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Characteristics of Chilling Symptoms of Cherry Tomato Compared to Beefsteak Tomato Harvested at Different Ripening Stages

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This study was carried out to determine the characteristics of chilling symptoms between cherry tomato 'Unicorn' and beefsteak tomato 'Madison' and to establish the equation for chilling symptoms. Each stage of breakers, turning, pink, light red, and red maturity tomato fruits were harvested from both tomato cultivars, and they were stored at 11°C and 5°C with 85% relative humidity for 21days separately to treat chilling stress and thereafter were stored for 4 days at 20°C with the same relative humidity for returning from chilling stress. The breakers, turning and pink stages of 'Unicorn' showed a lower color a*/b* value than 'Madison' in both temperatures. In addition, tomatoes stored 11°C had better color developmentthan tomatoes stored at 5°C in both cultivars. 'Unicorn' showed higher ion leakage than 'Madison' at both temperatures in all maturity stages. Moreover, 'Unicorn' appeared to have higher respiration rates and ethylene production than 'Madison' under chilling conditions. Also, 'Unicorn' had more sensitive reactions than 'Madison' under chilling conditions, 5°C treatments were more highly sensitive than 11°C, and lower maturity stages had higher chilling-sensitivity than higher maturity stages. Therefore, the identified characteristics of chilling symptoms, appropriate maturity stages and optimum temperature on this research could be useful for optimal fresh merchandising of cherry tomato 'Unicorn' and beefsteak tomato 'Madison'.

Key words: Ethylene production rate, Ion leakage, Respiration rate, Total chilling sensitivity.

Chilling injury (CI) is a physiological disorder of plants and plant organs caused by being exposed to low, but non-freezing, temperatures (>10°C)[1]. The main symptoms of chilling injury in tomato *(Lycopersicon esculentum)* fruit are abnormal ripening, pitting, and increased susceptibility[2-3], and these symptoms often develop only after transfer of the chilled fruit to non-chilling temperatures[4-5]. Cl can occur before or after the harvest, such as in the field or during transport, storage, and marketing[2]. Dysfunction of one or more cell membranes at chilling temperatures is thought to be the primary event that ultimately leads to chilling injury[6]. Mature-green tomato fruit stored at 5°C for 20 days had not begun to ripen and were essentially free of chilling injury symptoms; however, within 4 days of being returned to room temperature (25°C), the fruit had developed clearly visible symptoms of chilling injury[4]. Ion leakage is a parameter that has often been used to indicate physical damage to the plasmalema as a result of low temperature

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stress[7]. Chilled tomato fruit loses their ability to develop full color, shows increased sunken areas on the fruit (blemishes) and shows increased susceptibility to *Alternaria* rot and decay[8]. Care should be taken to ensure that fruits received at wholesale and retail destinations have not been exposed to chilling temperatures and exposure of fruits to such temperatures (less than 55°F) should be avoided at all times[9]. The objectives of this study were to find out the characteristics of chilling symptoms of cherry tomato 'Unicorn' compared with beefsteak tomato 'Madison' and to establish an equation for chilling symptoms.

MATERIALS AND METHODS

'Unicorn' and 'Madison' tomatoes (*Lycopersicon esculentum* Mill.) were grown hydroponically during summer to autumn at a plastic house in the northern part of Korea. Tomatoes were harvested at the breaker, turning, pink, light red and red maturity stages[10] of both cultivars at the same time. Ten fruits of each maturity stage were separately treated to measure for a particular parameter such as color development, ion leakage, respiration, and ethylene production rate. The harvested tomato fruits of two cultivars were stored separately in commercial sized carton boxes at 11°C and 5°C with 85% relative humidity for 21 days to treat chilling stress, and then these fruits were transferred and stored at 20°C with the same relative humidity condition for 4 days to observe chilling symptoms.

The color value of tomato skin was measured by Chroma Meter Model CR 400 (Konica Minolta Sensing, Inc., Japan). Measured values L*, a*, and b* represent the degree of lightness, redness, and yellowness respectively.

The Ion leakage measurement followed the slightly modified Kang *et al*. [11] method; each sample was taken from storage after 25 days. Mesocarp disks of tomatoes (4 mm thick) were prepared and put into 0.3 M mannitol solution, shaken at 100 cycles per min for 3 hours, and then measured for ion leakage by using a conductivity meter (SG3 - SevenGo™, Mettler Toledo Ltd., Switzerland). The total ion leakage read after frozen and thawed twice with shaking over the next few days. The % of ion leakage value was expressed as a percentage of 3hr values divided by total value. The difference of ion leakage between chilled and non-chilled temperatures (20°C) was calculated from the % ion leakage value in chilled temperatures (11 and 5°C) divided by the ion leakage value in non-chilled temperature.

For measuring respiration rates, the carbon dioxide was measured using a PBI Dan sensor Check Mate 9900. Ethylene production was measured using a GC 2010 Shimadzu equipped with a wax column and a flame ionization detector (FID). The detector and injector were operated at 127°C and the oven temperature was maintained at 50°C, while the carrier gas (N_2) flow rate was 0.67mL/s[12].

Total chilling sensitivity (TCs) at each stage was compared with the non-chilling stage (Red stage) using the following equation of the factors for color development (a*/b*), ion leakage, respiration rate, and ethylene production rate are well known as typical chilling injury symptoms of tomato fruits at both treated temperatures compared with the control (20°C).

$$\mathrm{TCs} = \frac{\Delta a^*/b^*{}_{20^\circ C\text{-}X^\circ C}\text{ of each stage}}{\Delta a^*/b^*{}_{20^\circ C\text{-}X^\circ C}\text{ of red stage}} \times 100 + \frac{\text{Ion leakage}_{X^\circ C/20^\circ C}\text{ of each stage}}{\text{Ion leakage}_{X^\circ C/20^\circ C}\text{ of red stage}} \times 100$$

$$+ \frac{\Delta\ \text{Respiration rate}_{20^\circ C\text{-}X^\circ C}\text{ of each stage}}{\Delta\ \text{Respiration rate}_{20^\circ C\text{-}X^\circ C}\text{ of red stage}} \times 100 + \frac{\Delta\ \text{Ethylene production rate}_{20^\circ C\text{-}X^\circ C}\text{ of each stage}}{\Delta\ \text{Ethylene production rate}_{20^\circ C\text{-}X^\circ C}\text{ of red stage}} \times 100$$

Statistical analysis

Graphs were produced using GraphPad prism 5 (GraphPad Software, Inc., USA). Color was analyzed by Dunnett's Multiple Comparison Test of 1 way ANOVA. Ion leakage, respiration, ethylene production, total chilling-sensitivity was analyzed by 2 ways ANOVA.

RESULTS AND DISCUSSION

Fruit color

Fruit color is one of the physical attributes of tomatoes that affect the consumer and buyer reference as well as market value. Chilling injured tomatoes showed uneven or partial ripening[13]. While the chilling conditions, the lower maturity stages (breaker, turning, and pink) of 'Unicorn' tomatoes showed lower color a*/b* values compared with lower maturity stages of 'Madison' tomatoes (Fig.1). The breakers and turning maturity stages stored at 5°C showed more uneven color development than those stored at 11°C in both cultivars. The stages of breakers, turning and pink at 5°C, breakers and turning at 11°C in 'Madison' tomatoes and the stages of breakers, turning, pink and light red of 'Unicorn' tomatoes at 11°C and 5°C showed a significant difference compared with red maturity stage. Chomchalow *et al.*,[14] indicated that tomatoes stored in lower temperature had less red color than tomatoes stored in higher temperature as lower storage temperature (5°C) delayed color development. In relation to this, Kader[15] indicated that temperature influences color infirmity and the softening rate of tomatoes. The lower maturity stages of 'Unicorn' tomatoes have shown the correct response of usual chilling stress as low development of color compared to the same stages of beefsteak 'Madison' tomatoes.

Ion leakage

'Unicorn' tomatoes showed higher difference of ion leakage between chilled temperatures (11°C or 5°C) and non-chilled temperature (20°C) than that shown with 'Madison' tomatoes (Fig. 2). The lower maturity stage took stronger effect from lower temperature compared to the higher maturity stage on the ion leakage in both 'Unicorn' and 'Madison' at 11°C and 5°C. In 'Unicorn' the tomatoes had a significant difference between 11°C and 5°C of all maturity stages, but 'Madison' tomatoes did not. Morris[2], who indicated that low temperatures induced chilling injury in tomatoes and this, becomes apparent in case the tomatoes are ripened at 20°C. In relation to Autio and Bramlage[16], less mature stage fruits are considered more susceptible to chilling temperature than ripe tomato fruits. Cherry tomato fruits showed higher ion leakage than beefsteak tomato fruits in all ripening stages and storage temperatures. Kang *et al.*,[17] reported that the ion leakage of mature green stage tomatoes was lower in mini size cultivar more than in normal size cultivar in low temperature storage.

Respiration rate

The respiration rate was higher in lower temperature (5°C) stored fruit than higher temperature (11°C) stored fruits in both cultivars. 'Unicorn' showed a higher respiration rate compared with 'Madison' tomatoes at both temperatures. Moreover, the breaker and turning maturity stages of 'Unicorn' showed a higher respiration rate between 11°C and 5°C compared with 'Madison' tomato. There was no significant difference of respiration rates between temperature and maturity stage in 'Madison' tomato (Fig. 3). Kang *et al.*,[11] found that chilling-induced increases the respiration in comparison with control fruit that appeared after 12 days at 10°C. Our results agreed with the results of Kang *et al.*,[11] showing that chilling-induced fruit displays higher respiration rates than non-chilled fruit. 'Unicorn' was more sensitive to chilling injury because they showed higher a respiration rate than 'Madison' tomatoes.

Ethylene production

Lower temperature (5°C) stored fruit presented higher ethylene production rates than higher temperature (11°C) stored fruit in both cultivars. 'Unicorn' showed higher ethylene production than 'Madison' tomatoes at 11°C and 5°C (Fig. 4). The 11°C and 5°C storage ethylene production rate did not show any significant difference at all maturity stages in both cultivars.

Table 1. A comparison of total chilling sensitivity of different maturity stages in two different cultivars at 11°C and 5°C storage temperature. Total chilling-sensitivity was subjectively assessed on fruits color, chilling injury index, respiration and ethylene production rate. The degree of chilling sensitivity was compared of each maturity stages with non-chilling stage (Red stage = 0 unit)

	'Unicorn'		'Madison'	
	11°C	5°C	11°C	5°C
Breakers	188.94*[z]	192.50*	110.35*	145.83
Turning	145.42	179.19**	105.92**	118.83
Pink	142.10	145.73	101.52	116.08
Lt. red	112.65	119.69	97.19	100.90

[z] Mean, *significant at 5% level and**significant at 1% level

This result agrees with the report of Klee[18], who indicated that as ethylene syntheses are lowered, tomato ripening is progressively delayed. Also McCollum *et al*.,[19] indicated that ethylene evolution was significantly higher in chilled fruit than in non-chilled fruit, even before the onset of visual symptoms of chilling injury. As a climacteric fruit, a tomato depends on ethylene for coordinated ripening[20]. Moreover, all maturity stages of 'Unicorn' showed a higher ethylene production rate difference between 11°C and 5°C compared with 'Madison' tomatoes. As 'Unicorn' tomatoes showed a higher ethylene production rate than 'Madison' tomatoes, the 'Unicorn' tomato was more sensitive to chilling injury.

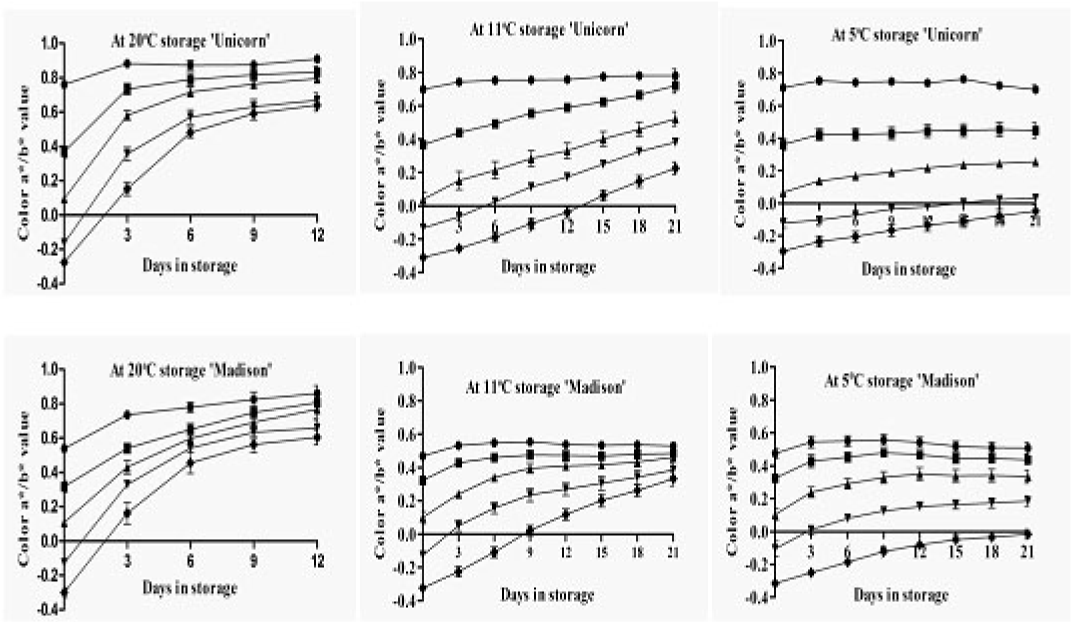


Fig. 1. Fruit color a*/b* value of different maturity stages of 'Unicorn' and 'Madison' at 20°C (as a control), 11°C and 5°C (♦: Breakers; ▼: Turning; ▲: Pink; ■: Light red; and ●: Red). Mean of five independent assays is reported with the standard error.

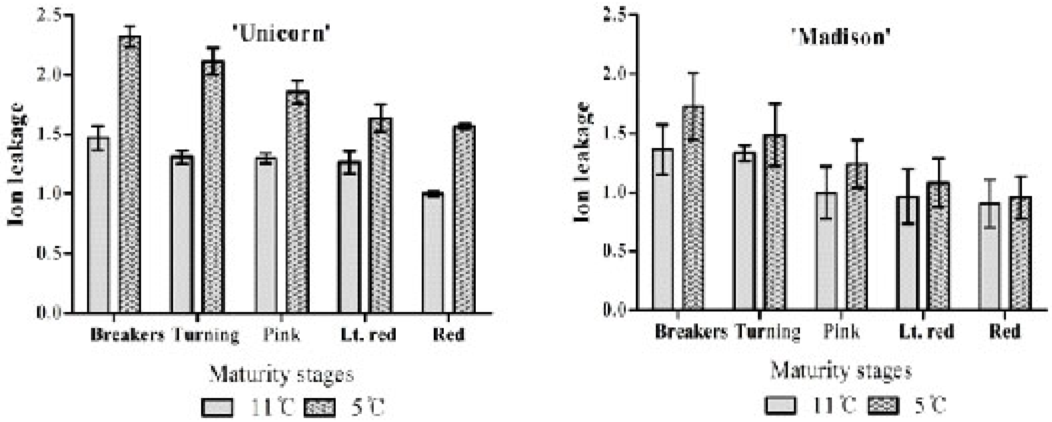


Fig. 2. Changes of ion leakage of different maturity stages of 'Unicorn' and 'Madison'(tomato stored at 11°C and 5°C for 21days separately and thereafter stored for 4 days at 20°C). Ion leakage values calculated from the % of total ion leakage: chilled temperature (11°C and 5°C) ion leakage value divided by non-chilled temperature **(**20°C) ion leakage value. Mean of five independent assays is reported with the standard error

Total chilling-sensitivity

Total chilling-sensitivity was calculated to get the chilling-sensitive temperature, chilling-sensitive maturity stages, and chilling-sensitive cultivar. The significant difference performed by breakers (<0.05) of 11°C 'Unicorn' and 11°C 'Madison', and breakers (<0.01) and turning (<0.05) of 5°C 'Unicorn' and 11°C'Madison' tomatoes (Table 1). In contrast, 11°C(<0.05) and 5°C(<0.05) 'Unicorn' displayed a significant between breakers

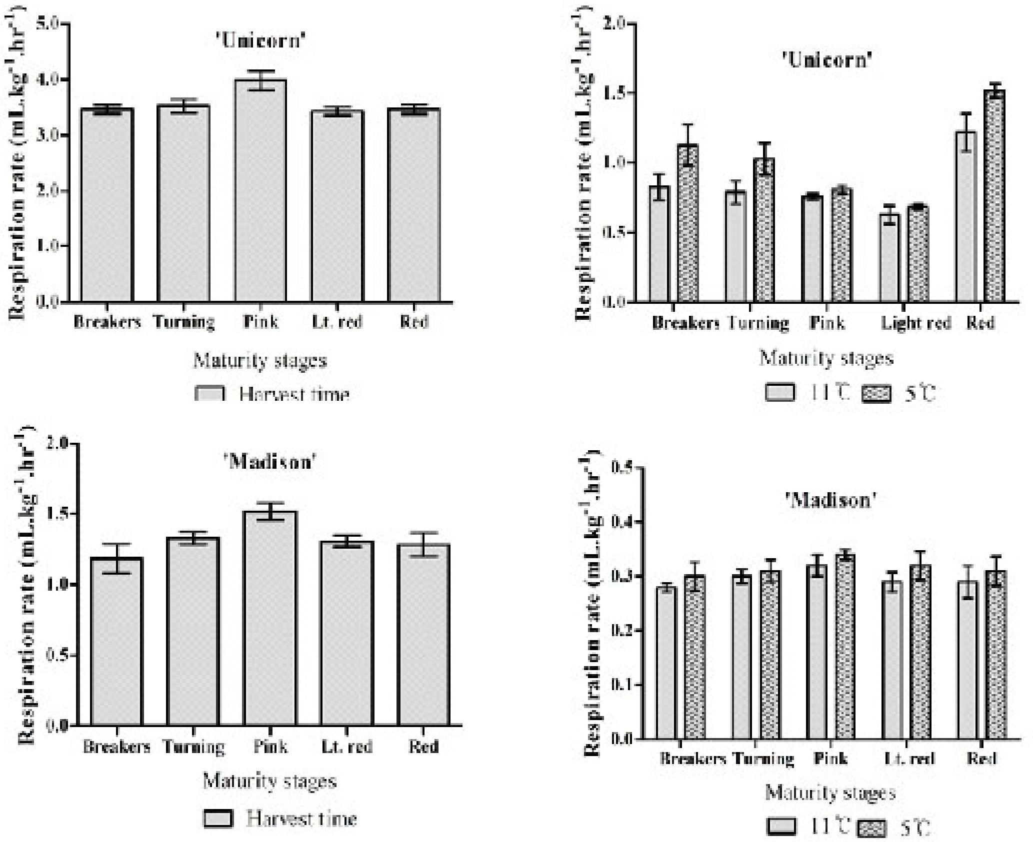
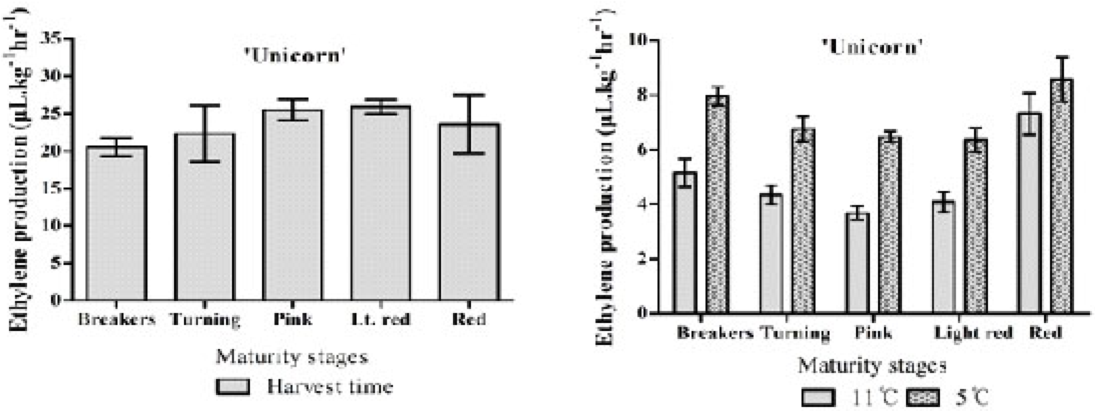


Fig. 3. Respiration rate of different maturity stages of 'Unicorn' and 'Madison'at the harvest time (20°C), 11°C and 5°C on the 25th storage day (tomato stored at 11°C and 5°C for 21days separately and thereafter stored for 4 days at 20°C). Mean of five independent assays is reported with the standard error.



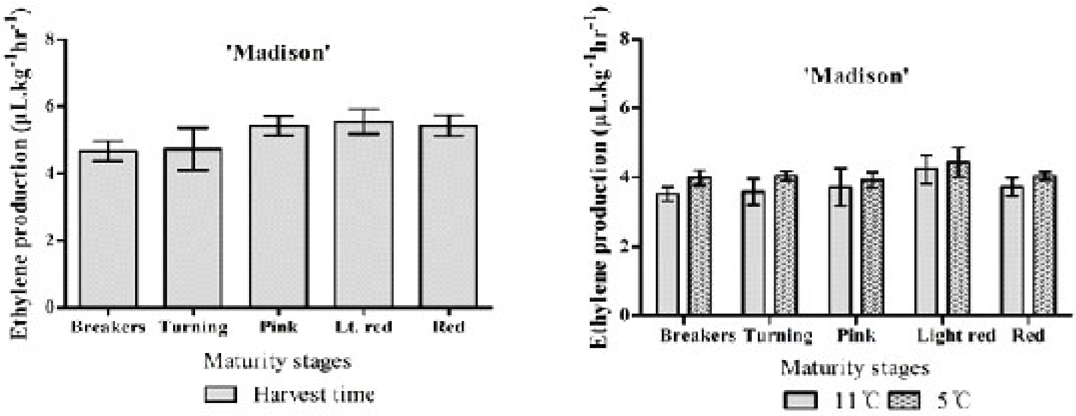


Fig. 4. Ethylene production rate of different maturity stages of 'Unicorn' and 'Madison'at the harvest time (20°C), 11°C and 5°C on the 25th storage day (tomato stored at 11°C and 5°C for 21days separately and thereafter stored for 4 days at 20°C). Mean of five independent assays is reported with the standard error.

and light red maturity. In addition, both cultivars were more sensitive to a storage temperature of 5°C than at a storage temperature of 11°C.

CONCLUSION

At 5°C tomatoes did not response to color development as much as at 11°C. However, 5°C tomatoes expressed more ion leakage, respiration, ethylene production as well as total chilling-sensitivity. In addition, earlier maturity stages of 'Unicorn' tomatoes evinced less color development and higher ion leakage and total chilling-sensitivity than 'Madison' tomatoes. Consequently, 'Unicom' cherry tomatoes should be handled at a later maturity stage than 'Madison' beefsteak tomatoes in cold chain circulation because cherry tomato fruits are more sensitive to chilling stress than beef steak tomato fruits.

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REFERENCES

1. Raison, J.K., and Lyons, J.M., Chilling injury: A plea for uniform terminology. *Plant Cell Environ*. 1986; **9**: 685–686.
2. Morris, L. I., Chilling injury of horticultural crops: an overview. *HortScience* 1982; **17**: 161-162.
3. Luengwilai K., Beckles, D.M., Saltveit. M. E., Chilling-injury of harvested tomato (SolanumlycopersicumL.) cv. Micro-Tom fruit is reduced by temperature pre-treatments. *Postharvest Biology and Technology* 2012; **63**:123–128
4. Sharom, M., Willemot, and Thompson, J. E., Chilling injury induces lipid phase changes in membranes of tomato fruit. *Plant Physiol*. 1994; **105**: 305-308.
5. Jing, Y., Fu, M., Zhao Y., and Mao, L., Reduction of Chilling Injury and Ultrastructural Damage in Cherry Tomato Fruits after Hot Water Treatment. *Agricultural Sciences in China*. 2009; **8**: 304-310
6. Nishida, I., and Murata, N., Chilling sensitivity in plants and cyanobacteria: the crucial contribution of membrane lipids. *Annual Review of Plant Physiology andPlant Molecular Biology* 1996; **47**: 541–568.
7. Tatsumi, Y., and Murata, T., Studies on chilling injury of fruits and vegetables. Part 1. Chilling injury of cucumber fruits with special reference to permeability of membrane of tissues. *J. Japan Soc. Hort. Sci*. 1978; **47**: 105-110.
8. Ding, C. K., Wang, C. Y., Gross, K. C., and Smith. D. L, Jasmonate and salicylate induce the expression of pathogenesis-related-protein genes and increase resistance to chilling injury in tomato fruit. *Planta* 2002; **214**: 895–901.
9. Kasmire, R. F., and Kader. A. A., Handling tomatoes at wholesale and retail: a guide for

better quality and greater profits. Outlook 1978; **5**: 5-12.

10. United States Department of Agriculture (USDA)., Color classification requirements in United States standards for grades of fresh tomatoes. U.S. Dept. Agr. Visual Aid TM-L-l, 1975.
11. Kang, H.M., Park, K.W., and Saltveit, M.E., Elevated growing temperatures during the day improve the postharvest chilling tolerance of greenhouse-grown cucumber (*Cucumissativus*) fruit. *Postharvest Biology and Technology* 2002; **24**: 9-57.
12. Park, K.W., Kang, H.M., and C.H. Kim, C.H., Comparison of storability on film sources and storage temperature for fresh Japanese mint in MA storage. *J. Bio. Env. Con*. 2000; **9**: 40-46 (in Korean).
13. Hobson, G. E., Low temperature injury and the storage of ripening tomatoes. *J. Hort. Sci*. 1987; **62**: 55-62.
14. Chomchalow, S., Assi, El N.M., Sargent, S.A., and Brecht, J.K., Fruit maturity and timing of ethylene treatment affect storage performance of green tomatoes at chilling and non-chilling temperatures. *Hort Technology.* January-March 2002; **12**:104-114.
15. Kader, A.A., Effects of postharvest handling procedures on tomato quality. *Acta Horticulturae* 1986; **190**: 209-221.
16. Autio, W. R., and Bramlage, W. J., Chilling sensitivity of tomato fruit in relation to ripening and senescence. *J. Amer. Soc. Hort. Sci*. 1986; **11**: 201-204.
17. Kang, H.M. and Park, K. W. andSaltveit, M.E., Chilling stress alleviation effect of pre-harvest heat treatment during cultivation of mature green tomato at low temperature storage. *J. Kor. Soc. Hort. Sci*. 1998; **40**:647-651.
18. Klee, H.J., Ripening physiology of fruit from transgenic tomato (*Lycopersicon esculentum*) plants with reduced ethylene synthesis. *Plant Physiol*. 1993; **102**: 911-916.
19. McCollum, T.G., D'Aquino, S., and McDonald, R.E., Heat treatment inhibits mango chilling injury. *HortScience* 1993; **28**:197–198.
20. Lu, J., Charles, M. T., Vigneaulta, C., Goyetteb, B., Raghavana, G.S. V., Effect of heat treatment uniformity on tomato ripening and chilling injury. *Postharvest Biology and Technology* 2010; **56**:155–162.