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Detection of Powdery Mildew Resistance Gene in Melon Cultivar Meloni Based on SCAR Markers

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Abstract. Powdery mildew is one of the diseases caused by fungal infections that can reduce the production of melon fruit worldwide including in Indonesia. A powdery mildew-resistant cultivar of melon is needed to increase melon yield crops. This study aimed to detect resistance gene linked to powdery mildew using a sequence characterized amplified region (SCAR) markers. The melon cultivar Meloni was used in this study. SL-3, PI 371795, and Aramis cultivar were used to compare. Amplification of the marker was performed employing a pair of primers. The result showed that Meloni had a powdery mildew resistance gene by the presence of a DNA target band at 1058 base pair (bp). Based on this result, it could be concluded that Meloni was an excellent melon cultivar because of its ability to overcome the powdery mildew infections naturally. SCAR markers have been used for various purposes, especially to detect resistance genes to plant diseases. The present study had provided information for plant breeders about Meloni as the new melon cultivar that was genetically resistant against powdery mildew infections. Furthermore, Meloni could be proposed as an alternative to native Indonesian superior melon seeds.

Key words: Meloni, Powdery Mildew, Resistance Gene, SCAR

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INTRODUCTION

Indonesia is a tropical country with abundant essential resources, and one of them is Melon (*Cucumis melo* L.). Melon is currently developed as a leading horticultural commodity in Indonesia. Melon fruit consumption is expected to continue rising. Agricultural statistics data showed that melon fruit production in Indonesia increased from 2011 with 103,840 tons to 125,447 tons in 2012, 125,207 tons in 2013, and 150,347 tons in 2014. However, in 2015, the fruit production declined to 137,879 tons with 7396 ha harvested land areas (Ministry of the Agriculture Republic of Indonesia, 2016). In the effort to produce melon in Indonesia, there are problems such as limited availability and soaring prices of seeds because they are generally imported from abroad. Most farmers use imported melon seeds for their superior quality. However, not all imported melons are in good quality, but some of them are susceptible to pest and disease infections.

Powdery mildew is a plant disease caused by fungal infections, and it is a big problem for farmers. Some species that have been identified to infect melon plants in Asia are *Podosphaera xanthii* and *Golovinomyces cichoracearum* (Hong et al., 2018). The initial symptom of this disease is the presence of white patches on the underside of the leaf. They are spreading to the upperside and finally covering all parts of the leaf. Furthermore, the leaf becomes brown and wrinkled while the stem becomes chlorotic and dies (Daryono & Maryono, 2017). Infection by these fungi results in reduced melon resistance that can lower the harvest quality, sugar content and aroma of the fruit. Various kinds of effort are carried out to overcome powdery mildew, one of which is using fungicides. However, excessive use of fungicides can affect the environment while continual use can cause fungi to become more resistant (Candido et al., 2013). For these reasons, the use of fungicides is considered to be ineffective and efficient. In addition to using fungicides, powdery mildew can be managed by plant breeding with crossing melon cultivars that are resistant to this disease. The development of new resistant cultivars is such a promising way to control powdery mildew (Cheng et al., 2012).

Meloni is a superior melon cultivar bred through a crossing between ♀ SL-3 and ♂ PI 371795 (Figure 1), assembled by the Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada (UGM) (Daryono et al., 2018). SL-3 is a result of Sun Lady planted up to 3rd generation which has superiorities such as oval round fruit shape, sweet and thick flesh, hard fruit, smooth fibers, and a nice aroma (Ishak & Daryono, 2018). Meanwhile, PI 371795 has superior properties against powdery mildew infections (Aristya & Daryono, 2013). The crossing between ♀ SL-3 and ♂ PI 371795 is expected to produce a new melon cultivar that inherits superior char-

acters from both parents, especially to the resistance of powdery mildew infections.

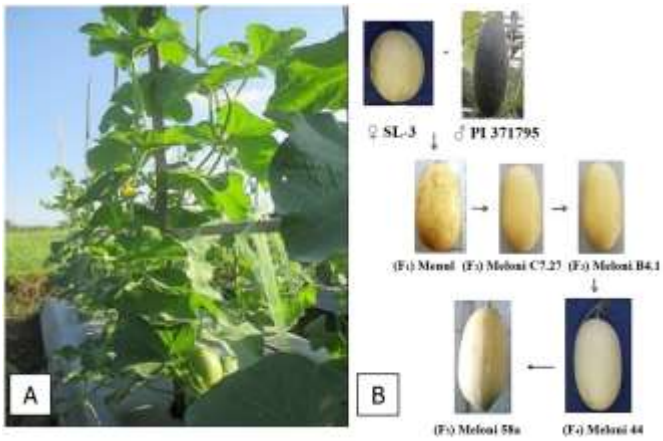

Figure 1. Plant of *Cucumis melo* L. cv. Meloni (A) and genealogy of cultivar Meloni (B)

Development in biotechnology especially in molecular biology has opened opportunities for its users to solve various problems in plant breeding. Breeding technology through conventional selection has been proven successful in increasing production and selecting a superior individual in a plant population. However, conventional selection has limitations especially in terms of the time required that is relatively long. Selection with phenotypic characters often gives inaccurate results, because phenotypic characters are mostly influenced by environmental factors. Thus, the use of molecular markers must be developed. SCAR is a PCR-based molecular marker where DNA fragments are amplified using a specific primer designed by nucleotide sequences of random amplified polymorphic DNA (RAPD) fragment clones related to the desired characteristics (Bhagyawant, 2016). SCAR primers have a size of 15-30 nucleotides. Besides being developed from RAPD, the molecular SCAR marker can also be developed from amplified fragment length polymorphism (AFLP) (Liang et al., 2011), inter simple sequence repeat (ISSR) (Yao et al., 2019), start codon targeted (SCoT) (Feng et al., 2018), and inter-retrotransposon amplified polymorphism (IRAP) (Mandoulakani et al., 2015). The advantages of SCAR are quick and easy to use, high reproducibility, specific loci, low quantities of DNA templates (10-50 ng per reaction), less sensitive to reactional condition, and codominant. The weakness of SCAR is needed for sequence data to design the PCR primers (Agrawal & Shrivastava, 2014). SCAR markers can be used for various purposes, such as cultivar identification (Jae-Han et al., 2014), detection of disease resistance genes (Sherin & Heba, 2019), and genetics mapping (Daryono et al., 2010).

Studies on the detection of powdery mildew resistance gene using SCAR markers in melon had been conducted. Daryono et al. (2011), designed SCAR markers from RAPD to detect powdery mildew resistance gen (*Pm-I*) in melon accurately. Based on their results, SCAR analysis in diverse melons detected a single 1058 bp linked to *Pm-I* in resistant melon PI 371795 and PMAR5. On the other hand, the primer failed to detect *Pm-I* in susceptible melon. Moreover, Fatmawati and Daryono (2016) also detected powdery mildew resistance gen in melon cultivar Tacapa Green Black. SCAR markers have also been used to identify pathogen-resistant genes in various plants, such as resistance to powdery mildew in common oat (Okon & Kowalczyk, 2012), aphid resistance gene in sorghum (Jin-hua et al., 2012), resistance to black rot disease (*Xca1Bo* gene) in cauliflower (Kalia et al., 2017) and Anthracnose diseases resistance (*Rca2* gene) in strawberry (Melinda et al., 2019). Therefore, the purpose of this study was to detect the powdery mildew resistance gene in Meloni using SCAR marker by PCR-method. This study was expected to provide information about Meloni as the new melon cultivar that was genetically resistant against powdery mildew infections.

METHODS

Study Area

This study was conducted in two different areas. Meloni was planted at the screenhouse of the Agrotechnology Innovation Centre (PIAT) UGM. While, detection of the powdery mildew resistance gene was carried out at the Laboratory of Genetics and Breeding, Faculty of Biology, UGM, Yogyakarta, Indonesia.

Preparation of Leaf Samples

This study used 6 samples of Meloni leaves taken from both 3 healthy and 3 powdery mildew-infected plants. Besides, leaf samples of SL-3, PI 371795 (the parents of Meloni) were used as a positive control while Aramis leaf samples were used as a negative control.

Detection of Powdery Mildew Resistance Gene

DNA Extraction

DNA was extracted using Nucleon PhytoPure kits. Each of the leaves samples was weighed to 0.3-0.5 grams and crushed with mortar and pestle. Reagent of Phytopure I (500 µl) was added slowly into the mortar, while continued to be crushed until the leaves were soft. The mixture of the sample was poured into a 1.5 µl tube. Afterward, 200 µl of Phytopure II reagent was added and slowly homogenized. These tubes were incubated at 65^{o}C for 20 minutes, and then placed in a refrigerator for 10 minutes. After that, 400 µl of cold chloroform was added to the tube, and then

was shaken slowly. Twenty microliter of Phytopure Resin was added by pouring it perpendicular to the tube. Then the tube was homogenized for about 30 minutes. After it was homogeneous, the mixture was centrifuged at 1300 rpm for 10 minutes. After centrifugation was finished, and the supernatant was moved into a 1.5 ml tube. The next step was adding cold isopropanol through the tube wall with a volume equal to the supernatant volume. It was shaken slowly and allowed to stand for 5 minutes, then centrifuged at 10.000 rpm for 10 minutes. The resulting supernatant was removed. Finally, the white DNA pellet was obtained at the base of the tube. Then DNA pellets were washed with 100 µl of 70% ethanol, and centrifuged at 10,000 rpm for 5 minutes. The washing was done 3 times. The ethanol was removed, then the pellets were air-dried. Fifty microliter 1X of TE buffer was added. Then DNA samples were obtained, later stored at -20^{o}C to be analyzed qualitatively and quantitatively.

A Qualitative and Quantitative Test of Isolated DNA

The qualitative test was carried out by electrophoresis method. Agarose gel of 0.16 gr (0.8%) was weighed, then put into a 100 ml Erlenmeyer. Forty milliliter of TBE 1X was added, then dissolved using a microwave for 40 seconds. It was ejected and allowed to stand until the heat was moderate. Two microliter of FloroSafe was added, then shaken. It was poured into a container that has been installed with a molding comb. This mixture was let stand until it formed a gel. After that, the comb was removed, and the container was transferred to the electrophorator. It was then poured with 1X TBE solvent until the gel surface was submerged.

DNA sample mixtures were put on a parafilm paper with 5 µl of DNA samples and 1 µl of loading dye. Using a micropipette, it was carried up and down. Afterward, DNA samples and 1 kb markers were put into the wells of the electrophoresis gel inside the electroporator. The electroporator was closed, the voltage was set at 100 volts. The migration was directed from - to +, and the ON button was pressed. After the DNA migration reached 2/3 of the gel length (visualized in blue color) with a time of about 30 minutes, the machine was turned off. After that, the gel was taken carefully. Then the electrophoresis gel was observed under UV light transilluminator.

The quantitative test of DNA samples was carried out using the UV-VIS Spectrophotometer method. The spectrophotometer was set at a wavelength of 260 nm and 280 nm, then the auto-zero button was pressed, so that the monitor would show a zero. The calibration was conducted using 1% TE buffer, by taking it as much as 2 µl then dropping it into the hole in the spectrophotometer. After that, the tool was wiped using tissue and dripped with 2 µl of DNA sample, resulting in DNA concentration and purity.

DNA Amplification Using SCAR Primers

SCAR primers used were the result of the development of RAPD primer pUBC411. Each primer contained the original 10 nucleotides of the RAPD primer plus 17 and 15 nucleotides for SCAPMAR5-F and SCAPMAS5-R, respectively (Daryono et al., 2011):

SCAPMARS-F:
5’ CAGACAAGCCCAGATAATTAACATCTC 3’

SCAPMAR5-R:
5’ CAGACAAGCCTAGGAGTTGTGGGCT 3’

DNA amplification was carried out by Polymerase Chain Reaction (PCR) method. The first step was preparation of PCR solution mix with a total volume of 25 µl, which contained 12.5 µL of Master Mix (PCR Kit Bioline), 2.5 µL of forward Primer (10 pmol), 2.5 µL of reverse primer (10 pmol), 2 µL of DNA template (250 ng) and 5.5 µL of double-distilled water. Then the tube with the PCR solution mix was amplified with a Thermal Cycler Bio-Rad machine and arranged according to the procedure, i.e. predenaturation at 95^{o}C for 5 minutes, denaturation at 95^{o}C for 1 minute, annealing at 60^{o}C for 30 seconds, elongation at 72^{o}C for 2 minutes and post-elongation at 72^{o}C for 10 minutes.

Electrophoresis

Agarose as much as 0.8 g (2%) was weighed. It was then put into a 100 ml Erlenmeyer and added with 1X TBE as much as 30 ml. It was dissolved using a microwave for 50 seconds. Every 10 seconds, it was taken and shaken. The Erlenmeyer was removed from the microwave and let stand until the heat was moderate. Then 5 µl of FloroSafe was added and shaken. The mixture was poured into a container that has been installed with a molding comb. Then the mixture was let stand until it formed a gel. After that, the comb was removed and the container was transferred to the electroporator. It was poured with 1X TBE solvent until the gel surface was submerged. DNA samples from PCR-SCAR and 100 bp marker were prepared. Using a micropipette, DNA samples and the marker were inserted into the well. The electroporator machine was closed. The voltage was set to 50 volts, the timer was set for 60 minutes. The migration was directed from - to +, and the ON button on the machine was pressed. Finally, the electrophoresis gel was observed under UV transilluminator.

RESULTS AND DISCUSSION

Management of diseases caused by powdery mildew infection is crucial to improve the quality and quantity of domestic melon production. Handling of powdery mildew using chemical fungicides is not effective because it can damage the environment. Moreover, excessive and sustainable use can cause the fungus to become more resistant. Plant breeding is one of the most effective ways to manage this disease because the trait of resistance can be genetically inherited. Mating of melons that are resistant and not resistant to powdery mildew can be used to study and determine the inheritance of resistance genes against powdery mildew (Daryono et al., 2011). This study was used Meloni that was a superior melon cultivar results from crossing between ♀ SL-3 and ♂ PI 371795. Its characters are ellipse fruit shape, orange yellow RHS 15B rind color, small to intermediate of fruit size (approximately 550-840 grams), sugar content at the flesh around 8.6-10.6% Brix, and fragrant aroma (Daryono et al., 2018).

Besides by observing directly, the resistance to powdery mildew can be determined through molecular analysis using genetic markers. Molecular markers are defined as certain segments of DNA that represent differences at the genome level. DNA is a potential and accurate source of genetic information. DNA can be found in almost all cell organisms. Molecular markers have an advantage compared to morphological test, which is stable, can be detected in all plant tissues, and are not influenced by the environment (Zulfahmi, 2013). Various types of molecular markers can be used, including RAPD, RFLP, AFLP, ISSR, SCAR, simple sequence repeat (SSR)/ Microsatellites, expressed sequence tags (EST) and single nucleotide polymorphism (SNP) and many others (Nadeem et al., 2018). This study utilized SCAR markers to detect resistance genes linked to powdery mildew. SCAR markers have high reproducibility and are more specific to one DNA band. When compared with the RAPD method, the SCAR method is less sensitive to reaction conditions while the RAPD marker is very sensitive to reaction conditions during PCR amplification (Yang et al., 2013).

The following steps were conducted in this study, i.e. DNA extraction, qualitative and quantitative DNA test, DNA amplification using SCAR primers linked to gene resistance of powdery mildew and visualization under UV transilluminator.

A Qualitative and Quantitative Test of Isolated DNA

DNA qualitative test was carried out by the agarose gel electrophoresis method that was visualized under UV transilluminator. The purpose of the qualitative test was to see the existence and quality of the DNA genome. The results of the qualitative DNA test are presented in Figure 2.

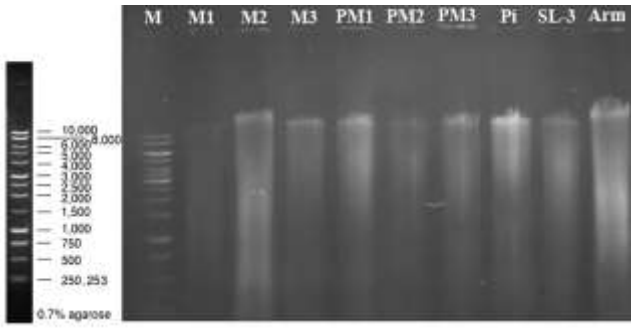

Figure 2. DNA genome electrophoresis results, M= marker; M1-M3= healthy Meloni; PM1-PM3= powdery mildew-infected Meloni; Pi= PI 371795; SL-3= Sun Lady generation-3; Arm= Aramis.

The DNA genome electrophoresis results revealed the DNA band was above 10.000 bp in size (Figure 2). This means that DNA was genomic DNA. The whole-genome size of melon is around 450 million base pairs (Mb) (Arumuganathan & Earle, 1991). On the other hand, the fragmented DNA was formed. It could be caused by insufficient isolation or small contaminants. The isolated DNA was analyzed further by a quantitative test.

A quantitative test of DNA was done by the spectrophotometric method. Spectrophotometry is a method for measuring the concentration and purity of a substance, which principle is based on the absorption of light by the substance using 2 types of wavelengths. The results of the quantitative test are presented in Table 1.

Table 1. DNA quantitative test results of Meloni, PI 371795, SL-3, and Aramis

Sample	Concentration (ng/ µl)	Purity	
Meloni 1	507.5	1.933	Healthy
Meloni 2	349	1.977	
Meloni 3	514	1.846	
Meloni 4	649.5	1.977	Infected
Meloni 5	1047	1.948	
Meloni 6	487.5	1.990	
PI 371795	760	1.954	
SL-3	2064	1.981	
Aramis	437	2.042	

Based on the DNA quantitative test results, the purity of DNA samples ranged from 1.990 to 2.042 (Table 1). The ratios of pure DNA were between 1.8-2. The purity below 1.8 indicated that there is a large molecular contamination such as protein or phenol, while if it is above 2, it is possible to have RNA con-

tamination (Brown, 1986; Fatmawati & Daryono, 2016). The results obtained showed that there was RNA contamination in the Aramis sample. Meanwhile, DNA extraction is said to be successful if the DNA has a high concentration. According to the recommendation of the Bioline protocol, the required concentration of the DNA template for eukaryotic PCR activities is 200 ng/µl or within the range of 100-500 ng/µl. According to the result, the concentrations of the isolated DNA were more than 200 ng/µl. The highest was 2064 ng/µl, while the lowest was 349 ng/µl. These isolation results can be used for the next process that was PCR-SCAR amplification linked to the gene resistant of powdery mildew disease.

Molecular Analysis Using SCAR Primers

SCAR marker used in this study was the modification of RAPD for the pUBC411 primer by adding 17 and 15 nucleotides from 10 RAPD nucleotides. DNA from these samples was amplified using a pair of forward and reverse SCAR primers linked to the powdery mildew resistance gene by the PCR method. The target of the gene could be observed by the emergence of amplified DNA at 1058 bp (Daryono et al., 2011). PCR-SCAR products of DNA samples are presented in Figure 3.

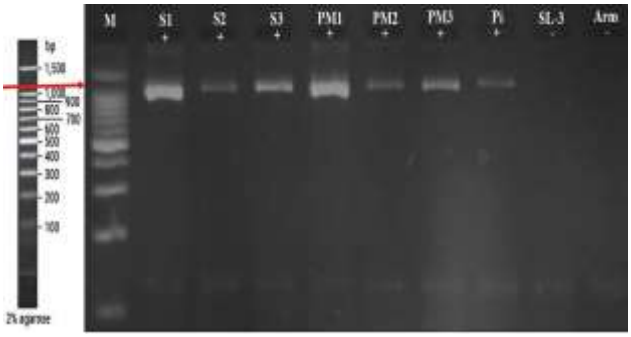

Figure 3. Amplified DNA with SCAR primers related to the gene resistant against powdery mildew disease. M= marker; M1-M3= healthy Meloni; PM1-PM3= powdery mildew-infected Meloni; Pi= PI 371795; SL-3= *Sun Lady* generation-3; Arm= Aramis; + = positive; -= negative of having resistant genes.

Based on amplified DNA, both healthy and infected Meloni samples have a resistance gene to powdery mildew (Figure 3). These were evidenced by the visualized target of the DNA band at 1058 bp. The results also correlated with the plant resistance test of powdery mildew that Meloni was the tolerant category (Ishak, 2018). This gene was also detected in PI 371795. Meanwhile, in SL-3 and Aramis there were no powdery mildew resistance gene. The selection of Aramis as a negative control (-) was based on Fatmawati and Daryono (2016), that Aramis had no resistance genes as evidenced by the absence of DNA bands at 1058 bp. This result was also supported by Daryono and Yembise (2018), that Aramis had no resistance gene of powdery mildew. While the selection of PI 371795 as a positive control (+) was based on previous studies, also it was one of the parents of Meloni. According to Daryono et al. (2011), SCAR markers have been used to analyze melon resistant to powdery mildew in several varieties of melon such as PMAR5, Harukei, WMR-29, PMR45, PMR5, Action 434, PI 414723, PI 124112, PI 124111, and PI 371795. The results showed that PI 371795 was one of the melons that had a resistance gene. It could also be known that the resistance trait of powdery mildew of Meloni was inherited from PI 371795. The resistance trait of powdery mildew is inherited from PI 371795 cultivar controlled by a single dominant gene (*Pm-I*) (Daryono & Qurrohman, 2009). While in SL-3 cultivar, there was no resistance gene because the phenotype traits were not resistant to powdery mildew. This was based on Saumi (2015) when SL-3 was planted in Block 1 PIAT UGM. SL-3 was infected with very severe powdery mildew.

Based on the result of BLAST analysis, it was known that the sequence of DNA amplification using SCAPMAR5 primers was part of *Cucumis melo* genomic chromosome 12 of nuclear DNA. However, the role of this resistance gene against powdery mildew in preventing infections remains unknown. Generally, plants use a wide range of mechanisms to resist infection and disease caused by pathogenic organisms. Mechanical or chemical barriers are the first line of defense that present in the epidermal layer of plant tissue to prevent the establishment and growth of many potential pathogens. If pathogens make it past this first line of defense, they will meet a second of defenses that is the interaction between plant resistance gen and pathogen avirulence (*Avr*) gene. Furthermore, Subsequent signal transduction events coordinate the activation of an array of defense responses associated with localized cell death (hypersensitive response), production of reactive oxygen species (ROS), lignification and callose formation, production of the antimicrobial compound, and many others (Hammod-Kosack & Jones, 1996; Eckardt, 2004). Several mechanisms of powdery mildew resistance in melon have been proposed. McCreight et al. (2011), reported that Melon PI 313970 has a rich source of genes for resistance to powdery mildew. This resistance is imparted by three different mechanisms: reduce conidial germination and appressorium formation, hypersensitive response, and resistant blisters. In barley, it has resistance gene locus *Mla* for powdery mildew that induce the resistant response of hypersensitive cell death. As with the *Mla* locus, different genes in melon PMR 45 and WMR 29 probably

cause the hypersensitive response (Kuzuya et al., 2006).

Studies on creating a new cultivar resistant melon to powdery mildew had been conducted. Aristya and Daryono (2013) crossed melons PI 371795 that was resistant with Action 434 that was not resistant to powdery mildew, but it has a good fruit quality. The result showed that all F_1 offspring had tolerant of powdery mildew. Then it was crossed by test cross with PI 371795 and developing F_2 offspring producing melon cultivar Tacapa. In developing Tacapa, It had resulted in 3 cultivars, such as Tacapa Silver, Tacapa Green Black dan Tacapa Gold. Based on the study of Fatmawati and Daryono (2016), Tacapa Green Black had a resistance gene of powdery mildew, while according to Ginting (2015) resistance gene also detected in Tacapa Silver.

The selection of powdery mildew-resistant plants is an effective way of preventing and controlling powdery mildew. In the present study, we found that a new melon cultivar Meloni had a resistance gene linked to powdery mildew. Because of the existence of the gene, it can handle pests and diseases by itself so that the use of pesticides could be reduced. Thus, this study had provided information for plant breeders about a new genetic source of melon resistant against powdery mildew.

CONCLUSION

Meloni has a powdery mildew resistance gene by the presence of a DNA target band at 1058 bp.

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