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A. Egrinya Eneji<sup>a</sup>, S. Inanaga<sup>b d</sup>, S. Muranaka<sup>c</sup>, J. Li<sup>a</sup>, T. Hattori<sup>d</sup>, P. An<sup>d</sup> & W. Tsuji<sup>d</sup>

<sup>a</sup> College of Agronomy and Biotechnology, China Agricultural University, Beijing, China

<sup>b</sup> Japan International Center for Agricultural Sciences, Tsukuba, Japan

<sup>c</sup> International Institute of tropical Agriculture, Kano Station, Nigeria

<sup>d</sup> Arid Land Research Center, Tottori University, Japan

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## Growth and Nutrient Use in Four Grasses Under Drought Stress as Mediated by Silicon Fertilizers

A. Egrinya Eneji,<sup>1</sup> S. Inanaga,<sup>2,4</sup> S. Muranaka,<sup>3</sup> J. Li,<sup>1</sup> T. Hattori,<sup>4</sup>  
P. An,<sup>4</sup> and W. Tsuji<sup>4</sup>

<sup>1</sup>College of Agronomy and Biotechnology, China Agricultural University,  
Beijing, China

<sup>2</sup>Japan International Center for Agricultural Sciences, Tsukuba, Japan

<sup>3</sup>International Institute of tropical Agriculture, Kano Station, Nigeria

<sup>4</sup>Arid Land Research Center, Tottori University, Japan

### ABSTRACT

Field water stress is a common problem in crop production, especially in arid and semi-arid zones and it is widely hypothesized that silicon (Si) could reduce water stress in plants. We set up a greenhouse study to evaluate some silicon sources—potassium silicate ( $K_2SiO_3$ ), calcium silicate ( $CaSiO_3$ ) and silica gel for growth and nutrient uptake by four grass species under adequate and deficit irrigation. The four species studied were Rhodes grass (*Chloris gayana*), Timothy grass (*Phleum pratense*), Sudan grass (*Sorghum sudanense*) and Tall fescue (*Festuca arundinacea*). For all species, the biomass yield response to applied silicon under deficit irrigation was significantly better than under adequate irrigation. The yield response of Rhodes grass across silicon sources was 205% under deficit irrigation compared with only 59% under adequate irrigation; for Sudan grass it was 49% compared with 26% and for Timothy, it was 48% compared with a mere 1%. The higher responses under deficit irrigation suggest that the plants relied more on silicon to endure drought stress. Biomass yield of individual plants also differed according to soil water levels with Timothy grass being the most sensitive to water stress as it exhibited the highest yield response (209%) to adequate irrigation. This was followed by tall fescue (122%) and Rhodes grass (97%). Sudan grass was the least affected by deficit irrigation, possibly on account of improved root mass and its natural drought tolerance. Strong associations were noted between the uptake of silicon and those of nitrogen (N) and phosphorus (P) irrespective of soil water condition, but the uptake of potassium (K) was more strongly correlated with that of Si under deficit than adequate irrigation. Improvements in plant growth following Si application could therefore be linked to enhanced uptake of major essential nutrients.

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Address correspondence to A. Egrinya Eneji, College of Agronomy and Biotechnology, China Agricultural University, Beijing, China. E-mail: aeeneji@cau.edu.cn

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## INTRODUCTION

Many plants deprived of Si suffer significant reductions in growth and yield as well as increased susceptibility to both biotic and abiotic stresses (Datnoff et al., 2001). It has been reported that silicon can increase plant defense systems against abiotic and biotic stresses, including water stress (Epstein, 1994; Belanger et al., 1995; Zhang and Schmidt, 1999). Leaves, stems and culms of plants, notably rice grown in soils treated with Si show an erect growth, thereby improving the distribution of light within the canopy (Ma and Takahashi, 1991; Savant et al., 1999). Although considerable research has been carried out on a particular stress much more is known in some areas (e.g., wind, Al and Mn toxicity stresses) than others, and some stresses (e.g., drought and radiation) have hardly been considered (Hodson and Sangster, 2002). In continuously cultivated agricultural soils, the plant available Si may become deficient. Calculations, from a sugarcane field showed that nearly 100 kg/ha of Si are removed from the soil each harvest cycle (Savant et al., 1999).

The correlation between silicon nutrition and water use has been studied in the past, but the results were inconsistent (Lewin and Reimann, 1969; Walker and Lance, 1991). Silicon can lower the electrolyte leakage from rice leaves and therefore promote greater photosynthetic activity in plants grown under water deficit or heat stress (Agarie et al., 1998). The rate of transpiration of Si-deficient plants increased by about 30% over that of control plants (Lewin and Reimann, 1969). Okuda and Takahashi (1965) reported similar results, except that in barley the effect was small (<10%). Higher water flux observed in silicon-applied sorghum in dry soil was ascribed to lowered hydraulic resistance resulting from increased root growth which favoured water movement from rhizosphere to roots (Hattori et al., 2005). Gao et al. (2004) observed that the reduction in transpiration following the application of silicon was largely due to a reduction in transpiration rate through stomata, indicating that silicon influences stomata movement.

Field water stress is a common feature, especially among forage crops in arid and semi-arid zones where animal grazing is a major occupation. Unlike for grain cereals (e.g. rice, wheat or barley), little work has done on the role of silicon on forage crops. This study was therefore performed to compare how silicon could mediate growth and nutrient uptake in some forage grasses under deficit soil water conditions.

## MATERIALS AND METHODS

The following grass species were studied between November 2003 and February 2004, at the Arid Land Research Center, Tottori University, Japan: Rhodes grass

(*Chloris gayana* Kunth. Cv. 'Asatsuyu'), Timothy grass (*Phleum pratense* L. cv. 'Kunpu'), Sudan grass (*Sorghum sudanense* Piper. Cv. Beru Sudan) and Tall fescue (*Festuca arundinacea* Schreb. Cv. 'Southern cross'). Two soil irrigation regimes: adequate irrigation at field capacity (6%) and deficit irrigation at half field capacity (3%) were used to compare the effects of three sources of Si (silica gel,  $\text{CaSiO}_3$  and  $\text{K}_2\text{SiO}_3$ ) on plant growth and nutrient (N, P, K and Si) uptake. The chemical composition of these Si sources were as follows: Calcium silicate ( $\text{SiO}_2$ -30%,  $\text{MgO}$ -5%,  $\text{CaO}$ -40%); potassium silicate ( $\text{K}_2\text{O}$ -20%,  $\text{SiO}_2$ -30%,  $\text{MgO}$ -5%,  $\text{B}_2\text{O}_3$ -0.1%,  $\text{CaO}$ -6%) and silica gel containing 80% acid-soluble silicic acid and 20% residual  $\text{H}_2\text{O}$ . The soil—a sandune regosol contained about 98% sand, 17 mg  $\text{kg}^{-1}$  available P, negligible organic carbon and nitrogen and a pH of 6.5. The soil was filled into plastic pots (h: 30 cm, d: 18 cm) at the rate of 4 kg/pot. All the Si fertilizer sources were applied and mixed with the soil at 1000 mg/kg prior to being transferred to the pots. Nitrogen, and P were basally applied at the rate of 400 and 300 mg/kg, while the K content in all pots were adjusted to the same level as that supplied from potassium silicate. Other nutrients-Mg and Ca were similarly adjusted.

The 4 plant species, 2 irrigation regimes and 4 Si sources were factorially combined and arranged into a completely randomized design in three replications. The experiment was conducted in a bio-hazard room set at room temperature with a relative humidity of 60%. Several seeds of each species were sown per pot and thinned to three uniform seedlings per pot 10 d after sowing. Starting from this time, pots were weighed every few days and water was applied to adjust soil moisture to 6 (wet treatment) and 3% (dry treatment) respectively. This was continued until the experiment was terminated. Water treatments (deficit and adequate irrigation) were initiated 3 weeks after sowing. We monitored plant growth for 6 weeks after water treatment before harvesting to determine the dry mass (shoot and root) yield by oven-drying at 80°C for 48 h.

To compare Si, N, P and K uptake and their relationships in the two irrigation regimes, dry shoot samples were first milled into fine powder. Silicon concentration was determined according to the procedure followed by Lux et al. (2002). Two hundred mg of the milled sample was transferred into Si-free beakers and ashed (500°C) in a muffle furnace for 5.5 hs. Upon the addition of 10 mL dilute HCl (1:1), the ashed sample was heated to dryness at 100°C. This was repeated thrice, followed by the addition of 15 mL HCl, heating at 100°C for 2 min. and filtering with an ash-free filter paper. The filter paper was washed thrice with 5 mL of 1:10 HCl, and 100 mL distilled water and again ashed (540°C) in a Si-free ceramic crucible for 5.5 h. The weight of Si was determined after cooling. Total N concentration was determined by dry combustion using the Sumi-graph CHN analyzer. Total P and K were determined as reported in Eneji et al (2001). Nutrient uptake was estimated as the product of concentration and shoot dry mass.

Biomass yield response to each Si source was calculated as follows:

$$\frac{+\text{Si yield} - \text{no Si yield}}{\text{no} - \text{Si yield}} \times 100\%$$

Biomass yield response to adequate water was calculated similarly as follows:

$$\frac{\text{Adequate irrigation yield} - \text{deficit irrigation yield}}{\text{deficit irrigation yield}} \times 100$$

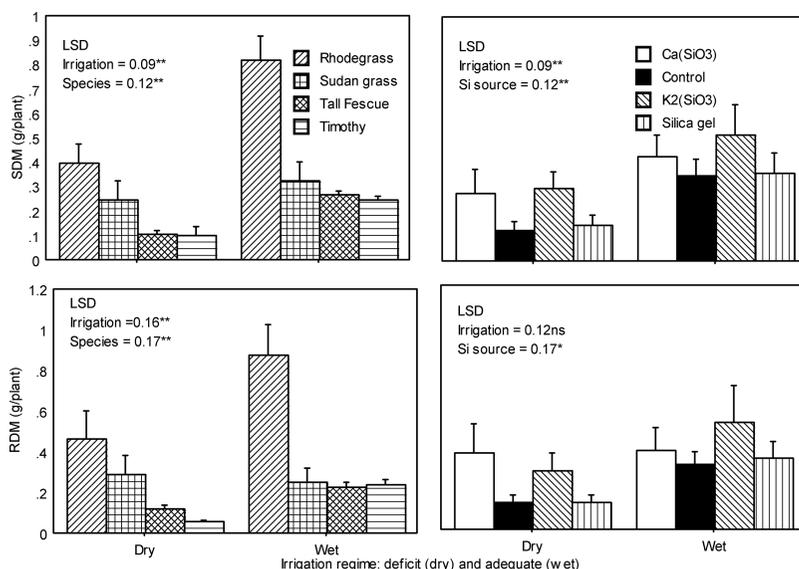
Analysis of variance was performed for all data using the Stat view software and treatment means were compared by LSD. Associations between Si uptake and the uptake of N, P and K were determined with linear correlations.

## RESULTS AND DISCUSSION

### Shoot and Root Dry Mass

The shoot and root dry mass (SDM and RDM) varied significantly according to irrigation, Si source and species (Fig. 1). Under adequate irrigation the SDM (0.4 g/plant) was about double that under deficit irrigation (0.2 g/plant).  $\text{K}_2\text{SiO}_3$  produced the best SDM (0.4 g/plant), followed by  $\text{CaSi}_2\text{O}_3$  (0.3 g/plant) but under adequate irrigation. Rhodes grass had much greater shoot mass than any of the species, irrespective of soil water conditions, while Timothy grass and Tall fescue performed poorly (Fig. 1). The poor performance may be ascribed to the soil; Timothy grass prefers wet, peaty, and heavily textured soils as opposed to the sandy soil used in this study. Although Tall fescue is a very adaptable species and grows well even in dry or wet conditions, the sandy soil was evidently not ideal.

For the sandy soil used in this study, silica gel seems to be an unsuitable source of Si. The root mass of plants fertilized with calcium silicate was markedly superior to all other sources under deficit irrigation, but potassium silicate was better under adequate irrigation (Fig. 1). As with SDW, Rhodes grass had much greater root mass than other species, but only Sudan grass produced greater root mass under deficit (0.32 g/plant) than adequate irrigation (0.25 g/plant). The interaction between species and Si was not significant, showing that the pattern of root development in any of the species was independent of the Si source. Matichenkov et al. (1999) reported that silicon substances accelerate the growth and development of the root system as observed for  $\text{CaSi}_2\text{O}_3$  in this study. Under field conditions, particularly in dense stands of sugarcane, Si stimulated growth and yield by decreasing mutual shading by improving leaf erectness, which decreases susceptibility to lodging (Savant et al., 1999). Leaf erectness which is mainly a function of Si depositions in the epidermal layers of



**Figure 1.** Variations in shoot and root dry mass in the various treatments (\* and \*\* indicate level of significance at  $p < 0.01$  and  $P < 0.05$ ).

the leaf panicle (Takahashi and Miyake, 1982), is an important factor affecting light interception in dense plant population and, hence, photosynthesis.

There were marked differences among species in the biomass yield responses to different silicate sources (Table 1). For all species, the response under deficit irrigation was significantly better than under adequate irrigation. The yield response of Rhodes grass across silicate sources was 205% under deficit irrigation compared with only 59% under adequate irrigation; for Sudan grass it was 49% compared with 26% and for Timothy, it was 48% compared with a mere 1%. The higher response to the silicate materials under deficit irrigation suggests that the plants relied more on these materials to endure or avoid drought stress. These trends also confirm earlier reports that Si application improved the tolerance of plants to water stress (Agarie et al. 1998; Ma et al 2001). Gong et al (2003) showed that silicon-applied wheat suffered no significant loss in dry mass even under drought conditions. As per the mechanism, Hattori et al. (2005) suggested that silicon application improved water uptake and hence, growth, through its effects on the stomatal conductance in sorghum.

On the average, the best silicate source for each irrigation level was  $K_2SiO_3$ , which may therefore be the recommended Si source for the sandy soil and grass species used in this study. However, the marked increases in soil pH associated with  $K_2SiO_3$  application must be addressed to avoid the expected problems of availability of certain nutrients and hence, their uptake by plants. Under deficit irrigation,  $CaSiO_3$  which also showed a high yield response (105%)

Table 1  
Biomass yield response (%) to Si application and irrigation among four grass species

Species	Adequate irrigation				Deficit irrigation				
	CaSiO <sub>3</sub>	K <sub>2</sub> SiO <sub>3</sub>	Silica gel	Mean	CaSiO <sub>3</sub>	K <sub>2</sub> SiO <sub>3</sub>	Silica gel	Mean	Irrigation
Rhodes grass	51.4	100.4	25.1	59.0	344.5	174.2	97.6	205.4	97.5
Sudan grass	24.9	89.1	-36.2	25.9	108.5	104.6	-66.1	49.0	7.1
Tall fescue	-25.5	-12.6	1.1	-12.3	4.0	53.7	-16.9	13.6	122.2
Timothy	1.1	-6.7	9.3	1.2	-36.8	153.5	26.3	47.7	209.0
Mean	12.9	42.5	-0.2	—	105.0	121.5	10.2	—	108.9

may be used. The observed variations in species response to silicon source must be considered in the choice of Si materials to improve plant growth or its resistance to stress.

Biomass yield of individual plants also responded differently to varying water levels as shown in Table 1. Timothy grass was the most sensitive to water stress as it showed the highest yield response (209%) to adequate irrigation. This was followed by tall fescue (122%) and Rhodes grass (97%). Sudan grass was the least affected by deficit irrigation, probably on account of improved root mass (Fig. 1) and its natural drought tolerance.

### Nutrient Uptake

The uptake of Si varied significantly between moisture regimes. Under the wet treatment, the uptake ( $17.5 \text{ g kg}^{-1}$ ) exceeded that in the dry treatment by 86% (Table 2). De Melo et al. (2003) found that Si accumulation in *Brachiaria* grasses was 1.4 times greater when the soil moisture was maintained at 80% field capacity than at 60% capacity. The increase in Si uptake with increasing soil water corroborates the hypothesis that Si is passively absorbed via mass flow, following the water during the transpiration process (Jones and Handreck, 1967). Differences in uptake among species and Si source were also significant. Among species, the Si uptake ranged from only  $5.8 \text{ g kg}^{-1}$  in Tall fescue to  $29.2 \text{ g kg}^{-1}$  in Rhodes grass and the uptake was greatest in  $\text{K}_2\text{SiO}_3$  ( $17.8 \text{ g kg}^{-1}$ ) compared with  $10.4 \text{ g kg}^{-1}$  in the silica gel treatment (Table 2). Some plants, notably dicots, are known as silicon non-accumulators and tend to have tissue concentrations of 0.5% or less (Marschner, 1995). Wetland grasses are known as Si accumulators because they tend to have relatively high concentrations (5% or higher) of tissue Si (Epstein, 1994). However, within a given plant species or cultivar, tissue levels of silicon vary in relation to soil Si availability (Datnoff et al., 1991). Silicon non-accumulators contain 0.5 percent to 1.5 % Si in their dry leaf tissues and absorb as much Si as is transported to their roots by the mass flow of transpirational water. Based on our Si uptake data, only Rhodes grass may be classed a silicon-accumulator.

Nitrogen uptake also varied highly according to soil moisture and plant species but was not much affected by Si source. The uptake in the dry treatment ( $8.4 \text{ g kg}^{-1}$ ) was about half that in the dry treatment ( $16.2 \text{ g kg}^{-1}$ ). Again the uptake was highest in Rhodes grass ( $20.9 \text{ g kg}^{-1}$ ) and lowest in timothy grass ( $8.8 \text{ g kg}^{-1}$ ). The uptake of P was severely reduced by deficit irrigation to only a third of that in the adequate treatment (Table 2). Highly significant differences were noted among species with Rhodes grass showing the highest P uptake. Silicon source did not have much influence on P uptake, although  $\text{CaSiO}_3$  and  $\text{K}_2\text{SiO}_3$  favoured greater uptake of P than silica gel, especially for Timothy and Rhodes grass. The mean uptake value of K was only  $1.4 \text{ g kg}^{-1}$  in the dry treatment compared with  $3.7 \text{ g kg}^{-1}$  in the wet treatment (Table 2). Among Si

Table 2  
Nutrient uptake (g/kg) among the four species in two moisture regimes and four silicon sources

Irrigation (I)	Si	N	P	K
Deficit	9.3	8.4	1.0	1.4
Adequate	17.5	16.1	3.3	3.7
CD <sup>¶</sup>	4.1**	3.4**	0.7**	1.3**
Si source (Si)				
K <sub>2</sub> SiO <sub>3</sub>	17.8	15.0	2.6	3.8
CaSiO <sub>3</sub>	14.9	13.8	2.4	2.7
Silica gel	10.4	10.3	1.9	1.9
Control	10.7	10.0	1.7	1.8
CD	5.8*	4.7*	ns	1.8*
Species (SP)				
Rhodes grass	29.3	20.9	4.3	5.1
Sudan grass	12.7	9.2	1.8	2.0
Tall fescue	5.8	10.2	1.4	1.8
Timothy grass	6.0	8.8	1.2	1.3
CD	5.8**	4.8**	0.9**	1.8**
I × Si	ns	ns	ns	ns
I × Sp	**	ns	**	ns
Si × Sp	ns	ns	ns	ns
I × Si × Sp	ns	ns	ns	ns

¶ = critical difference; \* and \*\* = significant at  $p < 0.05$  and  $0.01$  respectively; ns = not significant.

sources, K uptake varied from  $1.8 \text{ g kg}^{-1}$  in the control to  $3.8 \text{ kg}$  in  $\text{K}_2\text{SiO}_3$ . Rhodes grass had the highest K uptake ( $5 \text{ g kg}^{-1}$ ) while Timothy grass had the lowest ( $1.3 \text{ g kg}^{-1}$ ).

Reasonable correlations were noted between Si uptake and P uptake (Fig. 2) in both wet ( $r = 0.94$ ) and dry ( $r = 0.91$ ) treatments, indicating that Si application favours P uptake possibly because it can increase the water-soluble phosphorus in the soil (Mckeague and Cline, 1962). However, in a previous study with solution-cultured tomato, cucumber and bean, Miyake (1993) showed that Si application decreased P uptake. Gao et al (2004) also found that P concentration in xylem sap of maize treated with Si was significantly lower than in plants grown without Si, although the underlying mechanisms are still unclear. Also, the correlation between Si uptake and N uptake (Fig. 2) was reasonable ( $r = 0.88$  and  $0.84$  for the wet and dry treatments respectively). However, the uptake of Si under the wet treatment was less correlated ( $r = 0.54$ ) with K uptake than under the dry treatment ( $r = 0.74$ ). These relationships imply that Si application improves plant growth by balancing nutrient uptake, transport and distribution

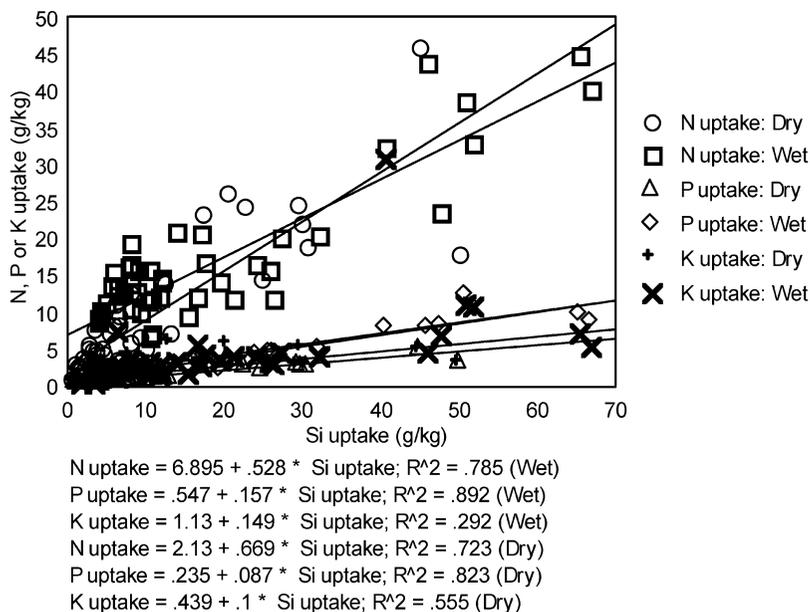


Figure 2. Relationships between the uptake of Si and that of N, P and K.

in plants. For rice and sugarcane, it increased yields by increasing growth, mineral nutrition, mechanical strength and resistance to mineral toxicities and biotic and abiotic stresses (Datnoff et al., 1997; Savant et al., 1999).

In conclusion potassium silicate was the best source of Si for the soil and species used in the study because it produced the greatest biomass yield responses across species. In the alternative calcium silicate may be used, but silica gel may be discarded as a Si source. Under deficit irrigation, plant biomass yield responded better to silicate application, suggesting their increased reliance on Si to avert moisture stress. The uptake of Si was positively correlated with those of N, P and K, but the variable responses of species to silicate sources must be considered in the choice of silicate materials for field application.

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