Proceeding the 1st International Conference on Sustainable Development and Green Economy in Small Islands (IC-SDGESI)
Ternate, North Maluku Province, Indonesia, 2024

Analysis For Flowers Pollinated Test in Several Inbred Melon (*Cucumis melo* L.)

A.L. Adiredjo[1]*, B.N Kholifah[1], R.B.P Ajidin[1]

[1]Plant Breeding Laboratory, Department of Agronomy, University of Brawijaya, Indonesia
*E-mail: al.adiredjo@ub.ac.id

Abstract

Melon (*Cucumis melo* L.) is an annual fruit plant from the Cucurbitaceae family. The economic value and diverse benefits make melon one of the commodities widely cultivated by various countries, including Indonesia. From 2010 to 2021, national melon consumption per capita increased. In addition, starting in 2022, melon became one of the nation's main export commodities. Therefore, melon has the potential to continue to be developed, one of which is through the development of superior seeds. The development of a superior seed aims to obtain a unique type of melon that meets market needs and can adapt to various environmental conditions. There are several methods to produce superior melon seeds, one of which is artificial hybridization. Initial considerations in selecting a hybrid parent include cross-pollination between inbreds. This research aimed to determine the percentage of flowers pollinated by the combination of cross-pollination and self-pollination in some melon inbreds and the yielding character of a combination of cross-pollination and self-pollination in some melon inbreds. The research was conducted at Brawijaya University, located in Donowarih Village, Karangploso Subdistrict, Malang Regency, in March–June 2021. The planting materials used are eight inbred melons, including three as female parents (ACL211390, ACL221402, and ACL231312) and five as male parents (ACD211303, ACD211254, ACD221362, ACD231380, and ACL211402). Some other materials include planting media (soil and manure), fertilizers, pesticides, EM4, and water. Pollination consists of five combinations of cross-pollination and self-pollination in each inbred female parent. The observational variables of this research are the percentage of flowers pollinated, fruit weight, fruit diameter, fruit length, thickness of fruit meat, number of seeds per fruit, and sweetness of fruit. Observational data is analyzed using unpaired student t-tests at the 5% level. The average percentage of flowers pollinated shows varied values and does not get a 100% value in the entire sample plant, but all pollination combinations have a value of > 0%. This is because pollination of three hermaphrodite flowers (the female parent) on each sample plant is done at different times. There is a combination of cross-pollination that has an average yield character (fruit weight, fruit diameter, fruit length, thickness of fruit meat, number of seeds per fruit, and sweetness of fruit) higher or lower than self-pollination. Differences in pollen sources are responsible for differences in the character of outcomes between pollination combinations with the same female parents. The t-test results between inbred ACL211390, ACL221402, and ACL231312 (female parent) showed significance in some yield characters. This indicates that inbred ACL211390, ACL221402, and ACL231312 can become inbred parents when producing hybrids of specific characters.

Keywords: *Pollination, flowers, inbred lines, melon*

1. Introduction

Melon (*Cucumis melo* L.) is an annual fruit plant that belongs to the Cucurbitaceae family. There are differences regarding the estimation of the center of melon domestication between Asia and Africa. Based on a comprehensive Cucumis DNA data set, recent studies have shown that domestication between Asian melon groups (*Cucumis melo* subsp. melo f. agrestis) and African melons (*Cucumis melo* subsp. meloides) occurred independently (Endl et al., 2018). The economic value and its various benefits make melon a commodity widely cultivated by multiple countries, including Indonesia. Based on the Statistics of Indonesian Seasonal Vegetables and Fruits for 2018, melon ranks second after watermelon as an annual fruit with the highest national production (Sub-Directorate of Horticulture Statistics, 2019).

Melon is one of the seasonal fruits that many people like. This is evidenced by an increase in the number of national melon consumption from 2010 to 2017. According to the Director General of Horticulture (2021a), national melon consumption in 2010, 2012, 2014, and 2017 was 0.156, 0.209, 0.417, and 0.521 kg per capita per year. Apart from having a domestic market, melons also have the potential to be a national export commodity. Based on the Director General of Horticulture (2021b) from 2017-2020, melon is one of the federal export commodities in the form of fresh products, with the highest total exports in 2018 of 179,825 tons, or US$309,674. Therefore, melon has the potential to continue to be developed, one of which is through the development of superior seeds. The development of superior seeds is an aspect that needs to be worked on. This aims to obtain unique melons that follow market needs and have the ability to adapt to various environmental conditions.

Several methods exist to produce superior melon seeds in plant breeding, including artificial hybridization. Artificial hybridization involves crossing two selected parents with different genetic makeups in a controlled manner (Acquaah, 2012). Mwangangi et al. (2019) explained that some of the goals of hybridization include (1) creating diverse plant populations as material for selecting hybrids, (2) combining certain desired characteristics in one individual, and (3) exploiting and utilizing hybrid varieties. The initial stage in forming superior hybrid seeds is the development of the primary population, which can be obtained through hybridization. The primary population resulting from hybridization at a later stage needs to be grouped and purified to get inbreds (pure lines) as material for selecting hybrid parents. Purification is carried out by self-pollinating the group until the 7th generation is obtained or until a homozygous (inbred) genotype is composed (Suwarno et al., 2017).

The initial consideration in selecting hybrid parents can be cross-pollination between inbreds currently in the purification stage, such as F3, F4, etc. This is to predict the success of cross-pollination and potential inbred candidates as hybrid parents by observing differences in fruit characters resulting from cross-pollination. The phenomenon of the effects produced by different pollen sources on observable characteristics such as size, color, shape, chemical composition, and development time of seeds and fruit from fertilization to germination is called the xenia effect (Denney, 1992). The results of this study are expected to provide information on the success rate of cross-pollination and yield characteristics for each combination of cross-pollination so that it can be used as a consideration in the selection of inbred candidates in the development of superior hybrid melons.

2. Material and Methods

The research was conducted at the Greenhouse of Brawijaya University, located in Donowarih Village, Karangploso District, Malang Regency, East Java Province. Donowarih Village is at an altitude of ±720 meters above sea level, with an average annual rainfall of 250 mm/month and an average temperature of 27°C (Irul, 2017). The research was carried out from March 2021 to June 2021. The materials used were planting media (soil and manure), fertilizers, pesticides, water, paper covers, labels, and eight melon inbreds consisting of three inbreds as female parents

(ACL211390, ACL221402, ACL231312) and five as male parents (ACD211303, ACD211254, ACD221362, ACD231380, ACL211402). The tools used are hoes, sprayers, buckets, spoons, tweezers, drinking water glasses, analytical scales, calipers, hand refractometers, and cameras.

The research was carried out by self-pollinating and cross-pollinating against inbreds ACL211390, ACL221402, ACL231312 (the female parent). Pollination was performed on three hermaphrodite (female parent) flowers per plant. The three flowers are not pollinated at the same time (day), but pollination is implemented when the hermaphrodite flowers have appeared and are ready to be pollinated. Before pollination, emasculation is undertaken on hermaphrodite flowers (female parents). The total number of hermaphrodite flowers pollinated in this study was 108.

The observed variables used were the percentage of flowers pollinated and several yield characters (fruit weight, fruit diameter, fruit length, fruit flesh thickness, number of seeds per fruit, and fruit sweetness). The observed data were then tested for normality using the Shapiro-Wilk test and the homogeneity test of variance using the Levene test. Data analysis used unpaired student T-test at a 5% level with the help of SPSS 25 software.

3. Results and Discussion

Pollination is known to be successful if the hermaphrodite (female parent) ovaries that have been pollinated remain green and develop to form fruit. Field results showed that the inbreds ACL211390, ACL221402, and ACL231312 had an average percentage of flowers pollinated of more than 0% in each combination (Table 1). It means that three inbreds can receive their pollen (self-pollination) and from 5 pollinated inbreds (cross-pollination). However, overall, the three inbreds showed that of the three hermaphrodite flowers (female parents) per plant pollinated, the average percentage of flowers pollinated was different. This is not related to the results of Leorentina's research (2019) Respatijarti et al. (2019), which showed that the percentage of cross-pollination success between Melindo X Glamor melons with pollination time (06.00-11.00) and the ratio of hermaphrodite flowers: male flowers (1:1) used was the same as this study showing the percentage results 100% pollination success on all sample plants. This is thought to be caused by differences in the pollination period of the three hermaphrodite flowers on the three pollinated inbreds because it was found in Leorentina's research (2019) that the two hermaphrodite flowers used as female parents were cross-pollinated at the same time. This was reinforced by the fact that two hermaphrodite flowers were pollinated in the same period, and both succeeded in forming fruit found in this study, which was indicated by the percentage of flowers pollinated equal to 67%.

Table 1.
Percentage of successful pollination (%).

Treatment	Percentage of successful pollination (%)		Average
	n1	n2	
Self pollination			
ACL211390 X ACL211390	67%	67%	67%
ACL221402 X ACL221402	33%	33%	33%
ACL231312 X ACL231312	33%	33%	33%
Cross pollination			
ACL211390 X ACD211303	67%	33%	50%
ACL211390 X ACD211254	33%	33%	33%
ACL211390 X ACD221362	33%	67%	50%
ACL211390 X ACD231380	33%	33%	33%

ACL211390 X ACL211402	33%	33%	33%
ACL221402 X ACD211303	67%	67%	67%
ACL221402 X ACD211254	33%	67%	50%
ACL221402 X ACD221362	33%	33%	33%
ACL221402 X ACD231380	67%	33%	50%
ACL221402 X ACL211402	33%	33%	33%
ACL231312 X ACD211303	33%	67%	50%
ACL231312 X ACD211254	33%	33%	33%
ACL231312 X ACD221362	33%	67%	50%
ACL231312 X ACD231380	33%	33%	33%
ACL231312 X ACL211402	67%	67%	67%
Average			44%

The difference in the pollination period is caused by the appearance of pollinated hermaphrodite flowers, with a difference of 1-3 days between the n-th and (n-1) pollination periods. Pollination in the second or third period shows that the ovary does not develop or is not successful if the previous pollination was successful. This is in accordance with Bomfim et al. (2016), who state that earlier-formed Cucurbitaceae fruit has the effect of inhibiting later fruit formation because the assimilate produced by plants is limited. The fruit formed earlier requires sufficient assimilation for the development of fruit tissue and seeds so that plants focus assimilating allocation on earlier started fruits and limit the incorporation budget for later fruit development. Based on Zulkarnain et al. (2019), other essential factors that determine the success of pollination, whether natural or artificial, are the readiness of the stigma to receive pollen (stigmatic receptivity) and pollen viability so that pollen can form a tube and fertilize an ovule. Receptive stigma and viable pollen cannot guarantee successful pollination or fruit formation in Cucurbitaceae plants.

Table 2.
Test Result = T Percentage of Successful Pollination (%) Inbreds ACL211390, ACL221402, and ACL231312

Inbred	T test results
ACL211390 and ACL221402	-0.949^{ns}
ACL211390 and ACL231312	-0.949^{ns}
ACL221402 and ACL231312	0.000^{ns}

ns: no significantly different

According to Mussen and Thorp (1997), in Cucurbitaceae plants, it takes at least 500-1000 viable pollen grains that pollinate the pistils to produce fruit, and the low number of ovule that is successfully fertilized can cause fruit not to form. This is because a series of pollination processes, the growth of the pollen tube, and finally, the fertilization of the ovule are responsible for the release of plant hormones, which stimulate the formation of the fruit of the Cucurbitaceae plant and fruit tissue so that the ovary can develop into fruit (Bomfim et al., 2016). The lack of viable pollen that pollinates the stigma and manages to fertilize the ovule can be caused by the pollinator's ability to transfer viable pollen onto the receptive stigma. Observations of yield characters in ACL211390, ACL221402, and ACL231312 showed different results between pollination combinations for each inbred (Tables 3, 4, and 5). Differences in yield characteristics between cross-pollination combinations can be considered when selecting hybrid parents. Based on five combinations of cross-pollination on ACL211390, ACL221402, and ACL231312 inbreds, it was shown that there were combinations that had higher or lower fruit weight, fruit diameter, fruit length, thickness of fruit flesh, number of seeds per fruit, and sweetness than self-pollination. Olfati et al. (2010) stated that differences in pollen sources (pollinator genetics) can cause fruit weight, length, and diameter produced in cucumber plants to be larger or smaller than self-pollinated cucumbers.

Table 3.
Average Result of ACL211390 Inbred Characters Self Pollination and Cross Pollination.

Treatment	FW (g)	FD (cm)	FL (cm)	FT (cm)	NS (seed)	SL (brix)
Self pollination						
ACL211390 X ACL211390	629.0	10.97	9.96	2.38	402.5	9.30
Cross pollination						
ACL211390 X ACD211303	571.5	10.89	8.70	2.10	101.5	11.00
ACL211390 X ACD211254	866.0	12.15	10.43	2.83	131.0	10.20
ACL211390 X ACD221362	640.5	10.73	10.17	2.38	428.5	13.00
ACL211390 X ACD231380	353.5	8.97	8.41	1.70	240.0	9.40
ACL211390 X ACL211402	964.5	12.53	10.92	2.85	275.0	10.10

FW = Fruit Weight, FD = Fruit Diameter, FL = Fruit Length, FT = fruit flesh thickness, NS = Number of seed per fruit, SL = Sweetness level

Table 4.
Average Result of ACL221402 Inbred Characters Self Pollination and Cross Pollination

Treatment	FW (g)	FD (cm)	FL (cm)	FT (cm)	NS (seed)	SL (brix)
Self pollination						
ACL221402 X ACL221402	749.5	11.02	10.88	2.48	184.0	12.70
Cross pollination						
ACL221402 X ACD211303	393.0	9.08	8.53	1.53	217.0	13.20
ACL221402 X ACD211254	616.0	10.25	10.85	1.85	394.0	11.90
ACL221402 X ACD221362	418.5	9.24	9.48	1.82	308.0	13.60
ACL221402 X ACD231380	579.5	10.60	9.18	2.07	315.5	14.10
ACL221402 X ACL211402	528.5	9.90	9.27	2.25	260.5	12.10

FW = Fruit Weight, FD = Fruit Diameter, FL = Fruit Length, FT = fruit flesh thickness, NS = Number of seed per fruit, SL = Sweetness level.

Table 5.
Average Result of ACL231312 Inbred Characters Self Pollination and Cross Pollination

Treatment	FW (g)	FD (cm)	FL (cm)	FT (cm)	NS (seed)	SL (brix)
Self pollination						
ACL231312 X ACL231312	507.5	10.50	8.75	2.17	358.5	10.73
Cross pollination						
ACL231312 X ACD211303	687.0	11.35	10.24	2.34	413.0	13.10
ACL231312 X ACD211254	658.5	10.93	10.37	2.27	498.5	12.80
ACL231312 X ACD221362	721.5	11.53	9.90	2.25	223.5	8.70
ACL231312 X ACD231380	422.0	9.22	9.72	1.93	311.5	10.40
ACL231312 X ACL211402	692.5	11.36	10.64	2.18	448.5	12.55

FW = Fruit Weight, FD = Fruit Diameter, FL = Fruit Length, FT = fruit flesh thickness, NS = Number of seed per fruit, SL = Sweetness level.

Table 6.
T-Test Results of Inbred Characters ACL211390, ACL221402, and ACL231312

Inbred	FW	FD	FL	FT	NS	SL
ACL211390 and ACL221402	2.076^{ns}	2.539*	0.577^{ns}	2.636*	-1.411^{ns}	-3.979**
ACL211390 and ACL231312	0.483^{ns}	0.325^{ns}	-1.092^{ns}	1.037^{ns}	-2.728*	-1.039^{ns}
ACL221402 and ACL231312	-2.240*	-2.647*	-2.005^{ns}	-2.512*	-1.988^{ns}	2.217*

FW = Fruit Weight, FD = Fruit Diameter, FL = Fruit Length, FT = fruit flesh thickness, NS = Number of seed per fruit, SL = Sweetness Level. "ns" = no significantly different, "*" = significant difference (t_{table} 5%), "**" = Very significant difference (t_{table} = 1%).

Differences in pollen sources are also known to cause differences in sugar content in fruit. Militaru et al. (2015) explained that differences in fruit sweetness were found between apples pollinated by different pollinators. The number of seeds per fruit shows various or different results between pollination combinations. This difference can be influenced by pollen viability and stigma receptivity during pollination. According to Harliani et al. (2014), reproductive success (seed production) is controlled by pollen viability, which is responsible for the amount of pollen capable of fertilizing ovules and producing full-fledged seeds. Based on the research results of Hayes et al. (2005) showed that inbred plants had male fitness (number of male flowers per plant, pollen count per flower, and pollen tube length after 30 minutes of pollination) and female fitness (number of fruits per plant, number of seeds per fruit, and seed germination per fruit) were lower when compared to cross-pollinated plants. The results of the T-test between the inbreds ACL211390, ACL221402, and ACL231312 showed several significantly different results with very significant differences in fruit weight, fruit diameter, fruit flesh thickness, number of seeds, and fruit sweetness (Table 6). The difference in the character of these results could be due to differences in the composition of the genetic material between the ACL211390, ACL221402, and ACL231312 inbreds. This result is similar to a study by Huda et al. (2017), which stated that genotype had a significant effect on the character of male flowering age, harvest age, fruit length, fruit diameter, fruit flesh thickness, fruit skin thickness, fruit weight, and plant sugar content Melon. Genotype, environment, and interactions between genotype and environment are the factors responsible for the diversity or differences in yield characters between inbreds (Hermanto et al., 2017).

4. Conclusion and Recommendations

The percentage of successful pollination had varying values between pollination combinations on ACL211390, ACL221402, and ACL231312 inbreds. We did not obtain a 100% pollination success percentage from all sample plants. A variety of cross-pollination with yield characters (fruit weight, fruit diameter, fruit length, fruit flesh thickness, number of seeds per fruit, and fruit sweetness) is better than self-pollination results. There are several differences in yield characters between the ACL211390, ACL221402, and ACL231312 inbreds.

5. Acknowledgments

The authors thank Hashfi and Radin for assisting with the research and providing great support for the project.

References

[1] Acquaah, G. 2012. Principles of Plant Genetics and Breeding (2 ed.). John Wiley & Sons, Ltd

[2] Bomfim, I. G. A., Freitas, B. M., de Aragao, F. A. S., & Walters, S. A. 2016. Pollination in Cucur bit Crop. In Handbook of Cucurbits: Growth, Cultural Practices, and Physiology (1 ed., hal. 181–200). CRC Press.

[3] Denney, J. O. 1992. Xenia Includes Metaxenia. HortScience, 27(7), 722–728. https://doi.org/10.21273/hortsci.27.7.722

[4] Endl, J., Achigan-Dako, E. G., Pandey, A. K., Monforte, A. J., Pico, B., & Schaefer, H. 2018. Repeated domestication of melon (*Cucumis melo*) in Africa and Asia and a new close relative from India. American Journal of Botany, 105(10), 1662–1671.

[5] Harliani, E. N., Palupi, E. R., & Wahyudin, D. S. 2014. Pollen Storage Potential in Seed Production of Hybrid Cucumber (*Cucumis sativus* L) Variety KE014. Journal of Indonesian Horticulture, 5(2), 104–117. https://doi.org/10.29244/jhi.5.2.104-117

[6] Hayes, C. N., Winsor, J. A., & Stephenson, A. G. 2005. A comparison of male and female responses to inbreeding in Cucurbita pepo subsp. Texana (Cucurbitaceae). American Journal of Botany, 92(1), 107–115. https://doi.org/10.3732/ajb.92.1.107

[7] Hermanto, R., Syukur, M., & Widodo. (2017). Estimation of Genetic Variance and Heritability of Yield Characteristics and Yield Components of Tomato (Lycopersicum esculentum Mill.) at Two Locations. Indonesian Horticulture Journal,8(1), 31. https://doi.org/10.29244/jhi.8.1.31-38

[8] Hortikultura, D. J. 2021a. Statistics: Consumption. http://aplikasi2.pertanian.go.id/konsumsi/tampil_susenas_kom2_th.php

[9] Hortikultura, D. J. 2021b. Statistics: Export Import. http://database.hortikultura.pertanian.go.id/eksim2012/hasilEksporHs.php

[10] Huda, A. N., Suwarno, W. B., & Maharijaya, A. 2017. Genetic Variation of Fruit Characteristics among 17 Genotypes of Melon (Cucucmis melo L.). Journal of Indonesian Horticulture, 8(1), 1. https://doi.org/10.29244/jhi.8.1.1-12

[11] Irul. 2017. Donowarih Village, Karangploso District, Malang Regency. http://donowarih.sideka.id/2017/11/08/desa-doniwarih/

[12] Leorentina, A. B. 2019. Hybridization of Several Melon (*Cucumis melo* L.) Varieties with Pollination Time and Flower Proportion Based on Nesting Design. Thesis. University of Brawijaya.

[13] Militaru, M., Butac, M., Sumedrea, D., & Chitu, E. 2015. Effect of Metaxenia on the Fruit Quality of Scab Resistant Apple Varieties. Agriculture and Agricultural Science Procedia, 6, 151–156.

[14] Mussen, E. C., & Thorp, R. W. 1997. Honey Bee Pollination of Cantaloupe, Cucumber, & Watermelon. California Digital Library. University of California. https://doi.org/10.3733/ucanr.7224

[15] Mwangangi, I. M., Kiilu Muli, J., & Neondo, J. O. 2019. Plant Hybridization as an Alternative Technique in Plant Breeding Improvement. Asian Journal of Research in Crop Science, 4(1), 1–11.

[16] Olfati, J. A., Sheykhtaher, Z., Qamgosar, R., Khasmakhi-Sabet, A., Peyvast, G., Samizadeh, H., & Rabiee, B. 2010. Xenia and metaxenia on cucumber fruit and seed characteristics. International Journal of Vegetable Science, 16(3), 243252. https://doi.org/10.1080/19315260903584167

[17] Respatijarti, Roviq, M., & Adiredjo, A. L. 2019. Pollination time and proportion of female to male flowers affecting pollination effectivity and yield characters of some melon (*Cucumis melo* L.) varieties. Annals of Biology, 35(1), 61–66.

[18] Subdirektorat Statistik Hortikultura. 2019. Statistics of Seasonal Vegetables and Fruits. Central Bureau of Statistics RI.

[19] Suwarno, W. B., Sobir, & Gunawan, E. 2017. Melon Breeding: Past Experiences and Future Challenges. Proceeding International Seminar on Tropical Horticulture 2016: The Future of

Tropical Horticulture.
[20] Zulkarnain, Z., Eliyanti, E., & Swari, E. I. 2019. Pollen viability and stigma receptivity in Swainso na formosa (G.Don) J. Thompson (Fabaceae), an ornamental legume native to Australia. Orn amental Horticulture, 25(2), 158–167.