doi: 10.17221/12/2015-CJGPB

Evaluation of Selected *Cucumis sativus* Accessions for Resistance to *Pseudoperonospora cubensis* in Egypt

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Abstract

Metwally E.I., Rakha M.T. (2015): Evaluation of selected *Cucumis sativus* accessions for resistance to *Pseudoperonospora cubensis* in Egypt. Czech J. Genet. Plant Breed., 51: 68–74.

Downy mildew [*Pseudoperonospora cubensis* (Berk. & Curt.) Rostov] is a major destructive disease of cucumber *(Cucumis sativus* L.) worldwide. Resistant cultivars were recently introduced into Egypt, but yield losses were high if no fungicides were used. The objective of this study was to identify sources of resistance to downy mildew among Plant Introduction (PI) cucumber accessions from the U.S. National Plant Germplasm System. We evaluated 133 such accessions for downy mildew resistance under natural field epidemics during the summer 2013 at El-Beheira Governorate, Egypt. Mean ratings for downy mildew leaf damage ranged from 1 to 9 on a 0 to 9 scale. We classified 18 PI accessions (13.5%) as highly resistant (rating of 1.0–2.0), 46 (34.5%) as moderately resistant (rating 2.1–4.0), 40 (30%) as intermediate (rating 4.1–6.0), 12 (9%) as moderately susceptible (rating 6.1–7), and 17 (13%) as highly susceptible (rating > 7.1). The most resistant PI accessions were PI 432870, PI 432873, PI 432878, PI 432884, and PI 432886 with a rating of 1, which originated from China. The most susceptible PI accessions were Ames7736, PI 211979, PI 288991, PI 288992 and PI 289698 with a rating of 9. The five most resistant and five most susceptible accessions were further evaluated in replicated experiments during the summer 2014. Results from the repeated test confirmed the results from the first screening. No PI accession was found immune to downy mildew. However, high levels of resistance were observed in several PI accessions that could be useful for the breeding for resistance to *P. cubensis* in cucumber.

Keywords: cucumber; cucurbit downy mildew, field resistance; germplasm

Cucumber (*Cucumis sativus* L.) is the fourth most important vegetable crop worldwide, with global production of 65.1 million tons in 2012 [United Nations Food and Agriculture Organization (FAO) statistics; http://faostat.fao.org/] and a net value of over US $12 billion. Downy mildew, a foliar disease caused by the oomycete *Pseudoperonospora cubensis* (Berk. and Curt.) Rostow., is by far the most devastating disease of cucumber and cause losses up to 70–100% in Egypt (El-Hafaz *et al.* 1990). The pathogen generally thrives in warm humid regions. The current control relies mainly on multiple fungicide applications that exert selection pressure on the fungus, increasing the risk of the development of fungicide resistance in the pathogen population (Holmes *et al.* 2006). Moreover, frequent use of fungicides can be harmful to the environment and detrimental to natural enemies (Kookana *et al.* 1998; Kibria *et al.* 2010; Komarek *et al.* 2010). Therefore, the use of resistant cultivars could provide farmers with economic and environmentally sound management strategies for downy mildew control.

While studies on wild *Cucumis* accessions have not resulted in the identification of new downy mildew resistance sources (Lebeda 1999), numerous studies have identified several resistant cucumber cultigens from different geographical regions. Perhaps the most comprehensive study was conducted over multiple years by Wehner and Shetty (1997) in which the whole U.S. germplasm cucumber collection was tested

doi: 10.17221/12/2015-CJGPB

for resistance to downy mildew in North Carolina. The most-resistant cultigens were Gy 4, Clinton, Plant Introduction (PI) 234517, Poinsett 76, Gy 5, Addis, M 21, M 27, and Galaxy. Another large downy mildew resistance screening trial of 1300 cultigens evaluated for 4 years in Poland led to the identification of only six cultigens (PI 330628, PI 197088, PI 197086, PI 197085, Ames 2353 and Ames 2354) that demonstrated high levels of downy mildew resistance (Klosinska *et al*. 2010; Call *et al*. 2012).

Several downy mildew resistant cucumber cultivars were recently introduced into Egypt from the USA, England, Denmark, and the Netherlands but most were highly susceptible. Besides the environmental conditions that play a fundamental role in determining disease intensity (Cohen 1977; Lebeda & Cohen 2011), shifts in pathogen populations can have a great impact on the effectiveness of downy mildew resistance. Therefore, evaluating resistance to downy mildew under diverse environmental conditions and with potentially different pathogen populations enhances our understanding of those sources offering broad and stable resistance, which improves the chances of developing cultivars with durable resistance. To our knowledge, there are no reports on the evaluation of large numbers of introduced *C. sativus* germplasm for downy mildew resistance in Egypt. The objective of this study was to evaluate a large number of diverse cucumber PIs for field resistance to downy mildew to identify potential sources of resistance.

MATERIAL AND METHODS

The study was conducted in two stages. Stage 1 screening included all 133 accessions and a susceptible check. Stage 2 screening included selected accessions from Stage 1 screening classified as "highly resistant" or "highly susceptible" based on symptom ratings described below. Stage 1 and Stage 2 trials were conducted at the Elkananah farm in El-Beheira Governorate, Egypt.

Stage 1 screening trial. Seeds of 133 *C. sativus* PIs were obtained from the USDA, ARS North Central Regional Plant Introduction Station (NCRPIS) in Ames, Iowa (Table 1) and included accessions from 19 countries. Most accessions were selected based on previous studies reporting moderate to high downy mildew resistance (Wehner & Shetty 1997; Call *et al*. 2012). Additional information on *C. sativus* PIs used in this study is available at http://www.ars-grin.gov/npgs/searchgrin.html.

Seeds of PIs and Beta alpha, a downy mildew susceptible cultivar grown in Egypt, were sown on 1 April 2012 in 96-cell seedling trays with 50 ml of peat moss-vermiculite mixture (1:1 volume) (Al Kalthoum Agricultural Co., El Mansoura, Egypt) per seedling and fertilized weekly. After three weeks, seedlings (two true leaves) were transplanted to the field and arranged in a randomized complete block design with four replications of eight plants per accession spaced 1.5 × 1 m between and within rows, respectively. The trial was established during a period of high natural downy mildew epidemics. The field trial was surrounded by 35 border rows of the susceptible cultivar Beta alpha and every fifth row in the trial was planted with the susceptible check as spreader rows. Cv. Beta alpha is highly susceptible to downy mildew and the infected leaves produce fungal lesions which sporulate heavily and provide a secondary inoculum. PI accessions, border and spreader rows were transplanted on the same day. Plants were grown under drip irrigation and additional weekly surface irrigation of the trial was carried out to promote the uniform disease development. The fertilizer was applied at a rate of 40N-17P-30K kg/fed (N-P-K) with additional 18 kg N/fed applied at the vine-tip-over stage (four to six true leaves). Horticultural practices were performed according to the recommendations of the Egyptian Ministry of Agriculture.

Susceptible plants usually showed significant downy mildew symptoms within about four weeks after planting. Plots were rated for symptoms five weeks after transplanting on a 0 to 9 scale based on the percentage of symptomatic leaf area (0 = 0%, 1 =1–5%, 2 = 6–10%, 3 = 11–20%, 4 = 21–30%, 5 = 31–50%, 6 = 51–65%, 7 = 66–80%, 8 = 81–99%, and 9 = 100%). The classification of accessions into resistant and susceptible groups is somewhat subjective but in keeping with previous studies (Wehner & Shetty 1997; Call *et al*. 2012) accessions were grouped by mean downy mildew rating as follows: ≤ 2.0 (highly resistant); 2.1 to 4.0 (moderately resistant); 4.1 to 6.0 (intermediate); 6.1 to 7.0 (moderately susceptible); 7.1 to 9.0 (highly susceptible).

Stage 2 screening trial. Based on results of the Stage 1 trial, the most resistant accessions were PI 432870, PI 432873, PI 432878, PI 432884, and PI 432886 with a mean rating of 1.0. The most susceptible PIs were Ames7736, PI 211979, PI 288991, PI 288992 and PI 289698 with a mean rating of 9.0. These accessions along with Beta alpha were re-evaluated under natural field epidemics at the El-

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Table 1. Mean downy mildew ratings for 133 Plant Introductions (PIs) of *Cucumis sativus* L. tested in the field in Egypt in the Stage 1 screening trial, April, 2013

C. sativus PI[Z]	Origin	Rating (1–9)[Y]	*C. sativus* PI	Origin	Rating (1–9)
PI 432870	China	1.0	PI 489752	China	3.3
PI 432873	China	1.0	PI 114339	Japan	4.0
PI 432878*	China	1.0	PI 163217	Pakistan	4.0
PI 432884	China	1.0	PI 255938	Netherlands	4.0
PI 432886*	China	1.0	PI 279467	Japan	4.0
PI 432874	China	1.5	PI 288238	Japan	4.0
PI 432877	China	1.5	PI 306180	Russian Federation	4.0
PI 618893	China	1.5	PI 321009*	Taiwan	4.0
PI 257487	China	2.0	PI 360939	Netherlands	4.0
PI 390241	Japan	2.0	PI 372893	Netherlands	4.0
PI 390247	Japan	2.0	PI 390240	Japan	4.0
PI 390255	Japan	2.0	PI 390266	Japan	4.0
PI 418962	China	2.0	PI 390268	Japan	4.0
PI 419214	Hong Kong	2.0	PI 400270	Japan	4.0
PI 432875*	China	2.0	PI 418963	China	4.0
PI 432876	China	2.0	PI 418964	China	4.0
PI 432889	China	2.0	PI 422182	Netherlands	4.0
PI 432891	China	2.0	PI 432851	China	4.0
PI 209069	USA	3.0	PI 432852	Japan	4.0
PI 227207	Japan	3.0	PI 432860	China	4.0
PI 227209	Japan	3.0	PI 432867	China	4.0
PI 249562	Thailand	3.0	PI 432872	China	4.0
PI 321011	Taiwan	3.0	PI 432893	China	4.0
PI 330628*	Pakistan	3.0	PI 432894	China	4.0
PI 358813	Malaysia	3.0	PI 432895	China	4.0
PI 358814	Malaysia	3.0	PI 483342	China	4.0
PI 390250	Japan	3.0	PI 163213	Pakistan	5.0
PI 419009	China	3.0	PI 164679	India	5.0
PI 419017	China	3.0	PI 178886	Turkey	5.0
PI 432853	China	3.0	PI 197085*	India	5.0
PI 432854	China	3.0	PI 271327	India	5.0
PI 432859	China	3.0	PI 279466	Japan	5.0
PI 432865	China	3.2	PI 288990	Hungary	5.0
PI 432868	China	3.2	PI 288996	Hungary	5.0
PI 432869	China	3.2	PI 321008	Taiwan	5.0
PI 432890	China	3.2	PI 390243	Japan	5.0
PI 436648	China	3.2	PI 390244	Japan	5.0
PI 436672	China	3.3	PI 390257	Japan	5.0
PI 390259	Japan	5.1	PI 113334	China	7.0
PI 390260	Japan	5.1	PI 173893	India	7.0
PI 419010	China	5.1	PI 209067	USA	7.0
PI 426170	Philippines	5.1	PI 220860	South Korea	7.0
PI 432855	China	5.2	PI 234517	USA	7.0

doi: 10.17221/12/2015-CJGPB

Table 1 to be continued

C. sativus PI[Z]	Origin	Rating (1–9)[Y]	*C. sativus* PI	Origin	Rating (1–9)
PI 432871	China	5.2	PI 279468	Japan	7.0
PI 436609	China	5.2	PI 288993	Hungary	7.0
PI 451976	Japan	5.2	PI 288994	Hungary	7.0
PI 464873	China	5.5	PI 321010	Taiwan	7.0
PI 478366	China	5.5	PI 489754	China	7.0
PI 483339	South Korea	5.5	PI 508457	South Korea	7.0
PI 483340	South Korea	5.5	Ames 2353*	USA	8.0
PI 483343	South Korea	5.5	Ames 2354*	USA	8.0
PI 508459	South Korea	5.5	Ames 7735	USA	8.0
PI 605924*	India	5.5	PI 103049	China	8.0
PI 605996*	India	5.5	PI 167223	Turkey	8.0
PI 618936	China	5.5	PI 169386	Turkey	8.0
PI 618937*	China	5.5	PI 175688	Turkey	8.0
PI 175121	India	6.0	PI 209064	USA	8.0
PI 197086*	India	6.0	PI 292010	Israel	8.0
PI 197088*	India	6.0	PI 292012	Israel	8.0
PI 227013	Iran	6.0	PI 357857	former Serbia and Montenegro	8.0
P I267942	Japan	6.0	PI 466922	Russian Federation	8.0
PI 271326	India	6.0	Ames 7736	USA	9.0
PI 288332	India	6.0	PI 211979	Iran	9.0
PI 288995	Hungary	6.0	PI 288991	Hungary	9.0
PI 372900	Netherlands	6.0	PI 288992	Hungary	9.0
PI 426169	Philippines	6.0	PI 289698	Australia	9.0
PI 105340	China	7.0			

[Z]All seeds of *C. sativus* L. accessions tested in this study were obtained from the USDA, ARS North Central Regional Plant Introduction Station (NCRPIS) in Ames, USA; additional information on these PI can be obtained from the Germplasm Resources Information Network online database at http://www.ars.usda.gov; the country column indicates from where the germplasm was collected; [Y]ratings assessed visually five weeks after planting, and are based on the percentage of leaf area affected using a 0 to 9 scale (0 – no damage, 9 – dead plant); *highly resistant accessions tested by Call *et al.* (2012)

kananah farm in El-Beheira Governorate, Egypt (Table 2). Seeds of selected accessions along with the susceptible check were sown on 1 April 2014 and transplanted to the field in plots arranged according to a randomized complete block design with four blocks. Plots included seven plants. Management practices and downy mildew severity ratings were carried out as described above in the Stage 1 screening. Rating scale data were analysed by the Kruskal-Wallis test using the PROCNPAR1WAY procedure of SAS (SAS Institute 2011) and the accessions were ranked using Wilcoxon ranking scores. Correlation coefficients were calculated to measure the association between the first and the second stage screening ratings using PROC CORR in SAS.

RESULTS

Stage 1 trial. All susceptible check plants (Beta alpha cv.) showed severe disease symptoms with disease severity rating of 8 or 9. Mean ratings for downy mildew leaf symptom severity at five weeks after planting in the germplasm screening ranged from 1.0 to 9.0 on a 0 to 9 scale (Table 1). None of *C. sativus* PIs showed immunity to downy mildew even though some accessions demonstrated high levels of resistance (Table 1). Of the 133 PIs evaluated in 2012, 64 (48.1%) were considered resistant: 18 (13.5%) and 46 (34.5%) accessions were highly resistant and moderately resistant, respectively (Table 1). The predominance of moderately resistant PIs

doi: 10.17221/12/2015-CJGPB

was confirmed by the skewness and kurtosis, which were 0.277 and 0.596, respectively. The relatively high proportion of highly resistant and moderately resistant accessions is explained by the choice of accessions for this study that was based on reports from previous downy mildew screening trials in Poland and in the USA. The five most resistant accessions were PI 432870, PI 432873, PI 432878, PI 432884, and PI 432886 with a mean disease severity rating of 1. Conversely, the five most susceptible PIs were Ames7736, PI 211979, PI 288991, PI 288992 and PI 289698 with a mean disease severity rating of 9.

Stage 2 trial. We tested the five most resistant PIs along with the five most susceptible PIs to confirm the reactions of the highly resistant accessions identified in the first screening (Table 2) to determine whether individual plants within accessions with even higher resistance could be selected. The five highly resistant PIs tested in the Stage 2 trial (mean rating of 1.3) showed slightly increased mean downy mildew ratings compared to their rating in the Stage 1 trial (mean rating = 1.0) (Table 2). It is possible that the inclusion of the five highly susceptible accessions in the trial increased downy mildew disease pressure. A highly significant positive correlation (r = 0.90, $P \leq 0.0001$) between the ratings of 10 PIs in the first and second tests was found. In addition, the mean Wilcoxon rank scores for each PI and the mean ratings on the 1 to 9 scale are presented in Table 2 for the retest with the selected PIs. The x^2 for the Kruskal-Wallis test was 26.93 ($Pr > x^2$ = 0.0072) indicating a significant difference (P = 0.05) between PIs.

DISCUSSION

Downy mildew is a major foliar disease of cucumber (*C. sativus* L.) in the open field and greenhouses in Egypt. There are currently no cultivars that show resistance to downy mildew in Egypt. In this study large differences between 133 *C. sativus* accessions in disease ratings were observed and highly resistant accessions were identified. Our findings were similar to those reported from Poland and North Carolina from 2005 to 2009 by Call *et al.* (2012), who found that only twenty of 1300 tested cucumber cultigens were highly resistant to downy mildew and none was immune.

The five most resistant PIs in this study (PI 432870, PI 432873, PI 432878, PI 432884, and PI 432886) consistently showed a mean rating of 1.0 in both

Table 2. Evaluation of selected *Cucumis sativus* Plant Introductions (PIs) tested for downy mildew resistance in Egypt in the Stage 2 field screening trial, April, 2014

C. sativus PI[Z]	Origin	First test	Second test on selected PIs	
		rating (1–9)[Y]	Wilcoxon rank score[X]	rating (1–9)[W]
PI 432870	China	1	6	1
PI 432873	China	1	11	1.5
PI4 32878	China	1	6	1
PI 432884	China	1	8	2
PI 432886	China	1	8	1
Ames 7736	USA	9	21	8
PI 211979	Iran	9	25	8.5
PI 288991	Hungary	9	25	8
PI 288992	Hungary	9	25	9
PI 289698	Australia	9	22	8

[Z]All the seeds of *C. sativus* L. tested in this study were obtained from the USDA, ARS North Central Regional Plant Introduction Station (NCRPIS) in Ames, USA; additional information on these PI can be obtained from the Germplasm Resources Information Network online database at http://www.ars.usda.gov; the country column indicates from where the germplasm was collected; [Y]the ratings for the selected PIs are based on the first screen using a 1 to 9 scale (0 – no damage, 9 – dead plant); [X]the data were analysed using the PROC NPAR1WAY analysis of SAS and the mean Wilcoxon rank scores for each PI are presented; the x^2 for the Kruskal-Wallis test was 26.93 ($Pr > x^2$ = 0.0072) indicating a significant difference (P = 0.05) between PIs; [W]the ratings for the selected PIs are based on the second screen using a 1 to 9 scale (0 – no damage, 9 – dead plant)

doi: 10.17221/12/2015-CJGPB

years. These accessions were also found to be highly resistant in North Carolina and Poland by Call *et al*. (2012). These five PIs originated from China and numerous studies have identified high levels of downy mildew resistance in *C. sativus* PIs originating from India, China, and Japan (Pitrat *et al*. 1989; Staub *et al*. 1989; Call *et al*. 2012). Intriguingly, some PIs that were resistant in other studies were observed to be intermediate or susceptible in this study. The most resistant PIs identified by Call *et al*. (2012) including PI 605924, PI 605996, PI 618937, PI 197086, and PI 197088 showed intermediate resistance in our study with mean ratings of 5 or 6. Ames 2353, Ames 2354 and PI 234517 were major sources of downy mildew resistance in the United States (Call *et al*. 2012), but were susceptible in this study with mean ratings of 7 or 8. Furthermore, PI 234517, which was determined to be highly resistant by Wehner and Shetty (1997), was susceptible in our test. Therefore, we recommend that cucumber cultivars developed in the U.S. be tested for resistance to *P. cubensis* using the Egyptian race(s) of the pathogen before release.

Although we did not study the pathotypes of *P. cubensis*, our results may indicate the presence of different races of *P. cubensis* in Egypt compared to the U.S. Shetty *et al*. (2002) proposed the existence of at least two races of downy mildew: a race in the People's Republic of China and India, which is different from a distinct a race present in the United States and Poland. Besides pathogen race differences, environmental conditions such as leaf wetness and temperature play a fundamental role in cucumber response to downy mildew (Call *et al*. 2012). It is also possible that a shift in the pathogen population has changed the resistance ranking of the PIs.

PI 432870 was highly resistant to downy mildew in this study and was also reported to be highly resistant to powdery mildew (caused by *Sphaerotheca fuliginea*) in a screening done in the USA by Block and Reitsma (2005). Although cucumber has a narrow genetic base (i.e. low diversity), downy mildew resistance has been identified in the USA. PIs germplasm collection in recent decades (Staub *et al*. 1989; Wehner & Shetty 1997; Klosinska *et al*. 2010; Call *et al*. 2012). In this study PIs with higher levels of resistance have been identified in several accessions. Breeders should utilize these PIs in their programs to develop cultivars with high resistance in Egypt.

Acknowledgements. The authors wish to thank Dr. P. Hanson and M. Mecozzi, AVRDC – The World Vegetable Center and Dr. A. Amiri, University of Florida, for manuscript reviewing and improvement.

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Received for publication January 21, 2015
Accepted after corrections April 22, 2015

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