formation. At 7 days after planting, the fertilizer applied

was Urea 14 g, SP36 (superphosphate containing 36% P₂O₅) 20 g; and KCl 14 g per plant. At the flowering stage, the dosage of fertilizer was Urea 10 g, SP36 15 g, and KCl 10 g per plant. During pod formation, NPK fertilizer (15: 15: 15) was applied at a rate of 10 g per plant. Irrigation was carried out (by opening a water channel once a week) only when there was no rain. Pests and plant diseases were controlled mechanically if the intensity was low, and using pesticides if the pest attack was moderate to severe.

Observations were made on both quantitative and qualitative characters.

The observed quantitative characters were: (i) days to first flowering (i.e. the number of days from planting to the date when the first open flower appeared in the plant); (ii) days to first green pod harvest (i.e. the number of days from planting until the date when the vegetable pods in a plant were judged to first reach harvestable stage); (iii) pod length based on the mean of ten pods per plant; (iv) pod width, based on the mean of ten pods per plant; (v) the weight of a pod, based on the mean of ten pods per plant; (vi) total number of pods per plant, calculated from the first to the 10th harvest; (vii) total weight of pods per plant; calculated from the number of pods multiplied by the weight of pod; (viii) mean number of seeds per pod; calculated from a random sample of ten pods. The samples used for in measurement were three plants in each replicate plot.

The observed qualitative characters were twelve in number, namely: stem color; leaflet shape; pod color; pod wing color; pod surface texture; pod shape; presence of pod specks; the anthocyanin color intensity in pods; calyx color; corolla color; seed color; and seed shape (see Table 2). These observations on qualitative characters were carried out using the IBPGR (1982) descriptor list for winged bean. In Indonesia, this descriptor list has been published as a modified Test Implementation Guide (PPU) to ensure uniqueness, uniformity, and stability in describing plant characteristics (PVTTP 2014).

Data analysis

For each qualitative character, the number of lines (n) expressing a particular trait as listed in Table 2 was converted into a percentage ($n \times 100/21$).

For quantitative characters, mean plot values were used as input to calculate the minimum, maximum, mean, standard deviation, and coefficient of variation for each line across its three replicate blocks, using the 19th edition of the Genstat software package. In addition, the XLSTAT version 2014 software package was used to construct boxplots for each quantitative character that illustrated in graphical terms the minimum, 1st quartile, median, third quartile, and maximum values across the 21 lines.

Table 1. The origins of the twenty-one winged bean lines evaluated in this study

Winged bean lines	Origin
SKB 1.5	Bengkulu, West Sumatra
PLB 1.1	Brebes, Central Java
PLB 2.3	Brebes, Central Java
KPN 3.2.1	Nganjuk, East Java
BNN 1.1	Nganjuk, East Java
MNN 1.1	Nganjuk, East Java
NSM 2.1	Malang, East Java
CKM 1.1.1	Malang, East Java
KePM 1.2.3	Malang, East Java
SWM 1.1	Malang, East Java
KaPM 2.1	Malang, East Java
MDM 1.2	Malang, East Java
KePM 2.5	Malang, East Java
KePM 2.2	Malang, East Java
DJB 1	Batu, East Java
DJB 2	Batu, East Java
KPJ 1.1.1	Jember, East Java
PTL 2.1	Lombok Utara, West Nusa Tenggara
MML 1.4	Luwu Timur, Southeast Sulawesi
UB 1.1	Plant Breeding Laboratory Collection, Faculty of Agriculture, Univ. Brawijaya, Malang, East Java
UB 1.2	Plant Breeding Laboratory Collection, Faculty of Agriculture, Univ. Brawijaya, Malang, East Java

Table 2. List of observed qualitative characters based on the terminology of the modified (IBPGR 1982) descriptor list incorporated in PVTTP (2014).

Characters	Expression
Stem color	Green (1), Greenish purple (2), Purple (3), Other (4)
Leaflet shape	Ovate (1), Deltoid (2), Ovate lanceolate (3), Lanceolate (4), Long lanceolate (5)
Pod color	Cream (1), Green (2), Pink (3), Purple (4), Other (5)
Color of wing pods	Green (1), Purple (2), Other (3)
Pod surface texture	Smooth (3), Medium (5), Rough (7)
Pod shape	Rectangular (1), Semiflat (2), Flat on side (3), Flat on suture (4)
Presence of pod specks	Absent (1), Present (9)
Anthocyanin color intensity in pods*	None (1), Weak (3), Medium (5), Strong (7), Very strong (9)
Corolla color	White (1), Blue (2), Purplish blue (3), Purple (4), Reddish purple (5)
Calyx color	Green (1), Greenish purple (2), Purple (3), Other (4)
Seed color	Cream (1), Light Brown (2), Brown (3), Purple (4), Black (5), Brown-black (6), Purplish black (7), White (8).
Seed shape	Round (1), Oval (2), Oblong (3)

Note: * indicates additional characters not listed in Descriptors for winged bean (IBPGR 1982) and PVTTP (2014)

A multivariate analysis was then carried out on the data set, employing the Multivariate Statistical Program (MVSP) software package version 3.1. The matrix of pairwise Pearson's correlation coefficients between the eight quantitative variables across the 21 lines provided the input into a principal component analysis (PCA). The PCA was used to determine to what extent each of the eight variables contributed to the total measured variation among the eight variables measured on the 21 lines. Of the eight orthogonal components generated by the analysis, only three components with an eigenvalue >1 were accepted as accounting for significant proportion of the total variation (Mattjik and Sumertajaya 2011). On these three principal components, a character was considered as making a significant contribution if its loading coefficient ≥ 0.6 , (Peres-Neto et al. 2003). The combined data set was then subjected to a cluster analysis using an *agglomerative hierarchical clustering* procedure applied to Euclidean distance and a UPGMA (Unweighted Pair Group Method Using Arithmetic Average) strategy to group the 21 lines and to represent these groupings in the form of dendrograms (Mohammadi and Prasanna 2003).

RESULTS AND DISCUSSION

Observations on qualitative characters

Investigations of the genetic variation within underutilized crop species are indispensable to planning useful breeding strategies to improve them (Cooper et al. 2001). In addition, comprehensive characterization provides a foundation for efficient germplasm conservation that enables future genetic improvement (Fitriana and Susandarini 2019).

Genetically based phenotypic variation in winged bean in Indonesia has been observed among local collections of accessions from several regions. A previous investigation characterized several local accessions of Indonesian winged bean and documented observed variation in several morphological characters e.g. corolla color, appearance of anthocyanin in pods, pod length, and days to first flowering (Permatasari et al. 2018).

In the present study, morphological observations were conducted on 12 qualitative and 8 quantitative characters among 21 Indonesian winged bean lines. The results are summarised in Table 3 for the qualitative characters and in Table 4 for the quantitative characters.

Among the qualitative characters, stem color was predominantly green (19 lines i.e. 90.47%) while greenish-purple and purple colors respectively were observed in just one line each (i.e. 4.76% each). This result is similar to that of Khan (1976) in Papua New Guinea who observed that the basic colors of winged bean stem were either green or purple, with green being the most common. The intensity of purple on the stem in our study varied from purple tinge with green-base to purple. Leaflet shapes were of three types (deltoid 85.71%, ovate-lanceolate 4.76%, and long lanceolate 9.52%). Three pod shapes were found (rectangular 61.90%, semi-flat 33.33%, and flat on the side

4.76%). Pod shape was observed by cross-cutting the pods. Pod surface texture was of two types (smooth 33.33% and medium 66.67%). Pod color was all green (100%). The pod wing color had two types (green 90.48%, and purple 9.52%). The pods had three basic colors i.e. green, pink, and pale yellow. On green pods, purple specks with various degrees of color intensity were observed, to the point that some pods could be considered as uniformly purple in color (Khan 1976). Pods without any anthocyanin were the most common (90.47%) while pods with anthocyanin were found in only two lines (9.52%). Of these two lines, one had pods with weak anthocyanin color intensity (4.76%) while the other line had a moderate anthocyanin color intensity (4.76%) (Figure 1). The pod wing color was generally green with variant variation from light purple to dark purple (Khan 1976). There were four corolla colors: blue (28.57%), purplish-blue (57.14%), purple (9.52%), and reddish-purple (4.76%) (Figure 2). The corolla base-color was purple and blue in which blue was more typically found than purple. In some cases, there were white and bright blue colors. The most common calyx color was green (85.71%), while two lines (9.52%) had greenish-purple calyces, and one line (4.76%) had dark purple calyces. Seed color also varied, namely light brown (9.52%), brown (80.95%), and black (9.52%). Seed shape was predominantly round (18 lines; 85.71%), while three lines had oval-shaped seed (14.29%).

In this study, purple coloration was found in several of our defined characters: calyx color; corolla color; pod specks; pod wing color; and anthocyanin intensity in pods. Some lines had purple or reddish-purple corollas, while the calyx color appeared dark purple. Thompson and Haryono (1980), in their study in Central Java of Indonesian winged bean germplasm, found evidence that dark purple corolla color was related to pigmentation in the calyx, pod specks, and the pod wings. In Papua New Guinea germplasm, Erskine and Khan (1977) carried out genetic analysis of five qualitative characters finding each of them to be strongly influenced by single gene loci; purple stem color was found to be completely dominant over green stem color, purple calyx over green calyx, purple over green pod wing color, purple specks over uniformly green pod color, and rectangular pod shape over flat pod shape.

Furthermore, significant genetic linkage was observed between stem color and calyx color and between wing color and pod specking. In the present study, it was found that there was a correspondence between purple or reddish-purple corolla and the appearance in pods of anthocyanin with weak to moderate intensity, and with purple color in the wings of pods. Two lines that had those character traits were KePM 2.2 and MDM 1.2. It needs to be emphasized that the phenotypic expression of a simple inherited character may differ depending on the genetic background within the particular population in which the character is observed. Even so, qualitative characters such as stem color, leaflet shape, calyx/corolla color, pod shape, and pod surface texture have proved to be very useful, relatively stable, markers for genetic studies (Erskine and Khan 1977).



Figure 1. Anthocyanin color intensity of winged bean pods. A. None; B. Weak; C. Moderate



Figure 2. Color variations of the corollas. A. Blue; B. Purplish blue; C. Purple; D. Reddish purple

Observations on quantitative characters

The results of observations on eight quantitative characters measured on the 21 winged bean lines are presented in Table 4, in which the values for the minimum, maximum, mean, and coefficient of variation for each quantitative character are listed.

On average, the 21 lines took 68 days from the time of planting to reach the first open flower stage. The line PTL 2.1 was the quickest to flower (58 days) while KPJ 1.1 was the slowest (81 days). The average time from planting to first harvestable green pods was 123 days with MML1.4 b being the fastest (99 days) and SKB 1.5 the slowest (151 days). The long time required by winged bean, with its viny plant architecture, to reach flowering and pod maturity has been reported in many studies (Eagleton 2019; Tanzi et al. 2019). Daylength and temperature have significant influences on growth and phenology of winged bean (Schiavianto and Valio 1996). Some Indonesian accessions

adapted to high rainfall and luxuriant growing conditions such as those that occur in Bogor, West Java, have a particularly extended vegetative growth phase before they reach flowering, followed by a long reproductive phase (Eagleton 2019). On the other hand, Erskine (1981) reported Papua New Guinea accessions that reached first open flower in as few as 44 days. In our study, we identified Indonesian lines in which the number of days to first flower was as short as 58 days, compared with the study in Bogor in which no flowering occurred in under 70 days.

Tanzi et al. (2019) have suggested that the interaction between morphology, growth, and yield is a potential target in breeding programs aimed at developing productive winged bean ideotypes. In this study, the line with shortest pod length (13.7 cm), line PLB 1.1 also had the lowest pod weight, 8.05 g/pod. while the line with the longest pods (38.4 cm), line NSM 2.1 also had the highest pod weight

(25.65 g/pod). The average number of pods per plant among the 21 lines ranged from 29 in line DJB 1 to 55 in line SWM 1.1. The total pod weight per plant among the 21 lines ranged from 372.7 g to 1169.5 g, with an overall mean of 673.1 g. The number of seeds per pod ranged from 11 in line PLB 1.1 to 18 in line NSM 2.1, with an average of 14.2 per pod across all 21 lines. For the character pod length, the variation across the 21 winged bean lines appeared to fall into three natural groupings, lines with short pods (8.0 cm - 15.0 cm), those with moderate pod length (15.1 cm - 24.0 cm), and those with long pods (>24.1 cm). Genotype NSM 2.1, with long pods (> 30 cm) and high number of seed per pod (> 15) produced the greatest weight of pods per plant (> 1000g). Kuswanto et al. (2016) have suggested that pod length is a pivotal character in the selection and improvement of winged bean varieties despite its higher yield, the long pods of NSM 2.1 are irregular in shape, thus lowering its aesthetic appeal as a vegetable variety. Careful consideration will need to be given to various pod characteristics in developing vegetable ideotypes for winged bean.

In planning plant breeding programs, a critical issue is an extent to which phenotypic variability in characteristics is a reflection of underlying genotypic variability, and thus is exploitable in genetic terms. One indicator of this underlying genotypic variability, in a germplasm evaluation of the kind undertaken in this study, is the coefficients of variation for the characters assessed (Table 4). Another is the evenness of distribution of this variation across the range for each character best expressed graphically in the form of box plots. Figure 3 presents box-plot graphs for each of the eight quantitative characters, in which the spread of values for each character is depicted in terms of the first quartile, median and third quartile values.

Coefficients of variation (CV) for the two characters number of days to first open flower and for pod width were less than 10%. For number of days to first harvestable green pods, for number of pods per plant, and for number of seed per pod, the CV lay between 13.9% and 20.7%; while for pod length, pod weight, and pod weight per plant CV exceeded 25%.

The box plot analyses give an indication not only of the spread but also the skew in the distribution of the 21 line means for each character. For number of days to first open

flower, for pod length, and for individual pod weight, there were more lines with values below the overall mean than above it. On the other hand, for number of days to first harvestable green pods and for number of pods per plant there were more lines above the mean than below it. Extreme values, such as in pod length for line NSM 2.1, are a cause of skewness, and draw attention to possible exploitable genetic variants.

Table 3. Variations in 12 qualitative characters based on the modified *Psophocarpus tetragonolobus* descriptor list (PVTTP 2014, derived from IBPGR 1982).

Characters	Description	No. of lines	% of lines
Stem color	Green	19	90.47
	Greenish purple	1	4.76
	Purple	1	4.76
Leaflet shape	Deltoid	18	85.71
	Ovate lanceolate	1	4.76
	Long lanceolate	2	9.52
Pod color	Green	21	100.00
Color of wing pods	Green	19	90.48
	Purple	2	9.52
Pod surface texture	Smooth	7	33.33
	Medium	14	66.67
Pod shape	Rectangular	13	61.90
	Semiflat	7	33.33
	Flat on side	1	4.76
Presence of pod specks	Absent	19	90.47
	Present	2	9.52
Anthocyanin color intensity in pods	None	19	90.47
	Weak	1	4.76
	Medium	1	4.76
Corolla color	Blue	6	28.57
	Purplish blue	12	57.14
	Purple	2	9.52
	Reddish purple	1	4.76
Calyx color	Green	18	85.71
	Greenish purple	2	9.52
	Dark purple	1	4.76
Seed color	Light brown	2	9.52
	Brown	17	80.95
	Black	2	9.52
Seed shape	Round	18	85.71
	Oval	3	14.29

Table 4. Minimum, maximum, mean, and coefficient of variation for eight quantitative characters of 21 winged bean (*Psophocarpus tetragonolobus*) lines.

Characters	Code	Min.	Lines	Max.	Lines	Mean	CV (%)
Days to first open flowers	DFF	58	PTL 2.1	81	KPJ 1.1	68	9.5
Days to first harvestable green pods	DHGP	99	MML 1.4	151	SKB 1.5	123	14.1
Pod length (cm)	PL	13.7	PLB 1.1	38.4	NSM 2.1	22.6	27.8
Pod width (cm)	PW	1.30	KPN 3.2.1	1.97	BNN 1.1	1.47	9.6
Individual pod weight (g)	IPW	8.05	PLB 1.1	25.65	NSM 2.1	16.09	20.7
Number of pods per plant	NPP	29	DJB 1	55	SWM 1.1	43	32.1
Total weight of pods per plant (g)	TWPP	372.7	UB 1.1	1169.5	NSM 2.1	673.1	33.4
Number of seeds per pod	NSP	11.1	PLB 1.1	17.6	NSM 2.1	14.2	13.9

Principal components analysis

The results of a principal components analysis (PCA) applied to the quantitative characters, are presented in Table 5. Three components with eigenvalues > 1, accounting in total for 85.57% of the data on the original eight characters, were considered adequate to describe the data and to allow for useful inferences about the structure of the complete data. Component 1, with an eigenvalue of 3.917, accounted for 48.97% of the variation in the original data set. Component 2 with an eigenvalue of 1.574, accounted for an additional 19.67% of the original variability, and component 3 accounted for another 16.9%. Several characters were closely aligned on component 1. These were days to first open flower and days to first harvestable green pod, along with pod length, pod weight, number of seeds per pod and the total weight of pods per plant. On the other hand, the two characters number of pods per plant and pod width, contributed significantly only to component 3, and their contribution was in opposite directions.

According to Afuape et al. (2011), principal component analysis is a technique useful for identifying plant characters that contribute the most to the observed variation in genotypic groups. It has practical applications in exploring germplasm for the identification of parents for possible inclusion in breeding purposes and for inferring hypotheses about the contribution that individual characters might be expected to make to breeding objectives. The results of our principal components analysis appear to be in general agreement with those of Erskine and Kesavan (1982) who found a strong heritable correlation between

pod length and pod weight, but little relationship of these variables with pod number per plant. The results also suggest that the quest for an early flowering winged bean genotype might be associated with a lower accumulative vegetable pod yield within the Javanese environment (Eagleton 2019).

Cluster analysis of winged bean lines

The cluster analysis of multivariate dissimilarity measures between the 21 winged bean lines based on observations of the 12 qualitative characters is shown in Figure 4 in the form of a dendrogram, with Euclidean dissimilarity coefficients ranging from 0 to 9.46. Low dissimilarity coefficients indicate close relationships among lines, and between clusters of lines. Eight lines had dissimilarity coefficients of 0 between them i.e. KaPM 2.1, MML 1.4, KPN 3.2.1, UB 1.2, KPJ 1.1. 1, KePM 1.2.3, BNN 1.1, SKB 1.5. This indicated they were identical in terms of the observed set of qualitative characters. Besides those eight lines, the two lines (PLB 1.1 and PLB 2.3) had a dissimilarity coefficient of 0 between them. The lines PLB 1.1 and PLB 2.3, both originated from Brebes, Central Java.

In the dendrogram in Figure 4, a Euclidean dissimilarity coefficient of 4.8 was used as a reference point in identifying two main clusters among the 21 lines based on qualitative characters. The similarity of characters in lines in clusters and the distinguishing of character between clusters in Table 6.

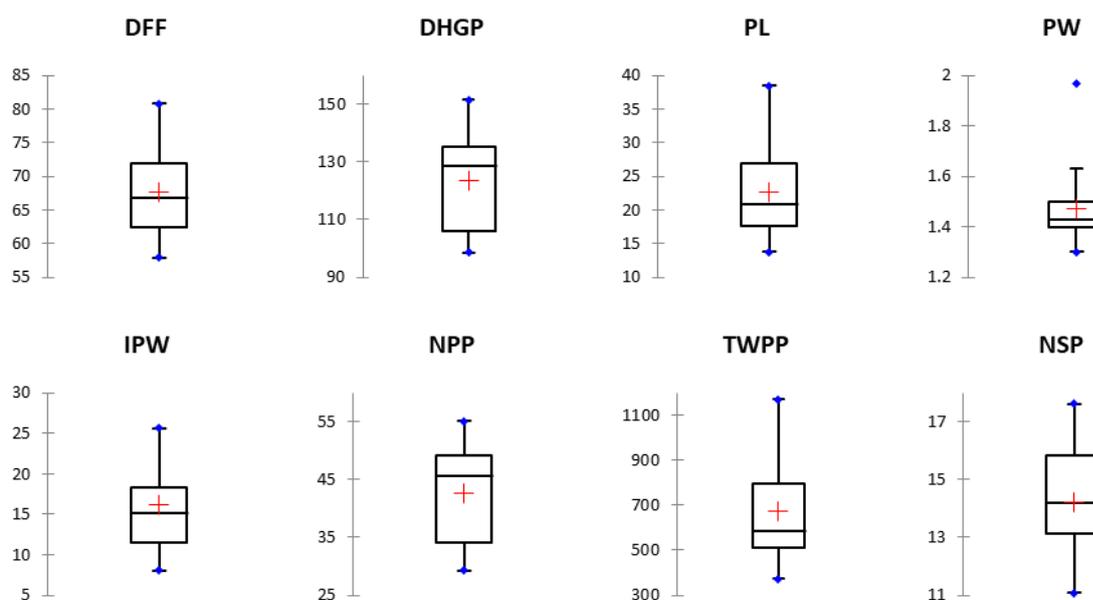


Figure 3. Boxplots of eight quantitative characters measured across 21 winged bean lines: DFF: days to first Flower; DHGP: days to first harvestable green pods; PL: pod length (cm); PW: pod width (cm); IPW: individual pod weight (g); NPP: number of pods per plant; TWPP: total weight of pods per plant (g); NSP: number of seeds per pod. In the box plot, the cross, is the mean value, the blue dots are the minimum and maximum values, the lower edge of the box is the first quartile value, the central line is the median value, and the upper edge of the box is the third quartile. The whiskers are the Tukey limits (lower and upper respectively)

Table 5. The coefficients of loading of quantitative character on the three principal components as the result of PCA

Characters	PC1	PC2	PC3
Days to first flowering	0.728	-0.565	0.233
Days to green pod harvesting	0.684	-0.606	0.137
Pod length	0.827	0.352	-0.329
Pod width	-0.381	0.290	-0.644
Weight per pod	0.917	0.265	-0.156
Number of pod per plant	-0.277	0.529	0.757
Weight of pods per plant	0.735	0.541	0.356
Seed per pod	0.795	0.191	-0.182
Eigenvalue	3.917	1.574	1.354
Variance (%)	48.970	19.670	16.930
Cumulative (%)			85.570

Appropriate breeding methods based on genetic variation and distance

Hybridization between distinctive genotypes followed by judicious selection in the segregating generations is a classical plant breeding method. If the prospective parent genotypes are very different from one another then it is to be expected that there will be a high level of genetic variation among the segregating generations that ensue from a cross between them. In our study, the evaluation of qualitative and quantitative characters on the 21 lines was an important step in determining the genetic diversity among our collection of Indonesian winged bean germplasm and in identifying potential parents for breeding improved cultivars.

Table 6. The similarity of characters in clusters and the distinguishing character between clusters

The similarity of character in cluster 1	Lines in cluster 1	The similarity of character in cluster 2	Lines in cluster 2	The distinguishing of character between clusters
Green stem color	MDM 1.2	Green pod color	NSM 2.1, CKM 1.1, MNN 1.1, PLB 2.3, PLB 1.1, SKB 1.5,	Pod wing color
Deltoid leaflet shape	KePM 2.2	Green pod wing color	BNN 1.1, KePM 1.2, KPJ 1.1.1, UB 1.2, KPN 3.2.1, MML 1.4, KaPM 2.1, KePM 2.5, PTL 2.1, DJB 2, SWM 1.1, DJB 1, UB 1.1	Presence of pod specks
Green pod color		Absence of pod specks		Anthocyanin color intensity in pods
Purple pod wing color				Seed color
Presence of pod specks				
Black seed color				

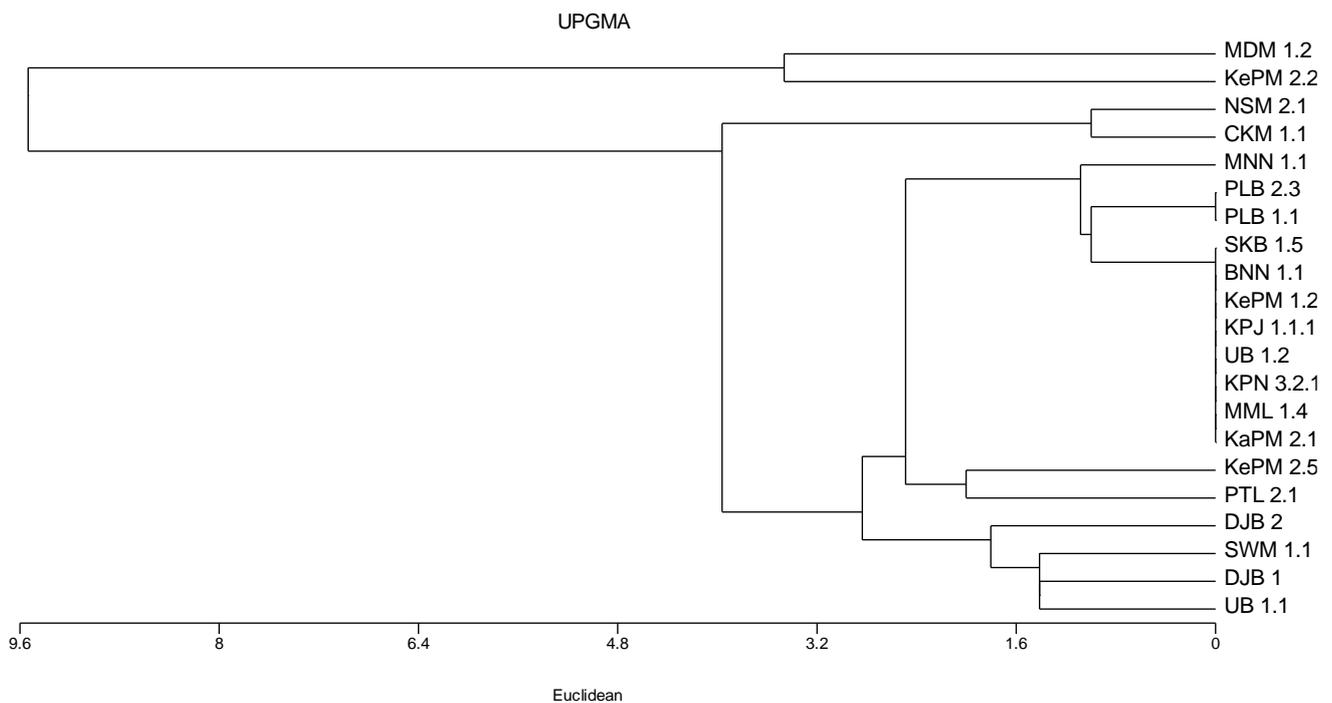


Figure 4. Genetic dendrogram of 21 winged bean genotypes analyzed by *Unweighted pair group method* (UPGMA) based on 12 qualitative characters

Differences in qualitative characteristics in clusters of lines are useful as markers in validating the effectiveness of crosses in a breeding program. In some cases, a particular qualitative characteristic may be linked as a marker to desired characteristics in a commercial cultivar. In other cases, particular qualitative characteristics or combinations of characteristics may be desired in their own right. Thus, for example, the genotype MDM 1.2 could be used as a candidate for introducing anthocyanin color with moderate color intensity, in order to generate novel, purple-winged pods into cultivars. Among the quantitative characters in our study, pod length and weight had high coefficients of variation, with line NSM 2.1 having an exceptional pod length of 38.4 cm (Table 4). Genetic analysis, involving crosses between lines like NSM 2.1 and MDM 1.2, followed by evaluation in F₁, F₂ and perhaps backcross generations, would help to elucidate the extent to which pod length and anthocyanin color are under genetic control and what influence they have on important productive parameters such as total yield of vegetable pods (Erskine and Kesavan 1982).

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