International Journal of GEOMATE, Dec., 2017, Vol. 13, Issue 40, pp.35-42
Special Issue on Science, Engineering & Environment, ISSN: 2186-2990, Japan
DOI: https://doi.org/10.21660/2017.40.2603

SCREENING TECHNIQUES FOR DOWNY MILDEW RESISTANCE IN GHERKIN CUCUMBERS

*Piyavadee Charoenwattana[1], Chanulak Khanobdee[2] and Artit Udomyotin[3]

[1]Faculty of Agricultural Technology, Rajamangala University of Technology Thanyaburi, Thailand;
[2,3] Agricultural Technology Research Institute**,** Rajamangala University of Technology Lanna, Thailand

*Corresponding Author, Received: 15 June 2016, Revised: 4 Aug. 2016, Accepted: 25 Nov. 2017

ABSTRACT: Downy mildew (*Pseudoperonospora cubensis*) causes significant losses in cucurbitaceous crops worldwide. *P. cubensis* isolates were obtained from infected cucumber leaves in Lampang province, Thailand during 2010-2012 in order to determine the downy mildew virulence. The isolates were tested for the presence of highly compatible reactions on specific hosts and categorized as the pathotype 3. This study determined a screening technique that could improve downy mildew resistance of gherkin cucumber lines (*Cucumis sativus* L.) grown under greenhouse and field conditions including inbred lines, gherkin varieties and hybrid parents of cucumbers. The pedigree method was applied to four generations of gherkin cucumber lines (F_2-F_5). The results showed an average mildew resistance score for 8 elite gherkin lines from the F_5 generation after 40 days of transplanting. The score of the 8 elite lines was less than an average score for other gherkin and commercial cucumber varieties and was equivalent to an average score for downy mildew resistant varieties. On average, these 8 elite lines from the F_5 generation had maximum yields as measured by a mean output of 26.9 tons per hectare (more than that of other gherkin cucumber varieties), mean fruits per plant of 32.6 fruits, and a mean weight of each fruit of 19.4 grams. It was concluded that a disease resistance breeding program using this screening technique and its application was successful in controlling and improving gherkin cucumber lines resistance to the downy mildew pathogen, and which led to increased productivity.

Keywords: Downy mildew, Pathotype, Gherkin, Screening, Resistance

1. INTRODUCTION

Gherkins and cucumbers belong to the same species (*Cucumis sativus*), but they are different cultivars [1]. Gherkins are fruits similar to cucumbers in form and nutritional value. They are usually picked when they are 4 to 8 cm (1 to 3 inches) in length and pickled in jars or cans with vinegar (often flavored with herbs, particularly dill; hence, “dill pickle”) or brine to resemble a pickled cucumber. Pickled gherkins are served together with other foods, often in sandwiches. They are associated with central European and European Jewish cuisine, but are now found more widely consumed. In Thailand, gherkins are currently produced in vinegar for exports [2]. They are commonly known as gherkins in the United Kingdom, Ireland, and Australia and as pickles in the United States and Canada. Cucumbers and gherkins can be grown in many areas, and their production can be optimized if relevant government bodies support the research and development of better seeds that combat such pests as fruit flies [1]. China has been the world's largest producer of cucumbers and gherkins, followed by Turkey and Iran and they have maintained their positions during the new millennium. According to the 2014 FAOSTAT, China produced 56.8 million tons of cucumbers and gherkins (accounting for about three-fourths of global output) while Thailand produced about 0.17 million tons of these vegetables [3].

Downy mildew [*Pseudoperonospora cubensis* (Berk.& Curt.) Rostov.] is an economically important disease in cucumber production, especially in humid regions [4]. The disease also develops in temperate as well as tropical areas with either high or low rainfalls that bring about sufficient leaf wetness. Inadequate control measures can cause major losses of cucumber, melon, squash, pumpkin, watermelon, and other cucurbits [5]. A host range of *P. cubensis* is reported to include 50 species from approximately 20 genera of the Cucurbitaceae family [6]. Pathotypes can be differentiated by observing physiological reactions on a diverse set of cucurbit genera. Six pathotypes of *P. cubensis* were analyzed based on their compatibility with specific hosts [7], [8]. The identification of downy mildew’s host specificities provided useful information for pathogen characterization [4]. Breeding programs were conducted to increase the level of resistance to downy mildew in cucumber. However, it is difficult to improve resistance to downy mildew in cucumber due to environmental variability and a narrow genetic base in cucumbers [4]. Efficient and accurate pathogenicity tests for measuring resistance is important for a successful disease resistance breeding program. To determine downy mildew resistance in cucumber, 155 cucumber

cultivars were screened using an intensity of sporulation method [9]. Plant breeders commonly use a rapid method with visual evaluation using a subjective rating scale to determine sporulation for selection of families resistant to downy mildew [10]. A set of 65 diverse cultivars were evaluated in the field using sporulation and other leaf and vine traits for resistance to downy mildew [11]. Downy mildew requires 4 to 12 - day sporulation under controlled conditions [12].

An efficient screening test was used to identify downy mildew resistance through the selection of gherkin cucumber lines under a disease resistance program known as the pedigree method. The test aims to improve the resistance of cucumber to downy mildew. All experiments were conducted at the Agricultural Technology Research Institute (ATRI), Rajamangala University of Technology Lanna, Lampang province during May 2010 – August 2012 to identify and improve resistance in gherkin cucumber lines for durable resistance and management.

2. OBJECTIVE

This study determined the pathotype of downy mildew isolates collected at ATRI, Lampang province. The screening technique was used to screen and select gherkin cucumber lines under greenhouse and field conditions to increase plant resistance to the pathogen using the pedigree method.

3. METHODOLOGY

3.1 Fungal Isolate, Plant Growth and Host Specificity Testing

Isolates of *P. cubensis* were collected from downy mildew lesions on heavily infected cucumber leaves at ATRI, Lampang province (Lampang isolate) to determine host specificities of the pathogens [7]. Inoculations were made to 15 cultivars representing 8 species within 5 genera of the family Cucurbitaceae, with 12 plants per cultivar per assay and repeated twice (Table 1). Plants were grown in a greenhouse in 9 cm^2 plastic pots using sterilized planting materials (Krassmann KTS2, Germany) composed of peat moss and vermiculite; watered twice daily and fertilized as needed. Once they reached a two-expanded-cotyledon stage, adaxial and abaxial leaf surfaces were inoculated with a suspension of *P. cubensis* (10^4 sporangia ml^{-1}) by spraying until incipient run-off. Inoculated plants were placed in darkness at 20 °C with high humidity for 21 hours. Subsequently, they were transferred to a greenhouse with a temperature of 24-28 °C and a relative humidity of 80-90%. The plants were observed daily for symptom development. Inoculated plants were evaluated for the presence of lesions and sporulation intensity. Sporulation was rated qualitatively as compatible or incompatible (Table 1). The disease reactions of the host plants were recorded on day seven after inoculation. The host plants without fungal inoculation were used as a control.

3.2 Screening Gherkin Cucumber Lines for Resistance to Downy Mildew Pathogen under Greenhouse and Field Conditions

To improve resistance to *P. cubensis* in gherkin cucumber lines, four generations of gherkin cucumber lines (F_2-F_5) were tested and screened, along with gherkin varieties, commercial varieties of cucumber and downy mildew resistant varieties using a screening technique. The pathogenicity test in this study originated from 169 gherkin cucumber lines of the F_2 generation. The disease resistance of the F_3 and F_4 generations were tested and screened in the greenhouse. The completely randomized design (CRD) was replicated twice with 12 plants per cultivar per assay. The plants were inoculated with a suspension of *P. cubensis* (Lampang isolate) as described above. Disease reactions of the plants were recorded on the 3rd, 5th and 7th day after inoculation. The host plants without fungal inoculation were used as a control.

The F_2-F_5 generations of gherkin cucumber lines were also tested and screened under field conditions. The field experiments deployed a randomized complete block design (RCB). 20 plants from each variety were transplanted in a field. During the growing season, the field was exposed to natural epidemics encouraged by a border row of susceptible varieties in each plot to help monitor and spread the inoculum by overhead irrigation. The disease reactions were recorded on the 20th, 30th and 40th day after transplanting.

All cucumber lines and varieties were rated for resistance to downy mildew after inoculation for foliar lesions with a 0-5 visual rating scale (0 – no foliar symptoms, 1 – 1-20 % symptoms, 2 – 21-40 % symptoms, 3 – 41- 60 % symptoms, 4 – 61-80 % symptoms, 5 – 81-100 % symptoms) with some modifications for greenhouse and field assessment [13].

3.3 Statistical Analysis

The quantitative data were examined using an analysis of variance (ANOVA) and means were compared at a significant probability level of $p<0.05$ using the Duncan's New Multiple Range Test (DMRT).

4. RESULTS AND DISCUSSION

Disease symptoms of *P. cubensis* (Lampang isolate) first appeared as small, slightly chlorotic to bright yellow areas on an upper leaf surface. Later, the lesions expanded; they remained chlorotic or yellow or became necrotic and brown (Fig. 1). The morphological characters of the Lampang isolate visualized under a light microscope and scanning electron microscope (Fig. 2-3) provided morphology of the pathogen.

Fig. 1 The downy mildew symptoms on cucumber leaves.

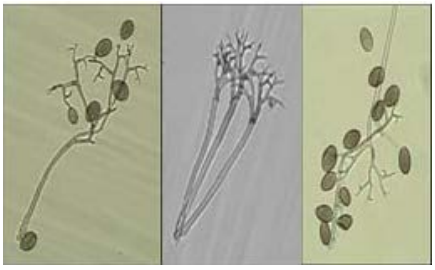

Fig. 2 Morphology of sporangiophores and sporangia of the Lampang isolate under a light microscope.

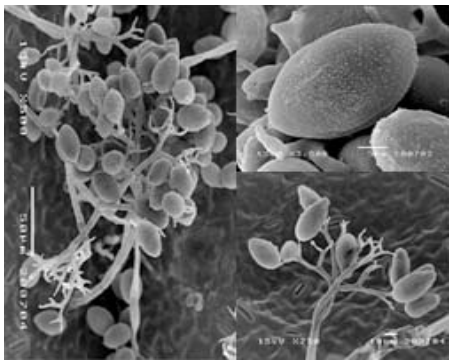

Fig. 3 Sporangiophores and sporangia of the Lampang isolate visualized by SEM.

4.1 Pathogenicity Testing

Inoculations of *P. cubensis* (Lampang isolate) to 15 cultivars representing 8 species within 5 genera of the Cucurbitaceae family showed physiological reactions of the Lampang isolate and was classified as pathotype 3. This isolate was highly compatible with *Cucumis sativus, C. melo* var. *reticulatus, C. melo* var. *conomon* and *C. melo* var. *acidulous* (Table 1 and Fig. 4**)**. A pathogenicity test showed that a host specificity of *P. cubensis* isolate could be used to identify downy mildew in cucumber. In 1987, Thomas et. al. found five pathotypes of *P. cubensis* including pathotype 1 and 2 in Japan, pathotype 3 in Israel, and pathotype 4 and 5 in the USA [7]. The pathotype 6 was reported in Israel as a new pathotype by Cohen et. al. [8]. The resistance of these hosts varied according to locations, suggesting that different pathotypes were present in various locations.

Table 1 Reactions of selected cultivars of cucurbit host species to the downy mildew pathogen.

Species	Cultivar	Reaction[1/]
Cucumis sativus	C1	+
	Malai 759	+
	Toto	+
	Ranthong	+
Cucumis melo var.*reticulatus*	Singto	+
	Chiatai	+
Cucumis melo var.*conomon*	Kamini	+
	PI420149*****	+
	PI420150*****	+
	PI532830*****	+
Cucumis melo var.*acidulus*	PI200819*****	+
Citrullus lanatus	Black watermelon	-
Cucurbita maxima	Singto	-
Lagenaria vulgaris	Advance	-
Luffa acutangula	Angle luffah	-

Note: ***** The seeds were obtained from National Plant Germplasm System, GRIN USDA.

[1/] Based on the presence of highly compatible reactions between *P. cubensis* and the most susceptible host genotype. + is highly compatible; - is incompatible, very low compatible, or lowly compatible.

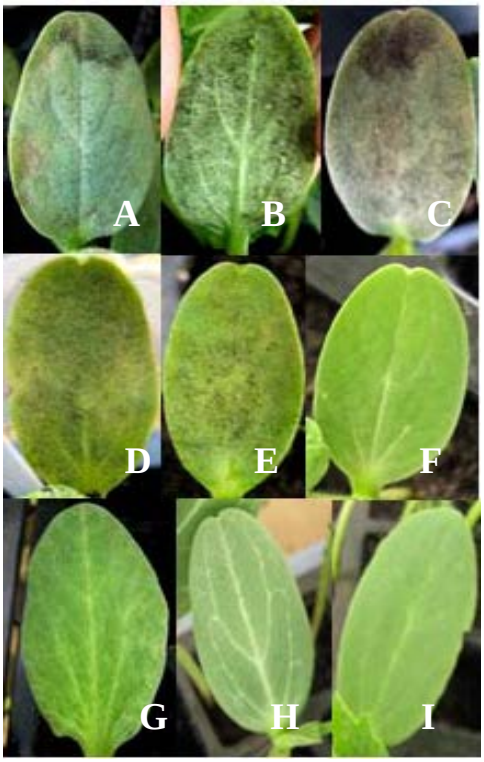

Fig. 4: Disease reactions of *P. cubensis* (the Lampang isolate) on cucurbit host species (A, B) *Cucumis sativus*; (C) *Cucumis melo* var. *reticulatus*; (D) *Cucumis melo* var. *conomon*; (E) *Cucumis melo* var. *acidulous*; (F) *Citrullus lanatus*; (G) *Cucurbita maxima*; (H) *Lagenaria vulgaris*; and (I) *Luffa acutangula* under greenhouse conditions at 7th day after inoculation.

4.2 Screening for Downy Mildew Resistance under Greenhouse and Field Conditions

169 gherkin cucumber lines from the F_2 generation were divided into 2 groups and were tested and screened, along with gherkin varieties, commercial varieties of cucumber and downy mildew resistant varieties. The pedigree selection applied to the F_2 generation in the field and yielded 37 lines of gherkin cucumbers with downy mildew resistance. 30 days after transplanting, 10 highly resistance lines were found to have an average score of 0.2 (compared to an average score of 0.3 for gherkin varieties, 0.2 for commercial varieties and 0.2 for downy mildew resistant varieties). 40 days after transplanting the 10 highly resistance lines had an average score of 0.4 (compared to 0.7 for gherkin varieties, 1.4 for commercial varieties and 0.9 for downy mildew resistant varieties) (Table 2) [14].

Table 2: Mean downy mildew resistance scores for 10 gherkin cucumber lines of the F_2 generation[1/] field tested during May-August 2010.

Line	DMR score (DAT)		
	20	30	40
Group1			
91	0.0	0.1	0.2
42	0.0	0.1	0.3
17	0.0	0.1	0.4
68	0.0	0.1	0.4
93	0.0	0.1	0.4
Group2			
1	0.0	0.1	0.3
55	0.0	0.1	0.4
40	0.0	0.3	0.5
24	0.0	0.1	0.8
56	0.0	0.8	0.9
GCL mean	0.0	0.2	0.4
GV mean	0.0	0.3	0.7
CV mean	0.0	0.2	1.4
DMRV mean	0.0	0.2	0.9

Note: [1/] According to Mendel's experiments, plants from the pure lines were named parental generation (P). The progeny generation from the cross of the parental generation (P) was named the first filial generation (F_1). The subsequent generations produced by self-pollination are symbolized F_2, F_3, F_4 and F_5, respectively [15].
DMR= Downy Mildew Resistance; DAT= days after transplanting; GCL=Gherkin Cucumber Lines; GV= Gherkin Varieties; CV=Commercial Varieties of cucumber; DMRV=Downy Mildew Resistance Varieties

Next, 332 gherkin cucumber lines of the F_3 generation were divided into 3 groups and were further tested for downy mildew resistance in the greenhouse by fungal inoculation for 7 days. The test results showed that average scores for lines and commercial varieties of cucumber were significantly different at $p < 0.01$. Average scores for lines less than 0.5 were lower than that for commercial varieties (0.7) after inoculation for 7 days. The test results showed that 56 lines (out of 110) in the first group had a downy mildew resistance score of less than or equal to 0.6, 46 lines (out of 95) in the second group had a mildew resistance score of less than or equal to 0.9 and 36 lines (out of 127) in the third group had a downy mildew resistance score of less than or equal to 0.5 after inoculation for 7 days (Table 3). 138 lines selected from these 3 groups of the F_3 generation were tested for disease resistance under field conditions. The results showed 46 lines of gherkin cucumber had downy mildew resistance, in which the 6 highly resistance lines (out of 46) had an average score of 0.7, compared to an average score of 1.1 for commercial varieties of cucumber after 30

days of transplanting. The 6 highly resistance lines had an average score of 0.8, compared to an average score of 1.8 for commercial varieties of cucumber after 40 days of transplanting (Table 4).

Table 3: Mean downy mildew resistance (DMR) scores for 3 groups of gherkin cucumber lines of the F_3 generation greenhouse tested during October-November 2010.

Line/ Variety	DMR score			No. of selected lines
	7 DPI	Min- Max	of selected lines	
Group1				
GCL mean	0.5	0.3-0.6	≤0.6	56
CV mean	0.7	0.6-0.8	-	
Group2				
GCL mean	0.8	0.5-0.9	≤0.9	46
Group3				
GCL mean	0.4	0.2-0.5	≤0.5	36
CV mean	0.7	0.6-0.7	-	

Note: DPI = days post inoculation
GCL=Gherkin Cucumber Lines; CV=Commercial Varieties of cucumber

Table 4: Mean downy mildew resistance (DMR) scores for 6 gherkin cucumber lines of the F_3 generation field tested during January-March 2011.

Line	DMR score (DAT)		
	20	30	40
71	0.6	0.9	0.6
117	1.0	0.6	0.8
72	0.6	0.7	0.8
122	1.1	0.7	0.9
100	1.4	0.9	1.0
123	0.9	0.7	1.0
GCL mean	0.9	0.7	0.8
CV mean	0.9	1.1	1.8

Note: DAT= days after transplanting
GCL=Gherkin Cucumber Lines; CV=Commercial Varieties of cucumber

The pathogenicity test of downy mildew resistance in a greenhouse for 128 gherkin cucumber lines of the F_4 generation was also carried out. These 128 lines were distinguished into two groups: 92 and 36 lines. An average score of downy mildew resistance lines, commercial varieties of cucumber and downy mildew resistance varieties in both groups were significantly different ($p < 0.01$). The test results showed that 54 lines (out of 92) in the first group had a mildew resistance score of less than or equal to 2.3 after inoculation for 7 days and that 28 lines (out of 36 in the second group) had a mildew resistance score of less than or equal to 1.5 after inoculation for 7 days (Table 5). Subsequently, downy mildew resistance of these 82 lines from the F_4 generation were tested and screened in field conditions. Average scores for lines and varieties were significantly different ($p < 0.01$). After 40 days of transplanting, 5 highly resistance lines (out of 24) had an average score of 1.0, compared to an average score of 2.6 for gherkin varieties, 2.2 for commercial varieties of cucumber, and 1.6 for downy mildew resistant varieties (Table 6). These 5 lines from the F_4 generation yielded an average output of 21.8 tons per hectare, produced 25.2 fruits per plant and weighed 19.9 grams per fruit, compared to the yields of gherkin cucumber varieties (26.8 tons per hectare, 31.8 fruits per plant and 19.6 grams per fruit (Table 7).

Table 5: Mean downy mildew resistance (DMR) scores for 3 groups of gherkin cucumber lines of the F_4 generation greenhouse tested in July 2011.

Line/ Variety	DMR score			No. of selected lines
	7 DPI	Min- Max	of selected lines	
Group1				
GCL mean	2.0	0.8-2.3	≤2.3	54
CV mean	2.3	2.0-2.6	-	-
DMRV mean	2.1	1.5-2.4	-	-
Group2				
GCL mean	1.0	0.5-1.5	≤1.5	28
CV mean	2.2	1.8-2.5	-	-
DMRV mean	1.9	1.5-2.5	-	-

Note: DPI = days post inoculation
GCL=Gherkin Cucumber Lines; CV=Commercial Varieties of cucumber; DMRV=Downy Mildew Resistance Varieties

Table 6: Mean downy mildew resistance (DMR) scores for 5 gherkin cucumber lines of the F_4 generation field tested during August-November 2011.

Line	DMR score (40 DAT)	
56	0.9	p-r [2/]
61	1.0	o-r
63	1.0	m-r
73	1.1	n-r
60	1.2	l-r
GCL mean	1.0	
GV mean	2.6	
CV mean	2.2	
DMRV mean	1.6	
F-test [1/]	**	
C.V. (%)	8.0	

Note: [1/] ** indicate significant difference at $p<0.01$, [2/] Means followed by different letters are significantly different at $p<0.05$ according to *DMRT*.

Table 7 Yield, number of fruits and weight of 5 gherkin cucumber lines for the F_4 generation field tested during August-November, 2011.

Line	Yield (t/ ha)	Number of fruits (fruits/ plant)	Weight (grams/fruit)
56	22.5 a-f [2/]	25.1 a-e	21.4 c-f
61	20.0 a-h	23.6 a-g	18.4 f-i
63	21.2 a-g	24.0 a-f	20.0 d-h
73	25.6 a-d	29.4 ab	18.5 f-i
60	20.6 a-c	23.6 a-g	21.5 c-f
GCL mean	21.8	25.2	19.9
GV mean	26.8	31.8	19.6
F-test [1/]	**	**	**
C.V. (%)	69.3	69.3	8.5

Note: [1/] *, ** indicate significant difference at $p<0.05$ and $p<0.01$, respectively.
[2/] Means followed by different letters are significantly different at $p<0.05$ according to ***DMRT.***

Lastly, the screening technique applied to the F_5 generation using pedigree selection in the field produced 8 elite lines (out of 52) of gherkin cucumbers with an average score of 1.7, compared to an average score of 2.7 for gherkin varieties, 1.9 for commercial varieties of cucumber and 1.7 for downy mildew resistant varieties after 40 days of transplanting (Table 8). These gherkin cucumber lines (P1-P8) can be used as parental lines to generate a hybrid variety and perform a combining ability test. These 8 elite lines from the F_5 generation yielded an average output of 26.9 tons per hectare, produced 32.6 fruits per plant and weighed 19.4 grams per fruit (compared to 23.1 tons per hectare, 27.5 fruits per plant and 19.8 grams per fruit for gherkin varieties) (Table 9). The results indicated that the screening technique used in the pedigree method increased downy mildew resistance and thus outputs of gherkin cucumber lines. This is consistent with the findings of Celetti et. al., that downy mildew reduced the quantity and quality of outputs by 30%- 100% [16].

This study also compared the DMR scores of the 8 elite lines from the F_5 generation to similar gherkin lines belonging to the F_2-F_4 generations. The results showed that, the 8 elite gherkin lines from the F_5 generation resisted to downy mildew (their average score was less than the average scores for gherkin varieties and commercial varieties of cucumber yet equal to downy mildew resistant varieties) and provided higher yields than that of the F_4 generation. Their average mildew resistance score was more than that of the F_4 generation; however, the yield was greater than that of the F_4 generation (Table 10).

Table 8: Mean downy mildew resistance scores for 8 gherkin cucumber lines of the F_5 generation field tested during January-March, 2012.

Line	DMR score (40 DAT)	
P1	1.2	h-j [2/]
P2	1.7	d-i
P3	1.2	h-j
P4	1.9	c-h
P5	2.0	c-h
P6	2.4	a-g
P7	1.3	g-j
P8	1.9	c-h
GCL mean	1.7	
GV mean	2.7	
CV mean	1.9	
DMRV mean	1.7	
F-test [1/]	**	
C.V. (%)	8.5	

Note: [1/] ** indicate significant difference at $p<0.01$,
[2/] Means followed by different letters are significantly different at $p<0.05$ according to *DMRT.*

Table 9: Yield, number of fruits and weight of 8 gherkin cucumber lines of the F_5 generation field tested during January-March, 2012.

Line	Yield (t/ ha)	Number of fruits (fruits/ plant)	Weight (gram/fruit)
P1	23.1 a-c [2/]	24.8 a-g	20.8 d-g
P2	26.2 a-c	30.7 a-e	17.9 e-j
P3	27.5 a-c	32.3 a-d	18.8 d-i
P4	22.5 a-d	26.7 a-g	18.8 d-i
P5	28.1 a-c	31.7 a-d	18.9 d-i
P6	37.5 a	42.8 a	20.4 d-h
P7	26.8 a-c	40.0 ab	21.2 d-g
P8	26.2 a-c	32.0 a-e	18.8 d-j
GCL mean	26.9	32.6	19.4
GV mean	23.1	27.5	19.8
F-test [1/]	*	**	**
C.V. (%)	13.3	15.3	4.5

Note: [1/] *, ** indicate significant difference at $p<0.05$ and $p<0.01$, respectively.
[2/] Means followed by different letters are significantly different at $p<0.05$ according to *DMRT.*

Table 10: Mean downy mildew resistance scores for 8 elite gherkin cucumber lines from four generations (F_2-F_5) field tested.

Line	DMR score (40 DAT)			
	F_2	F_3	F_4	F_5
P1	1.4	1.6	2.2	1.2
P2	2.5	1.5	1.2	1.7
P3	2.5	1.5	1.0	1.2
P4	2.5	1.5	1.2	1.9
P5	2.5	1.5	1.2	2.0
P6	2.5	1.5	1.2	2.4
P7	2.5	1.5	1.2	1.3
P8	2.5	1.5	1.2	1.9
GCL mean	2.4	1.5	1.3	1.7
GV mean	0.7	3.7	2.6	2.7
CV mean	1.4	1.8	2.2	1.9
DMRV mean	0.9	0.6	1.6	1.7

5. CONCLUSION

The cucumber downy mildew (*P. cubensis* - Lampang isolate) was identified as pathotype 3 for the presence of highly compatible reactions on four specific cucurbit host species. Eight elite gherkin cucumber lines of the F_5 generation were obtained from the screening technique used in the pedigree method where there was an average disease resistance score of less than or equal to 2.4 after 40 days of transplanting. These 8 elite lines yielded an average output of 26.9 tons/ hectare, which was more than the yield from gherkin cucumber varieties. The number of fruits and the weight obtained from 8 gherkin cucumber lines of the F_5 generation were 32.6 fruits per plant and 19.4 grams per fruit. The average mildew resistance score for the 8 elite gherkin lines from the F_5 generation after 40 days of transplanting was less than the average score of gherkin varieties and commercial varieties of cucumber and equal to downy mildew resistant varieties. Thus, the screening technique for downy mildew resistance under greenhouse and field conditions constitutes an efficient technique for developing gherkin cucumber lines with high resistance to downy mildew.

6. ACKNOWLEDGEMENTS

This study was financed by a research grant from National Center for Genetic Engineering and Biotechnology, Ministry of Science and Technology and Agro-on (Thailand) Co. Ltd. Their assistance and cooperation are gratefully acknowledged.

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