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Inheritance and comparison of phenotypic characters of cultivated butternut pumpkin (*Cucurbita moschata* (Duchesne) Poir 'Butternut')

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ABSTRACT. Butternut pumpkin (*Cucurbita moschata* (Duchesne) Poir 'Butternut') is a nutrient-rich horticulture plant which provide a high economic value. To meet the market's demand, butternut pumpkin must has a prime and maximum phenotypic characters both in quantity and quality. This research aims to examine the phenotypic characters and gene interactions that occurs in butternut pumpkin. Butternut pumpkin were cultivated in two different places (screen house and agricultural land) and using two different seed, cultivated seeds by Laboratory of Genetics and Breeding in Faculty of Biology Universitas Gadjah Mada (noncommercial) and Panah Merah seeds (commercial). Observation of phenotypic character was carried out for 3-4 months starting from planting to harvesting. About thirteen phenotypic characters was analyzed using quantitative data analysis of variance (one way ANOVA) and Chi-Square test. Based on the result, it is known that noncommercial and commercial butternut pumpkin have 10 different quantitative characters. Noncommercial butternut pumpkin has uniform phenotypic characters but unstable in size of plant (stem diameter 1.22 ± 0.21 and 1.37 ± 0.34 cm) and fruit's shape (dumbbell, pyriform, and disc). Noncommercial butternut pumpkin has dominant epistasis gene interaction with ratio of 12 (dumbbell): 3 (pyriform): 1 (disc). Commercial butternut pumpkin has uniform and stable phenotypic characters with dumbbell fruit's shape.

Keywords: *Cucurbita moschata* 'Butternut'; colour chart; gene interaction; phenotypic characters; recessive allele

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INTRODUCTION

Indonesia is a country that has high biodiversity both in flora and fauna. This biodiversity is often used by the community to develop the potential owned by flora and fauna in Indonesia. Pumpkin is a type of plants which has high biodiversity and has the potential to be developed as a source of food. This development is useful to overcome the food crisis faced by Indonesia nowadays. Pumpkin has a high diversity of cultivars, one of it being the butternut pumpkin.

Butternut pumpkin (*Cucurbita moschata* (Duchesne) Poir 'Butternut') is an herbaceous plant that has a branching and vining stem and almost all parts of the plant is covered by sharp fine hair (Sari *et al*., 2017; Suwanto *et al*., 2015). The stem is round or pentagonal with twisted tendrils (Tedianto, 2012). Single leaf, grayish green, heart-shaped (cordate), and its diameter can reach up to 20 cm (Tedianto, 2012). The flower is actinomorphic or irregular and bell-shaped (companulatus). Male and hermaphrodite flower can exist in one plant. Butternut pumpkin has a unique fruit's shape that resembles pear/dumbell (a perfect character standart of butternut pumpkin), contains high level of sweetness, and has a good taste. Fruit's color can be yellow, brownish yellow, or orange. The fruit can weigh up to 2-5 kg and can be harvested after 3-4 months (Tedianto, 2012).

Butternut pumpkin has a high nutrient content. The fruit contains β-karoten, carbohydrates, proteins, carotenoid, calcium, phosphor, iron, vitamin B, vitamin C, and a high fiber content (Ranonto *et al*., 2015). This high nutrient content makes butternut pumpkin very potential to be used as an alternative staple food, thus if optimized should be able to help overcome the food crisis (Suwanto *et al*., 2015).

Butternut pumpkin is cultivated in large areas of land. To meet the market's demand, butternut pumpkin must have prime and maximum phenotypic characters both in quantity and quality. In this research, butternut pumpkin that was previously cultivated in the screen house is moved to the agricultural land. This transition makes the plant must be able to adapt to new environment that is shown by the phenotypic and genotypic stability. This shift makes the plant produce certain variations. Variations can be either phenotypic variations or genotypic variations. Phenotypic variations can be seen by observation of morphological characters in plant organs. Meanwhile, genotypic variations can be seen by DNA observation. Genotypic variations will cause in diversity of the fruit's shape so continuous plant breeding needs to be done to get the perfect fruit's shape (Nopianasanti, 2018). Therefore, this research was conducted to examine the stability of butternut pumpkin. This research is expected to provide information and description about plant phenotypic variability butternut pumpkin for selection the ancestor to get high quality of seed production.

MATERIALS AND METHODS

This research was done in the screen house and agricultural land in Jamusan, Sleman, Yogyakarta. There were fourty five sampels of butternut pumpkin that cultivated using noncommercial and commercial seeds. About 15 noncommercial butternut pumpkin cultivated on the agricultural land, and 15 commercial butternut pumpkin cultivated on the agricultural land. The noncommercial seeds are the fourth pure strain seeds obtained from Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada. The commercial seeds were obtained from seeds company called PT. East West Seed Indonesia (EWINDO) with trademark of Panah Merah.

Preparation before planting. There are several steps to be done before planting, such as soil plowing, preparation of planting area, fertilization, and irrigation.

Seed germination and planting. Seeds are sown in a room with warm temperature with a tray of sand, cloth, water, and a 10-watt lamp for temperature regulation. Incubation is carried out for 4 days under the light. Sprouts that start growing are moved one by one into polybags. Sprouts that are 1 week old are moved to be planted on land. Noncommercial seeds are cultivated in the screen house and agricultural land. Meanwhile, commercial seeds are cultivated on the agricultural land.

Care and maintenance of plants. Plants in the screen house are watered every 3 days, while plants in the agricultural land are watered once when planting. The planting process is accompanied by the addition of lime to stabilize the pH of soil. Fertilization and pesticides spraying are carried out twice per month in the vegetative period. 3 weeks old plants are given a bamboo as support. The branches or leaves that are attacked by pests are trimmed. Every 3 days, the growing stem is wrapped around the bamboo so the plant can grow vertically. Some branches that are close to the ground are trimmed so the growth does remain vertically. Plants in the screen house were treated with castration and pollination, while plants on the agricultural land were treated with open pollination. Harvesting is done when the fruit is brown evenly, has a distinctive pumpkin aroma, and there is a loud sound when tapped. After harvesting, the seeds are taken and dried to be replanted.

Observation of phenotypic characters stability. The phenotypic characters that were observed consist of qualitative and quantitative characters. Qualitative characters include the shape and color of leaves, stems, flowers, seeds, and fruits. The fruit's shape is observed with IBPGR guide. Colors are determined using RHS Colour Chart. Taste of the fruits are determined using hand refractometer Atago. Quantitative characters include fruit length, fruit diameter, fruit weight, fruit skin thickness, fruit flesh thickness, and the amount of viable seeds per fruit.

Data analysis. Quantitative characters are analyzed using quantitative data analysis of variance (One way ANOVA) with randomized group design by calculating the range, mean, variety, standard deviation, and coefficient of phenotype diversity. Qualitative characters are analyzed with descriptive methods using multiple scoring. Fruit's shape data is analyzed using Chi-Square Test to observe the gene interaction.

RESULTS AND DISCUSSION

This research was conducted by observing quantitative and qualitative characters of noncommercial and commercial butternut pumpkin cultivated in the screen house and agricultural land. Plant breeding is an effort to obtain prime variety products through crossing and the use of intensive agricultural technology (Qaim, 2020; Dierig *et al*., 2011). Plant breeding through crossing produce a hybrid plant with nonidentical traits from the parental. This plant frequently segregates when crossed, necessitating strain purification (Gerald *et al*., 2013; Edlich-Muth *et al*., 2016). Pure strain is an individual with homozygous genotype resulting from repeated crossing and has stable characters (Daryono & Maryanto, 2017). A total of 45 samples are used in this research. Results of this research were fruit's shape characters, stability test using quantitative characters, number of fruit's shape population of noncommercial butternut pumpkin, and Chi Square test result on noncommercial butternut pumpkin.

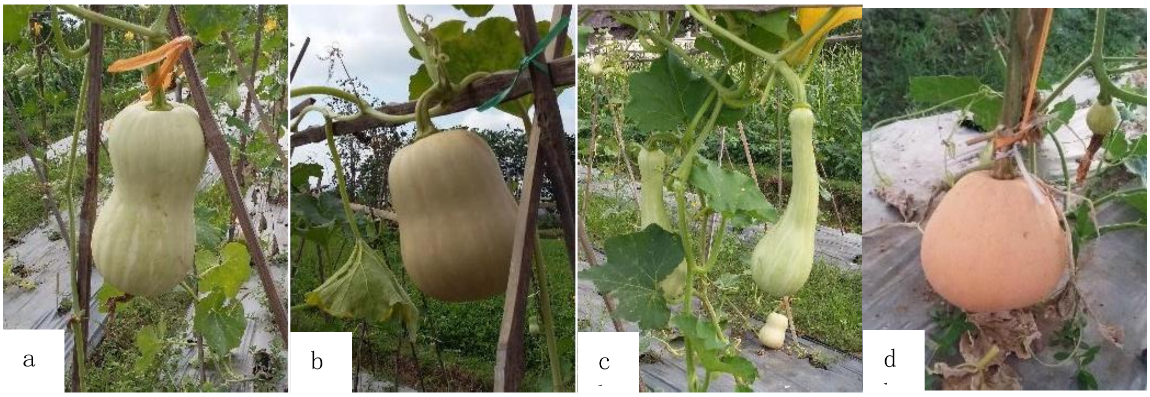

Fig. 1. Fruit's shape characters: a. Pear; b. Paprika; c. Pyriform; d. Disc.

Noncommercial butternut pumpkin produced three different shapes, which are dumbbell, pyriform, and disc. Meanwhile, commercial butternut pumpkin produced only one fruit shape, which is dumbbell. It is known that commercial butternut pumpkin has a stability in producing phenotypic characters especially in the fruit's shape because it has passed quality test of product.

Differences in productivity and phenotypic characters between land and screen houses are caused by different environmental variability such as sunlight intensity, humidity, air temperature, pests and plant pests (Muttaqien & Rahmawati 2019; Naim-Feil *et al*., 2017; Ping *et al*., 2013).

Table 1. Stability test using quantitative characters (One way ANOVA)

No	Phenotypic characters	Mean G4 Screen house Jamusan (NC)	G4 agricultural land Jamusan (NC)	G1 agricultural land Jamusan (C)
1	Stem diameter (cm)	1.22 ± 0.21b	1.37 ± 0.34b	0.78 ± 0.21a
2	Leaf length (cm)	17.85 ± 1.94c	15.07 ± 1.39b	13.08 ± 2.18a
3	Leaf width (cm)	23.65 ± 2.60c	20.21 ± 2.07b	18.07 ± 3.36a
4	Fruit weight (kg)	1.09 ± 0.35b	0.63 ± 0.24a	0.88 ± 0.42ab
5	Fruit horizontal circumference (cm)	28.10 ± 7.67b	22.59 ± 6.04a	21.35 ± 2.13a
6	Fruit vertical circumference (cm)	51.13 ± 8.14b	41.99 ± 6.06a	48.83 ± 8.35b
7	Fruit top diameter (cm)	2.67 ± 0.45b	2.20 ± 0.30a	2.25 ± 0.28a
8	Fruit bottom diameter (cm)	1.81 ± 0.57b	1.19 ± 0.32a	1.33 ± 0.25a
9	Fruit horizontal diameter (cm)	8.83 ± 1.93a	7.75 ± 1.62a	7.83 ± 0.90a
10	Fruit vertical diameter (cm)	17.64 ± 4.68b	14.96 ± 2.95a	18.77 ± 2.85b
11	Brix	9.73 ± 1.49b	8.47 ± 2.10ab	7.40 ± 2.03a
12	Fruit skin thickness (cm)	0.20 ± 0.05a	0.19 ± 0.35a	0.18 ± 0.04a
13	Fruit flesh thickness (cm)	8.48 ± 1.95a	7.93 ± 1.12a	7.63 ± 0.84a

Notes: NC= non-commercial; C= Commercoal; Data is attached with mean ± standard deviation value. Same letter on the same line indicates that the result is not significantly different based on Duncan Test with significance level of 5%

Based on the result of phenotypic characters observation, it is known that butternut pumpkin has three different shapes of fruit: pear or paprika (dumbbell), pyriform, and disc. Our previous research (Nopianasanti, 2018) showed that paprika-shaped fruit is only plastic and is the result of differences in nutrient transport and photosynthate translocation due to environmental influences which produce different shape of paprika and pear. Dumbbell still shown to be the dominant alleles in the expression of fruit's shape. Pyriform is a form of recessive phenotype expression from new genotype characters originating from the unification of dominant and recessive alleles. Disc is a newly formed shape caused by unification of recessive alleles because of segregation.

Based on Table 1, it is known that the qualitative phenotypic characters of noncommercial and commercial butternut pumpkin indicate no significant differences for fruit horizontal diameter, fruit skin thickness, and fruit flesh thickness. Phenotypic characters such as stem diameter, leaf length, leaf width, fruit weight, fruit horizontal circumference, fruit vertical circumference, fruit top diameter, fruit bottom diameter, fruit horizontal diameter, fruit vertical diameter, and brix indicate significant differences for noncommercial and commercial butternut pumpkin. This result shown that butternut pumpkin has uniform but unstable quantitative phenotypic characters at significance level of 5%.

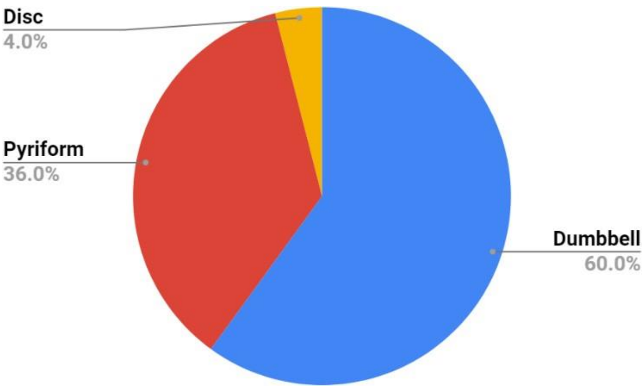

Fig. 2. Population of noncommercial butternut pumpkin.

On Fig. 2, it is known that dumbbell shape is the biggest shape produced by 60%. Meanwhile, pyriform is only 36% and disc only 4%. Based on the data, it is known that dominant allele is shown by dumbbell shape, while recessive allele is shown by disc shape. The value of dominant allele frequency is 0.71 and 0.29 for recessive allele.

Table 2. Chi-Square Test on noncommercial butternut pumpkin.

Characters	O	e	(o-e)	$(o-e)^2$	$(o-e)^{2/e}$
Screen house					
Dumbbell	16	11.73	4.27	18.19	1.55
Pyriform	6	9.39	-3.39	11.48	1.22
Disc	1	1.88	-0.88	0.77	0.41
				x2	3.18
Agricultural land					
Dumbbell	21	27.55	-6.55	42.92	1.56
Pyriform	30	22.04	7.96	63.35	2.87
Disc	3	4.41	-1.41	1.98	0.45
				x2	4.88

Notes: test was done using significance level of 5%, df = 2, p = 5.99.

The Chi-Square test was conducted using the ratio of dominant epistasis to determine the gene interaction happened in noncommercial butternut pumpkin. Chi-Square Test result using fruit's shape characters of noncommercial butternut pumpkin shown that the value of x2 is less than the critical value (p = 5.99). This indicates that there are no significant differences and noncommercial butternut pumpkin was proven to have a dominant epistasis gene interaction with ratio of 12 (dumbbell): 3 (pyriform): 1 (disc). Based on the result, it is concluded that noncommercial butternut pumpkin is already in the Hardy-Weinberg equilibrium. Hardy-Weinberg's law states that the frequency of alleles in a population is stable from one generation to the next if no external influences can affect it (Robinson, 2003).

Nonconformity in the results obtained can be caused by the number of populations that is too small thus causing genetic drift so that genetic variations are reduced (Reece *et al*., 2014; Lynch *et al*., 2016; Hedrick & Garcia-Dorado, 2016). In addition, this can also be caused imperfect segregation events in the first offspring so the results obtained are not in accordance with the desired characters (Daryono & Maryanto, 2017).

CONCLUSION

The noncommercial butternut pumpkin has an instability in producing phenotypic characters, while commercial butternut pumpkin has uniform and stable phenotypic characters. Gene interaction happened in noncommercial butternut pumpkin was proven to be dominant epistasis with ratio of 12 (dumbbell): 3 (pyriform): 1 (disc). Commercial butternut pumpkin is shown to have a stability in strain purification.

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