A Single Dominant Gene *Ch* for Chilling Resistance in Cucumber Seedlings

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ABSTRACT. An experiment was conducted to determine the genetics of chilling resistance in cucumber (*Cucumis sativus* L.) inbred NC-76 that was developed from PI 246930, an accession from the U.S. Department of Agriculture germplasm collection. NC-76 was crossed with 'Chipper' and breeding line Gy 14 to produce F_1, F_1 reciprocal, F_2, and BC_1 generations for evaluation. Cucumber seedlings at the first true leaf stage were placed in growth chambers set at 4 °C for 7 h and a photosynthetic photon flux of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Segregation in the F_2 fit a 3 : 1 inheritance pattern, with resistance being dominant. The backcross of the F_1 to the susceptible parent produced a 1 : 1 ratio, confirming that chilling resistance was from a single gene. The single dominant gene controlling chilling resistance in NC-76 was given the symbol *Ch*.

Tropical and subtropical plants exhibit a marked physiological dysfunction when exposed to low or nonfreezing temperatures below 10 °C. Lyons (1973) suggested that this physiological harm should be referred to as "chilling injury" (CI) to differentiate it from freezing damage. Such injury to susceptible plants has also been referred to as "low temperature injury" or "cold injury", and in apple (*Malus ×domestica* Borkh.) as "low-temperature breakdown." CI appears to be the preferable term (Ryall and Lipton, 1972) because it is not easily confused with freezing injury or with phenomena related to cold or winterhardiness (Levitt, 1972; Weiser, 1970).

Cucumber is one of the more chilling-sensitive crops. It does not grow at temperatures below 16 °C and is susceptible to CI, especially at temperatures below 6 °C. As a result, in many geographical regions where the crop is planted before the risk of frost has ended (e.g., northern Europe and eastern United States), the cucumber crop is at risk in early spring plantings.

Comparisons of genotypes for their resistance to low temperatures during the seedling stage of development in cucumber have been reported. Smeets and Wehner (1997) identified chilling resistance in AR75-79 (sometimes called 'Little John') and 'Chipper', and a method for testing the chilling resistance of cucumber seedlings was developed. Using this method, Chung et al. (2003) investigated the inheritance of CI in progenies of resistant 'Chipper' and AR75-79 crossed with susceptible Gy 14. Their data suggested that chilling resistance was maternally inherited. In a previous study (Wehner and Kozik, 2007), it was shown that there was low heritability for chilling resistance in two cucumber populations that were developed from elite cultivars not chosen for chilling resistance. A later screening of the cucumber germplasm

collection resulted in the identification of a high level of resistance in PI 246930.

The objective of this study was to determine the genetic basis of resistance in PI 246930. Information about the inheritance of resistance would help breeders to devise programs to incorporate resistance into new breeding lines and elite cultivars.

Materials and Methods

PLANT MATERIAL. The parental genotypes used in this study constituted three contrasting accessions of cucumber: inbred NC-76 (selected from PI 246930), inbred 'Chipper', and breeding line Gy 14. Accession PI 246930 is highly resistant, 'Chipper' is moderately resistant (Chung et al., 2003), and Gy 14 is susceptible. The resistant line NC-76 was crossed with 'Chipper' and Gy 14, both chosen as susceptible parents on the basis of their reaction to low temperatures in our previous phytotron studies (data not shown). The plant material studied consisted of reciprocal F_1, F_2, and backcross progenies from three groups of crosses between NC-76, 'Chipper', and Gy 14. All crosses were made by hand pollination in a greenhouse at North Carolina State University, Raleigh and at the Research Institute of Vegetable Crops, Skierniewice, Poland in 2003 and 2004.

CHILLING RESISTANCE TEST. Experiments were conducted under controlled environment conditions in the growth chambers of the Southeastern Plant Environment Laboratory at North Carolina State University (Thomas et al., 2005). Seeds were sown in peat pots (57 mm², 100 mL volume) filled with a substrate of gravel and peat in a 1 : 1 ratio and placed in flats. One seed was sown in each pot, with 54 pots contained in each flat.

After seeding the families, the flats were placed in growth chambers set at 26 °C day/22 °C night temperatures under 12 h of combined fluorescent and incandescent light (from 0800 to 2000 HR). Light intensity (photosynthetic photon flux) was 650 and 44 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. Plants were watered with the standard phytotron nutrient solution (Thomas et al., 2005).

After the plants reached the first true leaf stage, they were moved from the main growth chamber to the chilling chamber for treatment at 4 °C under a light intensity of 500 μmol·m^{-2}·s^{-1} photosynthetic photon flux for a duration of 7 h. After the chilling treatment, they were returned to the main growth chamber and placed under the same light and temperature regime as before. Plants were rated 14 d after chilling, rating the damage (chlorosis and necrosis) on the first true leaf. The scale was 0 to 9: 0 = no damage, 1 to 2 = trace of damage, 3 to 4 = slight damage, 5 to 6 = moderate damage, 7 to 8 = advanced damage, and 9 = plant dead. Data were collected as means over all leaves on the plants within each generation.

The experiment was a split-plot treatment arrangement in a randomized complete block design with 10 replications. Each flat that constituted the plot contained three plants of each parent, six F_1 plants, six BCP_1 plants, six BCP_2/BCP_3 plants, and 30 F_2 plants.

Data analysis. For purposes of data analysis, plants rated 0 to 6 were considered to be resistant, and those rated 7 to 9 were considered susceptible. Plants rated 7 to 9 will not recover from CI when returned to warm temperature, but those rated 0 to 6 will recover and grow out of their injury. Data were tested for goodness-of-fit to theoretical ratios using the chi-square tests for each of the F_2, BCP_1, and BCP_2 populations (Srb and Owen, 1955).

Results and Discussion

In initial studies, the original population of PI 246930 showed some variation (highly resistant and moderately resistant plants) in response to low temperatures. Individual plants with high resistance were selected, self-pollinated and progeny tested. After four cycles of inbreeding and selection, newly obtained lines of PI 246930 exhibited similar expression of high but nonabsolute resistance levels as their parental stock (data not shown), and were designated NC-76. The variation range of self-pollinated progenies of PI 246930 plants did not exceed those same categories (Table 1). In contrast, plants of 'Chipper' or Gy 14 exhibited susceptibility (rating of 9).

The amount of CI in the parental lines and their segregating crosses was similar, and chi-square tests for homogeneity indicated that the data could be pooled. Therefore, data for each generation were pooled for the two crosses. The F_1, reciprocal F_1, and BC_1 to the resistant parent NC-76 had all resistant plants, which is expected for a dominantly inherited trait (Table 1). The pooled F_2 population had 566 resistant plants and 170 susceptible plants. These data fit a ratio of three resistant : one susceptible with a probability of 0.491 (pooled $\chi^2 = 1.424$). This ratio suggests that

resistance is controlled by single completely dominant gene. This interpretation was confirmed by the segregation ratios in the backcross populations. The pooled BC_1 to resistant NC-76 parent was considered resistant (100% resistant). In the pooled BC_1 to susceptible parents 'Chipper' and Gy 14, there were 75 resistant and 77 susceptible plants. These data fit a ratio of one resistant : one susceptible, with a probability of 0.872 ($\chi^2 = 0.026$).

The genetics of the resistance to CI in cucumber were studied in this experiment. The results clearly confirm the presence of a high level of resistance to CIs in NC-76 (originating from PI 246930). Evidence was obtained on the inheritance of resistance to chilling temperatures from the segregation data of progenies of the resistant NC-76 crossed with the susceptible 'Chipper' and Gy 14. The variation in the F_2 could be explained by a single completely dominant gene. The resistance gene, particularly when homozygous, confers a high level of resistance to chilling temperatures useful for protecting the cucumber crop from CI in spring plantings.

The F_1, reciprocal F_1, and BC_1 to the resistant parent showed a similar pattern, but were less resistant than NC-76 (data not shown). The F_1 generation was resistant, but the damage ratings were higher than NC-76, indicating partial resistance in the heterozygote.

In previous studies (Chung et al., 2003), 'Chipper' was slightly resistant and Gy14 was susceptible. In our study, the slight resistance of 'Chipper' did improve the resistance of the progeny when crossed with highly resistant NC-76, with slightly lower damage ratings recorded (data not shown).

Table 1. Segregation for chilling resistance based on leaf damage in cucumber seedlings from crosses of NC-76 × 'Chipper' and NC-76 × Gy 14.

Parent or cross	Plants observed (no.)			Expected ratio	Chi-square	df	P
	Resistant	Susceptible	Total				
NC-76 × 'Chipper'							
P1 NC-76	53	0	53	1:0	–	–	–
P2 Chipper	1	56	57	0:1	–	–	–
F1 (P1 × P2)	56	1	57	1:0	–	–	–
F1r (P2 × P1)	55	1	56	1:0	–	–	–
BC1P1 (F1 × P1)	110	0	110	1:0	–	–	–
BC1P2 (F1 × P2)	56	53	109	1:1	0.08	1	0.78
F2 (F1 × self)	373	114	487	3:1	0.67	2	0.71
NC-76 × Gy14							
P1 NC-76	27	0	27	1:0	–	–	–
P3 Gy14	0	27	27	0:1	–	–	–
F1 (P1 × P3)	27	0	27	1:0	–	–	–
F1r (P3 × P1)	26	0	26	1:0	–	–	–
BC1P1 (F1 × P1)	27	0	27	1:0	–	–	–
BC1P3 (F1 × P3)	19	24	43	1:1	0.581	1	0.446
F2 (F1 × self)	193	56	249	3:1	0.847	2	0.655
Pooled (NC-76 × 'Chipper' with NC-76 × Gy14)^z							
P1 NC-76	80	0	80	1:0	–	–	–
P2 Chipper	1	56	57	0:1	–	–	–
P3 Gy14	0	27	27	0:1	–	–	–
F1 (P1 × P2,P3)	83	1	84	1:0	–	–	–
F1r (P2,P3 × P1)	83	1	84	1:0	–	–	–
BC1P1 (F1 × P1)	137	0	137	1:0	–	–	–
BC1P2,P3 (F1 × P2,P3)	75	77	152	1:1	0.026	1	0.872
F2 (F1 × self)	566	170	736	3:1	1.424	2	0.491

^{z}Chi-square tests for homogeneity indicated that the data could be pooled over families.

There were no reciprocal differences for high-level chilling resistance, indicating no cytoplasmic or maternal effects because the F_1 and reciprocal F_1 were resistant, even when the susceptible cultigen was used as the female parent. However, the lower-level resistance found in 'Chipper' was reported to be maternally inherited (Chung et al., 2003) based on observing reciprocal effects in the F_1 progeny. The differences in the two studies might be from some differences in the chilling test method. In our studies, seedlings at the first true fully expanded leaf were subjected to a chilling treatment of 7 h at 4 °C, whereas Chung et al. (2003) treated seedlings whose first true leaf were apparent but not fully expanded for 5.5 h at 4 °C. Younger seedlings (cotyledons stage) are less sensitive to CI than seedlings at the first true fully expended leaf (Smeets and Wehner, 1997). Thus, the tests they ran were less severe and 'Chipper' has slight resistance under those conditions.

The symbol *Ch* is proposed to designate the single completely dominant gene from NC-76 for resistance to chilling (Wehner, 1993). Resistance conferred by the gene was highly effective under severe chilling conditions. NC-76 is easily crossed with elite cucumber cultivars, and can be used routinely for the introduction of resistance into new cultivars.

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