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Abstract: Salinity stress affects photosynthate partitioning between sources and sinks of plants, but how it affects on these systems is less understood. Because sources and sinks are closely knitted, any adverse effect under sub-optimal condition on one part is often misinterpreted for the other. Knowledge on regulation of carbon partitioning is indispensable for stress resistance and good plant growth. In the present study, alteration of carbon partitioning in tomato plants (lycopersicon esculentum L. cv. Momotarou) under saline (NaCl) environment was studied by feeding radioactive 11C and stable 13C isotopes. Pulse chases were conducted for measuring spatial and temporal distributions of 13C. 13C was measured by standard conventional technique, but 11C distribution was monitored using by PETIS. Salt stress resulted in reduced carbon translocation towards roots. Majority of the photosynthate accumulated in the leaf. We have also observed that the reduction in translocation of carbon occurred well before salt stress symptoms of reduced photosynthesis and plant growth in salt exposed plants. The effect on sink activity also showed by decrease in stem diameter and reduced photosynthetic activity. In addition, PETIS analysis of 11C translocation

indicates that carbon translocation to roots was inhibited under salt conditions without direct effect of leaf Na accumulation and osmotic stress These results suggest that NaCl has direct effects on plants inhibiting carbon partitioning within few hours of salt solution exposure without inhibition of source activity.

Highlighting

In the present study, the effect of salinity stress on vegetative growth in tomato (lycopersicon esculentum L. cv. Momotarou) plants were examined in terms of source-sink relationship.
The effect of sourse-sink relation was analyzed using conventional techniques (photosynthesis, ^{13}C transport, change in stem-diameter) and Positron-emitting radionuclide ^{11}C was also used to study transport process in plants.
Time relation of the effect for these parameters by salinity stress suggested that salinity stress adversary effect sink activity rater than source activity in plant.

Greeting

Dear Editors of Plant Science.

Please find a manuscript on "Use of positron emitting tracer imaging system for measuring the effect of salinity on temporal and spatial distribution of ^{11}C tracer and coupling between source and sink organs." for possibility of publishing in Plant Science.
We are happy to conduct this new work to see how salinity affects plant physiological activities. We have chosen tomato as the test plant in our study.
We believe that this research will generate future research activities.

Thank you very much,

Sincerely,
Ryuichi Suwa

Running head: Tracing ^{11}C and source-sink coupling under salt stress

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Abstract

Salinity stress affects photosynthate partitioning between sources and sinks of plants, but how it affects on these systems is less understood. Because sources and sinks are closely knitted, any adverse effect under sub-optimal condition on one part is often misinterpreted for the other. Knowledge on regulation of carbon partitioning is indispensable for stress resistance and good plant growth. In the present study, alteration of carbon partitioning in tomato plants (lycopersicon esculentum L. cv. Momotarou) under saline (NaCl) environment was studied by feeding radioactive ^{11}C and stable ^{13}C isotopes. Pulse chases were conducted for measuring spatial and temporal distributions of ^{13}C. ^{13}C was measured by standard conventional technique, but ^{11}C distribution was monitored using by PETIS. Salt stress resulted in reduced carbon translocation towards roots. Majority of the photosynthate accumulated in the leaf. We have also observed that the reduction in translocation of carbon occurred well before salt stress symptoms of reduced photosynthesis and plant growth in salt exposed plants. The effect on sink activity also showed by decrease in stem diameter and reduced photosynthetic activity. In addition, PETIS analysis of ^{11}C translocation indicates that carbon translocation to roots was inhibited under salt conditions without direct effect of leaf Na accumulation and osmotic stress These results suggest that NaCl has direct effects on plants inhibiting carbon partitioning within few hours of salt solution exposure without inhibition of source activity.

Keyword

NaCl, PETIS, Photosynthesis, Source-Sink, Stem-diameter, Translocation

Introduction

Salt stress and drought stress are the two major physico-chemical factors currently limit agricultural productivity worldwide. Such abiotic stresses alone cause average yield loss exceeding more than 65% of the optimal value (Boyer 1982). No rainfall for an extended period during cropping seasons causes drought and crop failure. But, irrigation-water provides no complete relief from water stress. It contains several ions including Ca^{2+}, Mg^{2+} and Na^{+}; the first two elements precipitate as carbonates, but Na^{+} is left soluble in the soil solution that leads to salinization (Jacobsen & Adams 1958; Ashraf 1994). Both drought and salinity decrease free energy potential of water and cause loss of cell turgor.Salt stress and drought stress on plant photosynthesis has been well documented.

Plants produce assimilate by photosynthesis from light, CO_2, and water. Once they are made at source, the assimilate is translocated to the sink part via phloem. Though it is well known that salt affects photosynthesis, understanding the influence of salt stress in terms of source-sink relationship is still lacking. There is a close relationship between source supply and sink demand for assimilates through the hydrostatic pressure. This was demonstrated by stable and radio isotopes of carbon such as ^{13}C and ^{14}C by plant physiologist to study long and short distance transport process in terms of source-sink relationship (Salisbury &Ross 1992, Suwa et al 2006, Fujita et al 2004). But difficulties were encountered in sample preparation and monitoring spatial and temporal distribution of these types of isotopes (Dilkes *et al*. 2004). Therefore, using only standard techniques of 13 and 14 C method have limitation to providing transport in details of short-term response. Suwa et al (2006) reported using tobacco plants at vegetative stage that sink

activity of stem, the parameter was shown as stem expanded rate, is inhibited earlier than source, the parameter was shown as photosynthetic gas exchange, activity by salt stress. However, photosynthate transport in terms of source sink relation was not studied enough. At the report did not focused on tranlocation in terms of source-sink time relation. However, Tracking photosynthate transport in details is essential to detect the effect of salt on plant biomass production in terms of source-sink relationship.

Positron-emitting radionuclide ^{11}C was also used to study transport process in plants. the advantage is that it is short enough for repeated experiments in single plant within short time scale. The combination of positron-emitting ^{11}C tracer and positron emitting tracer imaging system (PETIS) has an advantage in plant physiology research by proving non-invasive and real-time imaging of ^{11}C distribution (Uchida et al. 1998, Matsuhashi et al. 2005). That is the advantage suit for analyze short-term response in plant. Based on these information, the present study was undertaken to examine the effect of salinity on tomato plants in terms of source-sink relationship, particularly, focused on time relation between salinity effect for assimilate production and carbon translocation. Other source-sink related parameters such as photosynthetic rate, stem diameter changes were also observed.

Materials and methods

Plant material and culture

Tomato (*Lycopersicon esculentum* L. cv. Momotarou) seeds were surface sterilized and germinated on half-strength MS agar at 25°C. Ten days after germination, the seedlings were transplanted to 500 ml pots filled with soil mixture containing granite regosol, perlite and peat moss at the ratio 2:2:1. The pots were kept in the growth chamber at Graduate School of Biosphere Science, Hiroshima University, Japan. The plants were grown under artificial light irradiance of 500 μmol m^{-2} s^{-1} at 14 h light and 10 h dark durations. The temperatures of light and dark periods were 25 and 20°C, respectively. The relative humidity of the growth chamber was 70%. The plants were irrigated with nutrient solution containing 7.1 mM N (KNO_3/$Ca(NO_3)_2$), 0.48 mM P (KH_2PO_4), 2.6 mM K (KH_2PO_4/KNO_3), 0.5 mM Ca ($Ca(NO_3)_2$), 1 mM Mg ($MgSO_4$.), 54 M Fe (Fe^{3+}EDTA), 18 M Mn ($MnSO_4$), 0.15 M Zn ($ZnSO_4$), 0.16 M Cu ($CuSO_4$), 4.6 M B (H_3BO_3) and 0.1 M Mo ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$). Sixty days after germination, the plants were used in the following experiments.

Tracking Photoassimilate through ^{13}C technique

Three salinity treatments (0, 75 and 150 mM) of NaCl were administered to the seedlings for a period of 5 days. The experiment was arranged in a randomized complete block design with five replications. The schedule of the following measurements was given in Fig. 9.

Measurement of plant biomass, Na, K, and Ca

The plants were harvested at 13 and 72 h after salinity treatment. Each plant was separated into roots, stem and leaves and lyophilized for estimation of dry weight. The dried plant parts were ground to powder with a vibrating sample mill (Model T1-100, Heiko Co Ltd., Fukushima, Japan). Aliquots of the powder were wet ashed with an acid mixture (HNO_3:$HClO_4$, 4:1, v/v) and concentration of Na, K and Ca were determined by flame photometer (ANA-135, Tokyo).

Measurement of gas exchange

Photosynthetic rate, transpiration, leaf inner CO_2 concentration and stomatal conductance of the 5th expanded leaf was measured with a portable IRGA (Model LI 6400, Li-COR Co Ltd., Lincoln, USA) at intervals as shown in Fig. 9. The PAR during the measurement was above 500 μmol m^{-2} s^{-1}. All measurements were recorded for three times and differences between observations were negligible.

^{13}C feeding

$^{13}CO_2$ feeding was given to the 5th matured leaf according to the schedule given in Fig.9. The leaf was enclosed in a transparent plastic bag and 350 μL of $^{13}CO_2$ (99 ^{13}C atom %) was introduced from a cylinder. The leaf was allowed to assimilate for one hour at PAR of more than 500 μmol photon m^{-2} s^{-1}. The plants were harvested at 17 and 96 h after salinity treatment. The plant was separated into fed leaf, other leaves, stems with petioles and roots. The plant parts were lyophilized and ground to powder for measurement of

^{13}C abundance.

Determination of ^{13}C abundance

The percentage of ^{13}C atoms in the powdered plant sample was determined using a mass spectrometer (model Delta plus, Finigan company, San Jose, Ca, USA) (Suwa et al., 2006)

The excess percentage of ^{13}C atom in the sample was calculated as the difference in ^{13}C atom percentage between the $^{13}CO_2$ fed and unfed samples. The amount of labeled C in the plant samples was calculated using the following equation.

^{13}C amount in the plant part from the feeding = ^{13}C atom% excess in the sample × amount of C in the plant parts. The amount of total C in the sample was determined using the element analyzer facilitated in the mass spectrometer.

^{13}C partitioning rate (%)=^{13}C amount in the plant part / ^{13}C amount in the whole plant ×100 (%).

Measurement of stem diameter

Changes in stem diameter at 5 cm above basal end of stem (Fig. 3, 4) were continuously recorded in both control and NaCl treatments during the period of 1 day before treatment to the end of the experiment with a shrinkage type micro-displacement detector (Fujita *et al*. 2003). The sensors were connected to a computerized data acquisition system (NEC, Sanei Kogyo Co. Ltd. Tokyo). The sensors were fastened to the growing stem and connected to the power system and data logger. A glass rod in place of plant sample was the blank for the measurements and the sensitivity in measurement was within a limit of 2 μm. All

measurements were recorded three times.

Tracking Photoassimilate by PETIS

Experimental plants received the control (0 mM NaCl) or salt (150 mM NaCl) treatment. The experiment was replicated 4 times. (4 plants each Control and NaCl treated plant)

Production and feeding of $^{11}CO_2$

Both the control and saline treated plants were fed with $^{11}CO_2$ according to time schedule given in Fig. 10. $^{11}CO_2$ was produced by bombarding pure nitrogen gas with an energetic proton beam delivered from cyclotron located at Quantum Beam Science Directorate, Japan Atomic Energy Agency (See reference of Ishioka et al. (1999) for detailed information).

Approximately 50-100 MBq of $^{11}CO_2$ was collected into a CO_2 trap, and used in each PETIS imaging. The feeding of gases to the gas-cell was controlled by the $^{11}CO_2$ gas circulation system. The system consists of pumps, mass flow controller, electric valves and a CO_2 trap with freshly prepared $^{11}CO_2$. A test plant was placed in the center between a couple of opposing PETIS detectors in a chamber, so as to be correctly placed on the focal plane. Ambient air was conditioned at temperature of 25 and at a humidity level of 65 % in the chamber. They were kept in dark for 2 h before feeding. 5th matured leaf of the plant was inserted into a ‘’gas-cell’’, a clear acrylic box with inside dimensions of 12 cm in length, 8 cm in width and

1 cm in depth, for feeding gases. The gas-cell was sealed at the petiole of the leaf with plastic clay to prevent leakage of the fed gases, and connected to a $^{11}CO_2$ gas circulation system. The light intensity was measured with a light meter (model LI-189, LI-COR, Inc., Lincoln, USA) and conditioned approximately 150 μmol photon $m^{-2}s^{-1}$ at the surface of the gas-cell.

Measurement of ^{11}C translocation using PETIS

Each run of imaging using PETIS was started at the same time of the beginning of feeding A pair of γ-rays produced by annihilation of an electron and positron emitted from ^{11}C was continuously recorded by the PETIS detectors and a time series of images for spatial distribution of the radioisotope (20 second per frame for 360 frames, 2 hours in total) was generated automatically by PETIS. The same plant was subjected to ^{11}C feeding and imaging for three times in a sequence (Fig. 9).

Region Of Interest (ROI) were set on the movie data obtained and the time course of radioactivity within each ROI was extracted from the data. We selected Two ROIs along the stem on the data images (Fig 6). Upstream ROI, ROI 1, was set on the stem to which the fed leaf was attached, and ROI 2 was set on the lower internodes both Control and NaCl treatment condition. Each obtained data (Time Activity Carve (TAC)) were subjected to the quantification of ^{11}C translocation velocity.

Here, ‘’ decay-correction’’ refers to the conversion of the observed activities into values under the assumption that decay did not occur.

Calculation of transport velocities of ^{11}C photoassimilates from the PETIS data

The ^{11}C translocation velocity was evaluated through estimation of a transit time of ^{11}C for the interval between the two ROIs from these TAC. The transit time was estimated as follows.

1. An approximate line (Y=aX+b) was made for the interval of consecutive 500 sec (25 flame) from each data point on the TAC.
2. X-intercept (X_0=－b/a) was determined from the approximate line.
3. All the estimated X_0 were gathered as histogram.
4. The highest frequent X_0 was defined as the arrival time of ^{11}C-labeled photoassimilates at the ROI.
5. Difference in arrival times of ROI 1 and 2 was defined as the transit time between them.

Similarly, these differences were estimated from the other sequences of same plants.When the difference in γ-ray arrival time between ROIs on first feeding is described as 1, quotient values of those differences at 2nd and 3rd feeding experiment compared with 1st feeding are shown as indicator of ^{11}C translocation velocity in Figure 8

When the quotient value is Low, the velocity from ROI1 to ROI 2 is high.

The value were estimated using equation below

$(B_t－A_t)/(B_1－A_1)$

A: the most frequent X_0 at ROI 1

B: the most frequent X_0 at ROI 2

t: Times of feeding

Statistical analysis

ANOVAs for variable were performed using GENSTAT (Williams & Matheson 1994). The significant difference between the means were tested by Least Significant Difference at 0.05 level.

RESULTS

Plant growth and concentration of minerals

The salt treatment for 72 h decreased dry mass of the plants (Fig. 1).

K concentrations of all plant parts were lower except in the leaf at 75 mM NaCl treatment. The concentrations of Ca and Na increased in the plant parts under saline treatment compared to the control (Table 1).

Photosynthesis

A decline in leaf photosynthetic and transpiration rate was observed by saline treatment of the plants (Fig. 2). The decline trend was more evident in the 150 mM NaCl compared to the 75mM treatment.

Photosynthetic rate was not affected at 7 h, but reduced to half at 72 h after 150 mM NaCl treatment.

Change in stem diameters

NaCl application, which started in the daytime, immediately affected the stem diameter and the inhibitive effect on stem expansion continued to increase until the end of the experiment (Fig. 3). However, NaCl treatment could not change the pattern of stem diameter dynamics; the diameter usually shrunk during the daytime and recovered during the night.

Stem diameter expansion also strongly inhibited after NaCl treatment (Fig 4).

^{13}C translocation

The translocation of ^{13}C assimilates was affected by the salinity treatments significantly.

Salinity treatment tended to inhibit the ^{13}C translocation from the fed leaf to other parts at the first feeding (Fig. 5A). As time progressed, the fed leaf accumulated more ^{13}C and distribution to roots in particular was significantly depressed (Fig. 5B).

Analysis of ^{11}C translocation using PETIS

Figure 6(a) gives example of the test plants and serial PETIS images after $^{11}CO_2$ feeding; these images were generated from integration of the corresponding images collected every 20 s. The advantage of PETIS measurement is that it enables the visualization of translocation in the intact plant. The scan sequence of

these serial images consisted of twelve 10 min.

It is evident that ^{11}C exported to petiole and stem part from ^{11}C fed leaf according to time sequence.

Figure 6(b) shows the PETIS image of Plant1. This image represents the integration of all the frames of the captured moving image. It demonstrates that ^{11}C-photoassimilates were transported via the petiole and stem toward the roots. Similar images were obtained with the other individual and when NaCl was fed (not shown). Time-Activity curves (TAC) in ROI 1 and ROI 2 of plant 1 at 1st feeding of control plant are shown in Fig 7.

Translocation velocity was shown in figure 8. The value was estimated using all acquired TAC. Detail is described in materials and methods. It was clearly noticed that salinity suppressed ^{11}C transport velocity towards the basal part of stem after its administration. The suppression was more evident after 5h compared with 2h after salt treatment.

Discussion

In present study, the relationship between source supply and sink activity of tomato plant under the influence of salinity was efficiently analyzed using standard conventional techniques and PETIS.

Salinity stress reduced photosynthetic rate (Fig 2). It was evident in our study that growth of tomato plant was inhibited by salinity (Fig. 1) support our earier findings (Suwa et al., 2006).

In terms of long term response of salinity to plant growth, This is clearly due to the effect of the stress on leaf photosynthesis. Although salinity did not alter photosynthesis within the first 7 h of the treatment,

1 significant reduction of photosynthesis occurred 24 h after the imposition of salinity stress and the impact
2 continued thereafter (Fig. 2). Previous study reported NaCl affected leaf photosynthesis, because it's
3 adverse impact on stomatal conductance and carboxylation capacity (Everard *et al*. 1994). In contrast to
4 photosynthesis, the effect of the stem diameter was quick and instantaneous. Salinity decreased stem
5 diameter immediately after exposure to salt and the magnitude of the impact increased divergence of the
6 curves depicting diameters for control and saline treated plants (Fig. 3). The results of the current study are
7 similar to the stress studies on Japanese persimmon and tobacco (Fujita *et al*. 2003; Suwa *et al*. 2006); salt
8 stress reduced photosynthesis, transpiration, water potential and stomatal conductance and increased Na
9 concentration in the plant parts. It is accepted that, low water potential and poor transpiration under saline
10 environment are responsible for the reduction in source activity. The present study showed that the stem
11 diameter is composed of two components, a ''reversible component" and an ''irreversible component"
12 (Sevanto et al. 2002). The reversible component" is a layer of extensible tissues surrounding the xylem that
13 comprises matured cells with rigid cell walls (Brough et al. 1986; Genard et al. 2001; Klepper 1971; Molz
14 et al. 1973). The results of the present study are consistent with the findings that the stem diameter shrinks
15 during the day because of the loss of water in transpiration and swells at night owing to the uptake and
16 storage of water (Hinekley et al. 1975). The current results also showed that salinity promoted stem
17 shrinkage during the daytime, probably because of a reduction in the water supply (Fujita et al 2003.).
18 In contrast, the ''irreversible component" of the stem may be related to stem growth, as observed by the
19 daily increase in stem diameter. Suwa et al (2006) reported that water potential was recovered at night time.

The potential saturated during the condition. Therefore difference of change in stem diameter at nighttime was treated as irreversible component, what is called sink growth.

In the present experiment, salinity treatment decreased stem growth. These results clearly indicate that the increase in the sink activity was suppressed by the salt stress before source activity reduction.

^{13}C fed to the leaf at this time demonstrated clearly discrimination in partitioning against the roots (Fig. 5A). This discrimination in partitioning was more evident at the second feeding (Fig. 5B); percentage of ^{13}C going into roots was reduced to half in the saline treated plants compared to the control.

It is said that under normal circumstances, flow resistance of the transport pathway does not exert any control over sink growth (Gifford & Evans 1981; Minchin *et al*. 1993) and the roots do functions near to saturation (Farrar & Williams 1990). However under perturbed situation, the flow resistance in the translocation pathway may lead to accumulation of assimilates in the leaf. There is possibility of a stress related stimuli occurring through alteration of sugars or phyto-hormone compositions of the phloem solution providing a signal to the source (Roitisch, 1999) for decrease of its activity.

To unravel the mechanism of salinity stress on phloem transport, positron-emitting isotope ^{11}C was used and pulse chase was monitored using PETIS. In order to prevent direct_influence of water stress under saline condition from transpiration, feeding of isotope and monitoring were done under the cover of darkness. Mainly Na is translocated from root to upper part via xylem. Under dark condition, Na related inhibition for leaf loading may excluded. Na concentration among the plant parts after NaCl treatment at

dark condition was not altered by the treatment (data not shown).

It was clearly noticed that salinity suppressed ^{11}C transport towards the basal part of stem within 2h of the salt treatment (Fig. 8). It was observed that the nighttime recovery of stem diameter of plant parts was marginally affected by salinity (Fig. 4), suggesting minimal effect of stress on plant water relations. And stem diameter expansion also strongly inhibited after NaCl treatment. When there was no osmotic stress and Na accumulation at source part in plant by transpiration under dark condition, the inhibition of ^{11}C translocation rate (Fig. 8), was solely due to NaCl toxicity for sink activity.

In case of plant growth reduction by salinity stress, there is possibility that perturbation of source sink relationship also exist as a parallel with other well known salinity induced growth inhibition mechanisms.

Minchin *et al* (2002) provided evidence for source-sink coupling through phloem loading of carbohydrates and a similar mechanism might have worked in our experiment. It might also be possible that a change in sugar composition in the sink end of the phloem might have affected assimilate flow from the source (Ayre *et al*. 2003).

Feed back inhibition of photosynthesis as a result of decreased sink demand is well-known phenomenon (Roitsch 1999)

This data suggested that depression of photosynthetic activity of the leaf occurred after the alteration of ^{13}C partitioning to roots. Similarly, it was observed that defoliation in wheat plant did not reduce photosynthesis, but reduced partitioning of ^{14}C to roots (Dilkes *et al*. 2004).

Our results were the first of its kind to distinguish the effects of osmotic stress and ion toxicity under saline environment in the translocation of carbon assimilates. In soybean plant NaCl toxicity was observed to induce permanent reduction in transport of ^{11}C-assimilates to roots (Wieneke & Fritz 1985; Fritz *et al*. 1987).

These evidences are in parallel observation on Japanese persimmon and tobacco under salt stress (Fujita et al. 2003; Suwa et al. 2006) suggest that NaCl toxicity directly hinders carbon translocation in the phloem. Moreover, the adverse effect of salinity on ^{11}C partitioning to roots due to modification of sink activity precedes the negative impact on source activity. Together current results and information, it can be concluded that salinity toxicity affects sink activities at earlier time and more severely than source activities in tomato plant.

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(mg g^{-1}DW)

Element	Parts	NaCl treatment (mM)		
		0	75	150
K	Leaf	8.3 ± 0.3	8.4 ± 0.2	7.2 ± 0.4
	Stem	52.2 ± 1.0	47.1 ± 1.1	47.9 ± 1.5
	Root	28.6 ± 0.3	23.6 ± 0.8	24.7 ± 0.8
Na	Leaf	0.4 ± 0.1	0.6 ± 0.1	0.8 ± 0.1
	Stem	1.8 ± 0.4	3.1 ± 0.3	4.4 ± 0.4
	Root	5.4 ± 0.4	7.8 ± 0.3	9.4 ± 0.6
Ca	Leaf	8.6 ± 0.3	10.1 ± 0.4	10.4 ± 0.6
	Stem	12.3 ± 1.1	14.5 ± 0.8	16.1 ± 1.3
	Root	7.1 ± 0.4	9.2 ± 0.3	9.7 ± 0.4

FIGURE CAPTIONS AND LEGENDS

Figure 1. Effects of salinity on dry weight of tomato plants. Salinity treatment was applied to 60 days old plants by adding NaCl to irrigative solution. Values are mean and stand deviation of 5 replicates.

Figure 2. Effects of salinity on photosynthetic (A) and transpiration rates (B) in tomato plants. Salinity treatment was applied to 60 days old plants by adding NaCl to irrigative solution. Values are mean ± stand deviation of 5 replicates.

Figure 3. Effects of salinity on stem diameter and circadian rhythm of stem diameter dynamics in tomato. The plants were kept in growth chamber and light was switched on and off at 6 am and 8 pm, respectively. The salinity treatment was applied to 60 days old plants by adding NaCl to irrigative solution. Changes in stem diameter were continuously recorded with a shrinkage type micro-displacement detector in three control and three salt-treated plants. The changes in stem diameter of different plants treated with the same treatment occur in a similar pattern. The figure shows the change in a representative plant from each treatment.

Figure 4. The shrinkage of stem diameter caused by salinity stress under dark condition in tomato. The

plants were grown in growth chamber for 60 days. The light was switched off four hours before the application of treatment solutions. Changes in diameter of stems (5 cm above the base) were continuously recorded with a shrinkage type micro-displacement detector in three control (0 mM NaCl) and three salt-treated (150 mM NaCl) plants. The shrinkage of stem in response to the application of NaCl occurred in a similar pattern for every plant, and the data from a representative one were shown in the figure.

Figure 5. Effects of salinity on ^{13}C partitioning to different plant parts in tomato. Salinity treatment was applied to 60 days old plants by adding NaCl to irrigative solution. $^{13}CO_2$ feeding was given to the 5^{th} matured leaf at 6 (A) and 72 h (B) after salinity treatment. Plant samples were collected at 17 and 96 hours after salinity treatment for the first and second feeding, respectively. Values are means of 5 replicates. The letters present the comparison (by lsd 0.05) between the treatments within the same plant part; values with the same letter are not significantly different.

Figure 6 (a) and (b). (a) Serial PETIS images of feeding after 11CO2 exposure. The scan sequence was 10-min scans. Images are arranged sequentially from left to right and top to bottom. All the images were corrected for the ^{11}C radioactive decay. (b) The image made by integrated γ-ray signal during the translocation of ^{11}C in the phloem of tomato. Sixty days old tomato plants were treated with 0 or 150 mM NaCl. ^{11}C was fed to the 5th leaf under a light source of 150 mmol m^{-2} s^{-1}, and then the plants were kept in

dark for 2 h. γ-ray signal was monitored by PETIS. The circles define the regions of interest (ROI), in which the data from two region (ROI 1-2) have been use to calculate translocation rates.

Figure 7. Typical γ-ray activity of region of interest at 1st feeding of control plant.
ROI 1 indicate upper side of the stem. ROI 2 indicate lower side of stem.
All corrected value was used to histogram analysis. That was described in result.

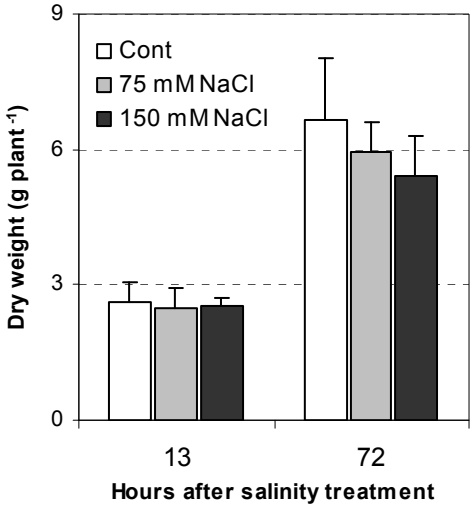
Figure 8. Effects of salinity on the relative translocation rate of ^{11}C in tomato. ^{11}C feedings were carried out sequentially three times in the same plant. At the first feeding, both control and salt-treated plants were irrigated with solution without NaCl. At the second and third feeding the salt-treated plants were irrigated with solution containing 150 mM NaCl. ^{11}C was fed to the 5th leaf for 2 min under a light source of 150 □mol m^{-2} s^{-1}, and then the plants were kept in dark. γ-ray emitted for ^{11}C was continuously recorded by PETIS for 2 hours (360 frames) at each time of feeding. The calculation of relative translocation rate was described in Materials and Methods. Values in the figure are mean and standard error of four plants.

Figure 9. Time schedule of Experiment 1. Sixty days old seedlings of tomato were exposed to 0, 75 and 150 mM NaCl for 96 h. The circles indicate the time when an event was carried out. The measurement of stem diameter was started 24 h before the application of NaCl and maintained until the end of experiment

period.

Figure 10. Time schedule of experiment 2. Sixty days old tomato plants were exposed to 0 or 150 mM NaCl under dark condition. ^{11}C was fed to the 5th leaf for 2 min under a light source of 150 □mol m^{-2} s^{-1}, and then the plants were kept in dark. γ-ray emitted for ^{11}C was continuously recorded by PETIS for 2 hours (360 frames) at each time of feeding.

Figures



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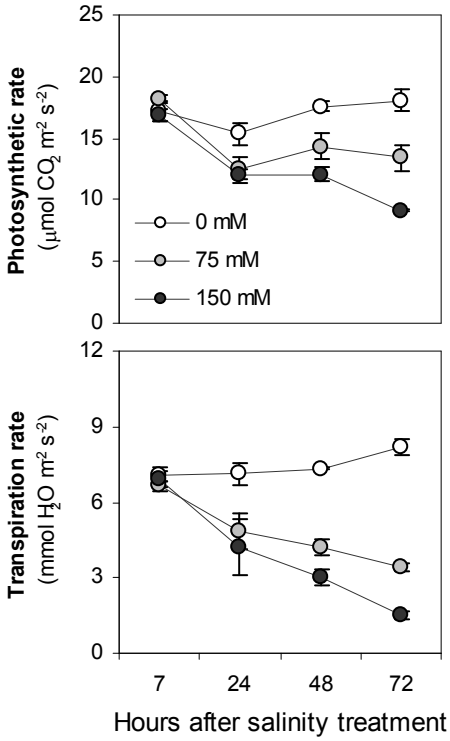

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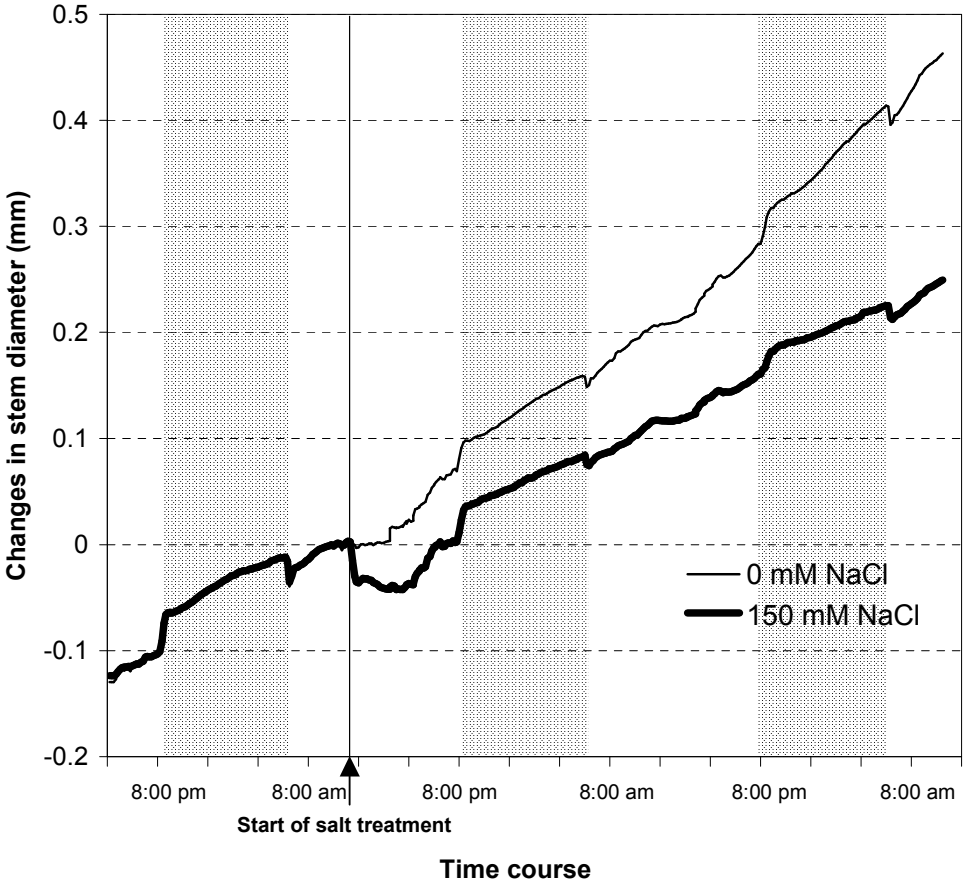

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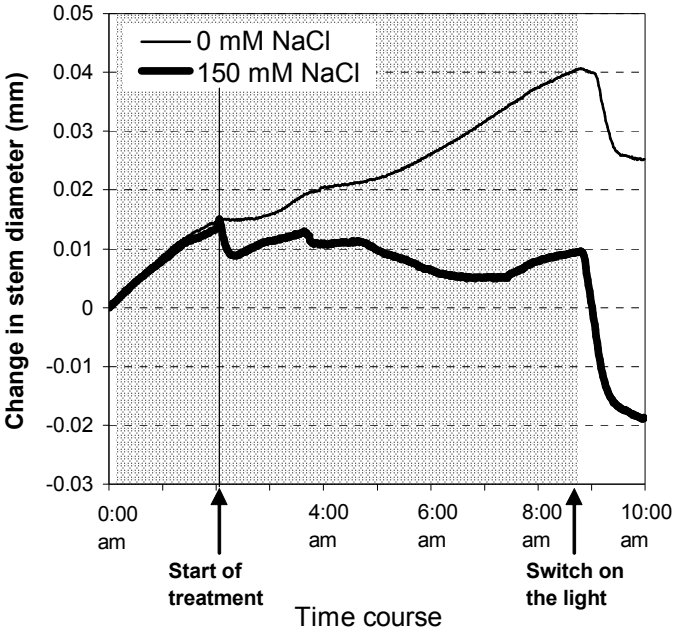

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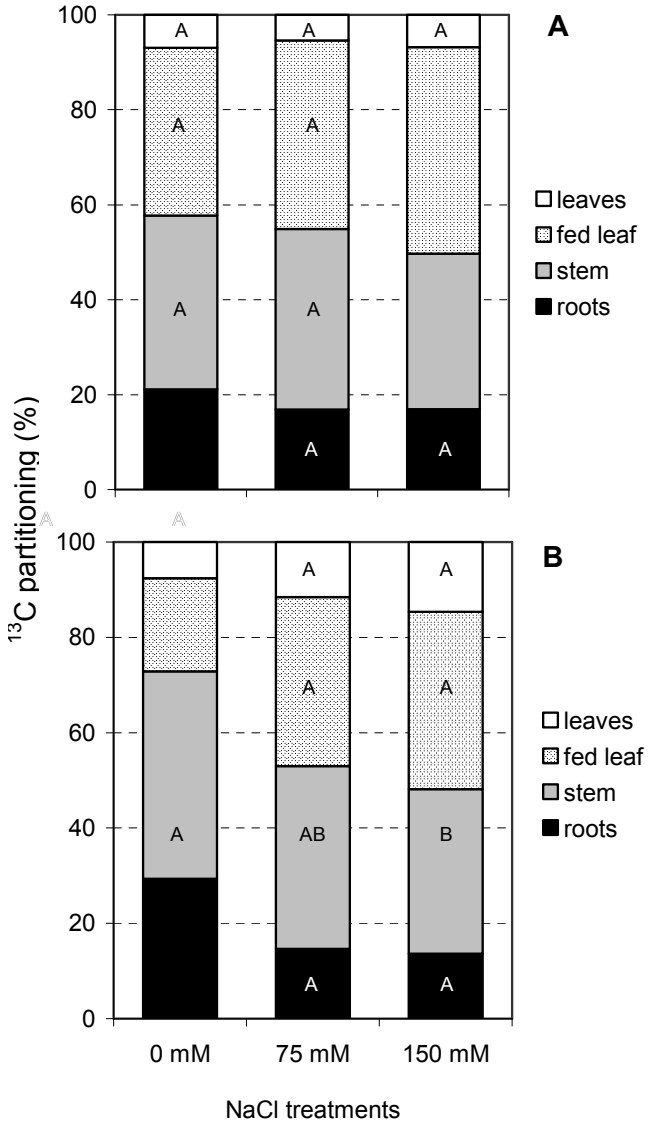

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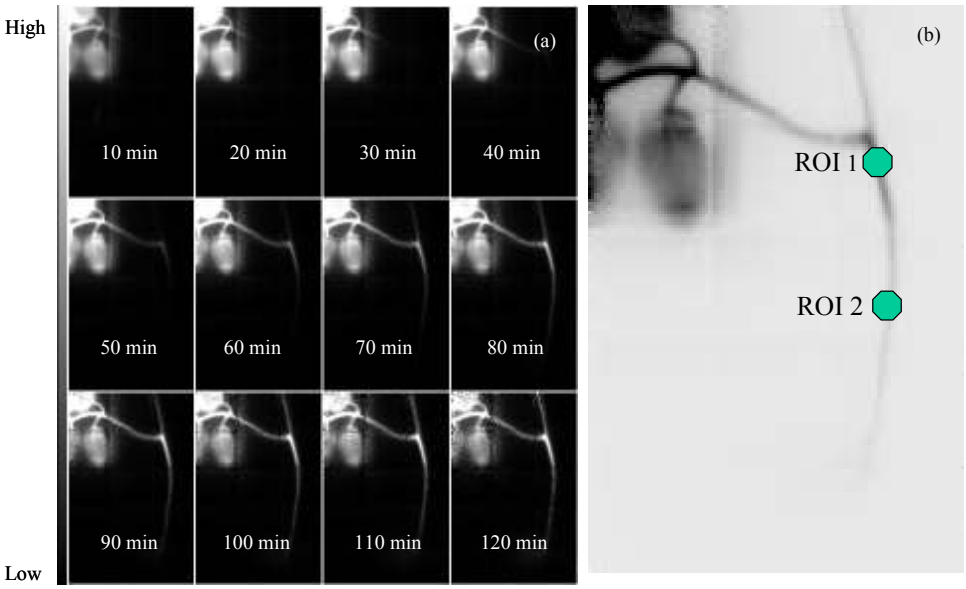

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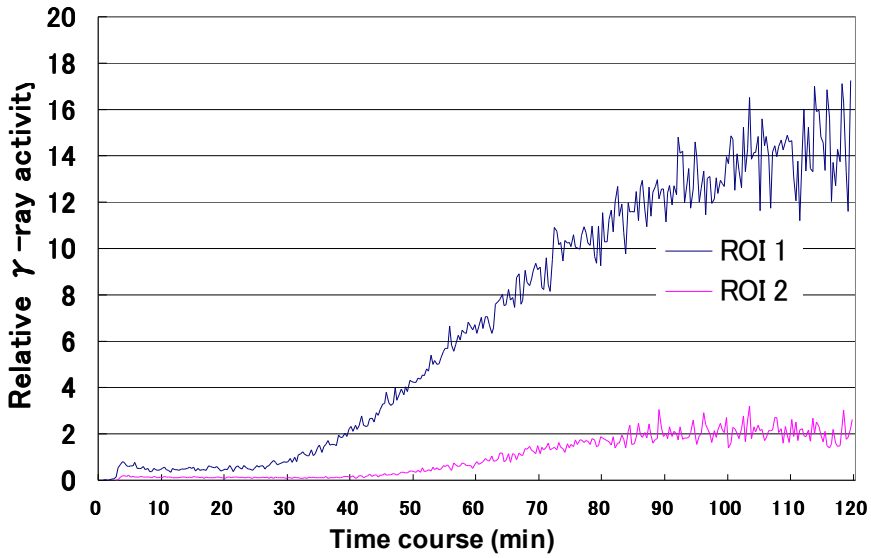

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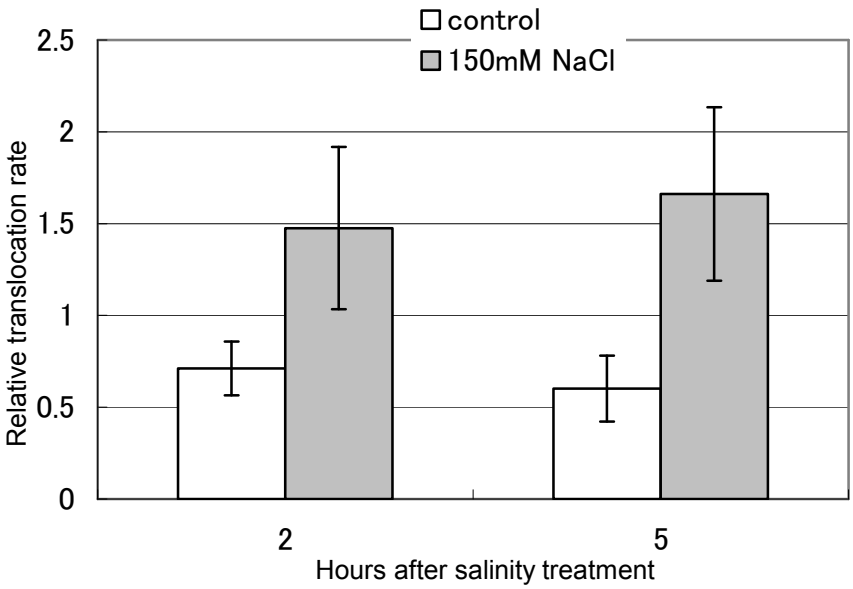

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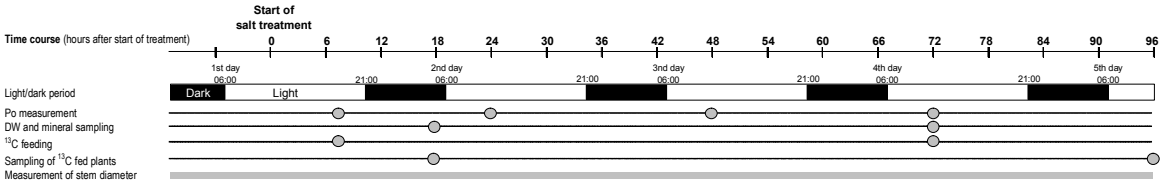
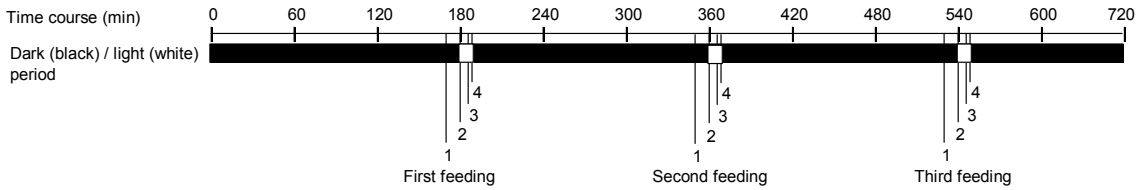


Figure 9. Time schedule of experiment 1
Sixty days old seedlings of tomato were exposed to 0, 75 and 150 mM NaCl for 96 h. The cirles indicate the time when an event was carried out. The measurement of stem diameter was started 24 h before the application of NaCl and maintained until the end of experiment period



1: Treatment solutions were applied 15 min before each feeding. At the first feeding, the control treatment solution (0 mM NaCl) was applied to both control and salt-treated plants. At the second and third feeding 150 mM NaCl was applied to salt-treated plants

2: Switch on the light (150 µmol m^{-2} s^{-1}) 5 min before each feeding

3: Start ^{11}C feeding and PETIS monitoring. The ^{11}C feeding and mornitoring lasted 2 min and 2 hours, respectively

4: Stop ^{11}C feeding and and witch off the light

Figure 11. Time schedule of experiment 2.
Sixty days old tomato plants were exposed to 0 or 150 mM NaCl mainly under dark condition. 11C was fed to 5th leaf and its stranlocation was mornitor by PETIS system

Table 1

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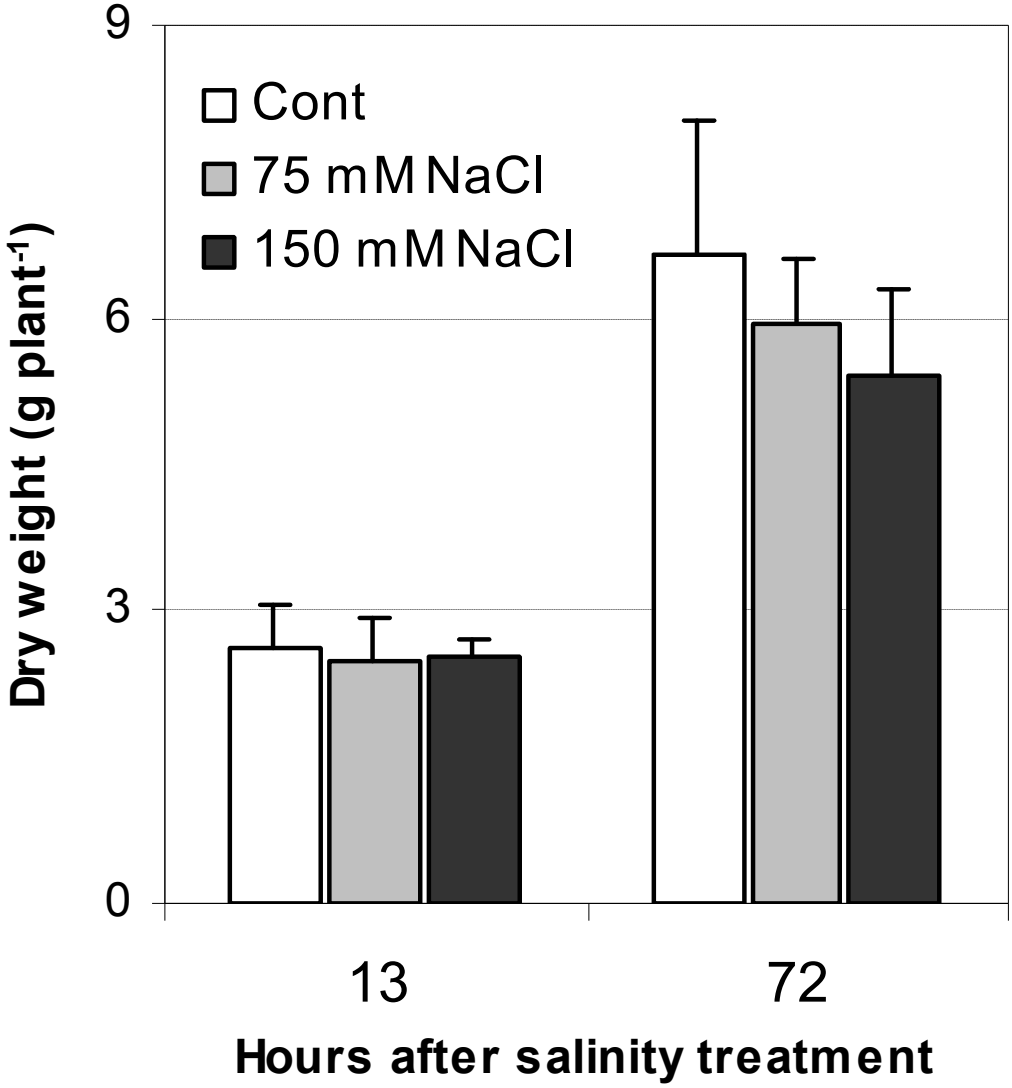

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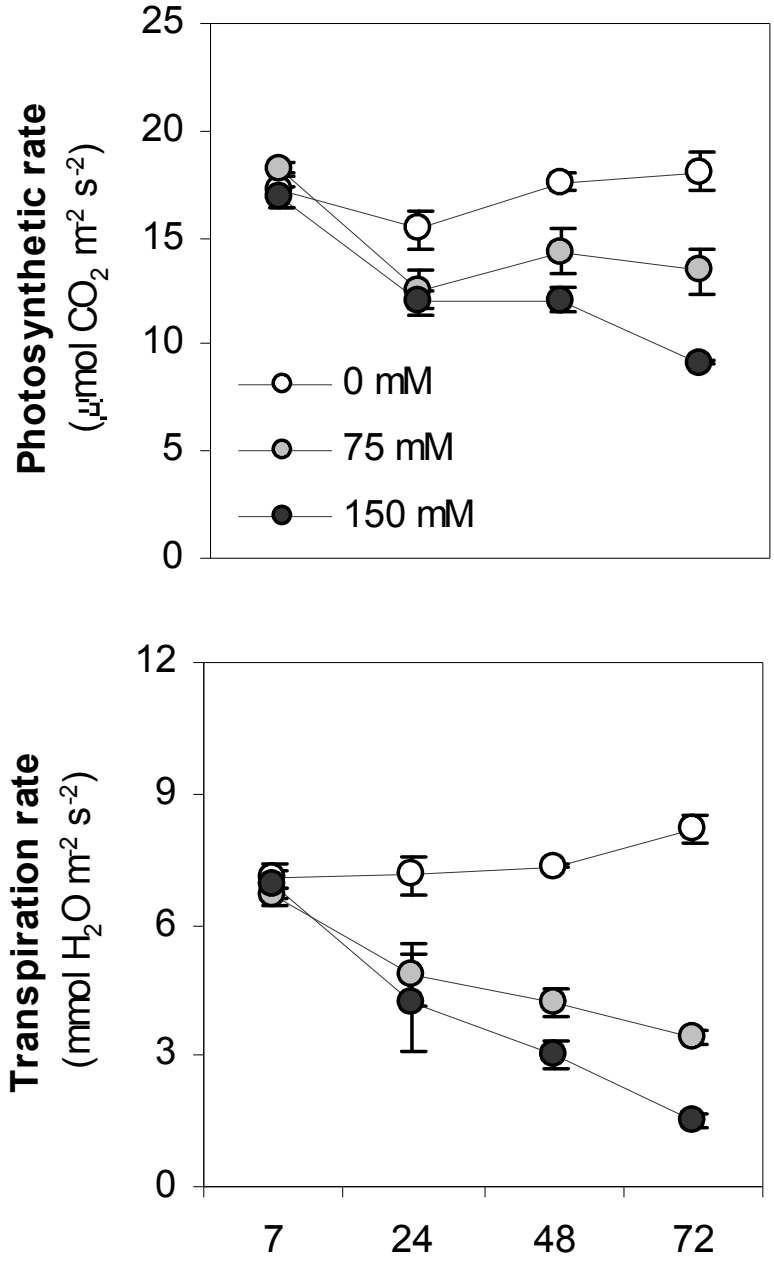

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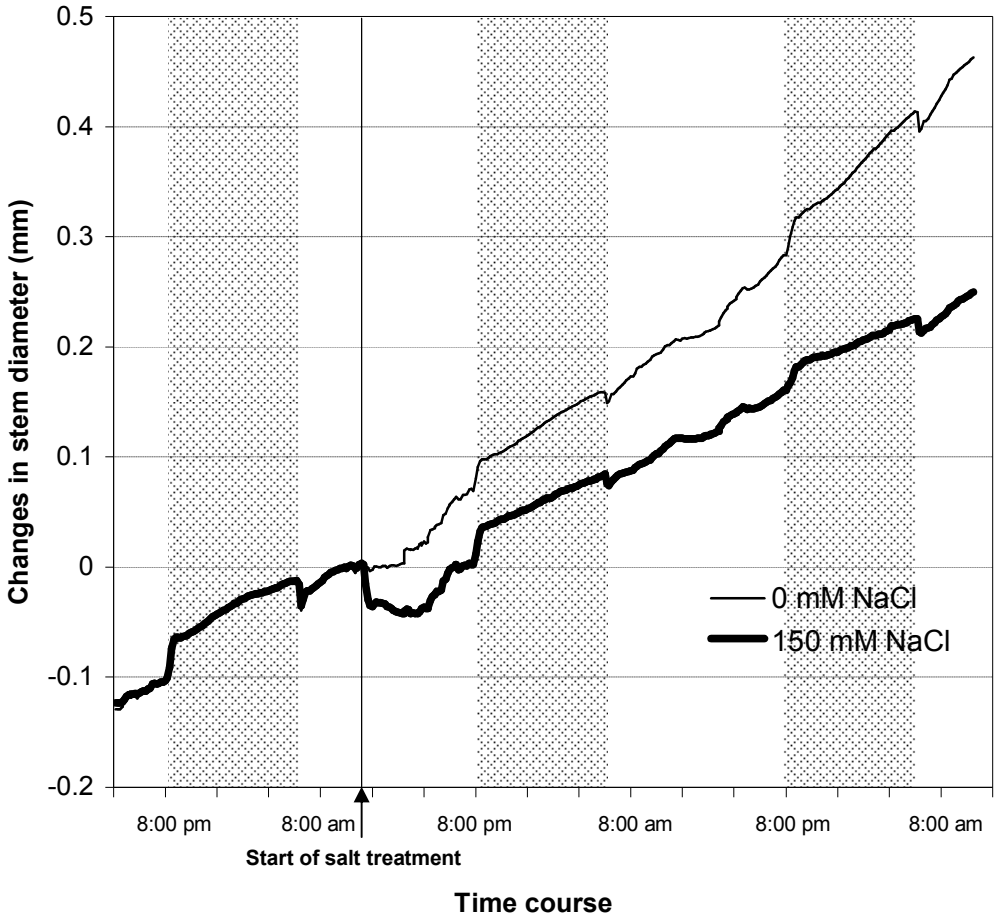

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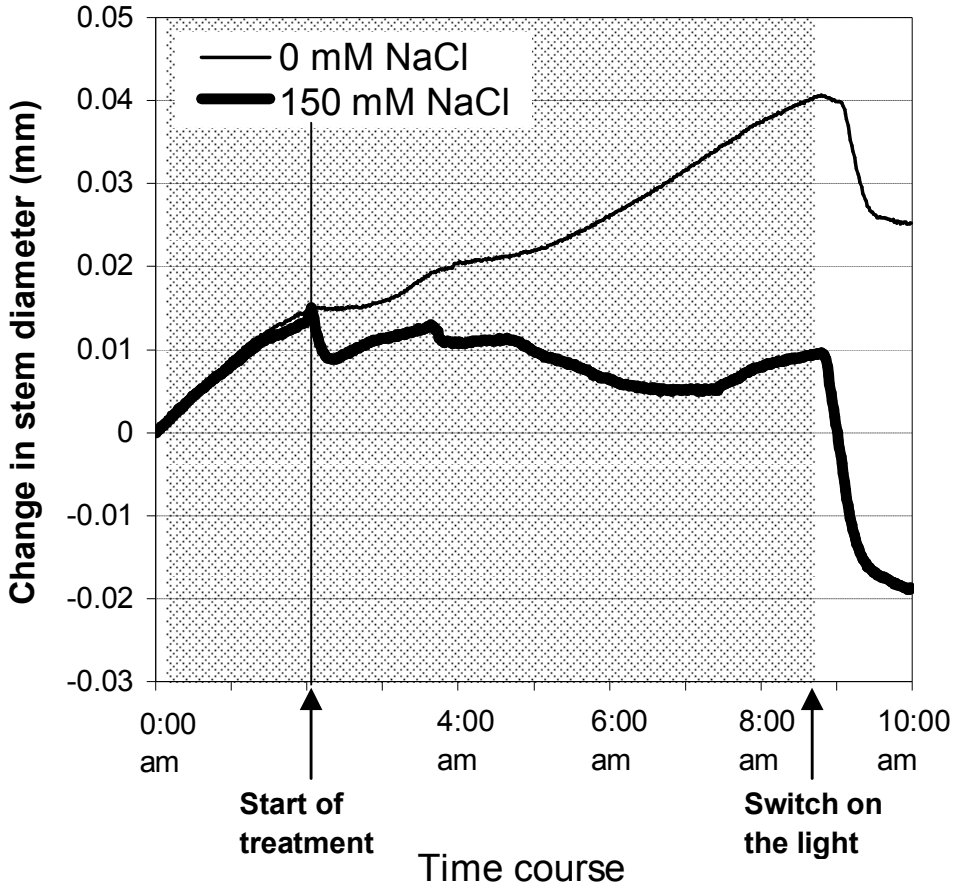

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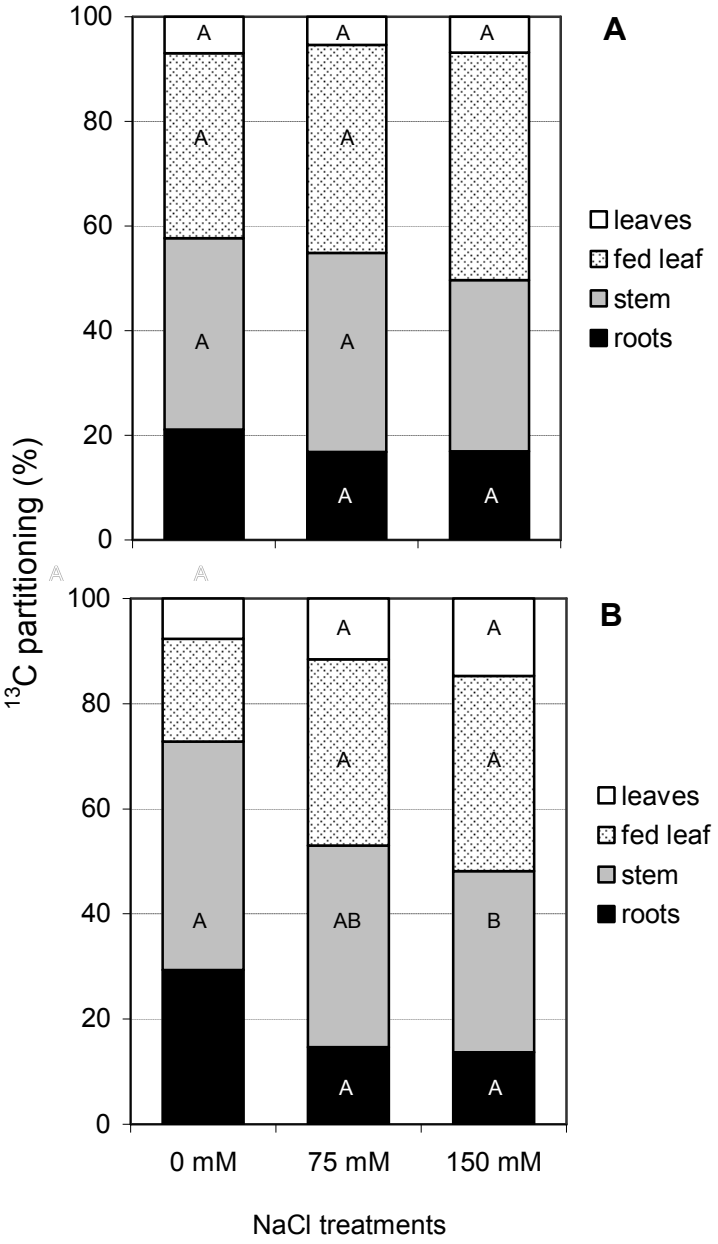

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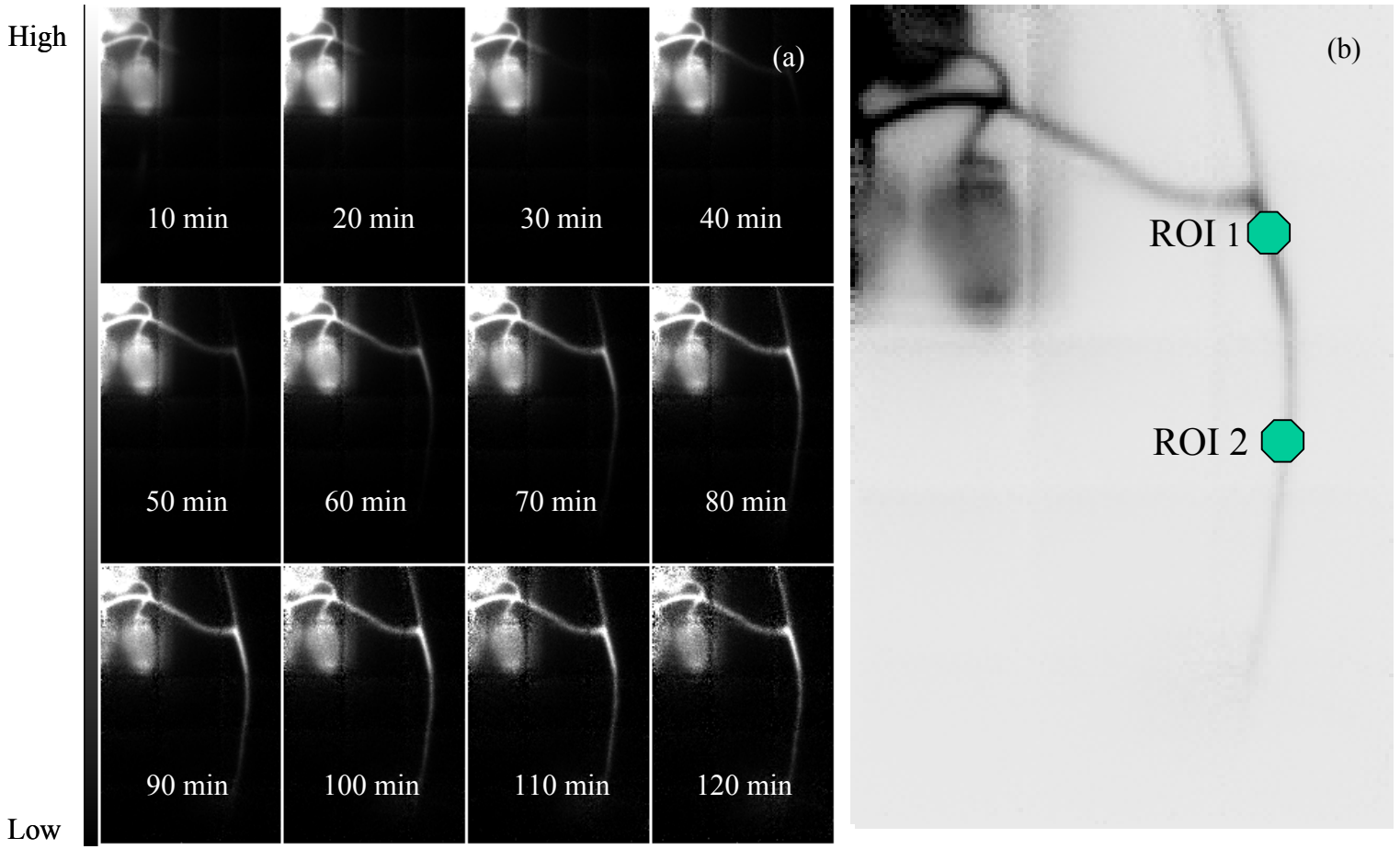

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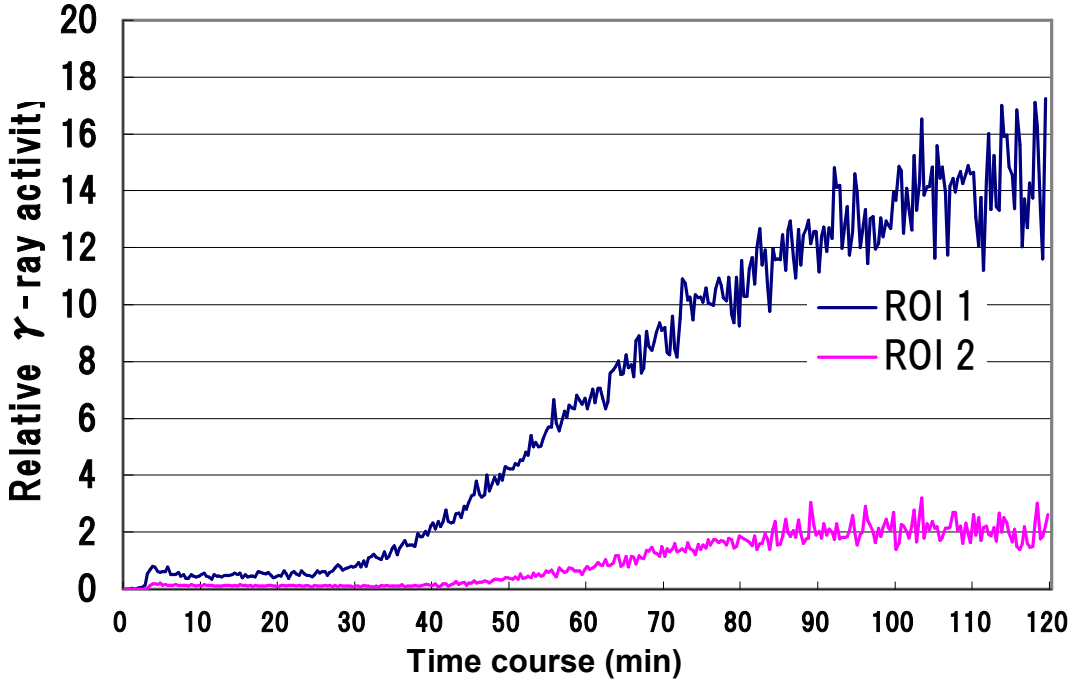

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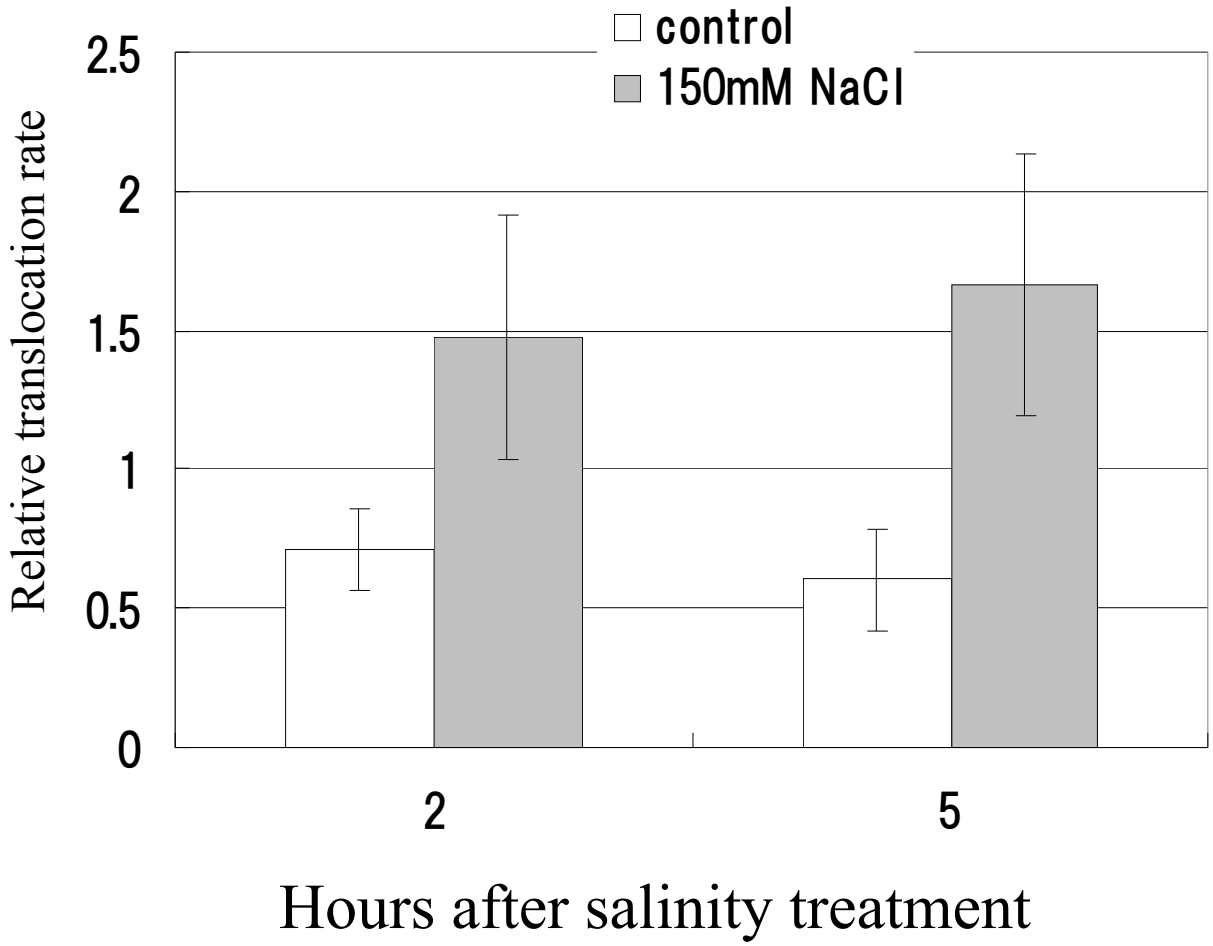

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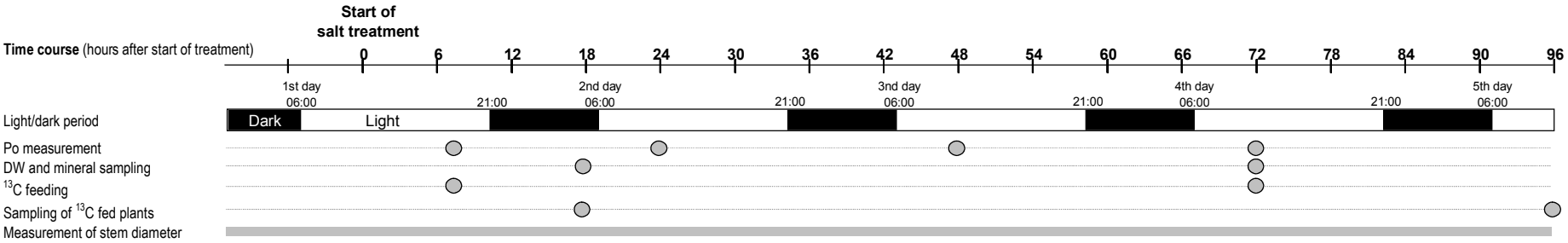

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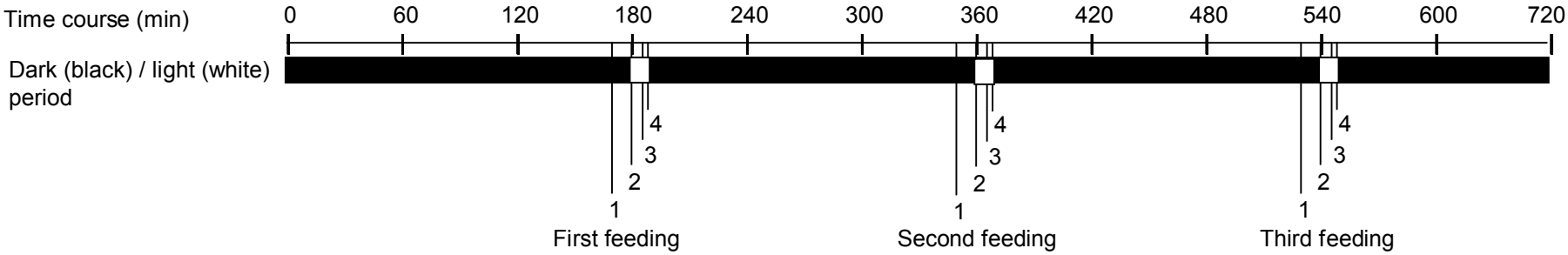

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