### Relationships between Yield, Mineral Content of Fruits, and Sap Bleeding Rate in Dutch and Japanese Tomato Cultivars

#### Akimasa NAKANO\*, Tadahisa HIGASHIDE and Dong-Hyuk AHN

Institute of Vegetable and Floriculture Science, National Agriculture and Food Research Organization (Tsukuba, Ibaraki 305-8519, Japan)

#### Abstract

We present the relationship between yield, mineral content, and the sap bleeding rate (SBR) caused by root pressure as an indicator of root activity in eight Dutch and eight Japanese tomato cultivars (including popular Japanese cultivar 'Momotaro') grown in a hydroponic system. Root dry weight in the rockwool slab was positively correlated with stem and leaf fresh weight. In the Dutch cultivars, the SBR remained high (over 20 mL·h<sup>-1</sup>) as the root dry weight increased. In contrast, the SBR of Japanese 'Momotaro' series cultivars decreased. Ca in the tomato fruits and the SBR were higher in the Dutch cultivars than in the 'Momotaro' series. The SBR was found to positively correlate with fruit or total fresh weight. The higher SBR, which supports Ca transport to the fruits, probably led to the higher yields. The SBR might be a simplified indicator of selecting high-yielding cultivars in long-term cultivation.

**Discipline:** Soils, fertilizers and plant nutrition / Horticulture **Additional key words:** calcium, high yield cultivar, root activity, shoot growth

#### Introduction

The annual yield of greenhouse tomatoes (Solanum lycopersicum) in the Netherlands has doubled from about 30 kg·m<sup>-2</sup> in 1983 to around 60 kg·m<sup>-2</sup> in 2005. However, Japanese greenhouse tomato yields have increased only slightly over the past 30 years (Higashide and Heuvelink, 2009). Therefore, a high-yielding greenhouse production system needs to be developed in Japan if it is to compete in global markets along with the Netherlands. The overall situation in both countries is guite different, however, as the greenhouses used for tomato production in Japan are mainly plastic houses with area smaller than that in the Netherlands. We suggest that high tomato yields in the Netherlands may be due to two main factors regarding the production system. The first factor is the use of hydroponics combined with rockwool and a drip irrigation system (the best media and nutrient supply system for hydroponics). The second factor is the selection of cultivars suitable for hydroponics.

High tomato yields have generally been achieved in the Netherlands, but the cultivars have low Brix soluble solid content. In contrast, consumer demand in Japan has prompted the development of low-yielding but high-quality cultivars (Higashide et al. 2012). Thus, a trade-off relationship exists between yield and quality. We need to improve productivity (high yield and high quality) by introducing the Dutch system to Japan.

Our top priority is to clarify the characteristics of high-yielding Dutch cultivars. The increased yield in Dutch tomatoes over the past 50 years has been due to increases in total dry matter (DM) production resulting from high light use efficiency (LUE) (Higashide and Heuvelink, 2009). The differences in photosynthetic rate and DM production between Dutch and Japanese cultivars have been studied (Matsuda et al. 2011; Matsuda et al. 2013) to determine whether these factors cause the low yields of Japanese tomatoes. Recently, Higashide et al. (2014) reported that the yield of 'Momotaro York' increased when grafted onto

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<sup>\*</sup>Corresponding author: e-mail anakano@affrc.go.jp

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a Dutch rootstock ('Maxifort') due to the improvement in LUE. However, there has been little evaluation of root development in rockwool hydroponics (Rijck and Schrevens 1998) on account of technical difficulties (Savvas and Lenz 2000).

In this study, we tried to clarify the relationship between the sap bleeding rate (SBR), a possible indicator of root activity (Morita et al. 2000), and yields from Dutch and Japanese tomato cultivars grown in a rockwool hydroponic system. Moreover, we determined the mineral content of the tomato fruits as one indicator of fruit quality. We also discuss the differences between cultivars and their relationships to root activity.

#### **Materials and Methods**

#### 1. Cultivars and cultivation conditions

Sixteen tomato cultivars (Solanum lycopersicum), consisting of eight Dutch cultivars: 'GERONIMO', 'MA-KARI', 'MATRIX', 'BLITZ', 'GRACE', 'MATCH', 'QUEST', and 'DUNDEE' (De Leuter Seeds, the Netherlands), and eight Japanese cultivars: 'Momotaro York', 'House Momotaro', 'Momotaro Colt', 'Momotaro 8' (Momotaro series, Takii Seeds, Japan), 'Bit jelly' (Asahi Industry, Japan), 'Reika', 'Reiyo' (Sakata Seeds, Japan), and 'Shifuku' (Kaneko Seeds, Japan), were grown using a hydroponic system and rockwool media. The tomato seeds were sown in seed trays filled with a nursery medium on 12 June 2006, and then placed in a seedling growth chamber (Seedling Terrace; Mitsubishi Plastics Agri Dream, Japan). The seedlings were fertilized every two days from below using commercial nutrient solution (High-Tempo; Sumitomo Chemicals, Japan). The solution consisted of 10.7 mM NO<sub>3</sub><sup>-</sup>, 6.3 mM K<sup>+</sup>, 5.4 mM Ca<sup>2+</sup>, 1.9 mM Mg<sup>2+</sup>, 2.4 mM H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, 3.8 mg·L<sup>-1</sup> Fe, 0.38 mg·L<sup>-1</sup> Mn, 0.26 mg·L<sup>-1</sup> B,  $0.15 \text{ mg}\cdot\text{L}^{-1}$  Zn,  $0.05 \text{ mg}\cdot\text{L}^{-1}$  Cu, and  $0.07 \text{ mg}\cdot\text{L}^{-1}$  Mo, adjusted to electrical conductivity of 1.8 dS·m<sup>-1</sup>. The seedlings were illuminated with fluorescent lamps using a 16-h day length and a photosynthetic photon flux density (PPFD) of 397  $\pm$ 39 µmol·m<sup>-2</sup>·s<sup>-1</sup>. They were grown at 900 µmol·mol<sup>-1</sup> CO<sub>2</sub> and at air temperatures of 23°C and 17°C (day and night, respectively). On 21 July 2006, the seedlings were transplanted into rockwool (Grodan expert, the Netherlands) in a plastic-covered greenhouse with a width of 972 m<sup>2</sup> and an eve height of 4 m, and located at the experimental station, NARO Institute of Vegetable and Tea Science (NIVTS), Taketoyo, Aichi, Japan (34°49'N, 136°54'E). Four tomato seedlings were planted on one rockwool slab (20 cm in width, 90 cm in length, and 6.5 cm in height), with a 22.5-cm inter-plant width. The planting density was two plants per m<sup>-2</sup>.

In the greenhouse, temperature ventilation and heating were set to turn on at 25°C and 15°C, respectively. Throughout the experiment, which ended on 5 October 2006, the tomato seedlings were supplied with a commercial nutrient solution (Otsuka-A; OAT Agri-Techno, Tokyo, Japan) that consisted of 12.4 mM NO<sub>3</sub><sup>-</sup>, 5.7 mM K<sup>+</sup>, 5.5 mM Ca<sup>2+</sup>, 2.0 mM Mg<sup>2+</sup>, 1.2 mM H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, 3.6 mg·L<sup>-1</sup> Fe, 1.6 mg·L<sup>-1</sup> Mn, 0.68 mg·L<sup>-1</sup> B, 0.12 mg·L<sup>-1</sup> Zn, 0.04 mg·L<sup>-1</sup> Cu, and 0.04 mg·L<sup>-1</sup> Mo, adjusted to electrical conductivity of 1.6 dS·m<sup>-1</sup>. Sufficient nutrient solution was supplied and the daily drain percentage was maintained at over 20% of the total nutrient solution supply.

The tomatoes were trained vertically using a high wiring planting system. The sap bleeding rate (SBR) was measured at the end of the experiment (on 5 October 2006) as described below, along with the shoot fresh weight and remaining fresh fruit weights. The total fresh fruit weights were calculated as the sum of the remaining fruit and harvested fruit from each plant.

# 2. Measurement of SBR and root weight in the rockwool medium

The SBR was measured for three hours (from 14:00 to 17:00) after the experiment ended. The tomato stem was cut at 5 cm from the surface of the medium, and then the cut stem surface was covered with about 10 g of cotton sheet to collect the bleeding sap. The cotton surface was covered with vinyl film to prevent the collected bleeding sap from evaporating. The cotton and vinyl film were fixed to the stems with rubber bands. After one hour, the cotton sheet was collected and weighed to calculate the SBR. Root weight in the rockwool was estimated based on the difference between the dry rockwool weight, including the roots, dried at 80°C for seven days, and the combusted rockwool weight at 600°C (Nakano et al. 2007).

#### 3. Mineral content of the tomato fruits

The tomato fruits from the fourth truss of each cultivar were randomly sampled and representative parts of the fruits were freeze-dried. The samples were pulverized, with 200 mg samples being digested in a mixture of 2 mL concentrated nitric acid and 1 mL hydrogen peroxide using a microwave sample digestion system (START D, Milestone General Inc.) at 180°C for 10 min. An aliquot of the samples was diluted with extra-pure water (Simpli Lab-UV, MILLIPORE Inc.). K, Ca, Mg, Fe, Mn, Cu, and Zn were then determined using inductively coupled plasma spectrometry (ICPM-8500, Shimadzu Inc.).

#### Results

#### 1. Root weight

There was a positive correlation between shoot fresh weight and root dry weight in the rockwool slab (R = 0.71). This indicated interaction between shoot and root growth

(Fig. 1). The eight Dutch cultivars had a wider range of root dry weight distribution than the Japanese cultivars. 'DUNDEE' had the lowest root dry weight (5.2 g plant<sup>-1</sup>) and 'MATRIX' had the highest (18.5 g/plant<sup>-1</sup>). Cultivar 'Reiyo' had the lowest Japanese root dry weight (5.2 g plant<sup>-1</sup>) and 'Reika' had the highest (14.4 g plant<sup>-1</sup>), except for the 'Momotaro' series. Among the 'Momotaro' series, cultivar 'Momotaro York' had the lowest root dry weight (7.5 g plant<sup>-1</sup>) and 'Momotaro Colt' had the highest (12.8 g plant<sup>-1</sup>). There was no significant difference between the Dutch and the Japanese cultivars in terms of root dry weight per plant.

#### 2. Sap bleeding rate (SBR)

All Dutch cultivars showed a high SBR (average of  $21.1 \text{ mL}\cdot\text{h}^{-1}$ ) that did not decline as the root dry weight increased (Fig. 2). The Japanese cultivars, except for the 'Momotaro' series, also did not show a decrease in SBR, although the overall SBR average was lower than for the Dutch cultivars (average of  $19.9 \text{ mL}\cdot\text{h}^{-1}$ ). However, the SBR of the 'Momotaro' series cultivars decreased in line with higher root dry weight, and their average SBR was  $17.9 \text{ mL}\cdot\text{h}^{-1}$ , which was lower than that of the Dutch and other Japanese cultivars.

#### 3. Relationship between SBR and productivity

Fruit yields and the SBR of the 'Momotaro' series were lower than those of the Dutch cultivars and the other Japanese cultivars, and except for the 'Momotaro' series,



▲ Dutch cultivars, ○ Japanese cultivars ('Momotaro' series), ● Japanese cultivars (not including 'Momotaro' series). Each dot indicates the average of four plants. Correlation coefficient (R) between root dry weight and stem and leaf fresh weight was 0.71.

were in between the results for the Dutch cultivars and those for 'Momotaro. Generally, there was a positive correlation between the SBR and fruit weight (Fig. 3). The SBR also had a positive correlation with total shoot weight, which included fruits, stems, and leaves (R = 0.58), and this correlation was higher than for fruit weight only.

#### 4. Mineral content

Generally, the differences in fruit mineral content (K, Mg, Fe, Mn, Cu, and Zn) were quite small among the cultivars (Table 1), except for Ca. The coefficients of variation (C.V.) among the cultivars were within 15% of the average. Ca and Mn concentrations were higher in the Dutch cultivars than in the Japanese cultivars. However, Cu was higher in the Japanese cultivars. Ca and Mn concentrations in the fruits, and the SBR were lower in the 'Momotaro' series, and Ca and Mn concentrations had positive correlations to the SBR (Ca, R = 0.65 and Mn, R = 0.75, respectively) (Fig. 4).

#### Discussion

#### 1. SBR and productivity

There were differences in tomato productivity among



## Fig. 2. Relationship between sap bleeding rate and root dry weight

▲ Dutch cultivars, ○ Japanese cultivars ('Momotaro' series), ● Japanese cultivars (not including 'Momotaro' series). Each dot indicates the average of four plants. Solid and dotted lines show the linear regression of the Dutch and Japanese cultivars (only 'Momotaro' series), respectively. Each correlation coefficient (R) between the sap bleeding rate to root dry weight of Dutch and 'Momotaro' series was 0.04 and 0.90, respectively.

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Fig. 3. Relationship between each plant part and sap bleeding rate
▲ Dutch cultivars, ○ Japanese cultivars ('Momotaro' series), ● Japanese cultivars (not including 'Momotaro' series). Each dot indicates the average of four plants. Each correlation coefficient (R) between the sap bleeding rate to fruits fresh weight, stem and leaf fresh weight and fruits, stem and leaf fresh weight was 0.46, 0.20, and 0.58, respectively.

the cultivars. In particular, root weights differed widely, and the Dutch cultivars differed more widely than the Japanese cultivars. Generally, the higher the root dry weight, the greater the nutrient uptake due to the increased root surface area. It could be that increased root dry weight leads to a greater SBR. In this experiment, however, there was no correlation between root dry weight and SBR. The SBR was almost constant at 20 mL·h<sup>-1</sup> and independent of root dry weight in the Dutch cultivars. However, in the 'Momotaro' series, the SBR decreased in line with higher root dry weight, but the SBR of the 'Momotaro' series, which is supposed to be more sensitive to physical confinement, needs to be further investigated.

Aboveground production was not significantly different between the Japanese and Dutch cultivars during the early stages of tomato growth. As the plants grew, Dutch cultivar production gradually overtook that of the Japanese cultivars (Ando et al. 2015). That means that Dutch cultivars are suitable for long-term production. The mechanisms supporting long constant production are not clear. However, in this experiment, the root growth of the 'Momotaro' series resulted in lower SBRs, which might cause the low productivity of Japanese cultivars when grown in long-term production systems. The roots were contained in the rockwool slab. This situation might induce physiological stress and/or physical stress (confinement) in the Japanese cultivars, and may have also reduced the SBR. The weakness of the 'Momotaro' series roots hinders constant productivity. Root grafting to a vigorous stock has

improved tomato productivity (Higashide et al. 2014), and such results support the "High SBR-High Productivity" hypothesis.

Seventy percent of the flux to the fruits is from phloem (Araki et al. 2004). Xylem also contributes 30% of the flux, which might also be important. Hardly any Ca translocation via phloem occurs compared to the transport of other elements (Ho and White 2005). This suggests that xylem transport is an important means of supplying Ca to fruits and may contribute to fruit enlargement at night (Asai et al. 2005). In this experiment, the SBR contribution to the plants was higher at night than during the day. Therefore, the increased SBR may also have contributed to Ca transport and fruit production. These results support the hypothesis that the higher SBR of the Dutch cultivars contributed to higher to mato yields. Therefore, maintaining a high SBR relative to increased root mass is necessary in order to achieve higher yields of 'Momotaro' Japanese cultivars.

#### 2. SBR and mineral content

Both Ca and Mn concentrations in the fruits, and the SBR of the 'Momotaro' series were lower than those of the Dutch cultivars, while the other Japanese cultivars were placed between them. Very little Ca is transported by phloem (Jeschke and Pate 1991) and such transport depends on the transpiration rate. This means that Ca transport to organs that have a relatively lower dependency on transpiration relies on root pressure (Masuda et al. 1996). The surface of a tomato fruit is covered by a cuticle; therefore,

	Cultiver	K	Ca	Mg	Fe	Mn	Cu	Zn
	Cultivar	mg g <sup>-1</sup>			μg g <sup>-1</sup>			
Japanese	Momotaro York	30.4	0.65	1.21	43.2	21.9	6.42	15.3
	House Momotaro	28.1	0.73	1.11	28.3	22.3	5.92	13.9
	Momotaro Colt	30.2	0.61	1.17	38.7	17.7	5.69	14.4
	Momotaro 8	33.1	0.68	1.42	43.2	21.9	6.54	15.9
	Bit jelly	34.2	0.92	1.25	39.3	26.0	6.26	17.0
	Reika	32.1	0.98	1.18	46.6	24.2	7.01	15.7
	Shifuku	31.4	0.77	1.40	43.7	23.4	5.43	14.8
	Reiyo	34.8	0.76	1.57	41.7	24.7	6.49	14.5
Dutch	GERONIMO	32.8	1.33	1.20	37.5	29.9	5.34	14.2
	MAKARI	31.1	1.10	1.20	37.4	30.0	5.36	15.2
	MATRIX	32.0	1.25	1.14	36.5	32.4	5.21	15.9
	BLITZ	32.9	1.07	1.18	34.4	22.8	5.30	14.9
	GRACE	31.3	0.87	1.09	32.3	22.0	4.35	13.3
	MATCH	32.1	1.31	1.22	33.2	27.2	5.34	14.0
	QUEST	33.0	1.08	1.10	37.2	25.5	4.97	14.5
	DUNDEE	33.8	1.04	1.29	41.8	28.6	5.93	17.0
Momotaro series		30.4 a	0.67 a	1.23	38.3	21.0 a	6.14 a	14.9
Japanese varieties except for Momotaro series	Tukey-Kramer test (P<0.05)	33.1 b	0.86 a	1.35 ns	42.8 ns	24.6 ab	6.30 a	15.5 ns
Dutch Varieties		32.4 ab	1.13 b	1.18	36.3	27.3 b	5.23 b	14.9
Japanese / Dutch		1.0	0.7	1.1	1.1	0.8	1.2	1.0
Japanese vs. Dutch	Fisher's LSD (P<0.05)	ns	*	ns	ns	*	*	ns
coefficient of variation (%)	Japanese	7.0	16.9	12.1	13.8	11.0	8.2	6.6
	Dutch	2.8	13.7	5.8	8.2	13.3	8.5	7.7
	total	5.2	24.8	10.6	12.7	15.2	12.1	7.0

Table 1. Mineral content of Japanese and Dutch tomato cultivars

Values represent the average of three fruits. Values with the same letter(s) in each row are not significantly different; ns: not significant (P < 0.05).

transpiration-dependent Ca transport to the fruits is lower than for the leaves. The fruits also compete with leaves for Ca, and defoliation improves blossom-end rot (BER) (Sato et al. 2004). The mitigation of excessive ion stress by fertilization reduced BER and improved the SBR (Nakano et al. 2001). These results show that root-pressure-dependent Ca transport at night could have important effects on fruit Ca concentration. The relationship that exists between SBR and Ca might play an important role in Ca transport to the fruits and the final Ca concentration of the fruits.

The mobility of Mn differs depending on its valency, especially when only available in its reduced form  $(Mn^{2+})$ .

The reduction is mediated by the combined effects of enzymatic reduction at the root surface and chemical reduction through the release of reductants, such as phenolics and malate (Löhnis 1951). The tolerance to toxicity also differs among plant species. Tomato plant roots accumulate more Mn than rice plant roots. Roots that have a strong ability to accumulate Mn turn brown in color (Godo and Reisenauer 1980). Moreover, root oxidation activity could also reflect Mn accumulation in the fruits. Competition from other cations could also be a possible mechanism (Tachibana and Inden 1976) that lowers Mn concentrations in the fruits of Japanese cultivars. However, there were no differences in



Fig. 4. Relationship between Ca or Mn concentration of fruits and sap bleeding rate

▲ Dutch cultivars, ○ Japanese cultivars ('Momotaro' series), ● Japanese cultivars (not including 'Momotaro' series). Each dot indicates the average of four plants in SBR and three fruits of Ca and Mn concentrations. Each correlation coefficient (R) between the sap bleeding rate to Ca and Mn was 0.65 and 0.75.

K concentration between the Japanese and Dutch cultivars. The results suggest that Mn uptake by Dutch cultivars was superior to that by Japanese cultivars. The Japanese cultivars are supposed to be bred for soil conditions that are less aerated than the hydroponic system. When soils lack oxygen, root oxidation ability becomes an important trait and Mn uptake can become restricted in Japanese cultivars compared to Dutch cultivars. A further study of oxidation ability is thus necessary.

The electron density map of the crystal structure of photosystem II at a resolution of 1.9 Å showed that five oxygen atoms served as oxo bridges linking the five metal atoms (four Mn and one Ca), and that four water molecules were bound to the  $Mn_4CaO_5$  cluster (Umena et al. 2011). This may play an important role in photosynthesis, even in high productivity tomato cultivars.

The Cu concentration of the Japanese cultivars was conversely higher than in the Dutch cultivars. Cu is easy to absorb from the soil. Thus, a relationship may exist between absorbability and fruit concentration. Phenolics are possible reductants for Mn, which may affect Cu uptake. Therefore, a further study on differences in cultivar exudation (e.g. phenolics or organic acid), including a study of oxidation ability, should be conducted.

These results showed that the SBR had a positive correlation to shoot fresh weight and fruit yield, which suggests that Ca transport is driven by root pressure at night and that Mn transport is related to root reductive activity, which may lead to higher fruit yields. Breeding new cultivars with effective Ca and Mn absorption and transport to the fruits might lead to high-yielding cultivars. Moreover, the SBR may be a simple marker that could be used to select highyielding cultivars in long-term cultivation.

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