KELLY DA SILVA COUTINHO DETMANN

ASPECTOS FISIOLÓGICOS E DA PRODUÇÃO DO ARROZ EM RESPOSTA AO SILÍCIO

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fisiologia Vegetal para obtenção do título de *Doctor Scientiae*.

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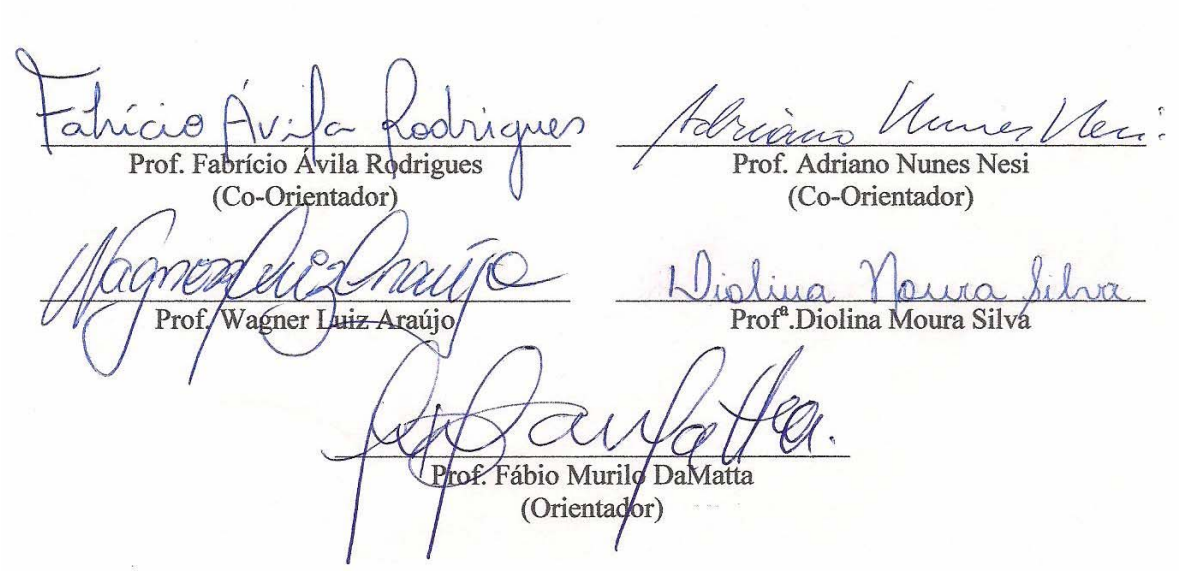

Ao meu amado e querido esposo Edenio Detmann,

Aos meus filhos Helga e Johan Claus

e com carinho a minha amiga Lílian Sanglard e a minha avó *Jovelina*:

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ÍNDICE

RESUMO

DETMANN, Kelly da Silva Coutinho, D.Sc., Universidade Federal de Viçosa, setembro de 2011. **Aspectos fisiológicos e da produção do arroz em resposta ao silício.** Orientador: Fábio Murilo DaMatta. Coorientadores: Adriano Nunes Nesi e Fabrício Ávila Rodrigues.

O presente estudo foi conduzido procurando-se analisar as contribuições da suplementação de silício (Si) no acúmulo de biomassa da planta e na produção de grãos de arroz (*Oryza sativa* L.). Para tal, diferentes experimentos foram conduzidos, submetendo-se plantas de arroz (subsp. *japônica* Oochikara - WT) e seu mutante defectivo para a absorção de Si – *lsi*1 (Low silicon rice 1) a diferentes condições de cultivo hidropônico com diferentes suplementações de Si (0 e 2 mmol L^{-1}). Para tal, no primeiro experimento, procedeu-se a análises alométricas e de crescimento, com o objetivo de avaliar o padrão de alocação de biomassa nas diferentes fases do ciclo do arroz, sob diferentes disponibilidades de Si. No presente trabalho, foi evidenciado que independentemente do genótipo, o Si não alterou a alocação de biomassa nas fases iniciais do ciclo fenológico, mas aumentou a produtividade de grãos em ambos os genótipos (34 % WT e 24 % *lsi*1). No segundo experimento, plantas foram cultivadas com e sem suplantação de Si durante a fase vegetativa, quando, então, no início da fase reprodutiva, metade das plantas em cada tratamento inicial (-Si e +Si) teve sua suplementação de Si modificada (-Si/+Si e +Si/-Si), formando quatro condições experimentais até o fim do experimento. Nos resultados, pode-se enfatizar que o Si, no estádio reprodutivo, é mais efetivo para aumentar a produtividade do que no estádio vegetativo. A remoção ou adição de Si na fase reprodutiva de arroz teve efeito significativo no aumento do dreno (número de grãos por panícula), com aumento da taxa fotossintética, mas sem alterações no ângulo foliar. Para investigar, os efeitos do Si sobre o crescimento, a produção e a fotossíntese em diferentes razões área foliar por número de grãos foram combinadas análises de trocas gasosas e fluorescência da clorofila a, medições incorporação de carbono marcado, conseguinte partição em diferentes frações e a análise do perfil metabólico. Para tal, o terceiro experimento consistiu na manipulação do dreno (remoção ou não da panícula) de plantas WT e *lsi* 1, com ou sem adição de Si. A presença deste elemento fez aumentar o número e a massa individual dos grãos. O silício aumentou o número e a massa individual dos grãos, consequentemente, modificou as relações fonte-dreno. Tais modificações no dreno estimularam a atividade fotossintética na folha-fonte, associado fundamentalmente ao aumento da condutância mesofílica por mecanismos ainda

desconhecidos. Além disso, a suplantação de silício alterou o metabolismo primário estimulando a remobilização de aminoácidos.

ABSTRACT

DETMANN, Kelly da Silva Coutinho, D.Sc., Universidade Federal de Viçosa, September 2011. **Physiological and productive aspects of rice plants according to silicon supplying.** Advisor: Fábio Murilo DaMatta. Co-Advisors: Adriano Nunes Nesi and Fabrício Ávila Rodrigues

The objective of this study was to analyze the role of supplemental silicon (Si) in the biomass accumulation and grain yield of rice (*Oryza sativa* L.). Different experiments were carried out in which "wild" rice plants (japonica subsp. Oochikara - WT) and mutant defective for the absorption of Si - lsi1 (Low silicon rice 1) were raised under hydroponics conditions with different Si supplementation (0 or Si 2 mmol Si L^{-1}). In the first experiment, it was performed an allometric analysis to evaluate the pattern of biomass allocation in different phenological stages according to Si supplying. In this study, regardless of genotype, it was shown that Si does not change the allocation of biomass in the first two phenological stages, but it increased grain yield in both genotypes (34% in WT and 24% in lsi1). In the second experiment, plants were allowed to grow with or without supplemental Si during the growing season. After that, when the early reproductive stage was reached, half of plants in each treatment (-Si and +Si) was crossed over with regard Si supplementation totalizing four treatments (-Si/-Si, -Si/+Si, +Si/-Si, and +Si/+Si). The Si was found to be more effective to increase productivity in the reproductive stage compared to vegetative stage. The addition of Si in the reproductive stage caused a prominent increase in grain production (number of grains per panicle). It was also observed an increase in photosynthetic rate but no changes in leaf angle was caused by Si supplying. Different ratios of grains number to leaf area were established to evaluate the effects of Si on the growth, production and photosynthesis. Such analyses were performed by linking advance gas exchange and chlorophyll *a* fluorescence measurements with carbon isotope labeling and metabolic profiling. Considering this, the third experiment consisted of WT and lsi plants with and without panicle, and raised with or without supplemental Si totalizing four treatments. Addition of Si increased the number and mass of individual grains. As a consequence, it seemed to cause some modifications on the source-sink relations. Such changes in the sink stimulated the photosynthetic activity in the leaf. That effect was primarily associated with an increased mesophilic conductance. However, the actual cause of that alteration still remains to be explained. In addition,

supplemental Si changed the primary metabolism by stimulating the remobilization of amino acids.

INTRODUÇÃO GERAL

O silício (Si) é um metalóide encontrado na tabela periódica no grupo 14 (IV) e terceiro período, o que lhe confere propriedades de doar ou receber elétrons, além da capacidade de fazer ligações químicas com outros elementos de várias formas. No solo, o Si é o segundo elemento mais abundante depois do oxigênio, encontrando-se principalmente na forma de silicatos, em estrutura similar à de um tetraedro, com o átomo do Si no centro e o oxigênio em cada vértice. Os tetraedros têm grande estabilidade porque os elementos envolvidos encontram-se no estado de oxidação mais estável. Na solução do solo, o Si está disponível para as plantas como ácido monosilícico, em concentrações da ordem de 0,1-0,6 mM (Epstein, 1999). Quando absorvido pelas plantas, o Si é depositado na forma amorfa nas paredes celulares, ligado a pectinas e polifenóis (Currie et al., 2007), nas quais as concentrações podem variar de 0,1-15%, dependendo da espécie (Epstein, 1999). Em geral, as monocotiledôneas apresentam altos níveis de Si na parte aérea (10-15%) comparadas com as dicotiledôneas (< 0,5%) (Hodson et al., 2005). Tais diferenças são atribuídas primeiramente aos diferentes transportadores de Si (Ma & Yamaji, 2008).

Os transportadores envolvidos na absorção e translocação de Si nas plantas têm sido identificados em espécies acumuladoras de Si, incluindo o arroz, o milho e a cevada (Ma et al., 2002; 2006). No caso específico do arroz, o transporte do Si na raiz ocorre por transportadores de influxo Lsi 1 (Low silicon rice 1) e efluxo Lsi 2 (Low silicon rice 2). Ambos transportadores são proteínas intrínsecas de membrana da subfamília das aquoporinas, localizando-se na membrana plasmática da exoderme e endoderme de raízes lateral e principal (Ma et al., 2002; 2006; Yamaji & Ma, 2009). Em nível celular, o transportador de influxo do Si tem localização distal, enquanto o de efluxo, localização proximal. Assim, o ácido monosilícico é transportado da solução externa para o interior da célula pelo Lsi1 saindo pelo Lsi2, gerando gradiente de concentração de Si, da solução do solo para o interior da raiz. Em adição, Lsi1 e Lsi2 também podem formar um gradiente de concentração de Si, entre a endoderme e a exoderme, visto que as raízes de arroz são caracterizadas pela formação do aerênquima, acompanhada pela destruição de células do córtex, com exceção da exoderme e endoderme. O Si atravessa a estria de Caspary na

endoderme através do Lsi2 e, então, alcança o estelo. Com efeito, mais de 90% do Si absorvido pelas raízes é translocado para a parte aérea (Ma e Takahashi, 2002). No xilema, o Si é translocado pela via transpiratória, na forma de ácido monosilícico (Mitani et al., 2009). Cumpre ressaltar que ambos transportadores são expressos constitutivamente na raiz, tendo, contudo, sua expressão aumentada durante o estádio de emborrachamento e/ou sob baixa disponibilidade de Si (Ma et al., 2002; 2007).

O ácido monosilícico, na parte aérea, é depositado, principalmente, em camadas de Si-cutícula, onde se polimeriza na forma de sílica gel ($SiO_2.nH_2O$) após desidratação (Ma & Takahashi, 2002; Fauteux et al., 2005). Recentemente, a proteína Lsi 6 (Low silicon rice 6) foi identificada como responsável pelo transporte de Si, do xilema para as células do parênquima do xilema, nas folhas de arroz (Yamaji & Ma, 2009). A proteína Lsi 6 apresenta 77% de homologia com a proteína Lsi1, ambas apresentam transporte ativo e movimento de influxo em estudos com oócitos de *Xenopus laevis* (Yamaji & Ma, 2009). Diferentemente do Lsi 1, o gene Lsi 6 é constitutivamente expresso nas folhas, mas pode ser expresso também nas raízes sob baixa disponibilidade de Si (Yamaji et al., 2008).

Evolutivamente, diferentes funções fisiológicas têm sido propostas para o Si nas plantas, dentre as quais se destacam estabilidade mecânica, resistência contra fungos, insetos e herbivoria (Jones & Handreck, 1967; Raven, 1983; Ma, 2004; Epstein & Bloom, 2005). De fato, o Si pode reduzir os efeitos de vários estresses abióticos (e.g., salinidade, toxidez por metal, desbalanço nutricional, déficit hídrico, altas temperaturas e congelamento) devido, principalmente, à sua deposição na parede das células da raiz, do colmo e da folha (Epstein, 1999). Além disso, a barreira física formada pela deposição de Si na parede celular pode, *per se*, aumentar a resistência física ao patógeno ou permitir maior tempo para a defesa do hospedeiro. De maneira complementar, o efeito do Si em diminuir a severidade de doenças, por exemplo, em milho, arroz e pepino, é atribuído não apenas à sua ação como barreira física, mas também pelo aumento da atividade de enzimas de defesa (peroxidase do guaiacol, oxidase do polifenol e fenilalanina amonialiase) (Yang et al., 2003; Liang et al., 2005; Cai et al., 2008). Nesse contexto, plantas de arroz infectadas por *Pyricularia grisea* apresentaram maior produção de duas fitolexinas quando em presença de Si (Rodrigues et al., 2004), enquanto uma classe de fitoalexinas foi identificada apenas no extrato de folhas de pepino inoculadas com *Podosphaeria xanthi* e supridas com

Si (Fawe et al., 2001). Em outro estudo, plantas de arroz tratadas com Si aumentaram e mantiveram a maior expressão de peroxidase em períodos de 12 h até 96 h após a inoculação, o que foi acompanhado pelo acúmulo de lignina conquanto as plantas sem Si apresentaram um pico único de expressão às 36 h após a inoculação (Rodrigues et al., 2005). Dessa forma, observa-se que o Si promove diferentes padrões de expressão das enzimas de defesa em arroz e pepino inoculados com *P. grisea* e *P. xanthi*, respectivamente. Tomados em conjunto, esses resultados sugerem que o Si, na forma solúvel, pode interferir na via de sinalização celular e desempenhar um papel importante como sinal molecular (Fawe et al., 2001).

O uso de técnicas de biologia molecular tem permitido progressos significativos no entendimento dos mecanismos de absorção e translocação de Si na planta (Ma et al., 2002; 2006; 2007; Yamaji & Ma, 2009) e no efeito do Si em diminuir a severidade de doenças (Cai et al., 2008; Ghareeb et al., 2011). No entanto, o mecanismo pelo qual esse elemento afeta a produtividade do arroz ainda continua pouco conhecido. O aumento na produção de biomassa observado nas plantas de arroz supridas com Si deve refletir-se na fotossíntese da planta inteira e/ou por área foliar, visto que esse é o processo pelo qual as plantas transformam energia luminosa em energia química, assimilando o CO_2 e outros compostos na forma de compostos orgânicos. Em dois estudos independentes, Takahashi et al. (1966) e Ma (1990) não encontraram diferenças significativas na razão entre a taxa de assimilação de CO_2 e a área foliar das plantas supridas ou não com Si, durante o estádio vegetativo. Ainda, Agarie (1992; 1998), estudando os efeitos do Si na taxa fotossintética por área de cada folha do colmo principal de arroz, cultivado por 40 dias, apenas identificou diferenças na taxa fotossintética na quarta folha abaixo da folha mais jovem completamente expandida. No entanto, tal diferença desaparecia ao expressar-se a taxa fotossintética por unidade de clorofila.

O aumento na assimilação de CO_2 por planta justificaria a maior produção de grãos (Takahashi et al., 1966) caso houvesse concomitante acréscimo de fotoassimilados no colmo e na bainha da folha durante a fase vegetativa, para posterior remobilização para os grãos. No entanto, isso não foi observado, e plantas supridas com Si apresentaram menores concentrações de sacarose na seiva do floema comparadas com as não supridas (Watanabe et al., 2001). Mesmo desconsiderando-se a ausência de ganho de fotoassimilados nos locais

de reserva, o possível aumento na produção de fotoassimilados poderia também ser utilizado para a formação do corpo vegetativo, geralmente maior nas plantas supridas com Si (Okuda & Takahashi, 1961; Ma et al., 1989). Além disso, plantas de arroz supridas com Si somente no estádio vegetativo apresentaram massa de panícula similar às não supridas durante todo o ciclo (Ma et al., 1989). Por sua vez, as plantas que estavam em presença constante de Si foram similares em número de grãos às que foram suplementadas apenas no estádio reprodutivo. Tomadas em conjunto, esses dados sugerem que o Si só afetaria a produção de grãos quando presente durante a fase reprodutiva.

A deposição de Si nas paredes celulares permite o desenvolvimento de folhas mais eretas, que intensificaria a taxa de fotossíntese da planta inteira, por melhorar a distribuição luminosa ao longo da copa (Epstein, 1999). Visto que o Si é um elemento imóvel, as plantas supridas com esse elemento, somente na fase reprodutiva, não apresentaram folhas mais eretas do que as suplementadas no vegetativo. Assim, tais resultados são contrastantes, ao menos em primeira instância, com a hipótese de que a modificação do ângulo foliar seria o único responsável pelo aumento da produtividade do arroz suprido com Si, conseqüentemente, no incremento da fotossíntese necessário para o enchimento do maior número de grãos nas plantas com Si.

A fotossíntese constitui-se em um processo metabólico altamente integrado e regulado, sendo que a síntese, o carregamento e o descarregamento de produtos finais exercem, em curto espaço de tempo, o controle no metabolismo. A força do dreno (capacidade de aumentar o tamanho ou a atividade de drenos) constitui fator determinante para o aumento da fotossíntese e, consequentemente, para o ganho de biomassa da planta (Paul & Floyer, 2001). Assim, a regulação da fotossíntese pela atividade do dreno é mediada por sinais que coordenam a capacidade fotossintética para o crescimento e estoque de carbono, sendo dependentes da fisiologia da planta inteira (Paul & Foyer, 2001). O tamanho final do dreno, ou a produção de grãos em arroz é definido pelo número total de espiguetas que florescem, ou seja, o número de grãos cheios e não cheios até a maturidade. As mudanças fisiológicas que se iniciam na segunda parte do estádio reprodutivo culminam com o crescimento da panícula (Horie et al., 2003). Essa é a fase central, na qual o número de drenos se torna dreno atuante, que corresponde à força do dreno da planta (Lafarge & Bueno, 2009). Entretanto, planta de arroz suprida ou não com Si durante a floração tem efeito significativo

no número de grãos (Ma et al., 1989). Assim, o Si modificaria a relação fonte-dreno da planta ao promover o enchimento de maior número de grãos. Considerando-se que grande parte do carbono presente no grão de arroz é assimilada nas folhas durante a fase de enchimento dos grãos (Yoshida, 1981; Murchie et al., 1999) e sendo a folha bandeira a de maior atividade fotossintética, o rendimento de grãos é potencialmente afetado por fatores que afetam a taxa fotossintética dessa folha (Dingkuhn et al., 1989). Dessa forma, ao aumentar o número de drenos, durante a fase reprodutiva, o Si alteraria a necessidade de fotoassimilados na fase de enchimento de grãos, a qual deve refletir-se na taxa fotossintética da folha-bandeira.

Atualmente, não existem evidências de que o Si possua papel catalítico no metabolismo do carbono e nitrogênio de plantas superiores. Diante do exposto, percebe-se que pouco, ou nada, é conhecido acerca dos efeitos do Si na fisiologia das plantas que culminam na produção de grãos. Sugere-se, aqui, que, o aumento da biomassa de arroz, promovido pelo Si, pode ser devido à alteração do metabolismo do carbono e da arquitetura da planta. Assim, como o efeito do Si para a produtividade é expressivo na fase reprodutiva (Ma et al., 1989) e esse não exerceria, durante a fase vegetativa, nenhum efeito na fotossíntese da fonte (Takahashi et al., 1966; Ma, 1990; Agarie, 1992; 1998), tem-se por hipótese que o Si interfira na força do dreno por aumentar o seu número durante a fase reprodutiva e, consequentemente, tendo reflexos diretos na taxa de fotossíntese. Tais reflexos residiriam principalmente na fisiologia da folha bandeira, por alterar a exigência de fotoassimilados durante o enchimento de grãos e não à maior capacidade da fonte *per se*. Pretendeu-se, portanto, (i) investigar o crescimento vegetativo, a produção, o metabolismo do carbono e os teores de nutrientes em plantas de arroz, supridas ou não com Si, e; (ii) determinar, em diferentes razões área foliar por número de grãos, os efeitos do Si sobre o crescimento, a produção e a fotossíntese.

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CAPÍTULO I

Efeito do Si aplicado em diferentes épocas do ciclo fenológico sobre o crescimento, a fotossíntese e a produção de arroz (*Oryza sativa* L. cv. 'Oochikara') e seu mutante defectivo para a absorção de Si (*lsi* 1)

Introdução

O arroz (*Oryza sativa* L.) requer grandes quantidades de silício (Si) para seu desenvolvimento adequado e, principalmente, para a manutenção de alta produtividade (Savant et al., 1997; Ma & Takahashi, 2002). O ácido monosilícico é absorvido pelas raízes, translocado para a parte aérea via xilema e depositado, principalmente, em camada de Si-cutícula, onde se polimeriza na forma de sílica gel ($SiO_2.nH_2O$) após desidratação (Ma & Takahashi, 2002; Fauteux et al., 2005). A deposição de Si nas paredes celulares das folhas está associada a diferentes funções fisiológicas nas plantas, como estabilidade mecânica, resistência contra fungos, insetos e herbivoria (Jones & Handreck, 1967; Raven, 1983; Epstein, 1999; Ma, 2004; Epstein & Bloom, 2005).

A capacidade do arroz de acumular Si tem sido atribuída à presença dos transportadores Lsi 1 ("Low silicon rice 1") e Lsi 2 ("Low silicon rice 2") na raiz (Ma et al., 2006; 2008). Lsi 1 e Lsi 2 são proteínas de membrana plasmática das células exodérmicas e endodérmicas da raiz, localizados nos sítios distal e proximal da célula, respectivamente (Ma et al., 2002; 2006; 2007a). Os genes *Lsi* 1 e *Lsi* 2 são expressos constituitivamente na raiz, tendo, contudo, sua expressão aumentada durante o estádio de emborrachamento e/ou sob baixa disponibilidade de Si (Ma et al., 2002; 2007a). Recentemente, a proteína Lsi 6 foi identificada como responsável pelo transporte de Si, do xilema para as células do parênquima do xilema nas folhas de arroz (Yamaji & Ma, 2009). Diferentemente do *Lsi* 1, o gene *Lsi* 6 ("Low silicon rice 6") é constitutivamente expresso nas folhas, mas pode ser expresso também nas raízes sob baixa disponibilidade de Si (Yamaji et al., 2008). Embora o uso de técnicas de biologia molecular tenha permitido avançar-se no entendimento dos mecanismos de absorção e translocação de Si na planta (Ma et al., 2002; 2006; 2007a;

Yamaji & Ma, 2009), o mecanismo pelo qual esse elemento afeta a produtividade do arroz ainda continua virtualmente desconhecido.

Nas primeiras pesquisas para a identificação do papel fisiológico do Si no arroz, observou-se que as plantas supridas com Si exibiam maior biomassa, folhas mais eretas e maior rendimento de grãos em comparação às não supridas (Ohkawa, 1936; Ishibashi, 1936; Okamoto et al., 1956). A hipótese mais aceita para o efeito do Si em aumentar a produtividade do arroz é que esse elemento promove o desenvolvimento de folhas mais eretas, as quais permitiriam intensificar a fotossíntese da planta inteira por melhorar a distribuição luminosa ao longo da copa (Takahashi et al., 1990; Epstein, 1999). Nesse contexto, além de proporcionar melhor distribuição luminosa ao longo da planta, folhas mais verticalizadas em relação ao solo poderiam não atingir a saturação luminosa durante todo seu ciclo diurno ou então passariam pouco tempo expostas ao estresse luminoso, resultando, portanto, em maior fotossíntese na planta inteira.

Embora, em primeira instância, a hipótese anteriormente apresentada pareça suportável, alguns resultados experimentais indicam que a alteração do ângulo foliar não poderia explicar totalmente a ação do Si sobre a produtividade. Tal suposição se baseia no fato de que o Si pode ser efetivo em aumentar a produtividade via efeitos ocorrentes durante a fase de formação e enchimento dos grãos das panículas (Okuda & Takahashi, 1961). Esse comportamento parece indicar que o Si seria determinante na fase de formação da panícula para o ganho de biomassa de grãos, número de espiguetas por panícula e para o enchimento das espiguetas (Ma et al., 1989). Como o Si é um elemento imóvel nas plantas e o arroz tem crescimento anual, as plantas supridas com esse elemento somente na fase de enchimento dos grãos não seriam capazes de gerar folhas mais eretas. Assim, caso houvesse efeito do Si sobre a fotossíntese, este não poderia ser atribuído a alterações no arranjo foliar, embora modificações sobre as taxas fotossintéticas não tenham sido avaliadas (Okuda & Takahashi, 1961; Ma et al., 1989).

Neste estudo, plantas de arroz (*Oryza sativa* L. subsp. *japonica* 'Oochikara') e o mutante defectivo para a absorção de Si - *lsi1* foram cultivadas na presença ou ausência de Si, objetivando-se avaliar os efeitos desse elemento no ângulo foliar, na transpiração foliar e na taxa fotossintética, e suas possíveis contribuições para o acúmulo de biomassa e produção de grãos.

Material e métodos

Material vegetal, condições de cultivo e desenho experimental

Foram conduzidos dois experimentos em casa de vegetação, em Viçosa (20°45'S, 42°54'W, altitude de 650 metros) Minas Gerais. As sementes de arroz (*Oryza sativa* L. subsp. *japonica* 'Oochikara') e seu mutante defectivo para a absorção de Si - *lsi1*) foram lavadas em solução de hipoclorito de sódio por 1,5 min, seguidas de lavagem em água desionizada por 3 min. As sementes germinaram em papel-filtro embebido em solução nutritiva com meia força iônica, modificada de Hoagland & Arnon (1950), constituída de: 1,0 mmol L^{-1} KNO_3; 0,25 mmol L^{-1} $NH_4H_2PO_4$; 0,1 mmol L^{-1} NH_4Cl; 0,5 mmol L^{-1} $MgSO_4.7H_2O$; 1,0 mmol L^{-1} $Ca(NO_3)_2$; 0,30 µmol L^{-1} $CuSO_4.5H_2O$; 0,33 µmol L^{-1} $ZnSO_4.7H_2O$; 11,5 µmol L^{-1} H_3BO_3; 3,5 µmol $L^{-1}MnCl_2.4H_2O$; 0,1 µmol L^{-1} $(NH_4)_6Mo_7O_{24}.4H_2O$; 25 µmol L^{-1} $FeSO_4.7H_2O$ e 25 µmol L^{-1} EDTA bisódico e foram mantidas a 25°C, por seis dias. A solução nutritiva foi trocada a cada quatro dias ou quando a condutividade elétrica atingia 85% do seu valor inicial. O pH foi verificado diariamente e mantido entre 5,5 e 6,0. O ácido monosilícico foi obtido pela passagem do silicato de potássio através de uma coluna contendo resina trocadora de cátions (Amberlite IRA 410), adicionado à solução nutritiva nas concentrações de 0 (-Si) ou 2 (+Si) mmol L^{-1}, conforme os tratamentos.

O primeiro experimento foi conduzido em delineamento inteiramente casualizado, em esquema fatorial 2 × 2 [adição ou não de Si (0 ou 2 mmol L^{-1}) na solução nutritiva; e dois genótipos de arroz [cv. 'Oochikara' (WT) e o mutante *lsi1*]. Com base na uniformidade das plantas, foram selecionados seis vasos por tratamento, totalizando 24 plantas, procedendo-se à análise de crescimento das plantas em três etapas do ciclo: na fase vegetativa (30 dias após transplantio); na fase reprodutiva, caracterizada pela emissão da panícula (60 dias após o transplantio); e na fase de enchimento dos grãos, durante a fase do grão leitoso (90 dias após o transplantio). Nos vasos selecionados para as avaliações de crescimento, metade das plantas foi separada para as coletas destrutivas de material vegetal e quantificação de Si na matéria seca da folha bandeira. Ainda, nas plantas adicionais foram realizadas avaliações de produtividade aos 110 dias após o transplantio.

O segundo experimento foi conduzido apenas com plantas da cv. 'Oochikara', em delineamento inteiramente casualizado em esquema fatorial 2 × 2 [adição ou não de Si (0 ou 2 mmol L^{-1}) na solução nutritiva durante a fase vegetativa e adição ou não de Si (0 ou 2 mmol L^{-1}) na solução nutritiva durante a fase reprodutiva]. As plantas foram supridas por 20 dias, quando, então, o desenvolvimento da panícula foi monitorado, em um microscópio de luz, a cada três dias. Após a identificação do início da fase reprodutiva, metade das plantas em cada tratamento inicial (-Si e +Si) teve sua suplementação de Si modificada, formando quatro condições experimentais até o fim do experimento, i.e., plantas continuamente supridas ou não com Si (-Si e +Si), plantas supridas com Si na fase vegetativa, mas não na fase reprodutiva (+Si/-Si), e plantas não-supridas com Si na fase vegetativa, porém supridas com esse elemento na fase reprodutiva (-Si/+Si).

Os vasos selecionados tiveram metade de suas plantas separadas para procederem-se às coletas destrutivas de material vegetal, ângulo foliar e parâmetros de trocas gasosas na fase vegetativa (28 dias de cultivo) e na fase de enchimento de grãos (90 dias). Ainda, nas plantas adicionais, foram realizadas avaliações de produtividade aos 120 dias após o transplantio.

Análise de crescimento, ângulo foliar e concentrações de Si

Os vasos selecionados para avaliações de crescimento tiveram suas plantas separadas após as análises de trocas gasosas e de ângulo foliar para procederem-se às coletas destrutivas de material vegetal. As plantas foram, então, separadas em folhas, colmo, raiz, panícula e grãos para se estimar as características de massa, número, comprimento, diâmetro e área. Os tecidos das plantas foram secos em estufa, a 70°C, por 72 h; posteriormente, o valor da massa seca das diferentes partes foi obtido. Baseado nos dados acima se calculou as seguintes características de crescimento: biomassa total (BT- g); fração de massa foliar (FMF- g de massa seca foliar g^{-1}de biomassa seca total), fração de massa do colmo (FMC - g de massa seca dos colmos g^{-1}de biomassa seca total), fração de massa radicular (FMR - g de massa seca radicular g^{-1}de biomassa seca total) e área foliar específica (AFE – m^2 de área foliar kg^{-1} de biomassa seca foliar).

A área foliar total foi mensurada com um medidor de área (Area Measurement System, Delta-T Devices, Cambridge, UK). A inclinação foliar, em relação ao horizonte, (=0°) foi mensurada ao meio dia utilizando-se de um angulômetro.

No fim do experimento, as folhas bandeira foram coletadas, lavadas em água desionizada e secas por 72 h, a 65°C. A concentração foliar de Si foi determinada por técnica colorimétrica em 0,1 g de matéria seca conforme Dallagnol et al. (2011).

Parâmetros de trocas gasosas

A taxa de assimilação líquida de carbono (*A*), a condutância estomática ao vapor de água (g_s), a concentração subestomática de CO_2 (C_i) e a taxa transpiratória (*E*) foram medidas entre 12:00 e 13:00 h, em sistema aberto, sob radiação fotossinteticamente ativa (RFA) de 1000 μmol fótons m^{-2} s^{-1}, à pressão parcial de CO_2 de 40 Pa, com um analisador de gases a infravermelho (LI-COR, Lincoln, NE, USA). Procedeu-se, ainda, à estimação da taxa de transporte de elétrons (TTE) utilizando-se do sistema de trocas gasosas mencionado anteriormente, acoplado a um fluorômetro (LI-6400-40; Li-Cor Inc.), conforme descrito em DaMatta et al. (2002). As avaliações de trocas gasosas foram realizadas nas folhas do perfilho principal quando completamente expandida em cada fase, quarta folha na fase vegetativa, quinta folha na fase reprodutiva e na folha bandeira na fase de enchimento de grãos.

Análises estatísticas

A unidade experimental foi composta pelo vaso contendo seis plantas. Os dados foram submetidos a análise de variância (ANOVA) e as comparações entre as médias dos tratamentos foram realizadas por intermédio do teste Tukey ($\alpha = 0,05$). As análises foram realizadas utilizando-se o Sistema de Análises Estatísticas e Genéticas (SAEG, versão 9.1).

Resultados

Experimento I

Efeito do Si na alocação de biomassa nas diferentes fases do ciclo fenológico

Na fase vegetativa e reprodutiva, as plantas da cv. Oochikara (WT) não diferiram quanto aos componentes de crescimento com a adição de Si ($P>0,05$; Tabela 1). As plantas

do mutante *lsi1* apresentaram maior área foliar durante a fase vegetativa quando supridas com Si comparada com as plantas do mutante não supridas, e menor fração de massa radicular comparadas com o WT na mesma condição de Si (Tabela 1). Os genótipos não diferiram na fase reprodutiva quanto aos componentes de crescimento, independentemente da adição de Si (Tabela 1).

Na fase de enchimento de grãos, os genótipos não diferiram ($P>0,05$) na alocação de biomassa para as folhas, colmo e raiz, e área foliar, independentemente da presença do Si (Tabela 1). A fração de massa de semente foi maior ($P<0,05$) para ambos genótipos na presença de Si na solução nutritiva (Tabela 1). As plantas WT apresentaram maior fração de massa de semente ($P<0,05$) em relação à do mutante *lsi* 1 na ausência de Si (Tabela 1).

Efeito do Si na produtividade

O número de panículas por planta e a umidade de semente não diferiram ($P>0,05$) entre os genótipos ou pela adição de Si (Tabela 2). A adição de Si nas plantas WT fez aumentar ($P<0,05$) a concentração de Si na matéria seca, o número de grãos por planta, a massa de mil grãos e o número de grãos por panícula comparados com plantas sem suplementação de Si (Tabela 2); no entanto, no genótipo *lsi 1* apenas o número de grãos por panícula foi ampliado ($P<0,05$) pela adição de Si (Tabela 2). Os genótipos não diferiram ($P>0,05$) na ausência do Si, mas com a adição desse elemento, WT apresentou maior percentagem de Si na matéria seca, maior número de grãos por planta e maior número de grãos por panícula em relação ao mutante ($P<0,05$; Tabela 2).

Tabela 1: Efeito do Si (0 ou 2 mmol, respectivamente, -Si e +Si) sobre parâmetros de crescimento em distintos estádios fenológicos em dois genótipos de arroz [cv. 'Oochikara' (WT) e seu mutante defectivo para a absorção de Si (*lsi 1*)]. Cada estádio foi analisado separadamente e, para cada parâmetro, médias seguidas de letra maiúscula representam o efeito de Si na mesma cultivar e dreno; as médias seguidas de letras minúsculas representam o comportamento dos genótipos quando submetidos ao mesmo tratamento de Si e dreno

Parâmetros	**Vegetativo**				**Reprodutivo**				**Enchimento**			
	WT		***Lsi* 1**		**WT**		***lsi* 1**		**WT**		***lsi* 1**	
	-Si	**+Si**	**-Si**	**+Si**	**-Si**	**+Si**	**-Si**	**+Si**	**-Si**	**+Si**	**-Si**	**+Si**
BT (g planta^{-1})	17Aa	22Aa	21Aa	19Aa	32Aa	32Aa	33Aa	31Aa	35Aa	39A	36Aa	39Aa
AF (m^2)	0,037Aa	0,032Aa	0,035Ba	0,038Aa	0,126Aa	0,101Aa	0,102Aa	0,079Aa	0,138Aa	0,141Aa	0,167Aa	0,141Aa
FMF (g g^{-1})	0,263Aa	0,221Aa	0,294Aa	0,309Aa	0,261Aa	0,258Aa	0,260Aa	0,269Aa	0,192Aa	0,176Ba	0,202Aa	0,202Aa
FMC (g g^{-1})	0,493Aa	0,559Aa	0,591Aa	0,554Aa	0,453Aa	0,462Aa	0,466Aa	0,410Aa	0,486Aa	0,462Aa	0,512Aa	0,475Aa
FMR (g g^{-1})	0,091Aa	0,081Aa	0,115Aa	0,137Ab	0,287Aa	0,280Aa	0,274Aa	0,321Aa	0,097Aa	0,097Aa	0,109Aa	0,092Aa
FMS (g g^{-1})	-	-	-	-	-	-	-	-	0,22Ba	0,26Aa	0,18Bb	0,23Aa

BT - biomassa total; AF - área foliar total; FMF – fração de massa seca foliar; FMC – fração de massa seca do colmo; FMR – fração de massa seca radicular; FMS – fração de massa seca de semente.

Tabela 2: Efeito da suplementação de Si (0 ou 2mmol, respectivamente, -Si e +Si) nos parâmetros de produção de dois genótipos de arroz, cv 'Oochikara' (WT) e seu mutante defectivo para a absorção de Si (*lsi 1*), após 120 dias de cultivo. Para cada parâmetro, médias seguidas de letra maiúscula representam o efeito de Si na mesma cultivar e médias seguidas de letras minúsculas comparam o comportamento dos genótipos quando submetidos ao mesmo tratamento de Si. ($n = 6$; F test, $P \leq 0.05$)

Parâmetro	**Produção**			
	WT		***lsi* 1**	
	-Si	**+Si**	**-Si**	**+Si**
Si (dagkg^{-1} MS)	1,14 Ba	5,10 Aa	0,99 Aa	2,91 Ab
N° Grão (u planta^{-1})	584 Ba	781 Aa	470 Aa	582 Ab
Massa 1000-grãos (g planta^{-1})	34 Ba	37 Aa	34 Aa	35 Aa
Panículas (u planta^{-1})	6,7 Aa	7,4 Aa	7,3 Aa	8,3 Aa
N° Grão (u panícula^{-1})	78,3 Ba	94,4 Aa	61,12 Bb	81,7 Ab
Espiguetas vazias (% panícula^{-1})	11,49 Aa	10,80 Ab	13,45 Aa	14,01Aa
Umidade (%)	10,46Aa	11,81Aa	10,58Aa	10,67Aa

1000 – grãos – massa seca de mil grãos por planta.

Experimento II

Silício na fase vegetativa não altera o crescimento da planta e afeta apenas marginalmente o ângulo foliar

As avaliações foram realizadas no estádio vegetativo (28 dias após o transplantio) e na fase de enchimento de grãos (100 dias após o transplantio; fase do grão leitoso). Não foram observados efeitos (P>0,05) no acúmulo de biomassa (Tabela 3) com a adição de Si durante a fase vegetativa. A arquitetura foliar não foi alterada pela suplementação de Si durante a fase vegetativa (45° em relação ao horizonte), exceto na folha mais jovem (90° em relação ao horizonte) durante os primeiros 20 dias de cultivo (Figura 1A e 1B).

Figura 1: Efeito do silício na arquitetura de plantas de arroz (*Oryza sativa* L. subsp. *japonica* 'Oochikara'). Em todas as figuras, da esquerda para a direita: plantas supridas sem adição de Si em todo ciclo (-Si), apenas no estádio vegetativo (-Si/+Si), apenas após o estádio vegetativo (+Si/-Si) e com adição de Si em todo o ciclo (+Si). A - Plantas no estádio vegetativo (26 dias após o transplantio); B - Plantas no início do estádio reprodutivo (35 dias após o transplantio).

Efeito da adição e remoção de Si nos diferentes estádios de crescimento

Não houve diferença significativa entre os tratamentos para biomassa total, área foliar total e área foliar específica durante a fase de enchimento de grãos (Tabelas 3 e 4). A fração de massa foliar e fração de massa do colmo foram menores nas plantas que tiveram sua suplementação de Si modificada após o estádio vegetativo (-Si/+Si e +Si/-Si) em relação às plantas dos demais tratamentos, sem associação com a adição de Si (Tabela 3).

Comparando-se plantas supridas com Si após o estádio vegetativo (-Si/+Si) com aquelas não supridas com Si em todo o ciclo (-Si), não foi observada alteração na biomassa total, na área foliar total, na área foliar específica, na fração de massa radicular e no número de panículas por planta (Tabela 3), porém a adição de Si fez reduzir as frações de massa foliar e do colmo, além de aumentar o número de grãos por planta, a massa de mil grãos e número de grãos por panícula (Tabela 3). A taxa de assimilação de carbono, a taxa de transporte de elétrons e a taxa transpiratória também não foram alteradas em plantas supridas com Si após o estádio vegetativo (-Si/+Si), enquanto a condutância estomática e a concentração de CO_2 na cavidade subestomática foram menores nas plantas não suplementadas com Si (-Si) (Tabela 4).

A remoção de Si após a fase vegetativa (+Si/-Si) não resultou em alterações nas variáveis estudadas (P>0,05), exceto as frações de massa foliar e do colmo e a taxa de

assimilação líquida de carbono, cujos valores foram menores que nas plantas supridas com Si durante todo o ciclo (+Si), (P<0,05; Tabelas 3 e 4).

Plantas continuamente suplementadas com Si (+Si), em relação às não suplementadas (-Si), exibiram maior (P<0,05) número de grãos por planta e por panícula, maior massa de mil grãos e maior taxa de assimilação líquida de carbono, mas sem alterações significativas (P>0,05) nas demais variáveis estudadas (Tabelas 3 e 4).

Não se procederam às comparações entre os estádios vegetativo e reprodutivo devido a grande variabilidade das condições ambientais (temperatura e UR) quando as variáveis relacionadas à fotossíntese foram medidas, em cada estádio fisiológico.

O efeito do Si sobre a produtividade é expressivo na fase reprodutiva

A adição de Si apenas na fase reprodutiva ou durante todo o ciclo fez aumentar (P<0,05) o número de grãos por panícula e por planta (Tabela 3), porém sendo menor (P<0,05) em plantas não supridas com Si durante todo o ciclo ou quando supridas na fase vegetativa (Tabela 3).

Tabela 3: Efeito da suplementação com Si (0 ou 2mmol, respectivamente, -Si e +Si) nos parâmetros de crescimento nos diferentes estádios fenológicos de arroz, cv. Oochikara. Cada estádio foi analisado separadamente e para cada parâmetro, médias seguidas de letra maiúscula representam o efeito de Si. (n = 6; F test, $P \leq 0.05$)

Fases	**Vegetativo**		**Enchimento**			
Parâmetros	**-Si**	**+Si**	**-Si**	**+Si/-Si**	**-Si/+Si**	**+Si**
BT (g planta^{-1})	25,1 A	25,0 A	28,95 A	34,5 A	36,8 A	35,78 A
AF (m^2)	0,157 A	0,148 A	0,124A	0,109 A	0,108 A	0,115A
AFE (m^2 kg^{-1})	18,86 A	17,81[a]	20,06 A	18,86 A	18,01 A	18,07 A
FMF (g g^{-1})	0,332 A	0,326 A	0,207 A	0,170 B	0,173 B	0,195 A
FMC (g g^{-1})	0,576 A	0,584 A	0,416 A	0,357 B	0,368 B	0,447 A
FMR (g g^{-1})	0,092 A	0,090 A	0,110 A	0,090 A	0,088 A	0,092 A
N° Grão (u planta^{-1})*	-	-	394,8 B	392,9 B	471,7 A	472,6 A
Massa 1000-grãos (g planta^{-1})*	-	-	25,76 B	33,43 A	30,30 A	35,2 A
N° Grão (u panícula^{-1})*	-	-	83,1 B	84 B	96,9 A	97,8 A
Panículas (u planta^{-1})*	-	-	4,6 A	5,0 A	5,17 A	4,75 A

BT - biomassa total; AF - área foliar total; AFE – área foliar específica; FMF – fração de massa seca foliar; FMC – fração de massa seca do colmo; FMR – fração de massa seca radicular; 1000 – grãos – massa seca de mil grãos por planta. (*) Análises realizadas após 120 dias de cultivo.

Tabela 4: Efeito da suplementação com Si (0 ou 2mmol, respectivamente, Si- e Si+) nos parâmetros de trocas gasosas nos diferentes estádios fenológicos de cv. Oochikara. Cada estádio foi analisado separadamente e para cada parâmetro, médias seguidas de letra maiúscula representam o efeito do fornecimento de Si. $n = 6$. F test, $P \leq 0.05$

Fase	**Vegetativo**		**Enchimento**			
Parâmetros	**-Si**	**+Si**	**-Si**	**+Si/-Si**	**-Si/+Si**	**+Si**
***A* (µmol CO_2 m^{-2} s^{-1})**	23,06 A	23,02 A	22,57 B	21,79 B	22,06 B	25,51 A
g_s (mmol H_2O m^{-2} s^{-1})	0,440 A	0,489 A	0,429 B	0,523 A	0,534 A	0,587 A
C_i (µmol mol^{-1})	291 A	299 A	286 B	301 A	308 A	300 A
***E* (mmol H_2O m^{-2} s^{-1})**	4,11 A	4,33 A	6,46 A	6,34 A	6,29 A	6,89 A
TTE (µmol elétrons m^{-2} s^{-1})	130 A	130 A	157 A	149 A	156 A	159 A

A – taxa de assimilação de carbono; g_s - condutância estomática; C_i – concentração de CO_2 na cavidade subestomática; *E* – taxa transpiratória; TTE – taxa de transporte de elétrons.

Discussão

No presente trabalho, foi evidenciado o efeito diferencial do Si nas fases do ciclo do arroz (*Oryza sativa* L. subsp. *japonica* 'Oochikara') e também para seu mutante defectivo para a absorção de Si (*lsi1*). Independentemente do genótipo, o Si não alterou a alocação de biomassa nas fases iniciais do ciclo fenológico, mas levou a aumentos na produtividade de grãos (Tabelas 1, 2 e 3), corroborando resultados prévios obtidos por diversos pesquisadores (Ishibashi, 1936; Okamoto *et al.*, 1956; Okuda & Takahashi, 1961; Tamai & Ma, 2008). No mutante *lsi* 1, a alanina da posição 132 é substituída por treonina, acarretando mudanças das interações da asparagina 108 (responsável pelo *loop* no poro do transportador), porém a mutação não inviabiliza o transporte do Si, mas sim a eficiência de sua eficiência (Ma et al., 2006), justificando as diferenças nas concentrações de Si na matéria seca (Tabela 2) e seus efeitos, principalmente, nas variáveis de produtividade em relação ao tipo selvagem (Ma et al., 2002). Além disso, em condição de baixa disponibilidade de Si, a expressão dos transportadores Lsi 2, nas células da endoderme e exoderme (Ma et al., 2007a), e do transportador Lsi 6, em todas as células das raízes finas, aumenta (Yamaji & Ma, 2007). Pode-se considerar que plantas *lsi* 1 apresentam menor influxo de Si pela proteína Lsi 1, mas possivelmente o padrão de expressão dos demais transportadores poderia ser modificado numa tentativa de aumentar a absorção de Si, de forma a manter a homeostase da concentração desse elemento na planta.

Peng et al. (1998) e Sheehy et al (2001) relacionaram altas taxas de crescimento relativo durante o estádio vegetativo com alta mobilização de reservas. O Si não alterou o crescimento vegetativo (Tabelas 1 e 3) e tampouco a taxa de assimilação de carbono por unidade de área foliar durante a fase vegetativa (Tabela 4), conforme previamente observado (Takahashi et al., 1966; Ma, 1990; Agarie et al., 1992). Em adição, o Si não modificou a área foliar (Tabela 1; 3) e o ângulo foliar da maioria das folhas (Figura 1) tornando pouco provável que ocorresse um maior acúmulo de fotoassimilados no colmo durante o estádio vegetativo, sugerindo não ser este um efeito primário do Si sobre a produtividade.

O fornecimento de Si após a fase reprodutiva foi determinante para o aumento do número de grãos (Tabela 3), em conformidade com os dados de Ma et al. (1989). Nos resultados aqui apresentados, pode-se enfatizar que o Si, no estádio reprodutivo, é mais efetivo para aumentar a produtividade do que no estádio vegetativo (Tabela 3). Além disso, as mudanças fisiológicas que se iniciam na segunda parte do estádio reprodutivo e culminam apenas com o crescimento da panícula (Horie et al., 2003), como a percentagem de espiguetas cheias por panícula e a massa de mil grãos (Tabelas 2 e 3), não foram afetadas pelo Si, sugerindo que o Si interfere no período entre a formação da panícula e o início de seu enchimento.

O tamanho final do dreno (número de grãos por panícula) na produção de fotoassimilados da folha bandeira de arroz é definido pelo número total de espiguetas que florescem, número de grãos cheios e não cheios até a maturidade. A remoção ou adição de Si na fase reprodutiva de arroz teve efeito significativo no número de grãos por panícula (Tabela 3), os quais se tornam drenos fortes. Assim, o Si deve modificar a relação fonte-dreno da planta ao promover a maior formação de grãos. Considerando-se que grande parte do carbono presente no grão de arroz é assimilada nas folhas durante a fase de enchimento dos grãos (Yoshida, 1981; Murchie et al., 1999) e, sendo a folha-bandeira a de maior atividade fotossintética, a maior taxa de assimilação de CO_2 por área observada nas plantas supridas com Si, sem acompanhamento da condutância estomática (Tabela 4), poderia ser justificada pelo aumento do dreno pelo Si.

A fotossíntese constitui-se num processo metabólico altamente integrado e regulado, sendo que a síntese, o carregamento e o descarregamento de produtos finais exercem, em

pouco tempo, o controle do metabolismo. A força do dreno (capacidade de aumentar o tamanho ou a atividade de drenos) constitui fator determinante para o aumento da fotossíntese e, consequentemente, para o ganho de biomassa da planta (Paul & Floyer, 2001). O tamanho final do dreno da produção de grãos de arroz foi definido pelo número total de espiguetas que floresceram e pela percentagem de espiguetas cheias. Esta, então, é a fase central na qual o número de drenos se torna dreno atuante, que corresponde à força do dreno da planta (Lafarge & Bueno, 2009). Dessa forma, ao aumentar o número de drenos, durante a fase reprodutiva, o Si alterou a necessidade de fotoassimilados na fase de enchimento de grãos, a qual deveria refletir-se na taxa fotossintética da folha bandeira. No entanto, os mecanismos pelo qual o Si aumentou o número de grãos por panícula, e como a folha fonte modificou metabolismo para garantir o enchimento dos grãos necessitam ser investigados.

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CAPÍTULO 2

Silicon nutrition increases grain yield, which in turn exerts a feed-forward stimulation of photosynthetic rates via enhanced mesophyll conductance and alters primary metabolism in rice

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SUMMARY

Silicon (Si) is not considered an essential element for higher plants and is believed to have no effect on primary metabolism in unstressed plants. In rice (*Oryza sativa* L.), Si nutrition improves grain production, particularly under stressed conditions. However, no attempt has been made to elucidate the physiological mechanisms underlying such responses. We combined advanced gas exchange analysis and chlorophyll *a* fluorescence measurements with carbon isotope labelling and metabolic profiling to measure the effects of Si nutrition on rice photosynthesis, along with the associated metabolic changes, by comparing wild-type rice (cv. 'Oochikara') with the low-Si rice mutant *lsi1*. Si did not affect plant growth but led to higher crop yield under unstressed conditions, paralleling an increased nitrogen use efficiency. Improved yields in Si-treated plants were associated with increases in both the number of spikelets and grain weight, with no significant effect of Si on the percentage of filled spikelets. Higher crop yields brought about an increased sink strength that, in turn, exerted a feed-forward effect on photosynthesis that was fundamentally associated with increased mesophyll conductance. By contrast, Si nutrition did not affect photosynthetic gas exchange during the vegetative growth phase or in de-grained plants. Additionally, Si nutrition altered primary metabolism by stimulating amino acid remobilisation.

INTRODUCTION

Silicon (Si) is the second most abundant element after oxygen in the Earth's crust. Because silicon dioxide comprises 50–70% of the soil solution, all plants grown in soil contain some Si in their tissues. However, Si is often assumed to be biologically unreactive and is not considered an essential element for higher plants. The most positive and consistent effects of Si nutrition have been found in the alleviation of both biotic (*e.g.*, pathogens and insects) and abiotic (*e.g.*, salt, heavy metals, light and drought) stresses in a wide variety of plant species (Epstein, 2009; Keeping and Reynolds, 2009). In fact, a growing body of evidence suggests that the benefits of Si fertilisation are minimal or even nonexistent unless the plant is under some form of imposed stress (Epstein, 2009). This has been recently demonstrated in molecular studies using *Arabidopsis thaliana* under unstressed conditions, where Si addition only altered the expression levels of two of the nearly 40,000 transcripts (Fauteux *et al.*, 2006). Even in high-Si-accumulating monocots, Si has limited effects on both the transcriptome (wheat; Chain *et al.*, 2009) and proteome (rice; Nwugo and Huerta, 2011) in the absence of stress, which lends further support to the general belief that Si has no effect on metabolism in unstressed plants, suggesting a nonessential role for this element.

Two genes encoding Si transporters (*Lsi1* and *Lsi2*) have been identified in rice roots (Ma *et al.*, 2006; 2007). Si is transported via Lsi1 and Lsi2 from the root epidermis into the root steles and then moves to the shoot by transpirational water flow via the xylem, after which it is polymerised and accumulated on the shoot tissues as silica (Ma *et al.*, 2006). Additionally, *Lsi6* is involved in Si distribution in rice shoots (Yamaji and Ma, 2009). These specific Si transporters are associated with the high ability of rice to actively take up Si in the form of monosilicic acid and could explain its high Si concentration on

shorts, which can reach values as high as 10% of shoot dry weight (Ma and Takahashi, 2002). Under field conditions, Si fertilisation is widely used to enhance rice production. This effect of Si has been traditionally attributed to its role in alleviating abiotic and biotic stresses as well as in improving resistance to lodging and increasing the erectness of leaves; these effects allow better light transmittance through plant canopies and thus indirectly improve whole-plant photosynthesis (Tamai and Ma, 2008). There is, however, evidence suggesting that Si addition scarcely affects the net CO_2 assimilation rate (*A*) *per se* and also has no impact on the tiller number, root dry weight or leaf area. By sharp contrast, rice grain yield is remarkably increased by Si fertilisation, as evidenced by rice mutants defective in Si uptake (Tamai and Ma, 2008). Increased production has chiefly been associated with (i) lower transpiration of the spikelets because high moisture conditions play a key role in the normal development of the husk and (ii) the protection against pathogen infection (Tamai and Ma, 2008). The omission of Si during the vegetative growth stage with a subsequent Si application following the beginning of the reproductive stage results in rice grain yields similar to those found when Si is added during the entire crop cycle (Okuda and Takahashi 1961; Ma *et al.*, 1989). Given this fact, improved photosynthesis associated with enhanced leaf erectness due to Si fertilisation can be ruled out because this trait is defined during the vegetative growth phase.

Taking into account the facts that, in rice, (i) Si has a significant effect on the percentage of filled spikelets and the number of spikelets per panicle and therefore on fertility (Ma *et al.*, 1989), (ii) most carbon in the rice grain comes from photoassimilate produced in leaves (especially the flag leaf) during the grain-filling period (Yoshida, 1981; Murchie *et al.*, 1999), and (iii) Si does not affect leaf area, it can be hypothesised that Si should modify the source-sink relationships through increased sink strength. These

relationships, in turn, will result in increased photosynthetic capacity of the flag leaf, with probable consequences on carbon metabolism. To test this hypothesis, source-sink imbalances were analysed via controlled de-graining experiments, which were expected to modulate photosynthesis in unstressed rice plants. Advanced gas exchange analysis and chlorophyll *a* fluorescence measurements with carbon isotope labelling and metabolic profiling were used to measure the effects of Si on photosynthesis and the process that governs metabolism in rice, and we did this by comparing wild-type (WT) rice (cv. 'Oochikara') and an *lsi1* mutant defective in Si uptake. Physiological and molecular studies using this mutant have helped to elucidate the Si uptake system in addition to increasing our knowledge about the importance of Si to rice physiology (Ma *et al.*, 2006). The results of the present study demonstrated that the increase in grain yield in Si supplied plants was due mainly to a positive effect on A via a mesophyll conductance (g_m)-mediated effect coupled with enhanced sink strength. Our results highlight the importance of Si nutrition in controlling the nitrogen (N)/ carbon (C) balance and amino acid homeostasis. The results are discussed in the context of current models of the metabolic regulation of the sink/source relationship and photosynthetic metabolism.

RESULTS

Measurements were performed in three different phenological phases: vegetative stage (25-26 days after transplanting), panicle emission (about 50 days after transplanting), and the milking grain stage where sink strength is believed to be maximum (about 90 days after transplanting). Regardless of genotype, no noticeable effect of Si on photosynthetic gas exchange parameters was detected in the first two which phenological stages. Therefore, data for these evaluations are not presented.

Silicon concentration is increased, whereas N concentration unaltered, upon Si nutrition

As expected, Si addition induced increased Si concentration in leaf tissues, which was 324% higher in WT and 241% higher in *lsi1* plants than in their Si-deprived (–Si) counterparts. On average, the leaf Si concentration was 80% higher in Si supplied (+Si) WT plants than in +Si mutant plants (Table 1). Regardless of treatment, N concentration remained unaltered in both flag leaves and grain tissues (Table 1).

Si nutrition does not affect plant growth but enhances crop yield

Regardless of treatment, there were no alterations in total biomass, total leaf area or specific leaf area (Table 1). Notably, Si nutrition significantly improved crop yield by increasing both the number of spikelets (43% in WT and 9% in *ls1* plants) and the 1000-grain weight (6% in WT and 22% in *ls1* plants) with no significant effect of Si on the panicle number and the percentage of filled spikelets (Table 1). The highest grain production was found in +Si WT plants. It should be noted that grain yield correlated positively with leaf Si concentration ($r = 0.74$, $P < 0.001$). In any case, a closer inspection of such a relationship revealed that +Si *lsi1* plants had a lower yield than –Si WT plants in spite of the larger Si concentration in the former. One possible explanation could be an intrinsic lower grain yield capacity in the mutant than in its WT counterpart.

Photosynthetic gas exchange parameters are affected by Si nutrition

Because +Si plants had a higher grain yield with no commensurate changes in leaf area, *A* per unit leaf area must increase to meet the photoassimilate demand of grains. In

fact, significant enhancements in A, ranging from 11 to 25%, were noted in plants with full grain load (+G) relative to de-grained plants (–G). Moreover, grain yield was correlated with A ($r = 0.64$, $P < 0.001$).

Although A correlated significantly with stomatal conductance (g_s) ($r = 0.67$, $P< 0.001$), no noticeable alterations in g_s among treatments were found (Table 2). Total transpiration, which largely depends on stomatal aperture, leaf area and canopy architecture, was also unaffected by the treatments (Table 2). In contrast to g_s, g_m significantly increased in +G plants relative to –G individuals (ranging from 49 up to 198%), and as a consequence, the chloroplast CO_2 concentration (C_c) tended to increase accordingly (Table 2). Notably, both g_m and C_c reached their highest values in +G+Si WT plants, with no significant difference in these parameters among plants from the other +G treatment (both WT and *lsi1* genotypes). In any case, g_m and C_c correlated positively to each other ($r = 0.54$, $P< 0.001$). Collectively, this information indicates that increases in A were largely associated with increases in g_m ($r = 0.71$, $P< 0.001$), which in turn translated into higher CO_2 availability around the rubisco environment. In the long term, a higher C_c mediated by a higher g_m should increase the ability of rubisco to discriminate $^{13}CO_2$, which was, in fact, reflected in the negative correlations of the carbon isotope composition ratio ($\delta^{13}C$) with both C_c ($r = -0.35$, $P = 0.025$) and g_m ($r = -0.52$, $P< 0.001$). In fact, the highest C_c, observed in +G+Si WT plants was accompanied by significantly more negative $\delta^{13}C$ in these plants when compared with plants from the other treatments (Table 2).

The maximum rate of carboxylation (V_{cmax}) and the maximum rate of carboxylation limited by electron transport (J_{max}), both on a C_c basis, were unaffected by the treatments, with the exception of grain-related increases in V_{cmax} (27%) and J_{max} (18%) in +Si WT plants (Table 2).

Overall, only marginal differences in gas exchange parameters were noted in the –G treatment when comparing Si and genotype effects.

Uptake of $^{14}CO_2$ and fractionation of ^{14}C-labelled tissue extracts

The rate of $^{14}CO_2$ uptake (assessed under saturating CO_2 and therefore in the absence of diffusion-mediated limitations of photosynthesis, which thus reflects the potential capacity for carbon fixation) in leaf segments isolated from the experimentally treated plants suggested that there was no effect of Si on the fixation or partitioning of the recently fixed ^{14}C among the various experimental treatments (Table 3). However, the rate of $^{14}CO_2$ uptake was generally lower in *lsi*1 plants than in their WT counterparts. Irrespective of treatment, approximately 87% of the assimilated $^{14}CO_2$ was recovered in soluble sugars, 0.4-0.6% in amino acids, 5-7% in organic acids plus hexoses, 3-6% in starch, 0.2-0.5% in proteins and 0.9-1.7% in insoluble cell-wall components (Table 3). Comparing +G with -G, some sink effects were noted, such as the decreased ^{14}C redistribution into amino acids and proteins (only in *lsi*1) and starch (regardless of genotype) irrespective of Si supply.

Enzyme activities

No effect of Si nutrition on the activities of 11 tested enzymes was noted in -G plants, regardless of genotype. Some Si effects were observed in +G plants: increases in Rubisco (initial activity and activation state) and NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in +Si WT plants, a decrease in NADP-GAPDH activity in +Si *lsi1* plants relative to their –Si counterparts, and +Si-related increases in ATP-dependent phosphofructokinase (PFK), PGA kinase and aldolase in both genotypes.

The most prominent changes in enzyme activities were related to sink effects as observed in the increased activities of phosphoglucomutase (PGM), NAD-GAPDH and NAD-dependent malate dehydrogenase (NAD-MDH) in +G plants when compared with -G plants. Also, aldolase and phosphoglucose isomerase (PGI) tended to increase in +G plants, although these increases were significant only for +Si plants from both genotypes (aldolase) and for –Si WT plants (PGI). NAD-GAPDH was consistently lower in *lsi1* than in WT plants regardless of Si and grain effects, whereas transaldolase activity was lower in WT than in the mutant, although the differences reached statistical significance only in the +G treatment. Triose-phosphate isomerase (TPI) did not change in response to the imposed treatments.

The plant metabolite profile is affected by Si nutrition and, most particularly, by Si-mediated increases in grain load

To obtain a broader overview of the major pathways of primary metabolism, a GC-MS-based metabolite profiling method (Fernie *et al.*, 2004) was used to quantify the relative metabolite levels in response to the imposed treatments. All values were normalised against the +G-Si WT plants. Among the 45 compounds successfully annotated, considerable changes in the concentrations of a wide range of organic acids, amino acids and sugars were evident (Figure 1a and Table S1). The data obtained were displayed in false colour in the heat map of Figure 1a to provide an easy overview (the full data set is available in Table S1). From this display, considerable changes in the levels of the analysed metabolites were noticeable. Interestingly, +G plants from both genotypes displayed reduced levels of several amino acids in the presence of Si, as observed for alanine, arginine, methionine, ornithine, and valine. In addition, +G+Si WT plants showed reduced

asparagine, aspartate, lysine, phenylalanine, proline, serine, threonine and tyrosine, which suggests a higher mobilisation of these amino acids to sustain the high grain yield and demand of +G+Si WT plants. Reductions in glutamate, glutarate and tryptophan in +G+Si *lsi*1 plants were also noted.

Only minor alterations in the levels of organic acids were noted in +G plants. However, isocitrate and dehydroascorbate (+Si WT) as well as citrate, lactate and pyruvate (+G *lsi1* plants) were reduced. In both genotypes a strong reduction in galactinol (40% in WT and 25% in *lsi1*) was observed under +G+Si treatment. Intriguingly, sucrose, fructose and glucose were consistently lower in +G+Si *lsi1* plants than in their –Si counterparts.

Proportionally smaller changes in the levels of metabolites were linked to Si nutrition when comparing -G with +G plants. Nevertheless, isocitrate was reduced in both genotypes supplied with Si. Methionine and shikimate were reduced, while tryptophan was increased, in WT plants. By contrast, dehydroascorbate, malate and serine pools were reduced, while both GABA and pyruvate increased, in the *lsi*1 plants.

When comparing the genotypes, it is notable that de-graining treatments significantly reduced ascorbate, glutamate, and valine and increased glutarate and shikimate in -Si *lsi*1 plants. Pyruvate, aconitate, isocitrate and malate were increased in -Si *lsi*1 plants, in contrast to reduced 2-oxoglutarate and GABA when compared with their WT counterparts. Notably, sucrose, fructose and glucose were consistently lower in *lsi*1 than in WT plants in the absence of Si. The -G treatment promoted much smaller alterations in +Si plants, with significant increases only in glutamine, methionine, glucose and sucrose, while lactate was decreased in *lsi*1 plants. In +G plants the absence of Si strongly affected plant metabolism, with reductions in arginine, asparagine, aspartate, and pyruvate, while citrate, fructose, galactose, glucose, lactate and tyrosine were significantly higher in *lsi*1 plants

than in WT (Figure 1a, Table S1). The +G+Si *lsi1* plants accumulated less pyruvate and more isocitrate, isoleucine, phenylalanine and tyrosine than +G+Si WT plants. Notably, galactinol was reduced in +Si plants under +G conditions in both genotypes.

To explore in more detail the effects of Si on plant metabolism, the metabolic data set was analysed using principal component analysis (PCA) (Figure 1b). This fingerprinting analysis revealed a clear trend of metabolic re-adjustment in response to +Si and +G conditions, particularly in *lsi*1 plants, as illustrated by the first two principal components (PC), which cover the major variance of the data set (Figure 1b, PC1 covers 66.5% of total variance and PC2 17%). It is clear that in -G WT plants, the relative importance of Si was limited and could not separate these treatments, while in +G plants, there was a relatively minor separation, which suggested a Si effect in these plants. Surprisingly, the results obtained by these analyses were more evident in *lsi*1 plants, where a clear separation between the effects of Si and grain load could be observed. It is, however, noteworthy that, in +G plants, the effects of Si were much more apparent, and the treatment effects could be easily distinguished. The metabolic events occurring in +G+Si conditions are best exemplified by the metabolites with the highest PCA scores and ANOVA *P*-values (*i.e.*, those metabolites with a main impact on the variance of the data set; Table S2). A number of amino acids, such as alanine, aspartate, ornithine, and threonine, as well as the sugars glucose and fructose, accounted for the main changes observed in primary metabolism.

Next was carried out a broad correlation analysis (between the relative level of each metabolite to the relative level of Si in all experimental samples) in an attempt to determine which changes were most closely associated with the change in Si concentration. When evaluating the strengths of these correlations and their significances, it becomes apparent that only 16 of the metabolic changes (those in alanine, arginine, glutamine, isoleucine,

methionine, ornithine, valine, dehydroascorbate, 2-oxoglutarate, isocitrate, pyroglutamate, quinic acid, fructose, glucose, galactinol and glycerol) were closely associated with concentrations in Si (Table S3). Amongst those, only 2-oxoglutarate was positively correlated with Si, suggesting that increases in Si concentration negatively affected the concentrations of a variety of metabolites, specifically amino acids (7 out of 16).

DISCUSSION

Si nutrition improves rice production (Ishibashi, 1936; Okamoto *et al.*, 1956; Tamai and Ma, 2008), but surprisingly, no attempt has been made to date to elucidate the physiological mechanisms underlying the responses of plants to Si. In this study, Si concentrations in leaf tissues were manipulated by omitting Si from the culture solution (-Si plants) as well as by using the low-Si rice mutant *lsi*1. This approach revealed new insights into the links between the Si-related improvement of rice crop yield and photosynthesis, along with the associated metabolic changes.

Silicon nutrition increases both rice grain yield and nitrogen use efficiency

Previous analyses of the rice yield components showed that Si supply improves crop yield by enhancing both the number of spikelets per panicle and, most particularly, the percentage of filled spikelets, with no significant effect on the panicle number or the 1000-grain weight (Ma *et al.*, 1989; Tamai and Ma, 2008). Despite the fact that the total number of spikelets was significantly increased in this study, especially in WT plants, there was found increase in grain weight (particularly in the mutant), with no significant effect of Si on the percentage of filled spikelets. A decreased percentage of filled spikelets in Si-deprived rice plants has been attributed to higher disease intensity and increased spikelet

transpiration, which is especially important if the rice crop encounters typhoon conditions during the spikelet-filling period (Tamai and Ma, 2008). Although we cannot rule out such a transpiration effect, we contend that it had only negligible importance in determining grain yield under our experimental conditions. Therefore, Si-related improvements in rice production under unstressed conditions should be more directly associated with differentiation and development of reproductive structures. Although Ma *et al.* (1989) have suggested that Si may ameliorate pollen viability, virtually nothing is known concerning the physiological basis of how Si affects rice production.

Taking into account that neither total biomass nor leaf N level varied across treatments (and assuming similar total plant N contents), N remobilisation to grains must have increased with grain load in +Si plants to maintain an unaltered N content spread over a higher grain biomass. The implication of this is that crop yield is effectively increased without impairing grain quality (in terms of protein content) in addition to improving N use efficiency.

Increased grain yield exerts a feed-forward stimulation on photosynthetic rates via enhanced mesophyll conductance

We showed for the first time that Si leads to increases in crop yield , which brings about an increased sink strength that, in turn, exerts a feed-forward effect on photosynthesis. These effects were fundamentally associated with increased g_m and were particularly pronounced in WT plants. In these plants, increases in C_c mediated by higher g_m apparently led to increases in both Rubisco initial activity and activation status (see Galmés *et al.*, 2011), coupled with increased V_{cmax} and J_{max}. In any case, alterations in actual A were not accompanied by significant changes in the rate of $^{14}CO_2$ uptake in +G

WT plants, regardless of Si supply, which suggest that when limitations to CO_2 diffusion are fully overcome by the super-saturated CO_2 supply, g_m-related differences in A are abolished. Earlier attempts to demonstrate an effect of Si nutrition on rice photosynthesis (*e.g.*, Nwugo and Huerta, 2008, 2011; Chen *et al.*, 2011) most likely failed because those investigations examined plants during their vegetative growth phase, when sink strength is relatively low. This was also noted in this study and was further corroborated by the similar A among plants from the -G treatment.

Recently, Centritto *et al.* (2009) posited that under drought conditions, g_m also plays an important role in determining photosynthesis because rice genotypes with inherently higher g_m are capable of maintaining a higher A. To the best of our knowledge, the current study is the first to report a direct effect of sink strength on g_m. The mechanisms underlying this relationship are not immediately evident. Although several investigators have attempted to explain the mechanistic bases of g_m variations, which may depend on leaf thickness, surface area of chloroplasts exposed to intercellular airspace, mesophyll cell wall thickness, membrane permeability to CO_2 and carbon anhydrase activity (Evans *et al.*, 2009; Tholen and Zu, 2011), our understanding of this subject remains far from clear. Accordingly, the limited progress in elucidating the mechanisms that govern g_m could be linked to the lack of a proper method to evaluate the contributions of both anatomical and biochemical components of g_m (Tholen and Zu, 2011).

A practical consequence of sink-related changes in g_m is on the link between $\delta^{13}C$ and water use efficiency (WUE). $\delta^{13}C$ in leaf dry matter has been used as a proxy for long-term WUE in a variety of studies because changes in $\delta^{13}C$ are related to changes in either or both A (carbon gain) and g_s (water loss), with a more negative $\delta^{13}C$ reflecting lower WUE (Farquhar *et al.*, 1989). Given that transpiration rates were unaffected by the treatments and

total biomass tended to be higher in +G +Si WT plants, a higher WUE is to be expected, in contrast to the lower WUE that would be predicted by lower $\delta^{13}C$ values. A lower $\delta^{13}C$ in +G +Si WT individuals should have arisen due to the proportionally higher increase in C_c compared to A, thus allowing Rubisco to increase discrimination against $^{13}CO_2$, a fact further supported by the negative correlation between $\delta^{13}C$ and C_c (and also g_m). Similar results were reported for tobacco by Flexas *et al.* (2006). As a consequence, the relationship between $\delta^{13}C$ and WUE is invalidated and hence may limit the applicability of $\delta^{13}C$ as a proxy for WUE. Overall, these findings match the theoretical calculation by Warren and Adams (2006), who reported that differences in g_m could induce a variation of up to 2 to 4‰ in leaf $\delta^{13}C$, with no noticeable difference in WUE.

Si nutrition affects source–sink strength relationships and stimulates amino acid remobilisation

In many cases, reductions of enzyme/protein levels do not lead to significant metabolic alterations, probably due to the induction of compensatory pathway(s) (Hodges, 2002). This seems not to be the case in our study because in the *lsi*1 knockdown lines, the leaf metabolite concentrations was much more affected than in WT plants under the same growth conditions. In fact, the changes observed in leaf metabolism cannot be directly associated with changes in the transcript levels of enzymes (Fauteux *et al.*, 2006) or enzyme activities (Table 4), suggesting post-transcriptional regulation as a major factor responsible for the metabolic changes observed in the current study. By sharp contrast, K deficiency has been associated with enzyme regulation at the levels of mRNA and protein by maintaining carbon flux into amino acids and proteins and increasing the carbon-to-

nitrogen ratio (N/C) in amino acids (Armengaud *et al*., 2009). Taken together, these data suggest that Si might act as a signal to promote amino acid remobilisation (as is believed to occur with diseased rice plants, in which Si nutrition may potentiate mechanisms of host resistance via alterations in plant metabolism; Dallagnol *et al*., 2011). Support for this assumption comes also from a recent study demonstrating that Si nutrition can modulate the expression of a leucine-rich repeat (LRR) family protein and can play a central role in perceiving an as yet uncharacterised Si signal (Fleck *et al*., 2011). LRR proteins belong to the receptor-like kinase family, a major protein family with more than 1100 members in rice (Morillo and Tax, 2006).

Our analysis of the metabolite profiles of +G+Si *lsi1* plants indicated that leaves were characterised by reduced sucrose, fructose and glucose, in accord with relatively small changes in *A*. Indeed, the reduced levels of several amino acids in +G+Si WT plants were associated with an increased sink strength mediated by Si nutrition, with little, if any, impact on growth. It has been suggested that amino acid export can be regulated by sucrose transport or metabolism (Barneix, 2007), and Winter *et al.* (1992) postulated that both sucrose and amino acid export to the sieve tube depend on photosynthetic metabolism in the source cell. By contrast to our results, increased seed sink strength in *Vicia narbonensis*, which was induced by over-expressing an amino acid permease (AAP-12), stimulated not only seed growth but also the growth of vegetative organs, indicating that the increased N uptake due to higher seed demand in AAP-12 plants is partly compensated for by growth stimulation of vegetative organs (Götz *et al*., 2007). However, an analysis of the metabolism of potato plants over-expressing the sucrose transporter *So*SUT1 demonstrated that leaves were characterised by reduced sucrose in the absence of major changes in *A* (Leggewie *et al*., 2003). Furthermore, the most likely explanation for the changes in the

levels of carbohydrates in that study is that *So*SUT1 was active *in vivo* and catalysed sucrose efflux from the leaves at an enhanced rate. When taken in the context of these studies, it is clear that the relationship among source-sink parameters and metabolite levels is rather complex and is likely influenced by multiple factors. Nevertheless, our evaluation of the metabolites that corresponded to the Si concentration revealed three sets of compounds: those intimately involved in respiration (isocitrate and 2-oxoglutarate), a handful of amino acids (alanine, arginine, glutamine, ornithine, isoleucine, methionine and valine), and four sugars/sugar derivatives (glucose, fructose, galactinol and glycerol). Notably, Si did not affect the actual rates of ^{14}C incorporation into amino acids. When taken together, these data clearly demonstrate that, at least under the conditions explored here, Si nutrition plays an important role in modulating the rate of flux from 2-oxoglutarate into amino acid metabolism, supporting the emergent view that amino acid metabolism is a tightly and intricately controlled network (Sweetlove and Fernie, 2005; Less and Galili, 2008). These data also support our view of a role for Si nutrition in orchestrating amino acid remobilisation (although such a remobilisation should be just beginning, because no detectable changes in leaf N concentration were found). Naturally, the metabolites we measured represent only a small part of the whole rice metabolome, and it is yet to be determined in future studies whether similar observations can be made across a broader spectrum of metabolites. In any case, Si *per se* may directly impact the metabolite profile of rice. Therefore, it could have some as yet unknown function in rice metabolism, even under unstressed conditions.

CONCLUSIONS

Here we demonstrate that Si nutrition leads to an improved crop yield, even under unstressed conditions, paralleling an increased nitrogen use efficiency in rice. We additionally demonstrate that Si nutrition results in altered primary metabolism, with Si clearly stimulating amino acid remobilisation. However, it is important to note that the mechanism by which this is achieved is, as yet, unknown. Overall, higher crop yields bring about an increased sink strength, which in turn exerts a feed-forward, mesophyll conductance-associated effect on photosynthesis. Therefore, our report identifies Si nutrition as an important target in attempts to improve the agronomic yield of rice.

EXPERIMENTAL PROCEDURES

Plant material, growth conditions and experimental design

Rice plants from cv. 'Oochikara' and the low-silicon 1 (*lsi1*) mutant (Ma *et al.*, 2006) were grown in a screen house in plastic pots with 5 L of nutrient solution containing 0 or 2 mM Si under naturally fluctuating environmental conditions. Further details have been given elsewhere (Dallagnol *et al.*, 2011). Maximum photosynthetic photon flux density (PPFD) inside the screen house was approximately 1500 μmol m^{-2} s^{-1}. The experiment had a completely randomised design, with eight treatment combinations, forming a 2^3 factorial (two genotypes, two Si levels and two grain loads, *i.e.*, 0 and full grain burden), with six plants in individual pots per treatment combination serving as conditional replicates. The experiments were repeated twice, yielding similar results for whole plant biomass, *in situ* gas exchange parameters and crop yield. Unless otherwise indicated, sampling and measurements were performed during the milking grain stage, *i.e.*, about 90 days after transplanting.

Si concentration

Flag leaves were collected, and their Si concentrations were colourimetrically determined according to Dallagnol *et al.* (2011).

Biomass and crop yield

At the end of the experiment, plants were harvested and separated into culms, leaves, roots and reproductive parts. Total leaf areas were measured with an area meter. Plant tissues were then oven-dried at 70°C for 72 h, after which the dry weights of the leaves, stems and roots were determined. Based on these data, total biomass, leaf mass ratio (LMR), stem mass ratio (SMR), root mass ratio (RMR) and total grain yield were obtained. The specific leaf area of flag leaves, the panicle number, the percentage of filled spikelets and 1000-grain weight were also determined.

Photosynthetic gas exchange measurements

A, g_s and intercellular CO_2 concentration (C_i) were measured on attached leaves (flag leaf) with a portable open-flow gas exchange system (LI-6400XT, LI-COR, Lincoln, NE, USA). Measurements were made from 1000 to 1300 hours (solar time), which is when A was at its maximum, under artificial PPFD, *i.e.*, 1000 μmol photons m^{-2} s^{-1} at the leaf level and 400 μmol CO_2 mol^{-1} air. During the measurements, the leaf-to-air vapour pressure deficit was about 1.0 kPa.

Leaf gas exchange parameters were also determined simultaneously with measurements of chlorophyll fluorescence using the above-mentioned gas exchange system

equipped with an integrated fluorescence chamber head (LI-6400-40, LI-COR Inc.). The actual photochemical efficiency of photosystem II (ϕ_{PSII}) was determined by measuring steady-state fluorescence and maximum fluorescence using a light-saturating pulse of approximately 8000 μmol m^{-2} s^{-1} following the procedures of Genty *et al.* (1989). The electron transport rate (J) was then calculated from $J = \phi_{PSII}\ \beta\ \alpha$ PPFD, where α is leaf absorptance and β reflects the partitioning of absorbed quanta between photosystems II and I. The product β α was determined according to Valentini *et al.* (1995), from the relationship between ϕ_{PSII} and ϕ_{CO2} obtained by varying light intensity under non-photorespiratory conditions. Estimations of g_m were performed using the method of Harley *et al*. (1992), as follows:

$$g_m = A/(C_i - (\Gamma^*(J + 8(A + R_l))/(J - 4(A + R_l))))$$

where A, C_i and J were taken from gas exchange and chlorophyll fluorescence measurements at saturating light, light respiration leaf (R_l) is the rate of mitochondrial respiration in the light not related to photorespiration and Γ* is the chloroplastic CO_2 photocompensation point in the absence of mitochondrial respiration. Leaf respiration in the dark was measured in early morning at PPFD = 0 μmol m^{-2} s^{-1} in dark-adapted leaves and taken as a proxy for R_l (Pinelli and Loreto, 2003; Centritto *et al.*, 2009). The conservative parameter Γ* for rice was taken from Li *et al.* (2009). To convert A–C_i curves into A–C_c curves, C_c was calculated according to Flexas *et al.* (2007). V_{cmax} and J_{max} were estimated by fitting the mechanistic model of CO_2 assimilation proposed by Farquhar *et al.* (1980) using the C_c-based temperature dependence of kinetic parameters of Rubisco (Bernacchi *et al.*, 2002). Fitting the model involved optimising the parameter values by adjusting them to minimise the sums of residuals between the observed and modelled

assimilation values over a range of C_c. This procedure was performed using the software package Solver in Microsoft Excel. Afterwards, the photosynthetic parameters V_{cmax}, J_{max} and g_m were normalised to 25°C using the temperature response equations from Sharkey *et al.* (2007). Corrections for the leakage of CO_2 into and out of the leaf chamber of the LI-6400 were applied to all gas exchange data, as described by Flexas *et al.* (2007).

Total canopy transpiration over the course of the day was gravimetrically measured using a balance (0.1 g precision).

Incubation of leaf discs with $^{14}CO_2$ and fractionation of ^{14}C-labelled tissue extracts

Flag leaves were detached from the main tiller in the morning and immediately brought to the laboratory. This material was used for the ^{14}C labelling of several cellular constituents, which was performed by illuminating leaf segments in a leaf chamber (LD2/2, Hansatech, Kings Lynn, UK) under a saturating CO_2 partial pressure (~5 kPa) at a PPFD of 1000 μmol m^{-2} s^{-1} at 30°C for 30 min. The CO_2 was supplied from 400 μL of 1 M $NaH^{14}CO_3$ (specific activity of 1.96 GBq $mmol^{-1}$), pH 9.3, placed on a felt mat at the base of the leaf chamber. Leaf discs were then flash-frozen and stored at –80°C until required.

The ^{14}C labelling of soluble sugars, starch, amino acids, organic acids and hexoses, proteins, cell wall and total uptake was performed as detailed by Lytovchenko *et al.* (2002).

Enzyme activities

Leaf flag tissues were harvested 6 h into the photoperiod. Enzyme extracts were prepared from these as described by Nunes-Nesi *et al.* (2007). The activities of the following enzymes were assessed: aldolase, NAD-GAPDH, ATP-PFK, PGI, TPI (all assayed as detailed in Fernie *et al.*, 2001), NADP-specific GAPDH (Leegood and Walker,

1980), NAD-MDH (Jenner *et al.*, 2001), Rubisco (Sulpice *et al.*, 2007), transaldolase (Debnam and Emes, 1999) and PGM (Nunes-Nesi *et al.*, 2005).

Metabolite levels

Leaf samples were collected at midday, immediately frozen in liquid nitrogen, and then stored at -80°C until further analysis. The samples were lyophilised at -48°C and crushed in a ball mill. All other metabolites were quantified by GC-MS–based metabolic profiling exactly as described previously (Lisec *et al.*, 2006), with the exception that the injected volumes were optimised for rice samples according to Kusano *et al.* (2011). Both chromatograms and mass spectra were evaluated using TAGFINDER (Luedemann *et al.*, 2008). Metabolites were identified in comparison to database entries of authentic standards (Kopka *et al.*, 2005; Schauer *et al.*, 2005). Identification and annotation of detected peaks followed the recommendations for reporting metabolite data described in Fernie *et al.* (2011).

Other assays

Plant tissues were oven-dried for 72 h at 70°C, after which total N and $\delta^{13}C$ were measured, as described previously (DaMatta *et al.*, 1999; 2002).

Statistical analysis

The data were analysed with a simple factorial analysis of variance (three-way maximum interactions), and comparisons between treatment means were carried out, using the *F* test at 5% probability. The Pearson's linear correlation technique and PCA were used

to examine the relationships among variables. Analyses were performed using SAS software version 9.1.

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SUPPORTING INFORMATION

Table S1. Relative metabolite contents in flag leaves of cv. 'Oochikara' (WT) and the *lsi1* mutant.

Table S2. Over-representation analysis of the principal component analysis loadings of metabolite data.

Table S3. Pairwise correlation coefficients calculated between the contents of Si and metabolites.

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Table 1. The effects of grain load and silicon (Si) supply (0 or 2 mM: –Si or +Si, respectively) on the concentrations of Si (flag leaves) and nitrogen (N; flag leaves and grains), growth parameters [total biomass, leaf area (LA), specific leaf area (SLA), leaf mass ratio (LMR), stem mass ratio (SMR) and root mass ratio (RMR)] and grain yield components (panicle number, total spikelet number, percentage of filled spikelets and 1000-grain weight) of two rice genotypes [cv. 'Oochikara' (WT) and the *lsi1* mutant defective for Si uptake] grown in nutrient solution. For each parameter, means followed by the same uppercase letter did not differ significantly between Si treatments within the same genotype; the means followed by the same lowercase letter did not differ significantly between genotypes within the same Si treatment. Asterisks indicate significant differences between –Grain and +Grain plants within the same Si treatment and genotype. $n = 6$. F test, $P \leq 0.05$.

Parameters	−Grain				+Grain			
	WT		*lsi*1		WT		*lsi*1	
	−Si	+Si	−Si	+Si	−Si	+Si	−Si	+Si
Silicon (dag kg^{-1} DW)	11.7 Ba	47.3 Aa	6.03Aa	25.5Bb	11.4 Ba	51.0 Aa	9.9 Ba	29.1 Ab
Leaf N (g kg^{-1} DW)	31.3Aa	26.3Ba	24.4Ab	28.9Ab	30.0Aa	30.0Aa	27.8Aa	27.1Aa
Grain N (g kg^{-1} DW)	---	---	---	---	15.3Aa	14.0Aa	14.1Aa	13.8Aa
Biomass (g plant^{-1})	36.08Aa	39.54Aa	39.90Aa	40.54Aa	39.74 Aa	47.00Aa	39.10 Aa	39.62 Aa
LA (m^2)	0.137 Aa	0.171Aa	0.132Aa	0.138Aa	0.136Aa	0.141Aa	0.143Aa	0.142Aa
SLA (m^2 kg^{-1})	17.55Aa	19.85 Aa	18.13 Aa	18.00 Aa	19.87 Aa	20.62 Aa	20.21 Aa	18.16 Aa
LMR (g g^{-1})	0.218Aa*	0.222Aa*	0.198Aa	0.205Aa	0.171Ab	0.146Ab	0.185Aa	0.203Aa
SMR (g g^{-1})	0.667Aa*	0.674Aa*	0.698Aa*	0.683Aa*	0.434 Aa	0.384 Aa	0.467 Aa	0.475 Aa
RMR (g g^{-1})	0.115Aa*	0.103Aa*	0.103Aa	0.112Aa*	0.092Aa	0.081Aa	0.100Aa	0.094Aa
Panicle number (plant^{-1})	---	---	---	---	6.7 Aa	7.4 Aa	7.3 Aa	8.3 Aa
Spikelet number (plant^{-1})	---	---	---	---	349 Ba	498 Aa	286Ba	312Ab
Filled spikelets (%)	---	---	---	---	88.5Aa	89.2Ab	86.5Aa	86.0Aa
1000-grain weight (g)	---	---	---	---	36.08Ba	38.24Aa	31.62Ba	38.64Aa

Table 2. The effects of grain load and silicon (Si) supply (0 or 2 mM: –Si or +Si, respectively) on photosynthetic gas exchange parameters obtained *in situ* [net CO_2 assimilation rate (A), stomatal conductance (g_s), internal CO_2 concentration (C_i) and total diurnal transpiration (E)], on those parameters derived from A–C_c curves [chloroplastic CO_2 concentration (C_c), mesophyll conductance (g_m), the maximum rate of carboxylation (V_{cmax}) and the maximum rate of carboxylation limited by electron transport (J_{max}), both on a C_c basis], and on the carbon isotope composition ratio (**$\delta^{13}C$**) of two rice genotypes [cv. 'Oochikara' (WT) and the *lsi1* mutant defective for Si uptake] grown in nutrient solutions. With the exception of E, which was determined on a whole-canopy level (and only in the +G treatment), all data were obtained in flag leaves. Statistics are shown in Table 1.

Parameters	−Grain				+Grain			
	WT		*lsi*1		WT		*lsi*1	
	−Si	+Si	−Si	+Si	−Si	+Si	−Si	+Si
A (µmol CO_2 m^{-2} s^{-1})	20.0Aa*	20.9*Aa	18.4Aa*	18.0Ab*	22.2Ba	25.3Aa	22.1Aa	22.8Ab
g_s (mmol H_2O m^{-2} s^{-1})	392Aa	371Aa	317Aa	324Aa	382Aa	394Aa	362Aa	380a
C_i (µmol mol^{-1})	275Aa	265Aa	251Aa	259Aa	263Aa	243Bb	250Aa	262Aa
E (mol H_2O g^{-1} $plant^{-1}$)	--	--	--	--	1.63 Aa	1.57 Aa	1.60 Aa	1.60 Aa
g_m (mmol CO_2 m^{-2} s^{-1})	184Aa*	155*Aa	142Aa*	144*Aa	264Ba	463Aa	212Aa	267Ab
C_c (µmol mol^{-1})	139Aa	106Aa*	113Aa	134 Aa	154 Aa	187 Aa	148 Aa	138 Ab
$V_{c,max}$ (µmol CO_2 $m^{-2}s^{-1}$)	124Aa	119Aa*	139Aa	123Aa	124Aa	151Aa	134Aa	143Aa
J_{max} (µmol e^- $m^{-2}s^{-1}$)	131Aa	119Aa*	116Aa	132Aa	141Aa	140Aa	139Aa	139Aa
$\delta^{13}C$ (-‰)	27.0 Aa	26.9 Aa*	26.4Aa	26.0Aa	26.9Ba	28.3Aa	26.8Aa	26.9Ab

Table 3. The effects of grain load and silicon (Si) supply (0 or 2 mM: –Si or +Si, respectively) on total tissue incorporated radioactivity and the partitioning of ^{14}C-labelled leaf tissues into several constituents in flag leaves of two rice genotypes [cv. 'Oochikara' (WT) and the *lsi1* mutant defective for Si uptake] grown in nutrient solutions. Statistics are shown in Table 1.

Parameters	-Grain				+Grain			
	WT		*lsi*1		WT		*lsi*1	
	-Si	+Si	-Si	+Si	-Si	+Si	-Si	+Si
Total ^{14}C uptake (kBq g^{-1})	25.2Aa	26.7Aa	24.6Aa	22.9Aa	28.9Aa	29.3Aa	20.9Ab	18.1Ab
Redistribution of radiolabel (as percentage of total assimilated)								
Soluble sugars	85.9Aa	88.0 Aa	83.7 Aa	86.6 Aa	88.3 Aa	87.7 Aa	86.1 Aa	87.9 Aa
Amino acids	0.52 Aa	0.40 Aa	0.37Ab*	0.37Aa*	0.42Ab	0.35Ab	0.59Aa	0.63Aa
Organic acids + hexoses	7.24 Aa	4.79 Aa	7.68 Aa	5.10 Aa	6.93 Aa	6.67 Aa	5.39 Aa	6.18 Aa
Starch	4.74 Aa*	5.42 Aa*	6.23 Aa*	6.39 Aa*	3.18 Aa	4.12 Aa	5.81 Aa	3.29 Aa
Proteins	0.28 Aa	0.27 Aa	0.31 Aa*	0.26 Aa*	0.22 Aa	0.22 Aa	0.44 Aa	0.54 Aa
Cell wall	1.26Ab	1.10Ab	1.70 Aa	1.27 Aa	0.91Ab	0.93Ab	1.70 Aa	1.43Aa

Table 4. The effects of grain load and silicon (Si) supply (0 or 2 mM: -Si or +Si, respectively) on enzyme activities, expressed as nmol g^{-1} FW min^{-1} in flag leaves of two rice genotypes [cv. 'Oochikara' (WT) and the *lsi1* mutant defective for Si uptake] grown in nutrient solutions. Statistics are shown in Table 1.

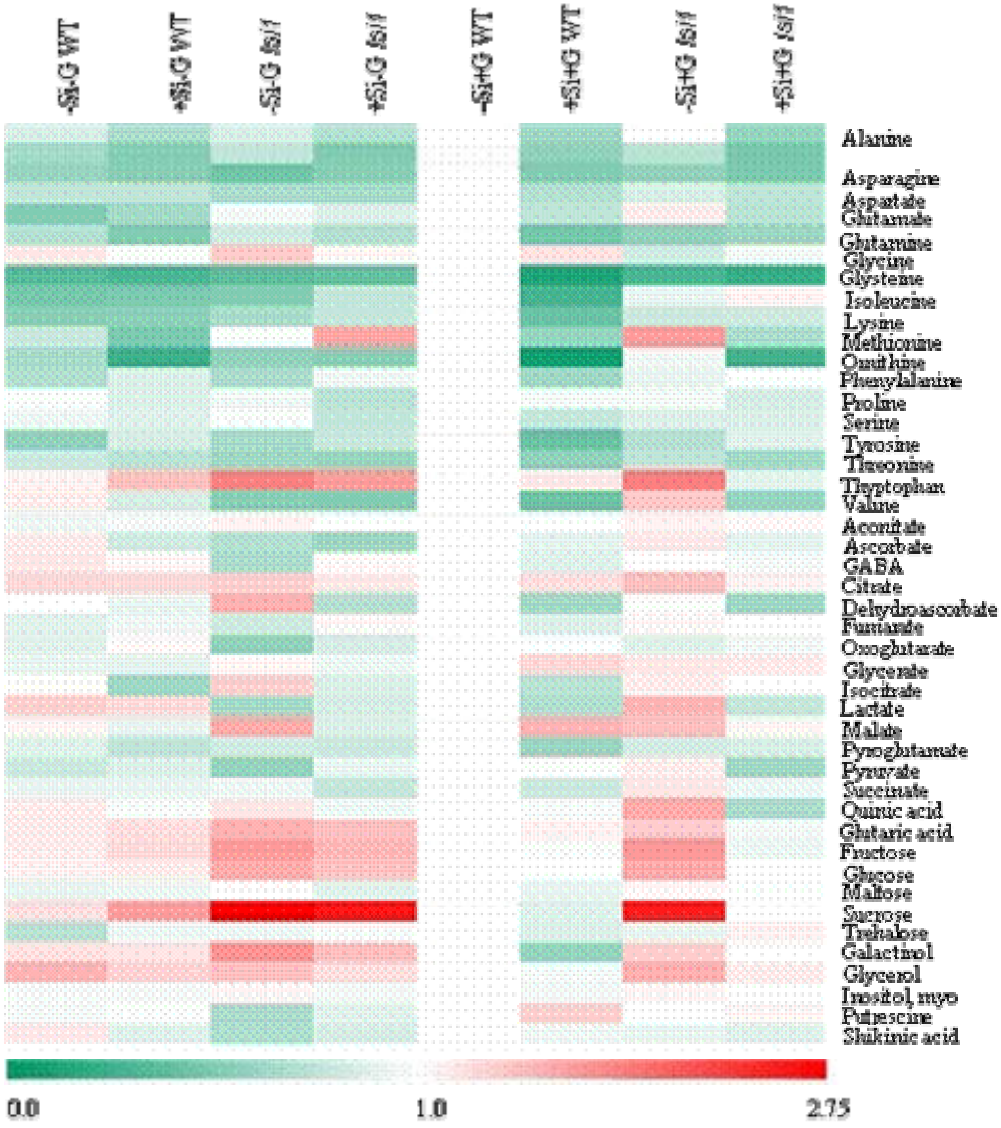
Parameters	-Grain				+Grain			
	WT		*lsi*1		WT		*lsi*1	
	-Si	+Si	-Si	+Si	-Si	+Si	-Si	+Si
Rubisco (initial activity)	5034Aa	4756Aa*	6061Aa	5176Aa	5279Ba	8452Aa	5482Aa	4711Ab
Rubisco (maximum activity)	8239Aa	8792Aa	9371Aa	8182Aa	9604Aa	10904Aa	8751Aa	7511Ab
Rubisco activation (%)	61.1Aa	54.0Aa*	64.4Aa	62.4Aa	55.2Ba	77.6Aa	62.6Aa	64.3Ab
NADP-GAPDH	1373Aa	1391Aa	759Ab*	823Ab	1187Ba	1693Aa	1089Aa	726Bb
NAD-GAPDH	3350Aa	4000Aa	2384Ab	2233Ab	3784Aa	3934Aa	2496Ab	2878Ab
PGM	177Aa*	284Aa*	358 Aa*	198Aa*	473Aa	410Aa	329 Aa	327Aa
PGI	5311Aa*	8243Aa	8604 Aa	7980 Aa	14408Aa	9398Aa	8114Aa	8178Aa
PFK	116Aa	91.5Aa	84.0Ab	82.9Aa	92.0Aa	72.7Ba	89.0Aa	60.5Ba
Aldolase	3959Aa	4591Aa	3941Aa	3890Aa	4617Aa	6314Aa	4515Ba	5368Aa
PGA Kinase	1.60Ba	1.82 Aa	1.62Aa	1.57Aa*	1.64Ba	2.09Aa	1.49Ba	1.84Aa
NAD-MDH	11241Aa*	13514 Aa*	6736Ab*	7320Ab*	23082 Aa	19445Aa	15023Ab	18371Aa
Transaldolase	26.1Aa*	30.1Aa*	35.2 Aa	45.0 Aa*	13.6Ab	15.4Ab	33.7 Aa	30.6 Aa
TPI	0.423 Aa	0.469Aa	0.448 Aa	0.392Aa	0.393 Aa	0.490 Aa	0.465 Aa	0.484Aa

LEGEND TO FIGURE

Figure 1. Changes in metabolite content in flag leaves of two rice genotypes [cv. 'Oochikara' (WT) and the *lsi1* mutant defective for Si uptake] under the effects of grain load (+G and -G) and Si supply (0 and 2 mM: –Si and +Si, respectively). (A) Overlay heat map of the metabolite profiles representing the changes in relative metabolite contents. The colours indicate the proportional content of each putatively identified metabolite among the samples, as determined by the average peak response area after normalisation against the +G–Si WT plants. The lowest normalised value receives green, and the highest receives red (see colour bar at the bottom). The exact values for each metabolite are provided in Table S1. (B) Principal component analysis (PCA) of the metabolite data. PCA was performed on the full data set obtained for all treatments analysed. Table S2 shows the analysis of the PCA loadings. Metabolites were determined as described in "Experimental Procedures." Values presented are means of six biological replicates.

Figure 1

A


-Si-G WT
+Si-G WT
-Si-G Isi1
+Si-G Isi1
-Si+G WT
+Si+G WT
-Si+G Isi1
+Si+G Isi1
Alanine
Asparagine
Aspartate
Glutamate
Glutamine
Glycine
Glysteine
Isoleucine
Lysine
Methionine
Ornithine
Phenylalanine
Proline
Serine
Tyrosine
Threonine
Thyptophan
Valine
Aconitate
Ascorbate
GABA
Citrate
Dehydroascorbate
Fumarate
Oxoglutarate
Glycerate
Isocitrate
Lactate
Malate
Pyroglutamate
Pyruvate
Succinate
Quinic acid
Glutaric acid
Fructose
Glucose
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Putrescine
Shikimic acid
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1.0
2.75

B

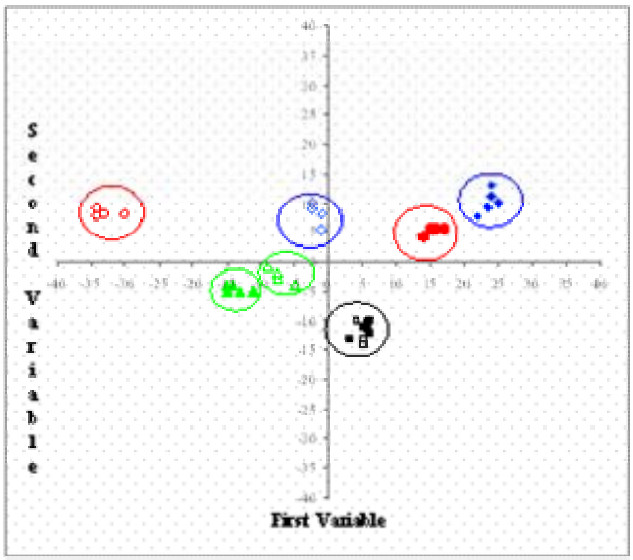
Second Variable
First Variable
-Si-G WT
+Si-G WT
-Si-G Isi1
+Si-G Isi1
-Si+G WT
+Si+G WT
-Si+G Isi1
+Si+G Isi1

Table S1. Relative metabolite contents in flag leaves of two rice genotypes. Samples of cv. 'Oochikara' (WT) and the *lsi1* mutant defective for Si uptake under the effects of grain load (+Grain and -Grain) and Si supply (0 and 2 mM: -Si and +Si, respectively) were analysed by GC-MS. For each parameter, means followed by the same uppercase letter did not differ significantly between Si treatments within the same genotype; the means followed by the same lowercase letter did not differ significantly between genotypes within the same Si treatment. Asterisks indicate significant differences between -Grain and +Grain plants within the same Si treatment and genotype. $n = 6$. F test, $P \leq 0.05$.

Parameters	-Grain				+Grain			
	WT		*lsi*1		WT		*lsi*1	
	-Si	+Si	-Si	+Si	-Si	+Si	-Si	+Si
Aconitate	0.941Ab	0.968Aa	1.093Aa	0.991Aa	1.000Aa	0.987Aa	1.089Aa	1.038Aa
Alanine	0.854Aa	0.678Aa	0.850Aa	0.706Aa	1.000Aa	0.633Ba	1.004Aa	0.590Ba
Arginine	0.645Aa*	0.530Aa	0.766Aa	0.508Aa	1.000Aa	0.567Ba	0.743Ab	0.477Ba
Ascorbatec	1.128Aa	0.820Aa	0.706Ab*	0.619Aa	1.000Aa	0.904Aa	1.156Aa	0.901Aa
Asparagine	0.604Aa*	0.542Aa	0.449Aa	0.536Aa	1.000Aa	0.522Ba	0.576Ab	0.486Aa
Aspartate	0.763Aa*	0.762Aa	0.684Aa*	0.658Aa	1.000Aa	0.706Ba	0.845Ab	0.748Aa
Butyrate	1.161Aa	1.061Aa	0.675Bb	1.054Aa	1.000Aa	0.880Aa	1.018Aa	0.961Aa
Citrate	1.272Aa	1.265Aa	1.318Aa	1.161Aa	1.000Ab	1.234Aa	1.429Aa	1.099Ba
Cysteine	0.349Aa	0.305Aa	0.374Aa	0.402Aa	1.000Aa	0.123Aa	0.302Aa	0.216Aa
Dehydroasorbate	1.008Ab	0.911Aa	1.543Aa*	0.719Ba	1.000Aa	0.639Ba	0.979Aa	0.626Aa
Fructose	1.119Ab	1.247Aa*	1.692Aa	1.486Aa*	1.000Ab	0.984Aa	1.696Aa	0.929Ba
Fumaratec	0.892Aa	0.944Aa	0.813Aa	1.061Aa	1.000Aa	0.872Aa	1.007Aa	1.007Aa
Galactinol	1.202Ab	1.192Aa	1.744Aa	1.447Aa*	1.000Ab	0.592Bb	1.337Aa	1.000Ba
Glucose	1.089Ab	1.131Ab	1.573Aa	1.421Aa*	1.000Ab	1.015Aa	1.590Aa	1.015Ba
Glutamate	0.520Ab*	0.643Aa	0.956Aa	0.869Aa	1.000Aa	0.746Aa	1.133Aa	0.727Ba
Glutamine	0.707Aa	0.510Ab	0.823Aa	0.708Ba	1.000 Aa	0.457Aa	0.580Aa	0.616Aa
Glutarate	1.128Ab	1.228Aa	1.526Aa	1.427Aa*	1.000Aa	1.090Aa	1.347Aa	0.950Ba

Parameters	-Grain				+Grain			
	WT		*lsi*1		WT		*lsi*1	
	-Si	+Si	-Si	+Si	-Si	+Si	-Si	+Si
2-Oxoglutarate	0.894Aa	1.037Aa	0.572Ab	0.848Aa	1.000Aa	0.991Aa	0.880Aa	0.954Aa
Glycerate	0.916Aa	0.905Aa*	1.070Aa	0.932A	1.000Ba	1.283Aa	1.161Aa	1.141Aa
Glycerol	1.515Aa*	1.298Aa*	1.429Aa	1.220Aa	1.000Ab	0.957Aa	1.549Aa	1.133Ba
Glycine	1.157Aa	0.978 Aa	1.364 Aa*	1.070 Aa	1.000 Aa	1.203Aa	0.824 Aa	0.952 Aa
Myo inositol	0.969 Aa	0.959 Aa	1.073 Aa	0.946 Aa	1.000 Aa	0.950 Aa	1.073 Aa	0.986 Aa
Isocitrate	1.028Ab	0.630Ba	1.318Aa	0.860Ba	1.000Aa	0.726Bb	1.124Aa	1.006Aa
Isoleucine	0.472Aa*	0.493Aa	0.513Aa*	0.773Aa*	1.000Aa	0.283Ab	0.915Aa	1.091Aa
Lactate	1.320Aa	1.249Aa*	0.654Ab*	0.844Ab	1.000Ab	0.696Aa	1.506Aa	0.782Ba
Lysine	0.542Aa*	0.557Aa	0.668Aa	0.780Aa	1.000Aa	0.418Ba	0.795Ab	0.814Aa
Malate	1.073Ab	0.908Aa*	1.571Aa	0.864Ba	1.000Ba	1.506Aa	1.426Aa	1.073Ab
Maltose	0.906Aa	0.931Aa	1.017Aa	0.883Aa	1.000Aa	0.909Aa	1.050Aa	0.991Aa
Methionine	0.787Ba	0.486Bb	0.983Aa*	1.620Aa*	1.000Ab	0.586Ba	1.672Aa	0.677Ba
Ornithine	0.656Aa	0.240Aa	0.569Aa	0.537Aa	1.000Aa	0.066Ba	0.943Aa	0.266Ba
Phenynalanine	0.699Aa*	0.864Aa	0.683Aa	0.939Aa	1.000Aa	0.630Bb	0.908Aa	0.994Aa
Proline	0.980Aa	0.870 Aa	0.943 Aa	0.742 Aa	1.000 Aa	0.955 Aa	0.954 Aa	0.839 Aa
Putrescine	0.964 Aa	0.976 Aa	0.681 Aa	0.885 Aa	1.000 Aa	1.317 Aa	1.018 Aa	1.066 Aa
Pyroglutamate	0.880 Aa	0.764 Aa	0.816 Aa	0.804 Aa	1.000Aa	0.611Bb	0.831Ab	0.854Aa
Pyruvate	0.796 Aa	0.868Aa	0.591 Ba*	0.919 Aa*	1.000 Aa	0.978 Aa	1.122 Aa	0.627 Bb
Quinate	1.124Aa	0.971Aa	1.174Aa*	0.944Aa	1.000Ab	0.972Aa	1.572Ab	0.681Ba
Serine	0.943Aa	0.876Aa	0.981Aa	0.773Ba	1.000 Aa	0.778 Ba	0.865 Aa	0.859Aa
Shikimate	1.147Aa	0.878Ba	0.687Ab	0.839Aa	1.000Aa	0.939Aa	0.916Aa	0.876Aa
Succinate	0.917Aa	0.922Aa	0.941Aa	0.785Aa	1.000Aa	0.800Aa	1.170Aa	0.931Aa
Sucrose	1.232Ab	1.675Ab*	2.739Aa	2.601Aa*	1.000Aa	0.877Aa	2.55Ab	1.005Ba
Threonine	0.804Aa	0.739Aa	0.632Aa	0.605Aa	1.000Aa	0.577Ba	0.753Ab	0.641Aa
Trehalose	0.727Aa	0.927Aa	0.928Aa	0.966Aa	1.000Aa	0.868Aa	0.906Aa	1.099Aa
Tryptophan	1.092Bb	1.443Aa	1.870Aa	1.682Aa*	1.000Ab	1.160Aa	1.883Aa	0.894Ba
Tryosine	0.584Aa	0.839Aa	0.662Aa	0.793Aa	1.000Aa	0.421Bb	0.720Aa	0.891Aa
Valine	1.112Aa	0.840Aa*	0.544Ab*	0.512Aa	1.000Ab	0.433Ba	1.393Aa	0.599Ba

Table S2. Over-representation analysis of the principal component analysis (PCA) loadings of metabolites with a main impact on the variance of the data set. ANOVA *P*-values express the statistical significance of the changes in primary metabolite contents.

Parameters		Canonical variable	
		First	Second
Amino acids	Alanine	-52.671	18.500
	Arginine	-14.210	-26.53
	Asparagine	26.830	6.484
	Aspartate	-87.910	-28.904
	Glutamate	17.627	4.803
	Glutarate	-30.147	-10.600
	Isoleucine	-7.792	12.694
	Methionine	29.253	12.883
	Ornithine	-42.341	-14.664
	Treonine	118.077	16.132
	Tryptophan	10.056	10.834
	Valine	22.852	4.173
Organic acids	Ascorbate	-3.307	-4.695
	Dehydroascorbate	35.496	0.951
	Glycerate	-12.410	-3.241
	Isocitrate	-0.392	1.469
	Lactate	-20.372	-10.369
	Malate	21.584	7.590
	Pyroglutamate	-35.931	20.863
	Pyruvate	4.757	-17.740
	Quinate	29.361	8.167
Sugars	Fructose	45.670	-8.522
	Glucose	-94.136	1.581
	Sucrose	12.722	0.063
Sugar alcohols	Galactinol	-12.647	-6.320
	Glycerol	22.410	-3.242
Others	Shikimate	18.296	-5.753
	Putrescine	-32.114	-9.963
Eigen value		331.78	85.10
Relative importance (%)		66.57	17.07

Table S3. Pairwise correlation coefficients, with their corresponding *P* values, calculated between the concentrations of Si and all other metabolites. Correlations were determined using a combined data set including data obtained from treated and control samples.

Parameters	***R***	***P***	Parameters	***r***	***P***
		Amino acids			
Alanine	-0.512	**0.001**	Lysine	-0.303	0.057
Arginine	-0.366	**0.020**	Methionine	-0.480	**0.002**
Aspartate	-0.204	0.207	Ornithine	-0.489	**0.001**
Asparagine	-0.099	0.542	Phenylalanine	-0.056	0.733
Glutamate	-0.268	0.095	Proline	-0.171	0.292
Glutamine	-0.403	**0.010**	Serine	-0.325	0.041
Glutarate	-0.105	0.520	Tyrosine	-0.177	0.275
Glycine	-0.014	0.930	Treonine	-0.236	0.145
Cysteine	-0.155	0.340	Tryptophan	-0.170	0.294
Isoleucine	-0.353	**0.025**	Valine	-0.375	**0.017**
		Organic acids			
Aconitate	-0.178	0.272	Isocitrate	-0.663	**<0.001**
Ascorbate	-0.198	0.222	Lactate	-0.156	0.335
GABA	0.066	0.686	Malate	-0.115	0.481
Citrate	-0.081	0.619	Pyroglutamate	-0.438	**0.005**
Dehydroascorbate	-0.407	**0.009**	Pyruvate	0.074	0.652
Fumarate	-0.026	0.876	Succinate	-0.253	0.115
2-Oxoglutarate	0.304	**0.057**	Quinate	-0.335	**0.035**
Glycerate	0.191	0.238			
		Sugars			
Fructose	-0.302	**0.058**	Sucrose	-0.265	0.098
Glucose	-0.299	**0.061**	Trehalose	0.083	0.612
Maltose	-0.205	0.204			
		Sugar alcohols			
Galactinol	-0.489	**0.001**	Myo-inositol	-0.266	0.097
Glycerol	-0.317	**0.046**			
		Others			
Putrescine	0.276	0.085	Shikimate	-0.027	0.869

CONCLUSÃO

Em contraste com a hipótese de que a modificação do ângulo foliar seria o principal responsável pelo aumento da produtividade do arroz (*Oryza sativa* L.) pelo Si, os resultados apresentados sugerem que a adição desse elemento, particularmente durante o estádio reprodutivo, promove mudanças fisiológicas que culminam no aumento da produção. O Si modifica as relações fonte-dreno (aumento do número de grãos por panícula) e, por conseguinte, estimula a atividade fotossintética da folha fonte, fato associado fundamentalmente a incrementos na condutância mesofílica. Adicionalmente, demonstrou-se que o Si altera o metabolismo primário em plantas de arroz intensificando a remobilização de aminoácidos para os drenos, especialmente nas plantas do tipo selvagem. Resta investigar os mecanismos fisiológicos associados com o Si e o aumento dos componentes da produção em arroz.