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Positive Impact of Similarity on Twice Single Seed Descent of Purification on Bambara Groundnut (*Vigna subterranea* L. Verdcourt)

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ABSTRACT

The main problem of local line Bambara groundnut (*Vigna subterranea* L. Verdcourt) genotype improving is the low of purity. The research on genetic purification of Bambara groundnut had finished in twice single seed descent. The objective of this research was to find out the uniformity gain level within lines each other after single seed descent applied twice. This research was conducted in Agriculture Research Station, Universitas Brawijaya, from 2014 to 2016. There were 20 selected local lines evaluated twice with single seed descent method. On the first and second evaluation, it was applied on previous 19 local lines and 1 check genotype. Similarity coefficient was analyzed with cluster analysis of qualitative characters within lines. Variability within line was described on quantitative characters. The local lines had different response on single seed descent selection. These differences depended on genetic of qualitative characters. On the first single seed descent, it got similarity coefficient of 0.704-0.832. On the second one, it got similarity coefficient of 0.804-1.00. Increasing of similarity coefficient from 0.10-0.168 will improve purification of local lines 15.76-20.19 %. Two circles of single seed descent selection were capable to purify local lines. Variability of quantitative character depended on kind of character.

INTRODUCTION

Bambara groundnut (*Vigna subterranea* L. Verdcourt) is one of underutilized crops that grows in South East Asia, like Indonesia, Malaysia and Thailand. This beans are more adaptive and drought tolerant in low fertility soil (Kuswanto, Waluyo, Pramantasari, & Canda, 2012). This plant is able to fix nitrogen in soil through a symbiosis with Rhizobium bacteria, like the other leguminous (Ntundu, Bach, Christiansen, & Andersen, 2004), so the nitrogen needed is always enough. This crop is a drought tolerant plant which plays its role in adapting to climate changes. It becomes a genetic resource to improve plant variety which has resistance to biotic and abiotic stress (Padulosi, Heywood, Hunter, & Jarvis, 2011).

Underutilized crop can increase food security by eliminating the risk of over dependency on the limited main crops. Besides that, it could

support plant diversification effort and increase sustainable agricultural development by reducing cultivation input such as reducing the application of nitrogen fertilizer so it can diminish agricultural sector contribution in increasing the greenhouse gas effect (Mayes et al., 2012). To enhance attainment of potential yield of the crop, it requires the production of Bambara groundnut in areas with similar environmental conditions (Pungulani, Kadyampakeni, Nsapat, & Kachapila, 2012).

Since the Bambara groundnut landraces are not homogenous and are only adapted to certain environments, there is a lack of uniformity in performance of these landraces if grown under varied environmental conditions. There is, therefore a need to come out with improved varieties, to promote and enhance cultivation of Bambara groundnut across the region and the country at large (Akpalu, Atubilla, & Oppong-Sekyere, 2013).

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The important issue to develop the local lines of Bambara groundnut is genetic diversity. There are some characters that showed diversity in one lines, such as seed color, seed shape or growth habit. The differences of its qualitative character show a different genetic trait (Kuswanto, Waluyo, Pramantasari, & Canda, 2012). Plant breeding on Bambara groundnut is exposed to some problems of local varieties. The main problem of Bambara groundnut genotype improvement is the low of purity. Purity is the most important part in developing self-pollinating crop in Indonesia. Bambara groundnut is a self-pollinating crop, so purity is important to be developed to create pure superior varieties. Purity will determine a uniformity of local varieties

The research was started in 2014 to purify the local line based on plant and seed characters. The previous research from Nuryati, Soegianto, & Kuswanto (2014) had 50 genotypes based on the selection of seed characters. Purification process had been done based on pod and seed characteristics. Pod and seed characteristic from every local line had high variability. According to Oyiga & Uguru (2011), the primary components of Bambara groundnut yield are the number of pods per plant and seed weight. Seed weight shows correlation with the number of flower per plant and the number of pod per plant (Jonah, 2011; Jonah, Aliyu, Jibung, & Abimiku, 2013). Study of correlation in character which has a high yield is more effective for selection of superior genotypes. Shape, color and texture of pods from one location show considerable variation. The same thing happens in the shape, color and texture of seed. This variability on pod and seed characteristics can be used as material for breeding program in developing new varieties.

The research on genetic purification of Bambara groundnut had finished in twice single seed descent. There were 20 selected genotypes from previous research Nuryati, Soegianto, & Kuswanto (2014). The plant selection was based on single seed descent so it will improve purity of the line gradually. Breeder would take one seed of plant to be planted on next planting. One seed is assumed as one genotype which heritage the same genetic characters. The application of single seed descent will improve purity. Purification will become

perfect if a single seed descent is applied more than once.

The research served a new information about possibility on single seed descent application to purify local varieties. So, the objective of this research was to evaluate the improving purity of local line of Bambara groundnut and to find out the uniformity gain level within lines after single seed descent applied twice.

MATERIALS AND METHODS

The research consisted of 3 observations: evaluation of qualitative character on collected lines, application first single seed descent and application of second single seed descent, respectively. This research was conducted in Agriculture Research Station, Universitas Brawijaya, Indonesia, from 2014 to 2016. The materials used in this research were 20 selected genotypes of Bambara groundnut. They were the selected line by single seed descent from previous research. Selection was conducted among collected 50 lines that had distinct characters and a sufficient number of seeds as material for evaluating genetic relationship and variability. The material of the first research was based on the same characters between parent and progenies that were selected by single seed descent. Selected seeds were planted as material of the second research. From all of researches, they were sowed and observed the qualitative and quantitative characters.

The observation on quantitative and qualitative characters of every individual plant was done following the International Plant Genetic Resources Institute descriptors for Bambara groundnut (IPGRI, 2000). Qualitative characters are the growth habit, terminal leaflet shape, color of terminal leaflet, pigmentation on hypocotyls, pigmentation on flower, stem hairiness, pod shape, pod color, pod texture, seed shape, and seed color. While quantitative characters are days of the first flowering, number of flowers per plant, age of harvest, number of pods per plant and fruit set.

Genetic uniformity was described by Simple Matching Coefficient. It analyzed on qualitative character using cluster analysis through Multivariate Statistical Package 3.1 program. Data analysis of quantitative character used evaluation of variability by coefficients of variance.

RESULTS AND DISCUSSION

Evaluation of 50 Selected Local Lines

The evaluation of genetic variability was from selected 50 local lines on 24 quantitative characters. The variation of each line had narrow variability for plant height, canopy width, number of leaves, petiole length, terminal leaflet length, terminal leaflet width, internode length, flowering age, banner length, peduncle length, banner length, days of fresh harvest, harvesting seed, pod length, pod width, number of stems, number of nodes per stem, seed length and seed width, 100 seeds weight and shelling percentage. Three other characters were included the number of flowers per plant, weight of fresh pod per plant and number of pod per plant which showed that there were lines which had narrow variability and there were lines that had broad variability.

Local line of GSG 3.5, GSG 2.4 and WSS 2.2.2 line had broad variability in number of flowers per plant character. There were 32 purified local lines that had broad genetic variability and 18 purified local lines that had narrow genetic variability in fresh pod weight per plant character. While for number of pods per plant, there were 37 purified local lines which had narrow genetic variability and 13 lines with broad genetic variability (Nuryati, Soegianto, & Kuswanto, 2014). Table 1 describes the total of 50 selected local lines from collected lines.

Increasing Genetic Similarity within Lines

These local lines were planted as material in this research and were observed the qualitative characters. There were 20 local lines selected from previous research were analyzed with cluster analysis. Cluster analysis applied to analyze genetic relationship among plant individually. Cluster analysis was based on qualitative characters. Qualitative characters controlled by monogenic (1-2 genes) and non-response to environmental factors. So in the case, variability of qualitative characters indicated a variability of genetic factors. On the first single seed descent, it got similarity coefficient of 0.704-0.832. On the second single seed descent, it got similarity coefficient of 0.804-1.00. The increasing of similarity coefficient from 0.10 until 0.168 will improve purification of local lines 15.76-20.19 %, and it depends on genotype.

On the first single descent, only 5 genotypes of Bambara groundnut (CCC 1.4.1, SS 2.2.2, GSG 1.5, BBL 10.1, and CCC 1.1.1) had similarity coefficient more than 0.80. This value explained a single seed descent must be continued to second selection. The higher of similarity coefficient, the more uniform of plants character of a genotype will be so that it is pure genetically. According to Pandin (2010) the degree of similarity which used genetic matrix can be divided into four categories: very close resemblance (very good) $r > 0.9$; good $0.8 < r < 0.9$; less good $0.7 < r < 0.8$; bad $r < 0.7$. The level of genetic similarity of a population can be described by a genetic distance from individual members of the population. The closer its genetic distance among individuals in a population, the more uniform the population will be. The farther its individual genetic distance in a population, the more population has become increasingly diverse.

On the second single descent, almost all of genotypes had similarity coefficient of more than 0.80. There was one genotype, GSG 312, had a perfect similarity coefficient, because it had value 1 (100 %). It could be said that line GSG 312 had full purified. Uniformity within population was determined with genetic distance among plant individually. The closer genetic distance among plants, the more uniform their genetic characters will be. Similarity coefficient is 1 (100 %) means the genetic distance within line GSG 312 is 0 %, so all characters of GSG 312 were same or uniform. Uniformity among individual plant within same line is assumed to be caused by a relative relationship (Austi, Damanhuri, & Kuswanto, 2014) as impact in line of parent (Putri, Sutjahjo, & Jambormias, 2014).

Table 2 also shows about the increasing similarity coefficient from first single seed descent to second one. The increasing from 0.032 until 0.217 and the average increasing is 0.095. Increasing of similarity is logical process in single seed descent. Plant selection based on one seed described the best selected genotype from previous population. One seed will produce many seeds and all of seeds have same genetic characters. But, sometimes, segregation process will make offspring diverse. A Single seed descent needs to be applied more than once, so it will be gained purified line.

Table 1. Selected 50 local lines with their qualitative pod characters

No	Name of lines	Pod shape	Seed color	Seed shape	Seed texture
1	BBL 10.1	two-seeded pod	Black	other	Smooth
2	BBL 2.1.1	without point	Black	round	Smooth
3	BBL 2.3.1	without point	dark brown	round	Smooth
4	BBL 2.4	without point	Brown	mixture	Smooth
5	BBL 5.3.1	without point	dark brown	round	Smooth
6	BBL 5.3.2	without point	dark brown	oval	Smooth
7	BBL 6.1.1	without point	Black	round	Smooth
8	BBL 6.2.1	without point	dark purple with spot	round	Smooth
9	BBL 6.3.1	without point	dark brown	round	Smooth
10	CCC 1.1.1	without point	Black	round	Smooth
11	CCC 1.3.1	without point	dark purple	round	Smooth
12	CCC 1.4.1	without point	dark purple	round	Rough
13	CCC 1.5	without point	Brown	mixture	Smooth
14	CCC 2.1.1	without point	Black	round	Smooth
15	CKB 1	Un-identified	Black	Un-identified	Smooth
16	GSG 1.1.1	without point	Black	round	Smooth
17	GSG 1.4	without point	Black	mixture	Rough
18	GSG 1.5	without point	dark purple	mixture	Rough
19	GSG 1.6	without point	Brown	mixture	Smooth
20	GSG 2.1.1	ending in a point,	Black	round	Smooth
21	GSG 2.2.1	ending in a point,	black with spot	round	Smooth
22	GSG 2.4	ending in a point,	Black	mixture	Rough
23	GSG 2.5	ending in a point,	dark purple	mixture	Rough
24	GSG 3.1.2	without point	Black	oval	Smooth
25	GSG 3.2.1	without point	black with spot	round	Smooth
26	GSG 3.3.1	without point	dark brown	round	Smooth
27	GSG 3.3.2	without point	dark brown	oval	Smooth
28	GSG 3.5	without point	Brown	mixture	Smooth
29	GTKB 1	Un-identified	Black	Un-identified	Smooth
30	JLB 1	Un-identified	Black	Un-identified	Smooth
31	LLB 1	Un-identified	Black	Un-identified	Smooth
32	MBG 1.1.2	without point	Black	oval	Smooth
33	MBG 1.2.1	without point	black with spot	round	Smooth
34	MBG 3.1.1	without point	Black	round	Smooth
35	MBG 3.3.1	without point	dark purple	round	Smooth
36	MBG 5.1.1	without point	Black	round	Smooth
37	MBG 5.3.1	without point	dark purple	round	Smooth
38	MBG 7.1	two-seeded pod	Black	other	Smooth
39	TKB 1	Un-identified	Black	Un-identified	Smooth
40	WSS 1.1.2	without point	Cream	oval	Smooth
41	WSS 1.2.2	without point	Brown	oval	Smooth
42	WSS 1.3.2	without point	dark brown	oval	Smooth
43	WSS 1.4.2	without point	dark purple	round	Smooth
44	WSS 2.2.2	ending in a point,	Brown	oval	Smooth
45	WSS 2.3.2	ending in a point,	dark brown	oval	Smooth
46	WSS 3.1.2	without point	Cream	oval	Smooth
47	WSS 3.2.2	without point	Brown	oval	Smooth
48	WSS 4.3.2	without point	dark purple with spot	oval	Smooth
49	WSS 6.3.2	ending in a point,	dark purple with spot	oval	Smooth
50	WSS 8.2	two-seeded pod	dark purple	other	Smooth

Table 2. Similarity Coefficient on first and second of single seed descent

No.	Genotype	First single seed descent	Second single seed descent	Increasing of similarity
1	BBL 10.1	0.825 - 1.00	0.875-1.00	0.050
2	BBL 2.1.1	0.756 - 0.957	0.856-1.00	0.100
3	BBL 6.1.1	0.709 - 1.00	0.875-1.00	0.064
4	CCC 1.1.1	0.808 - 1.00	0.846-1.00	0.038
5	CCC 1.4.1	0.807 - 1.00	0.856-1.00	0.049
6	CCC 2.1.1	0.743 - 0.957	0.875-1.00	0.032-0.043
7	CKB1	0.781 - 1.00	0.833-1.00	0.052
8	GSG 1.1.1	0.746 - 1.00	0.804-1.00	0.0574
9	GSG 1.5	0.814 - 1.00	0.875-1.00	0.061
10	GSG 2.1.1	0.704 - 0.957	0.875-1.00	0.171
11	GSG 2.4	0.750 - 1.00	0.875-1.00	0.105
12	GSG 2.5	0.760 - 1.00	0.856-1.00	0.096
13	GSG 3.1.2	0.783 - 1.00	1.00	0.217
14	JLB 1	0.715 - 1.00	0.844-1.00	0.129
15	PWBG 3.1.1	0.764 - 0.957	0.781-1.00	0.017
16	PWBG 5.1.1	0.720 - 0.957	0.859-1.00	0.139
17	PWBG 5.3.1	0.702 - 0.957	0.846-1.00	0.144
18	PWBG 7.1	0.761 - 1.00	0.807-1.00	0.046
19	TKB1	0.793 - 1.00	0.864-1.00	0.071
20	UB Cream	0.832 - 1.00	0.875-1.00	0.043
	Average	0.764-0.987	0.859-1.00	0.095

The main qualitative character support variability was a difference in seed color character. Seed color often disturbs uniformity of lines. On the first observation, the genotype of Bambara groundnut showed that there were differences between progenies and parents. For example, CCC 1.1.1 lines had black color of seed. The progenies of this line had seed with cream color (1.31 %), brown (4.34 %), black (59.57 %), black with brown spots (10.56 %), dark brown (11.86 %) and dark purple (12.36 %). It happened because there was a segregation of heterozygous genotype. Seed color of quantitative character was not influenced by the environment but controlled by a single gene (Nuryati, Soegianto, & Kuswanto, 2014). Second observation, in genotype CCC 1.1.1 plant number 3 with early planted seed color was black, and also the progenies had seed with brown reddish color 11.11 %, black 44.44 %, brown 5.56 % and dark purple 38.89 %. It was the reason why single seed descent was chosen in this research.

The difference in seed color on Bambara groundnut also found in other research, such as Zimbabwe Red, the seeds were quite uniform in color, with a low segregation into different red color. After three evaluation seasons, it showed less segregation in seed color. There were also

black seed color in Garborone Black (Africa), it segregated into other color, especially brown and the most common seed color was black and brown on a single pod (Heller, Begemann, & Mushonga, 1997). The same thing was described by Ouedraogo et al. (2008), there was a segregation seed color in progenies, farmers in Burkina Faso who used seeds from prior plantation period, stated that the color of seed change year by year.

Variation of Quantitative Characters

The evaluation of variability in phenotypic was done by coefficients of variation in each quantitative character (Table 3 and Table 4). Flowering age and (sometimes) harvesting age are controlled by monogenic, so both of them have minimum response to environmental factors. The late flowering has a detrimental effect on seed yield of in the savannah zone. In areas with marginal rains, earlier flowering confers an advantage of forming more pods and seeds and consequently higher yields (Abu & Saaka, 2011).

Table 3 shows that coefficient of variation in days of first flowering and harvesting age has a low value, less than 10 %. The low to medium value of variation coefficient shows a uniformity in those plants (Rozika, Murti, & Purwanti, 2013).

Nuryati, Soegianto, & Kuswanto (2014) stated that the average of flowering age of Bambara groundnut was 47 days and average of harvesting age was 128 days. According to Shaumi, Chandria, Waluyo, & Karuniawan (2012) if the variability is low, so selection have to be stricter in order to achieve a desired genotype uniformity on that character because the individual of its population is relatively uniform. But, it is not recommended that the selection of local line of Bambara groundnut to be based on quantitative characters. The selection strategy should be able to single out genotype which has excellent genes and is still expressed in every condition (Mustikarini, Ardiarini, Basuki, & Kuswanto, 2017).

The other characters had high response to environment and controlled by polygenic. The quantitative character from the observation had high coefficient of variation. Quantitative character was controlled by polygenic and each gene contributes little portion to phenotype. The quantitative characters relatively had a larger environmental component than the genetic component. The high value of variation coefficient showed a large

variety trait of the population. The high variability showed a diversity in plant character, so it still needed a selection of superior character to achieve uniform population (Shaumi, Chandria, Waluyo, & Karuniawan, 2012).

The difference of variation coefficient value gave a different information with analysis in qualitative character. On qualitative character, which cluster analyzed applied, stated that among individual within line was more uniform than quantitative characters. In other hand, the qualitative character analysis was better to evaluate uniformity or purity of Bambara groundnut, both of vegetative and reproductive characters.

The application of first single seed descent and second one had the same pattern of coefficient of variation. Although flowering age and harvesting age were the characters with low value, but other characters had a high value. The high of variation coefficient was supported by environment effect. It can be concluded that purification of Bambara groundnut was not suitable when evaluated from quantitative characters.

Table 3. Coefficient variation on first single seed descent

No.	Genotype	Flowering age	Flower number	Harvesting age	Pod number	Fruit set
1	BBL 10.1	10.52	30.28	6.12	32.30	27.25
2	BBL 2.1.1	11.33	31.02	5.91	42.21	46.08
3	BBL 6.1.1	9.39	44.33	6.30	63.13	44.75
4	CCC 1.1.1	9.77	36.65	7.53	53.62	55.96
5	CCC 1.4.1	9.42	47.07	5.89	64.57	41.71
6	CCC 2.1.1	5.22	47.75	6.16	45.80	49.99
7	CKB1	12.24	35.88	5.49	44.35	35.99
8	GSG 1.1.1	6.38	53.03	7.46	59.48	63.47
9	GSG 1.5	12.79	23.29	6.16	24.53	18.25
10	GSG 2.1.1	9.26	65.07	7.69	67.32	51.24
11	GSG 2.4	12.45	50.52	5.75	66.95	56.53
12	GSG 2.5	8.96	19.60	6.25	52.19	46.32
13	GSG 3.1.2	9.16	34.45	6.26	29.81	22.31
14	JLB1	5.30	34.03	6.93	39.02	33.48
15	PWBG 3.1.1	6.12	49.94	7.27	64.24	67.90
16	PWBG 5.1.1	10.73	61.16	6.05	51.82	37.73
17	PWBG 5.3.1	11.84	32.41	6.08	38.00	32.59
18	PWBG 7.1	6.87	27.82	6.51	38.05	26.48
19	SS 2.2.2	7.03	58.56	4.29	54.42	41.01
20	TKB1	8.39	38.69	6.54	54.47	39.10
	Average	9.16	41.08	6.33	49.31	41.91

Remarks: CV (low: 0-25 %), (medium: 25-50 %), (high: 50-75 %), (very high: 75-100 %)

Table 4. Coefficient of variation on second single seed descent

No.	Genotype	Flowering age	Flowers number	Harvesting age	Pod number	Fruit set
1	BBL 10.1	7.12	19.38	3.97	19.35	14.11
2	BBL 2.1.1	7.04	30.77	4.46	42.38	21.69
3	BBL 6.1.1	9.54	26.46	3.59	33.4	22.69
4	CCC 1.1.1	7.48	22.26	4.48	30.7	22.12
5	CCC 2.1.1	8.01	25.21	7.29	38.01	19.7
6	CCC1.1.1	8.84	29.9	4.29	34.9	35.94
7	CKB 1	17.09	38.46	6.89	40.38	17.85
8	GSG 1.1.1	5.61	15.53	3.2	19.83	17.38
9	GSG 1.5	7.09	19.75	4.48	31.46	15.07
10	GSG 2.1.1	5.88	29.97	3.68	29.91	16.04
11	GSG 2.4	8.16	21.61	3.8	20.07	9.88
12	GSG 2.5	8.57	25.98	4.88	43.57	26.21
13	GSG 3.1.2	8.25	23.87	2.79	32.52	14.71
14	JLB 1	7.21	22.87	2.74	30.42	14.56
15	PWBG 311	6.39	22.01	3.24	30.6	18.01
16	PWBG 511	7.19	17.06	3.85	25.51	17.49
17	PWBG 531	8.57	24.59	3.97	31.46	13.26
18	PWBG 7.1	8.58	24.85	2.42	25.45	20.15
19	TKB 1	7.69	32.29	2.96	51.21	30.18
20	UB Cream	3.31	17.81	2.12	40.46	20.22
	Average	7.88	24.53	3.96	32.58	19.36

CONCLUSION

Similarity of coefficient from the first single seed descent was less than 0.80, so it must be continued with the second one. Two circles of single seed descent selection were capable to purify Bambara groundnut of local lines until more than 0.80. Simple similarity coefficient of qualitative character described a genetic distance among plant individual within line population. The quantitative characters had high environment effect on phenotype so it could not be used to evaluate the purity of Bambara groundnut.

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