Endosperm color inheritance pattern of black Toraja rice

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Abstract. This study aims to obtain information about the endosperm color inheritance pattern of Toraja black rice. This research was conducted by the experimental farm, Hasanuddin University Faculty of Agriculture, from August 2017 to February 2018. This study was designed using a Randomized Block Design. The color of the seed as a treatment consists of full black (the outer and inner parts of rice), medium black (the outer part and a small portion of inner part of rice), and thin black (only the outer part of rice), with 8 replications so there were 24 experimental units. The seedling were planted in a bucket, one seedling for each bucket. The inheritance pattern of full black and thin black genotypes follows the Mendel inheritance pattern with a ratio of genotype 1 : 2 : 1. Percentage of full black endosperm color has a high heritability, while percentage of medium black and the percentage of thin black have moderate heritability.

1. Introduction

The types of rice consumed by Indonesian people include white rice, brown rice and black rice. Black rice contains vitamins and minerals that are better than white rice [1], contains low sugar content, has lots of fiber and vitamin E, and has anthocyanin content with high antioxidant activity. Some studies show that anthocyanin as an antioxidant has a protective effect on inflammation, atherosclerosis, carcinoma, and diabetes [2]. Black rice is a source of important genetic diversity in rice breeding efforts. Black rice is mostly obtained from local rice from several regions in Indonesia, one of which is in the Toraja Regency area.

Black Toraja rice is one of the germplasm sources in rice breeding. But it still has a very low level of purity that is there is a variety of color of seeds in one panicle. There are three levels of seed color in black Toraja rice, among others some rice seeds are black on the outside and inside of rice (full black), some seeds are black on the outside and little in the rice (medium black) and others are black only the outside (thin black) [3].

The success of breeding depends largely on genetic diversity, the role of genes and selection methods. The color properties of black rice endosperms can be identified by genes that control anthocyanins. The anthocyanin content is controlled by genes with different properties and amounts of each plant [4]. Genetic diversity between individuals or populations can be predicted using morphological characterization [5]. Morphological properties (phenotypes) can be used as tangible clues to specific genes and gene markers in chromosomes because the properties that affect morphology can be reduced

[6]. The morphological characterization used is based on Mendel's simple inheritance patterns, such as shape, color, size, and weight.

The inheritance pattern of the color characteristics of black rice can be predicted by looking at the segregation pattern in the population. Differences in inheritance patterns of color properties and the number of genes controlling the color properties of rice, are thought to be influenced by the choice of parents used [7]. Morphological characterization and inheritance patterns of the color properties of black rice are basic information to determine the right steps in assembling rice varieties in full black. This study aims to obtain information about the endosperm color inheritance pattern of Toraja black rice.

2. Materials and methods

2.1. Materials

Local black Toraja rice seeds taken on farmers 'land in North Ao' Gading Toraja Hamlet, land, sand and compost, size 27 buckets, hoes, spades, sprayers, trays, cameras, meters, shovel, knives and writing instruments

2.2. Methods

The research was conducted at the Laboratory of Plant Biosciences and Reproduction of Biotechnology, and Experimental Farm, Department of Agronomy, Faculty of Agriculture, Hasanuddin University, Makassar. The study took place from Agust 2017 until February 2018. This research was in the form of an experiment compiled based on a randomized block design (RBD) pattern, with genotype as a treatment consisting of 3 endosperm color groups, including full black (HP), medium black (HM), and thin black (HT). Each treatment was repeated 8 times and each replication consisted of 20 buckets.

The black rice seed used is the Pare Ambo variety obtained from a survey of farmers in Ao 'Gading Toraja North Hamlet. The seeds used as treatment are seeds with three levels of color grouping. Color grouping criteria based on the results of cutting each grain from the panicle. Color grouping consists of three groups, among others, full black (the outer and inner parts of the rice are black), medium black (the outside and a little part of the rice is black) and thin black (only the outside of the rice is black). To ensure the color of the seed, cut and strip the grain of the grain transversely into two parts. The part used in the study is embryonic seed pieces [3]. Pieces of embryonic seeds were sown on sand and compost 1: 1 (v / v) for 5 days. Before seeding, the seeds are treated with fungicide (Dithane 45) (1 g ml-1) to protect the seeds from disease. After the seeds germinate for 5 days, the seeds are transferred to the planting media in the form of topsoil paddy fields. The soil is put into a bucket-shaped pot. Each bucket contains 10 kg of soil. The seeds of the seedlings that are transplanted are the seeds that successfully grow to form the radicle. Each bucket is planted with one seed. The type of fertilizer used is Phonska (15: 15: 15) 1.5 g / bucket equivalent to 300 kg ha-1 and urea 1 g / bucket equivalent to 300 kg ha-1. The first fertilization is carried out at 1 week after transplanting. The second fertilization is done at the age of 1 month after transplanting. The observed parameters included the percentage of full black seeds (HP), percentage of medium black seeds (HM) and percentage of thin black seeds (HT). Data was analyzed using variance analysis (ANOVA) followed by Least Significant Difference (LSD). Data analysis was also performed with the Chikuadrat test to determine the Mendel ratio in population F2 [8]. Heritability is calculated based on the separation of components of variance by formula [9]:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2} \quad (1)$$

σ_g^2= Genetic of variance
σ_e^2= Environmental of variance

3. Result and discussion

3.1. Percentage of Full Black Seed (HP).

$LSD_{0.05}$ test on the average percentage of full black seed color (HP) was significantly different from thin black seeds (HT) with a percentage value of 10.58%, medium black (HM) with a value of 3.98% and full black (HP) with a value of 23.86%. The highest percentage of full black color (HP) is plants with full black genotype (HP) and plants with the lowest full black (HP) seed yield are medium black seeds (HM) (table 1). So it is possible that a full black genotype is a more homozygous dominant genotype.

Table 1. The average percentage of the color genotype of Toraja black rice seeds

Treatment	Average		
	PHP	PHM	PHT
HP	23,86[a]	55,93[a]	20,21[b]
HM	3,98[c]	67,67[a]	28,35[a]
HT	10,58[b]	58,81[a]	30,52[a]

The numbers followed by letters in the same line (a, b, c) mean that they are not significantly different from the test level $LSD_{0.05}$. HP (Full black), HM (Medium black), HT (Thin black), PHP (Percentage of full black), PHM (Percentage of middle black), PHT (Percentage of black is thin)

Chisquare value data calculates full black seed genotype (HP) obtained that, there are plants with full black seeds (HP), medium black (HM), and thin black (HT) with a ratio of 1: 2: 1. In accordance with the value X^2 count = 1.57 which is smaller than X^2 table = 5.99 (Table 2). This shows that the character is controlled by one locus with two alleles per locus and no dominance. So that black rice genotypes of full black seed color are hopeful genotypes to become superior seeds.

Table 2. The chisquare value calculates the average genotype of full black seed (HP) Toraja black rice

Fenotype	Observation (O)	Expectation (E)	$(O-E)^2/E$
HP	14.31	14.07	0.00
HM	31.80	28.13	0.48
HT	10.15	14.07	1.09
			X^2_{count} = 1.57
			X^2_{table} = 5.99

db =2, α = 5%. HP (full black seed), HM (medium black seed), HT (thin black seed)

3.2. Percentage of Medium Black Seed (HM).

The variance data showed that the genotype treatment had no significant effect on the percentage of medium black seed (HM). Most plants with the percentage of medium black seed (HM) are plants with medium black genotype (HM) with an average of 67.67%, and the lowest are full black seeds (HP) with an average of 55.93% per panicle (table 1). This corresponds to the color genotype of the seed planted with the most color genotypes produced. So it is expected that the medium black seed color genotype has a more heterozygous nature, resulting in segregation between thin black genotypic properties and full black genotypic properties.

The Chisquare value data calculates the medium black seed (HM) genotype obtained that, there are plants with full black (HP), medium black (HM), and thin black (HT) seeds with a ratio that deviates from the mandatory ratio 1: 2: 1, with a value X^2 count = 15.05 which is greater than X^2 table = 5.99

(table 3). The X^2 count results show that middle-black characters produce seeds with thin black characters, medium black, and black full of ratios that are not in accordance with Mandel's theory. Genes that regulate the character of a plant are controlled symmetgenically or polygenically. Simpelgenic means that the character is controlled by a few genes and the influence of genes on the expression of the character is high, while polygenic is controlled by many genes and the effect of these genes is small on the expression of a character [10]. A character controlled by a little gene will give a segregation pattern that follows Mendel's law and its modification. In contrast to characters controlled by many genes, because the influence of each small gene on a character then its inheritance is not simple and does not follow the inheritance pattern of Mendel's Law [11].

Table 3. The chisquare value calculates the average genotype of medium black seed (HM) Toraja black rice

Fenotype	Observation (O)	Expectation (E)	$(O-E)^2/E$
HP	2.70	14.74	9.84
HM	41.87	29.48	5.21
HT	14.39	14.74	0.01
			$X^2_{count} = 15.05$
			$X^2_{table} = 5.99$

db =2, α = 5%. HP (full black seed), HM (medium black seed), HT (thin black seed)

3.3. Percentage of Thin Black Seed (HT).

$LSD_{0.05}$ test results on the average percentage of full black seed color (HP) with a value of 20.21% were significantly different from black thin (HT) and medium black (HM). The percentage of thin black (HT) is not significantly different from the percentage of medium black (HM). Most plants with the highest percentage of thin black seed (HT) are plants with thin black genotype (HT) with an average amount of 30.52% per panicle, and plants with the lowest percentage of thin black seed (HT) are full black seeds (HP) with the average number is 20.21% per panicle (table 1). It can be expected that the black seed color genotype is slightly more homozygous recessive.

Chisquare value data calculates the genotype of thin black seed (HT) obtained that, there are plants with full black seeds (HP), medium black (HM), and thin black (HT) with a ratio of 1: 2 3. In accordance with the value X2 count = 3.99 which is smaller than X2 table = 5.99 (table 4). This indicates that the character is controlled by one locus with two alleles per locus and no dominance.

Table 4. The chisquare value calculates the average genotype of thin black seed (HT) Toraja black rice

Fenotype	Observation (O)	Expectation (E)	$(O-E)^2/E$
HP	7.22	12.49	2.23
HM	31.41	24.98	1.65
HT	11.34	12.49	0.11
			$X^2_{gcount} = 3.99$
			$X^2_{table} = 5.99$

db =2, α = 5%. HP (full black seed), HM (medium black seed), HT (thin black seed)

3.4. Analysis of Heritability

The heritability data of the characteristics of black rice rice in this study are presented in table 5. The results of the analysis of all characters in Toraja black rice rice are based on endosperm full black (HP), medium black (HM) and thin black (HT) color groupings It was found that the character of the percentage of full black endosperm (HP) color had a high heritability (0.782). The percentage character is medium black (HM) (0.247) and the percentage of thin black (HT) (0.256) has moderate heritability.

The character of the percentage of full black endosperm color has a high heritability indicating that the character is easy to inherit and its inheritance is more influenced by genetic factors than environmental factors. The percentage of middle black and the percentage of thin black has moderate heritability, indicating that the character has a moderate level of inheritance and inheritance is more influenced by genetic and environmental factors. Increased heritability can be caused by a decrease in the variety of environments or an increase in genetic diversity. Conversely, if the range of environments increases or genetic variability decreases, heritability will decrease. Appropriate heritability only applies to populations and locations where the value of heritability is calculated [12].

Table 5. The variable heritability value is the character of Toraja black rice

Variable	$\sigma^2 g$	$\sigma^2 e$	h^2	Criteria
PHP	80.80	22.550	0.782	High
PHM	27.013	82.360	0.247	Medium
PHT	9.080	26.340	0.256	Medium

$\sigma^2 e$: varians of environment, $\sigma^2 g$: varians of genetic, h^2 : heritability. Heritability criteria: high ($h^2 > 0{,}5$), medium ($0{,}2 \leq h^2 < 0{,}5$), low ($h^2 < 0{,}2$). Percentage of full black (PHP) (%), Percentage of middle black (PHM) (%), Percentage of thin black (PHT) (%)

4. Conclusion

The inheritance pattern of full black and thin black genotypes follows the Mendel inheritance pattern with a ratio of genotype 1: 2: 1, while the middle black genotype does not follow the Mandel inheritance pattern. The percentage character of full black endosperm color has a high heritability. The percentage character of medium black and thin black percentage has moderate heritability.

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