Independence between Scab Resistance and Morphological Traits in Cucumber

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Abstract. No linkage was detected in cucumber (*Cucumis sativus* L.) between the *Cuc* locus for scab resistance and the following eight morphological loci: heart leaf (*hl*), numerous fruit spines (*ns*), small fruit spines (*ss*), tuberculate fruit (*Tu*), uniform immature fruit color (*u*), dull fruit epidermis (*D*), gynoeious sex expression (*F*), and delayed flowering (*df*). All nine traits were monogenically inherited.

Sixteen disease resistance loci and >100 morphological marker genes have been identified in cucumber (Pierce and Wehner, 1989, 1990; Robinson et al., 1976; Vakalounakis, 1993). However, linkage relationships among most of these simply inherited traits have not been analyzed. If linkage relationships between resistance genes and morphological markers were known, breeding for disease resistance could be enhanced. Such information would allow researchers to use morphological markers instead of more conventional screening procedures and might permit faster and easier identification of resistant genotypes.

Scab or spot rot caused by *Cladosporium cucumerinum* Ell. & Arth. is a serious cucumber disease in many parts of the world. (Greelman, 1960; Sherf and MacNab, 1975). Scab resistance in cucumber is dominant and controlled by the *Ccu* locus (Abul-Hayja, 1975).

No linkage has been found between scab resistance (*Ccu*) and crinkled leaf (*cr*), divided leaf (*dvl*), nonbitter cotyledon (*bi*), stunted cotyledon (*sc*), mottled cotyledon (*mc*), yellow cotyledon (*yc-1* and *yc-2*), revolute cotyledon (*rc*), rosette (*ro*), light sensitivity (*ls*), glabrate (*glb*), glabrous (*gl*), long hypocotyl (*lh*), fruit spine color (*B*), compact plant habit (*cp*), powdery mildew resistance (*pm*), and watermelon mosaic virus-1 resistance (*wmv-1*) (Abul-Hayja et al., 1975; den Nijs and Boukema, 1983; Wang et al., 1987; Yorty, 1968; Zijlstra, 1987). Identifying tightly linked markers would be useful in selecting scab-resistant genotypes. Vakalounakis and Williams (1989) developed an efficient protocol for differentiating cucumber seedlings resis-

tant and susceptible to scab, without killing the susceptible genotypes. Thus, our study was designed to test for linkage between the *Ccu* gene and eight previously unevaluated morphological genes. This information would aid in genetic map construction and selection in cucumber.

Materials and Methods

The genetic traits examined were heart leaf (*hl*), numerous fruit spines (*ns*), small fruit spines (*ss*), tuberculate fruit (*Tu*), uniform immature fruit color (*u*), dull fruit epidermis (*D*), gynoeious sex expression (*F*), and delayed flowering (*df*).

The inbred lines Straight 8 and WI 2757 were used as parents in this study. Straight 8 possesses normal-shaped leaves (*hl+*), monoecious sex expression (*f*), normal flowering (*Df*), few and coarse fruit spines (*Ns* and *Ss*, respectively), warty fruit (*Tu*), mottled immature fruit color (*U*), dull fruit epidermis (*D*), and susceptibility to scab (*ccu*). WI 2757 possess heart leaves (*hl*) (Vakalounakis, 1992), gynoeious sex expression (*F*), delayed flowering (*df*), numerous and small fruit spines (*ns* and *ss*, respectively), smooth fruit (*tu*), uniform immature fruit color (*u*), glossy fruit epidermis (*d*), and resistance to scab (*Ccu*).

Both cultivar seeds were obtained from the U.S. Dept. of Agriculture, Agricultural Research Service and the Univ. of Wisconsin cucumber breeding programs in Madison. The F_1 hybrid of Straight 8 x WI 2757 was selfed, and the resulting F_2 progenies and parents were planted in a greenhouse at the Plant Protection Institute at Katsambas area, Heraklio, Greece, in Spring 1992. The soil type was silt loam at pH 7.5, with a total calcium carbonate concentration of 45%, and electrical conductivity of $2.6 \text{ mS} \cdot \text{cm}^{-1}$. During the crop period, the air mean maximum and minimum were 25 and 7°C, respectively; the mean maximum and minimum relative humidities (RH) were 97% and 65%, respectively.

To index for scab infection, F_2 seedlings at the cotyledon stage were inoculated with *C.*

cucumerinum isolate PHW 662 according to Vakalounakis and Williams's (1989) method. Seedlings grown at 20°C were inoculated 1 day after emergence by placing a 5- μl inoculum droplet (2×10^5 spores/ml) on the newly expanded cotyledons. Seedlings were incubated in a darkened dew chamber for 48 h at 20°C and $\approx 100\%$ RH and then grown for 6 days at 20°C. Scab evaluation started 8 days after pathogen inoculation according to a 10-point visual scale (0 = no symptoms; 1 = glossy spot with less than four necrotic flecks; 3 = more than five superficial necrotic flecks that may coalesce; 5 = necrotic spots surrounded by a light halo, adaxial leaf tissue collapsed; and 9 = sunken necrotic lesion larger than 8 mm in diameter surrounded by a light green halo, adaxial leaf tissue collapsed; even numbers were used to assess intermediate responses). Seedlings with disease ratings ≤ 4.0 were classified as resistant, whereas those with disease ratings > 5 were classified as susceptible (Vakalounakis and Williams, 1989).

Each plant was assessed for sex expression when it was ≈ 1.6 m long and 25 days thereafter. Plants bearing only pistillate flowers were classified as gynoeious, and plants bearing only staminate flowers on the lower nodes of the main axis and beyond these nodes groups of staminate nodes alternating with single pistillate nodes were classified as monoecious (Galun, 1961). When recording for femaleness, plants with mixed staminate and pistillate flowers in the lower seven nodes followed by continuous pistillate stage were occasionally observed and were classified as gynoeious. To assess the delayed flowering response, we recorded the node number of the first flower on the main stem. Plants without flowers at least up to the fifth node were classified as delayed flowering. Fruit 20 to 15 days old were assessed for spine frequency (few or many) and size (coarse or fine), immature fruit color (mottled or uniform), tuberculate fruit (warty or smooth), and dull fruit epidermis (dull or glossy). Leaf shape (heart or normal leaf) was assessed when plants were ≈ 1.6 m long and 25 days thereafter. Plants bearing mostly heart-shaped leaves were classified as heart leaf; others were classified as normal.

The F_2 data were analyzed using the χ^2 test for goodness of fit at $P \leq 0.05$. When a significant P value was obtained, linkage intensity was calculated from the F_2 data by the product-ratio method (Immer, 1930).

Results and Discussion

Ccu and all morphological loci tested demonstrated good agreement with the expected 3 : 1 F_2 segregation for monogenic inheritance (Table 1). In every case where the scab resistance gene was paired with one of the eight morphological markers, each pair showed an adequate fit to a 9 : 3 : 3 : 1 dihybrid ratio (Table 1); this would be the segregation predicted if traits were independent. To the best of our knowledge, this is the first report of the linkage relationship absence between the *Ccu* locus and *hl*, *ns*, *ss*, *Tu*, *u*, *D*, *F*, and *df* loci.

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Table 1. Lack of linkage (F₂ data) between scab resistance (*Ccu*; *Cladosporium cucumerinum*) and morphological loci of cucumber (*Cucumis sativus* L.)

Loci	Total no. plants	Segregation locus <i>Ccu</i> × locus <i>Y</i> ²			Single locus segregation				Joint segregation	
		Genotype locus <i>Ccu</i>	Genotype locus <i>Y</i>		Locus <i>Ccu</i>		Locus <i>Y</i>			
			<i>Y</i> –	<i>yy</i>	χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>
<i>Ccu-hl</i>	265	<i>Ccu</i> –	145	47	0.92	0.50–0.25	0.15	0.75–0.50	2.05	0.75–0.50
		<i>ccu ccu</i>	51	22						
<i>Ccu-ns</i>	199	<i>Ccu</i> –	100	45	0.48	0.50–0.25	2.29	0.25–0.10	3.26	0.50–0.25
		<i>ccu ccu</i>	40	14						
<i>Ccu-ss</i>	199	<i>Ccu</i> –	100	45	0.48	0.50–0.25	2.29	0.25–0.10	3.26	0.50–0.25
		<i>ccu ccu</i>	40	14						
<i>Ccu-Tu</i>	153	<i>Ccu</i> –	84	27	0.49	0.50–0.25	0.18	0.75–0.50	1.57	0.75–0.50
		<i>ccu ccu</i>	33	9						
<i>Ccu-u</i>	157	<i>Ccu</i> –	87	28	0.26	0.75–0.50	0.05	0.90–0.75	0.32	0.98–0.95
		<i>ccu ccu</i>	32	10						
<i>Ccu-D</i>	154	<i>Ccu</i> –	86	25	0.41	0.75–0.50	1.45	0.25–0.10	1.07	0.99–0.75
		<i>ccu ccu</i>	32	11						
<i>Ccu-F</i>	242	<i>Ccu</i> –	134	40	1.24	0.50–0.25	0.44	0.75–0.50	7.29	0.10–0.05
		<i>ccu ccu</i>	43	25						
<i>Ccu-df</i>	222	<i>Ccu</i> –	112	48	1.02	0.50–0.25	0.73	0.50–0.25	3.69	0.50–0.25
		<i>ccu ccu</i>	49	13						

²*Y* = dominant marker genotype, *y* = recessive marker genotype.

Vakalounakis (1992) reported that 11 disease resistance and morphological loci mapped to four linkage groups as follows: Group I, *hl-ss-ns-Tu-u-D-pm*; Group II, *B-R*; Group III, *F*; and Group IV, *bi*. Based on Vakalounakis' data (1992), the absence of linkage between the *Ccu* locus and the *B* locus for fruit spine color (Shanmugasundaram et al., 1971; Vakalounakis, 1993), the linkage between *Ccu* locus and *Fcu-1* locus (resistance to race 1 of *Fusarium oxysporum* f. sp. *cucumerinum*; Vakalounakis, 1993), and our results, we conclude that *Ccu* is located on a separate linkage group with *Fcu-1*. This finding agrees with Pierce and Wehners' findings (1990) that the *Ccu* locus is in its own linkage group with the *Fl* locus for fruit length.

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