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Breeding Sweet Basil for Chilling Tolerance

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Sweet basil (*Ocimum basilicum* L., Lamiaceae) is a tropical species native to East Africa (Simon et al. 1990; Vieira and Simon 2000, 2006; Vieira et al. 2003). Sweet basil can be damaged in the field by chilling temperatures in spring and fall, and when stored and transported. Temperatures below 5°C lead to significant leaf necrosis and damage has been observed when temperatures drop below 10°C (Lange and Cameron 1997). Chilling causes brown discoloration of interveinal leaf areas, an increase of leaf blade thickening, decrease of plant growth, a reduction of the postharvest shelf life, and deterioration of quality and marketability (Cantwell and Reid 1993; Lange and Cameron 1994). The present study was undertaken to identify and develop chilling tolerant basil.

METHODS

Development of Chilling Tolerant and Chilling Sensitive Populations

Two cultivars of sweet basil including 'Italian Large Leaf' (ILL) (Battistini seeds, Cesena, Italy) and 'Sweet Basil' (Meyer Seeds, Baltimore, Maryland) were used in this study. Both cultivars were popular Italian Sweet Basil types grown commercially in the US for the fresh culinary herb market. Both cultivars, as with all other sweet basils, were sensitive to chilling injury damage both in the field and during storage and transport. Seeds were sown into 96 cell packs (75 mL) flats filled up with coir mix (Scottes, Redi-earth). Plants at the 6 week stage D (Hao 1998) were subjected to chilling temperatures under controlled conditions in a cold chamber (4°C for 48, 72, 96 and 120 hr). Since 2002, over 6,000 basil plants were individually screened for chilling tolerance. From this screen, over 70 single plant selections (SPS) including 46 tolerant and 24 sensitive were identified, and populations were created from each on the basis of presence or absence of visual chilling injury after exposure to controlled cold/chilling temperatures. From these developed lines, 2 tolerant and 2 sensitive genotypes were selected for heritability studies. These 4 genotypes were both crossed and selfed and have been followed for 5 generations. Twenty F_2 lines were evaluated for chilling tolerance. The chilling tolerant germplasm created from the SPS were maintained, selfed and re-grown in the greenhouse to confirm relative tolerance to chilling conditions under controlled cold conditions. Each line was subjected to chilling temperatures at each generation to ensure stability in order to better understand the inheritance of this trait. Only those lines which exhibited little to no chilling injury were continued and maintained as advanced lines for further development.

Plants were visually rated for the degree of chilling injury after one week post-chilling exposure. Visual chilling injury (necrosis, dark stains) was scored using a modified system described by Meir et al. (1997). Leaf injury (1 = no damage; 2 = severe dark spots; 3 = black stains on 30% of the total leaf area; 4 = black stains on 30%–50% of the total leaf area; 5 = black stains on more than 50% of the total leaf area); content of water (leaf water content/dry weight basis); and general performance of the plant were assessed in a completely randomized design with four replications.

Field Evaluation of Chilling Tolerant Basil Lines and Hybrids

In 2006, 28 chilling tolerant basil lines from second, third, and fourth generations plus a commercial control (Sweet Basil of Meyer Seeds from the original parent seedstock) were field evaluated in a randomized block design with three replications at the Clifford and Melda E. Snyder Research and Extension Farm in Pittstown,

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New Jersey. This study was to evaluate the general plant performance, essential oil content, scent strength and aroma characteristics of the chilling tolerant lines under field conditions. Growth data was recorded in the field and quality assessments were evaluated immediately after harvesting. Essential oils were extracted and analyzed according to the method of Vieira and Simon (2000).

RESULTS AND DISCUSSION

Chilling damage to basil appears as leaf necrosis and spotting (Fig. 1) and in some plants, nonreversible wilting or death. Seedlings varied in tolerance to chilling, and single plants that exhibited little to no damage were identified (Fig. 1, 2). Chilling injury was associated with leaf water content/DW basis over the 5 generations of selfed lines subjected to chilling stress (4°C, 72 hr) (Fig. 3). The highest chilling tolerant lines (BCR31, BCR36) had higher leaf water content than low tolerant lines (Fig. 4). Crossing BCS08, a chilling sensitive line (seed parent) with a tolerant line BCR31 (pollen parent), produced an F_2 population of 23 plants of which 18 were undamaged suggesting dominance for chilling resistance (Table 1). We found that chilling tolerance in basil is a heritable character and can be incorporated into breeding programs. The degree of both sensitivity and tolerance to chilling conditions varied in plants and selection using the described screen is efficient to identify genetic materials with improved chilling tolerance.

Field Trial Evaluation of Chilling Tolerant Basils

From 2002–2006, our selections and progress was focused on chilling tolerance with other evaluations principally related to ensuring that the original phenotype of the parent populations were maintained for market acceptance. In 2006, for the first time, we evaluated the field performance of a selected sub-set of our chilling

Fig. 1. Different Sweet Basils, 'Italian Large-Leaf' selections showing different chilling damage based on genotypes and leaf age following exposure to chilling temperatures. Three chilling tolerant basils CR31 exhibiting no damage plus to the right, 2 chilling sensitive wilted plants (left). Chilling injury on a chilling sensitivity genotype varied by leaf age and leaf position (right).

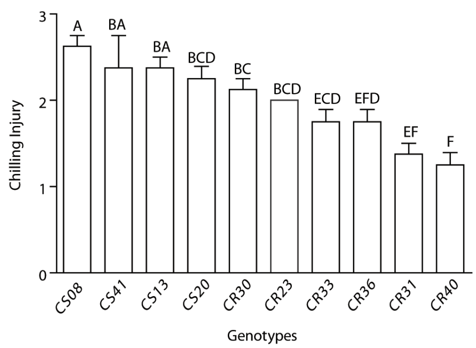

Fig. 2. Screening Sweet Basil, 'Italian Large-Leaf' basil lines to chilling injury (1=no damage, 2=low damage, 5=severe damage) following exposure of plants to low temperature stress.

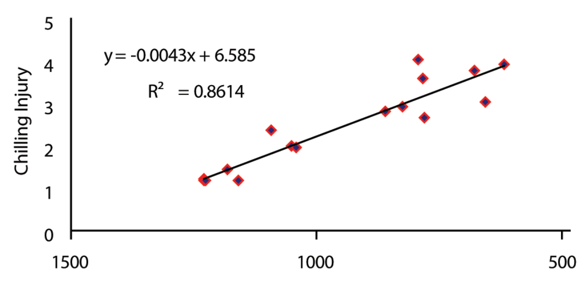

Fig. 3. Correlation between chilling injury (1=no damage, 2=low damage, 5=severe damage) and leaf water content/DW (LWC/DW) basis for sweet basil progenies (5 generations of selfed lines) subjected to chilling stress (4°C, 72 hr).

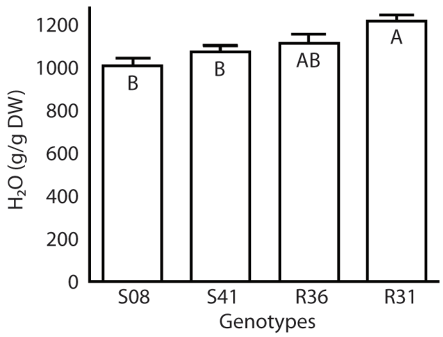

Fig. 4. Leaf water content (LWC/ DW) after chilling treatment (72 h/4°C) from 12 plants/line. P=0.005 (Duncan). Basil lines R31 and R36 are chilling tolerant; S08 and S41 are chilling sensitive.

Table 1. Chilling injury frequency for 'Sweet Basil' F_2 progenies from cross of BCS08 (mean=2.9) × BCR 31 (mean=1.3). Chilling grade means evaluated after exposure to low temperature stress (4°C for 72 h). N=23.

No. individual plants	Chilling injury grade[z]
18	1
2	1.5
2	3
1	4
0	5

[z]Visual chilling injury (necrosis, dark stains) scoring system adapted from Meir et al. (1997). Leaf injury (1 = no damage; 2 = severe dark spots; 3 = black stains on 30% of the total leaf area; 4 = black stains on 30%–50% of the total leaf area; 5 = black stains on more than 50% of the total leaf area).

tolerant lines and found that most of the chilling tolerant basil lines were superior in performance to 'Sweet Basil' of Meyer Seeds, the control. Final plant growth within this trial ranged from 434–970 g fresh wt/plant to 85–434 g dry wt/plant, and plants reached a maximum of 62 cm in height and 57 cm spread (data not presented). All of the chilling lines expressed the desired phenotype of 'Sweet' or 'Italian' basil (data not presented) and would be acceptable visually to the market. Lines varied in leaf size, leaf texture, and leaf surface shape, which will permit further selection. The chilling tolerant lines were of the Sweet Basil type and met the minimum requirements relating to growth and visual appearance. All lines exhibited a similar leaf color, flower color and spike color.

Each line was also assessed for aroma and taste. We had noted an association, but not a direct link, between the occurrences of a strong "licorice" aroma with improved chilling tolerance. However further studies (data not presented) found that that the high levels of methylchavicol were not linked genetically to chilling tolerance. Of the 28 chilling-tolerant lines, 16 exhibited an "acceptable" 'Sweet' basil/'Italian' basil aroma and taste, while the others exhibited varying degrees of a licorice aroma/taste (Table 2). The most promising chilling tolerant lines included CB6, CB11, CB25, and CB30. Among these most promising lines, variation was observed in actual leaf characteristics. The chilling tolerant lines grew well in the field and performed excellent at this site and there are now ample lines and hybrids from which to make further selections. Field testing of these selections across multiple sites is now needed.

REFERENCES

Cantwell, M.I. and M.S. Reid. 1993. Postharvest physiology and handling of fresh culinary herbs. J. Herbs Spices Med. Plants 1(3):93–127.

Hao, Z. 1998. Biosynthesis of methylcinnamate in *Ocimum basilicum*. PhD. Diss, Purdue Univ., West Lafayette, IN.

Lange, D.L. and A. Cameron. 1994. Postharvest shelf life of Sweet Basil. HortScience 29:102–103.

Lange, D.L. and A. Cameron. 1997. Pre- and postharvest temperature conditioning of greenhouse-grown Sweet Basil. HortScience 32:114–116.

Meir, S., R. Ronen, S. Lurie, and S.P. Hadas. 1997. Assessment of chilling injury during storage: Chlorophyll fluorescence characteristics of chilling-susceptible and triazole chilling tolerant basil leaves. Postharvest Biol. Technol. 10:213–220.

Simon, J.E., J. Quinn, and R.G. Murray. 1990. Basil: A source of essential oils. p. 484–489. In: J. Janick and J.E. Simon (eds.), Advances in new crops. Timber Press, Portland, Oregon.

Vieira, R.F., P. Goldsbrough, and J.E. Simon. 2003. Genetic diversity of basil (*Ocimum* spp.) based on RAPD markers. J. Am. Soc. Hort. Sci. 128:94–99.

Vieira, R.F. and J.E. Simon. 2000. Chemical characterization of basil (*Ocimum* spp.) found in the markets and used in traditional medicine in Brazil. J. Econ. Bot. 54(2):207–216.

Vieira, R.F. and J.E. Simon. 2006. Chemical characterization of basil (*Ocimum* spp.) based on aromatic volatiles. Flavor Frag. J. 21:214–221.

Table 2. Essential oil content and composition and organoleptic characteristics of the Rutgers chilling tolerant lines and hybrids field-grown in northern New Jersey, 2006.

Cultivar or line[z]	EO[y]	% of total EO: 1,8-Cineole	Linalool	MC[x]	Eugenol	Scent[w] strength	Aroma characteristic
Control							
Meyer	0.9±0.1	6.3±0.6	48.7±0.7	0.9±0.1	5.91±2.05	M	Sweet basil hint of licorice
Chilling sensitive lines							
CB1	0.9±0.1	3.4±0.3	44.9±4.6	1.1±0.2	2.73±0.35	M	Sweet basil
CB2	1.0±0.2	3.6±0.7	47.4±2.6	1.2±0.4	3.17±0.36	M	Sweet basil
CB3	1.0±0.1	3.4±0.5	40.6±8.6	9.2±0.1	8.15±9.60	M	Licorice aroma
CB10	0.7±0.0	4.6±0.2	37.0±4.8	1.9±1.4	4.36±0.48	Li	Sweet basil type
CB17	0.9±0.1	3.3±0.5	44.5±3.2	1.2±0.5	3.19±0.36	S	Sweet basil, aromatic
CB18	0.7±0.1	4.5±0.6	42.4±6.2	1.1±0.6	4.31±0.61	L	Sweet basil
CB22	0.8±0.2	3.2±1.1	40.9±10.7	0.8±0.4	2.77±0.15	M	Italian basil
CB23	1.1±0.1	3.8±1.1	42.6±11.9	1.5±0.1	2.07±0.47	M	Italian basil
Chilling tolerant lines							
CB4	1.1±0.2	2.2±0.2	29.7±4.5	43.0±3.9	0.05±0.03	L	Sweet basil/licorice
CB5	0.9±0.1	2.1±0.3	27.1±1.6	43.8±2.8	0.02±0	L	Sweet basil/licorice
CB6	0.6±0.4	5.9±0.7	45.0±3.4	2.0±1.3	3.97±3.79	M	Sweet Italian, licorice note
CB7	1.0±0.1	2.2±0.4	27.3±1.8	42.0±2.3	0.10±0.04	S	Italian basil w/mod licorice note
CB8	1.0±0.1	1.9±0.3	28.1±2.2	40.8±2.3	0.04±0.02	M	Methyl chavicol-licorice note
CB9	1.1±0.1	2.2±0.1	27.7±1.3	41.8±4.8	0.0±0.0	L	Mild methyl chavicol, licorice
CB11	1.0±0.1	3.2±0.7	39.4±3.4	17.0±6.8	0.98±0.70	L	Sweet basil type
CB14	0.8±0.1	4.1±0.7	42.3±3.2	0.9±0.1	3.42±0.80	L	Sweet basil type w/ licorice note
CB16	0.7±0.1	3.7±0.5	30.9±7.8	28.4±13	1.06±0.80	M	Sweet basil with licorice note
CB19	0.7±0.1	5.8±1.2	43.7±5.3	1.3±0.2	2.63±0.70	L	Sweet Italian basil
CB20	0.6±0.1	3.5±0.5	30.5±7.0	22.9±18	1.72±1.60	L	Sweet basil, trace of licorice
CB21	0.7±0.1	2.9±0.2	29.1±4.7	29.9±20	1.39±1.90	S	Licorice type
CB24	1.1±0.1	1.9±0.3	24.8±16.	37.1±1.4	0.0±0.0	S	Licorice aroma
CB25	1.2±0.1	1.6±0.7	29.3±2.0	41.7±2.1	0.01±0.0	L	Italian basil, licorice note
CB29	1.1±0.1	1.8±0.5	42.2±8.0	25.7±1.0	1.24±1.50	M	Sweet basil
CB30	0.7±0.1	5.0±0.5	49.2±2.6	1.5±0.4	2.96±0.80	M	Sweet basil, rich aroma

[z]The most promising chilling tolerant lines base on essential oil yield and overall flavor = CB6, CB11, CB25, CB29, CB30. The most promising chilling sensitive lines were CB2 and CB23.
[y] ml/100 g DW.
[x]MC=methylchavicol (estragole).
[w]Scent strength: L=light; M=moderate; S=strong.