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Short Communication: Characterization and nutrient analysis of seed of local cowpea (*Vigna unguiculata*) varieties from Southwest Maluku, Indonesia

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Abstract. *Karuwal RL, Suharsono, Tjahjoleksono A, Hanif N. 2021. Short Communication: Characterization and nutrient analysis of seed of local cowpea (*Vigna unguiculata*) varieties from Southwest Maluku, Indonesia. Biodiversitas 21: 85-91.* Cowpea (*Vigna unguiculata* (L.) Walp.) is a legume species that have many local varieties across regions in Indonesia, including in Southwest Maluku District. It has been utilized by the community as raw materials, but mainly for food sources of self-consumption. While there are rich local varieties of cowpea in Southwest Maluku, the data about its morphological characters and nutritional compositions are not available. The objectives of the research were to analyze morphological characters and nutritional compositions of local seven cowpea varieties from Southwest Maluku and classify these varieties according to such characters and compositions. Morphological characters observed consisted of shape, color, texture, eye pattern, eye color, size (length, width, thickness), and 100-grain weight. Nutritional composition consisted of proximate analysis tested using AOAC (Association of Official Analytical Chemist) method and folic acid content using HPLC (High-Performance Liquid Chromatography) method. The results showed that the varieties have significant variation in morphological characters and nutrition composition. There are differences in shape, color, texture, eye pattern, and color. Seed size in terms of length, width and thickness have range values of 5-9 mm, 4-6 mm, 3-4 mm, respectively while seed weight ranges 11-19 g. Furthermore, nutrition composition is significantly affected by varieties. Moisture content ranges between 11-17%, ash content of 3.13-3.97%, fat content of 0.58-1.42%, protein of 15.5-20.76%, carbohydrate of 58.46-63.48% and folic acid content of 100.13-131.57 µg/mL. There is significant correlation between morphology characters and nutrition composition. Principal Component Analysis (PCA) showed that local varieties can be classified into three clusters. Therefore, KM1, KM3, KM4 and KM7 varieties that have the highest size, weight, and nutrition composition can be selected in plant breeding.

Keywords: Local cowpea, morphology characters, nutritional composition

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is a species of local legume in Indonesia that has been cultivated across the country (Rukmana 2014). It is known to adapt well in dry tropical areas and on land with a porous texture with more than 85% sand and low organic phosphorus content. In addition, this plant has ability to fix nitrogen due to the presence of root nodules so that it brings benefits for soil fertility (Inebome et al. 2014).

One region in Indonesia that has cowpea germplasm is Southwest Maluku District in Maluku Province, Indonesia which is locally known as *kacang merah*. In this area, cowpea has been cultivated on dry land, usually intercropped with corn (Polnaya 2008). Seed of cowpeas is common to be consumed by community together with corn and rice. While cowpea has the great potential to be utilized and developed at large commercial scale as raw material for food products, such as soy and tempeh, the current uses in Southwest Maluku are mainly for self-consumption. This condition is understandable since there is still limited understanding of the basic information of the plant especially cowpea originated from Southwest Maluku District, including the morphological characteristics and the composition of nutrients.

Nutritional composition is very important for commercialization of plants as a food source. From chemical view, nutrients are composed of five main components, namely protein, lipid, carbohydrate, vitamin and mineral. Most legumes are important sources of protein, which is classified as macronutrient, which also applies to cowpea. In addition, cowpea is also a source of vitamins, one of them is folic acid (Saini et al. 2016). Folic acid is classified as a micronutrient that plays an important role in metabolism (Forges et al. 2007).

Previous research on the proximate composition of local cowpea varieties had been carried out in Africa by Owolabi et al. (2012) and reported that the local cowpea had water content of 7.9-9.10%, ash content of 3.38-3.62%, lipid content of 4.6-3.77%, protein content of 19.84-

22.13% and carbohydrate levels of 60.06-63.30%. On the other hand, Rychlik et al. (2007) researched folate levels in dried beans of legumes obtained from several countries reported that folate levels in cowpea ranged from 100-150 µg/100g. The difference in chemical compositions is influenced by processing and cooking, while physical characters are very much determined by the process of seed hydration (Henshaw 2008).

Study on the composition of nutrients in the form of proximate levels and folic acid content of local cowpea varieties in Indonesia is lacking. It, therefore, requires extensive research to fill the gap. This effort is very important since large variety of germplasm in plant breeding programs can help commercial development and utilization purposes. Further, information about the morphological characters and nutritional composition of local cowpea varieties are very much needed to serve as baseline information to screen germplasms with good seed quality. Therefore, this study aims to analyze the morphological characters and nutritional composition of local cowpea varieties in Southwest Maluku and to classify them according to variations in these characters and compositions. The results of this study are expected to obtain local varieties with high seed quality and nutrient composition.

MATERIALS AND METHODS

Plant materials

Seven local varieties of cowpea were used in this study, namely KM1, KM3, KM4, KM6, KM7, KM8, and KM9. Seeds from each local variety were obtained from farmers in Kisar, Southwest Maluku District. For comparison, we also used three cultivars, namely KT1, KT2, and KT7, obtained from Indonesian Legumes and Tuber Crops Research Institute (ILETRI), or locally known as BALITKABI, Malang, East Java, Indonesia.

Morphological observations

Seeds were selected and collected, and then put in waterproof plastic, and stored at 4°C. Observation of characters followed by cowpea descriptors (IBPGR 1983). Morphological characters consisted of qualitative characters, namely, shape, color, texture, eye pattern, and eye color. While quantitative characters included length, width, thickness, hundred weight grain. Observations were made on 10 seeds randomly selected except measuring weight which used 100 seeds.

Nutritional composition analysis

Analysis of nutritional composition focused on proximate analysis and folic acid content. Proximate analyses were carried out following the standard method of AOAC (2000). A total of 20 gram of seed samples was mashed and prepared for the measurement of the proximate composition. Water content was determined by drying in an oven at 105 °C for 24 hours. Ash content was measured by gravimetric, lipid content by soxletation method, protein content with the Kjeldahl method while carbohydrate content was determined by differences in water, ash, lipid, and protein content.

Folic acid content was determined by the HPLC method which modified from the method was used by Ekinci and Kadakal (2005). A total of 50 g of the seeds was mashed, macerated with 70% methanol, and filtered with Whatman No.1. The filtrates were sonicated and evaporated at 40°C until were formed rough extract. Furthermore, the extract was partitioned in stages based on polarity with n-hexane, chloroform, and n-butanol solvents. The butanol fraction was injected into an HPLC machine. Running HPLC used column C_{18} with eluent aquabidest which added tris flour acid 1% and acetonitrile (90: 10) at a flow rate of 0.3 mL/minute for 60 minutes. The detector used UV at a wavelength of 282 nm. The calculation of folate content was based on the folic acid series standard curve from Sigma Aldrich ran in HPLC.

Data analysis

The data were analyzed by ANOVA and continued with the Duncan Multiple Range Test (DMRT) test at a significant level of 5%. Correlation analysis used Pearson Correlation in the SPSS 16.0 program. Grouping local varieties used Principal Component Analysis (PCA) in PAST 3.0 software using clustering K-Mean.

RESULTS AND DISCUSSION

Morphological characters

In general, there are differences in the morphological characteristics of seeds across varieties (Table 1). The seeds shape are square (rhomboid) and kidney (like kidneys) with varying colors and have a smooth texture and different eye colors and patterns. The result of seed characterization is in line with the research of Adewale et al. (2011). Seed color determines acceptance by consumers (Mustapha 2008) and this is in accordance with the exploration of Polnaya (2008), which found that the community in Southwest Maluku rarely planted KM7 varieties. This is likely caused by the less attractive color and influences its appearance when served for food source. The seed stature of each variety can be seen in Figure 1.

The statistical analysis result shows that the length, width, thickness, and weight of 100 grains are influenced by the variety ($p <0.05$). Each variety has a significant difference in seed size (Figure 2). The highest seed length and seed thickness is found in KM4 variety while the biggest seed width is obtained in KM6 variety. Furthermore, KM3 variety has the highest weight of 100 grains. When compared with cultivars from ILETRI, the local varieties have higher seed size and weight. This finding is in line with Henshaw's (2008) study that the size of cowpea seeds ranged from 6-10 mm, 4-7 mm, 3-5 mm respectively for the length, width and thickness of the seeds. Seed weight ranges from 11-26 g. On the other hand, Ezeaku et al. (2015) explained that seed size is inherited and is less influenced by environmental factors.

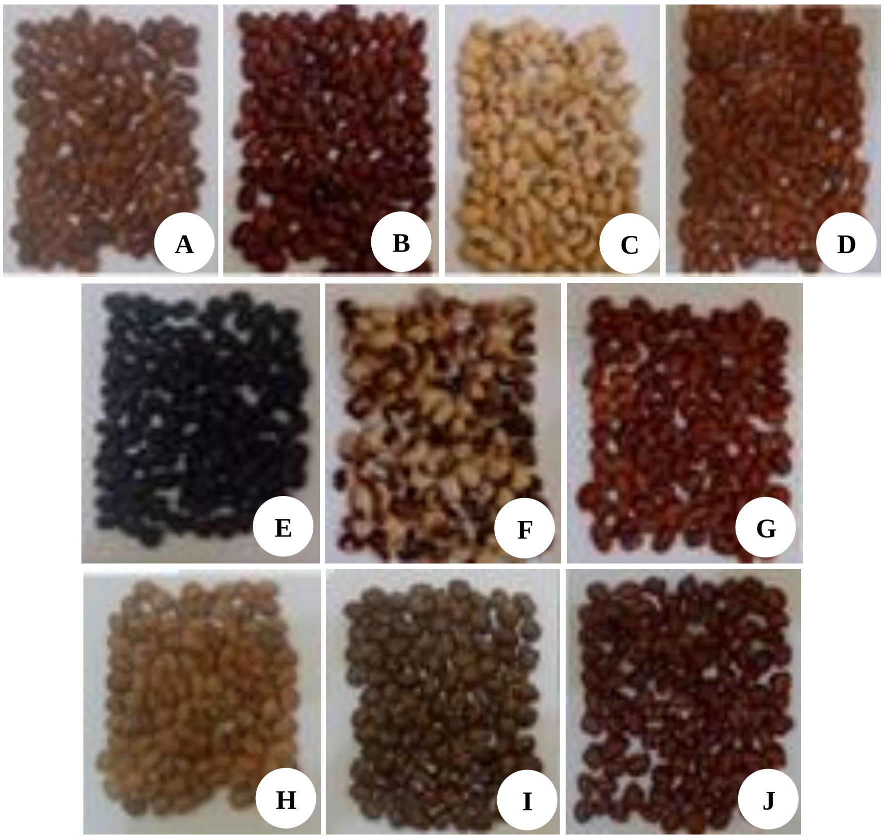

Figure 1. Stature of seed of local cowpea varieties in Southwest Maluku, Indonesia and cultivars from ILETRI. A. KM1, B. KM3, C. KM4, D. KM6, E. KM7, F. KM8, G. KM9, H. KT1, I. KT2, J. KT7

Table 1. Morphological characters of seed of local cowpea varieties in Southwest Maluku, Indonesia and cultivars from ILETRI

Varieties	Seed shape	Seed color	Seed texture	Eye pattern	Eye color
KM1	Rhomboid	Batik	Smooth	Very small	White
KM3	Kidney	Red	Smooth	Holstein	Red
KM4	Kidney	White	Smooth	Holstein	Black
KM6	Rhomboid	Brown	Smooth	Very small	Grey
KM7	Rhomboid	Black	Smooth	Very small	Black
KM8	Kidney	Red striped	Smooth	Very small	Black
KM9	Kidney	Yellowish-brown	Smooth	Very small	Grey
KT1	Rhomboid	Brown	Smooth	Holstein	Brown
KT2	Rhomboid	Gray-brown	Smooth	Holstein	Black
KT7	Rhomboid	Red	Smooth	Small	Red

Nutritional composition

Nutritional composition consists of proximate content and folic acid content. Table 2 shows there are differences in proximate content across varieties ($p < 0.05$). Proximate levels include water content ranging from 11-17%, ash content which is 3.13-3.97%, 0.58-1.42% in lipid content, 15.5-20.76% in protein content, and 58.46-63.48% in carbohydrate content. The highest moisture and ash content is found in KM8 variety while the lowest is KM1 and KM7. Conversely, KM7 variety has the highest lipid and protein content. The lowest lipid content is found in KM3 and protein content is in KM8 variety. The highest carbohydrate is obtained in KM1 variety and the lowest is found in KM7 variety. In addition, folic acid content of cowpea based on seed color ranged from 100.13-131.57 μg/mL (Figure 3). Similar to carbohydrate content, KM1 variety has the highest folic acid content and KM7 variety has the lowest. When compared with cultivars from ILETRI, the local varieties from Southwest Maluku have higher water, ash, lipid content, and lower protein content. This is in line with the research of Owolabi et al. (2012) that superior varieties have usually a higher protein content than local varieties.

Table 2. Proximate analysis of seed of local cowpea varieties in Southwest Maluku, Indonesia and cultivars from ILETRI

Varieties	WC (%)	AC (%)	LC (%)	PC (%)	CC (%)
KM1	13.79[e]	3.22[d]	0.97[cd]	18.54[d]	63.48[a]
KM3	16.1b[c]	3.19[d]	0.58[e]	17.95[d]	62.19[b]
KM4	17.43[a]	3.17[d]	1.1[c]	19.3[c]	59.01[d]
KM6	15.9[c]	3.33[cd]	1.31[b]	20.76[b]	58.71[d]
KM7	16.25[b]	3.13[d]	1.42[b]	20.76[b]	58.46[d]
KM8	17.47[a]	3.66[b]	1.11[c]	15.5[e]	62.28[b]
KM9	15.36[d]	3.23[d]	0.92[d]	19.46[c]	61.04[c]
KT1	10.7[h]	3.52[bc]	2.03[a]	21.03[b]	62.73[b]
KT2	11.05[g]	3.97[a]	1.89[a]	20.74[b]	62.36[b]
KT7	11.43[f]	3.62[b]	0.86[d]	22.84[a]	61.26[c]
Mean	14.55	3.40	1.22	19.69	61.15

Note: Different letters in the same column show significant differences (α=0.05). WC: water content; AC: ash content; LC: lipid content; PC: protein content; CC: carbohydrate content

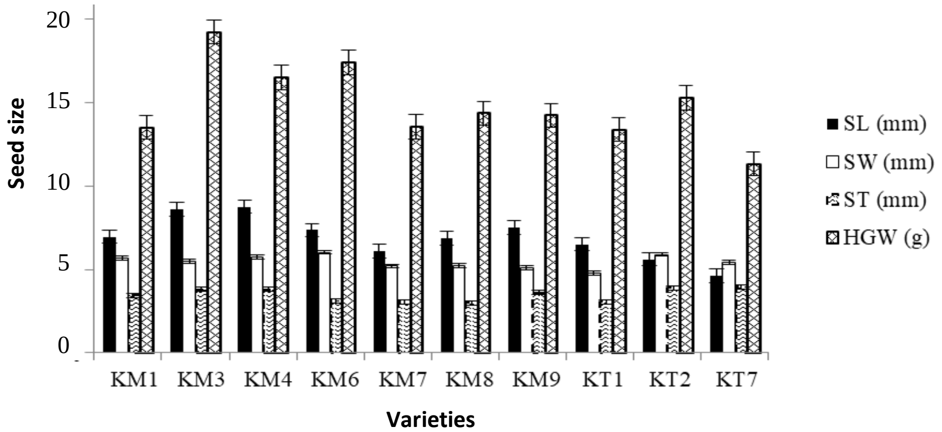

Figure 2. Morphological characters of cowpea varieties used in this study. SL: seed length; SW: seed width; ST: seed thickness; HGW: 100-grain weight

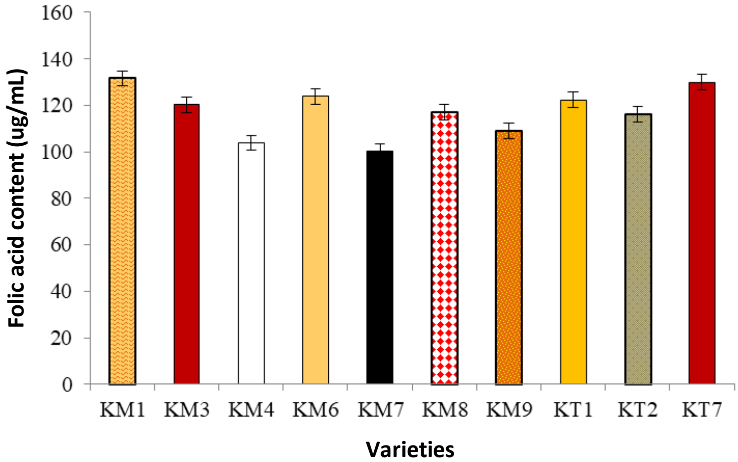

Figure 3. Folic acid content of seed of local cowpea varieties in Southwest Maluku, Indonesia and cultivars from ILETRI based on seed color

The carbohydrate content in this study is in line with the research of Owolabi et al. (2012), namely 56.24-63.30% for local varieties and superior varieties. Ash and fat contents are almost the same as Henshaw's (2008) study, which are 3-4% and 0.6-1%, respectively. The results showed that the water content ranged from 13-17% while the protein content was between 15-20%. This is different from the results of previous research by Animasaun et al. (2015) which stated that of, among others water content ranges from 8-10% and protein content is 22-26%. Furthermore, folate levels are in line with the research by Rhyclik et al. (2007) which stated that folate levels in dried beans of cowpea ranged from 100-150 µg/100 g. Based on the results of the study, it is shown that protein content was inversely proportional to carbohydrate content. This is in line with what Nielson et al. (1993).

Nutritional composition is one of the important characters in the development of superior varieties. The difference in proximate levels can be affected by plant varieties and the environmental factors where the plant is grown, including soil and climate (Henshaw 2008). In our study, the environmental conditions of the local varieties are different from the cultivars from ILETRI. Southwest Maluku District is located in the eastern part of Indonesia which has drier climate with low rainfall and humidity, and high temperatures (Susanto & Sirappa 2005). In addition, the cultivation technology applied by the farmers in Southwest Maluku is still simple with no use of fertilizers in their intercropping system (Polnaya 2008). In contrast, ILETRI Malang area has environmental conditions with high rainfall, low temperatures, and moderate organic content, and they applied mulch and fertilizer in its cultivation (ILETRI 2017). As such, it is understandable that these factors affected the proximate level.

In addition, the differences in levels and distribution of folate in plants are strongly influenced by physiological mechanisms and their balance in plant cell organelle compartments (Cossin 2000). The pathway of folate synthesis consists of three mechanisms, namely the conversion of guanosine triphosphate (GTP) to pterine in the cytosol, conversion of chorismate to amino deoxychorismate (ADC) and esterification to p-ABA (p-aminobenzoate) in plastids, condensation, glutamylation and reduction of pterin and p-ABA forms folate in mitochondria (Basset et al. 2005).

Thus, GTP and chorismate determine the level of folate. GTP is the product of the Krebs cycle in respiration originating from pyruvate, while chorismate is derived from the shikimate pathway (Nelson 2004). On the other hand, Basset et al. (2004) explained that the hydroxyl group of chorismate is replaced with an amino group to form the ADC. This can occur if there is a physiologically high concentration of NH_3. Furthermore, nitrogen group compounds also play an important role in folate synthesis. In legumes, nitrogen compounds can be fixed to NH_3^+ by fixing bacteria in root nodules and greatly affect plant growth and productivity (Schmidt et al. 2014). The role of soil microbes will be in effective when chemical fertilizers are used (Dar et al. 2018).

Correlation between morphological characters and nutrient composition

The results of the correlation analysis in Table 3 show that there is a correlation between morphological characters and nutrient composition. Positive correlations are found between water content and seed length (r = 0.68), ash and lipid content (r = 0.47), lipid content and seed shape (r = 0.55), protein content and seed shape (r = 0.69). In addition, seed color is correlated with carbohydrate content and folic acid content with a correlation coefficient of 0.74 and 0.50, respectively. The 100-grain weight is also correlated very significantly with seed length (r = 0.73), while seed thickness is significantly correlated with eye pattern (r = 0.57). Figure 4 shows a linear correlation between folic acid content and seed color with y = 3.0173x + 106.82.

Conversely, a negative correlation is found between water and ash content, protein content, and seed shape with a correlation coefficient of 0.58, 0.64, 0.67, respectively. Furthermore, there is correlation between water content and seed length (r = 0.59), lipid content and seed color (r = 0.45), protein content and seed length (r = 0.56) and seed length and seed shape (r = 0.69).

Cluster of cowpea varieties

Principal components analysis of the morphological characters and nutritional composition showed that the percentage variance in the first main component is 31.57% and in the second main component is 20.46% (Figure 5). Clustering using K-Mean classifies cowpea varieties in four clusters. Cultivars from ILETRI are clustered in one cluster whereas local varieties split into three clusters. The KM 1 variety form their own cluster based on carbohydrate content, folic acid content, seed color, and seed thickness. The KM3, KM8, and KM9 varieties are grouped in the second cluster based on the length and width of the seeds, 100-grain weight, and water content. The third cluster consists of KM 4, KM6, and KM7 varieties based on water content and eye color.

Table 3. The matrix of correlation between characters in cowpea varieties

	WC	AC	LC	PC	CC	FAC	SL	SW	ST	HGW	SC	SS	EP
AC	-0.58**												
LC	-0.51*	0.47*											
PC	-0.64**	0.15	0.35										
CC	-0.5*	0.39	0.02	-0.31									
FAC	-0.37	0.27	-0.08	0.07	0.42								
SL	0.68**	-0.59**	-0.36	-0.56*	-0.17	-0.19							
SW	-0.28	0.14	0.39	0.23	0.03	0.31	0.14						
ST	-0.25	0.12	-0.35	0.26	0.14	0.09	-0.01	0.12					
HGW	0.48*	-0.31	-0.22	-0.37	-0.17	-0.19	0.73**	0.32	0.12				
SC	-0.13	0.17	-0.45*	-0.42	0.74**	0.50*	-0.17	-0.16	0.08	-0.19			
SS	-0.67**	0.27	0.55*	0.69**	0.01	0.29	-0.69**	0.38	-0.17	-0.43	0		
EP	-0.44	0.3	0.22	0.28	0.21	0.04	0.04	0.34	0.57**	0.2	-0.26	-0.04	
EC	0.21	0.31	0.2	-0.13	-0.26	-0.43	-0.11	-0.15	0.09	0.05	-0.37	-0.22	0.34

Note: **: very significance correlation; *: significance correlation; WC: water conten, AC: ash content, LC: lipid content, PC: protein content, CC: carbohydrate content, FAC: folic acid content, SL: seed length, SW: seed width; ST: seed thickness; HGW: 100-grain weight, SC: seed color, SS: seed shape, EP: eye pattern, EC: eye color

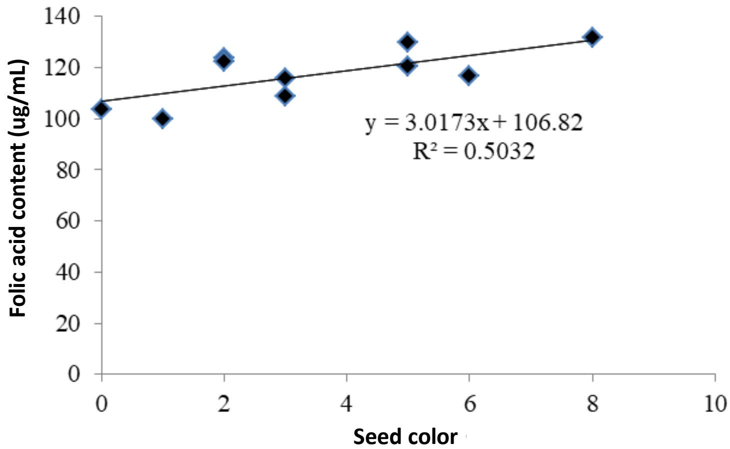

Figure 4. Correlation between folic acid content and seed color

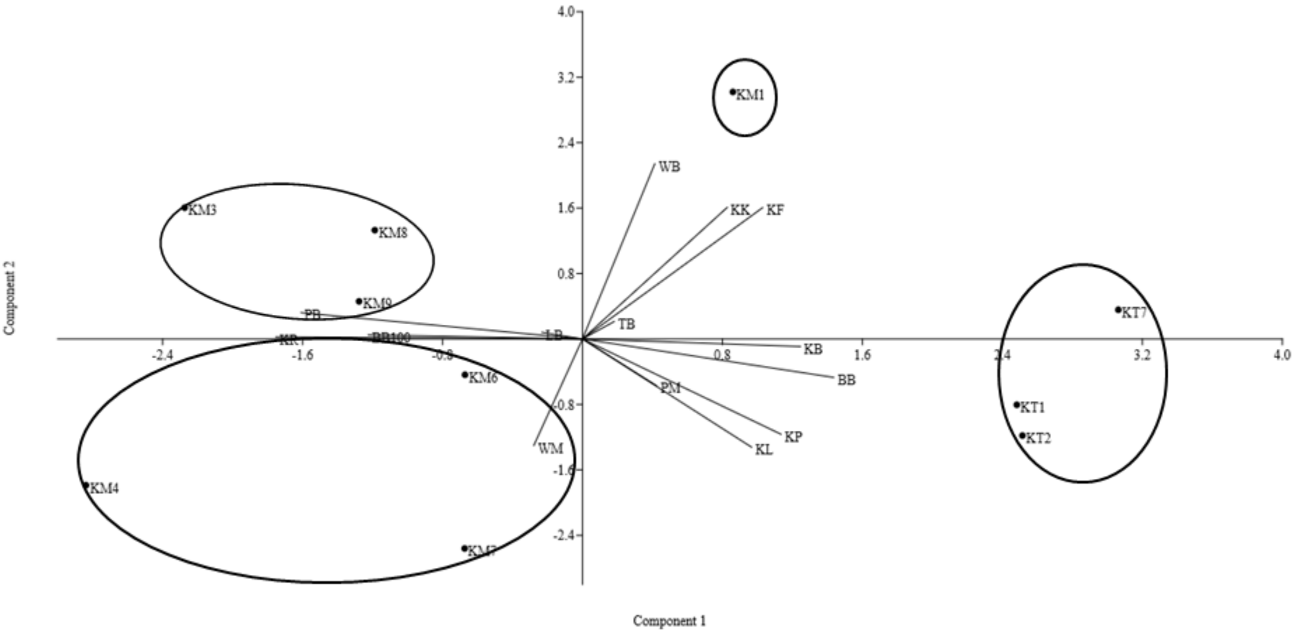

Figure 5. Cluster of local cowpea varieties based on morphology characters and nutritional composition using *clustering K-Mean*

Morphological characters and the nutrients composition are very important in genetic improvement of agricultural crops. This significant correlation is due to the existence of the source-sink relationship in plants that are genetically regulated and supported by environmental factors. The correlation analysis results can be continued with the principal components analysis, which is statistical tools to identify a number of populations so that they can be selected in plant breeding programs (Johnson, 1998). In cowpea, identification of accession was carried out based on plant morphological characters and proximate levels of seeds carried out by Animasaun et al. (2015) The percentage of cumulative variance in the first component was 26.63% and the second component was 49.67%.

In conclusion, local varieties of cowpea originated from Southwest Maluku have significant differences in morphological characters and nutrient composition in seeds. Our study found that KM3 and KM4 varieties have higher seed size and weight. The KM7 variety has high lipid and protein content, while KM 1 variety has high carbohydrate and folic acid content. There is a correlation between characters and the principal component analysis classifies local varieties of cowpea into three clusters. Varieties with high-value characters can be selected in plant breeding for beneficial development.

REFERENCES

Adewale BD, Adeigbe OO, Aremu CO. 2011. Genetic distance and diversity among some cowpea (*Vigna unguiculata* (L.) Walp) genotypes: Intl J Res Plant Sci 1 (2): 9-14.

Animasaun DA, Oyedeji S, Azeez YK, Mustapha OT, Azeez MA. 2015. Genetic variability study among ten cultivars of cowpea (*Vigna unguiculata* L. Walp) using morpho-agronomic traits and nutritional composition. J Agric Sci 10 (2): 119-130.

Basset GJC, Quinlivan EQ, Gregory III JF, Hanson AD. 2005. Folate synthesis and metabolism in plants and prospects for biofortification. Crop Sc. 45: 449-453.

Basset GJC, Ravanel S, Quiniliva EP, White R, Giovannoni JJ, Rebeille F, Nichols BP, Shinozaki K, Seki M, Gregory III JF et al. 2004. Folate synthesis in plants: the last step of the p-aminobenzoate branch is catalyzed by a plastidial aminodeoxychorismate lyase. Plant J 40: 453-461. DOI: 10.1073/pnas.0308331100.

Cossins EA. 2000. Canadian society of plant physiologists gold medal review the fascinating world of folate and one-carbon metabolism. Can J Bot 78 (6): 691-708.

Dar ZM, Masood M, Mughal AH, Asif M, Malik MA. 2018. Review on drought tolerance in plants induced by plant growth-promoting rhizobacteria. Intl J Curr Microbiol App Sci 7 (5): 412-422.

Ekinci R, Kadakal C.2005. Determination of Seven Water-Soluble Vitamins in Tarhana, A Traditional Turkish Cereal Food, by High-Performance Liquid Chromatography. Acta Chromatograph 15: 289-297.

Ezeaku IE, Mbah BN, Baiyeri KP. 2015. Planting date and cultivar effects on growth and yield performance of cowpea (*Vigna unguiculata* (L.) Walp). Afr J Plant Sci 9 (11): 439-448.

Forges T, Monnier-Barbarino P, Alberto JM, Gueant-Rodriguez RM, Daval JL, Gueant JL. 2007. Impact of folate and homocysteine metabolism on human reproductive health. Hum Rep Update 13 (3): 225-238.

Henshaw FO. 2008. Varietal differences in physical characteristics and proximate composition of cowpea (*Vigna unguiculata*). World J Agric Sci 4 (3): 302-306.

IBPGR [International Board Plant Genetic Resources]. 1983. Descriptors for Cowpea. IBPGR Secretariat, Rome.

Inobeme A, Nlemadim AB, Obigwa PA, Ikechukwu G, Ajai AI. 2014. Determination of proximate and mineral compositions of white cowpea beans (*Vigna unguiculata*) collected from markets in Minna, Nigeria. Intl J Sci Eng Res 5 (8): 502-504.

Jha AB, Kaliyaperumal A, Diapari M, Ambrose SJ, Zhang H, Ta'ran B, Bett KE, Vandenberg A, Warkentin TD, Purves RW. 2015. Genetic diversity of folate profiles in seeds of common bean, lentil, chickpea and pea. J Food Compos Anal. DOI: 10.106/j.jfca.2015.03.006.

Mustapha Y. 2008. Inheritance of seed colour in cowpea [*Vigna unguiculata* (L.) Walp.]. Intl J Pure Applied Sci. 2: 1-9.

Nelson D, Lehninger A, Cox MM. 2004. Principles of Biochemistry. 4th ed. WH Freeman, New York.

Nielsen SS, Brandt WE, Singh BB 1993. Genetic variability for nutritional composition and cooking time of improved cowpea lines. Crop Sci 33: 469-472.

Owolabi AO, Ndidi US, James BD, Amune FA. 2012. Proximate, antinutrient and mineral composition of five varieties (improved and local) of cowpea, *Vigna unguiculata*, commonly consumed in Samaru community, Zaria, Nigeria. Asian J Food Sci Technol 4 (2): 70-72.

Polnaya F. 2008. Eksplorasi dan karakterisasi plasma nutfah kacang tunggak (*Vigna unguiculata* L. (Walp.) di pulau Lakor. Jurnal Budidaya Pertanian. 4 (2): 115-121. [Indonesian]

Rukmana R. 2014. Sukses Budidaya Aneka Kacang Sayur di Pekarangan dan Perkebunan. Lily Publisher, Yogyakarta.

Rychlik M, Englert K, Kapfer S, Kirchhoff E. 2007. Folate contents of legumes determined by optimized enzyme treatment and stable isotope dilution assays. J Food Compos Anal 20: 411-419. DOI: 10.1016/j.jfca.2006.10.006.

Saini RK, Nile SH, Keum YS. 2016. Folates: chemistry, analysis, occurrence, biofortification and bioavailability. Food Res Intl 6-11. DOI: 10.1016/j.foodres.2016.07.013.

Schmidt R, Martina K, Amr M, Elshahat MR, Marlene M, Kenneth BJ, Rudolf B, Gabriele B. 2014. Effects of bacterial inoculants on the indigenous microbiome and secondary metabolites of chamomile plants. Front Microbiol 5: 64. DOI: 10.3389/fmicb.2014.00064.

Susanto AN, Sirappa MP. 2005. Prospek dan strategi pengembangan jagung untuk mendukung ketahanan pangan di Maluku. Jurnal Litbang Pertanian 24 (2): 70-79. [Indonesian]