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DEPENDENCE OF CALCIUM UPTAKE ON WATER ABSORPTION AND RESPIRATION IN ROOTS OF TOMATO PLANTS (*LYCOPERSICON ESCULENTUM* MILL.)

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KITANO M., ARAKI T., YOSHIDA S. and EGUCHI T. *Dependence of calcium uptake on water absorption and respiration in roots of tomato plants (Lycopersicon esculentum Mill.)*. BIOTRONICS **28**, 121–130, 1999. Root xylem sap exuded from the decapitated stem stumps of tomato plants (*Lycopersicon esculentum* Mill.) was collected in the daytime and the nighttime, and dependence of calcium uptake on water absorption and respiration in the tomato roots is examined by analyzing exudation rate (J_W; $\mu L\ s^{-1}$), calcium concentration ($[Ca^{2+}]$; $mmol\ L^{-1}$) in the xylem sap and root calcium uptake rate (J_{Ca}; nmol s^{-1}) in the root exudation. J_W from the stem stump was about one third of transpiration rate from the whole shoot before decapitation and was kept two to five times higher in the daytime than in the nighttime. The xylem sap $[Ca^{2+}]$ was about 3 to 4.5 times higher than that of the nutrient solution in the hydroponic pot, and the xylem sap $[Ca^{2+}]$ in the daytime was 1.5 times higher than that in the nighttime. Consequently, J_{Ca} in the daytime was 3 to 5.5 times higher than that in the nighttime. The daytime higher J_{Ca} was depressed in the restrictedly water absorbing plant, and the restriction of root respiration in the daytime lowered the xylem sap $[Ca^{2+}]$, which resulted in significant depression in J_{Ca}. Furthermore, the lower J_{Ca} in the nighttime was not enhanced by activating the nighttime root respiration. Thus, the daytime root calcium uptake was regulated by both of the water absorption and the respiration in roots, and the nighttime root respiration was not a rate-limiting factor for the lower calcium uptake in the nighttime. The observed dependence of calcium uptake on water absorption and respiration in roots was explained in relation to the important role of the root endodermis.

Key words: *Lycopersicon esculentum* Mill.; tomato roots; calcium uptake; water absorption; root respiration; endodermis.

INTRODUCTION

Calcium is required to stabilize cell walls and plasmamembranes and to maintain the membrane permeability (*7, 9*), and the local calcium deficiency in the distal fruit tissue causes blossom-end rot. Calcium is immobile in the phloem, and the transport of calcium through the plant is restricted to the xylem

transport, which is driven by the transpiration stream. Therefore, the calcium uptake into the xylem by roots and the distribution through the xylem network can be key processes regulating calcium accumulation into fruits, which is affected by environment conditions (*1, 2, 5, 7–11, 13*).

For the calcium uptake by roots, a model representing the important role of the endodermis with Casparian bands (i.e. water and Ca^{2+} impermeable structure) has been proposed by Clarkson (*3, 4*), where the endodermal cell is characterized by an arrangement of calcium channels in the outer membrane external to the Casparian bands and of the energy-dependent Ca^{2+} pumping units in the inner membrane: Ca^{2+} transported with the transpiration stream via the apoplast of the cortex is admitted into the cytoplasm of the endodermal cell through the Ca^{2+} channels in the outer membrane (*12*), and the free Ca^{2+} in the endodermal cytosol is actively discharged into the stelar apoplast by the energy-dependent Ca^{2+} pumping units in the inner membrane (*6*). Consequently, by balancing these influx and efflux of Ca^{2+}, concentration of Ca^{2+} in the endodermal cytosol is kept lower than the damaging levels, and the Ca^{2+} discharged from the endodermal cytosol into the stelar apoplast is loaded into the xylem with the transpiration stream. These processes can be considered to regulate the calcium uptake capacity by roots (*3, 4*), and further it has been reported that root exudation appears to be a suitable index of the calcium uptake capacity by roots (*5, 9*).

In the present study, therefore, root xylem sap exuded from the decapitated stem stumps of tomato plants was collected in the daytime and the nighttime, and dependence of the calcium uptake capacity on water absorption and respiration in tomato roots is examined in relation to the role of the endodermis.

MATERIALS AND METHODS

Plant materials

Tomato plants (*Lycopersicon esculentum* Mill. cv. Hausu-Momotaro) were grown hydroponically at a day/night air temperature of 23/18℃ and a relative humidity of 70% in a phytotron glass room. Young seedlings were planted in hydroponic pots (13 L) in which composition of the nutrient solution was Ca^{2+}, 1.8; K^+, 3.7; Mg^{2+}, 0.9; $H_2PO_4^-$, 0.8; NO_3^-, 7.6; NH_4^+, 0.8 mmol L^{-1} with iron EDTA and micronutrients. The seedlings were pinched at two leaves above the first truss before anthesis of the second truss, and the material plants which were fruiting only on the first truss were used for the experiment.

Analysis of root exudation

About 21 to 30 days after anthesis on the first truss, the plants were conducted into a growth chamber with the artificial light of metal halide lamps (DR400/T(L), Toshiba Corp., Tokyo, Japan) at a PPFD of 300 μmol m^{-2} s^{-1} in a photoperiod of 12 hours (6:00–18:00) under constant condition of an air temperature of 20℃ and a relative humidity of 70%. The nutrient solution described above was also used for the experiment of xylem sap collection.

Collection of xylem sap exuded from the decapitated stem stumps was started at 12:00 in the daytime and at 24:00 in the nighttime. A stem of a material plant was cut at 2 cm below the cotyledon, and the decapitated stem stump was connected to a measuring tube. Rapid exudation was found in the tube just after the decapitation, but the initial exudate during five minutes was removed because it was contaminated with the wounded cytosol. Thereafter, measurement of the exudation rate and sampling of the exuded xylem sap were repeated in course of time at intervals of 5 to 7 min in the daytime and 10 to 13 min in the nighttime: The exudation rate (J_W; $\mu L\,s^{-1}$) was evaluated by measuring the exudate level on the scale of the measuring tube, and the exuded xylem sap in the tube was sampled into a vial by using a microsyringe.

Concentration of Ca^{2+} ($[Ca^{2+}]$; $mmol\,L^{-1}$) in the exuded xylem sap was determined reflectometrically by using an analytical test strip (Refrectoquant Calcium-Test, Merck, Darmstadt, Germany) and a reflectometer (RQ flex plus, Merck, Darmstadt, Germany): A red complex is formed on the test strip by reaction of Ca^{2+} in the sample with glyoxal-bis(2-hydroxyanil). Flux of Ca^{2+} (J_{Ca}; $nmol\,s^{-1}$), i.e. calcium uptake rate by roots, was evaluated by $J_w \times [Ca^{2+}]$.

Treatments on water absorption and respiration in roots

To examine effects of water absorption and respiration on calcium uptake in the tomato roots, the root water absorption was restricted in the daytime, and the root respiration was restricted in the daytime and activated in the nighttime.

For the restriction of root water absorption during the xylem sap collection in the daytime, the whole shoot including all leaves was covered with a transparent polyethylene bag for six hours prior to the decapitation, where the restricted transpiration in the shoot resulted in restriction of the root water absorption.

For restriction of the root respiration during the xylem sap collection in the daytime, the hydroponic pot was kept airtight by covering with plastic film, and N_2 gas was flushed intermittently into the nutrient solution to keep the hypoxic condition under dissolved O_2 concentration lower than $9.4\,\mu mol\,L^{-1}$, where the dissolved O_2 concentration was monitored by a dissolved oxygen sensor (UC-12, Central Kagaku Co. Ltd, Tokyo, Japan).

For activating the root respiration during the xylem sap collection in the nighttime, sucrose was added to the nutrient solution at a concentration of 1% two hours before the xylem sap collection (22:00). The nutrient solution was aerated sufficiently before the time of the xylem sap collection (24:00), and the hydroponic pot was kept airtight, where dissolved O_2 concentration in the nutrient solution was measured in course of time as mentioned above. Respiration rate (J_{O_2}; $\mu mol\ O_2\ plant^{-1}\,s^{-1}$) in roots was evaluated from the decrease rate of the dissolved O_2 concentration in the pot.

RESULTS AND DISCUSSION

Figure 1 shows time course patterns of J_W, $[Ca^{2+}]$ and J_{Ca} in root exudation

from the stem stump after decapitating at the stem base in the daytime and in the nighttime. J_W from the stem stump was about one third of transpiration rate from the whole shoot. J_W in the daytime decreased to the half in 60 min after decapitation, but it was kept two to five times higher than the nighttime J_W which remained constant at about 1.4 μL s^{-1}. There was no significant difference in $[Ca^{2+}]$ just after decapitation between the daytime and the nighttime, where $[Ca^{2+}]$ in the exuded xylem sap was about 3 to 4.5 times higher than that of the nutrient solution in the pot (i.e. 1.8 mmol L^{-1}). $[Ca^{2+}]$ in the daytime, however, increased by 50% in 60 min after decapitation and became 1.5 times higher than the nighttime $[Ca^{2+}]$ which remained constant at about 5 mmol L^{-1}. This increase in the daytime $[Ca^{2+}]$ after decapitation is considered to be caused by reduction in the diluting effect under decreasing root water absorption after decapitation. Consequently, J_{Ca} in the daytime was kept 3 to 5.5 times higher than the nighttime J_{Ca} which remained constant at about 7 nmol s^{-1}. This suggests that the root calcium uptake rate (i.e. rate of Ca^{2+} loading into xylem sap) is remarkably higher in the daytime than in the nighttime.

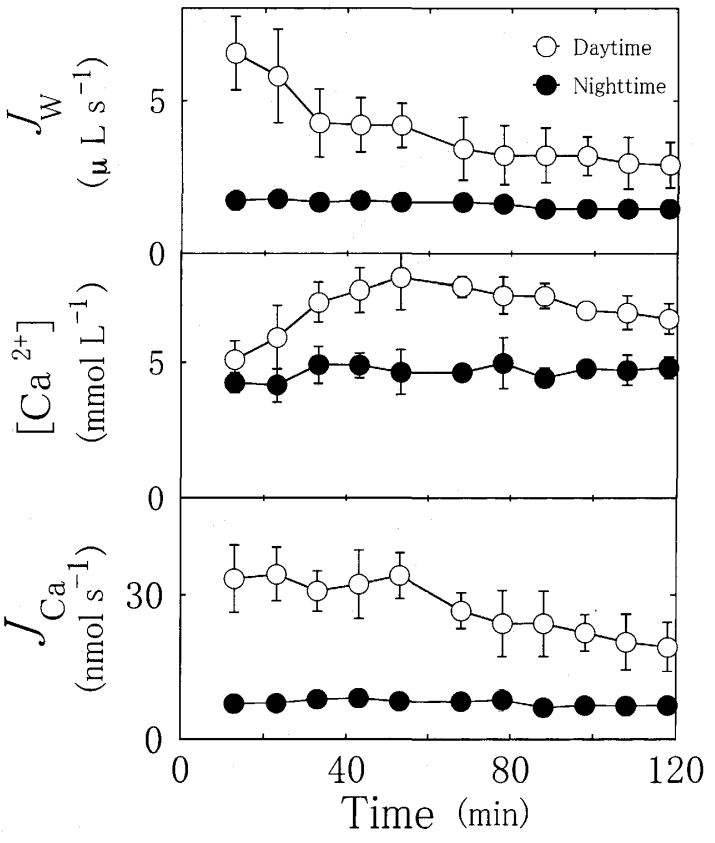

Fig. 1 Time course patterns of exudation rate (J_W), Ca^{2+} concentration ($[Ca^{2+}]$) in xylem sap and Ca^{2+} flux (J_{Ca}) in root exudation from the stem stump after decapitating at the stem base in the daytime and in the nighttime. At zero time (i.e. midday in the daytime and midnight in the nighttime), plants were decapitated at their stem bases. Means of three plants are shown with standard deviations.

Dependence of this daytime higher calcium uptake rate on root water absorption was examined by using the plant where daytime transpiration, i.e. root water absorption, was restricted by covering the whole shoot with a transparent polyethylene bag. Figure 2 shows time course patterns of J_W, $[Ca^{2+}]$ and J_{Ca} in the daytime exudation from the decapitated stem stump of the restrictedly transpiring plant in comparison with those of the freely transpiring plant with no polyethylene shoot cover. J_W in the restrictedly transpiring plant was kept lower than that in the freely transpiring plant. There was no significant difference in $[Ca^{2+}]$ just after decapitation between both the plants. $[Ca^{2+}]$ in the restrictedly transpiring plant was kept constant at the lower level of about 5 mmol L^{-1}, which was almost the same as $[Ca^{2+}]$ in the nighttime (Fig. 1) and was also about three times higher than $[Ca^{2+}]$ in the nutrient solution. J_{Ca}, therefore, was markedly depressed in the restrictedly transpiring plant as compared with the freely transpiring plant. This indicates that strength of transpiration stream from root surface to the stelar xylem is one of the rate-

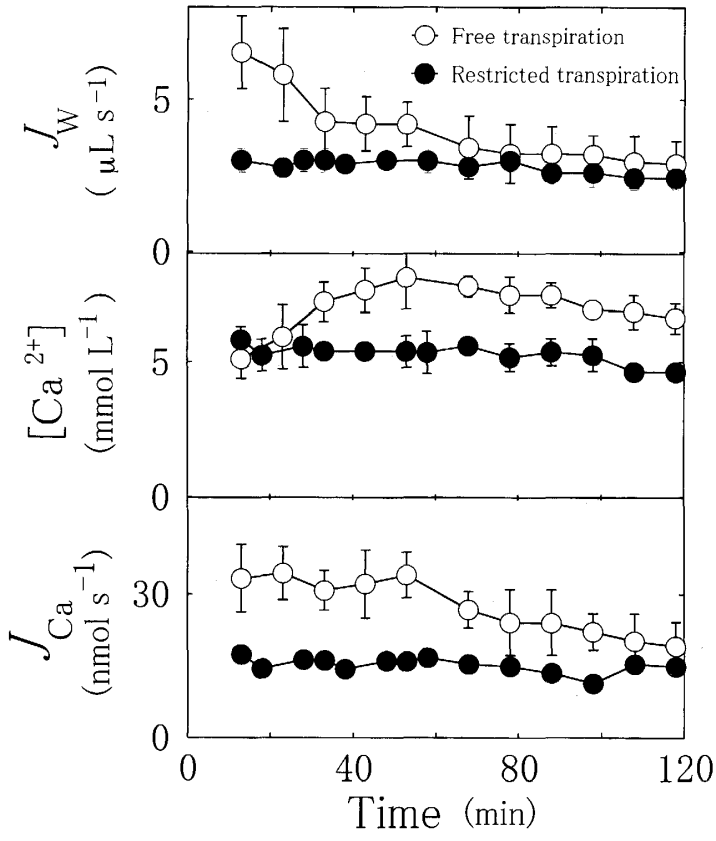

Fig. 2 Time course patterns of exudation rate (J_W), Ca^{2+} concentration ($[Ca^{2+}]$) in xylem sap and Ca^{2+} flux (J_{Ca}) in the daytime root exudation from the decapitated stem stumps in the restrictedly transpiring plant and in the freely transpiring plant. For restriction of the shoot transpiration, the all leaves were covered with a transparent polyethylene bag. At zero time (i.e. midday), plants were decapitated at their stem bases. Means of three plants are shown with standard deviations.

limiting factors regulating the daytime Ca^{2+} loading into xylem (*2, 5, 7, 9*).

Ca^{2+} flux (J_{Ca}) through root xylem can be considered to depend on not only exudation rate (J_W) but also on [Ca^{2+}] in xylem sap. The exuded xylem sap was found to be highly concentrated with Ca^{2+} and had [Ca^{2+}] about three times higher than that in the nutrient solution in the pot (Figs. 1 and 2). This fact suggests that there exists some physiological process actively pumping Ca^{2+} into the sap flow from the root surface to the stelar xylem. Therefore, effect of root respiration on the daytime calcium uptake by roots was examined by using the plant where the root respiration was restricted by root hypoxia. Figure 3 shows time course patterns of J_W, [Ca^{2+}] and J_{Ca} in the daytime exudation from the decapitated stem stump of the restrictedly respiring roots under root hypoxia in comparison with those of the freely respiring plant under sufficient dissolved O_2. Both of J_W and [Ca^{2+}] in the daytime were significantly lowered by the restriction in root respiration. In particular, under the restricted root respiration, xylem sap was not significantly concentrated with Ca^{2+}, and [Ca^{2+}] in the xylem

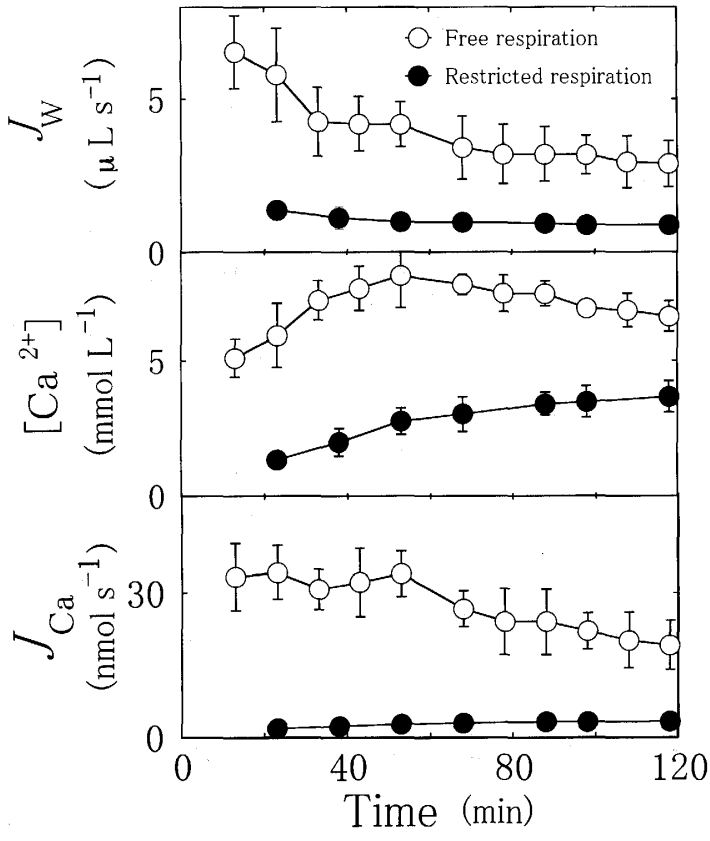

Fig. 3 Time course patterns of exudation rate (J_W), Ca^{2+} concentration ([Ca^{2+}]) in xylem sap and Ca^{2+} flux (J_{Ca}) in the daytime root exudation from the decapitated stem stumps in the plant with the restrictedly respiring roots and in the plant with the freely respiring roots. For restriction of root respiration, dissolved O_2 of the nutrient solution in the hydroponic pot was purged by N_2 gas. At zero time (i.e. midday), plants were decapitated at their stem base. Means of three plants are shown with standard deviations.

sap exuded for about 40 min after decapitation remained around $[Ca^{2+}]$ of the nutrient solution in the pot, whereas $[Ca^{2+}]$ in the xylem sap under sufficient root respiration was always about three times higher than that in the nutrient solution. This lowered J_W and $[Ca^{2+}]$ in the restrictedly respiring roots resulted in extreme depression in J_{Ca}. This suggests that root Ca^{2+} uptake depends on the respiration-dependent process contributing to Ca^{2+} concentration into root xylem sap. Thus, the higher rates of root Ca^{2+} uptake in the daytime requires not only the strong transpiration stream, i.e. the active water absorption, but also respiration-dependent Ca^{2+} pumping into the sap flow in roots.

To confirm the rate-limiting factor for the lower Ca^{2+} uptake rate found in the nighttime (Fig. 1), effect of root respiration on Ca^{2+} uptake in the nighttime was examined by activating the nighttime root respiration by the sucrose addition into the nutrient solution in the pot. Figure 4 shows effect of the sucrose addition on root respiration rate (J_{O_2}) in the nighttime. In the normal nutrient solution without the sucrose addition, J_{O_2} in the nighttime was lower than that in the daytime, and the difference was significant at 5% level. The nighttime root respiration, however, was significantly activated by the sucrose addition, and this activation in the nighttime root respiration was significant at 5% level. Figure 5 shows time course patterns of J_W, $[Ca^{2+}]$ and J_{Ca} in the nighttime exudation under the respective conditions with 1% sucrose and no sucrose in the nutrient solution. There found no significant effects of the sucrose addition on J_W, $[Ca^{2+}]$ and J_{Ca} in the nighttime. This indicates that the nighttime root respiration under the sufficient supply of dissolved O_2 cannot be a rate-limiting factor for the lower root calcium uptake in the nighttime and

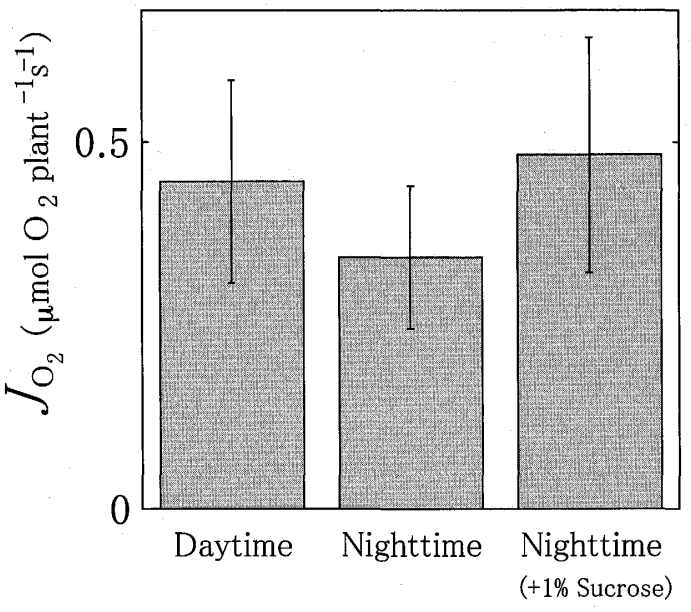

Fig. 4 Root respiration rates (J_{O_2}) in the daytime and in the nighttime and effect of 1% sucrose addition into the nutrient solution on the nighttime root respiration rate. Means of three plants are shown with standard deviations.

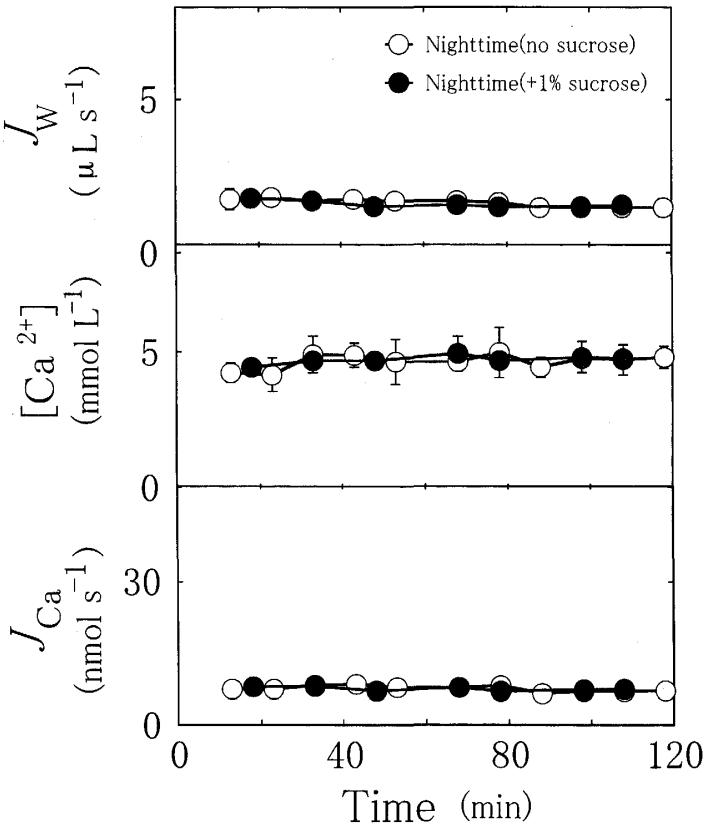

Fig. 5 Time course patterns of exudation rate (J_W), Ca^{2+} concentration ([Ca^{2+}]) in xylem sap and Ca^{2+} flux (J_{Ca}) in the nighttime root exudation from the decapitated stem stumps in the plant grown with the 1% sucrose added nutrient solution and in the plant grown with the normal nutrient solution without sucrose addition. The sucrose addition into the nutrient solution significantly activated the nighttime root respiration as shown in Fig. 4. At zero time (i.e. midnight), plants were decapitated at their stem base. Means of three plants are shown with standard deviations.

that the lower transpiration stream (i.e. the lower root water absorption) in the nighttime restricts the nighttime calcium uptake to the lower levels.

The calcium uptake rate by roots was much higher in the daytime than in the nighttime (Fig. 1). This higher rate of calcium uptake in the daytime can be attributed to the higher water absorption (i.e. the higher mass flow with the transpiration stream through root tissues) and to the higher concentration of calcium in the sap (*9*). The endodermal cells in roots play a key role for the transport of water and solutes from the cortex to the stelar xylem, since the endodermal cells possess Casparian bands impermeable to water and solutes. In the Clarkson's model (*3, 4*) representing the calcium transport across the endodermis from the cortex to the stele, the mass flow with the transpiration stream in the apoplast contributes to the calcium influx into the endodermal cytosol through the calcium channels in the endodermal outer membrane and also contributes to the calcium loading to the xylem through the stelar apoplast.

Furthermore, the energy-dependent calcium pumping units in the endodermal inner membrane, which discharge Ca^{2+} from the endodermal cytosol to the stelar apoplast, contribute to the higher calcium concentration in the xylem sap against the diluting effect by the transpiration stream. This energy-dependent calcium efflux from the endodermal cell needs to be balanced with the calcium influx through Ca^{2+} channels for keeping Ca^{2+} concentration in the endodermal cytosol lower than the damaging level. Therefore, for keeping the higher rate of calcium uptake by roots found in the daytime, the higher turnover rate of calcium across the endodermal cells is required, which is considered to be supported by both of the higher water absorption and the sufficient respiration in roots as mentioned above. On the other hand, the lower rate of root calcium uptake in the nighttime suggests that the lower water absorption brings the lower calcium influx to the endodermis, which can be balanced with the energy -dependent calcium pumping supported by the nighttime lower root respiration. Thus, dependence of calcium uptake on water absorption and respiration in tomato roots in the daytime and the nighttime was demonstrated on the basis of the analysis of root exudation and the model representing the important role of the root endodermis characterized by an arrangement of the Ca^{2+} channels and the energy-dependent Ca^{2+} pumping units.

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