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Two-gene Interaction and Linkage for Bitterfree Foliage in Cucumber

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ABSTRACT. A second gene for bitterfree foliage in cucumber (*Cucumis sativus* L.) was discovered. In a cross between two inbred lines having bitterfree foliage (NCG-093 and WI 2757), the F_1 progeny were bitter, the F_2 progeny segregation frequency fit a ratio of 9 bitter : 7 bitterfree, and the BC_1 segregation frequencies fit a ratio of 1 bitter : 1 bitterfree. Thus, a second factor nonallelic to the previous bitterfree gene, *bi*, controls the bitterfree trait. When F_2 and BC_1 progeny resulting from crosses of bitterfree NCG-093 with other bitter lines were studied, the second factor for bitterfree in NCG-093 fit a recessive, single-gene model. The existence of a second, recessive bitterfree gene was confirmed in additional crosses, and the gene was designated *bi-2*. Further analysis of two crosses indicated that *bi-2* was linked with the short petiole (*sp*) gene (map distance = 11 cM).

Cucumber (*Cucumis sativus* L.) normally has bitter foliage and fruit free of bitterness. Bitterfree is controlled by a single recessive gene, *bi* (Pierce and Wehner, 1990), and was discovered by Andeweg and DeBruyn (1959) in 1 out of 15,000 plants tested from >89 cultivars and breeding lines. The bitterfree gene was linked weakly to gene *F*, which conditions gynoecy, with a recombination frequency of 0.37 (Fanourakis and Simon, 1987). The *bi* gene makes the foliage bitterfree and prevents the fruit from becoming bitter under stress. Andeweg and DeBruyn (1959) noted that cultivars used in Dutch greenhouse production occasionally had bitter fruit, so the bitterfree gene has been used extensively to make them resistant to stress-induced bitterness.

Plants with the *bi/bi* genotype are less preferred by three species of cucumber beetles (DaCosta and Jones, 1971a, 1971b). However, most American pickling and slicing cucumber cultivars have the normal *Bi/Bi* genotype. Fruit of these cultivars have a more persistent "cucumber" taste than cultivars with the *bi/bi* genotype, and plants are resistant to spider mite (Soans et al., 1973). Another single dominant gene, Bitter fruit (*Bt*), identified in the wild 'Hanzil Medicinal' cucumber from India (PI 173889), makes the fruit (but not the foliage) extremely bitter and inedible (Barham, 1953).

Progeny of NCG-093 (bitterfree foliage) crossed with WI 2757 (bitterfree gene *bi*) (Peterson et al., 1982) were studied in 1986 (unpublished data). In that cross, the F_1 progeny had bitter foliage, and the F_2, BC_{1A}, and BC_{1B} had a trend toward two-gene interaction segregation ratios, indicating a second gene in NCG-093 that was not allelic to *bi*. The objective of this study was to determine the inheritance of a possible second bitterfree gene, its interaction with gene *bi*, and its linkage relationship to other genes in cucumber.

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Materials and Methods

The experiment was conducted from 1994 to 1996 in greenhouses at North Carolina State Univ. Three families were developed using lines possessing previously established morphological genetic markers (Pierce and Wehner, 1990): NCG-093 (short petiole, bitterfree foliage) x WI 2757 (bitterfree gene *bi*); NCG-093 x NCG-101 (long hypocotyl, white fruit, bitter foliage); and NCG-093 x NCG-094 (umbrella leaf, bitter foliage). NCG-093 was obtained as the short petiole mutant from den Nijs and Boukema (1985). Before use in crosses, the parental inbred lines of the three families were increased by self-pollination and checked for uniformity of gene expression. Parental inbreds were crossed, and the F_1 progeny were self-pollinated to produce the F_2 generation. The F_1 progeny were simultaneously backcrossed to each parent to produce the BC_{1A} (F_1 x P_A) and BC_{1B} (F_1 x P_B). All pollinations were made by hand in the greenhouse.

Seedlings were grown in a greenhouse (32 ± 8 °C in the summer, 27 ± 6 °C in the fall, and 23 ± 4 °C in the winter) in flats of vermiculite on benches heated to 32 °C. The experiment consisted of 18 plants of each parent and F_1, 144 of F_2, and 72 of each BC_1. Five days after seeding, plants were examined for bitterness by tasting one third of one cotyledon of each plant. Each taste-tester rinsed orally and ate a soda cracker after tasting a plant that had a bitter cotyledon to maintain accurate classification of bitter or nonbitter plants. The data were verified later by tasting one true leaf at the fifth node.

Linkage with *sp* was evaluated by measuring petiole length and using the normal and short petiole parents as guides. Normal petioles are 2.4 to 8.8 times longer than the short petioles of *sp/sp* plants (den Nijs and Boukema, 1985).

After collecting the seedling data, the plants were transplanted to the field at the Horticultural Crops Research Station near Clinton, N.C., for evaluation of other traits. Plants were grown on raised beds 1.5 m apart, with plants in rows by generation, and a density of 6 plants/m of row. Recommended cultural practices were used for plant production (Schultheis, 1990).

Data analysis. Data were analyzed using the SASGENE program (Liu et al., 1997), a SAS (SAS Institute Inc., Cary, N.C.) computer program for analysis of gene segregation and linkage

Table 1. Two-gene goodness-of-fit test for bitterfree foliage in NCG-093 x WI 2757 cucumber inbreds.[z]

Generation	No. observed (norm:btfree)	Ratio tested (norm:btfree)	χ^2	P
P_A (NCG-093, bitterfree)	0:18	0:1	---	---
P_B (WI 2757, bitterfree)	0:18	0:1	---	---
F_1	18:0	1:0	---	---
F_2	78:65	9:7	0.17	0.68
BC_{1PA}	33:39	1:1	0.50	0.48
BC_{1PB}	34:36	1:1	0.06	0.81

[z]Norm = normal bitter, btfree = bitterfree.

Table 2. Single-gene goodness-of-fit test for a second gene for bitterfree foliage of the crosses of NCG-093 x NCG-094 and NCG-093 x NCG-101 cucumber inbred lines.[z]

Generation	No. observed (norm:btfree)	Ratio tested (norm:btfree)	χ^2	P
NCG-093 x NCG-094				
P_A (NCG-093, bitterfree)	0:18	0:1	---	---
P_B (NCG-094, normal)	17:0	1:0	---	---
F_1	18:0	1:0	---	---
F_2	108:36	3:1	0.0	1.0
BC_{1PA}	34:38	1:1	0.22	0.64
BC_{1PB}	72:0	1:0	---	---
NCG-093 x NCG-101				
P_A (NCG-093, bitterfree)	0:18	0:1	---	---
P_B (NCG-101, normal)	18:0	1:0	---	---
F_1	18:0	1:0	---	---
F_2	104:37	3:1	0.12	0.73
BC_{1PA}	38:34	1:1	0.22	0.64
BC_{1PB}	72:0	1:0	---	---

[z]Norm = normal bitter, btfree = bitterfree.

relationship. SASGENE makes the following calculations: 1) single-gene or two-gene goodness-of-fit tests, which were based on comparisons of observed frequency data with the expected Mendelian segregation ratios of 3:1 and 1:1 or 1:0, or 9:7, 1:1 and 1:1, respectively, for F_1, F_2 and BC_1 generation data; 2) chi-square and probability values, analyzed for independent assortment or linkage likelihood; 3) recombination fraction (rf) and standard errors (SES), which were calculated using the formulae of Sinnott (1939) and Weir (1994) for coupling or repulsion phase. Consideration of repulsion or coupling phases depended on whether the two genes of interest were introduced separately or together from one parental line.

Results and Discussion

TWO-FACTOR DETECTION. Epistatic interaction analysis for segregation of the bitterfree foliage trait in the cross of NCG-093 x WI 2757 showed that both parents were bitterfree but produced all bitter F_1 progeny (Table 1). According to the two-gene goodness-of-fit test formula, the F_2 fit a 9:7 ratio ($p > 0.68$) and the BC_{1A} and BC_{1B} progeny segregations fit a 1:1 ratio ($p > 0.48$ and 0.81, respectively). The data fit the gene model for duplicate recessive gene epistatic interaction (Stansfield, 1991), where there are two loci (*a* and *b*) controlling leaf bitterness as follows: the four double-dominant genotypes (*A_B_*) produce the bitter phenotype; all other genotypes (*aaB_*, *A_bb*, and *aabb*) produce the bitterfree phenotype. It appears that the two bitterfree loci interrupt the biochemical pathway for production of cucurbitacin at two different and independent locations. Thus, either locus in homozygous recessive condition stops the production of bitter compounds in the leaves.

SINGLE GENE TEST. Using data from the F_2 and BC_1 progeny of NCG-093 x NCG-094 and NCG-093 x NCG-101, a single-gene goodness-of-fit test was used to detect the factor that controlled bitterfree in NCG-093 and that was nonallelic to *bi*. The parental inbreds used as males in these two crosses (i.e., NCG-094 and NCG-101, both bitter) had the contrasting phenotype from NCG-093. This was done to determine the inheritance of the putative second bitterfree gene separately from the WI 2757 inbred with the *bi* gene.

The second factor for bitterfree fit the expected single gene model of 1:0, 3:1, and 1:1 or 1:0 ratios in the F_1, F_2, and BC_1 generations (Table 2). Therefore, we propose a second, single recessive gene for bitterfree foliage, which is nonallelic to *bi*, to be designated *bi-2* (bitterfree 2).

GENE LINKAGE ANALYSIS. In the F_2 and BC_1 of NCG-093 x NCG-094 and NCG-093 x NCG-101, six genes were segregating (Pierce and Wehner, 1990): *bi-2* (bitterfree 2), *sp* (short petiole), *rc-2* (second revolute cotyledon 2), *w* (white immature fruit), *lh* (long hypocotyl), and *ul* (umbrella leaf). Recombination frequencies of *bi-2* and *sp* in those F_2 and BC_1 families ranged from 0.87 to 0.125 (Table 3), with an average map distance between *bi-2* and *sp* of 11 cM. The gene *bi-2* was not linked to *rc-2*, *w*, *lh*, or *ul* (data not presented).

Thus, a second gene for bitterfree foliage (*bi-2*) exists independent of *bi*. Those interested in *bi-2* for genetic studies can obtain seeds of NCG-093 from T.C.W.

Table 3. Linkage relationships between *bi-2* and *sp* in the F_2 and BC_1 generations of the crosses of NCG-093 x NCG-094 and NCG-093 x NCG-101 cucumber inbred lines.[z]

Generation	No. of plants					χ^2	*P*	rf	SE
	Total	*B_S_*	*B_ss*	*bbS_*	*bbss*				
NCG-093 x NCG-094									
F_2	144	103	5	12	24	57.2	<0.01	0.121	0.023
BC_1	72	32	2	6	32	44.0	<0.01	0.111	0.037
NCG-093 x NCG-101									
F_2	141	99	5	7	30	87.5	<0.01	0.087	0.020
BC_1	72	32	6	3	31	40.8	<0.01	0.125	0.039

[z]Both families had the gene pair in coupling phase. *B = Bi-2, b = bi-2, S = Sp*, and *s = sp*.

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