Defoliating to 12−15 leaves increases calcium concentration and decreases blossom-end rot incidence in fruit of tomato plant grown under moderate water stress

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The objectives of this study were to (i) determine the optimum number of whole leaves to retain on a tomato plant for effective blossom-end rot (BER) management and (ii) explore the relationship between shoot calcium (Ca) and fruit Ca in non-defoliated plants in two different sized fruit cultivars, a large-fruited cultivar 'Momotaro fight' and a medium-fruited cultivar 'Cindy sweet'. Treatments involved maintaining 18, 15 and 12 leaves on a plant. All lateral shoots were removed regularly throughout the growing period except the shoot closest to the flowering truss in the 18-leaf treatment. At the length of 10 cm, these shoots were sampled for real time Ca determination using a hand held Ca^{2+} meter. In the plants defoliated to 18 leaves, BER was higher in 'Momotaro fight' at 10% compared to 2% in 'Cindy sweet'. Fruit growth rate was significantly increased by defoliation in 'Momotaro fight', however no significant difference was observed among treatments in 'Cindy sweet'. Defoliating to 12 leaves increased daily Ca transport rate by 59% and 37% in 'Momotaro fight' and 'Cindy sweet', respectively. Defoliating to 12 leaves increased the water-soluble Ca concentration in the distal part of fruit by 34% and 14% in 'Momotaro fight' and 'Cindy sweet', respectively. In the plants defoliated to 18 leaves where only old yellowish leaves were removed, a significant steady decrease was observed in the concentration of water soluble Ca in the distal part of fruit with increase in truss order. There was a significant linear relationship between water-soluble Ca concentration in the distal part of the fruit and Ca concentration in the lateral shoot of plants defoliated to 18 leaves. We conclude that under moderate water stress by root zone restriction and also certain other BER inductive conditions, defoliation to 12–15 leaves on a tomato plant should be a promising approach for decreasing BER incidence in susceptible large fruit cultivars.

Key words : BER management, defoliation, water–soluble Ca, lateral shoot Ca, root zone restriction

Introduction

Tomato is an important horticultural crop that earns farmers income. Tomato is consumed in large quantities worldwide and makes substantial overall health and nutritional contributions to the human diet[3, 7]. Its production is not without challenges ranging from pests to diseases and physiological disorders, which may decrease yield and quality. In recent years, consumer needs and environ-mental concerns have pressured growers to supply high–quality tomatoes using sustainable production methods. In this regard, greenhouse tomato production is shifting to meet these emerging needs.

Root zone volume restriction is one production method that has been adopted by greenhouse tomato growers. Several studies have shown that this system can improve the quality of tomato fruit produced applying drip fertigation[14, 15, 20]. However, the use of root zone volume restriction predisposes the plant to water stress. Even though water stress in tomato can improve fruit quality by influencing the content and composition of soluble sugars, organic acids, and some amino acids[14, 15, 22], it is associated with the increased risk of blossom–end rot (BER) occurrence[1, 6, 18]. In tomato production, BER may cause severe economic loss because of the deterioration in fruit quality, and market acceptability[21]. Many studies have associated BER with systemic or localized calcium (Ca) deficiency[5, 17].

It has been reported repeatedly that excessive nitrogen nutrition and subsequent vigorous vegetative growth

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often causes severe BER incidence. Our result revealed that competition for water and Ca between leaves and reproductive organs may be a major cause of BER development. Recently, we reported that alternate leaflet removal for tomato plant grown under restricted root zone volume increased Ca transport into fruit and reduced BER incidence[11, 16]. We noted that this technique can be laborious in commercial tomato production. Here, first, we report results of a more practical defoliation technique suitable for commercial production for tomato plants grown under a system combining restricted root zone volume and solar-mediated fertigation control.

In our previous study, we observed tip-burn, another Ca deficiency symptom that develops in young leaves on apical and also lateral shoots in Ca-starved plants developing severe BER fruits (unpublished data). These facts may represent that young shoot and floral organs are similarly influenced by competition for Ca against mature leaves. It was supposed that Ca status of young lateral shoot may relate to that of young fruit sensitive to BER and reflect or indicate the risk of BER development. Cultural practices such as lateral shoot removal, defoliation, pruning, pinching and staking are some of the common practices performed in tomato production to ensure high yield and quality. The relationship between lateral shoot Ca and Ca in fruit as a diagnostic measure for fruit Ca status has not been explored. Thus, secondly, we attempted an exploration of the relationship between Ca status in lateral shoot and neighboring young fruits.

Materials and methods

An experiment was carried out in a glasshouse in the Field Science Center, Okayama University from January to July of 2018. Temperature in the glasshouse was maintained above 12℃ by a warm-air heater, and adequate ventilation was applied with windows when temperature exceeded 28℃. Two tomato cultivars, 'Momotaro fight' (Takii Co., Ltd., Kyoto, Japan) and 'Cindy sweet' (Sakata Seed Corp., Yokohama, Japan) were examined. On 5th January, the seeds were sown on vermiculite moistened with water in trays and placed in a growth chamber set at 25℃ for a 12 h day length. At the third true leaf stage, the seedlings were transplanted into plastic pots (12 cm in diameter) filled with 600 ml of tomato growing medium. The seedlings were then moved to a small plastic house within the glasshouse and fertigated daily with half strength Ohtsuka A solution having an EC of 120-130 $mS \cdot m^{-1}$ (OAT Agrio, Tokyo, Japan). At the growth stage where flower buds on the plants were visible, the plants were transferred to a solar mediated fertigation system within the glasshouse as previously described[20, 21].

Four plants were maintained for each treatment in each cultivar. Six leaves below the 1st truss were retained on all the plants before treatments commenced. All lateral shoots were removed regularly throughout the growing period except the shoot closest to the flowering truss in the control plants. At the length of 10 cm, these shoots were sampled for real time Ca determination using a hand held Ca^{2+} meter. At anthesis of 5th truss, defoliation treatments were commenced and continued until the 1st fruit on the 10th truss had been sampled for 'Momotaro fight' and 1st fruit on the 15th truss for 'Cindy sweet'. Treatments were :

1. Control where no defoliation was done except for the dead and yellowing leaves to maintain 18-21 leaves at any given time on the plant
2. 15 leaves maintained on the plant
3. 12 leaves maintained on the plant

In this study, the treatments are henceforth referred to as 18-leaf, 15-leaf and 12-leaf, respectively. Sampling was done on the 1st fruit on the 5th-10th truss at 14 days, and 5th-15th truss at 18 days after anthesis in 'Momotaro fight' and 'Cindy sweet', respectively. Defoliation treatment was done at anthesis of the 1st flower on the youngest truss. Flowers on each truss were thinned to retain 4 and 8 fruits on 'Momotaro fight' and 'Cindy sweet', respectively. Nutrient solution supplied to the plants during the day was monitored within the sampling period and drained solution over 6 hours was recorded (Fig. 1). At sampling, fruit fresh weight without calyx was measured to determine fruit growth rate. The fruit was then divided equatorially into two portions, proximal and distal, and prepared for Ca extraction. Sequential extraction to water- and hydrochloric acid (HCl)-soluble Ca fractions that served as representatives for (1) apoplastic and cytoplasmic Ca^{2+}, loosely wall-bound Ca ; (2) residual insoluble Ca, respectively, was done, as described in Yoshida *et al.* (2014)[21]. Ca concentration was determined using atomic absorption spectrometry (SPCA-6210, Shimadzu, Kyoto, Japan) and described as $\mu mol \cdot g^{-1}$ FW.

For shoot Ca determination, sap from 1 cm of the shoot of 18-leaf treatment was squeezed onto a hand held Ca^{2+} meter (LAQUA twin B-751, Horiba Ltd. Kyoto, Japan) and the concentration in ppm was recorded. Fruit growth rate was calculated as fresh weight divided by the number of days after anthesis. Daily Ca transport rate was determined by dividing the Ca amount in fruit by the number of days after anthesis. Concen-tration of the water-soluble Ca in the distal part of the fruit was determined as the quotient of the Ca concentration in the distal part of the fruit in μmol and the fresh weight of the distal tissue in grams. The incidence of BER was recorded as it appeared. Microsoft Excel spreadsheets were used for data analysis and comparison of means was done using Tukey's test.

Results

1. Nutrient absorption and BER incidence

As shown in Figure 1, the plants in all the treatments absorbed over 50% of the nutrient solution supplied in the daytime in both cultivars. The rate of BER occurrence in this study was generally low, however the 18-leaf in both cultivars had the highest rates (Fig. 2). No BER incidence was observed in the 12-leaf, or in the 12 and 15-leaf treatments in 'Momotaro fight' and 'Cindy sweet', respectively. In the 18-leaf, BER was higher in 'Momotaro fight' at 10% compared to 2% in 'Cindy sweet'. The earliest BER incidence was observed on the 7th truss of 'Momotaro fight' in the 18-leaf and 15-leaf

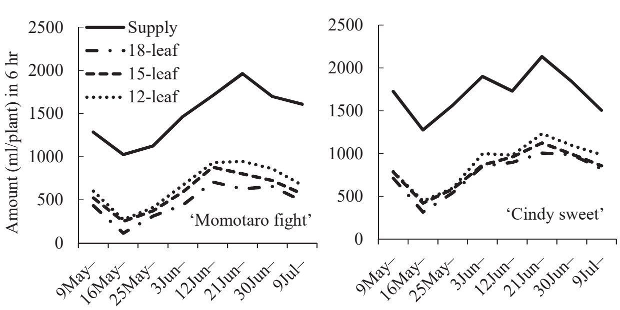

Fig. 1 Changes in the amount of nutrient solution supplied and drained during day time (6 hours) within the sampling period between 9th May to 17th July 2018 for two tomato cultivars.

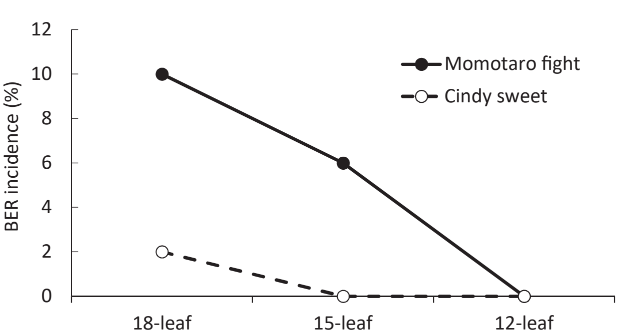

Fig. 2 BER incidence (%) in two tomato cultivars as influenced by number of retained leaves on a plant in Spring 2018.

treatment. The highest rate of BER incidence was observed in 18-leaf, 10th truss. In 'Cindy sweet', the earliest BER incidence was observed in the 10th truss, 18-leaf treatment.

2. Effect of number of retained leaves on Fruit growth rate

Fruit growth rate was higher in 'Momotaro fight' than in 'Cindy sweet' (Fig. 3). The increase in fruit growth rate in 12-leaf treatment was 21% and 6% in 'Momotaro fight' and 'Cindy sweet', respectively. However, this increase was not significant in 'Cindy sweet' at $P<0.05$.

3. Daily amount of Ca translocated into fruit

Daily amount of Ca transported into fruits increased as number of leaves decreased in the two cultivars, 'Momotaro fight' and 'Cindy sweet' (Fig. 4). The Ca transport rate was highest in 12-leaf 'Momotaro fight' and lowest in 18-leaf 'Cindy sweet'. There was a signifi-

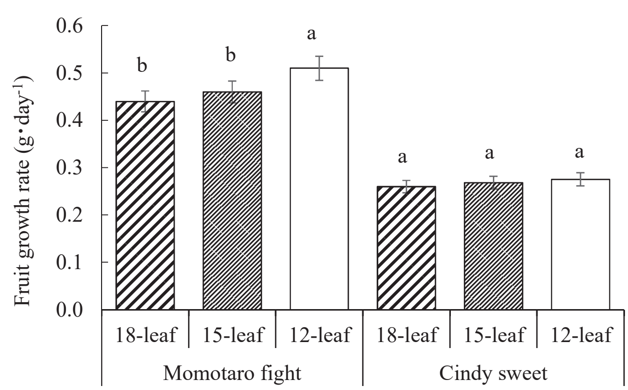

Fig. 3 Fruit growth rate of two tomato cultivars 'Momotaro fight' (n=24, 6 trusses × 4 plants) and 'Cindy sweet' (n=44, 11 trusses × 4 plants) as influenced by number of leaves on a plant. Different letters indicate significant mean differences among treatments within a cultivar by Tukey's test at $P<0.05$.

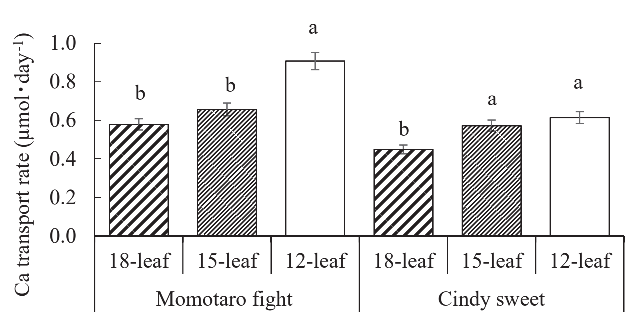

Fig. 4 Daily Ca translocated into fruit in two tomato cultivars 'Momotaro fight' (n=24, 6 trusses × 4 plants) and 'Cindy sweet' (n=44, 11 trusses × 4 plants) as influenced by number of leaves on a plant. Different letters indicate significant mean differences among treatments within a cultivar by Tukey's test at $P<0.05$.

cant difference in Ca transport rate between 18-leaf and 12-leaf in 'Momotaro fight'. However, there was no significant difference between 18-leaf and 15-leaf. Defoliating to 12-leaf increased Ca transport rate by 59% and 37% in 'Momotaro fight' and 'Cindy sweet', respectively.

4. Concentration of water-soluble Ca in the distal part of fruit

Defoliation increased Ca concentration in both cultivars (Fig. 5). Defoliating to 12 and 15 leaves increased the water-soluble Ca concentration in the distal part of fruit by 34% and 23% in 'Momotaro fight', and 14% and 9% in 'Cindy sweet', respectively.

5. Concentration of water soluble Ca in the distal part of fruit in non-defoliated plants as influenced by order of inflorescence

There was a significant steady decrease in the concentration of water-soluble Ca in the distal part of fruit in 18-leaf plants with increase in truss order (Fig. 6). The Ca concentration was highest in the 5th truss and lowest in the uppermost last sampled truss in both cultivars. The decrease in water-soluble Ca concentration between the 5th truss and the 10th truss was 55% and 43% in 'Momotaro fight' and 'Cindy sweet', respectively.

6. Real time Shoot Ca concentration in 18-leaves tomato plants

Results of the shoot Ca concentration in 18-leaf plants showed that there was more water-soluble, non-structural, Ca^{2+} in the shoots of 'Cindy sweet' than that in 'Momotaro fight' (Fig. 7). The Ca^{2+} meter recorded significantly lower concentrations in 'Momotaro fight' with a reading of as low as 1 ppm in the 10th truss compared to 4 ppm in the 15th truss of 'Cindy sweet'. However, in both cultivars, shoot Ca concentration and order of inflorescence revealed a significant linear relationship.

7. Relationship between shoot Ca concentration and water-soluble Ca concentration in the distal part of individual fruit in 18-leaf plants

There were significant linear relationships between shoot Ca concentration and water-soluble Ca concentration in the distal part of fruit in plants defoliated to 18 leaves (Fig. 8) in both cultivars. The coefficient of determination was 64 % and 81% in 'Momotaro fight' and 'Cindy sweet', respectively.

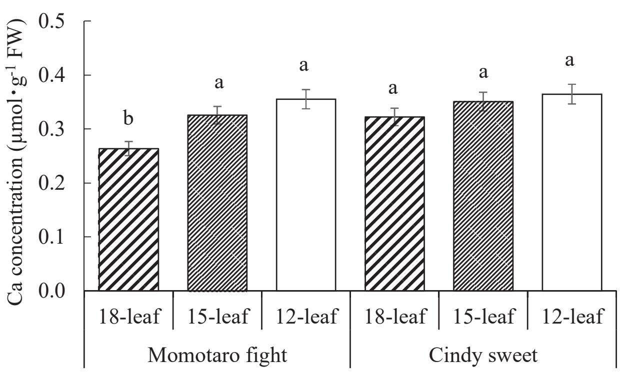

Fig. 5 Water-soluble Ca concentration in the distal part of fruit in two tomato cultivars 'Momotaro fight' (n=24, 6 trusses × 4 plants) and 'Cindy sweet' (n=44, 11 trusses × 4 plants) as influenced by number of leaves on a plant. Different letters indicate significant mean differences among treatments within a cultivar by Tukey's test at $P<0.05$.

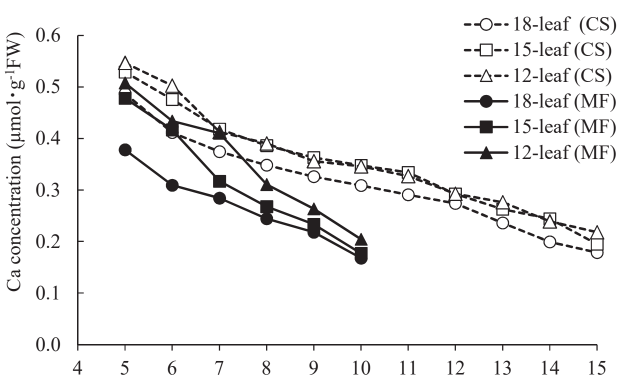

Fig. 6 Water-soluble Ca concentration in the distal part of individual fruit in two tomato cultivars 'Momotaro fight' (MF) (n=24, 6 trusses × 4 plants) and 'Cindy sweet' (CS) (n=44, 11 trusses × 4 plants) as influenced by number of leaves on a plant and order of inflorescence.

Discussion

The objective of this study was first, to determine the effect of number of whole leaves retained on a plant on Ca transport into fruit and BER incidence and second, to assess the relationship between shoot Ca concentration and water-soluble Ca concentration in the distal part of

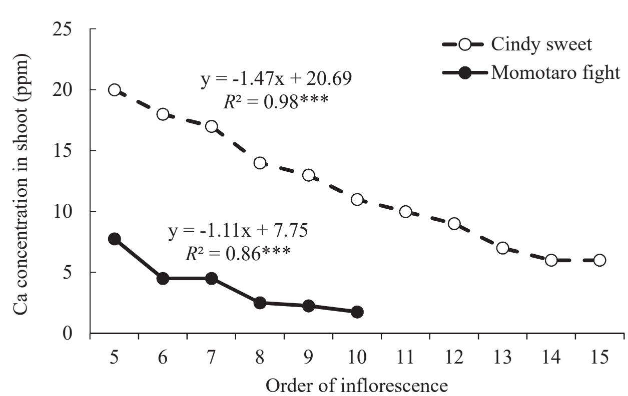

Fig. 7 Shoot Ca concentration of plants defoliated to 18 leaves in two tomato cultivars as influenced by order of inflorescence. ***$P<0.001$

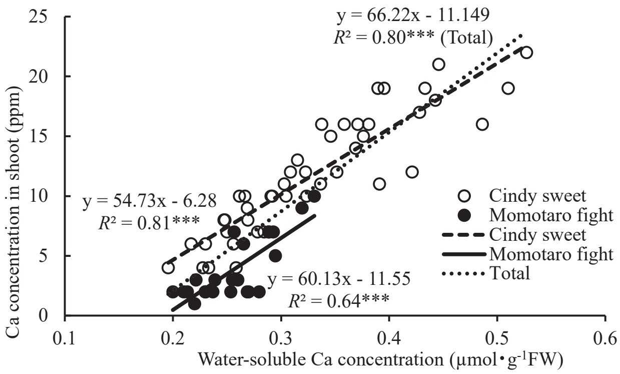

Fig. 8 Relationship between water-soluble Ca concentration in distal part of individual fruit and neighboring shoot Ca concentration in two tomato cultivars in 18-leaf plants. ***$P<0.001$

fruit in two tomato cultivars under moderate water stress. In this study, tomato plants were grown under 600 ml/plant of root zone volume in a solar mediated fertigation system (moderate water stress) and 18, 15 and 12 leaves were retained on the plant after anthesis of the 1st fruit on the 5th truss. It is well known that water stress is associated with the increased risk of BER occurrence[1, 6, 18)] by restricting water uptake which is the solvent for Ca^{2+} flux, therefore depressing Ca translocation along vascular vessels and then causing a lack of Ca in fruit required for cell structure maintenance. It further increases the risk of BER development by restricting Ca uptake and/or reducing transpiration rate, known to be a driving force of Ca transport together with water flow to fruit[1, 5, 18)]. However, water stress in the root zone is known to improve the fruit quality by influencing the content and composition of soluble sugars, organic acids, and some amino acids[14, 15, 22)]. On the other hand, defoliation has been found to reduce BER incidence in tomato plants grown under moderate water stress[11)] and also non stressed hydroponic condition[16)]. In this study, BER incidence was below 10%, and the incidence was more prevalent in non-defoliated plants (18-leaf) than in the defoliated plants (15-leaf and 12-leaf) in both cultivars (Fig. 2). Notably, BER incidence was observed in higher trusses as the season progressed which coincided with a decrease in Ca concentration in fruit (Fig. 6). BER development in tomato has been attributed to a low Ca level in the whole plant due to decreased soil Ca supply or root Ca uptake, low transport of Ca to and in the fruit, or an increased demand for Ca due to high growth rate of the fruit[8, 9)]. Although the decrease in Ca concentration in the upper trusses was expected to induce BER, its incidence was low. Studies have shown that for BER to occur, Ca concentration must fall below a certain threshold[12, 19)]. The water-soluble Ca concentration in the fruit ranged from 0.53–0.18 μmol・g^{-1} FW in both cultivars, and this may be within the threshold values reported by Vinh *et al.* (2018).

Cultivar differences in susceptibility to BER were also observed in this study. The large fruit cultivar 'Momotaro fight' which had a faster fruit growth rate was more susceptible than the medium fruit cultivar 'Cindy sweet' (Fig. 3). The importance of growth rate on the resulting Ca concentration of fruit is well demonstrated by several authors in BER development in tomato[11, 13, 19)]. In this study, Ca transport rate was higher in the large fruit cultivar than in the medium fruit cultivar and subsequently higher in defoliated plants than in the control (Fig. 4). In comparing the Ca transport rate of different size tomato fruit, the authors found that large fruit cultivars transported potentially more Ca than the small fruit cultivars and suggested that the Ca transporting potential into tomato fruit is proportional to fruit expansion, and that defoliation may have caused a compensatory physiological effect enabling the fruit to draw in more water, hence increasing the Ca transport into the fruit[11)].

In the 12-leaf plants, fruit had the highest water-soluble Ca concentration in both the large and medium fruit cultivars (Fig. 5). In terms of Ca^{2+} partitioning, it has been shown that conditions that affect the growth rate of storage organs might also affect vegetative plant parts. In other defoliation studies, photosynthate production and distribution into different parts depends on the magnitude of defoliation[4, 10)]. Since vegetative growth, as a powerful sink, consumes produced assimilates, limitation of vegetative growth enhances assimilate transport to fruits. Bangerth (1979)[2)] showed that competition between vegetative and storage organs could well have an influence on Ca distribution. The leaf possesses a higher transpiration rate than the fruit, and often acts as a competing sink with the fruit for directional Ca flow and accumulation[18)].

Ca accumulation in tomato fruit has been shown to be dependent on rates of xylem sap flow influenced by transpiration and growth rates[5, 8)]. In an effort to explore the relationship between shoot Ca and fruit Ca in plants defoliated to 18 leaves, this study revealed that both water-soluble Ca concentration of fruit (Fig. 6) and shoot Ca concentration (Fig. 7) decreased in upper trusses. Since there was a relationship between shoot Ca and fruit Ca (Fig. 8), shoot Ca may be used as a useful tool to predict the fruit Ca status allowing growers to improve this status before BER is triggered. This however is a preliminary study and more experiments need to be done to ascertain the model and further understand the changes in Ca concentration that were observed.

In conclusion, retaining 12-15 leaves on a tomato plant grown under moderate stress reduces BER incidence and increases daily Ca transport rate into fruit and water-soluble Ca concentration of both large and medium fruit tomato cultivars. There is a relationship between shoot Ca and fruit Ca that can be explored in determining the fruit Ca status for management of BER. Even though the number of possible interactions that can affect Ca uptake and distribution is so great, defoliation is one simple sustainable cultural practice that can be adopted by growers for management of BER.

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水ストレス条件下で発生するトマトの尻腐れ果は12〜15枚に摘葉することで軽減される

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トマト植物体の葉面積を制限すると，果実への Ca の転流が促進され，尻腐れ果の発生が軽減される．そこで，尻腐れ果発生に及ぼす摘葉強度の影響について検討した．大玉品種‘桃太郎ファイト’と中玉品種‘シンディースイート’を用い，第３花房開花後から実験期間を通じて葉数を12，15，18（対照区，老化して葉が黄変し始めた葉のみを摘除）で管理する３処理区を設けた．成熟葉18枚に調整した対照区の尻腐れ果発生率は‘桃太郎ファイト’で約10%，‘シンディースイート’で約２％であった．‘桃太郎ファイト’では摘葉によって果実肥大が促進されたが，‘シンディスイート’では差が認められなかった．12枚に摘葉すると果実への Ca 転流速度は‘桃太郎ファイト’で59%，‘シンディースイート’で39%高くなった. 果実先端部の水溶性 Ca 濃度は上位の果房ほど低くなる傾向にあった．また，果実の Ca 栄養診断を目的として開花中の花房直下の腋芽を採取して茎汁液中の Ca イオン濃度を測定したところ, 対照区では直近の果実中水溶性 Ca 濃度との間に有意な相関が認められた．以上のように，株当たりの葉数を12枚程度に摘葉することによって果実への Ca と水分の流入が促進され，尻腐れ果の発生が抑制されることが明らかになった．

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