Fruit morphological changes and yield of hybrid butternut squash (*Cucurbita moschata*) cultivated using its replanted seeds

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Abstract. Butternut squash is a horticultural commodity and is classified as an annual plant. This study aimed to evaluate the fruit morphological changes and yield of hybrid butternut squash grown from replanted seeds. This study was arranged in a randomized complete block design with two treatments,namely commercial hybrid seeds (F1) and seeds obtained from the first harvest generation (F2). The results showed that butternut squash cultivated from commercial hybrid seeds (F1) showed more vigorous vegetative growth than plants derived from replanted seeds (F2), particularly in the elongation and widening of lower, middle, and upper leaves. Fruit morphology also differed markedly between generations. Fruits produced from F1 plants were more uniform in shape and size, generally showing a jug-like form with a more bulging bottom part, whereas F2 plants produced fruits with more diverse and elongated shapes. Fruit circumference development in F1 increased rapidly at 11–12 days after fruit enlargement, while F2 showed a more linear enlargement pattern. In addition, F1 exhibited greater fruit shape uniformity, reflected by a higher coefficient of determination compared with F2. However, F2 plants produced significantly higher fruit number and fresh fruit weight than F1. These findings indicate that cultivation using replanted seeds (F2) altered fruit morphology and reduced uniformity, but increased yield potential compared with commercial hybrid seeds (F1).

1 Introduction

Butternut squash (*Cucurbita moschata*) is a horticultural plant native of Mexico, which produces fruits that vary from flattened, globular, round to oblong and cylindrical as well as pear-, heart-, and dumbbell-shaped [1]. The fruit of this plant resembles nuts, contains many nutrients, especially beta-carotene, vitamin A, high fiber, and low-fat content, making it good for consumption [2]. Butternut squash has good shelf life, can be stored for long periods of time, is often processed frozen or dried, and is flexible in culinary processing, making it increasingly demanded by domestic and international markets [3]. Butternut squash is used as a raw material for juice, jam and food coloring [4].

Butternut squash plants have not been massively cultivated due to people's assumptions that they can only be grown on large areas of land. However, this crop can actually be grown in a narrow area by maximizing vertical space. This is because Butternut squash is a creeping fruit plant that can be adjusted vertically [5]. In the midst of limited land in Indonesia, the cultivation of this crop is starting to become an attractive alternative in the development of productive and sustainable urban agriculture in the tropics [6].

The availability of sufficient quantities of high-quality seeds is essential for the successful cultivation of butternut squash and the improvement of crop productivity [7]. In cultivation practices, hybrid seeds (F1) are often preferred because they have advantages in terms of growth vigor, disease resistance, and high yield [8]. However, F1 seeds are relatively expensive and cannot be used repeatedly because their F2 crops often show genetic segregation, resulting trait diversity [9]. This raises the question of whether the F2 seeds, which are harvested seeds from F1 plants, can still be replanted and still provide decent growth and yield.

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This research is not only important from an agronomic perspective, but also has economic relevance, especially for smallholder farmers who want to utilize their own harvested seeds to reduce production costs. Thus, this study aims to evaluate the extent of growth and yield differences between Butternut squash from F1 and F2 generation cultivated in tropical land, as well as assess the potential for utilizing F2 seeds as an alternative seed source.

2 Materials and Methods

2.1 Research procedure

The study was carried out at the outdoor agriculture experimental farm, Universitas Sriwijaya, Indonesia. The farm is situated within the tropical lowland climate zone. The seeds of Butternut squash varieties used in this experiment were commercial hybrid seeds (F1) and seeds collected after the following harvest (F2). The seeds were sown in seedling trays filled with a mixture of soil and chicken manure (2:1 v/v). The seedlings with four leaves were transplanted from seedling trays to plastic pots. Transplanting was carried out at 21 days after sowing (DAS). The dimensions of the pots used are 37.6 cm in upper diameter, 32 cm in base diameter, and 26.2 cm in height. The pot was filled with a mixture of the tropical ultisol soil and chicken manure at 2:1 v/v. One seedling was planted at each pot. Butternut squash was fertilized one week after transplanting using the commercial compound NPK fertilizers (16:16:16) at doses of 5 g per plant. Additional fertilizer was added 4 and 6 weeks after planting with the same doses of 5 g per plant.

2.2 Data collection

The rates of leaf expansion were daily measured based on the length and width of leaf blade. Measurements were started from the time the leaf blades begin to unfold until the next 16 days. As the leaves grow larger, the stems also continue to lengthen and produce the next leaves. After the first leaf stopped to expand, the next newly unfolded leaf was selected as the second leaf to be measured for its expansion rate. The third leaf went thru the last similar measurements.

The fruit growth rate was measured daily for 21 days. The parts measured including fruit length, upper diameter (L1), middle diameter (L2), and lower diameter (L3). Other vegetative data measured includes the number of leaves, plant length, and number of fruits weekly counted. The first fruit harvest begins when the plant had reached 16 weeks old after planting. Furthermore, fruit harvesting is carried out at any time if there is ripe fruit. The first fruit harvest was conducted when the plants reached 16 weeks after planting. Thereafter, ripe fruits were harvested whenever they reached physiological maturity over a three-week harvesting period. All fruits harvested during this period were collected, weighed, and used to determine fruit yield as well as fruit length, width, and diameter.

Measurements of the inner dimensions of the fruit are carried out destructively, including the length and width of the fruit cavity. The thickness of the fruit flesh measured using a digital caliper. The fresh and dry weight of the fruit sample was weighted. The 3-mm slice of fruit fresh samples were dried in oven at temperature of 105°C until fully dried. The dried samples were measured using an analytical scale.

2.3 Experimental design and data analysis

This study was arranged in a randomized complete block design with two treatments, namely commercial hybrid seeds (F1) and seeds obtained from the first harvest generation (F2). The experiment was repeated six times, and each replication consisted of three plants. All data were organized and analyzed with analysis of variance (ANOVA). Significant differences between treatments were tested using the least significant difference (LSD) test at $P \leq 0.05$ in the R-studio software.

3 Results and Discussion

Leaf is an important vegetative organ that plays an important role in plant metabolic activities. Leaf growth was identified as very fast and easily visible starting from the first day of leaf opening until 16 days after leaf unfolding. Related to leaf width, measurements were divided into lower, middle, and upper leaf in each F1 and F2. The results showed that the middle leaves experienced a more dominant elongation compared to the lower or upper ones. There was a dramatic increase in the elongation of the middle leaves, especially at 7 and 8 days after complete leaf unfolding. Specifically, the rate of elongation of the lower, middle, and upper leaves, respectively, in F1 was clearly more active than that of F2. The F2 leaves tended to be shorter and stagnated quickly (Fig. 1).

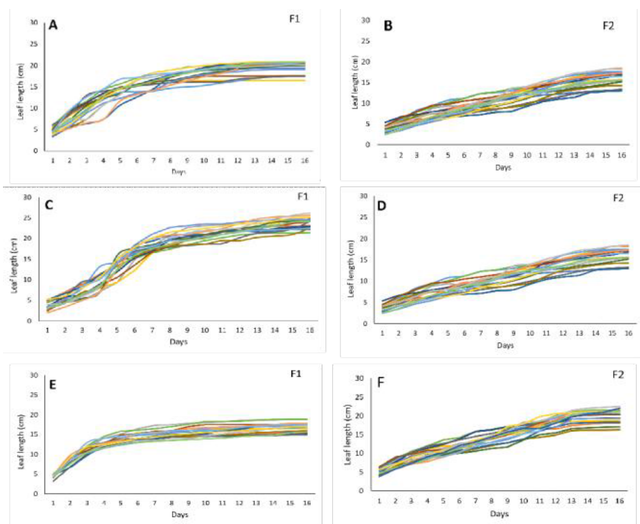

Fig. 1. Daily leaf length of Butternut squash on three leaf position cultivated through F1 and F2. The leaf position consists of lower (A, B), middle (C, D), and upper (E, F).

Regarding leaf width, it turns out that the rate of leaf widening of F1 and F2 had a different trend. The

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observations showed that the leaves of pumpkins cultivated with F1 were wider than those of F2. Consistently, this phenomenon occurred in the lower, middle, and upper leaves. Interestingly, leaf widening based on leaf position in each phenotype showed a similar trend (Fig. 2).

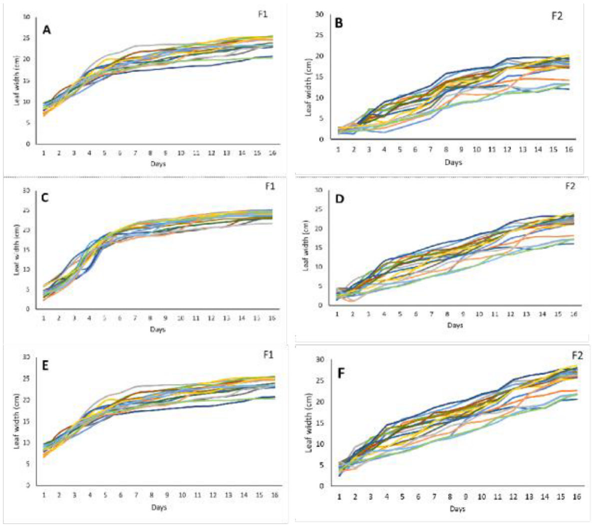

Fig. 2. Daily leaf width of Butternut squash on three leaf position cultivated through F1 and F2. The leaf position consists of lower (A, B), middle (C, D), and upper (E, F).

The cultivation of Butternut squash using F1 and F2 seeds did not show a decrease in its vegetative characteristics, especially with regard to plant length and number of leaves. Nevertheless, there was an increase in plant length and number of leaves in each week. Especially for the number of leaves, the increasing number of new leaves was not dramatic at 5 weeks after planting (WAP). This phenomenon occurred as the plants had entered the generative stage characterized by the appearance of flowers, so that vegetative growth such as plant length and leaf growth gradually slowed down (Fig. 3).

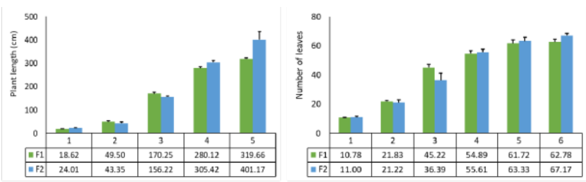

Fig. 3. Plant length and number of leaves of butternut cultivated through F1 and F2.

After passing the vegetative growth phase, butterhead pumpkin will enter the generative phase to produce fruit as a productive organ. Butterhead pumpkin has a distinctive morphology like a jug with different shapes on the bottom, middle, and upper. Thus, the fruit of this plant is also highly recommended to be cultivated to increase aesthetic value in addition to consumption because it has a distinctive shape. Cultivation of Butternut squash through F1 and F2 obtained different fruit characteristics. When Butternut squash was cultivated with F1, fruit shape and size are more uniform. The fruits produced on F1 Butternut squash are jug-like with the bottom more bulging than the top. In contrast, there was a more diverse fruit shape and size when Butternut squash was cultivated through F2. More specifically, the shape of the fruit produced on F1 and F2 seeds is as shown in Fig. 4.

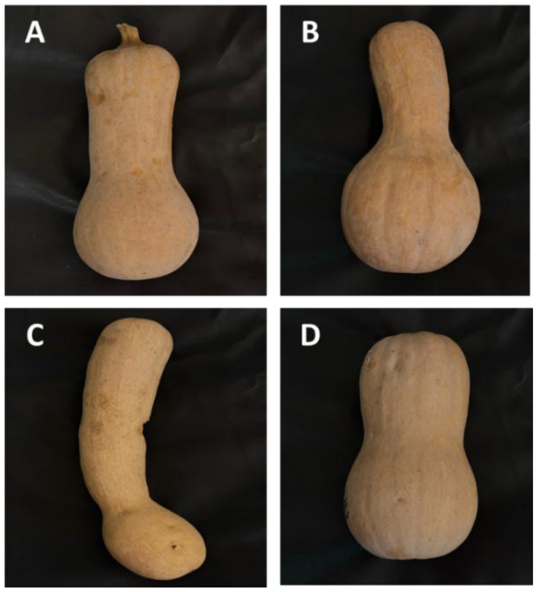

Fig. 4. Variation of Butternut squash when cultivated using F1 producing uniform shape (A) and F2 differentiated shape as “shape 1” (B), “shape 2” (C), and “shape 3” (D).

Fruit length and fruit circumference (bottom, middle, and top) are the most likely indicators of Butternut squash fruit growth. Fruit length tended to be more uniform in F1 than in F2. However, this fruit length pattern did not show significant differences between F1 and F2. In terms of fruit circumference, namely bottom, middle, and top fruit circumference, F1 had a dramatic increase especially at 11 and 12 days after fruit enlargement. On the other hand, in F2, fruit circumference enlargement tended to increase according to a linear pattern (Fig. 5).

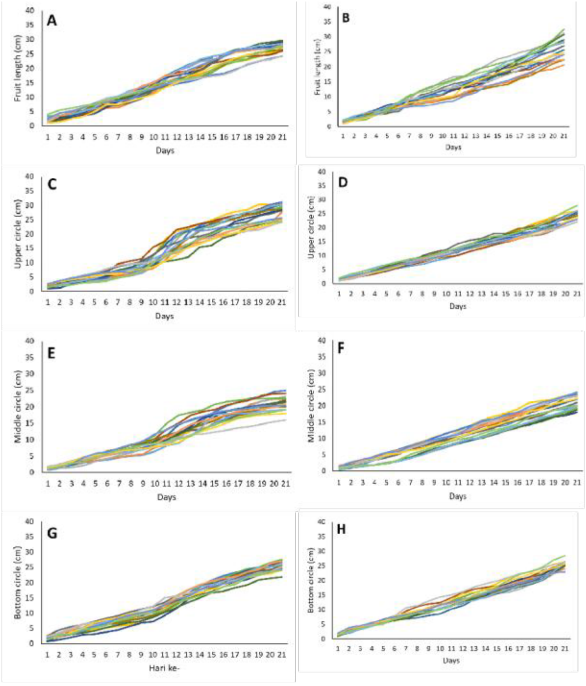

Fig. 5. Fruit growth rate of Butternut squash as indicated by fruit length and circumference on different phenotype. The phenotype consists of F1 (left) dan F2 (right).

As the fruits of Butternut squash are diverse, it is interesting to know the relationship between pumpkin length and Butternut squash diameter at the top, middle, and bottom, respectively. The analysis showed that Butternut squash cultivated through F1 had a higher relationship between fruit length and diameter. This means that any fruit elongation will be followed by fruit enlargement (Fig. 6). In contrast, the fruit produced by Butternut squash cultivated through F2 has a low length and circle relationship, for shape 1 (Fig. 7), shape 2 (Fig. 8), and shape 3 (Fig. 9). This phenomenon also confirms that the fruits produced in F2 tend to be more diverse and inconsistent.

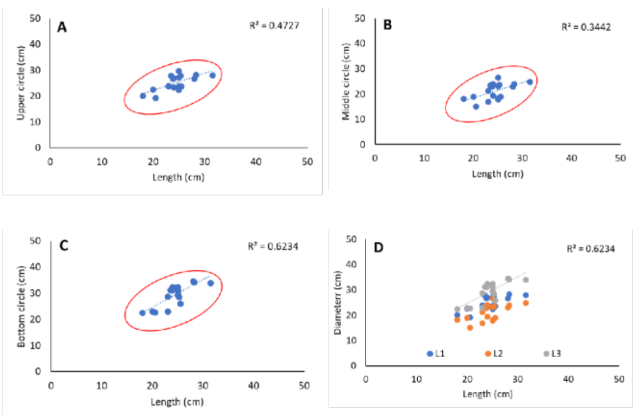

Fig. 6. Relationship between fruit length and circumference on Butternut squash cultivated using F1.

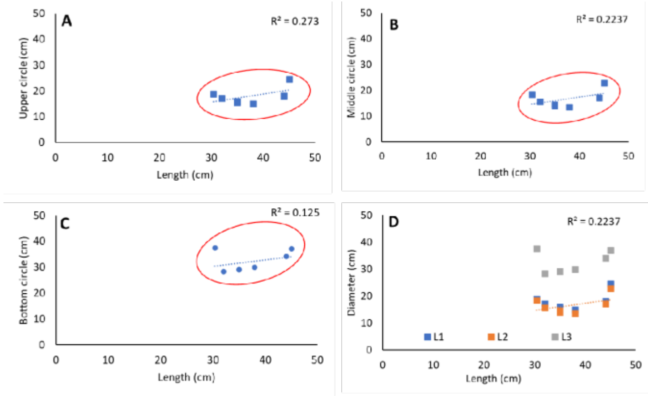

Fig. 7. Relationship between fruit length and circumference on Butternut squash cultivated using F2 as "shape 1".

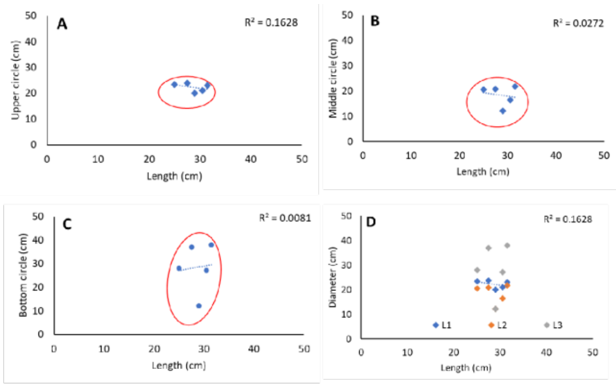

Fig. 8. Relationship between fruit length and circumference on Butternut squash cultivated using F2 as "shape 2".

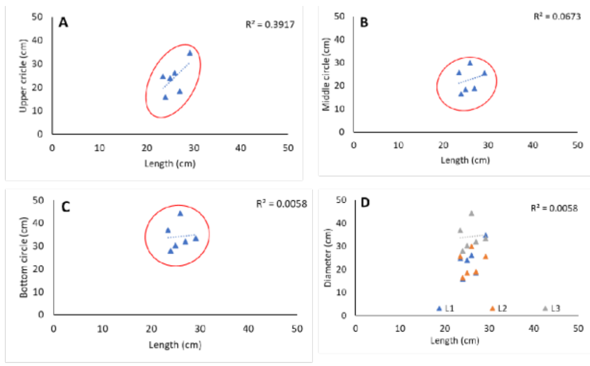

Fig. 9. Relationship between fruit length and circumference on Butternut squash cultivated using F2 as "shape 3".

Some variables showed significant differences when Butternut squash was cultivated through F1 and F2. Butternut squash cultivated with F1 has a more rounded fruit shape as represented through the upper circle of fruit and middle circle of fruit which are more dominant compared to F2. In contrast, when Butternut squash is cultivated through F2, the shape of the fruit to be more elongated. The number of fruits and fresh weight of fruits in F2 were significantly higher than F1. However, the perfection and quality of the fruit shape of Butternut squash cultivated with F1 is better than that of F2. This is mainly because Butternut squash fruit with a large shape reflects better fruit quality, as shown by the more dominant fruit circumference measurements (Table 1).

Table 1. Some variables that indicated differences of Butternut squash cultivated through F1 and F2 phenotype.

Variables	F1		F2		LSD
Number of fruits	1.44	b	3.44	a	0.80
Fruit fresh weight	890.08	b	1029.72	a	16.50
Fruit dry weight	171.37	b	236.44	a	16.23
Volume of fruit	880.97	a	864.55	a	20.22
Length fruit	24.35	b	30.97	a	3.08
Upper circle of fruit	25.16	a	21.15	b	2.57
Middle circle of fruit	21.2	a	19.06	b	2.07
Bottom circle of fruit	29.27	a	29.27	a	3.65
Number of seeds	225.77	a	182.88	a	17.09
Total weight of seeds	25.92	a	25.78	a	8.07
Fresh weight 100 seeds	14.18	a	12.14	a	3.32
Dry weight 100 seeds	6.82	a	5.50	a	2.65
Length of fruit cavity	7.58	a	8.52	a	1.12
Width of fruit cavity	5.58	a	6.19	a	0.73
Thickness of fruit cavity	18.2	a	19.48	a	2.41
Length of fruit split	19.81	a	21.72	a	3.17

Means followed by the same letters within columns are not significantly different, whereas means followed by different letters within columns are significantly different based on the LSD test at $p \leq 0.05$. *LSD* = least significant difference.

Butternut squash growth cultivated on F1 and F2 generation

The plant can propagate through vegetative and generative ways. Butternut squash is a generatively propagated plant using seeds. Seed propagation remains the most practical method for commercial production, and technology has continued to be developed as an alternative to improve seed quality to optimize plant growth [10].

Plant propagation through F1 and F2 generations has been found to have an impact on plant growth. F2 generation plants have been reported to have high variability compared to F1 [11]. Based on the results of this study, plants from the F2 generation exhibited greater morphological variation than those from the F1 generation. This variability was particularly evident in leaf shape and leaf width (Figure 2). Higher morphological variation in F2 compared to F1 is also seen through plant length. This statement is reinforced by the larger standard error of F2, especially at 5 weeks after planting (Figure 3). [12] states that standard error is one of the variables that describe the variation of data in the field. The higher the standard error means the more varied the data collected.

Despite the morphological variation of F2 compared to F1, the quantitative parameters measured tended not to have significant differences. These results suggest that although genetic segregation increased phenotypic diversity, it did not substantially affect overall vegetative growth. Leaf length, leaf width, plant length, and number of leaves between F1 and F2 were almost similar. This phenomenon is in line with [13] who reported that some morphological parameters did not show significant differences between F1 and F2 generations.

Butternut squash yield cultivated on F1 and F2 generation

The cultivation of Butternut squash using the F1 and F2 generations showed striking differences, especially in terms of yield. The fruit, which is the yield organ in this plant, appears to have a varied shape when cultivated through F2 (Figure 4). In contrast, the fruit on F1 tends to be more uniform. [14] reported that plants cultivated through F2 tend to experience high yield variability. This is because genetic segregation occurs so that there are differences in heritability in plants, The phenomenon of genetic segregation in F2 that affects yield has been proven in several crops such as tropical carrot tomato, and pumpkin [15]. Interestingly, despite the greater variability in fruit morphology, the F2 generation produced significantly higher fruit number and fresh fruit weight than the F1 generation. This may be attributed to genetic segregation in the F2 population, which allows the expression of favorable allele combinations associated with improved reproductive performance and yield potential.

Uniformity in F1 is also seen through the regularity of fruit shape on the top, middle, and bottom sides. This was confirmed by the higher coefficient of determination of F1 compared to F2 (Figures 6, 7, and 9). Fruit is the organ that has the most impact on genetic segregation.

4 Conclusion

The cultivation of butternut squash using different seed generations resulted in distinct growth, fruit morphology, and yield characteristics. Commercial hybrid seeds (F1) promoted better vegetative performance and produced fruits with higher uniformity in shape and size. In contrast, the use of replanted seeds (F2) increased variability in fruit characteristics, particularly fruit shape, but significantly improved fruit number and fresh fruit weight. Therefore, replanted seeds may provide higher production potential, although with reduced fruit uniformity compared to commercial hybrid seeds.

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