Original Paper Plant Biotechnology, **21** (2), 95-101 (2004)

Rapid Down and Upward Translocation of Salicylic Acid in Tobacco Plants

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Received 30 September 2003; accepted 14 November 2003

Abstract

Salicylic acid (SA) is a proposed signal for systemic acquired resistance to pathogen infection, while precise mode of SA translocation is little understood. To study it directly, ^{14}C-SA was fed to the petiole end and the signal was detected autoradiographically. In juvenile plants, the signal moved to the lower stem and root, then the vascular systems of upper, middle and lower leaves within 1 h. In adult plants with 13 developed leaves, it was detected in nodes of 6 upper and 3 lower leaves at 10 min, and all leaves at 1 h along the orthostichy of phyllotaxis after feeding from the 7th leaf. The signal dispersed to the leaf gap, cortex parenchyma, and epidermis. One and 7% of radioactivity recovered was detected as SA β-glucoside at 10 min and 1 h, respectively. Thus, SA can move rapidly at all nodes of plants as an emergency signal within 1 h.

Key words: epidermis, phloem, salicylic acid, translocation, tobacco plant.

Abbreviations

SA, salicylic acid; SAG, SA β-glucoside; TLC, thinlayer chromatography;
SAR, systemic acquired resistance.

Introduction

SA (salicylic acid) has been proposed as an endogenous induced signal in the resistance to pathogen infection in tobacco (Malamy *et al.*, 1990) and cucumber (Metraux *et al.*, 1990). Subsequent studies such as Yalpani *et al.* (1991) and Malamy and Klessig (1992) confirmed the importance of SA as the systemic signal using tobacco mosaic virus-infected tobacco plants. Known studies of SA translocation to upper leaves from lower leaves were examined using pathogen-infected plants; the translocation of SA to upper non-infected leaves from pathogen-infected lower leaves was studied 3 to 7 days after inoculation. The amount of accumulated SA is dynamically changing in pathogen infected lower leaves, then precise mode of SA translocation, especially the speed and mode of the movement throughout the plant would be difficult to elucidate from these experiments.

SA is thought to move to upper non-infected leaves from lower pathogen-infected leaves through phloem (Metraux *et al.*, 1990; Rasmussen *et al.*, 1991; Yalpani *et al.*, 1991). Transportation via phloem in veins, petiole, stems and roots makes up the major part of the phloem stretch. Nevertheless, the transport trajectory has gained a much less distinct profile than the phloem zones involved in loading and unloading in sources and sinks (van Bel, 2003). The sieve tubes are said to be essentially leaky, and the solute and solvent are lost and retrieved along the sieve tube from sources to terminal sinks such as root/shoot tips (Ayre *et al.*, 2003). Actually, 6% of photo-assimilates was reportedly lost, and 3-4% was retrieved every centimeter along the phloem pathway in bean plants (Minchin and Thorpe, 1987). However, translocation in phloem or leakage from phloem depends on both the characteristics of individual solute and the transporters as reported for sucrose transporters (Stadler *et al.*, 1995) and sulfate transporters (Yoshimoto *et al.*, 2003) in the plasma membrane of sieve elements and companion cells. These results suggest that the nature of the transportation of a solute should be studied in each case.

Thus we studied SA movement autoradiographically supplying ^{14}C-SA from the cut petiole and stem ends. To obtain clear results, early SA-translocation within 1 h after feeding was analyzed to minimize the possibility of degradation or con-

version of the SA to other substances after long incubation periods. We report here the mode of SA translocation throughout the plant after the feeding of ^{14}C-SA, showing that the majority of SA moved as free SA to all nodes of adult plants within 10 min to 1 h.

Materials and Methods

Plant materials and application of SA

Tobacco plants (*Nicotiana tabacum* cv. Samsun NN) were grown in a green house at 25℃. Tobacco seedlings used here were 3 weeks old, 15-30 mm in height, and 0.17-0.28 g-fresh weight per plant. For experiments, roots of plants were gently washed with tap water, and put into water in a vessel. An aliquot of SA solution was placed on the cut surface of stem or petiole, and an aliquot of water was successively added thereafter without a pause in the water supply. To prepare cross sections, a block of stem or petiole was kept on ice, and transversely cut to 200 μm in thickness using a hand microslicer (Cat. No. G-6263, NM type, Nakamura, Japan).

Dryness and autoradiography of plant materials

At 10, 20 or 60 min after SA application, the whole seedlings or cross sections of stem or petiole were quickly put between paper towels and pressed at 80℃ for complete dryness. The dried plant was exposed to X-ray film for the period indicated in the legend of the figures.

Extraction of SA and SAG

Stem segments were cut out at 10 min or 1 h after feeding, and kept until use at -80℃. Each segment was homogenized in liquid nitrogen in a mortar by hand, and extracted with 3 volumes of 90% methanol. An aliquot containing about 700 dpm was subjected to TLC for the separation of SAG from SA with authentic ^{14}C-SA and synthesized SAG as the standards (Edwards, 1994), and radioactivity of the spots corresponded to SA and SAG were detected by BAS 2000 image scanner (Fuji Film, Japan).

Results

Rapid systemic movement of SA in both lower and upper directions through vascular systems in tobacco seedlings

At first, the systemic translocation of SA within 1 h after ^{14}C-SA feeding was studied using whole tobacco plants at a juvenile stage. One microliter of SA solution (60 pmoles SA containing 1 kBq ^{14}C-SA) was fed to the cut end of stem or petiole of a 3 week-old tobacco seedling (4-5 leaf stage, 0.2-0.3 g fresh weight per plant), and water was successively added without interruption. The translocation of ^{32}P-phosphate supplied from a leaf, is known to be carried out through phloem (Buchanan *et al.*, 2000). Previous reports such as Yalpani *et al.* (1991) suggested SA may move via phloem. Thus we could expect SA-movement via phloem in this system. After 10, 20 or 60 min, the plants were dried and exposed to X-ray film. When ^{14}C-SA was supplied to the cut petiole end of the 3rd leaf as indicated by arrowheads in plants 1, 4, and 7 in **Fig. 1A,** radioactive signal was found in the lower stem and root after 10 min (plant 1 in **Fig. 1B**), extending gradually to the main vascular system of the 2nd and 4th leaves after 20 min(4) and 60 min(7). When supplied from cut petiole of the 2nd leaf (2, 5, 8), movement of the signal after 10 min(2) was likely slower than that in 1, but similar after 60 min(5, 8). Feeding from the stem cut end at the lowest position (3, 6, 9), in this case xylem is the expected route of solute translocation, resulted in movement firstly to the vascular system of the 3rd leaf (source leaf on photosynthesis) after 10 min (3), to that of the 4th after 20 min(6), and then to that of all younger sink

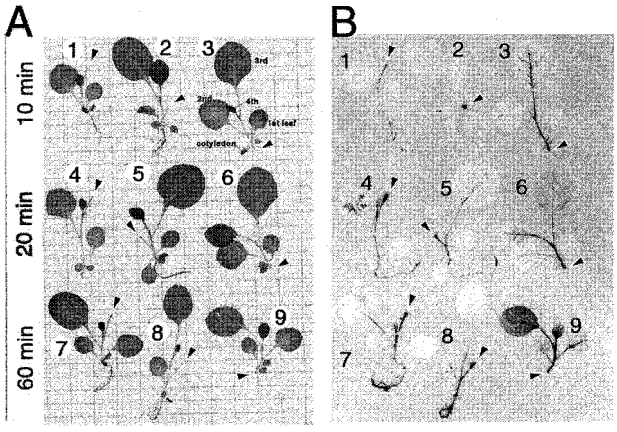


Fig. 1 Rapid SA-movement to both upper and lower directions within 10 min in tobacco seedlings.

One microliter of SA solution (1 kBq ^{14}C-SA and 60 pmoles SA) was put on the cut surface of stem or petiole of a 3 week-old tobacco seedling (ca. 0.2-0.3g fresh weight per plant), and water was successively added thereafter without interruption. After 10, 20 or 60 min, the plant was put between paper towels and held at 80℃ pressing for complete dryness. The dried plant was exposed to X-ray film. A: Photograph of dried plants. B: Autoradiogram of these plants. 1-3: 10 min, 4-6: 20 min, 7-9: 60 min after feeding. Arrowheads indicate the cut region from which SA solution was added. Exposure time to X-ray film was 12 days.

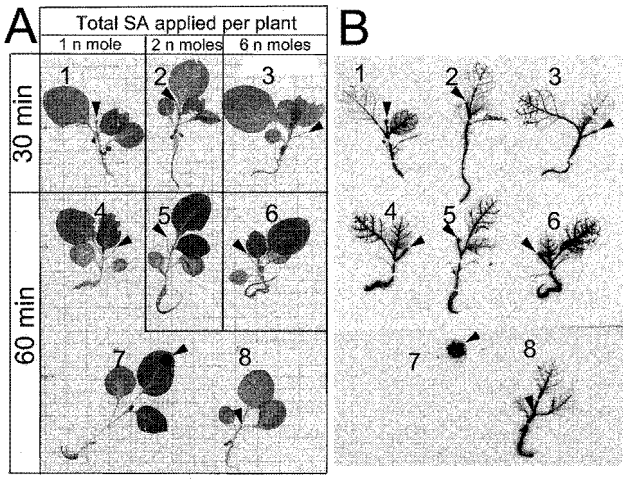


Fig. 2 SA-translocation in tobacco seedlings mainly through vascular system but not from leaf surface.

Two microliter of SA solution containing 3.7 kBq ^{14}C-SA and 1, 2 or 6 n moles cold SA, respectively, was put on cut petiole end of the 3rd leaf (1-6), on cut stem end just under apical meristem (8), and on leaf surface of the 3rd leaf (7). Plants were exhibited to dryness after 30 min (1-3) and 60 min (4-8). A: Photograph of dried plants. B: Autoradiogram of these plants. Arrowhead indicates the region at which the SA solution was applied except for plant 7, where it was applied in the region shown by a closed circle. Exposure time to X-ray film was 14 days.

leaves including the leaf blade after 60 min(9). As the net amount of ^{14}C-SA was the same in this experiment, these results indicate that the translocation of SA was likely via phloem when supplied from the cut petiole end, and via xylem when supplied from the cut stem end at the lowest position. The translocation via xylem was carried out more rapidly than that via phloem.

In the next experiment in **Fig. 2**, the total amount of SA applied was increased to 1, 2 or 6 nmoles from the 60 pmoles used in **Fig. 1**. Two microliter of SA solution containing 3.7 kBq ^{14}C-SA and cold SA with an appropriate concentration was supplied to the cut petiole end of the 3rd leaf (plants 1-6 in **Fig. 2A** and **B**). High levels of cold SA seemed to have no effect on the movement of ^{14}C-SA in the seedlings (compare 1, 2 and 3, or 4, 5 and 6) within 1 h. Together with the results in **Fig. 1**, predominant translocation of the signal from the 3rd leaf to younger sink leaves with time was observed. The supply of SA from the most upper stem end (8) resulted in a similar distribution pattern of the signal as 4. The concentration of 6 n moles SA per plant used here in 3 and 6 corresponds to a final mean concentration 3.7 μg SA g^{-1} fresh weight, which is enough to induce resistance to TMV-infection in tobacco plant (Willits and Ryals, 1998). Thus, translocation of SA from one part to the other in plants can be done smoothly at least within this SA range, ruling out a limit of capacity for SA-transport via endogenous putative SA-transporters working in the phloem stream. When the ^{14}C-SA solution was placed on the surface of the 3rd leaf indicated by a closed circle on plant 7 in **Fig. 2A**, almost no signal moved within 1 h to other parts of the plant but remained at around the original point (see plant 7 in **Fig. 2B**).

SA-movement through vascular bundles and parenchyma cells in adult tobacco plants

The results of **Fig. 1** and **2** provide limited information on SA-translocation, because the plants were too small for a precise analysis. Then, we used tobacco plants at the mid-vegetative stage 35 cm in height. As illustrated in **Fig. 3A**, 10 μl of SA solution (20 nmoles SA containing 37 kBq ^{14}C-SA) was added from the cut petiole end of the 7th leaf, and the regions indicated in **Fig. 3B**-1-4 were cut transversely to 200 μm in thickness at 5 and 10 min after feeding, respectively. In the experiments in **Fig. 3-5**, the numbering of leaves was altered from that in **Fig. 1** and **2**: the most upper 3 cm long leaf was named the1st.

Observations and autoradiograms of the transverse sections of the petiole, positioned 10 mm lower than the SA-applied cut end (**Fig. 3A**), showed that the signal was abundant in the vascular bundle with a dispersed pattern, and considerable in cortex parenchyma, and in epidermis (**Fig. 3B**, column 1) at 5 and 10 min after feeding. The signal in cortex seemed to move and condense in the epidermis (white arrowhead) after 10 min.

In the node of the 7th leaf (position 3 in **Fig. 3A**), a high level of the signal was found in the vascular system in the base of the petiole on the upper region of 7th node (**Fig. 3B**) 5 min after feeding, and it likely moved to the cortex parenchyma and then epidermis (white arrowhead) at 10 min. The signal preferentially moved to the node of the next lower 8th leaf (position 4 in **Fig. 3B**), but not to the next upper 6th leaf (position 2 in **Fig. 3B**). In the node of the 8th leaf (position 4), major signal was found only in the vascular system horizontally directed to the orthostichy of the base of the 7th leaf (red circle) at 5 min. At 10 min, the signal likely moved and accumulated in the epidermis in the same direction (position 4 in **Fig. 3B**). We noticed that the signal was located only in the direction of the orthostichy of the 7th leaf (the direction is indicated by a close arrowhead) within an angle of 72°

(illustrated in **Fig. 3C** as the orange region). This phenomenon reflected the orthostichy of phyllotaxis of tobacco (2/5) as shown in a spiral form in **Fig. 3C** (Yamamoto, 1967). In the transverse section of the upper 6th node (position 2 in **Fig. 3B**), no signal was detected within 10 min, indicating that the phloem flow is predominant in the lower part but not the upper part in the early stages after SA-feeding.

Rapid systemic movement of SA depending on phyllotaxis in adult tobacco plants

To further study long-distance movement of SA in stem, 10 μl of SA solution (20 nmoles SA containing 37 kBq ^{14}C-SA) was added from the cut end of the 7th petiole of tobacco plants at the late vegetative stage (80 cm in height), and the regions indicated as 1-13 in **Fig. 4A** were cross cut at both upper and lower positions of each node (for example, 7-1 and 7-2 on the node of the 7th leaf) to 200 μm in thickness at 10 min after feeding. Photographs and autoradiograms of the transverse sections of the petiole and stem are shown in **Fig. 4B**, with neighboring sections in parallel, and sections from the upper or lower position in the upper or lower line, respectively. Arrowheads show the horizontal direction to the center of the vascular system in the petiole region of the 7th node (refer to the red closed circle in **Fig. 3** and the vertical red line (3) in **Fig. 4A**), depending on orthostichy. Without exception, all signals in stem sections were horizontally directed toward the orthostichy of the base of the petiole in the 7th node as shown by black arrowheads. The levels of signal were likely highest in the node of the 7th leaf, and then the 8th, 6th, 9th, 5th, 2nd, 4th, 3rd, 10th, 11th, 1st, 0s, 12th, 13th, and 14s in that order. Thus, SA predominantly moves along the orthostichy of tobacco phyllotaxis of ^{14}C-SA-supplied leaf, and the movement was mostly in the lower direction at the beginning, and then the upper direction later. The signal was higher than expected in the 2nd node, which is positioned just above the 7th node along the orthostichy. However, in the 12th node which is just below the 7th node, no detectable signal was found. One possible reason for this may be enhanced senescence because of the lower position of the leaf in the plant. To our surprise, the signal was clearly found at all upper and lower nodes after 1 h (data not shown). These results indicate that ^{14}C-SA moves rapidly to all nodes in plants within 10 min to 1 h after the application from the 7th petiole depending on the orthostichy of tobacco phyllotaxis.

Translocation of SA in whole plant is dependent on the structure of tobacco stem

To study how the distribution of the signal is dependent on the structure, photographs and autoradiograms of the transverse sections around the 7th leaf-node (flamed with blue in **Fig. 4B**), were enlarged (**Fig. 4C**).

Referring to the illustration of tobacco stem with the vascular system in brown (**Fig. 4C**, right), we found a considerable level of signal in the leaf gap region (red arrows and arrowheads in **Fig. 4C**, respectively). To our knowledge, there is no report on the role of the leaf gap in the movement of low molecular weight compounds in plants. In the transverse sections of the petiole region of the 7th node, the signal is also detected in cortex parenchyma cells and epidermis (open arrowhead). We again found the signals in stem sections (7-1, 7-2, 7S) within an angle of 72 ° to the horizontal direction to the base of the petiole in the 7th node along the orthostichy.

After prolonged incubation of 1 h after feeding, the signal distribution in the transverse sections of individual nodes was very similar to the results in **Fig. 4**, while the signal in lower nodes of stem became clearer (data not shown).

Recovery of ^{14}C-SA and SA β-glucoside from stem

In plants, SA is found in both free acid form and its β-glucoside form (SAG), which is thought to be a storage type and accumulated in vacuoles. After pathogen-infection, both SA and SAG are accumulated. When free SA is ectopically added to a plant, it is converted rapidly to SAG (Hennig *et al.*, 1993). Generally 5 to 10-fold higher levels of SAG than SA were found in tobacco leaf tissue. To know whether the supplied ^{14}C-SA moves as the free SA form, we extracted the signal from the stem segments (a-e in **Fig. 5A**) after feeding ^{14}C-SA from the cut end of the 7th petiole as illustrated in **Fig. 4A** and **5A**. Then free SA and SAG were extracted and quantified. At 10 min after feeding, the relative radioactivity in the stem segment containing the 5th node (a) was 9% of that of the 7th node (b), from which ^{14}C-SA entered. In the segment at position c, which contains the 11th node, the level was 4%. When an aliquot of the extract containing about 700 dpm was separated to ^{14}C-SA and ^{14}C-SAG by the TLC method (Edwards, 1994), a major signal was detected at the position of free SA (**Fig. 5C**). The levels of ^{14}C-SAG at position a and b were 0.7% of the total radioactivity recovered, indicating the long distance translocation of SA within 10 min is likely carried out as the free SA form in tobacco plants. After prolonged incubation for 1 h, the possible

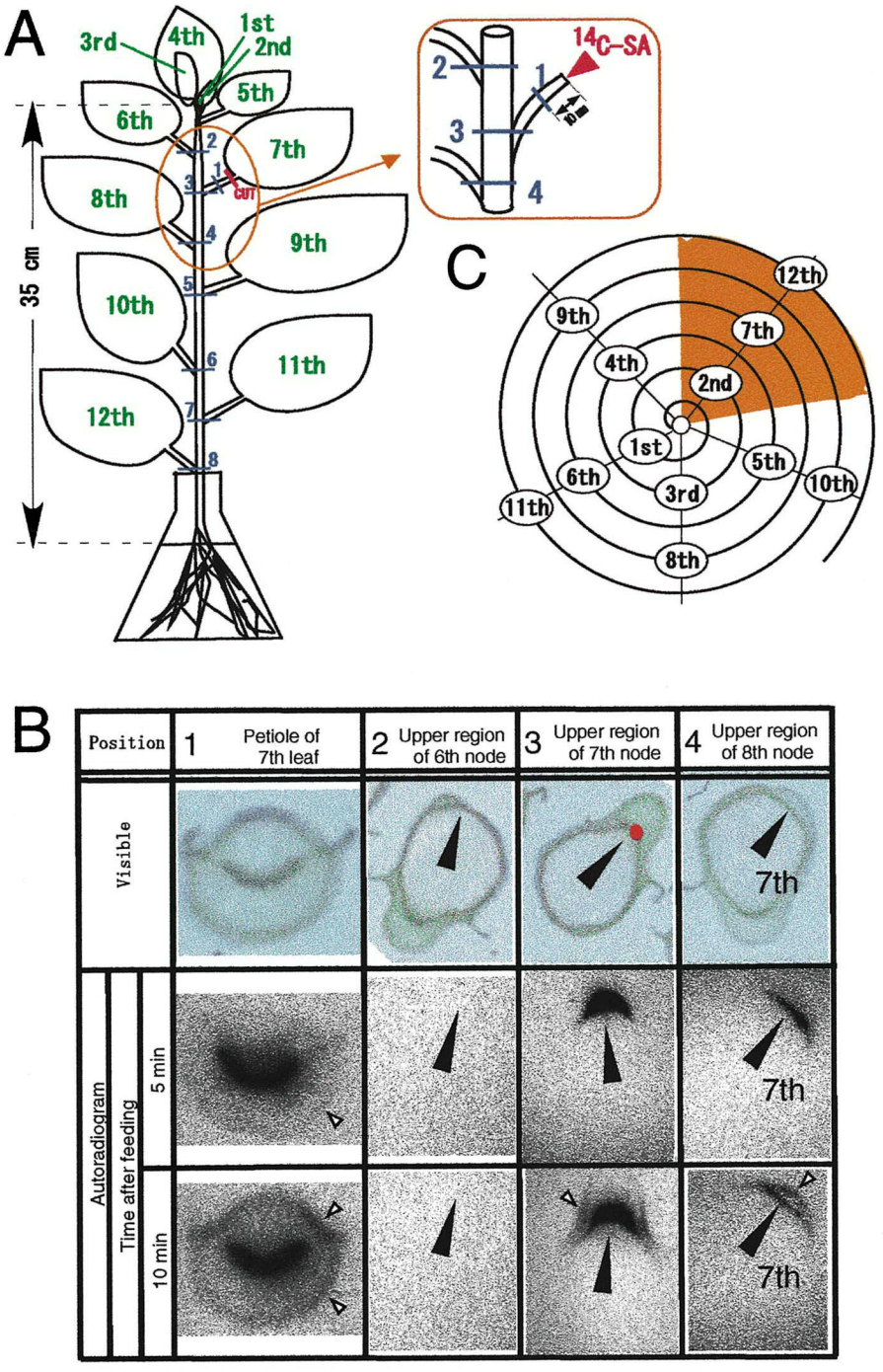


Fig. 3 Rapid SA-movement to vascular systems, parenchyma cells and eipdermis.
A : The 7th leaf of an adult tobacco plant (35 cm in height) was cut off as shown red bar. And 10μl of SA solution containing 3.7 kBq ^{14}C-SA in 20 nmoles cold SA was added at this cut end. Five to 10 min after SA-feeding, 200μm transverse sections were cut at positions 1-4, and subjected to dryness and then autoradiography. B : Visible observation and autoradiogram of transverse sections at positions 1-4 are shown. Arrowheads indicate the horizontal direction for the center of basal vascular system of the 7th node. Open arrowheads indicate epidermis to which radioactive signal was accumulated. Exposure time to X-ray film was 13 h. C : Phyllotaxis of tobacco (2/5) shown in spiral form. The area in orange indicates the horizontal direction for the center of basal vascular sysem of 7th node (red circle in "Visible" panel position 3) within 72°.

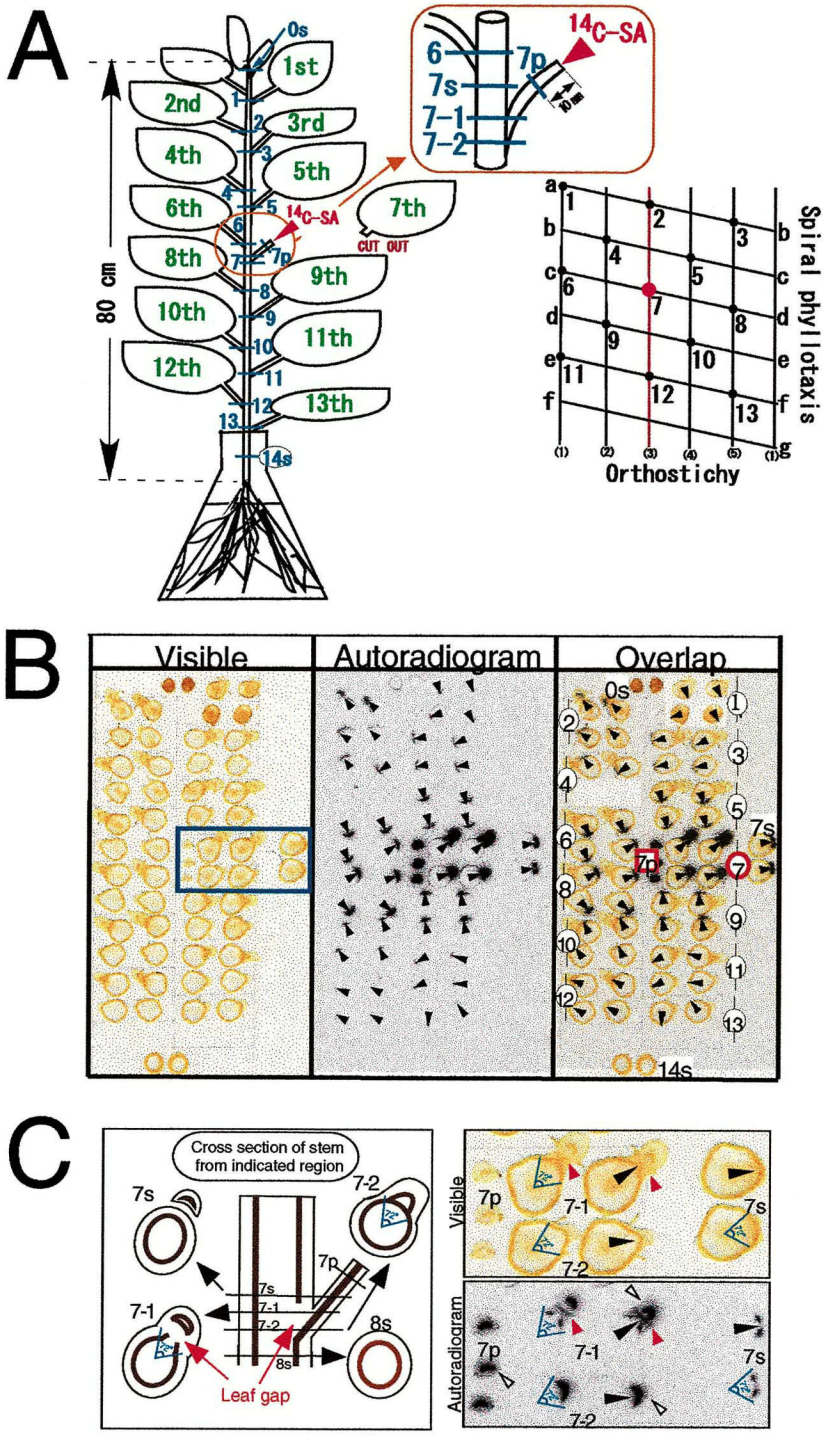
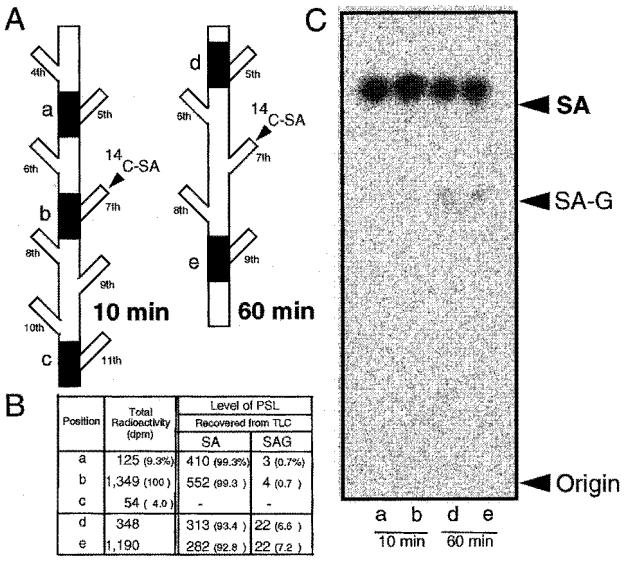


Fig. 4 Up and down SA-movement in stem of adult tobacco plants depending on the orthostichy of phyllotaxis.

A : The 7th leaf of an 80 cm-tobacco plants in late vegetable stage was cut off, at which ^{14}C-SA was fed as shown in the legend of **Fig. 3.** Ten minutes after SA-feeding, cross sections were prepared at positions indicated by blue bars. B : Visible (left), autoradiogram exposed for 220 h to X-ray film (middle), and the overlap feature (right). Arrowheads indicate the horizontal direction for the center of the basal vascular system of petiole in 7th node, to which ^{14}C-SA was supplied. C : Illustration of the structure of tobacco stem with vascular system in brown (left). Enlarged picture of visible (upper right) and antoradiogram (lower right) surronded by the blue frame in panel B. Exposure time to X-ray film was 19 h. Red arrows or arrowheads indicate the region of leaf gap. Black arrowheads show the direction to the base of petiole of the 7th node. Open arrowheads correspond the epidermis.



B

Position	Total Radioactivity (dpm)	Level of PSL Recovered from TLC	
		SA	SAG
a	125 (9.3%)	410 (99.3%)	3 (0.7%)
b	1,349 (100)	552 (99.3)	4 (0.7)
c	54 (4.0)	-	-
d	348	313 (93.4)	22 (6.6)
e	1,190	282 (92.8)	22 (7.2)

Fig. 5 Quantification of ^{14}C-SA and ^{14}C-SA β-glucoside in the stem by TLC method.

A: Illustration of the sampling of stem sections. At 10 min or 1 h after ^{14}C-SA feeding at cut petiole end of 7th leaf, stem segments indicated as a-e were prepared and radioactive materials recovered in the fractions of free SA and SAG were isolated, and the total radioactivity was determined. B: An aliquot containing 700 dpm was supplied to TLC to separate SAG from SA. As the standards, ^{14}C-SA and synthetic SAG were used. C: Total radioactivity in the stem extract was shown with relative ratio. The level of signal for SA and SAG recovered from the TLC plate was shown as PSL which is the unit of BAS 2000 scanner (Fuji Film, Japan) with relative ratio.

signal levels for ^{14}C-SAG at the nodes of both two upper (d) and two lower (e) leaves were increased to 7%, indicating that conversion of ^{14}C-SA to ^{14}C-SAG occurs with time.

Discussion

How does the translocation of SA occur in whole plants? This subject is important to understand the mechanism of SAR, which is a typical induced resistance characteristic of plants. We showed here that SA moves as free SA to all nodes within 10 min to 1 h using a model system; adult healthy tobacco plants by feeding of ^{14}C-SA from cut petiole end. As shown in **Fig. 3** and **4**, the radioactive signal was found mainly in vascular systems at early period. Phloem is believed to be the route of solute-translocation from the leaf (or cut petiole end in this experiment) to other parts of the plant. Actually, the autoradiograms in **Fig. 1** and **2** showed that SA likely moves via phloem to lower parts first and then to upper parts after ^{14}C-SA feeding from the cut petiole end. Xylem also can be an effective transport trajectory for SA as shown in **Fig. 1**, where the movement of the signal to upper parts from the lowest stem end was more rapid than from the cut petiole end. However, the contribution of xylem to SA-translocation on SAR in planta is not clear now.

The downward and upward translocation of SA observed here was dependent on the orthostichy of the tobacco phyllotaxis. It well coincides with the phenomenon on ^{14}C-assimilates translocation in tobacco plant reported by Yamamoto (1967). The data in **Fig. 4** showed the 2nd node contains more signal than expected from the position in the plant. It is well consistent with the observation that SAR was preferentially induced in the 5th upper leaf of pathogen-infected leaves (Shulaev *et al.*, 1995); the 5th upper leaf position is along the orthostichy of tobacco phyllotaxis (2/5) as shown in **Fig. 4**. Interestingly, the signal found in each node was directed horizontally toward the base of the petiole of the 7th node (red closed circle in **Fig. 3**) at an angle of 72°, confirming that the first SA-translocation occurs strictly along the orthostichy.

The radioactivity was found mainly in the vascular system but also in the cortex parenchyma and epidermis of transverse sections of stem and petiole, suggesting the possible movement of SA from phloem to parenchyma cells and then epidermis. The autoradiogram of the transverse section of petiole in position 1 in **Fig. 3A** and **B** indicated that the ^{14}C-SA directly moves to cortex parenchyma through the apoplasts of petiole from the SA-fed cut end, or that the signal leaks from phloem to parenchyma cells in the petiole. Although the sections were prepared 10 mm far from the cut petiole end as shown in **Fig. 3A**, the signal was clearly detected in cortex parenchyma and epidermis at 10 min after feeding (**Fig. 3B-1**). Similar phenomena were also found in the upper stem regions of the 7th and 8th nodes (**Fig. 4B-3** and **4**, respectively). The so-called volume flow model says that sieve tubes are essentially leaky and solute and solvent are lost and retrieved along the sieve tubes (Eschrich *et al.*, 1972), supporting the evidence of photo-assimilates along the phloem pathway in bean plant (Minchin and Thorpe, 1987). Together, SA could leak from phloem and distribute to the other parts in a short time in plants. It is interesting that SA, which can induce resistance to pathogen in many likely transports to apoplast from phloem, spreading to cortex parenchyma and then epidermis which is the most outer tissue in the plant with frequent exposure to pathogens.

One hour after feeding, 7% of radioactivity was found as SAG in the stem. As the translocation signal for SAR, SAG is a possible candidate. About 80% of SA was reportedly found as SAG in plant at 2 days or later after inoculation with pathogen (Hennig *et al.,* 1993; Lee and Raskin, 1998). In our system, the time course analysis of SA conversion to the conjugated form was done at 10 min and 1 h after feeding ^{14}C-SA. The results in **Fig. 5** show that in the stem 10 cm from the node attached to the SA-supplied petiole, 0.7% and 7% of radioactivity was found as ^{14}C-SAG at 10 min and 1 h after feeding, respectively. As SAG was recovered from apoplasts, SAG could directly enter to phloems from its opened sites in leaves. It is not clear that the SAG found in the stem segment was transported via phloem or converted to SAG from transported ^{14}C-SA in the petiole and stem cells. On the other hand, both SAG content and SA β-glucosidase activity in apoplasts increased within 1 h after exogenous feeding of SA to tobacco leaf, suggesting SAG is easily degraded to free SA in apoplasts (Seo *et al.,* 1995). Anyhow, almost all SA seems to move as free SA to all nodes at least within 10 min after SA-feeding in our model experiments, and SAG would be a possible solute for translocation in the later stages. Conversion to methyl SA from ^{14}C-SA is not clear in this system, while methyl SA has been reported as an active signal as free SA.

On the capacity of SA-translocation, we also obtained an interesting data. Results in **Fig. 2** showed that more than 4 μg of SA can be moved in 1 g of fresh plant, at which level SAR can be induced. Transporters and channels for sucrose and sulfate transfer were reported to reside in the plasma membrane of sieve elements and companion cells of transport phloem (Stadler *et al.,* 1995; van Bel, 2003; Yoshimoto *et al.,* 2003). Although the transporters and channels on SA transfer are not clear, our data suggested that the capacity to carry SA from one part to the other is a considerable level in tobacco plants.

SA rapidly moved from one petiole to all nodes in healthy tobacco plants to our surprise. It suggests that SA similarly moves as a systemic signal for SAR from the pathogen-infected lower leaves to healthy upper leaves inducing defense-related gene expression. Previous translocation studies employing ^{14}C-SA have suggested that SA is translocated from the lower leaves and also synthesized de novo in the upper leaves (Molders *et al.,* 1996). As the other signals for SAR, reactive oxygen species (Alvarez *et al.,* 1998) and lipids (Maldonado *et al.,* 2002) were proposed. Our data presented here would help to understand the role of SA, which may co-operate with these possible SAR signals, and mechanism of SAR. This is the first clear report to show rapid translocation of SA along the orthostichy of phyllotaxis in tobacco plants to our knowledge, and may also help to understand the mode of early translocation of other bioactive molecules in plants.

Acknowledgments

The authors thank Dr. Shigeo Tanaka of Tokyo Agricultural University for chemically synthesized SAG. This work was supported by PROBRAIN (Program for Promotion of Basic Research Activities for Innovative Biosciences) and Grand-in-aid for scientific research on priority area (A), Ministry of Education, Culture, Sports, Science and Technology.

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