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Silicon-induced root nodulation and synthesis of essential amino acids in a legume is associated with higher herbivore abundance

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Silicon-induced root nodulation and synthesis of essential amino acids in a legume is associated with higher herbivore abundance

Scott N. Johnson[1*], Susan E. Hartley[2], James M.W. Ryalls[1], Adam Frew[1], Jane L. DeGabriel[3], Michael Duncan[4] and Andrew N. Gherlenda[1]

[1] Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, NSW, 2751, Australia

[2] York Environment and Sustainability Institute, Department of Biology, University of York, York YO10 5DD, United Kingdom

[3]New South Wales Office of Environment and Heritage, PO Box 1967, Hurstville NSW, 1481, Australia

[4]School of Science and Health, Western Sydney University, Locked Bag 1797, Penrith, NSW, 2751, Australia

*Corresponding author: Scott Johnson, Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, NSW, 2751, Australia (Scott.Johnson@westernsydney.edu.au)

Running headline: Silicon indirectly promotes aphid abundance

Summary

1. Ecologists have become increasingly aware that silicon uptake by plants, especially the Poaceae, can have beneficial effects on both plant growth and herbivore defence. The effects of silicon on other plant functional groups, such as nitrogen-fixing legumes, have been less well studied. Silicon could, however, indirectly promote herbivore performance in this group if reported increases in N_2-fixation caused improvements in host plant quality for herbivores.
2. We tested how silicon supplementation in the legume *Medico sativa* affected plant growth rates, root nodulation and foliage quality (silicon content and amino acid profiles) for an insect herbivore (*Acyrthosiphon pisum*).
3. Plants supplemented with silicon (Si+) grew three times as quickly as those without supplementation (Si-), almost entirely in shoot mass. While root growth was unaffected by silicon uptake, root nodules containing nitrogen-fixing bacteria were 44% more abundant on Si+ plants. Aphid abundance was twice as high on Si+ plants compared to Si- plants and was positively correlated with silicon-stimulated plant growth.
4. Si+ plants accumulated more than twice as much silicon as Si- plants, but did not have higher silicon *concentrations* because of dilution effects linked to the rapid growth of Si+ plants. Si+ plants showed a 65% increase in synthesis of essential foliar amino acids, probably due to increased levels of root nodulation.
5. These results suggest that increased silicon supply makes *M. sativa* more susceptible to *A. pisum*, mainly because of increased plant growth and resource availability (i.e. essential amino acids). While silicon augmentation of the Poaceae frequently improves herbivore defence, the current study illustrates that this cannot be assumed for other plant families

where the beneficial effects of silicon on plant growth and nutrition may promote herbivore performance in some instances.

Key-words: amino acids, aphids, legume, nodulation, nitrogen fixation, plant defence, silica, silicon

Introduction

Plant silicon has multiple functional roles in plants and it is unusual in that it has been suggested to provide a particularly broad range of benefits to plants, including resistance to both abiotic and biotic stresses (Cooke, DeGabriel & Hartley 2016; Cooke & Leishman 2016). These include drought and salinity (Liang et al. 2007; Ma & Yamaji 2008), herbivores (Reynolds, Keeping & Meyer 2009; Hartley & DeGabriel 2016) and diseases (Fauteux et al. 2006). Silicon is the second most abundant element in the Earth's crust, which plants acquire from the soil via uptake of soluble silicic acid (Ma & Yamaji 2008). The Poaceae, in particular, often accumulate large amounts of silicon, sometimes in excess of 10% of dry mass, more than any other inorganic constituent (Epstein 1999). The process of silicon uptake and deposition is still incompletely characterised, but both active and passive transport mechanisms have been identified in a range of crop species (Ma et al. 2006; Hartley 2015; Deshmukh & Belanger 2016). Silicon can be deposited within or between cells, in the cell wall or as discrete opaline phytoliths (Cooke & Leishman 2011). Deposition patterns can be altered markedly by changes in silicon supply (Hartley et al. 2015).

Agronomists have recognised since the 1960s that applying silicon to soils can dramatically increase rates of plant growth with benefits for crop yield in a range of systems (Epstein 1999; Guntzer, Keller & Meunier 2012). However, most of these studies demonstrated these benefits in the Poaceae, where silicon can be used as a structural material to support more erect growth as well as increased rigidity and resistance to lodging (Schoelynck et al. 2010; Stromberg, Di Stilio &

Song 2016). It is also metabolically 'cheaper' than other structural biochemicals (e.g. lignin), so this silicon-supported upright growth potentially allows plants to increase photosynthetic efficacy and compete more effectively for light and space (Schoelynck et al. 2010; Stromberg et al. 2016). However, the existence of these benefits is less-well characterised in other plant functional groups such as nitrogen-fixing legumes, though there have been some studies which demonstrate growth increases in silicon supplemented legumes (Horst & Marschner 1978; Miyake & Takahashi 1985; Guo et al. 2006). In addition to improving plant structure, silicon supplementation may have another potential benefit for legume growth via increased rates of root nodulation and symbiosis with nitrogen fixing bacteria (Nelwamondo & Dakora 1999; Mali & Aery 2008). Increased nodulation and biological nitrogen fixation, however, frequently makes legumes more susceptible to herbivory via increased plant growth and nitrogen availability in the plant (e.g. Gerard 2001; Johnson & McNicol 2010; Guo et al. 2013). This seems especially true for fluid-feeding aphids, which benefit from fluxes in amino acid concentrations in the phloem sap (e.g. Johnson, Ryalls & Karley 2014; Ryalls et al. 2016). Silicon supplantation may therefore indirectly promote the performance of some herbivores if it causes such changes in the host plant.

In addition to promoting plant growth, silicon deposition in plant tissues has been shown to be an effective defence against both vertebrate (Massey & Hartley 2006; Wieczorek et al. 2015) and invertebrate herbivores (Keeping & Meyer 2006; Massey, Ennos & Hartley 2006; Frew et al. 2016). Silicon negatively affects herbivores via abrasive effects on herbivore mouthparts (Massey & Hartley 2009; but see Kvedaras et al. 2009), reduced digestive efficiency (Massey et al. 2006; Massey & Hartley 2006; Wieczorek et al. 2015) and induction of secondary metabolites (Reynolds et al. 2009), which can include metabolites involved in indirect defences (e.g. recruitment of herbivore natural enemies) (Kvedaras et al. 2010).

The range of effects of silicon supplementation on legumes presents a dichotomy whereby increasing silicon availability could either make legumes better defended against herbivores via increased silicon defences in the foliage (Reynolds et al. 2009) or make them more nutritious for herbivores via increased nodulation increasing nitrogen availability (Mattson 1980). The relative balance of these two effects, operating either directly (e.g. defence) or indirectly (e.g. nutritional quality), has not yet been tested experimentally and is the rationale for this study.

The objective of this study was to determine whether increasing silicon availability for lucerne (*Medicago sativa* L.) affected an insect herbivore (the aphid *Acyrthosiphon pisum*) either via increased silicon uptake into the foliage and/or altered concentrations of amino acids in the foliage. Based on observations that silicon can increase root nodulation in legumes (Nelwamondo & Dakora 1999; Mali & Aery 2008), we predicted that silicon supplementation would increase plant growth rates and root nodulation, which would increase foliar amino acid concentrations and consequently herbivore performance.

Materials and Methods

Experimental Procedure

Lucerne (*M. sativa* L., cv. Sequel) plants were grown from seed at 26/18ºC (day:night) in a glasshouse receiving supplemental light (15:9 light:dark) and humidity controlled at 55% (see Ryalls et al. 2013 for further technical details). Eighty eight plants were grown in 70 mm diameter pots that were 135 mm deep (allowing unrestricted root growth for the duration of the experiment). Each contained c. 700g of soil excavated from the Hawkesbury campus of Western Sydney University (latitude −33.608847, longitude 150.747016). The soil is typified as low-fertility sandy loam in the Clarenden Formation (Chromosol), which has low bioavailable Si content of 17 mg kg^{-1} (see Barton et al. 2010 for full details). Sandy loam soils typically contain c. 35 mg kg^{-1} of

bioavailable Si (Environmental Analysis Laboratory, Southern Cross University personal communication). Plants were irrigated with c. 70 ml of tap water (3ppm Si) three times a week. After two weeks of growth, half of the plants continued to receive tap water at the same intervals while the other half received 70 ml of 500 mg l^{-1} soluble Si in the form of $NaSiO_3.9H_2O$ (Sigma-Aldrich, Castle Hill, NSW, Australia) three times a week for six weeks. $NaSiO_3.9H_2O$ has been used in numerous studies to increase silicification of leaves and deter herbivores (reviewed by Reynolds et al. 2009). When plants were six weeks old, 22 of the plants receiving the silicon supplementation and 22 of the plants receiving tap water were each inoculated with two teneral female *A. pisum*. Plants were configured in a randomised fashion in the glasshouse. *Acyrthosiphon pisum* used in the experiment were taken from an established culture originating from an individual parthenogenetic adult female collected from a lucerne field on campus (Ryalls et al. 2017). Cultures were maintained on spare lucerne plants (cv. Sequel) for at least six generations prior to the inoculation period.

White mesh (organza) bags (125 x 170 mm) were applied tightly around the rim of all pots confining aphids to their allocated plants. After two weeks, bags were removed from all plants, aphid colonisation assessed (% plants with > 1 aphid present) and individuals removed with a fine paintbrush to be counted. Plants were separated and cleaned free of soil with water before measuring shoot height, maximum rooting depth and counting the number of root nodules (housing nitrogen-fixing bacteria). Plants were freeze dried for 48 hr and weighed. Leaves were separated from the stems and ball-milled to a fine power prior to chemical analysis.

Chemical analysis

Chemical analysis was conducted on insect-free plants (44 of the 88 plants). To provide enough dried leaf material to perform both amino acid and silicon analysis to be conducted on the same

plants it was necessary to pool foliar samples (2-3 plants per sample). This resulted in nine replicates of Si+ and Si- plants. Foliar silicon concentrations were analysed with X-ray fluorescence spectrometry according to the methodology set out in Reidinger, Ramsey & Hartley (2012). In brief, plant material was ground to a fine powder and pressed into 13mm-diameter pellets. Following the methods of Reidinger et al. (2012), foliar silicon concentration was determined using a Niton XL3t XRF analyzer (Thermo Fisher Scientific, Inc., MA, USA), for a measurement time of 30 seconds. Results were expressed as foliar silicon concentration (as % of dry mass), calibrated against plant-certified reference material of known silicon content (Garbuzov, Reidinger & Hartley 2011).

Soluble amino acids were extracted and analysed from milled foliar samples (10-15 mg) following the protocol set out by Ryalls et al. (2015). Foliar amino acid composition is tightly correlated with phloem amino acids in *M. sativa*, so analysing foliar material is a reliable proxy for phloem quality (Ryalls et al. 2017). Amino acid standards within the AAS-18 (Fluka, Sigma-Aldrich) reference amino acid mixture were supplemented with asparagine and glutamine (A0884 and G3126, respectively, from Sigma, Sigma-Aldrich). Nine essential amino acids (i.e. those that cannot be synthesised by insects *de novo*), including arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine (Morris 1991) and 10 non-essential amino acids (alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine and tyrosine) were detected using this method.

Statistical analysis

The effects of silicon supplementation and aphid inoculation and the interaction between these terms on plant dry mass, shoot height, rooting depth, root nodule abundance and root nodules per cm of roots were analysed with two way analysis of variance (ANOVA) tests. Differences in silicon content (log transformed) and concentrations between treatment groups were analysed with a

one-way ANOVA (silicon supplementation included as a fixed factor). The effects of Si addition on aphid colonisation success and aphid abundance were analysed with generalised linear models with binomial error structure and a logit link function and Poisson error structure with a log link function, respectively. Silicon supplementation was included as a fixed effect and the dispersion parameter was estimated. Pearson's correlation tests were used to explore relationships between plant parameters and aphid abundance. Where appropriate, data transformations (see Figure and Table legends for details) were chosen to meet model assumptions and give residual diagnostic plots which fitted a normal distribution and showed least heteroscedasticity. Permutational multivariate analysis of variance (PERMANOVA) was used to explore the impacts of silicon supplementation on amino acid composition and concentrations for total, essential and non-essential amino acids. ANOVA and generalised linear models were performed with Genstat (version 17, VSN International, UK), whereas the PERMANOVA was conducted in R v3.3.1 using the using the R-package vegan (Oksanen et al. 2017).

Results

Silicon supplementation (Si+ plants) increased plant biomass almost three-fold compared to those grown without silicon supplementation (Si- plants), whereas the presence of aphids had no significant impact on plant biomass (Fig. 1 A). This increase in biomass in Si+ plants was attributed to increased growth in the shoots rather than the roots, the latter being similar in Si+ and Si- plants (Table 1). Plants inoculated with aphids grew slightly less tall than those without (Table 1). Silicon supplementation also resulted in c. 44% increase in root nodulation (Fig. 1B), which was reflected in higher nodule density on the roots (Table 1). Nodulation was unaffected by aphids (Fig. 1B, Table 1).

Aphid colonisation success was similar on Si- and Si+ plants (73% and 64%, respectively; $F_{1,42}$ = 0.42, P = 0.517). Aphids were substantially more abundant (+112%) on Si+ plants than Si- plants (Fig. 2). Moreover, aphid abundance was positively correlated with plant biomass (Fig. 3A) and plant height (Fig. 3B).

Si+ plants accumulated over twice as much silicon as Si- plants (mean ± standard error: 262 ± 48.14 mg and 124.35 ± 14.05, respectively) ($F_{1,16}$ = 12.05, P = 0.003). However, because Si+ plants grew so much bigger than Si- plants, this diluted foliar silicon concentrations by 26% in Si+ plants (Fig. 4A). PERMANOVA analysis showed that Si+ plants had 65% higher concentrations of essential amino acids than Si- plants (Fig. 4B). Overall there was no difference in total and non-essential amino acid concentrations between the two groups, but there was a significant increase in the concentrations of 11 of the 18 amino acids tested in the silicon treated plants compared to controls (Fig. S1; Table S1).

Discussion

This study has shown that silicon supplementation caused a significant increase in root nodulation in a legume, despite no increase in root length. This contributed to silicon-induced increases in shoot biomass and enhanced foliar resources in the form of essential amino acids. While silicon accumulation increased substantially in silicon supplemented plants, foliar silicon concentrations were diluted due to this rapid increase in plant growth. These silicon-induced changes to plant physiology and chemistry were associated with significant increases in herbivore abundance.

Enhanced growth of the meristems and increased synthesis of essential amino acids were the most likely drivers of increased aphid abundance on Si+ plants. Aphids often feed on actively growing stems because nutrients, specifically amino acids, are being translocated to these tissues for growth

(Raven 1983; Dixon 1998; Johnson, Elston & Hartley 2003). We found a positive correlation between aphid abundance and plant growth (both dry mass and plant height), which supports the conclusion that aphids performed better on the faster growing plants. Silicon supplementation also increased concentrations of essential amino acids in the foliage by 65% relative to plants without silicon supplementation. The concentration of essential amino acids in the phloem sap of plants is comparatively low (10-30% of total protein amino acids; Douglas 2003), so silicon-induced increases could promote aphid performance. In particular, >90% of total amino acid content of aphid tissues are essential amino acids (Douglas 2003) so this group is very important for aphid nutrition. We did not attempt to directly relate aphid abundance to amino acid concentrations of specific plants on which aphids were feeding because aphids themselves have large qualitative and quantitative effects on phloem amino acids (Douglas 2003), particularly in *Medicago* spp. (Guo et al. 2013). This confounds interpretation of how silicon supplementation was affecting amino acid concentrations on aphid-infested plants, not least because aphid populations were much larger on Si+ plants (and possibly having larger impacts on amino acids than on Si- plants). Nonetheless, it seems likely that silicon-induced increases in foliar essential amino acids were at least partly responsible for increased aphid abundance.

While silicon has been shown to increase root nodulation and nitrogen fixation in legumes (Nelwamondo & Dakora 1999; Dakora & Nelwamondo 2003; Mali & Aery 2008), the mechanisms for this have yet to be demonstrated. The simplest explanation is that increased root growth increases potential invasion sites for rhizobial bacteria (Mali & Aery 2008), but we found that silicon increased nodule density and had little impact on root growth *per se*. Using comparable application rates (480 mg l^{-1}) as the present study (500 mg l^{-1}), Nelwamodo & Dakora (1999) similarly found that nodule density increased on sand-grown cowpea (*Vigna unguiculata*) plants.

In a follow up study, Nelwamondo et al. (2001) showed that silicon supplementation increased the abundance of bacteroids and symbiosomes (the plant-derived membrane that encases the bacteroids) in root nodules, which they suggested could explain enhanced N_2 fixation. They also found that silicon increased cell wall thickness of root nodules which appeared to decrease intercellular spaces which should, in theory, reduce N_2 fixation because of lower gaseous diffusion (Nelwamondo et al. 2001). The fact that N_2-fixation actually increased led Nelwamondo et al. (2001) to speculate that silicification reduced the need for lignin, which is costly in terms of carbon; carbon could then be channelled into bacteroid respiration to increase N_2-fixation. It is also possible that carbon could be channelled towards enhanced nodule organogenesis. Nelwamondo et al. (2001) also proposed that silicon supplementation had parallels with phosphorus fertilisation in terms of stimulating root nodulation, including increased production of compounds that upregulate nodulation genes (Dakora & Nelwamondo 2003). This has yet to be tested.

The efficacy of silicon defence against sap-feeders, such as aphids, appears less clear cut than on chewing herbivores and has been the subject of debate (Massey et al. 2006; Keeping & Kvedaras 2008; Kvedaras et al. 2009). Published studies have shown silicon has either no (e.g. Hogendorp, Cloyd & Swiader 2009; Cherry et al. 2012; Keeping, Miles & Sewpersad 2014) or negative (e.g. Gomes et al. 2008; Costa, Moraes & DaCosta 2011; Dias et al. 2014) effects on this feeding guild. The variation in these published findings probably reflects the fact that whilst silicon can negatively affect aphids via epidermal resistance to stylet penetration, aphids can circumvent some of the consistent negative impacts reported for chewing herbivores (e.g. mandible wear and impaired food breakdown) simply by virtue of their feeding behaviour (i.e. fluid acquisition via a stylet). Further experimental work and meta-analysis of existing studies would help to address the extent to which different herbivore guilds are affected by silicon defences. In the present study, silicon

application did not affect aphids adversely because Si+ plants did not have higher concentrations of silicon in the foliage.

In addition to promoting physical defences at the leaf surface, silicon can also stimulate phytohormonal pathways in the plant, such as the jasmonic acid (JA) pathway (Ye et al. 2013), which underpins synthesis of secondary metabolites with defensive functions (Wu & Baldwin 2010). Stimulation of the JA pathway is known to supress the salicylic acid (SA) pathway, which is often triggered by phloem-feeding herbivores and can result in plants mounting a defensive response against these herbivores (Ode, Johnson & Moore 2014). We still know very little about how silicon affects other plant defences, but if silicon application were to stimulate the JA pathway (and supress the SA pathway), this may have also contributed to the success of aphids on silicon supplemented plants in this study. We can conclude, however, that if defences were activated by silicon application, they had minimal impacts on aphids in this system.

This study has shown that lucerne plants benefitted from silicon supplementation in terms of both increased growth and resource acquisition via root nodulation, whereas the concentrations of silicon in the foliage tended to decrease in silicon supplemented plants. Aphid abundance increased substantially on these faster-growing plants. There a number of hypotheses considering the relationships between resources (nitrogen, water), plant growth and herbivory (reviewed by Hartley & Jones 1997). Our results are consistent with the resource availability hypothesis (Coley, Bryant & Chapin 1985), which predicts that faster growing plants, with increased access to resources (e.g. either through silicon fertilisation or biologically fixed nitrogen) will be less well defended against herbivores. Increasing the rate of silicon supplementation may increase foliar silicon concentrations, but increasing the concentrations of silicon application much beyond those used in this study would most likely inhibit nodulation and N_2-fixation, as has been reported for

cowpea plants (Mali & Aery 2008). In that study, high doses of silicon application reduced calcium concentrations in the plant; calcium increases biosynthesis of isoflavonoid nodulation signals in legumes (Dakora & Phillips 1996) so it was suggested this was the reason for high silicon application diminishing nodulation (Mali & Aery 2008).

To our knowledge, this is the first study to report beneficial effects of silicon addition on herbivore performance which arose indirectly via promotion of root nodulation and amino acid biosynthesis. Further work on non-grass species is needed, but our results suggest that in some functional groups of plants, such as legumes, the positive effects of silicon on plant growth and nitrogen acquisition may outweigh any additional defensive function of silicon supplementation. Our findings suggest that silicon supplementation of legumes may not provide the benefits that such applications provide to grasses such as cereals (Guntzer et al. 2012), at least in part because the positive effects on nodulation, nutrient content and yield may make these crops more susceptible to aphids pests and the diseases they vector. There is an increasing need to extend the use of legumes in agriculture because of their benefits to soil fertility, so understanding how silicon promotes nodulation and N_2-fixation and its indirect impact on plant susceptibility to herbivores could be a promising area of research.

Author contributions

SNJ, JMWR, AF and ANG conceived the experimental design. SNJ, JMWR, AF, JDG, MD and ANG acquired and processed data with JMWR and SEH specifically undertaking chemical analysis. SNJ and JMWR analysed the data and all authors contributed to the interpretation and drafting of the manuscript.

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Data accessibility

Data from this paper are available at the Dryad digital repository doi:

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462

463

465 **Table 1.** Plant (shoot) height, maximum rooting depth and number of root nodules per cm of root
466 length in response to silicon supplementation (Si+) and without silicon supplementation (Si-) and
467 aphid inoculation.

Silicon supplementation	Aphid inoculation	Plant (shoot)[1] height (cm)	Maximum rooting depth (cm)	Root nodules per cm of root[2]
Si-	Control	7.52 ± 0.76	10.06 ± 1.01	0.68 ± 0.10
	Aphids	6.70 ± 0.60	11.47 ± 0.79	0.35 ± 0.05
Si+	Control	13.84 ± 1.32	11.40 ± 1.17	0.78 ± 0.19
	Aphids	10.22 ± 0.93	9.92 ± 1.11	0.86 ± 0.20
Statistical analysis				
	Silicon	$F_{1,84}$ = 27.11, P < 0.001	$F_{1,84}$ = 0.01, P = 0.918	$F_{1,84}$ = 4.19, P = 0.044
	Aphids	$F_{1,84}$ = 3.99, P = 0.049	$F_{1,84}$ = 0.01, P = 0.970	$F_{1,84}$ = 0.71, P = 0.401
	Silicon x Aphids	$F_{1,84}$ = 1.12, P = 0.293	$F_{1,84}$ = 1.12, P = 0.164	$F_{1,84}$ = 1.82, P = 0.181

468 [1] log transformed
469 [2] log+1 transformed
470

471

Figure Legends

Figure 1. Impacts of silicon supplementation and aphid inoculation on the (A) biomass (log transformed) and (B) abundance of root nodules on *M. sativa*. Mean values ± standard error shown. N = 22.

Figure 2. Impact of silicon supplementation on aphid (*A. pisum*) abundance on colonised plants. Mean values per plant ± standard error shown. N = 14 (Si-) and N = 16 (Si+).

Figure 3. Correlations between aphid abundance and (A) plant biomass and (B) plant (shoot) height. N= 30. Solid lines represent linear regression through all the points. Dashed lines represent 95 % confidence intervals

Figure 4. Impacts of silicon supplementation on foliar (A) silicon concentrations (logit transformed) and (B) essential amino acids in *M. sativa*. Mean values ± standard error shown. N = 9.

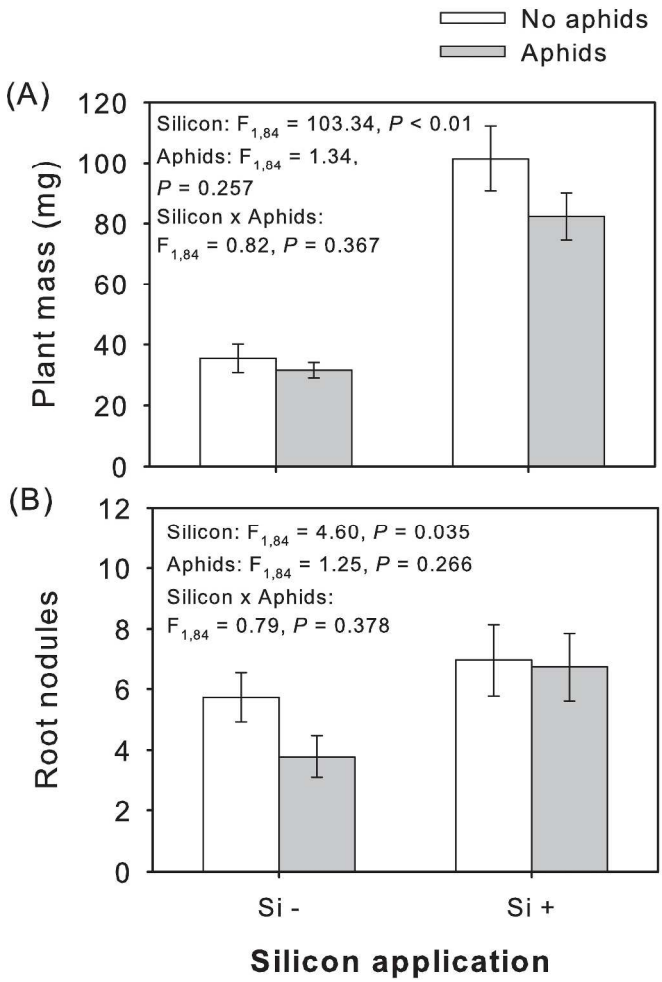

Figure 1

296x420mm (300 x 300 DPI)

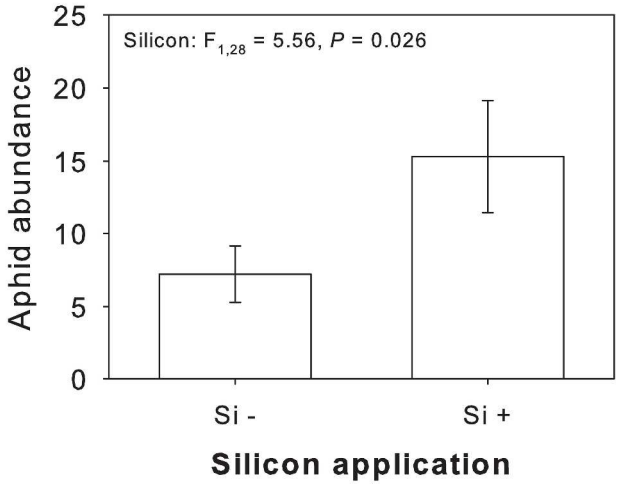

Figure 2

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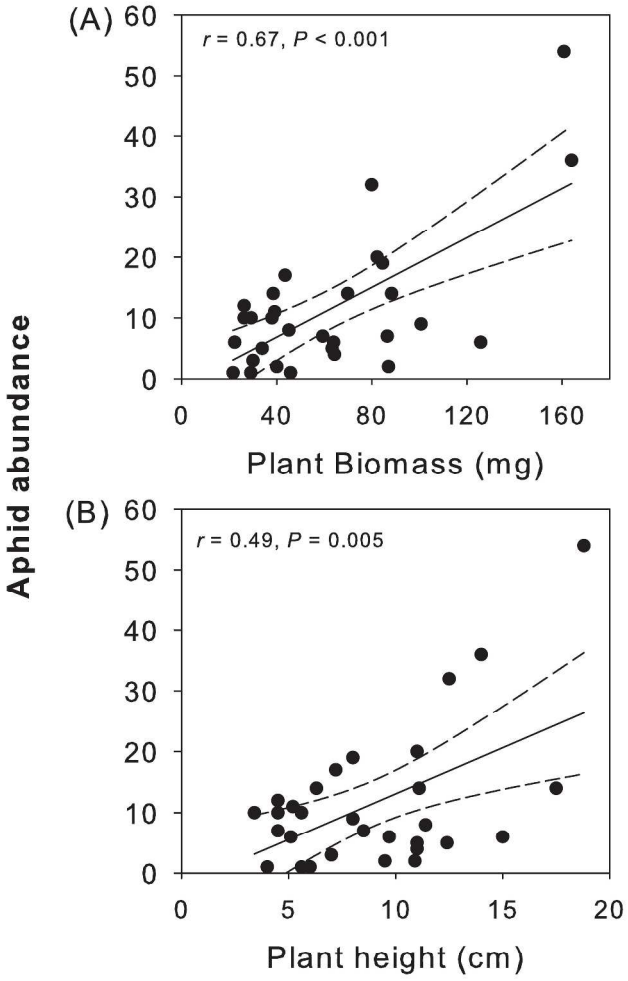


Figure 3

296x420mm (300 x 300 DPI)

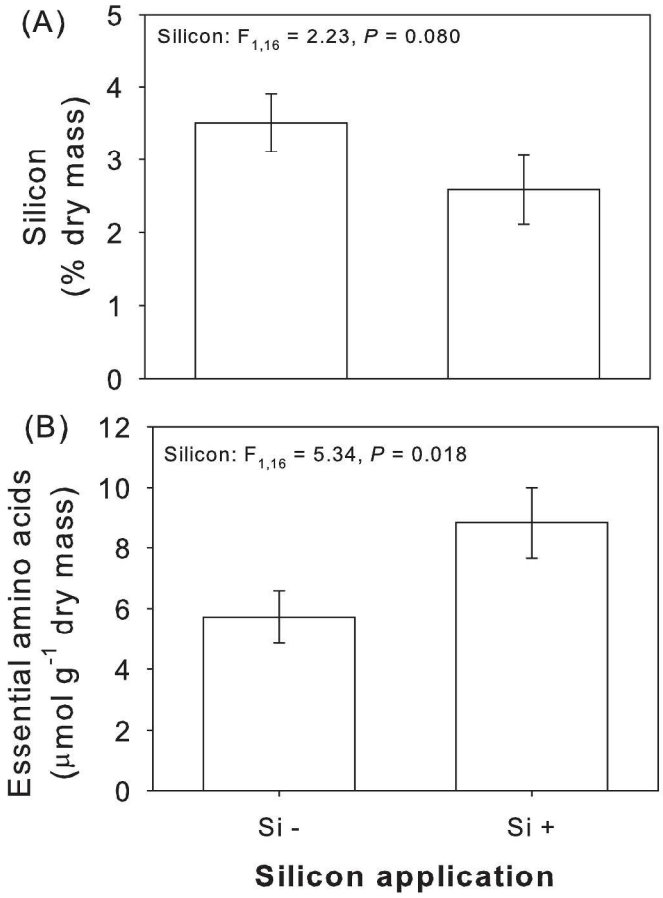


Figure 4

296x420mm (300 x 300 DPI)

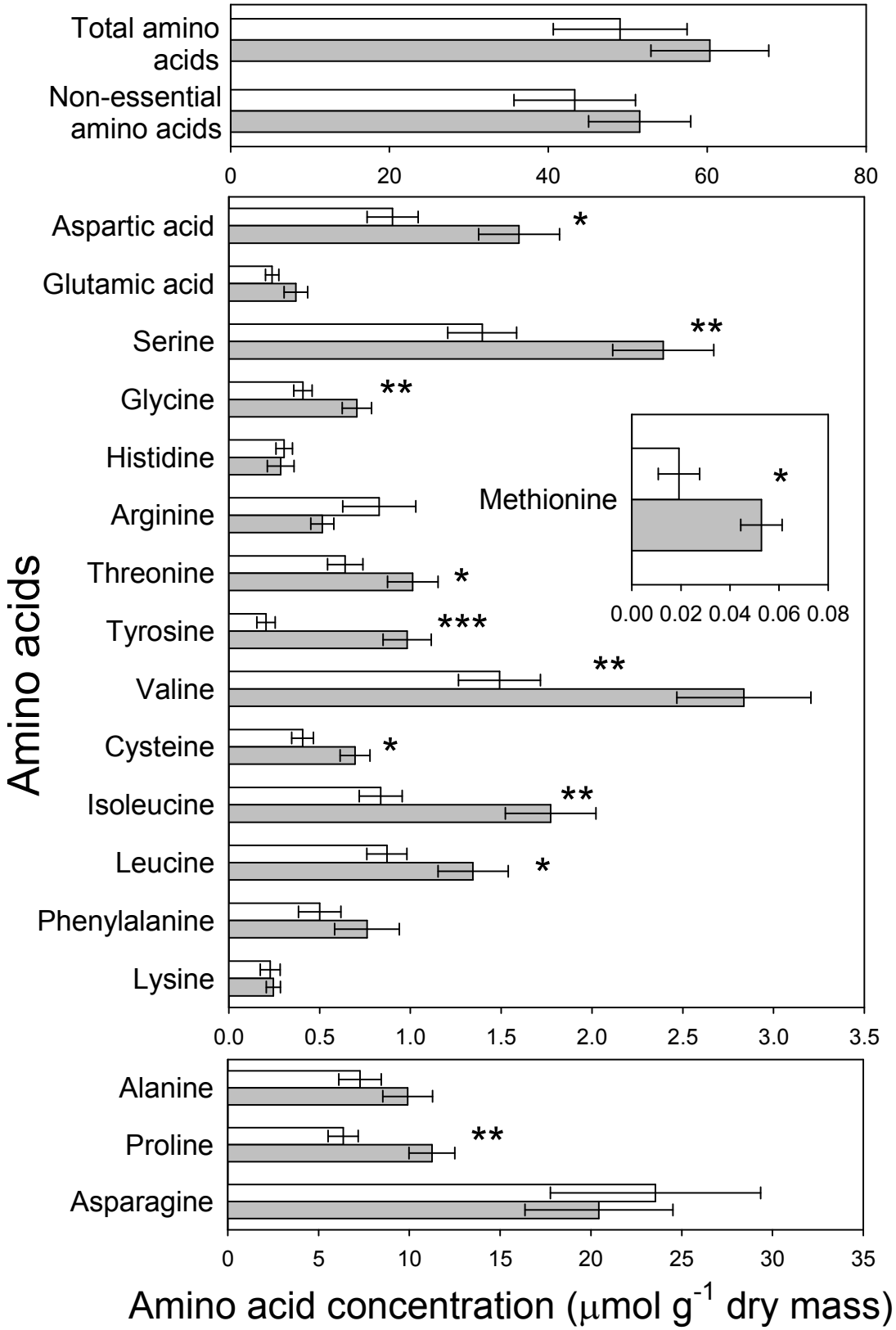
Supplemental Material

Table S1. Statistical analysis for amino acid concentrations shown in Figure S1. Statistically significant differences between silicon supplemented and non-supplemented plants shown in bold.

Amino acids	**$F_{1,16}$**	***P***
Total amino acids	1.82	0.163
Non-essential amino acids	1.44	0.244
Aspartic acid	**7.01**	**0.018**
Glutamic acid	3.02	0.102
Serine	**8.79**	**0.009**
Glycine	**9.63**	**0.007**
Histidine	0.05	0.829
Arginine	2.20	0.157
Threonine	**4.83**	**0.043**
Tyrosine	**29.99**	**< 0.001**
Valine	**9.69**	**0.007**
Cysteine	**8.15**	**0.01**
Isoleucine	**11.50**	**0.004**
Leucine	**4.54**	**0.04**
Phenylalanine	1.49	0.24
Lysine	0.62	0.81
Methionine	**5.69**	**0.03**
Alanine	2.11	0.17
Proline	**10.48**	**0.005**
Asparagine	0.19	0.67

Supplemental Figure

Figure S1. Impacts of silicon supplementation (filled bars represent Si+ plants, open bars are Si- plants) on foliar amino acids grouped by total amount, non-essential and individual amino acids. Mean values ± standard error shown. N = 9. Statistically significant difference between plants (see Table S1) indicated * P <0.05, ** P < 0.01, *** P < 0.001.


Total amino acids
Non-essential amino acids
0
20
40
60
80
Aspartic acid
Glutamic acid
Serine
Glycine
Histidine
Arginine
Threonine
Tyrosine
Valine
Cysteine
Isoleucine
Leucine
Phenylalanine
Lysine
Methionine
0.00 0.02 0.04 0.06 0.08
Amino acids
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Alanine
Proline
Asparagine
0
5
10
15
20
25
30
35
Amino acid concentration (μmol g-1 dry mass)