Chapter 5
Silicon-Mediated Tolerance to Metal Toxicity

Abstract Silicon (Si) has been well documented to mitigate phytotoxicity of metals including manganese (Mn), iron (Fe), aluminium (Al), cadmium (Cd), arsenic (As), chromium (Cr), lead (Pb), copper (Cu) and zinc (Zn). However, the mechanisms explaining this phenomenon are still not clear. In general, there are two mechanisms, i.e. an external (*ex planta*) and an internal (*in planta*) one, proposed for explaining why and how Si can regulate plant resistance and/or tolerance to metal toxicity. Among the interactions of Si with metals, the possible roles of Si in enhancing tolerance to Mn and Al have been most extensively investigated, while over the last decade more work has been done on the Si-mediated alleviation of toxicity of the most important heavy metals, i.e. Cd, As, Cr and Pb. Nevertheless, direct evidence is still lacking that can clearly dissect the mechanisms involved in Si-mediated tolerance against metal toxicity, especially at the molecular level.

Keywords Aluminium • External mechanisms • Heavy metals • Internal mechanisms • Manganese • Silicon

5.1 Silicon-Alleviated Metal Toxicity Symptoms

5.1.1 Manganese and Iron

Manganese (Mn) is an important essential micronutrient for plant growth, but it easily becomes toxic above physiological levels. Mn toxicity occurs frequently in highly reduced paddy soils (waterlogged lowland soils) or in highly weathered acidic soils of tropical and subtropical areas. In general, the visual symptoms of Mn toxicity will vary with the plant species and plant sensitivity to excess Mn supply. Toxicity occurs at leaf Mn concentrations ranging from 200 to 5,300 mg kg^{-1} (Edwards and Asher 1982; Clarkson 1988), depending largely on the species; genotype; environmental conditions such as temperature, humidity, pH, light and source of nitrogen; nutritional interactions with calcium (Ca), magnesium (Mg), iron (Fe), phosphorus (P), molybdenum (Mo) and Si; and the action of mycorrhizae (El-Jaoual and Cox 1998). Symptoms of Mn toxicity are quite diverse among plant species, but brown spots on older leaves near the main and secondary veins surrounded by

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chlorotic zones are typical symptoms of Mn toxicity (Morris and Pierre 1949; Foy et al. 1978, 1995; El-Jaoual and Cox 1998; Li et al. 2012a).

Mn toxicity symptoms are reported to be alleviated considerably by the addition of Si (Figs. 5.1 and 5.2). As early as 1957, Williams and Vlamis (1957a, b) showed that Si decreased Mn toxicity in barley (*Hordeum vulgare*) by causing Mn to be more evenly distributed instead of being concentrated in discrete necrotic spots, although the total Mn in the leaves was unaffected by Si treatment. Subsequently, alleviation of Mn toxicity by Si was reported in many crops (Table 5.1).

Okuda and Takahashi (1965) demonstrated significant alleviative effect of Si on Mn toxicity in rice plants and attributed this effect to Si-mediated inhibition of Mn uptake. It has been reported by Júnior et al. (2010) that dry matter weight of roots, sheaths, leaves and aerial parts of Mn-stressed rice (*Oryza sativa*) plants was significantly higher in plants treated with Si and the leaf erectness was significantly increased with addition of Si. Tavakkoli et al. (2011) reported that interactions of Si slag with phosphorus increased dry matter yield of rice grown on a highly weathered, acidic lateritic soil and probably mitigated Mn toxicity by decreasing Mn concentration. Li et al. (2012a) investigated the alleviative effect of Si on excess Mn in rice. Mn toxicity symptoms generally appeared as dark brown spots, necrotic lesions, chlorosis of leaf edges and tips and crinkle leaf. Clearly, Si supply could significantly alleviate Mn toxicity symptoms in both Mn-sensitive and Mn-tolerant cultivars (Fig. 5.2; Li et al. 2012a). Horst and Marschner (1978a) reported that without Si supply, bean growth depression and toxicity symptoms occurred at concentrations as low as 0.5 μM Mn in the nutrient solution, while the Si-treated plants could tolerate 5.10 μM Mn. They argued that the increase in Mn tolerance was not caused by a depressing effect of Si on the uptake or translocation of Mn but rather by an increase in Mn tolerance in leaf tissues. Iwasaki and Matsumura (1999) investigated Si-induced alleviation of Mn toxicity in two contrasting pumpkin (*Cucurbita*

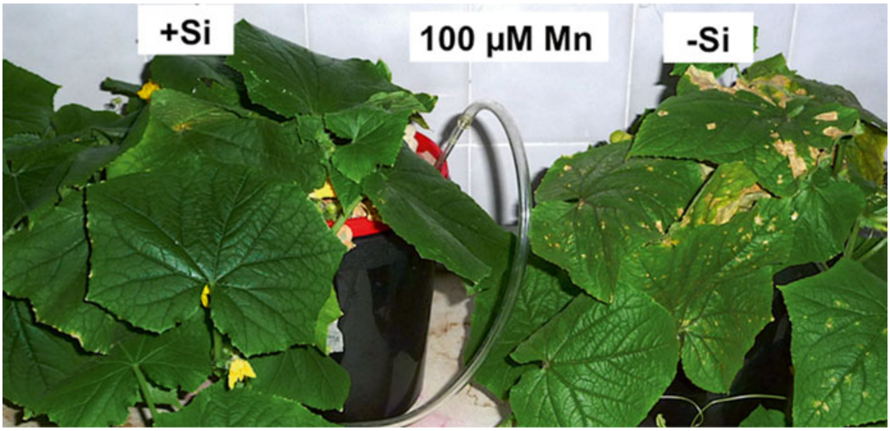

Fig. 5.1 Si alleviates symptoms of Mn toxicity in cucumber (Photograph by Miroslav Nikolic)

Fig. 5.2 Toxicity symptoms of two rice cultivars grown in a nutrient solution containing normal (6.7 μM) or high Mn (2.0 mM) with or without Si (1.5 mM) supply (From Li et al. 2012a)

moschata) cultivars (cultivar Shintosa, a bloom type, and cultivar Superunryu, a bloomless type). The results showed that high Mn significantly depressed root and shoot biomass of both cultivars in the absence of Si, while in the presence of Si, high Mn only inhibited the growth of Superunryu cultivar, but not that of Shintosa which accumulated more Si than Superunryu. Interestingly, Mn uptake was similar in both cultivars. Iwasaki et al. (2002a, b) and Führs et al. (2009) showed that in cowpea receiving 50 μM Mn, dark brown spots were the first visible Mn toxicity symptoms, which completely disappeared in Si-fed plants throughout the experiment. However, distinct brown spots were noted in the Si-pretreated plants grown hydroponically with excess Mn, although the symptoms were less severe in the Si-pretreated plants than in the untreated ones (Iwasaki et al. 2002b). Rogalla and Römheld (2002) reported that brown spots on leaves, a typical symptom of Mn toxicity, appeared at over 50 μM Mn in Si-deprived plants, but at 500 μM and above in Si-fed plants (Table 5.2). Both shoot and root fresh weights of plants grown with Mn levels ranging from 50 to 1,000 μM were significantly increased by the addition of Si (Rogalla and Römheld 2002; Wiese et al. 2007). The experiments with cucumber (*Cucumis*

Table 5.1 Plant species reported to be positively responsive to mitigation of Mn toxicity by Si

Plant species	References
Barley	Williams and Vlamis (1957a, b), Vlamis and Williams (1967), and Horiguchi and Morita 1987
Maize	Doncheva et al. (2009)
Rice	Okuda and Takahashi (1962), Júnior et al. (2010), Tavakkoli et al. (2011), and Li et al. (2012a)
Sugarcane	Clements (1965) and Fox et al. (1967)
Wheat	Vlamis and Williams (1967)
Oats	Vlamis and Williams (1967)
Rye	Vlamis and Williams (1967)
Sorghum	Galvez et al. (1987)
Sudan grass	Bowen (1972)
Bean	Horst and Marschner (1978a)
Cowpea	Horst et al. (1999), Iwasaki et al. (2002a, b), and Führs et al. (2009)
Cucumber	Rogalla and Römheld (2002), Shi et al. (2005a), Wiese et al. (2007), Feng et al. (2009), Dragisic Maksimovic et al. (2007, 2012)
Pumpkin	Iwasaki and Matsumura (1999)

Table 5.2 Mn toxicity symptoms of *Cucumis sativus* plants grown in a nutrient solution with 1.8 mM (+Si) or without (–Si) Si

	Manganese concentration (μM)				
–Si concentration	0.5	5	50	500	1,000
–Si	–	–	+	++	+++
+Si	–	–	–	+	++

From Rogalla and Römheld (2002)
– none; +, marginal; ++, strong; +++, severe Mn toxicity symptoms

sativus) by Shi et al. (2005a) confirmed that plants grown hydroponically with excess Mn (600 µM Mn) showed typical visible symptoms of Mn toxicity on leaves where severe chlorosis developed on both older and younger leaves, while limited chlorosis occurred on leaves of plants treated with Si. All variables including shoot height, root length, shoot and root biomass and plant biomass were greatly suppressed by excess Mn, but were significantly reversed by Si supply (Shi et al. 2005a). A series of experiments with cucumber by Dragisic Maksimovic et al. (2007) and Dragisic Maksimovic et al. (2012) also showed that although the Mn concentration in the leaves of cucumber plants treated with 100 µM was 10–40 times higher than the optimum, no Mn toxicity symptoms appeared in Si-fed plants, compared to Si-deprived plants. Both the root and shoot biomass of plants grown hydroponically in presence of high Mn was improved by the presence of Si (Dragisic Maksimovic et al. 2007; Dragisic Maksimovic et al. 2012).

Fe is another important essential micronutrient for plant growth. Like Mn toxicity, Fe toxicity is primarily a problem for plants growing in highly reduced conditions (e.g. waterlogged lowland soils, poorly drained, compacted or poorly aerated soils) or in highly weathered acid soils of tropical and subtropical areas. One of the most commonly visible Fe toxicity symptoms is leaf necrotic spots; additional symptoms include dark green foliage, stunted top and root growth, as well as leaf bronzing, especially on rice, which causes dark brown to purple spots on the foliage. Silicon has been reported to significantly mitigate Fe toxicity symptoms in rice (Okuda and Takahashi 1965; Fu et al. 2012; Dufey et al. 2014). Okuda and Takahashi (1965) speculated that Si-mediated inhibition of Fe uptake explained the positive effect of Si against Al. It was also reported that application of slag at a rate of 2 t ha^{-1} to soils containing high exchangeable Fe and Mn led to a decrease in both Fe and Mn contents in rice plants without producing deficiency symptoms (IRRI 1965, 1966). More recent findings showed that a whole range of toxicity symptoms such as reduction of root and shoot biomass, primary root length and root number, aberrant root morphology and destruction of cytoplasmic organelles and nuclei in root epidermal cells were successfully mitigated by the addition of Si in an Fe-sensitive rice cultivar grown hydroponically with 100 μM Fe^{2+} (Fu et al. 2012). The inclusion of Si to rice plants grown in presence of a toxic level of Fe was found to significantly reduce Fe toxicity symptoms (Dufey et al. 2014).

5.1.2 Aluminium

Aluminium (Al) is not only a major constituent of most mineral soils but also a major constraint for plant growth at pH values ranging from 3.5 to 4.5. When in excess, Al severely inhibits root growth and nutrient uptake and limits crop production on highly weathered acid soils of tropical and subtropical areas worldwide. A typical symptom of Al toxicity is growth inhibition of the root system (Rorison 1960). As early as the 1960s, Peaslee and Frink (1969) demonstrated that the uptake of Al and Mn by tomato (*Lycopersicon esculentum*) could be depressed by the addition of silicic acid to the soil although no explanation was given for this result. Over the last 20 years, the possible impacts of Si on Al toxicity in plants have been extensively investigated. So far, positive effects of Si on Al-induced inhibition of root growth have been reported in many plants (crops) (Table 5.3). Clearly, less work has been done on Si and Al interactions since one decade ago.

Despite the previous reports, not all the experiments have convincingly shown the benefits of Si against Al toxicity. Some indicated that the alleviative effect was genotype and time dependent (Li et al. 1989; Cocker et al. 1998a, b; Kidd et al. 2001; Zsoldos et al. 2003). Time-dependent changes in Si-induced amelioration of Al toxicity in maize (*Zea mays*) were first demonstrated for the first time by Kidd et al. (2001). In a 48-h experiment, they showed that after an 8-h exposure to high Al, inhibition of root elongation in an Al-sensitive cultivar was significantly alleviated by Si pretreatment (root elongation rate was 55 % of the corresponding controls, compared to only 12 % when not pretreated with Si), whereas in an Al-resistant cultivar such effects of Si were not observed. In a long-term experiment (after 4 days), the effects of Si pretreatment on Al toxicity subsided (Zsoldos et al. 2003). Wang et al. (2004) found that clear alleviative effects of Si on Al-induced inhibition

Table 5.3 Interactions between Al and Si in experiments

Plant species	Authors and/or references
Barley	Hammond et al. (1995), Liang et al. (2001), and Morikawa and Saigusa 2002
Sorghum	Galvez et al. (1987), Galvez and Clark (1991), Hodson and Sangster (1993), and Li et al. (1996)
Rice	Rahman et al. (1998), Hara et al. (1999), and Singh et al. (2011)
Mung bean	Yang et al. (1999)
Maize	Ma et al. (1997), Corrales et al. (1997), Kidd et al. (2001), and Wang et al. (2004)
Teosinte	Barceló et al. (1993)
Wheat	Cocker et al. (1998a, b) and Zsoldos et al. (2003)
Cotton	Li et al. (1989)
Soybean	Baylis et al. (1994)
Melastoma malabathricum	Watanabe et al. (1997)
Holcus lanatus	Kidd and Proctor (2001)
Norway spruce	Ryder et al. (2003)
Stylosanthes	Zhang et al. (2009)

of root elongation (after 12 h) were noted when Si was supplied both as a pretreatment and during the exposure to excessive Al, but essentially no such effects were observed when Si was supplied only as a pretreatment. On the other hand, working with five varieties of *Stylosanthes*, Zhang et al. (2009) found that both Si pretreatment and continuous Si supply significantly overcame the inhibitory effect of Al on root elongation, and more importantly, the phenotypical difference in Al tolerance among the five genotypes tested disappeared after Si treatment.

Furthermore, the benefits of Si on Al toxicity were found to be dependent on the Si source (Morikawa and Saigusa 2002). Also, Si effects on Al detoxification in plants varied greatly with both Al and Si levels used (Li et al. 1989; Liang et al. 2001; Ma et al. 1997; Yang et al. 1999). Hammond et al. (1995) found that the alleviative effect of Si on Al-induced root elongation increased with Si concentrations.

Cocker et al. (1998b) examined the effects of Si on Al toxicity in both Al-tolerant and Al-sensitive wheat cultivars. Interestingly, Si was found to alleviate Al toxicity at low Al concentrations, but Si supply failed to exhibit significant alleviative effect in the Al-sensitive cultivar exposed to higher Al concentrations. It thus seems to suggest that the effect of Si on Al detoxification depends largely upon the plant species, Al and Si levels and the duration of plants exposed to Al.

5.1.3 Cadmium

Cadmium (Cd) is a nonessential toxic heavy metal that is highly mobile in the environment. Exposure to Cd can cause serious problems to microorganisms, plants and animals even at trace concentrations and can be highly toxic to human beings through its bioaccumulation in the food chain (Song et al. 2009). Once absorbed, transported and accumulated into plant tissues in excess amounts, Cd will disturb plant metabolisms in multiple ways. Many studies have indicated that excess Cd accumulated in plant tissues not only causes Fe, Mg and Ca deficiency (Welch 1995) and reduces chlorophyll content (Feng et al. 2010) but also inhibits plant growth and respiration; destroys the ultrastructure of plant cells such as the nucleus, chloroplast and mitochondria; and alters the activity and quantity of the key enzymes involved in various metabolic pathways (Nwugo and Huerta 2008; Cunha and Nascimento 2009; Song et al. 2009). Over the last decade, growing evidence has been presented in the literature showing that Si can significantly mitigate Cd toxicity symptoms and improve the growth and development of higher plants (Table 5.4).

It was reported that typical Cd toxicity symptoms such as chlorosis, leaf rolls and stunting appeared in the leaves of maize grown under Cd stress (Liang et al. 2005; see Fig. 5.3). The addition of Cd (20 and 40 mg Cd kg^{-1}) considerably reduced shoot

Table 5.4 The beneficial effects of Si on mitigation of Cd toxicity in plants

Plant species	References
Rice	Shi et al. (2005b), Nwugo and Huerta (2008a, b, 2011), Zhang et al. (2008), Gu et al. (2011), Tripathi et al. (2012a), Liu et al. (2013b), and Kim et al. (2014b)
Maize	Liang et al. (2005), Cunha et al. (2008), Cunha and Nascimento (2009), and Vaculík et al. (2012)
Wheat	Rizwan et al. (2012)
Pakchoi	Song et al. (2009)
Cucumber	Feng et al. (2010)
Strawberry	Treder and Cieslinski (2005)
Cotton	Farooq et al. (2013)
Peanut	Shi et al. (2010)
Solanum nigrum	Liu et al. (2013a)
Mangrove	Zhang et al. (2013a)
Mustard	Vatehova et al. (2012)

Fig. 5.3 Silicon-enhanced shoot growth and silicon-alleviated Cd toxicity symptoms of maize grown on an oxisol treated with 0 (CK), 20 and 40 mg Cd kg^{-1} with or without 400 mg Si kg^{-1} (Photograph by Yongchao Liang)

Fig. 5.4 Silicon-enhanced root growth and silicon-alleviated Cd toxicity symptoms of maize grown on an oxisol treated with 0 (CK), 20 and 40 mg Cd kg^{-1} with or without 400 mg Si kg^{-1} (Photograph by Yongchao Liang)

dry weight of maize on an oxisol (by 54 % and 25 % respectively; Liang et al. 2005), a condition that was significantly alleviated by the addition of Si (see Fig. 5.3). The shoot dry weight of plants exposed to Cd20 and Cd40 treatments was increased by addition of Si by 69 % and 119 %, respectively (Liang et al. 2005).

Root growth under Cd stress was also found to be improved significantly by addition of Si (see Fig. 5.3). The root tips of maize exposed to Cd were blackish (more apparently in the higher Cd treatment, Cd40), but they remained normally white coloured in the Cd plus Si treatment (see Fig. 5.4). Working on the genotypic differences between two contrasting pakchoi (*Brassica chinensis*) cultivars grown with 5 mg Cd L^{-1} plus 1.5 mM Si, Song et al. (2009) found that, in the Cd-sensitive cultivar, the inclusion of Si increased the shoot and root dry weight by 25 % and 40 %, respectively. They also found that the alleviative effect of Si on plant growth was more significant in the Cd-tolerant cultivar than in the Cd-sensitive cultivar, suggesting that Si and Cd interactions are genotype dependent.

Nwugo and Huerta (2008a, b) showed that treatment with 2.5 μM Cd significantly reduced the growth of rice seedlings, while inclusion of Si significantly increased all growth variables of Cd-stressed rice plants. They further showed that the inhibitory effects of Cd toxicity on photosynthesis, water use efficiency and light use efficiency were significantly mitigated by the addition of Si (Nwugo and Huerta 2008a, b). Feng et al. (2010) also reported that the commonly present Cd toxicity symptoms in cucumber, i.e. leaf yellowing and necrosis caused by excess Cd-induced nutrient imbalances, were markedly prevented by the application of Si (see Fig. 5.5); this was in accordance with the increased levels of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid in Si-fed cucumber plants exposed to Cd stress. In addition, water use efficiency (WUE) was greatly decreased by exposure to Cd treatment for 10 or 15 days, but was significantly increased by the addition of Si in Cd-stressed plants (Feng et al. 2010).

5.1.4 *Arsenic*

Arsenic (As) is a nonessential highly toxic oxianionic metalloid that occurs naturally in all soils (Cullen and Reimer 1989). Human exposure to excess As will pose a major health risk, leading to a variety of health problems including skin conditions

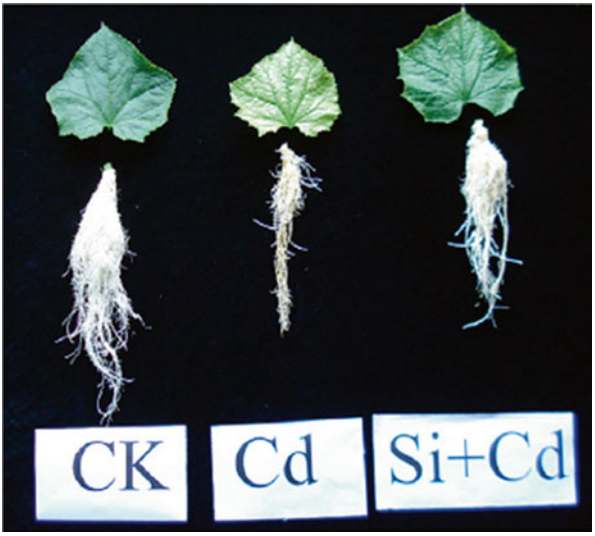

Fig. 5.5 Effects of exogenous silicon on the phenotype of cucumber leaves and roots treated with Cd (From Feng et al. 2010)

and respiratory, pulmonary, cardiovascular and neurological diseases (Mandal and Suzuki 2002). As toxicity to humans has been an ever-increasingly important public concern because As contamination events are frequently reported throughout the world, particularly in some developing Southeast Asian countries such as Bangladesh, India and China (Dhar et al. 1997; Nordstrom 2002). As contamination may occur in soil and water through repeated application of As-containing pesticides and fertilizers, atmospheric deposition from the burning of fossil fuels, disposal of industrial and animal wastes and mining activities (Meharg et al. 2009). Contamination of soil and irrigation water with As may consequently lead to As accumulation in rice grain or straw (Abedin et al. 2002; Meharg et al. 2009; Finnegan and Chen 2012).

Upon exposure of plant roots to As, the metalloid inhibits root extension and proliferation. Once taken up and transported into the cell, arsenate (As^V) can be readily converted to arsenite (As^{III}), which is more toxic to living organisms. It has been demonstrated that both forms of As (As^V and As^{III}) can disrupt plant metabolism and severely inhibit both plant vegetative and reproductive growth and consequently lead to fertility and yield losses (Garg and Singla 2011; Finnegan and Chen 2012).

So far, limited information is available on Si and As interactions as only a few papers have been published on this topic (Guo et al. 2005; Bogdan and Schenk 2008; Shi 2008; Tripathi et al. 2013). Guo et al. (2005) investigated the effect of silicate on the growth and arsenate uptake by rice seedlings grown hydroponically and found that shoot dry weights increased with increasing external Si concentrations in the culture solution. The addition of Si to the culture solution at rates of 0.5, 1.0 and 2.8 mM Si increased shoot dry weight by 24, 37 and 33 %, respectively, but had little effect on root dry weight.

In a pot experiment on rice grown with three Si levels (0, 200 and 400 mg Si kg^{-1}) and three As levels (0, 25 and 50 mg As kg^{-1}) Shi (2008) showed that on the average, compared with Si-untreated treatments, the application of Si at 200 and 400 mg kg^{-1} increased shoot biomass of rice plants by 22 % and 32 %, respectively, and root biomass by 22 % and 34 %, respectively. However, the response of rice grain yield of As-stressed plants was shown to depend on the application rate of Si (Shi 2008). The application of Si increased the grain yield by 53 % at a lower rate (200 mg kg^{-1}), but reduced the grain yield at a higher rate (400 mg Si kg^{-1}) (Shi 2008). In the case of the rice *Lsi1* mutant defective in Si uptake, the application of Si (200 and 400 mg Si kg^{-1}) increased shoot biomass by 55 % and 27 %, respectively, and root biomass by 14 % and 7 %, respectively. However, the application of Si (200 and 400 mg Si kg^{-1}) decreased grain yield by 35 % and 74 %, respectively. The reason why grain yield for the rice *Lsi1* mutant was reduced by the Si application is still unclear. On the other hand, Bogdan and Schenk (2008) investigated the effect of Si on As accumulation in the grain of rice grown on six paddy soils in the greenhouse condition. They found a significant inhibitory effect of indigenous silicic acid in the soil solution on As uptake and accumulation, implying that soils with high plant-available Si contents result in low plant As accumulation and that Si application to soils may further decrease the As content of rice.

5.1.5 Chromium

Chromium (Cr) is another highly toxic oxianionic metalloid. Cr contamination is now receiving increased public attention because it has become a serious environmental problem as a consequence of anthropogenic activities including mining or industrial activities and repeated use of metal-enriched chemical fertilizers, pesticides, sewage sludge and wastewater irrigation in agriculture (Zayed and Terry 2003). The three species of Cr (Cr^{III}, Cr^{IV} and Cr^{VI}) are all highly phytotoxic, while Cr^{VI} is toxic to all living organisms. For plants, exposure to excessive Cr leads to inhibition of seed germination and plant growth, nutrient and water imbalance, degradation of photosynthetic pigments, inactivation of mitochondrial electron transport and inhibition of antioxidant defence enzyme activities (Dixit et al. 2002; Shanker et al. 2005; Panda 2007). Recently, Si has been reported to be effective in mitigating Cr toxicity in rice (Zeng et al. 2011; Tripathi et al. 2012b), barley (Ali et al. 2013) and pakchoi plants (Zhang et al. 2013b). In a hydroponic experiment with two Cr levels (0 and 100 μM), three Si levels (0, 1.25, and 2.5 mM) and two rice genotypes differing in grain Cr accumulation, Zeng et al. (2011) showed that seedling height and dry biomass of the two rice varieties were significantly reduced under Cr stress, but the reduction in seedling height was significantly alleviated by the inclusion of Si to the solution, and this alleviative effect was particularly distinct in the highest Si treatment. Tripathi et al. (2012b) also investigated the Cr^{IV} and Si interactions in rice and found that Cr treatment inhibited plant growth and decreased photosynthetic pigment and protein contents, which was accompanied by a significant increase in Cr accumulation and lipid peroxidation (as malondialdehyde; MDA), whereas the addition of Si to the Cr treatment alleviated Cr toxicity and promoted growth of rice by decreasing Cr accumulation, root-to-shoot Cr transport and MDA level.

In a pot experiment with pakchoi plants grown at three levels of Cr (50, 100 and 200 mg kg^{-1} Cr) and three levels of Si (0.5, 1.0 and 1.5 g kg^{-1} Si), Zhang et al. (2013b) demonstrated that Si supply increased plant growth but only at the lowest Cr level (50 mg Cr kg^{-1}). Increasing Si supply decreased shoot dry weight at both 100 and 200 mg Cr kg^{-1}. Ali et al. (2013) showed that application of Si mitigated Cr toxicity in barley plants as reflected by a significant increase in growth and photosynthetic parameters. They also demonstrated that the alleviative effect of Si on Cr toxicity was stronger at a higher Si level than at a lower Si level (2 mM Si vs. 1 mM Si).

5.1.6 Lead

Lead (Pb) is also a nonessential but highly toxic heavy metal that tends to accumulate in the environment because of its strong sorption to the minerals in soils and sediments. Pb contamination in soil, water and sediment arising from agricultural and industrial activities is a worldwide environmental problem. Exposure to excessive Pb can cause serious health risks and represents a genuine public concern. In plant tissues, excessive Pb affects membrane structures, inhibits photosynthesis and causes nutrient, water and phytohormone imbalance. Typical toxicity symptoms include stunted growth, chlorosis and root blackening.

In recent studies, Si has been reported to be effective in mitigating Pb toxicity in plants (Araujo et al. 2011; Li et al. 2012b; Bharwana et al. 2013). Araujo et al. (2011) showed that Si supply alleviated the inhibitory effect of Pb on the growth of maize. Bharwana et al. (2013) found that the addition of Pb (50 μM and 100 μM) to the culture solution caused a severe inhibition of cotton growth, which was fully alleviated by addition of 1 mM Si. Li et al. (2012b) reported that soil treatment with 800 mg Pb kg^{-1} decreased both shoot and root dry weight of banana seedlings, while the amendment of Si at 800 mg kg^{-1} (as sodium metasilicate) significantly enhanced banana biomass.

5.1.7 Zinc and Copper

Both zinc (Zn) and copper (Cu) are essential micronutrients for plant growth with numerous important metabolic functions (Marschner 1995). However, exposure to excessive Zn or Cu concentrations can cause severe phytotoxicity. Commonly visible symptoms of Zn toxicity include dark pigmented spots or blotches on older leaves, particularly on petioles and around the margins and veins of leaf blades

(O'Sullivan et al. 1997). Yellowing and wilting of sweet potato leaves are typical consequences of severe damage to the roots exposed to high Zn concentrations. Symptoms of severe Fe deficiency induced by Zn toxicity include a pale yellow to white interveinal chlorosis and eventual necrosis of leaf blades and growing points (O'Sullivan et al. 1997). Cu toxicity symptoms observed typically in moderate cases include pale green to white chlorotic mottles occurring on some mature leaves with even minor veins remaining green. However, in more severe cases, slow growth and poor root system development were generally accompanied by prominent chlorosis (O'Sullivan et al. 1997). So far, it has been demonstrated that the addition of Si can mitigate Zn toxicity in maize (Cunha et al. 2008; Cunha and Nascimento 2009; Kaya et al. 2009) and rice (Song et al. 2011; Gu et al. 2012). On the other hand, Masarovic et al. (2012) have shown that Si supply did not significantly alleviate Zn toxicity in young sorghum with an exception of Si-stimulated growth of primary seminal roots. Kaya et al. (2009) reported that significantly lower chlorophyll content, leaf relative water content (RWC) and biomass were noted in maize plants growing under excess Zn (0.5 mM) stress than in the control plants, whereas the addition of 1.0 mM Si significantly reduced those symptoms. As shown in Fig. 5.6, Song et al. (2011) showed that symptoms of Zn toxicity in rice were typically defined by a yellow colour on the lower leaves starting from the tips and spreading towards the base of the leaves. However, all symptoms of Zn toxicity were significantly alleviated by the addition of Si (Fig. 5.6). Gu et al. (2012) also showed that the addition of Si overcame growth inhibition of rice seedlings subjected to high Zn. Moreover, the alleviative effect of Si on Zn toxicity was dependent on the Si level used. Cunha et al. (2008) found that the root and shoot biomass of maize was increased by 80 % and 59 %, respectively, by applying 200 mg Si kg^{-1} to an acidic tropical soil contaminated with 100 mg Zn kg^{-1}.

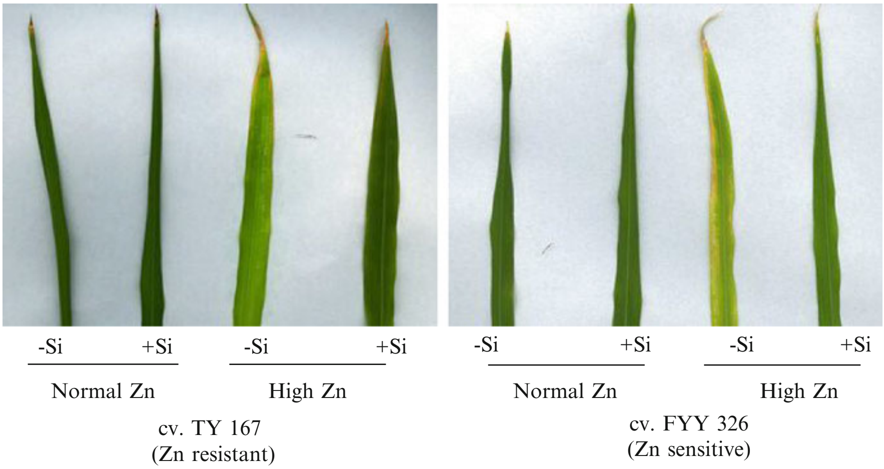

Fig. 5.6 Toxicity symptoms on two rice cultivars grown hydroponically with either normal (0.15 μM) or high (2 mM) Zn with (1.5 mM) or without Si for 7 days (From Song et al. 2011)

Cu toxicity has been recently reported to be mitigated by the addition of Si in *Arabidopsis thaliana* (Li et al. 2008; Khandekar and Leisner 2011), *Zinnia elegans* (Frantz et al. 2011), *Erica andevalensis* (Rossini et al. 2011) and rice (Kim et al. 2014a). Collin et al. (2014) reported that bamboo grown hydroponically with 100 mM Cu induced suffered a significant growth inhibition with severe visual toxicity symptoms (i.e. chlorotic leaves and brown root coloration); the addition of 1.1 mM Si significantly resorbed those symptoms. Frantz et al. (2011) investigated Si-mediated tolerance to Cu toxicity in both a Si-accumulating [zinnia (*Zinnia elegans*)] and a Si-non-accumulating [snapdragon (*Antirrhinum majus*)] species based on visible stress indicators and dry weight analysis and showed that initially Si had a positive effect on zinnia but not on snapdragon. However, enzymatic assays and nutrient analysis of plant tissues showed that both species responded to supplemental Si, showing evidence of alleviated stress and balanced nutritional status more similar to healthy, control plants than plants exposed to high Cu stress. Rossini et al. (2011) examined the influence of Si added at 0.5 and 1.0 mM on responses to Cu excess in *Erica andevalensis* grown in nutrient solutions containing 1 or 500 μM Cu. Plants treated with 500 μM Cu showed differences in growth and shoot water content depending on Si supply, and the addition of 1 mM Si to high-Cu nutrient solutions significantly improved plant growth and reduced water loss, thus preventing plant death related to Cu excess. Li et al. (2008) investigated the role of Si in plant response to Cu stress in *Arabidopsis thaliana* and found that leaf chlorosis and shoot and root biomass reduction caused by excess Cu were significantly alleviated by Si amendments.

5.2 Mechanisms of Alleviation of Metal Toxicity by Silicon

The alleviative effect of Si on metal toxicity has been well documented, but the underpinning mechanisms are still unclear and controversial. Based on the available information in the literature, two major mechanisms, i.e. external (*ex planta*) and internal (*in planta*), have been proposed for explaining how Si can mitigate metal toxicity in plants. The *ex planta* mechanism was proposed prior to the *in planta* mechanism. From a chemical point of view, the *ex planta* mechanism is much simpler and easier to understand than the *in planta* mechanism. Nevertheless, the latter is more fascinating for plant physiologists. .

*5.2.1 External (*Ex Planta*) Mechanisms*

5.2.1.1 Effect of pH

External (*ex planta*) mechanisms include pH effects and reduced bioavailability of metals and are based partly on both solution chemistry of metals and interactions of metals with Si that are strongly affected by pH values in solution. Although Al and

Si are considered to form biologically unavailable complexes in solution, it is still unclear what concentrations of Al and Si and what pH ranges are required to form these complexes (Cocker et al. 1998a). The formation of aluminosilicate (AS) and its species, like Al per se, depends upon pH and the AS species that are insoluble at near neutral pH values (Cocker et al. 1998a).

The activity or bioavailability of metals in the root growth medium is strongly associated with the pH value, while inclusion of Si increases pH, especially when the basic or alkaline silicates such as sodium silicate, potassium silicate, calcium, magnesium silicate, etc., are used as Si sources. This *ex planta* mechanism was the first proposed to explain how Si can mitigate Al toxicity in plants (Hodson and Evans 1995; Hammond et al. 1995; Cocker et al. 1998b).

Li et al. (1996) proposed that the mechanism of alleviation of Al toxicity by Si could be attributed to pH effects but not the direct interactive effects of Si on Al in solutions. Their conclusions were drawn mainly from the following observations: (1) in solutions containing 296 μM Al with no Si added, the growth of sorghum seedlings was significantly decreased compared to the controls; (2) the root growth of seedlings grown hydroponically with 296 μM Al and varying Si levels was improved significantly with increasing levels of Si, but such effects were observed only when Al was added to the basal nutrient solution following the addition of Si without lowering the solution pH; (3) the addition of Si to the basal nutrient solution led to a pH increase up to or over 10.0, which facilitated precipitation of Al with Si and consequently decreased Al phytotoxicity. However, if the solution pH was lowered to 4.0 following the addition of Si, prior to the addition of Al, Al toxicity was maintained in presence of Si in solutions containing Al ranging from 534 to 1602 μM (Li et al. 1996). However, it was reported that, prior to the addition of the Al salt, even when the solution pH was adjusted to be within the acidic pH range to avoid precipitation of Al with Si, Al toxicity in wheat was still significantly alleviated by Si (Cocker et al. 1998b), suggesting that the formation of hydroxyaluminosilicate (HAS) was limited in bulk solutions with free Al levels in solutions unaffected by Si, but rather the alleviation mechanisms of Al toxicity by Si might involve the formation of HAS in the root apoplast (*in planta* mechanism).

It is generally recognized that the *ex planta* mechanisms similar to Si-alleviated Al toxicity applies to Si-mediated detoxification of Mn, Cd, Cr, Pb, Zn and Cu in soil/plant systems, i.e. reduction in metal availability via metal immobilization arising from an increase of pH (Galvez et al. 1987; Chen et al. 2000; Liang et al. 2005; Gu et al. 2011; Li et al. 2012b; Zhang et al. 2013a, b). This is easy to understand and also true when basic sodium metasilicate, slag or alkaline Si-containing materials such as biosolids and coal fly ash are incorporated into metal-contaminated soils as Si sources (Chen et al. 2000; Liang et al. 2005; Gu et al. 2011, Zhang et al. 2013b). Chen et al. (2000) reported that furnace slag was more effective in suppressing Cd uptake by rice and wheat than $CaCO_3$ or steel sludge. They speculated that the pH rise effect caused by the furnace slag used together with the plant-available Si released from the furnace slag contributed to the reduction of Cd uptake by plants (Chen et al. 2000). In a pot experiment with maize, Liang et al. (2005) showed that the application of sodium metasilicate at a higher rate of Si (400 mg Si kg^{-1}) to an acidic soil increased soil pH by 1.2 units, which contributed partly to the alleviation of Cd toxicity by Si. However, such Si-induced pH rise effects could not sufficiently explain the mitigation of Cd toxicity in maize. To further reveal the whole picture of Si effects on Cd detoxification in maize, Liang et al. (2005) ran another pot experiment in which less amount of silicate (50 mg Si kg^{-1}) was incorporated into the same Cd-contaminated soil tested to keep the soil pH unchanged. They reported that no significant differences were found either in soil pH or in plant-available Cd concentrations (e.g. $CaCl_2$-extractable fraction and water-soluble fraction of Cd) between the Si-treated soil and the Si-untreated soil. Nevertheless, Cd concentration in xylem sap was significantly lower in Si-treated plants than in Si-deprived plants, and Cd toxicity in maize was still significantly mitigated. These findings clearly suggest that the Si-induced pH rise effects are insufficient to explain the Si-mediated Cd detoxification, and both *ex planta* and *in planta* mechanisms must be involved. Gu et al. (2011) reported that the pH value of an acidic soil tested was increased by 1–2 units following the incorporation of coal fly ash and steel slag, leading to an over 60 % decrease in plant-available concentrations of heavy metals (Cd, Zn, Cu and Pb) in the soil tested. They further demonstrated significant negative correlations between heavy metal concentrations and pH values. Li et al. (2012b) reported that the addition of 800 mg Si kg^{-1} as sodium metasilicate to a Pb-contaminated soil significantly increased soil pH and carbonate- and residual-bound fractions of Pb, but decreased exchangeable fraction of Pb, thus reducing the plant availability of Pb. By contrast, addition of 100 mg Si kg^{-1} did not influence soil pH but still exhibited similar effects of Pb immobilization. All these findings seem to suggest that, apart from Si-induced pH effects, metal immobilization or inactivation in the growth medium by complexation of metals with silicates incorporated is also attributed to Si-mediated metal detoxification in plants (see Sect. 5.2.2 in this chapter).

5.2.1.2 Reduced Metal Bioavailability

It has been demonstrated that the availability of Al to living organisms in natural waters could be limited due to the fact that, chemically, Al and Si can form metastable species of AS in dilute acidic solutions (Birchall 1990; Exley and Birchall 1992, 1993; Chappell and Birchall 1988). Birchall et al. (1996) agreed that as the precursors of amorphous minerals such as proto-imogolite and allophanes, hydroxyaluminosilicate (HAS) species can form in dilute Al solutions containing high concentrations of silicic acid at pH ranging from 4.5 to 7.5. By using membrane filtration, ion exchange and dialysis, Exley and Birchall (1992) were able to identify HAS formation in solutions of low Al concentration (4 μM) and Si concentration (0–500 μM). Birchall (1992, 1993) and Exley and Birchall (1992) provided evidence for the formation of HAS and concluded that the reduction of Al bioavailability by Si could be attributed to the formation of HAS species in solutions at pH ≥4.0, especially in the rhizosphere where a boundary layer of near neutral pH may exist and is likely to be enhanced in the case of plants grown in hydroponics with nitrate as a sole nitrogen source (Hodson and Evans 1995). Taylor et al. (1997)

reported that the affinity of silica for Al was at least a million times stronger in an oligomeric form of silica (a soluble low-molecular-mass form) than in a monomeric form of silica, suggesting that this oligomeric form of silica may be responsible for the amelioration of Al toxicity observed, but these compounds have yet to be detected in the natural environment.

Barceló et al. (1993) showed that Si-mediated alleviative effects on Al detoxification in teosinte were attributed to HAS formation, leading to a significant reduction in concentration of monomeric Al species in the nutrient solution in the presence of Si. Ma et al. (1997) also concluded that the amelioration of Al toxicity in maize by Si was due to the formation of Al and Si complexes in solution rather than a physiological effect of Si on the plant, because they measured a decrease of the concentration of toxic Al^{3+} in the presence of Si.

More recently, this mechanism of Al immobilization or inactivation by Si has also been considered with respect to other heavy metals such as Cd, Cr, Pb, Zn and Cu (Liang et al. 2005; Yang et al. 2005; Gu et al. 2011; Gu et al. 2011; Zhang et al. 2013b). Liang et al. (2005) reported that both water- and $CaCl_2$-extractable fractions of Cd were significantly lowered by the application of Si at a rate of 400 mg kg^{-1}, but no effect was detected when only 50 mg Si kg^{-1} was applied. The same group (Yang et al. 2005) found that the ameliorative effect of Si on Cd toxicity in maize was a silicate-induced alteration of Cd forms in soil, resulting in a reduction of Cd bioavailability. They found that, at pH 5.9, Si application increased the carbonate-bound and the residual fractions of Cd, but decreased the exchangeable and Fe/Mn oxide-bound fractions of Cd. Although Si was applied as sodium metasilicate, these authors neutralized the induced pH change before application and showed that the pH effects of Si application on Cd immobilization in soil were negligible (Yang et al. 2005). Similar conclusions were drawn by Cunha et al. (2008) where the Cd detoxification in maize by silicates was attributed to Cd immobilization, but not to the increase of soil pH driven by the application of calcium silicate. They showed that Si altered the Cd and Zn fractions in soil, decreasing the most labile pools and increasing the more stable fractions such as those bound to organic matter and crystalline Fe oxides. More recently, Gu et al. (2011) showed that Cd, Zn, Cu and Pb concentrations estimated by the diffusive gradients in thin films technique were significantly decreased (by at least 84 %) in the steel-slag treatments and metal fluxes from the soil solid phase to solution were significantly reduced by steel-slag amendments as well. X-ray diffraction (XRD) analysis of steel slag spiked with heavy metals showed that the heavy metal precipitates (depositions) were mainly composed of insoluble Cd, Zn, Cu and Pb silicates, phosphates and hydroxides, showing the immobilization of heavy metals by steel-slag amendments (Gu et al. 2011). Zhang et al. (2013b) reported that shoot accumulation of Cr in pakchoi grown in a soil contaminated with Cr was significantly decreased by application of Na_2SiO_3. The alleviative effect of Si was mainly ascribed to the reduction of exchangeable-bound Cr fractions in the soil and the formation of precipitate-bound and organic matter-bound Cr.

It has been suggested that a critical concentration of Si at which the formation of HAS occurs is 100 μM (Exley and Birchall 1992). However, Cocker et al. (1998b) demonstrated that Al toxicity in Al-sensitive wheat grown at 1.5 μM Al could be significantly alleviated by the addition of 5 μM Si, which is well below the suggested critical Si requirement. They also demonstrated that Al toxicity symptoms were reduced in wheat at the lower end of the pH range ($pH \leq 5.0$) in which HAS formation is believed to occur, and Si addition did not reduce free Al concentration in solution.

Clearly, it is not conclusive yet whether co-deposition or precipitation of metals with Si in nutrient solutions or solid growth media always plays an important role in Si-mediated reduction of metal toxicity. However, information available from the literature so far shows that the results depend largely upon the experimental conditions employed. Obviously, although the *ex planta* mechanisms can explain in part why silica, particularly the basic silicates or Si-containing materials applied to the growth medium, can effectively alter metal toxicity in some cases, it is undoubtedly not the sole mechanism involved in Si-mediated metal detoxification in plants.

*5.2.2 Internal (*In Planta*) Mechanisms*

As early as 1940, it was found that the concentrations of mineral nutrients were higher in aerial parts of Si-deficient plants than in those of Si-sufficient plants (Wagner 1940). Later, more researchers confirmed this phenomenon (Yoshida et al. 1959; Okuda and Takahashi 1962; Tanaka and Park 1966). This role of Si in influencing accumulation of Mn and Fe seemed to be the most apparent in rice because rice plants normally have five to ten times higher leaf Mn concentrations compared to other grasses (Vlamis and Williams 1967). Barley plants grown hydroponically developed necrotic spots on their leaves under excess Mn, while the Mn toxicity symptoms disappeared by the addition of Si, which was attributed to Si-mediated microdistribution of Mn in barley leaves (Williams and Vlamis 1957a, b). In a later study, it was shown that barley, rye (*Secale cereale*), rice and ryegrass (*Lolium perenne*) all developed Mn toxicity symptoms (i.e. brown necrotic spots) in the absence of Si, but Si addition prevented the development of necrotic spots (Vlamis and Williams 1967). Okuda and Takahashi (1962) demonstrated that the Si-mediated reduction of Fe and Mn toxicity in rice plants could be attributed to a decrease in uptake of Fe and Mn by the plants in the presence of Si. They also indicated that the reduced forms of Fe and Mn (i.e. Fe^{2+} and Mn^{2+}), the major forms of Fe and Mn, were much more readily oxidized by the roots in the Si-fed rice than in the Si-deficient rice, thus forming more insoluble coatings as Fe or Mn plaque on the surface of the Si-fed rice roots. Thus, Si is believed to promote the oxidative power of rice roots with the consequent apoplastic deposition of Fe and Mn oxides. This effect depended upon Si within the rice plants but not upon Si in the external solution.

Over the past two to three decades, more progress has been made in demonstrating *in planta* mechanisms of Si-mediated detoxification of metals in plants (e.g. Cocker et al. 1998a, b; Horiguchi and Morita 1987; Iwasaki and Matsumura 1999;

Iwasaki et al. 2002a, b; Rogalla and Römheld 2002; Wang et al. 2004; Liang et al. 2005; Nwugo and Huerta 2008a,b; Song et al. 2009; Song et al. 2011; Li et al. 2012a; Dragisic Maksimovic et al. 2012). For example, Cocker et al. (1998a) demonstrated that Si could reduce the inhibitory effects of Al on root elongation under conditions that were neither the result of Si-induced increase in solution pH nor the formation of HAS. In the experiments with the Al-sensitive maize (*Zea mays* L.) cultivar Lixis, Wang et al. (2004) showed that Si treatment but not Si pretreatment mitigated Al-induced root injury as indicated by less root growth inhibition and callose formation and Si treatment did not alter monomeric Al concentrations in the nutrient solution, suggesting an *in planta* mechanism. More recently, Gu et al. (2012) showed that, compared with the high Zn treatment alone (200 μM Zn), the addition of 0.5 or 1.8 mM Si to the high Zn treatment had no significant impacts on the activity of free Zn^{2+} in the nutrient solutions, although the Si treatments mitigated Zn toxicity in rice seedling.

5.2.2.1 Uptake and Translocation of Metals

The question whether Si can suppress the uptake of metals or not is still under debate. Okuda and Takahashi (1962) postulated that Si decreased Fe and Mn uptake in rice plants by increasing the oxidizing power of the roots grown under flooded conditions. However, Williams and Vlamis (1957a, b) and Vlamis and Williams (1967) clearly demonstrated that, at least in graminaceous species, the uptake of Mn was not reduced by Si application, but Si caused Mn to be more evenly distributed instead of being concentrated in discrete necrotic spots. This finding was supported by subsequent experiments in dicots (Horst and Marschner 1978a; Iwasaki and Matsumura 1999; Rogalla and Römheld 2002; Führs et al. 2009). Iwasaki and Matsumura (1999) demonstrated that Si alleviated Mn toxicity through a co-localized accumulation of Mn with Si in a metabolically inactive form around the base of the trichomes on the leaf surface, while Si addition did not affect the plant Mn content. Horiguchi (1988) reported that Si treatment altered the distribution of Mn by increasing Mn concentration in roots and decreasing that in shoots, thus reducing the leaf symptoms of Mn toxicity in rice. In cucumber grown under Mn excess, the leaf concentration and total content of Mn were found to be independent or even higher in the Si-fed plants than in Si-deprived plants (Rogalla and Römheld 2002; Shi et al. 2005a). On the contrary, Li et al. (2012a) showed that the addition of Si decreased shoot Mn concentration, but greatly increased root Mn concentration in a Mn-sensitive rice cultivar (XXY) (Table 5.5). Similar to the findings of Horiguchi (1988), this phenomenon indicates that Si-enhanced Mn tolerance is not due to restriction of Mn uptake from solutions, but to restriction of root-to-shoot Mn transport. However, Li et al. (2012a) also showed that the addition of Si greatly decreased both shoot and root Mn concentration in a Mn-tolerant rice cultivar (ZLY), suggesting that Si-enhanced Mn tolerance is due to restriction of Mn uptake from the nutrient solutions (Table 5.5). This would suggest that Si-mediated Mn tolerance is genotype dependent.

Table 5.5 Mn concentrations in shoots and roots of rice plants grown in nutrient solution containing normal (6.7 μM) or high (2.0 mM) Mn with or without Si supply

Variety	Mn treatment	Si supply	Shoot (μg g^{-1} DW)			Root (μg g^{-1} DW)		
XXY	Normal	–	415.80 ± 44.70			135.93 ± 5.26		
		+	490.51 ± 54.25			187.35 ± 12.86		
	High	–	4515.82 ± 175.58			2069.20 ± 39.76		
		+	4340.81 ± 62.06			3804.40 ± 137.82		
ZLY	Normal	–	335.11 ± 4.70			193.64 ± 12.96		
		+	445.79 ± 60.17			291.83 ± 61.98		
	High	–	5754.97 ± 82.31			3532.94 ± 345.31		
		+	2088.34 ± 185.40			1481.43 ± 81.31		
			Shoot			Root		
			df	*P*	LSD$_{0.05}$	*df*	*P*	LSD$_{0.05}$
	Cultivar		1	<0.001	149.918	1		200.263
Source of variation	Mn		1	<0.001	149.918	1	<0.001	200.263
	Si		1	<0.001	149.918	1		200.263
	Cultivar × Mn		1	<0.05	212.017	1		283.215
	Cultivar × Si		1	<0.001	212.017	1	<0.001	283.215
	Mn × Si		1	<0.001	212.017	1		283.215
	Cultivar × Mn × Si		1	<0.001	299.837	1	<0.001	400.526

From Li et al. (2012a). Data are means ± SD of 3 replicates

Apart from Mn, Si-mediated uptake and transport of Al and Cd are two typical case studies that have been extensively examined. Using X-ray microanalysis, Hodson and Sangster (1993) provided the first direct evidence showing that Al toxicity may be decreased through co-precipitation or co-deposition of Al with Si in the root epidermal walls of sorghum. Subsequent work in the same laboratory indicated that Al and Si were co-localized in the epidermal and hypodermal cells of the roots of wheat treated with Al and Si (Cocker et al. 1997). As is well-known, the root cell wall, which is mainly composed of cellulose and matrix polysaccharides including pectins and hemicelluloses, plays a crucially important role in Al tolerance and Al resistance (Horst 1995; Yang et al. 2008). It is in the cell wall that Al and Si interact actively, forming co-precipitation and/or co-deposition of Al with Si and leading to less Al transport into the symplast and less toxic effects of Al on cell morphology (Cocker et al. 1998a).

Chen et al. (2000) reported that the higher pH and increasing available Si from the furnace slag contributed to the reduced Cd uptake by rice and wheat, leading to a more effective mitigation of Cd toxicity by furnace slag than by calcium carbonate or steel sludge. The role of Si in minimizing uptake and root-to-shoot transport of metal ions has been confirmed in both monocotyledonous and dicotyledonous plants exposed to toxic Cd concentrations (Shi et al. 2005b; Liang et al. 2005; Zhang et al. 2008; Song et al. 2009; Shi et al. 2010; Farooq et al. 2013; Zhang et al. 2013a). In pot experiments with maize, Liang et al. (2005) showed that although soil pH and availability of Cd were unaffected by incorporation of Si at a rate of 50 mg Si kg^{-1}, Cd concentration in shoots of Si-treated plants was significantly decreased, but the total Cd uptake in shoots and roots was significantly increased due to its significantly higher shoot and root biomass compared with the Si-untreated plants. Shi et al. (2005b) reported that Si also significantly reduced the root-to-shoot transport of the apoplastic fluorescence tracer PTS (tri-sodium-8-hydroxy-1, 3, 6-pyrenesulphonate), suggesting that the heavy deposition of silica in the endodermis might partially physically block the apoplast bypass flow across the roots, thus restraining the apoplastic transport of Cd. After analysing Cd content in roots and comparing the total Cd content in plants, Zhang et al. (2008) found both Cd content in roots and the ratio increased significantly by Si treatment, indicating that the exogenous Si supply significantly enhanced Cd retention in the roots and inhibited the root-to-shoot Cd translocation.

Si-mediated Cd uptake and translocation were observed not only in Si-accumulating plant species such as rice and maize but also in non-Si accumulators such as pakchoi (Song et al. 2009) and strawberry (*Fragaria vesca*) (Treder and Cieslinski 2005). As can be seen in Fig. 5.7, the addition of 1.5 mM Si significantly decreased Cd concentration in shoots but increased Cd concentration in roots of pakchoi grown hydroponically with 0.5 or 5.0 mg Cd L^{-1} (Song et al. 2009).

It was also reported that Si used as soil amendment prior to planting was effective in preventing excessive Cd uptake by strawberry plants grown on sandy soil, and this effect was observed in stems, leaves and fruits but not in roots (Treder and Cieslinski 2005). However, foliar application of potassium silicate did not reduce the concentration of Cd in the individual parts of strawberry plants (Treder and Cieslinski 2005). More importantly, such inhibitory effects of Si on root-to-shoot Cd translocation were species dependent. For example, working with a

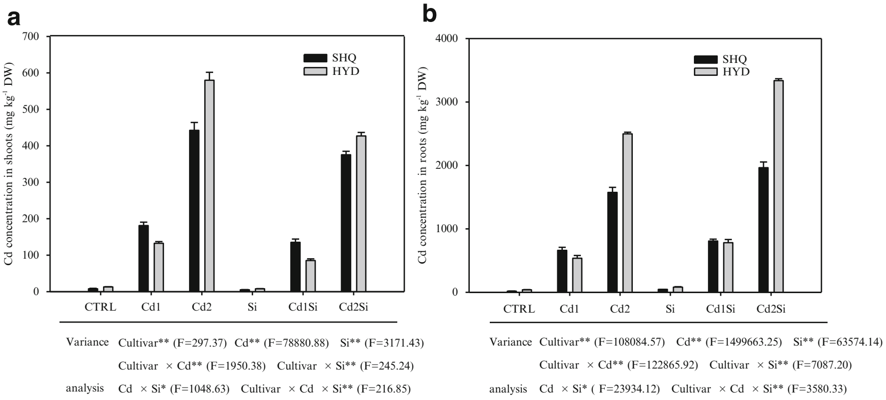

Fig. 5.7 Cd concentrations in shoots (**a**) and roots (**b**) of pakchoi plants grown hydroponically with various levels of Cd with (1.5 mM) or without Si for 7 days (From Song et al. 2009). Data are means ± SD ($n = 3$); P-value indicates significance level based on three-way ANOVA; $^*P < 0.05$, $^{**}P < 0.01$. *CTRL* treatment with neither Cd nor Si, *Cd1* treatment with 0.5 mg L^{-1} Cd, *Cd2* treatment with 5.0 mg Cd L^{-1}, *Si* treatment with 1.5 mM Si, *Cd1Si* treatment with 0.5 mg L^{-1} Cd plus 1.5 mM Si, *Cd2Si* treatment with 5.0 mg Cd L^{-1} plus 1.5 mM Si

heavy metal hyperaccumulator, *Solanum nigrum*, grown hydroponically with Cd, Liu et al. (2013a) have recently reported that the Cd concentration in both roots and shoots was significantly reduced by the addition of Si, especially in expanding and old leaves, while the relative proportion of ethanol-extractable Cd, water-extractable Cd and NaCl-extractable Cd in roots were increased, and the root-to-shoot Cd translocation was not decreased by the addition of Si. The differences in Cd translocation affected by Si between this study and those performed previously (e.g. Shi et al. 2005b; Liang et al. 2005; Zhang et al. 2008; Rizwan et al. 2012) could be attributed partly to the different plant species used. *Solanum nigrum* is a Cd hyperaccumulator, but none of rice, cucumber or durum wheat is not.

Inhibition of As uptake and root-to-shoot transport by Si has also been proposed as Si-mediated mitigation of As toxicity in rice (Guo et al. 2005; Tripathi et al. 2013). Guo et al. (2005) demonstrated that As concentrations in shoots and roots and total As uptake by rice seedlings grown with toxic level of As were significantly decreased by the inclusion of Si into the nutrient solution. The inhibitory effect of indigenous silicic acid in the soil solution on As uptake by rice was clearly shown, implying that soils with high plant-available Si contents resulted in low plant As contents and that Si application to soils may decrease the As content of rice (Bogdan and Schenk 2008).

Uptake and transport of Zn in maize and rice (Kaya et al. 2009; Song et al. 2011), Pb in banana (Li et al. 2012b), Cr in rice (Tripathi et al. 2012b) and Cu in *Erica andevalensis* (Oliva et al. 2011) have recently been reported to be significantly decreased by the incorporation of Si. Tripathi et al. (2012b) showed that Si addition alleviated Cr toxicity and promoted growth of rice by decreasing Cr accumulation and root-to-shoot Cr transport. Zn toxicity in rice (Song et al. 2011) and maize (Kaya et al. 2009) mitigated by Si added was also attributed partly to silicon-suppressed uptake and transport of Zn in plants. For example, Song et al. (2011) reported that the addition of Si significantly decreased Zn concentrations in shoots but increased Zn concentrations in roots of both Zn-sensitive (cv. FYY 326) and Zn-resistant (cv. TY 167) rice cultivars grown hydroponically with high Zn supply (2 mM Zn) (Fig. 5.8). On the contrary, addition of Si considerably increased root Zn concentrations in both cultivars to different degrees, especially in the resistant cultivar (Song et al. 2011). Oliva et al. (2011) showed that the addition of Si to high-Cu nutrient solutions significantly decreased leaf Cu concentration and increased Cu concentration in roots of *E. andevalensis*. EDX analysis of phytoliths isolated from leaves further showed that the phytoliths consisting of silica deposits were associated with Cu and other elements (K, Ca and P). The leaf phytoliths formed in Si-treated plants might have partly contributed to tolerance by Cu immobilization and inactivation. However, contrasting results have recently been reported by Collin et al. (2014) that Cu concentrations in leaves, stems or roots of bamboo grown with 100 µM Cu in a hydroponics system were not affected by Si, but the proportion of Cu adsorbed on roots was 61.5 % in the Cu plus Si treatment compared to 42 % in the Cu treatment only, suggesting that biologically active Cu was reduced by Si supplementation.

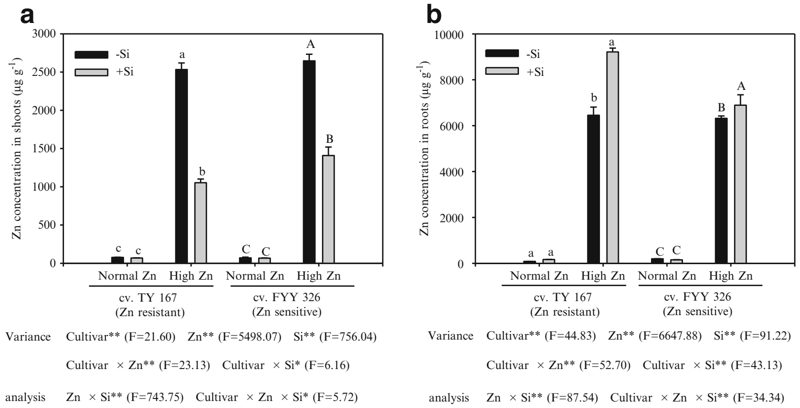

Fig. 5.8 Zn concentration in shoots (**a**) and roots (**b**) of two rice cultivars grown hydroponically in either normal (0.15 μM) or high (2 mM) Zn supplied nutrient solutions with (1.5 mM) or without Si for 7 days (From Song et al. 2011). Data are means ± SD of three replicates; *P*-values indicate significance level based on three-way ANOVA. $^*P<0.05$, $^{**}\ P<0.01$; data followed by different letters within the same cultivar are significantly different ($P<0.05$)

5.2.2.2 Binding Properties of Cell Walls

As is well known, cell walls consisting of cellulose and matrix polysaccharides (hemicellulose and pectins) play an important role in metal retention and immobilization in plants and thus in metal tolerance and/or resistance, as the cell wall is the first barrier or compartment for any ions including heavy metals to enter the cells (Horst 1995; Yang et al. 2008; Lux et al. 2011). Horst et al. (1999) observed that Si lowered the apoplastic Mn concentration in cowpea, suggesting that Si may modify the cation-binding capacity of the cell wall. Working on cowpea plants grown hydroponically with high Mn (50 μM), Iwasaki et al. (2002a,b) found that the Mn concentration in the leaf apoplastic washing fluids (AWFs) was significantly higher in plants receiving a continuous supply of Si and not showing typical Mn toxicity symptoms than in plants not receiving Si supply and showing distinct Mn toxicity symptoms. These results suggest that Si alleviates Mn toxicity not only by decreasing the concentration of soluble apoplastic Mn through enhanced binding of Mn to the cell walls, but soluble Si plays a role in the detoxification of apoplastic Mn. By working on Si and Mn tolerance in cucumber, Rogalla and Römheld (2002) reported that Mn concentrations in the AWF were significantly higher in Si-untreated plants than in the Si-treated plants, especially in the $BaCl_2$- and DTPA-extractable fraction of the leaf apoplast although the total Mn content in the leaves was unaffected by Si treatment. The Mn concentration of the AWF was correlated positively with the severity of Mn toxicity symptoms but negatively with the Si supply. Furthermore, less Mn was located in the symplast (<10 %) and more Mn was bound to the cell walls (>90 %) in the leaves of Si-supplied plants, while a uniform distribution of Mn (about 50 % in each compartment) was found in the Si-untreated plants. These authors concluded that the Si-mediated tolerance to excess Mn is a consequence of stronger binding of Mn to the cell walls which lowers Mn concentration within the symplast. However, the Si-mediated strong binding of Mn to the cell wall was detectable only when the plants received a simultaneous supply of Si and high Mn concentrations in the nutrient solution, and no alteration in Mn binding capacity was observed in Si-treated plants when isolated cell walls were loaded with Mn during a short period of time (Rogalla and Römheld 2002). Moreover, supply of Si to roots did not change the CEC of the cell wall materials isolated from cucumber leaves in the high Mn-treated plants (Dragisic Maksimovic et al. 2012). On the other hand, studies in cowpea suggest that the alleviation of Mn toxicity cannot be explained only by a decrease in free leaf apoplastic Mn through its enhanced binding by the cell wall macromolecules in Si-treated plants (Iwasaki et al. 2002b; Führs et al. 2009). It is not possible, thus, to generalize that Si-enhanced cell wall binding of Mn demonstrated in cucumber (Rogalla and Römheld 2002; Wiese et al. 2007; Dragisic Maksimovic et al. 2012) constitutes the universal mechanism of Si alleviation of Mn toxicity in all plant species. Sequestration of Mn into the vacuoles might play an important role in Si-mediated Mn tolerance in some plant species such as bean (Horst and Marschner 1978b), but again this mechanism has yet to be confirmed in others such as cowpea (Horst et al. 1999).

There is convincing evidence showing Al and Si co-deposition in plant cell walls (Hodson and Evans 1995; Wang et al. 2004). Al and Si are co-localized in the epidermis of the needles of white spruce (Hodson and Sangster 1998). By using electron energy loss spectroscopy, Turnau et al. (1993) demonstrated that Al and Si co-deposition in polyphosphate granules in the fungal symbiont *Paxillus involutus* associated with the roots of *Pinus sylvestris* is the possible mechanism of mycorrhizal amelioration of Al toxicity. Wang et al. (2004) also drew a conclusion from their data that Si treatment led to the formation of hydroxyaluminosilicates (HASs) in the apoplast of the root apex, thus detoxifying Al. Neumann and zur Nieden (2001)) also reported that Zn was co-precipitated with Si as zinc silicate in the leaf epidermal cell walls of a Zn-tolerant plant, *Minuartia verna*, naturally grown in a mining area. In a solution culture experiment with maize grown with Cd supply, more Cd was bound to the root cell walls in Si-treated plants than in non-Si-treated plants (Liang et al. unpublished), suggesting an important role of the root apoplast in Si-mediated detoxification of excess Cd. More recently, Zhang et al. (2013a) have reported the effect of Si on Cd subcellular distribution in the leaves and root tips of mangrove seedlings. They found that the mechanisms of Si amelioration of Cd stress were tissue dependent. In the leaves and root tips, Si reduced Cd concentration in subcellular fractions, Cd mobility and the concentration of biologically active Cd in the cell wall. Si did not change the distribution of Cd between compartments in the leaves, but it increased the proportion of Cd in the cell walls and reduced the proportion of Cd in the symplast of the root tips. This result is consistent with the earlier report by Wang et al. (2004) and Prabagar et al. (2011), who suggested that Si treatment reduced the concentration of biologically active Al within the cell walls of maize and Norway spruce, which decreased Al penetration and cell damage. In a vermiculite pot experiment, Ye et al. (2012) also found that Cd concentrations in different fractions extracted from the cell walls of root tips of

Kandelia obovata were greatly affected by Si supply. Silicon treatment restricted the apoplastic transport of Cd with more Cd adsorbed on the root cell walls of *K. obovata*, thus leading to a reduction of the ratio of Cd in the symplast and alleviation of Cd toxicity to the cytoplasm. This result suggests that Si enhances the Cd-binding capacity of the cell walls and restricts the apoplastic transport of Cd, resulting in Si-mediated amelioration of Cd toxicity in plants. More recently, using suspended rice cells and protoplasts as materials, Liu et al. (2013a) have demonstrated that most Si accumulated in the cell walls was present as a wall-bound organosilicon compound. At moderate concentrations of Cd in the culture medium, the protoplast Cd concentrations in Si-accumulating cells were significantly decreased compared to those from Si-limited cells (Liu et al. 2013a). In situ analysis of cellular fluxes of the Cd^{2+} in suspension cells and root cells of rice exposed to Cd^{2+} and/or Si treatments showed that + Si cells significantly inhibited the net Cd^{2+} influx, compared to –Si cells. All these findings suggest that co-deposition of Si and Cd in the cell walls via a [Si-hemicellulose] Cd co-complexation may explain why Cd ion uptake in rice can be suppressed by Si (Liu et al. 2013a; Ma et al. 2015). Working on alleviative effect of Si on Zn toxicity in rice grown hydroponically and its underpinning mechanism, Gu et al. (2012) showed that, compared with Zn treatment only, the addition of Si to the high-Zn treatment resulted in a lower Zn loading in the free symplastic and free apoplastic space and cytoplasm/vacuole and higher share of Zn in the cell-wall-bound fraction of the whole rice plant. This is further supported by the Zinpyr-1 fluorescence test and energy-dispersive X-ray spectroscopy analysis showing that the concentrations of biologically active Zn^{2+} were decreased, with Zn and Si co-localized in the cell wall of metabolically less active tissues, especially in the root sclerenchyma (Gu et al. 2012).

However, contrasting results have been reported (Shi et al. 2005b; Collin et al. 2014). For instance, Collin et al. (2014) have recently observed that Si supplementation decreased the visible damage in bamboo grown hydroponically with high Cu exposure (100 μM Cu) for 4 months, but Si was not directly involved in Cu speciation in roots, which is contrary to the findings of Wang et al. (2004) who associated Si-ameliorated Al toxicity in rice to the formation of HAS in the roots (Wang et al. 2004). Collin et al. (2014) also showed that Si increased the proportion of Cu(I) S-ligands (organic and inorganic) in leaves of bamboo exposed to high Cu stress and may have enhanced the capacity of the plant to produce S ligand available to bind Cu, thereby increasing the Cu sequestration in a less toxic form. In an earlier report, Shi et al. (2005b) showed that most of the total root Cd (87 %) was localized in the symplast of rice grown hydroponically with Cd, whereas the apoplast Cd fraction accounted for only 13 % of the total Cd irrespective of Si treatment. Furthermore, it was shown that the distribution ratios of Cd in both symplast and apoplast of the leaves of Cd-stressed rice were not changed significantly by Si treatment. X-ray microanalysis (EDX) showed that Cd was deposited mainly in the vicinity of the endodermis and epidermis, and Si deposition was heavier in the endodermis than in the epidermis (Shi et al. 2005b). The results from EDX, however, cannot explain the mechanisms underlying the Si-enhanced Cd tolerance in rice seedlings because such low Cd concentrations in the roots were far below the detection limit of EDX (Shi et al. 2005b).

Recently, Khandekar and Leisner (2011) have investigated the expression of three MT (metallothionein) genes, *MT1a*, *MT2a* and *MT2b* encoding for cysteine-rich metal-binding proteins, in *Arabidopsis* treated with toxic level of Cu and Si and found that while all three MT genes were induced by Cu, the levels of expression either remained elevated or were boosted to even higher levels in plants subjected to treatment with Si and phytotoxic doses of Cu. The elevated production of MT in the Si-treated plants under excessive Cu stress may suggest that Si may be promoting gene expression of MTs to keep the levels of soluble Cu low within the cell to alleviate Cu toxicity.

5.2.2.3 Si-Mediated Synthesis and Release of Organic Acids for Chelation of Metals

Al-induced release of organic acids (e.g. malic acid and oxalic acid) has been considered a potential mechanism of Al-tolerant plants as the acids have the potential to chelate Al ions (Ma et al. 1997; Cocker et al. 1998b; Larsen et al. 1998; Klug and Horst 2010). Barceló et al. (1993) report that the concentration of malic acid was substantially higher in roots of teosinte (a wild maize) grown hydroponically with 60 μM Al and 4 μM Si than in roots of plants with supply of 60 μM Al only. These roots containing high malic acid neither showed Al-induced growth reduction nor exhibited Al-toxicity symptoms, suggesting that chelation of Al by malic acid within plant roots contributed partly to Al tolerance of teosinte grown in Si-containing nutrient solutions. Cocker et al. (1998b) suggested that exudation of malate (or other organic compounds) into the cell walls may promote the formation of HA and/or HAS. However, Kidd et al. (2001) reported that Si-enhanced exudation of phenolic compounds (catechol and flavonoid-type phenolics: catechin and quercetin), leading to complexation and detoxification of Al, was responsible for the Si-mediated enhancement of Al resistance in an Al-resistant maize cultivar. By contrast, Wang et al. (2004) concluded that the Al-induced exudation of organic acid anions and phenols from the root apices was unaffected by Si treatment, whereas Si accumulation in the cell wall fraction was greatly enhanced by Al treatment, thereby reducing the mobility of apoplastic Al. Cocker et al. (1998b) established a schematic model showing five processes involved in the proposed mechanism of reduction of Al toxicity by Si in root tips. One of these processes inferred that malate and other organic compounds (such as phenolic acids) were secreted into the bulk media and the cell walls of roots under Al stress and some of these organic compounds promoted the formation of AS and/or HAS. This way, far less Al was available for uptake into the symplast thereby alleviating Al toxicity.

Apart from the extensive research on Si-mediated synthesis and release of organic acids for chelation of Al, little information is available regarding the Si effects on other heavy metals. It is unknown whether Si can mediate the metabolism of organic acids, amino acids, polyamine, phenolic acids and phytochelatins (PCs) and metallothioneins (MTs) to chelate heavy metal ions via *ex* or *in planta* mechanisms. More recently, Khandekar and Leisner (2011) reported that the relative transcript levels of *MT2a*, *MT2b* (the type 2 metallothioneins genes) and *PCS1* (the major phytochelatin synthase gene) in the leaves of *Arabidopsis thaliana* Sf-1 plants under Cu stress were higher in the presence of Si than in the absence of Si, but a reverse result was obtained for relative transcript levels of *MT1a* (the type 1 metallothioneins gene).

5.2.2.4 Si-Mediated Antioxidative Defence Responses

It was reported that Si could increase antioxidant defence activity and decrease oxidative damage in cucumber under Mn stress (Shi et al. 2005a). Several subsequent studies confirmed this finding in Mn-stressed rice (Li et al. 2012a) and cucumber (Dragisic Maksimovic et al. 2012). For instance, Li et al. (2012a) showed that in Mn-sensitive rice cultivar, high Mn significantly increased superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities but decreased nonprotein thiols (NPT) and glutathione (GSH) concentrations, leading to accumulation of hydrogen peroxide (H_2O_2) and MDA. The addition of Si significantly counteracted high Mn-elevated MDA and H_2O_2 concentrations and enhanced plant growth in the Mn-sensitive cultivar. In contrast, in Mn-tolerant rice cultivar, high Mn considerably raised SOD activities and GSH concentrations, thus leading to relatively low oxidative damage, while the addition of Si mainly affected nonenzymatic antioxidants. Thus, it seems that the role of Si in mediating antioxidative defence activities in high-Mn-stressed plants is genotype dependent. Dragisic Maksimovic et al. (2012) investigated the role of Si in reducing Mn toxicity caused by elevated production of hydroxyl radicals (OH) in the leaf apoplast of cucumber grown under excessive Mn concentrations. The apoplastic concentration of free Mn^{2+} and H_2O_2 in high-Mn-treated plants was significantly decreased by Si supply, whereas the addition of Si suppressed the Mn-induced increased abundance of POD isoforms in the leaf apoplastic fluid and rapidly suppressed guaiacol-POD activity under excess Mn (Fig. 5.9). Although supplying Si markedly decreased the accumulation of $\cdot OH$ in the leaf apoplast with excess Mn, adding silicic acid to the Mn^{2+}/H_2O_2 reaction mixture did not directly affect the Fenton reaction ($Mn^{2+} + H_2O_2 \rightarrow Mn^{3+} + OH^- + \cdot OH$) in vitro. These results indicate that Si contributes indirectly to a decrease in $\cdot OH$ in the leaf apoplast by decreasing the free apoplastic Mn^{2+}, thus regulating the Fenton reaction. A direct inhibitory effect of Si on guaiacol-POD activity (also demonstrated in vitro) may also contribute to decreasing the POD-mediated generation of $\cdot OH$.

Dragisic Maksimovic et al. (2007) also showed that the concentrations of phenolic compounds, such as coniferyl alcohol and coumaric and ferulic acids, in the leaf extracts tended to be lower in the Si-treated plants at high Mn supply. On the other hand, application of Si induced a significant increase in the concentrations of chlorogenic and caffeic acids in the leaf extracts of high Mn-treated plants. The POD and PPO activities were enhanced by the high Mn supply in both root and leaf extracts, while the root application of Si decreased POD and PPO activities in both roots and leaves. These results suggested that Si nutrition modulated the metabolism

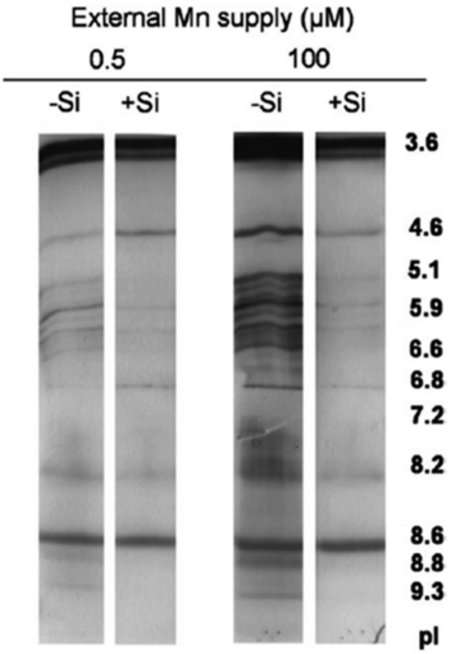

Fig. 5.9 Separation of the POD isoforms from the LAF by IEF (From Dragisic Maksimovic et al. 2012). PODs were detected by staining with 9.2 mM guaiacol and 5 mM H_2O_2. The cucumber plants were grown in nutrient solutions with normal (0.5 µM) and high (100 µM) supply of Mn, with (1.5 mM) or without $Si(OH)_4$

and utilization of phenols mainly at the leaf level by stimulating the formation of Si polyphenol complexes. Concomitantly, lower concentrations of phenolic compounds available to act as substrates for PPO and POD in Si-treated Mn-stressed plants may thus be responsible for depressing the generation of potential toxic intermediates that induce leaf browning (Dragisic Maksimovic et al. 2007). It was reported by Führs et al. (2009) that in Si-treated cowpea, a high Mn supply (not showing Mn toxicity symptoms) led to increased concentrations of benzoic acid in the apoplastic washing fluid (AWF) fraction and to decreased abundance of ferulic acid and ρ-hydroxybenzoic acid compared with non-Si-treated plants, which showed symptoms of Mn toxicity.

Numerous experiments have recently been performed to examine whether Si-mediated antioxidant defence capacity observed in Mn-stressed plants is a universal mechanism for the alleviation of metal toxicity in plants (Nwugo and Huerta 2008a; Song et al. 2009; Feng et al. 2010; Shi et al. 2010; Song et al. 2011; Zeng et al. 2011; Khandekar and Leisner 2011; Tripathi et al. 2012a, b, 2013; Liu et al. 2013a, b). Song et al. (2009) investigated the roles of Si in enhancing Cd tolerance in two contrasting non-Si-accumulating pakchoi cultivars grown hydroponically with 0.5 and 5 mg L^{-1} Cd without or with Si supply. Their results indicated that SOD, CAT and APX activities decreased, but MDA and H_2O_2 concentrations increased at the higher Cd level, which were counteracted by the addition of Si. Furthermore, ascorbic acid (AsA), GSH and NPT concentrations increased at the higher Cd level and even more so with the addition of Si. The effects of Si and Cd on the antioxidant enzyme activity were further verified by SOD and CAT isoenzyme analysis (Fig. 5.10). The Si effect was more effective in enhancing Cd tolerance in the Cd-tolerant cultivar than in the Cd-sensitive cultivar. All these results suggest that Si can mediate antioxidant defence capacity to reduce membrane lipid peroxidative damage in pakchoi plants exposed to Cd stress. Similar results regarding Si-enhanced antioxidant defence capacity were also obtained in Cd-stressed peanut, rice and *S. nigrum* (Shi et al. 2010; Tripathi et al. 2012a; Liu et al. 2013b), in Cr-stressed rice and pakchoi (Zeng et al. 2011; Tripathi et al. 2012b; Zhang et al. 2013b) and in Zn-stressed rice (Song et al. 2011). Song et al. (2011) reported that SOD, CAT and APX activities were significantly increased, whereas MDA and H_2O_2 concentrations were decreased in Si-supplied plants of both Zn-sensitive and Zn-resistant rice cultivars exposed to excess Zn. These alleviative effects of Si, further confirmed by histochemical staining methods (Fig. 5.11), were more prominent in the Zn-resistant cultivar than in the Zn-sensitive one. From Fig. 5.11, it can be clearly seen that for the Zn-resistant cultivar (TY-167), the roots treated with 2 mM Zn alone were strained extensively, while the roots became lighter straining in the Zn plus Si treatment compared with the Zn treatment alone, suggesting that Si had prophylactic effects on cell membranes against Zn-induced oxidative damage. Similar changes were also observed in the roots of the Zn-sensitive FYY-326 (Fig. 5.11) although the Si beneficial effects on the protection of cell membrane against Zn-induced oxidative damage were more significant in the Zn-resistant plant roots than in the Zn-sensitive plant roots.

Taken together, Si appears to enhance enzymatic and nonenzymatic antioxidant defence activities in plants to protect the cell organs such as chloroplasts, mitochondria and nuclei from oxidative damage, thereby leading to Si-mediated detoxification of heavy metals (see Nwugo and Huerta 2008; Song et al. 2009; Shi et al. 2010).

5.2.2.5 Molecular Aspects of Si-Mediated Metal Resistance

So far, interactive effects of Si on metal toxicity in plants have been extensively investigated at the physiological and/or biochemical level and rapid progress has been made in this field. However, very limited information is available on molecular aspects of Si and metal interaction.

Li et al. (2008) investigated two genes related to Cu transport in *Arabidopsis*, Cu transporter 1 (*COPT1*) and heavy metal ATPase subunit 5 (*HMA5*), which were induced by high levels of Cu, and found that the RNA levels of *COPT1* and *HMA5* were significantly decreased by elevated Si supply (Li et al. 2008). Khandekar and Leisner (2011) found that Cu/Zn-SOD activity was induced by Cu toxicity but SOD activity was increased even more if plants were provided with extra Si and toxic levels of Cu. This is in line with the findings showing that expression of both *CSD1* and *CSD2* is induced by high levels of Cu, while expression of both *CSD1* and *CSD2* was brought to even higher levels if plants were provided with elevated doses of Cu and Si. This is interesting, since the *CSD1* gene product is cytoplasmic, whereas that of *CSD2* is localized in the chloroplast. The increases in SOD RNA levels matched well with increases in enzyme activity, suggesting that at high doses of Cu, some of the heavy metal must be entering *Arabidopsis* leaf cells, and

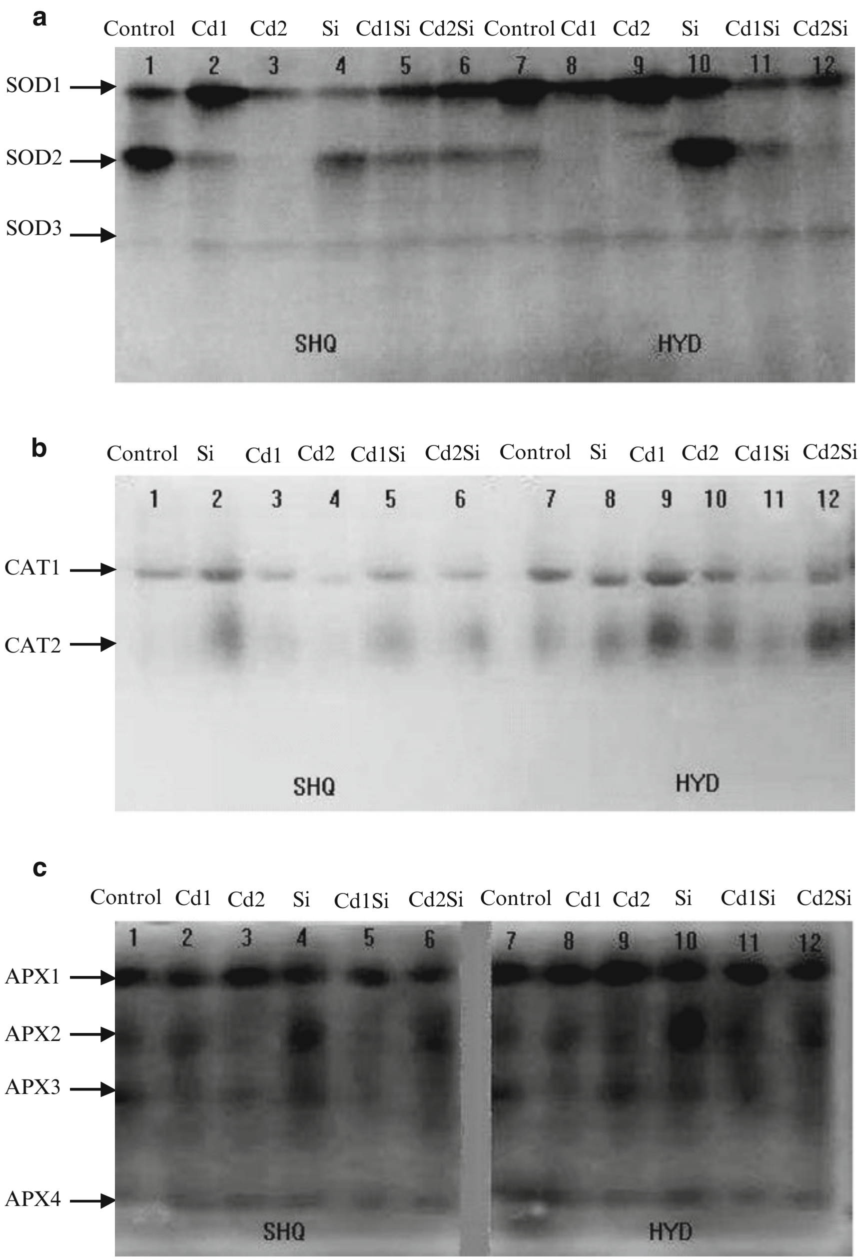

Fig. 5.10 Effect of Si on SOD (**a**), CAT (**b**) and APX (**c**) isoform activities of the leaves of two pakchoi cultivars grown under Cd stress (From Song et al. 2009). Proteins from leaves were extracted and loaded into the native PAGE. Following the electrophoresis, the gels were strained and photographed. Lanes 1–6 represent the Cd-sensitive cultivar (SHQ), while lanes 7–12 represent the Cd-tolerant cultivar (HYD). *Control*: treatment with neither Cd nor Si. *Cd1*: treatment with Cd at 0.5 mg L^{-1}. *Cd2*: treatment with Cd at 5.0 mg L^{-1}. Si: treatment with 1.5 mM Si. *Cd1Si*: treatment with 0.5 mg Cd L^{-1} plus 1.5 mM Si. *Cd2Si*: treatment with 5.0 mg Cd L^{-1} plus 1.5 mM Si

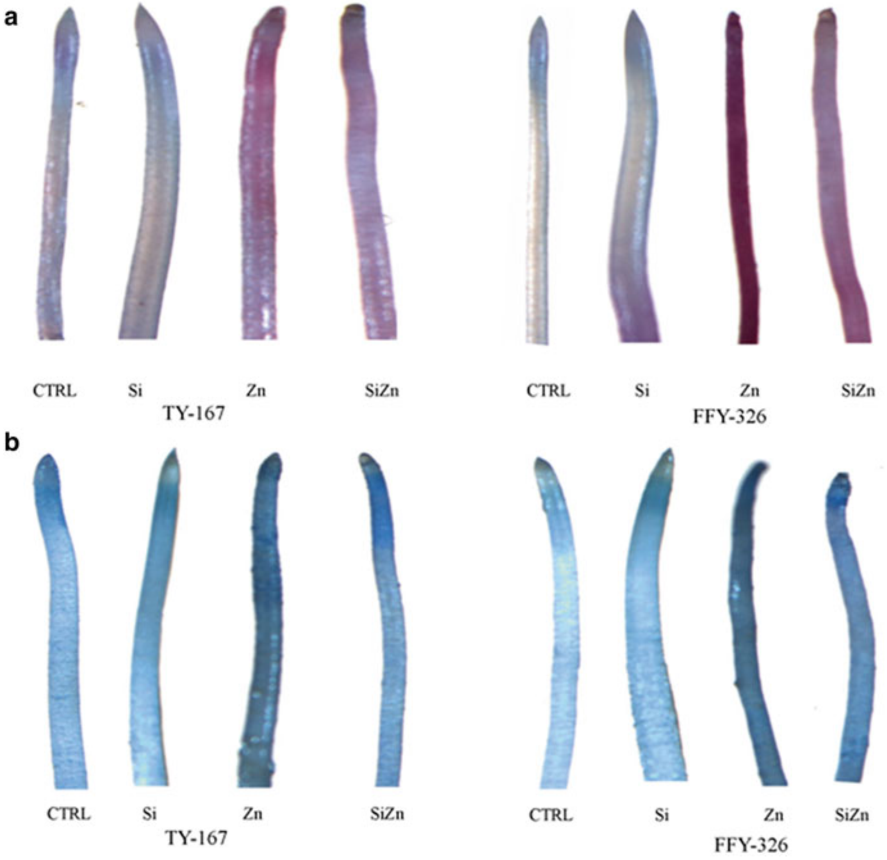

Fig. 5.11 Lipid peroxidation (**a**) and loss of plasma membrane integrity (**b**) in the root tips of Zn-sensitive rice cultivar (FFY-326) and Zn-tolerant rice cultivar (TY-167) grown hydroponically with either normal (0.15 μM) and high (2 mM) Zn, with (1.5 mM) or without Si for 7 days (Song et al. 2011). The roots were rinsed with 0.5 M HCl solution and were stained with Schiff's reagent (**a**) or Evans blue (**b**) and immediately photographed under a light microscope. The scale bar in the graph indicates 1.0 cm

Si is permitting plants to more effectively deal with Cu by enhancing expression of detoxifying genes. Plants treated with elevated Cu showed increased phenylalanine ammonia-lyase (PAL) activity that was reduced when the plants were also provided with extra Si. The three genes, *PAL1*, *PAL2* and *PAL3*, all showed similar patterns of gene expression that matched previous enzymatic data, while *PAL4* was elevated by the presence of high Cu irrespective of whether Si was present or not (Khandekar and Leisner 2011). Kim et al. (2014a) investigated the expressions of *HMA* genes (*OsHMA2* and *OsHMA3*) in rice plants grown hydroponically for 1, 5 and 10 days with six treatments (i.e. control, 1.0 mM Si, 100 μM Cu, 100 mM Cd, 100 µM Cu plus 1.0 mM Si and 100 µM Cd plus 1.0 mM Si). Their results showed that compared with control and Si treatment only, the mRNA expression of *OsHMA2* and *OsHMA3* encoding heavy metal transporters (*OsHMA2* and *OsHMA3*) was significantly upregulated by treatment with Cu or Cd, but was significantly and greatly downregulated by the combined treatment with Cu/Cd with Si. These expression patterns of *OsHMA2* and *OsHMA3* are in line with the significantly lower concentration of Cu and Cd in the combined treatment with Cu/Cd with Si as compared with the Cu/Cd treatment alone. To elucidate the mechanisms of Si involved in Cd detoxification at the molecular level, Nwugo and Huerta (2011) investigated the effect of Si on the leaf proteome of rice plants exposed to Cd stress. In total 60 protein spots were identified that were differentially regulated by Cd and/or Si treatments. Among these, 18 matching to 11 proteins were related to CO_2 assimilation/photosynthesis processes, representing the largest functional category group of proteins that were differentially expressed due to Cd and/or Si treatments. More recently, based on the results regarding Si-improved photosynthesis parameters and chloroplast ultrastructure under high-Zn (2 mM) stress, Song et al. (2014) have analysed the results of high-throughput sequencing and selected the main photosynthesis-related genes that were differentially expressed (see Fig. 5.12). According to the results of high-throughput sequencing (Fig. 5.12), Song et al. (2014) investigated the expression levels of the photosynthesis-related genes by using fluorescent real-time qPCR and showed that the expression levels of photosynthesis-related genes including *Os08g02630* (*PsbY*), *Os05g48630* (*PsaH*), *Os07g37030* (*PetC*), *Os03g57120* (*PetH*), *Os09g26810* and *Os04g38410* decreased in Si-deprived rice plants under high-Zn (0.2 mM Zn) stress, while the addition of 1.5 mM Si increased the expression levels of these genes in plants under high-Zn stress at 72 h, and the expression levels were higher in Si-treated plants than in Si-deprived plants. Similar work was also done by Li et al. (2011). They showed that a group of genes responsible for photosynthesis showed differential expression in rice grown under excess Mn (2 mM). The relative expression levels of *PsbP* and *ATPase* protein genes were significantly increased in plants treated with excess Mn, but were maintained at even higher levels in plants treated with both excess Mn and 1.5 mM Si. By contrast, expression levels of *HemD*, *Lhcb*, *pyrophosphatase* and *phosphoribulokinase* were significantly decreased under excess Mn, while the addition of Si significantly increased the expression level of these genes. Expression levels of *PsaH* were significantly increased by excess Mn, but were significantly reduced by Si.

It seems that more direct and convincing evidence needs to be accumulated to uncover the mechanisms of Si effects on plant resistance to heavy metals at a transcriptional or proteomic level. It may be interesting to investigate whether Si is involved in stimulating the efflux and inhibiting the influx of heavy metals in plasma membranes under heavy metal stress (Wu et al. 2013).

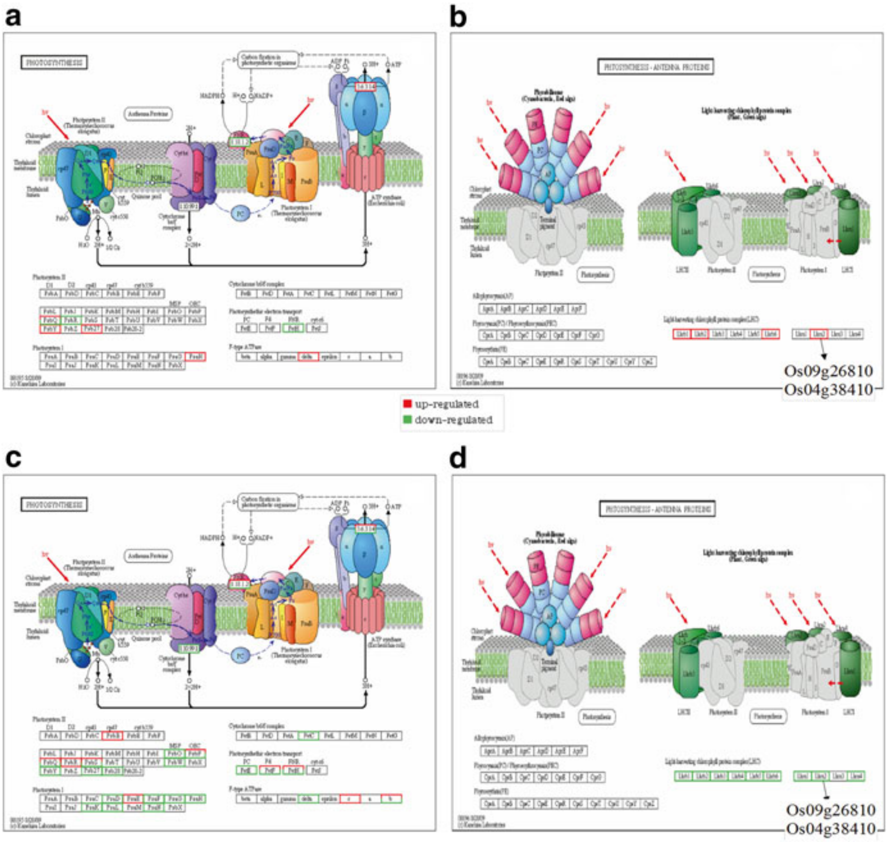

Fig. 5.12 The pathway analysis of photosynthesis (**a** and **c**) and photosynthesis-antenna proteins (**b** and **d**) of Zn-sensitive rice cultivar grown hydroponically with either normal (0.15 μM) or high (2 mM) Zn with (1.5 mM) or without Si for 72 h through high-throughput sequencing (From Song et al. 2014)

5.3 Conclusion and Perspectives

In a review paper, Ma (2001) summarized the possible beneficial effects of Si on crop growth in relation to biotic and abiotic stresses. The beneficial roles of Si were attributed mainly to silica gel deposited in the leaves and stems of plants and partially to the interactions between silicic acid and other elements such as Al, but no evidence was then available showing the involvement of Si in plant metabolism (Ma 2001; Ma et al. 2001). As discussed above, however, rapid progress has recently been made in demonstrating that Si not only plays some mechanical roles in providing resistance to stressful environments but, more importantly, is actively involved in physiological and biochemical processes. Growing evidence suggests that Si is

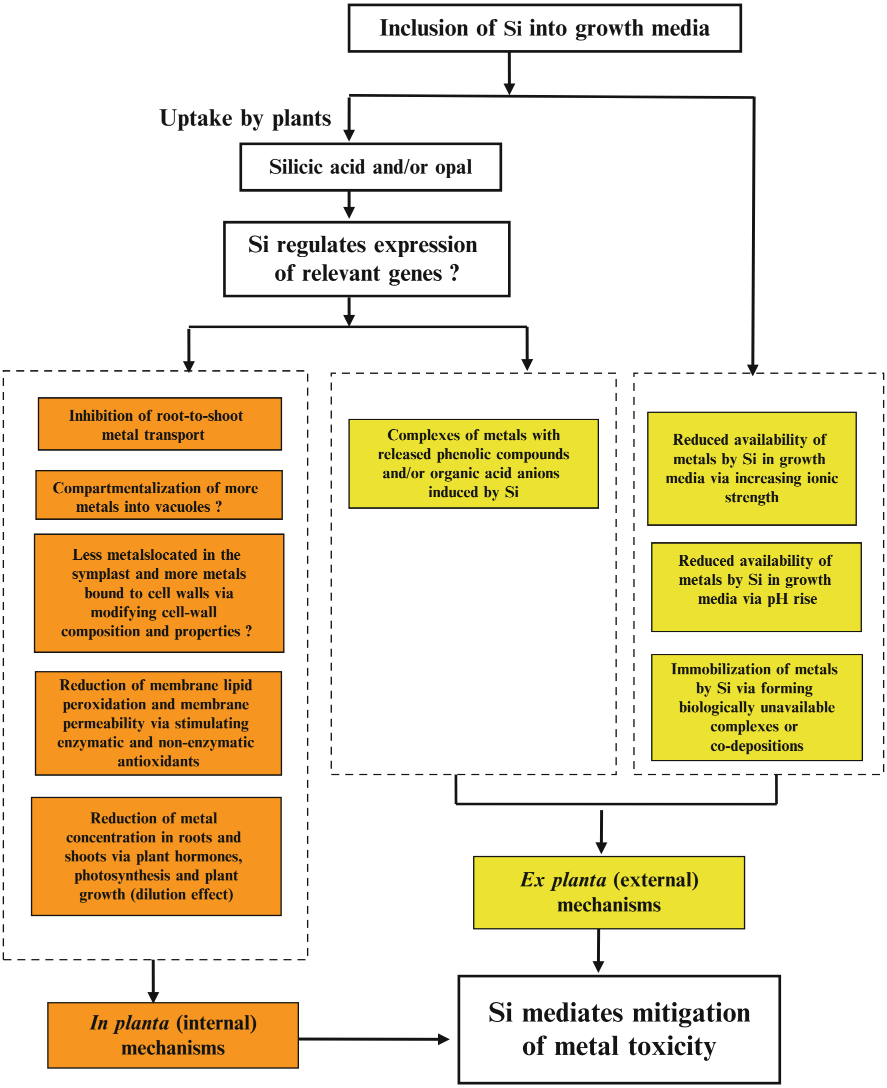

Fig. 5.13 Schematic diagram of proposed mode of action of Si-mediated alleviation of metal stress in plants

involved in regulating the expression of genes responsible for many plant metabolic processes, especially under heavy metal stress conditions. Based on the current information in the literature (Epstein 1994, 1999; Ma 2001; Liang et al. 2007; Wu et al. 2013), a schematic diagram of action model of Si-mediated alleviation of metal stress in plants is summarized in Fig. 5.13. The key question is to ascertain whether Si can regulate the expression of genes related to the metabolic processes in plants exposed to excess metals as shown in Fig. 5.6. The interactions of Si with

Al have been most clearly elucidated at the chemical and physiological or biochemical levels, but over the last 10 years, little research has been done at the molecular or gene level. While sound research has been done on the alleviative effect of Si on heavy metal toxicity, most studies have been descriptive. Future work should focus on mechanistic investigations of Si and metal interactions at the molecular level with an emphasis on transcriptomic or proteomic studies.

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