Silicon promotes nodule formation and nodule function in symbiotic cowpea (Vigna unguiculata).

New Phytol. (1999), **142**, 463–467

Silicon promotes nodule formation and nodule function in symbiotic cowpea (*Vigna unguiculata*)

A. NELWAMONDO AND F. D. DAKORA*

Botany Department, University of Cape Town, Private Bag, Rondebosch 7701, South Africa

Received 16 July 1998; accepted 18 February 1999

SUMMARY

Applying silicon in the form of metasilicic acid (H_4SiO_3) or silicic acid (H_4SiO_4) to *Bradyrhizobium*-infected, hydroponically grown cowpea seedlings resulted in a significant ($P \leqslant 0.05$) increase in the number of nodules, nodule dry matter, and nitrogen fixed on a per plant basis. Total dry matter of plants increased with silicon supply, and the differences were significant ($P \leqslant 0.05$) at the higher silicon concentrations. Cowpea plants cultured in sand were also assessed for their response to silicic acid. Nodule number and nodule mass increased with silicon supply to sand cultured plants, though nitrogen fixation was unaltered. Although silicon is not essential for growth of cowpea, it is important for nodule formation and nodule functioning in hydroponically grown plants. Consequently, data collected and conclusions drawn from earlier glasshouse experiments, which have excluded silicon from nutrient solutions, may be flawed. Future studies on nodulation and nitrogen fixation using legumes in liquid culture must therefore include silicon as a nutrient element.

Key words: silicon, cowpea, nodulation, N_2 fixation, nutrient solution.

INTRODUCTION

Most glasshouse studies involving legumes have been conducted using various nutrient solutions for growth of plants in liquid and sand culture (Smith *et al.*, 1983). However, these solutions usually lack silicon. Whether the omission of silicon as a nutrient affected the quality of data collected, and/or the conclusions drawn, is still open to question, and a recent report suggests that silicon stimulates growth of plants (Epstein, 1994). With legumes, nodulation and nitrogen (N_2) fixation is dependent on an adequate supply of both macro- and micro-nutrients (Munns, 1977; Smith, 1982). These nutrients are not only essential for the symbiotic interaction but also for the host plant and its microbial partner (Munns, 1977; Smith, 1982). Although silicon is an element that occurs abundantly in soil solution and in the dry matter of many plants (Epstein, 1994), its role in the symbiotic process has not been assessed.

In non-legume species, such as rice, barley and beet, silicon has been shown to be essential for both plant growth and increased yields (Okuda & Takahashi, 1965; Deren *et al.*, 1994; Winslow *et al.*, 1997). In legumes such as common bean and soybean, silicon is reported to increase total biomass, root length, and root mass when supplied in the root medium (Horst & Marschner, 1978; Miyake & Takahashi, 1985). In legumes which have the potential to form nodules this increase in root length and root mass with added silicon would imply an increase in the number of potential infection sites for rhizobial invasion, which would in turn, lead to increased nodulation and N_2 fixation. Unfortunately, these studies on silicon nutrition of the two nodulating legumes (Horst & Marschner, 1978; Miyake & Takahashi, 1985) focus only on plant growth and not on its symbiotic performance. The purpose of this study was to examine the symbiotic response of cowpea to silicon nutrition under glasshouse conditions using both liquid and sand culture.

*Author for correspondence (fax 00 27 21 650 4041; e-mail Dakora@botzoo.uct.ac.za).

MATERIALS AND METHODS

The effects of metasilicic acid (H_4SiO_3) and silicic acid (H_4SiO_4) on growth and symbiotic performance of cowpea in liquid culture

Seeds of cowpea (*Vigna unguiculata* L. Walp. cv 8586) were sown in freely draining sand, inoculated with a peat-based inoculant of *Bradyrhizobium* strain CB756, and left to germinate in a naturally lit glasshouse during the summer in Cape Town. At 7

d after planting, the seedlings were rinsed, and cultured hydroponically in N-free modified Hoagland nutrient solution (Hewitt, 1966), using 3.5 l pots. The solution was adjusted to contain different concentrations of silicon, either as metasilicic acid (H_4SiO_3) or silicic acid (H_4SiO_4). The concentrations used were 0, 0.04, 0.08, 0.20, 0.40 and 0.80 g l^{-1} for metasilicic acid and 0, 0.048, 0.096, 0.24, 0.48 and 0.96 g l^{-1} for silicic acid. The nutrient solution was aerated by bubbling with air and changed after every 3 d. Four replicate pots were used for each treatment with four plants per pot. The plants were harvested 36 d after planting (DAP). Data collected included nodule numbers, nodule dry weights, and total biomass. The shoots, roots and nodules of plants were oven dried at 80°C, weighed, finely ground, and digested for N determination by the Kjedahl technique. The amount of N fixed was estimated as the difference between plant total N and seed N and specific N_2-fixing activity calculated as mg N fixed per g nodule dry matter (DM).

The effects of exogenous supply of silicon on cowpea growing in sand culture

Sand is unweathered silica material. Plants growing in sand culture can therefore potentially obtain silicon from the sand used as the rooting medium (Brady, 1990). An experiment was therefore conducted to determine whether, as in the hydroponic system, plants growing in sand culture require an exogenous supply of silicon for growth. Seeds of cowpea were sown in freely draining sand contained in 3.5 l plastic pots and inoculated with *Bradyrhizobium* strain CB756. After germination, seedlings were thinned to four per pot and plants exposed to silicic acid as indicated. Four pots were used per treatment. The plants were grown under the glasshouse conditions already described and each silicon concentration was applied four times a week. Accumulated salts from frequent nutrient application were flushed out once a week. The plants were harvested at 36 DAP, and analysed for growth and symbiotic performance.

Statistical analysis

The effects of different silicon concentrations on growth and symbiotic performance of cowpea were analysed statistically by a one-way ANOVA using the STATISTICA package.

RESULTS

Growth and symbiotic response of hydroponically cultured cowpea plants to metasilicic acid (H_4SiO_3)

Supplying metasilicic acid to cowpea significantly ($P \leqslant 0.05$) increased nodule number per plant in all treatments except the control and 0.04 g l^{-1} (Fig. 1a). Nodulation in silicon-treated plants also varied

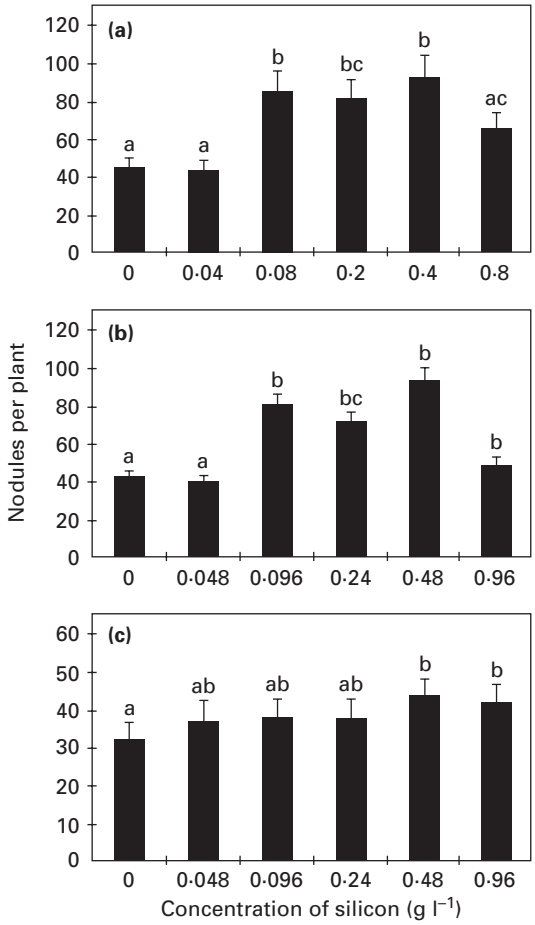

Fig. 1. Effects of the concentration of silicon on nodulation of 36-d-old cowpea plants. (a) Plants grown hydroponically and supplied with H_4SiO_3, (b) grown hydroponically and supplied with H_4SiO_4, and (c) grown in sand and supplied with H_4SiO_4. Vertical lines on bars represent SD ($n=4$). Different letters show significant differences at $P \leqslant 0.05$ using one-way ANOVA.

significantly with the concentration of metasilicic acid in the root medium. Nodule number per plant was significantly ($P \leqslant 0.05$) higher from 0.08 to 0.40 g l^{-1} but not at 0.96 g l^{-1} (Fig. 1a). Nodule DM followed a similar pattern to that of nodule number, with the highest mass found in plants receiving 0.08–0.40 g l^{-1} of metasilicic acid (Figs 2a, 3a).

The total DM of plants also increased with silicon supply in the nutrient solution (Fig. 3a), but this was significant only at the higher silicon concentrations. Consequently, the amount of N fixed per plant was significantly ($P \leqslant 0.05$) greater in all plants receiving metasilicic acid except in those given 0.04 g l^{-1} (Table 1). However specific N_2-fixing activity in nodules was the same in all treatments except at the highest silicon concentration, where it differed significantly (Table 2).

Growth and symbiotic response of hydroponically cultured cowpea plants to silicic acid (H_4SiO_4)

Plants grown with silicic acid as the main source of silicon showed a similar response to those provided

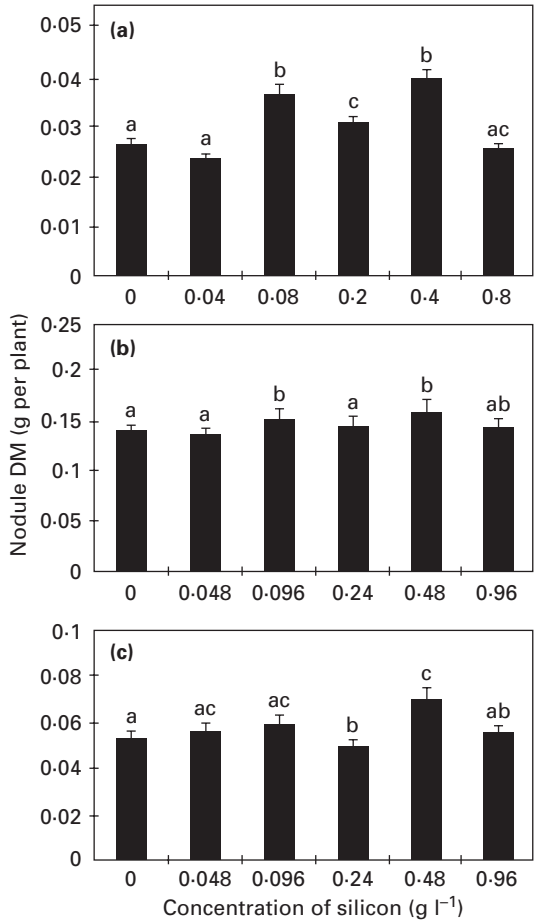

Fig. 2. Effects of the concentration of silicon on nodule dry matter (DM) in 36-d-old cowpea plants. (a) Plants growth hydroponically and supplied with H_4SiO_3, (b) grown hydroponically and supplied with H_4SiO_4, and (c) grown in sand and supplied with H_4SiO_4. Vertical lines on bars represent SD ($n=4$). Different letters show significant differences at $P \leqslant 0.05$ using one-way ANOVA.

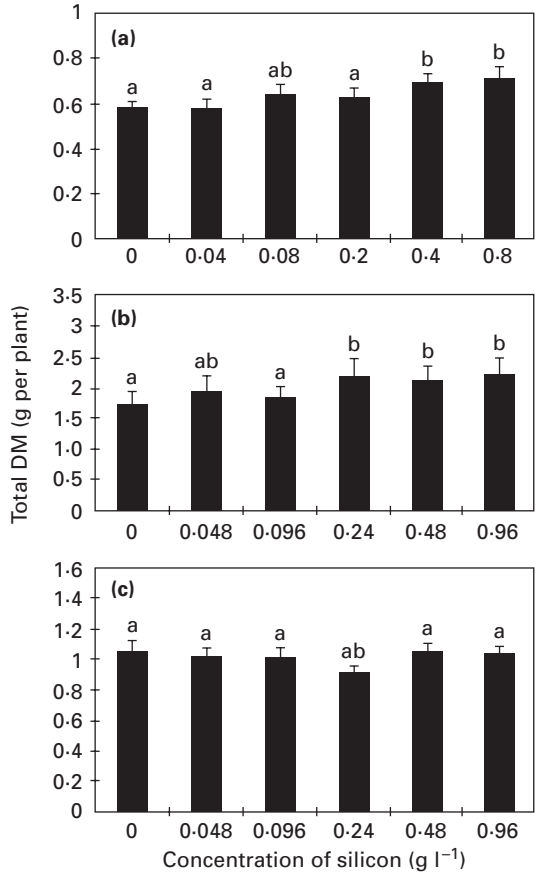

Fig. 3. Effects of the concentration of silicon on total plant dry matter (DM) in 36-d-old cowpea plants. (a) plants grown hydroponically and supplied with H_4SiO_3, (b) grown hydroponically and supplied with H_4SiO_4, and (c) grown in sand and supplied with H_4SiO_4. Vertical lines on bars represent SD ($n=4$). Different letters show significant differences at $P \leqslant 0.05$ using one-way ANOVA.

with metasilicic acid. The number of nodules per plant increased markedly ($P \leqslant 0.05$) with increasing silicon supply (Fig. 1b). Nodule DM also showed an increase which was significant at higher concentrations of silicic acid (Fig. 2b). As with metasilicic acid, total DM of plants also increased markedly ($P \leqslant 0.05$) in the presence of higher concentrations of silicic acid (Fig. 3b). Specific N_2-fixing activity was generally the same for all treatments except at 0.48 g l^{-1} (Table 2).

Growth and symbiotic response of sand-cultured cowpea plants to silicic acid (H_4SiO_4)

Applying silicic acid to sand-grown cowpea plants promoted an increase in nodule number, especially at the higher concentrations (Fig. 1c). Consequently, nodule DM per plant also increased with increasing silicon supply to the roots (Fig. 2c). However, plant growth in sand culture was not stimulated by silicic acid (Fig. 3c). The amount of N fixed was not affected by application of silicic acid to the roots (Table 1).

DISCUSSION

Contrary to the generalized notion that silicon is not important for plant growth (Epstein, 1994), the total biomass of nodulated cowpea increased significantly ($P \leqslant 0.05$) when silicon was supplied to hydroponically grown plants (Fig. 3a,b). Nevertheless, the view that silicon is not needed for plant growth has led to the exclusion of this element from most nutrient solutions used in glasshouse experiments (Smith *et al.*, 1983). The increased biomass in silicon-fed cowpea is consistent with the findings of other studies which show that exogenous supply of silicon can promote growth, and even increase yields of certain agricultural crop plants (Okuda & Takahashi, 1961; Horst & Marschner, 1978; Miyake & Takahashi, 1985, 1986; Ma & Takahashi, 1993). As an extreme case, vegetables such as cucumber fail to grow in the absence of silicon (Miyake & Takahashi, 1983), indicating that this element is an

Table 1. *Effect of application of silicon on the amounts of N fixed in 36-d-old nodulated cowpea plants grown in liquid culture with metasilicic acid (H_4SiO_3) or in sand culture with silicic acid (H_4SiO_4)*

Silicon concentration (g l^{-1})		N fixed (mg per plant)	
Liquid culture (H_4SiO_3)	Sand culture (H_4SiO_4)	Liquid culture (H_4SiO_3)	Sand culture (H_4SiO_4)
0	0	73.4 ± 14.0 a	49.2 ± 8.3 a
0.04	0.048	70.6 ± 10.0 a	47.8 ± 10.1 a
0.08	0.096	85.6 ± 15.0 bc	53.1 ± 11.2 a
0.20	0.24	80.5 ± 16.0 b	48.5 ± 9.6 a
0.40	0.48	92.4 ± 6.0 c	52.0 ± 12.3 a
0.80	0.96	84.9 ± 17 b	51.4 ± 6.8 a

Values followed by dissimilar letters are significantly different at $P \leqslant 0.05$ using one-way ANOVA. Mean ± SD ($n = 4$).

Table 2. *Effect of silicic acid on specific N_2-fixing activity of 36-d-old nodulated cowpea plants grown hydroponically with metasilicic acid (H_4SiO_3) or in sand culture with silicic acid (H_4SiO_4)*

Silicon concentration (g l^{-1})		mg N fixed per g Nodule DM	
Liquid culture (H_4SiO_3)	Sand culture (H_4SiO_4)	Liquid culture (H_4SiO_3)	Sand culture (H_4SiO_4)
0	0	751.29 ± 217.05 ab	933.04 ± 167.51 a
0.04	0.048	744.26 ± 112.15 ab	851.38 ± 281.34 ab
0.08	0.096	591.11 ± 87.88 a	901.71 ± 203.77 a
0.20	0.24	671.24 ± 86.91 ab	994.10 ± 138.67 a
0.40	0.48	582.55 ± 47.17 a	739.62 ± 126.13 b
0.80	0.96	861.81 ± 174.17 b	945.83 ± 221.59 a

Values followed by dissimilar letters are significantly different at $P \leqslant 0.05$ using one way ANOVA. Mean ± SD ($n = 4$). Silicon concentration in liquid culture is the same as in sand culture, but the actual amounts used differ because of the molar mass of silicon source.

essential nutrient for these species. Many upland rice ecotypes also show significant decreases in yield with inadequate silicon supply in acid soils (Deren *et al.*, 1994; Winslow *et al.*, 1997). The significant ($P \leqslant 0.05$) increase in total DM of silicon-treated symbiotic cowpea plants (Fig. 3a,b) therefore suggests that while silicon may not be essential for growth of this species, it is nevertheless important for promoting increased biomass. However whether this increment in growth of cowpea translates into higher pod yields, as observed in soybean (Miyake & Takahashi, 1985), was not tested.

The promotion of nodule formation by silicon in hydroponically grown cowpea plants (Fig. 1a,b) has a close parallel with the effect of phosphorus (P), boron (B), molybdenum (Mo) and other nutrient elements on symbiotic legumes (Israel, 1987; Le Roux, 1994; Muofhe, 1994). The amounts of N fixed also increased with silicon application to plants (Table 1) as did tissue concentration of silicon (Nelwamondo A, & Dakora FD, unpublished), a finding consistent with the effects of various other essential elements on the symbiotic process (Munns, 1977; Smith, 1982). Although N_2 fixation was not measured in an earlier study (Miyake & Takahashi, 1985), the N content of leaves, stems and roots of soybean was consistently higher in the nodulating isoline than in the non-nodulating isoline when silicon was provided. This agrees with our data. The stimulatory effect of P on the symbiotic performance in legumes has been attributed to many factors including increased production of compounds that induce nodulation genes (Dakora & Le Roux, 1995). Calcium and magnesium are also known to increase the biosynthesis of isoflavonoid nodulation signals in symbiotic legumes (Richardson *et al.*, 1988; Dakora & Phillips, 1996). However, it remains to be determined whether the increase in nodule formation that follows supply of silicon to cowpea is a consequence of greater signal production. The increased root mass observed with silicon application to cowpea (data not shown) could double the number of infection sites on root hairs, ultimately leading to enhanced invasion of rhizobium and increased

symbiotic performance. Additionally, an increase in root surface area would result in more efficient nutrient uptake, which could in turn, improve plant growth. In the field, nodulating legumes probably benefit in all of these different ways from the absorption and assimilation of silicon in soil solution.

Nodule number and nodule DM, though not N fixed, were stimulated by provision of silicic acid to sand-cultured plants (Figs 1c, 2c, Table 1). Since silicon is available in sufficient quantities in soil solution for uptake by plants (Epstein, 1994), it is unlikely that studies involving the use of soil culture would require exogenous supplement of silicon. The lack of any effect of exogenous silicon on growth, specific N_2-fixing activity or N_2 fixation in sand-cultured plants also suggests possible release of silicon from the sand itself.

This study is significant because silicon has so far been excluded from all conventional nutrient solutions used in glasshouse experiments with plants. Our findings show that exclusion of silicon would have an adverse effect on growth of hydroponically cultured plants, and consequently may have affected the quality and perhaps interpretation of the data collected in earlier experiments. Future studies on legume nodulation and N_2 fixation in liquid culture must therefore include silicon as a nutrient element. Our data demonstrate that, though not essential for cowpea growth, silicon is important for the symbiotic process in this species However, how and where silicon exerts its influence on nodulation and N_2 fixation is yet to be determined.

ACKNOWLEDGEMENTS

This work was supported by funds from the Foundation of Research and Development, Pretoria, and the University Research Committee, University of Cape Town.

REFERENCES

Brady NC. 1990. *The nature and properties of soils, 10th edn*. New York, USA: MacMillan Publishing Company.

Dakora FD, Le Roux RJ. 1995. Phosphorus nutrition alters root flavonoid content, nitrogen fixation and phosphorus partitioning in cowpea. In: Tihonovich IA, Provorov NA, Romanov VI, Newton WE, eds. *Nitrogen fixation: fundamentals and applications*. London, UK: Kluwer Academic Publishers.

Dakora FD, Phillips DA. 1996. Diverse functions of isoflavonoids in legumes transcend anti-microbial definitions of phytoalexins. *Physiological and Molecular Plant Physiology* **49**: 1–20.

Deren CW, Datnoff LE, Snyder GH, Martin FG. 1994. Silicon concentration, disease response, and yield components of rice genotypes grown on flooded organic histosols. *Crop Science* **34**: 733–737.

Epstein E. 1994. The anomally of silicon in plants. *Proceedings of the National Academy of Sciences, USA* **91**: 11–17.

Hewitt EJ. 1966. *Sand and water culture methods used in the study of plant nutrition, 2nd revised edition*. Commonwealth Bureau of Horticultural and plantation Crops, East Malling. Technical Communication No. 22. Farnham Royal, UK: Commonwealth Agricultural Bureau.

Horst WJ, Marschner H. 1978. The effect of silicon on manganese tolerance of bean plants (*Phaseolus vulgaris* L.). *Plant Soil* **50**: 287–303.

Israel DW. 1987. Investigation of the role of phosphorus in symbiotic dinitrogen fixation. *Plant Physiology* **84**: 835–840.

Le Roux RJ. 1994. Honours thesis. Botany Department, University of Cape Town, South Africa.

Ma JF, Takahashi E. 1993. Interaction between calcium and silicon in water-cultured rice plants. *Plant Soil* **148**: 107–113.

Miyake Y, Takahashi E. 1983. Effect of silicon on the growth of solution-cultured cucumber plant. *Soil Science Plant Nutrition* **29**: 71–83.

Miyake Y, Takahashi E. 1985. Effect of silicon on the growth of soybean plants in a solution culture. *Soil Science Plant Nutrition* **31**: 625–636.

Miyake Y, Takahashi E. 1986. Effect of silicon on the growth and fruit production of strawberry plants in a solution culture. *Soil Science Plant Nutrition* **32**: 321–326.

Munns DN. 1977. Mineral nutrition and the legume symbioses. In: Hardy RWF, Gibson AH, eds. *A Treatise on Dinitrogen Fixation. Section iv*. New York, USA: John Wiley and Sons, 353–391.

Muofhe ML. 1994. Effects of boron nutrition and cotyledon manipulation on symbiotic performance of Bambara groundnut (*Vigna subterraneum* L.). Honours thesis, Botany Department, University of Cape Town, South Africa.

Okuda A, Takahashi E. 1961. Studies on the physiological role of silicon in crop plants. I. Discussion on the silicon deficient culture method. *Journal of Science and Soil Manure* **32**: 475–480.

Okuda A, Takahashi E. 1965. The role of silicon. In: *The mineral nutrition of the rice plant*, IRRI. Baltimore, MD, USA: Johns Hopkins Press, 123–146.

Richardson AE, Djordjevic MA, Rolfe BG, Simpson RJ. 1988. Effects of pH, Ca and Al on the exudation from clover seedlings of compounds that induce the expression of nodulation genes in *Rhizobium trifolii*. *Plant Soil* **109**: 37–47.

Smith FW. 1982. Mineral Nutrition of Legumes. In: Vincent JM, ed. *Nitrogen fixation in legumes*. Sydney, Australia: Academic Press, 155–172.

Smith GS, Johnston CM, Cornforth IS. 1983. Comparison of nutrient solutions for growth of plants in sand culture. *New Phytologist* **94**: 537–548.

Winslow MD, Okada K, Correa-Victoria F. 1997. Silicon deficiency and the adaptation of tropical rice ecotypes. *Plant Soil* **188**: 239–248.