Chapter 9

Silicon and Plant–Pathogen Interactions

Abstract The beneficial effects of silicon (Si) at preventing plant diseases have been known for many decades, and the list of plant–pathogen interactions influenced by Si keeps expanding. However, it is quite evident that the prophylactic properties of Si will vary greatly depending on the plant and the pathogen. The recent discovery of Si-specific transporters in rice roots has been instrumental in identifying plants that possessed such transporters and were thus genetically disposed to accumulate Si in their tissues and benefit more from Si amendments. For the same reason, soil applications of Si lead to significantly more Si accumulation in plant tissues than foliar applications and are likely to yield better results. An issue that has received less attention is the specificity of Si with respect to plant pathogens. Based on the literature, there is an overwhelming abundance of reports describing the positive effects of Si against fungal pathogens compared to bacteria, virus or nematodes. Among fungal pathogens, those described as biotrophic or hemibiotrophic, such as powdery mildews and *Magnaporthe grisea,* appear to be better controlled by Si. The reasons behind this apparent specificity are still unknown but recent data suggest that Si would interfere with effector proteins released by those pathogens, thus allowing the plant to mount better defence reactions. Our recent progress at understanding Si properties will contribute to optimizing its use in the context of sustainable agriculture.

Keywords Absorption • Biotrophic pathogens • Effector proteins • Mode of action • Silicon properties

9.1 Introduction

Experimental evidence dating back to 1860 has convincingly shown that most plants, with the notable exception of horsetail, can complete their life cycle without silicon (Si) (Epstein 1999). The nutritional role of Si in plant metabolism appears to be limited, and certainly, its essentiality in plant growth has not been clearly established (Epstein 1994, 1999, 2001). Nevertheless, there has been accumulating evidence that Si absorption plays an important function in alleviating biotic and abiotic stresses. Many reports have implicated Si with improved plant growth in situations of nutrient deficiency or excess (Bloemhard and Van Moolenbroek 1994; Datnoff

Y. Liang et al., *Silicon in Agriculture*, DOI 10.1007/978-94-017-9978-2_9

et al. 2001), and more commonly, Si fertilization has been linked to increased resistance of plants to diseases, namely, in the case of powdery mildew pathogens on wheat (*Triticum aestivum*) (Leusch and Buchenauer 1989; Bélanger et al. 2003), barley (*Hordeum vulgare*) (Jiang et al. 1989), rose (*Rosa rugosa*) (Shetty et al. 2012), cucumber (*Cucumis sativus*) (Wagner 1940; Miyake and Takahashi 1983a; Adatia and Besford 1986; Menzies et al. 1991; Samuels et al. 1991a, b), muskmelon (*Cucumis melo*), zucchini squash (*Cucurbita pepo*) (Menzies et al. 1992), grape (*Vitis vinifera*) (Bowen et al. 1992) and dandelion (*Taraxacum mongolicum*) (Bélanger et al. 1995) and for other diseases such as blast (*Pyricularia grisea*) and brown spot (*Bipolaris oryzae*) on rice (*Oryza sativa*) (Datnoff et al. 1997; Rodrigues et al. 2003a, b; Rodrigues et al. 2004; Sun et al. 2010; Ning et al. 2014), Fusarium wilt (Miyake and Takahashi 1983b) and root rots (Chérif et al. 1992a, 1994).

Despite the accumulating scientific evidence of the benefits of Si in agriculture, use of Si products or amendments is still misunderstood because of the many intricacies surrounding Si properties, absorption and efficacy. In this chapter, we will review the latest scientific developments in terms of Si research and plant protection, in an effort to clarify the prophylactic role of Si.

9.2 Soil Versus Foliar Applications

One of the most controversial issues surrounding Si use and properties relates to its application. Given the difficulties in applying Si in the form of silicic acid to the root system, it has been suggested that foliar applications could overcome these limitations and confer the same benefits. Some companies have developed some Si-based products specifically for foliar applications.

The current debate regarding the efficacy of Si as foliar applications lies in two main areas: absorption and mode of action. As explained above, root transporters that carry Si to the plant's upper parts mediate Si absorption. It is well known that plants that absorb the most Si are the ones that respond the best to Si feeding, so it would be fair to conclude that Si absorption is essential for a plant to derive benefits from it. Herein lies the problem because there is no strong evidence that plants can absorb Si through the leaves, which would render foliar applications much less useful. As a matter of fact, recent studies have shown that foliar applications will not lead to significant amounts of Si being absorbed in plants compared to root applications. This was particularly true in the case of wheat where plants sprayed with a Si solution did not accumulate more Si than controls (Guével et al. 2007). Expectedly, the prophylactic effects of Si were significantly more beneficial on plants where Si was fed through the roots and that had absorbed high amounts of Si. Similar results were recently reported with soybean in trials against soybean rust (Rodrigues et al. 2009).

Despite the overwhelming evidence that root applications of Si will lead to higher absorption and better prophylactic results, there is still a movement, both commercial and scientific, to promote the benefits of foliar applications of Si. This situation has created a climate of confusion about Si and its properties because, under the premise of a similar active ingredient, we have two completely distinct phenomena at play. It is therefore important to categorize each one of them in order to better understand how foliar and root applications of Si interact with the plant.

In the case of foliar applications, the literature does indeed contain reports of disease control with Si-based solutions. In most cases, the best results have been obtained against foliar pathogens such as powdery mildews. However, very few studies have looked into the mechanisms behind the process, or even if treated plants absorbed Si. Those that did showed conclusively that plants did not absorb Si under foliar treatments (Liang et al. 2005; Guével et al. 2007; Rodrigues et al. 2009). In an interesting experiment where Hoagland's solution was used as a control, the authors showed that powdery mildew repression was similar with the latter solution as with the solution made from potassium silicate. Incidentally, salt sprays, including potassium salts such as potassium carbonates and potassium phosphates, have been reported many times to reduce disease incidence with particular emphasis on powdery mildews (Reuveni and Reuveni 1995; Bélanger and Labbé 2002; Ehret et al. 2002), an observation in line with the results obtained with Hoagland's solution. Liang et al. (2005) suggested that disease reduction caused by foliar sprays of potassium silicate was the result of an osmotic effect on spores germinating at the leaf surface. Based on this evidence, it thus appears that any foliar sprays of salts, and more specifically potassium salts, including potassium silicate, can afford a certain control of disease incidence as a result of a direct effect on the pathogen rather than one mitigated by the plant. As such, while it is acknowledged that Si foliar sprays can yield positive effects against some pathogens, these effects do not appear to be related to the unique and intrinsic properties of Si *in planta* and should therefore be discussed in a separate context.

9.3 Silicon Specificity with Plants

As discussed in Chap. 4, the protective role of Si against plant pathogens will be greatly influenced by the ability of the plant species under treatment to absorb the element. For this reason, some plant species will not respond to a Si treatment and results will often be interpreted as a failure by Si to confer protection, rather than a biological limitation. It is therefore always important to make sure that a plant is Si competent before exposing it to the element. As a general rule, all monocots are Si accumulators, and all studies done to date confirm that they do carry the influx genes for Si transport. For dicots, the picture is not as clear as most dicots are unable to accumulate Si due to the absence of the specific NIPs required for Si influx. For instance, the model plant Arabidopsis will only accumulate limited amounts of Si if fed with the element because it lacks NIPIIIs (Montpetit et al. 2012). Notable exceptions among dicots are the Cucurbitaceae that are well known to benefit from Si feeding. Not surprisingly, the first Si transporters in dicots were found in that family (Mitani and Ma 2005). More recently, Si transporters have been described in soybean, a result congruent with reports of Si accumulation and protective effect in the species (Deshmukh et al. 2013). As more genomic data become available, it will become easier to precisely classify a plant as Si accumulator or not, simply on the presence of aquaporins permeable to silicic acid.

9.4 Silicon Specificity with Pathogens

Another confusing issue about Si relates to its specificity in terms of plant pathogens that are controlled by a treatment. Assuming a starting premise with a plant that is Si competent, the prophylactic properties of Si appear to be more efficient against biotrophic or hemibiotrophic fungal pathogens. Interestingly, very few studies have ever compared the relative efficiency of Si against different plant pathogens simultaneously but the literature contains a lot more reproducible and convincing data against plant pathogens that are classified as having a biotrophic phase. For instance, powdery mildews, strict biotrophs, are particularly well controlled by Si, and the list of plants benefiting from this effect is only limited by the host range of powdery mildews and the ability of a given plant to absorb Si. Among hemibiotrophs, rice blast, caused by the fungus *Magnaporthe grisea*, and brown spot caused by *Bipolaris oryzae* (Ning et al. 2014) are arguably the most commonly reported diseases to be controlled by Si. However, even though less numerous, there are reports of efficacy against necrotrophs. More specifically, pathogens such as *Rhizoctonia solani* on rice (Rodrigues et al. 2003b) and *Pythium ultimum* on cucumber (Chérif and Bélanger 1992) have been delayed in their development when inoculated on plants treated with Si. In the latter case, the prophylactic effects were rather modest and short lasting, but measurable. These limited examples associating Si with protection against necrotrophs have been sufficient to label Si as conferring broad-spectrum disease resistance in plants (Van Bockhaven et al. 2013), but the reality remains that there is an overwhelming bias toward biotrophs and hemibiotrophs.

Interestingly, the literature contains limited examples of plant pathogens other than fungi that are controlled by Si. For instance, very few cases of control of bacterial diseases have been associated with Si, with the notable exception of *Ralstonia solanacearum* on tomato (Dannon and Wydra 2004) and bacterial blight on rice (Xue et al. 2010a, b). While tomato is considered a Si non-accumulator species, it is nonetheless noteworthy that *R. solanacearum* has a hemibiotrophic lifestyle with a complex repertoire of Type III effector proteins (see Section 9.7). Recent evidence does suggest that priming would play a role in this interaction (Ghareeb et al. 2011), as observed with fungi.

It is uncertain if this bias for pathogens with a biotrophic phase is simply the result of the plant–pathogen interactions that have been studied or the fact that Si amendments tend to be more efficient against this particular type of pathogens. As a matter of fact, very little attention has been given to the specificity, or lack thereof, of Si with regard to the pathogens it controls. In the context of optimizing the use of

Si in agriculture, it would certainly be appropriate to define with greater precision the spectrum of pathogens that are indeed more susceptible to a Si treatment. Part of this answer may come from a better understanding of the exact mode of action of Si *in planta*.

9.5 The Mode of Action of Si

Among all the intricacies related to Si properties, its mode of action remains arguably the most controversial and debated. Several review papers have proposed a commensurate number of hypotheses, and although a consensus has started to emerge, many unanswered questions persist. The initial theory concerning the mode of action of Si in plant's prophylaxis involved a mechanical barrier impeding fungal progress. Indeed, over the years, it had been generally accepted that polymerized Si in the plant cell wall and apoplast prevented pathogen penetration (Wagner 1940; Heath and Stumpf 1986; Carver et al. 1987, 1994; Ishiguro 2001). However, as early as 1965, this theory was put into doubt by Okuda and Takahashi (1965) citing Yoshi's results (Yoshi 1941) of noncorrelation between Si treatment and leaf toughness as measured by a needle-puncture method: 'From this result, it seemed that Si protected the rice plant against blast disease, but the increase in mechanical toughness of the plant tissue resulting from absorbed Si is not sufficient to explain the mechanism of protection'. Nevertheless, this theory has survived over the years and is still cited. Carver et al. (1987) upon the observation of Si accumulation in papillae consistent with findings from Kunoh and Ishizaki (1975) stated that polymerized Si at attempted sites of penetration might provide an additional mean of resistance against penetration. In other works, Kim et al. (2002) proposed the reinforcement of cell walls in rice as a mechanism for enhanced resistance provided by Si treatment. However, no evidence has ever directly linked cell wall reinforcement with penetration failure by the fungus. It should be noted that the logical association proposed between Si deposition and pathogen resistance stems from the fact that Si has been reported in several pathosystems to accumulate at infection sites (Samuels et al. 1991a, b) (Fig. 9.1). This probably derives from a higher transpiration rate at sites, where the cuticle is damaged, rather than a defensive process, as Si accumulation after pathogen penetration would be too late to prevent penetration. As a matter of fact, Chérif et al. (1992a) observed the accumulation of Si in needle-punctured leaf holes and showed the absence of such deposits when plants were grown under saturated humidity. Even though Si is effectively deposited at preferential sites of penetration, and is also continuously deposited at higher rates after penetration has occurred, the hypothesis of cell wall reinforcement by Si to explain enhanced resistance of plants against pathogenic fungi has been strongly contested in recent years.

Assuming that Si does not act exclusively as a physical barrier, the possibility that Si played an active role in the resistance process remained speculative. However, the opportunity for exploring this new hypothesis first came with the pathosystem cucumber–*Pythium* spp. Si applied at a concentration of 1.7 mM in the nutrient

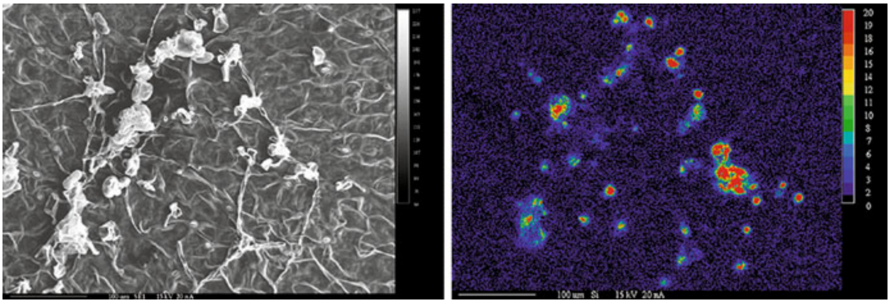

Fig. 9.1 Scanning electron (*left*) and X-ray (*right*) microanalysis showing that the accumulation of Si is coincident with *E. cichoracearum* presence on Si-treated *A. thaliana* leaves. The concentration of Si is indicated by color (see inset), where *red* represents the highest concentration of Si and *black* indicates no Si

solution was found to provide resistance to *Pythium ultimum* in cucumber (Chérif and Bélanger 1992). As in the case of the foliar interaction described by Samuels et al. (1991a, b), the plants reacted more promptly to the infection by *P. ultimum* and the number of infected cells accumulating an electron-dense, phenolic-like material was far greater (Chérif et al. 1992b). This material hindered the propagation of the parasite into the vascular system (Chérif et al. 1992b). Interestingly, none of these deposits, nor the cell walls at sites of penetration of the fungus, contained any trace of Si (Chérif et al. 1992b) as determined by SEM and scanning X-ray analysis. This excluded the possibility of Si functioning as a mechanical strengthener of cell walls to limit *P. ultimum* progress.

Corroborating evidence that Si played more than a mechanical role *in planta* was provided when the interaction between cucumber and powdery mildew was further analyzed at the chemical level (Fawe et al. 1998). The detection and analysis of antifungal compounds in infected cucumber leaves led to the determination of their nature and to the estimation of the effect of Si amendment on their appearance and accumulation (Fawe et al. 1998). Some of these metabolites, identified as flavonoids and phenolic acids, were specifically and strongly induced in a pattern typical of phytoalexins. Thus, Si was hypothesized to play an active role in disease resistance by stimulating the defence mechanisms, namely, phytoalexins, of cucumber in reply to fungal attack Fig. 9.2). This hypothesis was originally received with scepticism by the scientific community who argued that (1) monocots, for which the most important effects of Si feeding were reported, behaved differently and (2) direct genetic evidence to support the hypothesis was still lacking. Accordingly, subsequent efforts focused on the demonstration that monocots responded to Si feeding in the same manner as dicots did. Through electron microscopy studies and subsequent chemical analyses of rice plants, it was shown that rice plants treated with Si fended off rice blast infection through production of electron dense material that was fungitoxic to *Pyricularia oryzae* inside leaf cells (Rodrigues et al. 2003a). This finding was in direct contradiction with a previous publication that argued that amorphous Si deposition had prevented fungal penetration (Kim et al. 2002).

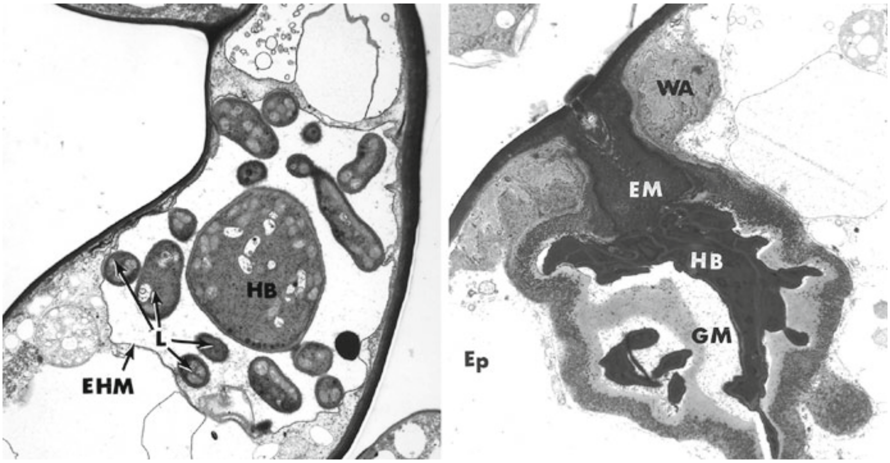

Fig. 9.2 Transmission electron micrographs of ultra-thin sections of *A. thaliana* leaves infected by *E. cichoracearum* (From Ghanmi et al. 2004). Si treatment (*right*) led to more efficient defence compared to control plants (*left*). *EHM* extrahaustorial membrane, *HB* haustorial body, *Ep* epidermis, *GM* granular material, *WA* wall apposition, *EM* electron-dense material, *L* lobes

Rodrigues et al. (2004) subsequently showed that the fungitoxic material was composed of momilactones, known as rice phytoalexins. At the same time, evidence was provided that Si amendments significantly reduced powdery mildew infection on wheat and that this phenomenon was once again attributable to a cellular reaction to fungal presence (Bélanger et al. 2003).

Since the deposited part of Si forming a physical barrier does not account for all the protection afforded by the element, it has been suggested that the soluble part of Si, silicic acid, could be involved in the prophylactic role of the element (Samuels et al. 1991a, b). Soluble Si has been reported inside the cell, in the cytosol, in chloroplastic membranes as well as in association with RNA and DNA (Aleshin et al. 1987). Also, high amounts of soluble Si have been detected in epidermal cells of barley at the beginning of their defence reaction against *Erysiphe graminis*, where it could play an important biochemical or physiological role during the penetration of the parasite (Zeyen et al. 1993). These data on the presence of Si inside the cells are scarce and debated, notably because of the poor measurement techniques available at the time. However, they suggest that Si can have a series of intracellular sites of action to explain its stimulating properties in plant disease resistance. Among others, it could alter directly or indirectly gene expression, by association with genes and modification of their configuration or by acting on the signal transduction pathway(s) activated by infection (Fawe et al. 2001). This second hypothesis is particularly interesting, as Si could represent, among other things, a natural activator of plant disease resistance. On the basis of their observations with cucumber, Fawe et al. (2001) suggested a model to explain how Si would play a role in induced resistance. According to their model, Si bioactivity was compared to that of known activators/secondary messengers of systemic acquired resistance (SAR) whereby it would act as a modulator influencing the timing and extent of plant defence responses. Like secondary messengers, the effects of Si on secondary metabolism are significant only after elicitation; both Si and known activators are characterized by a saturable effect. A difference between known SAR activators and Si is the loss of activity when Si feeding is interrupted (Samuels et al. 1991a, b), because polymerization of Si leads to its inactivation as an inducer of resistance. These points of comparison prompted the authors to propose that Si acted as a signal in inducing defence responses.

Induced resistance (IR) is a mechanism allowing plants to synthesize new defence compounds in response to the presence of a pathogen (Glazebrook 2005; Walters et al. 2013). During a pathogen attack, plants initiate active defences by the perception of an elicitor signal, followed by transduction of the signal to the nucleus by a network of mitogen-activated kinase (MAPK) cascades and the production of defence proteins (Jones and Dangl 2006; Benhamou 2009). In the case of biotrophic pathogens, most plants establish a type of IR called ‘systemic acquired resistance’ (SAR) that requires the activation of the salicylic acid (SA) signalling pathway (Delaney et al. 1994; Zhou et al. 1998; Glazebrook 2005) and the presence of the defence regulatory protein nonexpressor of pathogenesis-related protein1 (NPR1; Durrant and Dong 2004). Also, the IR mechanism is often associated with the augmented capacity to mobilize cellular defence responses only after a contact with pathogens (Conrath et al. 2002). This phenomenon, often called ‘priming’, allows the plant to respond more quickly and effectively to an attack, with minimal metabolic cost (Katz 1998; van Hulten et al. 2006). In this context, several studies have now shown that a pretreatment with Si will prime plants to better respond to pathogen infections (Fauteux et al. 2005; Chain et al. 2009; Van Bockhaven et al. 2013). However, the molecular mechanisms underlying such priming associated with Si remain poorly understood.

9.6 Transcriptomic Analyses

In an effort to better understand how Si affected the plant’s metabolism, a microarray study was performed in 2006 (Fauteux et al. 2006) on both control and powdery mildew-stressed Arabidopsis plants, with or without Si application (Fig. 9.3). The expression of all but two genes was unaffected by Si in control plants, a result contradicting reports of possible direct effect of Si on plant physiology as a fertilizer. In contrast, inoculation of plants, treated or not with Si, altered the expression of a set of nearly 4,000 genes. Following functional categorization, many of the upregulated genes were defence-related, whereas a large proportion of downregulated genes were involved in primary metabolism. Regulated defence genes included R genes, stress-related transcription factors, genes involved in signal transduction, the biosynthesis of stress hormones (SA, JA, ethylene) and the metabolism of reactive oxygen species. In inoculated plants treated with Si, the magnitude of downregulation was attenuated by over 25 %, an indication of stress alleviation. Those results

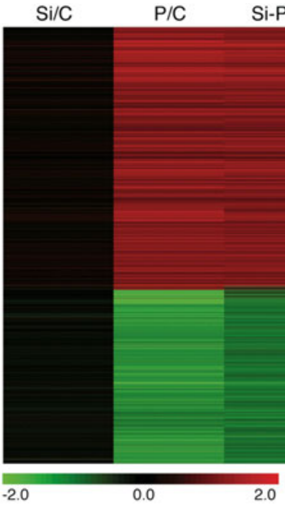

Fig. 9.3 Differential gene expression in Arabidopsis leaves following Si treatment and/or pathogen inoculation (From Fauteux et al. 2006). The columns represent the contrasts between the treatments: control (C), silicon (Si), *Erysiphe cichoracearum* (P) or a combination of both (Si-P). Each of the 3,970 differentially expressed genes ($p<0.01$, ≥1.5-fold change) in at least one contrast is represented by a *colored* line indicating the mean ($n=6$) relative transcript level: green corresponds to a Log_2 ratio of −2 (*down*regulation) and red corresponds to a Log_2 ratio of 2 (*up*regulation)

suggested that Si treatment had no effect on the metabolism of unstressed plants but that it had beneficial properties attributable to modulation of a more efficient response to pathogen stress.

However, following the discovery of Si transporters (Ma et al. 2006) that confirmed the inability of *Arabidopsis* to absorb large quantities of Si because of a lack of Lsi1 transporters, the previous results had to be interpreted with caution until similar studies with high Si-accumulating plants could be carried out. Incidentally, a large transcriptomic analysis (55,000 unigenes) with wheat, a high Si-accumulating plant (Chain et al. 2009), was reported a few years later with plants under both control and pathogen stress (*Blumeria graminis* f.sp. *tritici* (Bgt) (Fig. 9.4). The response to the supply of Si on control (uninfected) plants was limited to 47 genes of diverse functions, mainly related to stress, providing little evidence of regulation of a specific metabolic process. Plants reacted to inoculation with Bgt by an upregulation of many genes linked to stress and metabolic processes and a downregulation of genes linked to photosynthesis. Supplying Si to inoculated plants largely prevented disease development, a phenotypic response that translated into a nearly perfect reversal of genes regulated by the effect of Bgt alone. These results confirmed that Si played a limited role in a plant's transcriptome in the absence of stress, even in the case of a high Si-accumulating monocot such as wheat. On the other hand, the benefits of Si, in the form of biotic stress alleviation, were remarkably aligned with a counterresponse to transcriptomic changes induced by the pathogen Bgt.

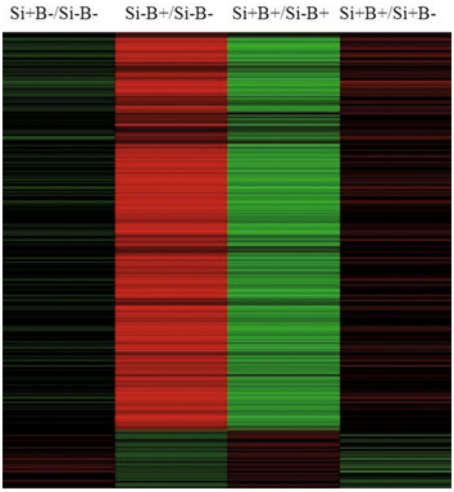

Fig. 9.4 Visual representation of differential gene expression in wheat leaves for plants having received or not Si amendment and/or *Blumeria graminis* f. sp. *tritici* inoculation (Chain et al. 2009). The columns represent contrasts between the treatments: control (Si–B–), Si alone (Si + B–), inoculation alone (Si–B+) or a combination of both (Si + B+). In total, 880 genes have been identified as differentially expressed ($p < 0.01$, ≥1.5-fold change) in at least one contrast. Each gene corresponds to a colored line indicating the mean ($n = 3$) relative transcript level: green corresponds to a $\log_2$ ratio of –2 (down-regulation), and red corresponds to a $\log_2$ ratio of 2 (upregulation)

9.7 The Search for a Better Model

While the transcriptomic analyses provided unique insights into how Si alleviated plant stress, it did not offer direct evidence of a biochemical role for Si. In the meantime, many additional reports came to support that Si did not directly induce immunity but rather primed plants for a better defence response against pathogens (Van Bockhaven et al. 2013). This property even extended to insects with studies showing that Si offered protection through a better arsenal and deployment of defence responses (Reynolds et al. 2009; Ye et al. 2013). Interestingly, this also represented a deviation from the initial hypothesis that Si acted as a mechanical barrier in plant–herbivore interactions. In spite of this mounting evidence of a positive role in priming plants, the mode of action of Si *in planta* remained unresolved.

For the most part, all studies that have shown better defence responses in presence of Si are strictly based on correlative observations. Hypotheses that Si can act as a secondary messenger, a modulator of defence responses or a priming agent have never been fully tested in the absence of a proper genetic model. In this context, Arabidopsis offers a wide range of accessions and mutants that could be helpful in assessing the role of Si. However, as convenient and versatile as Arabidopsis can be as a model plant, its usefulness for Si studies is compromised by its limited absorption of the element owing to the absence of influx transporters. As mentioned earlier, since the discovery of influx transporters in rice in 2006, it is now clear that

Arabidopsis lacks the aquaporins carrying the necessary features for Si permeability and is therefore considered a Si non-accumulator (Deshmukh et al. 2013). To circumvent this problem, Montpetit et al. (2012) proposed to increase Si absorption in Arabidopsis by the insertion of heterologous influx transporters, such as one from wheat in this specific situation.

The concept that it is possible to transform a non-accumulator plant species into an accumulating one opens up a wide array of possibilities to exploit the beneficial properties of Si. However, this presupposes that such transformed plants will display the expected phenotype in presence of Si. Vivancos et al. (2015) presented the first demonstration of this outcome whereby transformed plants were a lot more resistant to powdery mildew when fed with Si compared to control plants or transformed plants deprived of Si. This suggests that beneficial effects of Si are universal among plant species as long as a plant can absorb the element through the presence of influx transporters.

Considering the impressive body of information available on the Arabidopsis–powdery mildew interaction, Vivancos et al. (2015) took advantage of these resistant phenotypes to investigate the probable mechanisms behind the protective role of Si. As stated earlier, Si was initially described as providing a mechanical barrier impeding fungal penetration (Fauteux et al. 2005), and for a long period, this mode of action stood uncontested. However, this hypothesis was challenged by Samuels et al. (1991a, b) and Chérif et al. (1992a, b, 1994) who associated the protective role of Si with the elicitation of defence mechanisms. For these reasons, it was relevant to analyze the specific and well-described markers of resistance in the Arabidopsis–powdery mildew interaction and determine if Si was involved in their expression. Results clearly showed an increase in expression of genes encoding enzymes involved in the SA pathway directly associated with Si feeding and resistant phenotypes. At the same time, SA concentrations were also augmented, thus strengthening the hypothesis that priming occurred through that pathway. Interestingly, the production of camalexin remained unchanged, a result consistent with reports that camalexin production is useful against necrotrophs and not involved with biotrophs (Rogers et al. 1996). These results thus suggest that the response is aligned with the specific pathogen under study and that Si somehow facilitates this response but does not elicit directly the priming machinery.

Other factors militate in support of this indirect role of Si associated with the manifestation of priming. Silicic acid is an uncharged molecule for which no evidence of biochemical activity has ever been obtained. It has been argued that soluble Si could somehow be directly involved in the elicitation of defence responses, namely, as a secondary messenger, much in the same manner as salicylic acid (Fawe et al. 2001; Fauteux et al. 2005; Van Bockhaven et al. 2013). However, the presence of silicic acid in the symplastic environment and subsequent interactions with key defence molecules has simply not been corroborated by scientific data and remain speculative. Furthermore, the fact that the expression of *NPRI* and other defence-related genes was unchanged in *pad4* and *sid2* Arabidopsis mutants under Si treatment (Vivancos et al. 2015) would indicate that silicic acid does not act as a surrogate for salicylic acid as previously suggested (Fawe et al. 2001; Van Bockhaven et al. 2013).

The large array of available Arabidopsis mutants offered the unique opportunity to validate the hypothesis that priming of defence reactions explained how Si protected plants against diseases. Indeed, by using mutants able to absorb larger quantities of Si but deficient in the activation of the SA pathway, Vivancos et al. (2015) were able to test directly if the protective effect of Si became null or significantly altered, given the presumed inability of the plant to mount defence reactions against powdery mildews. It was therefore quite surprising to observe that plants transformed for high Si absorption and fed with Si displayed resistant phenotypes in spite of having lost the ability to produce defence reactions through the SA pathway. These observations strongly suggest that other factors than defence reactions are at play.

Based on these observations, it might be tempting to conclude that resistance conferred by Si on SA-deficient mutants supports the concept of a physical barrier. However, a mechanical barrier physically stopping a germinating spore would not lead to elicitation of defence mechanisms as observed here and in countless recent papers (Qin and Tian 2005; Bi et al. 2006; Kanto et al. 2007). On the other hand, these results bring a unique perspective as they show that if the priming state associated with Si feeding is altered, one can still obtain resistant phenotypes.

If these results appear contradictory at first, they provide an opportunity to consider an alternative hypothesis that would unify the modes of action behind the observed phenomena. It is well known that the prophylactic role of Si has been more documented and is more efficient against pathogens with a biotrophic phase (e.g. powdery mildews, oomycetes, rice blast). In the last few years, with the advent of high-throughput sequencing, the annotation of plant pathogen genomes has highlighted the presence and importance of effector proteins, most notably in the case of biotrophs and hemibiotrophs. Effectors will modify host cell structure, metabolism and function and interfere with signal pathways required for host invasion or for triggering host resistance (Giraldo and Valent 2013). Recent developments have located effectors in the apoplast, the extrahaustorial matrix or the cytoplasm after translocation across the plant membrane. Interestingly, amorphous Si deposition in plants is located in the apoplast and more precisely at the interface of the plasma membrane and the cell wall (Bauer et al. 2011; Zhang et al. 2013). This area is the site of intense interactions of many effectors with plant targets and sites of attempted penetration by biotrophic fungi (Bozkurt et al. 2012). As a matter of fact, the appressorium and the haustorium of powdery mildew fungi are structures of active release of effectors (Giraldo and Valent 2013); the appressorium will release effectors in the apoplastic compartment to prevent the action of plant proteases and the haustorium will release them into the cytoplasm through the extrahaustorial matrix (EHMx) to alter plant defences. Given that the apoplast and the EHMx are within the confines of Si deposition (Ghanmi et al. 2004), and based on our observations, it thus seems not only plausible but also logical that Si would interfere with effectors reaching their targets. This would thus prevent the invading fungus from inhibiting the plant defence, which results in the expression of the complete array of defence mechanisms as observed in this work and elsewhere. In addition, the intercellular space is a hostile environment for a fungal pathogen, and the latter will rely on apoplastic effectors to inhibit the release of a wide array of proteases and other plant molecules that would adversely affect its development (Win et al. 2012; Giraldo and Valent 2013). From our results, it does appear that this initial barrier is indeed quite efficient and has significantly delayed fungal infection. In consideration of the superior prophylactic role of Si against biotrophs, the heavy reliance of biotrophs on effectors to maintain their virulence and the site of Si deposition coinciding with effectors release, our results support a link between Si and effectors and certainly future efforts in testing this hypothesis.

In conclusion, the latest studies of the role of Si in plant–pathogen interactions have confirmed the association between Si and priming but have also uncovered a new phenomenon suggesting that mechanisms other than priming are involved in the way Si protects plants. These unforeseen results may be helpful in defining a unifying theory explaining the elusive and debated mode of action of Si in alleviating biotic stresses.

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