

Phenotypic and genetic diversity of watermelon (*Citrullus lanatus*) in East Java, Indonesia

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Abstract. Amzeri A, Badami K, Pawana G, Alfian Syah M, Daryono BS. 2021. Phenotypic and genetic diversity of watermelon (*Citrullus lanatus*) in East Java, Indonesia. *Biodiversitas* 22: 5223-5230. The assembly of hybrid watermelon (*Citrullus lanatus* Thumb.) varieties with superior characters is an effort to meet the needs of watermelon seeds in Indonesia and reduce dependence on imports of watermelon seeds. The morphological characterization of exploratory watermelon plants is needed to support the assembly of superior varieties because morphological characterization will reveal the characteristics of each watermelon tested. In addition, the genetic and phenotypic diversity coefficients, heritability, and correlation between characters are needed to support the assembling of the desired variety. This research aimed to determine morphological diversity, genetic and phenotypic diversity coefficients, heritability, and correlation among characters of watermelon from East Java, Indonesia. The research used a randomized complete block design with ten genotypes as treatment and three replications. The observed morphological characters were quantitative and qualitative characters. The number of characters was 60 characters. Eight quantitative characters, i.e., flowering date, harvesting date, fruit length, fruit diameter, skin thickness, fruit total soluble solids, number of seeds, and fruit weight were used to calculate the genetic and phenotypic diversity coefficient, heritability, and correlation between characters. Quantitative character data were subjected to analysis of variance, followed with a Duncan Multiple Range Test ($p < 0.05$). The results showed that (i) Grouping based on morphological characters produces dendrograms with similarity coefficients ranging from 0.58 to 0.86 or there was a morphological diversity of 0.14 to 0.42, (ii) The phenotypic diversity coefficient (PDC) was greater than the genotypic diversity coefficient (PDC) in all observed quantitative characters, (iii) The broad sense Heritability values of the tested watermelon genotypes ranged from 0.33 to 0.99, (iv) Fruit weight was significantly and positively correlated with fruit diameter, skin thickness, and number of seeds, (v) G1, G2, and G6 were the genotypes that can be used as parents to assemble superior watermelon varieties.

Keywords: Genetic variance, heritability, morphological diversity, phenotypic variance, watermelon

INTRODUCTION

Watermelon (*Citrullus lanatus* Thumb.) is an annual fruit plant with high economic value and is widely grown in various countries worldwide. This plant is widely cultivated in tropical and subtropical regions including Southeast Asia, Africa, the Caribbean, and the southern part of the United States (Saediman et al. 2020; Vinhas et al. 2021). In Southeast Asia, Indonesia is the second-largest watermelon producer, after Vietnam, with total production in 2017 of 499,475 tons (planted area of 32,558 ha), in 2018 of 481,727 tons (planted area of 31,699 ha), and 2019 of 523,355 tons (planted area of 34,505 ha) (FAO 2021). This amount of production can still meet the national needs, even Indonesia exported 165.01 tons of watermelon in 2018 (BPS-Statistics Indonesia 2018).

The problem with watermelon cultivation in Indonesia is the insufficient supply of watermelon seeds which caused the importation of seeds from Japan, Taiwan, and Europe (Jasmine et al. 2014). The need for watermelon seed in Indonesia is about 14.70 tons while the domestic watermelon seed production is around 12.50 tons, thus,

imported seeds of 2.20 tons are required to fulfill the national demand (BPS-Statistics Indonesia 2015). The scarcity of superior watermelon seeds causes the price of watermelon seeds to be very expensive so that it is not profitable for farmers in Indonesia. The assembly of hybrid watermelon varieties with superior characters is an effort to meet the needs of watermelon seeds in Indonesia and reduce the dependence on imports of watermelon seeds from abroad.

The first step in assembling superior watermelon varieties is to explore local cultivars in the watermelon planting centers. Plant exploration aims to collect germplasm as a source of genes in the assembly of plant varieties in plant breeding programs (Samadia and Haldhar 2020). The use of local varieties in the assembly of superior varieties is often recommended to expand the genetic background of the resulting improved varieties (Casanas et al. 2017). The use of local varieties that contain genes controlling resistance to various environmental stresses can increase the superiority of superior varieties to be produced through a plant breeding program (Levi et al. 2017; Khairullah et al. 2021; Walters et al. 2021).

Exploration of local watermelons in East Java-Indonesia has been carried out in 2019. East Java Province was selected as the place for watermelon plant exploration because it is Indonesia's largest watermelon producing province, accounting for 26.31 percent of the country's total watermelon production (BPS-Statistics Indonesia 2017). One of the watermelon planting centers in East Java is Madura Island. Madura Island has a large sub-optimal land area with low annual rainfall of 1346.89 mm (Suhartono et al. 2020), hence local cultivars obtained from Madura Island have the potential to be resistant to environmental stress and early maturity. These local cultivars can assemble varieties resistant to environmental stress and early maturity, which are needed for planting in areas with low rainfall (Amzeri et al. 2020). The number of local cultivars obtained in Madura Island was three genotypes. In addition, there were five local cultivars in Lamongan Regency, so that eight local cultivars were obtained from exploration in East Java Province.

The morphological characterization of exploratory watermelon plants is needed to support the assembly of superior varieties because morphological characterization will reveal the characteristics of each watermelon tested. Morphological information, for plant breeders, is not only used to see the morphological similarities between the watermelon plants tested, but also to obtain genetic information about the relationship between the watermelon plants tested (Gichimu et al. 2009). In the assembly of varieties, information on the relationship between breeding materials plays an important role in the efficient selection of parents through plant breeding programs (Choudhary et al. 2012; Kartahadimaja et al. 2021). Breeding materials with distant relatives are needed to determine the parents of the cross to assemble the desired variety (Gbotto et al. 2016). Crosses between parents of distant relatives will produce offspring with wide genetic segregation, making it easier to choose the desired variety (Pessoa et al. 2015). In addition, the genetic and phenotypic diversity coefficient, heritability, and correlation between characters are needed to support the assembling of the desired variety (Kuswanto 2017; Wehner et al. 2017). This research aimed to determine the morphological diversity, genetic and phenotypic diversity coefficients, heritability, and correlation among characters of watermelon from East Java, Indonesia.

MATERIALS AND METHODS

Plant materials

Plant materials used were ten genotypes of watermelon consisting of eight locally explored genotypes and two-hybrid varieties as checks (Legyta and Gonzales). Exploration and collection of eight local watermelons in East Java Province, Indonesia (Table 1, Figure 1) was conducted from April to August 2019. The main annual rainfall data and temperature are taken from the meteorology station at the research location. The eight genotypes were purified five times by selfing from September 2019 to December 2020 and tested for their morphological diversity from January to March 2021.

Field experiment

The research was conducted in Bangkalan Regency, Madura, Indonesia. The research location is located at latitude: 7°09'14.8" S, longitude: 112°44'01.6" E, altitude: 5 m, average annual rainfall: 269 mm, temperature: 28-32°C, Grumusol soil type, pH: 7.1. The research used a randomized complete block design with ten genotypes as treatment and three replicates, so there were 30 experimental units. Each experimental unit consisted of ten plants. Ten-day-old watermelon plants were transferred to beds with dimensions of 3.0 m x 3.5 m x 0.7 m (length x width x height) with a spacing of 60 cm x 250 cm. Basal fertilization was carried out during soil preparation at the rate of 150 kg NPK ha⁻¹ (2:2:1), and organic manure was applied at the rate of 10 tons ha⁻¹. NPK fertilizer was also applied at weekly intervals at the rate of 2 g per plant starting at 14 days after planting. After the plants had entered the generative phase, NPK fertilization was performed at the rate of 3 g per plant at weekly intervals. Plant pests and diseases are controlled according to plant conditions. Each plant is kept one watermelon on segment number eight.

The observed morphological characters were quantitative and qualitative characters. The number of characters measured was 60 characters. Eight quantitative characters, i.e., flowering date, harvesting date, fruit length, fruit diameter, skin thickness, fruit total soluble solids, number of seeds, and fruit weight were used to calculate the genetic and phenotypic diversity coefficient, heritability, and correlation between characters.

Table 1. Data of location and climate of watermelon exploration

Genotype	Location	Latitude longitude	Alt (m asl.)	Mean annual rainfall (mm)	Temp. (°C)
G1	Gellaman, Arjasa, Sumenep	6°57'00.6"S, 115°19'35.2"E	30 m	1395	21.10 - 35.40
G2	Saobi, Kangayan, Sumenep	6°59'26.8"S, 115°26'27.5"E	5 m	761	21.00 - 35.00
G3	Tlogoretno, Brondong, Lamongan	6°54'42.1"S, 112°12'38.5"E	18 m	2023	27.44 - 33.60
G4	Tlogoretno, Brondong, Lamongan	6°55'21.9"S, 112°12'28.1"E	12 m	2023	27.44 - 33.60
G5	Bulu tengger, Sekaran, Lamongan	7°04'17.8"S, 112°16'37.8"E	6 m	3129	19.70 - 32.10
G6	Miru, Sekaran, Lamongan	7°04'00.6"S, 112°16'23.5"E	6 m	3129	19.70 - 32.10
G7	Pamaroh, Kadur, Pamekasan	7°06'11.1"S, 113°30'23.9"E	121 m	1287	28.00 - 30.00
G8	Sukorame, Sukorame, Lamongan	7°20'53.3"S, 112°06'39.8"E	38 m	2738	27.44 - 33.60

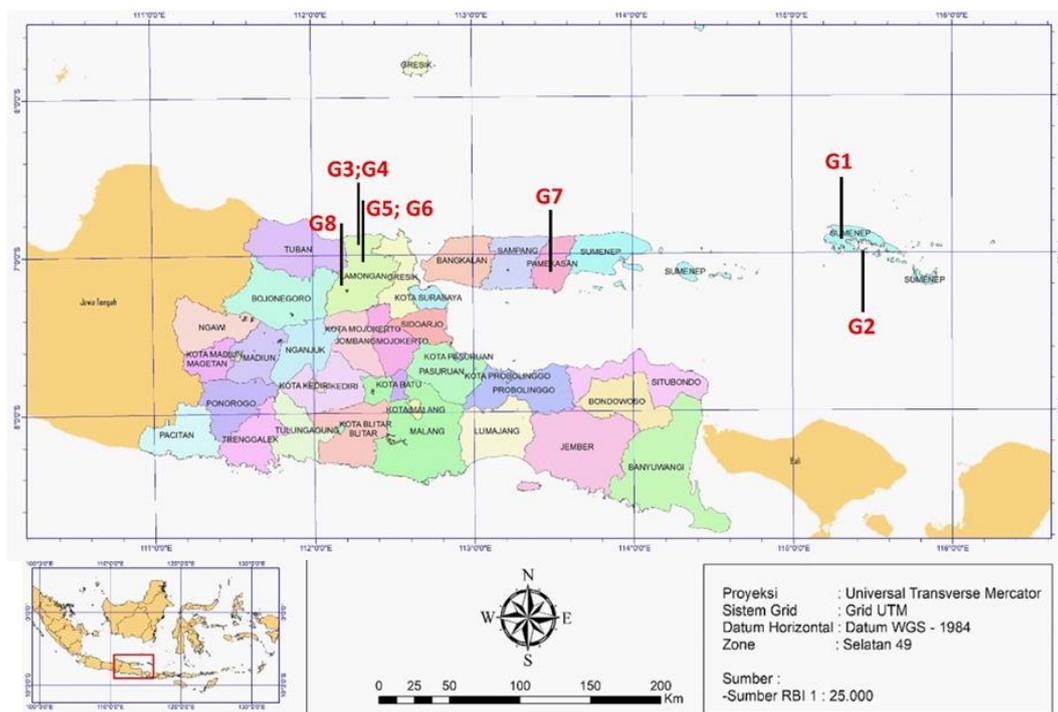


Figure 1. Location of local watermelon collection in East Java Province, Indonesia. G1-G8 indicates the location of the watermelon collection in Table 1

Data analysis

The modus method was used to analyze the qualitative data collected. Furthermore, the kinship analysis was carried out with the UPGMA (Unweighted Pair Group Method With Arithmetic Mean) method using the Numerical Taxonomy and Multivariate Analysis System (NTSYS version 2.1) software. Before being analyzed using software, the qualitative data was compiled into binary data. The characters that the watermelon genotype lacked were given the number 0, whereas the characters that the watermelon genotype held were given the number 1.

Quantitative character data was subjected to ANOVA and DMRT post hoc test ($p < 0.05$), performed using SPSS software version 22.0. Estimates of environmental, genetic, and phenotypic variances were calculated based on the expected value of the mean square of each parameter (Singh and Chaudary 1979). Based on Allard (1960), estimation of broad-sense heritability value (h^2_{bs}) was carried out. Pearson correlation coefficient analysis based on Walpole (1982) to determine the close relationship between the observed characters.

RESULTS AND DISCUSSION

Morphological characters

The cluster analysis results produced from the morphological similarity matrix of watermelon which is shown in Table 2 provided no grouping based on the origin or exploration outcomes, but rather on the similarity of 60 morphological traits. Genetic drift and selection in different

environments can cause genetic diversity greater than the distance from the area where the plant grows, meaning that even if a watermelon genotype comes from the same place, especially on quantitative characters, if the environment where it grows is different, it will affect genetic diversity (Munisse et al. 2011; Guo et al. 2013). According to Uslan et al. (2020), genotypes originating from the same area are not always in the same group. Genotypes with a lot of morphological character similarities have a tighter association, while genotypes with a few morphological character similarities have a more distant relationship (Basyuni and Jayusman 2019).

Grouping based on morphological characters produced dendrograms with similarity coefficients ranging from 0.58 to 0.86 or there is a morphological diversity of 0.14 to 0.42 (Figure 2). The morphological diversity is shown in Figures 3, 4, and 5. The large similarity between the genotypes indicates that these genotypes have a close relationship. At a similarity of 0.58, there are two main groups. Group 1 consisted of G1, G2, and G7, while group 2 consisted of G3, G4, G5, G6, Legyta variety, and Gonzales variety. Group 1 was united by the similarity of the character of the flower bud shape, depression at fruit base, depression at the fruit apex, size of pistil scar, flesh main color, number of seeds, seed size, and ground color of seed testa, while group 2 was united by the similarity of characters in fruit shape in longitudinal section and depression at the fruit apex. Group 2 formed two subgroups, i.e., group A consisted of G3, G4, G5, G6, Legyta variety, and Gonzales variety, while G8 separated from group A to form its own group (Group B) due to differences in petiole color, basic skin color intensity, and pericarp color.

Table 2. The similarity index value of ten watermelon genotypes (%)

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
G1	100.00									
G2	71.60	100.00								
G3	70.00	68.30	100.00							
G4	55.00	65.00	65.00	100.00						
G5	61.60	56.60	70.00	65.00	100.00					
G6	60.00	58.30	68.30	56.60	78.30	100.00				
G7	71.60	85.00	68.30	60.00	53.30	56.60	100.00			
G8	52.00	55.00	63.00	53.30	66.60	66.60	58.30	100.00		
G9	60.00	53.30	76.60	75.00	65.00	65.00	50.00	58.30	100.00	
G10	61.60	48.30	60.00	63.30	63.00	63.30	51.60	55.00	65.00	100.00

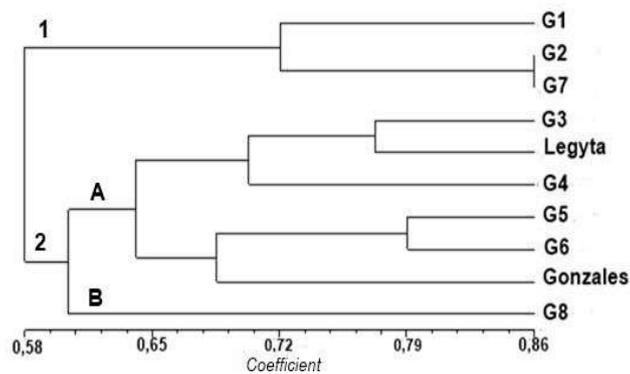


Figure 2. Dendrogram of watermelon genotypes from East Java-Indonesia based on morphological characters

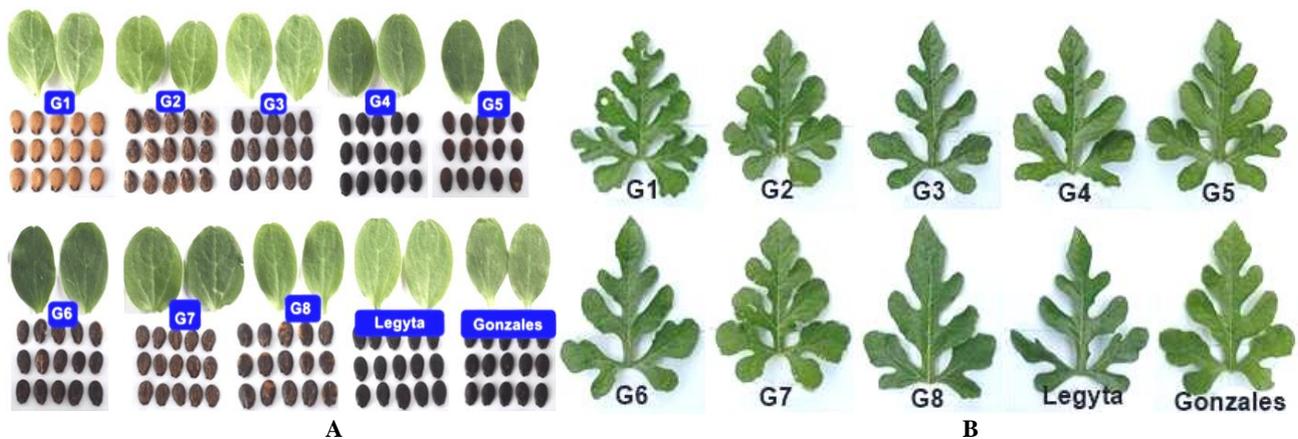


Figure 3. Morphological characters of 10 watermelon genotypes. A. Cotyledons and seeds. B. Leaf shapes



Figure 4. Flower morphological characters of 10 watermelon genotypes. A. Male flower. B. Female flower

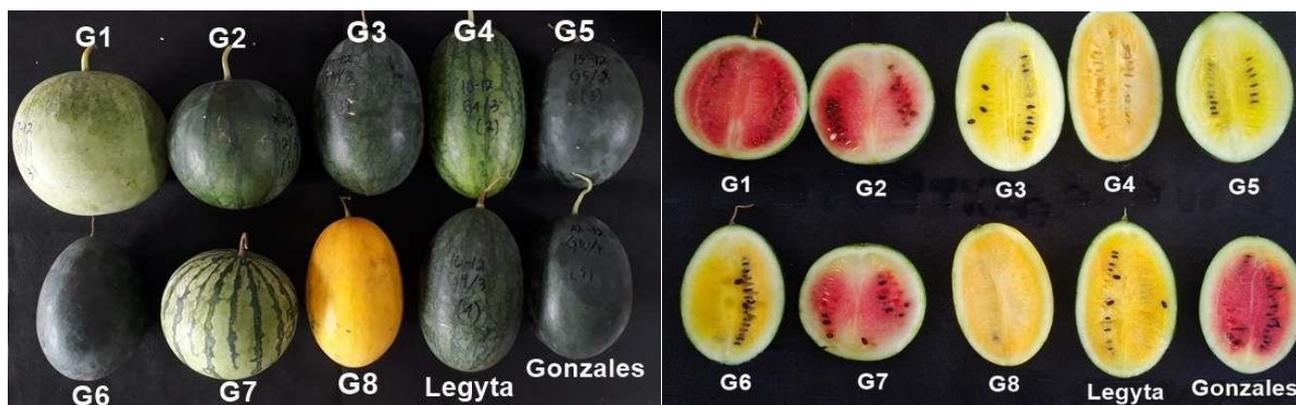


Figure 5. Fruit morphological appearance of ten watermelon genotypes

Yield component and yield variables

The Anova dan post hoc DMRT showed that flowering date, harvesting date, fruit length, fruit diameter, epidermal thickness, fruit total soluble solids, number of seeds, and fruit weight varied significantly among genotypes (Table 3; Table 4). All genotypes tested had an average harvesting date of fewer than 80 days, so all genotypes had a short harvest life. The G1 variety had the highest mean fruit weight (3.20 kg) while Gonzales had the lowest (2.18 kg). The average fruit weight of G1, G2, G6, and G7 was higher than the two check varieties (Legyta and Gonzales).

G1 had the highest average epidermal thickness of 1.23 cm, and the lowest average epidermal thickness was G2 and G8 (0.63 cm). G1 had the average ideal epidermal thickness mostly preferred by farmers and had the highest average value as compared to the two check varieties. All tested genotypes had a fruit total soluble solids value of more than 8 °Brix except G7.

The watermelon variety assembly program aims to create superior varieties with early maturity, high production, thick skin, and high total soluble solids in the fruit. Superior watermelon varieties are expected to have a harvest age of fewer than 80 days (Sunyoto et al. 2006), fruit weights ranging from 4-5 kg, skin thickness of 1.1-1.4 cm (Makful et al. 2019), and fruit total soluble solids of more than or equal to 8 °Brix (United Nation Economic Commission for Europe 2017). High production is described in the character of fruit weight. Fruit length and fruit diameter are supporting characters for fruit weight characters, where there is a positive correlation between the two characters with fruit weight (Mulyani and Waluyo, 2019; Bhagyalekshmi et al. 2020).

Genetic parameter analysis

The phenotypic diversity coefficient (PDC) was greater than the genotypic diversity coefficient (GDC) in all observed quantitative characters (Table 5). PDC values greater than GDC indicate that selection can be made based on the appearance of these characters (Rabou and El-Sayed 2021). Furthermore, Ene et al. (2016) suggested that PDC, which is greater than GDC, indicates that environmental factors rather than genetic factors much more influence the character. The PDC value, which is almost the same as

GDC, shows that the environment has very little influence on the appearance of the character. Characters with almost the same PDC and GDC values were fruit length, fruit diameter, epidermal thickness, fruit total soluble solids, and number of seeds per fruit.

Heritability values in the broad sense of the watermelon genotypes tested for the evaluated characters ranged from 0.33 to 0.99. Based on heritability criteria, the watermelon genotypes tested had moderate to high values. Characters that have high heritability values were fruit length, fruit diameter, epidermal thickness, fruit total soluble solids, and number of seeds. Characters with moderate heritability values were flowering date, harvesting date, and fruit weight. Characters with high heritability values imply that genetic influences are greater than environmental in determining character variance between genotypes (Ayaine et al. 2012; Adjoumani et al. 2016; Anburani et al. 2019; Badami et al. 2020). Selection of these characters has a high chance of genetic advance because genetic factors strongly control the observed characters so that they will be passed on to their offsprings (Ullah et al. 2012) and selection of characters that have high heritability values can be done in the early generations (Hakim and Suyamto 2017).

Correlation between quantitative characters

Fruit weight (yield) is the main component of an important melon plant because it has economic value. Breeding programs often use an indirect selection approach to improve yield characters by selecting characters that correlate with yield. The correlation between characters will facilitate selection because a decrease or increase will follow changes in the value of a character in the character (Said and Fatiha 2015). Selection will be more effective if there is a correlation between the characters to be selected (Nisha et al. 2018). The close relationship between the characters studied was estimated by using the correlation coefficient.

Positively correlated characters indicate that an increase in the value of a character will be followed by an increase in the value of other characters, while negatively correlated characters indicate that a decrease will follow an increase in the value of another character in the value of other

characters. Fruit weight was significantly positively correlated with fruit diameter, epidermal thickness, and number of seeds (Table 6). Fruit total soluble solids were not significantly correlated with all the observed characters. Epidermal thickness was significantly positively correlated with fruit diameter. Harvesting date had a highly significant positive correlation with flowering date. The use of genotypes with large fruit diameter, thick skin, and a large number of seeds is a breeding approach for increasing fruit weight. The breeding strategy is to increase the thickness of the epidermis by using a genotype with a large fruit diameter while getting an early maturity by using a genotype with a short flowering date.

Selected genotypes for plant breeding program

The watermelon varieties to be assembled must have the characteristics of early maturity, high production, thick epidermis, and high fruit total soluble solids. Based on the research results, all the tested genotypes had a short harvesting date (<80 days), so that the eight tested genotypes could be used to assemble early maturity watermelon varieties. G1, G2, G6, and G7 had higher average fruit weights than the two check varieties (Legyta and Gonzales), so they could be used to assemble watermelon varieties with high production characteristics. G1 had a thick fruit epidermal character as compared to nine tested genotypes (including comparison varieties). Furthermore, all tested watermelon genotypes had high fruit total soluble solids values (>8 °Brix) except G7.

Determination of genotypes that will be used as parents in the assembly of watermelon varieties in this research was based on the dendrogram results of morphological characters, assessment of quantitative characters, analysis of genetic parameters, and correlations between correlations quantitative characters. The genotypes that can assemble watermelon varieties with red flesh are G1 and G2 because they have early maturity, high fruit weight, and high fruit total soluble solids. In addition, G1 has thick fruit epidermis. The genotype that can be used to assemble watermelon varieties with yellow and/or orange flesh is G6 because it has early maturity, high fruit weight, high fruit total solids, and tends to be high in skin thickness.

The variety assembly method used to assemble the watermelon varieties produced in this research was by selection and hybridization. In watermelons with red flesh

color, the selection of certain characters in the G1 and G2 lines will be very effective because it will increase these characters. The effectiveness of the selection was caused by the heritability values for the characters of harvest age, fruit weight, epidermal thickness, and total soluble solids of fruit, which had moderate to high values, so that selection on these characters had a high chance of genetic advancement.

Table 3. Characteristics of flowering date, harvesting date, fruit length, and fruit diameter of ten watermelon genotypes

Genotype	Flowering date (days)	Harvesting date (days)	Fruit length (cm)	Fruit diameter (cm)
G1	23.00 ab	65.00 ab	20.23 e	62.03 a
G2	22.00 b	64.00 b	18.27 f	56.47 b
G3	23.00 ab	65.00 ab	30.30 b	45.33 e
G4	24.00 a	66.00 a	32.70 a	42.00 f
G5	23.00 ab	65.00 ab	28.33 c	48.47 d
G6	24.00 a	66.00 a	26.27 d	47.17 d
G7	21.00 b	63.00 b	18.87 ef	56.73 b
G8	23.00 ab	65.00 ab	30.17 b	41.03 g
Legyta	23.00 ab	65.00 ab	32.37 a	49.00 c
Gonzales	25.00 a	67.00 a	24.97 d	40.10 g

Note: The numbers followed by the same letter in the same column are not significantly different according to the 5% DMRT test

Table 4. Characteristics of epidermal thickness, fruit total soluble solids, number of seeds, and fruit weight of ten watermelon genotypes

Genotype	Epidermal thickness (cm)	Fruit total soluble solids (°Brix)	Number of seeds per fruit	Fruit weight (kg)
G1	1.23 a	9.00 d	254.44 a	3.20 a
G2	0.63 c	9.33 c	178.11 b	2.69 ab
G3	0.93 b	10.44 a	98.56 bc	2.41 bc
G4	0.67 c	8.76 e	118.33 bc	2.41 bc
G5	0.97 b	10.56 a	59.44 d	2.46 bc
G6	0.93 b	10.00 b	121.56 bc	2.60 bc
G7	0.70 c	7.11 f	164.67 b	2.58 bc
G8	0.63 c	10.44 a	97.44 bc	2.40 bc
Legyta	0.93 b	10.33 a	93.11 bc	2.48 bc
Gonzales	0.90 b	9.89 b	70.78 bc	2.18 c

Note: The numbers followed by the same letter in the same column are not significantly different according to the 5% DMRT test

Table 5. Values of environmental variance, genetic variance, phenotypic variance, heritability, GDC, and PDC of ten watermelon genotypes

Character	σ^2_e	σ^2_g	σ^2_p	GDC	PDC	h^2_{bs}
Flowering date	1.01	0.67	1.68	3.54	5.61	0.40
Harvesting date	1.01	0.67	1.68	9.53	15.10	0.40
Fruit length	0.74	29.88	30.62	20.83	21.08	0.98
Fruit diameter	0.54	54.70	55.24	15.14	15.22	0.99
Epidermal thickness	0.01	0.04	0.05	22.15	25.06	0.78
Fruit total soluble solids	0.02	1.17	1.19	1.96	1.98	0.98
Fruit weight	0.09	0.04	0.13	12.98	23.40	0.33
Number of seeds	1176.44	3042.89	4219.33	41.48	48.84	0.72

Note: σ^2_e : environmental variance, σ^2_g : genetic variance, σ^2_p : phenotypic variance, GDC: genotypic diversity coefficient, PDC: phenotypic diversity coefficient, h^2_{bs} : heritability in the broad sense. Heritability criteria: high ($h^2_{bs} \geq 0,5$), moderate ($0,2 < h^2_{bs} < 0,5$), low ($h^2_{bs} \leq 0,2$)

Table 6. The linear correlation coefficient between characters in the watermelon genotypes tested

	FD	HD	FL	FD	ST	FTSS	NS	FW
FD	1.00							
HD	1.00**	1.00						
FL	0.48	0.48	1.00					
FD	-0.66*	-0.66*	-0.73*	1.00				
ST	-0.03	-0.03	0.37	0.64*	1.00			
FTSS	0.31	0.31	0.51	-0.31	-0.07	1.00		
NS	-0.43	-0.43	-0.68*	0.85*	0.77*	-0.42	1.00	
FW	-0.03	-0.03	-0.37	0.64*	1.00**	-0.07	0.77*	1.00

Note: FA: flowering date, HA: harvesting date, FL: fruit length, FD: fruit diameter, ST: skin thickness, FTSS: fruit total soluble solids, NS: number of seeds, FW: fruit weight. *,**significant at 5% and 1% level of probability, respectively

In addition, the assembly of watermelon varieties using the hybridization method between G1 and G2 can be carried out. G1 and G2 have a distant relationship (28.4%) even though the two genotypes are in the same group. A cross between the two genotypes will produce a wide variation in offspring and no depression inbreeding occurs. G1 and G2 are lines that can be used as parents to assemble superior watermelon varieties with a red flesh color, while G6 are lines that can be used as parents to assemble superior watermelon varieties with an orange flesh color.

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