Molecular Plant Pathology

MOLECULAR PLANT PATHOLOGY (2015) 16(8), 811–824 DOI: 10.1111/mpp.12236

Primary metabolism plays a central role in moulding silicon-inducible brown spot resistance in rice

JONAS VAN BOCKHAVEN[1], KATHY STEPPE[2], INGVAR BAUWERAERTS[2], SHOSHI KIKUCHI[3], TAKAYUKI ASANO[3], MONICA HÖFTE[1] AND DAVID DE VLEESSCHAUWER[1,*]

[1]*Laboratory of Phytopathology, Faculty of Bioscience Engineering, Ghent University, B-9000 Ghent, Belgium*
[2]*Laboratory of Plant Ecology, Faculty of Bioscience Engineering, Ghent University, B-9000 Ghent, Belgium*
[3]*Plant Genome Research Unit, Agrogenomics Research Center, National Institute of Agrobiological Sciences, Tsukuba 305-8602, Ibaraki, Japan*

SUMMARY

Over recent decades, a multitude of studies have shown the ability of silicon (Si) to protect various plants against a range of microbial pathogens exhibiting different lifestyles and infection strategies. Despite this relative wealth of knowledge, an understanding of the action mechanism of Si is still in its infancy, which hinders its widespread application for agricultural purposes. In an attempt to further elucidate the molecular underpinnings of Si-induced disease resistance, we studied the transcriptome of control and Si-treated rice plants infected with the necrotrophic brown spot fungus *Cochliobolus miyabeanus*. Analysis of brown spot-infected control plants suggested that *C. miyabeanus* represses plant photosynthetic processes and nitrate reduction in order to trigger premature senescence and cause disease. In Si-treated plants, however, these pathogen-induced metabolic alterations are strongly impaired, suggesting that Si alleviates stress imposed by the pathogen. Interestingly, Si also significantly increased photorespiration rates in brown spot-infected plants. Although photorespiration is often considered as a wasteful process, recent studies have indicated that this metabolic bypass also enhances resistance during abiotic stress and pathogen attack by protecting the plant's photosynthetic machinery. In view of these findings, our results favour a scenario in which Si enhances brown spot resistance by counteracting *C. miyabeanus*-induced senescence and cell death via increased photorespiration. Moreover, our results shed light onto the mechanistic basis of Si-induced disease control and support the view that, in addition to activating plant immune responses, Si can also reduce disease severity by interfering with pathogen virulence strategies.

Keywords: *Bipolaris oryzae*, photorespiration, photosynthesis, plant immunity, plant–microbe interactions, rice, silicon.

**Correspondence*: Email: david.devleesschauwer@ugent.be

INTRODUCTION

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. Orthosilicic acid is readily taken up by plant roots and loaded into the xylem by a specific transporter system (Ma and Yamaji, 2006). Silicic acid is transported to the shoots via the xylem, where it is constantly polymerized, either as silica in the cell or as an insoluble, subcuticular silica layer outside the cell (Ma *et al*., 2011). To date, dozens of studies have documented the ability of Si to enhance plant growth and yield, and it is the only nutrient that is not detrimental when accumulated in excess (Epstein and Bloom, 2005). Moreover, Si-treated plants often display enhanced resistance against a wide variety of biotic and abiotic stresses (Epstein, 2009; Fauteux *et al*., 2005; Guntzer *et al*., 2011; Ma and Yamaji, 2006; Van Bockhaven *et al*., 2013). As such, Si amendment is one of the only plant protection strategies that enables plants to maximize the efficiency of their response to the exact set of environmental conditions encountered, at the same time as conserving resources for growth and development.

Although much remains to be discovered about the underlying mechanisms, a number of recent microarray and proteome analyses have begun to elucidate the molecular basis and regulation of Si-induced stress resistance (Brunings *et al*., 2009; Chain *et al*., 2009; Fauteux *et al*., 2005; Fleck *et al*., 2011; Ghareeb *et al*., 2011; Nwugo and Huerta, 2011). Emerging from these studies is the view that, rather than warding off pathogen attack through the formation of a physical silica barrier, Si mainly acts as a biological inducer of a wide variety of plant immune responses. Yet, Si does not appear to activate resistance *per se*, but rather intensifies and/or accelerates basal defence responses following stress perception. Moreover, several reports have documented the ability of Si to increase photosynthesis rates in plants subjected to either biotic or abiotic stresses, suggesting that Si also impinges on the primary metabolism of stressed plants (Chen *et al*., 2011; Dallagnol *et al*., 2013a; Farooq *et al*., 2013; Nwugo and Huerta, 2008; Perez *et al*., 2014; Resende *et al*., 2012; Shen *et al*., 2010).

In plants, primary metabolism encompasses both energy- and carbohydrate-providing processes, such as photosynthesis, gluconeogenesis and respiration, as well as nitrogen-associated

reactions accounting for the incorporation of inorganic and organic nitrogen in amino acids (Rontein *et al*., 2002). Plant–pathogen interactions are generally determined by the pathogen's need for nutrients and carbohydrates, and the plant's requirement for energy to fuel defence mechanisms (Berger *et al*., 2007; Bolton, 2009). As plant primary metabolism is at the crossroads of these contrasting interests, central metabolic processes are believed to play a key role in moulding pathological outcomes (Rojas *et al*., 2014). Moreover, current concepts suggest that these infection-induced metabolic alterations lie on a continuum between 'endurance' and 'evasion', two opposing physiological states during which plant cell death is suppressed or facilitated, respectively (Seifi *et al*., 2013). The endurance strategy is mostly effective against pathogens with a necrotrophic lifestyle, whereas evasion generally confers resistance towards biotrophic pathogens (Glazebrook, 2005; Mur *et al*., 2013; Seifi *et al*., 2013).

Interestingly, pathogens seem to have evolved sophisticated mechanisms to exploit these opposing metabolic states for their own benefit. For instance, archetypal necrotrophic pathogens, such as *Botrytis cinerea* and *Sclerotinia sclerotiorum*, are well known to trick the plant into activating certain types of programmed cell death, thus facilitating their own infection process (Govrin and Levine, 2000; Kabbage *et al*., 2013; Levine, 2007; Williams *et al*., 2011). Other necrotrophs have likewise been hypothesized to co-opt plant apoptotic pathways in order to cause disease, including the rice fungal pathogen *Cochliobolus miyabeanus* (Xiao *et al*., 1991). Also known as *Bipolaris oryzae* (anamorph; Breda de Haan), *C. miyabeanus* is the causal agent of the devastating rice brown spot disease (Dela Paz *et al*., 2006). Consistent with plant cell death facilitating *C. miyabeanus* infection, brown spot is especially prevalent in rainfed ecosystems in which plants are prone to premature senescence as a result of suboptimal growth conditions (Leung *et al*., 2003; Ou, 1985; Zadoks, 2003). Although semi-resistant rice cultivars are available, brown spot management still relies heavily on the application of hazardous fungicides (Castell-Miller and Samac, 2012). In this context, the application of Si holds great potential for environmentally friendly, economically sound and sustainable control of brown spot disease. However, despite some recent progress (Dallagnol *et al*., 2009, 2011a), much remains to be learned about the precise mechanisms through which Si protects rice from brown spot attack.

Aiming to shed more light on the rice–*C. miyabeanus* interaction and the beneficial effect of Si on plant disease resistance, we performed a microarray experiment comparing the transcriptome of control and Si-treated plants during the early stages of infection [12 h post-inoculation (hpi)]. Our data support a central role of nitrogen- and photosynthesis-related metabolic processes in shaping the outcome of rice–brown spot interactions. Moreover, we propose that Si protects rice from brown spot attack by preventing the pathogen from hijacking the plant's primary metabolism.

RESULTS

Si amendment promotes growth and development of non-stressed rice plants

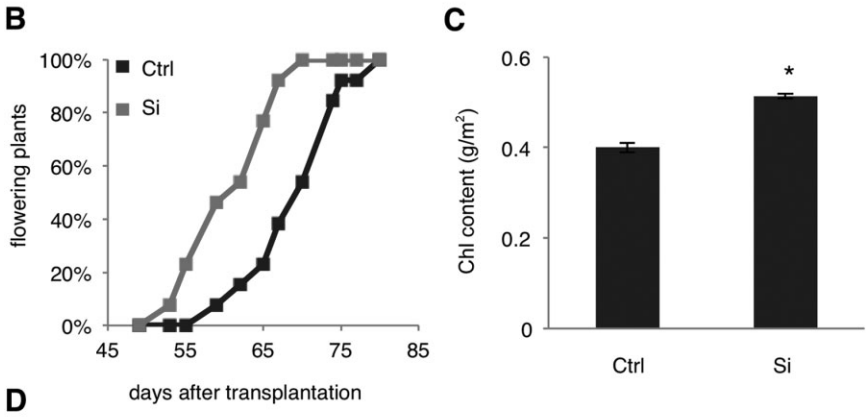
To assess the impact of Si on the growth phenotype of rice (cultivar Nipponbare), we employed a hydroponic gnotobiotic system, wherein plants were continuously supplied with 2 mM silicic acid. Compared with control non-treated plants, Si-supplied plants not only accumulated more biomass, but also displayed early flowering and improved tillering and ear filling (Fig. 1A, B). Furthermore, leaf gas exchange measurements showed that, although the level of respiration (R_d) was similar for both treatments, Si application increased net photosynthesis as well as the level of photorespiration (Fig. 1D and Table S1, see Supporting Information). Similarly, Si-treated plants showed higher levels of photochemical quenching (q_P), which is a measure of the amount of light energy used by the plants. In addition, and as suggested by the significant increase in photosystem II (PSII) efficiency (Φ_{PSII}), Si-treated plants also seemed to be able to channel more light energy into the reaction centre of PSII, a phenomenon which is probably explained by the increased chlorophyll content in these plants (Fig. 1C). Considering that net photosynthesis was improved without altering the transpiration rates, Si treatment also seemed to positively influence plant water-use efficiency.

Si enhances resistance to *C. miyabeanus*

Consistent with previous results (Dallagnol *et al*., 2009, 2013a, b; Rezende *et al*., 2009; Silva *et al*., 2012), the application of Si resulted in a substantial reduction in brown spot disease severity in both intact plant and detached leaf assays (Fig. 2A–D). Si-treated rice leaves not only displayed slower disease development, but also significantly smaller lesions and substantially less chlorosis and necrosis on brown spot infection compared with inoculated control plants. The prophylactic role of Si has been mechanistically explained through the combined effects of passive protection offered by the hard, subcuticular silica layer and Si-mediated recruitment of active plant defence mechanisms. To investigate the importance of passive and active defences in Si-inducible brown spot resistance and to assess the time span during which Si is effective, the influence of different Si treatments on brown spot resistance was investigated (Fig. 2E). All Si treatments were routinely compared with control plants that were grown in the absence of Si (Si–/Si–). Plants receiving Si were either continuously treated with Si throughout the course of the experiment (Si+/Si+), supplemented with Si up to 3 days before inoculation (Si+/Si–) or short term treated with Si starting 3 days prior to inoculation (Si–/Si+). Interestingly, depriving plants of Si for 3 days prior to inoculation (Si+/Si–) significantly attenuated the level of Si-inducible brown spot resistance, despite having no discernible impact on the silicification of trichomes and the subcuticular silica layer of Si-treated

A

characteristics	Ctrl mean	Ctrl SE	Si mean	Si SE	
dry plant weight (g)	3.86	± 0.31	9.71	± 0.91	*
# tillers / plant	1.23	± 0.14	2.46	± 0.28	*
ear weight (g)	0.91	± 0.09	1.82	± 0.14	*
ear filling (%)	0.67	± 0.04	0.83	± 0.13	*



D

Leaf gas exchange measurements	Ctrl mean	Ctrl SE	Si mean	Si SE	
net photosynthesis (A_n; μmol CO_2/m^2/s)	9.61	± 1.14	12.88	± 1.42	*
respiration (R_d; μmol CO_2/m^2/s)	3.45	± 0.23	3.61	± 0.16	
photorespiration (R_i; μmol CO_2/m^2/s)	3.44	± 0.18	3.99	± 0.23	*
photosystem II efficiency (Φ_{PSII})	0.14	± 0.01	0.17	± 0.00	*
photochemical quenching (q_P)	0.30	± 0.02	0.36	± 0.01	*
transpiration (T_r; mmol H_2O)	2.36	± 0.22	2.88	± 0.13	

Fig. 1 (A) Yield characteristics of control (Ctrl) and silicon (Si)-treated rice plants. (B) Si treatment induces early flowering. (C) Impact of Si feeding on the plant chlorophyll (Chl) content. (D) Leaf gas exchange and chlorophyll *a* fluorescence measurements reveal a positive effect of Si on the photosynthetic abilities of treated plants. Data are means ± standard error (SE) of at least three independent biological repetitions. Asterisks indicate statistically significant differences compared with non-treated controls ($n \geq 24$, *t*-test, $\alpha = 0.05$).

plants. In contrast, plants treated with Si for 3 days only (Si–/Si+) were as resistant as continuously treated plants (Si+/Si+). Considering that such a short time Si application did not lead to visible silicification of the plants, these results strongly suggest that active Si effects outweigh the importance of Si-imposed physical barriers in the development of Si-inducible brown spot resistance. Consistent with this hypothesis, microscopic analysis of the infection process in control and Si-treated plants revealed no significant differences in the number of successful penetration events, indicating that Si does not impede pre-penetration development of *C. miyabeanus* (Van Bockhaven *et al*., 2015).

Microarray experiment

To shed further light on the molecular basis underpinning Si-induced resistance against *C. miyabeanus*, a microarray experiment was performed in which we compared the transcriptome of control and Si-treated plants following pathogen infection (2 × 2 factorial design: control and Si-treated; mock-treated and infected). To maximize the chance of detecting causal resistance mechanisms, we chose to sample at 12 hpi, which is 6 h prior to the appearance of the first disease symptoms and 6 h post-pathogen penetration. Of the 43 494 gene probes on the microarray, 2906 were significantly differentially expressed among the four comparisons (false discovery rate < 0.01; $-2 > \log_2$ fold change > 2) (Fig. 3A). The Volcano plots shown in Fig. 4 illustrate the distribution of the data points, the significance of the measured changes in gene expression levels and the number of differentially expressed genes for each treatment comparison. The dark grey dots represent genes with a low significance [$-\log_{10}(P$ value) < 2], whereas the light grey dots are genes with a high significance [$-\log_{10}(P$ value) > 2], but a fold change < 4. The black dots are the genes selected as differentially regulated [$-\log_{10}(P$ value) > 2 and fold change > 4]. Using the Venny

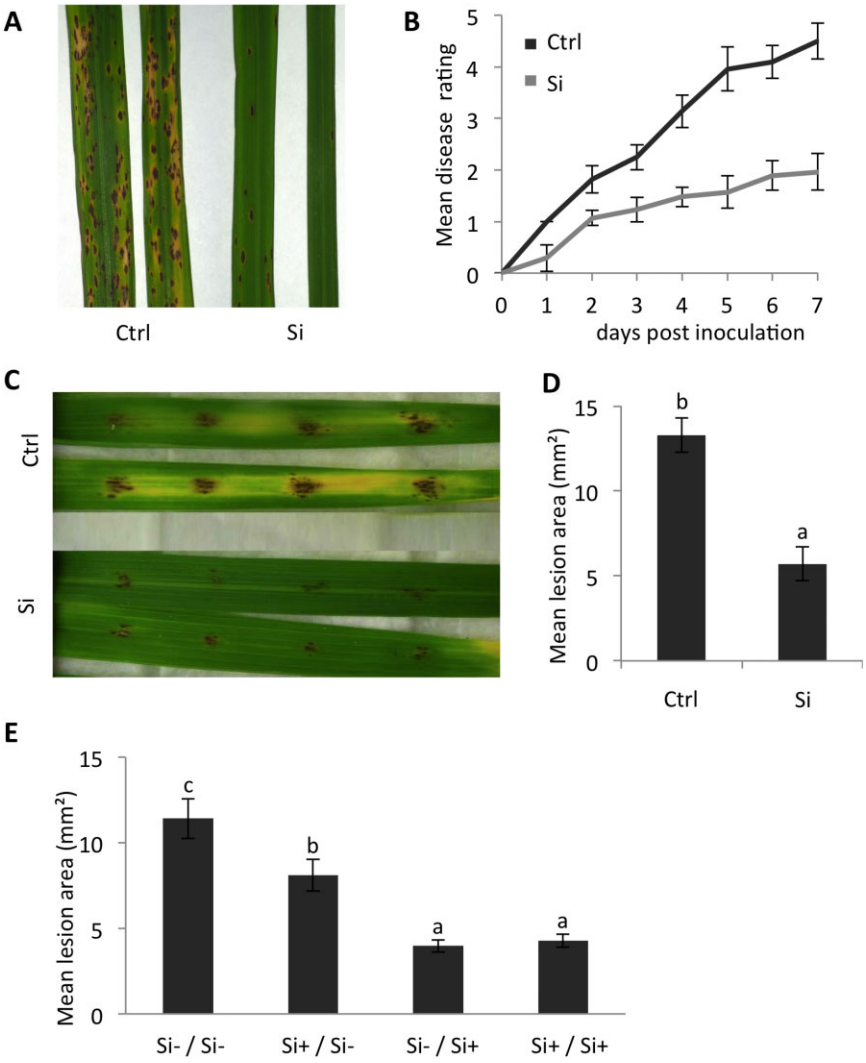


Fig. 2 Silicon (Si) induces resistance against the rice brown spot fungus *Cochliobolus miyabeanus*. (A, B) Effect of Si feeding on symptom development following spray inoculation of intact plants. Disease was scored daily using a 1–5 disease severity scale as outlined in Experimental procedures. The photograph shown in (A) depicts the representative disease symptoms on control (Ctrl) and Si-fed plants at 3 days post-inoculation. (C, D) Effect of Si feeding on the mean lesion area 3 days after inoculation of detached leaf pieces with four 15-μL droplets of spore solution (n = 20, Mann–Whitney, α = 0.05). (E) Impact of different Si treatments on the level of brown spot resistance at 3 days post-*C. miyabeanus* infection. Plants were either continuously treated with Si (Si+/Si+), deprived of Si 3 days prior to inoculation (Si+/Si–) or short-term treated with Si starting 3 days prior to inoculation (Si–/Si+). Plant non-treated with Si (Si–/Si–) served as controls (n = 24, Mann–Whitney, α = 0.05). Different letters indicate statistically significant differences. All experiments were repeated at least twice with similar results.

algorithm, we also constructed a Venn diagram to visualize the number of uniquely and commonly regulated genes for each pair of comparisons (Fig. 3B). Interestingly, Si had a fairly similar impact on uninfected (Si,m vs. Ctrl,m) and infected (Si,I vs. Ctrl,I) leaves, directing transcriptional reprogramming of 1822 and 2214 genes, respectively, most of which were down-regulated (Fig. 3A and Tables S2 and S4, see Supporting Information). Brown spot infection, however, had a comparatively less pronounced effect, with 483 and 362 genes differentially expressed in control (Ctrl,I vs. Ctrl,m) and Si-treated (Si,I vs. Si,m) leaves, respectively (Tables S3 and S5, see Supporting Information). Moreover, hierarchical clustering analysis (Fig. 3C) showed that the influence of Si in non-inoculated leaves (Si,m vs. Ctrl,m) was similar to that in inoculated leaves (Si,I vs. Ctrl,I), whereas *C. miyabeanus* affected less genes in Si-treated plants (Si,I vs. Si,m) than in non-treated controls (Ctrl,I vs. Ctrl,m). Moreover, Si treatment appeared to reverse the effect of *C. miyabeanus* on the gene expression profile, whereby genes that were up-regulated in inoculated control plants were now down-regulated, and vice versa (Fig. 3C; comparison Ctrl,I vs. Ctrl,m and Si,I vs. Ctrl,I).

To validate our microarray results, quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed on 15 genes that were differentially regulated on Si feeding. As shown in Table S6 and Fig. S1 (see Supporting Information), qRT-PCR and microarray data were linearly correlated (R^2 = 0.91).

Gene ontology (GO) enrichment analysis

GO enrichment analysis using MapMan software (Usadel *et al*., 2009) identified a wide variety of metabolic processes (Fig. 5 and Table S7, see Supporting Information) and defence response pathways (Fig. 6 and Table S7) that were significantly influenced by brown spot infection and/or Si application.

Impact of Si on non-inoculated leaves (Si,m vs. Ctrl,m)

The application of Si led to an increased expression of genes that mediate chlorophyll biosynthesis, light-dependent reactions

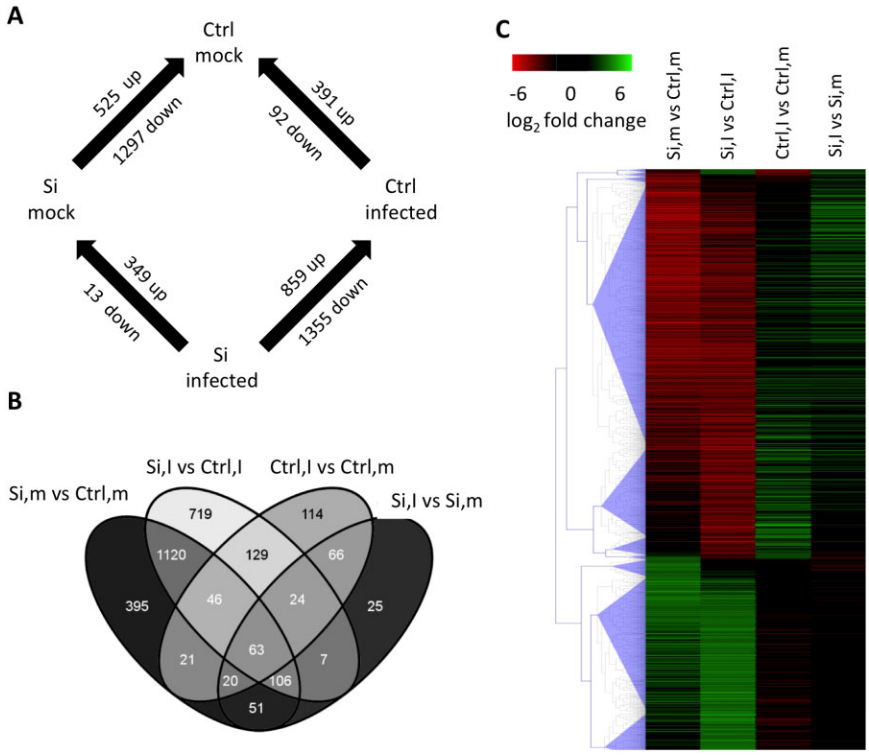


Fig. 3 (A) Experimental design and the number of differentially expressed genes for each treatment comparison. (B) Venn diagram (Oliveros, 2007) showing genes differentially expressed for each comparison and all possible combinations. (C) Hierarchical clustering analysis identified similar expression patterns in Si-treated vs. non-treated rice leaves and in brown spot-infected vs. uninfected samples (Euclidean distance, complete linkage clustering, false discovery rate < 0.01). Si, silicon; Ctrl, control; I, inoculated; m, mock-inoculated.

and nitrate reduction. Genes involved in glycolysis, cell wall biosynthesis and degradation, and nitrogen metabolism and transport were differentially expressed. Moreover, Si application down-regulated amino acid metabolism-related and a number of defence-associated genes involved in lipid desaturation, the phenylpropanoid pathway, the biotic stress response and the metabolism of the defence hormones ethylene (ET), jasmonic acid (JA) and salicylic acid (SA).

Impact of C. miyabeanus *(Ctrl,I vs. Ctrl,m)*

One of the major influences of *C. miyabeanus* on primary metabolic processes was the down-regulation of genes involved in photosynthetic processes, such as chlorophyll biosynthesis, light-dependent reactions, the Calvin cycle and photorespiration. In addition, brown spot infection also weakly up-regulated amino acid biosynthesis, whereas enzymes involved in nitrate reduction were down-regulated. With respect to defence-related pathways, brown spot infection resulted in the up-regulation of genes involved in JA metabolism, cell wall biosynthesis and degradation, and the phenylpropanoid pathway. Finally, consistent with ET functioning as a virulence factor of *C. miyabeanus* (De Vleesschauwer *et al*., 2010), infected plants also displayed increased expression of various genes related to ET metabolism.

Impact of Si on infected leaves (Si,I vs. Ctrl,I)

The influence of Si on infected leaves almost completely mirrored its effect in non-inoculated plants, the only notable difference being the higher number of differentially expressed genes associated with light-dependent reactions, the Calvin cycle, photorespiration and nitrate reduction in infected tissues. Conspicuously, Si treatment induced relatively few defence-related genes in infected leaves, suggesting that Si-inducible brown spot resistance is not associated with widespread activation of plant resistance responses.

Impact of C. miyabeanus *on Si-treated leaves (Si,I vs. Si,m)*

Brown spot infection did not significantly affect the defence-related transcriptome of Si-treated leaves, except for a slight increase in the number of genes implicated in defence-related processes, such as the phenylpropanoid pathway, cell wall biosynthesis, JA and ET metabolism and biotic stress responses. Moreover, contrary to its strong effect on the primary metabolism of control inoculated leaves, *C. miyabeanus* failed to down-regulate photosynthesis-associated reactions and nitrate reduction in Si-treated leaves.

Detailed analysis of the influence of *C. miyabeanus* and Si on the expression of central metabolic genes

Brown spot-infected leaves are hallmarked by the formation of necrotic lesions surrounded by a chlorotic halo, whereas Si-treated leaves demonstrated smaller lesions and less chlorosis (Fig. 2A, C). Linking these phenotypes to the results of the GO enrichment analysis (Figs 5 and 6; Table S7) strongly suggests that central

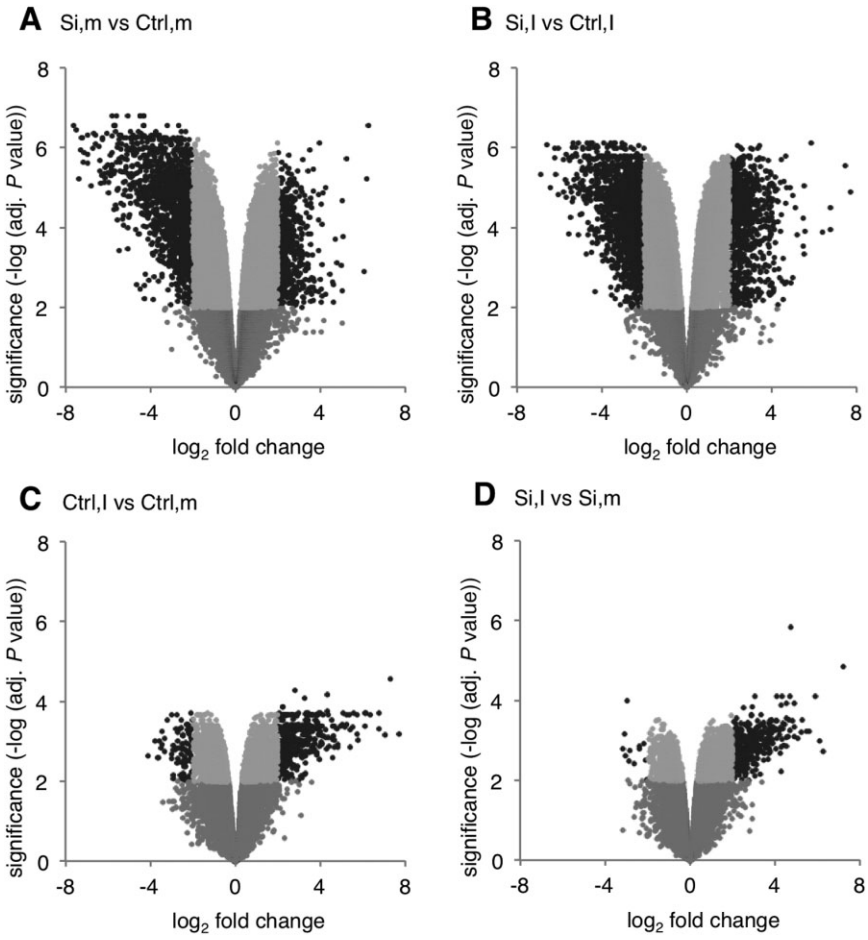


Fig. 4 Volcano plots showing gene expression changes and associated significance values across the different treatment comparisons. Each array spot is represented by a single dot. Black dots represent differentially expressed genes [$-2 > \log_2$-based signal ratio > 2 and $-\log_{10}(P \text{ value}) > 2$]. Si, silicon; Ctrl, control; I, inoculated; m, mock-inoculated.

metabolism plays a central role in shaping the outcome of rice–*C. miyabeanus* interactions. In the following sections, the impact of Si and/or *C. miyabeanus* on a subset of primary metabolism genes is discussed in more detail.

Impact of Si on central metabolism in non-inoculated leaves (Si,m vs. Ctrl,m)

As shown in Fig. 7A and Tables S2 and S7, Si application increased the expression of photosynthetic genes that mediate light harvesting and the Calvin cycle, which corroborates the observed increase in photosynthesis in Si-treated plants (Fig. 1D). Interestingly, the increased photosynthetic ability of Si-treated plants coincided with a down-regulation of genes involved in mitochondrial ATP synthesis. As Si-treated plants are more efficient in using light energy (Fig. 1D), it is tempting to hypothesize that chloroplastic ATP synthesis may be sufficient for the plant's energy requirements, resulting in decreased ATP synthesis in the mitochondria. However, further proof is clearly needed. Meanwhile, Si feeding also led to an up-regulated expression of several nitrate and nitrite reductases, as well as the nitrate transporters *NRT1.1C*, *NRT1.4* and *CLC1*. As AtNRT1.1, the homologue of OsNRT1.1C, has been reported to act as a nitrate receptor/transporter (Gojon *et al*., 2011), and AtCLCa, the homologue of OsCLC1, accounts for the transport of nitrate from the vacuole to the cytosol (De Angeli *et al*., 2006; Geelen *et al*., 2000), these findings may point towards increased cytosolic nitrate concentrations that could fuel the predicted nitrate reduction in Si-treated leaves. In addition, the ammonium transporters *AMT2.2* and *AMT3.3* were down-regulated by Si treatment in infected leaves. The localization of most ammonium and nitrate transporters in rice leaves remains to be elucidated (Gaur *et al*., 2012; Martinoia *et al*., 2007; Suenaga *et al*., 2003), but their differential expression suggests that Si may induce a shift in nitrogen transport.

The impact of Si on the transcriptome and metabolism of non-stressed rice plants has been investigated in a number of previous studies. In agreement with our findings, Brunings *et al*. (2009) found Si to significantly alter the basal expression level of more than 220 rice genes, which leaves open the possibility that Si is essential for rice. In contrast, Watanabe *et al*. (2004) found only 20 genes to be differentially expressed on Si treatment, despite using a similar hydroponic rice-growing system. Although differences in rice cultivars, microarray platforms and statistical settings used in the different studies cannot be excluded, none of these factors justifies a 10- to 100-fold difference in the number of

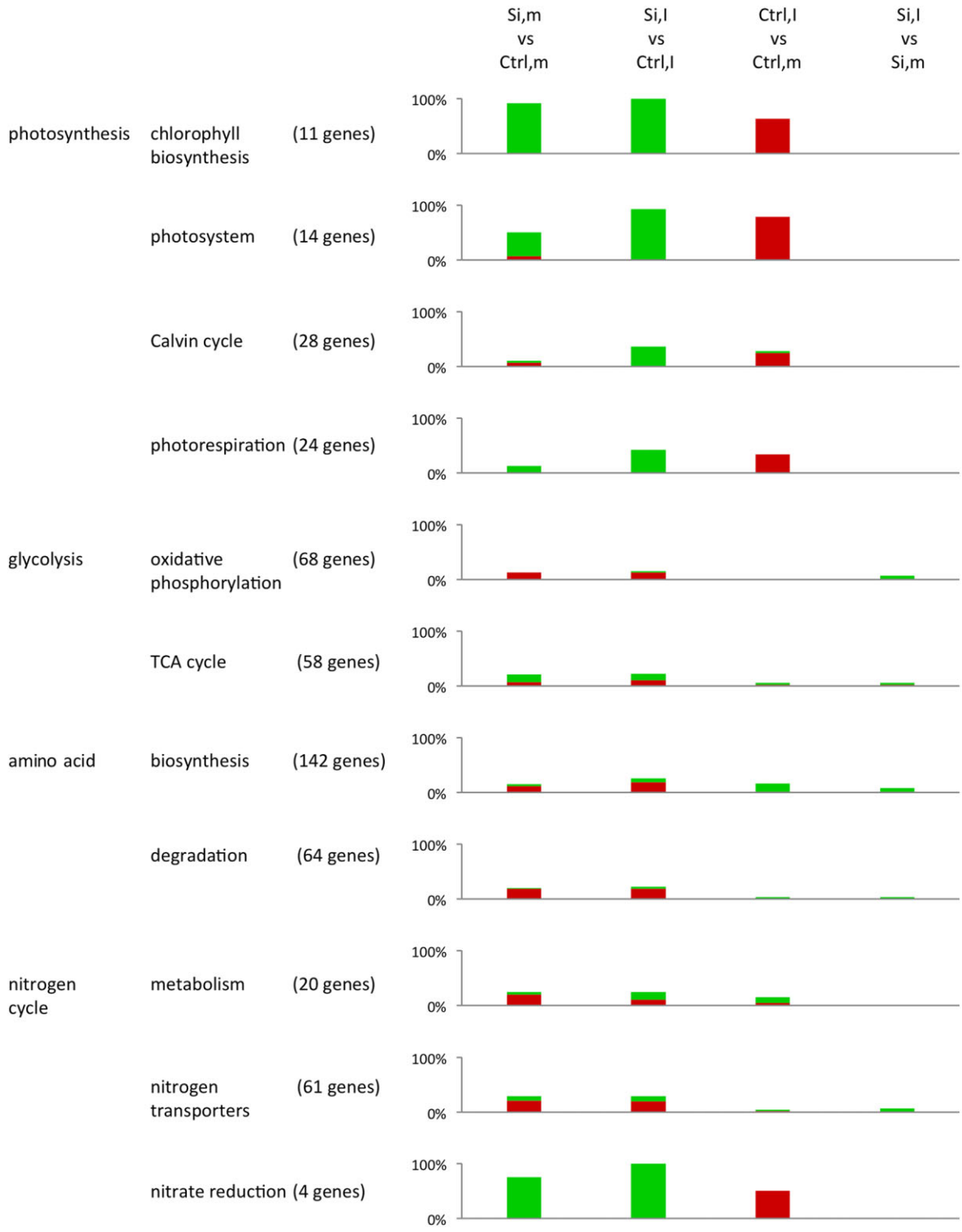


Fig. 5 Gene ontology (GO) enrichment analysis demonstrating the impact of silicon treatment and brown spot infection on primary metabolic processes in rice (LIMMA; false discovery rate < 0.01). For each GO term, the total number of genes represented on the microarray are shown, whereas the coloured bars indicate the relative number of genes showing a significant difference in expression across the different treatment comparisons (green bars, up-regulation, $\log_2$ fold change > 2; red bars, down-regulation, $\log_2$ fold change < −2). GO terms were derived from the MapMan database (Usadel *et al*., 2009). Si, silicon; Ctrl, control; I, inoculated; m, mock-inoculated.

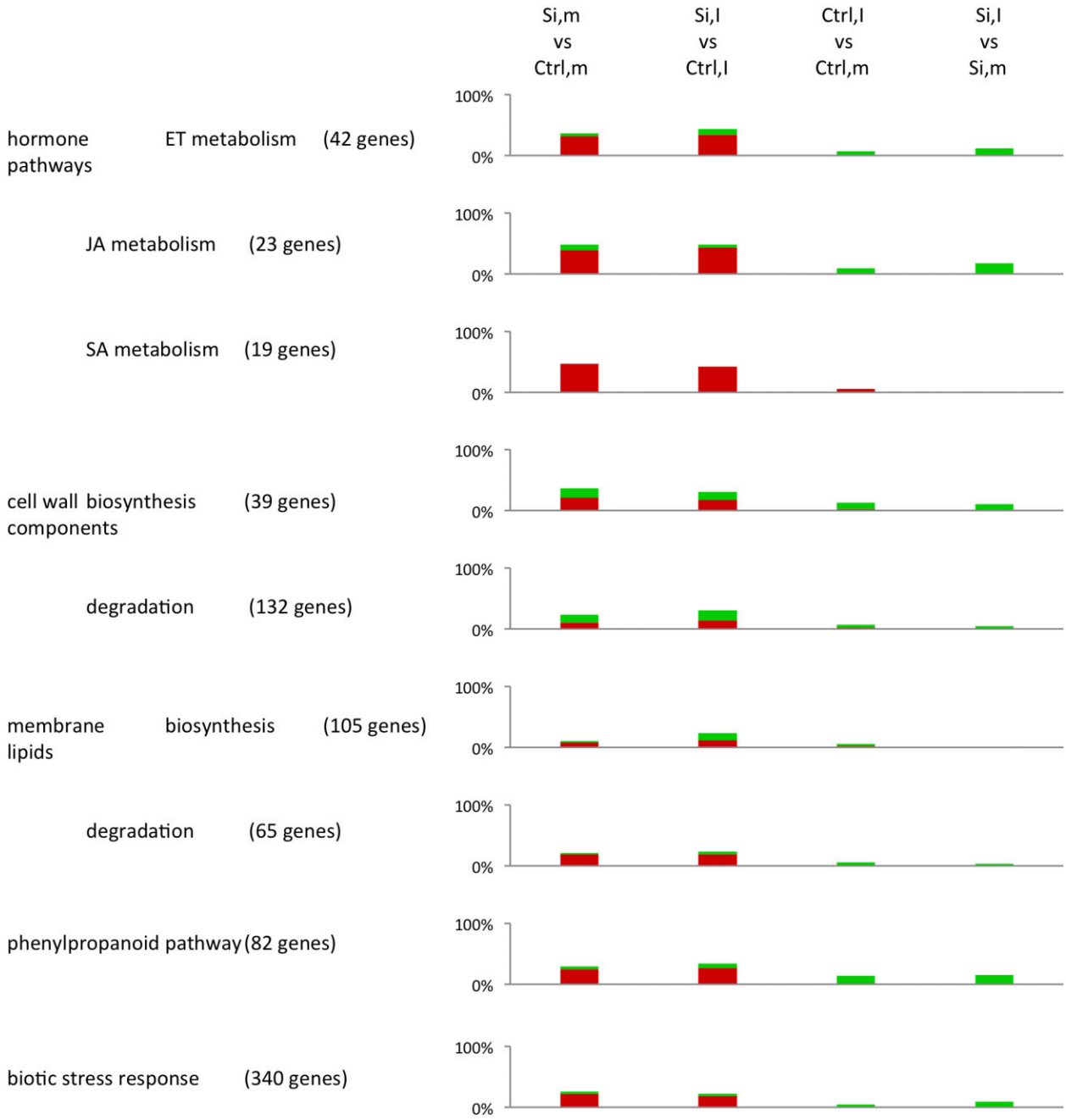


Fig. 6 Enrichment analysis of plant defence-related gene ontologies (GOs) (LIMMA; false discovery rate < 0.01). For each GO term, the total number of genes represented on the microarray are shown, whereas the coloured bars indicate the relative number of genes showing a significant difference in expression across the different treatment comparisons (green bars, up-regulation, $\log_2$ fold change > 2; red bars, down-regulation, $\log_2$ fold change < −2). GO terms were derived from the MapMan database (Usadel *et al*., 2009). Si, silicon; Ctrl, control; I, inoculated; m, mock-inoculated.

Si-responsive genes. Another confounding factor, however, involves the plant growth conditions and, especially, the light conditions. Agarie *et al*. (1992) found that, under suboptimal light conditions, Si treatment leads to a stronger growth increase than under optimal light conditions. Considering that all our assays were performed under growth chamber conditions, it is not inconceivable that the relatively low light intensities to which our plants were exposed amplified the impact of Si on the plant's basal transcriptome.

Impact of C. miyabeanus *on central metabolism (Ctrl,I vs. Ctrl,m)*

The transcriptional changes in central metabolic genes after brown spot infection (Fig. 7B) indicated a down-regulation of pho-

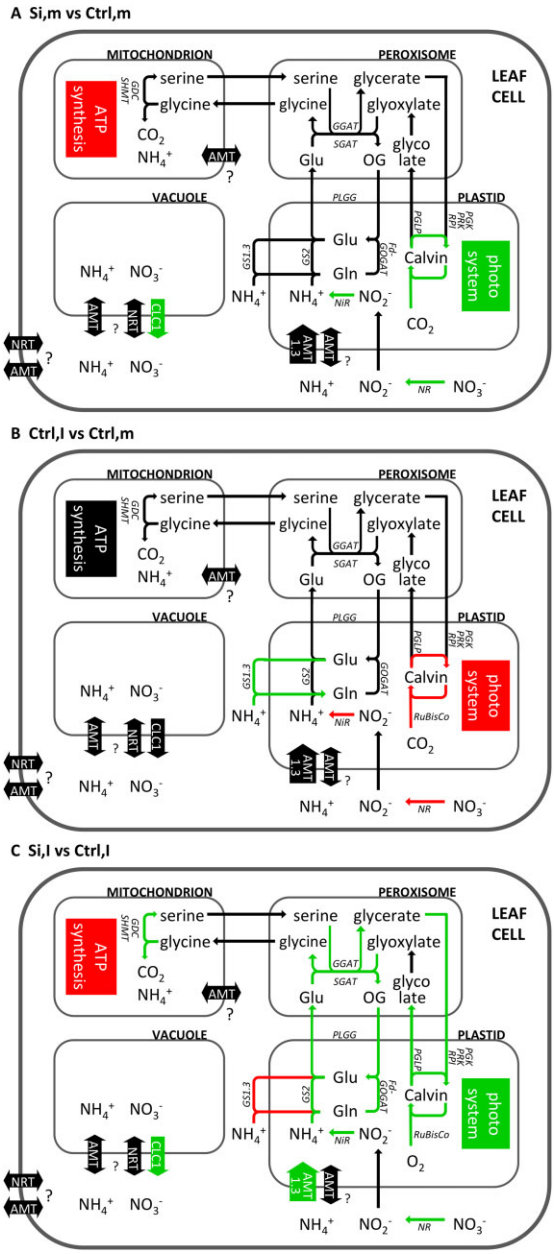


Fig. 7 Influence of silicon (Si) application (A), *Cochliobolus miyabeanus* infection (B) and combined Si and *C. miyabeanus* treatment (C) on the expression of genes involved in central carbon/nitrogen (C/N) metabolism. Arrows in red and green indicate down- and up-regulation of gene expression, respectively. *AMT*, *ammonium transporter*; *CLC1*, *nitrate transporter*; *NRT*, *nitrate transporter*; *GDC*, *glycine dehydrogenase*; *GGAT*, *glutamate:glyoxylate aminotransferase*; *GOX*, *glycolate oxidase*; *GS*, *glutamine synthetase*; *NADH- or Fd-GOGAT*, *NADH- or Ferredoxin-dependent glutamate synthase*; *NiR*, *nitrite reductase*; *NR*, *nitrate reductase*; *PGK*, *phosphoglycerate kinase*; *PGLP*, *phosphoglycolate phosphatase*; *PLGG*, *plastidic glycolate/glycerate translocator*; *PRK*, *phosphoribulokinase*; *RPI*, *ribose-5-phosphate isomerase*; *RuBisCo*, *ribulose-1,5-bisphosphate carboxylase/oxygenase*; *SGAT*, *serine:glyoxylate aminotransferase*; *SHMT*, *serine hydroxymethyltransferase*.

tosynthetic genes involved in light-dependent reactions and the Calvin cycle (Tables S3 and S7). Brown spot infection in rice leaves is characterized by the occurrence of large necrotic lesions surrounded by a chlorotic halo (Ahn *et al*., 2005; Dallagnol *et al*., 2011b). Depending on the aggressiveness of the pathogen strain and the intrinsic level of resistance of the rice cultivar used, disease symptoms start to appear from 18 hpi onwards. Interestingly, we found *C. miyabeanus* to down-regulate rice photosynthetic genes at 12 hpi, a time point at which disease lesions are not yet visible. Rather than being the consequence of symptom development, such photosynthetic impairment may thus be causally involved in the *C. miyabeanus* infection process.

Interestingly, photosynthetic inhibition could promote fungal pathogenesis in different ways. First, inhibition of photosynthesis could deprive invaded rice leaves from the ATP necessary to fuel energy-demanding defence mechanisms. Furthermore, delinking light capture and the Calvin cycle results in an over-reduction of electron transfer and oxidative damage to chloroplast membranes, which is well known to promote susceptibility to necrotrophic pathogens (Kangasjärvi *et al*., 2012). Finally, a decrease in photosynthesis may also induce metabolic changes that cause enhanced nutrient leakage, thus favouring *C. miyabeanus* pathogenicity. In line with this notion, our findings showed a strong up-regulation of cytosolic *glutamine synthetases* (*GS1.3*) and down-regulation of several *nitrate* and *nitrite reductases* in brown spot-infected leaves, which is suggestive of senescence-linked nitrogen remobilization (Masclaux *et al*., 2001; Pageau *et al*., 2006). Moreover, in keeping with our transcriptional data, a recent proteomics study has shown that *C. miyabeanus* also induces various enzymes involved in amino acid biosynthesis (Kim *et al*., 2014). Therefore, it is tempting to speculate that *C. miyabeanus* disrupts the plant's photosynthetic apparatus to induce host senescence and nitrogen remobilization, thereby securing access to nutrients throughout the course of infection.

Interestingly, brown spot infection also seemed to attenuate photorespiration, as evidenced by the down-regulation of several photorespiratory marker genes, including several orthologues of *ribulose-1,5-bisphosphate carboxylase/oxygenase* (*RuBisCo*). Although RuBisCo plays an essential role in carboxylation during photosynthesis, transient alterations in the expression of *RuBisCo* genes are often attributed to photorespiration during which RuBisCo fixes oxygen rather than carbon dioxide (Lakshmanan *et al*., 2013; Wang *et al*., 2012). Previously, various groups have shown that victorin, a toxin produced by *Cochliobolus victoriae*, induces susceptibility in oat plants by inhibiting the photorespiratory enzyme glycine decarboxylase (Navarre and Wolpert, 1995, 1999; Tada *et al*., 2005). Moreover, we have demonstrated previously that *C. miyabeanus* is able to produce ET *in planta*, resulting in stomatal opening and reduced photorespiration activity (J. Van Bockhaven, unpublished data). Although further proof is clearly needed, these findings raise the

possibility that, in addition to reducing photosynthesis rates, *C. miyabeanus* may also interfere with photorespiratory processes in order to cause disease.

Impact of Si on central metabolism of infected leaves (Si,I vs. Ctrl,I)

One of the most peculiar findings in this study was the observation that Si application does not result in a major activation of plant defence responses, such as the phenylpropanoid pathway or SA-, JA- and ET-dependent defences. Together with the apparent down-regulation of mitochondrial ATP synthesis in Si-treated plants, these observations strongly suggest that Si may not confer brown spot resistance via the activation of classic immune mechanisms, but rather may impinge on the central metabolism of treated rice plants (Figs 5, 6 and 7C). Most conspicuously, Si application seemed to reverse most metabolic processes that are associated with *C. miyabeanus* infection. First, the decreased expression of the ammonium transporters *AMT2.2* and *AMT3.3* and the up-regulation of several nitrate and nitrite reductases, as well as the nitrate transporter genes *NRT1.1C*, *NRT1.4* and *CLC1*, suggest that Si counteracts brown spot-induced nitrogen remobilization. In addition, Si treatment protected infected rice leaves from *C. miyabeanus*-mediated down-regulation of photosynthetic genes. Safeguarding the photosynthesis machinery appears to be a common aspect of Si-induced broad-spectrum stress tolerance, as many reports have shown unanimously that Si protects and promotes photosynthesis during stress situations (Chen *et al*., 2011; Dallagnol *et al*., 2013a; Nwugo and Huerta, 2008, 2011; Perez *et al*., 2014).

Although the underlying mechanisms remain elusive, the Si-induced up-regulation of several photorespiratory marker genes, including *glutamate:glyoxylate aminotransferase* (*GGAT*), *phosphoglycolate phosphatase* (*PGLP*) and *plastidic glycolate/glycerate translocator* (*PLGG*), suggests that increased photorespiration rates may be one of the driving forces behind the protective role of Si on photosynthesis. Often considered to be a wasteful process, evidence is accumulating that photorespiration also prevents the over-reduction of photosynthetic electron transport and subsequent damage to the photosynthetic apparatus during abiotic stress (Bauwe *et al*., 2012; Cai *et al*., 2011; Cheng *et al*., 2007; Hoshida *et al*., 2000; Rivero *et al*., 2009; Wang *et al*., 2012). In addition, photorespiration is increasingly being implicated in pathogen defence, an effect which is mostly linked to the photorespiration-induced production of reactive oxygen species (ROS) and associated hypersensitive response (HR)-like cell death (Kangasjärvi *et al*., 2012; Rojas *et al*., 2014; Sørhagen *et al*., 2013; Wingler *et al*., 2000). However, considering that ROS formation and HR are largely ineffective against *C. miyabeanus* (Ahn *et al*., 2005), the potential role of photorespiration in Si-induced resistance against *C. miyabeanus* is probably caused by safeguarding of the photosynthetic apparatus.

Intriguingly, increased photorespiration rates may also provide a mechanistic framework for the increased nitrate reduction and chloroplastic ammonium transport and re-assimilation seen in Si-treated plants (Fig. 5; Bloom *et al*., 2010; Foyer *et al*., 2009; Naik, 2006; Rachmilevitch *et al*., 2004). Photorespiration is a complex and energy-consuming interorganellar process that is usually initiated by high temperatures and stomatal closure resulting in low CO_2 and/or high O_2 levels *in planta* (Bauwe *et al*., 2012; Foyer *et al*., 2009). In C3 plants, such as rice, these conditions give rise to the addition of oxygen rather than CO_2 to ribulose-1,5-bisphosphate by RuBisCo. The resulting glycolate is converted in the peroxisomes to glycine, which is then catalysed in the mitochondria with the resultant release of CO_2 and NH_3. Photorespiratory CO_2 feeds back into the Calvin cycle, whereas excess NH_3 is re-assimilated in the cellular nitrogen cycle (Linka and Weber, 2005; Nunes-Nesi *et al*., 2010; Wingler *et al*., 2000). Interestingly, application of Si not only up-regulated the ammonium transporter *AMT1.3* (Foyer *et al*., 2009; Li *et al*., 2012; Tanaka *et al*., 2004), but also chloroplastic *glutamine synthetase 2* (*GS2*) and *Ferredoxin-dependent glutamate synthase* (*Fd-GOGAT*). Both enzymes play an essential role in nitrogen metabolism by catalysing the conversion of ammonium and glutamate to form glutamine (Lam *et al*., 1996; Lea and Miflin, 2010). In the light of the above-mentioned induction of photorespiratory genes, these data may be interpreted to suggest that the Si-induced increase in nitrate reduction and photorespiratory ammonium transport results in enhanced ammonium levels in the chloroplast, which are subsequently re-assimilated by GS2 and Fd-GOGAT.

Si-induced broad-spectrum resistance: a case of disarming the enemy?

Over the past few years, various other transcriptomic and proteomic studies have been conducted to explain the protective effects of Si in a number of pathosystems (Chain *et al*., 2009; Fauteux *et al*., 2006; Ghareeb *et al*., 2011; Nwugo and Huerta, 2011; Watanabe *et al*., 2004; Zargar *et al*., 2010). One of the most salient results of these studies is that Si negates many of the transcriptional changes induced by pathogen inoculation. For instance, in one of the first studies aimed at the investigation of the molecular basis of Si-induced plant resistance, Fauteux *et al*. (2006) found that the inoculation of Arabidopsis with the powdery mildew fungus *Erysiphe cichoracearum* resulted in transcriptional reprogramming of an extensive gene set of nearly 4000 genes. Remarkably, comparing control and Si-treated plants, no major changes were found in either the number or expression level of up-regulated genes. In contrast, many of the down-regulated genes were less severely affected when plants were treated with Si. Similar findings were obtained by Chain *et al*. (2009) when studying the effect of Si application on the transcriptome of wheat plants inoculated with the biotrophic pathogen *Blumeria graminis*

f. sp. *tritici*. In this case, Si-treated plants displayed an almost perfect mirror image of the expression changes seen in inoculated control plants. Moreover, contrary to the nearly 900 genes responding to *Blumeria* infection in control leaves, very few genes were regulated by the pathogen in Si-treated wheat plants, indicating that Si almost eradicated the stress imposed by the pathogen (Chain *et al*., 2009). In rice, the study by Brunings *et al*. (2009) and our data on the effect of Si on rice blast and brown spot resistance, respectively, similarly show that the impact of pathogen infection on the plant's transcriptome is diminished by Si treatment. Therefore, rather than creating resistance by directing massive transcriptional reprogramming of defence-related genes, Si appears to nullify the impact of pathogen inoculation on the plant's transcriptome. One interesting extrapolation is that Si may condition plant disease resistance by preventing the exploitation of pathogen virulence factors. Whether such impairment of pathogen virulence is a result of Si boosting the plant perception of and/or response to so-called pathogen-associated molecular patterns or, alternatively, results from the inhibition of the production and/or delivery of specific virulence factors remains to be elucidated.

CONCLUSIONS

In summary, our data have provided novel insights into the myriad cellular responses that enable rice to fend off *C. miyabeanus* attack, and support a model whereby the pathogen suppresses photosynthesis and nitrate reduction in order to trigger premature senescence in infected tissues and induce a state of susceptibility. Si application, however, may confer brown spot resistance, at least in part, by redirecting the plant's central metabolism in a photorespiration-dependent manner. Moreover, in conjunction with other microarray studies on Si-induced disease resistance, our data suggest that, rather than acting as an initiator of induced defence responses, Si may confer disease resistance by interfering with microbial virulence factors.

EXPERIMENTAL PROCEDURES

Plant material and growth conditions

All rice plants (*Oryza sativa* L.) were *japonica* cultivar Nipponbare. Rice seeds were surface sterilized with 70% ethanol for 1 min and 1% sodium hypochlorite solution for 10 min, rinsed three times with sterile distilled water and germinated at 28 °C for 5 days on wet sterile filter paper in Petri dishes sealed with parafilm (≥92% relative humidity). The seedlings were transplanted on vermiculite in half-strength modified Hoagland solution (Hewitt and Smith, 1975). Five days later, the plantlets were transferred to a gnotobiotic hydroponic growing system in full-strength modified Hoagland solution [pH 6.5; 1 mM KNO_3, 0.25 mM $NH_4H_2PO_4$, 0.1 mM NH_4Cl, 0.5 mM $MgSO_4$, 1 mM $Ca(NO_3)_2$, 0.025 mM $FeSO_4$, 0.025 mM ethylenediaminetetraacetic acid (EDTA)-bisodium salt, 0.0003 mM $CuSO_4$, 0.00033 mM $ZnSO_4$, 0.01150 mM H_3BO_3, 0.0035 mM $MnCl_2$, 0.0001 mM $(NH_4)_6Mo_7O_{24}$, 0.01 mM $MnSO_4$]. Silicic acid was prepared from potassium silicate by means of a cation exchanger (Amberlite IR120, Acros organics, Geel, Antwerpen, Belgium) and added to the nutrient solution to a final density of 2 mM. The Hoagland solution was replaced every 7 days. Control and Si-treated rice plants were grown together in a completely randomized design under growth chamber conditions (28 °C; 12 h/12 h light regime; light intensity, 600 μmol/m²/s) for 5 weeks until they reached the seven-leaf stage.

Pathogen inoculation and disease rating

Cochliobolus miyabeanus isolate Cm988 (De Vleesschauwer *et al*., 2010) was routinely grown on potato dextrose agar (PDA, Difco (Becton, Dickinson and Company), Franklin Lakes, NJ, USA) at 28 °C in darkness. To induce sporulation, 7-day-old mycelium was exposed to blue light (12 h photoperiod; Philips TLD 18W/08 and Philips TLD 18W/33, Philips, Amsterdam, Noord-Holland, the Netherlands) for 3 days. Conidia were harvested as described by Thuan *et al*. (2006) and suspended in 0.5% gelatin (type B from bovine skin; Sigma-Aldrich, Diegem, Vlaams-Brabant, Belgium G-6650) to a final density of 1×10^4 conidia/mL. For inoculation, leaves of 5-week-old plants (seven-leaf stage) were misted with conidial suspension (1 mL per plant) using a compressor-powered airbrush gun. Control plants were sprayed evenly with a 0.5% gelatin solution. Inoculated control and Si-treated plants were kept together in a humid and warm infection chamber (28 ± 4 °C, 92% or greater relative humidity) for 18 h to promote fungal penetration, and thereafter transposed to growth chamber conditions (28 °C, 12 h photoperiod) for disease development. Disease severity was assessed at 3 days post-inoculation (dpi) by calculating the diseased leaf area using APS assess 2.0 imaging software (APS, St Paul, MN, USA). Alternatively, disease was scored using a 1–5 disease severity scale following De Vleesschauwer *et al*. (2010): 1, no infection or less than 2% of the leaf area infected with small brown specks of less than 1 mm in diameter; 2, less than 10% of the leaf area infected with brown spot lesions with grey to white centres, about 1–3 mm in diameter; 3, average of about 25% of the leaf area infected with brown spot lesions with grey to white centres, about 1–3 mm in diameter; 4, average of about 50% of the leaf area infected with typical spindle-shaped lesions, 3 mm or longer, with necrotic grey centres and water-soaked or reddish brown margins, with little or no coalescence of lesions; 5, more than 75% of the leaf area infected with coalescing spindle-shaped lesions.

For detached leaf assays, the two youngest fully developed leaves of 5-week-old plants were detached, cut into 7-cm segments, and floated overnight on sterile distilled water to eliminate residual wounding stress. The next day, leaf segments were placed in square Petri dishes lined with moist paper towels and drop inoculated with four 15-μL droplets of suspension (1×10^4 conidia/mL in 0.25% gelatin). After 24 h, the droplets were removed with a laboratory tissue, and resistance was quantified at 72 hpi by measuring the mean lesion area using APS assess 2.0 imaging software (APS). All infection trials were repeated at least three times with similar results.

Plant phenotyping

All plants were phenotyped using a LI-6400XT leaf gas exchange/chlorophyll fluorescence instrument fitted with a 6400-40 Leaf Chamber

Fluorometer (Li-Cor Biosciences, Lincoln, NE, USA). The LI-6400XT cuvette temperature was set to match the temperature in the growth chamber at the start of the measurement. The cuvette CO_2 concentration was maintained at 400 ppm. Dark respiration was measured pre-dawn and in the dark, with the light source of the LI-6400XT disabled. Light-acclimated gas exchange measurements were performed after turning on the light in the growth chamber, and allowing the plants to acclimate for 30 min. During measurements, the light source of the cuvette was maintained at 1500 μmol photosynthetically active radiation (PAR)/m^2/s (0.1:0.9 mix of 460 nm and 640 nm).

For chlorophyll fluorescence measurements, dark-adapted chlorophyll *a* fluorescence [before (F_0) and after (F_m) a 1-s 7000 μmol/m^2/s saturating light flash] was quantified immediately after recording dark respiration. Light-acclimated PSII efficiency ($F_{v'}/F_{m'} = 1 - F_{0'}/F_{m'}$; Valentini *et al*., 1995; Maxwell and Johnson, 2000), effective PSII quantum yield ($\Phi_{PSII} = 1 - F_s/F_{m'}$) and photochemical quenching, which relates $F_{v'}/F_{m'}$ to Φ_{PSII} and is a non-linear measure for the proportion of open PSII centres ($q_P = [F_{m'} - F_s]/[F_{m'} - F_{0'}]$), were calculated from steady-state fluorescence (F_s), maximum fluorescence after a saturating light flash ($F_{m'}$ after 1 s at 7000 μmol/m^2/s) and minimum fluorescence after a far-red pulse ($F_{0'}$ after 3 s at 9 μmol/m^2/s peaking at 740 nm).

Microarray analysis and data processing

Rice plants (cultivar Nipponbare) were grown and infected with *C. miyabeanus* isolate Cm988 as described previously. Samples from mock-infected and brown spot-inoculated plants were taken at 12 hpi. Three independent biological repetitions were included in the analysis, each repetition representing a pool from at least six individual plants. Total RNA was extracted using the spectrum plant total RNA kit (Sigma-Aldrich, Diegem, Vlaams-Brabant, Belgium) and subsequently Turbo DNase treated according to the provided protocol (Ambion (Thermo Fisher Scientific Inc.), Waltham, MA, USA). First-strand cDNA was synthesized from 2 mg of total RNA using Multiscribe reverse transcriptase (Applied Biosystems (Thermo Fisher Scientific Inc.), Waltham, MA, USA) and random primers following the manufacturer's instructions. For microarray analysis, a previously described two-dye method, allowing direct comparison between two samples on the same microarray, was used (Satoh *et al*., 2010). In brief, cyanine 3- or cyanine 5-labelled cRNA samples were synthesized from 850 ng of total RNA using a low-input RNA labelling kit (Agilent Technologies, Santa Clara, CA, USA) and hybridized to commercially available 60-mer 44 K Agilent rice arrays according to the manufacturer's protocols (Agilent Technologies, Santa Clara, CA, USA). A full list of the expressed sequence tags (ESTs) represented on the array can be found at http://ricexpro.dna.affrc.go.jp/rice-44k-microarray.html. Following washing, slide image files were generated using a DNA microarray scanner (G2505B; Agilent Technologies) and signal intensities were extracted and normalized within each array using Feature Extraction version 9.5 (Agilent Technologies). Signal intensities among all arrays were normalized according to the quantile method for standardization (global scaling) using EXPANDER 6 (Shamir *et al*., 2005). All data are available in the Gene Expression Omnibus (NCBI-GEO; http://www.ncbi.nlm.nih.gov/geo) database under the reference GSE55330. Significance analysis was performed using a fixed linear model (LIMMA) implemented in Multiple Experiment Viewer (MeV; Saeed *et al*., 2003). Differentially expressed genes were defined as genes with a false discovery rate of less than 0.01 (Tamura *et al*., 2011) and a four-fold difference in signal ratio. Hierarchical linkage clustering was performed (complete linkage, Euclidian distance) using MeV. GO analysis was performed with MapMan version 3.5.1 (Usadel *et al*., 2009).

Validation of microarray results

Real-time qRT-PCR analysis was performed on 15 genes that were differentially regulated on Si feeding. All PCR amplifications were conducted in optical 96-well plates with the Mx3005P real-time PCR detection system (Stratagene (Agilent Technologies), Santa Clara, CA, USA), using Sybr Green master mix (Fermentas (Thermo Fisher Scientific Inc.), Waltham, MA, USA) to monitor double-stranded DNA synthesis. The expression of each gene was assayed in duplicate in a total volume of 25 μL including a passive reference dye (ROX) according to the manufacturer's instructions (Fermentas (Thermo Fisher Scientific Inc.), Waltham, MA, USA). The thermal profile used consisted of an initial denaturation step at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 57 °C for 30 s and 72 °C for 30 s. To verify the amplification of one specific target cDNA, a melting curve analysis was included according to the thermal profile suggested by the manufacturer (Stratagene (Agilent Technologies), Santa Clara, CA, USA). The amount of plant RNA in each sample was normalized using elongation factor eIFα (LOC_Os03g08020) as internal control. All primer sequences are listed in Table S8 (see Supporting Information).

ACKNOWLEDGEMENTS

This work was supported by a specialization fellowship of the Flemish Institute for the stimulation of Scientific–Technological Research in Industry (IWT, Belgium) given to JVB, grants from the Special Research Fund of Ghent University (GOA 01GB3013), the Research Foundation Flanders (FWO G.0833.12N) and a FWO postdoctoral fellowship to DDV.

REFERENCES

Agarie, S., Agata, W., Kubota, F. and Kaufman, P.B. (1992) Physiological roles of silicon in photosynthesis and dry matter production in rice plants. *Jpn. J. Crop Sci.* **64**, 200–206.

Ahn, I.P., Kim, S., Kang, S., Suh, S.C. and Lee, Y.H. (2005) Rice defense mechanisms against *Cochliobolus miyabeanus* and *Magnaporthe grisea* are distinct. *Phytopathology*, **95**, 1248–1255.

Bauwe, H., Hagemann, M., Kern, R. and Timm, S. (2012) Photorespiration has a dual origin and manifold links to central metabolism. *Curr. Opin. Plant Biol.* **15**, 269–275.

Berger, S., Sinha, A.K. and Roitsch, T. (2007) Plant physiology meets phytopathology: plant primary metabolism and plant–pathogen interactions. *J. Exp. Bot.* **58**, 4019–4026.

Bloom, A.J., Burger, M., Rubio Asensio, J.S. and Cousins, A.B. (2010) Carbon dioxide enrichment inhibits nitrate assimilation in wheat and Arabidopsis. *Science*, **328**, 899–903.

Bolton, M.D. (2009) Primary metabolism and plant defense: fuel for the fire. *Mol. Plant–Microbe Interact.* **22**, 487–497.

Brunings, A., Datnoff, L.E., Ma, J.F., Mitani, N., Nagamura, Y., Rathinasabapathi, B. and Kirst, M. (2009) Differential gene expression of rice in response to silicon and rice blast fungus *Magnaporthe oryzae*. *Annu. Appl. Biol.* **155**, 161–170.

Cai, Y., Cao, F., Wei, K., Zhang, G. and Wu, F. (2011) Genotypic dependent effect of exogenous glutathione on Cd-induced changes in proteins, ultrastructure and antioxidant defense enzymes in rice seedlings. *J. Hazard. Mater.* **192**, 1056–1066.

Castell-Miller, C.V. and Samac, D.A. (2012) Population genetic structure, gene flow and recombination of *Cochliobolus miyabeanus* on cultivated wildrice (*Zizania palustris*). *Plant Pathol.* **61**, 903–914.

Chain, F., Côté-Beaulieu, C., Belzile, F., Menzies, J.G. and Bélanger, R.R. (2009) A comprehensive transcriptomic analysis of the effect of silicon on wheat plants under control and pathogen stress conditions. *Mol. Plant–Microbe Interact.* **22**, 1323–1330.

Chen, W., Yao, X., Cai, K. and Chen, J. (2011) Silicon alleviates drought stress of rice plants by improving plant water status, photosynthesis and mineral nutrient absorption. *Biol. Trace Elem. Res.* **142**, 67–76.

Cheng, C., Yun, K.Y., Ressom, H.W., Mohanty, B., Bajic, V.B., Jia, Y., Yun, S.J. and Reyes, B.G. (2007) An early response regulatory cluster induced by low temperature and hydrogen peroxide in seedlings of chilling-tolerant *japonica* rice. *BMC Genomics*, **8**, 175.

Dallagnol, L.J., Rodrigues, F.A., Mielli, M.V.B., Ma, J.F. and Datnoff, L.E. (2009) Defective active silicon uptake affects some components of rice resistance to brown spot. *Phytopathology*, **99**, 116–121.

Dallagnol, L.J., Rodrigues, F.A., DaMatta, F.M., Mielli, M.V.B. and Pereira, S.C. (2011a) Deficiency in silicon uptake affects cytological, physiological, and biochemical events in the rice–*Bipolaris oryzae* interaction. *Phytopathology*, **101**, 92–104.

Dallagnol, L.J., Rodrigues, F.A., Martins, S.C.V., Cavatte, P.C. and DaMatta, F.M. (2011b) Alterations in rice leaf physiology during infection by *Bipolaris oryzae*. *Australas. Plant Pathol.* **40**, 360–365.

Dallagnol, L.J., Rodrigues, F.A., Chaves, A.R.M., Vale, F.X.R. and DaMatta, F.M. (2013a) Photosynthesis and sugar concentration are impaired by the defective active silicon uptake in rice plants infected with *Bipolaris oryzae*. *Plant Pathol.* **62**, 120–129.

Dallagnol, L.J., Rodrigues, F.A. and Mielli, M.V.B. (2013b) Silicon improves the emergence and sanity of rice seedlings obtained from seeds infected with *Bipolaris oryzae*. *Trop. Plant Pathol.* **38**, 478–484.

De Angeli, A., Monachello, D., Ephritikhine, G., Frachisse, J.M., Thomine, S., Gambale, F. and Barbier-Brygoo, H. (2006) The nitrate/proton antiporter AtCLCa mediates nitrate accumulation in plant vacuoles. *Nature*, **442**, 939–942.

Dela Paz, M.A.G., Goodwin, P.H., Raymundo, A.K., Ardales, E.Y. and Cruz, C.M.V. (2006) Phylogenetic analysis based on *ITS* sequences and conditions affecting the type of conidial germination of *Bipolaris oryzae*. *Plant Pathol.* **55**, 756–765.

De Vleesschauwer, D., Yang, Y., Cruz, C.V. and Höfte, M. (2010) Abscisic acid-induced resistance against the brown spot pathogen *Cochliobolus miyabeanus* in rice involves MAP kinase-mediated repression of ethylene signaling. *Plant Physiol.* **152**, 2036–2052.

Epstein, E. (2009) Silicon: its manifold roles in plants. *Ann. Appl. Biol.* **155**, 155–160.

Epstein, E. and Bloom, A.J. (2005) Mineral nutrition of plants: principles and perspectives, Sunderland: Sinauer Associates.

Farooq, M.A., Ali, S., Hameed, A., Ishaque, W., Mahmood, K. and Iqbal, Z. (2013) Alleviation of cadmium toxicity by silicon is related to elevated photosynthesis, antioxidant enzymes; suppressed cadmium uptake and oxidative stress in cotton. *Ecotoxicol. Environ. Saf.* **96**, 242–249.

Fauteux, F., Rémus-Borel, W., Menzies, J.G. and Bélanger, R.R. (2005) Silicon and plant disease resistance against pathogenic fungi. *FEMS Microbiol. Lett.* **249**, 1–6. doi: 10.1016/j.femsle.2005.06.034.

Fauteux, F., Chain, F., Belzile, F., Menzies, J.G. and Bélanger, R.R. (2006) The protective role of silicon in the Arabidopsis-powdery mildew pathosystem. *Proc. Natl. Acad. Sci. USA*, **103**, 17 554–17 559.

Fleck, A.T., Nye, T., Repenning, C., Stahl, F., Zahn, M. and Schenk, M.K. (2011) Silicon enhances suberization and lignification in roots of rice (*Oryza sativa*). *J. Exp. Bot.* **62**, 2001–2011.

Foyer, C.H., Bloom, A.J., Queval, G. and Noctor, G. (2009) Photorespiratory metabolism: genes, mutants, energetics, and redox signaling. *Annu. Rev. Plant Biol.* **60**, 455–484.

Gaur, V.S., Singh, U.S., Gupta, A.K. and Kumar, A. (2012) Understanding the differential nitrogen sensing mechanism in rice genotypes through expression analysis of high and low affinity ammonium transporter genes. *Mol. Biol. Rep.* **39**, 2233–2241.

Geelen, D., Lurin, C., Bouchez, D., Frachisse, J.M., Lelièvre, F., Courtial, B., Barbier-Brygoo, H. and Maurel, C. (2000) Disruption of putative anion channel gene AtCLCa in Arabidopsis suggests a role in the regulation of nitrate content. *Plant J.* **21**, 259–267.

Ghareeb, H., Bozsó, Z., Ott, P.G., Repenning, C., Stahl, F. and Wydra, K. (2011) Transcriptome of silicon-induced resistance against *Ralstonia solanacearum* in the silicon non-accumulator tomato implicates priming effect. *Physiol. Mol. Plant Pathol.* **75**, 83–89.

Glazebrook, J. (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu. Rev. Phytopathol.* **43**, 205–227.

Gojon, A., Krouk, G., Perrine-Walker, F. and Laugier, E. (2011) Nitrate transceptor(s) in plants. *J. Exp. Bot.* **62**, 2299–2308.

Govrin, E.M. and Levine, A. (2000) The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea*. *Curr. Biol.* **10**, 751–757.

Guntzer, F., Keller, C. and Meunier, J.-D. (2011) Benefits of plant silicon for crops: a review. *Agron. Sustain. Dev.* **32**, 201–213.

Hewitt, E.J. and Smith, T.A. (1975) Plant mineral nutrition. In: *Experimental Methods for the Investigation of Plant Nutrient Requirements* (Hewitt, E.J. and Smith, T.A., eds), pp. 176–222. London: English Universities Press Ltd.

Hoshida, H., Tanaka, Y., Hibino, T., Hayashi, Y., Tanaka, A. and Takabe, T. (2000) Enhanced tolerance to salt stress in transgenic rice that overexpresses chloroplast glutamine synthetase. *Plant Mol. Biol.* **43**, 103–111.

Kabbage, M., Williams, B. and Dickman, M.B. (2013) Cell death control: the interplay of apoptosis and autophagy in the pathogenicity of Sclerotinia sclerotiorum. *PLoS Path.* **9**, e1003287.

Kangasjärvi, S., Neukermans, J., Li, S., Aro, E.M. and Noctor, G. (2012) Photosynthesis, photorespiration, and light signalling in defence responses. *J. Exp. Bot.* **63**, 1619–1636.

Kim, J.Y., Wu, J., Kwon, S.J., Oh, H., Lee, S.E., Kim, S.G., Wang, Y., Agrawal, G.K., Rakwal, R., Kang, K.Y., Ahn, I.P., Kim, B.G. and Kim, S.T. (2014) Proteomics of rice and Cochliobolus miyabeanus fungal interaction: Insight into proteins at intracellular and extracellular spaces. *Prot.* **14**, 2307–2318.

Lakshmanan, M., Mohanty, B. and Lee, D.Y. (2013) Identifying essential genes/reactions of the rice photorespiration by *in silico* model-based analysis. *Rice (N.Y.)*, **6**, 1–5. doi: 10.1186/1939-8433-6-20.

Lam, H.M., Coschigano, K.T., Oliveira, I.C., Melo-Oliveira, R. and Coruzzi, G.M. (1996) The molecular genetics of nitrogen assimilation into amino acids in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 569–593.

Lea, P.J. and Miflin, B.J. (2010) Nitrogen assimilation and its relevance to crop improvement. In *Annual Plant Reviews Volume 42: Nitrogen Metabolism in Plants in the Post-Genomic Era* (Foyer, C.H. and Zhang, H., eds), doi: 10.1002/9781444328608.ch1. Oxford: Wiley-Blackwell.

Leung, H., Zhu, Y., Revilla-Molina, I., Fan, J.X., Chen, H., Pangga, I., Cruz, C.V. and Mew, T.W. (2003) Using genetic diversity to achieve sustainable rice disease management. *Plant Dis.* **87**, 1156–1169.

Levine, B. (2007) Cell biology: autophagy and cancer. *Nature*, **446**, 745–747.

Li, S., Li, B. and Shi, W. (2012) Expression patterns of nine ammonium transporters in rice in response to N status. *Pedosph.* **22**, 860–869.

Linka, M. and Weber, A.P.M. (2005) Shuffling ammonia between mitochondria and plastids during photorespiration. *Trends Plant Sci.* **10**, 461–465.

Ma, J.F. and Yamaji, N. (2006) Silicon uptake and accumulation in higher plants. *Trends Plant Sci.* **11**, 392–397.

Ma, J.F., Yamaji, N. and Mitani-Ueno, N. (2011) Transport of silicon from roots to panicles in plants. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* **87**, 377–385.

Martinoia, E., Maeshima, M. and Neuhaus, H.E. (2007) Vacuolar transporters and their essential role in plant metabolism. *J. Exp. Bot.* **58**, 83–102.

Masclaux, B.C., Quilleré, I., Gallais, A. and Hirel, B. (2001) The challenge of remobilisation in plant nitrogen economy. A survey of physio-agronomic and molecular approaches. *Ann. Appl. Biol.* **138**, 69–81.

Maxwell, K. and Johnson, G.N. (2000) Chlorophyll fluorescence: a practical guide. *J. Exp. Bot.* **51**, 659–668.

Mur, L.A.J., Simpson, C., Gay, A., Smith, J.A., Paveley, N., Sánchez-Martin, J. and Prats, E. (2013) Stomatal lock-up following pathogenic challenge: source or symptom of costs of resistance in crops? *Plant Pathol.* **62**, 72–82.

Naik, R.M. (2006) Dependence of in vivo nitrate reduction on photorespiration and mitochondrial respiration in leaves of *Nicotiana sylvestris*. *J. Plant Biochem. Biotechnol.* **15**, 59–61.

Navarre, D.A. and Wolpert, T.J. (1995) Inhibition of the glycine decarboxylase multienzyme complex by the host-selective toxin victorin. *Plant Cell*, **7**, 463–471.

Navarre, D.A. and Wolpert, T.J. (1999) Victorin induction of an apoptotic/senescence-like response in oats. *Plant Cell*, **11**, 237–249.

Nunes-Nesi, A., Fernie, A.R. and Stitt, M. (2010) Metabolic and signaling aspects underpinning the regulation of plant carbon nitrogen interactions. *Mol. Plant.* **3**, 973–996.

Nwugo, C.C. and Huerta, A.J. (2008) Silicon-induced cadmium resistance in rice (*Oryza sativa*). *J. Plant Nutr. Soil Sci.* **171**, 841–848.

Nwugo, C.C. and Huerta, A.J. (2011) The effect of silicon on the leaf proteome of rice (*Oryza sativa* L.) plants under cadmium-stress. *J. Proteome Res.* **10**, 518–528.

Oliveros, J. (2007) VENNY. An interactive tool for comparing lists with Venn diagrams. *BioinfoGP, CNB-CSIC*. Available at http://bioinfogp.cnb.csic.es/tools/venny/index.html

Ou, S.H. (1985) *Rice Diseases*. Kew: Commonwealth Mycological Institute.

Pageau, K., Reisdorf-Cren, M., Morot-Gaudry, J.F. and Masclaux-Daubresse, C. (2006) The two senescence-related markers, *GS1* (cytosolic glutamine synthetase) and *GDH* (glutamate dehydrogenase), involved in nitrogen mobilization, are differentially regulated during pathogen attack and by stress hormones and reactive oxygen species in *Nicotiana tabacum* ana tabacum L. leaves. *J. Exp. Bot.* **57**, 547–557.

Perez, C.E.A., Rodrigues, F., Moreira, W.R. and Damatta, F.M. (2014) Leaf gas exchange and chlorophyll a fluorescence in wheat plants supplied with silicon and infected with *Pyricularia oryzae*. *Phytopathology*, **104**, 143–149.

Rachmilevitch, S., Cousins, A.B. and Bloom, A.J. (2004) Nitrate assimilation in plant shoots depends on photorespiration. *Proc. Natl. Acad. Sci. USA*, **101**, 11 506–11 510.

Resende, R.S., Rodrigues, F., Cavatte, P.C., Martins, S.C.V., Moreira, W.R., Chaves, A.R.M. and Damatta, F.M. (2012) Leaf gas exchange and oxidative stress in sorghum plants supplied with silicon and infected by *Colletotrichum sublineolum*. *Phytopathology*, **102**, 892–898.

Rezende, D.C., Rodrigues, F.Á., Carré-Missio, V., Schurt, D.A., Kawamura, I.K. and Korndörfer, G.H. (2009) Effect of root and foliar applications of silicon on brown spot development in rice. *Australas. Plant Pathol.* **38**, 67–73.

Rivero, R.M., Shulaev, V. and Blumwald, E. (2009) Cytokinin-dependent photorespiration and the protection of photosynthesis during water deficit. *Plant Physiol.* **150**, 1530–1540.

Rojas, C.M., Senthil-Kumar, M., Tzin, V. and Mysore, K.S. (2014) Regulation of primary plant metabolism during plant–pathogen interactions and its contribution to plant defense. *Front. Plant Sci.* **5**, 1–7. doi: 10.3389/fpls.2014.00017.

Rontein, D., Dieuaide-Noubhani, M., Dufourc, E.J., Raymond, P. and Rolin, D. (2002) The metabolic architecture of plant cells. Stability of central metabolism and flexibility of anabolic pathways during the growth cycle of tomato cells. *J. Biol. Chem.* **277**, 43 948–43 960.

Saeed, A.I., Sharov, V., White, J., Li, J., Liang, W., Bhagabati, N., Braisted, J., Klapa, M., Currier, T., Thiagarajan, M., Sturn, A., Snuffin, M., Rezantsev, A., Popov, D., Ryltsov, A., Kostukovich, E., Borisovsky, I., Liu, Z., Vinsavich, A., Trush, V. and Quackenbush, J. (2003) TM4: a free, open-source system for microarray data management and analysis. *Biotechniques*, **34**, 374–378.

Satoh, K., Kondoh, H., Sasaya, T., Shimizu, T., Choi, I.R., Omura, T. and Kikuchi, S. (2010) Selective modification of rice (*Oryza sativa*) gene expression by rice stripe virus infection. *J. Gen. Virol.* **91**, 294–305.

Seifi, H.S., Van Bockhaven, J., Angenon, G. and Höfte, M. (2013) Glutamate metabolism in plant disease and defense: friend or foe? *Mol. Plant–Microbe. Interact.* **26**, 475–485.

Shamir, R., Maron-Katz, A., Tanay, A., Linhart, C., Steinfeld, I., Sharan, R., Shiloh, Y. and Elkon, R. (2005) EXPANDER–an integrative program suite for microarray data analysis. *BMC Bioinformatics*, **6**, 232.

Shen, X., Zhou, Y., Duan, L., Li, Z., Eneji, A.E. and Li, J. (2010) Silicon effects on photosynthesis and antioxidant parameters of soybean seedlings under drought and ultraviolet-B radiation. *J. Plant Physiol.* **167**, 1248–1252.

Silva, M.R.J., Pereira, S.C., Rodrigues, F.A., Antônio, L., Júnior, Z., Fontes, L.F. and Oliveira, M.G.A. (2012) Silicon and manganese on the activity of enzymes involved in rice resistance against brown spot. *Trop. Plant Pathol.* **37**, 339–345.

Sørhagen, K., Laxa, M., Peterhänsel, C. and Reumann, S. (2013) The emerging role of photorespiration and non-photorespiratory peroxisomal metabolism in pathogen defence. *Plant Biol. (Stuttg).* **15**, 723–736.

Suenaga, A., Moriya, K., Sonoda, Y., Ikeda, A., Wirén, N.V., Yamaguchi, J. and Yamaya, T. (2003) Constitutive expression of a novel-type ammonium transporter OsAMT2 in rice plants. *Plant Cell Physiol.* **44**, 206–211.

Tada, Y., Kusaka, K., Betsuyaku, S., Shinogi, T., Sakamoto, M., Ohura, Y., Hata, S., Mori, T., Tosa, Y. and Mayama, S. (2005) Victorin triggers programmed cell death and the defense response via interaction with a cell surface mediator. *Plant Cell Physiol.* **46**, 1787–1798.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**, 2731–2739.

Tanaka, N., Fujita, M., Handa, H., Murayama, S., Uemura, M., Kawamura, Y., Mitsui, T., Mikami, S., Tozawa, Y., Yoshinaga, T. and Komatsu, S. (2004) Proteomics of the rice cell?: systematic identification of the protein populations in subcellular compartments. *Mol. Genet. Genomics*, **271**, 566–576.

Thuan, N.T.N., Bigirimana, J., Roumen, E., Straeten, D. and Höfte, M. (2006) Molecular and pathotype analysis of the rice blast fungus in North Vietnam. *Eur. J. Plant Pathol.* **114**, 381–396.

Usadel, B., Poree, F., Nagel, A., Lohse, M., Czedik-Eysenberg, A. and Stitt, M. (2009) A guide to using MapMan to visualize and compare omics data in plants: a case study in the crop species, maize. *Plant Cell Environ.* **32**, 1211–1229.

Valentini, R., Epron, D., Angelis, P., Matteucci, G. and Dreyer, E. (1995) In situ estimation of net CO_2 assimilation, photosynthetic electron flow and photorespiration in Turkey oak (*Q. cerris* L.) leaves: diurnal cycles under different levels of water supply. *Plant, Cell Environ.* **18**, 631–640.

Van Bockhaven, J., De Vleesschauwer, D. and Höfte, M. (2013) Towards establishing broad-spectrum disease resistance in plants: silicon leads the way. *J. Exp. Bot.* **64**, 1281–1293.

Van Bockhaven, J., Spichal, L., Novak, O., Strnad, M., Asano, T., Kikuchi, S., Höfte, M. and De Vleesschauwer, D. (2015) Silicon induces resistance to the brown spot fungus *Cochliobolus miyabeanus* by preventing the pathogen from hijacking the rice ethylene pathway. *New Phytol.* In press. doi: 10.1111/nph.13270.

Wang, H., Zhang, M., Guo, R., Shi, D., Liu, B., Lin, X. and Yang, C. (2012) Effects of salt stress on ion balance and nitrogen metabolism of old and young leaves in rice (*Oryza sativa* L.). *BMC Plant Biol.* **12**, 1–11. doi: 10.1186/1471-2229-12-194.

Watanabe, S., Shimoid, E., Ohkamab, N., Hayashid, H., Yoneyamad, T., Yazakie, Y., Fujiie, F., Shinboe, K., Yamamotoe, K., Sakatae, K., Sasakie, T., Kishimotoe, N., Kikuchi, S. and Fujiwara, T. (2004) Identification of several rice genes regulated by Si nutrition. *Soil Sci. Plant Nutr.* **50**, 1273–1276.

Williams, B., Kabbage, M., Kim, H.J., Britt, R. and Dickman, M.B. (2011) Tipping the balance: Sclerotinia sclerotiorum secreted oxalic acid suppresses host defenses by manipulating the host redox environment. *PLoS Pathog.* **7**, e1002107.

Wingler, A., Lea, P.J., Quick, W.P. and Leegood, R.C. (2000) Photorespiration: metabolic pathways and their role in stress protection. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **355**, 1517–1529.

Xiao, J.Z., Tsuda, M., Doke, N. and Nishimura, S. (1991) Phytotoxins produced by germinating spores of *Bipolaris oryzae*. *Phytopathology*, **81**, 58–64.

Zadoks, J.C. (2003) Fifty years of crop protection, 1950–2000. *NIAS Wageningen J. Life Sci.* **50**, 181–193.

Zargar, S.M., Nazir, M., Agrawal, G.K., Kim, D.W. and Rakwal, R. (2010) Silicon in plant tolerance against environmental stressors: towards crop improvement using omics approaches. *Curr. Proteomics*, **7**, 135–143.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig. S1 Correlation of microarray (*x* axis) and real-time reverse transcription-polymerase chain reaction (RT-PCR) data (*y* axis) for a subset of 15 differentially expressed genes.

Table S1 Overview of physiological parameters analysed during leaf gas exchange measurements of control and silicon-treated plants.

Table S2 List of differentially expressed genes in non-inoculated silicon-treated plants (Si,m vs. Ctrl,m).

Table S3 List of differentially expressed genes in *Cochliobolus miyabeanus*-infected control plants (Ctrl,I vs. Ctrl,m).

Table S4 List of differentially expressed genes in *Cochliobolus miyabeanus*-infected and silicon-treated plants vs. infected control plants (Si,I vs. Ctrl,I).

Table S5 List of differentially expressed genes in silicon-treated plants following *Cochliobolus miyabeanus* infection (Si,I vs. Si,m).

Table S6 Validation of microarray data by quantitative reverse transcription-polymerase chain reaction (qRT-PCR).

Table S7 Central metabolism-associated genes differentially expressed after silicon (Si) application and/or *Cochliobolus miyabeanus* infection.

Table S8 Primers used for microarray validation.