Hybridization and yield test of reciprocal watermelon in the greenhouse scale

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Abstract. This study aims to select reciprocal watermelon crosses in a new superior variety breeding program using the single cross method. The male and female parents used in the study consisted of 12 pure lines (WM 08-19-1 ♂x♀ WM 06-27-4; WM 01-3-3-4-1 ♂x♀ WM 04-12-11-1-1; WM 11-1-2-2-8 ♂x♀ WM 10-1-1-9-10; WM 03-27-2 ♂x♀ WM 08-6-3; WM 12-1-5 ♂x♀ WM 04-1-5-10; WM 16-1-5-6-3 ♂x♀ WM 06-1-11-5). The study used a one-factor randomized block design (RBD) with 3 replications. Data analysis used analysis of variance and the least significant difference test (LSD) at the 5% level. The fastest harvest age was found in the WM 2110-1011 line and the fruit with the highest sweetness level was found in the WM 2110-1011 line. The results of the reciprocal hybridization research conducted, watermelon lines that have a plain striped type on the fruit are lines WM 06-27-4, WM 04-12-11-1-1 and WM 10-1-1-9-10. The thin striped type is found in the WM 08-6-3 line and the thick striped type is found in the 04-1-5-10 line and WM 06-1-11-5. The line that has yellow flesh is WM 04-1-5-10. The results of this study indicated that the collection of pure lines and reciprocal crosses are used as selection materials for the F1 hybrid watermelon breeding program.

1 Introduction

Watermelon has many benefits when consumed, such as preventing dehydration, maintaining skin moisture, and maintaining healthy hair [1]. Indonesia has a fairly large export opportunity for watermelon, namely 1,000 ton while world market demand for watermelon reaches 169,746 ton/year and is not yet sufficient for international market demand [2]. The development of watermelon productivity in Indonesia according to the Statistics Indonesia has not experienced a significant increase in the last three years, in 2018 watermelon production was 481,744 ton, in 2019 it was 523,333 ton and in 2020 it was 523,335 ton [3].

Most of the hybrid watermelon seeds used by Indonesian farmers are varieties originating from abroad (introductions), so superior pure lines are needed to create quality local hybrid watermelons that are suitable for cultivation in Indonesia [3]. The concept of pure lines itself emerged from a series of experiments on soybeans carried out by the Danish botanist W.L Johannsen in 1903 [4]. The introduction of hybrid seeds is often a hope for Indonesian

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farmers in increasing fruit production [5]. The development of superior watermelon varieties can use plant breeding techniques such as crossing, selection and polyploidization [6].

The increasing demand for watermelons can be met by producing higher quality plants with higher potential than their parents from plant breeding activities [7]. The development of superior cultivars that have high yield potential can be done in various ways, including through hybridization or crossing [8]. Reciprocal crosses are carried out to see the viability value of the population, the results of reciprocal crosses are used as a reference for selection. A reciprocal cross is a cross between two parents, where the parent acts as the female in one cross, but also becomes the male in another cross. The aim of watermelon plant breeding is to produce hybrid varieties that can be developed quickly and produce high yields [9].

Genetic diversity plays a very important role in plant breeding activities, from watermelon lines that have previously been produced it is necessary to carry out a selection process, one of which is selection of pure lines. The lines that have been previously obtained can be identified for their respective agronomic and morphological characters, so that their genetic potential can be known [10]. The aim of this research is to carry out reciprocal crosses of six pairs of pure lines to produce single cross hybrid seeds which will become new varieties with high levels of sweetness, fast maturity and high yield potential for greenhouse scale.

2 Research Method

The research was conducted in June-September 2021 at the Greenhouse Seed Teaching Farm. The materials that will be used are black silver plastic, cocopeat, mulch, drum, fertilizer 2 $kg.m^{-2}$, agricultural lime 1 $kg.m^{-2}$, insecticide (material active profenofos 500 $g.l^{-1}$, prevathon active ingredient chlorantaniliprol concentration 0.5-2 $ml.l^{-1}$, and furadan 3G with active ingredient (karbofuran), fungicide (dithane M45 with active ingredient mankozeb M-45 dose 2 $g.l^{-1}$), bactericide (nordox active ingredient copper oxide 56%), fertilizer (urea with a concentration of 2 $g.l^{-1}$, NPK Mutiara 15:15:15 per plant as much as 20 g and white KNO_3 as much as 5 $g.l^{-1}$, oil paper, paper clips, markers, labels and seeds of six pairs of pure lines of watermelon. The six pairs of pure lines can be seen in Table 1.

The tools used in this research include agricultural tools for tilling the land, namely hoes. Tools for maintenance such as buckets, roll meters, scissors, stakes, trolleys, sprayers, sickles, corsets, a set of irrigation tools (drip stick). Other complementary tools include writing instruments for observation, lid paper for pollination, mica plastic for labeling, filters, knives, tiles for the extraction process and a refractometer which is used to measure sweetness levels. Seed packaging uses aluminum foil Ziplock plastic.

The method used in this research is a Randomized Block Design for observing quantitative data with one factor, namely the pure strain of watermelon. This experiment used six pairs of watermelon lines. Three samples were taken from each bed to obtain 18 experimental units.

Qualitative character data was observed based on Union International of Protection Observation Vegetable (UPOV) [11] and for color observations using the RHS Color Chart. Three plant samples were taken from each variable for each replication. The data obtained will be analyzed using analysis of variance, if there is a significant difference in the mean value of the treatment, then continue using the LSD-test at the 5% level.

2.1 Quantitative observation (9 variables)

1) Age of flowering, observed from the total flowering of 50% of each line. Observations were made on each sample, in units of days after transplanting (DAT).
2) Harvest time is seen when the fruit is fully ripe. Observations were made on each sample, in units of days after transplanting (DAT).

3) Weight per fruit, weigh each fruit. Observations on each sample.
4) Length of fruit, measure each fruit using a ruler longitudinally.
5) Fruit diameter measure each fruit using a ruler crosswise.
6) Thickness of the fruit skin, measured with a ruler looking at the distance from the fruit flesh to the outer skin.
7) Sugar content or sweetness level measured using a refractometer. Observations were made on each sample. with ^{o}brix units.
8) The weight of the seeds must be observed on each sample.
9) Weight 100 seeds, each fruit is counted as 100 seeds, do 3 repetitions.

Table 1. Watermelon pure lines parental

No	Female (♀)	Plant characteristic	Male (♂)	Plant characteristic
1	WM 06-27-4	Green skin color, thick stripes, red flesh color, oval shape, harvest age 70 days after planting.	WM 08-19-1	Dark green skin, plain stripes, red flesh color, oval shape, harvest age 67 days after planting.
2	WM 04-12-11-1-1	Dark green skin color, plain stripes, red flesh color, oval shape, harvest age 70 days after planting.	WM 01-3-3-4-1	Dark green skin color, plain stripes, red flesh color, oval shape, harvest age 72 days after planting.
3	WM 10-1-1-9-10	Dark green skin color, plain stripes, red flesh color, oval shape, harvest age 69 days after planting.	WM 11-1-2-2-8	Dark green skin color, thin strips, red flesh color, oval shape, harvest age 70 days after planting.
4	WM 08-6-3	Bright green skin color, thin strips, red flesh color, oval shape, harvest age 70 days after planting	WM 03-27-2	Bright green skin color, thin strips, red flesh color, oval shape, harvest age 71 days after planting.
5	WM 04-1-5-10	Green skin color, thick stripes, yellow flesh color, oval shape, harvest age 70 days after planting.	WM 12-1-5	Bright green skin color, thin strips, yellow flesh color, oval shape, harvest age 70 days after planting.
6	WM 06-1-11-5	Green skin color, thick stripes, red flesh color, oval shape, harvest age 72 days after planting.	WM 16-1-5-6-3	Green skin color, thick stripes, red flesh color, oval shape, harvest age 63 days after planting.

2.2 Qualitative observation (9 variables)

1) Leaf type observations are made when the plant begins to enter the generative phase.
2) Leaf color Observation of leaf color is seen when the plant enters the vegetative phase.
3) Stem color, seen when the plant enters the vegetative phase.
4) The color of the flower crown is seen from the color of the crown when the plant has entered the flowering phase.
5) Fruit shape, observe the shape of the fruit when the fruit is fully ripe.
6) Fruit skin color observed when the fruit is harvested.
7) Striated type, observed when the fruit is harvested.

8) The color of fruit flesh is observed when the fruit has been harvested and then seen the color of the flesh.
9) The seed color observed when the seeds have been counted and weighed and the color of the seeds is seen. The color of the seeds is observed when the fruit is harvested.

3 Results and Discussion

A reciprocal cross itself is a cross between two parent plants, where the parent acts as the male in one cross, and as the female in another cross. Reciprocal crosses are carried out to see the viability value of the population, the results of reciprocal crosses are used as a reference for selection.

The results of observations in the research are presented in a variance analysis of several observed observation variables. The results of the analysis show that there are real differences in each observation variable observed. The observation results can be seen in Table 2.

Table 2. Recapitulation of F-value and Coefficient of Variance in Reciprocal Hybridization.

No	Parameter	F-value		Coefficient of variance (CV) (%)
1	Age of flowering (DAT)	2.43	*ns*	6.75
2	Harverst time (DAT)	1.29	*ns*	2.29
3	Weight per fruit (kg)	21.39	**	13.67
4	Length of fruit (cm)	136.65	**	7.42
5	Diameter of fruit (cm)	6.76	**	7.63
6	Thickness of the fruit skin (cm)	14.54	**	3.93
7	Sugar content (°brix)	3.15	*ns*	4.90
8	The weight of the seed	435.98	**	1.95
9	Weight of 100 seeds	4.23	*	7.84

Remark: ns: not significant * : significant different * * : very significant different

Flowers on watermelon plants mostly appear when the plants are 30 days old and above. The flowering age of plants can be influenced by environmental factors such as rainfall, sunlight, humidity and temperature [12]. Observation of flowering age began when the female flowers first appeared. The line that flowers appear first is the WM 10-1-1-9-10 line, namely at 31 days after planting, and the line that takes the longest to flower is the WM 04-12-11-1-1 line, namely at 37 days after planting (Table 3). Table 4. shows that there is a significant difference in hybridization results, but there is no significant difference in the observation parameter of harvest age.

Table 3. Observation Variable Flowering Age Reciprocal Hybridization

No	Lines	Age of flowering (DAT)
1	WM 06-27-4	34.89
2	WM 04-12-11-1-1	37.33
3	WM 10-1-1-9-10	31.22
4	WM 08-6-3	34.00
5	WM 04-1-5-10	35.78
6	WM 06-1-11-5	33.40

Remark: Numbers followed by the same letter in the same column are not significantly different according to the LSD test at the 5% level.

Table 4. Observation of Fruit Results from Reciprocal Hybridization.

No	Lines	Harvest	Weight per fruit		Length of fruit		Diameter Of fruit	
1	WM 2110-0608	70.22	1.91	b	19.77	cd	13.28	b
2	WM 2110-0401	70.00	1.08	a	16.17	b	11.06	a
3	WM 2110-1011	68.78	1.74	b	21.12	d	12.87	b
4	WM 2110-0803	70.33	1.68	b	18.74	c	12.77	b
5	WM 2110-0412	70.89	0.86	a	13.19	a	10.74	a
6	WM 2110-0616	72.00	0.80	a	14.20	ab	10.00	a
BNT 5%			0.43		3.33		1.54	
KK %		2.29	13.67		7.73		7.63	

Remark: Numbers followed by the same letter in the same column are not significantly different according to the LSD test at the 5% level.

The results of the reciprocal hybridization of six pairs of watermelon lines obtained the results as in Table 4. The fastest harvest time is the WM 2110-1011 line, which can be harvested at 68 days after planting. Physiologically mature plants can be seen from the physical condition of the plant. The leaves on the plant's vines begin to dry out. The plant line that has the longest harvest time is the WM 2110-0608 line, which can be harvested at 72 days after planting. The success of artificial pollination is influenced by several factors including parental compatibility, timeliness of female receptive maturity and male anthesis, plant fertility and environmental factors such as rainfall, sunlight, humidity and temperature [12].

In chili plants fruit weight is used as a character for selecting hybrids because it describes the productivity per hectare. Observation of fruit weight is carried out by weighing each fruit that has been harvested using a scale. Most consumers prefer medium-sized fruit with a fruit weight of around 1.5-2 kg [13]. The fruit in this study that had the heaviest weight was the WM 2110-0608 strain, with a fruit weight of 1.91 kg and the fruit with the lightest weight was the WM WM 2110-0616 strain. The results of these observations can be used to determine fruit production per hectare.

Fruit diameter has a real positive correlation with fruit length [3]. The length of the watermelon was observed by dividing the watermelon into two parts, then measuring the length using a ruler. The length and width of the watermelon affect the size of the fruit. The fruit with the longest size is strain WM 2110-1011 and the smallest fruit is strain 2110-0616. The fruit with the largest diameter is on the WM 2110-0608 line and the fruit with the smallest diameter is on the WM 2110-0616 line.

Table 5. Variable Observation of skin thickness and sweetness level of fruit resulting from reciprocal hybridization

No	Lines	Thickness of fruit skin		Sugar content
1	WM 2110-0608	0.97	cd	9.26
2	WM 2110-0401	0.88	b	9.43
3	WM 2110-1011	0.98	d	10.08
4	WM 2110-0803	0.91	bc	8.77
5	WM 2110-0412	0.80	a	8.94
6	WM 2110-0616	1.00	d	9.60
LSD 5%		0.06		
CV %		3.93		4.90

Remark: Numbers followed by the same letter in the same column are not significantly different according to the LSD test at the 5% level.

The function of the thickness of the fruit skin is to keep the inside of the fruit in good condition. When shipping fruit over long distances, the thickness of the fruit skin will play

an important role. The level of fruit sweetness affects the quality of the fruit, most consumers like fruit with a high level of sweetness (Table 5). Observe the level of sweetness using a refractometer on the middle and edges of the fruit. The results of reciprocal hybridization on the skin thickness parameter obtained significantly different results, while the results of the analysis on the level of fruit sweetness showed that the results were not significantly different.

The thickness of the fruit skin is very important for fruit durability when shipped long distances [14]. The skin thickness observed in this study was the highest, namely 0.98 cm, namely in the WM 2110-1011 strain, while the average fruit skin thickness with the lowest value was in the WM 2110-0412 strain, namely 0.80 cm thick. Most consumers like watermelon with thin skin. Watermelon contains 8-10% solids and 20-25% sugar or sucrose [15]. Measuring the sweetness level of the fruit is carried out using a refractometer. The sweetness level is measured at the edges and middle of the fruit and then averaged. Most consumers like fruit with a high level of sweetness. The fruit with the highest level of sweetness is the WM 2110-1011 strain with a sweetness level as high as 10.08 ^{o}brix and the fruit with the lowest sweetness level is the WM 2110-0803 strain with a sweetness level of 8.77 ^{o}brix. Observations on the level of sweetness made after analysis did not show any differences in the level of sweetness and were not significantly different.

The seeds in watermelon plants function as material for generative plant propagation. Watermelon plants have a variety of seed colors, some are black and some are brown. Seed observations were the number of seeds, weight of seeds per fruit and weight of 100 seeds. The results of the observations showed interactions and were significantly different and then analyzed using a follow-up test with a level of 5%. The results of the observations can be seen in Table 6.

The number of seeds in watermelon plants is divided into three classes, namely those with many seeds, medium seeds and few seeds [15]. As a result of this reciprocal hybridization, the fruit that produced the most seeds was in the WM 2110-0803 line, namely 217 seeds and the fruit that produced the fewest seeds was in the WM 2110-0616 line, namely 132.7 seeds. The heaviest seed weight per fruit in this study was the WM 2110-0401 strain, namely 9.29 g and the lowest was the WM 2110-0616 strain, namely 5.0 g. The weight of 100 seeds from each strain is the heaviest, namely the WM 2110-0608 strain, weighing 4.3 g and the ligh test, namely the WM 2110-0616 strain, weighing 3.6 g.

Table 6. Variable Observation of Reciprocal Hybridization Seed Results

No	Lines	Number of seeds		Weight of seeds per fruit (g)		Weight of 100 Seeds (g)	
1	WM 2110-0608	195.67	e	9.22	d	4.30	c
2	WM 2110-0401	153.00	c	9.29	d	3.43	a
3	WM 2110-1011	158.67	d	8.11	c	3.97	bc
4	WM 2110-0803	217.00	f	9.03	d	3.60	ab
5	WM 2110-0412	114.33	a	6.03	b	4.23	c
6	WM 2110-0616	132.70	b	5.00	a	3.60	ab
LSD 5%		4.93		0.62		0.52	
CV %		1.77		1.95		7.84	

Remark: Numbers followed by the same letter in the same column are not significantly different according to the LSD test at the 5% level.

Qualitative observations on watermelon plants include leaf color, leaf shape, fruit flesh color, fruit skin color, fruit shape, sweetness level and flower crown color. Qualitative observations were guided by UPOV [11] and for observing color characters used the RHS Color Chart as a reference in determining the characters of watermelon plants. Observations on the leaves can be seen in Figure 1.

The leaves of the watermelon plant are a very important part of the plant, because they are where the photosynthesis process occurs. The leaf shapes on watermelon plants are strong, weak and medium types [11]. From the results of observations in research that has been carried out, the average type of watermelon leaf is medium and strong. The leaves of the watermelon plant are finger-shaped with a hairy surface, heart-shaped base, tapered tip, wavy edges and a green color [7]. Seed production and watermelon cultivation activities in order to obtain maximum results need to pay attention to the number of leaves and the length and width of the leaves, because the leaves are quite an important part and are the site of photosynthesis. The ToPAS method was used in this research to optimize the growth of watermelon plants. The observed leaf image can be seen in Figure 1. The observed color of leaves and flower crowns can be seen in Table 8.

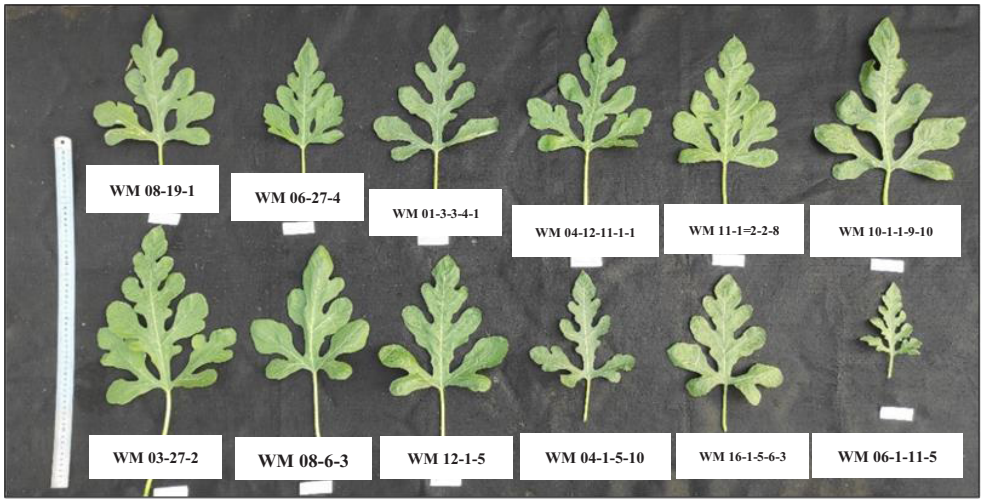

Fig 1. Observation of leaf characters in reciprocal hybridization

Fig 2. Observation of flower characters in reciprocal hybridization

Watermelon plants have three types of flowers, namely male flowers, female flowers and perfect flowers (hermaphrodite). The male flowers on watermelon plants have stalks with a bright yellow crown, while the female flowers have a stalk 45 mm long and the crown is bright yellow. Watermelon plant flowers will emerge from the leaf axils and bloom in the morning [16]. The results of observing the color of the flower crown, all flowers have the same crown color, namely bright yellow. The observed flower image can be seen in Figure 2.

Table 7. Shape Characters of Fruit Results from Reciprocal Hybridization

Lines	Leaf type	Leaf shape
WM 06-27-4	Medium	Oval
WM 04-12-11-1-1	Medium	Oval
WM 10-1-1-9-10	Medium	Oval
WM 08-6-3	Medium	Oval
WM 04-1-5-10	Medium	Oval
WM 06-1-11-5	Strong	Oval

Table 8. Color of Leaves and Flower Crowns

Lines	Leaf color	Flower crowns color
WM 06-27-4	RHS green group 138C	RHS yellow group 2C
WM 04-12-11-1-1	RHS green group 138C	RHS yellow group 2B
WM 10-1-1-9-10	RHS green group 138D	RHS yellow group 2A
WM 08-6-3	RHS green group 138D	RHS yellow group 2B
WM 04-1-5-10	RHS green group 138C	RHS yellow group 2B
WM 06-1-11-5	RHS green group 138 D	RHS yellow group 2C

Table 9. Fruit Skin Color, Striation Type and Fruit Flesh Color

Lines	Fruit skin color	Striation type	Fruit flesh color
WM 06-27-4	RHS green group 135B	no striation	RHS red group 39A
WM 04-12-11-1-1	RHS green group 137A	no striation	RHS red group 40D
WM 10-1-1-9-10	RHS green group 137A	no striation	RHS orange red 33C
WM 08-6-3	RHS yellow green group 144A	Smooth	RHS red group 45D
WM 04-1-5-10	RHS yellow green group 144D	Thick	RHS yellow group 6B
WM 06-1-11-5	RHS yellow green group 144A	Thick	RHS red group 39B

The shape of watermelon is important in market demand. In general, watermelons have three shapes, namely oval, oblong and round [15]. In Table 7, the shape of all watermelons is oval with different types of striations. Strains WM 06-27-4, WM 04-12-11-1-1, WM 10-1-1-9-10 and have a plain striated type. The WM 08-6-3 strain has a thin striated type. The WM 04-1-5-10, WM 06-1-11-5 strains have a thick striated type. Fruit observation variables use UPOV guidelines [11].

There are three types of watermelon rind in the Balitbu Tropika collection, namely striped light green, plain light green and plain dark green [8]. The skin color of watermelon is dark green and light green. In Table 9, the strains that have a dark skin color are WM 06-27-4 (green group 235B), WM 04-12-11-1-1 (green group 137A), WM 10-1-1-9-10 (green group 137A). The watermelon strains that have light skin color (Table 9) are the WM 08-6-3 (yellow green group 144A), WM 04-1-5-10 (yellow green group 144D), WM 06-1-11-5 strains (yellow green group 144A). The color of the flesh of the fruit produced in this study was all red, there were only a pair of lines that had yellow flesh, namely the WM 04-1-5-10 strain (Table 9). These results can be seen in Figure 3.

Fig 3. Fruit resulting from reciprocal hybridization

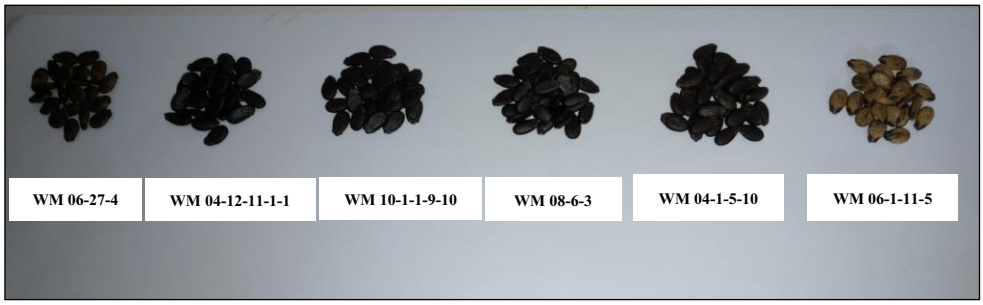

Fig 4. Watermelon seeds resulting from reciprocal hybridization

Table 10. Seed color of watermelon

Lines	Seed color
WM 06-27-4	RHS *black group* 203B
WM 04-12-11-1-1	RHS *black group* 203A
WM 10-1-1-9-10	RHS *grey brown* N194B
WM 08-6-3	RHS *black group* 203A
WM 04-1-5-10	RHS *grey brown* N199B
WM 06-1-11-5	RHS *black group* 203B

Seed colors are divided into seven colors, namely white, cream, green, red, red brown, brown and black [11]. The WM 10-1-1-9-10 strain and the WM 06-1-11-5 strain have different colors, namely grey, while the other four lines have black seeds (Figure 4 and Table 10). This data was used as additional data in this research to determine the success rate of hybridization that had been carried out previously. Yield potential is the ability of plants to produce optimally under optimal soil, water and plant conditions. The following are the results of the Metro Garden greenhouse scale yield test.

Table 11. Weight Per Fruit Yield Ability Test Greenhouse Metro Garden

No	lines	Weight per fruit	
1	WM 2110-0608	1.91	b
2	WM 2110-0401	1.08	a
3	WM 2110-1011	1.74	b
4	WM 2110-0803	1.68	b
5	WM 2110-0412	0.86	a
6	WM 2110-0616	0.80	a
LSD 5%		0.43	
CV %		13.67	

Remark: Numbers followed by the same letter in the same column are not significantly different according to the LSD test at the 5% level.

Watermelon fruit weight is divided into three classes, class I fruit which weighs more than 4 kg, class II fruit which weighs 2-4 kg and class III fruit which weighs less than 2 kg [15]. The results of observing the fruit weight parameters showed that the average fruit weight for each line was as shown in Table 11. The greatest potential yield was tested for this yield, namely the WM 2110-0608 line which had the heaviest weight, namely 1.91 kg. These results can be even higher if the environment is controlled and in optimal conditions.

4 Conclusion

Watermelon seed production was carried out using six pairs of watermelon lines from several generations of selection, the WM 10-1-1-9-10 line can be said to be superior because it has the highest sweetness level of 10.08 ºbrix, and the fastest harvest age is 68- 69 DAT. From the results of reciprocal hybridization carried out, each line has advantages, including the plain striated type, namely the lines WM 06-27-4, WM 04-12-11-1-1 and WM 10-1-1-9-10, the thin striated type is in line WM 08-6-3 and the thick striated type is in line 04-1-5-10 and WM 06-1-11-5. In total, there are six pairs of lines, one line has yellow flesh, namely the WM 04-1-5-10 line.

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