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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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Running headline: Silicon indirectly promotes aphid abundance

1   **Summary**

2   1. Ecologists have become increasingly aware that silicon uptake by plants, especially the

3   Poaceae, can have beneficial effects on both plant growth and herbivore defence. The

4   effects of silicon on other plant functional groups, such as nitrogen-fixing legumes, have

5   been less well studied. Silicon could, however, indirectly promote herbivore performance in

6   this group if reported increases in N_2-fixation caused improvements in host plant quality for

7   herbivores.

8   2. We tested how silicon supplementation in the legume *Medico sativa* affected plant growth

9   rates, root nodulation and foliage quality (silicon content and amino acid profiles) for an

10   insect herbivore (*Acyrthosiphon pisum*).

11   3. Plants supplemented with silicon (Si+) grew three times as quickly as those without

12   supplementation (Si-), almost entirely in shoot mass. While root growth was unaffected by

13   silicon uptake, root nodules containing nitrogen-fixing bacteria were 44% more abundant

14   on Si+ plants. Aphid abundance was twice as high on Si+ plants compared to Si- plants and

15   was positively correlated with silicon-stimulated plant growth.

16   4. Si+ plants accumulated more than twice as much silicon as Si- plants, but did not have

17   higher silicon *concentrations* because of dilution effects linked to the rapid growth of Si+

18   plants. Si+ plants showed a 65% increase in synthesis of essential foliar amino acids,

19   probably due to increased levels of root nodulation.

20   5. These results suggest that increased silicon supply makes *M. sativa* more susceptible to *A.*

21   *pisum*, mainly because of increased plant growth and resource availability (i.e. essential

22   amino acids). While silicon augmentation of the Poaceae frequently improves herbivore

23   defence, the current study illustrates that this cannot be assumed for other plant families

24   where the beneficial effects of silicon on plant growth and nutrition may promote herbivore

25   performance in some instances.

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

27

28   **Introduction**

29   Plant silicon has multiple functional roles in plants and it is unusual in that it has been suggested to

30   provide a particularly broad range of benefits to plants, including resistance to both abiotic and

31   biotic stresses (Cooke, DeGabriel & Hartley 2016; Cooke & Leishman 2016). These include drought

32   and salinity (Liang et al. 2007; Ma & Yamaji 2008), herbivores (Reynolds, Keeping & Meyer 2009;

33   Hartley & DeGabriel 2016) and diseases (Fauteux et al. 2006). Silicon is the second most abundant

34   element in the Earth's crust, which plants acquire from the soil via uptake of soluble silicic acid (Ma

35   & Yamaji 2008). The Poaceae, in particular, often accumulate large amounts of silicon, sometimes in

36   excess of 10% of dry mass, more than any other inorganic constituent (Epstein 1999). The process

37   of silicon uptake and deposition is still incompletely characterised, but both active and passive

38   transport mechanisms have been identified in a range of crop species (Ma et al. 2006; Hartley 2015;

39   Deshmukh & Belanger 2016). Silicon can be deposited within or between cells, in the cell wall or as

40   discrete opaline phytoliths (Cooke & Leishman 2011). Deposition patterns can be altered markedly

41   by changes in silicon supply (Hartley et al. 2015).

42

43   Agronomists have recognised since the 1960s that applying silicon to soils can dramatically

44   increase rates of plant growth with benefits for crop yield in a range of systems (Epstein 1999;

45   Guntzer, Keller & Meunier 2012). However, most of these studies demonstrated these benefits in

46   the Poaceae, where silicon can be used as a structural material to support more erect growth as

47   well as increased rigidity and resistance to lodging (Schoelynck et al. 2010; Stromberg, Di Stilio &

48   Song 2016). It is also metabolically 'cheaper' than other structural biochemicals (e.g. lignin), so this

49   silicon-supported upright growth potentially allows plants to increase photosynthetic efficacy and

50   compete more effectively for light and space (Schoelynck et al. 2010; Stromberg et al. 2016).

51   However, the existence of these benefits is less-well characterised in other plant functional groups

52   such as nitrogen-fixing legumes, though there have been some studies which demonstrate growth

53   increases in silicon supplemented legumes (Horst & Marschner 1978; Miyake & Takahashi 1985;

54   Guo et al. 2006). In addition to improving plant structure, silicon supplementation may have

55   another potential benefit for legume growth via increased rates of root nodulation and symbiosis

56   with nitrogen fixing bacteria (Nelwamondo & Dakora 1999; Mali & Aery 2008). Increased

57   nodulation and biological nitrogen fixation, however, frequently makes legumes more susceptible

58   to herbivory via increased plant growth and nitrogen availability in the plant (e.g. Gerard 2001;

59   Johnson & McNicol 2010; Guo et al. 2013). This seems especially true for fluid-feeding aphids,

60   which benefit from fluxes in amino acid concentrations in the phloem sap (e.g. Johnson, Ryalls &

61   Karley 2014; Ryalls et al. 2016). Silicon supplantation may therefore indirectly promote the

62   performance of some herbivores if it causes such changes in the host plant.

63

64   In addition to promoting plant growth, silicon deposition in plant tissues has been shown to be an

65   effective defence against both vertebrate (Massey & Hartley 2006; Wieczorek et al. 2015) and

66   invertebrate herbivores (Keeping & Meyer 2006; Massey, Ennos & Hartley 2006; Frew et al. 2016).

67   Silicon negatively affects herbivores via abrasive effects on herbivore mouthparts (Massey &

68   Hartley 2009; but see Kvedaras et al. 2009), reduced digestive efficiency (Massey et al. 2006; Massey

69   & Hartley 2006; Wieczorek et al. 2015) and induction of secondary metabolites (Reynolds et al.

70   2009), which can include metabolites involved in indirect defences (e.g. recruitment of herbivore

71   natural enemies) (Kvedaras et al. 2010).

72

4

73   The range of effects of silicon supplementation on legumes presents a dichotomy whereby

74   increasing silicon availability could either make legumes better defended against herbivores via

75   increased silicon defences in the foliage (Reynolds et al. 2009) or make them more nutritious for

76   herbivores via increased nodulation increasing nitrogen availability (Mattson 1980). The relative

77   balance of these two effects, operating either directly (e.g. defence) or indirectly (e.g. nutritional

78   quality), has not yet been tested experimentally and is the rationale for this study.

79

80   The objective of this study was to determine whether increasing silicon availability for lucerne

81   (*Medicago sativa* L.) affected an insect herbivore (the aphid *Acyrthosiphon pisum*) either via

82   increased silicon uptake into the foliage and/or altered concentrations of amino acids in the

83   foliage. Based on observations that silicon can increase root nodulation in legumes (Nelwamondo

84   & Dakora 1999; Mali & Aery 2008), we predicted that silicon supplementation would increase plant

85   growth rates and root nodulation, which would increase foliar amino acid concentrations and

86   consequently herbivore performance.

87

88   **Materials and Methods**

89   *Experimental Procedure*

90   Lucerne (*M. sativa* L., cv. Sequel) plants were grown from seed at 26/18°C (day:night) in a

91   glasshouse receiving supplemental light (15:9 light:dark) and humidity controlled at 55% (see Ryalls

92   et al. 2013 for further technical details). Eighty eight plants were grown in 70 mm diameter pots

93   that were 135 mm deep (allowing unrestricted root growth for the duration of the experiment).

94   Each contained c. 700g of soil excavated from the Hawkesbury campus of Western Sydney

95   University (latitude −33.608847, longitude 150.747016). The soil is typified as low-fertility sandy

96   loam in the Clarenden Formation (Chromosol), which has low bioavailable Si content of 17 mg kg^{-1}

97   (see Barton et al. 2010 for full details). Sandy loam soils typically contain c. 35 mg kg^{-1} of

98   bioavailable Si (Environmental Analysis Laboratory, Southern Cross University personal

99   communication). Plants were irrigated with c. 70 ml of tap water (3ppm Si) three times a week.

100   After two weeks of growth, half of the plants continued to receive tap water at the same intervals

101   while the other half received 70 ml of 500 mg l^{-1} soluble Si in the form of $NaSiO_3.9H_2O$ (Sigma-

102   Aldrich, Castle Hill, NSW, Australia) three times a week for six weeks. $NaSiO_3.9H_2O$ has been used in

103   numerous studies to increase silicification of leaves and deter herbivores (reviewed by Reynolds et

104   al. 2009). When plants were six weeks old, 22 of the plants receiving the silicon supplementation

105   and 22 of the plants receiving tap water were each inoculated with two teneral female *A. pisum*.

106   Plants were configured in a randomised fashion in the glasshouse. *Acyrthosiphon pisum* used in

107   the experiment were taken from an established culture originating from an individual

108   parthenogenetic adult female collected from a lucerne field on campus (Ryalls et al. 2017). Cultures

109   were maintained on spare lucerne plants (cv. Sequel) for at least six generations prior to the

110   inoculation period.

111

112   White mesh (organza) bags (125 x 170 mm) were applied tightly around the rim of all pots

113   confining aphids to their allocated plants. After two weeks, bags were removed from all plants,

114   aphid colonisation assessed (% plants with > 1 aphid present) and individuals removed with a fine

115   paintbrush to be counted. Plants were separated and cleaned free of soil with water before

116   measuring shoot height, maximum rooting depth and counting the number of root nodules

117   (housing nitrogen-fixing bacteria). Plants were freeze dried for 48 hr and weighed. Leaves were

118   separated from the stems and ball-milled to a fine power prior to chemical analysis.

119

120   *Chemical analysis*

121   Chemical analysis was conducted on insect-free plants (44 of the 88 plants). To provide enough

122   dried leaf material to perform both amino acid and silicon analysis to be conducted on the same

6

123   plants it was necessary to pool foliar samples (2-3 plants per sample). This resulted in nine

124   replicates of Si+ and Si- plants. Foliar silicon concentrations were analysed with X-ray fluorescence

125   spectrometry according to the methodology set out in Reidinger, Ramsey & Hartley (2012). In brief,

126   plant material was ground to a fine powder and pressed into 13mm-diameter pellets. Following the

127   methods of Reidinger et al. (2012), foliar silicon concentration was determined using a Niton XL3t

128   XRF analyzer (Thermo Fisher Scientific, Inc., MA, USA), for a measurement time of 30 seconds.

129   Results were expressed as foliar silicon concentration (as % of dry mass), calibrated against plant-

130   certified reference material of known silicon content (Garbuzov, Reidinger & Hartley 2011).

131

132   Soluble amino acids were extracted and analysed from milled foliar samples (10-15 mg) following

133   the protocol set out by Ryalls et al. (2015). Foliar amino acid composition is tightly correlated with

134   phloem amino acids in *M. sativa*, so analysing foliar material is a reliable proxy for phloem quality

135   (Ryalls et al. 2017). Amino acid standards within the AAS-18 (Fluka, Sigma-Aldrich) reference amino

136   acid mixture were supplemented with asparagine and glutamine (A0884 and G3126, respectively,

137   from Sigma, Sigma-Aldrich). Nine essential amino acids (i.e. those that cannot be synthesised by

138   insects *de novo*), including arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine,

139   threonine and valine (Morris 1991) and 10 non-essential amino acids (alanine, asparagine, aspartic

140   acid, cysteine, glutamic acid, glutamine, glycine, proline, serine and tyrosine) were detected using

141   this method.

142

143   *Statistical analysis*

144   The effects of silicon supplementation and aphid inoculation and the interaction between these

145   terms on plant dry mass, shoot height, rooting depth, root nodule abundance and root nodules per

146   cm of roots were analysed with two way analysis of variance (ANOVA) tests. Differences in silicon

147   content (log transformed) and concentrations between treatment groups were analysed with a

148   one-way ANOVA (silicon supplementation included as a fixed factor). The effects of Si addition on

149   aphid colonisation success and aphid abundance were analysed with generalised linear models

150   with binomial error structure and a logit link function and Poisson error structure with a log link

151   function, respectively. Silicon supplementation was included as a fixed effect and the dispersion

152   parameter was estimated. Pearson's correlation tests were used to explore relationships between

153   plant parameters and aphid abundance. Where appropriate, data transformations (see Figure and

154   Table legends for details) were chosen to meet model assumptions and give residual diagnostic

155   plots which fitted a normal distribution and showed least heteroscedasticity. Permutational

156   multivariate analysis of variance (PERMANOVA) was used to explore the impacts of silicon

157   supplementation on amino acid composition and concentrations for total, essential and non-

158   essential amino acids. ANOVA and generalised linear models were performed with Genstat (version

159   17, VSN International, UK), whereas the PERMANOVA was conducted in R v3.3.1 using the using

160   the R-package vegan (Oksanen et al. 2017).

161
162   **Results**

163   Silicon supplementation (Si+ plants) increased plant biomass almost three-fold compared to those

164   grown without silicon supplementation (Si- plants), whereas the presence of aphids had no

165   significant impact on plant biomass (Fig. 1 A). This increase in biomass in Si+ plants was attributed

166   to increased growth in the shoots rather than the roots, the latter being similar in Si+ and Si- plants

167   (Table 1). Plants inoculated with aphids grew slightly less tall than those without (Table 1). Silicon

168   supplementation also resulted in c. 44% increase in root nodulation (Fig. 1B), which was reflected in

169   higher nodule density on the roots (Table 1). Nodulation was unaffected by aphids (Fig. 1B, Table

170   1).

171

172   Aphid colonisation success was similar on Si- and Si+ plants (73% and 64%, respectively; $F_{1,42}$ =

173   0.42, P = 0.517). Aphids were substantially more abundant (+112%) on Si+ plants than Si- plants

174   (Fig. 2). Moreover, aphid abundance was positively correlated with plant biomass (Fig. 3A) and

175   plant height (Fig. 3B).

176

177   Si+ plants accumulated over twice as much silicon as Si- plants (mean ± standard error: 262 ±

178   48.14 mg and 124.35 ± 14.05, respectively) ($F_{1,16}$ = 12.05, P = 0.003). However, because Si+ plants

179   grew so much bigger than Si- plants, this diluted foliar silicon concentrations by 26% in Si+ plants

180   (Fig. 4A). PERMANOVA analysis showed that Si+ plants had 65% higher concentrations of essential

181   amino acids than Si- plants (Fig. 4B). Overall there was no difference in total and non-essential

182   amino acid concentrations between the two groups, but there was a significant increase in the

183   concentrations of 11 of the 18 amino acids tested in the silicon treated plants compared to controls

184   (Fig. S1; Table S1).

185

186   **Discussion**

187   This study has shown that silicon supplementation caused a significant increase in root nodulation

188   in a legume, despite no increase in root length. This contributed to silicon-induced increases in

189   shoot biomass and enhanced foliar resources in the form of essential amino acids. While silicon

190   accumulation increased substantially in silicon supplemented plants, foliar silicon concentrations

191   were diluted due to this rapid increase in plant growth. These silicon-induced changes to plant

192   physiology and chemistry were associated with significant increases in herbivore abundance.

193

194   Enhanced growth of the meristems and increased synthesis of essential amino acids were the most

195   likely drivers of increased aphid abundance on Si+ plants. Aphids often feed on actively growing

196   stems because nutrients, specifically amino acids, are being translocated to these tissues for growth

197   (Raven 1983; Dixon 1998; Johnson, Elston & Hartley 2003). We found a positive correlation

198   between aphid abundance and plant growth (both dry mass and plant height), which supports the

199   conclusion that aphids performed better on the faster growing plants. Silicon supplementation also

200   increased concentrations of essential amino acids in the foliage by 65% relative to plants without

201   silicon supplementation. The concentration of essential amino acids in the phloem sap of plants is

202   comparatively low (10-30% of total protein amino acids; Douglas 2003), so silicon-induced

203   increases could promote aphid performance. In particular, >90% of total amino acid content of

204   aphid tissues are essential amino acids (Douglas 2003) so this group is very important for aphid

205   nutrition. We did not attempt to directly relate aphid abundance to amino acid concentrations of

206   specific plants on which aphids were feeding because aphids themselves have large qualitative and

207   quantitative effects on phloem amino acids (Douglas 2003), particularly in *Medicago* spp. (Guo et al.

208   2013). This confounds interpretation of how silicon supplementation was affecting amino acid

209   concentrations on aphid-infested plants, not least because aphid populations were much larger on

210   Si+ plants (and possibly having larger impacts on amino acids than on Si- plants). Nonetheless, it

211   seems likely that silicon-induced increases in foliar essential amino acids were at least partly

212   responsible for increased aphid abundance.

213

214   While silicon has been shown to increase root nodulation and nitrogen fixation in legumes

215   (Nelwamondo & Dakora 1999; Dakora & Nelwamondo 2003; Mali & Aery 2008), the mechanisms

216   for this have yet to be demonstrated. The simplest explanation is that increased root growth

217   increases potential invasion sites for rhizobial bacteria (Mali & Aery 2008), but we found that silicon

218   increased nodule density and had little impact on root growth *per se*. Using comparable application

219   rates (480 mg l^{-1}) as the present study (500 mg l^{-1}), Nelwamodo & Dakora (1999) similarly found

220   that nodule density increased on sand-grown cowpea (*Vigna unguiculata*) plants.

221   In a follow up study, Nelwamondo et al. (2001) showed that silicon supplementation increased the

222   abundance of bacteroids and symbiosomes (the plant-derived membrane that encases the

223   bacteroids) in root nodules, which they suggested could explain enhanced N_2 fixation. They also

224   found that silicon increased cell wall thickness of root nodules which appeared to decrease

225   intercellular spaces which should, in theory, reduce N_2 fixation because of lower gaseous diffusion

226   (Nelwamondo et al. 2001). The fact that N_2-fixation actually increased led Nelwamondo et al. (2001)

227   to speculate that silicification reduced the need for lignin, which is costly in terms of carbon; carbon

228   could then be channelled into bacteroid respiration to increase N_2-fixation. It is also possible that

229   carbon could be channelled towards enhanced nodule organogenesis. Nelwamondo et al. (2001)

230   also proposed that silicon supplementation had parallels with phosphorus fertilisation in terms of

231   stimulating root nodulation, including increased production of compounds that upregulate

232   nodulation genes (Dakora & Nelwamondo 2003). This has yet to be tested.

233

234   The efficacy of silicon defence against sap-feeders, such as aphids, appears less clear cut than on

235   chewing herbivores and has been the subject of debate (Massey et al. 2006; Keeping & Kvedaras

236   2008; Kvedaras et al. 2009). Published studies have shown silicon has either no (e.g. Hogendorp,

237   Cloyd & Swiader 2009; Cherry et al. 2012; Keeping, Miles & Sewpersad 2014) or negative (e.g.

238   Gomes et al. 2008; Costa, Moraes & DaCosta 2011; Dias et al. 2014) effects on this feeding guild.

239   The variation in these published findings probably reflects the fact that whilst silicon can negatively

240   affect aphids via epidermal resistance to stylet penetration, aphids can circumvent some of the

241   consistent negative impacts reported for chewing herbivores (e.g. mandible wear and impaired

242   food breakdown) simply by virtue of their feeding behaviour (i.e. fluid acquisition via a stylet).

243   Further experimental work and meta-analysis of existing studies would help to address the extent

244   to which different herbivore guilds are affected by silicon defences. In the present study, silicon

245   application did not affect aphids adversely because Si+ plants did not have higher concentrations

246   of silicon in the foliage.

247

248   In addition to promoting physical defences at the leaf surface, silicon can also stimulate

249   phytohormonal pathways in the plant, such as the jasmonic acid (JA) pathway (Ye et al. 2013),

250   which underpins synthesis of secondary metabolites with defensive functions (Wu & Baldwin 2010).

251   Stimulation of the JA pathway is known to supress the salicylic acid (SA) pathway, which is often

252   triggered by phloem-feeding herbivores and can result in plants mounting a defensive response

253   against these herbivores (Ode, Johnson & Moore 2014). We still know very little about how silicon

254   affects other plant defences, but if silicon application were to stimulate the JA pathway (and

255   supress the SA pathway), this may have also contributed to the success of aphids on silicon

256   supplemented plants in this study. We can conclude, however, that if defences were activated by

257   silicon application, they had minimal impacts on aphids in this system.

258

259   This study has shown that lucerne plants benefitted from silicon supplementation in terms of both

260   increased growth and resource acquisition via root nodulation, whereas the concentrations of

261   silicon in the foliage tended to decrease in silicon supplemented plants. Aphid abundance

262   increased substantially on these faster-growing plants. There a number of hypotheses considering

263   the relationships between resources (nitrogen, water), plant growth and herbivory (reviewed by

264   Hartley & Jones 1997). Our results are consistent with the resource availability hypothesis (Coley,

265   Bryant & Chapin 1985), which predicts that faster growing plants, with increased access to

266   resources (e.g. either through silicon fertilisation or biologically fixed nitrogen) will be less well

267   defended against herbivores. Increasing the rate of silicon supplementation may increase foliar

268   silicon concentrations, but increasing the concentrations of silicon application much beyond those

269   used in this study would most likely inhibit nodulation and N_2-fixation, as has been reported for

12

270   cowpea plants (Mali & Aery 2008). In that study, high doses of silicon application reduced calcium

271   concentrations in the plant; calcium increases biosynthesis of isoflavonoid nodulation signals in

272   legumes (Dakora & Phillips 1996) so it was suggested this was the reason for high silicon

273   application diminishing nodulation (Mali & Aery 2008).

274

275   To our knowledge, this is the first study to report beneficial effects of silicon addition on herbivore

276   performance which arose indirectly via promotion of root nodulation and amino acid biosynthesis.

277   Further work on non-grass species is needed, but our results suggest that in some functional

278   groups of plants, such as legumes, the positive effects of silicon on plant growth and nitrogen

279   acquisition may outweigh any additional defensive function of silicon supplementation. Our

280   findings suggest that silicon supplementation of legumes may not provide the benefits that such

281   applications provide to grasses such as cereals (Guntzer et al. 2012), at least in part because the

282   positive effects on nodulation, nutrient content and yield may make these crops more susceptible

283   to aphids pests and the diseases they vector. There is an increasing need to extend the use of

284   legumes in agriculture because of their benefits to soil fertility, so understanding how silicon

285   promotes nodulation and N_2-fixation and its indirect impact on plant susceptibility to herbivores

286   could be a promising area of research.

287

288   **Author contributions**

293

294   **Acknowledgements**

13

299

300   **Data accessibility**

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

302

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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

472   **Figure Legends**

473   **Figure 1.** Impacts of silicon supplementation and aphid inoculation on the (A) biomass (log

474   transformed) and (B) abundance of root nodules on *M. sativa*. Mean values ± standard error shown.

475   N = 22.

476   **Figure 2**. Impact of silicon supplementation on aphid (*A. pisum*) abundance on colonised plants.

477   Mean values per plant ± standard error shown. N = 14 (Si-) and N = 16 (Si+).

478   **Figure 3.** Correlations between aphid abundance and (A) plant biomass and (B) plant (shoot)

479   height. N= 30. Solid lines represent linear regression through all the points. Dashed lines represent

480   95 % confidence intervals

481   **Figure 4**. Impacts of silicon supplementation on foliar (A) silicon concentrations (logit transformed)

482   and (B) essential amino acids in *M. sativa*. Mean values ± standard error shown. N = 9.
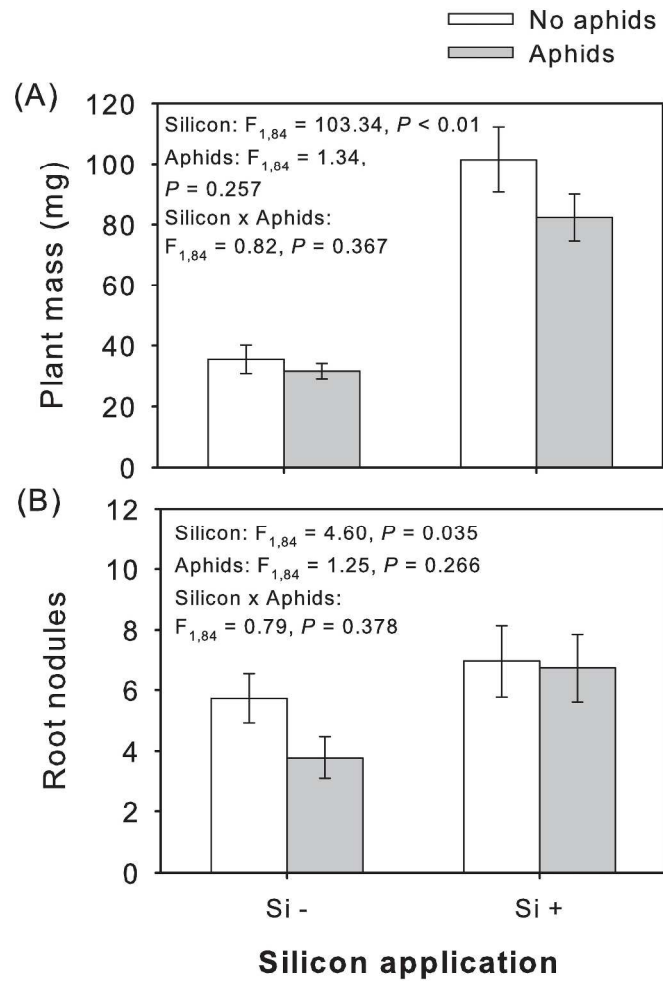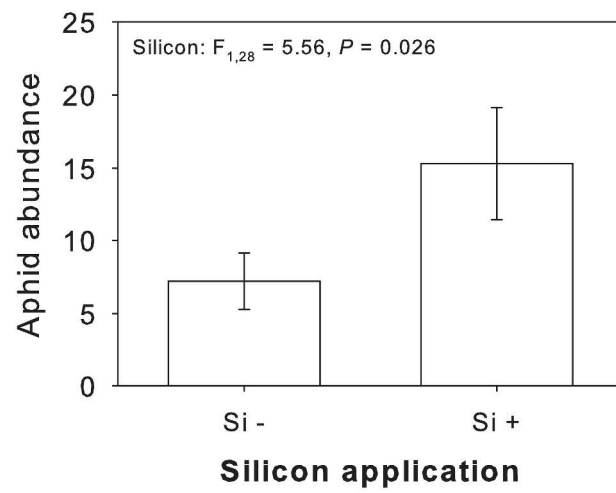
Figure 1

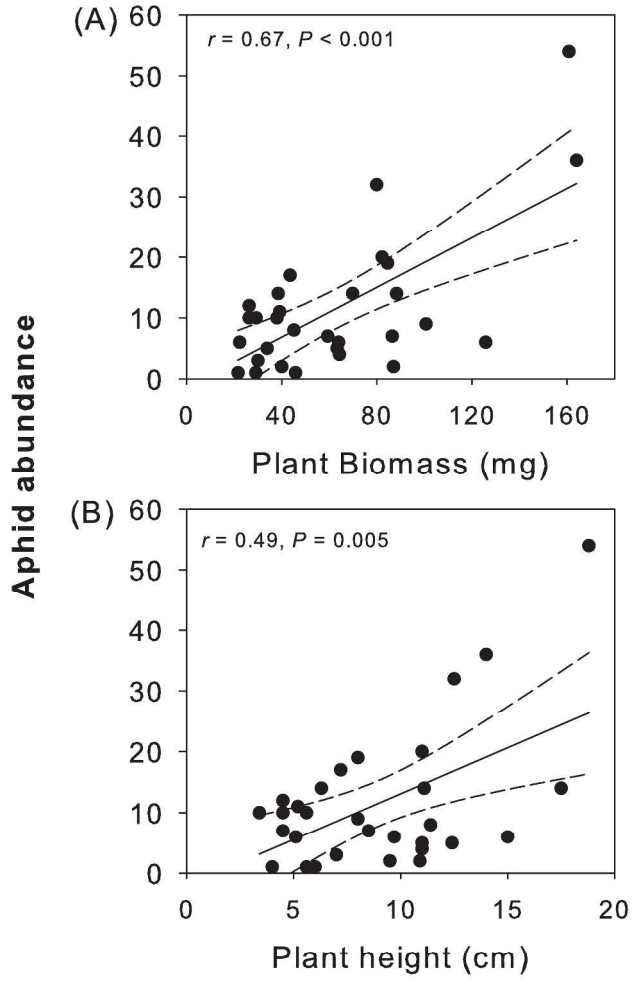296x420mm (300 x 300 DPI)

Figure 2

296x420mm (300 x 300 DPI)

Figure 3

296x420mm (300 x 300 DPI)

(A) Silicon: $F_{1,16} = 2.23$, $P = 0.080$

Silicon (% dry mass)

(B) Silicon: $F_{1,16} = 5.34$, $P = 0.018$

Essential amino acids (μmol g^{-1} dry mass)

Si -   Si +
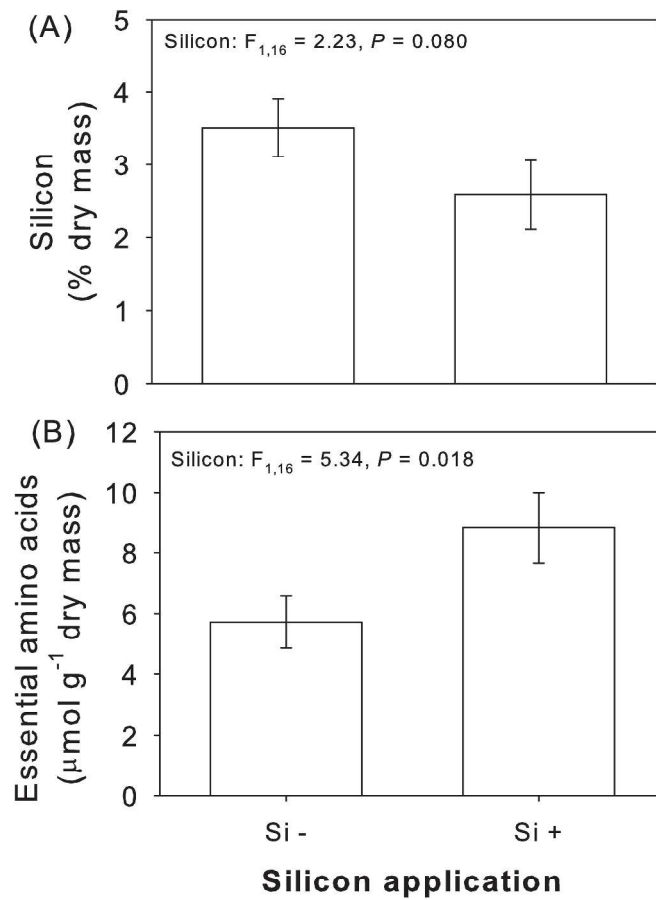
Silicon application

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 * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Amino acid concentration (μmol g^{-1} dry mass)