Nonparametric Stability Analysis of Starch Content of Gamma Irradiated Cassava at Three Locations in West Java, Indonesia

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Abstract

Cassava is one of the largest starch producing tuber crops in Indonesia. Tapioca from cassava starch can be used as a raw material for a variety of foods including biscuits, instant porridge, meatball, sausage, nuggets, and flour condiment. Breeding programs to improve cassava yield and starch content by using mutagen gamma rays irradiation was tested in four cassava generations (M1-V4). Cassava is propagated by stem cuttings and the new characteristic obtained from gamma irradiation mutation is stable and can be passed from one generation to the next. Cassava mutants were obtained by evaluating the performance in different environmental conditions. The testing of yield adaptability and stability through a series of multi location is an important step before a new variety can be released. The aim of this research was to compare nonparametric stability and to evaluate the stability of the starch content of 16 genotypes (14 mutants and 2 varieties) in three locations in West Java Province i.e. Tapos (Depok), Cikarawang (Bogor), and Ciseeng (Bogor). Experiments were conducted in a completely randomized block design with three replications nested in each environment. Testing of yield stability in this study used four approaches with 10 parameters of nonparametric method. Starch content was estimated using gravimetric method. Based on frequency stability ranking of starch content from gravimetric method the G63142 genotype had the highest starch content estimation (29.99%), and the top five genotypes with high starch content were G63142, G61142, “Manggu”, G62151, and G63124. G61142 was categorized as genotype with static and dynamic stability, therefore this genotype is a very potential mutant to be released, whereas G63124 is categorized as a genotype with a static stability. Genotypes G63142, G62151, and “Manggu” had variable but relatively high starch content.

Keywords: gravimetric, mutant, static stability, dynamic stability

Introduction

Cassava is one of the important carbohydrate producing crops after wheat, rice, corn, potato, and barley (Lebot, 2009). FAO (2017) showed that Indonesia ranks as the fourth largest cassava producing country after Nigeria, Thailand, and Brazil in 2016. Cassava is used as raw material for food and non-food industries, including feed, flour, and fuel.

Cassava contributed the largest percentage of starch from tuberous crops (29%), followed by sweet potato (26%) and potato (5%). Cassava starch has granule form which are round with a truncated end; so it is different from corn starch which is hexagonal, and sagoo starch which is oval (Murtiningrum, 2012). Tapioca from cassava starch can be made into biscuits, instant porridge, processed foods such as meatball, sausage, nuggets, and flour condiment. Hydrolisis of modified cassava flour can be processed into soft drink, milk, and ketchup (Haloho, 2014).

Indonesian cassava starch export value in 2017 was US$ 5.60 billion, whereas the imports value was US$ 126.4 billion (BPS, 2017). The Indonesian cassava starch exports decreased to US$ 5.28 billion in 2018, whereas the import value increased to US$ 185.6 billion at the same period. Even though Indonesia has exported cassava starch and chips, the national production faces uncertainties of productivity because of pest outbreak and weather fluctuations.

Efforts to obtain new cassava varieties through conventional breeding have limitations when using stem cuttings as the genetic material. Cassava is usually propagated vegetatively because it only flowers and produce seeds at 800 m above sea level, therefore cassava has a relatively low genetic variance. Vegetative propagation through stem cuttings has many advantages; the new characteristic obtained from gamma irradiation are stable and can be passed...
from one generation to the next. Genetic variance can be increased by gene recombination, hybridization, genetic manipulation, mutation induction, or polyploidy (Syukur et al., 2012). Breeding through mutation induction on cassava has been used to develop new superior varieties; Fahreza (2014) and Maharani (2015) have reported an improved yield and starch content in cassava through mutation breeding using mutagen gamma ray irradiation.

According to the regulation by Indonesian Ministry of Agriculture (2011) the new crop genotypes have to be characterized for yields, adaptability to different environments and locations prior. Different genotypes might express different characters in different environments (Harahap, 1982).

This study evaluated yield stability of different genotypes of cassava at three locations in West Java Province. The locations were selected based on the ideal topology and topography for growing cassava. Genotype performance might change in different environments known as interaction of genotype and environment (G^E). Interaction of genotype and environment is very important when a mutant genotype is to be grown in different environments. Baye et al (2011) stated the importance of developing varieties that are specifically adapted to a particular environment (Baye et al., 2011).

Yield stability studies based on the interaction of genotype x environment have been widely conducted, e.g. Finlay-Wilkinson (1963), Eberhart-Russell (1966), Francis and Kannenberg (1978), and Gauch (2006). The interaction of genotype and environment can also hinder the progress of selection, disrupt the selection of excellent varieties in a testing of varieties and difficult to make appropriate conclusion if a genotype test is performed in a wide range of environments. The testing of yield stability in this study used four approaches with 10 parameters of nonparametric method. Huehn (1990) suggests that the nonparametric procedure has several advantages compared to the parametric stability. These benefits are the reduction of bias caused by outliers, no assumptions needed of the observed values and ease of use in interpreting, adding, or deleting unsuitable genotypes.

The starch content of cassava tubers was usually measured using a gravimetric method. Subekti et al. (2018) evaluated starch content of cassava “Gajah”, a mutant derived from mutation induction using gamma ray irradiation. The value of gravimetric method was included to the formula and the percent of starch content was measured. The starch content estimation (expressed in %) from gravimetric method had similar results with laboratorium test.

The aim of this research was to compare non parametric stability measures and to evaluate the starch content stability of 16 genotypes of cassava that consists of 14 mutants and two varieties, in three locations in West Java Province i.e. Depok (Tapos), Bogor (Cikarawang), and Bogor (Ciseeng).

Materials and Methods

The genetic materials used for this experiment were 14 putative mutant lines (G61122, G61142, G61143, G61151, G62143, G62132, G62151, G62153, G631111, G63121, G63124, G63142, G63143, G63153) and two national varieties (“Gajah” and “Manggu”). The putative mutants were selected from the previous generation (Subekti et al., 2018). Breeding programs to improve cassava yield and starch content was conducted using mutagen gamma ray irradiation with five doses (0, 15, 30, 45, 60 Gy), the crop performance was evaluated in four generations. Based on the regulation by Indonesian Ministry of Agriculture (2011), genotype performances have to be stable in different environment.

The research was conducted at Cikarawang (Bogor) with altitude of 192 m above sea level, Ciseeng (Bogor) with altitude of 125 m above sea level, and Tapos (Depok) with altitude of 110 m above sea level. The experiment was conducted in a randomized complete block design with three replication in each location. Plant maintenance involved regular watering, fertilizing, and weeding. Pest and disease control was conducted only when required. Gravimetric analysis on fresh tubers was conducted twice at harvest. Cassava tubers were harvested 11 months after planting.

Starch content was analyzed using a gravimetric method by Kawano et al. (1987). Tubers with length of > 20 cm and diameter of >4 cm were weighed in the air (x) and in the water (y). Specific gravity was calculated by the formula (x/y). The value of specific gravity was included to the formula (112.1 x SG) – 106.4 to get the percentage of starch content.

Nonparametric stability analysis was based on ranks classification (Ackura et al., 2006). Ackura et al. (2006) showed that nonparametric procedure has an advantage of reducing bias caused by outliers. Rahadi et al. (2013) used the nonparametric stability analysis based on two different concepts, static and dynamic stability, of yield for nine chili pepper genotypes in eight environments.
Data analysis consisted of the analysis of variance followed by nonparametric stability analysis with 10 methods of $S_{i}^{1}$, $S_{i}^{2}$, $S_{i}^{3}$, and $S_{i}^{6}$ (Nassar and Huehn, 1987), RS (Kang, 1988), Top (Fox et al., 1990), and $NPI_{1}$, $NPI_{2}$, $NPI_{3}$ and $NPI_{4}$ (Thennarasu, 1995) against the production characteristics of each plant using the following formula (Syukur et al., 2012):

$$S_{i}^{(1)} = 2 \sum_{j} \sum_{i=1}^{n} \frac{r_{ij} - r_{yj}}{\sqrt{n(n-1)}}; S_{i}^{(2)} = \sum_{j=1}^{n} \left( \frac{\hat{r}_{yj} - \bar{r}_{yj}}{\bar{r}_{yj}} \right) / (n-1);$$

$$NP_{i}^{(0)} = \frac{\sum_{j=1}^{n} \left( r_{ij} - M_{ai}^{*} \right)}{n}; NP_{i}^{(2)} = \frac{\sum_{j=1}^{n} \left( \frac{r_{ij} - M_{ai}^{*}}{M_{ai}} \right)}{n};$$

$$NP_{i}^{(0)} \cdot \frac{n}{n(n-1)} \sum_{j=1}^{n} \left( \frac{r_{ij} - \bar{r}_{y}}{\bar{r}_{y}} \right)^{2};$$

where $n$ is the number of location, $r_{ij}$ is genotype ranks-i in environments-j, $\bar{r}_{yj}$ is the rank of the genotype from a corrective values, $M_{ai}^{*}$ is median from corrective value, and $M_{ai}$ is median from all environments.

**Result and Discussion**

**Interaction of Genotype and Environment (GXE)**

Starch content from fresh tubers were analyzed by the combined variant analysis. Cassava genotypes have different percentage of starch content, whereas the environment and interaction of genotype x environment had no significant effect on starch content (Table 1), therefore each genotype could perform equally well at the three locations.

Subekti et al. (2018) reported that in terms of yield stability, the M1V4 generation mutants had stable yields, however different mutants were different in their starch content. Anasari et al. (2017) showed that there was no significant interaction of genotype x environment in affecting Pakchoy yields in three different locations, and the genotypes tested had high diversity with high adaptability to each location.

Figure 1 showed the interaction response indicated by the starch content fluctuations at each location. A high diversity of macro environment is equal with high environment diversity for each genotypes (Satoto et al. (2009). Three cassava genotypes, G61142, G61143, and G62132, had similar starch content at every location.

Chahal and Gosal (2002) showed that the qualitative interaction of genotypes were identified by yield frequency ranking in each location. Based on frequency ranking in three locations, some genotype were stable only on two locations (Table 2). G63142 had the highest starch content estimation (29.99%), whereas G62132 had the smallest starch content estimation (18.22%). Both showed good performance in Depok and Cikarawang. The last generation was tested by Subekti et al. (2018) and 23.86% had the highest and 12.60% had the smallest starch.

**Table 1.** Variant analysis of the combined starch content (%) from three geographic locations

| Sources of variants | DF | SS  | MS  | F calc | Pr > F     |
|---------------------|----|-----|-----|--------|------------|
| Environment (E)     | 2  | 157.4353 | 78.7176 | 3.84 | 0.1123*ns |
| Replication/Environment | 3  | 43.9847   | 14.6615 | 1.31 | 0.5105*ns |
| Genotype (G)        | 15 | 832.5972  | 55.5064 | 2.26 | 0.0281*    |
| G x E               | 30 | 737.5985  | 24.5866 | 1.31 | 0.2020*ns  |
| Errors              | 45 | 844.1654  | 18.7592 | 0.2020*ns |
| Total               | 95 | 2648.249  |       |       |            |

Note: *= significantly different; ns= not significantly different at $\alpha=5%$
content estimation. These genotypes demonstrated an increase in starch content in the three locations compared to those in the previous location.

**Nonparametric Stability Analysis of Starch Content at Three Locations in West Java, Indonesia**

The testing of yield adaptability and stability through a series of multi location evaluation is an important step before a new variety is released (Syukur et al., 2012). Stability studies based on GxE interaction need to be complemented with nonparametric methods to reduce bias caused by outliers. In addition, this method had no assumption of the values, easy to interpret and to add, or to delete unsuitable genotypes without destroying the population (Huehn, 1990).

De Vita et al. (2010) suggested that the released genotypes should have high adaptability and high stability. Mohammadi et al. (2010) separated the different methods based on two concepts of stability, dynamically stable (adaptation followed the environmental index) and statically stable (yield stable in each environment). The farmers need stable and high yielding genotypes categorized by dynamic stability (Zulhayana, 2010). The stability values obtained from the Nassar and Huehn (1987) method showed that G63142 genotype had the biggest value with 29.99% starch content estimation (Table 3), followed by G61142 and local genotype “Manggu”. The smallest starch content estimation was found in G62153 (18.23%). Sabaghnia et al. (2006) showed the smallest parametric stability genotype was more stable compared to the other genotypes. Based on S1\(^{(1)}\) parameter of stability index (Nassar and Huehn, 1987) “Gajah” and G61142 genotype were stable, whereas S1\(^{(2)}\), S1\(^{(3)}\) and S1\(^{(4)}\) parameter showed that G62153 genotype was stable. “Gajah” was the most stable compared to the local genotype “Manggu” in each environment. Fox et al. (1980) identified that genotypes in the top ranks from each environment were categorized as stable.

Table 3 shows that three cassava mutants, G61142, G62151, and G63142, had the highest ranks in TOP layer, therefore these mutants are recommended and adaptive. Rank-sum method by Kang (1988) was the sum of ranking in starch content estimation (%) obtained by Shukla method. The smallest value indicates a stable genotype, and G61142 was the most stable genotype. Table 4 shows all data from 10 parameters of 16 cassava genotypes in three environments.

**Table 2. Starch content estimation (%) of 16 cassava genotypes at three geographical locations in West Java, Indonesia**

| No | Genotype | E1 Rank | E2 Rank | E3 Rank | Average Rank | Average |
|----|----------|---------|---------|---------|--------------|---------|
| 1  | G61122   | 24.28   | 9       | 22.25   | 8            | 16      | 21.10cd |
| 2  | G61142   | 23.18   | 10      | 27.67   | 3            | 3       | 25.45abc|
| 3  | G61143   | 19.00   | 15      | 21.41   | 11           | 19.81   | 20.07cd |
| 4  | G61151   | 21.17   | 13      | 21.08   | 13           | 21.49   | 8       | 21.25bcd |
| 5  | G62143   | 21.68   | 11      | 21.47   | 10           | 25.76   | 1       | 22.97bcd |
| 6  | G62132   | 16.93   | 16      | 17.49   | 16           | 20.25   | 12      | 18.22d  |
| 7  | G62151   | 29.40   | 4       | 26.67   | 4            | 21.23   | 9       | 25.77abc|
| 8  | G62153   | 20.54   | 14      | 20.53   | 15           | 18.83   | 15      | 19.97cd |
| 9  | G63111   | 21.20   | 12      | 23.14   | 6            | 19.76   | 14      | 21.36bcd|
| 10 | G63121   | 24.44   | 8       | 21.48   | 9            | 25.76   | 2       | 23.97bcd |
| 11 | G63124   | 32.66   | 2       | 23.27   | 5            | 24.61   | 6       | 25.72abc|
| 12 | G63142   | 39.29   | 1       | 29.75   | 2            | 20.93   | 10      | 29.99a  |
| 13 | G63143   | 25.29   | 7       | 21.27   | 12           | 19.81   | 4       | 23.93bcd|
| 14 | G63153   | 30.05   | 3       | 20.70   | 14           | 20.26   | 11      | 23.67bcd|
| 15 | “Gajah”  | 28.12   | 5       | 22.28   | 7            | 25.20   | 5       | 25.20abc|
| 16 | “Manggu” | 27.06   | 6       | 29.79   | 1            | 21.92   | 7       | 26.25ab |
|    | Environment average | 25.27   | 23.14   | 21.89   |             | 23.77   |         |

Note: E1= Depok (Tapos), E2= Bogor (Cikarawang), E3= Bogor (Ciseeng); values followed by the same letter within the same column was not significantly different based on Duncan’s test at α=5%.
Table 3. Average starch content estimation and nonparametric stability values of 16 cassava genotypes in three locations

| Geno   | SCE (%) | S1 (1) | S1 (2) | S1 (3) | S1 (4) | TOP | MID | LOW | RS | NP1 | NP2 | NP3 | NP4 |
|--------|---------|--------|--------|--------|--------|-----|-----|-----|-----|-----|-----|-----|-----|
| G61122 | 20.10   | 10.67  | 17.33  | 14.81  | 5.08   | 0.00| 33.33| 66.67| 27  | 4.33| 0.43| 0.52| 0.49|
| G61142 | 26.95   | 3.00   | 3.00   | 5.25   | 3.25   | 66.67| 33.33| 0.00| 3   | 1.33| 0.44| 0.47| 0.44|
| G61143 | 20.08   | 5.67   | 1.33   | 1.04   | 1.45   | 0.00| 0.00 | 100.00| 21  | 3.33| 0.30| 0.36| 0.32|
| G61151 | 21.22   | 6.00   | 8.33   | 6.54   | 3.63   | 0.00| 33.33| 66.67| 15  | 2.33| 0.18| 0.25| 0.22|
| G62143 | 22.97   | 9.67   | 50.33  | 48.67  | 8.55   | 0.00| 0.00 | 100.00| 27  | 5.00| 0.50| 0.72| 0.64|
| G62132 | 18.23   | 6.67   | 5.33   | 4.04   | 2.85   | 33.33| 0.00 | 66.67| 23  | 4.33| 0.27| 0.37| 0.32|
| G62151 | 25.77   | 7.00   | 10.33  | 9.74   | 4.35   | 66.67| 33.33| 0.00| 11  | 3.33| 0.83| 0.84| 0.79|
| G62153 | 19.97   | 5.67   | 0.33   | 0.56   | 1.02   | 0.00| 0.00 | 100.00| 16  | 1.33| 0.09| 0.11| 0.09|
| G63101 | 21.37   | 10.00  | 17.33  | 24.60  | 6.31   | 0.00| 33.33| 66.67| 16  | 3.33| 0.28| 0.41| 0.38|
| G63121 | 24.04   | 5.33   | 16.33  | 14.16  | 5.37   | 33.33| 0.00 | 66.67| 16  | 3.33| 0.37| 0.61| 0.50|
| G63124 | 25.37   | 3.33   | 1.00   | 1.20   | 1.20   | 33.33| 66.67| 0.00| 8   | 2.00| 0.40| 0.50| 0.44|
| G63142 | 29.99   | 9.33   | 24.33  | 23.97  | 6.97   | 66.67| 0.00 | 33.33| 17  | 5.00| 2.50| 1.41| 1.18|
| G63143 | 23.93   | 6.67   | 16.00  | 18.00  | 4.50   | 33.33| 33.33| 33.33| 33.33| 16  | 3.00| 0.38| 0.47| 0.42|
| G63153 | 23.67   | 11.67  | 39.00  | 74.44  | 12.22  | 33.33| 0.00 | 66.67| 21  | 4.67| 0.42| 0.65| 0.59|

“Gajah” 25.20 3.00 1.33 2.30 2.12 0.00 100.00 0.00 21 3.33 0.67 0.79 0.75

“Manggu” 26.26 6.33 12.00 20.80 6.40 33.33 66.67 0.00 14 4.00 0.57 1.00 0.89

Note: SCE= starch content estimation per plant (%); S1 (1), S1 (2), S1 (3), S1 (4) (Nassar and Huenh, 1987); Top (Fox et al., 1990); RS= Rank-sum by Kang (1988); NP1, NP2, NP3, and NP4 by Thennarasu (1995).

Table 4. Ranking of starch content estimation (%) and nonparametric stability of 16 cassava genotypes in three locations

| Geno   | SCE (%) | S1 (1) | S1 (2) | S1 (3) | S1 (4) | TOP | MID | LOW | RS | NP1 | NP2 | NP3 | NP4 |
|--------|---------|--------|--------|--------|--------|-----|-----|-----|-----|-----|-----|-----|-----|
| G61122 | 13      | 15     | 12     | 10     | 10     | 12  | 16  | 12  | 16  | 10  | 9   | 9   |
| G61142 | 2       | 1      | 5      | 6      | 6      | 2   | 1   | 1   | 11  | 7   | 8   |
| G61143 | 14      | 5      | 3      | 2      | 3      | 12  | 10  | 6   | 12  | 3   | 4   |
| G61151 | 12      | 7      | 7      | 7      | 7      | 12  | 5   | 4   | 2   | 2   | 2   |
| G62143 | 10      | 13     | 16     | 15     | 15     | 12  | 16  | 15  | 5   | 12  | 12  |
| G62132 | 16      | 9      | 6      | 5      | 5      | 7   | 12  | 13  | 3   | 4   | 3   |
| G62151 | 4       | 11     | 8      | 8      | 8      | 2   | 3   | 7   | 15  | 14  | 14  |
| G62153 | 15      | 6      | 1      | 1      | 1      | 12  | 6   | 2   | 1   | 1   | 1   |
| G63111 | 11      | 14     | 13     | 14     | 12     | 12  | 6   | 8   | 4   | 5   | 5   |
| G63121 | 7       | 4      | 11     | 9      | 11     | 7   | 6   | 9   | 6   | 10  | 10  |
| G63124 | 5       | 3      | 2      | 3      | 2      | 7   | 2   | 3   | 8   | 8   | 7   |
| G63142 | 1       | 12     | 14     | 13     | 14     | 2   | 7   | 16  | 16  | 16  | 16  |
| G63143 | 8       | 10     | 10     | 11     | 9      | 7   | 6   | 5   | 7   | 6   | 6   |
| G63153 | 9       | 16     | 15     | 16     | 16     | 7   | 10  | 14  | 9   | 11  | 11  |

“Gajah” 6 2 4 4 4 12 10 10 10 14 13 13

“Manggu” 3 8 9 12 13 7 4 11 13 15 16

Note: SCE= starch content estimation per plant (%); S1 (1), S1 (2), S1 (3), S1 (4) (Nassar and Huenh, 1987); Top (Fox et al., 1990); RS= Rank-sum by Kang (1988); NP1, NP2, NP3, and NP4 by Thennarasu (1995).
Syukur et al. (2012) showed that $S_1^{(1)}$ and $S_1^{(2)}$ parameter were placed in the same rank, whereas these parameters showed different values (Table 3). Only two genotypes, G61151 and G63143, were placed in the same rank. This shows that the tested genotypes behave similarly in different environment, whereas $S_1^{(3)}$ and $S_1^{(6)}$ parameters combined the yield and stability in each environment.

Nine genotypes (G61122, G61142, G61151, G62143, G62132, G62151, G62153, G63153, and Gajah) have stable starch content. G61151 has the highest rank of starch content estimation stability (21.22%). Spearman’s correlation demonstrated the relationship of various methods of static or dynamic stability of starch content estimation (%).

Starch content estimation (%) has highly significant and positive correlation with Top, $NP_i^2$, $NP_i^3$, and $NP_i^4$, but is negatively correlated with RS (Table 5). The stability values were only obtained by Huehn ($S_1^{(1)}$, $S_1^{(2)}$, $S_1^{(3)}$, $S_1^{(6)}$) method and did not have correlation with Thennarasu’s ($NP_i^1$, $NP_i^2$, $NP_i^3$, $NP_i^4$) method.

Table 5. Spearman correlation of nonparametric stability parameter against the starch content estimation (%) of 16 genotypes in three environments

|         | SCE (%)  | $S_1^{(1)}$ | $S_1^{(2)}$ | $S_1^{(3)}$ | $S_1^{(6)}$ | TOP  | RS   | $NP_i^1$ | $NP_i^2$ | $NP_i^3$ |
|---------|----------|-------------|-------------|-------------|-------------|------|------|----------|----------|----------|
| $S_1^{(1)}$ | -0.170   | 0.530       |             |             |             |      |      |          |          |          |
| $S_1^{(2)}$ | 0.133    | 0.770**     | 0.624       | 0.000       |             |      |      |          |          |          |
| $S_1^{(3)}$ | 0.142    | 0.754**     | 0.905**     | 0.601       | 0.001       | 0.000|      |          |          |          |
| $S_1^{(6)}$ | 0.242    | 0.783**     | 0.895**     | 0.957**     | 0.366       | 0.000| 0.000| 0.000   |          |          |
| TOP     | 0.703**  | -0.122      | -0.017      | 0.035       | 0.151       |      |      | 0.592*  | 0.713**  |          |
| RS      | -0.515*  | 0.585*      | 0.486       | 0.365       | 0.317       | 0.041|      | 0.164   | 0.231    | 0.016    |
| $NP_i^1$ | 0.058    | 0.718**     | 0.708**     | 0.620*      | 0.688**     | 0.013|      | 0.713** | 0.963    | 0.002    |
| $NP_i^2$ | 0.712**  | 0.213       | 0.235       | 0.134       | 0.261       | 0.541*|      | 0.027   | 0.450    | 0.002    |
| $NP_i^3$ | 0.793**  | 0.222       | 0.377       | 0.315       | 0.456       | 0.528*|      | 0.019   | 0.863**  | 0.000    |
| $NP_i^4$ | 0.790**  | 0.216       | 0.367       | 0.316       | 0.454       | 0.516*|      | 0.019   | 0.832**  | 0.995**  |

Note: SCE= starch content estimation (%), * and **= significant at the levels of 5% and 1%; $S_1^{(1)}$, $S_1^{(2)}$, $S_1^{(3)}$, $S_1^{(6)}$ (Nassar and Huehn, 1987); Top (Fox et al., 1990); RS= Rank-sum by Kang (1988); $NP_i^1$, $NP_i^2$, $NP_i^3$, $NP_i^4$ (Thennarasu, 1995).

These results were similar to those reported by Rahadi et al. (2013), but are different from Sabaghnia et al. (2012) who reported that Thennarasu method had a high correlation with yield.

Principal component analysis (PCA) was described by Spearman’s correlation. Zulhayana (2010) showed the high and positive value of nonparametric stability index identified the dynamic stability genotype. The result of principal component analysis (PCA) in Figure 2 divides the nonparametric stability methods into three groups, C1, C2, and C3. Top, $NP_i^2$, $NP_i^3$, and $NP_i^4$ parameter and average starch content estimation (Y) are in the same group (C1), $S_1^{(1)}$, $S_1^{(2)}$, $S_1^{(3)}$, $S_1^{(6)}$, and $NP_i^2$ parameters are in group C2, whereas RS (Rank-Sum) is in group C3. Group C1 shows the concept of dynamic stability because Top, $NP_i^2$, $NP_i^3$, and $NP_i^4$ method has the highest and positive correlation with the starch content estimation. Mut et al. (2008) showed that Top and RS methods were included as dynamic stability, however RS in this study has the negative correlation. The
Figure 2. Principal component analysis of starch content estimation (Y) and 10 nonparametric stability methods in cassava genotypes in three environments, C1, C2, and C3.

Table 6. Ranking of starch content estimation (Y) and nonparametric stability of 16 genotypes in 3 environments

| Genotype   | Dynamic stability | Frequency | Static stability | Frequency |
|------------|-------------------|-----------|------------------|-----------|
| Y          | Top               | NP_i^2    | NP_i^3           | NP_i^4    |
| G61122     | 13                | 12        | 10               | 9         | 9         | 0         | 15        | 12        | 10        | 10        | 16        | 12        | 0         |
| G61142     | 2                 | 2         | 11               | 7         | 8         | 4         | 1         | 5         | 6         | 6         | 1         | 1         | 5         |
| G61143     | 14                | 12        | 12               | 3         | 4         | 0         | 5         | 3         | 2         | 3         | 10        | 6         | 3         |
| G61151     | 12                | 12        | 2                | 2         | 2         | 5         | 7         | 7         | 7         | 5         | 4         | 1         |
| G62143     | 10                | 12        | 5                | 12        | 12        | 0         | 13        | 16        | 15        | 15        | 16        | 15        | 0         |
| G62132     | 16                | 7         | 3                | 4         | 3         | 4         | 9         | 6         | 5         | 5         | 12        | 13        | 1         |
| G62151     | 4                 | 2         | 15               | 14        | 14        | 0         | 11        | 8         | 8         | 8         | 3         | 7         | 1         |
| G62153     | 15                | 12        | 1                | 1         | 1         | 5         | 6         | 1         | 1         | 1         | 6         | 2         | 6         |
| G63111     | 11                | 12        | 4                | 5         | 5         | 4         | 14        | 13        | 14        | 12        | 6         | 8         | 1         |
| G63121     | 7                 | 7         | 6                | 10        | 10        | 2         | 4         | 11        | 9         | 11        | 6         | 9         | 1         |
| G63124     | 5                 | 7         | 8                | 8         | 7         | 2         | 3         | 2         | 3         | 2         | 2         | 3         | 3         |
| G63142     | 1                 | 2         | 16               | 16        | 16        | 0         | 12        | 14        | 13        | 14        | 7         | 16        | 0         |
| G63143     | 8                 | 7         | 7                | 6         | 6         | 2         | 10        | 10        | 11        | 9         | 6         | 5         | 1         |
| G63153     | 9                 | 7         | 9                | 11        | 11        | 2         | 16        | 15        | 16        | 16        | 10        | 14        | 0         |

"Gajah"     | 6                 | 12        | 14               | 13        | 13        | 0         | 2         | 4         | 4         | 4         | 10        | 10        | 3         |

"Manggu"    | 3                 | 7         | 13               | 15        | 16        | 0         | 8         | 9         | 12        | 13        | 4         | 11        | 1         |

Note: Y = starch content estimation (%); S1^1, S1^2, S1^3, S1^6 (Nassar and Huehn, 1987); Top, Mid, and Low by Fox et al. (1990); RS by Kang (1988); NP_i^1, NP_i^2, NP_i^3, and NP_i^4 (Thennarasu, 1995).
effect of high environmental diversity caused the changes in stability value.

Static stability concept can be used to select the best genotype with the highest production in different environmental conditions, the stability of genotype was also determined by ranking frequency (Table 6). Ranking frequency of two stability methods (dynamic and static) shows the information about genotype stability with the starch content estimation (\%).

Static and dynamic stability concepts depend on range of region and testing sites, the more extensive the range of testing sites the more diversity in site conditions. Table 6 shows the top five ranks based on starch content estimation (\%) are G61142, G62151, G63124, G63142, and "Manggu". The nine genotypes categorized as statically stable (yield stable in each environment) are G61151, G62153, G61142, G62132, G63111, G63121, G63124, G63143, and G63153. The static stability concept is highly dependent on the range of region and testing sites.

Twelve cassava genotypes are categorized as dynamically stable (adaptation followed the environmental index); they are G62153, G63124, G61142, G61143, Gajah, G61151, G62132, G62151, G63111, G63121, G63143, and "Manggu". The dynamic stability concept is more appropriate as the basis of selecting genotypes at the time of releasing genotype varieties because can explain the stability and adaptability of a genotype. G61142 was categorized as statically and dynamically stable, the most stable genotype and potential to released as new variety. This is related to the purpose of releasing varieties that still need improvement in starch content estimation quantity.

**Conclusion**

Variant analysis of the combined 16 cassava genotypes tested in three environments showed that the interaction between genotype and location did not have significant effects in each environment. Cassava genotypes, however, demonstrated different starch content estimation measured by gravimetric method. G63142 had the highest starch content estimation of 29.99\%, whereas the top five genotypes based on the percentage of starch content estimation are G63142, G61142, local variety "Manggu", G62151, and G63124. G61142 was categorized as statically and dynamically stable, the most stable genotype, and has potentials to be released as a new variety. G63124 was categorized as statically stable, whereas G63142, G62151, and local "Manggu" had the highest diversity and high percentage of starch content estimation.

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