Research Article

Cluster Analysis Based Selection in Seedling Population of Cassava Clones

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Abstract | The research was conducted in the rainy season in early 2018, in the Jambegede Research Station, Kepanjen sub-district, Malang, East Java, Indonesia. Clones that selected in this study were 1,016 clones from 42 families. Cassava selection in the early stages involved a large number of clones, therefore cluster analysis (the K-means cluster analysis method) was carried out to classify clones based on tuber yield and harvest index and to facilitate the selection process. The aim of this study was to select high yielding cassava clones as selection material for the next stage of selection in the highlands. The F_1 seedlings population selected, had a wide range of tuber yield. The results of this study indicated that the average of tuber yield of all families were lower than the check varieties, because the F_1 plants' ability to form tubers was not optimal, and there were even plants that only produce roots. However, there were F_1 plants which had higher tuber yields than the average yield of the check varieties. The harvest index in F_1 population ranged from 0.25 to 0.96 with an average of 0.75. The analysis cluster based on fresh tuber yield and harvest index of the F_1 families formed five clusters. The characteristics of these cluster were fresh tuber yield ranged from 4.84 kg to 7.4 kg with average of 6.05 kg and harvest index ranges from 0.71 to 0.82 with average of 0.79. The cluster that consisting superior clones were cluster 4.

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Introduction

According to FAOSTAT (2020), Indonesia is the 4th biggest cassava producer in the world after Nigeria, Congo, and Thailand. Badan Pusat Statistik (2015) states that the national cassava production reached 21 801 415 t in 2015, with Lampung province as the largest contributor followed by Central Java, East Java, and West Java.

Cassava propagation is by stem cutting for the commercial production, but for breeding programs, cassava propagation in the first cycle is by seeds. Full-sib and / or half-sib populations are the basic material in cassava breeding, which are then evaluated through phenotypic mass selection (Ceballos *et al.*, 2015). One of the mating designs that had been widely used by cassava breeder to generate full-sib progeny for genetic studies is diallel analysis (Kulembeka *et al.*, 2012; Zacarias and Labuschagne, 2010). Conventional breeding to produce new high yielding cassava varieties is still dominant. Germplasm evaluation is the initial stage in conventional breeding, followed by hybridization to increase genetic diversity and clonal selection. Cassava hybridization which aims to obtain seeds as a selection material is usually carried out for 1 yr to 2 yr (yr=years). Mutation can also be done to increase genetic diversity (Sholihin *et al*., 2019).

The selected clones to be tested during 4 yr to 6 yr for field evaluations, commonly divided into selection stages such as clonal evaluation trials, preliminary yield trials, advanced yield trials, and multi environment trials (Ceballos *et al*., 2016; Wolfe *et al*., 2017). Selection at F_1 seedling stage is primarily based on high heritability traits such as plant type, branching habits, and reaction to certain diseases (Ceballos *et al*., 2004), including certain traits such as storage root yield, harvest index, and dry matter content (Ojulong *et al*., 2010).

Cassava is generally grown in tropical lowlands and requires about 8 mo (month) of warm weather to mature. But in some areas, cassava is widely planted in highlands (600 m to 1,000 m above sea level – [m.s.a.l.]). In the highlands, cassava requires a longer harvest time (15 mo to 24 mo) in order to obtain high tuber yields. When cassava grown in cooler zones such as tropical highland and in lowland sub-tropics, leaf net photosynthetic rate is greatly reduced and growth slowly. Thus, the crops require longer period for a reasonable productivity (El-Sharkawy, 2006, 2012). Noerwijati *et al*. (2017) reported that 15 clones tested in Ponorogo (altitude 800 m.a.s.l) and harvested at 10 mo had an average yield of 7.79 t ha^{-1}, while the average yield in Kediri (altitude 80 m.a.s.l) was 54.84 t ha^{-1}. Tuber yield in Ponorogo was very low, because the location was high altitude (above 800 m.a.s.l). El-Sharkawy (2006) states that in highland, there was a decrease the average of photosynthesis ability so that cassava tuber yield was decreased. Noerwijati and Budiono (2015) reported that at highland cassava yield could decrease around 86 %.

Cluster analysis is a multivariate analysis that have the function of minimizing differences within clusters and maximizing differences between clusters (Oliveira *et al*., 2016). Genotype selection in large numbers have a high level of difficulty. Cluster analysis can be used to classify genotypes and determine the best cluster (Kozak *et al*. 2008). Cluster analysis had been done for selection on cassava, among them were Avijala *et al*. (2015) who did the study on estimation the genetic diversity among 21 cassava genotypes and Oliveira *et al*. (2016) had conducted cluster analysis on cassava accessions based on quantitative characteristics.

The aim of this study was to select high yielding cassava clones as selection material for the next stage of selection in the highlands.

Materials and Methods

Experimental location

The crossing activity to get seeds was carried out in 2017 in Tlekung Village, Jun Rejo sub-district, Batu District, East Java, Indonesia. The altitude of crossing location is around 900 m.a.s.l. Then the seedling populations were planted in the rainy season in early 2018, in the Jambegede research station, Kepanjen sub-district, Malang, East Java, Indonesia. The research location had an association soil type between Alfisol and Inceptisol, climate type C3 with an average rainfall of 2,300 mm per year. The minimum air temperature is 23.5 °C and a maximum of 32 °C, with a relative humidity of about 79 %.

Plant materials

The materials used in this study were 1 016 cassava seedlings. The source of origin of cassava seedlings were from Faroka, Litbang UK 2, Kaspro Ijo, Gajah Ungu, Malang 4, Malang 6, Adira 4, Kaspro Putih, and Tlekung Ungu as crossing parents.

Climatic observation

In the research location, monthly average climatic data on rainfall, temperature, and relative humidity were shown at Table 1. Highest rainfall was occurred in January, while the lowest in May. The average of relative humidity and temperature were 84.8 % and 26 °C, respectively.

Experimental design and treatment details

The experimental design used in this study was augmented design. It used when the number of entries to be tests is large and there is no replication. Asante and Dixon (2009) stated that augmented designs were efficient in the identification of superior cassava genotypes with desirable traits. The F1 seedlings were devided in some blocks. Some parental lines namely UK 1 Agritan, Litbang UK 2, Malang 4, Adira 4, Lokal Tlekung Ungu, and Gajah Ungu were used as check variety.

Table 1: *Monthly average climatic data on rainfall, temperature, and relative humidity in the research location.*

Month	Rainfall (mm)	RH (%)	Temperature (°C)
January	357.0	84.9	27.1
February	225.9	84.6	25.6
March	181.1	84.8	26.1
April	92.0	84.5	26.6
May	38.0	84.2	26.7
June	0.0	85.2	25.1
July	5.0	85.1	24.7
August	0.0	84.3	23.4
September	0.0	83.8	24.9
October	0.0	84.8	27.3
November	70.9	84.1	27.1
December	178.8	87.6	26.8
Total/average	1 148.7	84.8	26.0

Cultural practices

Seedlings were planted in the field with a spacing of 100 cm × 80 cm within and between rows. Plants are fertilized with a dose of 135 kg N+60 kg P_2O_5+30 K_2O ha^{-1} (Saleh *et al*., 2016). Fertilizer is given twice (1 mo and 3 mo after planting). Weeding and repairs of ridges was done manually and carried out before fertilization. Irrigation was carried out only at planting, then relying on rainfall.

Data collection

Harvest of plants done at 10 mo after planting. The parameters observed were fresh tuber yields and harvest index. Harvest index was calculated by dividing fresh root yield by total biomass (Ojulong *et al*., 2010; Tumushimbise *et al*., 2014).

Data analysis

The data obtained were analyzed using descriptive statistics and cluster analysis (K-means cluster analysis method) with R software (Idlette-Wilson, 2018).

Results and Discussion

Among 1,016 individuals in cassava seedling populations, fresh root yield ranged from 0.20 kg to 9.20 kg with an average of 1.80 kg $plant^{-1}$ (Table 2). The average yield of each F_1 family lower than the average of the check varieties. The tubers yield in F_1 population cannot be optimal because the plants come from seeds, there were even F1 plants that do not produce tubers. However, there were F_1 individuals that had tuber yield per plant above the average of check varieties. That clone came from an open pollinated with Kaspro female parent. This is in line with Ceballos *et al*. (2004) statement that changes in the shape and size of roots/tubers in F1 plants (from seeds) often occur when planted clonal/ vegetatively in the following year. Clone selection at the single plant selection stage in the cassava breeding program is often inefficient because the yield of fresh roots between seedlings and the advanced clonal multiplication stage had no linear relationship.

For the harvest index, the selected family had a harvest index above 0.5, which means the family had good ability to produce tubers. Badewa *et al*. (2020) stated that the genotypes that had high harvest index was able to partition dry matter to the storage root well. Harvest index of cassava seedlings in this study ranged from 0.25 to 0.96 with an average 0.75. While Ojulong *et al*. (2010) reported that from the seedling stage, harvest index estimates ranged from 0.05 to 0.90. The distribution of dry matter to the roots can be measured by harvest index and can be used as a selection criterion for higher yield potential in cassava. Harvest Index (HI) represents the efficiency of storage root production and is usually determined by the ratio of storage root weight to the total plant weight (Badewa *et al*., 2020).

Table 2: *Yield and harvest index of F1 population and check varieties.*

Genotypes	Yield/plant (kg)					Harvest index			
	n	Min	Max	Mean	Stdev	Min	Max	Mean	Stdev
F1 population	1,016	0.20	9.20	1.80	1.67	0.25	0.96	0.75	0.12
Adira 4	14	2.70	9.00	5.98	2.31	0.62	0.89	0.80	0.08
UK 1 Agritan	14	2.00	12.00	7.40	2.99	0.62	0.88	0.79	0.07
Litbang UK 2	14	1.00	16.20	5.78	3.45	0.62	0.92	0.82	0.08
Malang 4	14	2.40	7.80	4.84	1.54	0.67	0.89	0.79	0.05
Lokal Tlekung Ungu	14	5.00	9.20	6.96	1.24	0.56	0.83	0.71	0.08
Gajah Ungu	14	3.40	8.10	5.34	1.32	0.74	0.89	0.81	0.04
Average of check varieties				6.05			0.88		

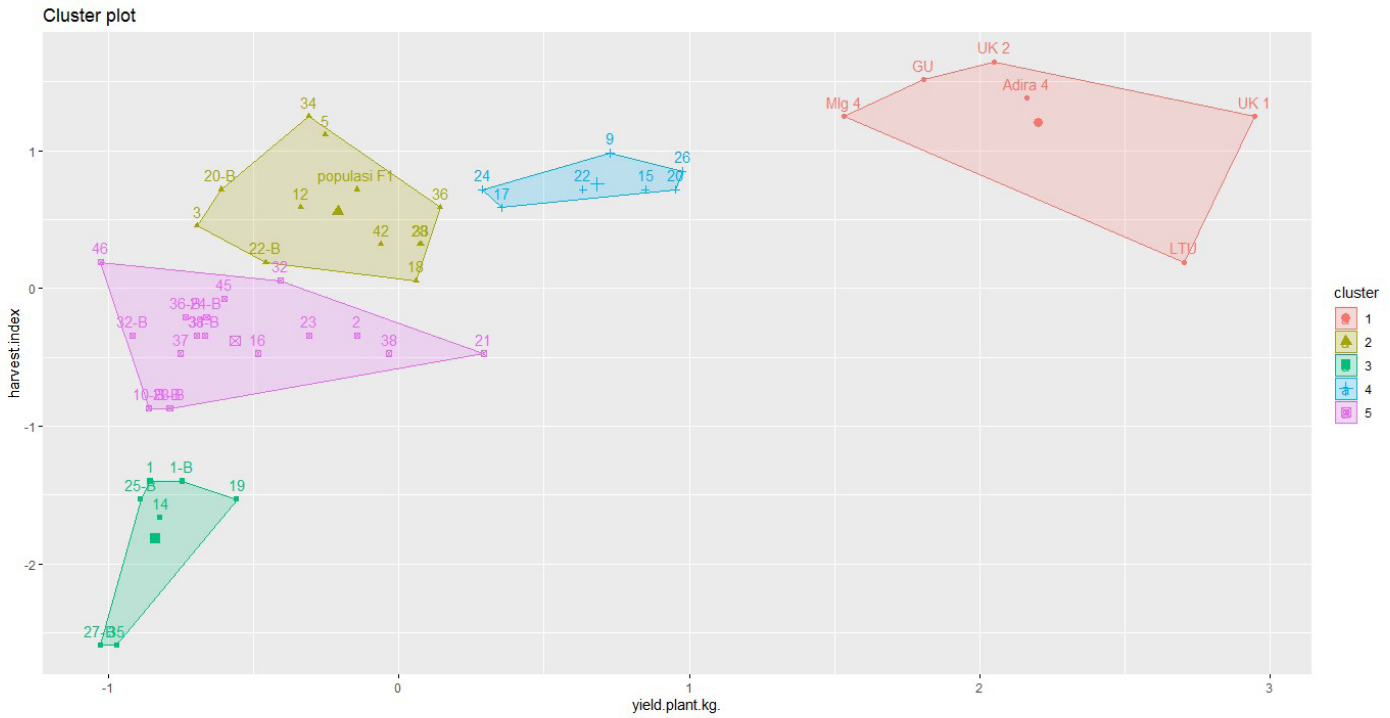

Figure 1: *Cluster plot of tuber yield and harvest index mean of F1 families.*

Cluster analysis was carried out based on tuber yield data and harvest index and produced five clusters. The check varieties form separate clusters from other clusters, with characteristics of the clusters was the tuber yield ranges from 4.84 to 7.4 kg with an average of 6.05 kg (Figure 1). Check varieties are genotypes that had high production. Accoding to Okogbenin *et al.* (2013), high tuber yield plants are associated with high levels of bulking ability over a long period of time, whereas plants with low tuber yield are associated with low bulking rates for a short or long period of time.

Tuber yield in cluster 1 ranged between 4.84 kg to 7.40 kg with an average of 6.05 kg, while the harvest index ranged from 0.71 to 0.82 with an average of 0.79. Members of Cluster 1 are all check varieties namely Adira 4, UK 1 Agritan, Litbang UK 2, Malang 4, Local Tlekung Ungu, and Gajah Ungu. The average of tuber yield of UK 1 Agritan variety was the highest, while the lowest was Malang 4 (Table 3). The check varieties were genotypes that had stable yield, so that all of check varieties could produce high tuber yield.

Cluster 2 had tuber yield ranged from 0.20 kg to 2.70 kg with an average of 1.68 kg, while the harvest index ranged from 0.70 to 0.79 with an average of 0.73 (Table 4). Members of cluster 2 were 15 families and the value of population mean was included in this cluster. Cassava clones that had the highest average tuber yield in Cluster 2 came from the crossing between Malang 6 (female parent) × Gajah Ungu (male parent). Malang 6 was cassava variety that had average tuber yield around 36.41 t ha^{-1} and belong to bitter cassava, while Gajah Ungu is local variety that had high yield potential (could reach 100 t ha^{-1}) and belong to sweet cassava.

Table 3: *Genotypes in the cluster 1.*

No	Cluster 1		
	Genotypes	Yield plant^{-1} (kg)	Harvest index
1	Adira 4	5.98	0.80
2	UK 1 Agritan	7.40	0.79
3	Litbang UK 2	5.78	0.82
4	Malang 4	4.84	0.79
5	Local (Tlekung Ungu)	6.96	0.71
6	Local (Gajah Ungu)	5.34	0.81

Table 4: *Mean of yield plant^{-1} and harvest index in each cluster.*

Cluster	Yield plant^{-1} (kg)			Harvest index		
	Min	Max	Mean	Min	Max	Mean
Cluster 1	4.84	7.40	6.05	0.71	0.82	0.78
Cluster 2	0.20	2.70	1.68	0.70	0.79	0.73
Cluster 3	0.20	1.05	0.54	0.50	0.59	0.56
Cluster 4	3.21	3.83	3.56	0.75	0.77	0.76
Cluster 5	0.40	2.59	1.08	0.63	0.69	0.66

Different with cluster 2, cluster 3 had tuber yield ranged from 0.20 kg to 1.05 kg with an average of 0.54 kg, while the harvest index ranged from 0.50 to 0.59 with an average of 0.56 (Table 4). Cluster 3 with seven families had average of tuber yield lower than the second cluster. The family that had the highest average of tuber yield in cluster 3 came from the crossing with Malang 6 as female parent and UJ 3 as male parent, but the average of tuber yield in this family was lower than the population mean (Table 4).

Cluster 4 had five families and it had the highest average of tuber yield among the other clusters, except cluster 1. Cluster 4 characteristics were the tuber yield ranged from 3.21 kg to 3.83 kg with an average of 3.56 kg, while the harvest index ranged from 0.75 to 0.77 with an average of 0.76 (Table 4). In cluster 4, the family that had the highest average of tuber yield was come from open-pollinated with Kaspro as female parent. The average of tuber yield from this family was higher than the population mean but lower than the average of check varieties (Table 5). In this cluster, eight genotypes had tuber yields above the average of check varieties, i.e. from family number 9, number 20, and number 26. There was one genotype in this cluster that had highest tuber yields among other genotypes with tuber yield around 9.2 kg per plant (Table 6).

Table 5: *The families that have highest average tuber yield in each cluster.*

Cluster	Family number	Crossing parents	Yield plant^{-1} (kg)	Harvest index
2	17	Malang 6×Gajah Ungu	2.70	0.74
3	19	Malang 6 × UJ 3	1.05	0.58
4	26	Kaspro (Open pollinated)	3.83	0.76
5	21	Malang 6 (Open pollinated)	2.59	0.66

For the last cluster (cluster 5), the tuber yield ranged between 0.40 kg to 2.59 kg with an average of 1.08 kg, while the harvest index ranged from 0.63 to 0.69 with an average of 0.66 (Table 4). Similar with cluster 2, cluster 5 was also containing fifteen families. Families that had the highest average tuber yields in cluster 5 were come from open-pollinated with female parents Malang 6.

The data in Table 5 showed that all families that had the highest average yield in each cluster had Malang 6 as female parent except family number 26. This showed that the Malang 6 variety had a very good genetic potential. Malang 6 was a progeny from a cross between MLG 10071 (female parent) and MLG 10032 (male parent).

Table 6: *Genotypes that have tuber yields above the average tuber yield of check varieties (6.05 kg plant^{-1}) and have a high harvest index (> 0.5).*

No	Genotypes	Yield plant^{-1} (kg)	Harvest index	External color of root	Color of root cortex	Color of root pulp
1	CMM 17009-1	6.8	0.84	DB	P	W
2	CMM 17020-2	6.6	0.83	DB	W	W
3	CMM 17020-5	7.2	0.78	DB	C	W
4	CMM 17020-16	8.4	0.81	DB	P	W
5	CMM 17021-14	8.6	0.70	DB	C	W
6	CMM 17021-28	7.7	0.75	W	W	W
7	CMM 17021-29	7.6	0.73	B	W	W
8	CMM 17021-31	8.0	0.50	B	C	W
9	CMM 17021-53	6.1	0.60	B	W	W
10	CMM 17021-57	6.9	0.74	W	W	W
11	CMM 17021-67	8.1	0.84	B	C	W
12	CMM 17021-88	6.2	0.78	W	PK	W
13	CMM 17021-94	7.0	0.71	DB	W	W
14	CMM 17024-6	6.3	0.85	DB	W	W
15	CMM 17024-19	7.5	0.84	DB	W	W
16	CMM 17026-21	7.2	0.87	W	W	W
17	CMM 17026-27	8.0	0.85	DB	W	W
18	CMM 17026-30	7.8	0.76	DB	W	W
19	CMM 17026-51	9.2	0.84	W	W	W
20	CMM 17042-21	7.8	0.86	DB	P	W
21	CMM 17042-48	8.6	0.91	W	W	W
22	CMM 17042-55	6.4	0.83	DB	W	W
23	CMM 17042-58	8.1	0.79	DB	W	W
24	CMM 17042-66	7.8	0.87	LB	W	W
	Yield mean of check varieties	6.05	0.88			

Noted: External color of root: W: White; LB: Light Brown; B: Brown; DB: Dark Brown. Color of root cortex: W: White; C: Cream; PK: Pink; P: Purple. Color of root pulp: W: White.

A total of 24 clones had higher tuber yield than average of check varieties (Table 6). These genotypes came from six families, namely family number 9, number 20, number 21, number 24, number 26, and number 42. The external color of the tuber of these clones was classified into four groups viz white (six clones), light brown (one clone), brown (four clones), and dark brown (13 clones). The color of root cortex was classified into four groups viz white

(16 clones), cream (four clones), pink (one clone), and purple (three clones), while the color of the root pulp is all white.

Harvest index (HI) is highly correlated with root yield and had a high heritability. Indirect selection for yield through HI at earlier stages of selection is more effective than direct selection using yield itself (Ojulong *et al*., 2010). However, this did not match with the results of this study. In this selection, for example, progenies from controlled pollinated between Malang 6 and Adira 4 had harvest index ranged from 0.47 to 0.87, however tuber yield variation was very high (Figure 2). These result showed that harvest index in this stage cannot be used yet as a selection criterion. A similar result shown in Figure 3 for progenies from open-pollinated with Kaspro as female parent. One clone with harvest index of 0.82 had fresh tuber yield of 1.80 kg, while the other clone with harvest index of 0.84 had the highest tuber yield of 9.20 kg.

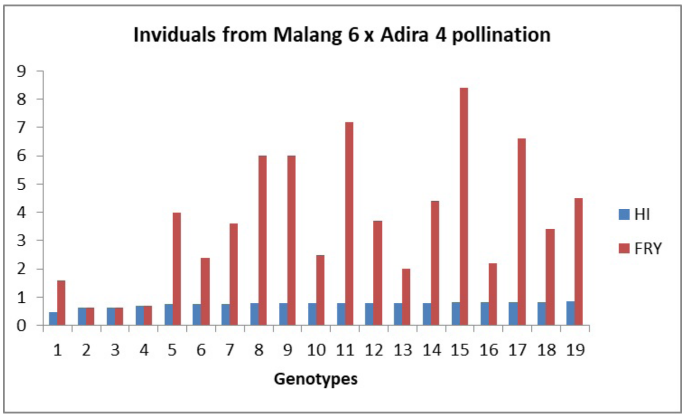

Figure 2: *Fresh root yield and harvest index performance at 10 mo after planting of cassava clones from controlled pollinated with Malang 6 as female × Adira 4 as male parent.*

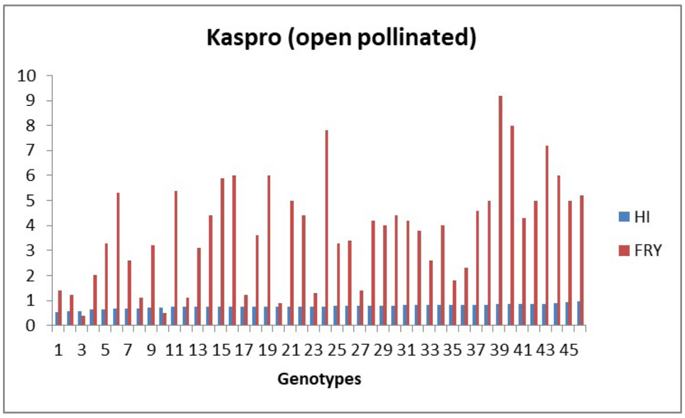

Figure 3: *Fresh tuber yield and harvest index performance at 10 mo after planting of cassava clones from open pollinated with Kaspro as female parent.*

Figure 4 showed performance of fresh tuber yield and harvest index from cassava clones that had higher tuber yield than check varieties at 10 mo after planting. Tuber yields of these clones ranged from 6.1 kg to 9.2 kg. The highest yield (9.2 kg plant^{-1}) was achieved by the CMM 17026-51 clone which came from an open pollinated with Kaspro (local variety) as female parent. The lowest harvest index in these clones was 0.5 and the highest was 0.91 (Figure 4). This showed that high tuber yield should had a high harvest index value, however high harvest index did not necessarily had high tuber yield. Ojulong *et al*. (2010) reported that harvest index estimates ranged from 0.05 (GM 252B-215) to 0.90 (GM 853-13) at the seedling stage. Simultaneous selection of yield and quality traits (such as harvest index) can be carried out at earlier stages of selection.

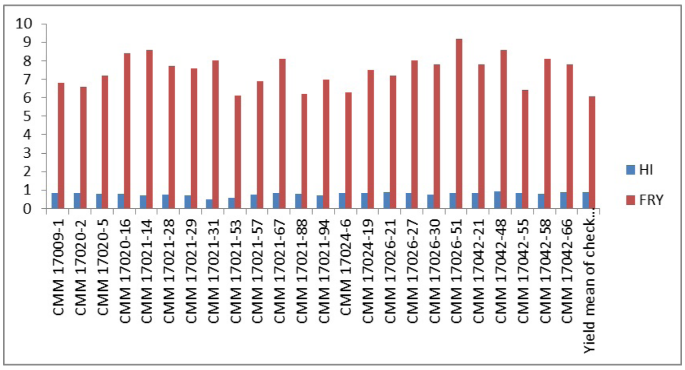

Figure 4: *Fresh tuber yield and harvest index performance at 10 mo after planting of cassava genotypes that had higher tuber yield than check varieties.*

In Indonesia, there is no cassava variety that had been specifically classified as adaptive to highland. The cassava yield is known to be influenced by the altitude. For maximum growth and yield of cassava, the cassava plant requires a warm, humid climate. Temperature is important, as all growth stops at about 10 °C. The crop is typically grown in areas that are frost-free all year round. Cassava requires a warm and humid climate (del Rio and Simpson, 2014). The highest root production can be expected in the tropical lowlands, below 150 m altitude, where temperatures average 25 °C to 27 °C, but some varieties grow at altitudes up to 1 500 m.a.s.l (del Rio and Simpson, 2014; Moore and Lawrence; 2005). Therefore, it is necessary to select genotypes / clones that can produce well in the highlands. Selection of the initial stage (single plant selection) is done at a location with altitude of about 400 m.a.s.l. The next stage of selection is planned to be done at a location with altitude of about 700 m.a.s.l. If the selected clones had reached the advanced yield test stage, an adaptability test will be carried out, and to determine clones that had broad or narrow adaptability, a GGE analysis can be carried out as had been done by Noerwijati *et al*. (2014).

Conclusions and Recommendations

The average tuber yield from the F_1 families in this study was lower than the average tuber yield of check varieties, because the clones were derived from seeds and the yield were not yet stable. There were 24 clones that had tuber yield above the tuber yield average of check varieties (6.05 kg plant^{-1}) and had high harvest index (> 0.5). These result of this study showed that harvest index cannot be used yet as a selection criterion at initial stage. There was an opportunity to obtain high yielding cassava clones as selection material for the next stage of selection.

Novelty Statement

Cassava varieties that had been released in Indonesia does not yet have specifications for environmental adaptation at medium to high altitude. For this reason, this study was a series of research to obtain cassava varieties with high yield and high starch content that is adaptive to medium to high land.

Author's Contribution

Kartika Noerwijati: Concepts, design, definition of intellectual content, literature search, experimental studies, data acquisition, data analysis, statistical analysis, and manucript preparation.
Sholihin Sholihin and Tinuk Sri Wahyuni: Definition of intellectual content, literature search, experimental studies, and manuscript review.
Rohmad Budiono and Nguyen Van Minh: Literature search, definition of intellectual content, and manuscript review
Roy Hendroko Setyobudi and Zane Vincēviča-Gaile: Literature search, definition of intellectual content, manuscript editing, manuscript review, and guarantor.
Lulu Husna: Experimental studies, data analysis, statistical analysis.
All authors read and approved the final manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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