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Short Communication

Silicon deficiency promotes lignin accumulation in rice

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Abstract Although both lignin and silica accumulate on cell walls and confer rigidity, mechanical strength, and resistance to pathogen invasion in rice, it remains unclear whether silicon deficiency affects lignin accumulation. We demonstrate that low silicon rice mutated in a silicon influx transporter, Lsi1, or a silicon efflux transporter, Lsi2, contained larger amounts of lignin in the rice straws. Furthermore, wild-type rice cultivated on low silicon media also accumulated larger amounts of lignin in shoots. Significant accumulation of guaiacyl lignin upon silicon deficiency was determined by nitrobenzene oxidation analysis. These data indicate a negative correlation between silicon accumulation and lignin deposition.

Key words: Lsi1, Lsi2, silicon, lignin, rice

Silicon is accumulated as opaline silica in rice plant bodies and is essential for growth and production. Silica helps rice plants overcome both biotic and abiotic stresses such as pathogen infections, pests, lodging, drought and nutrient imbalance (Ma et al. 2011). In rice, silica accumulates mainly on cell walls, forming silica-cuticle double layers and silica-cellulose double layers in the surface of leaves, stems and hulls (Ma et al. 2006). It was also suggested that silica is combined in some way with phenol-polysaccharide or lignin-polysaccharide compounds (Inanaga and Okasaka 1995). In fact, *in vitro* simulation experiments showed that silica deposition in aqueous borax solution is induced by the addition of soluble lignin from rice (Fang and Ma 2006). In roots of gramineous plants, a sequential chain of events involving polyphenols, suberin, lignin and silica deposition may lead to a strengthening of the endodermal cell walls (Parry and Kelso 1975). These data suggest that a chemical and physiological interaction between silica and cell wall constituents including lignin exists.

Lignin is a complex polymer formed by the oxidative radical coupling of 4-hydroxycinnamyl alcohols (monolignols) and related compounds. To date, three types of lignins have been characterized, namely, *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) lignins. H-lignin is composed of *p*-hydroxyphenyl units and is characteristic of grasses. G-lignin is derived from coniferyl alcohol and is a major component of gymnosperm lignin. S-lignin is present in grasses and angiosperms, and is derived from sinapyl alcohol (Dixon et al. 2001; Higuchi 1985; Ralph 2010; Umezawa 2010).

Because lignin, like silica, confers to cell walls rigidity, mechanical strength, and resistance to pathogen invasion, it can be rationalized that the deposition of silica and lignin may be coordinately regulated in rice plants. However, it remains unclear whether silicon deficiency affects lignin accumulation.

To address the question, we first analyzed the lignin content of straws of wild-type rice and the mutants defective in silicon uptake, *lsi1*, *lsi2*, *G139*, *D85* and *10-64*. Here, *lsi1*, *G139* and *10-64* are allelic mutants defective in silicon influx transporter Lsi1, which were isolated from the cultivars Oochikara, Koshihikari and Nipponbare, respectively (Ma 2007; Ma et al. 2006). On the other hand, *D85* and *lsi2* are allelic mutants defective in silicon efflux transporter Lsi2, whose corresponding wild type cultivars are Koshihikari and Taichung-65, respectively (Ma 2007; Ma et al. 2007). Both mutant

Abbreviations: AIR, alcohol-insoluble residue; G-lignin, guaiacyl lignin; H-lignin, *p*-hydroxyphenyl lignin; Hyd, *p*-hydroxybenzaldehyde; HydA, *p*-hydroxybenzoic acid; Lsi1, Low silicon rice1; Lsi2, Low silicon rice2; S-lignin, syringyl lignin; Syr, syringaldehyde; SyrA, syringic acid; Van, vanillin; VanA, vanillic acid.

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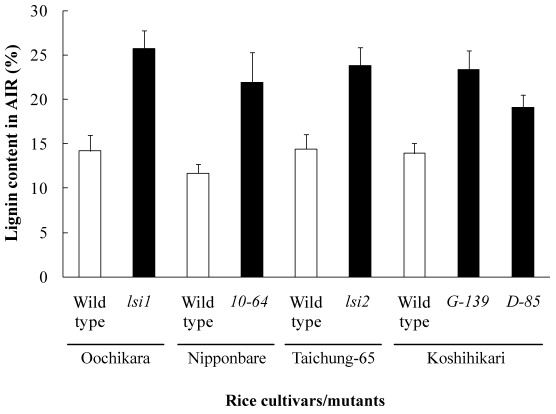

Figure 1. Lignin content in wild-type and mutant rice. Lignin was determined as thioglycolic acid lignin in AIR prepared from straw of parent cultivars and their mutants defective in silicon uptake grown in a paddy field. Error bars represent standard error of ten biological replicates. Lignin contents of all mutants showed statistically significant difference from those of the corresponding wild types by Students's *t*-test at $p<0.001$.

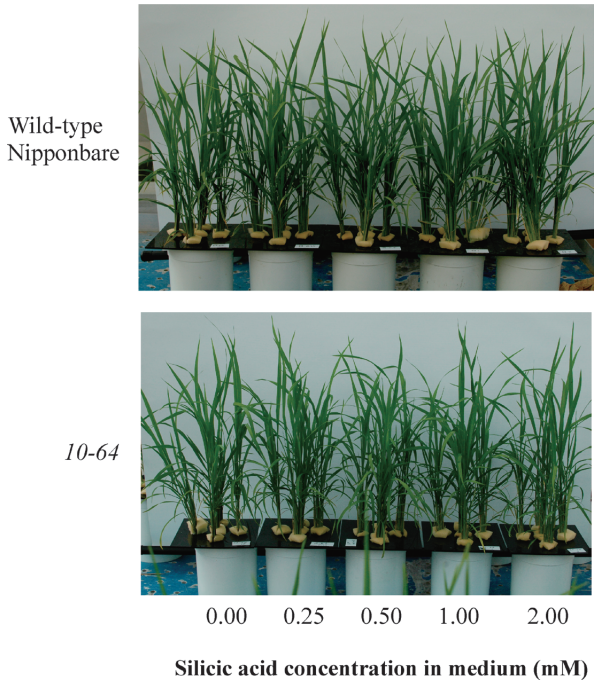

Figure 2. Growth of wild-type rice and mutant at different silicic acid concentrations. Both wild-type rice (cv. Nipponbare) and *Lsi1* mutant (*10-64*) plants were cultivated in a nutrient solution containing different concentrations of silicic acid for one month.

types cannot properly take up silicon from soil, leading to a lower content of silicon (Ma et al. 2006, 2007). These lines were grown on a paddy field at the Institute of Plant Science and Resources, Okayama University, Kurashiki, from mid June to end of September in 2006. The rice straws without panicles were first dried in a 60°C oven for 2 days. Next, each tiller was excised by scissors and pulverized for 2 min at 25 Hz in stainless steel ball mills using TissueLyzer (Qiagen, Valencia, CA). The resulting

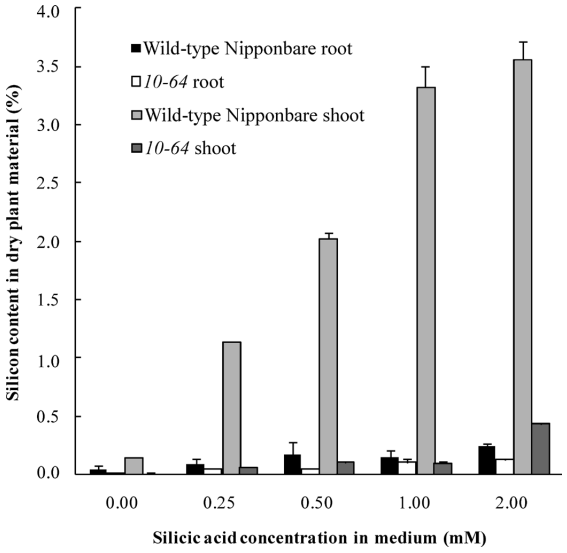

Figure 3. Silicon content in the shoots and roots of wild-type and mutant rice. Samples were taken from wild-type rice (cv. Nipponbare) and *Lsi1* mutant (*10-64*) plants grown in a nutrient solution containing different concentrations of silicic acid. Error bars represent standard error of three biological replicates.

powder was repeatedly extracted with methanol to give alcohol-insoluble residue (AIR), and lignin in AIR was measured as thioglycolic acid lignin using a previously described method (Suzuki et al. 2009).

Regardless of which cultivar or gene mutation was analyzed, lignin content was higher in the mutants than in the corresponding wild type (Figure 1). Recently, a similar result showing that a low silicon mutant, *lsi1*, accumulated larger amounts of lignin was independently reported (Yamahata et al. 2010).

Our data (Figure 1) clearly indicated that the low silicon mutants contained larger amounts of lignin, suggesting a connection between silicon deficiency and higher lignin accumulation in rice. To prove this, both the wild-type rice (cv. Nipponbare) and *Lsi1* mutant (*10-64*) were grown in a nutrient solution containing 0.00, 0.25, 0.50, 1.00, or 2.00 mM Si as silicic acid as previously described (Ma et al. 2006). After one-month growth (Figure 2), the shoots and roots were harvested separately, and the silicon content in different organs was determined as previously described after digest with HF-H_2O_2 and HNO_3 (Tamai and Ma 2008). Analysis of silicon content of these plants revealed increasing silicon content in shoots and roots of the wild type with increasing medium silicic acid concentration; however, in *10-64*, the silicon content was much lower compared with the wild type (Figure 3).

Because silicon content in the shoots was drastically affected by medium silicic acid concentration (Figure 3), we determined lignin in AIR from the shoots as thioglycolic acid lignin as above to assess the correlation between silicon content and lignin accumulation. As

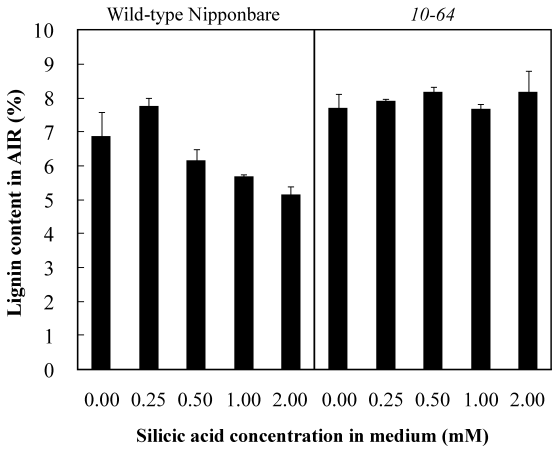

Figure 4. Lignin content in the shoots of wild-type and mutant rice. Both wild type (cv. Nipponbare) and *Lsi1* mutant (*10-64*) were grown in a nutrient solution containing different silicic acid concentration for one month. Lignin in the shoots was determined as thioglycolic acid lignin in AIR. Error bars represent standard error of three biological replicates.

shown in Figure 4, thioglycolic acid lignin analysis revealed that lignin content in the shoots of wild-type plants decreased as the silicic acid concentration increased in the medium. In contrast, lignin content in *10-64* was not significantly affected by medium silicic acid concentration. These results indicate that silicon deficiency promotes lignin accumulation, which is contrast to the result by Fleck et al. (2011). They proved that silicon enhanced suberization and lignification in rice roots. The regulatory mechanism of lignin accumulation as a response to silicon content might be different between roots and shoots in rice.

Finally, to examine which type of lignin accumulated in low silicon conditions, we completed nitrobenzene oxidation analysis (Katahira and Nakatsubo 2001) with slight modifications (Umezawa et al. 2007) of AIR from the shoots of wild-type and mutant *10-64* plants cultured in different silicic acid concentrations (Figure 2). As shown in Figure 5, yields of vanillin (Van) and vanillic acid (VanA) from the shoots of wild-type rice plants cultured under no silicic acid (0.00 mM) or low silicic acid (0.25 and 0.50 mM) concentrations were about double of those from plants cultured in a higher silicic acid concentration (2.00 mM). Additionally, the ratios of Van+VanA to syringaldehyde (Syr) + syringic acid (SyrA) and *p*-hydroxybenzaldehyde (Hyd)+*p*-hydroxybenzoic acid (HydA) were higher at low silicic acid concentrations (Table 1). This indicates that in the wild type, G-lignin is predominantly synthesized under low silicon conditions. A similar tendency was observed in the yield of Van+VanA (Figure 5) and the ratio of Van+VanA to Syr+SyrA and Hyd+HydA (Table 1) in *10-64* plants.

Although the lignin content was similar between wild type and *10-64* on the same low silicon medium (0.25 mM) (Figure 4), the amount of Van from *10-64* was much lower than that from wild type, but the yield of other products was not largely different after nitrobenzene oxidation (Figure 5). The reason is not clear. One possible explanation is that such substructures resistant to nitrobenzene oxidation as 5,5-dilignol or *β*,*β*-dilignol moieties (Chang and Allan, 1971) might be more produced in *10-64* than in wild type.

In conclusion, we demonstrated silicon deficiency promotes lignin accumulation in the shoots of rice plants. The major accumulated lignin was G-lignin. Our results might give a hint to produce ash-free Gramineae energy crops with large amount of lignin that generate high energy by combustion. The molecular mechanism

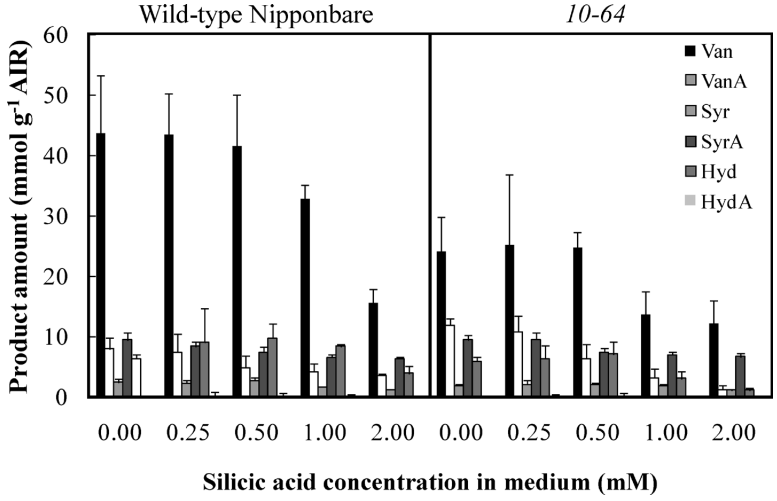

Figure 5. Amount of nitrobenzene oxidation products from wild-type and mutant rice. Both wild type (cv. Nipponbare) and *Lsi1* mutant (*10-64*) were grown in a nutrient solution containing different concentrations of silicic acid for one month, and AIR prepared from the shoots was subjected to the analysis. Van, vanillin; VanA, vanillic acid; Syr, syringaldehyde; SyrA, syringic acid; Hyd, *p*-hydroxybenzaldehyde; HydA, *p*-hydroxybenzoic acid. Error bars represent standard error of three biological replicates.

Table 1. Ratios of vanillin (Van)+vanillic acid (VanA), syringaldehyde (Syr)+syringic acid (SyrA), and *p*-hydroxybenzaldehyde (Hyd)+*p*-hydroxybenzoic acid (HydA) produced after nitrobenzene oxidation of the shoots. The values were calculated from the average values indicated in Figure 5.

Silicic acid concentration in medium (mM)	Wild-type Nipponbare			*10-64*		
	Van+VanA	Syr+SyrA	Hyd+HydA	Van+VanA	Syr+SyrA	Hyd+HydA
0.00	0.74	0.17	0.09	0.68	0.21	0.11
0.25	0.72	0.15	0.13	0.67	0.22	0.12
0.50	0.70	0.15	0.15	0.65	0.20	0.15
1.00	0.69	0.15	0.16	0.59	0.31	0.10
2.00	0.62	0.25	0.13	0.60	0.35	0.05

linking silicon deficiency and lignin accumulation remains to be elucidated; the identification of the specific tissues and cells that accumulate G-lignin and the up-regulated genes upon silicon deficiency may offer a clue to elucidate the mechanism.

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