

# Chilling-tolerant U.S.-processing Cucumber (*Cucumis sativus* L.): Three Advanced Backcross and Ten Inbred Backcross Lines

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Environmental stresses such as chilling temperatures can reduce seed germination rate, seedling emergence rate, flower and fruit development, marketable yield, and postharvest fruit storage longevity in cucumber (*Cucumis sativus* L.) (Staub and Bacher, 1997; Staub and Wehner, 1996). Chilling temperatures occur in unpredictable patterns, making it difficult to implement management practices for crop protection. Moreover, response of cucumber seedlings to chilling depends on pre- and postchilling environment. Therefore, breeding for tolerance to chilling is an attractive management tool to minimize crop loss.

Chilling injury at the first true-leaf stage in cucumber is controlled by simple plastidic

(maternal; Chung et al., 2003) and nuclear (parental; Kozik and Wehner, 2008) factors. While Chung et al. (2007) identified three putative plastidic single-nucleotide polymorphisms (SNPs) associated with chilling tolerance in processing cucumber ‘Chipper’, Kozik and Wehner (2008) characterized one dominant nuclear gene, *Ch*, in line NC-76 (derived from PI 246930). The response of chilling tolerant ‘Chipper’ and line NC-76 seedlings challenged at 4 °C for 5.5 h under 270  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  photosynthetic photon flux irradiance are similar (Gordon and Staub, 2011).

No chilling-tolerant U.S.-processing cucumber varieties are commercially available. Although the processing cultivar Chipper (released in 1968 from Clemson University, Clemson, SC) possesses plastid genes for chilling tolerance in the seedling stage, its yield and quality are substantially lower than current cultivars. Therefore, experiments were undertaken to introgress the plastid chilling tolerance of ‘Chipper’ (donor parent) into the commercially acceptable processing cucumber line M29 [recurrent parent; experimental inbred line ( $S_{11}$ ), North Carolina State University, Raleigh, NC] via backcrossing and self-pollination (Gordon and Staub, 2013). As a result, a series of three advanced backcross (ABL;  $BC_5$ ) and 10 inbred backcross (IBL;  $BC_2S_{3-5}$ ) chilling-tolerant U.S.

processing lines possessing acceptable yield and quality traits are being released by the Agricultural Research Service (ARS), U.S. Department of Agriculture (USDA). The chilling tolerance of backcross progeny is characterized by their response to chilling stress (5.5 h at 4 °C in 270  $\mu\text{mol}\cdot\text{s}^{-2}\cdot\text{m}^{-2}$  cool white lighting) at the first true-leaf stage under controlled environmental conditions. These lines provide chilling-tolerant genetic stocks suitable for immediate use by cucumber improvement programs.

## Origin

Breeding lines were developed through initial reciprocal matings made between chilling tolerant ‘Chipper’ (CH is used hereafter to indicate tolerant cytoplasmic constitution) and chilling sensitive processing line M29 (M designates sensitive constitution) to produce  $F_1$  progeny for backcrossing to the recurrent parent, line M29 (Gordon and Staub, 2013). NCSU M 29 was developed from the cross of two accessions, PI 222783 (‘Khar Sabz’)  $\times$  PI 222985 (‘Khar’). These PIs were crossed in 1980 and their progeny selected for cold germination ability in 1982–1986 in controlled-germination chambers, and finished as an  $S_{11}$  inbred. Chilling-sensitive and tolerant advanced backcross (ABL;  $BC_1$ - $BC_5$ ) and inbred backcross (IBL;  $BC_2S_{1-5}$ ) lines were created from  $F_1$  reciprocal chilling-sensitive and tolerant lines by backcrossing to a cloned recurrent parent of the alternate chilling response type [i.e., tolerant (‘Chipper’) and sensitive (M29), respectively] parental line. Exact reciprocal  $F_1$  progeny for comparative genotypic and phenotypic analyses were developed by crossing ‘Chipper’ and M29 to produce germplasm in chilling-tolerant (CH; ‘Chipper’  $\times$  M29) and in sensitive (M; M29  $\times$  ‘Chipper’) maternal plastidic backgrounds.

Reciprocal  $F_1$ s intended to produce chilling-tolerant ABL were backcrossed to their paternal genetic donor five times to produce  $BC_1$ - $BC_5$  progeny. For production of IBL, 240 randomly chosen reciprocal ABL  $BC_2$  progeny (i.e., 120 chilling-sensitive ABL  $BC_2$ , and 120 chilling-tolerant ABL  $BC_2$ ) were self-pollinated five times to produce the sensitive and tolerant [containing the M29 plastid (MIBL), and tolerant ‘Chipper’ plastid (CHIBL), respectively]  $BC_2S_5$  progeny. Attrition due to seedling death and lack of seed set during reciprocal backcrossing yielded a set ( $BC_1$ - $BC_5$ ) of 10 ABL (i.e., five each—chilling tolerant and sensitive) and 163 (i.e., 57 CHIBL and 106 MIBL) IBL for molecular genotyping, chilling tolerance testing, and field evaluation.

## Line Evaluation

Initially, progeny within the  $F_1$  and  $BC_1$ - $BC_5$  generations of the two ABL chilling response types were phenotyped for chilling response in the University of Wisconsin Biotron-controlled environmental facility

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Table 1. Descriptive statistics of yield component traits of cucumber (*Cucumis sativus* L.) 'Vlasset', advanced backcross (ABL; BC<sub>3</sub>-BC<sub>5</sub>), and inbred backcross (IBL; BC<sub>2</sub>S<sub>4</sub>) lines, and their parents as assessed at Hancock WI field trials in 2006 and 2007 (mean values represent replication averages taken over four harvests in each of two plantings).

Inbred line designation <sup>z</sup>	Sex type <sup>y</sup>			Days to anthesis <sup>s</sup>			Fruit number per plant <sup>w</sup>			Fruit length (cm) <sup>y</sup>			Fruit diam (cm) <sup>y</sup>			Fruit L:D ratio <sup>u</sup>							
	G	PF	M	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max				
<b>2006</b>																							
Gabrielle ARS ABL CHBC <sub>3</sub>	X			35.2	1.10	34	37	1.6	0.32	1.0	2.1	12.4	1.80	8.3	14.5	3.9	0.60	2.5	4.7	3.19	0.15	2.75	3.43
Saira ARS ABL CHBC <sub>4</sub>	X			34.8	0.70	34	36	1.4	0.39	0.8	2.0	12.3	2.50	5.6	14.2	3.9	0.80	1.9	4.8	3.21	0.20	2.80	3.50
Chipper		X		36.8	1.10	35	38	1.0	0.17	0.8	1.3	12.3	2.00	4.6	14.0	4.2	0.70	1.4	5.4	2.95	0.20	2.50	3.30
Line M29	X			35.2	0.70	34	36	1.5	0.43	1.2	2.3	12.7	1.50	7.6	14.5	4.0	0.50	2.4	4.6	3.20	0.10	2.80	3.40
Vlasset (Control cultivar)	X			34.2	0.40	34	35	1.7	0.13	1.5	1.9	11.6	1.30	7.2	12.7	4.0	0.60	2.4	5.2	2.93	0.20	2.40	3.20
Grand Mean—2006				35.2	0.8	34.2	36.4	1.4	0.3	1.1	1.9	11.9	1.8	6.7	14.0	3.9	0.6	2.1	4.9	3.1	0.2	2.7	3.4
Coefficient of variation (CV %)				2				17				2				4				5			
Mean separation within columns by LSD* (0.05)								0.3				0.8 <sup>NS</sup>				0.3							0.26
<b>2007</b>																							
Gabrielle ARS ABL CHBC <sub>3</sub>	X			48.0	1.30	46	50	3.0	0.73	2.6	4.6	12.1	0.29	11.7	12.6	3.9	0.15	3.7	4.1	3.13	0.06	3.10	3.20
Kodie ARS ABL CHBC <sub>5</sub>		X		48.7	1.70	46	51	2.3	0.90	1.3	3.7	11.4	1.90	8.0	15.5	3.6	0.50	3.0	4.8	3.16	0.40	2.25	3.71
Saira ARS ABL CHBC <sub>4</sub>	X			49.7	3.30	45	54	2.5	0.48	1.7	3.1	11.7	0.59	10.8	12.6	3.7	0.21	3.4	4.0	3.14	0.15	3.00	3.50
ARS IBL CHBC <sub>2</sub> S <sub>4</sub> -10		X		44.0	0	44	44	2.9	0.39	2.5	3.4	13.8	3.10	6.2	14.7	4.4	0.90	2.3	4.6	3.17	0.20	3.00	3.44
ARS IBL CHBC <sub>2</sub> S <sub>4</sub> -14		X		40.0	2.00	38	42	7.3	1.71	5.5	9.5	12.7	1.90	9.9	14.5	3.7	0.60	2.5	4.4	3.48	0.34	3.17	3.92
ARS IBL CHBC <sub>2</sub> S <sub>4</sub> -17		X		44.0	0.00	44	44	7.0	6.06	1.7	14.3	13.7	2.50	7.5	14.4	4.2	0.80	2.3	4.6	3.27	0.23	3.09	3.60
ARS IBL CHBC <sub>2</sub> S <sub>4</sub> -34	X			44.0	2.00	38	42	2.6	0.21	2.4	2.9	13.0	3.00	5.2	14.4	4.0	0.80	1.9	4.2	3.22	0.15	3.09	3.42
ARS IBL CHBC <sub>2</sub> S <sub>4</sub> -64	X			44.5	0.50	44	45	3.4	0.58	2.9	4.2	13.1	3.40	4.1	14.5	4.1	1.10	1.3	4.3	3.21	0.17	2.97	3.35
ARS IBL CHBC <sub>2</sub> S <sub>4</sub> -81	X			40.0	2.00	38	42	2.5	0.10	2.4	2.6	13.7	2.80	6.2	14.9	4.2	0.50	2.9	4.3	3.25	0.18	3.00	3.44
ARS IBL CHBC <sub>2</sub> S <sub>4</sub> -96	X			41.0	3.00	38	44	2.3	0.30	1.9	2.5	14.5	4.90	2.2	15.7	4.5	1.40	0.8	4.8	3.23	0.05	3.16	3.26
ARS IBL CHBC <sub>2</sub> S <sub>4</sub> -101	X			38.0	0.00	38	38	3.0	0.67	2.5	3.9	13.0	2.90	5.8	15.0	4.0	0.80	1.9	4.6	3.26	0.16	3.13	3.48
ARS IBL CHBC <sub>2</sub> S <sub>4</sub> -105	X			43.0	1.00	42	44	2.1	0.87	1.0	2.9	13.6	1.20	11.9	15.0	4.2	0.20	4.1	4.6	3.21	0.28	2.85	3.53
ARS IBL CHBC <sub>2</sub> S <sub>5</sub> -28		X		44.0	0.00	44	44	2.2	0.56	1.5	2.7	13.8	0.70	13.1	15.0	4.2	0.30	3.8	4.7	3.32	0.28	2.89	3.48
Chipper		X		48.2	1.30	46	50	1.8	0.33	1.5	2.3	12.7	1.13	8.9	11.7	4.4	0.35	3.1	4.2	2.93	0.10	2.70	3.00
Line M29	X			47.8	1.50	45	49	2.2	0.68	1.6	4.3	12.8	1.31	8.0	11.8	4.2	0.44	2.5	3.8	3.10	0.14	3.10	3.50
Vlasset (Control cultivar)	X			48.2	0.90	47	49	2.7	0.69	2.3	3.3	12.9	0.69	9.2	11.0	4.4	0.39	3.3	4.6	2.95	0.22	2.20	2.80
Grand Mean—2007				44.3	1.3	42.7	45.8	3.1	1.0	2.2	4.4	13.0	2.0	8.0	14.0	4.1	0.6	2.7	4.4	3.2	0.2	2.9	3.4
Coefficient of variation (CV %)				8				51				5				6				4			
Mean separation within columns by LSD (0.05)								0.5				1.2				0.4							0.24

<sup>z</sup>ARS = USDA, Agricultural Research Service; ABL = advanced backcross line developed by repeated backcrossing (BC; M29 recurrent parent); IBL = inbred backcross line developed by backcrossing followed by repeated selfing (S); CH denotes chilling-tolerant plastome of 'Chipper' as the maternal parent.  
<sup>y</sup>Sex type calculated based upon percentage of the first 10 flowering nodes bearing female flowers: monoecious (M; < 50%), predominantly female (PF; 50% to 99%), and gynoecious (G; 100%) as averaged over plots and harvests. Nodes with both male and female flowers were classified as "male."  
<sup>x</sup>Days to anthesis was recorded as the number of days between transplanting and the appearance of the first fully expanded corolla averaged over replications and planting dates.  
<sup>w</sup>Fruit number per plant was recorded as the average cumulative fruit yield per plant over harvests and planting dates.  
<sup>u</sup>Fruit length (L) and diameter (D) measured as the mean length of 5–10 randomly chosen U.S. Department of Agriculture (USDA) Grade 2B-3B cucumber fruit averaged over harvests and planting dates.  
<sup>t</sup>Fruit length (L):diameter (D) ratio.  
<sup>s</sup>Year 1 (2006) and 2 (2007) produced significant differences within lines trialed across both years. Thus, averages across plantings and reps were contained and presented in a "within year" fashion.  
<sup>r</sup>LSD = least significant difference.  
<sup>NS</sup> = not significant.



Fig. 1. Fruits of chilling tolerant (4 °C) cucumber (*Cucumis sativus* L.) advanced backcross lines (ABL) 'Gabrielle' (Panel A; ARS-ABL CHBC<sub>3</sub>), 'Saira' (Panel B; ARS-ABL CHBC<sub>4</sub>), and 'Kodie' (Panel C; ARS-ABL CHBC<sub>5</sub>), and tolerant inbred backcross lines (IBL) CHBC<sub>2</sub>S<sub>4</sub>-28 (Panel D), CHBC<sub>2</sub>S<sub>4</sub>-81 (Panel E), and CHBC<sub>2</sub>S<sub>4</sub>-105 (Panel F). Plastid chilling tolerance derived from 'Chipper' (CH) as the donor parent.

(Madison, WI) to confirm their predicted chilling response based on their cytoplasmic constitutions (Gordon, 2009; Gordon and Staub, 2011; Gordon and Staub, 2013). Subsequently, both chilling-tolerant and chilling-sensitive ABL [i.e., BC<sub>1</sub>-BC<sub>5</sub> generations (5) in each of 2 lineages] and CHIBL (i.e., 10 of 57 BC<sub>2</sub>S<sub>4-5</sub> generated from 1 BC<sub>2</sub>) were genotyped using 11 simple sequence repeat (SSR) codominant markers, and then evaluated in field plots for their general horticultural performance (i.e., yield and quality) and general combining ability (GCA) for four economically important traits. Genetic relationships among ABL, CHIBL, and parents were determined on the basis of Principal Component Analysis (PCA). Predictably, PCA results indicated that ABL were genotypically similar to line M29 and IBL were genetically diverse, but more similar to M29 than to 'Chipper'.

To estimate GCA estimation, ABL and CHIBL were crossed with elite processing cultigens (i.e., lines H-19, 7012, WI 1983G, 5551, Gy 14, and 'Addis') (Gordon and Staub, 2013). Parents, progeny (BC<sub>1</sub> and F<sub>1</sub>), and control cultivar Vlasset were evaluated for days to anthesis, sex expression type, fruit length (L):diameter (D) ratio (L:D), and yield in replicated trials at each of four harvests in 2006 and five harvests in 2007, at the University of Wisconsin Experiment Station at Hancock, WI. In addition, 44 CHIBL were also evaluated for these traits

to determine their horticultural acceptability. Some ABL (10) and IBL (12) differed from their nonrecurrent and both parents, respectively, in many of the traits measured. The phenotypic variation among IBL was remarkably broad (Table 1).

### Description

On the basis of genotypic and phenotypic analysis, large portions of the nuclear genome of the recurrent parent (and its phenotype) were recovered by the BC<sub>2</sub> generation, with nearly complete recovery of recurrent parental traits and its nuclear genome occurring by the BC<sub>3</sub> in ABL (Gordon and Staub, 2013). The GCA of IBL was significant ( $P < 0.001$ ) for yield, fruit size, fruit length, fruit diameter, and L:D ratio. Given their GCA, and genotypic and phenotypic characteristics when compared with their parents and the control cultivars, three chilling-tolerant ABL (designated 'Gabrielle' ARS CHBC<sub>3</sub>, 'Saira' ARS CHBC<sub>4</sub>, and 'Kodie' ARS CHBC<sub>5</sub>), and 10 chilling-tolerant IBL (designated ARS CHBC<sub>2</sub>S<sub>4</sub>-10, -14, -17, -34, -64, -81, -96, -101, -105, and CHBC<sub>2</sub>S<sub>5</sub>-28) are being released for commercial use (Table 1). The ABL and IBL are intended for the development of elite chilling-tolerant cultivars (Fig. 1). The quality (e.g., size) and yield characteristics of chilling-tolerant ABL Gabrielle, Saira, and Kodie and some IBL (e.g., CHBC<sub>2</sub>S<sub>4</sub>-14 and -17) are similar to or

better than the control cultivar Vlasset under Wisconsin growing conditions.

Though not tested directly for chilling tolerance, all IBLs were molecularly genotyped against the set of three previously described plastidic tolerance-associated SNPs (Chung et al., 2007; Gordon and Staub, 2013). Their phenotypic response to chilling (resistant or sensitive) is predictable based upon previous chilling studies conducted on the original parents and other chilling-tolerant germplasm. With the presence of the tolerant plastid and SNP-based genotype assessment, as well as the known nuclear response to chilling of the recurrent parent (susceptible; M29), the ABLs and IBLs resulting from the initial 'Chipper' × M29 cross are chilling tolerant (i.e., no visible necrotic symptoms) at the first true-leaf stage under the chilling conditions under which the parents were evaluated (i.e., 5.5 h at 4 °C).

### Availability

Seed of chilling tolerant ABL and IBL from a hand-pollinated greenhouse increase may be obtained by addressing requests to P.W. Simon, Vegetable Crops Research, U.S. Department of Agriculture, Agricultural Research Service, Department of Horticulture, University of Wisconsin, Madison, WI 53706.

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