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Chilling Tolerance and Field Performance of an F_1 Cooking Tomato Cultivar, Nitaki-Koma, Relative to Its Parents

Yasser M.M. Moustafa[1], Susumu Yui[2] and Matsuo Uemura*[1,3]

[1] *United Graduate School of Agricultural Sciences, Iwate University*, 3-18-8 Ueda, Morioka, Iwate 020-8550, Japan

[2] *Horticultural Breeding Laboratory, National Agricultural Research Center for Tohoku Region*, 4 Akahira, Shimokuriyagawa, Morioka, Iwate 020-0123, Japan

[3] *Division of Biosystem and Bioresource Technology, Cryobiosystem Research Center, Faculty of Agriculture, Iwate University*, 3-18-8 Ueda, Morioka, Iwate 020-8550, Japan

Chilling is one of the major environmental stresses for plants including tomato. A newly established F_1 hybrid cooking tomato cultivar, *Lycopersicon esculentum* Mill cv. Nitaki-Koma, has been recognized as a promising hybrid to satisfy consumers' demands in Japan. However, there are few reports on agronomical and physiological characteristics, which are necessary to increase its cultivation. Thus, we evaluated the responses of Nitaki-Koma to chilling temperatures as well as its agronomical characteristics in the field and compared the results with those of its parents. The maternal parent cultivar, Coudoulete (referred to as 'P1'), had some good agronomical characteristics, germinated faster, and was more tolerant to chilling temperatures than the paternal parent cultivar, Piline (referred to as 'P2'). P2 had, however, some desirable agronomical characteristics but germinated slowly under chilling conditions and was sensitive to chilling. The F_1 hybrid, Nitaki-Koma, showed the better-parent heterosis in some favorable characteristics, such as the number of clusters/plant, the total number of fruits/plant and the total fruit production/plant. It also showed the mid-parent heterosis in other characteristics, such as average fruit weight, plant height and chilling tolerance. Furthermore, seeds of F_1 germinated faster under chilling conditions than the paternal parent (P2). Therefore, F_1 was considered as a chilling tolerant tomato cultivar good for fruit production. These results further suggest the usefulness of tomato breeding for chilling tolerance by producing the F_1 hybrids by crossing chilling-tolerant cultivars.

Key Words: tomato (*Lycopersicon esculentum* Mill), F_1 hybrid, heterosis, chilling tolerance, field performance.

Introduction

The performance of crop genotypes under various environmental conditions must be evaluated to determine the desirability and adaptability of those genotypes in agriculture (Poysa *et al.* 1986). Tomato (*Lycopersicon esculentum* Mill) is cultivated in approximately 4.0 million hectares and is the second largest producing vegetable crop in the world, approximately 108.5 million metric tons in 2002 (FAO 2002). Although the performance of various genotypes of tomato has been well studied worldwide and hybrid vigor and high commercial value of the tomato hybrids have been reported by many investigators (Owen and Ang 1990, Hassan and Khalaf-Allah 1995, Abd-Allah 1999), tomato production is still greatly affected by various environmental stresses.

Chilling injury is considered as one of the most important environmental constraints to determine crop productivity and occurs in many agriculturally important plants that originated in the tropical and subtropical regions (e.g. banana, cotton, maize, rice and tomato) when they are exposed to non-freezing temperatures below 12°C (Lyons 1973, Saltveit and Morris 1990). Especially, it is known that seed germination and early seedling growth are the stages most sensitive to environmental stresses (Cook 1979). In the cultivated tomato, chilling temperatures over the range of 0 to 12°C result in significant delay in the onset of germination, reduction of germination rate and considerable variance of seedling growth (Jones 1986, Foolad and Lin 1998). Because large areas of land for tomato production are established by sowing seed directly in the field instead of transplanting (Liptay and Schopfer 1983), environmental stresses, such as chilling, restrict the establishment of direct-seeded tomato crops considerably.

Furthermore, the physiological disorders, including reduced growth and vigor, altered respiration and development, abnormal fruit ripening, accelerated water loss and senescence, surface pitting and increased disease susceptibility, often occur at various growth stages. These symptoms

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*Corresponding author (e-mail: uemura@iwate-u.ac.jp)

often develop during the exposure to chilling temperatures and are further enhanced when plants are subsequently re-warmed to normal growth temperatures (Saltveit 2002). Thus, the detailed, comprehensive studies to determine chilling tolerance of particular plant species are still necessary to apply data to breeding and horticultural fields.

In the present study, we evaluated chilling tolerance and field performance of an F_1 cooking tomato cultivar, Nitaki-Koma, established with parent cultivars (cvs. Coudoulete and Piline) at the National Agricultural Research Center for Tohoku Region in Japan (Ishii *et al.* 2001). Nitaki-Koma has been cultivated in Iwate Prefecture of the Tohoku Region. To meet the increasing demand of cooking tomato production in Japan, it may be possible to expand the cultivating area of Nitaki-Koma in Iwate and other places. However, because the cool temperatures in the growing season in Iwate Prefecture could reduce the growth and production of Nitaki-Koma, it is desirable to analyze the chilling tolerance of this cultivar in detail to encourage the cultivation of Nitaki-Koma to farmers. Furthermore, because preliminary observations in the field suggest that chilling tolerance of the maternal parent cultivar (cv. Coudoulete) may be greater than that of the paternal parent cultivar (cv. Piline) (Yui, personal communication), it is of interest to determine how the chilling tolerance trait is inherited from the parent cultivars with a differential extent of tolerance to the F_1 offspring. Thus, we evaluated comprehensively the chilling responses of Nitaki-Koma at various growth stages under laboratory conditions and compared the results with those obtained from the two parent cultivars. Furthermore, we determined the agronomical characteristics of Nitaki-Koma in the field where this particular F_1 cultivar has been cultivated to add economical values and provide useful information for cultivation to farmers. These results are discussed together to help elucidate the usefulness of the traits of Nitaki-Koma related to its parent cultivars for improvement of tomato chilling tolerance.

Materials and Methods

Plant materials

Three tomato cultivars, Coudoulete (P1), Piline (P2) and Nitaki-Koma (their "F_1" hybrid) were used in the present study. The two parental cultivars are homozygous lines. Brief descriptions of all cultivars are shown below.

Coudoulete: the plants are determinate, moderate foliage density and resistant to *Fusarium* wilt (*I2*) and *Verticillium* wilt (*Ve*). The fruits are red, block shaped, 80 g, uniform ripening, jointless, and have excellent firmness and vine storage, along with high soluble solid content, and high processing quality. The fruit size, concentration and uniformity of ripening make it suitable for machine harvest (Damidaux 1988).

Piline: the plants are determinate, resistant to *Verticillium* wilt (*Ve*), *Fusarium* wilt (*I*) and *Stemphylium* (*Sm*), and partially resistant to *Phytophthora infesatans* (*Ph-2*). This cultivar is similar to Europeel cultivar as a peeling tomato. The fruits are red, uniform ripening, elongated shape, smaller than Coudoulete and similar to Europeel cultivar type (Laterrot 1987). More details of the pedigree of the cultivar can be found elsewhere (Damidaux 1988, Laterrot 1987).

Nitaki-Koma: the plants are determinate and have a jointless pedicel. The fruits are plum-shaped with 2–3 locules. The fruit weight is about 70 g. Fruit yield is more than 70 tons/ha. The soluble solid content is 5.2% and the fruit quality is suitable for cooking. Nitaki-Koma is resistant to *Fusarium* wilt (race 1, 2) and *Verticillium* wilt (Ishii *et al.* 2001).

To increase the number of seeds of the F_1 hybrid "Nitaki-Koma" to use for our experiments, we crossed field-grown plants of Coudoulete as maternal parent and Piline as paternal parent.

Seed germination

Seeds of the three cultivars were surface-sterilized with 70% (v/v) ethanol for 1 min followed by 1% (v/v) NaOCl solution for 15 min, and rinsed with sterile, distilled water several times. Seeds were then plated onto Murashige-Skoog medium supplemented with 0.8% (w/v) agar in Petri dishes (15 cm in diameter) under aseptic conditions (three Petri dishes/treatment and 50 seeds/Petri dish). Seeds were exposed to chilling temperatures in Petri dishes arranged in a completely randomized design in an incubator maintained in the dark at 2 ± 0.5°C or 10 ± 0.5°C for various durations. Non-chilled seeds were maintained at 23 ± 1.0°C for the same durations.

The germination response was scored visually as radical protrusion at 12-h intervals for 5 days at 23°C, for 2 weeks after transfer to 23°C from 2°C for 2 weeks, or for 20 days at 10°C. When calculating the time required for germination, seeds that germinated within a particular interval were assumed to have germinated at the midpoint of that interval. All experiments were repeated two times and similar results were obtained.

Chilling tolerance of tomato plants

To determine the chilling tolerance of the two parent cultivars (P1 and P2) and their F_1 hybrid, 4-week-old tomato plants (five plants/cultivar) grown in 25 cm-round pots in an air-conditioned glass-house at 23°C were transferred to incubators at 2°C or 4°C under 16 hr light (about 120 μmol m^{-2} s^{-1})/8 hr dark regimes (Hsieh *et al.* 2002) for various durations and then returned to glass-house at 23°C. The experiment was repeated three times and we obtained similar results in all times. Photos were taken 1 week after returning to the 23°C-conditions to record the symptoms of chilling injury of the plants.

Electrolyte leakage of tomato leaves

To evaluate the chilling tolerance of the tomato leaves, 6-week-old tomato plants of the two parental cultivars and their F_1 hybrid (three plants/cultivar for every time point) grown in an air-conditioned glass-house at 23°C (non-chilled conditions) were transferred to incubators at 2°C or 4°C under 16 hr light (about 120 μmol m^{-2} s^{-1})/8 hr dark regimes for various durations. At the specified time point, one leaf from each plant was collected and leaf discs were then prepared. After washing with distilled water, the discs were pooled. From the pooled leaf discs, five replicates of the sample (0.5 g) were prepared. After sampling, the plants were returned to the glass-house at 23°C. Leaf discs for the re-warmed plants were prepared after incubation for 24 hrs in the glass-house. Leaf discs were samples at various time points during the chilling-stress treatment. The experiment was repeated twice.

The extent of chilling injury was evaluated by the electrolyte leakage from leaf discs as follows. After washing with de-ionized water, the leaf discs were immersed in 15 ml of de-ionized water in 50 mm-plastic tubes and incubated for three hours on a mechanical shaker (MMS-3010, EYELA, Japan). Then, the extent of electrolyte leakage in the incubation solution was determined using a conductivity meter (B-173, Horiba, Japan) and considered as leakage No. 1. Tubes containing the tomato leaf discs were then placed in a 80°C-hot water bath with proper agitation for 60 min followed by shaking for 30 min at room temperature on the mechanical shaker. Then, electrolyte leakage was determined again using the same conductivity meter, which was designated as maximum electrolyte leakage or leakage No. 2. Percentages of electrolyte leakage from chilled and chilled/re-warmed leaves were determined using the following equation:

% of electrolyte leakage = (leakage No. 1/leakage No. 2) × 100

Agronomical characteristics

At the National Agricultural Research Center for Tohoku Region, tomato seeds of the two cultivars and their F_1 hybrid were sown in germination trays in an air-conditioned glass-house in April 2002, and after 35 days from sowing, the tomato seedlings were transplanted in the field through black polyethylene mulch. All the common cultural practices for tomato production with mulch system were followed throughout the experiment. At the flowering stage, Coudoulete (P1) and Piline (P2) were crossed to obtained seeds of Nitaki-Koma (F_1). Agronomical characteristics of the parents and their hybrid grown under field conditions were determined two times independently (10 plants/cultivar in each experiment). Data were recorded on the number of branches/plant, the number of clusters/plant, the number of set fruits/cluster, the plant height (cm), the number of fruits/plant, the average fruit weight (g), and the total plant fruit production (kg). Heterosis of the F_1 hybrid was determined according to the following equation (Geleta *et al.* 2004):

Mid-parent heterosis (MPH, %) = $\{(F_1-\mathrm{MP})/\mathrm{MP}\} \times 100$

Better-parent heterosis (BPH, %) = $\{(F_1-\mathrm{HP})/\mathrm{HP}\} \times 100$

where F_1 is the F_1 performance, MP = (P1 + P2)/2 in which P1 and P2 are the performances of inbred parents, respectively, and HP is the high parent value.

Statistical analyses

Agronomical characteristics and electrolyte leakage data were subjected to the analyses of variance procedure and treatment means were compared among cultivars using Tukey-Kramer test at the $P = 0.05$ level as described by Gomez and Gomez (1984). Standard deviations were shown in all data except for visual evaluation of whole plant chilling tolerance in Fig. 3.

Results

Tomato seed germination

When seeds of P1 and P2 cultivars along with their F_1 hybrid were germinated at 23°C in a growth chamber, there were no statistical differences among the two parents and their F_1 hybrid (Fig. 1A). Under these conditions, more than 90% and nearly 100% of the seeds of all three cultivars germinated after 3 and 4 days from the initiation of imbibition, respectively.

To determine the effect of chilling temperatures on seed germination, seeds of the three cultivars were first incubated in an incubator at 2°C for 2 weeks and then transferred to an incubator at 23°C for another 2 weeks (see Materials and Methods). Because no seeds germinated at 2°C, the number of germinated seeds was counted after the transfer to 23°C (Fig. 1B). Under these conditions, the rate and percentage of germination were significantly greater in P1 and F_1 than in P2. The final percentage of germinated seeds under these conditions was 93.3% in P1, 91.6% in F_1, but 80.0% in P2. After 4 days from transfer of the seeds to 23°C, there were significant differences in seed germination percentage between P1 (81.6%) and F_1 (70.0%). On the other hand, only 33.3% of P2 seeds germinated at the same time with highly significant differences with both of P1 and F_1, indicating better performance of P1 and F_1 in seed germination after the chilling.

Under 10°C conditions, seeds of P1 showed better performance than those of P2 and F_1 (Fig. 1C). There were significant differences in germinating rate among the cultivars after 13 days of the beginning of the seed incubation at 10°C; 83.0% of P1 seeds were germinated comparing to 44.0% in P2 and 71.0% in F_1. Furthermore, the final percentages of seed germination under 10°C conditions were 97.0, 80.0, and 82.0% for P1, P2, and F_1, respectively, with significant differences between P1 and both of P2 and F_1.

Chilling tolerance of whole tomato plants

Chilling tolerance of tomato plants was evaluated using three different experiments: (1) 4-week-old plants were transferred to a 4°C incubator for 4 days (Fig. 2A), (2) 6-week-old-plants were transferred to a 4°C incubator for 2 weeks (Fig. 2B), and (3) 8-week-old tomato plants were transferred to 2°C incubator for 1 week (Fig. 2C). All chilled plants were subsequently returned to a 23°C glass-house and

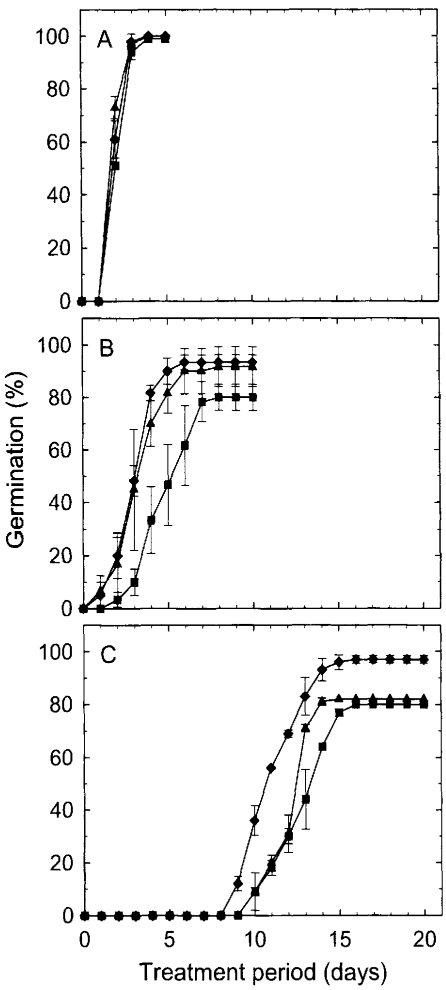

Fig. 1. Seed germination of P1 (diamond), P2 (square), and F_1 (triangle) tomato cultivars under non-chilling and chilling conditions. (A) Seed germination at 23°C (non-chilling); (B) seed germination under 23°C after exposure to 2°C. Seeds incubated on MS agar medium were maintained at 2°C for 2 weeks and then transferred to 23°C for another 2 weeks; (C) seed germination at 10°C. Error bars indicate the SDs of the three replicates at each data point.

grown for 1 week before chilling injury was evaluated. Fig. 2A shows that P2 plant was severely affected by the chilling treatment showing necrosis in some leaves. In contrast, leaves of the F_1 hybrid were slightly affected and P1 plants were as healthy as plants before chilling. In Fig. 2B, P2 plants were severely damaged after a prolonged chilling treatment (4°C for 2 weeks) compared with F_1 plants, which were less damaged, or P1 plants, which were not damaged at all. Chilling at 2°C for 1 week resulted in complete death of P2 plants, while F_1 plants showed severe damage but survived after rewarming (Fig. 2C). P1 plants were only slightly damaged and survived after rewarming. Collectively, these results clearly indicated that P1 plants have much greater chilling tolerance than P2 plants. In addition, chilling tolerance of F_1 plants was intermediate between that of P1 and P2 plants showing the mid-parent heterosis and the partial dominance of its parents in this trait.

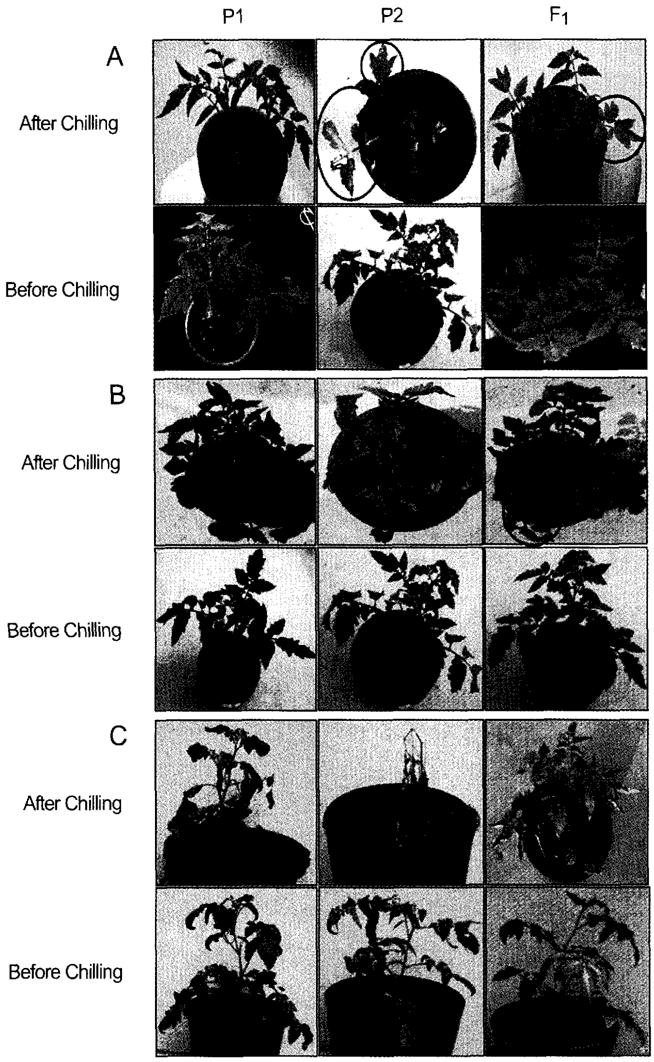

Fig. 2. Chilling tolerance of P1, P2 and F_1 tomato plants. Tomato plants from the three cultivars grown in an air-conditioned glass-house at 23°C were transferred to incubators at 2 or 4°C for various durations. Photos were taken before chilling or after chilling followed by incubation at 23°C for 1 week. (A) Four-week-old plants before chilling or after chilling at 4°C for 4 days followed by incubation at 23°C for 1 week; (B) six-week-old tomato plants before chilling or after chilling at 4°C for 2 weeks followed by incubation at 23°C for 1 week; (C) eight-week-old tomato plants before chilling or after chilling at 2°C for 1 week followed by incubation at 23°C for 1 week Open circles point the leaflets showing necrosis in P2 and F_1 plants.

Electrolyte leakage from tomato leaves

Electrolyte leakage from leaves of the two parents and their F_1 hybrid were determined to evaluate the chilling tolerance of the leaves after incubation at 2°C (Fig. 3) or 4°C (Fig. 4). Electrolyte leakage from leaves immediately after chilling at 2°C was less in P1 than in P2 or F_1 at day 1 and 3 ($P < 0.05$) but there were no or little differences in electrolyte leakage after chilling treatment for 5 days or longer (Fig. 3). When electrolyte leakage was determined with chilling at 2°C for 1 day and subsequent rewarming at 23°C for 1 day, there was a decrease in the extent in P2 and F_1 leaves with

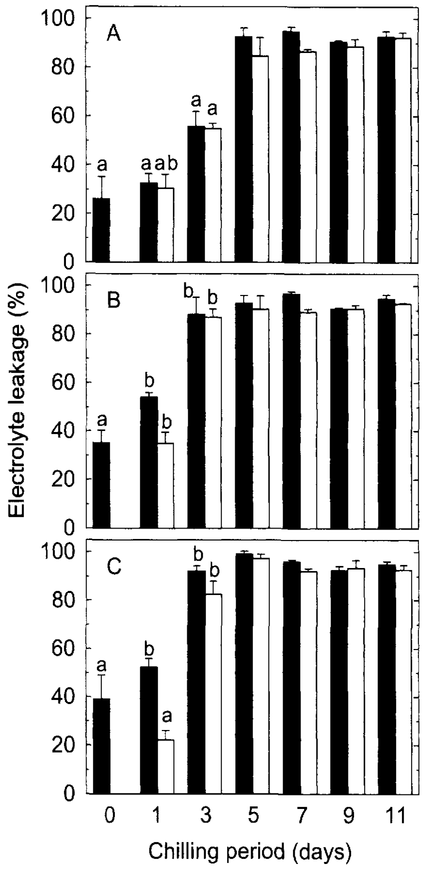

Fig. 3. Chilling tolerance of the tomato plants after exposure to 2°C. Six-week-old tomato plants were transferred from an air-conditioned glass-house at 23°C to a 2°C-incubator and incubated for specified periods. Electrolyte leakage from leaves of P1 (A), P2 (B), and F_1 (C) plants was determined immediately after the chilling treatment (black box) or after subsequent rewarming for 24 hours (white box). Error bars indicate the SDs of the three replicates of each data point. Groups with different letters are significantly different when the Tukey-Kramer test at P=0.05 was performed among the three cultivars with the same treatment. When no letters are shown (*i.e.*, samples that were chilled for 5 days or longer), no statistical differences were observed.

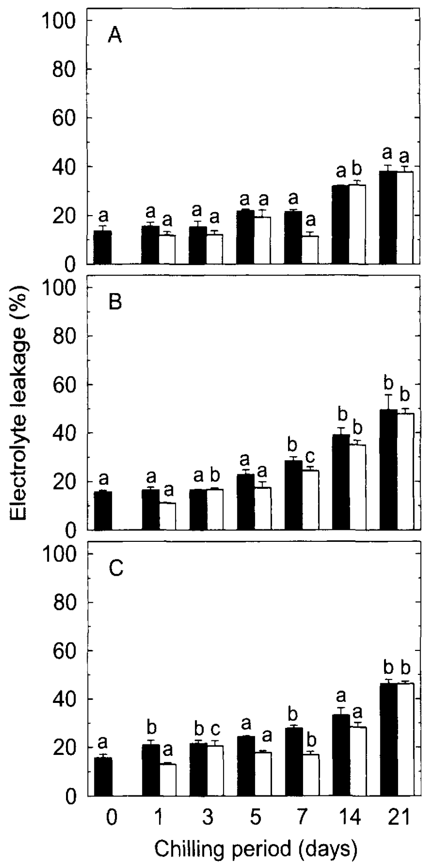

Fig. 4. Chilling tolerance of the tomato plants after exposure to 4°C. Six-week-old tomato plants were transferred from an air-conditioned glass-house at 23°C to a 4°C-incubator and incubated for specified periods. Electrolyte leakage from leaves of P1 (A), P2 (B), and F_1 (C) plants was determined immediately after the chilling treatment (black box) or subsequent returning to the glass-house for 24 hours (white box). Error bars indicate the SDs of the three replicates of each data point. Groups with different letters are statistically different when the Tukey-Kramer test at P=0.05 was performed among the three cultivars with the same treatment.

unknown reasons. The extent of electrolyte leakage was the least in F_1, the greatest in P2, and the intermediate in P1 with significant differences between F_1 and P2. At day 3, the extent of electrolyte leakage after rewarming was still much lower in P1 than either of P2 or F_1 ($P<0.05$). There were no differences in the electrolyte leakage from leaves when the plants were chilled for 5 days or longer before rewarming because almost all electrolytes were leaked out after the chilling treatment.

When the chilling temperature was raised to 4°C, the extent of electrolyte leakage from leaves became much less than that of leaves chilled at 2°C (Fig. 4). Although there were no apparent differences in the extent and the kinetics of electrolyte leakage from leaves of the three cultivars during chilling treatment, P1 had less leakage from leaves than P2 and F_1 plants when the plants were chilled for a longer period (e.g., 21 days, $P<0.05$). With shorter chilling periods, there was a tendency that the extent of electrolyte leakage was somewhat less in P1 than in P2 and F_1. Thus, electrolyte leakage tests of the three tomato cultivars chilled at 2 or 4°C collectively indicate that P1 leaves seem to be more tolerant against chilling temperatures than P2 or F_1 leaves, which is consistent with the results of whole plant systems described above.

Agronomical characteristics

Determination of the field performance of the two tomato cultivars (P1 and P2) and their F_1 hybrid (Fig. 5A to 5G) showed that P1 and F_1 preformed better than P2 in all categories except for the number of clusters/plant (Fig. 5B) and the total fruit production/plant (Fig. 5F), in which F_1 hybrid but not P1 showed significantly better performance than P2. P1 and F_1 performed similar except for a single category: P1 was greater than F_1 in the average fruit weight. F_1 showed the better-parent heterosis in the number of clusters/plant, the number of fruits/plant and the total plant fruit production

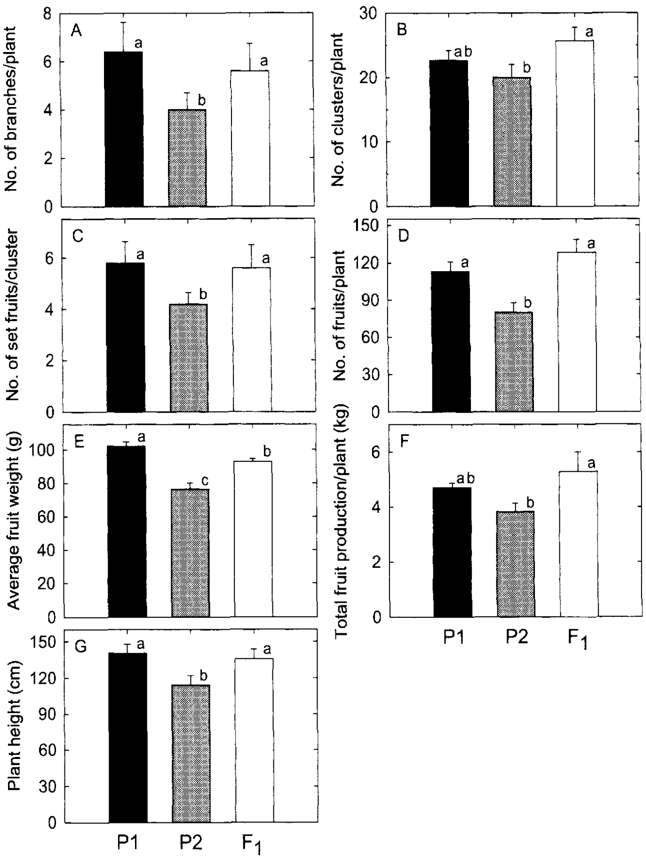

Fig. 5. Agronomical characteristics of P1 (black box), P2 (shaded box), and F_1 (white box) tomato cultivars. (A) Number of branches/plant; (B) number of clusters/plant; (C) number of set fruits/cluster; (D) total number of fruits/plant; (E) average fruit weight; (F) total fruit production/plant; (G) plant height (cm). Error bars indicate the SDs of the five replicates of each data point and different letters above the columns indicate significant difference with the Tukey-Kramer test performed at P= 0.05 among the three cultivars.

and the mid-parent heterosis in the other characteristics (Table 1).

Discussion

After evaluating two tomato cultivars, we concluded that the P1 (Coudoulete) cultivar is superior to the P2 (Piline) cultivar in some agronomical characteristics along with chilling tolerance. Although P2 had some desirable agronomical characteristics, it was sensitive to chilling stress. Furthermore, their F_1 hybrid (Nitaki-Koma) expressed the partial dominance of the paternal and maternal parents in chilling tolerance and showed the hybrid vigor and better-parent heterosis in the total number of clusters per plant, the total number of fruits/plant and the total plant fruit production. By taking a new approach that is to examine a combination of the studies of responses to chilling treatments at various growth stages (i.e., seed germination, seedling growth and electrolyte leakage of detached leaves along with the whole-plant systems) under laboratory conditions and the field evaluation of agronomical characteristics of the two tomato cultivars and their F_1 hybrid, we found a good combination between chilling tolerance and desirable agronomical characteristics in P1 and F_1. These two tomato cultivars can be useful as germplasm to obtain desirable characteristics in tomato breeding programs, especially for chilling tolerance.

In tomato, to our knowledge, there have been no reports on chilling tolerance analyzed at various growth stages with emphasis on hybrid vigor and heterosis. For salt tolerance, Monforte *et al.* (1997) reported the existence of heterosis in tomato. With corn, chloroplast structure and dimension have been reported to show some extent of heterosis under chilling conditions although chilling tolerance *per se* was not reported with the lines examined (Kutík *et al.* 2004). Furthermore, with tomato, there are many reports on heterosis on nutrition, growth and other agronomical characteristics (see a review by Makesh *et al.* 2002). Our results on heterosis in chilling tolerance of the F_1 hybrid (Nitaki-Koma) in the present study will add a valued approach for breeding programs of more chilling tolerant cultivars. To do so, we need to conduct a comprehensive study to evaluate chilling tolerance of a greater number of genetic lines of tomatoes systematically.

Levitt (1980) described the electrolyte leakage method as one of the most reliable protocols to evaluate chilling and freezing tolerance in plants. In the present study, evaluation of the chilling tolerance of the two cultivars and their F_1 hybrid by determination of electrolyte leakage after subjecting the plants to chilling stress conditions showed that the maternal parent "P1" is more chilling tolerant than the paternal

Table 1. Mean values and range of heterosis (%) for 7 characteristics of the F_1 hybrid, Nitaki-Koma

Heterosis	No. of branches/plant	No. of clusters/plant	No. of set fruits/plant	No. of fruits/plant	Average fruit weight (g)	Total fruit production/plant (kg)	Plant height (cm)
MPH[1]							
Mean	13.3±26.6	20.4±11.4	12.4±19.0	32.8±11.5	4.2±2.9	24.4±21.2	7.2±12.6
Range	−27.3 to 33.3	11.6 to 33.3	−9.09 to 40.0	24.4 to 45.8	1.8 to 7.4	2.3 to 44.5	−2.3 to 21.5
BPH[2]							
Mean	−12.3±39.9	11.6±3.2	−5.8±24.0	11.6±3.2	−9.8±1.5	9.5±16.1	−3.8±10.6
Range	−75.0 to 33.3	8.0 to 14.3	−40.0 to 16.7	8.0 to 14.3	−11.4 to −8.6	−8.9 to 21.3	−10.9 to 8.3

[1] Mid-Parent Heterosis
[2] Better-Parent Heterosis

one "P2" (Fig. 3 and Fig. 4). Furthermore, the F_1 hybrid expressed the partial dominance of its parents in chilling tolerance. Chilling tolerance of whole plants (Fig. 2) was in general consistent with that of excised leaves (Fig. 3 and Fig. 4). Thus, these results suggest that chilling tolerance of tomato plants can be evaluated simply with excised leaves rather than complicated, time-consuming procedures with whole plants.

Our results clearly demonstrated that differences in seed germination percentages of the three tomato cultivars were only apparent under chilling conditions (Fig. 1). These observations are consistent with previous reports that selection for rapid seed germination was effective under cold stress and salt stress, but not under non-stress conditions (Foolad and Jones 1991, Foolad *et al.* 1999). A better performance at the seed germination stage under environmental stress conditions would result in advantages in subsequent growth and, hence, production of crops. Thus, a good performance of Nitali-Koma seeds under chilling conditions is surely one of the suitable traits to increase cultivation and production of this cultivar in the Iwate area. Furthermore, in general, our results suggest that it is necessary to carefully evaluate the effect of specific environmental conditions on the performance of tomato plants in the area where tomato plants are cultivated.

Furthermore, a close evaluation of the results revealed that the relationships of chilling tolerance among the three cultivars are not always in the same order. Evaluation of chilling tolerance in general indicated that F_1 plants were chilling-tolerant to the extent similar to or only slightly less than P1 and greater than P2 (Fig. 1 to Fig. 4). However, there are some variations in this trait. For example, although F_1 seeds behaved similar to that of P1 seeds when seed germination was scored at 23°C after chilling treatment at 2°C for 2 weeks, the behavior of F_1 seeds was somewhat similar to P2 (not P1) when germinated at 10°C (Fig. 1). These findings suggest that F_1 seeds under different chilling temperatures may have different patterns of gene expression that comes from either of the two parents. Although the detailed mechanisms for these differential responses at seed germination under different chilling conditions need to be carefully analyzed, it is of interest to pursue further experiments to understand this phenomenon so that we may be able to use these tomato cultivars as genetic resources.

Our results provided us basic information on field performance in association with differences in response to chilling temperatures. The most common method of reducing plant sensitivity to chilling is by the development and use of more chilling tolerant cultivars (Walker *et al.* 1990). To efficiently produce chilling-tolerant cultivars through tomato breeding programs, it is necessary to elucidate the physiological and molecular backgrounds for the differential responses to chilling in the three tomato cultivars. Our preliminary observations (Moustafa *et al.* in preparation) revealed that chilling-induced accumulation of soluble sugars (sucrose, glucose, and fructose) was greater in leaves of P1 and F_1 than in those of P2. Soluble sugars have been shown to increase the stability of proteins and membranes at low temperatures (Carpenter and Crowe 1988), which may contribute to chilling tolerance in the P1 and F_1 cultivars. Furthermore, expression of a cold-responsive transcription factor (*C-repeat/dehydration responsive element binding factor 1, CBF1*) gene was greater in leaves of P1 and F_1 than in P2 after chilling (Moustafa *et al.* in preparation). Because the expression of *CBF1* has been known to have a close association with the acquirement of freezing tolerance in several plant species (Thomashow 1998) and the *CBF1* induction in a tomato cultivar was reported previously (Hsieh *et al.* 2002, Zhang *et al.* 2004), this gene is likely to play a role in determination of the extent of chilling tolerance in tomato.

In summary, our results clearly showed that an F_1 hybrid tomato cultivar (Nitaki-Koma) was chilling tolerant the extent of which was similar to the maternal parent cultivar (Coudoulete, P1), indicating that Nitaki-Koma is considered to be a good germplasm when used as a breeding material. The present method may be useful as an effective evaluation protocol for selecting chilling-tolerant tomato cultivars with desirable agronomic characteristics.

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Literature Cited

Abd-Allah, E.M.M. (1999) Combining ability, heterosis and correlation studies for some quantitative characters of tomato in the late summer planting. Zagazig J. Agric. Res. 26: 623–636.

Carpenter, J.F. and J.H. Crowe (1988) The mechanism of cryoprotection of proteins by solutes. Cryobiology 25: 244–255.

Cook, R.E. (1979) Patterns of juvenile morbidity and recruitment in plants. *In* "Topics in Plant Population Biology" Solbrig, O.T., S. Jain, G.B. Johnson and P.H. Raven (eds), Columbia Univ. Press, Los Angeles. p. 207–301.

Damidaux, R. (1988) COUDOULET, high soluble solids processing tomato. Station d/Amelioration des Plantes Maraicheres — INRA/CTCPA — Montfavet, France. Release on December 21, 1987.

FAO (2002) Agricultural Statistics Records. http://apps.fao.org/page/collections?subset=agriculture

Foolad, M.R. and R.A. Jones (1991) Genetic analysis of salt tolerance during germination in *Lycopersicon*. Theor. Appl. Genet. 81: 321–326.

Foolad, M.R. and G.W. Lin (1998) Genetic analysis of low temperature tolerance during germination in tomato, *Lycopersicon esculentum* Mill. Plant Breed. 117: 171–176.

Foolad, M.R., J.R. Hyman and G.Y. Lin (1999) Relationships between cold-and salt-tolerance during seed germination in tomato: analysis of response and correlated response to selection. Plant Breed. 118: 49–52.

Geleta, L.F., M.T. Labuschagne and C.D. Viljoen (2004) Relationship between heterosis and genetic distance based on morphological traits and AFLP markers in pepper. Plant Breed. 123: 467–473.

Gomez, K.A. and A.A. Gomez (1984) Statistical procedures for agricultural research A. Wiley-Interscience Publication, New York. 678 p.

Hassan, H.M. and A.M. Khalaf-Allah (1995) New promising tomato hybrids for plastic-house and open field growing conditions. Al-Azhar J. Agric. Res. 22: 153–163.

Hsieh, T., J. Lee, P. Yang, L. Chiu, Y. Charng, Y. Wang and M. Chan (2002) Heterology expression of the *Arabidopsis C-repeat/dehydration response element binding factor 1* gene confers elevated tolerance to chilling and oxidative stresses in transgenic tomato. Plant Physiol. 129: 1086–1094.

Ishii, T., M. Fujino, Y. Sato, D. Ishiuchi, S. Yui, Y. Yanoguchi, K. Ito, T. Uchiumi and M. Okimura (2001) 'Nitaki-Koma', a new hybrid tomato cultivar for cooking. Bull. Natl. Inst. Veg. Ornam. Plants & Tea, Japan 16: 311–320 (in Japanese with English summary).

Jones, R.A. (1986) High salt tolerance potential in *Lycopersicon* species during germination. Euphytica 35: 576–582.

Kutík, J., D. Holá, M. Kočová, O. Rothová, D. Haisel, N. Wilhelmová and I. Tichá (2004) Ultrastructure and dimensions of chloroplasts in leaves of three maize (*Zea mays* L.) inbred lines and their F_1 hybrids grown under moderate chilling stress. Photosynthetica 42: 447–455.

Laterrot, H. (1987) Piline. Station d/Amelioration des Plantes. Maraicheres, INRA-Avignon, France. Released on Decmber 21, 1987.

Levitt, J. (1980) Plant Responses to Environmental Stresses. Academic Press, New York. 497 p.

Liptay, A. and P. Schopfer (1983) Effect of water stress, seed coat restraint and abscisic acid upon different germination capabilities of two tomato cultivars at low temperatures. Plant Physiol. 73: 935–938.

Lyons, J.M. (1973) Chilling injury in plants. Annu. Rev. Plant Physiol. 24: 445–466.

Makesh, S., M. Puddan, S. Ashok and B.M. Rizwana (2002) Heterosis studies for quality and yield in tomato (*Lycopersicon esculentum* Mill.). Adv. Plant Sci. 15: 597–601.

Monforte, A.J., M.J. Asins and E.A. Carbonell (1997) Salt tolerance in *Lycopersicon* species. 5. Does genetic variability at quantitative trait loci affect their analysis? Theor. Appl. Gen. 95: 284–293.

Owen, H.R. and L.H. Ang (1990) Genotypic and chemical influences on fruit growth of tomato. HortScience 25: 1255–1257.

Poysa, V.W., R. Garton, W.H. Courtney, J.G. Metcalf and J. Muehmer (1986) Genotype-environment interaction in processing tomato in Ontario. J. Amer. Soc. Hort. Sci. 111: 293–297.

Saltveit, M.E. and L.L. Morris (1990) Overview of chilling injury of agronomical crops. *In* "Chilling Injury of Agronomical Crops" Chien, Y.W. (ed.), CRC Press, Boca Raton, p. 3–15.

Saltveit, M.E. (2002) The rate of ion leakage from chilling-sensitive tissue does not immediately increase upon exposure to chilling temperatures. Postharvest Biol. Technol. 26: 295–304.

Thomashow, M.F. (1998) Role of cold-responsive genes in plant freezing tolerance. Plant Physiol. 118: 1–8.

Walker, M.A., D.M. Smith, K.P. Pauls and B.D. McKersie (1990) Development of a chlorophyll fluorescence screening test to evaluate chilling tolerance in tomato. HortScience 25: 334–339.

Zhang, X., S.G. Fowler, H. Cheng, Y. Lou, S.Y. Rhee, E.J. Stockinger and M.F. Thomashow (2004) Freezing-sensitive tomato has a functional CBF cold response pathway, but a CBF regulon that differs from that of freezing-tolerant *Arabidopsis*. Plant J. 39: 905–919.