Classifying cassava (*Manihot esculenta* Crantz.) clones based on principal component analysis of specific characters for use as selection criteria

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**Abstract.** There is an abundance of cassava (*Manihot esculenta* Crantz.) genetic resources in Indonesia, and the local accessions are inseparable from the community of Indonesia. Several of the cultivars have cultural significance and over time have been bred for specific uses and products. The specific use and combination of traits encourages the use of local cultivars or aims for genetic improvement of the local cultivars. The objective of this study was to measure character variability and to categorize cassava clones based on specific characteristics to better inform selection criteria. A total of 156 cassava clones collected from all over Indonesia were evaluated along with three clones of the local cultivar Jatinangor as checks. This is basic research, so the data information can be a complement to the cassava germplasm in Indonesia. The experiment was performed as an augmented block design. The variability of characteristics was analyzed using principal components analysis with a Pearson correlation. Grouping of clones was accomplished using a symmetric biplot function. Three first principal components contributed to the maximum variability of cassava by 87.85 %, and characters that contributed variability had factor loadings>0.6. Having variability in characteristics suggests that there is an opportunity for performance-based clone selection. In this study, nine cassava clones with desirable trait combinations were identified based on PCA, of which four were identified as the best performing clones.

1. Introduction
Cassava (*Manihot esculenta* Crantz) is the world’s fourth most important staple crop as designated by the UN FAO, and it is a source of food, animal feed, industrial raw material, and renewable energy. Indonesia has a wealth of cassava germplasm in the form of local cultivars managed traditionally by the community as well as modern elite cultivars released from industry [1]. Cassava production in Indonesia is culturally significant, and each specific cultivar has been selected over time for a specific process and product [2].

Globally, 200 million metric tons of cassava is produced each year, and the demand for cassava in the form of products, especially as flour and starch, is increasing. Starch is used in the food industry, but it also has several commercial uses in the chemical and pharmaceutical industries as well as in...
textiles, paper, and adhesive materials [3]. In developing countries, the demand for cassava is projected to grow 2.0% per year for food and 1.6% per year for feed uses, while, based on current production levels, the total cassava production is estimated to be 256 million tons at 2020 [4].

Starch content is typically 30% in fresh root or 80% in dry product (flour), and this high starch content is what makes it so valuable [5]. For industrial uses, a starch content of 22.5% is standard [6]. Additionally, energy and fuel needs continue to increase, leading to governmental energy substituent programs encouraging the use of cassava in energy production as mineral resources (oil and coal) diminish. Moreover, cassava has potential as a major commodity for producing biofuel, specifically fuel grade ethanol. As market demand increases, there is great opportunity in producing cassava more effectively and efficiently.

Indonesia is considered a secondary center for diversity of cassava. This causes Indonesia to have a variety of cassava; however, farmers do not distinguish specific varieties. In plant breeding, genetic and phenotypic diversity are required for producing improved selections[7]. Parental selection is conducted on accessions that are genetically different, and if such genetic differences come from distinct populations, they may lead to improved hybrid production in plant breeding programs, especially in cassava since it experiences heterosis [8]. Evaluation of germplasm includes detecting potential new traits that can fit into the selection criteria [9].

The methods used in cultivar development programs, and their probability of success, depend on the number of characters to be improved simultaneously [10]. In cassava improvement, the breeder’s primary concern is to increase the root yield of varieties and enhance resistance to major diseases and pests [11, 12]. To determine the highest priority traits, the breeder needs to clearly understand the requirements of all stakeholders, be they farmers, manufactures, or the end users [10].

Indirect selection on traits with higher heritability and strong correlation to morphological and yield characteristics with low heritability is more efficient than direct selection on genetic improvement of desirable traits [13]. Assessing relatedness using correlation coefficient analysis permits breeders to distinguish a significant relationship between characteristics. Indirect selection in early generations via correlated traits has the highest indirect effect on dependent variables. For instance, root number, a trait affecting yield in cassava, is known to be fixed early on during the plant’s breeding cycle [17, 18], which can be used as an early selection criterion. Quality, however, is difficult to evaluate since it is a composite trait of many root characteristics [18]. These characteristics are usually determined by means of statistical procedures like correlation, regression, and path analysis [14].

The genetic resources of cassava in Indonesia are abundant. The geographic condition of Indonesia is a diverse archipelago, which affects the diversity of varieties. Some varieties have local names, but names are not indicative of the genetic background of a variety [15], [16]. The specific use of cassava encourages direct utilization of local cultivars for genetic improvement. Measuring trait variability and identifying cultivars or clones of cassava collected from different regions in Indonesia is essential in order to classify elite germplasm and select specific raw materials. The objective of this study was to evaluate the variability of multiple traits and categorize cassava clones based on specific characteristics in order to improve selection criteria.

2. Materials and Methods
The research was conducted at the Experimental Field Station, Universitas Padjadjaran in Jatinangor, Sumedang, Indonesia, from August 2013 to July 2014. A total of 156 cassava clones were collected from all over Indonesia and used as research materials (Fig.1). The research was carried out using an augmented block design. Three clones of local Jatinangor cultivars, i.e., Perak Raweuy, Peuteuy, and Jalang, were included in each block, so that in each block there were 17 clones and three checks. The check accessions were selected since they are locally adapted and they perform well at the research location.
yield and morphology characteristics were analyzed based on descriptor manuscripts [12, 19, 20, 21, 22, 23]. this study evaluated 23 characteristics, i.e., extent of root peduncle, root constrictions, root shape, external color of storage root, color of root pulp (parenchyma), color of root cortex, cortex ease of peeling, texture of root epidermis, root taste, cortex thickness, postharvest deterioration, average number of roots per plant, average root weight per plant, average weight per root, root weight per plot, concentration of yield, specific gravity, dry matter content, starch content, dry matter content per plant, starch content per plant, concentration of dry matter, and concentration of starch.

trait variability was analyzed by principal component analysis (PCA) with Pearson correlation using Microsoft Excel 2007 / XLSTAT Version 2009.3.02. Principal component analysis is a method used to identify some characteristics and classify variability. The currently used PCA method is a modification of the statistical technique [24]. Principal component analysis widely used in plant science to reduce the number of variables and to group them [25], and principal components (PCs) with an eigenvalue >1.00 are generally accepted as major components [26]. Characteristics with each PC with a value (PC > |0.6|) have a major contribution to the PC [27]. Clones were grouped with a symmetric biplot using the Biplot function.

The Hotelling test was used to specify clones and specific characteristics and, when a research variable consisted of two or more variables, to examine correlation, difference, and effect between two variables or more. The pattern of trait distribution and accessions on a PCA biplot are determined based on a polygon, sector, and center area [28]. The polygon is made by connecting the outermost coordinates of PC1 and PC2. The right angles of each polygon represent the main characteristics contributing to the diversity of a sector. The sector is an area between two lines (0.0) that are perpendicular to the line connecting the two outermost points of the polygon. The central area is characterized by the maximum distance of the ellipse radius from a central point expressed by Hotelling’s test approach [29]:

Figure 1. Indonesia map chart of cassava materials
\[ r_i = \pm \sqrt{\frac{p(n-1)}{(n-p)} F_{p,n-p,a} \frac{s_{pCi}^2}{n}} \]

Where, \( r_i \) = ellipse radius for PC\( i \),
\( p \) = PC components used,
\( n \) = the number of genotypes tested,
\( s_{pCi}^2 \) = variance of PC\( i \) score.

Based on the mathematical equations for the ellipse, the genotype that resides in the region within the ellipse is stable, as follows:

\[ \frac{(PC1)^2}{(r_1)^2} + \frac{(PC2)^2}{(r_2)^2} < 1 \]

3. Results and Discussion

3.1. Principal component analysis

Eigenvectors, variability (%), the first three PCs, and factor loadings were calculated (Table 1). The statistical description of 11 agronomic characters (i.e., shoot weight [young leaves] per plant (g), root number per plant, root weight per plant (kg), individual root weight (g), root yield potential [t/ha], specific gravity, dry matter content [%][fresh basic], starch content [%][fresh basic], shoot yield potential [kg/ha], dry matter yield potential [t/ha], starch yield potential [t/ha]) from 156 cassava clones of all islands in Indonesia (i.e., Java, Madura, Sumatera, Kalimantan, Sulawesi, Maluku, East of Nusa Tenggara, West of Nusa Tenggara, and Papua Islands) were observed.

Table 1. Eigenvalues, variability, the first three principal components (PC), factor loadings, and descriptive statistics of the 11 traits evaluated in 156 cassava clones

| Characters                              | PC1   | PC2   | PC3   | Min    | Max    | Mean   | Std. dev |
|-----------------------------------------|-------|-------|-------|--------|--------|--------|----------|
| Shoot weight (young leaves) per plant (g)| 0.34  | -0.42 | 0.84  | 0.50   | 107.40 | 32.54  | 22.74    |
| Root number per plant                   | 0.63  | -0.15 | 0.11  | 2.00   | 19.00  | 8.59   | 3.37     |
| Root weight per plant (kg)              | 0.95  | -0.15 | -0.19 | 0.18   | 12.80  | 4.04   | 2.32     |
| Individual root weight (g)              | 0.59  | -0.09 | -0.20 | 51.11  | 3530.00| 511.44 | 386.51   |
| Root yield potential (ton/ ha)           | 0.95  | -0.15 | -0.19 | 1.80   | 128.00 | 40.38  | 23.22    |
| Specific gravity                        | 0.19  | 0.95  | 0.23  | 1.01   | 1.00   | 1.13   | 0.08     |
| Dry matter content (%) (fresh basic)     | 0.19  | 0.95  | 0.23  | 17.73  | 127.39 | 37.53  | 12.05    |
| Starch content (%) (fresh basic)         | 0.19  | 0.95  | 0.23  | 6.71   | 84.37  | 20.73  | 8.54     |
| Shoot yield potential (kg/ ha)           | 0.34  | -0.42 | 0.84  | 5.00   | 1074.00| 325.36 | 227.40   |
| Dry matter yield potential (ton/ ha)     | 0.97  | 0.07  | -0.15 | 0.91   | 50.34  | 15.13  | 9.40     |
| Starch yield potential (ton/ ha)         | 0.96  | 0.12  | -0.14 | 0.51   | 28.17  | 8.35   | 5.39     |

The PCA correlation matrix allows for the reduction of dimensionality of interrelated variables [30]. Genetic diversity shown in the PCA is based on eigenvalues, which are equal to the number of variables [31]. Eigenvalues are used to show the dominant number of main components. Furthermore, PCA represents the contribution of the variability of each characteristic to the total variability. Using
an eigenvalue > 1 as the cutoff, there were three PCs that contributed to the maximum variability of cassava by 87.85%.

The first principal component (PC1) had an eigenvalue of 4.76, contributing to 43.26% of the variability. Characteristics contributing to the variability with factor loadings > 0.6 [27] were the number of roots per plant, weight of root per plant, root yield potential, root dry matter yield potential, and starch yield potential. The second principal component (PC2) had an eigenvalue of 3.18, contributing to 28.87% of the variability. Characteristics that contributed to variability of PC2 were specific gravity, dry matter content, and starch content. The third major principal component (PC3) had an eigenvalue of 1.73, contributing to 15.72% of the variability. Characteristics that contributed to PC3 were the shoot weight per plant and the potential yield of shoots or young leaves.

Variability of desired traits indicates that there is opportunity for clone selection based on performance. Selected clones could be used as materials to increase the genetic capacity of a particular character. Genetic capacity can be improve with plant breeding programs and selection is one of stage of plant breeding. If a selected clone has a superior root yield, that clone could be developed into an elite, locally adapted cultivar. Genetic variability can be used as an early parameter in identifying or predicting desirable characteristics of a plant. Evaluation of germplasm will not only identify elite germplasm that will be useful in breeding [10], but also determine clones with a high value of variability [7]. Moreover, identifying genetic variability of accessions collected in different geographic regions is expected to have a significant impact on promoting conservation and utilization of germplasm.

Both PCA and cluster analysis showed a natural grouping of cassava accessions, and the different measurements were correlated with the grouping of accessions. Positive (+) or negative (-) value of a factor indicates a positive or negative correlation between the component and the characteristic [32]. Characteristics with high loading values both positively and negatively contribute more than characteristics with low loading values to genetic variability. Morphological characteristics contributed a relatively low value to diversity even though the clones originated from geographically distinct areas. The genetic variability on _Manihot_ spp. was high, but the diversity within a set geographic area may be low [33]. The Wide range of genetic diversity in plant is explained by several suggested, ie. because generated through centuries of farmer selection, natural hybridisation, introduction processes. The range of genetic diversity in _Manihot_ spp is high, but maybe (possibility) can low depend on geographical region, selection from the local farmer, etc. Which in this case is likely related to the exchange of cassava cuttings among local farmers and the selection of specific desired traits.

### 3.2 Agronomic traits

This research included simultaneous measurements of many variables. Hotelling’s test is a standard tool for inferring the center of a multivariate normal distribution and was used to compensate for the multiple measurements. There were nine cassava clones with desirable trait combinations but only four clones were specifically identified as potentially elite material (Fig.2). Four cassava clones with the most desirable trait combinations were Ubi Kuning (Mentega), Mentega Bogor 4, Singkong Roti in Group 6, and Kefha 2 in Group 3.

The other nine clones were spread into clustered groups with varying levels of identification, as listed in Fig.2. The most prominent clones in Group 1 were Pagar Dewa1 and Baumata1, with the most desirable characteristics related to yield, while clones of Kefha1 and Apuy1 in Group 2 had desirable starch traits, and Kuning2 located in Group 9 had desirable traits related to shoot weight. Differences in shoot and flower traits, which appeared to be significantly different in Kuning2, can be seen from the condition of cassava, which can naturally self-pollinate, open pollinate, and have a proteogenic flower type [34]. The other 143 clones evaluated did not have particularly desirable trait combinations.

Traits related to carbohydrate storage tend to be highly variable in cassava. Among the storage root characteristics, a wide range of variation was observed in dry matter content (24.25%–47%), fresh
storage root weight (0.68–6.84 kg), starch yield (6.98%–18.67%), commercial storage root number (>20%) (1–7) length and storage root number (1.45%–7.89%) [39]. Based on a study of three local Brazilian accessions starch content reaching 21.76% [6]. In Indonesia, the genetic variation for starch content in a Kasesart clone from Lampung where the starch content reached 23.6% [37]. Furthermore, also reported genetic variation for dry matter (37.30%–45.26%) and starch yield (15.04%–24.97%) on a fresh weight basis within accessions [38]. While the concentration of starch in cassava is important, the quality and performance characteristics define the uses for a clone. The original cassava starch from the Malawian cassava genotype is indispensable to the starch industry in Malawi [36].

Cassava roots are an excellent source of carbohydrates. The economic value for cassava products for farmers and industrial uses is based on the dry matter content and yield [22]. High dry matter content is also a principal quality factor used by most farmers and researchers in selecting cassava varieties [39]. Yield of cassava roots is related to root volume and dry matter content. Yield, therefore, can be improved by increasing dry matter content [40]. Furthermore, the study suggested that variability among dry matter content within accessions is important for genetic improvement. Relatively high fresh storage root weight and starch content were recorded from the accessions introduced from IITA. Interestingly, all these accessions are relatively high in starch content coupled with high dry matter content [39]. The clones within this group have high potential for improving starch related traits.

The difference between yields in young cassava leaves is a quantitative characteristic that is highly dependent on environmental and genetic factors, as are many traits [42]. Indirectly, the quality and quantity of young cassava leaves is correlated to the formation of stems and roots. However, some studies have shown that the harvest of leaves can be performed without significantly impacting the growth of cassava roots. The yield of young cassava leaves is highly variable. Predicted yield is calculated based on productivity of young leaves in a unit area. The potential yield of young cassava leaves varies depending on cultivar, age of plant, soil fertility, harvest frequency, and climate [43].

With regards to agronomic traits, there was wide genetic variability. Agronomic traits have to do with phenotypic performance, which is highly affected by the environment. The high genetic variability of cassava in East Africa is linked to environmental adaptability factors to biotic and abiotic stresses, agronomic mechanisms, and post-harvest use [34]. This research is in accordance with their findings in that the accessions collected from several regions across Indonesia had more diverse agronomic traits than morphological characteristics, that this was most likely due to the locally adapted nature of the clones, and that quantitative traits were highly variable. Interestingly, some accessions that were genetically from the same background shared local names across regions, elucidating how some clones may have moved historically. By better understanding the target traits and their heritability, selection efficiency and the selection of elite parental lines can be improved [35]. Furthermore, selected clones can be used to increase yield and more efficiently develop locally adapted accessions with a high yield potential, and simultaneous selection of yield and quality characters could be carried out at earlier stages of selection with the help of simple statistical methods like stepwise regression and principal component analysis [18].

3.3 Biplot of principal component analysis
Cassava clones were grouped using a biplot analysis, and the PCA biplot is asymmetric because it represents the samples and variables of X, while asymmetric form mainly represents covariance or correlation. PCA is essentially directed to reduce dimensionality of a data set consisting of a large number of interrelated variables, while retaining as much variation as possible presented in the data [24]. One particular form of a scaling/ordination plot is called a biplot [44], where both objects and variables are included in a single scaling plot [45]. Usually, the first two components explain the importance of a larger number of variables in the total variation, and the first component is the most important because it has the greatest contribution to the data variation plot. The length of the genotype
vector, which is the distance between a genotype and the biplot origin, measures the difference of the genotype from the average genotype or from any treatments or variables [46].

Figure 2. Biplot grouping of 156 cassava clones based on 11 characteristics. K1 = Shoot weight (young leaves) per plant (g); K2 = Root number per plant; K3 = Root weight per plant (kg); K4 = Individual root weight (g); K5 = Root yield potential (t/ha); K6 = Specific gravity; K7 = Dry matter content (%) (fresh basic); K8 = Starch content (%) (fresh basic); K9 = Shoot yield potential (kg/ha); K10 = Dry matter yield potential (t/ha); and K11 = Starch yield potential (t/ha)

Biplot analysis can be used to estimate correlation among characters with one of the double variable techniques. Biplot analysis also explains the proximity between objects and descriptions of variables, both about its variability and its correlations, as well as the interrelationships between objects and their variables through graphs [47]. The biplot graphic [48], which represents the physical or chemical variables and individuals (genotypes) in a Cartesian plane (or in the three-dimensional space), originated from the PCA [30]. In the biplot depicting the vector length of each genotype, which shows the uniqueness, the relationship to each characteristic being measured is shown. The characteristics in the ellipse region are common characters possessed by each genotype [49]. Verifying how genotypes and variables are grouped is also possible because both positions of an observation are relative to each other and to the nine principal components of each variable for each genotype [30].

Root yield potential, root weight per plant, root number per plant, dry matter yield potential, and starch yield potential in PC1 and character specific gravity, dry matter content (fresh basic), and starch content (fresh basic) in PC2 were the major contributing characteristics, and the PCs indicate their relative importance to root yield [18]. However, yield is a complex trait resulting from the expression and association of different components [9]. PCA also used in the physical, physicochemical, and chemical characterization of true cajá genotypes [50]. They aimed to conduct breeding research and identify industrial-interest materials, and they observed that the skin and pulp mass, total soluble solids and sugars, and industrial yield characteristics were contributed mostly from the PC1 and PC2 components [30].
4. Conclusion

Based on the results of the research, one hundred and fifty-six cassava clones were evaluated and based on 11 characteristics, have a total variability of 87.85% following PCA. Clones were clustered into nine different groups based on specific characteristics. Four elite clones were identified along with an additional potential nine clones that also had desirable trait combinations. This grouping mechanism may result in an opportunity for a breeding program to improve locally available accessions of cassava in Indonesia. Furthermore, it will require producing a specific cultivar for a specific process and product as a source of food, animal feed, industrial raw materials, or renewable energy.

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