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Effects of Cold Durations on Chilling Injury in Lagenaria Germplasm

Nebahat Sari
Department of Horticulture, Cukurova University, 01330, Adana, Turkey

Emily Silverman, Danny Reiland, and Todd C. Wehner
Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609

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Abstract. **Cucurbit plants usually are sensitive to chilling and easily damaged. Although bottle gourds, which are members of the Cucurbitaceae family, are considered as fresh vegetables in some Asian countries, their main use in recent years is to be used as rootstocks in grafted watermelon cultivation. We tested 163 bottle gourd accessions of the U.S. Department of Agriculture (USDA) genebank for cold tolerance in the early seedling stage. The experiment was conducted using controlled environment chambers with 3 chilling durations (36, 48, and 60 hours) at 4 °C. Chilling damage was rated 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, 9 = plant totally dead). We rated damage separately for the cotyledons, true leaf, and growing point. Cold damage was higher at a chilling duration of 60 hours, and decreased at 48 and 36 hours. Most tolerant cultigens were PI 491272, PI 491280, PI 491281, PI 491286, and PI 491326. Most susceptible were PI 381845, PI 381846, PI 534556, PI 636137, and PI 668365.**

As one of the main environmental factors, low temperature is limiting for crop production. Chilling injury (CI) is a stress caused by low, non-freezing temperatures (0 to 12 °C) (Dong et al., 2019). Temperatures below 10 °C may injure tropical and subtropical crops, such as species of the Cucurbitaceae (Raison, 1974). Although some plants can withstand cold stress, low temperatures during the production of most summer vegetables such as cucurbits can cause reduced growth, leaf yellowing, and foliar necrosis.

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] is an economically important cucurbit species belonging to the family Cucurbitaceae. It is cultivated throughout the world and total production is more than 118 million t (FAO, 2017). Grafting of watermelon is an established production practice that contributes resistance to soilborne diseases or tolerance to abiotic stresses (Bertucci et al., 2018). Watermelons are grafted to increase fruit and seed yield and plant growth by enhancing water and plant nutrient uptake (Kombo and Sari, 2019; Oda, 1995; Yetisir and Sari, 2003). The most common rootstocks for watermelon grafting are *Cucurbita* interspecific hybrids (*C. moschata* × *C. maxima*), the bottle gourd (*Lagenaria siceraria*) accessions that are highly resistant to soilborne fungi (Liu et al., 2015) and wild watermelon (*C. amarus*) (King et al., 2010; Kombo and Sari, 2019; Kong et al., 2014),

Chilling tolerance has been studied in cucumber (Chung et al., 2003; Kozik et al., 2007; Kozik and Wehner, 2008; Smeets and Wehner, 1997), watermelon (Ghanbari et al., 2018; Kozik and Wehner, 2014; Spalholz and Kubota, 2017), and melon (Diao et al., 2019; Li, 2012; Zhang et al., 2017); however, few studies have been done on bottle gourds (Ko et al., 2006; Liu et al., 2003; Xing et al., 2017), and *Lagenaria* genetic resources have not been screened for low temperature conditions.

A method for screening seedlings of cucumber for chilling tolerance was developed by Smeets and Wehner (1997) using controlled environmental conditions and cultigens that were tolerant (AR75-79, 'Chipper', 'Pixie', and 'Wisconsin SMR 18') or susceptible (Gy14, 'Marketmore 76', NCSU M28, NCSU M29, and 'Poinsett 76'). Researchers concluded that genetic variation for chilling damage was greater at the first true leaf than at the cotyledon stage. Kozik and Wehner (2014) used a similar method for screening of watermelons. They tested 16 watermelon cultigens using four chilling durations (6, 12, 24, or 36 h) and 2 chilling temperatures (2 or 4 °C). They found 36 h at 4 °C or 24 h at 2 °C as optimal conditions for chilling treatment; PI 244018 was the most tolerant cultigen and NH Midget and Golden were the most susceptible cultigens.

USDA–Agricultural Research Service, Plant Genetic Resources and Conservation Unit in Griffin, GA (http://www.ars-grin.gov) has more than 235 PIs of *Lagenaria siceraria* (Levi et al., 2009). In this study, 163 *Lagenaria* accessions in USDA were evaluated for tolerance to low temperature.

Materials and Methods

Plant material. One hundred eighty *Lagenaria siceraria* accessions from USDA were

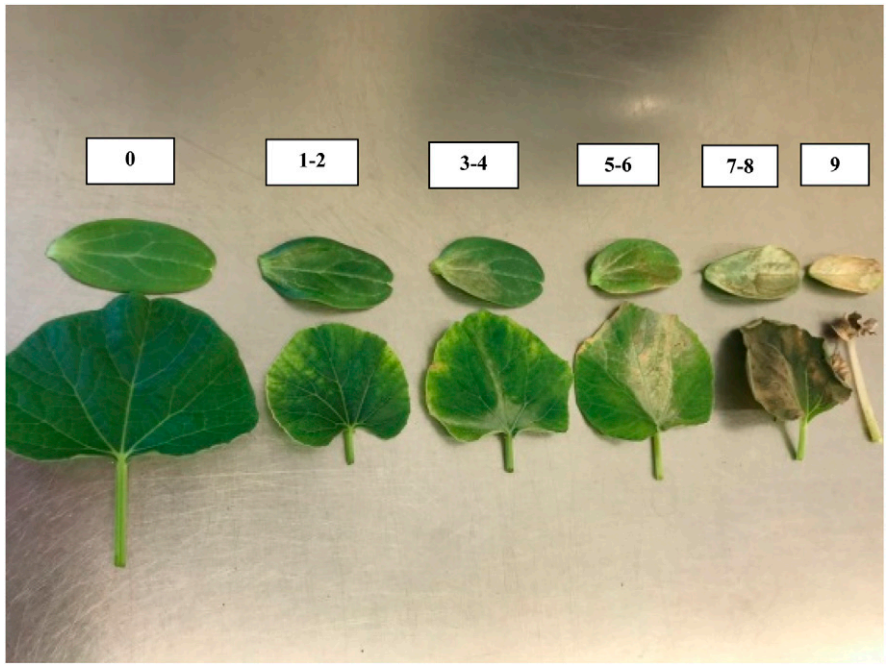

Fig. 1. Rating scale for chilling damage of 0 to 9: 0 = no damage, 1 to 2 = trace of damage, 3 to 4 = slight damage, 5 to 6 = moderate damage, 7 to 8 = advanced damage, 9 = plant dead (left).

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N.S. is the corresponding author. E-mail: nesari@cu.edu.tr.

Table 1. Seedling damages on growing point, first true leaf, and cotyledons with the observation of first and second week after 36-, 48-, and 60-h chilling treatments in 163 *Lagenaria* germplasms.[z]

	36-h duration			48-h duration			60-h duration			36-h duration			48-h duration			60-h duration		
	First week observation			First week observation			First week observation			Second week observation			Second week observation			Second week observation		
Cultigen	GP[y]	Lf[x]	Ct[w]	GP[y]	Lf[x]	Ct[w]	GP[y]	Lf[x]	Ct[w]	GP[y]	Lf[x]	Ct[w]	GP[y]	Lf[x]	Ct[w]	GP[y]	Lf[x]	Ct[w]
Grif 970	2.7	7.0	7.0	3.0	7.0	7.0	3.0	8.0	8.3	0.0	7.0	6.0	0.0	7.0	7.0	6.0	9.0	8.0
Grif 15945	2.7	6.3	5.3	1.7	6.0	4.7	3.7	7.0	6.0	0.0	6.7	6.7	0.0	7.0	7.0	0.0	6.0	6.0
PI 170463	2.0	5.7	7.3	1.0	6.0	7.0	4.7	7.0	8.0	0.0	8.0	9.0	0.0	5.0	9.0	9.0	9.0	9.0
PI 181913	1.7	6.0	6.0	2.0	5.0	7.0	3.0	8.0	6.7	0.0	6.3	5.3	0.0	3.7	7.0	0.0	7.0	5.7
PI 194994	2.0	6.3	6.0	1.0	6.0	5.7	3.7	7.3	7.0	0.0	5.3	4.7	0.0	6.3	6.0	0.0	7.3	7.7
PI 195321	2.7	6.0	5.7	2.3	5.3	5.0	3.7	7.7	6.7	0.0	5.3	5.0	0.0	6.0	6.0	0.0	7.0	7.0
PI 269505	3.0	5.3	7.3	3.0	7.0	6.7	5.0	7.0	8.0	0.0	4.3	6.7	0.0	7.3	7.0	0.0	6.7	8.3
PI 269506	3.0	7.0	5.7	2.7	7.0	6.0	6.7	7.3	8.3	0.0	6.0	5.0	0.0	8.0	8.0	9.0	9.0	9.0
PI 270456	4.0	5.3	5.0	2.7	7.0	7.0	5.0	8.0	8.0	0.0	3.7	3.7	0.0	8.0	8.0	0.0	8.0	7.7
PI 271356	3.0	3.0	7.0	3.0	4.0	8.0	4.0	7.0	7.7	0.0	2.7	6.3	0.0	3.7	7.7	0.0	5.7	7.7
PI 271360	3.3	6.0	5.7	2.3	6.7	5.3	3.7	7.0	6.7	0.0	5.7	5.0	0.0	7.0	5.7	0.0	5.7	6.3
PI 287533	4.0	5.7	5.7	4.0	6.3	6.0	4.0	7.7	7.0	0.0	5.7	5.3	0.0	6.3	6.0	0.0	8.7	6.7
PI 287534	1.7	2.0	4.0	3.3	6.0	5.7	4.0	8.0	7.0	0.0	3.0	5.0	0.0	6.0	5.7	0.0	8.3	7.7
PI 288497	2.3	5.3	6.7	2.7	5.7	7.3	5.0	5.0	6.0	0.0	5.3	6.3	0.0	5.7	7.3	0.0	5.0	6.0
PI 288499	2.7	6.3	6.7	2.0	8.0	8.0	3.3	8.0	8.0	0.0	6.0	6.0	0.0	8.0	8.0	0.0	8.0	8.0
PI 288500	3.0	7.0	7.0	3.0	9.0	7.0	5.0	8.3	7.7	0.0	6.7	6.7	0.0	9.0	8.0	0.0	9.0	8.0
PI 288503	2.0	2.0	6.0	2.0	4.0	6.0	3.0	5.0	7.0	0.0	2.0	6.0	0.0	8.0	8.0	0.0	8.0	8.0
PI 358045	3.3	5.7	6.3	3.3	5.3	5.7	4.0	8.0	7.3	0.0	7.0	5.7	0.0	7.0	7.0	0.0	8.0	9.0
PI 358046	2.7	6.0	8.0	2.7	7.0	8.0	6.0	8.0	8.0	0.0	6.0	8.3	0.0	7.0	7.7	0.0	8.0	8.3
PI 358048	2.0	5.7	5.3	2.0	8.0	7.3	3.0	8.0	7.0	0.0	7.0	6.0	0.0	7.3	6.7	0.0	8.0	7.0
PI 358049	2.3	6.0	7.3	2.0	7.0	8.0	4.0	6.7	8.0	0.0	7.0	8.0	0.0	7.0	7.0	0.0	6.7	8.0
PI 358050	1.7	4.0	4.3	3.0	7.0	7.0	2.7	6.3	6.0	0.0	5.0	5.0	0.0	7.7	7.3	3.0	7.0	7.7
PI 358051	2.0	5.7	5.3	3.0	7.0	7.0	3.0	7.0	7.0	0.0	7.0	8.0	0.0	8.0	6.7	0.0	7.0	7.0
PI 358052	1.7	6.0	6.3	2.3	8.0	7.0	4.0	6.3	8.3	0.0	7.0	7.0	0.0	7.7	7.0	0.0	7.7	9.0
PI 358053	2.0	6.0	8.0	2.0	5.7	8.0	4.3	6.0	7.0	0.0	7.0	9.0	0.0	6.7	8.0	0.0	8.0	9.0
PI 358059	2.0	5.3	7.3	2.0	6.0	8.0	3.3	5.7	7.7	0.0	7.0	9.0	0.0	7.0	8.0	0.0	7.0	9.0
PI 368635	1.7	5.0	4.0	1.7	5.3	6.0	5.7	6.0	7.7	0.0	5.0	4.0	0.0	7.0	7.0	3.0	7.7	8.0
PI 368638	1.7	6.0	5.0	2.0	5.3	4.7	3.7	5.7	6.0	0.0	6.0	5.0	0.0	8.0	8.0	0.0	8.0	7.0
PI 368639	2.0	5.3	7.0	2.0	6.0	7.0	3.7	7.0	7.3	0.0	5.7	6.7	0.0	6.7	7.8	0.0	8.0	9.0
PI 368640	1.0	3.0	2.0	1.0	4.0	2.7	1.7	5.0	5.3	0.0	5.0	5.0	0.0	6.7	7.3	0.0	7.0	8.0
PI 370474	2.0	4.3	4.7	2.3	5.0	4.7	3.0	7.0	7.0	0.0	6.0	4.0	0.0	7.0	7.0	0.0	7.0	6.3
PI 381822	1.7	4.7	2.7	2.0	5.7	4.0	2.7	5.3	5.0	0.0	5.0	2.7	0.0	6.3	6.0	0.0	7.0	7.0
PI 381823	2.7	4.3	3.0	3.3	4.7	4.7	3.0	6.3	7.0	0.0	4.0	4.0	0.0	4.7	4.7	0.0	5.3	7.0
PI 381825	2.0	5.7	5.7	3.0	4.3	5.3	3.7	5.7	6.0	0.0	6.0	6.0	0.0	6.0	6.0	0.0	7.0	9.0
PI 381826	3.0	7.0	7.0	2.7	6.7	5.7	4.0	6.3	7.7	0.0	7.0	7.0	0.0	6.3	5.7	0.0	7.0	7.3
PI 381827	2.3	5.3	4.7	2.7	7.0	5.0	5.7	6.7	7.7	0.0	5.0	5.3	0.0	7.0	5.3	3.0	8.3	9.0
PI 381828	2.0	5.7	3.7	3.0	7.0	5.0	3.7	6.7	6.3	0.0	7.0	4.0	0.0	6.7	5.0	0.0	7.3	7.0
PI 381829	3.0	7.0	6.0	3.3	7.0	6.3	4.0	7.0	6.0	0.0	7.0	6.0	0.0	7.0	5.3	0.0	8.0	5.3
PI 381830	2.7	7.0	7.0	2.7	8.0	6.7	4.3	7.3	7.7	0.0	8.0	7.0	0.0	8.0	7.0	3.0	9.0	7.7
PI 381831	3.0	5.0	6.0	3.0	8,0	7.0	3.0	8.0	7.0	0.0	5.0	6.0	0.0	8.0	7.0	0.0	8.0	7.0
PI 381832	2.7	4.7	5.0	3.0	7.7	6.7	4.0	8.0	6.0	0.0	3.3	3.7	0.0	8.0	7.0	0.0	8.0	7.0
PI 381834	3.0	7.0	6.3	3.0	7.7	7.0	3.7	7.7	6.7	0.0	8.0	6.0	0.0	7.3	6.0	0.0	8.0	7.0
PI 381835	3.3	7.3	5.3	3.3	7.7	6.7	3.7	7.3	6.7	0.0	7.0	7.0	0.0	7.7	6.0	0.0	7.3	5.7
PI 381836	3.0	7.3	6.3	3.0	7.7	6.7	4.7	8.0	6.7	0.0	6.7	6.7	0.0	7.7	7.0	0.0	8.7	7.3
PI 381837	3.3	7.0	6.3	4.0	7.3	7.3	4.0	7.7	7.3	0.0	7.0	6.0	0.0	7.0	8.0	0.0	8.0	8.0
PI 381838	3.7	7.3	6.7	3.0	7.0	8.0	5.7	8.0	8.0	0.0	7.0	6.0	0.0	6.3	8.0	0.0	8.7	8.0
PI 381839	3.0	6.7	6.3	2.7	7.0	7.0	3.7	6.7	6.0	0.0	7.0	6.0	0.0	6.7	6.0	0.0	7.0	7.0
PI 381840	4.0	7.0	7.0	4.0	7.0	7.0	3.7	7.7	7.0	0.0	7.0	7.0	0.0	7.0	6.3	0.0	8.0	8.0
PI 381842	4.3	7.0	6.3	3.3	7.0	6.7	3.7	6.0	6.0	0.0	7.0	6.3	0.0	6.7	6.7	0.0	7.0	8.0
PI 381843	3.0	7.3	5.7	3.0	8.0	6.3	8.0	8.0	9.0	0.0	8.0	7.3	0.0	8.0	7.3	0.0	8.0	9.0

(*Continued on next page*)

Table 1. (*Continued*) Seedling damages on growing point, first true leaf, and cotyledons with the observation of first and second week after 36-, 48-, and 60-h chilling treatments in 163 *Lagenaria* germplasms.[z]

	36-h duration			48-h duration			60-h duration			36-h duration			48-h duration			60-h duration		
	First week observation			First week observation			First week observation			Second week observation			Second week observation			Second week observation		
PI 381844	3.0	7.3	4.7	2.7	7.0	6.0	3.7	7.0	5.0	0.0	7.7	5.0	0.0	7.7	5.7	0.0	7.7	5.3
PI 381845	4.0	6.7	7.0	4.0	8.0	8.0	6.7	8.0	8.3	0.0	8.0	9.0	0.0	8.0	8.0	9.0	9.0	9.0
PI 381846	5.3	7.7	7.7	5.3	9.0	7.3	7.3	8.0	8.7	0.0	9.0	8.0	0.0	8.0	9.0	6.0	8.7	8.7
PI 381847	3.7	6.0	5.7	4.0	6.3	6.3	4.7	7.7	8.0	0.0	7.0	5.0	0.0	7.0	6.3	0.0	8.0	8.0
PI 381848	4.3	7.3	7.7	4.3	8.0	7.3	5.7	8.0	8.0	0.0	8.0	7.3	0.0	8.0	7.7	0.0	8.3	8.0
PI 381849	2.0	6.3	7.0	5.0	7.3	7.3	4.7	7.0	7.0	0.0	8.0	7.0	0.0	8.0	7.0	0.0	8.0	7.7
PI 381850	5.0	7.3	7.0	4.0	8.0	7.0	7.7	8.0	8.0	0.0	7.0	8.0	0.0	8.0	7.0	0.0	8.0	7.7
PI 381851	2.7	6.7	5.0	3.3	7.7	8.0	5.0	8.0	8.0	0.0	7.0	5.0	0.0	8.0	7.0	0.0	8.3	8.3
PI 381854	4.0	7.0	6.0	4.0	8.0	7.0	6.0	8.7	7.7	0.0	7.0	6.0	0.0	8.0	7.0	0.0	9.0	7.0
PI 438844	3.3	7.0	7.0	5.0	8.0	8.0	5.0	8.0	7.7	0.0	6.7	5.0	0.0	7.7	7.7	0.0	7.0	7.7
PI 438846	2.0	5.0	5.0	2.0	8.0	5.0	5.0	7.7	8.0	0.0	6.3	5.0	0.0	7.7	5.3	0.0	8.3	8.0
PI 442368	2.0	6.0	6.3	2.0	5.7	7.3	5.0	7.3	8.0	0.0	5.3	5.3	0.0	6.0	7.3	9.0	8.3	8.3
PI 458736	2.0	6.3	6.7	3.0	7.3	6.7	4.7	6.0	7.7	0.0	4.3	6.0	0.0	7.0	6.3	0.0	8.3	8.3
PI 470260	3.7	6.0	6.0	3.7	8.0	7.0	3.7	7.3	7.3	0.0	6.0	8.0	0.0	8.0	8.0	0.0	8.0	8.0
PI 487482	1.0	3.7	4.0	2.0	4.0	5.0	4.3	7.0	8.0	0.0	3.3	3.7	0.0	5.0	7.0	0.0	8.3	8.3
PI 491252	1.7	4.7	4.7	2.0	4.0	6.0	2.0	4.0	5.0	0.0	4.7	4.7	0.0	5.0	6.0	0.0	6.0	6.0
PI 491266	1.7	3.3	2.7	2.0	5.0	5.0	3.7	8.0	4.7	0.0	3.3	2.7	0.0	6.0	6.0	0.0	8.7	9.0
PI 491267	1.7	3.0	3.0	2.0	4.0	4.0	3.0	5.0	6.3	0.0	2.3	3.7	0.0	3.7	3.7	0.0	7.7	6.3
PI 491269	1.7	1.7	2.0	3.0	5.0	7.0	3.0	5.3	6.0	0.0	2.0	4.0	0.0	6.0	7.0	0.0	5.7	6.0
PI 491270	2.3	3.3	3.7	2.7	4.0	3.7	4.3	5.7	6.0	0.0	2.7	3.7	0.0	5.0	3.7	0.0	7.7	7.7
PI 491272	1.3	2.0	2.7	1.0	1.0	3.0	2.0	5.3	4.0	0.0	0.0	2.0	0.0	2.0	3.0	0.0	3.3	3.3
PI 491273	1.7	4.0	4.0	2.0	5.0	4.3	2.0	5.7	3.3	0.0	3.7	3.3	0.0	5.3	5.0	0.0	7.0	6.0
PI 491274	1.0	2.7	2.7	3.3	3.7	6.7	3.7	4.0	6.7	0.0	3.3	3.7	0.0	3.7	6.7	0.0	4.3	6.7
PI 491275	2.0	4.0	3.7	2.3	6.0	4.0	2.7	5.0	3.0	0.0	6.0	7.0	0.0	5.3	5.3	0.0	5.3	4.0
PI 491276	2.0	4.0	4.0	2.0	5.0	5.3	3.0	5.0	6.0	0.0	5.0	5.0	0.0	6.0	4.0	0.0	5.0	6.0
PI 491277	1.7	3.0	3.3	2.0	6.0	5.0	2.7	5.7	4.7	0.0	3.3	3.3	0.0	7.0	3.3	0.0	7.0	5.0
PI 491278	1.7	3.7	3.0	2.0	5.0	5.0	2.0	6.7	4.3	0.0	2.3	3.3	0.0	6.0	5.0	0.0	6.3	3.3
PI 491280	1.3	2.3	2.7	1.0	3.0	2.0	2.0	5.0	6.7	0.0	2.0	4.0	0.0	4.7	5.0	0.0	5.0	6.3
PI 491280	1.0	3.0	2.3	2.0	3.0	4.0	2.0	4.0	4.0	0.0	2.3	2.0	0.0	2.0	4.0	0.0	4.0	6.7
PI 491282	1.0	4.7	2.7	2.0	4.0	6.0	3.0	5.0	5.7	0.0	5.0	7.0	0.0	6.0	4.0	0.0	6.0	5.7
PI 491283	1.7	3.0	3.0	2.0	4.0	4.0	3.7	6.0	5.7	0.0	2.0	3.3	0.0	4.3	5.3	0.0	5.7	5.3
PI 491286	1.3	3.0	1.3	1.0	2.0	1.0	2.3	5.0	5.3	0.0	4.0	4.0	0.0	3.0	2.0	0.0	5.7	5.3
PI 491287	2.3	3.0	3.7	2.3	4.0	4.0	7.0	9.0	9.0	0.0	5.0	4.0	0.0	4.0	8.0	3.0	9.0	9.0
PI 491288	2.7	5.0	4.0	2.7	4.7	3.7	2.7	3.7	3.3	0.0	4.7	4.0	0.0	6.0	5.0	0.0	4.3	4.0
PI 491289	2.0	4.3	4.0	2.0	6.0	6.3	3.0	4.7	6.3	0.0	6.3	6.3	0.0	7.0	6.0	0.0	7.0	6.3
PI 491290	2.0	4.0	3.7	3.0	4.0	4.0	2.7	6.3	6.7	0.0	4.0	5.0	0.0	4.0	5.0	0.0	5.7	6.3
PI 491291	2.0	4.3	3.7	3.0	4.0	7.0	2.0	6.0	6.7	0.0	5.0	6.0	0.0	4.7	6.7	0.0	7.0	7.0
PI 491292	2.0	4.0	4.0	2.0	4.3	6.0	2.7	6.7	6.7	0.0	4.0	4.0	0.0	3.7	5.7	0.0	6.3	6.3
PI 491293	2.0	6.0	3.7	3.7	6.7	5.7	3.7	6.7	6.3	0.0	5.3	4.7	0.0	6.7	6.0	0.0	7.0	9.0
PI 491294	1.7	3.0	3.0	2.0	4.0	5.0	2.0	4.7	4.7	0.0	4.0	4.0	0.0	4.7	5.7	0.0	4.7	4.3
PI 491295	2.0	4.0	3.7	2.0	5.0	5.0	3.0	5.7	6.0	0.0	6.7	6.0	0.0	6.0	5.0	0.0	7.0	7.0
PI 491296	1.7	2.7	2.3	2.0	3.0	4.0	2.0	4.0	4.0	0.0	6.0	5.0	0.0	5.0	3.7	0.0	5.0	4.3
PI 491297	2.0	4.0	3.7	2.0	6.0	4.0	2.3	6.3	4.0	0.0	5.0	4.0	0.0	6.0	3.7	0.0	6.3	6.0
PI 491298	2.3	5.7	4.0	2.3	7.0	6.3	2.7	6.7	6.3	0.0	6.3	6.0	0.0	7.0	6.3	0.0	6.3	6.0
PI 491299	2.0	4.0	3.0	2.0	6.0	7.0	3.3	6.3	7.0	0.0	5.0	4.3	0.0	6.7	6.7	0.0	6.3	8.7
PI 491300	2.0	3.7	3.0	2.0	6.3	7.0	3.0	7.0	7.0	0.0	5.3	4.0	0.0	6.0	6.3	0.0	7.0	7.0
PI 491302	2.0	4.3	3.3	2.0	6.0	4.0	3.0	7.0	7.0	0.0	5.0	4.0	0.0	6.0	4.0	0.0	7.0	6.7
PI 491303	2.0	4.7	5.0	2.0	5.0	5.0	2.0	5.3	5.0	0.0	7.0	7.0	0.0	6.0	4.7	0.0	5.3	5.3
PI 491304	2.0	4.0	2.7	2.0	4.0	4.0	3.0	6.3	6.0	0.0	7.0	6.0	0.0	5.0	5.0	0.0	6.0	9.0
PI 491305	2.7	3.7	3.7	3.0	5.3	5.0	3.0	7.0	5.0	0.0	3.3	4.3	0.0	5.3	5.0	0.0	7.7	7.7

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Table 1. (*Continued*) Seedling damages on growing point, first true leaf, and cotyledons with the observation of first and second week after 36-, 48-, and 60-h chilling treatments in 163 *Lagenaria* germplasms.[z]

	36-h duration			48-h duration			60-h duration			36-h duration			48-h duration			60-h duration		
	First week observation			First week observation			First week observation			Second week observation			Second week observation			Second week observation		
PI 491306	2.0	5.0	4.7	3.0	6.0	6.0	5.3	7.3	7.0	0.0	7.0	6.0	0.0	6.0	6.0	0.0	7.3	7.3
PI 491308	2.0	6.0	4.0	2.0	6.7	5.7	5.0	8.0	7.0	0.0	7.0	6.0	0.0	6.0	4.7	0.0	8.0	8.3
PI 491309	2.0	5.7	5.7	2.0	7.3	7.0	3.7	6.7	6.7	0.0	6.0	6.0	0.0	7.3	6.3	0.0	7.0	6.7
PI 491311	2.7	7.0	5.7	4.0	8.0	7.0	5.3	7.0	6.7	0.0	7.0	6.0	0.0	8.0	7.0	0.0	7.0	8.0
PI 491312	2.3	5.3	6.0	3.0	6.0	8.0	3.0	7.0	7.0	0.0	4.7	7.0	0.0	7.0	8.0	0.0	7.0	8.0
PI 491313	2.3	6.0	6.0	3.0	6.7	6.7	4.0	7.7	8.0	0.0	7.0	5.3	0.0	6.7	6.7	0.0	7.3	8.3
PI 491314	2.0	5.0	4.7	2.0	5.0	4.7	2.0	6.0	4.7	0.0	6.0	7.0	0.0	6.0	6.0	0.0	6.0	6.0
PI 491315	2.3	5.7	5.0	3.0	7.0	7.0	3.0	6.7	6.7	0.0	5.7	6.0	0.0	6.7	6.7	3.0	7.7	7.3
PI 491316	2.0	5.3	3.7	2.0	6.7	7.0	2.0	4.0	4.0	0.0	6.0	6.0	0.0	7.0	8.0	0.0	6.7	7.7
PI 491317	2.7	5.0	3.7	3.0	5.0	4.0	3.0	4.3	4.0	0.0	7.0	4.0	0.0	6.0	6.0	0.0	4.7	4.3
PI 491318	1.3	1.7	1.7	3.0	5.0	5.0	2.7	5.7	4.0	0.0	6.0	4.0	0.0	3.7	7.0	0.0	6.0	7.3
PI 491319	1.0	4.0	3.0	3.0	5.7	6.0	3.0	6.0	6.0	0.0	7.0	5.0	0.0	7.0	6.0	0.0	6.7	7.3
PI 491320	1.0	3.0	3.0	2.0	4.0	5.7	2.0	4.0	6.0	0.0	4.0	4.0	0.0	3.7	6.7	0.0	4.0	6.7
PI 491321	2.0	4.0	3.7	2.0	6.3	6.0	2.7	5.3	6.0	0.0	5.0	4.0	0.0	5.7	5.3	0.0	5.3	6.0
PI 491322	1.3	1.7	2.7	2.0	7.0	7.0	2.7	6.0	6.0	0.0	4.0	4.0	0.0	6.7	5.7	0.0	4.0	8.3
PI 491323	1.7	3.7	3.3	2.0	4.0	4.0	2.0	5.0	4.0	0.0	4.0	4.0	0.0	4.0	4.0	0.0	5.0	4.0
PI 491324	2.0	2.0	2.0	2.0	5.0	7.0	2.0	5.0	7.0	0.0	2.0	2.0	0.0	6.3	7.3	0.0	5.3	7.0
PI 491325	1.7	2.3	3.7	2.0	7.0	5.0	2.0	7.0	4.7	0.0	4.0	4.0	0.0	7.0	5.0	0.0	5.7	4.3
PI 491326	1.3	1.7	1.7	2.0	3.0	3.0	1.3	4.0	2.7	0.0	3.0	2.0	0.0	3.0	3.3	0.0	3.0	2.7
PI 491329	2.0	3.7	2.3	2.7	6.7	5.3	2.3	5.3	3.0	0.0	5.0	4.0	0.0	7.7	4.7	0.0	5.0	4.3
PI 491330	2.0	3.7	2.3	2.0	7.0	3.0	2.0	6.3	4.3	0.0	4.7	3.0	0.0	7.0	6.0	0.0	7.0	5.0
PI 491331	1.7	3.3	3.3	2.0	6.3	6.3	3.0	5.3	5.7	0.0	4.0	3.3	0.0	7.0	5.3	0.0	7.0	6.3
PI 491332	1.0	2.7	3.3	2.7	6.0	6.0	2.7	5.3	5.3	0.0	3.0	5.0	0.0	6.3	6.0	0.0	6.3	5.0
PI 491333	1.7	3.7	3.0	2.0	6.0	6.3	2.7	5.7	5.0	0.0	4.0	3.0	0.0	6.7	6.7	0.0	4.0	5.7
PI 491335	1.7	2.7	3.0	2.0	5.0	5.0	2.0	5.0	4.7	0.0	2.7	2.7	0.0	5.0	4.3	0.0	3.0	6.3
PI 491336	1.7	3.7	4.0	2.0	6.0	6.0	2.0	5.7	6.3	0.0	7.0	7.0	0.0	7.0	6.3	0.0	7.0	7.0
PI 491337	1.0	3.3	4.0	2.0	5.0	6.0	2.0	5.7	4.0	0.0	6.0	5.0	0.0	5.0	6.0	0.0	7.0	5.7
PI 491339	1.7	4.7	4.0	2.0	7.0	5.0	2.0	5.3	5.7	0.0	6.7	6.3	0.0	6.7	5.7	0.0	5.3	6.3
PI 491343	1.7	4.0	5.7	3.0	6.3	6.3	3.0	6.0	4.7	0.0	7.0	6.7	0.0	7.0	6.3	0.0	7.0	6.7
PI 491346	2.3	4.0	4.3	3.0	5.0	5.3	2.7	5.3	5.7	0.0	6.0	6.0	0.0	7.0	5.3	0.0	6.0	5.3
PI 491348	2.3	4.3	4.7	3.0	7.0	6.7	2.7	5.7	5.3	0.0	4.0	5.0	0.0	8.0	6.0	0.0	7.3	8.0
PI 491349	3.0	6.0	6,0	3.0	6.7	6.0	3.0	6.0	6.3	0.0	7.0	5.7	0.0	6.0	5.3	0.0	6.0	6.3
PI 491350	3.0	7.0	5.0	3.0	7.0	7.0	3.0	6.7	6.7	0.0	7.0	6.0	0.0	6.7	6.7	0.0	7.0	7.0
PI 491351	2.0	5.3	5.3	2.0	6.0	6.0	2.0	6.0	5.7	0.0	4.0	5.0	0.0	6.0	5.3	0.0	8.3	9.0
PI 491352	2.0	6.0	5.3	2.0	6.0	6.0	2.7	2.7	5.3	0.0	6.0	5.0	0.0	6.0	6.0	0.0	4.3	5.3
PI 491353	2.0	6.0	5.0	2.0	5.7	5.0	3.3	5.3	5.3	0.0	6.0	4.5	0.0	5.7	5.0	0.0	8.0	7.0
PI 491354	3.0	6.0	6.0	3.0	6.0	6.0	4.0	5.7	6.0	0.0	6.0	6.0	0.0	6.0	6.0	0.0	6.0	6.0
PI 491355	2.0	6.0	4.0	2.0	6.0	4.0	3.3	5.7	5.7	0.0	6.0	8.3	0.0	6.0	4.0	0.0	5.7	5.7
PI 491356	3.0	6.7	6.0	3.0	6.7	6.0	3.0	6.7	6.7	0.0	7.0	7.0	0.0	5.3	7.3	0.0	6.7	6.7
PI 491357	3.0	5.0	4.7	2.7	4.0	5.0	3.0	5.0	5.7	0.0	7.0	6.0	0.0	3.7	4.7	0.0	7.0	7.0
PI 491358	3.0	6.0	4.7	2.7	5.7	4.7	2.7	6.7	6.7	0.0	6.0	5.0	0.0	5.0	4.7	0.0	8.0	7.0
PI 491358	3.0	6.3	5.0	2.0	5.3	5.0	2.0	4.7	4.7	0.0	5.7	4.3	0.0	5.7	5.0	0.0	5.0	8.0
PI 491360	1.7	3.7	4.0	2.0	2.3	2.7	2.0	3.0	3.0	0.0	3.7	3.7	0.0	2.7	4.3	0.0	3.0	3.0
PI 491361	2.0	6.0	5.0	2.0	6.0	5.0	2.0	6.0	5.0	0.0	5.0	7.0	0.0	6.0	6.0	0.0	6.0	9.0
PI 491363	3.0	5.3	5.7	3.0	5.0	5.0	2.0	5.0	5.0	0.0	5.0	6.0	0.0	5.0	4.7	0.0	5.0	5.0
PI 491365	2.0	3.7	2.3	1.7	3.3	2.3	1.7	3.3	2.3	0.0	5.0	4.0	0.0	3.7	5.7	0.0	2.7	6.0
PI 491366	2.0	5.3	3.7	2.3	5.3	4.3	2.3	5.3	5.0	0.0	5.0	6.7	0.0	4.0	5.7	0.0	5.7	5.3
PI 491367	2.0	5.0	5.0	3.0	6.0	5.0	3.0	5.3	5.0	0.0	5.0	5.0	0.0	5.7	5.3	0.0	5.3	5.3
PI 494865	2.3	5.7	4.3	3.0	5.7	4.0	2.7	5.7	6.0	0.0	5.7	7.0	0.0	5.7	4.0	0.0	5.3	7.0
PI 500808	2.3	5.7	6.3	3.0	6.0	6.0	3.0	5.7	6.0	0.0	5.3	7.0	0.0	5.7	6.3	0.0	6.7	6.0

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Table 1. (*Continued*) Seedling damages on growing point, first true leaf, and cotyledons with the observation of first and second week after 36-, 48-, and 60-h chilling treatments in 163 *Lagenaria* germplasms.[z]

	36-h duration			48-h duration			60-h duration			36-h duration			48-h duration			60-h duration		
	First week observation			First week observation			First week observation			Second week observation			Second week observation			Second week observation		
PI 500820	2.0	6.3	6.0	3.0	6.3	5.0	4.0	6.3	5.7	0.0	6.7	5.7	0.0	5.7	5.3	0.0	7.0	7.0
PI 500828	2.7	7.0	7.3	2.7	8.0	7.7	3.0	6.7	6.7	0.0	7.0	9.0	0.0	7.7	7.7	0.0	7.7	8.0
PI 534552	2.0	7.0	8.0	2.0	7.3	8.0	6.7	8.0	9.0	0.0	7.0	8.0	0.0	6.3	8.0	3.0	8.3	9.0
PI 534556	2.0	6.7	8.7	4.0	8.0	9.0	3.7	7.7	8.7	0.0	8.0	9.0	0.0	7.7	8.7	0.0	8.0	9.0
PI 636137	2.3	7.7	8.7	4.0	8.0	9.0	5.0	7.3	9.0	0.0	9.0	9.0	0.0	8.0	9.0	0.0	9.0	9.0
PI 639723	1.7	5.3	9.0	2.0	6.0	8.0	4.0	7.0	7.7	0.0	6.7	9.0	0.0	7.0	9.0	0.0	7.0	8.3
PI 641946	1.7	6.7	7.3	4.0	8.0	8.0	5.0	8.0	8.3	0.0	7.0	7.0	0.0	8.0	8.0	0.0	7.0	8.3
PI 642045	1.7	4.3	6.3	4.0	6.0	6.0	4.0	6.0	7.0	0.0	6.7	6.7	0.0	8.0	8.0	0.0	8.0	8.0
PI 658555	1.7	6.0	7.3	2.0	7.0	8.0	3.7	7.7	8.0	0.0	7.3	6.7	0.0	7.0	8.0	0.0	7.7	8.3
PI 666109	1.7	4.0	8.0	3.0	7.0	8.0	3.0	7.0	8.0	0.0	7.0	9.0	0.0	7.0	8.3	0.0	6.0	7.7
PI 675112	2.0	4.7	6.0	2.0	7.0	7.0	2.7	7.0	7.0	0.0	6.0	6.0	0.0	6.7	6.7	0.0	7.0	7.0
PI 668365	2.7	7.3	8.0	3.0	8.0	8.0	3.0	8.0	8.0	0.0	8.0	9.0	0.0	7.3	9.0	0.0	7.0	9.0
PI 668366	2.7	7.3	7.3	3.0	8.0	8.0	3.0	8.0	8.0	0.0	8.0	7.3	0.0	8.7	8.3	0.0	8.0	6.0
F ratio	3.835	3.083	3.016	5.339	6.047	5.364	4.357	5.032	6.234	5.098	6.236	4.966	3.654	6.550	3.005	4.564	5.679	4.550
Prob>f	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Min	1.0	1.7	1.3	1.0	1.0	1.0	1.3	2.7	2.3	0.0	0.0	2.0	0.0	2.0	2.0	0.0	2.7	2.7
Max	5.3	7.7	9.0	5.3	9.0	9.0	8.0	9.0	9.0	0.0	9.0	9.0	0.0	9.0	9.0	9.0	9.0	9.0
Mean	2.3	5.1	5.0	2.6	6.0	5.9	3.5	6.4	6.4	0.0	5.6	5.6	0.0	6.3	6.3	0.4	6.8	7.0

[z]Damage rating 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, 9 = plant dead.
[y]Growing point.
[x]Leaf.
[w]Cotyledons.

planted; however, only 163 accessions germinated sufficiently to be evaluated in this experiment. Seeds were sown in 48-cell flats filled with a mixture of sand and peat in a 1:1 ratio. After sowing, the flats were placed in a 37 m^2 environmentally controlled greenhouse at the North Carolina State University Phytotron. The greenhouse was set to maintain the air temperature at 26/22 ± 3.0 °C (day/night), 400 ppm CO_2, 50% relative humidity, and a 14-h photoperiod that was supplemented using sixteen 1000-W metal halide lamps. Plants were watered twice daily, in the morning with a standard phytotron nutrient solution (Thomas et al., 2005) and afternoon with reverse osmosis water. The seeds of each accession were sown with five replications of two seeds each with randomized block experimental design. Seedlings were kept in the greenhouse until the first true leaf stage.

Chilling treatments. Chilling tests were performed according to the method of Kozik and Wehner (2014) with some modifications. The seedling flats were moved from the greenhouse to the chilling chambers when the seedlings reached the first true leaf stage. Chilling treatments were conducted in environmentally controlled plant growth chambers set to maintain a constant 4.0 ± 0.5 °C with a constant light intensity of ≈450 $mmol{\cdot}m^{-2}{\cdot}s^{-1}$. Seedlings were kept under chilling conditions for a duration of 36, 48, or 60 h before being returned to the greenhouse. Control plants were left unchilled (without moving to a chilling room, in the same greenhouse) as a reference for the rating scale (damage rating of 0). The manifestation of chilling damage as water-soaked patches afterward turning yellow or necrotic occurred some days after chilling treatments on the shoots (cotyledons, true leaves, and growing points). Plants were rated 7 and 14 d after chilling for damage on the cotyledons, first true leaf, and growing point for each plant in each plot. The scale used was 0 to 9: 0 = no damage (based on the unchilled control plants), 1 to 2 = trace of damage, 3 to 4 = slight damage (20% to 50% of tissue necrotic), 5 to 6 = moderate damage (50% to 70% of tissue necrotic), 7 to 8 = advanced damage (70% to 90% of tissue necrotic), 9 = plant dead (90% to 100% tissue necrotic) (Kozik and Wehner, 2014). Figure 1 shows the scale of 0 to 9 on the cotyledons and first true leaves in *Lagenaria*.

Experiment design and data analysis. The experiment was conducted in a randomized complete block design with three replications. The effects of the chilling treatments were subjected to analysis of variance by 36, 48, or 60 h. Data were analyzed by the TABULATE and GLM procedures of SAS v9.0 Statistical Software Package (SAS Institute, Cary, NC). Mean separations of cultigens were tested using Fisher's protected least significant difference at $\alpha = 0.05$. The most susceptible or tolerant cultigens were chosen based on damage rating.

Results and Discussion

Damage rating after 1 and 2 weeks CI are shown on Table 1 and Fig. 2. At 36 h chilling duration, seedling damage on growing point ranged 1.0 to 5.3 (average 2.3), true leaf ranged 1.7 to 7.7 (average 5.1), and cotyledons ranged 1.3 to 9.0 (average 5.0). At 48 h chilling duration, seedling damage on growing point ranged 1.0 to 5.3 (average 2.6), true leaf ranged 1.0 to 9.0 (average 6.0), and cotyledons ranged 1.0 to 9.0 (average 5.9). At 60 h chilling duration, seedling damage on growing point ranged 1.3 to 8.0 (average 3.5), true leaf ranged 2.7 to 9.0 (average 6.4), and cotyledons ranged 2.3 to 9.0 (average 6.4). As chilling duration increased, seedling damage increased.

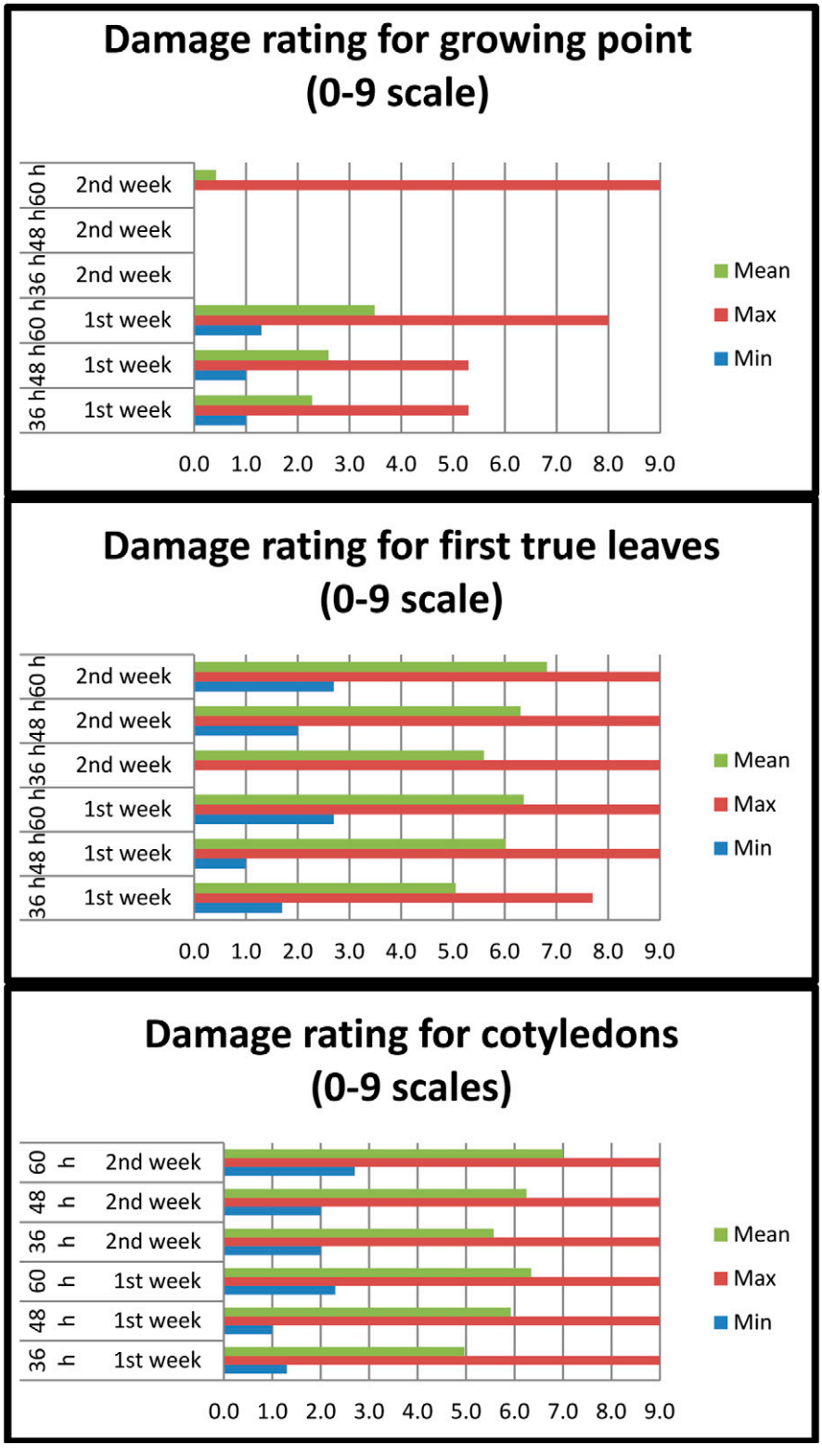

Fig. 2. Minimum, maximum, and mean of seedling damages on growing point, first true leaves, and cotyledons with the observation of first and second week after 36-, 48-, and 60-h chilling treatments.

The accessions tested in this research were mostly tolerant to the cold injury. The most tolerant were PI 491272, PI 491280, PI 491281, PI 491286, and PI 491326 and the most susceptible were PI 381845, PI 381846, PI 534556, PI 636137, and PI 668365 (Fig. 3A). Average chilling rating for the most tolerant and most susceptible PI accessions on first true leaf with the observation of first week after 36-h, 48-h, and 60-h treatments is shown in Table 2. As can be seen from the Table 2, although the scale value varies between 0 and 4 in tolerant accessions, these values were observed between 7 and 9 in susceptible accessions.

Two weeks after chilling treatment, *Lagenaria* seedlings no longer showed damage to the growing point, and had recovered in the greenhouse (Fig. 3B) from the 36-h and 48-h applications. Plants treated for 60 h had only a small amount of damage to the growing point in a few of the accessions. At 36 h chilling duration, seedling damage on true leaf ranged from 0.0 to 9.0 (average 5.6), and cotyledons ranged from 2.0 to 9.0 (average 5.6). At 48 h chilling duration, seedling damage on true leaf ranged from 2.0 to 9.0 (average 6.3), and cotyledons ranged from 2.0 to 9.0 (average 6.3). At 60 h chilling duration, seedling damage on true leaf ranged from 2.7 to 9.0 (average 6.8); and cotyledons ranged from 2.7 to 9.0 (average 7.0).

In our study, most of the bottle gourd accessions were highly tolerant, even at the longest chilling duration (60 h), at 4 °C and full light conditions. Spalholz and Kubota (2017) reported that watermelon (*Citrullus lanatus*) seedlings were generally chilling sensitive and therefore difficult to hold at low temperatures. However, the rootstocks used for watermelon grafting, interspecific squash (*Cucurbita maxima* × *C. moschata*) and bottle gourd (*L. siceraria*), are chilling tolerant. In our study, seedlings of bottle gourd (especially growing points) recovered from chilling damage, after they were transferred from the chilling chamber to the greenhouse for a few weeks, even if there was significant damage to the cotyledons and true leaves. These results are similar to the watermelon chilling results of Kozik and Wehner (2014), showing that chilling damage was lowest on the growing point and higher on the leaves and cotyledons.

In conclusion, the most tolerant bottle gourd accessions of the 163 tested were PI 491272, PI 491280, PI 491281, PI 491286, and PI 491326. In future studies, grafting success and compatibility tests should be evaluated for those accessions, as well as the effects of those rootstocks on yield and fruit quality of watermelon scions. However, gourd is also consumed as a vegetable in different parts of the world. It is also suggested that in some Asian and African countries where bottle gourd is consumed as a vegetable, these tolerant accessions can be

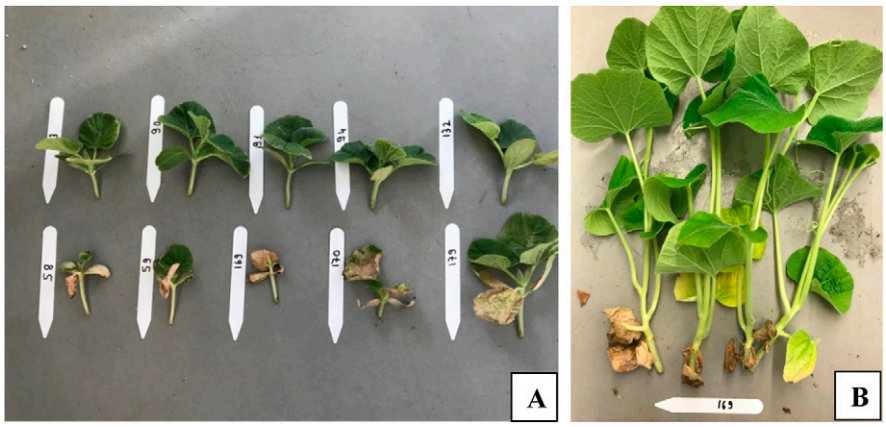

Fig. 3. Most tolerant (upper): 83 = PI 491272, 90 = PI 491280, 91 = PI 491281, 94 = PI 491286, 132 = PI 491326, and most susceptible (lower) accessions: 58 = PI 381845, 59 = PI 381846, 169 = PI 534556, 170 = PI 636137, 179 = PI 668365 after 1 week of chilling treatment (**A**); recovering of plant growing points 2 weeks after chilling treatment (**B**).

Table 2. Average chilling rating for the most tolerant and most susceptible PI accessions on first true leaf with the observation of first week after 36-, 48-, and 60-h treatments in 163 *Lagenaria* accessions.

		36 h		48 h		60 h	
Cultigen code	Accession	Mean	SD	Mean	SD	Mean	SD
		Most tolerant PI accessions					
83	PI 491272	0.0	0.0	2.0	0.0	3.3	2.5
90	PI 491280	2.0	0.0	4.7	0.6	5.0	0.0
91	PI 491281	2.3	1.2	2.0	0.0	4.0	0.0
94	PI 491286	4.0	0.0	3.0	0.0	5.7	1.2
132	PI 491326	3.0	0.0	3.0	0.0	3.0	1.7
		Most susceptible PI accessions					
58	PI 381845	8.0	0.0	8.0	0.0	9.0	0.0
59	PI 381846	9.0	0.0	8.0	0.0	8.7	0.6
169	PI 534556	8.0	0.0	7.7	0.6	8.0	0.0
170	PI 636137	9.0	0.0	8.0	0.0	9.0	0.0
179	PI 668365	8.0	0.0	7.3	1.2	7.0	1.7
F ratio		17.338	—	13.446	—	8.841	—
Prob > F		<0.0001	—	<0.0001	—	<0.0001	—
LSD (0.05)		1.106	—	0.644	—	1.327	—
Minimum		0.0	—	1.0	—	1.0	—
Maximum		9.0	—	9.0	—	9.0	—

LSD = least significant difference.

used to develop cold-tolerant bottle gourd varieties.

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