Journal of Experimental Botany, Vol. 64, No. 5, pp. 1281–1293, 2013
doi:10.1093/jxb/ers329 Advance Access publication 18 December, 2012

Journal of Experimental Botany
www.jxb.oxfordjournals.org

REVIEW PAPER

Towards establishing broad-spectrum disease resistance in plants: silicon leads the way

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Received 23 July 2012; Revised 11 October 2012; Accepted 15 October 2012

Abstract

Plants are constantly threatened by a wide array of microbial pathogens. Pathogen invasion can lead to vast yield losses and the demand for sustainable plant-protection strategies has never been greater. Chemical plant activators and selected strains of rhizobacteria can increase resistance against specific types of pathogens but these treatments are often ineffective or even cause susceptibility against others. Silicon application is one of the scarce examples of a treatment that effectively induces broad-spectrum disease resistance. The prophylactic effect of silicon is considered to be the result of both passive and active defences. Although the phenomenon has been known for decades, very little is known about the molecular basis of silicon-afforded disease control. By combining knowledge on how silicon interacts with cell metabolism in diatoms and plants, this review describes silicon-induced regulatory mechanisms that might account for broad-spectrum plant disease resistance. Priming of plant immune responses, alterations in phytohormone homeostasis, regulation of iron homeostasis, silicon-driven photorespiration and interaction with defence signalling components all are potential mechanisms involved in regulating silicon-triggered resistance responses. Further elucidating how silicon exerts its beneficial properties may create new avenues for developing plants that are better able to withstand multiple attackers.

Key words: *Cochliobolus miyabeanus*, *Magnaporthe oryzae*, *Rhizoctonia solani*, *Xanthomonas oryzae* pv. *oryzae*, biotic stress, induced systemic resistance, plant hormones, rice.

Introduction

In the absence of adaptive immunity displayed by animals, plants fend off microbial pathogens via complex resistance mechanisms providing several layers of constitutive and inducible defences. Many of these defences are controlled by a series of signalling pathways within which plant hormones play central roles. Intimately connected to each other via a network of positive and negative interactions, hormones are thought to provide flexibility to the defence signalling network by enabling plants to adaptively tailor their immune system to the type of attacker encountered (Pieterse *et al.*, 2009; Robert-Seillaniantz *et al.*, 2011). Salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) are the archetypal defence hormones and their importance in the hard wiring of the plant innate immune system is well established, especially in the model plant *Arabidopsis thaliana*. In this plant species, SA is generally associated with resistance to biotrophic pathogens, whereas JA and ET are generally associated with resistance to necrotrophic pathogens. Although there is evidence for both positive and negative relationships between these pathways (Mur *et al.*, 2006; Truman *et al.*, 2007), the primary mode of interaction appears to be mutual antagonism with corresponding trade-offs between resistance to

Abbreviations: ABA, abscisic acid; ET, ethylene; IAA, indole-3-acetic acid; ISR, induced systemic resistance; JA, jasmonic acid; SA, salicylic acid.

biotrophic pathogens on the one hand, and resistance to necrotrophs on the other (Spoel and Dong, 2008; Koornneef and Pieterse, 2008).

Recently, other hormones such as abscisic acid (ABA), gibberellin, cytokinin, auxin and brassinosteroid emerged as critical modulators of plant–microbe interactions as well (Asselbergh *et al.*, 2008; Robert-Seilaniantz *et al.*, 2007, 2011; Pieterse *et al.*, 2009; De Vleesschauwer *et al.*, 2012). Like SA, JA and ET, most of these hormones are differentially active against attackers with diverse modes of infection and, accordingly, influence disease outcomes by interfering with the SA–JA–ET backbone of the plant immune system (Robert-Seillaniantz *et al.*, 2007; Pieterse *et al.*, 2009). As a result, activation of defences against one type of pathogen often enhances susceptibility against others. While significant progress has been made in engineering plants that are resistant to specific classes of pathogens and various chemical and biological elicitors have been identified that are effective in inducing attacker-specific immunity (Boller and Felix, 2009), examples of plant broad-spectrum disease resistance are scarce. One exception, however, is the application of silicon as a plant-protection strategy. One of the most abundant elements on earth, silicon is well known to protect plants against a suite of pathogens with different lifestyles and modes of infection (Currie and Perry, 2007; Epstein, 2009; Fauteux *et al.*, 2005; Guntzer *et al.*, 2012; Ma *et al.*, 2006). To date, however, very little is known about the molecular basis and regulation of silicon-mediated disease resistance. In this review, we survey recent progress in deciphering the immune-regulatory role of silicon, thereby focusing on the genes and molecular pathways that govern the resistance response. First illustrating the economic importance of resistance trade-offs by using rice as a case study, we then combine knowledge on silicon-induced responses in diatoms and plants to propose several novel regulatory mechanisms that can help explain this element's extraordinary ability to protect plants from a multitude of stresses.

The importance of resistance trade-offs in rice production systems

Rice is the most important food crop of the developing world and the staple food of more than half of the world's population. Diseases caused by microbial pathogens have always had a significant impact on rice production. Historically, severe epidemics have led to serious food shortages, claiming the lives of millions (Ou, 1985). Nowadays, diseases are still among the major constraints on high rice productivity. Fungal diseases such as rice blast (caused by *Magnaporthe oryzae*), sheath blight (*Rhizoctonia solani*), brown spot [*Cochliobolus miyabeanus* (sexual stage), also called *Bipolaris oryzae* (asexual stage)] and bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) are the most serious constraints on high productivity (Webster and Gunnell, 1992). *R. solani* and *B. oryzae* are necrotrophic pathogens, while *M. oryzae* and *X. oryzae* pv. *oryzae* are considered to be hemi-biotrophic pathogens. Studies by Savary *et al.* (2000*a*, 2000*b*) demonstrate that, among the many diseases occurring in rice fields, brown spot and sheath blight account for the highest yield losses across all production systems (5 and 6%, respectively). In comparison, estimated yield losses for rice blast and bacterial blight are 0.3–5% and less than 1%, respectively. These figures indicate that the sustained efforts of resistance breeding against blast and bacterial blight have paid off (Leung *et al.*, 2003). Diseases such as sheath blight and brown spot have become more prominent due to either a lack of effective resistance in the germplasm or a lack of breeding effort. According to Leung *et al.* (2003) the challenge ahead is to develop broad-spectrum resistance and production systems that suppress multiple biotic stresses. Among these strategies, approaches based on the plant's own defensive repertoire seem very promising for sustainable rice production (Song and Goodman, 2001).

Table 1 summarizes our current knowledge on the role of plant hormones and other selected plant activators on disease resistance in rice against its four major pathogens. SA or compounds that act up- or downstream of SA in the SA-signalling pathway such as the plant activator probenazole or the SA analogues benzothiadiazole and triadinil are effective against the rice blast fungus *M. oryzae* and the bacterial blight pathogen *X. oryzae* pv. *oryzae* but do not induce resistance to *C. miyabeanus* or *R. solani* (Takatsuji *et al.*, 2010; De Vleesschauwer *et al.*, 2010; Iwai *et al.*, 2006; Shen *et al.*, 2011). JA plays a positive role in the resistance to *M. oryzae*, *X. oryzae* pv. *oryzae* and *R. solani* (Taheri and Tarighi, 2010), but is ineffective against *C. miyabeanus*. Application of ABA enhances susceptibility to rice blast (Koga *et al.*, 2004; Jiang *et al.*, 2010) and bacterial blight (Xu *et al.*, unpublished work), has no effect on *R. solani*, but induces resistance to the brown spot fungus *C. miyabeanus* (De Vleesschauwer *et al.*, 2010). ET is involved in rice blast resistance and it was shown that ET biosynthesis, but not ET itself is necessary for resistance to rice blast in young rice plants (Iwai *et al.*, 2006). A recent study by Shen *et al.* (2011) suggests, however, that ET has a negative role on resistance to bacterial blight. De Vleesschauwer *et al.* (2010) have likewise demonstrated that ET is involved in susceptibility to brown spot and that ABA-induced suppression of the ET response is involved in induced resistance to brown spot. The plant hormone auxin also plays contrasting roles in the interaction of rice with blast and bacterial blight on one hand, and brown spot on the other hand. Exogenous application of indole-3-acetic acid (IAA) increased susceptibility to bacterial blight (Ding *et al.*, 2008) and blast (Fu *et al.*, 2011), while it increased resistance to brown spot (Fonteyne, 2011). A rice line overexpressing *OsGH3.1*, a gene encoding an IAA amido synthetase that inactivates IAA by conjugating it to amino acids, was more resistant to *M. oryzae* (Domingo *et al.*, 2009), but Fonteyne (2011) revealed that this line is very susceptible to *C. miyabeanus*. These data clearly show that rice requires distinct signal transduction pathways to defend itself to its major pathogens and that trade-offs are especially apparent between pathogens with a contrasting life style such as *M. oryzae* and *C. miyabeanus*. Although both *R. solani* and *C. miyabeanus* are necrotrophic fungal pathogens, accumulating evidence suggests that also against the

Table 1. Effect of plant activators on the defence response of rice against its major fungal and bacterial pathogens.

	Magnaporthe oryzae	*Xanthomonas oryzae* pv. *oryzae*	*Cochliobolus miyabeanus*	*Rhizoctonia solani*	References
Plant hormones or analogues					
ET	+	–	–	NT	De Vleesschauwer *et al.*, 2010; Iwai *et al.*, 2006; Shen *et al.*, 2011
SA, benzothiadiazole, tiadinil	+	+	0	0	Ahn *et al.*, 2005; Babu *et al.*, 2003; Takatsuji *et al.*, 2010
JA	+	+	0	+	Ahn *et al.*, 2005; Mei *et al.*, 2006; Schweizer *et al.*, 1998; Taheri and Tarighi, 2010; Tao *et al.*, 2009
ABA	–	–	+	0	Jiang *et al.*, 2010; De Vleesschauwer *et al.*, 2010
Auxin	–	–	+	NT	Domingo *et al.*, 2009; Fonteyne, 2011; Fu *et al.*, 2011;
Plant activators					
Probenazole	+	+	0	0	Takatsuji *et al.*, 2010; Watanabe 1977;
Riboflavin	+	NT	–	+	Aver'yanov *et al.*, 2000; Taheri and Tarighi, 2010
Bacterial elicitors					
Pseudobactin	+	NT	NT	0	De Vleesschauwer *et al.*, 2008
Pyocyanin	+	NT	–	–	De Vleesschauwer *et al.*, 2006; De Vleesschauwer *et al.*, 2009
Silicon	+	+	+	+	Chang *et al.*, 2002; Dallagnol *et al.*, 2011; Rodrigues *et al.*, 2003; Rodrigues *et al.*, 2004

+, Positive effect on resistance; –, negative effect on resistance; 0, no effect on resistance; NT, not tested.

latter pathogens distinct resistance mechanisms are operative. Exogenous ABA, although highly effective against *C. miyabeanus*, failed to reduce sheath blight severity, whereas application of riboflavin, a water-soluble B vitamin thought to function via activation of JA-dependent defences (Taheri and Tarighi, 2010), induces resistance to *R. solani* while increasing susceptibility to *C. miyabeanus* (De Vleesschauwer *et al.*, unpublished work).

A similar picture emerges from our studies on rhizobacterium-mediated resistance in rice. The root-colonizing *Pseudomonas aeruginosa* strain 7NSK2 was found to induce induced systemic resistance (ISR) against *M. oryzae* and the blue phenazine pigment pyocyanin appeared to be an essential determinant of 7NSK2-mediated ISR. However, pyocyanin acts as a two-faced ISR elicitor, positively modulating protection against *M. oryzae* but repressing *R. solani* resistance. Transient generation of low-level micro-oxidative bursts by redox-active pyocyanin in planta most likely accounts for the dual role of this compound in 7NSK2-ISR because exogenous application of H_2O_2-quenching sodium ascorbate alleviated the contrasting effects of pyocyanin on *R. solani* and *M. oryzae* pathogenicity (De Vleesschauwer *et al.*, 2006). Later it was shown that topical application of pyocyanin also triggers susceptibility to *C. miyabeanus* (De Vleesschauwer *et al.*, 2009). Similar findings were obtained in response to root treatment with *Serratia plymuthica* strain IC1270. Although highly effective against *M. oryzae*, arresting the pathogen in its biotrophic phase by boosting infection-induced H_2O_2 accumulation in the epidermis, IC1270 colonization resulted in enhanced tissue colonization by both *R. solani* and *C. miyabeanus* (De Vleesschauwer *et al.*, 2009). The effect of reactive oxygen species-fueled hypersensitive-response-like cell death thus clearly varies according to the mode of infection of the invading pathogen. In this context, it can be hypothesized that the widespread circulation of high-yielding, semi-dwarf varieties carrying multiple blast-resistance genes might be an important factor driving the overall increase in sheath blight incidence that is typically observed in intensified rice production systems (Mew *et al.*, 2004). In this respect we have observed that pre-inoculation with an avirulent hypersensitive-response-triggering *M. oryzae* isolate favours subsequent infection with *R. solani* (De Vleesschauwer *et al.*, unpublished work).

Table 1 reveals that there is not a single hormone, plant activator or resistance elicitor that is active against all four major rice pathogens. Moreover, in many cases resistance to one pathogen is coupled to enhanced susceptibility against others, clearly demonstrating the occurrence of resistance trade-offs. The notable exception, however, is silicon, which triggers broad-spectrum resistance against all four pathogens.

Silicon: broad-spectrum inducer of resistance against biotic and abiotic stress

Background

The second most abundant element in the Earth's crust, silicon (Si) can comprise up to 70% of the soil mass in the form of silicate minerals and water-soluble orthosilicic acid [$Si(OH)_4$] (Epstein, 1994; Savant *et al.*, 1997; Ma and Yamaji, 2006). The concentration of orthosilicic acid in the soil solution averages over 0.1–0.6 mM and is affected

by its dissolution from soil minerals and its adsorption or resorption by the soil (McKeague and Cline, 1963; Epstein, 1994; Savant *et al.*, 1997). Extreme conditions including high temperatures and rainfall increase the release of orthosilicic acid, explaining why most weathered soils in the tropics are silicon-deficient (Savant *et al.*, 1997; Richmond and Sussman, 2003). Orthosilicic acid is taken up by plant roots and constantly polymerized into insoluble silica in cell walls, intercellular spaces and a subcuticular layer outside the cell in the leaves (Sangster and Hodson, 2001; Ma *et al.*, 2011). Silicon is known to increase the tolerance against both abiotic and biotic stresses in many plant species and it is the only nutrient which is not detrimental when collected in excess (Epstein, 1994; Fauteux *et al.*, 2005; Ma and Yamaji, 2006). According to the universally accepted criteria for the essentiality of a nutrient published by Arnon and Stout (1939), silicon is not essential for plants. An important criterion herewith is the intrinsic occurrence of silicon in the structure or metabolism of the plants, which to date has not been confirmed. Yet, a more recent definition of essential nutrients by Epstein and Bloom (2005) defines silicon as essential because silicon-deficient plants exhibit abnormalities in growth, development and reproduction. Amidst the ongoing debate on the essentiality of silicon, most authors refer to silicon as a 'semi-essential' nutrient (Epstein, 1999; Ma and Yamaji, 2006; Liang *et al.*, 2007).

Silicon uptake in plants

Silicon is readily absorbed by plant roots in the form of non-charged monosilicic acid [$Si(OH)_4$] (Ma and Yamaji, 2006). Various plant species, especially monocotyledons, are known to actively absorb silicon (Liang *et al.*, 2005). Since rice is a well-known silicon accumulator and an important scientific model organism, the silicon uptake mechanism has been most intensively studied in this plant species. In rice roots two silicon transporters with a different mode of action are responsible for the transport of silicic acid past the casparian strips in exo- and endodermis cells (Fig. 1). The influx transporter Lsi1 is located on the plasma membrane at the distal side of exo- and endodermis cells. Silicic acid is transported out of the exo- and endodermis cells through the Lsi2 transporters at the proximal side of these cells (Ma *et al.*, 2006, 2007, 2011). The uptake of silicic acid by Lsi1 is a passive process, while the transport via the Lsi2 transporters is actively driven by an ATP-consuming H^+ pump. Once taken up by Lsi1 in the exodermis and released by Lsi2, silicic acid diffuses through the apoplast of the aerenchyma.

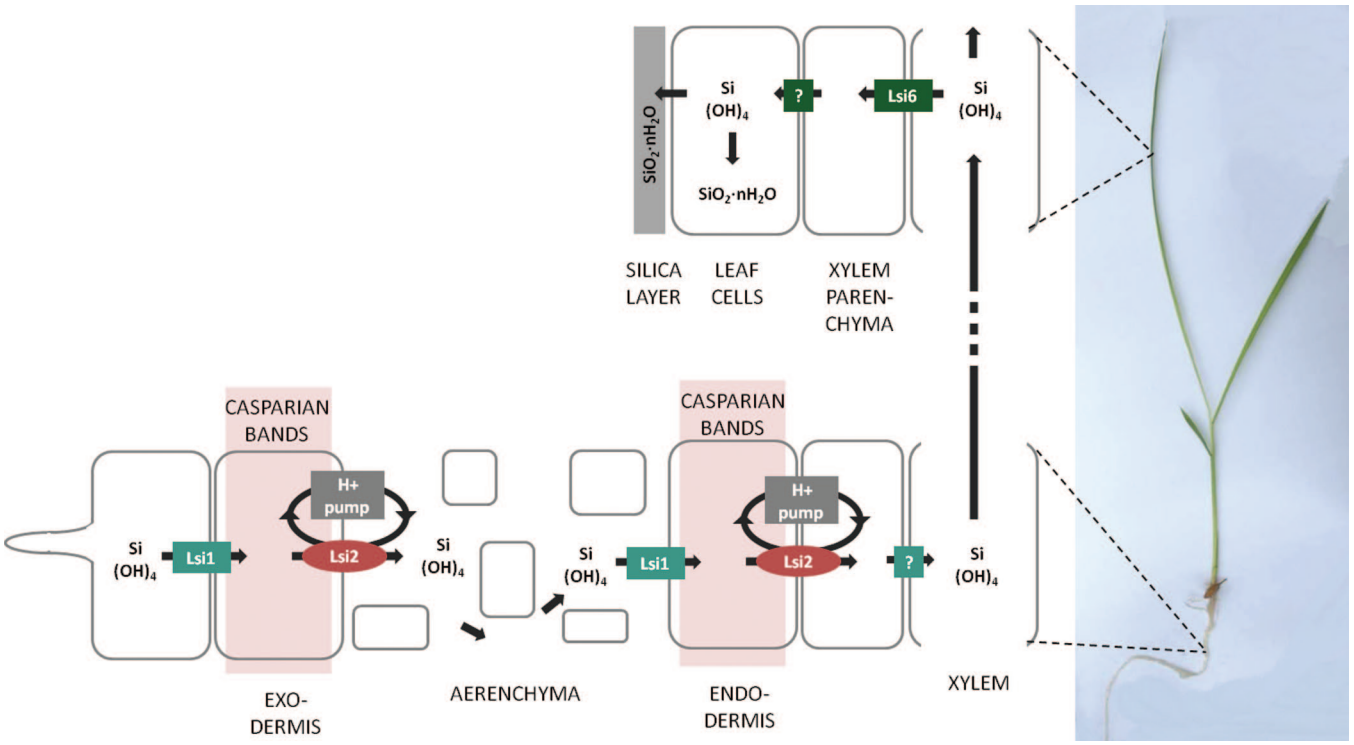

Fig. 1. General uptake of silicon in rice plants from root to shoot. From root epidermis cells silicic acid is transported through exodermis cells by the passive Lsi1 and the active Lsi2 silicon transporters. In the aerenchyma silicic acid moves apoplastically until it reaches the endodermis where the Lsi1 and Lsi2 transporters load silicic acid into the stele. An undefined transporter loads the silicic acid in the xylem. Via the xylem silicic acid arrives in the shoots, where the Lsi6 transporter unloads the silicic acid into the xylem parenchyma cells. An undefined protein transports the silicic acid in the leaf cells where it is polymerised either as silica in the cell or as a subcuticular silica layer outside the cell. Reprinted from *Trends in Plant Science* 11, Ma JF and Yamaji N, Silicon uptake and accumulation in higher plants, pp. 392–397, copyright 2006, with permission from Elsevier, and from Ma *et al.* (2011) from *Proceedings of the Japan Academy Series B-Physical and Biological Sciences* with permission.

Lsi1 transporters in endodermis cells take up the silicic acid from the aerenchyma and Lsi2 transporters load it into the stele. An unknown transporter is responsible for the xylem loading of silicic acid. Leaf cells take up silicic acid from the xylem by means of a Lsi1-like transporter, Lsi6. Inside the leaf cells, a natural polymerization process takes place, transforming water-soluble silicic acid into insoluble silica ($SiO_2 \cdot nH_2O$) either inside the cell, as phytoliths and colloidal cytoplasmic silica, or outside the cell as a silica layer or silica bodies located just beneath the cuticle (Yamanaka *et al.*, 2009; Ma *et al.*, 2011).

In maize, barley, pumpkin and wheat, orthologues of rice Lsi1 and Lsi2 have been shown to be involved in Si absorption (Chiba *et al.*, 2009; Mitani *et al.*, 2009*a*, 2009*b*, 2011; Montpetit *et al.*, 2012). Although the silicon transporters in different plant species are homologous to OsLsi1 and OsLsi2 in rice, the uptake of silicon differs considerably between rice and other plant species due to differences in root architecture (Mitani *et al.*, 2009b). In contrast with rice, the roots of most other plants lack both exodermal casparian strips and aerenchyma (Ma *et al.*, 2011). The Lsi1 transporter in barley, maize and pumpkin occur at the distal side of all root cells between the epidermis and hypodermis, whereas Lsi2 is localized in endodermal cells without polarity. In the latter plant species silicon appears to be taken up from the soil solution by Lsi1 outside the endodermis, whereas in rice Lsi1 transports silicon exclusively at the exodermis (Mitani *et al.*, 2009*a*, 2009*b*; Bauer *et al.*, 2011; Ma *et al.*, 2011).

Prophylactic effects of silicon treatment

To date, dozens of reports have documented the ability of silicon to alleviate biotic and abiotic stress. As such, silicon increases the tolerance towards both (hemi-)biotrophic and necrotrophic pathogens, not only in rice and other monocotyledons, but also in numerous dicotyledon plant species (Fauteux *et al.*, 2005; Cooke and Leishman, 2011). Moreover, silicon application is also effective against a suite of abiotic stresses including salinity, drought, heat, cold and metal toxicity. Most importantly, silicon protects plants against a multitude of stresses without the occurrence of resistance trade-offs and/or growth and yield penalties (Epstein, 1999, 2009; Fauteux *et al.*, 2005; Ma and Yamaji, 2006; Currie and Perry, 2007). As such, silicon amendment is one of the only plant-protection strategies that enables plants to maximize efficiency in responding to the exact set of environmental conditions encountered, at the same time as conserving resources for growth and development. These traits make silicon nutrition one of the most promising approaches for sustainable, environmentally sound and broad-spectrum disease control in various agricultural contexts. Originally, the prophylactic role of silicon treatment was attributed to the deposition of silica in the leaves, which was believed to act as a physical barrier that hampers pathogen penetration into the epidermis (Jones and Handreck, 1967). Although important, accumulating evidence indicates that this passive role of silicon is not solely determinant for the silicon-elicited stress protection. Indeed, analyses of different plant species showed that silicon nutrition can boost the expression of a large spectrum of inducible defence responses.

In cucumber roots, silicon treatment ensures an enhanced activity of chitinases, peroxidases, polyphenol oxidases and flavonoid phytoalexins after infection with *Pythium* spp. (Chérif *et al.*, 1994), whereas in leaves an increased concentration of antifungal components protects the plant against the powdery mildew pathogen *Podosphaera xanthii* and the necrotrophic fungus *Colletotrichum lagenarium* (Fawe *et al.*, 1998; Liang *et al.*, 2005). Kauss *et al.* (2003) discovered that a strongly cationic protein reinforces the cell wall at the site of attempted pathogen ingress by enhancing silica deposition, thus preventing infection by *C. lagenarium*. Similarly, recent research on the beneficial effect of silicon in roses clearly shows that the heightened resistance against *Podosphaera pannosa* is the result of an increased formation of papillae and deposition of callose and H_2O_2, along with an upregulation of the phenylpropanoid pathway producing antimicrobial phenolic compounds and flavonoids (Shetty *et al.*, 2011, 2012). Silicon treatment also protects *Arabidopsis* from powdery mildew (*Erysiphe cichoracearum*) due to the accumulation of fungitoxic phenolic compounds and silica depositions at the site of infection (Ghanmi *et al.*, 2004; Fauteux *et al.*, 2005). In wheat, silicon-induced resistance against *Blumeria graminis* f. sp. *tritici* is associated with increased papillae formation and accumulation of callose, fungitoxic phenolic compounds and methylated forms of *trans*-aconitate (Bélanger *et al.*, 2003; Rémus-Borel *et al.*, 2005, 2009). Likewise, the positive effect of silicon on rice resistance against the blast pathogen *M. oryzae* is characterized by increased accumulation of defence-related enzymes including glucanase, peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase, as well as by an accumulation of antimicrobial glycosylated phenolics and diterpenoid phytoalexins (Rodrigues *et al.*, 2003, 2004, 2005; Cai *et al.*, 2008). Finally, silicon-induced resistance against the rice brown spot pathogen *C. miyabeanus* seems to be the result of higher levels of chitinase, peroxidase, lignin and phenolics and lower lipid peroxidation and electrolyte leakage (Dallagnol *et al.*, 2011).

Although the significance and causal roles of many of these responses remain to be resolved, the wide variety of immune responses influenced by silicon amendment clearly demonstrates its potential to act as a biological inducer of plant innate defence responses. Moreover, the observation that all prophylactic effects are lost within a short period of time after silicon feeding is interrupted clearly suggests that the role of silicon as a modulator of basal defence responses is dominant over its function as a mechanical barrier (Samuels *et al.*, 1991; Fawe *et al.*, 1998; Fauteux *et al.*, 2005, 2006; Chain *et al.*, 2009; Zargar *et al.*, 2010; Ghareeb *et al.*, 2011).

Mechanisms of silicon action

Although the beneficial effects of silicon on disease resistance in plants have been known for years, few reports in the literature have focused on understanding the mechanistic basis and regulation of this response. Here we aim to propose several potential mechanisms that can explain the

prophylactic role of silicon by approaching this enigma from two different sides; that is, from the points of view of both a diatom and a plant.

Even though the essentiality of silicon in plant biology is still heavily debated (see above), in a few primitive life forms, such as diatoms, silicon is required for growth and development (Martin-Jézéquel *et al.*, 2000; Kinrade *et al.*, 2002). Diatoms are encased by a silica-containing cell wall, called a frustule, and the polymerization of silicon to a viable frustule is an energy-consuming process that depends on photorespiration (Martin-Jézéquel *et al.*, 2000). However, diatoms also depend heavily on silicon for many non-cell-wall-related processes, including protein phosphorylation, DNA replication and DNA–protein interactions (Sullivan and Volcani, 1973; Reeves and Volcani, 1984; Okita and Volcani, 1978). Like plants, diatoms contain several silicon transporters, often arranged in gene families, but these transporters are different in both their structures and functions from their plant counterparts (Hildebrand *et al.*, 1998; Ma *et al.*, 2004). Most tellingly, ectopic expression of a silicon transporter gene from diatoms in transgenic tobacco had no significant impact on silicon uptake, indicating fundamental differences in silicon absorption between plants and diatoms (Ma *et al.*, 2004). These differences notwithstanding, insights into the significance and regulation of silicon-mediated processes in diatoms may potentially shed new light on the poorly understood role of silicon in plant stress responses. In the subsequent parts of this review, we therefore aim to uncover silicon-mediated regulatory mechanisms in diatoms that also may apply to higher plants and evaluate whether these processes can contribute to broad-spectrum disease resistance (Raven, 2003; Thamatrakoln *et al.*, 2006; Currie and Perry, 2007; Pondaven *et al.*, 2007). Moreover, building upon recent progress in identifying and characterizing the genes and molecular pathways that are involved in regulating silicon-induced plant defence, we propose five hypothetical mechanisms that may explain how silicon elicits broad-spectrum disease resistance.

Silicon-induced priming for enhanced defence Over the past decade, a number of transcriptomic and proteomic studies have been performed to explain the protective role of silicon in various pathosystems (Watanabe *et al.*, 2004; Fauteux *et al.*, 2006; Chain *et al.*, 2009; Zargar *et al.*, 2010; Ghareeb *et al.*, 2011; Nwugo and Huerta, 2011). One of the most salient results of these studies is that silicon has very little impact on the metabolism of non-stressed plants. In rice, for instance, silicon treatment was found to alter the abundance of as few as four proteins in the absence of stress, as compared to 57 in plants responding to both silicon and cadmium stress (Nwugo and Huerta, 2011). Similar findings were obtained in several microarray studies on the effect of silicon in rice, wheat, *Arabidopsis* and tomato (Watanabe *et al.*, 2004; Fauteux *et al.*, 2006; Chain *et al.*, 2009; Ghareeb *et al.*, 2011). Together with the ability of silicon-treated plants to adapt to multiple types of stresses without the occurrence of resistance trade-offs, these data are compatible with the view that silicon application does not directly induce immunity but rather primes plants for enhanced defence in response to pathogen attack.

One notable exception, however, is a study by Brunings *et al.* (2009) in which silicon was shown to significantly alter the basal expression level of more than 220 rice genes. This result strikingly contrasts with previous work by Watanabe *et al.* (2004) who, using a similar hydroponic rice growing system, found approximately 10 times fewer genes to be differentially expressed. Although differences in rice cultivars, microarray platforms and statistical settings used in both studies cannot be excluded, none of these factors justifies a 10-fold difference in the number of silicon-responsive genes. Another confounding factor, however, involves the plant growth conditions. The literature is replete with reports that silicon promotes plant growth and development especially when the plant is under some form of stress (Epstein, 1999; Fauteux *et al.*, 2005, 2006; Ma and Yamaji, 2006). In line with this, one could speculate that silicon-treated plants display very little differential gene expression when grown under optimal conditions, whereas short and/or moderate stress episodes that may go unnoticed at the phenotypic level potentially amplify the influence of silicon on the plant's basal transcriptome.

Because priming initiates a state of readiness that does not confer resistance *per se*, but allows for accelerated induced resistance once an attack occurs, one presumed benefit of priming is that it entails fewer fitness costs than direct activation of defence (van Hulten *et al.*, 2006). Moreover, priming is thought to confer flexibility to adapt the defence response to a specific challenge, leading to a less costly and broader spectrum of resistance (Van der Ent *et al.*, 2008; Conrath, 2011). Although the molecular aspects of priming are still poorly understood, the induction of priming is increasingly associated with a subtle increase in the level of inactive signalling components such as mitogen-activated protein (MAP) kinases and transcription factors (Conrath, 2011). After perception of a second, pathogen-derived signal, the enhanced signalling capacity in primed plants would facilitate a faster and stronger immune response. Interestingly, using a quantitative PCR-based transcription profiling approach that is substantially more sensitive than microarrays and thus ideally suited to detect minor changes in gene expression (Caldana *et al.*, 2007), we were able to show that application of silicon to non-stressed rice plants results in the consistent up- and downregulation of 35 and 121 transcription factors, respectively (Van Bockhaven *et al.*, 2012). The reported role of many of these transcription factors in various plant defence responses is consistent with the ability of silicon to protect rice from multiple stresses. Therefore, it is not inconceivable that the broad-spectrum disease resistance in silicon-treated rice is at least in part the result of priming due to differential accumulation of defence-regulatory transcription factors, a process that is sufficient to prime defence genes, but too weak to activate them directly.

Silicon–hormone interactions An additional mechanism by which silicon may impact pathological outcomes is by influencing endogenous hormone balances. Corroborating

this concept, mounting evidence suggests that silicon is intimately associated with plant hormone signalling. In soybean, for instance, silicon treatment reportedly induces synthesis of gibberellic acid, while silicon-treated rice accumulates slightly higher levels of gibberellin and JA and lower levels of ET (Lee *et al.*, 2010; Hwang *et al.*, 2007).

However, consistent with its putative role as a biological priming agent (see above), major effects of silicon on plant hormone responses are only seen upon pathogen attack. In one of the first microarray studies on silicon-treated plants, Fauteux *et al.* (2006) demonstrated the stimulating effect of silicon on the biosynthesis of the stress hormones SA, JA and ET in leaves challenged with the powdery mildew pathogen *Erysiphe cichoracearum*. Similarly, microarray analysis of rice infected with *M. oryzae* showed that silicon triggers activation of the ET signalling pathway, the role of which in resistance to blast is well established (Iwai *et al.*, 2006; De Vleesschauwer *et al.*, 2008; Brunings *et al.*, 2009). Furthermore, in silicon-treated tomato plants infected with *Ralstonia solanacearum* both JA and ET signalling pathways were found to be induced, leading to increased resistance (Zhang *et al.*, 2004; Chen *et al.*, 2009; Kawamura *et al.*, 2009; Ghareeb *et al.*, 2011).

Although the underlying molecular mechanisms remain poorly understood, these data clearly demonstrate the potential of silicon to interfere at multiple levels with hormone biosynthesis and response pathways. Moreover, these findings suggest that silicon does not impose continuous changes in phytohormone homeostasis, but rather primes hormone biosynthesis and signalling processes, creating a flexible signalling network that allows the plant to finely tune its defence response to the invaders encountered. Verifying whether the versatile role of silicon may indeed be attributed to high-dimensional interactions with the plant's hormone signalling network is an important challenge ahead.

Targeted alterations in iron homeostasis: an alternative mechanism for silicon-induced disease resistance? Iron (Fe) is a ubiquitous redox-active element and an essential micronutrient for plants and associated microorganisms. Despite its paramount importance for plant growth and reproduction, iron has only recently been identified as a central factor regulating plant pathogen defences. Consistent with disease-related alterations in iron homeostasis in animals (Rouault, 2006; Brissot *et al.*, 2011), Liu *et al.* (2007) proposed a model whereby pathogen attack elicits the targeted redistribution of Fe to the apoplast, leading to Fe depletion in the cytosol of attacked cells and resultant activation of redox-dependent defence gene expression. Interestingly, plant Fe titres have also been shown to be a central factor in the induction of systemic resistance by beneficial rhizobacteria (De Vleesschauwer *et al.*, 2008; Van der Ent *et al.*, 2008). Many rhizobacteria competitively acquire ferric iron by producing large amounts of low-molecular-weight compounds or siderophores, called pyoverdins or pseudobactins. Given the scarcity of bio-available iron and the high affinity of pseudobactins for this ferric iron, pseudobactin-producing rhizobacteria are thought to interfere with the iron acquisition of other soil organisms, including the host plant. Accordingly, we recently showed a strict correlation between the resistance-inducing potential of bacterial pseudobactins and their ability to deprive young rice seedlings from iron (De Vleesschauwer and Höfte, 2009). Considering that the total iron content of silicon-treated plants is reduced by on average 20% (Islam and Saha, 1969; Ma and Takahashi, 1990), it is tempting to speculate that silicon may likewise induce disease resistance by perturbing iron homeostasis.

In favour of this assumption, transcriptome analysis of silicon-treated rice leaves revealed transcriptional reprogramming of several genes implicated in regulating intracellular iron homeostasis (Brunings *et al.*, 2009). Moreover, the expression patterns of these genes mirrors those observed in iron-deficient rice leaves, further supporting our hypothesis (Gross *et al.*, 2003; Kobayashi *et al.*, 2005; Dos Santos and De Oliveira, 2007; Walker and Connolly, 2008). It is important to note, however, that silicon amendment does not impose severe levels of iron stress, as evidenced by its growth-promoting abilities. Rather, silicon application may trigger dynamic yet subtle changes in plant Fe status, thereby preconditioning naïve tissues to respond faster and more strongly upon subsequent pathogen attack.

Several mechanisms can explain silicon-induced alterations in iron homeostasis. First, silicon application is well known to protect plants from iron toxicity by enhancing the oxidizing power of root tissues, which leads to increased oxidation of iron into iron oxides (Savant *et al.*, 1997; Fleck *et al.*, 2011). Being water-insoluble, these oxides cannot be absorbed by the roots and thereby lower the total amount of bio-available iron in the rhizosphere, potentially resulting in intracellular iron depletion (Okuda and Takahashi, 1964). Secondly, silicon and iron are able to interact and many iron molecules can be co-precipitated in silica. There are, for instance, reports of Fe^{2+} binding directly to silica and also Fe^{3+}-chelating siderophores can bind silicon (Perry and Keeling-Tucker, 1998; Saeki, 2004; Liang *et al.*, 2007; Schmiederer *et al.*, 2011). Finally and as indicated by aforementioned microarray data (Brunings *et al.*, 2009), silicon may impinge on the plant's Fe status by interfering either directly or indirectly with specific components of the iron-uptake and -signalling machinery.

Linking silicon-driven photorespiration to plant immunity The polymerization of silicon in diatoms is essential for the formation of cell walls and is therefore determining for the growth and viability of diatoms. The driving force behind silicon polymerization is respiration rather than photosynthesis. In particular, photorespiration is essential in providing ATP, serine and glycine, all of which are necessary for the polymerization of silicic acid in diatoms (Martin-Jézéquel *et al.*, 1998, 2000). Photorespiration is generally considered a wasteful process that occurs in C_3 plants under specific conditions. Mounting evidence, however, suggests that photorespiration might also be an important mechanism for many C_3 plants to cope with abiotic and biotic stress by maintaining electron flow to prevent photoinhibition. Many processes are involved in photorespiration-mediated stress defence, including the production of oxygen radicals,

increased assimilation of ammonium and replenishment of mitochondrial respiration. The outcome of these processes are extremely diverse, ranging from rapid cell death to increased longevity of the plants (Wingler *et al.*, 2000; Foyer *et al.*, 2009; Guan and Gu, 2009; Kangasjarvi *et al.*, 2012). Whereas induced cell death is often effective against biotrophic pathogens, an increase in cell viability generally leads to increased resistance against necrotrophic pathogens (Glazebrook, 2005). Accordingly, recent advances have brought several exciting new molecular links to support a central role of photorespiration in biotic and abiotic stress-response signalling. For instance, transgenic rice lines with increased chloroplastic glutamine synthetase activities were recently shown to be more resistant towards salt stress, an effect which the authors attributed to the increased photorespiration capacity associated with the transgenic phenotype (Hoshida *et al.*, 2000; Cai *et al.*, 2009). Similarly, expression of the photorespiratory enzymes, formate-tetrahydrofolate ligase and hydroxypyruvate kinase was found to be increased under cadmium stress in *Arabidopsis* cells and pea plants (Romero-Puertas *et al.*, 2007; Sarry *et al.*, 2006). In a different example, overexpression of three key photorespiratory genes encoding glycolate oxidase, serine:glyoxylate aminotransferase and glutamate:glyoxylate aminotransferase increased resistance in melon and *Arabidopsis* against the (hemi-)biotrophs *Pseudoperonospora cubensis* and *Pseudomonas syringae*, respectively (Kenigsbush and Cohen, 1992; Taler *et al.*, 2004; Rojas *et al.*, 2012). In accordance with this, loss-of-function mutations in another important photorespiratory enzyme, serine hydroxyl-methyltransferase, resulted in broad-spectrum susceptibility of *Arabidopsis* against the biotroph *P. syringae* pv. *tomato* DC3000 and the necrotrophic fungi *Alternaria brassicicola* and *Botrytis cinerea* (Moreno *et al.*, 2005).

Evidence connecting photorespiration to silicon-afforded stress tolerance comes from Nwugo and Huerta (2011), who reported that the beneficial effect of silicon in protecting rice from cadmium stress is associated with enhanced accumulation of the photorespiratory enzymes phosphoglycolate phosphatase and glycine dehydrogenase. Furthermore, intensive screening of the photosynthetic capacities in silicon-treated rice plants revealed significant increases in photorespiratory ability due to silicon treatment (Van Bockhaven *et al.*, unpublished work). Additional evidence supporting a role of photorespiration in silicon's mechanism(s) of action is currently missing; however, given the fact that photorespiration is important for disease resistance against various pathogens (see above), it is not unlikely that silicon-driven photorespiration may be an important mechanism leading to broad-spectrum disease resistance.

Interaction of silicon with signalling components Although complexation of orthosilicic acid by certain sugars and hydroxyl-amino acids has been demonstrated *in vitro* (Jugdaohsingh *et al.*, 2008), there is no definitive evidence yet that silicon binds to proteins or has direct biochemical functions at physiological pH. Nevertheless, consistent with the emerging role of silicon as a biologically active element capable of inducing plant defence responses, silicon is increasingly being associated with modulation of primary signal transduction. Fauteux *et al.* (2005) hypothesized that silicon's mode of action in signalling events could result from interactions with phosphorus and/or cationic metals such as Mn and Fe, which act as cofactors for many enzymes. Alternatively, though less parsimoniously, it was suggested that silicon may impinge on protein activity and/or conformation by binding hydroxyl groups on amino acid residues, thereby interfering with the phosphorylation status of these signalling proteins (Fauteux *et al.*, 2005). As for other molecular interactions, complexation and/or interaction of cellular components and silicic acid may alter the location, activity, transport and/or selectivity of the complexed molecules. In this scenario, silicon could influence the plant's defence responses at the post-translational stage, providing yet another mechanistic framework for how silicon improves disease resistance without inducing major changes in the transcriptome and proteome of non-stressed plants. Additional research is essential in exploring this train of thought, but more advanced proteomics analyses on silicon-treated plants might shed more light on the potential roles of silicon as a post-translational modifier of plant defence signalling.

Conclusions

Although many treatments are reported to induce resistance against plant pathogens, there are very few strategies that induce broad-spectrum disease resistance without trade-offs. Silicon is one of the only exceptions, rendering plants more resistant towards a wide range of abiotic and biotic stresses. The prophylactic role of silicon is the result of both passive and active effects. Many studies on silicon-induced broad-spectrum resistance report that the active effect is prevalent. However, the molecular underpinnings of silicon-mediated broad-spectrum disease resistance are still poorly understood.

By combining knowledge on silicon metabolism in diatoms and higher plants, we propose five potential mechanisms that may explain how silicon activates plant innate immune responses. First, evidence is accumulating that silicon induces resistance against a wide range of stress factors by modifying the intensity and/or timing of basal defence responses. The differential expression of several transcription factors in silicon-treated rice plants strengthens the hypothesis that silicon primes the plant's own defensive repertoire, resulting in rapid deployment of natural defence mechanisms upon pathogen attack. In a similar vein, silicon application may induce disease resistance by affecting plant hormone homeostasis, the role of which in shaping the outcome of plant–pathogen interactions is well established (Robert-Seilaniantz *et al.*, 2011). Third, the diverse beneficial role of photorespiration in plants under biotic stress, the dependency of silicon polymerization on photorespiration in diatoms and the accumulation of photorespiratory enzymes in silicon-treated plants following stress treatment argues that photorespiration may be an important factor in silicon-induced disease

resistance. Another putative mechanism involves the role of silicon in maintaining and adjusting cellular iron homeostasis. Silicon treatment seems to be accompanied by subtle yet dynamic changes in iron homeostasis, a phenomenon which shows strong commonalities to the mechanism of action of several resistance-inducing rhizobacteria. Finally, silicon may interact either directly or indirectly with various signal transduction components, resulting in enhanced signalling capacity in silicon-treated plants and fortified defence responses. Hypothetical in nature, each of these mechanisms requires extensive experimental validation, but may serve as a primer for future research aimed at delineating the molecular basis and regulation of silicon-afforded disease control.

Given the huge potential and value of silicon nutrition in stress management, the application of a range of biotechnological strategies based on the modulation of silicon content and its signalling effects could provide a unique tool for the genetic improvement of crop productivity in a sustainable manner. Classic genetic approaches and genome-wide transcriptional analyses are now beginning to unveil large numbers of silicon targets, shedding light on the complexity and diverse activity of silicon in plants. An important challenge in the coming years, however, will be unraveling the exact mechanisms of silicon-induced pathogen defences in a systems biology-based manner. This is especially important as studying how silicon is able to induce plant broad-spectrum disease resistance without inducing resistance trade-offs and appreciable fitness penalties will require profound knowledge of both the transcriptional and post-transcriptional fate of the target response. Special efforts should also be paid to uncovering the crosstalk mechanisms between silicon and other plant growth regulators. At the same time, controlled field experiments will be critical in understanding the physiological behaviour of silicon-induced plants under various stress conditions. By combining all of above-mentioned approaches, we may finally make sense of silicon-induced disease control.

Acknowledgements

This work was supported by grants from the Special Research Fund of Ghent University, by a specialization fellowship of the Flemish Institute for the stimulation of scientific-technological research in industry (to JVB) and by a post-doctoral fellowship of the Research Foundation-Flanders (to DDV).

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