RESEARCH

Heritability and Genetic Variance Estimates for Resistance to Downy Mildew in Cucumber Accession Ames 2354

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ABSTRACT

Downy mildew (DM) of cucumber (*Cucumis sativus* L.), caused by *Pseudoperonospora cubensis*, is a devastating disease of cucurbits. Resistance is available but is not sufficient to eliminate the need for fungicides to control the disease. Previously, the USDA Plant Introduction collection of cucumber germplasm was screened in Poland and North Carolina, and Ames 2354 was identified as highly resistant. The objectives of this study were to develop an effective method for measuring resistance to DM and to determine the type of gene action controlling resistance in Ames 2354. Plant Introduction 175695 was used as the susceptible parent in crosses with Ames 2354 to make seven generations for study: P_1, P_2, F_1, F_1 reciprocal, F_2, BC_1P_1, and BC_1P_2. Additive effects were more important than dominant effects in our study with resistance attributed to a few major loci. Greenhouse testing methods were identified that can be used in selecting for improved resistance. Selection using the best test methods could lead to a gain of at least 2.5 points (on a 10 point scale) per generation even under the lower selection intensities (i.e., 20%) typically used in recurrent selection programs.

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Abbreviations: DM, downy mildew; DSI, disease severity index.

Downy mildew (DM) of cucumber, caused by the oomycete *Pseudoperonospora cubensis*, is a devastating, worldwide disease of cucurbit crops both in field and protected culture. *Pseudoperonospora cubensis* outbreaks over the past several decades have been responsible for annual yield losses of up to 95% in the United States (Colucci et al., 2006). In Central Europe, it has been a serious problem since 1984 (Lebeda, 1991), and in Poland, the pathogen was first reported in 1985. Since that time, it continues to occur every year in cucumber growing areas and has the potential to cause severe damage to the foliage with associated yield losses. Through intensive breeding, several resistant cucumber hybrids have been developed in Poland since 1990 (Klosinska et al., 2010) but none with high enough resistance to eliminate the need for fungicides to control the disease.

Previously, the available USDA Plant Introduction collection of cucumber germplasm was studied to identify new accessions having a higher level of resistance under natural field epidemics in Poland and North Carolina (Klosinska et al., 2010; Call et al., 2012). Among about 1300 cucumber cultigens tested in Poland, six of them, PI 330628, PI 197088, PI 197086, PI 197085, Ames 2353, and Ames 2354, showed the highest level of resistance to DM over 4 yr of study. These six lines were more resistant than the currently available resistant Polish F_1 hybrids Rodos and Aladyn and U.S. cultivars Poinsett 76 and Slice. Interestingly, data for all cultigens from Poland showed a greater range of mean DM ratings compared

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with data from North Carolina (0.3 to 9.0 compared with 1.0 to 7.3, respectively) probably indicating that European populations of *P. cubensis* are highly variable and may have many pathotypes (Lebeda and Urban, 2004).

In previous research, different inheritance patterns for resistance to DM were observed: three recessive genes (Doruchowski and Lakowska-Ryk, 1992; Shimizu et al., 1963), three partially dominant genes (Pershin et al., 1988), an interaction between dominant susceptible and recessive resistance genes (Badr and Mohamed, 1998; El-Hafaz et al., 1990), one or two incompletely dominant genes (Petrov et al., 2000), and a single recessive gene (Angelov, 1994; Fanourakis and Simon, 1987; Van Vliet and Meysing, 1974, 1977). Different results in these studies were likely due to multiple factors including different pathogen populations, different resistant lines used, the resistance trait evaluated (chlorosis vs. necrosis vs. sporulation etc.), and different environmental conditions.

Several methods of estimating heritability and predicting selection response are available. Primarily, these methods partition the total variance into genetic and environmental variances and the genetic variance into additive and dominance components and interallelic interaction effects whenever the population structure and composition allows (Holland et al., 2003; Nyquist, 1991). Among others, a design based on the measure of variance from six generations (P_1, P_2, F_1, F_2, BC_1P_1, and BC_1P_2) can be used to estimate environmental, genetic, and additive variances. The variance of the F_2 provides an estimate of phenotypic variance whereas the mean variance of the nonsegregating generations (P_1, P_2, and F_1) gives an estimate of environmental effects (Wright, 1968). The additive variance is derived by subtracting the variances of the backcrosses from twice the phenotypic (F_2) variance as an extension of the single locus model under the hypothesis of absence of linkage and genetic × environment interactions (Warner, 1952). The broad- and narrow-sense heritability and the predicted gain from selection can then be calculated from the available estimates of genetic, additive, and phenotypic variances.

The objectives of this study were (i) to develop an effective method for measuring resistance to DM as means for detecting differential responses between DM resistant and susceptible seedlings and (ii) to determine the type of gene action controlling resistance *P. cubensis* in the resistant cultigen Ames 2354.

MATERIALS AND METHODS

Plant Material

The parental genotypes used in this study were resistant Ames 2354 and susceptible PI 175695, which were chosen on the basis of their reaction to DM in our previous studies (Klosinska et al., 2010; Call et al., 2012). Ames 2354 is a tolerant selection from PI 234517 (Carroll Barnes breeding line SC 50) made in 1982 by Gregory Tolla from a field infested with Verticillium wilt. Plant Introduction 175695 is a plant introduction accession identified as susceptible in a screening by Klosinska et al. (2010). The plant material studied consisted of F_1, reciprocal F_1, F_2, and backcross populations from the cross between Ames 2354 and PI 175695. All crosses were made by hand pollination in a greenhouse of The Research Institute of Horticulture, Skierniewice, Poland.

Experiment Conditions and Disease Evaluation

Resistance screening tests were conducted under controlled environment conditions in the growth chambers and in a greenhouse. Seeds were sown in plastic pots (10 cm diameter) filled with a peat substrate Kronen-Klasmann. Seeds were pregerminated on petri dishes followed by planting in pots (one seed per one pot). Seedlings were grown at 26/22°C (day/night) and 12 h of light.

Cucumber leaves heavily infected with *P. cubensis* were collected from experimental fields in Skierniewice, Poland, that had not been sprayed with fungicides. In the laboratory infected leaves were soaked in distilled water and rubbed gently with a glass rod to dislodge sporangia. The concentration of sporangia suspension was determined with the use of hemocytometer and adjusted to a final concentration of 5×10^4 sporangia mL^{-1}. Plants were inoculated at the first or two- to three-leaf stage depending on a test by misting the adaxial side of leaves with the sporangial solution until runoff using a hand-sprayer bottle (1 L size). The inoculated seedlings were incubated in a dark growth chamber for 48 h at 20°C and 100% relative humidity. After incubation plants in methods III and V were then removed from the moist chamber and placed on the greenhouse bench, where temperatures ranged from 25 to 30°C. Comparatively, seedlings in remaining methods (I, II, and IV) were grown at 24°C (day/night) with 12 h of light in growth chambers during a whole test. The specific formulas used in each method are described in Table 1.

Disease Ratings and Data Analyses

Disease ratings were made 8 to 20 d after inoculation using a scale of 0 to 9 (0 indicating no disease, 1–2 indicating trace, 3–4 indicating slight, 5–6 indicating moderate, 7–8 indicating severe, and 9 indicating dead) (Jenkins and Wehner, 1983). Ratings are based on percentage of infected leaf area, from which a disease severity index (DSI) was calculated.

We tested the validity of the inheritance of a single Mendelian gene after classifying each plant as susceptible or resistant based on their rank relative to the mean value of the disease assessment scale adopted (4.5). A mean value of 3.5 was also tested, since the resistant parent was rated below 3.5 in all but three experimental units (145 total). We performed segregation analysis and goodness-of-fit tests with the SAS-STAT statistical package (SAS Institute, 2004) and the SASGene 1.2 program (Liu et al., 1997). All χ^2 tests were performed at the 95% confidence level. Since there was strong evidence against the single gene hypothesis, we verified the distribution of the F_2 data for each family using the UNIVARIATE procedure of SAS-STAT and by plotting the disease ratings against their frequency before analyzing resistance to DM as a quantitative trait.

We tested the F_2 data for homogeneity of variances using the Bartlett's method (Ostle and Malone, 1988; Steel et al., 1997). Variances were not homogeneous among tests (F_2 Bartlett's $\chi^2 = 26.43$; *P*-value = <0.0001) so data is presented separately for each test.

Phenotypic (P), environmental (E), genotypic (G), and additive (A) variances were estimated from generation variances as follows (Warner, 1952; Wright, 1968):

Table 1. Measurement of downy mildew resistance on cucumber leaves using five different testing methods.

Method	Agenda†	Inoculation stage‡	Evaluation	
			Plant stage§	Days after inoculation
I	Growth chamber Inoculation on 17 Oct. 2011 Frozen sporangia for 1 mo.	First leaf	4–6 leaves¶	16 to 18
II	Growth chamber Inoculation on 17 Oct. 2011 Frozen sporangia for 1 mo.	2 to 3 leaves	6–10 leaves#	16 to 18
III	Greenhouse chamber Inoculation on 17 Aug. 2011 Fresh sporangia	2 leaves	6–7 leaves††	13
IV	Growth chamber Inoculation on 5 Nov. 2011 Frozen sporangia for 2–3 mo.	First leaf	5–6 leaves‡‡	18 to 20
V	Greenhouse chamber Inoculation on 13 Sept. 2009 Mixed fresh and frozen (1 mo.) sporangia	First leaf	3–4 leaves‡‡	8

†Infection cycle location (growth vs. greenhouse chamber), date of inoculation, and fresh vs. frozen inoculum.

‡Stage of plant at inoculation.

§Stage of plant at evaluation.

¶Evaluation was performed on whole plants (ignoring uppermost leaves) being at the time of evaluation at four- to six-leaf stage.

#Evaluation was performed on whole plants (ignoring uppermost leaves) being at the time of evaluation at 6- to 10-leaf stage.

††Mean of three leaves evaluated separately (second leaf and third leaf, eliminating readings of the first senescence leaf).

‡‡Evaluation was performed on the first leaf only, being inoculated.

$$\sigma^2(P) = \sigma^2F_2$$

$$\sigma^2(E) = \sigma^2P_1 + \sigma^2P_2 + (2 \times \sigma^2F_1)/4$$

$$\sigma^2(G) = \sigma^2(P) - \sigma^2(E)$$

$$\sigma^2(A) = (2 \times \sigma^2F_2) - (\sigma^2BC_1P_1 + \sigma^2BC_1P_2).$$

Heritability was estimated using the ratio of genotypic or additive variances to phenotypic variance. A large variation associated with variance component estimates is intrinsic in all quantitative genetic studies. With the experiment design used in our study, negative estimates for genetic variances and heritability estimates outside the expected range of 0 to 1 are possible. Negative estimates should be considered equal to zero (Robinson et al., 1955) but should be reported as actual values that can be used by future studies for unbiased estimation (Dudley and Moll, 1969).

The number of effective factors, an estimate of the genetic factors determining a quantitative trait (Mendelian genes or quantitative trait loci), was estimated using the following methods (Lande, 1981; Wright, 1968):

Lande's method I: $(P_1 - P_2)^2/8(\sigma^2F_2 - \sigma^2F_1)$

Lande's method II: $(P_1 - P_2)^2/8[\sigma^2F_2 - (1/2\sigma^2F_1 + 1/4\sigma^2P_1 + 1/4\sigma^2P_2)]$

Lande's method III: $(P_1 - P_2)^2/8(2\sigma^2F_2 - \sigma^2BC_1P_1 - \sigma^2BC_1P_2)$

Lande's method IV: $(P_1 - P_2)^2/8[\sigma^2BC_1P_1 + \sigma^2BC_1P_2 - (\sigma^2F_1 + 1/2\ \sigma^2P_1 + 1/2\sigma^2P_2)]$

Wright's method: $(P_1 - P_2)^2 \times \{1.5 - [2 \times (F_1 - P_1)/(P_2 - P_1) \times (1 - (F_1 - P_1)/(P_2 - P_1))]\} /\{8 \times [\sigma^2F_2 - (\sigma^2P_1 + \sigma^2P_2 + (2 \times \sigma^2F_1))/4]\}$

The assumptions for the estimates of number of effective factors were as follows: (i) with respect to all relevant loci, one parent is fixed with the alleles increasing the trait of interest and the other parent is fixed with alleles decreasing the trait of interest, (ii) additive gene effects, (iii) unlinked loci, and (iv) equal allelic effects at all loci.

The possible gain from selection per cycle was predicted as narrow-sense heritability $h^2 \times [\sigma^2(P)]^{1/2}$ multiplied by the selection differential in standard deviation units k for selection intensities of 5, 10, or 20% (Hallauer and Miranda, 1988). The statistical analysis was performed using the SAS-STAT statistical package (SAS Institute, 2004).

RESULTS AND DISCUSSION

Disease Reaction

No significant interaction between inoculation procedures with *P. cubensis* and populations was observed (Table 2). Seedlings of the inbred Ames 2354 (P_1) showed a low degree of disease symptoms measured by the DSI of infection (DSI = 1.0–1.8 depending on the test). In contrast, seedlings of PI 175695 (P_2) exhibited high susceptibility (DSI = 7.2–8.0). The highest disease incidence appeared on all tested populations in method III, where the mean DSI of infection was the highest (DSI = 5.1), since in other tests index of infection was lower (DSI = 4.0–4.4).

As our tests were conducted in greenhouse and growth chambers at different periods of time, the variability of pathogenicity of *P. cubensis* isolates may also reflect some environmental differences prevailing in the greenhouse chambers during each test. Some authors noted that even small environmental differences might make consistent identification and qualification of resistant genotypes difficult (Oerke et al., 2006; Lebeda and Cohen, 2011). This might affect the differences in the interaction between genotype and *P. cubensis* isolate in our experiments. Similar values for disease incidence and disease severity in all tests, and for parents and their crosses particularly, show methodological regularity of performed research. Repeatable and reliable results obtained in this part of our study allow genetic analysis of resistance to DM in cucumber.

Table 2. Mean disease severity index and number of seedlings tested from six generations of cucumber following the controlled inoculation with *Pseudoperonospora cubensis*.

Method†	P_1	Generation						Mean
		P_2	F_1	RF_1	F_2	Bc_1P_1	Bc_1P_2	
I	1.7	7.4	4.2	4.3	3.9	2.1	6.1	4.2
II	1.8	7.8	4.2	3.5	4.3	2.2	5.9	4.4
III	1.6	8.0	4.8	4.7	5.2	3.0	6.7	5.1
IV	1.0	7.3	2.7	2.7	4.7	2.5	5.9	4.0
V	1.4	7.2	3.0	3.7	4.9	3.0	6.8	4.4
Mean	1.5	7.5	3.8	3.8	4.6	3.0	6.3	4.4
Plants tested‡	155	213	204	160	853	250	227	NA§

†As described in Table 1.

‡Total number of plants tested by means all methods applied (sum).

§NA, not applicable.

Inheritance of Resistance

The F_2 population showed a continuous segregation in all tests and did not segregate into distinct categories, indicating that the inheritance of the disease reaction of *P. cubensis* is a quantitative trait. The F_1 and reciprocal F_1 populations showed no difference (mean of 3.8 for each population), indicating no maternal effects. Comparison of the F_1 mean (2.7–3.8) with parental midpoint (4.2–4.8) demonstrated a significant difference, indicating dominance in the direction of resistance (Table 2).

In our analysis, the variances of the six generations tested were generally consistent across tests (Table 3). Genetic variance was larger than environmental variance in all tests (Table 4). Over all tests, environmental variance was low (0.46 to 0.67). The large genetic variance (1.43 to 4.22) found in our study indicates that the hypothesis of a quantitative trait is more likely than the hypothesis of a single gene with large environmental variation.

Additive genetic effects were estimated (Table 4). Dominance variance could have been estimated by subtraction of genetic and additive variances from the phenotypic variance, but this estimate would be indirect and imprecise. Additive effects in our experiment were moderate or large for tests I, II, and IV (3.60, 5.30, and 2.51, respectively) but small for tests III and V (1.21 and 1.47, respectively) due to less phenotypic variation in the F_2's of tests III and V.

The broad-sense heritability was high for all tests (0.68 to 0.88) (Table 4). The narrow-sense heritability was very high in tests I, II, IV, and V (0.70 to 1.11) but moderately low (0.45) in test III, due to the small estimate of additive variance for that test. With the exception of test III, narrow- and broad-sense heritabilities were similar for all tests, indicating that dominance effects are likely small.

Our data showed that broad-sense heritability for resistance to DM in cucumber can be high, indicating more importance of genetic than environmental variability in all tests. Greenhouse testing should be used to capitalize on the higher additive components and increase the narrow-sense heritability for population improvement. The use of more uniform and controlled environments, such as greenhouse or growth chamber tests, helps to enhance the genotypic effect and to allow more precise selections of resistant parents for the next generation. In addition, the overall large heritability estimates confirm that genotype has a large effect in this testing environment. This further supports our hypothesis of that resistance to DM is mediated by a few genes with additive effects.

Estimates of the minimum number of effective factors (genes) for resistance may be biased, due to the fact that we had no direct estimates of dominance effects. We used five estimates indicating that few (1–3) genetic factors were involved in the inheritance of resistance to DM in cucumber (Table 5). Even though these estimates are not precise, they support the indication of a few loci regulating resistance to DM. This is corroborated by the large genotypic effects and the high heritability estimates. Our results indicating a small number of genes controlling resistance to DM in Ames 2354 agree with previous research reporting few genes involved (Badr and Mohamed, 1998; Doruchowski and Lakowska-Ryk, 1992; El-Hafaz et al., 1990; Pershin et al., 1988; Petrov et al., 2000; Shimizu et al., 1963).

Table 3. Phenotypic variances by generation for Ames 2354 (P_1), PI 175695 (P_2), and their progenies screened for resistance to downy mildew.†

Method‡	$\sigma^2(P_1)$	$\sigma^2(P_2)$	$\sigma^2(F_1)$	$\sigma^2(F_2)$	$\sigma^2(BC_1P_1)$	$\sigma^2(BC_1P_2)$
I	0.32	0.44	0.53	3.67	1.10	2.64
II	0.40	0.63	0.60	4.78	1.40	2.87
III	0.42	0.71	0.78	2.72	2.74	1.49
IV	0.85	0.71	0.63	3.26	1.72	2.29
V	0.89	0.49	0.63	2.10	1.96	0.76

†Data are ratings from five tests of *Cucumis sativus* cultigens Ames 2354 (P_1), PI 175695 (P_2), and their progenies. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = no disease, 1–2 = trace, 3–4, slight, 5–6 = moderate, 7–8 = severe, and 9 = dead).

‡As described in Table 1.

Table 4. Variance and heritability estimates for Ames 2354 (P_1), PI 175695 (P_2), and their progenies screened for resistance to downy mildew.†

Method‡	$\sigma^2(P)$§	$\sigma^2(E)$¶	$\sigma^2(G)$#	$\sigma^2(A)$††	H^2‡‡	h^2§§
I	3.67	0.46	3.21	3.60	0.88	0.98
II	4.78	0.56	4.22	5.30	0.88	1.11
III	2.72	0.67	2.05	1.21	0.75	0.45
IV	3.26	0.71	2.55	2.51	0.78	0.77
V	2.10	0.66	1.43	1.47	0.68	0.70

†Data are ratings from five tests of *Cucumis sativus* cultigens Ames 2354 (P_1), PI 175695 (P_2), and their progenies. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = no disease, 1–2 = trace, 3–4, slight, 5–6 = moderate, 7–8 = severe, and 9 = dead).

‡As described in Table 1.

§$\sigma^2(P)$, phenotypic variance, = σ^2F_2.

¶$\sigma^2(E)$, environmental variance, = $\sigma^2P_1 + \sigma^2P_2 + (2 \times \sigma^2F_1)/4$.

#$\sigma^2(G)$, genetic variance, = $\sigma^2(P) - \sigma^2(E)$.

††$\sigma^2(A)$, additive variance, = $(2 \times \sigma^2F_2) - (\sigma^2BC_1P_1 + \sigma^2BC_1P_2)$.

‡‡H^2, broad-sense heritability.

§§h^2, narrow-sense heritability.

Table 5. Estimates of number of effective factors and predicted gain from selection under different selection intensities for Ames 2354 (P_1), PI 175695 (P_2), and their progenies screened for resistance to downy mildew.†

	Effective factors						Gain§		
Method‡	L1¶	L2#	L3††	L4‡‡	W§§	Mean	5%	10%	20%
I	1.2	1.2	1.1	1.4	1.2	1.2	3.9	3.3	2.6
II	1.1	1.1	0.8	1.4	1.1	1.1	5.0	4.3	3.4
III	2.4	2.3	3.1	1.8	2.3	2.4	1.5	1.3	1.0
IV	1.9	1.9	2.0	1.9	2.2	2.0	2.9	2.4	1.9
V	2.6	2.8	2.2	3.8	3.1	2.9	2.1	1.8	1.4
Pooled	2.7	2.2	2.1	2.4	2.3	2.3	3.1	2.6	2.1

†Data are ratings from five tests of *Cucumis sativus* cultigens Ames 2354 (P_1), PI 175695 (P_2), and their progenies. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = no disease, 1–2 = trace, 3–4, slight, 5–6 = moderate, 7–8 = severe, and 9 = dead).

‡As described in Table 1.

§Gain from selection = $k \times h^2 \times [\sigma^2(P)]^{1/2}$, in which k is the selection differential in standard deviation units, h^2 is the narrow-sense heritability, and $\sigma^2(P)$ is the phenotypic variance.

¶Lande's method I as described in the Method section.

#Lande's method II as described in the Method section.

††Lande's method III as described in the Method section.

‡‡Lande's method IV as described in the Method section.

§§Wright's method as described in the Method section.

The resistant parent (Ames 2354) used in this study is a selection from self-pollination of PI 234517, also known as SC-50. Van Vliet and Meysing (1977) reported that the resistance found in Poinsett and PI 234517 was controlled by the same single recessive gene, designated as *dm* and likely originating from PI 197087. Therefore, it is probable that Ames 2354 contains at least the *dm* gene and likely one or more additional resistance genes. Plant Introduction 234517 (and likely Ames 2354) was highly resistant in the United Stated before a change in the pathogen population in 2004. This change resulted in a reduction of resistance in many previously highly resistant lines tracing resistance to PI 197087 (*dm* gene). From 2004 onward, these lines, including Ames 2354, have been only moderately resistant to the current DM in the United States (Call et al., 2012). This is evidence for differences in races between the United States and Poland. Call et al. (2012) reported similar results from both locations in their germplasm screening in 2005 to 2007. Shetty et al. (2002) also stated that there is no evidence for race differences between the United States and Poland. Klosinska et al. (2010) reported Ames 2354 as highly resistant in Poland from 2005 to 2009, and it was highly resistant in this study as well. There is, therefore, evidence for different races between locations.

Testing Methods

Five different testing methods were used in this study. The main differences between methods were fresh vs. frozen vs. mixed inoculum, plant stage of inoculation, days after inoculation that plants were rated, and method of rating (Table 1). Both fresh and frozen sporangia used for inoculum worked well in causing disease. For method IV, in which sporangia frozen for 2 to 3 mo were used, the slowest disease progression was observed. Sporangia maintained at −80°C for longer than 1 mo remain pathogenic, but their virulence decreased (data not shown). Given that enough time is allowed for the test plants to sporulate resulting in secondary infection, inoculation at the first true leaf compared to two or three leaves does not seem to be a major factor. It is probably better to inoculate more leaves to maximize disease and potential for secondary infection.

Plants were rated from 8 up to 20 d after inoculation among methods. It is difficult to determine the optimum time after inoculation to rate, as it is likely affected by environmental factors. If rating leaves that were not inoculated, enough time must be allowed for the inoculated leaves to produce inoculum for secondary infection. The best methods (I and II) were rated 16 to 18 d after inoculation, which allows enough time for this to occur. If rating only the first leaf, as was done in methods IV and V, it is not necessary to wait for any secondary infection, and plants can be rated when disease symptoms appear. It may be disadvantageous to try to rate only the first leaf long after inoculation, as there is an opportunity for natural or disease-induced senescence making it difficult to ensure the correct leaf is rated.

The most effective methods in our study rated all leaves on the plant (methods I and II). Method III used the mean of the individually rated leaves (second and third) and was the least effective of the methods used. This may be due to the variability in the spread of secondary infection in the canopy, which would cause variability among the rated leaves. For this reason as well as speed it is probably better to rate whole plants.

Our analysis showed that progress can be made using greenhouse or growth chamber selection. Selection using the best test methods (I and II) could lead to a gain of at least 2.5 points (on a 10 point scale) per generation even under the lower selection intensities (i.e., 20%) typically used in recurrent selection programs (Table 5). Methods IV and V could also be applied to make progress by selection but are less effective than methods I and II. We would recommend using method II for selection, in which plants are inoculated at two to three true leaves and disease is evaluated on the entire plants after allowing time for a secondary infection cycle. This method is closer to field conditions, where natural infection of the *P. cubensis* occurs in older plant stage.

CONCLUSIONS

Our study indicates that resistance to DM in cucumber should be regarded as a quantitative trait for breeding purposes, with a few major genes controlling the trait. This was estimated by use of a subjective resistance scale (based on the percentage of the leaf area damaged by the pathogen) to the segregating hybrid populations of cucumber using multiple testing methods. Transformation of the disease rating is sometimes recommended since the rating cannot be lower than 0

or higher than 9, but that was not useful in this case. Greenhouse and growth chamber testing of the breeding material is an effective method to select for improved resistance. Benefits of testing under controlled conditions include uniformity of environment as well as the ability for multiple tests per year. Field testing under natural infection should be used at later stages of selection to confirm resistance of improved material.

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References

Angelov, D. 1994. Inheritance of resistance to downy mildew, *Pseudoperonospora cubensis* (Berk. and Curt.) Rostow. Rep. 2nd Natl. Symp. Plant Immun. (Plovdiv) 3:99–105.

Badr, L.A.A., and F.G. Mohamed. 1998. Inheritance and nature of resistance to downy mildew disease in cucumber (*Cucumis sativus* L.). Ann. Agric. Sci. Moshtohor 36(4):2517–2544 (Abstract).

Call, A.D., A.D. Criswell, T.C. Wehner, U. Klosinska, and E.U. Kozik. 2012. Screening cucumber for resistance to downy mildew caused by *Pseudoperonospora cubensis* (Berk. and Curt.) Rostov. Crop Sci. 52:577–592.

Colucci, S.J., T.C. Wehner, and G.J. Holmes. 2006. The downy mildew epidemic of 2004 and 2005 in the eastern United States. In: G.J. Holmes, editor, Proceedings of The Cucurbitaceae 2006, Asheville, NC. 17–21 Sept. 2006. University Press, Raleigh, NC. p. 403–411.

Doruchowski, R.W., and E. Lakowska-Ryk. 1992. Inheritance of resistance to downy mildew (*Pseudoperonospora cubensis* Berk. and Curt.) in *Cucumis sativus*. In: R.W. Doruchowski, E. Kozik, and K. Niemirowicz-Szczytt, editors, Proceedings of the 5th EUCARPIA Cucurbitaceae Symposium, Warsaw, Poland. 27–31 July 1992. Res. Inst. Veg. Crops, and Warsaw Univ. Agric., Warsaw, Poland. p. 132–138.

Dudley, J.W., and R.H. Moll. 1969. Interpretation and use of estimates of heritability and genetic variances in plant breeding. Crop Sci. 9:257–262. doi:10.2135/cropsci1969.0011183X000900030001x

El-Hafaz, A., B. El-Din, H.H. El-Doweny, and M.M.W. Awad. 1990. Inheritance of downy mildew resistance and its nature of resistance in cucumber. Ann. Agric. Sci. Moshtohor. 28(3):1681–1697.

Fanourakis, N.E., and P.W. Simon. 1987. Analysis of genetic linkage in the cucumber. J. Hered. 78:238–242.

Hallauer, A.R., and J.B. Miranda. 1988. Quantitative genetics in maize breeding. 2nd ed. Iowa State Univ. Press, Ames, IA.

Holland, J.B., W.E. Nyquist, and C.T. Cervantes-Martinez. 2003. Estimating and interpreting heritability for plant breeding: An update. Plant Breed. Rev. 22:9–113.

Jenkins, S.F., Jr., and T.C. Wehner. 1983. A system for measurement of foliar disease in cucumbers. Cucurbit Genet. Coop. Rpt. 6:10–12.

Klosinska, U., E.U. Kozik, A.D. Call, and T.C. Wehner. 2010. New sources of resistance to downy mildew in cucumber. In: J.A. Thies, S. Kousik, and A. Levi, editors, Proceedings of The Cucurbitaceae 2010, Charleston, SC. 14–18 Nov. 2010. Am. Soc. Hortic. Sci., Alexandria, VA. p. 135–138.

Lande, R. 1981. The minimum number of genes contributing to quantitative variation between and within populations. Genetics 99:541–553.

Lebeda, A. 1991. Resistance in muskmelons to Czechoslovak isolates of *Pseudoperonospora cubensis* from cucumbers. Sci. Hortic. (Amsterdam) 45(3–4):225–260. doi:10.1016/0304-4238(91)90071-6

Lebeda, A., and Y. Cohen. 2011. Cucurbit downy mildew (*Pseudoperonospora cubensis*) biology, ecology, epidemiology, host-pathogen interaction and control. Eur. J. Plant Pathol. 129:157–192. doi:10.1007/s10658-010-9658-1

Lebeda, A., and J. Urban. 2004. Disease impact and pathogenicity variation in Czech populations of *Pseudoperonospora cubensis*. In: A. Lebeda and H.S. Paris, editors, Progress in cucurbit genetics and breeding research. Proceedings of Cucurbitaceae 2004, the 8th EUCARPIA Meeting on Cucurbit Genetics and Breeding, Olomouc, Czech Republic. 12–17 July 2004. Palacký University, Olomouc, Czech Republic. p. 267–273.

Liu, J.S., T.C. Wehner, and S.B. Donaghy. 1997. SASGene: A SAS computer program for genetic analysis of gene segregation and linkage. J. Hered. 88:253–254. doi:10.1093/oxfordjournals.jhered.a023099

Nyquist, W.E. 1991. Estimation of heritability and prediction of selection response in plant populations. Crit. Rev. Plant Sci. 10:235–322. doi:10.1080/07352689109382313

Oerke, E.-C., U. Steiner, H.-W. Dehne, and M. Lindenthal. 2006. Thermal imaging of cucumber leaves affected by downy mildew and environmental conditions. J. Exp. Bot. 57(9):2121–2132. doi:10.1093/jxb/erj170

Ostle, B., and L.C. Malone. 1988. Statistics in research. 4th ed. Iowa State Univ. Press, Ames, IA.

Pershin, A.F., N.I. Medvedeva, and A.V. Medvedev. 1988. Quantitative approach to studying the genetics of disease resistance. IV. Interaction of the genetic systems for resistance to powdery and downy mildews in cucumber. Geneticka, USSR. 24(3):484–493 (Abstract).

Petrov, L., K. Boodert, L. Sheck, A. Baider, E. Rubin, Y. Cohen, N. Katzir, and H.S. Paris. 2000. Resistance to downy mildew, *Pseudoperonospora cubensis*, in cucumbers. Acta Hortic. 510:203–209.

Robinson, H.F., R.E. Comstock, and P.H. Harvey. 1955. Genetic variances in open pollinated varieties of corn. Genetics 40:45–60.

SAS Institute. 2004. SAS 9.1.3 help and documentation. SAS Institute Inc., Cary, NC.

Shetty, N.V., T.C. Wehner, C.E. Thomas, R.W. Doruchowski, and K.P.V. Shetty. 2002. Evidence for downy mildew races in cucumber tested in Asia, Europe, and North America. Sci. Hortic. (Amsterdam) 94:231–239. doi:10.1016/S0304-4238(02)00013-4

Shimizu, S., K. Kanazawa, A. Kato, Y. Yokota, and T. Koyama. 1963. Studies on the breeding of cucumber for the resistance to downy mildew and other fruit characters. Engei Shikenjo Ho Koku 2:65–81 (Abstract).

Steel, R.G.D., J.H. Torrie, and D.A. Dickey. 1997. Principles and procedures of statistics: A biometrical approach. 3rd ed. WCB/McGraw-Hill, Boston, MA.

Van Vliet, G.J.A., and W.D. Meysing. 1974. Inheritance of resistance to *Pseudoperonospora cubensis* Rost. in cucumber (*Cucumis sativus* L.). Euphytica 23:251–255. doi:10.1007/BF00035865

Van Vliet, G.J.A., and W.D. Meysing. 1977. Relation in the inheritance of resistance to *Pseudoperonospora cubensis* Rost and *Sphaerotheca fuliginea* Poll. in cucumber (*Cucumis sativus* L.). Euphytica 26:793–796. doi:10.1007/BF00021708

Warner, J.N. 1952. A method for estimating heritability. Agron. J. 44:427–430. doi:10.2134/agronj1952.00021962004400080007x

Wright, S. 1968. The genetics of quantitative variability. In: S. Wright, editor, Evolution and genetics of populations, Vol. 1, 2nd ed. University of Chicago Press, Chicago, IL. p. 373–420.