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Structural basis for a root silicon-based barrier against cadmium, chromium and salt stress in plants

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Brief heading

Abiotic stresses such as salinity and toxic elements are threatening plant growth. Silicon (Si) deposition in the roots of Poaceae plants enhances stress resistance, but most dicots cannot efficiently form such Si barriers. This study reveals the role of mixed-linkage glucan (MLG) in the formation of root Si barriers. Introducing the rice MLG synthase gene may enable Si non-accumulators to benefit more from Si fertilization. Further development of strategies to strengthen plant Si barriers is expected to improve the sustainability of agriculture and forestry.

Summary

- Silicon (Si) forms a defensive barrier in roots of Si accumulators such as rice, blocking the absorption of toxic metals and salt, but many dicotyledons do not accumulate Si, limiting the Si application in agriculture. This may be related to differences in their cell walls: mixed-linkage glucan (MLG) exists in cell walls of monocotyledons but is absent in dicotyledons.
- *In vivo* and *in vitro* experiments, material characterization, and genetic modification approaches were employed to investigate the structural roles of MLG in root Si barriers and its effects on plant stress resistance.
- Both the roots of Si transporter Lsi2 (low silicon rice 2) defective rice mutants and dead rice continued to accumulate Si, showing root Si deposition is controlled by cell wall components, but not transporters. MLG induced Si polymerization *in vitro* and *CslF6* (encoding an MLG synthase) controlled root MLG and Si concentrations. Overexpression of rice *CslF6* in *Arabidopsis*, an Si non-accumulator, enabled them to form Si barriers, enhancing resistance to cadmium, chromium and salt stress.
- These findings suggest that MLG serves as a structural basis for root Si barriers and that genetic modification combined with Si supply is a promising strategy to endow Si non-accumulators with Si-mediated stress resistance.

Keywords

Silicon; CslF6; Mixed cross-linked glucan; Toxic metals; Salt; Rice; Si non-accumulators

Introduction

Plants inevitably encounter a range of abiotic stresses during their growth. Silicon (Si) has demonstrable benefits for plants in terms of resisting these stresses (Cooke & Leishman, 2016; Thorne *et al.*, 2020) and Si-accumulators cope with soil-based stresses such as toxic metal and salt stress by forming Si-based defense barriers (Liang *et al.*, 2007; Ranjan *et al.*, 2021; Asgher *et al.*, 2024; Pang *et al.*, 2024a). Silicon absorbed by roots in the form of silicic acid is deposited in the apoplast, particularly on the cell wall, to form a barrier (Epstein, 1994; Shivaraj *et al.*, 2022; Mitani-Ueno *et al.*, 2023) that reduces the entry of toxic elements and salt into cells and their translocation to the shoot through increasing adsorption and blocking diffusion (Coskun *et al.*, 2019; Pang *et al.*, 2024b; Yuan *et al.*, 2024).

Toxic metal pollution and salt stress have posed a systemic threat to global food security (Zhu, 2016; Hou *et al.*, 2020; Sanchez-Munoz *et al.*, 2025). Approximately 14%-17% of arable land worldwide is contaminated with toxic metals (Hou *et al.*, 2025), with cadmium (Cd) and chromium (Cr) being of particular concern, as their exceedance rates against agricultural thresholds (1 and 100 mg/kg) reach 9% and 3.2%. Approximately 1.1 billion hectares of arable land are affected by salinization (Guo *et al.*, 2025); about 60% of salinized land is highly sodic (Zhang *et al.*, 2023). These stresses not only reduce crop yields, but some toxic metals also endanger human health through the food chain, placing nearly 1 billion people in these regions with elevated public health and ecological hazards (Rezapour *et al.*, 2023; Hou *et al.*, 2025). Therefore, there is an urgent need to explore effective methods to reduce plants' absorption of toxic elements and salt and alleviate the impacts on crop growth they cause.

Many globally significant Poaceae crops are Si accumulators, and currently, Si fertilizers have been widely applied to rice, wheat, maize, sorghum, and sugarcane to enhance their yield and stress resistance (Galindo *et al.*, 2021; Yang *et al.*, 2024; Pang *et al.*, 2025a). However, in contrast, many dicotyledonous plant families, such as Brassicaceae, are Si non-accumulators (Hodson *et al.*, 2005; Trembath-Reichert *et al.*, 2015) and fail to form effective Si-based defense barriers (Wang *et al.*, 2020; Shivaraj *et al.*, 2022). As a result, the beneficial effects of Si in these plants are far less pronounced than in Si-accumulators, significantly limiting the scope of Si fertilizers to improve performance in Si non-accumulators, such as *Arabidopsis* and pakchoi (Vivancos *et al.*, 2015; Wang *et al.*, 2020; Pang *et al.*, 2024c). Therefore, the development of methodologies to enable Si utilization in Si non-accumulators is pivotal for broadening the agricultural benefits derived from the application of Si fertilizers and enhancing Si use efficiency.

This endeavor is hindered by our lack of understanding of the mechanisms which underpin the formation of Si defensive barriers in plant roots, and the reasons why this process is absent in some plant species. The difference of Si utilization between Si-accumulators and Si non-accumulators could be attributed to differences in the expression level, localization, and polarity of Si transporters that promote the uptake and transport of silicic acid (Ma & Yamaji, 2015; Deshmukh *et al.*, 2020). A series of proteins regulating Si uptake and transport (Lsi1, Lsi2, Lsi3, Lsi6, and SIET4) have been reported in Si-accumulators such as rice, while *Arabidopsis thaliana* lacks these proteins (Ma *et al.*, 2006; Ma *et al.*, 2007; Mitani-Ueno *et al.*, 2023). However, the silicon transporter Lsi1, which significantly influences silicon concentration in rice shoots, has no effect on root silicon concentration (Ma *et al.*, 2002). Roots play a critical role in plant responses to toxic element stress, as they serve as the primary site for toxic element uptake and the first point of contact for toxic element exposure (Shahid *et al.*, 2017; Ivanov & Zhukovskaya, 2021). Many crops' roots exhibit substantial Si deposition on the cell walls of both their surface and endodermis (Lux *et al.*, 1999; Lux *et al.*, 2020; Pang *et al.*, 2023a), and this trait plays a crucial role for blocking toxic elements from entering shoots via the apoplastic pathway (Thorne *et al.*, 2022; Pang *et al.*, 2025b). To better mitigate the challenges associated with toxic metals and salt stress, it is essential to elucidate the mechanisms of Si accumulation in roots and develop strategies to strengthen the root Si-based defense barriers.

Several mechanisms involving different cell wall components have been suggested to play a role in biosilicification (Guerriero *et al.*, 2016; Sheng & Chen, 2020; Zexer *et al.*, 2023). Specific cell wall components of Si-accumulators proposed as promoting silicic acid polymerization. include callose (Law & Exley, 2011; Brugiére & Exley, 2017); hemicellulose (He *et al.*, 2015) lignin (Zexer & Elbaum, 2020; Zancajo *et al.*, 2022), and protein (Ayieko *et al.*, 2023). Compared to Si non-accumulating dicotyledons, cell walls of monocotyledons such as Poaceae (Si-accumulators) contain a small amount of xyloglucan and pectin, but more mixed-linkage glucan (MLG, i.e. glucose connected by bonds 1, 3, and 1, 4) (Burton & Fincher, 2009) and has been suggested as a structural template for Si deposition (Fry *et al.*, 2008; Kido *et al.*, 2015), although mechanistic evidence to support this remains lacking, especially in roots. Here we test the hypothesis that MLG could serve as the structural basis for the formation of root Si-based defense barrier in Si-accumulators. Using *Oryza sativa* L. cv. ZH11 (a typical Si accumulator), and *Arabidopsis thaliana* L. cv. Columbia (a typical Si non-accumulator), this study sought to answer the following questions:

1) Is it cell wall components rather than Si transporters that affect Si accumulation in roots and the formation of root Si barriers?
2) Does MLG specifically induce Si polymerization and if it does, what is the underlying mechanism?
3) Can root Si barriers in Si non-accumulators be enhanced through genetic modification and, if so, does this lead to improved stress resistance?

Materials and Methods

Plant growth conditions

Rice seeds (*Oryza sativa* L. cv. ZH11) were disinfected with 10% hydrogen peroxide for 10 minutes, washed three times with deionized water, placed on moist filter paper, and germinated for five days under dark conditions at 30°C (Pang *et al.*, 2023b). The rice seedlings were then transferred into 1/4 strength Kimura B nutrient solution (pH adjusted to 6) and replaced every seven days. No additional Si was added to the nutrient solution, except in the Si treatment group. The temperature of the artificial climate chamber was 30°C, humidity was 55%, and light and dark hours were 14 and 10 hours.

All *Arabidopsis* seeds (*Arabidopsis thaliana* L. cv. Columbia) were surface sterilized with 10% (v/v) NaClO and 1% (v/v) Triton X-100 for five minutes, then treated with 75% (v/v) ethanol for 1 min. Seeds were rinsed for three times with deionized water and stored at 4°C for two days before sowing. Then, the sterilized seeds were sown on solid half-strength Murashige and Skoog (MS) medium containing 1% (w/v) agar and 0.5% (w/v) sucrose (pH 5.7) (Sun *et al.*, 2022). All *Arabidopsis* seedlings were grown vertically at 22°C with a day/night cycle of 12/12 h. After seven days, they were transferred to 1/2 Hoagland solution renewed every seven days.

Comparison of Si deposition in roots of different plants after excluding Si transporters

Dead plants were used to rule out the possible impact of root Si transporters on root Si concentration. To compare the differences in root Si deposition of dead rice and *Arabidopsis*, rice and *Arabidopsis* seedlings grown for 21 days were heated for 10 minutes in a boiling water bath to kill them. Some killed plants were dried and used to determine the initial Si concentration in roots. Then, other killed rice and *Arabidopsis* plants were transferred to water containing 1.0 mM silicic acid (produced by passing sodium silicate [CAS: 1344-09-8, Aladdin] solution through a column packed with ion exchange resin; 1.0 mM concentration of silicic acid is widely used in plant physiology experiments) (Pang *et al.*, 2023a; Pang *et al.*, 2024b). After five days, the plant roots were repeatedly cleaned with EDTA and deionized water and then dried. For determination of Si in plant roots, the roots were digested in the Microwave Digestion System using a mixture of 4 ml of HNO_3, 4 ml of H_2O_2, and 1 ml of HF

(Mitani-Ueno *et al.*, 2023). The Si concentration was determined by an inductively coupled plasma optical emission spectrometer (ICP-OES; PE-7000DV, USA) after eliminating the acid and setting a constant volume to 10 ml. In addition, rice roots were cut into 1 cm segments, which were fixed on the sample stage of scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDS) (Xiao et al., 2022). The root segments were placed longitudinally and transversely relative to the root axis, respectively. After freeze-drying and gold-sputter coating, SEM-EDS (ZEISS Gemini 300, Germany; EHT = 3 kV, Mag = 10 KX, WD = 5.5mm, Signal A = InLens) was used to observe the morphological characteristics of Si deposition on the root surface (Pang *et al.*, 2023a); meanwhile, the point scanning mode of EDS was employed to quantitatively analyze the mass fraction of Si in the root interior. The specific testing method was as follows: three detection sites were randomly selected from each root segment, and three technical replicates were set for each site to ensure data reliability.

In addition, 21-day-old live *Lsi2*-defective mutants of rice and their wild-type counterparts (cv. Taichung-65) were grown in 1/4 Kimura B nutrient solution containing 1 mM silicic acid for 5 days (mutant seeds were obtained through mutagenesis breeding and provided by Prof. Jian Feng Ma from Okayama University, Japan) (Ma *et al.*, 2007). Subsequently, the roots were repeatedly rinsed with EDTA and deionized water. After drying, the tissues were digested, and Si concentration was determined using the same method as described above.

Determination of the ability of MLG to induce silicic acid polymerization

Cellulose is the most common component in the cell walls of all plants, and in this study, it was used as a comparative reference for MLG. (1,3;1,4)-β-glucan (mixed linkage glucan, CAS: 9041-22-9, Purity: 80%) and (1,4)-β-glucan (cellulose, CAS: 9004-34-6, Purity: 99%) were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. In the experiment, 0.05 g of mixed linkage glucan (MLG) and cellulose were mixed separately with 10 mL of silicic acid solutions at concentrations of 1 mM, 2 mM, 5 mM, and 10 mM, respectively. As silicic acid undergoes spontaneous polymerization above 2 mM, the solutions were freshly prepared to minimize pre-polymerization. These concentrations were employed to test whether MLG induces silicic acid polymerization below its spontaneous threshold and accelerates it above the threshold. Meanwhile, a blank control group (CK) consisting of pure silicic acid without adding cell wall components was set up. All treatments were incubated on a shaker at 28°C (simulating the optimal growth temperature for rice) with a rotation speed of 200 rpm.

Unpolymerized silicic acid can react with molybdenum reagent to form silicomolybdic acid, while polymerized silicic acid cannot (Bao, 2000). Therefore, after 5 days of treatment, the concentration of unpolymerized silicic acid in the solution was determined by the molybdenum blue spectrophotometric method, with absorbance measured at 660 nm (Zhu *et al.*, 2023). In addition, the mixed solutions of 10 mM silicic acid with MLG/cellulose were centrifuged, and the supernatants were poured off. The residues were dried using a freeze dryer, and then the Si deposition on the surface of the residues was observed by SEM-EDS.

The binding of MLG and 10 mM silicic acid was investigated through material characterization methods. First, the X-ray photoelectron spectroscopy (XPS, Thermo K-Alpha, USA) was used to compare the dried precipitate of glucan-induced silicic acid polymerization (MLG+Si) with the dried pure silicic acid precipitate (Si) (Avila et al., 2001). The C 1s peak at 284.8 eV was used as the reference calibration peak, and fine scanning of the Si2p orbital was performed, with signal accumulation for at least five cycles. Subsequently, Fourier transform infrared spectroscopy (FTIR; Thermo Scientific, USA) was used to compare the precipitate of the MLG+Si group with pure glucan (Bourgeat-Lami et al., 2002). Before performing the infrared spectroscopy test, the sample powder was mixed evenly with dry potassium bromide powder and pelletized.

Evolutionary analysis and gene expression in different parts of rice

We first conducted a phylogenetic analysis of MLG synthase CSLF6. Amino acid sequences of CSLF6 homologs in 30 plant species with available sequences were obtained by BLAST search (https://blast.ncbi.nlm.nih.gov/). Sequence alignment by ClustalW and phylogenetic analysis by Maximum Likelihood method with 1000 bootstraps were conducted using MEGA-X (Stecher *et al.*, 2020).

Information on the *CslF6* expressions through transcriptome sequencing in different parts of rice (including root, leaf, flower, and seed) was obtained from the Ensembl Plants database (https://plants.ensembl.org/), represented by Transcripts Per Million (TPM). In addition, the difference of *CslF6* expressions in roots and leaves was further verified by RT-qPCR. Roots and leaves were collected from 21-day-old rice seedlings and immediately frozen in liquid nitrogen. Total RNA was extracted from the samples using a plant RNA extraction kit (EASY-DO, Zhejiang, China), and its purity and concentration were verified using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). High-quality RNA was reverse-transcribed into cDNA using a reverse transcription kit (EASY-DO, Zhejiang, China). The relative expression level of *CslF6* was analyzed by RT-qPCR (Bustin et al., 2005), with *Actin1* used as the internal reference gene. The primers used are listed in Table S1 (qPCR-CslF6 and qPCR-Actin). Relative expression levels were calculated by the ΔΔCt method.

Construction of *CslF6* deletion and over-expressing rice

Two *cslf6* mutants of ZH11 (*cslf6-1* and *cslf6-2*) were obtained using CRISPR/Cas9 technology, targeting TGAACCACCCGAGCCACGCACGG and GCCGACGGGTCGCAGTGGGAGGG. They are missing the T and C bases, respectively. We used Cas9 plant expression vector (pU6-gRNA) and single guided RNA expression vector (pZDgRNA_Cas9ver.2_HPT). To genotype these mutants, we extracted genomic DNA from leaves of transgenic rice plants using the CTAB method (Doyle & Doyle, 1987). Briefly, leaf tissues were frozen in liquid nitrogen, ground using a tissue grinder, and then incubated in CTAB extraction buffer. After chloroform extraction and isopropanol precipitation, the DNA pellet was washed and dissolved in sterile distilled water. Then, PCR amplification was performed using primer pairs flanking the designed target sites as listed in Table S1 (CslF6-1 and CslF6-2). The PCR products were sequenced directly using internal specific primers. Sequencing results were analyzed using DSDecodeM, and two homozygous mutants were selected for subsequent experiments (Xie *et al.*, 2017).

In addition, we constructed *CslF6* over-expression lines in ZH11 background. First, a 35S promoter-driven *CslF6* overexpression vector was constructed via PCR cloning. Specifically, the full-length CslF6 coding sequence (CDS) was amplified from rice cDNA using gene-specific primers listed in Table S1 (CslF6-OE) and then inserted into the binary vector pBWA(V)BS downstream of the 35S promoter. The recombinant vector was verified by Sanger sequencing. Subsequently, the verified construct was introduced into ZH11 rice calli using the Agrobacterium-mediated transformation method (Hiei *et al.*, 1994). Agrobacterium strain EHA105 harboring the recombinant vector was co-cultivated with the calli. After hygromycin screening and regeneration culture, positive transgenic plants were regenerated from the resistant calli. To confirm the integration of the exogenous CslF6 gene, genomic DNA was extracted from young leaves of the regenerated plants using the CTAB method and amplified via PCR with primers specific to the 35S promoter and *CslF6* CDS (Table S1). Finally, agarose gel electrophoresis was used to confirm whether the exogenous vector containing the target gene was successfully integrated into the rice genome, and two lines (*CslF6-OE3* and *CslF6-OE12*) were selected for subsequent experiments.

Determination of MLG content of *CslF6* deletion and over-expressing rice

Offspring of lines were collected for further experiments. Seed disinfection and germination were the same as described before. Wild-type (WT), knockout (*cslf6-1* and *cslf6-2*) and over expressing (*CslF6-OE3* and *CslF6-OE12*) rice seedlings were grown in 1/4 Kimura B nutrient solution for 21 days. The MLG contents in shoots and roots of 21-day-old WT, *cslf*6-1, *cslf*6-2, *CslF6-OE3*, *CslF6-OE12*) rice were determined using a kit from Suzhou Grace Biotechnology Co., Ltd. The detection principle is as follows: lichenase can specifically hydrolyze MLG into oligosaccharides, and β-glucosidase further hydrolyzes the oligosaccharides into glucose; after specific oxidation, glucose reacts with a chromogenic reagent to form a pink product, which has a maximum absorption peak at 510 nm (McClear & Glennie-Holmes, 1985). The content of MLG can be calculated by measuring the absorbance. Reagent blanks were included in the analysis, and the certified reference oat flour containing 7.5% MLG was used for quality control, with its results within the expected ranges.

Effect of MLG on Si deposition and cell wall mechanical properties in rice roots

21-day-old wild-type (WT), knockout (*cslf6-1* and *cslf6-2*) and over expressing (*CslF6-OE3* and *CslF6-OE12*) rice seedlings were transplanted into the 1/4 Kimura B nutrient solution containing 1.0 mM silicic acid for 14 days. The nutrient solution was replaced every 7 days. Subsequently, the plants were washed and digested using the same method as described above, and the Si concentration was determined.

In addition, root segments from the mature zone, located 1 cm away from the root tip, of WT, *cslf6-1*, and *CslF6-OE3* were taken for the hardness test. The crushed root was added to the ethanol solution for ultrasonic treatment, and then the dispersed solution was added to the mica sheet. Samples were tested using the tap mode of AFM (Bruker Dimension Icon, USA, the spring constant of Si_3N_4 cantilevers was 40 N m^{-1}) at room temperature (25°C) (He *et al.*, 2013). The spring constant (K value) of the sample is determined by the slope of the linear part of the curve, which can be used to represent the root hardness. A higher K value indicates a harder material.

MLG content and Si concentration of *CslF6* over-expressing *Arabidopsis*

First, we generated *CslF6* overexpressing *Arabidopsis* lines. The vector construction method was identical to that used for rice. Subsequently, the verified construct was introduced into inflorescences of the Col-0 ecotype via *Agrobacterium*-mediated transformation. Specifically, *Agrobacterium* strain GV3101 harboring the recombinant vector was resuspended in infiltration buffer (containing 0.02% Silwet L-77). Whole inflorescences of *Arabidopsis* plants were dipped into the bacterial suspension for 2-3 seconds, then sealed with plastic wrap to maintain humidity >90% and incubated in the dark at 25°C for 24 hours. The infiltration process was repeated three times at 7-day intervals. After harvesting T_0 seeds and screening on hygromycin-containing medium, resistant seedlings were transplanted into nutrient soil to generate T_1 plants, which were grown at 23°C under a 16h light/8h dark photoperiod. Approximately 20 days later, the CTAB method was used to extract genomic DNA from *Arabidopsis* leaves, which was then used for PCR verification to determine whether the exogenous vector containing *OsCslF6* was successfully integrated into the *Arabidopsis* genome. Positive T_1 plants were self-pollinated to produce T_2 seeds for subsequent experiments.

The wild type *Arabidopsis* (Col-0) and two overexpression lines (*OE11*, *OE17*) were cultured for 21 days, and the MLG contents in their shoots and roots were determined using a kit method. In addition, 21-day-old *Arabidopsis* were planted in 1/2 Hoagland nutrient solution with Si (1.0 mM silicic acid) and without Si, respectively. After 5 days of cultivation, the shoot length, root length, fresh weight, shoot Si concentration, and root Si concentration were determined.

Apoplast pathway and stress resistance of *CslF6* over-expressing *Arabidopsis*

Given that the apoplastic pathway has been proven to play an important role in plants' absorption of toxic elements/salt and Si's blocking of toxic elements/salt from entering plants, the apoplastic tracer PTS (Trisodium-8-hydroxy-1,3,6-pyrenetrisulfonic acid) was used to evaluate the effects of different treatments on the apoplastic absorption pathway in *Arabidopsis*. 21-day-old Col-0, *OE11*, and *OE17* were planted in 1/2 Hoagland nutrient solution with Si (1.0 mM silicic acid) and without Si. After 5 days of cultivation, the

Arabidopsis were planted in nutrient solution containing 50 mg/L PTS for 24 hours. Then, the previous solution was replaced with 1/2 Hoagland solution for another 24 hours, so that the transpiration stream could transport the remaining PTS to shoots. After that, the fresh shoot samples were chopped and incubated in distilled water at 90°C for 2 hours to extract PTS. Col-0 not planted in PTS were used as blanks. A fluorescence spectrophotometer (F-4600, Japan) was used to measure the PTS concentration, with an excitation wavelength of 403 nm and an emission wavelength of 510 nm.

The resistance of different *Arabidopsis* to cadmium stress (10 μM Cd^{2+}, as a representative of cationic toxic metals), chromium stress (50 μM $Cr_2O_7^{2-}$, as a representative of anionic toxic metals), and salt stress (100 mM Na^+) was tested. For 21-day-old Col-0, *OE11*, and *OE17* with comparable growth, two treatments were set up: Stress, where *Arabidopsis* grew in 1/2 Hoagland nutrient solution containing stress substances; and Stress + Si, where *Arabidopsis* were planted in nutrient solution containing stress substances and 1 mM silicic acid. In addition, a stress-free treatment was set as the control. After 5 days, photos were taken, and the shoot length, root length, and fresh weight were measured. The plants were then washed and digested following the same procedure, and the concentrations of Cd, Cr, and Na were measured. In addition, the metal translocation factor (TF) was calculated using the following equation:

Metal TF = shoot metal concentration / root metal concentration.

Quality control and statistical analysis

Three technical replicates were conducted for each sample during the determination of element concentrations by ICP-OES and spectrophotometry, gene expression levels by qRT-PCR, and root hardness by AMF. The certified reference material (GSB10010a) was used for quality control, which was within the expected ranges. Blanks and standard solutions were used for each batch. The data corresponded to the average of three or five biological replicates with standard deviation. Bar charts were drawn using Origin (Seifert, 2014). Statistical comparisons among different treatments for all experiments were performed in R software using Welch's two-sample t-test or the least significant difference (LSD) post-hoc test (Team, 2011). The significant differences were tested at the $p < 0.05$ level.

Results

Si deposition in dead plant roots and the effect of mixed-linkage glucan on Si polymerization

21-day-old rice and *Arabidopsis* seedlings cultured in Si free nutrient solutions were first killed in a boiling water bath and then soaked in a solution containing 1 mM silicic acid for 5 days. In this way, the increased Si in their roots can only come from the deposition of silicic acid and this method ensures we exclude the influence of transport proteins. The Si concentration of dead rice roots soaked in silicic acid solution (+Si) increased by 55.2% compared to before soaking (-Si), while the root Si concentration of dead *Arabidopsis* remained unchanged (Fig. 1a). Further results from SEM-EDS analysis showed that: the surface of dead rice roots in the -Si group was smooth, and no Si was detected in their longitudinal sections; in contrast, a large number of Si particles were deposited on the surface of dead roots in the +Si group, and the mass fraction of Si in the root interior was measured at 0.09% via EDS detection (Fig. 1a, S1). In addition, the results of a 5-day Si addition experiment conducted on live *Lsi2*-deletion mutant rice plants and their wild-type counterparts (cv. Taichung-65) showed that the Si concentration in the shoots of the mutant (*lsi2*) was 37.6% lower than that of the wild type (WT), while there was no significant difference in Si concentration in the roots between the two (Fig. S2).

Based on the above results, we hypothesized that cell wall components may affect the formation of the root Si-based barrier. A major difference between rice and *Arabidopsis* cell walls is the presence of MLG. Therefore, we examined the effect of

MLG on silicic acid polymerization *in vitro*. The results showed that 5 mM and 10 mM silicic acid could undergo obvious spontaneous polymerization; after adding MLG, the polymerization degree of 5 mM and 10 mM silicic acid was further enhanced (Fig. 1b, S3). By the 5th day, the concentrations of unpolymerized silicic acid were 9.0% and 12.1% lower than those in the control group (CK, without MLG addition), respectively. Moreover, after adding MLG, 1 mM and 2 mM silicic acid, which originally showed insignificant spontaneous polymerization (unsurprisingly as silicic acid only starts to polymerize above 2mM), also underwent polymerization. By the 5th day, the concentrations of unpolymerized silicic acid in the 1 mM and 2 mM silicic acid groups with MLG addition were 19.7% and 18.9% lower than those in CK. In contrast, the addition of cellulose had no significant effect on the concentrations of unpolymerized silicic acid (Fig. 1b). The morphology shown in the SEM-EDS micrographs further confirmed that more silicon particles were present on the surface of MLG residues than on that of cellulose residues (Fig. 1c).

In addition, the results of X-ray photoelectron spectroscopy (XPS) showed that compared with pure silicic acid precipitate (Si), MLG+Si caused a change in the electron binding energy of the 2p orbital of Si (from 103.5 eV to 103.3 eV) (Fig. 1d). This peak shift indicated an increase in electron density around Si atoms, suggesting the presence of interfacial interactions or charge transfer between Si and MLG (Moulder et al., 1992). Infrared spectroscopy data showed that the MLG+Si sample exhibited characteristic peaks at 798 and 1084 cm^{-1}, which could be assigned to Si-O-Si bonds, and Si-O-Si/Si-O-C/C-O-C bonds (Fig. 1e).

Mixed cross-linked glucan provides a structural basis for Si polymerization in live rice roots

The results of our first experiment have clearly shown that even after death, the cell walls of Poaceae plants could still induce Si polymerization, and MLG had a strong ability to induce Si polymerization (Fig. 1). MLG is synthesized by cellulose synthase-like F or H families of proteins, with CSLF6 being the best-characterized

MLG synthase (Burton & Fincher, 2009; Vega-Sánchez *et al.*, 2012). Phylogenetic analysis of proteins encoded by *CslF6* shows that its homologues are limited to Poaceae (Fig. S4). Evidence from transcriptomics and qPCR suggests that the *CslF6* gene was mainly expressed in roots, with much lower expression in leaves and other tissues (Fig. S5).

To investigate the role of MLG synthesized by CSLF6 protein in Si deposition in rice, we generated two rice *CslF6* knockout mutants (*cslf6-1* and *cslf6-2*) (Fig. S6) and 24 *CslF6* over-expression lines (Fig. 2a). Among the overexpression lines, *CslF6-OE3* and *CslF6-OE12* were selected for subsequent experiments. Measurement of 21-day-old rice plants from each group revealed that the MLG contents in the shoots and roots of the two knockout mutants were 45.2–60.3% lower than those of the wild type (WT), whereas the MLG contents of the two overexpression lines were 35.9–59.5% higher than those of WT (Fig. 2b, S7a). Additionally, after 14 days of cultivation in 1/4 Kimura B nutrient solution containing 1.0 mM silicic acid, no significant differences in shoot Si concentrations were observed between the knockout/overexpression lines and WT (Fig. S7b). However, compared with WT, the root Si concentrations of *cslf6-1* and *cslf6-2* decreased by 49.7% and 35.9%, whereas those of *Cslf6-OE3* and *Cslf6-OE12* increased by 27.1% and 17.3%, respectively (Fig. 2c). The results of hardness testing of root segment from the mature zone, located 1 cm away from the root tip using atomic force microscopy (AFM) showed that the K value (indicating hardness) of *cslf6-1* root was 15.4 N m^{-1}, which was 18.6% lower than that of *WT* (Fig. S8) (He *et al.*, 2013). In contrast, the K value of *CslF6-OE3* root was 43.1% higher than that of *WT* ($p < 0.001$).

MLG-induced Si defense barrier enhances stress resistance in *Arabidopsis*

Thirty transgenic *Arabidopsis* lines expressing *OsCslF6* under the control of the 35S promoter were obtained, and *OE11* and *OE17* were selected for subsequent experiments (Fig. 2d). The results showed that the content of MLG in shoots and roots of 21-day-old wild-type *Arabidopsis* (Col-0) was extremely low, less than 0.2 mg/g;

in contrast, the MLG content in shoots and roots of the two overexpression lines increased significantly, reaching 4.5-8.8 mg/g (Fig. 2e, S7c). Further analysis revealed that under non-stress conditions, there were no significant differences in shoot length, root length, or fresh weight between *OE11*, *OE17* and Col-0 at 21 days of age (Fig. 3a, b). In addition, 5-day Si addition treatment had no significant effect on the fresh weight, plant height, root length, or shoot Si concentration of these three *Arabidopsis* lines under non-stress conditions (Fig. 3a, b, S7d). For Col-0, Si addition also had no significant effect on its root Si concentration; however, Si addition significantly increased the root Si concentration of *OE11* and *OE17* by 60.1% and 92.9%, respectively (Fig. 2f). The results of the apoplastic tracer experiment showed that overexpression of *OsCslF6* had no significant effect on the shoot PTS concentration (Fig. 3c). Further analysis revealed that, compared with the non-Si treatment, Si addition had no significant effect on the shoot PTS concentration of Col-0, but it reduced the shoot PTS concentrations of *OE11* and *OE17* by 21.3% and 34.9%, respectively (Fig. 3c).

Subsequently, we investigated the effects of *OsCslF6* overexpression and Si addition on the growth status of *Arabidopsis* under cadmium (Cd), chromium (Cr), and salt stress. The results indicated that all three stresses significantly inhibited the plant height, root length, and fresh weight of *Arabidopsis* (Fig. 4, 5, Table S2). Under Cd stress, compared with the non-Si treatment, Si addition only increased the plant height of Col-0 by 19.4% and had no significant effect on its root length, fresh weight, or Cd concentrations in the shoots and roots (Fig. 4). However, Si addition significantly improved the plant height, root length, and fresh weight of *OE11* and *OE17* under Cd stress, with increases ranging from 16.4% to 54.5%. Meanwhile, although Si addition had no significant effect on the root Cd concentrations of both lines, it reduced the shoot Cd concentrations by 21.5% and 15.2%, and decreased the Cd translocation factors by 12.5% and 18.4%, respectively (Fig. 4, S9a).

Under Cr stress, Si addition had no significant effect on the growth status or Cr concentration in Col-0 but significantly enhanced the Cr resistance of *OE11* and *OE17*. Specifically, Si addition increased the plant height, root length, and fresh weight of *OE11* and *OE17* by 21.8%–57.8%, reduced the shoot Cr concentrations by 22.0% and 29.6%, and decreased the Cr translocation factors by 12.0% and 20.3%, respectively (Table S2). Under salt stress, Si addition only reduced the root Na concentration of Col-0 by 7.4% and had no significant effect on its plant height, root length, fresh weight, or shoot Na concentration (Fig. 5). In contrast, Si addition significantly improved the growth of *OE11* and *OE17*, increasing their plant height, root length, and fresh weight by 19.3%–117.9%, reducing the Na concentrations in the shoots and roots by 30.6%–76.5%, and decreasing the Na translocation factors by 41.7% and 66.2%, respectively (Fig. 5, S9b). In summary, Si had a limited effect on enhancing the resistance of Col-0 to toxic metals and salt stress but significantly improved the stress tolerance of the *OsCslF6*-overexpressing lines.

Discussion

This paper aims to address three fundamental questions regarding the formation of Si barriers in plant roots: the impact of cell wall components and Si transporters on the root Si barrier, the role of MLG in silicic acid polymerization, and the regulatory effect of genetic modification to increase Si deposition on the stress resistance of Si non-accumulators (Fig. 6). Through a series of *in vitro* and *in vivo* experiments, we demonstrate that the cell wall component MLG in rice roots is a structural scaffold for Si deposition, acting to induce silicic acid polymerization, possibly through its exposed hydroxyl groups. Introducing rice *CslF6* into *Arabidopsis* increases root Si deposition, which enhances stress resistance in this Si non-accumulating plant. These findings demonstrate the key role of the plant cell wall components in the formation of Si defense barriers in roots. This study also suggests a potential route, through a combination of genetic modification and Si supply, to enhance plant resistance to abiotic stress, even in plant species which are not naturally Si accumulators, with significant benefits for food security.

The cell wall components important in root Si deposition

The research conducted on dead plants and *lsi2* mutants identified that differences in cell wall components, rather than Si transporters, was the key mechanism leading to differences in the process of Si deposition on roots between rice and *Arabidopsis* (Fig. 1a, S1, S2). Poaceae and Equisetaceae are typical Si-accumulators (Hodson *et al.*, 2005). Compared with Si non-accumulators such as certain dicotyledons, the cell walls of Poaceae and Equisetaceae consist of MLG with a small portion of xyloglucan and pectin (Fry *et al.*, 2008; Vega-Sánchez *et al.*, 2012). In the study on the cell wall components of horsetails, researchers hypothesized that MLG may play a central role in regulating the location and/or quality of Si deposition (Fry *et al.*, 2008).

The present study offers a range of empirical evidence to substantiate this hypothesis. First, the *in vitro* experiment showed that MLG can strongly induce silicic acid polymerization (Fig. 1b). In contrast, the commercial cellulose used in this study has no significant effect on silicic acid polymerization. Structurally, MLG is mainly composed of cellotriosyl and cellotetraosyl units, which are linked by β -1,3 bonds; these β -1,3 bonds introduce flexible kinks into the polysaccharide chain at irregular intervals (Burton & Fincher, 2009). The β-1,3 linkages in MLG confer an open structure, which fully exposes the active hydroxyl groups at the C2, C3, and C6 positions of glucose residues and thereby promotes silicic acid polymerization (Purushotham *et al.*, 2022). These hydroxyl groups may bind to silicic acid molecules via hydrogen bonds or mediator ions/molecules, or directly undergo dehydration condensation with the active silanol groups of silicic acid to form Si-O-C covalent bonds—all interactions anchor free silicic acid molecules on the MLG surface, leading to local supersaturation of silicic acid (He *et al.*, 2013; Soukup *et al.*, 2020; Pang et al., 2023a). Furthermore, these interactions may alter the electron cloud distribution of silicic acid molecules, thereby weakening the intramolecular O-H bonds and accelerating the dehydration condensation of silicic acid (Dove *et al.*, 2019). The XPS results showed that the Si 2p binding energy of the MLG+Si group was lower than that of the Si group, while no obvious peak at 102.7 eV assigned to Si-O-C bonds was observed (Avila et al., 2001; Sung *et al.*, 2021). This suggests the possibility of Si-O-C bond formation, although further evidence is needed to confirm this. Due to the relatively low electronegativity of C, its ability to attract electrons from O is weak, which causes the electron cloud of O to be more inclined toward Si (Sorarù *et al.*, 1996). This increases the outer electron density of Si, reduces the binding energy of inner 2p electrons, and shifts the spectral peak toward the lower binding energy direction (Sorarù *et al.*, 1996). In addition, since physical adsorption usually only causes very slight fluctuations, this 0.2 eV shift indicates a higher possibility of chemical adsorption (Bao *et al.*, 2022; Greczynski *et al.*, 2023). The infrared spectroscopy results revealed that the MLG+Si group exhibited characteristic peaks at 798 cm^{-1} and 1084 cm^{-1} compared to MLG, indicating the possible presence of Si-O-Si and Si-O-C bonds (Bourgeat-Lami *et al.*, 2002), which *further* supports the possibility of chemical bond formation between Si and MLG through oxygen bridges. Previous work has shown that Si can form Si-O-C covalent bonds with hemicellulose and pectin in plant cell wall, which plays a key role in the structure and remodeling of plant cell wall (Sheng & Chen, 2020; Zexer *et al.*, 2024). It should be noted that our model for the interaction between MLG and silicic acid remains hypothetical. Further experimental evidence is required to confirm the presence of Si-O-C bonds between MLG and Si, and other possible interaction modes cannot be excluded.

The β-glucan synthase encoded by the *CslF6* gene not only serves as a key enzyme in the biosynthesis and accumulation of mixed-linkage glucan (MLG) in plants (Vega-Sánchez *et al.*, 2012), but also exhibits the unique ability to independently catalyze the synthesis of (1,3;1,4)-β-glucan—a catalytic activity that has been confirmed in cell-free systems *in vitro* (Purushotham *et al.*, 2022). Evidence from transcriptomics and qPCR in this study and other research indicates that CslF6 is highly expressed in rice roots but poorly expressed in leaves (Jin *et al.*, 2015). Then, two rice *cslf6* mutants and two *CslF6* over expression lines were generated to verify the role of MLG in inducing Si deposition. The absence of *CslF6* leads to a pronounced decrease in MLG content, while over expression of *CslF6* can promote MLG accumulation (Fig. 2, S7). In this in vivo experiment, no effect of *CslF6* deficiency or over-expression on shoot Si concentration was observed (Fig. S7), which is consistent with previous studies (Kido *et al.*, 2015). This is because Si accumulation in shoots is primarily regulated by Si uptake and transport systems (Yamaji *et al.*, 2024), whereas MLG only has an impact on the distribution pattern of Si in shoots (Kido *et al.*, 2015). However, results showed that *CslF6* had significant effects of root Si concentration and root hardness (Fig. 2, S8), indicating that MLG is a key component contributing to Si deposition in roots. However, all plants in this study were grown in Si-containing nutrient solution, and the changes in root hardness may also be attributed to the difference in MLG content. Previous studies have shown that MLG can strengthen shoot cell walls while providing flexibility, although its specific role in root remains to be further investigated (Vega-Sánchez et al., 2012). In addition, cell wall composition differs between different parts of the root structure, so a fuller understanding of the association between MLG and Si within roots, including the distribution of MLG across the epidermis and endodermis (where Si is concentrated in rice), requires further study.

This study found that although the MLG content in the roots of *CslF6*-defective mutant rice has dropped to an extremely low level, there is still a certain amount of Si deposition in their roots. In addition, although the increase in root MLG content in *OsCslF6*-overexpressing *Arabidopsis* can enhance the root Si concentration, the root Si concentration of *Arabidopsis* still does not reach the level of rice. This may be related to the regulatory effect of other cell wall components on Si deposition; in addition to MLG, the cell walls of rice and *Arabidopsis* also exhibit numerous differences in terms of their framework structure, component types, and proportions (Yokoyama & Nishitani, 2004). For instance, the effects of tricin, which is widely present in lignin of Poaceae plants, on root Si barriers remain to be investigated (Lam et al., 2021). In addition, environmental conditions during plant growth may also influence the Si barrier.

This work enhances our understanding of the role of MLG in silicification, but research has demonstrated the role of other cell wall components in the Si biomineralization processes which occur in marine algae and terrestrial plants (He *et al.*, 2014; Trembath-Reichert *et al.*, 2015; Mizuta *et al.*, 2021; Kajino *et al.*, 2025). For example, silaffins (a cationic polyamine) on the cell wall of diatoms and Siliplant1 (also a positively charged protein) in sorghum dumbbell-shaped epidermis cells may be scaffolds for Si deposition, as they contain abundant amine groups with the ability to induce silicic acid polymerization (Kröger *et al.*, 2000; Adiram-Filiba *et al.*, 2020; Ayieko *et al.*, 2023). In addition, Si can bind to hemicellulose or lignin in the form of Si-O-C bonds (Fang & Ma, 2006; Ma *et al.*, 2015; Pan *et al.*, 2017; Soukup *et al.*, 2020; Radotić *et al.*, 2022; Palakurthy *et al.*, 2024). Research on cucumbers has found that a lignin-based cell wall structure called the "neck strip" is crucial for Si deposition on their glandular trichomes (Hao *et al.*, 2024). Co localization of Si and callose has been found in horsetail and in epidermal trichomes of *Arabidopsis* (Law & Exley, 2011; Brugiére & Exley, 2017; Guerriero *et al.*, 2018); genetic evidence also suggests callose is indispensable for Si deposition in *Arabidopsis trichomes* (Kulich *et al.*, 2018).

Increasing Si utilization in non-accumulators and its benefits

The sources of Cd, Cr, and Na in soil include both natural geological background and inputs from human activities such as industrial emissions, fertilization, and irrigation (Hassani *et al.*, 2021; Hou *et al.*, 2025) and Cd, Cr, and Na remain common abiotic stress factors for plants in soil (Lei *et al.*, 2025; Mai *et al.*, 2025), with the apoplastic pathway playing a more important role in their uptake (Redjala *et al.*, 2009; Pasricha *et al.*, 2021). The effect of Si on enhancing plant resistance to abiotic and biotic stresses has been widely demonstrated in Si-accumulators (Liang *et al.*, 2007; Massey & Hartley, 2009; Debona *et al.*, 2017; Vaculík *et al.*, 2020; Pang et al., 2026).

However, this study found that for wild-type *Arabidopsis*, Si addition had a limited effect on enhancing resistance to toxic elements and salt stress (Fig. 4, 5, Table S2). Similarly, Si also performs suboptimally in reducing Cr uptake by pakchoi (Pang *et al.*, 2024c). The effect of Si on enhancing resistance to powdery mildew in *Arabidopsis* and strawberry was also not obvious (Palmer *et al.*, 2006; Vivancos *et al.*, 2015). These plants, along with many other food plants used by humans from the Liliaceae, Solanaceae, Rutaceae, and Fabaceae families, exhibit weak Si accumulation and deposition capacity (Fig. S10). This trait severely limits the benefits of Si application and hinders the widespread use of Si fertilizers in agricultural production.

Both the present study and previous research have demonstrated that wild-type *Arabidopsis* contains extremely low levels of MLG (Burton *et al.*, 2006), and no significant growth inhibition is observed in *Arabidopsis* following MLG accumulation (Doblin *et al.*, 2009). Similarly, overexpression of *OsCslF6* in rice exerts no negative impact on growth, whereas its knockout results in developmental retardation (Jin *et al.*, 2015; Dai *et al.*, 2023). MLG accumulation can play a positive role by enhancing cell wall thickness and stimulating defense signals (Dai *et al.*, 2023), but excessive accumulation that cannot be metabolized or regulated may lead to growth inhibition by hindering the transport of water and nutrients or causing energy consumption (Burton *et al.*, 2011). Further studies showed that the introduction of the *OsCslF6* gene alone did not directly improve the resistance of *Arabidopsis* to Cd, Cr, and salt stress; however, when combined with Si supply treatment, the root Si concentration in *Arabidopsis* increased, while the concentrations of toxic elements and salts in the above-ground parts decreased. The mechanism behind this phenomenon is that Si supply after overexpression of *OsCslF6* can significantly inhibit the apoplastic uptake pathway of toxic elements and salts in *Arabidopsis*, whereas overexpression of *OsCslF6* alone has little effect on the apoplastic uptake pathway. Studies on Si-accumulators such as rice have shown that Si can reduce the migration of harmful substances through apoplastic blocking (Coskun *et al.*, 2019; Pang *et al.*, 2024b). The mechanisms underlying this blocking effect include: the Si barrier formed in the roots can reduce the diffusion of toxic elements and salts to the xylem via the apoplastic pathway; Si can immobilize these substances in cell walls through adsorption and co-precipitation, thereby reducing their transport into cells and to above-ground parts (Flam-Shepherd *et al.*, 2018; Pang *et al.*, 2024a). In addition, Si may further enhance apoplastic blocking by promoting suberization, lignification, and Casparian band formation in the roots; however, some studies have shown that Si can delay or even have no effect on the formation of these structures (Fleck *et al.*, 2011; Kreszies *et al.*, 2020). Misplaced and abnormal shoot Si deposition mediated by Si transporter SIET4 can lead to death in rice (Mitani-Ueno et al., 2023). However, our results showed no negative effects on plant growth after strengthening the root Si-based barrier. This may be because MLG mainly affects Si deposition in roots, without interfering with Si uptake and transport.

This combined strategy of "genetic modification + Si supply" provides an effective and efficient solution for enhancing the root Si defense barrier in Si non-accumulators. So far, we have only tested the benefits of combining genetic modification and additional Si supply in one Si non-accumulating species, *Arabidopsis*. If this approach is effective in Si non-accumulating crops in the same plant family, the Brassicaceae, which includes many widely grown and important crops such as pakchoi, oil seed rape, broccoli, kale and turnip, it will be highly significant for improving food security in the face of a range of environmental threats. This strategy may also be applicable to forest trees, helping to accelerate afforestation in harsh environments. In addition to its role in resistance to abiotic stress, Si also plays an important role in resistance to biotic stress such as herbivore feeding and pathogen colonization (Zellner *et al.*, 2021; Johnson *et al.*, 2024; Pang et al., 2025c). Many of the most serious crop pests and pathogens are root-feeding and soil borne, making them particularly hard to control through conventional pesticide spraying (Chadfield *et al.*, 2022), so the strategy identified here of "genetic modification + Si supply" may have even greater benefits for sustainable agriculture and future food security.

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Competing interests

The authors declare no competing interests.

Author contributions

Z.P. and Y.L. designed this project. Z.P., N.S., Y.W., T.C., and W.Z. conducted the experiments. Z.P. and A.S. analyzed the data. S.E.H., R.R.B., and A.L. gave advice from a theoretical perspective. Z.P. and S.E.H. wrote the paper with inputs from all co-authors. Y.L. supervised this project and revised the paper.

Data availability

The Gene ID of *OsCslF6* on NCBI is 4344719. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Main text figure legends

Fig. 1. Si deposition in dead plant roots and in the purchased chemical components of cell wall. (a) Si concentration in the roots of dead rice and *Arabidopsis* with/without 1.0 mM Si supply. Data represent the mean and standard deviation of 3 replicates. A t-test was performed on different treatments, with ** indicating $p < 0.01$ and ns representing $p > 0.05$. The surface morphology of dead rice roots with/without Si supply is shown in the upper right corner. (b) The concentrations of unpolymerized silicic acid with the addition of purchased mixed linked glucan (MLG) and cellulose, and without the addition of cell wall components (CK). The initial concentrations of unpolymerized silicic acid are 1 and 10 mM. The data represent the mean and standard deviation of 3 replicates. LSD multiple comparisons were performed on the concentrations of unpolymerized silicic acid in each treatment on day 5. Different letters indicate significant differences at $p < 0.05$. (c) The morphology of Si deposition on the surfaces of MLG and cellulose shown in the SEM-EDS micrographs. The initial concentration of silicic acid is 10mM. (d) XPS information for MLG+Si and Si. (e) Infrared spectral data of MLG+Si and MLG.

Fig. 2. Effect of *CslF6* on root MLG content and Si concentration of rice and *Arabidopsis*. (a) Identification of *CslF6* overexpression vector in rice genome. Each numbered band comes from a plant. M stands for the marker used to indicate the molecular weights of DNA bands. + and - represent positive and negative controls, respectively. O stands for blank control (water). (b) and (c) Root MLG content and Si concentration of rice. (d) Identification of *OsCslF6* overexpression vector in *Arabidopsis* genome. (e) and (f) Root MLG content and Si concentration of *Arabidopsis*. Each group has 3 or 5 biological replicates. LSD multiple comparisons were performed among different groups. Different letters indicate significant differences at $p < 0.05$.

Fig. 3. Effect of Si on the growth and apoplastic uptake pathway of three *Arabidopsis* lines under non-stress conditions. (a) Growth status of *Arabidopsis* under non-stress conditions. The left and right parts show the treatments without and with Si addition, respectively; from upper to lower are Col-0, *OE11*, and *OE17*. (b) Shoot height, root length, and fresh weight of *Arabidopsis*. Each group has 5 biological replicates. (c) Shoot PTS concentrations in each *Arabidopsis* line without and with Si addition. PTS serves as an apoplastic pathway tracer. The data represent the mean and standard deviation of 3 replicates. LSD multiple comparisons were performed among different groups. Different letters indicate significant differences at $p < 0.05$.

Fig. 4. Effect of *CslF6* on the resistance of *Arabidopsis* to divalent cadmium (Cd). (a) Growth status of *Arabidopsis* under 10 μM Cd stress. The upper and lower parts show the treatments without Si addition and with Si addition, respectively; from left to right are Col-0, *OE11*, and *OE17*. (b-d) Shoot height, root length, and fresh weight of *Arabidopsis*. Col-0 without stress was used as the control (CK). Our previous studies have shown that there was no significant difference in the growth of Col-0, *OE11*, and *OE17* under non-stress conditions. (e) and (f) Cd

concentrations in the shoots and roots of *Arabidopsis*. Data are presented as the mean ± standard deviation of 3 or 5 replicates. LSD multiple comparisons were performed among different groups, and different letters indicate significant differences at $p < 0.05$.

Fig. 5. Effect of *CslF6* on the resistance of *Arabidopsis* to sodium (Na). (a) Growth status of *Arabidopsis* under 100 mM Na stress. From left to right are Col-0, *OE11*, and *OE17*. (b-d) Shoot height, root length, and fresh weight of *Arabidopsis*. Col-0 without stress was used as the control (CK). (e) and (f) Na concentrations in the shoots and roots of *Arabidopsis*. Data are presented as the mean ± standard deviation of 3 or 5 replicates. LSD multiple comparisons were performed among different groups, and different letters indicate significant differences at $p < 0.05$.

Fig. 6. The difference in Si deposition between Si accumulators and Si non-accumulators and the effect of the "genetic modification + Si supply" methodology on plant resistance to toxic metals and salt.

Legend for Supporting Information

Fig. S1 Determination of silicon mass fraction in dead rice roots via SEM-EDS point scanning mode.

Fig. S2 Shoot and root Si concentrations in live rice *Lsi2* deletion mutants and their wild type.

Fig. S3 The concentrations of unpolymerized silicic acid with the addition of mixed linked glucan (MLG) and cellulose, and without the addition of cell wall components (CK).

Fig. S4 Phylogenetic analysis of proteins encoded by *CslF6*.

Fig. S5 Expression levels of *CslF6* in different parts of rice.

Fig. S6 Gene structure of *CslF6* and its mutated sequences in CRISPR/Cas9 mutants.

Fig. S7 Shoot MLG content and Si concentration of rice (a, b) and Arabidopsis (c, d).

Fig. S8 Representative force-distance curves for WT, *cslf6-1* and *CslF6-OE3* rice roots.

Fig. S9 Effect of Si on Cd (a) and Na (b) translocation factors of three *Arabidopsis*.

Fig. S10 The average leaf Si concentration of the families to which the main grains, vegetables, and fruits belong.

Table S1 Primer sequences used in this study.

Table S2 Effect of *CslF6* on Arabidopsis resistance to hexavalent chromium (Cr).