

Low concentration silver ion decreases endogenous indole-3-acetic acid concentration in Arabidopsis root and increases root mass

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Abstract

Chemical biology research using chemicals has elucidated several mechanisms underlying the endogenous biosynthesis of indole-3-acetic acid (IAA) in plants. However, there has been little chemical biology research focused on the regulation of endogenous IAA concentrations in plant tissues, and only a few studies using silver ion (Ag^+) as a chemical have been reported to investigate how IAA concentrations in plant tissues change when this metal is applied to plant cultivation media. Previous studies have predominantly utilized Ag^+ concentrations in the range of several mg/L (tens of μM), the levels known to affect plant physiology. In this study, we conducted cultivation experiments with *Arabidopsis thaliana* to investigate how the presence of Ag^+ at low concentrations of below 2 μM , which do not induce silver-ion toxicity for usual plants, affects endogenous IAA concentrations in plant tissues and, consequently, root growth. Our experimental data showed that Ag^+ at low concentrations of 0.46–1.85 μM reduced excess IAA concentration in roots, thereby promoting root growth in *A. thaliana*.

1 INTRODUCTION

Indole-3-acetic acid (IAA), named auxin by Went in 1928 and subsequently isolated and identified by Kögl and Haagen-Smit et al. in 1934, is a plant hormone that plays an important role in plant growth at appropriate concentrations [1, 2]. IAA concentration in plant tissues is thought to be regulated by three coordinated pathways: biosynthesis, metabolic inactivation, and polar transport via PIN carrier proteins, which alter the direction and amount of transport depending on their intracellular location [3, 4]. The AUX1 transporter, which is responsible for the uptake of exogenous IAA into cells, is also thought to be involved in this concentration regulation [5, 6]. Meanwhile, the activity of the ABCB transporter, which is thought to be responsible for the extracellular export of endogenous IAA, is also thought to regulate IAA concentration in plant tissues [7, 8]. However, how this transporter's activity is controlled remains unknown.

While chemical biology research using chemical substances has elucidated several mechanisms underlying the endogenous biosynthesis of IAA in plants [9, 10], there has been little chemical biology research focused on the regulation of endogenous IAA concentrations in plant tissues. In particular, only a few studies using silver ion (Ag^+) have been reported to investigate how auxin concentrations in plant tissues change when Ag^+ is applied to plants, and how these changes in auxin concentrations affect the differentiation and growth of plant tissues [11–12].

Most of the chemical biology research utilizing Ag^+ was conducted to investigate the phenomena in which plant tissue differentiation and growth are promoted by using Ag^+ to inhibit the signaling function of ethylene, a hormone involved in plant maturation and senescence. In many of these studies, the silver ion concentrations employed were in the range of several mg/L (tens of μM) levels sufficient to affect ethylene signaling and plant physiology. [19, 20]

In this study, we conducted cultivation experiments using *Arabidopsis thaliana* with adapting low-concentration Ag^+ that does not affect ethylene signaling. On the basic experimental results obtained in this study, we report change of endogenous IAA concentration in plant roots and shoots, and how these changes in concentration of IAA affect the growth of plant tissues, although the results are preliminary and rudimentary.

2 MATERIALS AND METHODS

To elucidate the effects of Ag^+ on the changes in the concentration of endogenous IAA produced within plant tissue and the resulting changes in growth of *Arabidopsis*, plant cultivation experiments were conducted using two methods: short-term cultivation using gellan gum medium and long-term cultivation using commercially available peat-solidified medium and horticultural soil and pots. The materials and methods for the short-term and long-term cultivation experiments are as follows.

Cultivation experiments to examine the effects of Ag^+ on *A. thaliana* were conducted using two methods: short-term cultivation using gellan gum medium (15 days of cultivation after cold vernalization) and long-term cultivation using commercially available peat-solidified medium (47 days of cultivation after cold vernalization). The materials and methods for the short-term and long-term cultivation experiments are outlined below.

In this study, *Arabidopsis thaliana* Col-0 (isolated and named by G. P. Redei, compiled in RIKEN BRC Experimental Plant Web Catalog, and assigned in List of Natural Accession as BRC Number sja05801) seeds (RIKEN BioResource Research Center (BRC), Wako, Japan) were used under the Japanese national guidelines. All *Arabidopsis* cultivation after three days-treatment of vernalization in a refrigerator at 4 °C of the sowed seeds was done in artificial weather chambers (Nippon Medical & Chemical Instruments Co., LH220350SV, Osaka, Japan) that were controlled at 20 °C and around 5500 Lux lightning with white fluorescent lamps for 16 hours and dark condition for 8 hours intervals.

2.1 Short-term cultivation of *A. thaliana* Col-1

Cultivation experiments to examine the effects of Ags on *A. thaliana* were conducted using two methods: short-term cultivation using gellan gum medium (15 days of cultivation after cold vernalization).

For short-term (15 days) cultivation experiments, *A. thaliana* Col-1 seeds were directly sowed and grown on Murashige-Skoog gellan gum solid media (0.2% gellan gum) in a 350 mL transparent polycarbonate plastic box (Kenis Co., Plant Box 310071, Osaka, Japan). The gellan gum solid media were supplemented with purified water, a silver nitrate solution with a specified Ag^+ concentration of 0.46 μM or 1.85 μM .

2.2 Long-term cultivation of *A. thaliana* Col-1

For the long-term (47 days) cultivation experiments, peat compressed into plates (SAKATA SEED Co., Peat Ban72000078, Yokohama, Japan) were swelled with purified water or a silver nitrate solution with a specified concentration of Ag^+ , and used as the medium for sowing, vernalization and raising seedlings. Eleven days (safter the vernalization) old *Arabidopsis* seedlings were transplanted and cultivated using a 2:1 mixture soil with vermiculite (Nittai Co., Vermiculite GS30L, Osaka, Japan) and Standard Horticultural Soil No. 1 (Nihon Hiryo Co., Nippi Horticultural Soil No. 1, Fujioka, Gunma, Japan), autoclaved at 121 °C for 30 minuets and packed into ethanol-sterilized box-shaped plastic horticultural pots (W and D 55mm (bottom)-75mm (top), H 75mm) abiotically.

A commercially available pre-mixed fertilizer solution (1000 times diluted HYPONEX) (HYPONEX Co., Osaka, Japan) was supplemented to the horticultural pots once every three or four days. The same volumes of pure water (for control) or a silver nitrate solution with Ag^+ concentration of 0.46 μM or 0.92 μM was supplemented as necessitated to wet the pot soil.

2.3 Sampling cultured plants

In the short-term cultivation experiments, the plants were sampled 15 days after the cold vernalization of *Arabidopsis* seeds finished and the collected plants (9 plants from each horticultural pot) were cut into root and shoot parts using sterilized scalpels, and the weights of each part were measured. Then, each part was composited and placed in a 2 mL freezing tube with screw-cap, frozen in liquid nitrogen, and then stored in a -80°C freezer for later IAA extraction from the plant specimens.

In the long-term cultivation experiments, the plants were carefully removed from the pots at 47day of the cultivation after the three days cold vernalization of *Arabidopsis* seeds. After removing soil gently, rinsing and adsorbing moisture from the roots, the size of roots and above-ground parts were measured. After cutting the roots from the above-ground parts off, both wet weights were measured separately. Roots parts were placed in freezing tubes and four sections up to the top three nodes were also placed in freezing tubes for later IAA extraction and analysis, and frozen in liquid nitrogen, and then stored in a -80°C freezer.

2.4 IAA extraction

The *Arabidopsis* specimens stored in a -80°C freezer were sawed in room temperature and an appropriate amount of zirconium beads (Merck, BSMD113201TP, Rahway, NJ. USA) were added to the specimens for grinding using a vortex mixer at maximum vibration for 3 minutes. After grinding, 500 μL of 50% methanol was added to each crushed specimen, and then the tubes were reciprocally shaken at 120 spm at room temperature for 1 hour to extract plant ingredients including IAA. Then the tubes were centrifuged (4°C, 12,000 rpm, 10 minutes) and the supernatant was transferred to a new sterile microtube. This 50% methanol extraction was repeated twice and the resulting extract supernatants were collected in the same microtubes.

Each 500 μL of the supernatant was placed in an ultrafiltration spin column (3K, APRO Science, Naruto, Japan) and centrifuged (4°C, 14000 G, 10 minutes) to remove high molecules such as proteins. The 100

µl of ultrafiltered extracts were diluted with 900 µl of 50% methanol (10-fold dilution) and then the diluted extracts were kept in a refrigerator one night. The diluted samples were filtered through a 0.22 µm microfilters (ADVANTEC, DISMIC:13CP020AS, Tokyo, Japan) right before the analyzation.

2.5 IAA analysis

IAA and other indole derivatives in the *Arabidopsis* extracts were determined by a ultra-high-performance liquid chromatograph (UPLC, ACQUITY H-Class system, Waters, Milford, MA, USA) equipped with a fluorescence detector and a BEH C18 column (Waters). Chromatographic separation was performed on a UPLC system at a constant flow rate of 0.4 mL/min, with a total run time of 6.5 min. The mobile phase consisted of methanol (A), ultrapure water (B), and 100 mM sodium dihydrogen phosphate aqueous solution (C). The gradient program was initiated with 25% A, 65% B, and 10% C. The organic composition was increased linearly to 80% A, 10% B, and 10% C at 3.0 min (Curve 6), and maintained until 6.5 min, at which point it was immediately returned to the initial conditions (Curve 1) to complete the analysis.

3 RESULTS

3.1 Results from the short-term cultivation experiment

The IAA concentrations and wet biomasses data obtained from the short-term experiment are shown in Fig. 1. The average IAA concentrations of the root sections of 9 composite 15 days-old *A. thaliana* collected from the plants cultivated with Murashige-Skoog gellan gum solid media amended with 0.46 µM and 1.85 µM of Ag⁺ were 25.7% and 30.2% less than that of average concentration of IAA of the root sections of 9 composite same days-age *A. thaliana* plants taken from the same solid media without Ag⁺ amendment, respectively. On the contrary, total wet biomass of the root sections increased 1.35 times and 3.97 times higher when the *Arabidopsis* were cultivated using the solid media amended with 0.46 µM and 1.85 µM of Ag⁺ than that of *A. thaliana* cultured without Ag⁺ amendment medium, respectively.

Figure 1 also shows the average IAA concentrations and wet biomasses of the shoot sections of 9 composite 15 days-old *A. thaliana* plants collected from the experimental plant cultivation. The analysis data of IAA concentrations of the shoot sections were not much different between those when *A. thaliana* were cultivated using the gellan gum solid media amended with 0.46 µM of Ag⁺ and 1.85 µM of Ag⁺ and without Ag⁺. Wet biomasses of the shoot sections were 5.4% and 35.1% higher when the *Arabidopsis* were cultivated using the solid media amended with 0.46 µM of Ag⁺ and 1.85 µM of Ag⁺ than that without Ag⁺ amendment.

3.2 Results from the long-term cultivation experiment

The IAA concentrations and wet biomasses data obtained from the long-term experiment are shown in Fig. 2. The average IAA concentrations of the root sections of 2 samples of 47days-old *A. thaliana* plants taken from the plants cultivated with 2:1 mixture soil of vermiculite and standard horticultural soil amended with 0.46 µM of Ag⁺ and 0.92 µM of Ag⁺ were 60.0% and 50.5% less than that of average

concentration of IAA of the root sections of 47days-old *A. thaliana* plants taken from the same mixed soil cultivation without Ag^+ amendment, respectively. On the contrary, total wet biomass of the root sections increased 1.61 times and 1.41 times higher when the *A. thaliana* were cultivated using the soils with 0.46 μM of Ag^+ and 0.92 μM of Ag^+ than that of the *A. thaliana* cultured without Ag^+ amendment soil, respectively.

Figure 2 also shows the average IAA concentrations of the shoot sections of 2 samples of 47days-old *A. thaliana* plants taken by the experimental plant cultivation. The analysis data of IAA concentrations and wet biomass of the shoot sections were not much different between those when the *A. thaliana* were cultivated using the solid amended with 0.46 μM of Ag^+ and 0.92 μM of Ag^+ and without Ag^+ .

4 DISCUSSION

Most of the chemical biology research utilizing Ag^+ was conducted to investigate the phenomena in which plant tissue differentiation and growth are promoted by using Ag^+ to inhibit the signaling function of ethylene. However, the results of research on the effects of Ag^+ to the signaling functions of plant growth hormone auxin and ethylene suggest the possibility that Ag^+ can be used to regulate endogenous IAA concentrations in plant tissues and thereby control plant growth.

Several studies have investigated how IAA concentrations within plant tissues change when plants are treated with silver ions during cultivation. A study reported that Ag^+ did not only inhibit ethylene signaling but also possess the ability to promote the efflux of exogenous IAA from the plant body, [21] and Ag^+ effectively functioned to optimize plant rooting, growth and flowering. [14, 17, 18, 22] However, relationship of change in endogenous auxin concentration and plant growth affected by Ag^+ was not elucidated.

The data sets obtained from our plant cultivation experiments on the effects of Ag^+ to the endogenous IAA concentrations in roots and shoots of *A. thaliana* showed that the concentration of IAA in roots decreased when the plants were cultivated with the solid media or soil containing 0.46–1.85 μM of Ag^+ in both of the short-term cultivation experiment and the long-term cultivation experiment. The data sets also showed that the concentration of IAA in shoots was not different much each other when the plants were cultivated by the media or soil with or without 0.46–1.85 μM of Ag^+ in both of short-term and long-term cultivation experiments.

On the contrary, biomass of the roots of *A. thaliana* plants increased when the plants were cultivated with the media or soil containing 0.46–1.85 μM of Ag^+ in both of the short-term cultivation experiment and the long-term cultivation experiment. However, there was no much differences in biomass of the shoots of *A. thaliana* plants when the plants were cultivated by the media or soil with or without this range of Ag^+ .

5 CONCLUSION

From these observations got from this study, it is concluded that low concentration of silver ion (0.46–1.85 μM) has a function to reduce endogenous IAA concentration in root and to adjust it in appropriate range for enhancement of root elongation of the tested *A. thaliana* Col-1.

Declarations

Funding

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Ethics, Consent to Participate and Consent to Publish

The corresponding author concluded the Material Transfer Agreement (MTA) between RIKEN BioResource Research Center (BRC), a Japanese national bioresources culture collection center, to permit the use of *Arabidopsis thaliana* Col-0 (isolated by George. P. Redei at University of Missouri-Columbia, USA) seeds (selectively cultivated, harvested, identified307 and stocked by RIKEN BioResource Research Center, and assigned in List of Natural Accession as BRC Number sj05801). The authors used the experimental plant in compliance with the guidelines defined by the MTA and Guidelines on Access to Genetic Resources and the Fair Equitable Sharing of Benefits arising from their Utilization (the ABS Guidelines) under the Nagoya Protocol on Access to Genetic Resources and the Fair Equitable Sharing of Benefits arising from their Utilization to the Convention on Biological Diversity.

Conflict of interest;

No potential conflict of interest was reported by authors.

This paper is not relation to clinical trials

Author Contribution

Conceived and designed the plant cultivation experiments: G. Endo and E. Matsuyama. Performed the plant cultivation experiments: E. Matsuyama and G. Endo. Analyzed the IAA data: W. Ikeda-Ohtsubo and E. Matsuyama. Contributed reagents/materials/analysis tools: G. Endo, E. Matsuyama and W. Ikeda-Ohtsubo. Wrote the paper: G. Endo and W. Ikeda-Ohtsubo. Finally, all authors approved the manuscript.

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Data Availability

The data underlying this article are available in the article and in its online supplementary material (DOI: 10.5281/zenodo.22879196).

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Figures

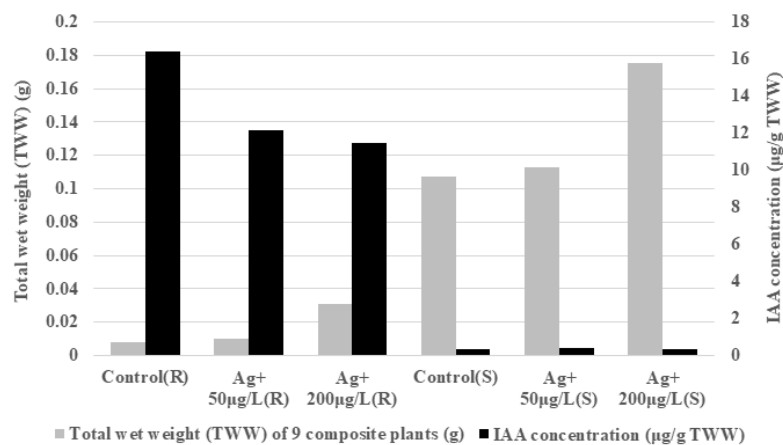


Fig.1 IAA concentrations and wet biomasses weight data of roots(R) and shoots(S) obtained from the short-term (15 days cultivation) experiment

Figure 1

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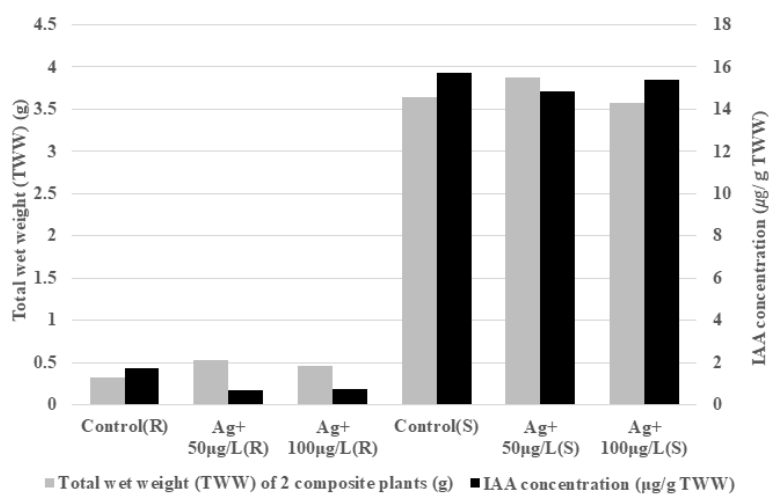


Fig.2 IAA concentrations and wet biomasses weight data of roots(R) and shoots(S) obtained from the long-term (47 days cultivation) experiment

Figure 2

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