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Original Article

Potency of silicon in reducing cadmium toxicity in Cempo Merah rice

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Abstract

Cadmium (Cd) is a toxic and harmful heavy metal that contaminates agricultural soil when released into the environment. The beneficial element silicon (Si) can be used to reduce Cd stress. However, the mechanism for reducing Cd toxicity in Cempo Merah rice remains unclear. In this study, we conducted an experiment to determine the required potency of Si for reducing Cd toxicity in the plant. We used a randomized design with two factorials, i.e., calcium silicate and cadmium sulfate at various concentrations (0 mg kg^{-1}, 50 mg kg^{-1}, and 100 mg kg^{-1}) with three replicates. The plants were maintained for 8 weeks. The measured parameters included the Cd, Si, and malondialdehyde (MDA) contents, superoxide dismutase (SOD) activity, and plant biomass. Using microscopy, we observed the anatomical root structure, including the thicknesses of the exodermis, sclerenchyma, and endodermis cell walls. The experimental results showed that an excess of Cd inhibited plant growth and increased the MDA content. Increased plant tolerance against the effect of Cd is required for their growth and survival in overcoming the negative impact of Cd exposure. As silicon is involved in the formation by roots of an apoplast barrier to limit Cd uptake, a combination of Cd and Si treatment in plants was found to produce lower Cd contents in the plant shoots and a minimum Cd translocation factor. The addition of Si was also observed to increase the SOD activity in plants under Cd stress. Therefore, Si is suggested to have potency in reducing Cd toxicity in Cempo Merah rice and increasing rice growth.

Keywords: Cadmium, Growth, Cempo Merah rice, Silicon, Toxicity

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Introduction

Agricultural soil contamination due to the accumulation of cadmium (Cd) causes toxicity in many crops (Nazar et al., 2012). Excessive use of phosphate fertilizer and industrial activities lead to Cd contamination in paddy soils and reductions in crop yields (Roberts, 2014). In non-contaminated soil, the Cd concentration is typically less than 0.5 ppm (Nazar et al., 2012), but the concentration may increase due to natural and anthropogenic factors. In high concentrations, Cd inhibits plant growth (Nazar et al., 2012). Liu et al. (2013) reported that the Cd uptake in sorghum grown in Cd-contaminated soil (50 mg kg^{-1}

and 100 mg kg^{-1} of soil) significantly suppressed growth. Other studies have reported that Cd exposure decreased the growth of peas (*Pisum sativum* L.) exposed for a period of 20 days (Hattab et al., 2009) and *Lemna polyrrhiza* L exposed for a period of 30 days (John et al., 2007). With a longer exposure period, Cd can lead to cell death (Benavides et al., 2005). This growth inhibition is associated with decreased chlorophyll synthesis and the rate of photosynthesis. Hédiji et al. (2010) found there to be a significant decrease of carotenoids and chlorophyll in old tomato leaves exposed to Cd for 90 days.

Another effect of Cd is lipid peroxidation, which is characterized by a high malondyaldehyde (MDA) level (Guo et al., 2017). A Cd-exposed plant experiences oxidative stress, and a consequent increase in reactive oxygen species (ROS) molecules, such as superoxide ($\bullet O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH) (Gill and Tuteja, 2010). Cd is a non-redox heavy metal, that is, it is only indirectly involved in redox reactions, but it can induce oxidative stress by interrupting antioxidant systems and some metabolic processes. The high ROS molecule can have an oxide lipid membrane consisting of poly unsaturated fatty acid, which will easily decompose to MDA (Gill and Tuteja, 2010). Therefore, MDA is often used as an oxidative stress marker.

Certain plants have a protection mechanism when exposed to a heavy metal, such as the ability to deploy an antioxidant defense system or to restrict metal translocation into their shoots by forming an apoplastic barrier in the roots (Benavides et al., 2005; Lux et al., 2011). Superoxide dismutase (SOD) is an antioxidant enzyme that has a role as a first line of defense, whereby it converts the more reactive superoxide ($\bullet O_2^-$) molecule into the less reactive hydrogen peroxide (H_2O_2) molecule (Gill and Tuteja, 2010). Among other plant defense strategies, silicon (Si) is effective in reducing Cd toxicity (Liang et al, 2007; Tang et al., 2015; Farooq et al., 2016). Si is beneficial to plants and has become essential to some plants, especially rice. Reducing mechanisms can work via both enzymatic and non-enzymatic antioxidant stimulation, and that of Si works by stimulating SOD activity (Tang et al., 2015). Si also has role in the formation of root apoplastic barriers to reduce Cd uptake (Liang et al., 2007).

In this study, we used Cempo Merah rice (*Oryza sativa* L.), an original cultivar from Sleman, Yogyakarta, Indonesia. The seed from this cultivar is fluffier and has higher in vitamin and mineral contents than other cultivars (Kristamtini and Purwaningsih, 2009). Rice growth requires the support of an irrigation system, and heavy-metal contamination is unavoidable. The mechanism by which Si reduces Cd toxicity in this plant remains unclear. The goal of this study was to conduct an experiment to determine the potential of silicon in reducing the toxicity of Cd.

Material and Methods

The experiment was conducted in the greenhouse of the Faculty of Agriculture, Universitas Gadjah Mada, Indonesia, with calcium silicate ($CaSiO_3$, Sigma Aldrich) and cadmium sulfate ($3CdSO_4.8H_2O$) as the Si and Cd sources. The growth medium was silt loam soil, the properties of which are listed in Table 1. We used a randomized design with two factorials, i.e., $CaSiO_3$ and $3CdSO_4.8H_2O$ at concentrations of 0 mg kg^{-1}, 50 mg kg^{-1}, and 100 mg kg^{-1}. Calcium silicate was added to the soil during the preparation stage. The selected 'Cempo Merah' rice seedlings were planted in polybags after 2 weeks of germination. The watering volume was adjusted to the water holding capacity. Cd was applied to the soil three times, on the 18^{th}, 27^{th}, and 36^{th} day after the seedlings were planted in the polybags, and the plants were maintained for 8 weeks. We measured the plant biomass (shoots and roots dry weights) to determine the plant growth.

Table-1. Soil properties

Properties	Value
Texture	Silt loam soil
Sand	38%
Silt	55%
Clay	7%
C-organic	0.56%
pH	6.82
Humidity	80–90%
Organic matter	1.37%
Total N	0.12%
Available N	107.28 ppm
Total P	0.04%
Available P	18.04 ppm
Total K	0.05%
Available K	0.72 me.100 g^{-1}
Cation Exchange Capacity	24.07 me.100 g^{-1}
Cd content	<0.01 ppm
SiO_2 content	1921.69 ppm

The measurements of the Cd content in the shoots, roots, and soil were performed using a modified method from that of Hédiji et al. (2010). The Cd

content was determined using flame atomic absorptions spectrometry (ContrAA ®300 Analytic Jena). The Cd translocation factor was obtained by measuring the ratio of the Cd contents in the shoots and roots. Using a spectrophotometer (UV-1800 Shimadzu) at λ 650 nm, we adopted a modified method from that of Wei-min et al. (2005) to measure the Si contents of the shoots and roots. The MDA content was also measured using a spectrophotometer (Genesis UV- Scanning Thermo-Scientific) at λ 532 and 600 nm and the thiobarbituric acid method with some modifications (Velikova et al., 2000).

We measured the SOD activity using a method modified from that of Shi et al. (2005) and a nitroblue tetrazolium (NBT) reagent. Increases in the SOD activity of the sample were indicated by a decrease in the formazan-blue color intensity. One unit of SOD activity in the extract was defined as that causing a 50% inhibition of NBT photo-reduction (Beauchamp and Fridovich, 1971). We prepared a cross section of the root 2 cm from the base using the embedding method (Ruzin, 1999). The anatomical structure of the root, including the thicknesses of the exodermis, sclerenchyma, and endodermis cell walls, was observed using a binocular light microscope (BOECO BM-180). All the presented data are means obtained from three replicate measurements. The data were analyzed using analysis of variance and SPPS Statistic 19 software. We used Duncan's test to evaluate the importance of the discrepancy among measures with p values < 0.05.

Results and Discussion

Cd stress has a negative impact on the life cycle of plants by suppressing their growth. Researchers have reported that a high Cd uptake inhibits plant growth in a variety of species, including for example tomato seedlings (Hédiji et al., 2010), *Pisum sativum* L. (Hattab et al., 2009), and *Lemna polyrrhiza L.* (John et al., 2007). The growth of three sorghum cultivars was reported to be significantly reduced by Cd stress (Liu et al., 2011). The growth parameter in this study was measured based on the plant biomass. Excess Cd was found to significantly reduce the dry weights of shoots and roots (Table 2), and the addition of exogenous Si to Cd-exposed plants significantly increased these weights.

The results indicate that Cd reduced the biomass of Cempo Merah rice (Table 2). The growth inhibition in Cd-stressed plants may occur because Cd affects the plant's metabolism by interfering with the water and mineral uptakes by its roots (Nazar et al., 2012). Because of its high mobility, the Cd^{2+} ion is easily absorbed and transported to aerial organs (Benavides et al., 2005).

Table-2. Dry weights of shoots and roots of Cempo Merah rice treated with Si and Cd

Treatment	Dry weight (g)	
	Shoots	Roots
Si 0 Cd 0 (Control)	12.76 ± 0.36^{d}	1.87 ± 0.21^{cd}
Si 0 Cd 50	11.18 ± 0.69^{b}	1.54 ± 0.07^{b}
Si 0 Cd 100	10.22 ± 0.12^{a}	1.05 ± 0.06^{a}
Si 50 Cd 0	13.75 ± 0.19^{e}	1.96 ± 0.08^{de}
Si 50 Cd 50	12.93 ± 0.24^{d}	1.70 ± 0.06^{bc}
Si 50 Cd 100	11.94 ± 0.27^{c}	1.58 ± 0.05^{b}
Si 100 Cd 0	14.17 ± 0.44^{e}	2.10 ± 0.08^{e}
Si 100 Cd 50	13.73 ± 0.16^{e}	1.90 ± 0.10^{d}
Si 100 Cd 100	12.97 ± 0.23^{d}	1.64 ± 0.11^{b}
Interaction of Si X Cd	+	+

Numbers on columns followed by the different letters indicate a significant difference based on Duncan's test ($p < 0.05$). The (+) symbol indicates interaction between Si and Cd.

This ion does not have its own transporter, however, so it must compete with other ions for the same transporter, such as the ZIP family, NRAMP, and Ca^{2+} channel (Gao et al., 2016). For example, excess Cd was found to inhibit nitrate and K^{+} ion uptakes in wheat (*Triticum durum*) seedlings (Veselov et al., 2003) and to affect the distributions of K, Fe, Zn, Mn and Mg in *Iris lacteal* (Guo et al., 2017). Niazy and Fouda (2016) studied the role of Si in alleviating the effect of Cd in lettuce (*Lactuca sativa* L.) The application of Si (100 mg kg^{-1} and 150 mg kg^{-1} of soil) was found to increase the dry weight of plants grown in Cd-contaminated soil with Cd concentrations of 5 mg kg^{-1}, 10 mg kg^{-1}, and 15 mg kg^{-1} of soil.

Silicon may have sufficient potency to reduce the negative effect of toxic heavy metals in plants, as described by Liang et al. (2007) and Bath et al. (2019). These mechanisms may include: 1) immobilizing the heavy-metal ion in growth media by increasing the soil pH and the co-precipitation of the toxic ion and Si in growth media; 2) stimulating both enzymatic and non-enzymatic antioxidant systems; 3) establishing the co-precipitation of toxic metal ions and Si in cell walls; and/or 4) stimulating compartmentation of the toxic ion in vacuoles by the formation of a phytochelatine–metal ion complex. Ma and Yamaji (2006) reported that Si could interact with several

components in plant cell walls to form silica (SiO_2), which means that this substance promotes cell wall strength and rigidity, increases the mechanical support of the aerial parts of plants, and enhances plant growth.

In this study, we investigated the role of Si in reducing Cd toxicity in Cempo Merah rice based on the following parameters: the Cd contents in shoots, roots, and soil; the Cd translocation factor value; shoot and root Si contents, some biochemical parameters were also determined, including the MDA level and SOD activity, as well as the root anatomy with respect to the thickening of cell walls, which has been suggested as having a role in the Cd-blocking mechanism.

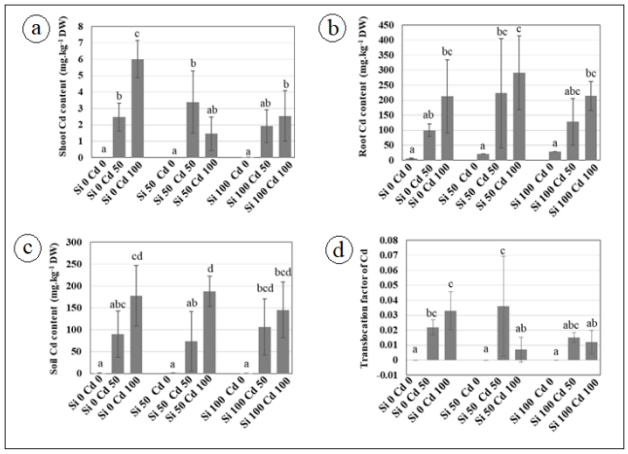

Figure 1. Cd contents in: (a) shoots and (b) roots and (c) soil treated with Si and Cd; (d) Cd translocation factor values in plants. According to the results of Duncan's test, bars with different letters indicate significant differences at $p < 0.05$.

Figures 1a–1c show the Cd uptake and translocation into plants based on the Cd contents of the shoots, roots, and soil. The shoots of plants treated with Cd (100 mg kg^{-1}) showed the highest Cd contents (Figure 1a) and this content was lower in plants treated with a combination of Cd and Si. The Cd content in the roots of the control, Si-treated, and Cd-treated plants (100 mg kg^{-1}) were significantly different (Figure 1b), however, the Cd contents in the roots of plant treated with only Cd and with the Si–Cd combination showed no significant difference ($p < 0.05$). A trend can be observed in which Cd had accumulated more in the roots of plants treated with the Si–Cd combination than in those treated with Cd only. This result might be related to the lower Cd translocation factor in plants treated with the Si–Cd combination than in plants treated only with Cd (Figure 1d). The Cd contents in soils of the control and Cd-treated plants were significantly different ($p < 0.05$) (Figure 1c), with the Cd content in plants treated with Si 50 Cd 100 being the highest.

The Cd content that accumulates in plants might be correlated with the stress level in Cempo Merah rice. The authors of one study reported that the application of exogenous Si into Cd-exposed media decreased the Cd content in ramie (Tang et al., 2015). However, that finding seems to contradict the results obtained here. In our study, the addition of Si to Cd-stressed plants resulted in higher Cd contents in roots than those to which Si had not been added (Figure 1b). The high accumulation of Cd in the roots might have occurred due to the internal role of Si in blocking the Cd apoplast pathways (Liang et al., 2005). Si treatment could decrease the Cd translocation factor in Cd-stressed plants and limit the Cd accumulation in shoots (Figures 1d and 1a). Tripathi et al. (2012) and Shi et al. (2015) investigated the role of Si in reducing the Cd contents in the shoots of Cd-exposed maize and rice. In addition, the protective role of Si in limiting the Cd translocation ability in ramie was reported by Tang et al. (2015). Liu et al. (2013) noted that silicon could accumulate in cell walls. The co-deposition of Si and Cd in cell walls might explain the limitation on the uptake of the Cd ion (Liu et al., 2013). Vaculík et al. (2012) also reported that Si could enhance the binding of Cd to the apoplasmic fraction in maize. Thus, it is possible that more Cd had accumulated in the roots of the rice treated with Si.

The Si contents in the shoots and roots fluctuated (Figure 2). The Si content in the shoots of plants treated with Cd showed a significant difference from those with other treatments ($p < 0.05$) (Figure 2a). However, unexpectedly, the Si content of shoots in the control plants were the highest. The Si contents of the roots showed no significant difference among the treated plants, except for those treated with Si (50 $mg.kg^{-1}$) (Figure 2b). The Si content in plants fluctuated due to the Si uptake by roots having been influenced by the level of abundantly available Si already present in the paddy soil. In addition, because rice is Si accumulator, each individual plant has a different Si uptake ability. The addition of exogenous Si into the soil thus tended to increase the Si content in the rice, which served to reduce the negative effects of Cd in Cempo Merah rice.

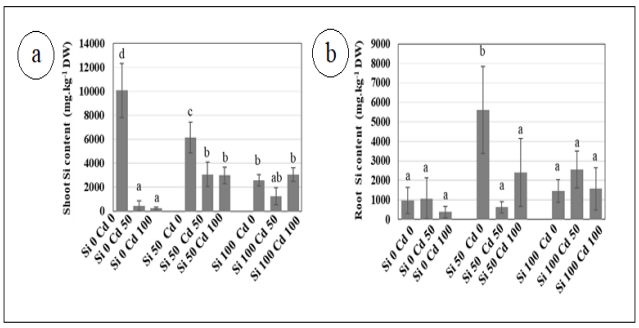

Figure 2. Si contents in: (a) roots and (b) shoots of plants treated with Si and Cd. According to Duncan's test, bars with different letters differ significantly at $p < 0.05$.

ROS molecules are transiently generated in plant cells during photosynthesis and cellular respiration, but their production will increase as an indirect consequence of heavy-metal toxicity (Gill and Tuteja, 2010; Stańczyk et al., 2005). Despite being an integral part of signal transduction and essential for intercellular communication, when produced in high quantities, they damage proteins, lipids, and nucleic acid, which ultimately causes oxidative stress. The Cd^{2+} ion is a non-redox heavy metal that is indirectly involved in redox reactions (Hossain et al., 2012). Thus, Cd contributes to the stress of plants and MDA is one of the important lipid peroxidation products.

The experimental results showed that excess Cd tended to increase the MDA contents (Figure 3a), but the addition of exogenous Si to Cd-exposed plants significantly decreased their MDA contents ($p < 0.05$). Plant treated with Cd (100) exhibited the highest MDA content, whereas plants treated with the Si (100) and Cd (50) combination exhibited the lowest MDA content. Many studies have reported that Cd stress increases the MDA content in various species, including for example *Iris lactea* L. (Guo et al., 2017) and *Populus canescens* (Dai et al., 2012). The addition of Si has been suggested to protect rice by reducing the MDA content (Figure 3a), and this role has been observed in Cd-exposed rice and ramie (Tripathi et al., 2012; Tang et al., 2015).

The main function of SOD is to scavenge ROS molecules to reduce the impact of Cd toxicity. Plants treated with Cd showed increased SOD activity (Figure 3b). SOD is an antioxidant enzyme that has crucial role as the first line of defense in scavenging ROS molecules by converting the superoxide radical ($\bullet O_2^-$) into hydrogen peroxide (H_2O_2), a moderately reactive molecule (Gill and Tuteja 2010; Stańczyk et al., 2005).

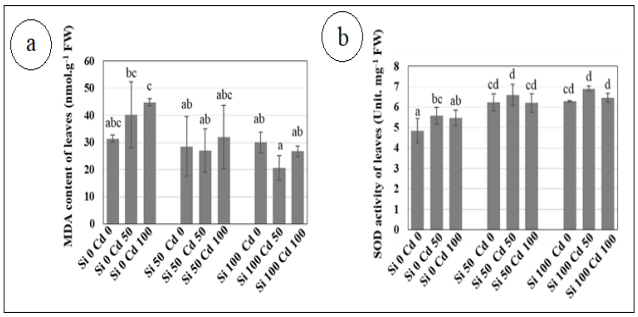

Figure 3. (a) MDA levels and (b) SOD activities in leaves of red rice (Oryza sativ L. 'Cempo Merah') treated with Si and Cd. According to Duncan's test, bars with different letters differ significantly at $p < 0.05$.

Cd treatment increased the SOD activity of the plant for its own defense. Exogenous Si application in Cd-exposed plants resulted in an increase in SOD activity, with the highest SOD activity observed in plants treated with Si (100) and Cd (50) (Figure 3b). Silicon has been reported by Tang et al. (2015) to increase SOD activity in supporting the defense of plants under Cd stress.

Table-3. Exodermis, sclerenchyma, and endodermis cell walls, and root diameters of 'Cempo Merah' red rice with Si and Cd treatments

Treatments	Thickness of cell walls (µm)		
	Exodermis	Sclerenchyma	Endodermis
Si 0 Cd 0	1.43 ± 0.03^{a}	2.26 ± 0.19^{abc}	3.02 ± 0.99^{a}
Si 0 Cd 50	1.51 ± 0.05^{ab}	2.04 ± 0.16^{ab}	3.30 ± 0.12^{ab}
Si 0 Cd 100	1.41 ± 0.02^{a}	1.68 ± 0.31^{a}	3.02 ± 0.23^{a}
Si 50 Cd 0	1.63 ± 0.07^{bcd}	2.77 ± 0.26^{bc}	3.10 ± 0.21^{a}
Si 50 Cd 50	1.70 ± 0.09^{cd}	2.84 ± 0.37^{c}	3.30 ± 0.31^{ab}
Si 50 Cd 100	1.74 ± 0.18^{cd}	2.93 ± 0.07^{c}	3.36 ± 0.07^{ab}
Si 100 Cd 0	1.80 ± 0.07^{d}	2.96 ± 1.03^{c}	4.06 ± 0.36^{b}
Si 100 Cd 50	1.63 ± 0.06^{bc}	3.05 ± 0.34^{c}	3.58 ± 0.52^{ab}
Si 100 Cd 100	1.52 ± 0.11^{ab}	2.52 ± 0.04^{bc}	3.65 ± 0.66^{ab}
Interaction of Si X Cd	+	-	-

Numbers in columns followed by the different letters show significant differences based on the Duncan's test ($p < 0.05$). (+) interaction between Si and Cd; (-) indicates no interaction between Si and Cd.

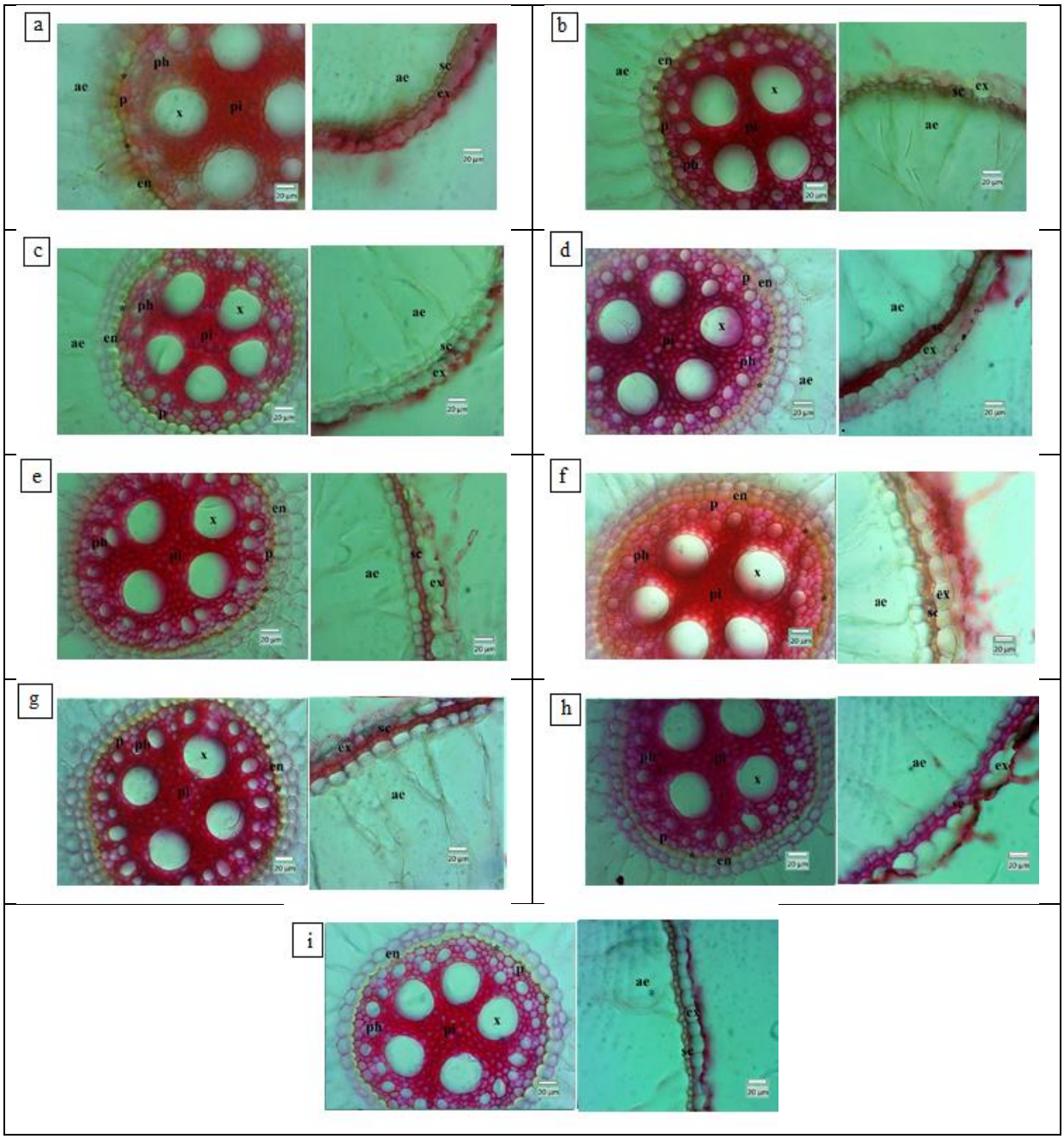

Figure 4. Root anatomical structure of rice plants treated with: (a) control, (b) Cd 50, (c) Cd 100, (d) Si 50, (e) Si 50 and Cd 50, (f) Si 50 and Cd 100, (g) Si 100, (h) Si 100 and Cd 50, and (i) Si 100 and Cd 100 mg kg^{-1}. From left to right: stele and peripheral regions. Abbreviations: x (xylem), ph (phloem), p (pericycle), en (endodermis), pi (pith), ae (aerenchyme), sc (sclerenchyme), and ex (exodermis). (*): U-type endodermic wall thickening. Bars: 20 µm.

Figure 4 shows transverse cross sections of rice roots. As we can see, thickening occurred in the cell walls of the endodermis, sclerenchyma, and exodermis of roots treated with Si and a combination of Si and Cd. This descriptive analysis was confirmed by the measurement of the thicknesses of these cell walls (Table 3).

The role of roots is to absorb water and nutrients, but they can also absorb undesirable compounds such as toxic metals. Although plants absorb Cd^{2+} ions through symplast and apoplast pathways, they also have tolerance mechanisms for limiting the

translocation of Cd into their shoots. In the symplast pathway, the uptake of Cd is limited by Cd chelators known as phytochelatins, which play a Cd detoxification role in plants by chelating and sequestering Cd ions in vacuoles (Gao et al., 2016). In the apoplast pathway, Cd uptake is restricted by the thickening of the exodermis, endodermis, and sclerenchyma cell walls of the roots (Lux et al., 2011). The exodermis and endodermis enable roots to establish and maintain selectivity with respect to ion uptakes.

The suberization of the cell walls of the exodermis and endodermis and the lignification of cell walls of the sclerenchyma are reported to result in an important barrier in the apoplast pathway and the means by which nutrient uptakes are controlled (Lux et al., 2011). The observed root anatomy in Cempo Merah rice treated only with Si and that treated with a combination of Si and Cd showed thickenings of the cell walls of the roots' endodermis, sclerenchyma, and exodermis (Figure 4, Table 3). The addition of Si is thought to increase the suberization and lignification activities of these cell walls. In maize roots, Si is reportedly deposited within the cell walls of the endodermis and pericycle, which seemed to serve as a tolerance mechanism in maize against Cd stress (Da Cunha and Nascimento, 2009). In this study, suberized and lignified rice roots enabled the plants to limit the Cd translocation from their roots to their shoots.

Conclusion

In conclusion, excess Cd in the soil was found to inhibit rice growth and increase the MDA content in Cempo Merah rice. To deal with the negative impacts of Cd exposure, plant tolerance to Cd must be increased to ensure its survival and growth. We suggest that silicon is involved in the formation of a root apoplast barrier that limits Cd uptake and increase SOD activity. Therefore, we suggest that Si has potency in reducing Cd toxicity in *Oryza sativa* L. Cempo Merah rice plants and increasing their growth.

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Contribution of Authors

Khasanah RAN: Conceived idea, designed research methodology, data collection, data interpretation, statistical analysis, manuscript writing and manuscript final editing
Rachmawati D: Conceived idea, designed research methodology, manuscript writing, manuscript final reading and approval