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Phenotypic response and SCAR marker for powdery mildew resistance genes detection in 'Gama Melon Parfum'

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Abstract Powdery mildew is caused by *Podosphaera xanthii* and *Golovinomyces cichoracearum* which poses a significant challenge in melon cultivation leading to reduce melon crop productivity and quality. 'Gama Melon Parfum' (GMP) is a hybrid melon resulting from a cross between 'Natsu no Omoide' ♀ from Turkmenistan and 'Miyamauri' ♂ from Japan. This melon is shown to promise as a valuable raw material for cosmetic products. The use of molecular markers has revolutionized breeding programs and successful in the evaluation and development of disease-resistant melon crops. The morphological observation provided evidence of notable color differences in the qualitative phenotypes of powdery mildew-infected 'GMP' plants. This was accompanied by a decrease in size across all quantitative phenotypic parameters compared to healthy plants (significance level 0.05). 'GMP' melons were categorized as tolerant cultivars, with powdery mildew infection percentages below 50% at the leaf, plant, and population levels. A SCAR marker linked to the powdery mildew resistance gene (*Pm-I* locus) was successfully detected in GMP melons, characterized by a 1058 bp band. This marker is indicated the presence of the powdery mildew resistance gene, conferring moderate tolerance to the disease.

Keywords: Disease index score, Powdery mildew infection, Symptoms stages, Sequence characterized amplified region

Introduction

Powdery mildew is a disease caused by infection with biotropic fungi of the order Ersyphales (Křístková *et al*., 2009). *Sphaerotheca fusca*, renamed *Podosphaera xanthii* (Castag.) U. Braun and N. Shish, and *Erysiphe orontii*, which has been renamed *Golovinomyces cichoracearum* have been identified as the ectoparasitic fungus responsible for powdery mildew infection (Vakalounakis *et al*., 1994). This fungal disease leads to a range of symptoms and detrimental effects, including stunted plant growth, reduced foliage, lower yields, diminished quality, and decreased nutrient content (Lebeda and Sedlakov,

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2010). Different melon genotypes exhibit varying responses to powdery mildew infection, which typically manifests as powdery white spots on leaves, branches, and trunks, severely affecting photosynthetic efficiency (Candido *et al*., 2014).

Controlling powdery mildew in melon crops can be challenging. Manual removal of infected leaves is labor-intensive and often ineffective due to the widespread nature of fungal spores. Chemical control using fungicides is another approach, but excessive use can lead to fungal resistance, negative ecological impacts, health risks to humans, and increased production costs (Ishak and Daryono, 2020). Therefore, breeding for resistance to powdery mildew presents a more sustainable and effective solution (Dreiseitl, 2020).

Advances in molecular biology have revolutionized the breeding of disease-resistant crops (Aroca and García, 2023; Meena *et al*., 2023). Marker-assisted selection (MAS) allows breeders to identify and select resistance genes more efficiently and easily. Specific information on these loci is crucial in breeding (Collard and Mackill, 2008), necessitating the development of MAS under certain conditions (Jiang, 2013). The use of molecular markers enables precise tracking of resistance genes in breeding populations, accelerating the development of resistant cultivars by reducing the time and resources required for phenotypic screening (Habe *et al*., 2023; Kalia *et al*., 2017; Qv *et al*., 2024).

Sequence Characterized Amplified Region (SCAR) markers, based on simpler polymerase chain reaction (PCR) techniques using specific primers of 18–26 base pairs linked to the target gene (Bortoloto *et al*., 2020; Huang *et al*., 2018; Yang *et al*., 2014), have been widely used to identify plant resistance genes. SCAR markers offer advantages in primer specificity and reproducibility compared to RAPD and ISSR markers (Joshi and Chavan, 2012). The use of the SCAR provides a genetic marker which can be easily and rapidly applied.

SCAR markers have been used to detect resistance genes in various plant species, including tomato (Mutlu *et al*., 2015; Park *et al*., 2013; Zhang and Panthee, 2021), wheat (Das *et al*., 2006; Wankhade and Wadikar, 2018), Indian mustard (Negi *et al*., 2000), oat (Chong *et al*., 2004; Okon and Kowalczyk, 2012), strawberry (Lerceteau-Köhler *et al*., 2005), pea (Janila and Sharma, 2004), chilli (Wankhade and Wadikar, 2018) and apple (Evans and James, 2003). In melon species, SCAR markers have been used to detect resistance genes against Begomovirus (Nidianti *et al*., 2023), *Fusarium oxysporum f. sp. cucumerinum* in cucumber (Jaber *et al*., 2020), and powdery mildew (derived from AFLP marker) (Shengli *et al*., 2005).

Cucumis melo L. 'Gama Melon Parfum' (GMP) is one of the excellent local melons that has been under development by Laboratory of Genetic and Breeding, Universitas Gadjah Mada since 2011 (Daryono and Maryanto, 2018). 'GMP' is distinct from other melon varieties due to its smaller size, bitter flavor, and

perfumed aroma upon ripening. This unique variety not only offers exceptional sensory qualities but also boasts high levels of total phenolics, calcium, and amino acids (Hasbullah *et al*., 2019). The bitter taste of 'GMP' is attributed to its cucurbitacin content, a compound known for its anti-tumor, anti-inflammatory, anti-atherosclerotic, and anti-diabetic properties (Saputri *et al*., 2020).

Additionally, 'GMP' exhibits significant antioxidant activity, with 73.78% RSA content, 27.19 mg/100g vitamin C, and 81.87 µg/g total flavonoids (Zulfikar *et al*., 2020). Since 2019, 'GMP' melon has been successfully developed as a raw material for the cosmetics industry through the collaboration of the Gama melon research team with a national cosmetics company (Saputri *et al*., 2020). Despite its potential, the production of 'GMP' melon faces challenges due to diseases caused by fungi, bacteria, viruses, and insects, among which powdery mildew is significant (Grumet *et al*., 2021).

Breeding new cultivars with powdery mildew resistance is an efficient, sustainable, and environmentally friendly method (López-Martín *et al*., 2022). The development of powdery mildew-resistant 'GMP' melon lines holds promise for enhancing the sustainability and profitability of melon production. However, achieving durable resistance remains a challenge due to the genetic diversity and adaptability of the powdery mildew fungi. This study aimed to examine the phenotypic characteristics of powdery mildew infection on 'GMP' melon plants by determining the level of resistance and the presence of resistance genes to powdery mildew infection.

Materials and methods

Research area

The study was carried out between July 2022 and January 2023. Phenotypic characterization, scoring the resistance level and sample collection were carried out at Greenhouse Mutihan, Madurejo Village, Prambanan District, Sleman Regency, Yogyakarta Special Region. Molecular analysis was carried out at the Genetics and Breeding Laboratory, Faculty of Biology, Universitas Gadjah Mada.

Melon cultivation

'GMP' and 'Meloni' melon seeds were obtained from Gama Melon Research Group and 'Aramis' melon seeds were obtained from reliable markets. To germinate, the melon seeds are soaked in water for 4-6 hours. The seeds are then placed on a tray which is protected from light. After 2-3 days of incubation, the seeds are transferred to small polythene bags containing a mixture of soil and

natural fertilizer. The seeds are grown in the polybags for about 7-12 days. The planting stage is carried out by transferring the seeds to the field. Plant maintenance included weeding, watering, tying stems to bamboo stakes at 12 days, cutting side branches, spraying insecticides, and fertilizing with NPK fertilizer.

Powdery mildew inoculation and scoring

Plants were inoculated with powdery mildew 14 days after planting (DAT). Six individual plants of each variety are inoculated by rubbing the fungal spores. Scoring at the leaf level was carried out using the 20 x 20 cm box line method, which contained small 0.5 x 0.5 cm boxes. Plant and population levels were scored by calculating the percentage of infection (Fukino *et al*., 2004). The values of the disease index were measured as described in Table 1. Every 3 days for 36 days, the percentages of leaf populations, plants and infection levels were calculated. Once the scores were obtained, the infection scale was calculated using the following Table 1.

Table 1. Disease index score of melon plants to powdery mildew infection on resistance test

Disease Index Score	% Leaf Area Affected
0	No Symptom
1	1-10
2	11-30
3	31-50
4	51-80
5	81-100

DNA extraction and quantitative analysis

From 3 healthy and 3 infected plants, leaf samples were taken from the 3rd to 5th stem segments. The samples were stored in a freezer at -20˚C. The Geneaid Isolation Kit was used for DNA isolation. The DNA isolation kit consists of GP 1 buffer, RNAse A, elution buffer, GP 2 buffer and GP 3 buffer. DNA isolation was performed according to manufacture protocols. Next, the concentration and purity of the DNA product was quantitatively tested using a Nanodrop UV-Vis spectrophotometer (NanoVue 4282 V2.0.4 Beckman).

PCR-SCAR amplification

DNA was amplified using SCAR primers linked to the powdery mildew resistance gene, following the My Taq HS Red Mix 2x Bioline procedure. The

SCAR primer results from the development of the RAPD pUBC411. Each SCAR primer contains the original 10 nucleotides of the RAPD primer plus 17 and 15 nucleotides for SCAPMAR5-F and SCAPMAR5-R respectively as follows in the Tabel 2.

Table 2. Sequence of oligonucleotide primers for SCAR locus

Locus	Primer	Sequence (5 - 3)
$SCAPMAR5_{1061}$	SCAPMAR5-F	CAGACAAGCCCAGATAATTAACATCTC
	SCAPMAR5-R	CAGACAAGCCTAGGAGTTGTGGGGGCT

DNA was sequentially amplified using a Bio-Rad thermal cycler following pre-denaturation and denaturation at 95ºC for 5 minutes and 60 seconds respectively. Annealing steps were performed at 50.1ºC for 30 seconds. This temperature was based on the primer optimization previously achieved. Extension and final extension were performed at 75ºC for 2 minutes and 10 minutes, respectively. Amplified DNA was analyzed by 1% agarose gel electrophoresis at 50 volts for 60 minutes. A GelDoc UV transilluminator was used to visualize the electrophoresis results. The presence of the powdery mildew resistance gene was indicated by the appearance of a 1058 bp size band on the electrophoretic gel (Daryono *et al*., 2009; Daryono and Yambise, 2018).

Data analysis

Qualitative phenotypic character data were analysed descriptively, while quantitative phenotypic character data were analysed using independent t-test with Microsoft Excel. The scoring of the resistance level of 'GMP' melon plants against powdery mildew was analysed using Microsoft Excel. The analysis of the molecular character data resulting from the GelDoc visualisation was carried out using the Photoshop program.

Results

Phenotypic characteristics of 'GMP'

Based on qualitative characteristics, differences between samples were in the colour of stems, leaves and fruits, as shown in Table 3. An independent t-test was performed (Table 4). Thev healthy plants were significantly larger than infected plants based on these analyses.

Table 3. Comparison of qualitative phenotypical characters of healthy 'GMP' melon plants and infected with powdery mildew

Parameters	Health plant	Infected plant
Stem shape	Cylindrical	Cylindrical
Stem colour*	RHS 136C	RHS 136D
Leaf shape	Reniform	Reniform
Leaf colour*	RHS 137A	RHS 137C
Flower shape	Rotate	Rotate
Crown colour ♂	RHS 9A	RHS 9A
Crown colour ♀	RHS 9A	RHS 9A
Petal colour ♂	RHS 144B	RHS 144B
Petal colour ♀	RHS 144B	RHS 144B
Stamen colour	RHS 6A	RHS 6A
Pistil colour	RHS 144 A	RHS 144 A
Fruit shape	Turbined Globular	Turbined Globular
Young fruit base color*	RHS 141C	RHS 145B
Young fruit pattern color*	RHS N 189A	RHS N 189B
Ripe fruit base color*	RHS 21A	RHS 21C
Ripe fruit pattern color*	RHS N 167B	RHS 166D

Tabel 4. Quantitative phenotypic characters of healthy and powdery mildew infected 'GMP' plants

Parameters	Mean ± SD		tStat	P(T<=t) two-tail
	Health Plant	Infected Plant		
Habitus length (cm)	193.59 ± 3.33	156.4 ± 4	21.43	0,000029
Stem diameter (cm)	0.93 ± 0.04	0.72 ± 0.02	13.41	0,000082
Stem circumference (cm)	1.97 ± 0.1	1.87 ± 0.04	2.91	0,009337
Leaf length (cm)	12.57 ± 0.35	11.78 ± 0.52	3.8	0,001309
Leaf width (cm)	19.41 ± 0.27	18.49 ± 0.3	6.81	0,000002
Fresh fruit weight (g)	232.5 ± 18.1	202.6 ± 26.2	2.82	0,011425
Fruit circumference (cm)	23.21 ± 0.63	21.92 ± 0.48	4.88	0,000120
Fruit horizontal diameter (cm)	7.29 ± 0.19	6.729 ± 0.31	4.58	0.000233
Fruit vertical diameter (cm)	7.23 ± 0.21	6.44 ± 0.36	5.74	0.000019
Horizontal diameter of cavity (cm)	5.06 ± 0.14	4.27 ± 0.28	7.48	0.000001
Vertical diameter of cavity (cm)	5.28 ± 0.2	4.66 ± 0.37	4.36	0,000378
Flesh fruit thickness (cm)	1.46 ± 0.05	1.28 ± 0.19	2.84	0,010772
Fruit skin thickness (cm)	1.18 ± 0.1	0.99 ± 0.08	4.44	0,000318

Morphological response and disease index of powdery mildew infection

The appearance of white fungal colonies on the surface of the leaves indicates the results of powdery mildew inoculation on 'GMP' melon plants (Figure 1). Depending on the resistance of the variety, the percentage of plants affected by powdery mildew varies. Resistance levels are determined using DI (disease index) analysis, which is based upon leaf, whole plant, and population

scores. The fungal colonies spread over an entire leaf and were covered with white flanks of spores (Figure 1C and 1D). At the end of cultivation, the leaves dried out and the plants died (Figure 1E).

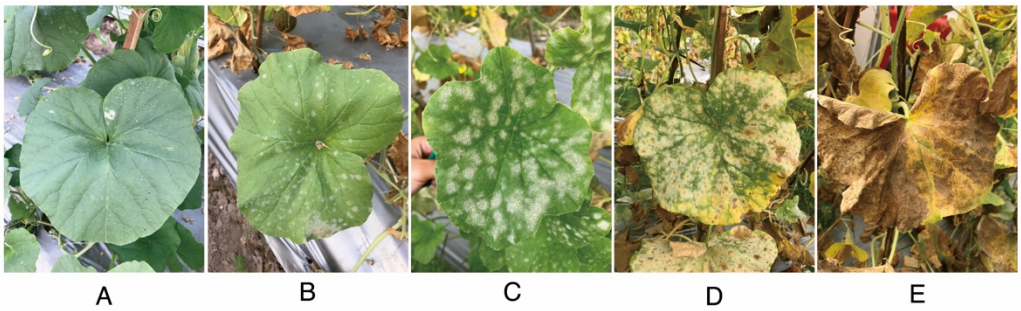

Figure 1. Stages of powdery mildew infection on leaves of 'GMP' melon, A) the leaf starts from a single point of infection, B) the infection spreads over the leaf surface in several white spots, C) the infection of the white spots spreads widely over the leaf surface, D) the entire leaf surface is infected, E) leaves infected with powdery mildew dry out

Table 5. Percentage of powdery mildew infection

Scoring day	Powdery Mildew Infection		
	Leaf level	Plant level	Population level
3	0.06	0.56	0.04
6	0.11	2.11	0.14
9	0.30	8.73	0.61
12	0.44	8.59	1.16
15	0.93	8.65	2.47
18	1.24	9.57	3.29
21	1.46	10.80	4.88
24	1.95	17.50	9.10
27	3.38	18.78	15.75
30	3.63	21.28	26.65
33	4.03	24.24	31.79
36	4.16	30.58	47.16

The increase in the percentage of infection in leave level was not significant (less than 10%), as shown in Table 5. Powdery mildew infection of 'GMP' at plant level was 0.56% on day 3 and increased to 30.58% at the end of the observation period. The percentage of infection at population level increased from day 3 (0.04%) to day 36 (47.16%). By converting the percentage of infection into a disease index (DI), the resistance of 'GMP' plants to powdery mildew can be determined. Plant resistance to powdery mildew disease, based

on the Disease Index (DI) Score Scale, is classified into resistant groups with DI Scale values of 0-1 or infection levels of 0-10%, moderately resistant groups (tolerant) with DI Scale values of 2-3 or infection levels of 11-50% and susceptible groups with DI Scale values of 4-5 or infection levels of 51-100% (Fukino *et al*., 2004). DI for powdery mildew infection at leaf, plant and population level of 'GMP' is shown in Figure 2.

The DI score at leaf level on days 3 to 18 showed no disease symptoms (Figure 2). From day 21 onwards, the DI scores increased with a score of 1, therefore 'GMP' plants were classified as resistant at leaf level. At the plant level, 'GMP' plants reached a DI score of 2 at the end of the observation period. Therefore, 'GMP' plants are classified as a mildly resistant group (tolerant). At population level, infection started on day 15 with a DI score of 2. Based on these results, 'GMP' plants can be classified as a mildly resistant group (tolerant) at population level. Overall, there was an increase in the DI score as the melon plants approached harvest age.

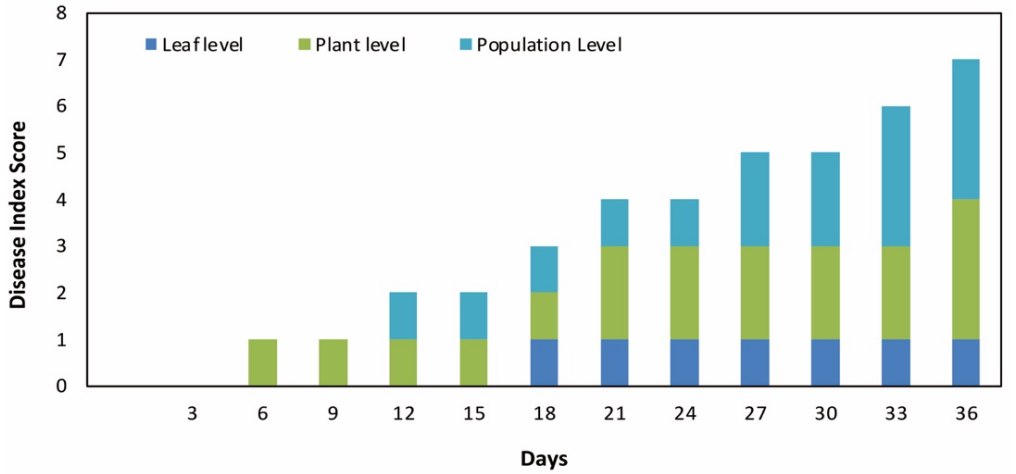

Figure 2. Disease Index scores of 'GMP' plants at the leaf, plant, and population levels

Detection of powdery mildew resistance genes with SCAR molecular markers

The resistance of 'GMP' plants to powdery mildew was validated by molecular analysis based on SCAR markers, as detailed in the Table 6. The results of the quantitative DNA assays obtained. The purity of the DNA isolated from the eight samples ranged from 1.8151 to 1.998. In accordance with the requirements of molecular analysis (Dewanata and Mushlih, 2021), good DNA purity values in the range of 1.8-2.0 were obtained.

Molecular analysis for the detection of powdery mildew resistance genes was performed on three healthy 'GMP' leaf samples (S1-S3) and three infected 'GMP' leaf samples (P1-P3). 'Meloni' (Mi) and 'Aramis' (Arm) leaves were used as positive and negative controls, respectively. Figure 3 shows the results of DNA amplification using the SCAR marker linked to the powdery mildew resistance gene. Powdery mildew resistance genes were found in healthy 'GMP' plants (S1-S3) and infected plants (P1-P3) in Figure 3. The powdery mildew resistance genes were also detected in the 'Meloni' plant. The presence of the powdery mildew resistance gene was indicated by the appearance of a target DNA band of 1058 bp. The 'Aramis' cultivar did not contain any powdery mildew resistance genes, as indicated by the absence of the 1058 bp target DNA band.

Table 6. Quality of DNA samples

Sample	Code	DNA Concentration (ng/µL)	DNA Purity (A260/A280)
Healty 'GMP'	S1	10.6	1.898
Healty 'GMP'	S2	18.7	1.928
Healty 'GMP'	S3	9. 0	1.998
Infected 'GMP'	P1	10.2	1.957
Infected 'GMP'	P2	12.1	1.981
Infected 'GMP'	P3	22.0	1.913
'Meloni'	Mi	7.8	1.815
'Aramis'	Arm	19.4	1.959

Discussion

Powdery mildew is an obligate biotrophic fungus. It has no ability to develop independently and grows only by extracting nutrients from its living host (Spanu, 2012; Takamatsu, 2013). Powdery mildew species are widely distributed in different genera. This leads to a wide range of niches in each species. The wide range of niches may be due to genetic changes driven by adaptive evolution of the fungi (Liang *et al*., 2018). The appearance of white spots on organs such as leaves and stems is the common response of plants affected by powdery mildew. Severe infestation causes white powdery pustules on the surface of the leaves which turn brown and die back (Takamatsu, 2013). Despite the large number of species, the symptoms of many plant families are very similar, and even the effects are species-specific (Ridout, 2009).

Pathogen-host interactions were studied on barley by *Blumeria graminis hordei* (Lambertucci *et al*., 2019), on wheat by *Blumeria graminis f.sp. triciti* (Bourras *et al*., 2018), on Arabidopsis by *Golovinomycesorontii* (Kuhn *et al*., 2016) and on grapevine by *Plasmopara viticola* and *Erysiphe necator* (Ruiz-García *et al*., 2021). Melon powdery mildew is caused by *Podosphaera xanthii*

and *Golovinomyces cichoracearum* (Kasiamdari *et al*., 2016) caused whitish colonies on both sides of the leaf, leaf yellowing, necrosis, incomplete fruit ripening and premature death that are common symptoms on cucurbits crops (Caligiore-Gei *et al*., 2022).

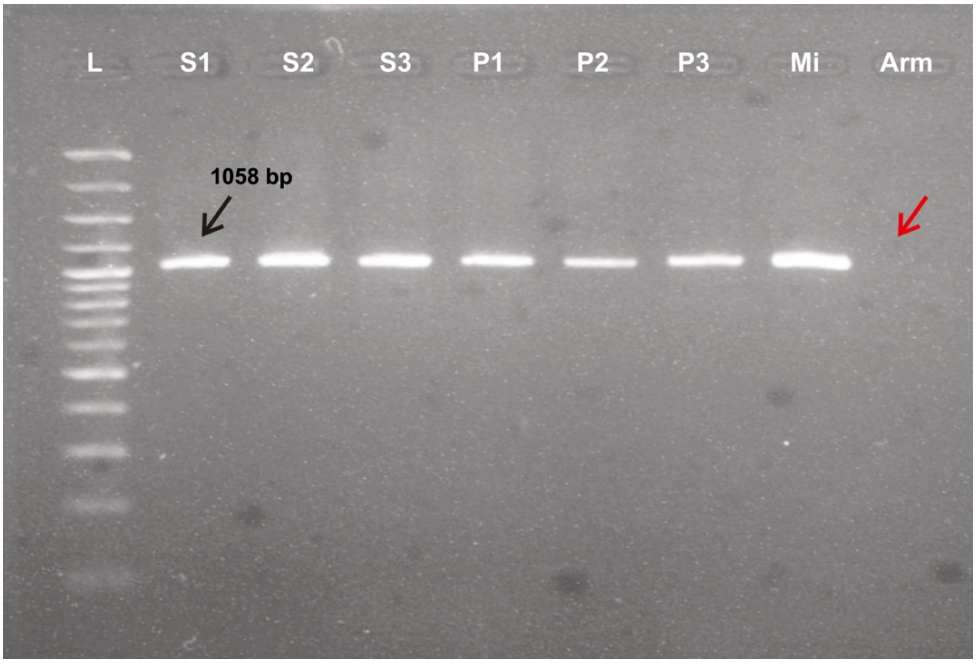

Figure 3. DNA amplification with SCAR primers linked to powdery mildew resistance genes. L = DNA ladder; S1-S3 = healthy 'GMP' plant; P1-P3 = powdery mildew-infected 'GMP' plant; Mi = 'Meloni'; Arm = 'Aramis'

The mechanism of powdery mildew infection is through cell wall breakdown by the appressorium structure. The structure is the development of the conidium, which is the infection structure of the powdery mildew spore. The appressorium penetrates the plant cell and forms a feeding structure (haustorium) surrounded by an extrahaustorial membrane. It is a plant native membrane, which has a unique composition due to modifications (Feechan *et al*., 2011).

Preventing powdery mildew infection remains a concern in the agricultural and plant breeding world because it drastically reduces production. The widespread use of insecticides causes health and ecological problems (Pathak *et al*., 2022). Another approach to control powdery mildew is the use of cadmium to alter the microbiome of the phyllosphere (Xu *et al*., 2023). Systemic resistance in *Brassica napus* and *Raphanus alboglabra* can also be increased by the use of microbial pesticides such as *Trichoderma harzianum* (Alkooranee *et al*., 2015). A more effective and environmentally friendly control strategy would be the

development of plant resistance gene to specific diseases. Many powdery-mildew resistance genes have been identified in various plant species such as in *Cannabis sativa* (Mihalyov and Garfinkel, 2021), African melon accession TGR-1551 (López-Martín *et al*., 2022), wheat (Cheng *et al*., 2022; Mapuranga *et al*., 2022; Mu *et al*., 2022; Zhang *et al*., 2023), melon (Cui *et al*., 2022; Ishak and Daryono, 2020).

The SCAR primer in this study was the result of the development of the RAPD marker ($pUBC411_{1061}$), which was evaluated for the first time and can discriminate between the resistant genotype PI-371795 and the susceptible 'Action-434'. The SCAR marker was then tested against different genotypes: PMAR5, 'Harukei', WMR-29, PMR45, PMR5, Nigeashi-1, PI-414723, PI-124112, PI-124111, Sunrise, Kohimeuri, PI-161375, Nigeashi-2, PI-371795 and 'Action434', which shows the ability to distinguish the presence of the *Pm-I* locus (Daryono *et al*., 2009). Using this marker, differences in resistance to powdery mildew have been shown between resistant 'Tacapa' and susceptible genotypes, 'Aramis', with and without a 1058 bp amplification band (Daryono and Yambise, 2018). This gene was also found in 'Meloni', but it was not found in the 'SunLady-3' cultivar (Ishak and Daryono, 2020). The 'GMP' melons in this study are also included in the resistance group as they have a gene for powdery mildew resistance. The use of SCAR primers can facilitate the initial screening of progeny in the assembly of new varieties and allow farmers to truly evaluate the ability of plants to resist powdery mildew.

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