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Bismuth Affects ROS Concentration and Cell Cycle in *Arabidopsis thaliana*

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Abstract

Bismuth (Bi) is a minor metal used in semiconductors as a lead substitute. We have previously demonstrated that Bi inhibits plant growth and causes cell death. However, the mechanism of growth inhibition by Bi remains unclear. Reactive Oxygen Species (ROS) are involved in the phytotoxicity of heavy metals. The effect of Bi on ROS production in plants is unknown. We examined $O_2^{\cdot-}$ and H_2O_2 involvement in Bi-treated *Arabidopsis thaliana* and found that these have decreased production in Bi-treated leaves and roots. SOD is involved in ROS metabolism, and the *FeSOD* expression level was not affected upon Bi treatment. In Bi-treated root meristems, the cell cycle was arrested. However, the genomic DNA of Bi-treated plants was not digested. Therefore, Bi may inhibit enzymes related to the cell cycle. These findings indicate that Bi phytotoxicity causes ROS disruption and cell cycle arrest.

Discipline: Agricultural Environment
Additional key words: FeSOD, meristem zone, S phase, superoxide ion

Introduction

Bismuth (Bi) is considered a minor metal. In Japan, it is used as an ingredient in some pharmaceuticals and is included in the Japanese Pharmacopoeia 18th edition (JP18) (Pharmaceutical and Medical Device Regulatory Science Society of Japan 2021). Bi subnitrate is an antiulcer drug with gastrointestinal mucosa convergence due to the protection of the alimentary canal mucosa (Srinarong 2014). Bi is also used in Sri Lanka, including Bi subsalicylate, which is used as a gastrointestinal medicine (Shanika et al. 2018). Additionally, Bi is used in semiconductors and water pipes as a lead (Pb) substitute in Japan.

The toxicity of excess Bi in animals was previously reported and indicated that Bi has lower toxicity than other metals, including Pb (Kubota et al. 1988, 1990). To date, no environmental quality standard for Bi is available in Japan. There are some reports about Bi concentration in the soil worldwide. In Japan, Bi is distributed in mines, such as the Ikuno Mine (Hyogo Prefecture) and the Houbenzan (Yamaguchi Prefecture) area (Ishihara 2008). Bi was detected in the soil and river around Bi smelting areas (Kubota et al. 1990). Furthermore, Bi drained from metalliferous mining and smelting areas was also detected in a rice paddy (Kubota et al. 1988) or soil and pasture herbage in England (Li & Thornton 1993). In Brazil, Bi was detected in fertilizers (Machado et al. 2017).

Recently, we reported Bi accumulation in *Arabidopsis thaliana* (*A. thaliana*) and *Solanum lycopersicum* (*S. lycopersicum*) (Nagata 2015, Nagata & Kimoto 2020). In these plants, root elongation and shoot growth were significantly inhibited by Bi in a dose-dependent manner. In *A. thaliana*, the Bi concentration in the root was sevenfold higher than in the shoot in 2 μM Bi-treated plants (Nagata 2015).

The stem cell niche in the *A. thaliana* root tip contains four quiescent center cells and is involved in root tissue maintenance (Dinneny & Benfey 2008). The cells of the meristem zone (MZ) give rise to all root tissues, including the epidermis, lateral root cap, cortex and endodermis, pericycle, vasculature, and distal columella initials (Di Mambro et al. 2019). A transition zone at the base of the MZ prevents cell division, allowing root cells to initiate elongation and differentiation and

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complete the cell differentiation cycle. Cell death was previously observed in Bi-treated root MZ (Nishimura & Nagata 2021), but the mechanism involved remains unclear.

Reactive oxygen species (ROS) include superoxide ($O_2^{\cdot-}$), singlet oxygen ($^1O_2$), lipid peroxides (ROO·), H_2O_2, and the highly reactive hydroxyl radical (OH·) (Kliebenstein et al. 1998). ROS homeostasis is a crucial factor in plant root growth and development because ROS acts as internal signals at low concentrations (Mittler 2017). High ROS concentrations can cause oxidation and damage proteins, lipids, and other cellular macromolecules (Apel & Hirt 2004, Jin et al. 2021). Lipids in the cell membrane are more sensitive to H_2O_2 exposure, a key ROS with high toxicity to plants (Mittler 2017, Jin et al. 2021). ROS are involved in the phytotoxicity of heavy metals, such as cadmium (Cd) (Hendrix et al. 2020). Generally, heavy metals induce ROS production, leading to oxidative damage (Dutta et al. 2018). Thus, we hypothesized that Bi-induced ROS caused cell death in the roots.

Superoxide dismutase (SOD) is an important ROS scavenging enzyme in plants; it catalyzes the conversion of $O_2^{\cdot-}$ to H_2O_2 (Kliebenstein et al. 1998). The plant SOD isoenzymes differ in their subcellular location. MnSOD (MSD) localizes to mitochondria and has a single isoform, MSD1. FeSOD (FSD) localizes to plastid and cytoplasm in *A. thaliana* and has three isoforms: FSD1, FSD2, and FSD3. FSD1 has the highest expression level in the rosette leaves of mature *A. thaliana* (Kliebenstein et al. 1998), while FSD2 and FSD3 play key roles in early chloroplast development (Myouga et al. 2008).

H_2O_2 produced by environmental stresses can affect the cell cycle (Mase & Tsukagoshi 2021). Root growth is caused by cell division at the root tip of *A. thaliana.* The cell cycle includes the S, M, and G phases, and genomic DNA is replicated in the S phase (Weimer et al. 2016). A previous study demonstrated the presence of a redox cycle within the plant cell cycle and that the redox state of the nucleus is an essential factor in cell cycle progression (de Simone et al. 2017). Additionally, sustained oxidation restricts nuclear functions and impairs progression through the cell cycle, leading to fewer cells in the root apical meristem (de Simone et al. 2017).

Cd-induced ROS production causes DNA damage in various plant species, including Arabidopsis (de Simone et al. 2017, Wang et al. 2016, Cao et al. 2018), wheat (Mutlu & Mutlu 2015), rice (Zhang X. et al. 2015), onion, and lettuce (Silveira et al. 2017).

This study examined ROS production and SOD expression levels in Bi-treated *A. thaliana* and the effect of Bi on DNA damage and the cell cycle.

Materials and methods

1. Plant material and growth conditions

Seeds of *A. thaliana* (L.) Heynh. Col-0 were obtained from Inplanta Innovations, Inc. (Kanagawa, Japan) and surface sterilized in 70% (v/v) ethanol for 2 min and in 10% (v/v) commercial bleach with detergent (Kitchen Haiter, Kao, Tokyo, Japan)—which included sodium hypochlorite (NaOCl), alkyl ether sulfate sodium salt, and NaOH—for 7 min. The seeds were subsequently rinsed with sterilized water five times and planted in a Murashige and Skoog (MS) medium containing B5 vitamins with 0.8% (w/v) agar and 1% (w/v) sucrose (Murashige & Skoog 1962). The pH of these MS agar media was prepared at 5.80. $Bi(NO_3)_3$ solution was added before the MS agar medium solidified. 1.00 g/L $Bi(NO_3)_3$ in 0.5 mol/L HNO_3 solution was obtained from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). MS agar plates were supplemented with various concentrations of $Bi(NO_3)_3$. Seeds were sown on a Bi-containing medium. A Bi-free medium was used as a control. All cultures were maintained at 23°C under a 16-h light/8-h dark cycle and grown for two weeks in an MLR-350 growth chamber (Sanyo, Osaka, Japan). All experiments conducted in this paper used Arabidopsis two weeks after sowing.

2. ROS quantification using MCLA

2-Methyl-6-(*p*-methoxyphenyl)-3,7-dihydroimidazo[1,2-α]pyrazin-3-one hydrochloride (MCLA) can be used for ROS quantification, especially for $O_2^{\cdot-}$ and $^1O_2$ (Nakano 1998). ROS measurements were conducted according to Kobayashi et al. (2004). After treatment, each root was washed once with 0.1 mM $CaCl_2$ (pH 4.75) and preincubated with 25 μM MCLA (Tokyo Kasei, Tokyo, Japan) for 10 min. Subsequently, the roots were washed once with 0.1 mM $CaCl_2$ (pH 4.75) and cut with a razor blade to obtain 10-mm sections from the root tip. One root section was placed in a tube containing 200 μL of 0.1 mM $CaCl_2$ (pH 4.75), and MCLA-dependent luminescence was measured with a luminometer (model LB9506; EG&G, Berthold, Germany).

3. ROS staining

For *in situ* H_2O_2 detection, samples were stained with 3,3′-Diaminobenzidine (DAB) (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) following a previously described method (Bindschedler et al. 2006). H_2O_2 can oxidize DAB to generate a dark brown precipitate in plant tissues. Roots of Bi-treated or untreated control plants were stained with a DAB solution via vacuum infiltration for 5 min. The roots were subsequently covered with

aluminum foil and incubated at room temperature for 4 h. Next, they were transferred to a bleaching solution of ethanol:acetic acid:glycerol (3:1:1) and heated at 98°C for 15 min. Heated samples were then placed in a fresh bleaching solution and allowed to stand for 30 min. All figures represent staining detected in ten independent repetitions for each tissue.

4. Anthocyanin quantification

Anthocyanin was extracted from Arabidopsis leaves using MeOH:HCl (99:1, v/v) for 48 h in the dark at 4°C. The optical density of the supernatant was measured at 535 nm using a UV/Vis spectrometer. The concentration of anthocyanin in the supernatant was calculated using a previously provided equation (Abdel-Aal & Huncl 1999).

5. SOD expression levels

After incubation under the treatment described above, total RNA from roots was isolated using an RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. First-strand complementary DNA was synthesized from 1 μg of total RNA using an oligo dT (18) primer and random primers (ReverTra Ace, TOYOBO, Japan).

PCR reactions were performed using *Act8* (At1G49240) as the reference gene. *MnSOD1* (AT3G10920), *FeSOD2* (AT5G51100), and *FeSOD3* (AT5G23310) sequences from *A. thaliana* are available in the NCBI database (http://www.ncbi.nlm.nih.gov/pubmed). *Act8* primers were designed using Primer3 software (Rozen & Skaletsky 2000). Primers for *MnSOD1*, *FeSOD2*, and *FeSOD3* were used according to the method of Gharari et al. (2014). The primer sequences used for DNA amplification were *MnSOD1*, F 5′- ACCCTAGCCGGCTTGAAGGAGAC -3′ and R 5′- GGCCGGTTCCAATGCGCCATA -3′; *FeSOD2*, F 5′- ACTCCCAATGCTGTGAATCC -3′ and R 5′- CTTCGGTGATGCAGAACTCA -3′; *FeSOD3*, F 5′- GGATGTGTGGGAGCACTCTT -3′ and R 5′- GATTGGGATGTTGGGTTCAC -3′; and *Act8*, F 5′- GATCACAGCTCTTGCCCCG -3′ and R 5′- ACAGTCCAATTTTACCTGCTGGA -3′. The amplified products were separated into a 1% agarose gel containing GelRed (Wako Pure Chemical Ind., Osaka, Japan). The intensity of each band was estimated using ImageJ (rsb.info.nih.gov/ij). The expected sizes for *MnSOD1*, *FeSOD2*, *FeSOD3*, and *Act8* were 108, 242, 157, and 130 bp, respectively.

6. Morphological analysis

Five seeds were grown in MS agar plates, including 2-μM Bi, and 20-μM KI was added to the medium. After 2 weeks of incubation, plants were separated into shoots and roots, and the root length was measured. The fresh weight (FW) of each tissue was measured after harvesting.

7. EdU staining

5-ethynyl-2′-deoxyuridine (EdU) can be taken up in place of thymine in the cell cycle and arrest the S phase (Li & Sheen 2016). EdU staining was performed as described by Kotogany et al. (2010) using an EdU detection cocktail (Thermo Fisher Scientific Inc., Tokyo, Japan) with minor modifications. Seedlings were treated with 10 μM EdU for 30 min and fixed in a 4% (w/v) formaldehyde and 1% (w/v) glutaraldehyde solution in PBS with 0.1% Triton X-100 for 30 min. Fixer was washed with PBS (3 × 10 min), incubated in the EdU detection cocktail for 30 min in the dark, and washed with PBS again before microscope observation. Yi et al. showed that 2 mM Hydroxyurea (HU) can break DNA strands (Yi et al. 2014) and was used as a control for the DNA replication inhibitor. The images were captured using an Olympus IX83 microscope (Olympus, Tokyo, Japan). The root cap was visualized under epifluorescence illumination (excitation, 495 nm; emission, 519 nm). All figures are representative of staining detected in the roots of ten independent experiments.

8. DNA extraction and gel electrophoresis

Fresh shoot and root tissues were ground in liquid nitrogen using a mortar and pestle. The samples were incubated at 65°C in an aliquot of cetyltrimethylammonium bromide (CTAB) buffer [2% (w/v) CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl pH 8.0] for 60 min, and genomic DNA was isolated. DNA samples (2 μg) were separated on a 2% agarose gel (Murray & Thompson 1980) containing GelRed (Wako Pure Chemical Ind., Osaka, Japan) and photographed under 500 nm light.

9. Statistical analysis

All experiments in this study were repeated ten times. In each study, five plants were used in the experiment. Data are presented as the means of ten technical replicates. Differences between treatments were statistically assessed using Dunnett's test, and statistical significance was inferred at $P < 0.05$.

Results

1. Bi effect on ROS production

ROS generation caused by aluminum (Al) in roots was previously elucidated using MCLA (Kobayashi et al. 2004). MCLA was used to quantify ROS in the shoots and roots of *A. thaliana* grown on an agar medium

containing various Bi concentrations for two weeks. With the increase in Bi concentrations, ROS production in the leaves was significantly reduced compared to the control (Fig. 1A). ROS production in 2 µM Bi-treated leaves was approximately half that of the control. Although not significantly different, ROS in the roots showed a decreasing trend. ROS production in 2 µM Bi-treated roots was approximately 30% lower than in the control. Moreover, 3,3′-Diaminobenzidine (DAB) can be oxidized by H_2O_2 in the presence of heme-containing proteins, such as peroxidases, to generate a dark brown precipitate (Bindschedler et al. 2006).

Huang et al. (2014) previously demonstrated that Al can induce ROS production and programmed cell death (PCD) in higher plants. Furthermore, we demonstrated by DAB staining that Strontium (Sr) can induce ROS production (Nagata 2023). The results of DAB staining indicated ROS production in the leaves and roots of the control (Fig. 1B). In addition, the 0.1 µM Bi-treated leaves and roots were slightly stained. In contrast, DAB staining was not observed in the leaves and roots of plants treated with 1- and 2-µM Bi.

2. Bi effect on anthocyanin production

Anthocyanins are water-soluble plant pigments involved in plant protection against ROS (Nakabayashi et al. 2014, Zhang Y. et al. 2015). ROS-induced anthocyanin production generates a feedback-scavenging reaction (Xu & Rothstein 2018). The anthocyanin concentrations in the leaves were determined (Fig. 2). No significant difference was observed in 0.1- and 1-µM Bi-treated plants compared with control plants. However, anthocyanin concentrations in 2 µM Bi-treated plants increased by approximately 25% relative to the control plants.

3. Bi effect on *SOD* expression levels in *A. thaliana*

SOD mRNA contents were quantified in each treated plant to determine *SOD* expression levels in the shoots and roots (Fig. 2). A significant decrease in the

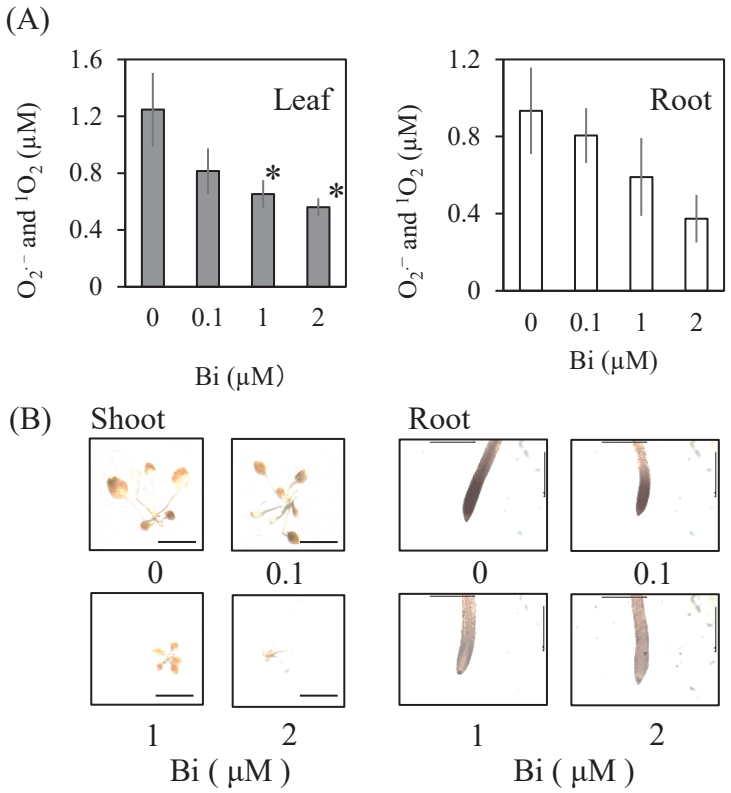

Fig. 1. Detection of ROS production in Bi-treated *A. thaliana*
(A) The ROS production was determined with MCLA. Data are means±SE (n = 10). Asterisks indicate a significant difference between Bi-treated plants and control plants ($P < 0.05$). (B) The ROS production was stained with DAB. The scale bar indicates 100 µm. Ten biological and technical replications were performed.

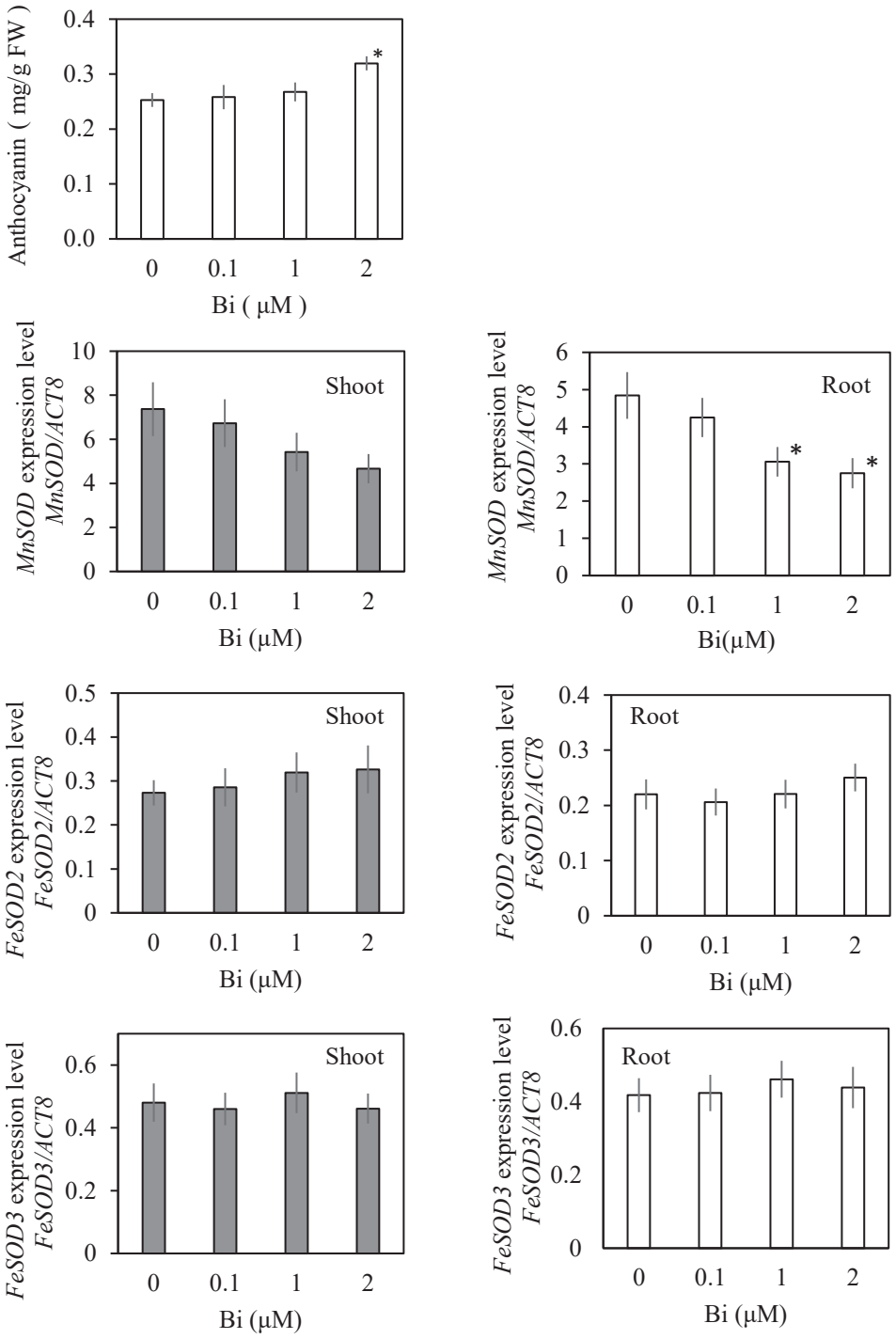

Fig. 2. Effect of Bi to anthocyanin concentration and *SOD* expression levels in *A. thaliana*
Anthocyanin was extracted and measured with a spectrometer. Expression levels of *MnSOD1*, *FeSOD2*, and *FeSOD3* were determined using qRT-PCR. Transcript accumulation was quantified as a value relative to *Act8* transcript accumulation. Data are means±SE (n = 10). Asterisks indicate a significant difference between Bi-treated plants and control plants ($P < 0.05$).

MSD1 expression level was observed only in 1- and 2-μM Bi-treated shoots compared with control plants. No significant differences were observed in the levels of *FSD1* and *FSD2* between Bi-treated shoots and roots and control plants.

4. Effect of KI on Bi-induced growth inhibition in *A. thaliana*

We confirm the growth defects caused by Bi-induced ROS using chemical reagents. Potassium iodide (KI) is a ROS scavenger, including H_2O_2, and its application against ROS production facilitates lateral root emergence (Orman-Ligeza et al. 2016) and decreases the root meristem size in *A. thaliana* (Hashem et al. 2021). Recently, we reported that Sr-induced root growth inhibition was partially restored by KI treatment (Nagata 2023). After 2 weeks of incubation, the restoration effects of KI on the Bi-induced growth inhibition were determined (Fig. 3). No KI-dependent restoration effect was observed on the growth of shoots treated with 2 μM Bi. However, the root fresh weight and length increased by approximately 22% and 45%, respectively, in KI-supplemented conditions compared to non-KI-supplemented conditions.

5. Effect of Bi on DNA replication in *A. thaliana*

An EdU-based proliferation assay was reported to compare cell division in seedling roots (Hsieh et al. 2015). Here, we attempted to use the same assay to examine whether the cell cycle is arrested in Bi-exposed Arabidopsis roots. Using the thymidine analog EdU to detect the S-phase entry, the root meristems displayed fluorescence in the Bi-untreated roots (Fig. 4). However, half of the roots treated with 0.1 μM Bi displayed no fluorescence. No EdU-induced fluorescence was observed in root meristems treated with 1- and 2-μM Bi and no fluorescence was observed in the HU-treated root meristems.

6. Damage of Bi to genomic DNA in *A. thaliana*

DNA ladders were detected under waterlogging stress, and PCD was observed in maize (Chen et al. 2014). In contrast, DNA ladder was not detected in tomatoes upon mercury exposure (Nagata 2014). After 2 weeks of incubation, the isolated genomic DNA was separated on a 2% agarose gel to investigate genomic DNA digestion (Fig. 5). The genomic DNA of control and Bi-treated plants was not digested.

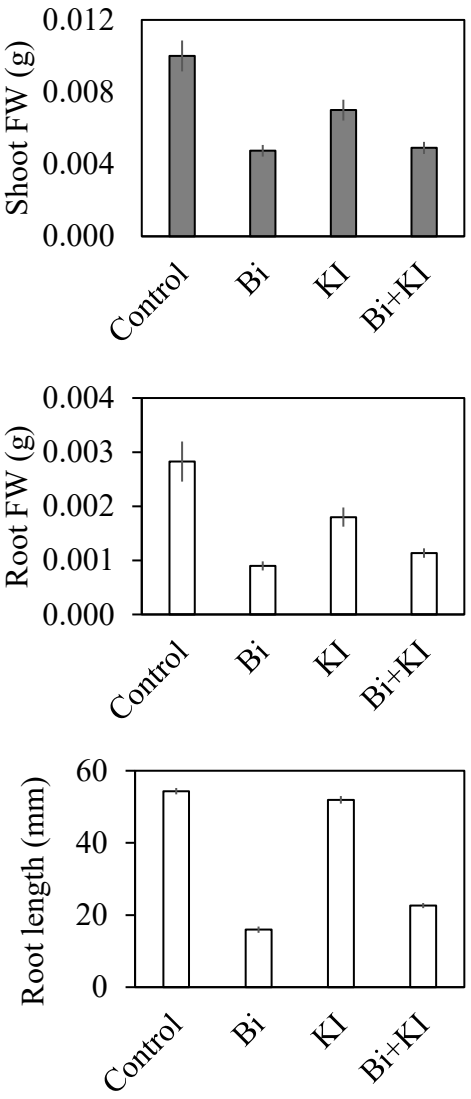

Fig. 3. Effects of KI on the Bi toxicity in *A. thaliana*
Two-week-old plantlets in 2 μM Bi containing agar medium supplemented with or without 50 μM potassium iodide (KI) were analyzed to determine the fresh weights of shoots, roots, and root length. Data represent means±SE (n = 10).

Discussion

We have previously reported Bi phytotoxicity; Bi disturbs Fe accumulation and induces cell death in *A. thaliana*. However, the process of Bi-induced cell death is not yet understood. Additionally, Cd increases ROS and causes DNA damage (Dutta et al. 2018). This study examined ROS involvement and DNA damage in Bi-treated *A. thaliana*.

To analyze the effect of Bi on ROS production, we

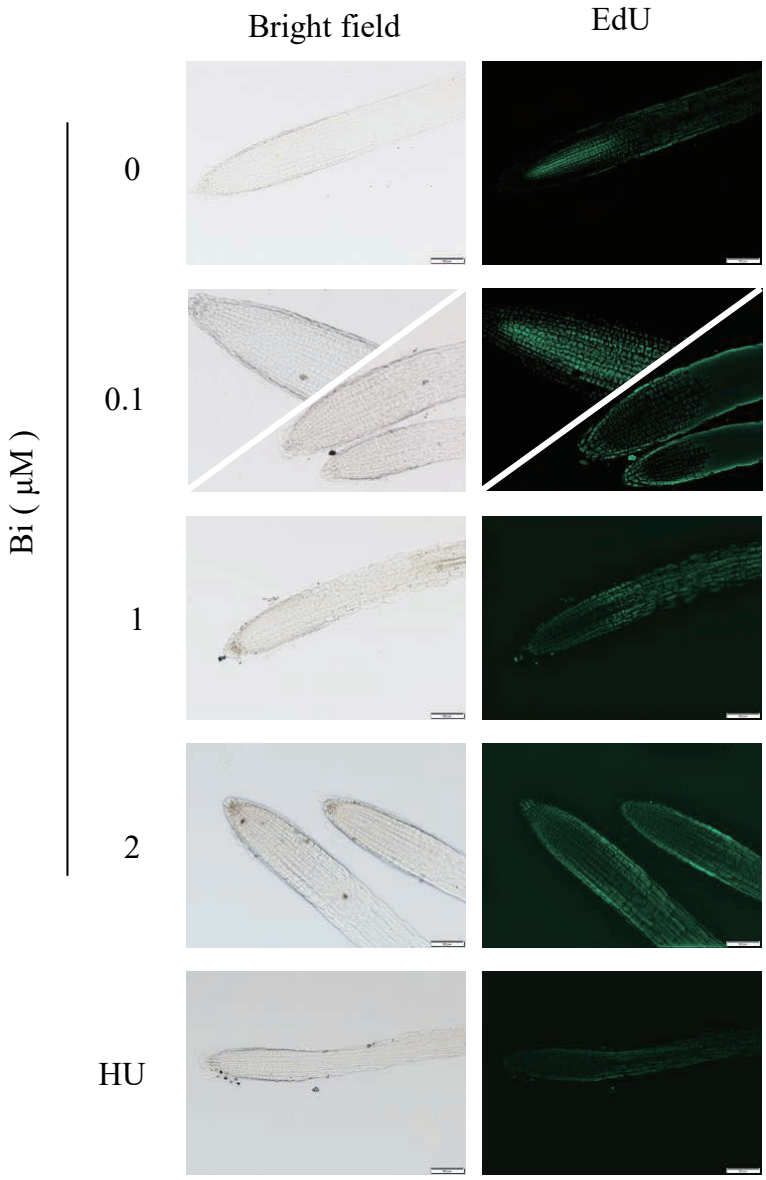

Fig. 4. Effect of Bi on the cell cycle in *A. thaliana*
Cell cycle S-phase entry was detected with 5-ethynyl-2′-deoxyuridine (EdU). The EdU fluorescence was observed by microscope. Roots treated with 0.1 μM Bi that showed or did not show EdU fluorescence are indicated in the upper left and lower right of the white line. The scale bar indicates 100 μm. All figures are representative of staining detected in the roots of ten independent experiments.

quantified $O_2^{\cdot-}$ and $^1O_2$ concentrations in tissues using MCLA (Fig. 1A). These concentrations in the leaves and roots were reduced compared with the control, suggesting that $O_2^{\cdot-}$ were reduced and metabolized into H_2O_2 by SOD-catalyzed reactions. We also observed H_2O_2 by DAB staining (Fig. 1B). No staining was observed in the leaves and roots of plants treated with 1- and 2-μM Bi. Unexpectedly, this result indicates that H_2O_2 concentrations decrease with increasing Bi. It suggests that the Bi accumulated in the root cells inhibits peroxidase activity. Heavy metals, such as Cd, are known to increase H_2O_2 (Gupta et al. 2017, Dutta et al. 2018). Bi reduced $O_2^{\cdot-}$ and H_2O_2 concentrations, which indicates that Bi causes different oxidative damage than Cd.

Next, we analyzed the expression of the ROS scavenging system in Bi-treated plants (Fig. 2).

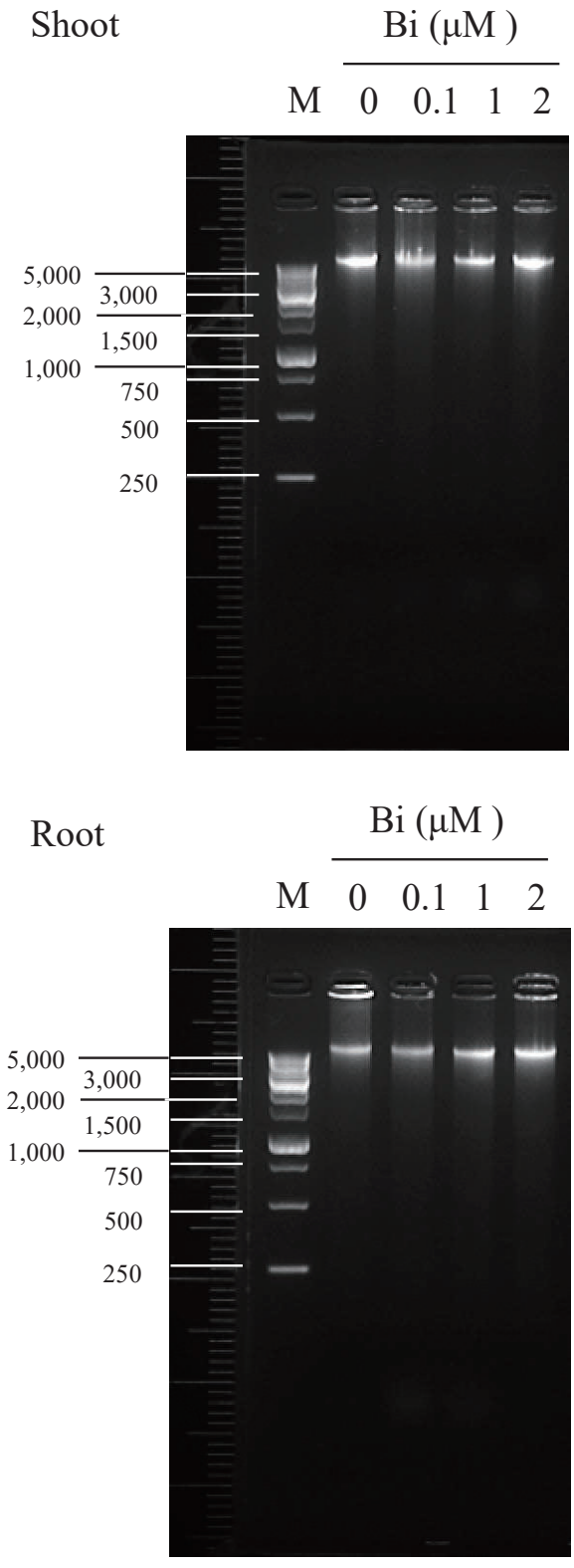

Fig. 5. Damage of Bi to genomic DNA in Bi-treated *A. thaliana*
The genomic DNA was separated on a 2% agarose gel. M indicates DNA ladder marker. Ten biological and technical replications were performed.

Anthocyanins are antioxidants that protect plants against ROS (Nakabayashi et al. 2014, Zhang Y. et al. 2015). The anthocyanin concentrations in 2 μM Bi-treated plants increased compared to control plants. ROS-induced anthocyanin production provides cytoprotection by scavenging ROS (Xu & Rothstein 2018). In addition, we measured the expression levels of *SOD*, which plays a central role in the ROS scavenging system (Fig. 2). *MSD1* expression levels were decreased by Bi treatment. However, *FSD2* and *FSD3* expression levels were not affected by Bi treatment. These results indicate that Bi might have a greater effect on the amount of transcription of mitochondrion-expressed proteins than that of plastid-expressed proteins. Furthermore, $O_2^{\cdot-}$, $^1O_2$, and H_2O_2 concentrations were reduced by Bi (Fig. 1), which may have suppressed *MnSOD* expression. NADPH oxidase (respiratory oxidase homolog) has been identified as a ROS-generating enzyme in *A. thaliana* (Kaya et al. 2019). Bi might nonspecifically inhibit enzyme activity in plant cells. Thus, we hypothesized that Bi inhibited the enzymatic activity of NADPH oxidase. However, our results were obtained after 2 weeks of growth, and it is unclear whether ROS is involved in the growth process.

KI is a chemical ROS scavenger in *A. thaliana* (Orman-Ligeza et al. 2016, Hashem et al. 2021). Its scavenging potential on Bi toxicity was estimated on agar medium (Fig. 3). Addition of KI alone inhibited shoot and root growth, suggesting that KI affects plant growth as a reducing agent. Arabidopsis grown in an environment with both Bi and KI increased fresh weight and root length compared to Arabidopsis grown on Bi-containing media without KI. These results suggest that 2 μM Bi could induce ROS generation in roots during growth. We believe that Bi causes ROS production in the early stages of culture and later inhibits the activity of enzymes, including NADPH oxidase, causing a decrease in ROS and growth inhibition. However, it is not only the catalytic reaction of NADPH oxidase that produces ROS. Fe produces hydroxyl radicals, other types of ROS, by the Fenton reaction (Halliwell & Gutteridge 1992). We previously reported that Bi causes an excess accumulation of Fe in roots (Nagata 2015, Nishimura & Nagata 2021). Fe-induced hydroxyl radicals may also contribute to growth inhibition.

The presence of a redox cycle within the plant cell cycle and the redox state of the nucleus are important factors in cell cycle progression (de Simone et al. 2017). In Bi-treated root meristems, the cell cycle was arrested (Fig. 4), suggesting that DNA repair is inhibited, at least in the S phase. The cell cycle is regulated by cyclins and cyclin-dependent proteins (CDKs). Bi might also affect cyclins D, E, and A, or the cyclin D–CDKA complex that

acts during the transition from the G1 to the S phase. Also, Hydroxyurea causes DNA damage by breaking the double strand of DNA (Rickman et al. 2020). We hypothesized that Bi might arrest the cell cycle by breaking the DNA double strand. Therefore, we attempted to elucidate whether DNA double-strand breaks caused DNA laddering.

In rice, DNA laddering occurs in salt stress-induced PCD (Lu et al. 2016). In our DNA damage experiments, the genomic DNA of Bi-treated plants was not digested (Fig. 5), indicating that PCD did not cause Bi toxicity to shoot and root cells. We previously reported Bi can cause cell death (Nishimura & Nagata 2021). Our findings suggest that Bi may cause cell death without DNA damage.

In the previous report (Nagata 2015), we showed that 0.1 μM Bi has no negative effect on growth but has some positive effects. In this report, it is shown that 0.1 μM Bi has some effects on ROS accumulation and cell cycles. We think that weak stress from low concentrations of Bi may induce resistance in Arabidopsis. In the future, we would like to investigate the physiological effects of low concentrations of Bi.

In conclusion, Bi reduced $O_2^{\cdot-}$ and H_2O_2 concentrations in leaves and roots. *MSD1* expression levels in Bi-treated *A. thaliana* were decreased. However, *FSD2* and *FSD3* expression levels were not affected by Bi treatment. Bi may induce hydroxyl radicals. In addition, Bi arrested the S phase of the cell cycle. However, the genomic DNA of Bi-treated *A. thaliana* was not digested. To our knowledge, this is the first study to elucidate that Bi disrupts ROS levels and arrests the cell cycle.

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Conflict of interest

The author has no competing interests to declare that are relevant to the content of this article.

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