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Evaluation of five commercial cucumber hybrids for Downy Mildew disease using RAPD, SCoT and SRAP molecular markers

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Downy mildew (DM) is the most devastating leaf disease of cucumber (*Cucumis sativus* L.) caused by the *Oomycete Pseudoperonospora cubensis*. The resistance mechanism of DM is still poorly understood in cucumber plants. In the present investigation, one moderately tolerant (Elnems), two susceptible (Barika F1and Basimah F1) and two tolerant (234 and SV4196CD) cucumber hybrids to DM were used. Genetic diversity was assessed using three types of molecular markers RAPD, SCoT and SRAP. In addition, selected candidate PCR bands were sequenced to investigate the possibility of identifying anonymous genes in cucumber hybrids related to DM tolerance. The results showed that the total number of bands produced by RAPD, SCoT and SRAP were187, 237 and 208, respectively. The polymorphism percentage revealed by RAPD, SCoT and SRAP was 80.75%, 75.95% and 86.54%, respectively. The resulting dendrograms successfully clustered the two tolerant hybrids 234 and SV4196CD in the same cluster. These two hybrids appear to be genetically related, although the sources of the hybrids are different. Elnems, Barika F1 and Basimah F1were also clustered together. The polymorphic fragments present only in the tolerant hybrids have been isolated, sequenced and aligned with the nucleotide sequences in the database. The alignment sequences revealed that one amplicon (M1E7D1-ME1) showed homology with Photosystem I P700 chlorophyll a apoprotein A1 (PsaA) protein, which could be related to DM tolerance. More studies are required to clarify the mechanism of DM resistance in cucumber.

Keywords: Cucumber; Downy mildew; Molecular markers; RAPD; SCoT and SRAP

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INTRODUCTION

Cucumber (*Cucumis sativus* L.) is among the most consumed vegetables worldwide (Paris *et al*., 2011) and has been cultivated for several thousand years. Cucumber plants are grown in a wide range of climates, open fields, and greenhouses. Cucumber has a chromosome number of 2n = 2x = 14 chromosomes. Cucumber developed from its extinct 2n = 24 ancestor through dysploid chromosome reduction (translocations, fusions, and inversions) except for cucumber chromosome number 7 which remained largely intact during the entire evolution of *Cucumis* (Weng, 2021). China is the largest producer, and Egypt is the tenth largest producer (FAOSTAT, 2023). Besides the importance of cucumber as an economic crop, it's a model system for studying plant vascular biology and sex determination (Huang *et al*., 2009). It is also used to study organellar genetics' genetic and physical bases (Bartoszewski *et al*., 2007).

Downy mildew (DM) is a devastating disease caused by the air-borne oomycete *Pseudoperonospora cubensis* (Wang *et al*., 2016; Berg *et al*., 2020 and Sun *et al* 2022). Due to the variation and mutation of *Pseudoperonospora cubensis* races, DM has become the most devastating leaf disease of cucumber worldwide which led to huge damage to cucumber (Wan *et al*., 2010; Berg *et al*., 2020 and Zhuo *et al*., 2024). The disease has also been recorded in about 40 genera in the Cucurbitaceae family (Palti and Cohen, 1980). The genetic and molecular study of DM resistance in cucumbers is essential for improving resistant cultivars (Mirzwa-Mróza *et al.,*2024). Molecular markers are considered valuable tools for crop improvement (Torres, 2010) and have proven useful tools for genetic mapping applications, diversity studies, cultivars identification, and phylogenetic relationships. Moreover, molecular marker techniques have been developed in recent years to evaluate the plant reaction to different diseases such as leaf rust in wheat (Hussien et al., 2024) rice blast (Yadav *et al*., 2017) and fusarium in cotton (Mahmoud *et al*., 2019). Also, for identifying molecular markers linked to resistant genes (Li *et al*., 2010; Devran *et al*., 2011). Different molecular markers have different properties including reproducibility, reliability, and cost, such as Random Amplified Polymorphic DNA (RAPD) markers, which are arbitrary nucleotide sequences. These markers allow rapid identification and isolation of chromosomes. Specific DNA fragments (Williams *et al*., 1990). These markers have been widely used in cucumber studies (Horejsi *et al*., 1999; Horejsi *et al*., 2000; Maliki *et al*., 2003; Duca *et al*., 2008; Onto *et al*., 2008; Smiech *et al.,* 2008c Faisal *et al*., 2011 and Panyanitikoon *et al*., 2018).

Start Codon Targeted (SCoT) was developed based on the short, conserved region surrounding the ATG

translation start codon in plant genes. These markers are reproducible and cheap. SCoT markers were used for three main applications: QTL mapping, bulked segregant analysis, and genetic diversity studies (Collard and Mackill, 2009). These markers were validated with several crops such as grape (Guo *et al*., 2012), Cicer species (Amirmoradi *et al*., 2012) orchardgrass (Zeng *et al*., 2014), sugarcane (Que *et al*., 2014), summer squash (Xanthopoulou *et al*., 2015), ramie (Satya *et al*., 2015), bottle gourd (Bhawna *et al*., 2016), watermelon (Vasudevan *et al*., 2017) and cotton (Mahmoud *et al*., 2019). Also, sequence–Related Amplified Polymorphism (SRAP), which targets the open reading frame, has been tested with several crops such as brassica, citrus, apple, plum, lettuce, celery, potato, cherry, garlic, and rice. These crops are easily amplified by SRAPs (Li and Quiros, 2001). In addition, the SRAP marker was used to identify the fruit shape of cucumber at the commercial fruit stage (Meng *et al*., 2012). Also, the SRAP technique has been used in a wide range of applications in different studies, including QTL mapping, genetic diversity studies, genetic linkage mapping, and identification of molecular markers linked to specific traits (Pan *et al*., 2005; Long-Zhou *et al*., 2008 and Aydemir, 2009).

Objectives

Determine the genetic diversity among five cucumber hybrids, evaluate them according to their tolerance and susceptibility to downy mildew, and identify polymorphic amplicons related to downy mildew tolerance among the studied hybrids.

MATERIALS AND METHODS

Plant materials

The plant materials used in the present investigation consisted of five commercial cucumber hybrids (Elnems, Barika F1, Basimah F1, 234 and SV4196CD). These hybrids are cultivated in Egypt and their selection was based on their reaction to DM disease. This data is provided by the produced companies (Table 1).

Methods

DNA extraction

According to Saghai-Maroof *et al*. (1984), DNA was isolated from the young leaves of the five cucumber hybrids using the CTAB extraction method.

PCR Analysis

In the present investigation, three different marker techniques, Random Amplified Polymorphic DNA (RAPD), Start Codon Targeted (SCoT), and Sequence – Related Amplified Polymorphism (SRAP), were employed to characterize the five cucumber hybrids at the DNA level.

RAPD-PCR analysis

Fourteen RAPD primers, according to (Horejsi *et al*., 1999; Onto *et al*., 2008 and Smiech *et al*., 2008) were applied. Macrogene, Corp, U.S.A. Synthesized These primers. The primer names, annealing temperatures and sequences are shown in Table 2. The PCR reaction was performed in 20µl volume composed of 1µl DNA, 1µl primer, 10 µl of master mix comprising (*Taq* DNA polymerase, 20 mM Tris buffer, 100 mM KCL, 0.1 mM EDTA, 1 mM DTT, glycerol, $(NH_4)_2SO_4$, KCL, dNTPs, gel loading dyes and $MgCL_2$) and the volume was adjusted to 20 µl with distilled water. The RAPD PCR amplification was performed as follows: an initial denaturation cycle at 95°C for 5 min followed by 35 cycles consisting of a denaturation step at 95°C for 1 min, an annealing step at temperatures ranging from 31°C to 37°C and an extension step at 72°C for 1 min. The final extension was at 72°C for 5 min. The amplified products were resolved in 1.5% agarose gel containing ethidium bromide (0.5 mg/mL) and then visualized on a transilluminator UV light.

SCoT-PCR analysis

Thirteen SCoT primers according to (Xanthopoulou *et al.,* 2015 and Bhawna *et al*., 2016) were used in the present investigation. These primers were synthesized by Macrogene, corp, USA. The primer names, annealing temperatures and sequences are shown in Table 2. The PCR reaction performed in 25µl volume includes 1µl of the DNA template, 1µl of the primer DNA (20 µM), 12.5 µl of master mix including (*Taq* DNA polymerase, 20 mM Tris buffer, 100 mM KCL, 0.1 mM EDTA, 1 mM DTT, glycerol, $(NH_4)_2SO_4$, KCL, dNTPs, gel loading dyes, $MgCL_2$) and 10.5 of sterile distilled water. The SCoT PCR amplification was as follows: The initial denaturation at 94°C for 5 min followed by 35 cycles of: denaturation step at 94°C for 50 sec, the annealing temperature ranged from 49 °C to 53°C for 1 min and 72°C for 2 min. The final extension step was extended to 5 min at 72°C. PCR products were visualized using the same procedure as in the RAPD technique.

SRAP-PCR analysis

Sixteen SRAP primer combinations were used in the analysis of the five hybrids. The primer names and sequences are displayed in Table 3. The primers were provided by Alpha DNA, Canada. The PCR reaction

performed in 20µl volume included: 1µl of the DNA template, 1µl of the forward primer DNA (20 µM), 1µl of the reverse primer DNA (20 µM), 10 µl of master mix comprising (*Taq* DNA polymerase, 20 mM Tris buffer, 100 mM KCL, 0.1 mM EDTA, 1 mM DTT, glycerol, $(NH_4)_2SO_4$, KCL, dNTPs, gel loading dyes, $MgCL_2$). The volume was adjusted to 20 µl with distilled water. The SRAP-thermocycling profiles were performed as follows: The initial denaturation at 94°C for 5min followed by 5 cycles of denaturation step at 94°C for 1 min, annealing step at 35°C for 1 min and extension at 72°C for 1min. Then 35 cycles of denaturation step at 94°C for 1 min, annealing at 56 °C except for one combination ME10-EM4 at 55°C for 1 min and the extension at 72°C for 1 min. The final extension was at 72 °C for 7 min. PCR products were visualized using the same procedure as in the RAPD technique.

Data analysis

The banding patterns obtained from fourteen RAPD, thirteen SCoT primers and sixteen SRAP primer combinations were analyzed using gel analyzer program version 3 (Alzohairy, 2008) for the presence (1) and absence (0). Then the resulting data was analyzed using Multi-Variate Statistical Package (MVSP) program version 3. The dendrograms for the five hybrids using RAPD, SCoT, SRAP and the combined data were constructed using UPGMA and similarity matrices according to Nei & Li's coefficient (Nei and Li., 1979).

Sequencing of some PCR polymorphic fragments in tolerant hybrids

The primers that characterized polymorphic fragments that were determined to be present in the tolerant hybrids were used in other PCR reactions by doubling the volumes of the main PCR reactions, and the resulting polymorphic DNA bands in the tolerant hybrids only were isolated and purified using QIAquick Gel Extraction Kit provided by Qiagen. The DNA sequences were determined using the Sanger sequencing method by Macrogen, Korea. Then the sequence analysis was performed by blasting with the available sequences of the National Center for Biotechnology Information (NCBI) (http://www.NCBI.nlm.nih.gov/) using BLASTX.

RESULTS

RAPD markers analysis

Fourteen RAPD primers have been used in this investigation, and the profile patterns are illustrated in Figure 1. The total number of amplicons produced by 14 RAPD primers was187 ranging from 3 to 27 with an average of 13.4 per primer. The BC526 primer produced the highest number of bands (27). The number of monomorphic amplicons was 36, averaging 2.6 per primer. Of 187 bands, 151 were polymorphic, ranging from 0 to 27, with an average of 10.8 per primer. The total percentage of polymorphism for the RAPD analysis was 80.75%. The primer polymorphism ranged from 0% for OPA-11 to 100% for OPC-05 and BC526 primers. A total of 95 unique markers (88 positives and 7 negatives) were produced, as shown in Tables 4 and 5. The RAPD similarity matrices among five cucumber hybrids according to Nei & Li's coefficient ranged from 0.480 to 0.723 as shown in Table 6. The highest similarity was 0.723 between the hybrid Elnems and Barika F1. In comparison, the lowest genetic similarity was 0.480 between Barika F1 and SV4196CD. The genetic similarity using Nei & Li's coefficient was used to construct a dendrogram using the UPGMA method (Figure 4). The generated dendrogram divided the five cucumber hybrids into two clusters. Cluster 1 consisted of three hybrids: Elnems, which has moderate tolerance to downy mildew, Barika F1 and Basimah F1, which are susceptible to downy mildew. Cluster 2 comprised the tolerant hybrids to downy mildew 234 and SV4196CD. The first cluster was further divided into two sub-clusters, the first sub-cluster included Elnems and Barika F1. The second sub-cluster included Basimah F1.

SCoT markers analysis

In the present investigation, thirteen SCoT primers were used, and the patterns of the profiles are shown in Figure 2. The total number of bands produced by the SCoT primers was 237, ranging from 9 to 36, with an average of 18.23 per primer. The number of polymorphic bands was 180, ranging from 1 to 35, with an average of 13.85 per primer. The total polymorphism percentage was 75.95%, ranging from 11.11% for SCoT 66 and SCoT 70 to 100% for SCoT 26 and SCoT 34. The total number of SCoT unique markers identified in the five cucumber hybrids was 134 bands; 131 of them were unique positive markers, and 3 were unique negative markers, as shown in Tables 4 and 5. The data obtained from thirteen SCoT primers has been used to detect the similarity among the five cucumber hybrids. The genetic similarity matrices were constructed according to Nei & Li's coefficient. The genetic similarity ranged from 0.556 to 0.702, as displayed in Table 6. The highest genetic similarity was 0.702 between the hybrid Barika F1 and Basimah F1.

Table 1. The name, degree of downy mildew infection and source of the five cucumber hybrids used in this study

Hybrid name	The infection degree to DM disease	Provided by
Barika F1	Susceptible	East West Seeds International Ltd., Thailand.
Basimah F1	Susceptible	East West Seeds International Ltd., Thailand.
Elnems	Moderate tolerant	Seminis Vegetable Seeds.Inc., USA.
234	Tolerant	Gento Seeds Ltd., Turkey.
SV4196CD	Tolerant	Seminis Vegetable Seeds.Inc., USA.

Table 2. The primer names, sequences of the RAPD and SCoT primers and their corresponding annealing temperatures

Primer Name	The sequence from 5'to 3'	Annealing temperature Cº	Primer Name	The sequence from 5'to 3'	Annealing temperature Cº
OP-G14	GGATGAGACC	36	SCoT13	ACGACATGGCGACCATCG	52
OP-X15	CAGACAAGCC	36	SCoT70	ACCATGGCTACCAGCGCG	51
OP-AS5	GTCACCTGCT	36	SCoT14	ACGACATGGCGACCACGC	50
BC519	ACCGGACACT	36	SCoT66	ACCATGGCTACCAGCGAG	51
BC526	AACGGGCACC	34	SCoT61	CAACAATGGCTACCACCG	49
B6	TGCTCTGCCC	34	SCoT33	CCATGGCTACCACCGCAG	49
E15	ACGCACAACC	34	SCoT34	ACCATGGCTACCACCGCA	50
E20	AACGGTGACC	34	SCoT52	ACAATGGCTACCACTGCA	50
D13	GGGGTGACGA	34	SCoT71	CCATGGCTACCACCGCCG	50
OPA-04	AATCGGGCTG	34	SCoT77	CCATGGCTACCACTACCC	53
OPA-05	AGGGGTCTTG	37	SCoT24	CACCATGGCTACCACCAT	49
OPA-11	CAATCGCCGT	31	SCoT26	ACCATGGCTACCACCGTC	50
OPC-04	CCGCATCTAC	36	SCoT31	CCATGGCTACCACCGCCT	50
OPC-05	GATGACCGCC	34	-	-	-

Table 3. The primer names and the sequences of the SRAP primers used in the SRAP analysis

Forward primers	Sequence 5'-3'	Reverse primers	Sequence 5'-3'
SA4	TTCTTCTTCCTGGACACAAA	EM4	GACTGCGTACGAATTTGA
ME8	TGAGTCCAAACCGGTGT	EM14	GACTGCGTACGAATTCAG
ME1	TGAGTCCAAACCGGATA	EM7	GACTGCGTACGAATTATG
ME10	TGAGTCCAAACCGGGAC	EM5	GACTGCGTACGAATTAAC
ME12	TGAGTCCAAACCGGGGT	EM10	GACTGCGTACGAATTCAT
ME14	TGAGTCCAAACCGGCTA		
ME4	TGAGTCCAAACCGGACC		
OD3	CCAAAACCTAAAACCAGGA		

Table 4. Number of primers and primer combinations (NP), total number of amplicons (TA), number of bands per primer (NB/P), band molecular weight (BMW), polymorphic amplicons (PA), percentage of polymorphism (%P), polymorphism rang (PR), unique positive (Pos), unique negative (Neg) bands and total (Tot) and genetic similarity (GS) as revealed by RAPD, SCoT, SRAP and combined data

Marker type	NP	TA		NB/P		BMW			PA		PR		Unique Bands			GS	
		Tot	Aver	High	Low	High	Low	Tot	Aver	%P	High	Low	Pos	Neg	Tot	High	Low
RAPD	14	187	13.4	27	3	4.545	0.091	151	10.8	80.75	100	0%	88	7	95	0.723	0.480
SCoT	13	237	18.23	36	9	4.391	0.067	180	13.85	75.95	100	11.11	131	3	134	0.702	0.556
SRAP combinations	16	208	13	22	7	7.365	0.053	180	11.25	86.54	100	22.22	92	24	116	0.871	0.352
Combined data																0.747	0.471

At the same time, the lowest genetic similarity was 0.556 between Elnems and 234. The genetic similarity using Nei & Li's coefficient was used to construct a dendrogram using the UPGMA method (Figure 4). This dendrogram divided the five cucumber hybrids into two main clusters. The first cluster consisted of three hybrids, further divided into two sub-clusters. Elnems is in the first sub-cluster, while the second sub-cluster includes Barika F1 and Basimah F1, which are susceptible to DM. The second cluster includes 234 and SV4196CD, which are tolerant hybrids.

SRAP analysis

In the present investigation, sixteen SRAP primer combinations have been used. The pattern profiles are shown in Figure 3. The total number of bands produced was 208 bands ranging from 7 to 22, with an average of 13 bands per primer. The number of polymorphic bands was 180, ranging from 2 to 22, with an average of 11.25 per primer. The percentage of polymorphism was 86.54%, ranging from 22.22% for the combination SA4-EM10 to 100% for the

Table 5. Primer name (PN), total number of amplicons (TA), monomorphic amplicons (MA), polymorphic amplicons (PA) and percentage of polymorphism (%P) as revealed by RAPD, SCoT and SRAP markers among the five cucumber hybrids

RAPD markers					SCoT markers					SRAP Markers				
PN	TA	MA	PA	P (%)	PN	TA	MA	PA	P (%)	PN	TA	MA	PA	P (%)
B6	16	1	15	93.75	SCoT13	32	3	29	90.63	ME1-EM7	13	1	12	92.31
BC519	16	1	15	93.75	SCoT14	27	1	26	96.30	ME1-EM10	18	0	18	100
BC526	27	0	27	100	SCoT24	18	9	9	50	ME1-EM14	9	2	7	77.78
D13	25	2	23	92	SCoT26	12	0	12	100	ME4-EM4	12	4	8	66.67
E15	11	3	8	72.73	SCoT31	36	1	35	97.22	ME4-EM5	10	2	8	80
E20	8	1	7	87.50	SCoT33	13	3	10	76.92	ME4-EM14	12	1	11	91.67
OPA-04	7	5	2	28.57	SCoT34	21	0	21	100	ME8-EM14	10	2	8	80
OPA-05	7	5	2	28.57	SCoT52	22	6	16	72.73	ME10-EM4	7	4	3	42.86
OPA-11	3	3	0	0	SCoT61	10	7	3	30	ME10-EM7	8	1	7	87.50
OP-AS5	10	4	6	60	SCoT66	9	8	1	11.11	ME12-EM4	12	0	12	100
OPC-04	12	1	11	91.67	SCoT70	9	8	1	11.11	ME14-EM5	20	1	19	95
OPC-05	16	0	16	100	SCoT71	13	9	4	30.77	ME14-EM14	11	1	10	90.91
OP-G14	19	3	16	84.2	SCoT77	15	2	13	86.67	OD3-EM5	16	1	15	93.75
OP-X15	10	7	3	30	-	-	-	-	-	OD3-EM14	22	0	22	100
-	-	-	-	-	-	-	-	-	-	SA4-EM4	19	1	18	94.74
-	-	-	-	-	-	-	-	-	-	SA4-EM10	9	7	2	22.22
Total	187	36	151	80.75		237	57	180	75.95		208	28	180	86.54
Average	13.4	2.6	10.8			18.23	4.38	13.85			13	1.75	11.25	

Table 6. Similarity matrices resulted from RAPD analysis (a), SCoT analysis (b), SRAP analysis (c) and combined analysis (d)

a) Elkem's		Barika F1	Basimah F1	234	SV4196CD
Elnems	1				
Barika F1	0.723	1			
Basimah F1	0.591	0.658	1		
234	0.535	0.55	0.606	1	
SV4196CD	0.488	0.48	0.526	0.64	1
b) Elnems		Barika F1	Basimah F1	234	SV4196CD
Elnems	1				
Barika F1	0.673	1			
Basimah F1	0.637	0.702	1		
234	0.556	0.608	0.63	1	
SV4196CD	0.557	0.563	0.585	0.67	1
c) Elnems		Barika F1	Basimah F1	234	SV4196CD
Elnems	1				
Barika F1	0.691	1			
Basimah F1	0.746	0.871	1		
234	0.448	0.532	0.586	1	
SV4196CD	0.352	0.446	0.457	0.58	1
d) Elnems		Barika F1	Basimah F1	234	SV4196CD
Elnems	1				
Barika F1	0.693	1			
Basimah F1	0.658	0.747	1		
234	0.513	0.564	0.607	1	
SV4196CD	0.471	0.499	0.524	0.627	1

combinations ME1-EM10, ME12-EM4 and OD3-EM14. 116 unique SRAP combination markers were identified in the five cucumber hybrids, of which 92 were positive, and 24 were negative, as shown in Tables 4 and 5. The scoring data from the sixteen SRAP combinations primers was used to compute similarity matrices according to Nei & Li's coefficient, as illustrated in Table 6. Genetic similarity ranged from 0.352 to 0.871. The highest genetic similarity was 0.871 between the hybrid Barika F1 and Basimah F1, followed by 0.746 between Elnems and Basimah F1. In contrast, the lowest genetic similarity was 0.352 between Elnems and SV4196CD. The genetic similarity using Nei & Li's coefficient was used to construct a dendrogram using the UPGMA (Figure 4). The resulting dendrogram divided the five cucumber hybrids into two clusters.

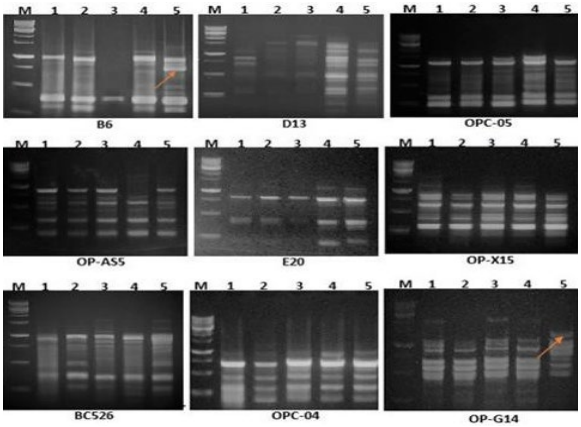

Figure 1. RAPD profiles of five cucumber hybrids amplified with the RAPD primers (M =1Kb marker, 1= Elnems, 2= Barika F1, 3=Basimah F1, 4=234 and 5= SV4196CD). Red arrows depict the PCR bands selected for sequencing

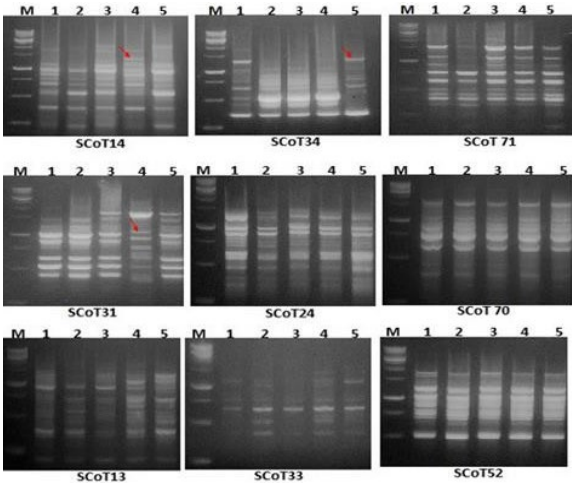

Figure 2. SCoT profiles of five cucumber hybrids amplified with SCoTprimers (lane M =1Kb marker, 1= Elnems, 2= Barika F1, 3= Basimah F1, 4=234 and 5= SV4196CD). Red arrows depict PCR bands selected for sequencing

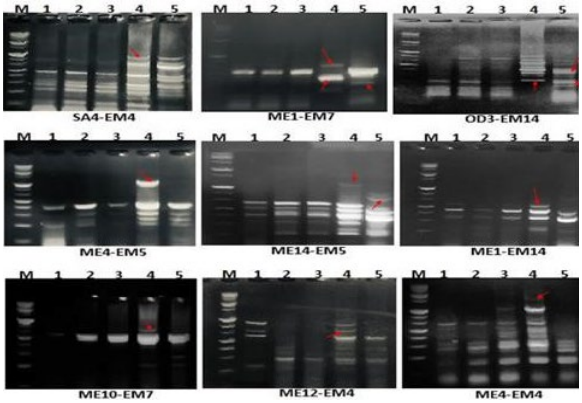

Figure 3. SRAP profiles of five cucumber hybrids amplified with SRAP combinations (lane M=1Kb marker, 1= Elnems, 2= Barika F1,3= Basimah F1, 4=234 and 5= SV4196CD). Red arrows depict the PCR bands selected for sequencing

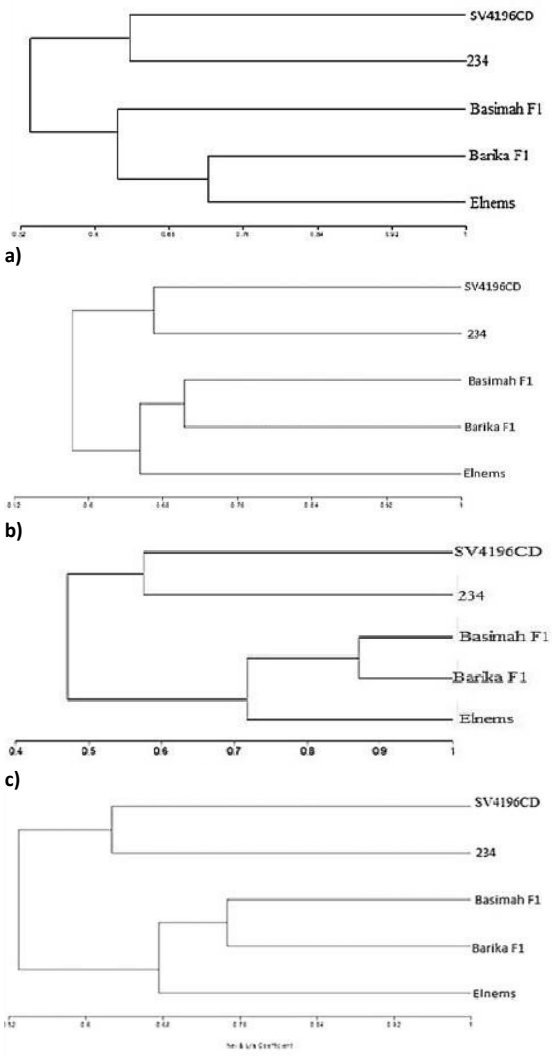

Figure 4. Dendrograms for the five cucumber hybrids constructed from the RAPD (a), SCoT (b), SRAP (c) and combined data (d) using UPGMA and similarity matrices according to Nei & Li's coefficient

a)

AATTTAGGGAATCAGTACTTGCTTGGATAGGCTATGAGGGGCTTCATGTC
ATATAGCCTTTCAAATCCACGTCTTCTTATGGGACTGTGGAAGTCAAGTC
CATTTCTGTCCCAGTTCCGTTTCATCTGGAAAATGCGTAATGATGTTTGG
GGCAGCGCAACCGAGGACGGGCTCAAAGTCCTATTACTGAGAAGAAACTT
TGCCTTTAAATTTATTATTATCCCAGGGTGTGGACTCAATTTCTTCTTGC
CACTAGCCTCCCCCGCAAATCAGACACATTAATCTTCATTCCCTGCCTAT
GGCCTGTTTTTCCTAAGTGCTCATTTTGTATGGGCTTTTAATTTAATGTT
TCTATTCACCGGGCGTGGTTATTGGCAAGAACTTATTGAATCCATCGTTT
GGGCTCATAATAAATTAAAAGTTGCTCCTGCTACTCAGCCTAAAGCCTTG
ACCATTGTCCAAGGACGTGCTGTAGGAGTAACCCATTACCTTCTGGGTGG
AATTGCCACAACATGGGCGTTCTTCTTAGCAAGAATTATTGCAGTCGGAT
AATGGCTAGGAGGATTTGAAAGGCATTATGGCACTAACATTTCCAAGGTT
TACCCAAGGCTTAGCTCAAGACCCCACTACTCTTCGTATCCGGTTTGGAC
TCAA

b)

Figure 5. Alignment of the nucleotide sequences for the SRAP fragment (M1E7D1_ME1) using BLASTX (a) and the nucleotide sequences for the SRAP fragment (M1E7D1_ME1) (b).

The first cluster consisted of three hybrids, further divided into two sub-clusters. Elnems was separated in the first sub-cluster. At the same time, Barika F1 and Basimah F1 were placed in the second sub-cluster. The second cluster comprised 234 and SV4196CD.

Combined genetic relationships as revealed by RAPD, SCoT and SRAP markers

The genetic similarity matrix resulting from the combined analysis using RAPD, SCoT and SRAP markers ranged from 0.471 to 0.747, as shown in Table 6. The highest similarity was 0.747 between Barika F1 and Basimah F1. The lowest genetic similarity, 0.471, was detected between Elnems and SV4196CD. As a result, the dendrogram, as shown in Figure 4, divided the five cucumber hybrids into two clusters. Cluster 1 comprised three hybrids, Elnems, Barika F1 and Basimah F1, further divided into sub-clusters. The first sub-cluster included Elnems, while the second sub-cluster consisted of Barika F1 and Basimah F1. Cluster 2 consisted of hybrids 234 and SV4196CD.

Sequence analysis of some polymorphic PCR bands

In the present investigation, two RAPD, three SCoT and thirteen SRAP polymorphic bands were characterized, isolated, purified and sequenced (Macrogen Company, Korea). The sequence analysis was performed by blasting with the available sequences of the National Center for Biotechnology Information (NCBI) (http://www.NCBI.nlm.nih.gov/). The sequence comparison of these polymorphic fragments against sequences in the NCBI database revealed that one fragment (M1E7D1_ME1) with length 654 bp, which resulted from the SRAP combination ME1-EM7 showed homology with Photosystem I P700 chlorophyll a apoprotein A1 (PsaA) protein (Figure 5). The query coverage was 82% and 74%, E value was 58 and 50, the percentage of identity was 89.38% and 90.72% in *Vicia sativa* and *Oreocnide trinervis* respectively. This protein could be related to DM tolerance.

DISCUSSION

The growing field of molecular marker techniques and the different types of molecular markers with differing properties widen their use in various applications, including genetic diversity studies and marker-assisted selection (Mishra *et al*., 2014; Hayward *et al*., 2015). In addition, they provide powerful tools for rapid genetic tagging of genes of interest for linked marker-assisted selection (Devran *et al*., 2011; Meng *et al*., 2012). In the present investigation, we used three types of molecular markers with differing properties (RAPD, SCoT and SRAP) to detect genetic diversity among five cucumber hybrids with different reactions to DM disease and to identify new sequences related to downy mildew tolerance in cucumber. The genetic mechanism for downy mildew resistance in cucumber is poorly understood and needs more studies to elucidate the mechanism (Wang *et al*., 2016). According to our results, SRAP markers produced the highest percentage of polymorphism, and the lowest percentage of polymorphism were produced by SCoT markers. In addition, SRAP markers showed more reproducibility than the other two markers and produced many unique bands. Also, SCoT markers produced the highest number of unique bands in the present investigation. Moreover, SCoT markers have been used as an effective tool in genetic diversity studies in different plants by several researchers (Guo *et al*., 2012; Rathore *et al.*, 2014; Que *et al*., 2014; Zeng *et al*., 2014; Satya *et al.,* 2015; Bhawna *et al*., 2016; Agarwal *et al*., 2018; Gupta *et al*., 2019; and Kumar and Agrawal., 2019). Further, the results of the present investigation using RAPD primers agreed with other cucumber genetic diversity studies conducted by different authors, which produced a polymorphism percentage of 66.7% (Faisal *et al*., 2011) and ~77% (Onto *et al*., 2008). Also, RAPD markers were used to differentiate between susceptible and resistant cucumber varieties to DM disease (Panyanitikoon *et al*., 2018; Smiech *et al*., 2008).

The data resulted from fourteen RAPD, thirteen SCoT primers, and sixteen SRAP primer combinations were used to construct genetic similarity matrices according to Nei & Li's coefficient. Across the three types of markers, the highest genetic similarity (0.871) was revealed by SRAP, followed by 0.723% in RAPD and then 0.702 in SCoT. The lowest was 0.352, as revealed by SRAP markers. Genetic similarities between the five cucumber hybrids were used to construct dendrograms using the UPGMA method. The dendrograms generated by RAPD, SCoT, SRAP and the combined data showed that the two tolerant hybrids formed a distinct cluster. Meanwhile, the moderately tolerant and susceptible hybrids were clustered together in the dendrograms of all markers. In addition, Elnems, Barika F1 and Basimah F1 in all dendrograms are further subdivided into two sub-clusters. The first sub-cluster included Elnems (moderately tolerant). In contrast, the second

sub-cluster consisted of Barika F1 and Basimah F1 (susceptible), except for the dendrogram resulting from the RAPD analysis, which subdivided the previous hybrids into two sub-clusters. The first sub-cluster included Elnems, Barika F1, and the second sub-cluster consisted of Basimah F1. According to our results, SCoT and SRAP markers successfully differentiated the five cucumber hybrids according to their reactions to downy mildew, and RAPD partially succeeded. The results indicated by the present study showed the potential ability to use these markers for further studies to understand the mechanism behind the resistance process. These results are based on Panyanitikoon et al. (2018), who mentioned that the RAPD marker is positively associated with a plant's reaction to DM disease. Based on RAPD markers, the dendrogram clustered the cucumber germplasm into clusters A and B, which corresponded well with plant disease reaction to downy mildew. In addition, SRAPs were used to screen resistant and susceptible cucumber parents for molecular markers linked to the Javanese root-knot nematode (*Meloidogyne javanica*) resistance gene (*mj*). They identified two molecular markers flanking the *mj* gene (Devran *et al.,* 2011).

Sequence analysis of some polymorphic PCR bands

Analysis of polymorphic DNA marker sequences followed by bioinformatics analysis could provide useful insights into the possible functions of these molecular markers and their relationships to important traits (Liu *et al*., 2012). In the present investigation, two RAPD, three SCoT and thirteen SRAP combinations polymorphic bands were characterized, isolated, purified and sequenced (Macrogen Company, Korea). The sequence analysis was performed by blasting with the available National Center for Biotechnology Information (NCBI) sequences. The sequence comparison of these polymorphic fragments against sequences in the NCBI database revealed that one fragment (M1E7D1_ME1) showed homology with Photosystem I P700 chlorophyll a apoprotein A1 (PsaA) protein. This protein could be related to DM tolerance. In this regard, Harith-Fadzilah, *et al*. (2021) reported that the six proteins (PsaA, PsbB, REM, DIR19, PHGPX and CAB5) might be candidate biomarkers for developing a molecular-based system for detecting red palm weevil (RPW)-infested (*Rhynchophorus ferrugineus*) oil palm (*Elaeis guineensis*; OP) trees before symptoms manifestations. These proteins could provide useful insights into the vital processes in the host's infestation response and identify the potential biomarkers for an early detection technique. In contrast, in several studies, the QTL on chromosomes 5 and 4 was identified as having the most considerable effect on DM resistance. However, the contribution of the different identified QTL to overall DM resistance varied greatly from study to study possibly reflecting differences in inoculum strains in other parts of the world and/or differences in environmental conditions between studies (Caldwell *et al.,* 2011; Yoshioka *et al*., 2014; Li *et al*., 2018 and Wang *et al*., 2018).

In conclusion, three molecular markers RAPD, SCoT, and SRAP were used in terms of their informativeness and efficiency in studying the relationships among five cucumber hybrids for their tolerance to DM disease. Based on the data obtained from these markers, including the data obtained by the combined use of the three markers, it was highly observed that the tolerant cucumber hybrids to DM were clustered together in the same cluster. At the same time, the susceptible and moderately tolerant genotypes were placed in the other cluster. The results revealed that the two cucumber hybrids which are tolerant to DM separated in the same cluster despite their different origins. The NCBI BLAST tool was used to align some polymorphic amplicons with sequences in the NCBI database. One DNA amplicon M1E7D1_ME1 generated from the primer combination ME1-EM7 showed similarity to photosystem IP700 chlorophyll a apoprotein A1. This suggests that the DNA amplicon M1E7D1_ME1 may play a role in the DM tolerance. In addition, the results indicated that this marker could be useful for selecting individuals carrying the tolerance gene in cucumber breeding programs.

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