Effect of boron on the microcracking of tomato fruit

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Summary (English)

Microcracking (MC) downgrades the quality of tomato fruits (Solanum lycopersicum) because it allows entry of fungi, and reduces the shelf life of the fruits by damaging the fruit cuticle membrane (CM). Boron sprayed at early fruit developmental stages reduces MC in common tomato fruit and MC incidence has been thought to be influenced by climatic conditions. However, the mechanism of how B affects the incidence of MC in cherry tomatoes is still unknown. This study was therefore initiated to investigate the effect of B on the occurrence of MC on cherry tomato, using a susceptible and non-susceptible cultivar of cherry tomato. In the first study, Cherry tomato 'Chika' seedlings were grown under three different B concentrations (C5: 4.5 mg·L-1, C90: 90 mg·L-1, C0: 0 mg·L-1) during summer. Each truss was harvested when the fruit at proximal position became full ripe stage. Fruits at middle to distal position were still orange (O) to green (G). C90 reduced both fruit size and MC incidence and the MC was also found to appear at G stage. Thus, B treatment was effective for the inhibition of MC when it was applied at 90 ppm in early growing stage. However, it was not yet clear if the inhibition of MC under B excess treatment is the result of reduction of fruit growth or B directly blocks the occurrence of MC. Therefore, in the second study, we try to clarify the relationship between fruit growth, B deficiency and MC in tomato fruit. Experiments were conducted to evaluate the effect of boron (B) deficiency on MC in tomato (Solanum lycopersicum) fruits. Seedlings of two cherry tomato that differ in their susceptibility to MC— 'Regina', an MC-resistant cultivar, and 'Chika', an MC susceptible cultivar were grown hydroponically and treated with 0, 5 and 10 mg·L-1 of B in summer and autumn. 'Regina' fruit that were B deficient showed a higher incidence of

MC than fruit treated with B 15 Days After Anthesis (DAA). While no significant difference was observed in ‘Chika’ fruit under the same treatment, we found them to be susceptible to B deficiency when treated at an earlier stage (0 DAA). In both cultivars, B treatment did not affect CM deposition or fruit size. However, under B deficiency, MC incidence, and cell enlargement increased, especially in summer, when fruit growth rate was high. This indicates that the additive effects of fruit growth rate and B deficiency on the CM increased the incidence of MC in tomato fruit during summer.

Summary (Japanese)

トマト（Solanum lycopersicum）の果実に発生する生理障害であるつやなし果では，果実表面のクチクラ膜に微細な亀裂（MC）が生じる．つやなし果では水分の損失が 促進され，また病原体の侵入を許しやすくなることから棚持ちが著しく悪くなる．これまでに， 果実の発達初期段階においてホウ素を葉面散布することで大玉品種におけるつやなし果発生が 低減されることが明らかになっているが，ミニトマトにおけるホウ素処理の影響や，ホウ素が つやなし果発生を抑制する機構は明らかになっていない．本研究では，つやなし果が発生しや すい品種と発生しにくい品種を材料とし，ホウ素処理を施すことでミニトマトのつやなし果発 生にホウ素が与える影響を検討した．実験 1 では，つやなし果が発生しやすい‘千果’ を 3 つのホウ素濃度条件で水耕栽培した（C5: 4.5 mg·L -1 , C90: 90 mg·L -1 , C0: 0 mg·L -1 ）. C90 では果実サイズおよび MC 発生率が対照区（C5）と比較して減少したことから，ミニトマト でもホウ素処理がつやなし果発生率に影響を与えることが確認された．実験 2 では，‘千果’ および，つやなし果が発生しにくい‘レジナ’を用い，0, 5 および 10 mg·L -1 のホウ素濃度 で水耕栽培することで，ホウ素欠乏がつやなし

果発生に与える影響をさらに検討した．夏作の ‘レジナ’では，ホウ素欠乏処理（R0）を開花 15 日後（15DAA）に開始した際に対照区 （R5）よりもつやなし果発生率が上昇した．夏作の‘千果’では，15DAA にホウ素欠乏処理 を開始してもつやなし果発生率に影響が出なかったものの，0DAA に処理を開始すると C0 で つやなし果発生率が減少し，C10 で上昇した．一方で，秋栽培では両品種において，ホウ素処 理はつやなし果発生率に影響を与えなかった．夏栽培では，ホウ素処理は両品種の果実サイズ および単位面積あたりのクチクラ沈着量に影響を与えなかったが，ホウ素欠乏処理の果実では 果実表皮細胞のサイズが増加していた．ホウ素欠乏処理は，果実表皮細胞の肥大促進を通じて クチクラ膜にストレスを与えていることが示唆され，特にその影響は果実肥大速度の高い夏作 において顕著になると考えられた．

Chapter I. General Introduction

1.1 Description of Microcracking

Microcracking (MC) of the cuticle, also called cuticle cracking, russeting, hair cracking, swell cracking, shrink cracking, rain check, crazing, and cuticle blotch is a physiological disorder characterized by micro cracks of 1–2 mm observed in many fruits, such as apple (Malus domestica), cherry (Prunus avium), and tomatoes (*Solanum lycopersicum*). It occurs on the cuticle membrane (CM) in the peduncular the sides regions of the fruit (Opara et al., 1997; Dorais et al., 2004). MC reduces the esthetic appearance of tomatoes (*Solanum lycopersicum*) (Peet and Willits 1995) and their post-harvest shelf life (Dorais et al., 2001) owing to infection by infiltration of pathogens through these lesions (Peet, 1992). MC is distinguished from radial macrocracking or shoulder check by a smaller crack size and shallow depth (Dorais et al., 2004). Severe cases of MC in tomatoes can spread to the entire surface of the fruit and form corky tissues following the healing of wounds caused by this phenomenon. For tomato-grower, MC in tomato fruit means a lower selling price that can cause significant economic losses. It is, therefore, important to identify the causes and mechanisms involved in the occurrence of MC.

1.2 Cuticle Membrane

The cuticle membrane (CM) forms the outermost layer of the epidermis and is composed of a cutin polymer matrix, consisting mainly of esterified fatty acids and embedded

cuticular and surface-deposited epicuticular waxes (Peschel et al., 2007). It acts as the barrier which prevents excessive water lost by evaporation from the plant to its environment. MC defect is expressed within the epicarp and CM of the fruit (Snapp et al., 2001). Many studies have tried to linked MC incidence with CM properties. In cherry tomato, cracking resistance fruit was thought to correlate with CM thickness. It was observed that fruit with thicker CM deposition were less susceptible to cracking (Matas et al., 2004).

1.3 Physiological explanation of the occurrence of microcracking

Many studies toward the incidence of cracking and MC in tomato fruits, as well as other fruit such as apple and pear (Opara et al., 1997) have been conducted. Some studies focused on physiological and genetics aspect of some MC resistant tomato cultivar (Peet, 1992; McAvoy, 1995). Others studies looked for the effect of climatic conditions or cultivation practices on the occurrence of MC on tomato fruits (Dorais et al., 2001 and 2004; Simard, 2001). The physiological explanation for MC is usually associated with rapid transportation of water and sugars into fruits while the elasticity and resistance of their CM are weak (Cotner et al., 1969; Guichard et al., 2001). Fruits with high sugar content usually have a larger water supply (Bussières, 1995), thus causing an increase in the pressure applied against the CM and greater vulnerability of the fruit to MC (Corey and Tan, 1990). Since the CM of the tomato is 97% impermeable to gas exchange (Corey and Tan., 1990), an increased pressure inside the fruit would create tension on the CM. As a result of the extreme variations

and continuous of the internal pressure of the fruit, there would be a weakening of the resistance of the CM and consequently the occurrence of MC.

1.4 Climatic conditions conducive to Microcracking

Greenhouse growing climatic conditions have been shown to play an important role in MC occurrence (Peet, 1992; Peet and Willits, 1995; Opara et al., 1997; Ehret, 1993; Dorais et al., 2001 and 2004; Simard, 2001). Simard (2001) demonstrated that 21 days before harvesting, some factors such as the average daytime temperature (18 and 25ºC); average night temperature (14 and 21ºC); the average daily temperature (17 and 23ºC); and the day/night temperature difference (4 and 13ºC); influenced the incidence and severity of the MC. In addition, sudden temperature variations promoted MC of fruits (Peet, 1992; McAvoy, 1995). Corey and Tan (1990) demonstrated that a high day/night temperature difference promoted variations of the pressure inside the fruit and thus the incidence of MC. Peet (1992) observed that under high temperatures and light intensity, the incidence of the MC is often larger. These climatic conditions would promote a greater flow of photo assimilates towards the fruits and a heating of plant tissues following solar radiation, causing an increase in internal pressure and increased tension on the CM of the fruits. Under high humidity conditions, transpiration of plants is restricted and root pressure increases, thus promoting MC (Peet and Willits, 1995). In addition, under high temperatures, inhibition of evapotranspiration and maintaining of the heat balance would cause an increase in the internal pressure of the fruits and the MC (Peet, 1992; Corey and Tan, 1990).

1.5 Role of Boron on MC incidence

Boron (B) is an important micronutrient involved in cell wall development, cell division, phloem development, and movement of sugars, metabolism of nitrogen and phosphorus and absorption of salts (Dale and Krystyna, 1998). It also plays an important structural role in plants and are involved in cell division and cell elongation in the fruit epidermis. Spraying B fertilizers 3-4 times during flowering and early fruit development periods can reduce fruit cracking in 'Duwei' pomelo. Wang and Qin (1987) reported that the difference in the effective B content between normal fruit peels and cracking fruit peels was not significant; however, higher effective B content in leaves could help to reduce fruit cracking.

B deficiency significantly alters the activity of certain enzymes and thus affect the metabolism of higher plants. Yamauchi et al. (1986) demonstrated that B plays an important role in calcium metabolism of the cell wall. B deficiency can cause decrease in Ca concentrations associated with peptide compounds. It also results in the development of corky lesions near the calyx (Jarvis and McKeen 1991; Winsor and Adams, 1987). It is known that in the most severe cases of MC of the CM of tomatoes greenhouses are characterized by the appearance of related lesions. Huang et al. (2004) showed that a weekly foliar spray of 300 $mg \cdot L^{-1}$ of B on common tomato 'Mountain spring' cultivar reduces the shoulder check defect (MC around the shoulder area of tomato fruit) by 42%, compared to 150 $mg \cdot L^{-1}$ of B, which reduced the defect by only 9% when grown at a temperature of 25 °C/20 °C (day/night). However, the mechanism of how B affects the incidence of MC in cherry tomatoes is still

unknown. It was hypothesized that B deficiency could increase MC by affecting CM deposition or epidermal cell division and cell enlargement. This study was therefore initiated to investigate the effect of B on the occurrence of MC on cherry tomato.

1.6 Objectives

The objective of this study is to evaluate the effect of B on the occurrence of MC and investigate its possible mechanism of action using a susceptible and non-susceptible cultivar of cherry tomato. Greenhouse climatic conditions have been shown to play a role in the incidence of MC (Dorais et al., 2001 and 2004). Therefore, the experiments were conducted in both summer and autumn, focusing on the effect of temperature and B deficiency on 1) formation of microcracks in the CM of tomato fruit, 2) and the deposition of the CM, 3) epidermal cell size.

Chapter II. Effect of boron on the microcracking of 'Chika' cultivar cherry tomato

Abstract

Microcracking (MC) downgrades the quality of tomato because it provides entry for fungi, and reduces the shelf life of tomato fruit. Huang et al., (2004) found that B could reduce MC incidence in common tomato but it's known that MC occurs also in cherry tomato. Therefore, in this study the effect of B on MC of cherry tomato was evaluated. Cherry tomato cv. Chika seedlings were grown under three different B concentrations (C5: 4.5 $mg \cdot L^{-1}$, C90: 90 $mg \cdot L^{-1}$, C0: 0 $mg \cdot L^{-1}$) during summer. Each truss was harvested when the fruit at proximal position became full ripe stage. Fruits at middle to distal position were still orange (O) to green (G). C90 reduced both fruit size and MC incidence (less than 2%) and the MC was also found at G stage. Thus, B treatment was effective for the inhibition of MC when it was applied at 90 ppm in early growing stage.

2.1 Introduction

Improving the quality and appearance of fruit is the foundation for successful fresh market tomatoes production. Tomato fruit quality and appearance are determined by a wide range of environmental and genetic factors, where nutrition plays an important role. Potassium (K), calcium (Ca), and boron (B) are the key nutritional factors controlling fruit development and maturation (Marschner, 1995).

Huang and Snapp, (2004) investigate the effect of foliar spray of Ca (2 $g \cdot L^{-1}$), B (15; 30 $mg \cdot L^{-1}$) Ca + B, on 'Mountain Spring' tomato. Temperature was kept at 25°/ 20°C (day/ night). Twenty- eight percentage of reduction in defect was associated with Ca + B spray or B alone. Incidence of defect was low with highest rate of B foliar spray in common tomato. Based on these observations it was hypothesized that B deficiency could induce MC incidence. It has been known that MC also occurred in cherry tomato. Therefore, this study was conducted to evaluate the effect of B on the MC of cherry tomato. Apart from the primary objective to determine the effect of low B levels on tomato fruit development and quality, the possible toxicity reactions of high B-levels were also investigated.

2.2 Material and Method

All experiments were carried out at the experimental field of Yamagata University Tsuruoka, Japan.

2.2.1 Plant material and Boron treatment.

On 3 April, 2017, seeds of tomato cv. Chika were sown in a moisturized petri dish and incubated at 24-26°C for inducing germination. On 5 April, 2017 the germinated seeds were sown in a cell tray with 125 ml cells, filled with planting media (Baido 300, Tsuruoka city Agr. Coop, Tsuruoka, Japan) containing 220 $mg \cdot L^{-1}$ of N, 550 $mg \cdot L^{-1}$ of P, and 210 $mg \cdot L^{-1}$ of K, at a pH of 5.5–6.5. The cell tray was then placed in an incubator set at 24 ± 2 °C and 16 h day length. The light intensity of the incubator was approximately an average of 90–100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the canopy of the seedlings. On 24 April, (19 DAS) the seedlings with 4-5 leaves were transplanted in 1L plastic pot and grown in a glass-house. On 11 May (17DAT) the tomato plants with 7-8 leaves were transplanted in 50L Styrofoam boxes filled with nutrient solution which was prepared using chemical fertilizers (Otsuka 1, 2 and 5, OAT AGRIO Co., Ltd, Tokyo, Japan) described in Table 1 and grown hydroponically in a plastic house until the B treatment started.

Table 1. Composition of Otsuka fertilizer.

	Amount of nutrient (%)										
Fertilizer	N	P_2O_5	K_20	MgO	Mn	B_2O_3	CaO	Fe	Cu	Zn	Mo
N°1	10	8	27	4	0.1	0.1		0.18	0.002	0.006	0.002
N°2	11	0	0	0	0	0	23	0	0	0	0
N°5	6	0	9	0	2	2	0	5.7	0.04	0.08	0.043

To avoid heating, the containers were covered by aluminum foil. Air was supplied by an air pump (LP-60AHC, Yasunaga air pump inc., Tokyo, Japan) throughout the growing period. On 18th may, 15 Days After Anthesis. B treatment was conducted until the end of the cultivation. The Experiment design was a randomized complete block. Three different treatments (C0 C5 and C90) were conducted. Among them, C5 was 4.5 $mg \cdot L^{-1}$ B; C90, 90 $mg \cdot L^{-1}$ and C0 $mg \cdot L^{-1}$. The nutrient solution (Table 2) was completely changed once a week.

Table 2. Concentration of nutrients in solution used for hydroponics.

	Concentration		
Nutrient	mM	mEq	ppm
K	5.82	5.82	588
Ca	3.13	6.26	739.5
Mg	1.51	3.02	372.3
P	1.02	2.04	117.6
Fe	0.05	0.05	22.05
Cu	0.003	0.006	0.8
Zn	0.001	0.002	0.33
Mn	0.012	0.024	0.15
Mo	0.006	0.024	3
B	0.07	0.07	4.46
NH4-N	1.02	1.02	117.6
NO3-N	8.95	8.95	1327.5

Each truss was harvested when the fruit at proximal position because full ripe (R) (Fig.2). Fruits at middle and distal position were still orange (O) to green (G) stage. Three trusses were allowed to bear, because on the fourth truss C90 treatment didn't set fruits. Each fruit was independently analyzed.

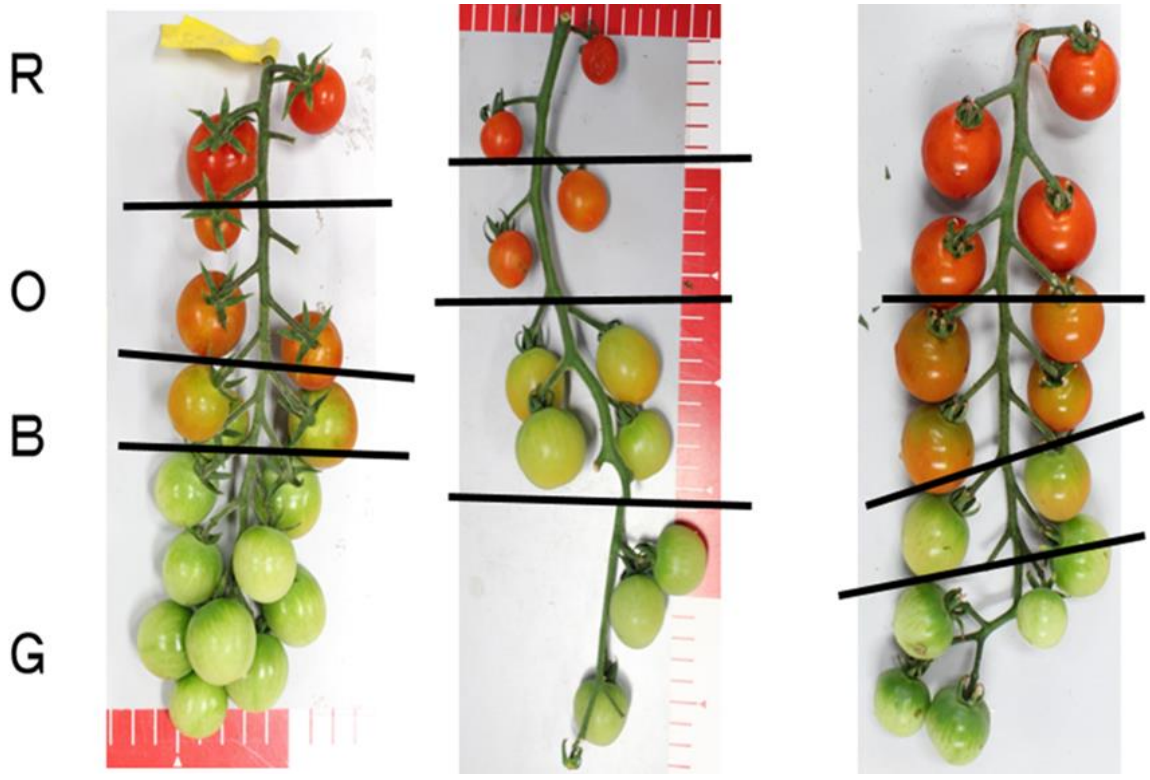

Fig.1. Fruit at harvested stage and Severity of MC.

R: red, O: orange, B: breaker and G: green stage.

Fruit size

Harvested fruit were weighed, and their equatorial diameter and heights were measured using a digital caliper. Surface area was calculated from the radius (equivalent to the average of fruit height and its diameter), assuming a spherical shape.

2.2.2 Microcracking

Each fruit (both sides) was observed under a digital microscope (Leica DMS 1000, Tokyo, Japan) to determine the incidence of MC. To evaluate the severity of MC, all fruit were sorted into three categories: MC0, no MC; MC1, few MC oriented parallel to each other; and MC2, fruit surface covered with a network of MC (Fig. 2).

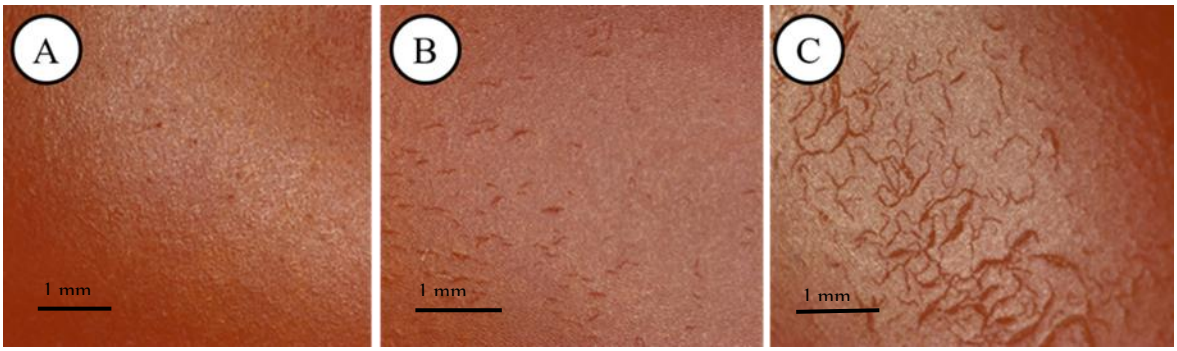

Fig. 2. Severity of MC. Representative image of tomato skin with different categories of MC.

A: No MC (MC0); B: MC oriented parallel to each other (MC1), and C: Fruit surface covered with a network of MC (MC2). Magnification: 1.25×; scale bar: 1 mm

2.3 RESULTS AND DISCUSSION

2.3.1 Plant growth, fruit size and Microcracking

C90 and C0 treatments significantly affected plant growth. C90 treated plants showed lower shoot growth, smaller trunk diameter and necrosis of root tissues compare to control. In C0 treatment, leaves were thick, curled, discolored and root tissues were short (Fig.3).

Fig.3. Effect of B deficiency and toxicity in tomato plant.

A: Effect of B deficiency and toxicity on plant growth

B: Effect of B deficiency and toxicity on tomato root development

C: Effect of B deficiency and toxicity on tomato leaves

Similar result was found in melons that were grown under B-deficient conditions, where leaves yellowed prematurely owing to chlorophyll losses (Com-brink, et al., 1995). Regarding the fruit size, C90 inhibited fruit growth but C5 and C0 did not show significant difference (Table 3). These results suggested that B deficiency induced 15 DAA did not influence 'Chika' fruit growth. We should note that a conventional concentration of B (5 $mg \cdot L^{-1}$) was applied before the start of treatment. However, it was observed that B deficiency induced some physiological disorders such as the inhibition of plant growth. Smaller fruit size observed under excess B treatment, might be due to poor water uptake following the root necrosis caused by B toxicity (Fig.3 C).

Regarding the MC incidence, no significant difference was found between C5 and C0. In the opposite, regardless of fruit position on the truss, 90 $mg \cdot L^{-1}$ treatment dramatically reduced MC incidence (less than 2%). Thus, B was effective for the inhibition of MC when it was applied at 90 $mg \cdot L^{-1}$. However, it was not yet clear if the inhibition of MC under B treatment is the result of the reduction of fruit growth or B directly blocks the occurrence of MC. Gill and Nandpuri (1968) found that MC could be related to fruit size and shape.

Furthermore, MC was found to occurs in early growing stage of tomato fruit (G stage). Similar result was shown in Young (1947) and den Outer (1987) where, based on field observation MC of tomato fruit was reported to occur most often at G stage. This suggested that B deficiency applied more earlier could have affect MC in 'Chika'. Additionally, based on the observation in greenhouse production, 'Chika' seems to be a MC susceptible cultivar.

The effectiveness of B deficiency in inducing MC could therefore appear in very early growing stage.

Table 3. Effect of B concentration on the occurrence of MC at each growing stage.

Treatments	Fruit size (cm^3) [z] ± SE				MC incidence (%) [x] ± SE			
	G	B	O	R	G	B	O	R
C0	17.0 ± 2.3 a	16.0 ± 2.5 a	19.0 ± 2.4 a	17.0 ± 2.6 a	28.0 ± 3.7 a	36.0 ± 3.9 a	28.0 ± 3.3 a	53.0 ± 4.8 a
C5	17.0 ± 2.5 a	19.0 ± 2.9 a	19.0 ± 2.9 a	15.0 ± 2.6 a	27.0 ± 3.7 a	33.0 ± 3.1 a	38.0 ± 2.7 a	44.0 ± 5.0 a
C90	12.0 ± 1.9 b	12.0 ± 1.8 b	12.0 ± 2.5 b	16.0 ± 2.3 b	2.0 ± 0.0 b	2.0 ± 0.0 b	0.0 ± 0.0 b	2.0 ± 1.6 b

[z] Fruist volume were measured using volume meter. Means (R n: C0=59, C5=47, C90= 40; O n: C0=58, C5=56, C90= 47; B n: C0=42, C5=43, C90= 18; G n: C0=57, C5=55, C90= 44 with different letters in each column are significantly different by Tukey's HSD test at $P<0.05$.

[y] MC: Micro Cracking. MC was determined by observing tomato fruit on digital microscope. Fruits were sorted according to their growing stage.

C: 'Chika', G: Green, B: Breaker, O: Orange, R: Red

2.4 CONCLUSION

The results of this study indicated that B deficiency and B toxicity affect negatively plant growth. B at 90 $mg \cdot L^{-1}$ was effective for the inhibition of MC. However, it also inhibited fruit growth. Thus, it is not yet clear if the inhibition of MC under B excess treatment is the result of reduction of fruit growth or B directly blocks the occurrence of MC. Therefore, in the chapter 2, we will try to clarify the relationship between fruit growth, B deficiency and MC in tomato fruit.

Chapter III. Boron deficiency enhances microcracking in tomato fruit during Summer.

Abstract

Experiments were conducted to evaluate the effect of boron (B) deficiency on microcracking (MC) in tomato (*Solanum lycopersicum*) fruits. Seedlings of two cherry tomato cultivars 'Chika' and 'Regina' were grown hydroponically and treated with 0, 5 and 10 $mg \cdot L^{-1}$ of B in summer and autumn. 'Regina' fruit that were B deficient showed a higher incidence of MC than fruit treated with B 15 Days After Anthesis (DAA). While no significant difference was observed in 'Chika' fruit under the same treatment, we found them to be susceptible to B deficiency when treated at an earlier stage (0 DAA). In both cultivars, B treatment did not affect CM deposition or fruit size. However, under B deficiency, MC incidence, and cell enlargement increased, especially in summer, when fruit growth rate was high. This indicates that the additive effects of fruit growth rate and B deficiency on the CM increased the incidence of MC in tomato fruit during summer.

3.1 INTRODUCTION

Microcracking (MC) of the cuticle is a physiological disorder characterized by micro cracks of 1–2 mm observed in many fruits, such as apple, berries, and tomatoes. It occurs on the cuticle membrane (CM) in the peduncular region and the sides of the fruit (Opara et al., 1997; Dorais et al., 2004). MC reduces the esthetic appearance of tomatoes (*Solanum lycopersicum*) (Peet and Willits 1995) and their post-harvest shelf life (Dorais et al., 2001) owing to infection by infiltration of pathogens through these lesions (Peet, 1992). For tomato-growing greenhouses, MC in tomato fruit means a lower selling price that can cause significant economic losses. It is, therefore, important to identify the causes and mechanisms involved in the occurrence of MC.

It has been demonstrated that MC is difficult to control in greenhouse-grown tomatoes because of the complex interplay of several factors in the occurrence of this phenomenon (Dorais et al., 2001). Boron (B) and calcium (Ca) have been studied in relation to cracking because they play an important structural role in plants and are involved in cell division and cell elongation in the fruit epidermis. Huang et al. (2004) showed that a weekly foliar spray of 300 $mg \cdot L^{-1}$ of B on common tomato 'Mountain spring' cultivar reduces the shoulder check defect (MC around the shoulder area of tomato fruit) by 42%, compared to 150 $mg \cdot L^{-1}$ of B, which reduced the defect by only 9% when grown at a temperature of 25 °C/20 °C (day/night). It was, therefore, hypothesized that B deficiency could increase MC. However, the mechanism of how B affects the incidence of MC in cherry tomatoes is still unknown.

The objective of this study is to evaluate the effect of B on the occurrence of MC and investigate its possible mechanism of action using a susceptible ‘Chika’ and non-susceptible ‘Regina’ cultivar of cherry tomato. Greenhouse climatic conditions have been shown to play a role in the incidence of MC (Dorais et al., 2001 and 2004). Therefore, the experiments were conducted in both summer and autumn, focusing on the effect of temperature and B deficiency on 1) formation of microcracks in the CM of tomato fruit, 2) deposition of the CM and 3) epidermal cell size.

3.2 Material and Methods

3.2.1 Plant material and culture

Two experiments were conducted in summer and autumn of 2020 and 2021. The first experiment was conducted in both summer and autumn 2020. Two cherry tomato cultivars that differ in their susceptibility to MC— 'Regina', an MC-resistant cultivar, and 'Chika', an MC-susceptible cultivar—were studied to evaluate the effect of season and B treatment on the occurrence of MC. In summer, on March 5, 2020, the seeds of both cultivars were sown in a moisturized Petri dish and incubated at 24 ± 2 °C to induce germination. Five days later, the germinated seeds were sown in a cell tray filled with 125 mL cell suspension and a planting medium (Baido 300, Tsuruoka city Agr. Coop, Tsuruoka, Japan) containing 220 $mg \cdot L^{-1}$ of N, 550 $mg \cdot L^{-1}$ of P, and 210 $mg \cdot L^{-1}$ of K, at a pH of 5.5–6.5. The cell tray was then placed in an incubator set at 24 ± 2 °C and 16 h day length. The light intensity of the incubator was approximately an average of 90–100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the canopy of the seedlings. Twenty days after sowing (DAS), seedlings with 4–5 leaves were transplanted in 1 L plastic pots and grown in a glass house. Thirty days after transplanting (DAT), seedlings with 7–8 leaves were transplanted in 25 L Styrofoam boxes filled with nutrient solution, which was prepared using chemical fertilizers (Otsuka 1, 2 and 5, OAT AGRIO Co., Ltd, Tokyo, Japan) (Table 1), and grown hydroponically in a plastic house until the B treatment was started.

To avoid heating, the containers were covered with aluminum foil. Air was supplied to the roots by using an air pump (LP-60AHC, Yasunaga air pump inc., Tokyo, Japan)

throughout the growing period. B treatment started 15 days after anthesis (DAA), on May 15, 2020, and the fruit were harvested when fully ripened, between July 8 and August 10, 2020. For autumn production, seeds of both the cultivars were sown on July 24, 2020, transplanted to nutrient solution on September 23, 2020. B treatment was started on October 7, 2020, 15 DAA. The fruit were harvested between November 15 and December 15, 2020, when fully ripened.

In the second experiment conducted in summer 2021, the same method as the first experiment was followed. Seeds of 'Chika' cultivar was sown on February 23. The seedlings were transplanted to nutrient solution on April 20, followed by B treatment on May 12. Because we aimed to investigate the effect of growth stage and B treatment on 'Chika' cultivar, B treatment started when the fruit in the first, second, and third truss completed 20 DAA, 7 DAA, and 0 DAA, respectively; the fruit were also harvested when fully ripened, between June 7 and the end of July.

3.2.2 Boron treatment

The experimental design was randomized with five replicates of each treatment. However, in the second experiment, unfortunately, owing to a strong typhoon, two replicates were lost in which B treatment was started 20 DAA.

Three different treatments (C0, Rg0; C5, Rg5; C10, Rg10; C is for 'Chika' and Rg is for 'Regina') were applied to seedlings of both cultivars. Among them, C0 and Rg0 were treated with 0 $mg \cdot L^{-1}$ of B which aimed to induce B deficiency in the seedlings. C5 and Rg5

were treated with 5 $mg \cdot L^{-1}$ of B, which is the optimum quantity of B required for normal growth of the seedlings. This treatment is considered as a control. C10 and Rg10 were treater with 10 $mg \cdot L^{-1}$ of B. It was observed in preliminary experiments (data not shown) that MC occurred even under a normal concentration of B. The high B concentration (10 $mg \cdot L^{-1}$) was applied to determine whether it could reduce the incidence of MC in tomato fruit. The pH and electric conductivity (EC) of the nutrients were maintained at 5.5–6.5 and 1.52–2 mS cm-1, respectively, and the nutrient solution (Table 2) was changed weekly. Three trusses were used, because on the fourth truss, B deficit plants did not bear fruit. Each fruit was analyzed independently.

3.2.3 Fruit size

Harvested fruit were weighed, and their equatorial diameter and heights were measured using a digital caliper. Surface area was calculated from the radius (equivalent to the average of fruit height and its diameter), assuming a spherical shape.

3.2.4 Microcracking

Each fruit (both sides) was observed under a digital microscope (Leica DMS 1000, Tokyo, Japan) to determine the incidence of MC. To evaluate the severity of MC, all fruit were sorted into three categories: MC0, no MC; MC1, few MC oriented parallel to each other; and MC2, fruit surface covered with a network of MC (Fig. 1).

3.2.5 Cuticle isolation

Cuticle was enzymatically isolated (Orgell, 1955; Yamada et al., 1964). Fruits were cut into two halves and the mesocarp was removed. The exocarp was incubated in a 50 mM acetic acid buffer solution (pH 4.0) containing pectinase (6 $g \cdot L^{-1}$) (Pectinase SS 18%, dextrin 82%, Yakult Pharmaceutical industry Co., Ltd, Tokyo, Japan) and cellulose (3 $g \cdot L^{-1}$) (Cellulase Y-NC 33%, dextrin 67%, Yakult Pharmaceutical industry Co., Ltd, Tokyo, Japan). Vacuum and ultrasonic waves were used to facilitate enzyme penetration, and the samples were incubated at 50 °C for 30 days. The enzyme solution was changed weekly, and isolated CMs were thoroughly rinsed in deionized water, dried at 40 °C, and weighed.

3.2.6 Epidermal cell size

Ten digitized calibrated images of individual CM were prepared at 20× (DM 2500 LED, LEICA, Tokyo, Japan). Cell width (W) and length (L) of five cells per sample were measured, and the cell size was calculated by assuming a spherical shape of the cell, using the following formula:

Cell size = $(W/2 \times L/2) \times 3.14$

3.2.7 Data analysis

Statistical analysis was performed using the software package SPSS 24.0 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). Data were then subjected to a one-way analysis of variance, and significance of the differences was determined using Tukey's honestly significant difference at 0.05 probability level. A Bonferroni/Dunn test was used to determine the effect of B on incidence of MC.

3.3 Results

3.3.1 Effect of season and boron treatment on ‘Chika’ and ‘Regina’ tomatoes (Experiment 1)

3.3.1.1 Plant growth, fruit weight, microcracking

Regardless of the season, plant growth was affected by B deficiency. In B deficient plants (Rg0 and C0), leaves were thick, curled, discolored, and dried when compared to those of the control (Rg5 and C5). In the case of 10 $mg \cdot L^{-1}$ of B treatment (Rg10 and C10), plants showed a lower shoot growth, and the borders of old leaves appeared dried (Fig. 3). However, fruit weight was not affected by B treatment in both cultivars (Table 2.1).

Table 2.1. Effect of B treatment on fruit weight, MC incidence and CM deposition.

Seasons	Treatments	Fruit weight (g)	MC incidence (%) [z]			CM deposition (µg.mm^{-2}) [y]
			MC0	MC1	MC2	
Summer	Rg0	14.5 ± 0.4 a	30.2 ± 4.1 b	20.7 ± 3.4 a	44.1 ± 3.9 a	11.3 ± 1.5 a
	Rg5	14.8 ± 0.5 a	56.4 ± 4.2 ab	13.4 ± 2.7 a	39.6 ± 5.7 ab	10.8 ± 1.4 a
	Rg10	14.9 ± 0.4 a	71.2 ± 2.9 a	12.0 ± 1.7 a	15.7 ± 1.9 b	10.5 ± 1.1 a
	C0	14.7 ± 0.6 a	8.9 ± 3.8 a	12.8 ± 1.7 a	78.1 ± 3.9 a	21.4 ± 2.3 a
	C5	16.8 ± 0.5 a	8.6 ± 2.6 a	21.4 ± 3.5 a	69.7 ± 3.6 a	19.3 ± 2.1 a
	C10	13.7 ± 0.5 a	11.4 ± 3.4 a	16.0 ± 2.8 a	72.5 ± 4.1 a	20.7 ± 2.3 a
Autumn	Rg0	10.0 ± 2.2 a	87.5 ± 3.6 a	11.2 ± 3.3 a	1.2 ± 1.6 a	8.3 ± 0.7 a
	Rg5	7.3 ± 1.9 a	88.7 ± 4.0 a	10.0 ± 4.1 a	1.2 ± 1.6 a	8.7 ± 1.0 a
	Rg10	8.5 ± 2.0 a	93.7 ± 2.6 a	6.2 ± 2.7 a	0.0 ± 0.0 a	8.1 ± 0.6 a
	C0	10.5 ± 2.5 a	79.9 ± 3.9 a	6.2 ± 2.9 a	12.5 ± 3.4 a	13.8 ± 0.8 a
	C5	12.7 ± 2.5 a	55.7 ± 3.9 a	11.2 ± 2.8 a	33.6 ± 4.3 a	14.2 ± 0.9 a
	C10	12.7 ± 2.3 a	74.6 ± 5.1 a	10.8 ± 3.6 a	19.5 ± 3.9 a	17.3 ± 2.4 a

Rg: 'Regina'; C: 'Chika'

Means (In Summer n: Rg0 = 81, Rg5 = 73, Rg10 = 78; C0 = 118, C5 = 113, C10 = 125. In Autumn Rg0 = 80, Rg5 = 54, Rg10 = 55; C0 = 77, C5 = 80, C10 = 81) with different letters between B treatment, within each cultivar are significantly different by Tukey's HSD test at $P<0.05$.

[z] MC: Microcracking. MC was determined by observing tomato fruit under digital microscope. Fruits were sorted according to their severity in MC0, 1 and 2. 0: No MC; 1: few MC oriented parallel to each other, and 2: Fruit surface covered with a network of MC.

[y] CM: Cuticle Membrane. CM deposition was determined by dividing each fruit CM weight per fruit surface area.

In the autumn, MC did not differ based on treatment in either cultivar. However, in summer, we observed that the frequency and severity of MC was significantly increased in Rg0 compared to that in Rg10, while the frequency and severity of MC was not affected by treatment in 'Chika' (Table 2.1). Although not significant, the frequency and severity of MC was slightly higher in fruit of Rg0 plants than those of Rg5 plants because a conventional concentration of B (5 mg·L^{-1}) was applied before the start of treatment (15 DAA). It is

possible that Rg0 plants accumulated enough B before the treatment started to mask the differences between Rg0 and Rg5.

3.3.1.2 Effect of season and boron treatment on cuticle membrane, fruit surface area, and epidermal cell size

In both seasons, no significant difference was observed in the CM deposition between the cultivars under any of the treatment conditions (Table 2.1). Thus, CM deposition was considered to not be involved in the increase in MC due to B deficiency in 'Regina'. By contrast, fruit from seedlings cultivated in summer showed higher CM deposition than from those grown in autumn. Consistent with the results of fruit weight (Table 2.1), no significant difference among the treatments was observed in the case of fruit surface area in both seasons (Table 2.2).

Table 2.2. Effect of B treatment on tomato fruit surface area and epidermal cell size.

Seasons	Treatments	Fruit surface area (cm^2) [z] ± SE			Epidermal Cell size [y] (μm^2) ± SE			
		MC0	MC1	MC2	MC0	MC1	MC2	Average
Summer	Rg0	25.2 ± 2.4 a	25.6 ± 1.3 a	24.6 ± 1.1 a	1486.3 ± 16.5 a	1523.9 ± 15.6 a	1463.8 ± 16.4 a	1491.4 ± 18.4 a
	Rg5	27.8 ± 1.5 a	28.7 ± 1.8 a	21.5 ± 1.6 b	1435.4 ± 21.1 a	1221.1 ± 15.1 a	1423.2 ± 13.1 a	1357.3 ± 15.1 ab
	Rg10	27.5 ± 0.7 a	25.6 ± 2.1 a	25.6 ± 2.3 a	1121.2 ± 17.1 b	1053.3 ± 11.7 b	1547.2 ± 18.6 a	1247.0 ± 19.1 b
	C0	20.3 ± 3.7 a	36.3 ± 1.8 a	24.9 ± 1.8 b	1197.4 ± 17.8 c	1383.4 ± 17.5 b	1753.8 ± 16.8 a	1453.4 ± 19.4 a
	C5	33.6 ± 1.4 a	33.8 ± 1.4 a	25.8 ± 1.6 b	1146.4 ± 10.4 c	1354.8 ± 15.5 b	1658.6 ± 19.9 a	1434.9 ± 18.6 a
	C10	30.5 ± 2.6 a	32.8 ±1.7 a	22.2 ± 1.0 b	902.1 ± 16.2 d	1399.7 ± 18.3 b	1776.2 ± 19.1 a	1413.3 ± 21.6 a
Autumn	Rg0	20.3 ± 2.0 a	22.3 ± 1.1 a	ND	955.9 ± 16.2 b	1451.7 ± 16.5 a	ND	1176.3 ± 19.0 a
	Rg5	15.6 ± 1.7 a	15.6 ± 0.9 a	ND	924.4 ± 16.2 b	1147.2 ± 16.8 b	ND	988.1 ± 16.7 a
	Rg10	17.3 ± 1.3 a	27.3 ± 3.3 a	ND	843.5 ± 14.8 b	1033.7 ± 9.7 b	ND	906.9 ± 14.3 a
	C0	20.3 ± 2.4 a	22.8 ± 3.1 a	19.1 ± 2.7 a	1190.1 ± 15.3 a	1923.9 ± 22.7 a	2648.6 ± 38.1 a	1825.4 ± 32.1 a
	C5	23.9 ± 1.8 a	27.4 ± 3.0 a	22.2 ± 2.5 a	863.1 ± 9.0 b	1674.5 ± 19.8 ab	2035.8 ± 20.9 a	1494.4 ± 24.6 b
	C10	17.9 ± 2.7 a	23.5 ±3.2 a	16.3 ± 3.5 a	882.1 ± 11.9 b	1477.1 ± 18.1 b	2064.3 ± 20.8 a	1474.4 ± 24.3 b

Rg: 'Regina'; C: 'Chika'; ND: No Data

Means (In Summer n: Rg0 = 81, Rg5 = 73, Rg10 = 78; C0 = 118, C5 = 113, C10 = 125. In Autumn Rg0 = 80, Rg5 = 54, Rg10 = 55; C0 = 77, C5 = 80, C10 = 81) with different letters between MC0,1 and 2, within each row are significantly different by Tukey's HSD test at $P<0.05$. For the average value, means followed by different letter within the column are significantly different by Tukey's HSD test at $P<0.05$.

[z] Fruit surface area was calculated from the radius (equivalent to the average of fruit height and diameter), assuming the spherical shape.

[y] Epidermal cell size was calculated by assuming a spherical shape of the cell, following this formula: Cell size = (W/2×L/2) ×3.14. W=cell width, L: cell lengh. n=10

However, fruit from 'Regina' plants treated with 0 $mg \cdot L^{-1}$ of B showed larger average epidermal cell size than that in fruit treated with 5 or 10 $mg \cdot L^{-1}$ of B (Fig. 2.1 and Table 2.2). In 'Chika', no significant difference was found in average epidermal cell size between fruit from plants that received different treatments. When cell size was compared between MC levels, it was found that the cell size increased with the severity of MC. This indicates a positive correlation between MC severity and epidermal cell size (Table 2.2).

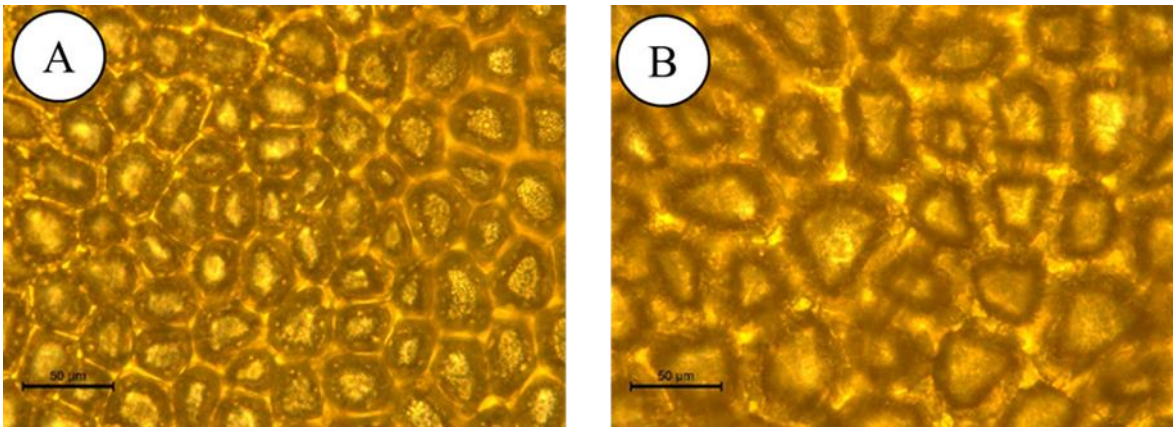

Fig. 2.1. Effect of B on epidermal cell expansion on tomato fruit.

Representative light microscopy images of CM with no MC and CM with MC.

A: Epidermal cell of intact tomato fruit (MC0), B: epidermal cell of microcracked tomato fruit (MC2).

Magnification: 40×; Scale bar: 50 µm

3.3.2 Effect of boron treatment at different fruit growth stages on the occurrence of MC in 'Chika'

In the second experiment, we started B treatment in 'Chika' when the anthesis in the third truss just occurred (0 DAA). At that time, flowers in the first and second truss completed 20 DAA and 7 DAA, respectively. Thus, three trusses in different growing stages were simultaneously exposed to B treatment. Similar to the results of the first experiment, fruit weight was not affected by B treatment (Table 2.3). However, in the third truss, incidence of MC was significantly higher in C0 than in C5. This difference was not evident in the first and second truss. The results of the first experiment suggested a low reactivity to B-deficient treatment in 'Chika'; however, starting B treatment at the very early stage of fruit development could reduce MC occurrence in this cultivar (Table 2.3). Additionally, MC incidence decreased with higher B concentration regardless of the growing stage at which the treatment was applied.

Table 2.3. Effect of B deficiency at different growing stage on Chika fruit weight and MC.

Growing stage (DAA) [z]	Treatments	Fruit weight (g) ± SE	MC incidence (%) ± SE [y]		
			MC0	MC1	MC2
0	C0	15.1 ± 2.8 a	13.7 ± 4.5 b	6.3 ± 2.7 a	80.0 ± 4.3 a
	C5	20.3 ± 2.3 a	47.9 ± 4.2 ab	10.4 ± 3.6 a	41.7 ± 4.4 b
	C10	14.9 ± 2.9 a	68.3 ± 4.0 a	0.0 ± 0.0 b	31.7 ± 4.0 b
7	C0	18.5 ± 2.0 a	30.0 ± 5.0 a	15.0 ± 3.5 a	55.0 ± 6.4 a
	C5	12.6 ± 1.4 a	50.0 ± 6.0 a	12.5 ± 3.0 a	37.5 ± 6.7 a
	C10	12.8 ± 2.2 a	35.5 ± 5.5 a	6.4 ± 2.0 a	35.5 ± 5.0 a
20	C0	14.4 ± 2.0 a	13.7 ± 4.5 b	6.4 ± 2.5 a	72.7 ± 6.4 a
	C5	18.7 ± 2.6 a	55.4 ± 6.6 ab	16.0 ± 3.7 a	28.5 ± 5.6 a
	C10	21.7 ± 1.3 a	78.3 ± 1.7 a	0.0 ± 0.0 b	21.7 ± 1.7 a

[z] Treatments started at 0, 7 or 20 Days After Anthesis.

Means (C0 n: 0 DAA = 31, 7 DAA = 31, 20 DAA = 31; C5 n: 0 DAA = 32, 7 DAA = 25, 20 DAA = 32. C10 n: 0 DAA = 20, 7 DAA = 30, 20 DAA = 20) with different letters between B treatment, within each growing stage are significantly different by Tukey's HSD test at $P<0.05$.

[y] MC: Microcracking. MC was determined by observing tomato fruit under digital microscope. Fruits were sorted according to their severity in MC0, 1 and 2. 0: no MC; 1: few MC oriented parallel to each other, and 2: Fruit surface covered with a network of MC.

DAA = Day After Anthesis.

3.4 Discussion

This study established that under B deficiency in summer, both MC susceptible ('Chika') and non-susceptible ('Regina') cultivars showed high incidence of MC compared to those that received B treatments, suggesting that B deficiency promotes occurrence of MC in tomato fruit. According to previous research, cracking resistance in cherry tomato fruit was correlated with CM thickness (Matas et al., 2004). It was observed that fruit with thicker CM deposition were less susceptible to cracking (Matas et al., 2004). In apple fruit, MC susceptibility was found to be related to CM with a lower fracture strain and larger epidermal and hypodermal cell sizes and cell numbers. Additionally, at maturity, in non-susceptible cultivars, fruit showed a higher surface area than that in the susceptible cultivars (Khanal et al., 2020). It was possible that B deficiency could induce the occurrence of MC in tomato fruit by affecting these factors. However, in this study, we observed that B deficiency did not affect fruit size and CM deposition in either tomato cultivar (Table 2.1). On the contrary, we found a significant difference in cell size with change in B treatment. Here, we have discussed the possible role of B in MC development.

When the treatment started 15 DAA, irregular epidermal cell expansion was observed in 'Regina' cultivars under B deficiency compared to those that received B treatment (Table 2.2), whereas for 'Chika', no significant difference was found. The average epidermal cell size was positively correlated with MC severity (Table 2.2). Additionally, fruit with MC showed high variability and irregularity in the distribution of epidermal cell size (Fig. 2.1). Khanal et al. (2020) reported similar results when comparing MC incidence

between a non-susceptible and susceptible cultivar of apple fruit. This suggested that B deficiency could have promoted MC by inducing abnormal cell expansion and/or irregular cell distribution. Several mechanisms could explain how MC can result from abnormal cell expansion or irregular size distribution under B deficiency.

First, the CM could weaken if cell size is heterogenous and/or if the cells are large. This was confirmed by Eccher et al. (1975) who showed that the irregularity of epidermal and hypodermal cells increased the incidence of MC in 'Golden Delicious' apple cultivar. Bell et al. (1937) and Meyer et al. (1944) reported that if the fruit expands during development without adequate cell division, the periclinal cell walls expands, which increases the strain. To accommodate the expansion in periclinal cell wall, the anticlinal cell walls of neighboring cells must separate and reorient themselves to be a part of the periclinal cell wall. Maguire (1998) found that this cell wall reorientation concentrates cuticular strain on the areas immediately above the anticlinal cell wall and that the separation of anticlinal cell wall is followed by weakening of the CM in the tangential plane (Khanal et al., 2020). Variation in cell size is likely to induce concentrated stress. Smaller cells will be firmer than larger cells causing accumulation of tangential stresses at interfaces between smaller and larger cells. This could lead to a thinning of the epidermal cell wall and, thus, weakening the CM (Khanal et al., 2020). This decrease in CM strength could promote MC occurrence. B deficiency could promote MC through the same process.

Another explanation may be related to epidermal cell turgor pressure. Oey et al. (2007) reported decreased fracture strain in apple fruit tissues with high cell turgor. The

epidermal cells of tomato fruit under B deficiency could have high turgor. This could lead to weakening of the CM and the occurrence of MC. However, additional investigation is needed to confirm this hypothesis.

Finally, B deficiency is known to induce a reduction in cell wall elasticity, which causes the cell wall to crack more easily and/or cells to separate from one another under tension along the middle lamellae (Opara et al., 1997, Dorais et al., 2004 and Huang et al., 2004). A separation of epidermal cells, as shown in Fig. 2.1, could weaken the cellular support of the CM and, therefore, increase MC incidence (Ganie et al., 2013).

It is notable that, in summer, both cultivars, showed reduced epidermal cell size when treated with 10 $mg \cdot L^{-1}$ of B compared to that with 0 $mg \cdot L^{-1}$ of B in MC0 fruit (Table 2.3). In autumn, MC occurrence did not show any significant difference between the treatments (Table 2.1). This indicates that changes in cell size under B deficiency alone does not explain MC occurrence in both the cultivars and that another factor might be involved in this process. Peet (1992) observed that under high temperatures and light intensity, the incidence of the MC is often larger. These climatic conditions would promote a greater flow of photo assimilates towards the fruits, and a heating of plant tissues due to solar radiation, thus increasing internal pressure of the fruit and inducing greater tension in fruits CM. Under high humidity conditions, transpiration of plants is restricted and root pressure increases, thus promoting MC (Peet and Willits, 2013). Pearce et al. (1993) found that the fruit growth rates are positively related to fruit temperature between 10 °C and 30 °C. Based on these observations, besides B deficiency, the other factor influencing the occurrence of MC in

tomato fruit could be fruit growth rate, which differed in summer and autumn, due to the variation in the temperature, light intensity, and relative humidity. MC has been found to result from CM strain (Si et al., 2021), suggesting that higher fruit growth could induce greater strain on the CM, thus, increasing the rate of MC occurrence.

Tomato fruit development takes place in four stages: fruit set stage at anthesis; cell division stage, from 3 to 10 DAA; cell expansion stage from 12 to 35 DAA; and fruit ripening stage from 35 DAA to 40 DAA (Azzi et al., 2015). In this study, when the treatment started at 0 DAA in 'Chika' cultivar, fruit under B deficiency treatment showed higher MC than those that received B treatments (Table 2.3). However, when the treatment started 15 DAA, no significant difference was found (Table 2.2). In the 'Regina' cultivar, even if the treatment started 15 DAA, B deficiency showed high MC incidence. This suggested that B deficiency could have promoted MC incidence in both the cultivars by affecting cell division and/or cell expansion. Fruit with high severity of MC had smaller surface area in both cultivars and larger epidermal cell size (Table 2.2) in the 'Chika' cultivar, suggesting that B deficiency could have promoted MC in 'Chika' fruit by inhibiting cell division. MC occurrence could, therefore, result in abnormal cell expansion through the inhibition of cell division during fruit development. In the 'Regina' cultivar, MC incidence can be explained by two possibilities. First, B deficiency could have affected cell division. In that case, cell division could have continued even after 15 DAA. We should note that 'Regina' cultivar is morphologically different from common cultivars of cherry tomato. It's an ornamental tomato, that has a maximum of 15 to 20 cm height, while 'Chika' can grow over 2 m. Second, if we consider that cell division duration was the same as that of common cherry tomatoes,

B deficiency could have promoted MC incidence by affecting cell expansion in the ‘Regina’ cultivar. B deficiency could have affected cell wall elasticity and, therefore, could have led to decreased cellular support for the CM and, hence, increased MC incidence (Ganie et al., 2013). However, further studies are needed to confirm this hypothesis.

3.5 Conclusion

In summary, we conclude that under B deficiency in summer, both MC susceptible (‘Chika’) and non-susceptible (‘Regina’) cultivars showed high incidence of MC compared to those that received B treatments, suggesting that B deficiency promotes occurrence of MC in tomato fruit. A simultaneous effect of B deficiency and high fruit growth rate in summer could promote MC incidence in both tomato cultivars. Additionally, high MC incidence under B deficiency could be the result of abnormal cell expansion and/or irregular epidermal cell size distribution. However, additional investigations are needed to evaluate the optimal B concentration and temperature for reducing MC incidence in tomato fruit.

Chapter IV. General discussion

The objective of this research was to evaluate the effect of B on the occurrence of MC and investigate its possible mechanism of action using cherry tomato. In order to achieve this goal, the hypothesis was that B deficiency would induce MC on tomato fruit. The result of the chapter two showed that both B deficiency and B toxicity severely affected plant growth (Fig.3). However, tomato fruit treated with normal B concentration and B deficiency treatment had higher incidence of MC compared to fruits treated with 90 $mg \cdot L^{-1}$ of B (Table 3). This indicated that the MC was not affected by the changes observed on the plants under B deficiency and B toxicity treatment and that B concentration was involve in the difference of MC incidence within the treatments. It was found that fruit size was smaller under B toxicity plant compared to the others treatments (Table 3). Thus, it was not clear whether the reduction on MC observed in case of excess B treatment was due to the reduction of the fruit size or the effect of B concentration. The chapter three was therefore set to further analyze the effect of the fruit growth and B deficiency on tomato fruits, using two cherry tomato cultivars that differ in their susceptibility to MC— 'Regina', an MC-resistant cultivar, and 'Chika', an MC-susceptible cultivar. The result showed that B deficiency could enhance MC in both cultivars (Table 2.1 and 2.3) regardless of the fruit size. However, the effectiveness of B deficiency occurred in earlier growing stage in case of 'Chika' cultivar (Table 2.3). 'Chika' cultivar, is known to be susceptible to MC. This could explain why MC incidence was not significantly different between the C0 and C5 plants when the treatment

was applied at 15 DAA. MC could have occurred in earlier growing stage as shown in the table 3.

Growing the fruit in summer and autumn, showed that B deficiency was more effective on increasing MC incidence in summer than in autumn. Suggesting that, B deficiency alone did not increase MC in both cultivars. The fruit growth rate, that differ between the seasons was thought to act simultaneously with B deficiency and induced higher rate of MC in tomato fruits. Peet (1992) observed that under high temperatures and light intensity, the incidence of the MC was often larger. These climatic conditions would promote a greater flow of photo assimilates towards the fruits, and a heating of plant tissues due to solar radiation, thus increasing internal pressure of the fruit and inducing greater tension in fruits CM. Under high humidity conditions, transpiration of plants is restricted and root pressure increases, thus promoting MC (Peet and Willits, 2013). Pearce et al. (1993) found that the fruit growth rates are positively related to fruit temperature between 10 °C and 30 °C.

Regarding, the mechanism of occurrence of MC, the table 2.2 and the fig. 2.1 showed a significant difference in cell size with change in B treatment. When the treatment started 15 DAA, irregular epidermal cell expansion was observed in ‘Regina’ cultivars under B deficiency compared to those that received B treatment (Table 2.2), whereas for ‘Chika’, no significant difference was found. The average epidermal cell size was positively correlated with MC severity (Table 2.2). Additionally, fruit with MC showed high variability and irregularity in the distribution of epidermal cell size (Fig. 2.1). B deficiency was thought to increase MC incidence through many possible mechanisms.

First, the CM could weaken if cell size is heterogenous and/or if the cells are large. This was confirmed by Eccher et al. (1975) who showed that the irregularity of epidermal and hypodermal cells increased the incidence of MC in 'Golden Delicious' apple cultivar. Bell et al. (1937) and Meyer et al. (1944) reported that if the fruit expands during development without adequate cell division, the periclinal cell walls expands, which increases the strain. To accommodate the expansion in periclinal cell wall, the anticlinal cell walls of neighboring cells must separate and reorient themselves to be a part of the periclinal cell wall. Maguire (1998) found that this cell wall reorientation concentrates cuticular strain on the areas immediately above the anticlinal cell wall and that the separation of anticlinal cell wall is followed by weakening of the CM in the tangential plane (Khanal et al., 2020). Variation in cell size is likely to induce concentrated stress. Smaller cells will be firmer than larger cells causing accumulation of tangential stresses at interfaces between smaller and larger cells. This could lead to a thinning of the epidermal cell wall and, thus, weakening the CM (Khanal et al., 2020). This decrease in CM strength could promote MC occurrence. B deficiency could promote MC through the same process.

Another explanation may be related to epidermal cell turgor pressure. Oey et al. (2007) reported decreased fracture strain in apple fruit tissues with high cell turgor. The epidermal cells of tomato fruit under B deficiency could have high turgor. This could lead to weakening of the CM and the occurrence of MC. However, additional investigation is needed to confirm this hypothesis.

Finally, B deficiency is known to induce a reduction in cell wall elasticity, which causes the cell wall to crack more easily and/or cells to separate from one another under tension along the middle lamellae (Opara et al., 1997, Dorais et al., 2004 and Huang et al., 2004). A separation of epidermal cells, as shown in Fig. 2.1, could weaken the cellular support of the CM and, therefore, increase MC incidence (Ganie et al., 2013).

Chapter V. General Conclusion

In summary it can be conclude that, regardless of fruit size and phenotypes of tomatoes B deficiency increases MC incidence. The effectiveness will appear during early growing stage of the fruit in case of susceptible cultivars.

Regarding the mechanism of occurrence of MC, a simultaneous effect of B deficiency and high fruit growth rate in summer could promote MC incidence in both susceptible and non-susceptible tomato cultivars. Additionally, high MC incidence under B deficiency could be the result of abnormal cell expansion and/or irregular epidermal cell size distribution. However, additional investigations are needed to evaluate the optimal B concentration and temperature for reducing MC incidence in tomato fruit.

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