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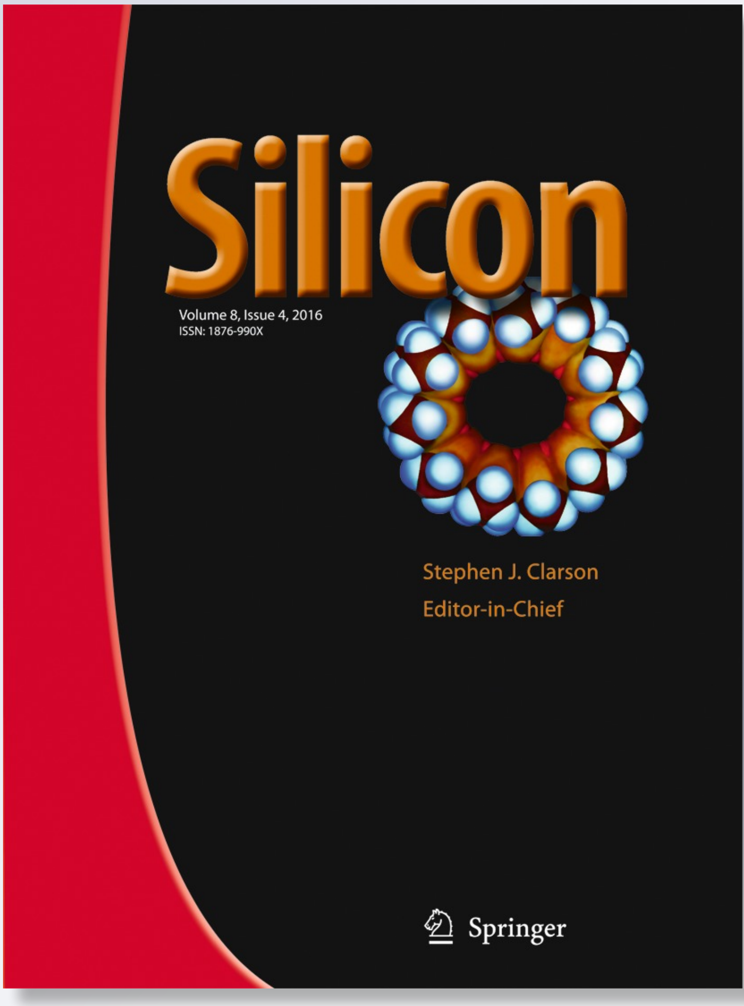

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ORIGINAL PAPER

The Effect of Seasonal Variations, Covariations with Minerals and Forage Value on Itchgrass' Foliar Silicification from Sudanian Benin

Changes in Itchgrass leaf silicification co-vary with minerals and forage value

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Abstract Silica (SiO_2) in forage grasses has been found in reducing cell-wall digestibility. This study investigates whether: (i) the seasonal variability affects the silica and minerals accumulation and forage values of leaves of *R. cochinchinensis* and (ii) silica concentration is correlated with minerals and fodder value. In an itchgrass population selected in the W Biosphere Reserve, leaves were collected on 90 marked plants from May to October 2003 and 2004, at 15 days intervals except May, June and October. Some 300 g of fresh blades from the 3rd most recently expanded leaves were oven dried and analyzed for dry mass, SiO_2, ash, N, Na, Ca, P, K, and Mg. Digestible Nitrogen Matter (DNM) and Fodder Energetic Value (FEV) were calculated using the Demarquilly formula. Apart from SiO_2, ash and forage value, data were log-transformed to restore homoscedasticity before statistical analyses. SiO_2 ranges from 5.69 % to 9.95 %, i.e. varying 1.4 fold between May and October, reaching 1.75 fold at mid-September. SiO_2 is positively related to Ca but negatively to K, P, N, DNM and FEV. The negative correlations suggest that SiO_2 concentration in *R. cochinchinensis* could be reduced with a significant increase in energy and accumulation of important nutrients such as N, P and K. Therefore, leaf silicification and nutritive value relationship should be conclusive in the case of itchgrass.

Keywords *Rottboellia cochinchinensis* · Silicification · Minerals · Seasonal variations · Forage value · Covariations · Sudanian Benin

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1 Introduction

Plant silicification has recently received increasing attention, especially where the economy is agriculture dependent. Grasses can have high silica content (1–13 %) [1] which, is taken up as monosilicic acid and deposited in the cell walls of leaves, especially on the leaf perimeter

[2]. Plant silica content is greatly influenced by soil type, water uptake and grass species. There can be over a 4-fold range in silica content in grass simply due to soil type. Clay soils have much more available soluble silica than other soil types.

Silicon plays important roles in plants. Its physiological benefits include nutrient uptake [3], photosynthesis and solar tracking [4]. The management benefits include aluminium (Al) tolerance and heavy metal toxicities control [3], diseases and pests' resistance, and minimising lodging [5]. However, the excess uptake of Si by grasses provides a higher mechanical resistance to degradation [6]. It also affects the quality of plant fibres [6, 7]. Silica is mostly deposited in organs through high transpiration [8, 9].

Leaf silica deposits may result from evaporation, because silica content increases where transpiration is high [10, 11]. Silica is the largest mineral component of perennial grasses. Grasses containing high levels of silica react to variation in precipitation.

Several studies have already shown that fodder species differ in silica content and this may affect their palatability and digestibility [10, 12, 13]. Silica may reduce livestock preference or palatability for certain plants [14]. Silicon may also reduce digestibility of fodder grass species [8] by: (i) acting as a varnish on the plant cell wall and reducing access to rumen microflora; (ii) complexing trace elements like Zn and reducing their availability to rumen microflora; or (iii) complexing enzymes that are integrally involved in rumen metabolism [12, 15]. Other reports indicate that a water soluble form of Si inhibits activities of some digestive enzymes, but the insoluble form is chemically inert [14].

Since tropical grass species were found to be highly silicified [11, 16], how to reduce leaf silica concentration to improve their palatability, digestibility and nutrient value for animal high productivity become questionable. There is a wide variation in the constituents of plant species grown under various conditions and particularly in the case of pasture grasses grown on tropical ferric acrisols. Investigations have focused on itchgrass, an annual grass, which is widely dominant in sudano-sahelian savanna [17, 18].

In the tropics, the harvesting of straw in the dry season replaces the hay and fodder conservation [19] and *R. cochinchinensis* is one of the preferred species [20]. This grass species ecology, germination and control management strategies are well documented [21–24]. Indeed, itchgrass is present in more than 30 warm-climate countries of Africa, America, Asia and Oceania. It thrives in moist, permeable heavy-textured soils. Itchgrass as a weed is particularly troublesome in crops [25]. This plant species is an erect grass of 285 cm high, showing one single axis with reduced basal branching, no shelf of tillers and dotted distribution of ground cover [16]. Its adventitious roots with lower nodes are as stilt. It is very well known and encountered in good economy water stations. It grows along roadsides and densely in some well-drained stations [26].

While it is feared by farmers because of its difficult control and progress [27–30], *R. cochinchinensis* largely contributes to grassland biomass production for feeding herbivores in West African tropical grasslands including the northern Benin as its maximum biomass in the studied area is 1018 ± 678 g DM ha^{-1} [16, 18]. The species and a complex of other grass and broadleaved plants are routinely grazed by herbivores [16, 31] and its fruits are used in feeding poultry [32]. At maturity, the stem is robust which decreases its appetite for cattle. It is consumed between May and November [16]. *R. cochinchinensis* is found to be a useful fodder grass when young and suitable for ensiling. When tall, its stiff hairs cause irritation and unpalatability [33]. Tanzanian farmers rank it third behind *Pennisetum purpureum* and *Megathyrsus maximus* for growth, milk yield and general animal health. It can be mixed with other grasses to feed cattle, which results in a higher dry matter intake [34]. In Pakistan, itchgrass was not found nutritive enough for animal requirements and supplementation was recommended [35]. In Samoa, wilting itchgrass gave better results than hay as dry season forage for goats and resulted in higher voluntary dry matter intake, energy intake, and crude protein digestibility [36]. The species is highly and diversely browsed by ruminants in northern Benin. But, until now, very few data exist on the nutritional aspects at the green stage. Moreover, SiO_2 accumulates with the plant age and high SiO_2 concentrations are generally associated with sclerophylly (i.e. a high proportion of vascular tissue and sclerenchyma), and a low specific leaf area (SLA) and relative water content [1]. However, the functional significance of the differences in SiO_2 content within the growth period is still little studied.

Variations in SiO_2 concentrations in relation to other leaf traits were recently analysed in *Andropogon gayanus* var. *bisquamulatus, Elymandra androphila, Hyparrhenia subplumosa*, *Panicum maximum* C_1 and *Panicum maximum.* Silica was correlated negatively with carbon and positively with relative water content, nitrogen, soluble ash and specific leaf area [37]. Furthermore, leaf traits differed with rainfall and species from drier sites showed lower SLA and higher N, as a response to stronger average irradiance in arid habitats [38]. But, there has been no attempt yet to correlate variation in SiO_2 concentration with variation in the nutritional value of the leaves of grasses.

In this work, we investigated: (i) the seasonal variability in silica and minerals concentrations and forage values of leaves of *R. cochinchinensis* throughout its growth season; and (ii) leaf silica concentration covariations with other minerals and fodder value.

2 Material and Methods

2.1 Study Area

The study was conducted in the W Biosphere Reserve in Benin (WBR), 11°26'-12°26'N and 2°17'-3°05'E (Fig. 1). It covers 6,102 km^2 representing 56.32 % of the Transboundary Biosphere Reserve shared by Benin, Niger and Burkina-Faso. There is a trade wind from April to November in a south-west direction and Harmattan from November to March in a north-east direction. This generates very low air humidity and environmental dry conditions. Minimum temperature decreases by 17 °C during the period of Harmattan in which the air dryness is the highest and the relative humidity is lower than 30 %. Climate is sudanian and characterized by a rainy season and a dry season. Rainy season occurs from May to October with average annual rainfall from 900 to 1100 mm [39]. Monthly mean temperature ranges from 25 °C to 35 °C and the relative moisture is the highest in August (81 %) and the lowest in February (26 %). Relative irradiance averages 2950 hours [39] and determines the environmental water balance. Annual earlier rains provide soil humidification followed by the humid period and the period of establishment of the active herbaceous vegetation. At the end of the rainy season, soil still remains relatively humid and some herbaceous species may still use available water for maximum growth.

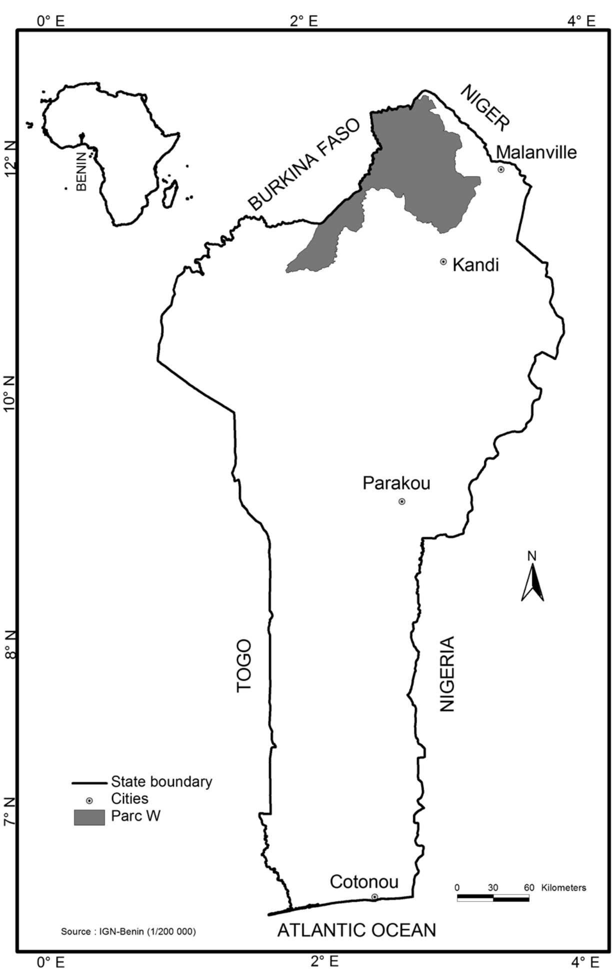

Fig. 1 The study area in northern Benin

Agriculture, cattle breeding and hunting are the most important socio-economic activities which highly threaten the landscapes conservation of the WBR [40]. The human density is about 18.2 inhabitants/km^2 and the most dominant ethnic groups are Batonou (32.6 %), Peul (22.1 %) and Dendi (18.2 %) [41]. The geological substratum rock is composed of quartzite, basilar rock, micaschist, schist, granite, gneiss and sandstone. Mineral, ferruginous and gley soils occur [19]. The main vegetation type encountered is shrubby and grass savannah and dry forest.

2.2 Materials and Samplings

A *Rottboellia cochinchinensis* pasture was sampled in the protected area at the establishment of the earlier native pasture on 25th March 2003. Plants that showed good shape were sampled among the species population in the W National Park (2°35'10"E; 12°20'38"N). Leaves were collected on 90 various plants sampled among the grassland, i.e. in 90 small plots of 1 m^2. Standardized leaves were marked on the same internodes on each plant stem. Blades were collected from the 3rd most recently expanded leaves from May to October at 15 days intervals except May, June and October. The leaves were washed to remove dust and stored in envelopes, sun dried for three days, oven dried for 2 days and used for mineral analyses. Ninety samples were harvested (i.e. 3 plants × 5 months × 2 treatments × 3 replicates). Soil and subsoil samples were taken for hydrogen ion determinations at the time the grass samples were collected, but no analyses were yet made and available for these samples. These collections were repeated from May to October 2004 on the same site.

2.3 Measurement of Leaf Traits

Sections were cut 6 cm above the ground and weighed. For each plot of 1 m^2, fresh material harvested at each date of cuttings was registered (FM). A sample of about 300 g of fresh material was taken on each plot, weighed in fresh (FM) and in dry (DM) after 48 hours oven dried at 65 °C. The dry matter content in the sample was calculated by the ratio DM/FM. Harvested dry matter is the product of FM by % DM.

Silica (SiO_2), nitrogen (N) and soluble ashes (SA) concentrations were analyzed in the samples. Nitrogen was analyzed by the Kjeldahl method. Silica was analyzed gravimetrically by dry ashing. Samples were oven-dried for 48 h at 105 °C and ground with a mill (Retsch ZM 100, Germany). The samples were ashed in crucibles at 550 °C in a muffle furnace (Lenton LCO4-1.06 Eurotherm 2416CG temperature/programmer; multi-program version 2416P8, Brussels, Belgium) for about 12 hours. Ashes were weighed (total ashes) and dissolved in hydrochloric acid (36–38 %) on a sand bath at 100 °C for 2 h, and filtered with ash-free filters (Schleicher and Schüll ashless, 589/2, 90 mm diameter). Filters were ignited in the muffle furnace for 12 hours. The residue (i.e. silica) was weighed and soluble ashes were calculated as (total ashes – silica). Soluble ashes and silica concentrations were expressed on an organic matter basis as follows: ODM = DM-SiO_2 (1); %SA = 100 × (TA-SiO_2)/ODM (2); %SiO_2 = 100× SiO_2/ODM (3); DM = dry mass, ODM = Organic Dry Mass; TA = Total Ashes.

Ninety samples of blades were analyzed for silica and soluble ashes, Na, K, Ca and Mg were analyzed in filtered solutions by Inductively Coupled Plasma Atomic Emission Spectrometry ICP-AES (Varian Vista MPX). Percentage based on dry mass was calculated for each parameter. Fodder value traits i.e. Digestible Nitrogen Matter (DNM) and Fodder Energetic Value (FEV) were calculated using the Demarquilly formula, i.e. DNM = [(%N × 6.25) – 4.5] (4).

2.4 Data Analysis

Data were examined with the Dixon test to detect outliers [42]. Chemical concentrations were compared to standards of Epstein & Bloom [43]. Statistical analyses were performed with STATISTICA 7.1 software (StatSoft Inc. 2005). Most data (except SiO_2 and SA), were log-transformed before analysis to restore homoscedasticity, i.e. N, P, K, Na, Ca, and Mg. ANOVAs were performed on the whole chemical and nutritional traits (N, P, K, Mg, Na, Ca, FEV, DNM, Ash, and SiO_2). One-way ANOVAs with the date of cutting mimicking the growth period as the main factor were performed to test differences between date's treatments. No transformation was performed for nutritional traits, i.e. FEV and DNM. Relationships between SiO_2 and the other traits (Ca, Mg, K, Na, DNM and FEV) were also assessed by means of Pearson correlation coefficients at 5 %. As there are no significant differences between the results obtained with materials collected from the same site in 2003 and 2004, only results from 2004 are shown in the present study as no significant difference was found among years.

3 Results

3.1 Dry Mass Production

The leaf dry mass production ranged from 20.3 to 44.27 % depending on the growth season with the highest value at the

Table 1 Seasonal change in foliar dry matter content, mineral concentrations and nutritional value of *Rottboellia cochinchinensis* from sudanian Benin during 2003 and 2004

Dates	DM (%)	SiO_2 (%DM)	Ash (%DM)	K (%DM)	Mg (%DM)	Ca (%DM)	P (%DM)	N (%DM)	Na (ppm)	Forage Energy (100 kg^{-1}DM)	DNM (g kg^{-1}DM)
End May	20.30±2.05a	5.69±0.63a	13.93±1.51ab	3.96±0.50e	0.28±0.05b	0.15±0.02a	0.28±0.04b	1.42±0.10d	122.67±12.06c	98.53±3.41c	46.70±4.20d
End June	23.60±1.70ab	8.56±0.10b	15.00±2.00b	3.09±0.50d	0.20±0.05ab	0.26±0.03bc	0.32±0.02b	1.05±0.04abc	130.00±13.01c	87.75±8.00c	25.78±3.00b
Mid-July	27.43±1.85bc	9.35±0.34b	15.59±2.18bc	2.96±0.25d	0.44±0.05c	0.22±0.03ab	0.27±0.05b	1.02±0.06abc	256.67±25.17d	72.45±3.00b	23.73±3.00b
End July	29.33±1.90cd	9.06±0.90b	16.00±2.00bc	2.71±0.20cd	0.26±0.04ab	0.24±0.03b	0.27±0.06b	0.96±0.03ab	62.83±7.37a	50.50±3.00a	20.33±2.14b
Mid-August	30.73±4.45cd	9.15±0.64b	15.00±1.20b	2.01±0.20ab	0.22±0.03ab	0.26±0.05b	0.25±0.04b	1.19±0.11c	64.83±6.43a	65.20±6.00b	33.63±2.03c
End August	33.47±1.86de	9.69±1.00b	15.00±3.00b	2.34±0.20bc	0.15±0.04a	0.32±0.04c	0.32±0.03b	1.02±0.10abc	75.00±7.21ab	61.20±9.00b	24.02±3.00b
Mid-September	36.07±3.04e	9.95±0.94b	13.00±2.00a	1.36±0.12a	0.16±0.04a	0.21±0.02b	0.25±0.04b	1.02±0.06bc	53.00±4.36a	62.50±6.00ab	25.55±2.01b
End September	40.40±1.80f	9.58±1.10b	15.00±3.00b	1.41±0.12a	0.16±0.02a	0.21±0.03ab	0.24±0.03b	1.02±0.09abc	92.17±5.13b	75.17±4.93b	23.76±2.00b
End October	44.27±1.16f	8.04±0.81b	12.00±2.00a	1.56±0.20a	0.22±0.04a	0.22±0.03ab	0.15±0.02a	0.84±0.08a	117.33±9.29c	94.13±5.95c	13.551.02a

M±SD (means plus and minus standard deviations); letters corresponded to post hoc groups at 5 %; Mid: Middle

Table 2 One way ANOVA of the effect of the growth season on mineral and forage value traits of *Rottboellia cochinchinensis*; $F_{8,18}$ value and significance at 5 % threshold

Source of variation	df	DM	SiO_2	Ash	K	Mg	Ca	P	N	Na	Energy	DNM
Growth season	8, 18	31.37	8.43	1.05	28.01	14.38	6.39	5.12	12.23	87.74	23.41	37.18
Probability	–	10^{-6}	$9.5\ 10^{-5}$	0.43	10^{-6}	$2.\ 10^{-6}$	$5.5\ 10^{-4}$	0.002	$7.\ 10^{-6}$	10^{-6}	10^{-6}	10^{-6}
Significance	–	*****	****	ns	*****	****	***	**	****	*****	*****	*****
CV	–	24.18	16.12	15.10	37.09	40.03	22.33	22.34	15.79	56.52	22.11	34.81

CV: coefficient of variation; CV= SD/Means; **:p<0.01; ***: P<0.001; ****: P<0.0001; *****: P<0.00001; ns: non-significant

End-October and the lowest at the End-May (Table 1). The general one way ANOVA showed a significant effect of the growing season on the dry mass production ($F_{8,18} = 31.37$; $P < 0.000001$; CV=24.2). The oldest leaves of *Rottboellia cochinchinensis* produced 118 % leaf dry mass more than the earliest.

3.2 Silica Concentration

The value of the leaves SiO_2 concentration of *Rottboellia cochinchinensis* ranging over the growing period are given in Table 1. The leaves are rich in SiO_2 (>0.1 %DM), as SiO_2 concentration ranged from 5.69 % to 9.95 %, depending on the growth period. There was a highly significant growth period effect (Table 2). End of May generally showed lower values (< 6 %), whereas from July to September showed much higher values (>8 %) (Table 1). Silica concentration varied 1.4 fold between the earliest and the latest growth periods, i.e. End-May and End-October respectively, reaching 1.75 fold at the Middle-September and there was a strong correlation among the harvest dates during the growth period (Table 2; $F_{8,18} = 8.43$; $p < 0.000095$; CV = 16.12 %).

3.3 Other Leaf Traits

As for silica, there was a highly significant season effect in all other *R. cochinchinensis* leaf traits except for ash (Table 2). This was largest for K, N and Na concentrations and for nutritional traits.

In Table 1 the mineral concentrations of *R. cochinchinensis* during the growth period indicate that these leaves are: (a) rich in Na (> 10 ppm DM) and K (> 1 %DM); (b) poor in Ca (< 0.5 %DM) and N (<1.5 %DM); (c) rich in Mg (> 0.2 %DM) except leaves that are harvested from the End-August to End-September; (d) rich in P (> 0.2 %DM) except the leaves that are harvested at the End-October.

Compared to other periods, the End-May generally showed higher values of N (1.42 ± 0.10 %DM), K

Table 3 Covariations between Minerals and forage value of leaves of *Rottboellia cochinchinensis*

	DM	SiO_2	Ash	K	Mg	Ca	P	N	Na	FE	NDM
DM	1										
SiO_2	**0.45***	1									
Ash	−0.30 ns	0.32 ns	1								
K	**−0.86*****	**−0.57****	0.26ns	1							
Mg	**−39***	−0.15ns	0.16ns	**0.43***	1						
Ca	0.17ns	**0.38***	0.06ns	−0.18ns	−0.34ns	1					
P	**−0.68*****	0.07ns	**0.44***	**0.44***	0.03ns	0.14ns	1				
N	**−0.63*****	**−0.50****	0.04ns	**0.58****	0.14ns	−0.25ns	0.26ns	1			
Na	−0.28ns	−0.15ns	0.07ns	**0.42***	**0.80*****	−0.19ns	−0.02ns	0.03ns	1		
FE	-0.12ns	**−0.69*****	−0.24ns	0.32ns	0.11ns	−045*	−0.26ns	0.22ns	0.37ns	1	
NDM	**−0.69*****	**−0.55****	0.14ns	**0.56****	0.09ns	**−039***	0.34ns	**0.88*****	−0.01ns	0.28ns	1

Data in bold: Significant correlation i.e. Coefficient of correlation and probability of significance. *: p<0.05; **: p<0.01; ***: P<0.001; ns: non-significant

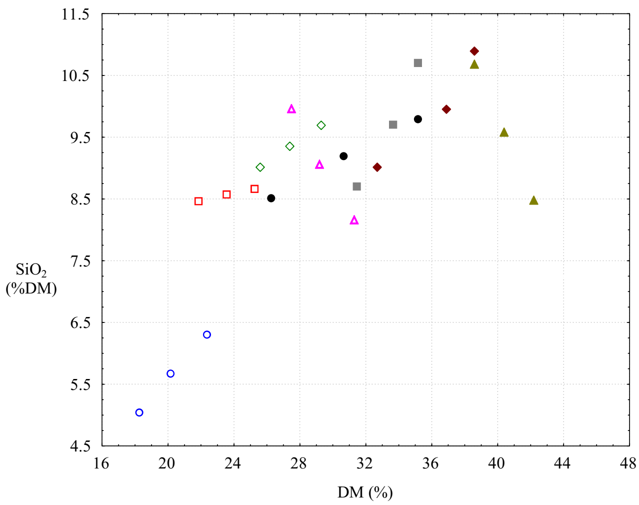

Fig. 2 Relationships between foliar concentrations in SiO_2 (%DM) and Dry mass (%); Where $R = 0.45$; $p < 0.05$; $N = 30$ (5 months. × 2 treatments × 3 replicates); Symbols: *: end October; ▲: end September;⧫: middle September; ■: end August;●: middle August; △: end July; ◊: middle July; □: end June; O: end May

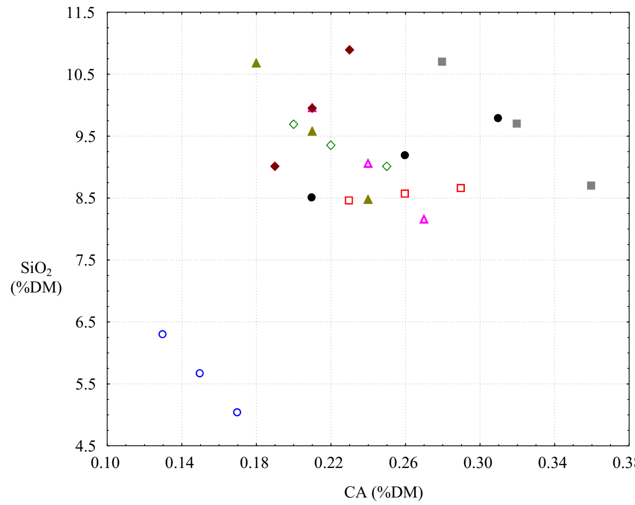

Fig. 4 Relationships between foliar concentrations in SiO_2 (%DM) and Calcium (%DM); Where $R = 0.38$; $p < 0.05$; $N = 30$ (5 months. × 2 treatments × 3 replicates); Symbols: *: end October; ▲: end September; ⧫: middle September; ■: end August; ●: middle August; △: end July; ◊: middle July; □: end June; O: end May

(3.96±0.5 %DM), Mg (0.28±0.10 %DM), FEV (98.53± 3.41g/100kgDM) and DNM (46.70±4.20 g.kg^{-1}DM). End-October showed lower values of ash (12±2 %DM), N (0.84±0.08 %DM), P (0.15±0.02 %), DNM (13.55±1.02 g.kg^{-1}DM) (Table 1). The leaves at the highest silica value (9.95±0.94 %DM) showed the lowest Na (53±4.36 ppm DM), K (1.36±0.12 %DM) and Mg (0.16±0.04 %DM) in the Middle-September.

3.4 Silica Concentrations Relationship with Other Traits

Table 3 shows Pearson coefficients of correlation between silica and the other parameters. Across the whole data set, silica concentrations are correlated positively with Ca and dry mass, but negatively with N, K, FEV and DNM (Table 3).

SiO_2 negatively correlated respectively with N concentrations (Fig. 2), K (Fig. 3), FEV (Fig. 4) and DNM (Fig. 5).

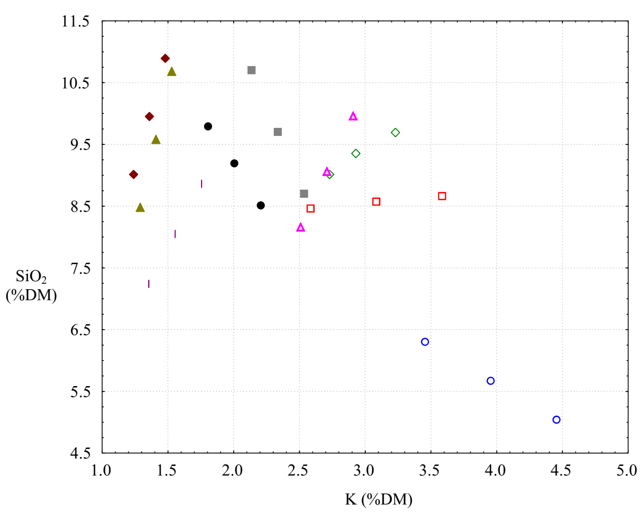

Fig. 3 Relationships between foliar concentrations in SiO_2 (%DM) and Potassium concentrations (%DM); Where $R = -0.57$; $p < 0.01$; $N = 30$ (5 months. × 2 treatments × 3 replicates); Symbols: *: end October; ▲: end September; ⧫: middle September; ■: end August; ●: middle August; △: end July; ◊: middle July; □: end June; O: end May

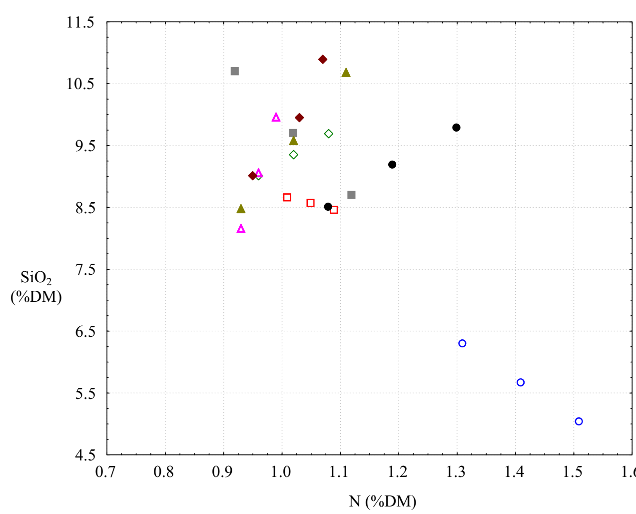

Fig. 5 Relationships between foliar concentrations in SiO_2 (%DM) and Nitrogen (%DM); Where $R = -0.50$; $p < 0.05$; $N = 30$ (5 months. × 2 treatments × 3 replicates); Symbols: *: end October; ▲: end September; ⧫: middle September; ■: end August; ●: middle August; △: end July; ◊: middle July; □: end June; O: end May

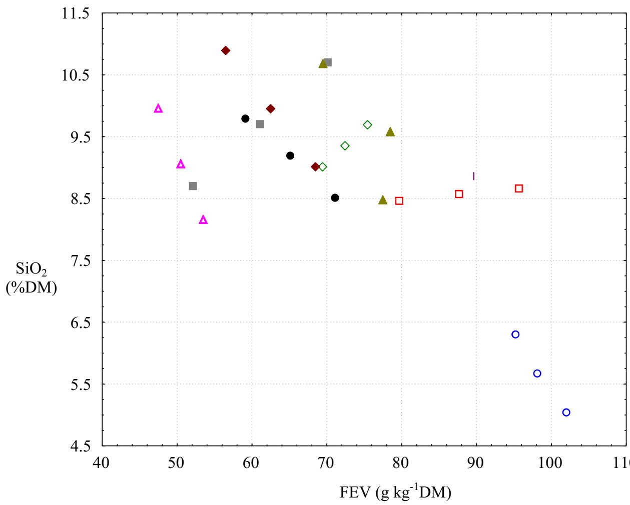

Fig. 6 Relationships between foliar SiO_2 (%DM) and Forage energetic value (g kg^{-1}DM); Where R = −0.69; p < 0.001; N = 30 (5 months. × 2 treatments × 3 replicates); Symbols: *: end October; ▲: end September; ⧫: middle September; ■: end August; •: middle August; △: end July; ♢: middle July; □: end June; O: end May

The general negative trend is due to the leaves which were harvested in September and August and showed the highest SiO_2 concentration (> 9.5 %) with the lower N (< 1.30 %), K (< 1.80 %) and FEV (< 90/100 kg DM), and the opposite trend with the leaves from End-May which showed lower values of SiO_2 (< 6.5 %) and higher values of N (> 1.30 %) and K (> 1.8 %).

Figures 2 and 4 respectively show the positive correlation between SiO_2 concentration and dry mass and

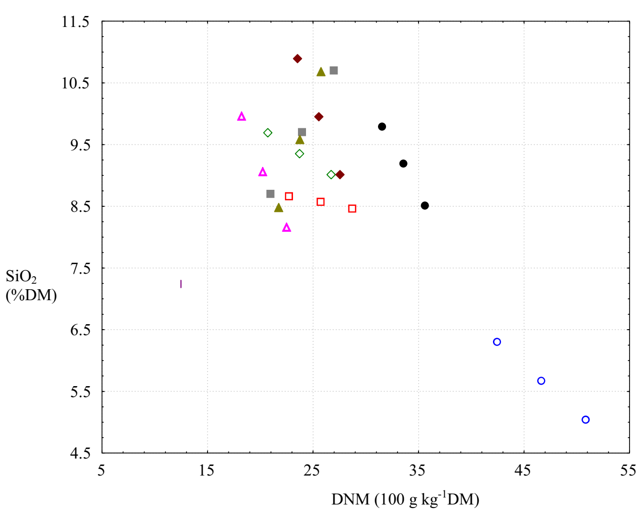

Fig. 7 Relationships between foliar SiO_2 (%DM) and Digestible Nitrogen Matter (100 g kg^{-1}DM). Where R = −0.55; p < 0.01; N = 30 (5 months. × 2 treatments × 3 replicates); Symbols: *: end October; ▲: end September;⧫: middle September; ■: end August; •: middle August; △: end July; ♢: middle July; □: end June; O: end May

Ca. Generally, the positive trend is mostly due to the leaves which are harvested at the End of May and show lower values for SiO_2, dry mass (< 21 %), Ca (< 0.20 %DM), and those which are harvested in August and September with highest values (respectively > 9.50 %DM, and 0.32 %DM). The pattern is not consistent for FEV and DNM. In fact, FEV and DNM values in the leaves of *R. cochinchinensis* varied significantly within the growth period (Tables 1, 2). While both traits highlighted the highest values at the End-May, FEV showed the highest values at the End-October and DNM at the End-May. SiO_2 showed strong negative correlation with FEV and DNM (Fig. 6–7). It appears that the leaves that are harvested at the Middle-May generally showed higher values of N (> 1.30 %), K (> 1.80 %), FEV (> 90/100kg DM) and DNM (> 40 g.kg^{-1}DM). At the End-October, they showed lower values of N (< 0.85 %), P (< 0.20 %), DNM (< 15 g.kg^{-1}DM) and higher values of dry mass (> 40 %), FEV (> 90/100 kgDM) and Na (> 110 ppm).

4 Discussion

4.1 Effect of Season on Foliar Silica Accumulation

Our results confirm that silica concentration often increases with the growth season. There have been relatively few tests of the response of silica content to phenology. Takahashi and Miyake [44] and Sinsin [16] are often quoted as field demonstrating the character of silica increase with the growing season. However, among the species they examined only *Eustachys paspaloides* and *Andropogon schirensis* showed enhanced silica accumulation with the growth period. Most other authors found lower concentrations of silica in plant leaves grown in the season [45–47, 49].

We found concentrations depending on the phenological stage. Phenological stage is well known to influence silica accumulation [48]. The higher leaf SiO_2 content of *R. cochinchinensis'* is consistent with the hypothesis that silica concentration increases with the plant's age [10, 15]. Our study indicates that *R. cochinchinensis'* increased its SiO_2 concentrations from May to September. Increasing SiO_2 concentration with age has already been reported [49–51]. Our study examines other leaf parameters. Interestingly, the growing season affected other leaf traits. A recurring pattern was the production of leaves with lower nitrogen concentration. Why could silica concentration increase in those leaves?

It was observed that on the poorer pastures, the plants were characterized by their high content of silica [16]. We have also found the phosphorus and nitrogen contents of itchgrass are relatively high, which suggests that this plant requires a relatively high fertility level for its successful growth. *R. cochinchinensis* is higher in phosphorus, nitrogen and silica than *Loxodera ledermannii* [52]. These data suggest that *L. ledermannii* will tolerate a lower fertility level than will itchgrass. This is consistent with the findings that itchgrass is a weed species for food crops such as maize and cassava [27–30], which require important investments for high productivities in the tropics. However, the ash and nitrogen contents did not change significantly from May to August. But these decreased in Middle-September and End-October which should be a good measure of the relative tolerance of this plant to various soil fertility levels.

Higher nutritional quality and lower silica content were found as a pattern in grass from heavily grazed sites [53, 54]. Increased silica accumulation might be a protective mechanism in leaves that would otherwise be palatable. Alternatively, enhanced silica accumulation might be an inevitable response. It is well known that silica accumulation is correlated to transpiration [15, 55]. Tropical leaves with a high photosynthetic capacity may have higher transpiration rates, thus increasing the silica deposition rate.

4.2 Silica Correlations with Other Leaf Traits

Correlations between the various constituents of the species are given in Table 3. In some cases, the response of other traits is similar to that of silica. However, the results are complex. Globally, there is a highly significant effect of growth period on leaf chemical accumulation (Table 2). SiO_2 was negatively correlated to K ($R_{SiO2/K} = -0.57$; $P<0.01$) and N ($R_{SiO2/N} = -0.50$; $P<0.01$). The correlation was positive with Ca ($R_{SiO2/Ca} = 0.38$; $P < 0.05$) or not significant with others. There is a high positive correlation between K, Mg, P, N and Na. This might be expected in case either of these is a limiting factor in plant growth. P and N are in the same family of elements. Generally, the pattern of foliar SiO_2 concentration is not clear throughout the growth period. Increased SiO_2 accumulation during the growth period might also result from interaction with uptake of other minerals. The positive correlation between SiO_2 and Ca suggests that the pattern of *R. cochinchinensis* foliar SiO_2 accumulation is identical to that of Ca. This is consistent with previous results on tropical grass species [1]. SiO_2 and Ca might be synergistic in these leaves. The more silicified leaves (harvested in September) show extreme values for Ca in the season and the lowest N, P, Mg and K. The low silicified leaves (i.e. harvested in May) hold the highest values of N, K, Mg, P and DNM and FEV early in the season. These elements show strong decreasing concentrations throughout the growth period, which might result from mineral dilution in a higher biomass production.

Both Ca and SiO_2 passively accumulated in plant organs having a high transpiration, and little mobility in the phloem. Indeed, SiO_2 was in synergy with Ca in rice iron-stressed conditions [56]. In contrast SiO_2 is antagonist to N, P, K, and Mg. Van der Vorm [57] observed that high SiO_2 content in nutrients solutions exerted suppressive effects on the contents of Ca and Mg in the leaves of rice and sugarcane. Clarifications are needed in the case of tropical grass silicification.

Otherwise, K is necessary for moving the sap [5]. If SiO_2 is accumulated through the transpiration stream [58], the plants that are poor in K might show lower SiO_2 concentration. K might be a functional regulator that reduces plant water loss. We found high K values in leaves harvested in April and August. This may imply that SiO_2 is either excluded for preferential uptake of K. Indeed, SiO_2 and K antagonistic and synergistic relationships have been documented [56]. Moreover, more silicified leaves i.e. harvested in September, show extreme values for Ca and the lowest values in N, P and K. The low silicified leaves i.e. harvested in May, showed the highest values of N, K, P and DNM early in the season. Negative correlation of SiO_2 with DNM and N suggests that silica might reduce the nutritional value of forage. Further studies are needed for analyze covariations between SiO_2 and structural and organic compounds and model tropical grass nutritional performances.

Plant silica generally increased during the growth period, as well as Ca. The mechanism of the apparent response is not clear. The intensity of the transpiratory stream could be an important determinant of the silica concentration during the growth season.

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