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Fruit growth of tomato associated with water uptake and cell expansion

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This experiment was carried out to study the growth mechanisms of tomato fruits in relation to water uptake and enlargement of cells in fruit pericarp. Growth parameters of tomato fruit such as cell layers, cell diameter and thickness of fruit pericarp, functional xylem vessels in fruit pedicel, water uptake rate and growth rate of fruits were investigated during entire growth period of tomato fruit grown hydroponically in a greenhouse. The results showed that fruit growth was strongly correlated with xylem functionality, water uptake rate and expansion of cells in fruit pericarp. This study also confirm that the cell division period in developing tomato fruit after pollination and fertilization was completed before 6 days after flowering (DAF) and following increment of fruit pericarp thickness was only due cell enlargement. Moreover, fruit growth was continued even after excision from plant which was coincided with water uptake rate and xylem functionality in fruit pedicel. The distribution of local relative growth rates and local water status of fruits indicated that the water potential gradient was existed in developing tomato fruit. This result also revealed that the most actively growing zone of fruit was located at the base of fruit from 0-5 mm at DAF 10.

Key words: Functional xylem vessels; growth; local water status; tomato; water potential gradient; water uptake

Introduction

Tomato (*Solanum lycopersicum* L.) is a most popular and economically important vegetable in the world. The popularity of tomato among consumers has made it an important source of vitamins A and C in diets. The growth of fleshy fruits like tomato fruit involves many physiological processes including fertilization followed by pollination, cell division and cell expansion. Some research works has been done elsewhere in the world and they reported that cell division is continued in the fruit ovary for 7-10 days after fertilization, thereafter, fruit is enlarged in the next several weeks by cell expansion (Mapelli *et al.,* 1978; Bohner and Bangerth, 1988). But it is still debatable that until what time after cell division, cell expansions become the key factor for fruit growth.

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In general, once cell division ends, cell expansion become the dominant way to increase fruit size (Bertin, 2005). Final fruit size is directly related to the number of cells produced in the period just immediately following pollination (Zhang *et al.,* 2006). Fruit cell expansion is necessarily important to the final yield and quality of fruit crops.

In tomato, fruit growth and quality depend on water and assimilates import in it. The amount of water uptake by tomato fruit is more than 90% of the total fruit weight. Johonson *et al.* (1992) have provided the direct evidence that the fruit growth is closely linked to the movement of water to the fruit. There are two distinct pathways for transport of water, minerals and photo assimilates in to fruit. Mostly water and minerals are transported through xylem while photo assimilates are moved via phloem tissue. However, some of studies have shown that phloem also serves as the main route for water into sink organs. It was estimated that 60-95% of water enters through phloem, while only 5-40% of water enters through the xylem in fruits of tomato (Ho *et al.,* 1987). In this experiment a dye will be introduced through fruit pedicel to allow staining of lignin wall around the xylem vessel that could ensure the functionality and/or conductivity of xylem vessels in fruit pedicel. The rate of water uptake by fruit per day will be determined from the excised fruit. At the same time growth rate of intact and excised fruit will be determined during this study. Moreover, cell layers as well as cell diameter in developing fruit pericarp will also be counted during the whole growth period. The research work has been undertaken to study the relation of fruit enlargement with development of xylem tissue in pedicel, cell expansion in pericarp and the rate of water uptake of developing tomato fruit.

Materials and Methods

Plant materials

Tomato plants (*Solanum lycopersicum* L. cv. Momotaro) were grown hydroponically in the greenhouse using Otsukahouse nutrient solution No. 1 & 2 (Otsuka House Fertilizer, Otsuka Chemical Co. Ltd., Osaka, Japan). Tomato seeds (Takii & Co., Ltd., Kyoto, Japan) were germinated in dark condition at 25 ± 1^{o}C temperatures in the laboratory on September 15, 2008. At 4-5 true leaves stage, healthy and uniform seedlings were transplanted in 15 L size plastic pot containing nutrient solution (EC 1.0 mS cm^{-1}, pH 6.25) in the greenhouse on October 20, 2008. Nutrient solution in pot was replaced once a week with adjusted EC and pH. The concentration of nutrient solution was increased from 1.0 to 1.2 mS cm^{-1} during first flowering to third flowering time. After third flowering to fruit harvest, the electrical conductivity of nutrient solution was

adjusted 1.5 mS cm^{-1}. Flowers were artificially pollinated by using hormone (Tomatotone) and days after flowering (DAF) was counted from the date of pollination.

Measurement of growth rate, local growth rate and local water status of fruit

For the measurement of fruit growth, volume of intact fruit was measured from plants in the greenhouse. All of fruits for this purpose were selected from first fruit truss. Two equatorial diameters and polar diameter were measured by using digital caliper (DIGI-KANON EMA-20, Nakamura Mfg. Co., Tokyo, Japan) at 2 days intervals from DAF 6 to DAF 46. Thereafter, fruit volume was determined assuming that the fruit was ellipsoid shape by using following equation:

$$V = \frac{4}{3}\pi \cdot abc \qquad (1)$$

Where a, b and c are the lengths of the semi axes of the ellipsoid. Then the growth rate of fruit was calculated from the changes in fruit volume as a function of time ($\frac{dV}{dt}$, $m^3\ s^{-1}$).

In order to determine local growth rate along fruit length as a function of distance from the fruit base to tip, 6 tomato fruits at DAF 10 were selected for the measurement while it was intact with plant in the greenhouse. Fruits were marked at approximately 1.5 mm intervals gently with Indian ink from the base to tip and measured the distance between the marks. Fruits were kept next 24 hr for growing then the intervals between the marks were measured again accurately by using a digital caliper. The relative rate of elongation was calculated for each interval from the length increased during the growth period. The water status of different location of fruit at DAF 10 was determined along fruit length with a psychrometer according to the methods described by Boyer and Knipling (1965), Nonami *et al.* (1987).

Determination of fruit growth

Growth rate of excised tomato fruit was determined with an extensiometer (Nonami and Boyer, 1990) that consisted of a rotary variable differential transformer (R30, Schaevitz Engineering, Pennsauken, N.J) under three base solutions namely pure water, -2 bars sucrose solution and -3 bars sucrose solution. Fruits at DAF 14 were excised from selected plant in the greenhouse and immediately fruit pedicel was inserted into distillated water containing glass tube and brought to the laboratory. Fruit pedicel was re-cut

under distilled water and set with another conical flask containing base solution thus fruit pedicel was in solution and fruit tip out side. Then fruit base as well as calyx was tightly fixed with container top by cloth adhesive tape. An aluminum bar attached to the rotary variable differential transformer was kept on fruit top to measure vertical expansion of fruit. The output of the transformer was recorded as the displacement in length with a recorder (OMNIACE II, RA 1200, NEC San-ei Instrument Ltd., Japan). The growth rate of fruit was calculated from the slope of the length displacement as a function of time.

Measurement of thickness, number of cell layers and cell diameter of fruit pericarp

For measurement of pericarp thickness, fruit pericarp was separated from the equatorial side of fruit and its thickness was measured with digital caliper. Since the thickness of pericarp was not uniformly distributed in fruit so that data collected from four locations of equatorial side and then averaged value was used as thickness of fruit. Thereafter, number of cell layers and cell diameter from same pericarp tissue were counted from a free hand cross section under a light microscope (Nikon Co., Japan).

Determination of water uptake rate of fruit

Water uptake rate of excised tomato fruits was determined by submerging fruit pedicel in distilled water for 24 hours. Before setting the treatments for 24 hr, the weight gain of fruit which only had their pedicel in water was measured periodically to check the kinetics of fruit hydration. Fruit with pedicel in distilled water was kept in humidified chamber with 100% RH. Weight change was recorded after fruit was quickly surface blotted with kimwipe tissue paper and immediately returned to water. This weight change of fruit was checked until 24 hr. The results showed that fruit weight was increased until 24 hours and then water uptake rate was determined after 24 hr from DAF 6 to DAF 46. Fruits were excised form plant with long peduncle and immediately kept in distilled water. After brought to the laboratory, fruit petals were discarded carefully and fruit pedicel was re-cut under distilled water before record initial weight. After that pedicel was submerged under distilled water in a glass tube kept in a humid chamber. The final weight was taken after 24 hr of hydration. Water uptake rate of fruits was determined by using the following equation:

$$\text{Water uptake rate (g/day)} = \frac{W_2 - W_1}{T_2 - T_1} \qquad (2)$$

Where, W_1, T_1 and W_2, T_2 were initial and final fruit weight and time, respectively. Relative weight change was determined from the weight difference divided by initial weight.

Determination of growth rate of excised fruit

Growth rate of excised tomato fruit was determined with an extensiometer (Nonami and Boyer, 1990) that consisted of a rotary variable differential transformer (R30, Schaevitz Engineering, Pennsauken, N. J) under three base solutions namely pure water, -2 bars sucrose solution and -3 bars sucrose solution. Fruits at DAF 14 were excised from selected plant in the greenhouse and immediately fruit pedicel was inserted into distillated water containing glass tube and brought to the laboratory. Fruit pedicel was re-cut under distilled water and set with another conical flask containing base solution thus fruit pedicel was in solution and fruit tip out side. Then fruit base as well as calyx was tightly fixed with container top by cloth adhesive tape. An aluminum bar attached to the rotary variable differential transformer was kept on fruit top to measure vertical expansion of fruit. The output of the transformer was recorded as the displacement in length with a recorder (OMNIACE II, RA 1200, NEC San-ei Instrument Ltd., Japan). The growth rate of fruit was calculated from the slope of the length displacement as a function of time.

Determination of functional xylem vessels in fruit pedicel

The ability of xylem vessels in fruit pedicel to conduct water was examined by dye infusion method. Fruits were excised form plant with long pedicel and immediately kept in distilled water then brought to the laboratory for dye infusion experiment. Fruit pedicel was re-cut under water and immediately inserted into glass tube containing dye (0.1% Toluidine blue). In most cases dye infusion through cut pedicel was allowed to continue for 6-7 hrs under laboratory condition but in some cases the duration was 24 hours. Pedicel was then cross and vertical sectioned and the number and diameter of stained xylem vessels were determined under a light microscope (Olympus Co., Ltd., Japan). Water flow rate per conductive xylem vessel was calculated dividing water uptake rates of excised fruit by the number of functional xylem vessels.

Results

Cell expansion and fruit growth

Cell diameter, cell layers and thickness of pericrp of developing tomato fruits were estimated form DAF 6 to DAF 46. The thickness of fruit pericarp was increased from 0.65 mm (DAF 6) to 6.86 mm (DAF 46) and this increment of thickness was rapid from DAF 10 to DAF 34 (Fig 1A). The cell layers of same pericarp were found almost unchanged during the entire growth period (Fig 1B). The average ranges of number of cell layers in fruit pericarp tissue were 22.85 to 25.65; the difference of this number was statistically non significance. Cell diameter in fruit pericarop as also determined from same pericarp tissue. The diameter of cell was increased as fruit became bigger from DAF 6 to DAF 40 (Fig 1C). At the early stage of fruit development, cell diameter increased uniformly from outer to inner pericarp cell but it become haphazard at later stage. At this stage, the tendency of cell expansion rapidly increased near the inner side as compared to outer side of pericarp. These results indicated that since number of cell layers was found unchanged but cell diameter and thickness of pericarp increased as fruit become mature, so fruit growth is solely depend on cell expansion and cell expansion further depend on water accumulation in it.

When three dimensional fruit volume was plotted against days after flowering (DAF) they form a sigmoid curve (Fig 2A). Fruit growth rate was calculated from volume change as a function of time (day) throughout the growth period of fruit. The result showed that the growth rate was increased sharply from DAF 6 to DAF 21 and maximum growth rate was observed at DAF 21 (Fig 2B) after that growth rate declined as fruit become mature.

The local relative growth rates and water status were determined to know the distribution of growth and water status along the fruit length from fruit base to tip. From the result it was found that the most active growing zone of fruit was located between 0 and 5 mm from the fruit base at DAF 10 (Fig 3A). Water status of different location along the fruit length was measured with a psychrometer. The components of water status of fruit were showed lower in the most growing zone. Water potential found significantly lower in the active growing zone then increased as growth rate become slower in comparatively mature zone of the fruit top (Fig 3B). Turgor of pericarp tissue was subtracted value of water potential from osmotic potential. It was exhibited similar trend like water potential and found lower at the active growing zone and became higher gradually when measured location were moved toward the mature zone of fruit (Fig 3C). On the other hand, osmotic potential was more negative in the rapid growing zone but it was not significantly different between active growing and comparatively mature zone of fruit (Fig 3B).

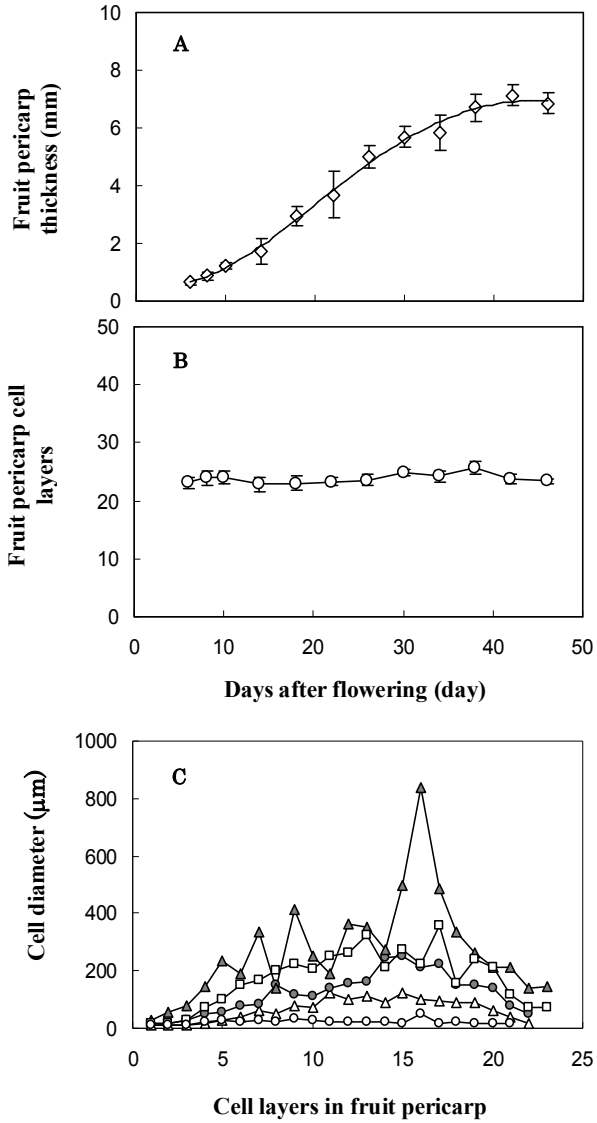

Fig 1. Thickness (A), cell layers (B) and cell diameter (C) of tomato fruit pericarp at different DAF. In C, DAF 6 (○), DAF 10 (Δ), DAF 20 (●), DAF 30 (□) and DAF 40 (▲). Vertical bars in A & B indicate 95% confidence intervals calculated from Student's t-distribution.

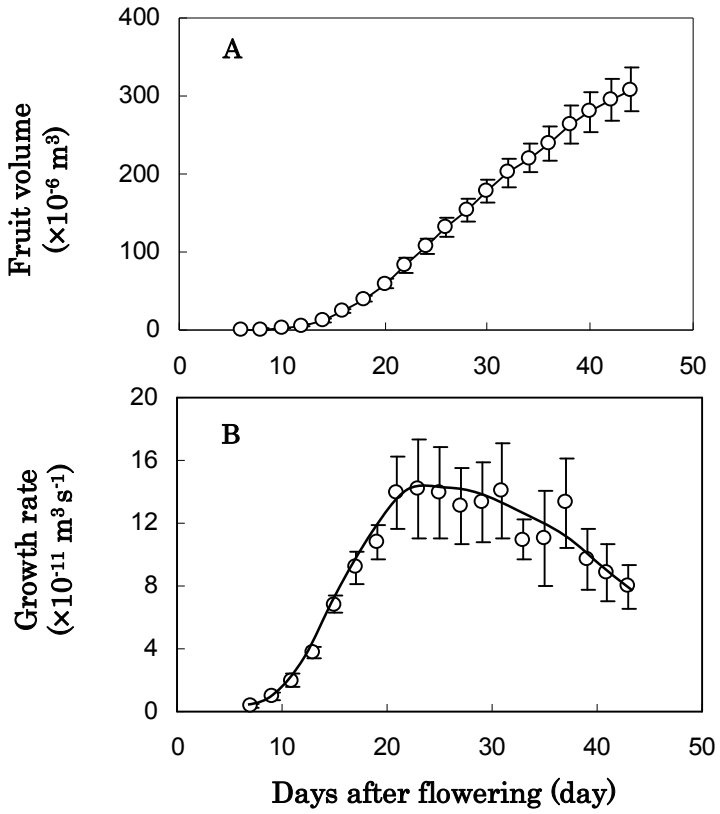

Fig 2. Volume (A), and growth rate (B) of tomato fruit at different days after flowering (DAF). In A & B, vertical bars indicate 95% confidence intervals calculated from Student's t-distribution

Water uptake rate and fruit growth

There are two distinct path ways to transport water, minerals and photo assimilates into developing tomato fruit. The rate of water uptake of fruit pedicel of excised tomato fruit was determined by hydration method throughout the entire growth period. The periodical fruit weight change was found 1:1 relation with time after pedicel submerged under distilled water (Fig 4A). This result assured that water uptake rate of excised tomato fruit can be determined

at least up to 24 hr of pedicel submerged. Water uptake rate of tomato fruit from DAF 6 to DAF 46 was determined from excised fruit by pedicel submerged in distilled water. The result showed that water uptake rate in fruit began to increase rapidly from DAF 6 to DAF 30 and then bit declined until DAF 46 but this reduction was not significantly different from DAF 30 (Fig 4B).This result suggested that since fruit was kept in a water saturated humid box during hydration experiment thus water uptake by fruit was mostly for cell expansion during growth of fruit. At the same time, we also determined the growth rate of excised fruit with the rotary variable differential transformer to ensure whether fruit was continued to grow after excision (Fig 4C). In this case, DAF 14 fruit was used under three different base solutions (pure water, -2 bars sucrose solution and -3 bars sucrose solution) treatment. From the result it was clearly found that the vertical expansion of excised fruit was continued to grow over 10 hours period under pure water condition with a little declined at the beginning. Growth rate was declined when fruit pedicel submerged in sucrose solution (Fig 4C). At -2 bars sucrose solution, growth rate reduced initially until about one hour of pedicel submerged then gradually became stable and come to the parallel of pure water treatment. Growth rate was significantly reduced while fruit pedicel experienced -3 bars sucrose solution (Fig 4C).Water uptake of fruit was related with functionality or conductivity of xylem vessels in fruit pedicel. The functionality of xylem vessels in the fruit pedicel was examined by dye infusion test from DAF 6 to DAF 46. Toluidine blue (0.1%) was infused through fruit pedicel for several hours and the lignified xylem vessels number and diameter were measured from a free hand cross section under microscope. Result showed that the number of conductive xylem vessels in fruit pedicel was increased from DAF 6 to DAF 38 after that conductive vessels number remained stable up to DAF 46 (Fig 5A). Diameter of these conductive xylem vessels was found to increase rapidly from DAF 6 to DAF 18 after that remained steady until fruit maturity (Fig 5B). These findings indicated that water conducting xylem vessels were functional throughout the growth period of developing tomato fruit. The anatomical study of dye infused fruit pedicel showed that the lignin in the vessels wall was stained due to absorbed dye until DAF 42. This result also confirmed the functionality or conductivity of xylem tissue in the fruit pedicel. Water flow rate of each conductive vessel was determined from the amount of water uptake of fruit per day divided by number of conductive xylem vessels. The result showed that flow rate of water by conductive xylem vessel was increased rapidly from DAF 6 to DAF 22 after that declined a bit but not significantly until 30 DAF and finally rapidly down as fruit become mature (Fig. 5C).

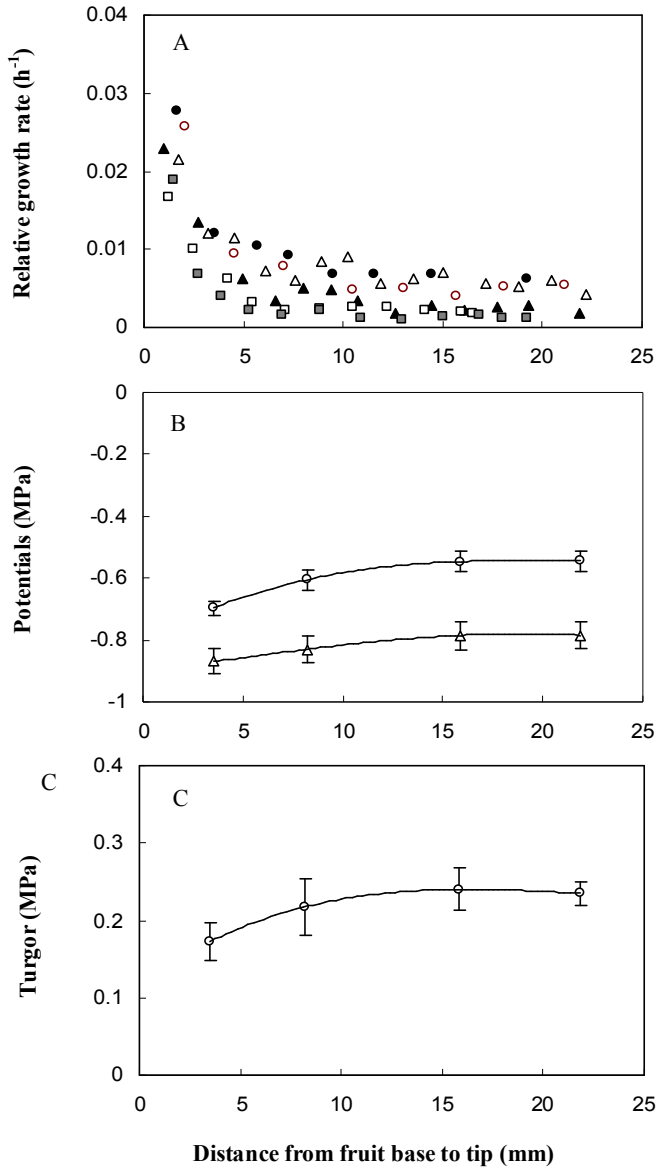

Fig 3. Local relative growth rates (A), water potential (ψ_w) & osmotic potential (ψ_s) (B) and turgor (ψ_p) (C) of tomato fruit at DAF 10. In A, six replications of DAF 10 fruits indicated by six different symbols. Vertical bars in B & C indicate 95% confidence intervals calculated from Student's t-distribution.

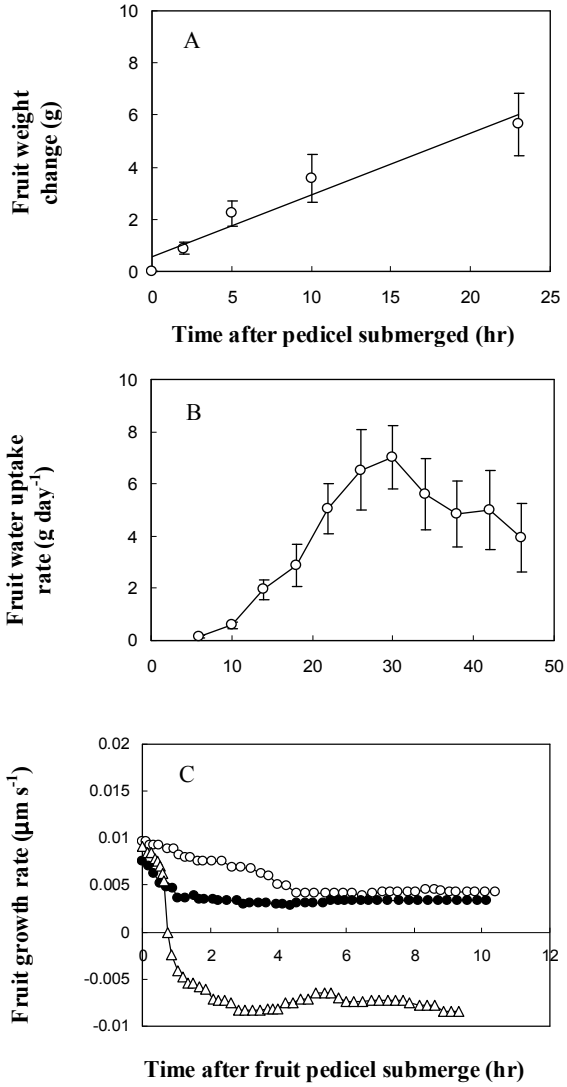

Fig 4. Periodical weight change (A), water uptake rate (B) and growth rate of excised fruit (C). In A, the regression line is $y = 0.2368x + 0.5681$, $r = 0.972$. Vertical bars in A & B indicate 95% confidence intervals calculated from Student's t-distribution.

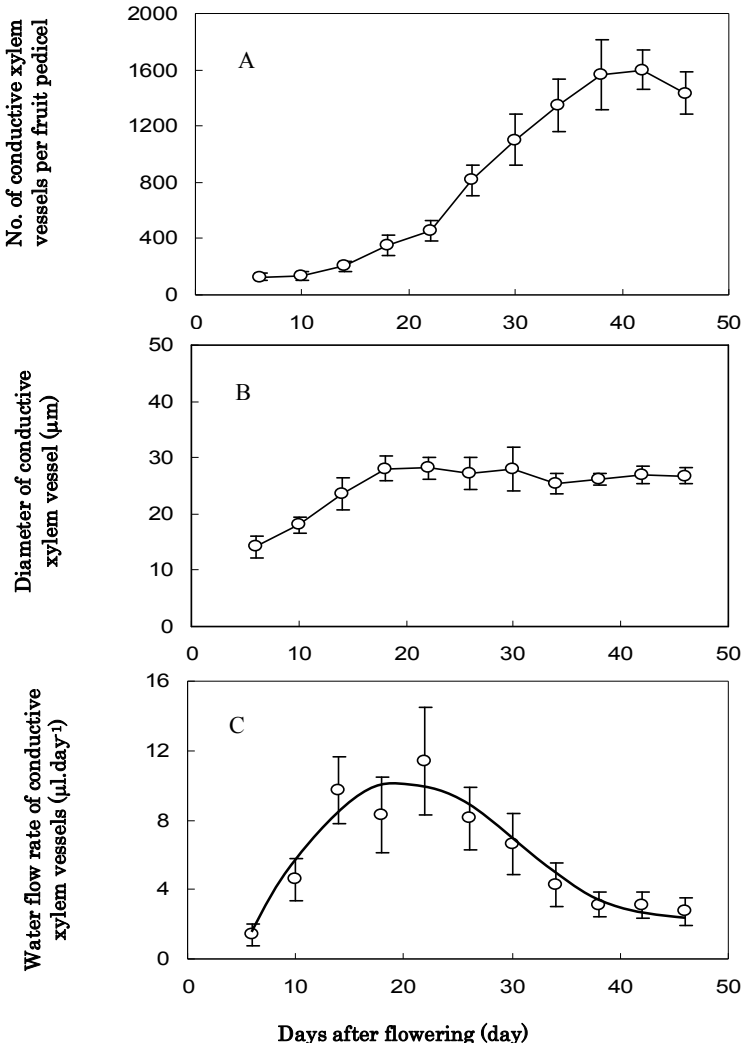

Fig 5. Number (A), diameter (B) and water flow rate (C) of conductive xylem vessels in fruit pedicel at different days after flowering (DAF). Vertical bars indicate 95% confidence interval calculated from Student's t-distribution.

Discussion

Water accumulation by fruit is important for both of fruit size and quality. Fruit growth has a strong relation with water entry in it. Therefore, tomato fruit growth and its enlargement can be explained in relation to xylem functionality, water uptake and cell expansion. Water and photo assimilates are imported into fruit through fruit pedicel. When the availability of assimilates is unlimited, the amount of water given to tomato fruit generally increased from pollination to next three weeks after that this rate decrease (Ho, *et al.,* 1987). Present study was undertaken to determined water uptake rate of excised tomato fruit by hydration method as used by Bondada, *et al.* (2005). The result showed that the water uptake rate was increased gradually from DAF 6 and continued to DAF 30 after that little declined. Dye infusion test exhibited that xylem vessel was functional throughout the growth period of fruit. As a result water uptake rate of developing tomato fruit was found related with xylem functionality in the fruit pedicel. Bussieres (2002) reported that the decrease in water import rate in tomato might be a limiting transfer rate in fruit pedicel. Significant reduction of xylem in the knuckle zone of fruit pedicel and increase in phloem cross sectional area are the limiting factor for decreasing water uptake rate reported by Lee (1989). In the pedicel of tomato fruit (Starck, *et al.,* 1990) and apple (Lang and Ryan, 1994), increase in phloem cross_sectional area have been found during the fruit growth period and they suspect that increase in pedicel phloem cross-sectional area might occur before increase fruit size. In this experiment growth rate of intact fruit was found to increase rapidly from DAF 6 with increased number of functional xylem vessels and also diameter of vessels. At the same time % relative water content of fruit was found to increase with increased fruit volume. Thus it can be conclude that water was imported into developing tomato fruit mostly through xylem. This result corroborated with the most recent findings of Windt, *et al.* (2009). They demonstrate NMR (nuclear magnetic resonance) flow imaging of xylem and phloem transport of developing tomato fruit truss and reported that at least 75% of the net influx into fruit occurred through the external xylem and about 25% via the perimedullary region, which contain both phloem and xylem.

Fruit growth and further development occurred due to cell expansion and this expansion was mostly related to water uptake into fruit during growth. Cell diameter in fruit pericarp enlarged as fruit become bigger which has correlation with pericarp thickness and the values were increased from 0.65 mm to 6.86 mm. The number of cell layers in pericarp was more or less similar from DAF 6 to DAF 46 and the values were 23 to 26 (average). These results confirmed that

cell division was ended before DAF 6 and following enlargement of fruit was solely for cell expansion in this tomato cultivar. This result is agreement with the results of (Mapelli, *et al.,* 1978). They reported that cell division is continued in the fruit ovary for 7-10 days after fertilization after that fruit enlarged through cell expansion. The local distribution of growth rates and water status of developing tomato fruits clearly indicated the presence of the gradient of water potential between rapidly growing zone and mature zone of fruit. Nonami, *et al.* (1997) reported that cell enlargement depends on the growth-induced gradient in water potential to move water into cells.

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