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Trend of Changes in Quantitative and Qualitative Traits in the Next Generation of BC3F2 Genotype from Crosses of Parent of High Protein Corn with Local Waxy Corn

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Abstract**—** *Corn can be used as an alternative staple food, but it is underdeveloped because it generally does not taste fluffier like rice. Therefore, it is necessary to develop corn that tastes fluffier, has high protein and has high production. The purpose of this study was to determine the trend of changes in quantitative (production) and qualitative (protein and amylopectin levels) traits in the next generation of BC3F2 genotype from a cross between high protein maize parents (Srikandi Putih variety) and high amylopectin maize (Local variety of Waxy corn). Seeds of Waxy corn, Srikandi Putih Varieties, BC2F1 and BC3F1 will be used as basic materials in this study. The study was designed in the form of a Randomized Block Design. The treatments consisted of genotypes BC2F1, BC3F1, Srikandi Putih Variety and Local variety of Waxy Corn. For the BC3F1 genotype, 210 plants were self-treated to produce the BC3F2 genotype. The results showed that there was an increasing trend of high protein and production characters from the inheritance of the Srikandi Putih Variety to the BC3F1 and BC3F2 genotypes. This was supported by an increase in ear length, ear diameter, weight of 100 seeds, seed weight per plot and seed production per hectare and an increase in protein content. The same trend also occurred in the character of amylopectin levels from the inheritance of local waxy corn parents to the BC3F1 and BC3F2 genotypes which were increasing.*
Keywords**—** *corn, protein, amylopectin, selfing*

I. INTRODUCTION

The need for basic food in Indonesia is increasing along with the increase in population. This shows that the agricultural food sector is still a very important sector. Dependence on rice as the only staple food in Indonesia needs to be anticipated by providing alternative staple foods such as corn. Generally, corn is used for animal feed, only a small part is used for food and industry. Generally, Indonesian people do not like corn as a staple food because the texture of corn rice is rough and hard. Generally only used as a snack. For this reason, it is necessary to improve the quality and taste so that it is delicious and nutritious. The combination of two good traits from two different maize varieties can be done if the genetic diversity is available. High quality protein maize (QPM) is available, but has a rough texture so it is not popular for food.

On the other hand, local waxy corn is also available in South Sulawesi, it is very popular both when the seeds are young and when the seeds are old because of its high amylopectin content, but the drawback is that the production is very low. The shortcomings of these two types of corn need to be eliminated by certain plant breeding techniques in order to obtain high protein corn and fluffier texture. Corn with these special characteristics can be formed through repeated and programmed plant breeding programs [1]. In waxy corn there is a recessive gene wx in a homozygous state (wxwx) which affects the chemical composition of starch causing a delicious and savory taste. Backcross breeding method can be applied to introgress donor genes from special corn with high amylopectin content (waxy corn) to high protein and high productivity (Srikandi Putih variety) in order to obtain corn that has the desired special characteristics (Varieties with high amylopectin and high protein).

The protein content of corn generally ranges from 8-11%, but the content of lysine and tryptophan is low, 0.225% and 0.05%, respectively, so it is still less than half that recommended by the Food and Agriculture Organization [2]. The QPM varieties released for the first time in Indonesia were Srikandi Kuning-1 and Srikandi Putih-1 free-polluted corn with a productivity of 7.0 t/ha [3]. Srikandi Kuning contains 10.38% protein, 0.477% lysine and 0.093% tryptophan, while Srikandi Putih contains 10.44% protein, 0.410% lysine and 0.087% tryptophan [4].

Generally, corn kernels have amylose content that is higher than their amylopectin content, except for waxy corn. The amylopectin content in waxy corn is almost 100%. Ordinary corn endosperm consists of a mixture of 72% amylopectin and 28% amylose [5]. Almost all of the endosperm content of waxy corn is amylopectin [6]. In waxy corn, there is a recessive gene wx in a homozygous state (wxwx) which affects the chemical composition of starch, causing a delicious and savory taste. Waxy corn yields are generally low, only 2-2.5 t/ha and are not resistant to downy mildew. Corn taste fluffier is closely related to the high content of amylopectin in seed starch. Starch consists of two glucose polymer compounds, namely amylose and amylopectin. The molecular weight of amylose and amylopectin depends on the botanical source of amylose which is a component with straight chains, while amylopectin with branched chains. Amylose is a helical straight chain polysaccharide with -1,4 glycosidic bonds. The branching point of amylopectin is the -1,6 bond. The number of glucose molecules in the amylose chain ranges from 250-350 units [7]. Starch is composed of at least three main components, namely amylose, amylopectin, and intermediates such as lipids and proteins. These components affect the functional and amylographic properties of corn flour [8]. The results showed that the protein content of Maize Pulut Takalar and Corn Pulut Gorontalo were relatively the same, 0.78% and 0.79%, respectively [9]. The composition of amylose and amylopectin in corn kernels is genetically controlled. In general, corn endosperm type horse teeth and pearls contain amylose 25-30% and amylopectin 70-75% of the total starch. Pulut corn has a starch content of almost 100% amylopectin. The presence of an epistatic recessive waxy (wx) gene located on chromosome nine affects the chemical composition of starch so that amylose accumulation is very little [10]. Corn contains about 10% protein, higher than rice (7.5%), and lower than wheat 14%. Other nutrients that corn contains are fat and fiber at 5% and 2%, respectively. The nutritional content per 100 g of seeds is 45 mg calcium, 3 mg iron, 24 mg phosphorus, 11 mg sodium, and 78 mg potassium [11]. Indonesia imports corn as feed, but efforts to meet the needs of corn as a staple food need to be encouraged. The higher the amylopectin content, the softer the corn taste.

II. RESEARCH METHOD

This research was conducted at the Cereal Crops Research Institute Allepolea Village, District. Lau, Maros Regency, South Sulawesi Province, Indonesia, from February to December 2021. The materials used in this study were: Seed of Srikandi Putih variety, waxy corn, genotypes BC2F1, and BC3F1. This study was designed in the form of a randomized block design. The treatments consisted of genotypes BC2F1, BC3F1, Srikandi Putih variety and waxy corn. Repeated 3 times. Specifically for the BC3F1 genotype, 210 plants were selfing to produce the BC3F2 genotype. The soil was treated with a tractor and hoe, then a plot of 8 m x 6 m was made, with 12 experimental units. Seeds of Srikandi Putih, local pulut, BC2F1, and BC3F1 before planting were given the fungicide Saromil to prevent downy mildew. Seeds are planted in a single way as much as 2 seeds / hole with a spacing of 70 cm x 20 cm. Fertilization of Urea, SP-36, KCl and NPK Ponska was carried out after corn plants were 7 days after planting (DAP) with a dose of 200 kg Urea/ha, 150 kg SP-36/ha and 100 kg KCl/ha. Specifically for Urea, it was given 2 times, 50% at 7 DAP and 50% urea at 30 DAP. After the plants were 7 DAP, thinning was done, leaving 1 plant per hole. 210 plants of BC3F1 were selfing to obtain BC3F2 seeds. Observation variables include: (1) plant height (cm), (2) number of leaves (strands), (3) male flowering age 50% (days), (4) female flowering age 50% (days), (5) ear length (cm), (6) ear diameter (cm), (7) dry weight 100 seeds moisture content 15% (g), (8) dry weight of seeds per plot moisture content 15% (g) and (9) Production seeds per hectare moisture content 15% (tons/ha), (10) Protein content (%) and (11) Amylopectin content (%).

III. RESULTS AND DISCUSSION

3.1. Plant height and number of leaves. The analyze of variance showed that the varieties and genotypes had no significant effect on the variables of plant height and number of leaves. Figure 1 shows that both the plant height and the number of leaves of the Srikandi Putih variety tend to be higher than other varieties and genotypes.

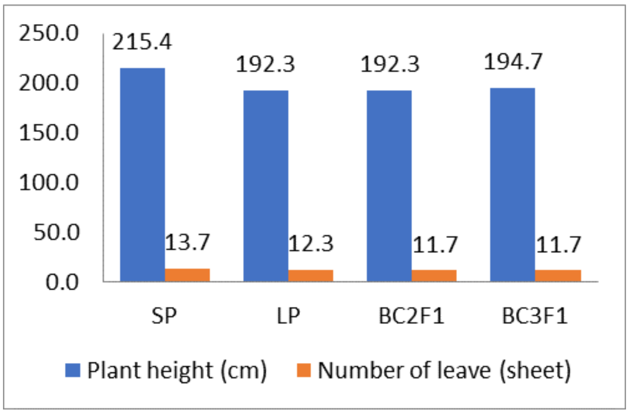

Fig.1. Plant height and number of leaves

Note: SP: Srikandi Putih variety, BC3F1: Genotype Backcross 3 fillial 1, BC2F1: Genotype backcross 2 fillial 1, LP: waxy corn

There was no difference in plant height and number of leaves of Genotypes BC3F1 and BC2F1 with the parent of Srikandi Putih and the parent of waxy corn. This is because the two parents also have no differences in the characters of plant height and number of leaves, as shown in Figure 1. Characters that are passed on by parents to their offspring can be dominant, recessive or codominant. The size of the character of the parents passed on to their offspring is also influenced by the method of crossing used. This is in accordance with the description of the Srkandi Putih variety that the plant height is approximately 195 cm [14]. Another study showed that the number of leaves of the Srikandi Putih variety and waxy corn was not significantly different, namely between 12-14 strands [15].

3.2. Flowering age. The analyze of variance showed that the varieties and genotypes tested had a significant effect on male and female flowering age. Table 1 shows the fastest male flowering age at local pulut and significantly different from all varieties tested except for the BC2F1 genotype. Furthermore, the genotypes of BC2F1 and BC3F1 were not significantly different, but both male flowers released faster and were significantly different from the Srikandi Putih variety. Table 1 also shows the flowering age of local pulut females, BC2F1 and BC3F1 out faster and significantly different from the Srikandi Putih variety, but between the three there is no significant difference.

Table 1. Age of male flowering and female flowering age 50%

Treatment	Average age of flowering males 50% (day)	Average age of flowering female 50% (day)
SP	47.0a	48.0a
LP	43.3c	44.3b
BC2F1	44.0bc	45.3b
BC3F1	44.3b	45.3b
$LSD_{0.05}$	0.88	1.15

Note:

Numbers followed by the same letter were not significantly different at LSD test $_{0.05}$.

SP: Srikandi Putih variety, BC3F1: Genotype Backcross 3 filial 1, BC2F1: Genotype backcross 2 filial 1, LP: waxy corn

The flowering age of males and females in the genotypes BC2F1 and BC3F1 came out faster than the parent of the Srikandi Putih variety. This shows that the characteristics of male and female flowering age are quantitative traits inherited by waxy corn parent. This is in line with the results of previous studies where the male and female flowering age characters which are faster than the parent Srikandi Putih have been slowly inherited from Genotype F1 then to F2 and to F3 which flower faster than the parent Srikandi Putih [16]. This also shows that the age of flowering of males and females remains faster even though they are backcrossed by the parent of Srikandi Putih as much as 2 to 3 times, then selfing 2 times.. This indicates that these two traits have started to stabilize in each generation produced. There is a difference in the data due to environmental influences. This is also in line with research showing the flowering age of males ranging from 43-48 days after planting and female flowering age between 45-52 days after planting [17].

3.3. Ear length and ear diameter. The analyze of variance showed that the varieties and genotypes tested had a significant effect on the length of the ear and the diameter of the ear. Table 2 shows the Srikandi Putih variety, genotypes BC3F1 and BC2F1 all three were higher, such as the length of the ear and the diameter of the ear and significantly different from the waxy corn. Between the Srikandi Putih variety, the genotypes BC3F1 and BC2F1 were not significantly different to the length and diameter of the ears.

Table 2. Ear length and ear diameter

Treatment	Average length of ear (cm)	Average diameter of ear (cm)
SP	15.7a	4.4a
LP	13.7b	3.4b
BC2F1	15.5a	4.3a
BC3F1	15.6a	4.3a
$LSD_{0.05}$	1.44	0.44

Note:

Numbers followed by the same letter were not significantly different at LSD test $_{0.05.}$

SP: Srikandi Putih variety, BC3F1: Genotype Backcross 3 filial 1, BC2F1: Genotype backcross 2 filial 1, LP: waxy corn

The length and diameter of the ear of the Srikandi Putih variety, genotypes BC2F1 and BC3F1 were higher and significantly different from the waxy corn. There was no significant difference between the Srikandi Putih with the BC2F1 and BC3 F1 genotypes. This indicates that the character of the length and diameter of the ear has been inherited by Srikandi Putih as the parent to his offspring, namely the BC2F1 and BC3F1 genotypes. This is also in line with the results of previous studies which showed the length and diameter of the ear of the Srikandi Putih variety were longer and significantly different from the waxy corn [15].

3.4. Weight of 100 Seeds, Weight of Seeds per Plot and Production per Hectare. The analyze of variance showed that the varieties and genotypes tested had a significant effect on 100 seed weight, seed weight per plot and seed production per hectare. Table 3 shows the highest weight of 100 seeds was found in the Srikandi Putih variety, followed by the genotype BC3F1 then BC2F1 and the lowest was in the waxy corn. All varieties and genotypes tested were significantly different to the weight of 100 seeds. Table 3 also shows the highest seed weight per plot and seed production per hectare in the Srikandi Putih variety, genotype BC3F1, genotype BC2F1 and significantly different from waxy corn. There was no significant difference between the Srikandi Putih variety, BC3F1 genotype, and BC2F1 genotype.

Table 3. Weight of 100 seeds, weight of seeds per plot and seed production per hectare

Treatment	Weight of 100 seeds (g)	Seed Weight per plot (kg)	Seed production per ha (t)
SP	28.5a	38.4a	8.0a
LP	25.7d	16.8c	3.5c
BC2F1	27.2c	35.4b	7.4b
BC3F1	27.9b	36.9ab	7.7ab
$LSD_{0.05}$	0.48	1.72	0.38

Note:

Numbers followed by the same letter were not significantly different at LSD test $_{0.05.}$

SP: Srikandi Putih variety, BC3F1: Genotype Backcross 3 filial 1, BC2F1: Genotype backcross 2 filial 1, LP: waxy corn

The highest character weight of 100 seeds started with the Srikandi Putih parent then followed by its derivatives, namely Genotype BC3F1 then Genotype BC2F1 and the lowest was waxy corn parent. This indicates that the more often backcross is carried out with the parents of Srikandi Putih and selfing is carried out 2 times, the inheritance of the character weighing 100 seeds is getting closer to the parent of Srikandi Putih. This is in line with the description of the Srikandi Putih variety with a potential of 32.5 g per 100 seeds under optimum conditions [14]. The character of seed weight per plot and seed production per hectare showed that the Srikandi Putih variety and BC3F1 genotype were not significantly different but both were higher and significantly different from the waxy corn parents. There was no significant difference between BC3F1 and BC2F1 but both were higher and significantly different from the waxy corn parents. This indicates that the more often backcross are carried out with the Srikandi Putih parent and then followed by selfing, the accumulation of inheritance of traits to their offspring occurs. This is in line with the results of previous studies which stated that the seed production per hectare of genotypes F1 and F2 was generally inherited from the Srikandi Putih variety which had high production potential compared to the waxy corn parent which had low production potential [15].

3.5. Protein and Amylopectin Levels. The analyze of variance showed that the varieties and genotypes tested had a significant effect on the variables of protein and amylopectin levels.

Table 4 shows the highest protein content of the BC3F2 genotype and significantly different from all varieties and genotypes tested. There was no significant difference between the Srikandi Putih variety with Waxy corn (LP), BC2F1, BC3F1. Table 4 also shows the highest levels of amylopectin content of waxy corn (LP) and significantly different from all varieties and genotypes tested except for the BC3F2 genotype which was not significantly different. Genotypes BC2F1 and BC3F1 were not significantly different from the Srikandi Putih Variety (SP).

Table 4. Protein and Amylopectin levels

Treatment	Average protein content (%)	Average anilopectin content (%)
SP	9.65b	68.39b
LP	9.49b	82.50a
BC2F1	9.53b	68.92b
BC3F1	9.60b	70.18b
BC3F2	10.42a	79.73a
$LSD_{0.05}$	0.49	6.27

Note:

Numbers followed by the same letter were not significantly different at LSD test $_{0.05.}$

SP: Srikandi Putih variety, BC3F1: Genotype Backcross 3 filial 1, BC2F1: Genotype backcross 2 filial 1, LP: waxy corn

Table 4 shows that there is a trend of increasing protein content as the generation continues to follow the traits of the elders of the Srikandi Putih variety, even the BC3F2 genotype exceeds the protein content of the Srikandi Putih Variety (SP). This is in line with the results of the study, that the protein content of the Srikandi Kuning variety of maize seeds increased as the generation continued from F1, F2 and BC1 with protein content of 7.30 ± 4.31%, 9.54 ± 0.82% and 13.30 ± 2.13% [18]. Another study on the quality diversity of several corn varieties showed that the protein content of extracted corn starch was influenced by the variety, an average of 0.92% with a range of 0.72-1.12% [9]. Furthermore, research on the analysis of protein, lysine and tryptophan levels in the seeds of several corn varieties showed that the Srikandi Kuning variety contained 10.38% protein, 0.477% lysine and 0.093% tryptophan, while the White Srikandi variety contained 10.44% protein, 0.410% lysine and 0.410% protein. tryptophan 0.087%, both higher than other varieties [20].. Another study reported that protein content was increased from 10.9% (original population) to 26.6% in the "Illinois High Protein" maize strain [21]. Likewise with the trend of increasing levels of amylopectin which tends to increase in the next generation which is close to the amylopectin levels of waxy corn elders. The results showed that Gene wx in waxy corn was easily transferred to non-sticky maize [22].

IV. CONCLUSION

The conclusions from the results of this study are as follows:

1. Inheritance of production characters from the parents of the Srikandi Putih Variety to the BC3F1 genotype was formed, which was indicated by an increase in the length of the ear, diameter of the ear, weight of 100 seeds, weight of seeds per plot, and production of seeds per hectare.
2. Inheritance of the protein content of the seeds from the parents of the Srikandi Putih Variety to the genotypes BC3F1 and BC3F2 was formed, which was indicated by an increase in the protein content of the seeds.
3. Inheritance of the character of amylopectin levels from local variety of waxy corn parents to BC3F1 and BC3F2 genotypes has been formed, which is indicated by an increase in seed amylopectin levels.

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