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Effect of Moisture Stress on Leaf Silicification of Three Tropical Fodder Grass Species (*Pennisetum purpureum*, *Panicum maximum* Cv. C1 And *P. Maximum* Jacq.) In Republic of Benin (West Africa)

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Abstract: Silica accumulation in plants was found to be influenced by environmental factors and growth conditions. But, the processes and patterns were reported to be extremely complex. To examine whether inter-specific variations in silica concentration and other traits and relationships exist in response to moisture stress, we created different moisture levels in 3 tropical fodder grass species (*Pennisetum purpureum*, *Panicum maximum* cv. C1 and *P. maximum* Jacq.) by watering pots every 3 days (moisture-stressed) or every day (control), from January 4 to March 15, 2002. Leaf biomass of all species was decreased in response to moisture stress and leaves were yellow and wilted. A lower leaf biomass under moisture stress was associated with blades of higher specific leaf area and water content and lower silica concentration in *P. purpureum* but not in the *Panicum* accessions. Silica concentration ranged from 2.03% to 5.2% in blades and from 1.95% to 3.4% in sheaths. *P. maximum* C1 had the lowest values, while *P. purpureum* showed the highest values. Increased silica deposition in well watered plants may result from higher transpiration rates. Silica was highly correlated with soluble ash in both species.

Key words: Water stress • Grass • Silica • Specific leaf area • Relative water content • Carbon • Soluble ashes

INTRODUCTION

Grasses can have high silica content (1-13%), which is taken up as monosilicic acid and deposited in the cell walls of leaves, especially on the leaf perimeter. Silicon plays important roles in plants. Its physiological benefits included nutrient uptake [1], photosynthesis and solar tracking [2]. The management benefits included aluminium (Al) tolerance and heavy metal toxicities control [1], diseases and pests' resistance and minimising lodging [3]. It also affected the quality of plant fibres [4, 5]. Several studies have showed that fodder species differ in silica content, which might affect their palatability and digestibility [6-8]. Silica may reduce livestock preference for and intake of certain plants [9] and may reduce digestibility of forage by: (i) acting as a varnish on the plant cell wall and reducing access to rumen microflora; (ii) forming insoluble compounds with trace elements

like Zn, reducing their availability to rumen microflora; or (iii) forming compounds with enzymes involved in rumen metabolism [7, 10]. Other reports indicate that a water-soluble form of Si inhibits activities of some digestive enzymes, while the insoluble form is chemically inert [9].

The inducible character of grass silicification in response to moisture status is still a subject of debate. Silica is mostly deposited in organs through high transpiration [11, 12] and is an agent for drought tolerance, reducing leaf transpiration [1]. The silicified cell wall of the epidermis behaves as a barrier against excessive water loss [13]. Leaf silica deposits may result from evaporation, because silica content increases where transpiration is high [14, 8]. However, silica concentrations in *Festuca scabrella* and *Stipa comata* in arid areas were similar to those at moist locations in Alberta [15]. On the other hand, *Carex filifolia*, which is

more silicified than *S. comata*, showed higher SiO₂ content at a moist location than at an arid one, suggesting that grasses containing high levels of silica react to variation in precipitation. Information is lacking on whether differences in silica concentration between species under various growth conditions affect their forage performance and value. The challenge is to develop pasture management strategies, which will improve both palatability and digestibility of tropical fodder grasses.

Co-variations between leaf anatomy and chemical composition in temperate grasses result in associations of high specific leaf area - low carbon concentration - high leaf water content - high nitrogen concentration - reduced sclerenchyma [16, 17]. In tropical species, variations in SiO₂ concentrations in relation to other leaf traits were recently analysed in *Andropogon gayanus* var. *bisquamulatus*, *Elymandra androphila*, *Hyparrhenia subplumosa*, *Panicum maximum* C1 and *Panicum maximum* [18]. Silica was correlated negatively with carbon and positively with relative water content (RWC), nitrogen (N), soluble ash (SA) and specific leaf area (SLA). Furthermore, leaf traits differed with rainfall; species from drier sites had lower SLA and higher N, as a response to stronger average irradiance in arid habitats [19]. Since silica appears to vary with leaf morphology, largely SLA, among species [20], will silica increase within a species under wet and dry conditions as SLA and LWC decrease with increasing water stress [21]? Whether plants from low-water environments are more sclerified than their relatives on higher rainfall sites as an adaptation to resist wilting and so mitigate cell damage [19], do species experiencing higher transpiration accumulate more silica? What are the consequences for their forage performance and values? In this study, leaves of 3 tropical grasses, widespread in various climates and grown in pots kept outdoors, were analysed to examine whether: (i) variations in silica concentration, morphological and structural traits are induced by water stress; (ii) this induction is correlated with changes within these traits; and (iii) inter-specific differences in silica concentration are correlated with differences in these traits between the species.

MATERIALS AND METHODS

Pennisetum purpureum, *Panicum maximum* and *Panicum maximum* cv. C1 are widespread in Sudano-guinean savanna [22] and contribute significantly to forage production in west Africa [23, 24]. Eight tillers from each of 5 clumps cut at 25 cm height were collected per species from the field and transplanted into the garden of the Faculty of Agronomic Sciences (Abomey Calavi University) in November 2000 - December 2001.

The experiment was conducted in southern Benin, from January to March 2002 (dry season), when precipitation was 78 mm and temperatures ranged from 20.3°C to 35.2°C. Monthly sunshine hours varied from 226.5 to 270.8 h, with a range in relative humidity of 34 - 94.4%. The evapotranspiration (ETP) of Penman calculated using the Penman-Monteith equation ranged from 112 to 183 mm (Table 1; [25]).

The soil on the experimental site is a sandy loam, which is slightly acid and poor in exchangeable bases, nitrogen and phosphorus. The C:N ratio is 10.6 (Table 2; [26]).

Twenty-four circular pots (6.61 dm³) were assigned to 3 species and 2 treatments with 4 replications. Eight plants of each grass species from the faculty garden were transplanted into pots filled with 6.5 kg of soil from the experimental site on January 4, 2002. Treatments were: watering pots daily (control); and watering every 3 days (moisture-stressed). Pots were randomly arranged with a spacing of 30 cm x 30 cm and all received the natural rainfall as well as artificial watering. Tillers grew until they had produced 2 expanded leaves and 5 tillers were marked for sampling to obtain material of a standardised age. Tillers were harvested on March 15, 2002.

For each plant per replication, 10 standardised leaf blades, a bulk sample of sheaths and a bulk sample of blades were sampled, washed and stored in envelopes. The standardised leaves were sampled at the 2nd internode from the base on *P. purpureum* and at the 3rd internode on *Panicum* accessions, to provide material of the same age. Blades were collected from the 2nd most recent fully expanded leaves on the 5 marked plants

Table 1: Climatic data (January - March 2002). P = precipitation; Tmi and Tma = minimum and maximum temperature; TM = mean temperature; RHmi and RHma = minimum and maximum relative humidity; SH = sunshine hours; ETP = evapotranspiration

Months	P (mm)	Tmi (°C)	Tma (°C)	TM (°C)	RHmi (%)	RHma (%)	SH (h)	ETP (mm)
January	37.7	20.3	32.8	26.6	34.0	94.2	270.8	112
February	0.0	22.7	35.2	28.9	40.1	94.1	226.5	165
March	40.6	24.2	35.1	29.7	45.0	94.4	255.9	183

Table 2: Soil parameters in the experimental garden of the Faculty of Agronomic Sciences of the Abomey Calavi University

pH (H ₂ O)	64
pH (KCl)	5.4
CEC (meq/100g)	6.23
Ca ⁺⁺ ech.meq/100g	2.58
Mg ⁺⁺ ech.meq/100g	1.32
K ⁺ ech.meq/100g	0.47
Na ⁺ ech.meq/100g	0.10
P (ppm)	6.89
N (%)	0.08
Organic matter (%)	1.40
C (%)	0.81
C:N ratio	10.6

Source: Sinsin and Kindomihou (1995)

(i.e., from 10 tillers) for *P. purpureum* and the 3rd most recently expanded leaves for *Panicum* accessions. Rectangular samples from the midpoint of each leaf blade were collected. These rectangular leaf portions were weighed fresh, measured for length and width and dried, before SLA and carbon concentrations were determined.

Ten sheaths and 10 blades per species from the leaf bulk samples from various internodes were also harvested, weighed fresh, oven-dried at 65°C for 48 h, ground and analysed for dry matter, silica and soluble ash concentrations. As no leaf area meter was available, the specific leaf area (SLA; ratio of leaf area to dry mass), as a key structural and functional leaf trait, was estimated on the portions of the standardized leaf blades. The mean of 10 samples per replication was calculated.

To determine the dry matter and leaf water concentrations, fresh samples were weighed just after harvest, blotted after washing and oven-dried for 48 h at 65°C.

Bulk samples were oven-dried for 48 h at 105°C and ground. Carbon was analysed on standardised blades by volumetric combustion using a Strohleim carbon analyser. Silica and soluble ash were analysed on bulk samples, gravimetrically by dry ashing. Samples were ashed in crucibles at 550°C in the muffle furnace Lenton LCO4-1.06 Eurotherm 2416CG, 2416P8 for 12 h. Dry ash was weighed to provide total ash content and then dissolved in hydrochloric acid (0.1 N) on a sand bath at 100°C for 2 h and filtered. Filters were ignited in a muffle furnace for 12 h. The residue corresponding to silica was weighed. Soluble ash and silica were calculated using expressions:

$\%SA = 100 \times (TA - SiO_2) / OM$; $\%SiO_2 = 100 \times SiO_2 / OM$; and $OM = DM - SiO_2$; where SA= soluble ash; TA = total ash; OM=organic matter; DM = dry matter; and SiO₂= silica. SA and Si were calculated as a percentage of organic matter.

Twenty-four (3 spp. x 2 treatments x 4 replications) bulk samples of both blades and sheaths were analysed for SiO₂ and SA and 24 samples of standardised leaves for carbon.

The Dixon test detected outliers [27] and statistical analyses were carried out using STATISTICA. Two-way ANOVA tested the influence of treatment (moisture stress) and species, as well as the interaction. One-way ANOVA tested differences between treatments within species and between species for each treatment. Post-hoc comparisons used Newman and Keul's test. Relationships between silica and other leaf parameters (C, SA, LWC and SLA) were assessed by means of the Pearson correlation coefficient.

RESULTS

Dry Mass Production: Leaf dry mass production ranged from 175.6 to 430.4 g/m², depending on treatment and species (Figure 1), with the highest values for *Pennisetum purpureum* and the lowest for *Panicum maximum* C1 (P<0.05). The well watered treatments produced more leaf dry mass for all species (Figure 1), with *P. purpureum* showing 430.4 g/m², *P. maximum* 265.8 g/m² and *P. maximum* C1 222.2 g/m². The general ANOVA showed a significant interaction of species x treatment. Moisture-stressed plants of *P. purpureum* produced 17.5% leaf dry mass than well watered plants, while corresponding reductions were 20.9% in *P. maximum* C1 and 8.4% in *P. maximum*.

Leaf Silica Concentrations: SiO₂ in blades ranged from 2.03% to 5.2%, depending on treatment and species (Figure 2a). Highly significant (P<0.001) variations existed among species, with *P. maximum* C1 having lower values (<2.4%) and *P. purpureum* higher values (>5%). In sheaths, SiO₂ ranged from 1.95% to 3.4%. *P. maximum* Jacq. showed the lowest value (1.95%) and *P. purpureum*

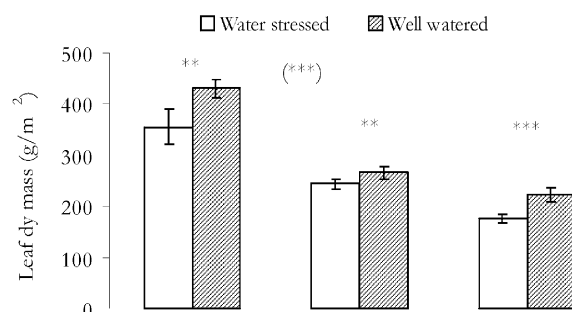


Fig. 1:

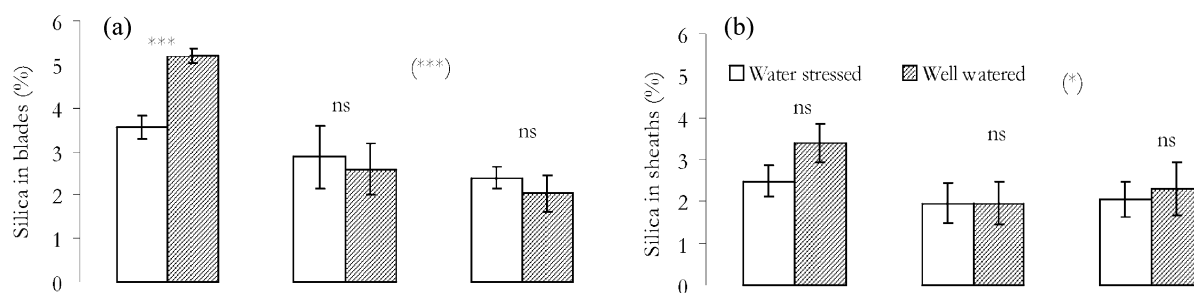


Fig. 2(a,b):

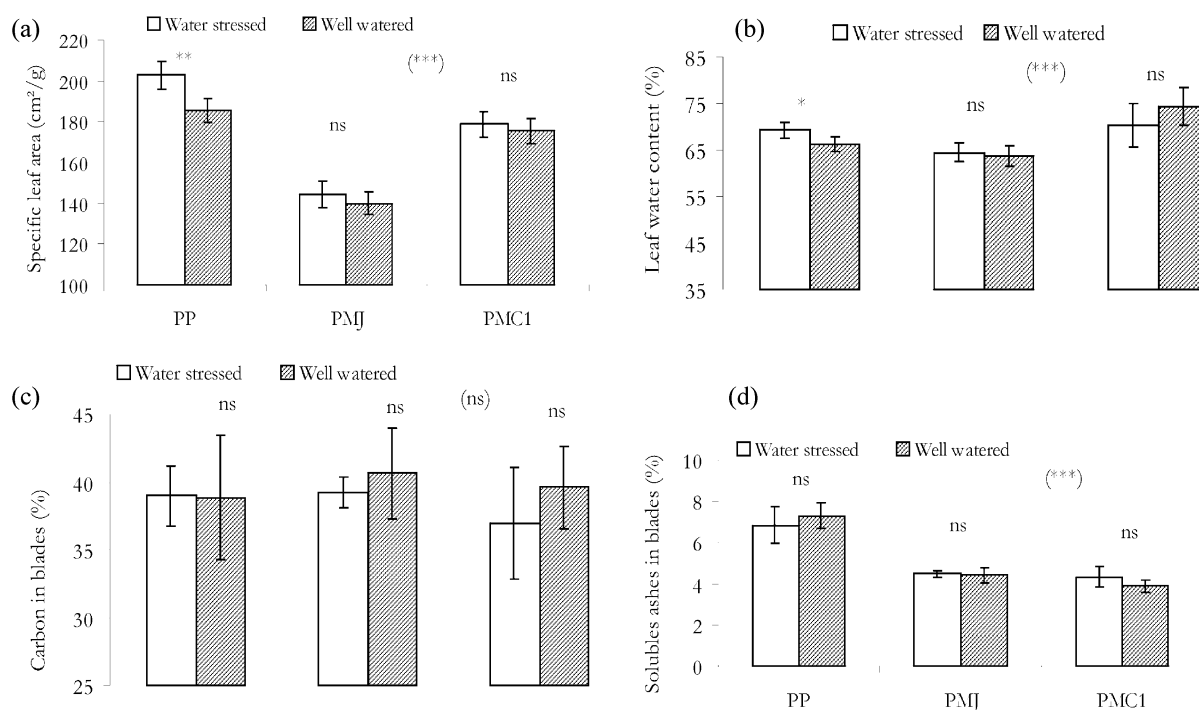


Fig. 3(a,b,c,d):

Figures: Chemical components and structural traits; Significance level of species effects in parentheses; PMJ: *Panicum maximum* Jacq.; PMC1: *Panicum maximum* C1; PP: *Pennisetum purpureum*; ns: non significant; ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$ Bars represents standard errors.

the highest (>3%) (Figure 2b). There was a highly significant interaction of species x treatment for SiO_2 in leaf blades, with silica levels unaffected by watering regimen in either *Panicum* accession, but decreasing by 35% ($P < 0.05$) in the moisture-stressed treatment for *Pennisetum*.

SiO_2 concentrations in leaf blades were correlated positively with soluble ash levels ($R_{\text{SiO}_2/\text{SA}} = 0.77$; $P < 0.001$).

Other Leaf Traits: There was a significant interaction of species x treatment for SLA and LWC. In general, SLA

was increased with moisture stress, but the differences were significant only for *Pennisetum* ($P < 0.01$) (Figure 3a), while LWC was increased with moisture stress in *Pennisetum* only ($P < 0.05$) (Figure 3b). As for silica, there was a highly significant species effect in other leaf traits except C (Figure 3c). Among the 3 species, *P. maximum* C1 generally had the lowest value for SA (<4.4%) (Figure 3d) and the highest for LWC (>70%) (Figure 3b), while *P. maximum* showed the lowest LWC in blades (<66.3%) (Figure 3b) and lowest SLA (<145 cm^2/g) (Figure 3a). SA in *P. purpureum* was higher than in the *Panicums*.

DISCUSSION

This study showed the relative influences of moisture stress on plant growth and silica concentration, with variable responses in the different species.

Does the moisture stress influence the plant silica accumulation?

The marked decrease in silica concentration in blades of *P. purpureum* following moisture stress contrasted with the small (but non-significant) increases in *Panicum* accessions. Wynn Parry and Smithson [28] suggested that soil moisture might influence SiO₂ uptake and its deposition as phytoliths in tropical grasses. The much lower silica levels in moisture-stressed plants of *P. purpureum* than in well watered ones are consistent with results from some field experiments, in which SiO₂ concentration in leaves of *Carex filifolia* at a moist location was higher than that at an arid location [15]. However, the finding contrasts with the hypothesis that increased transpiration rates under arid conditions should result in elevated rates of silica precipitation [29]. Similarly, it contrasts with results from a field experiment in which, under high rainfall, SiO₂ concentrations in leaves of *Festuca scabrella* and *Deschampsia caespitosa* were considerably lower than those of similar plants grown under low precipitation [15]. By comparison, silica levels in *Panicum* accessions were largely unaffected by moisture availability. Obviously, the two different genera have different mechanisms for coping with moisture stress. The drought-induced decrease in silica concentration in *P. purpureum* was also accompanied by marked increases in SLA and LWC. These results suggest a non-specific response to moisture stress in contrast with previous findings [8, 13]. Variations in SLA can result from either differences in leaf biomass [30] or changes in leaf cell wall constituents, *i.e.*, lignin and cellulose [31]. Otherwise, silicification would represent an alternative cheap structural compound that might substitute for energy-requiring organic compounds in species from arid environments, which would sclerify vasculature to resist wilting [32, 6, 19]. A negative relationship between silica and lignin concentrations in one case and lignin and cellulose in the other case, was found within 53 cultivars of rice (*Oryza sativa*) and 2 cultivars of *Bromus* [33, 34]. It is possible that silica accumulates less in grasses, which already invest considerable resources in supportive tissues, because the slow-growing species, generally poor in water and carbon, accumulate silica more in the epidermis for mechanical resistance [35, 36].

P. purpureum and *Panicum* accessions responded to moisture stress by producing much less leaf biomass (*i.e.*, 8-21% reduction) and leaves which were yellow and wilted, with higher SLA than in well watered plants. In contrast to previous findings [19], the ability of *P. purpureum* to display increased LWC in response to moisture stress probably reflects a higher ability for osmotic adjustment, or is a specific resource-use strategy that requires further investigation. The similar LWC in both wilted and turgid leaves in *Panicum* accessions might reflect a relative ability to escape some stresses [37, 38, 39], or some other mechanism since membrane elasticity, reduction of cell size and protoplasmic resistance (*i.e.*, ability of cells to resist mechanical damage and protein denaturising) are factors which maintain cell turgidity under drought conditions [40, 41]. All plants received 68 mm of rainfall from January to March 15, 2002 (Table 1), which may have masked the true influence of moisture stress on silica concentration in this study. The largest differences in soluble ash and silica concentrations in leaf blades of *P. purpureum* and *Panicum* accessions in this study appeared to be a nutritional discriminator. While high leaf production is a desirable attribute in a pasture grass, high silica concentration is undesirable from the point of view of reduced palatability.

Relationship Between Leaf Silica and Soluble Ash Concentrations: Silica and soluble ash (SA) concentrations were positively correlated in leaf blades. It has been well established that silicification is directly related to transpiration. As water evaporates, it becomes enriched in solutes including silicic acid. Consequently, SiO₂ preferentially precipitates where water has been removed, once concentration of silicic acid exceeds saturation level or pH conditions become favourable [29]. As lower water use reduces the uptake of silicic acid by plants (*i.e.*, SiO₂ and ash levels are decreased), Samson and Mehdi [42] suggested that ash is positively related to silica. Raven [12] found that the quantity of silica deposited per unit dry matter gained depends on the quantity of silicic acid per unit water transpired and the quantity of water transpired per unit dry matter gain. He concluded that the silicic acid transport system was constitutive rather than inducible. It is of interest that SA did not respond significantly to moisture stress in this study. For both species in pooled treatments, the significant and positive relationship between silica and soluble ash in leaf blades suggested

that the patterns of accumulation might be similar for silica and soluble ash. While high silica concentration is undesirable for reduced palatability, high ash concentration is desirable from the point of view of nutritive value of pasture grass.

CONCLUSION

This study found that changes in silica concentration in plant tissue in response to moisture stress varied with species. Our results showed that the decreased silica concentration would not represent a specific response to the moisture stress. Thus, leaves produced under moisture stress differed in some agronomic parameters (*i.e.*, lower leaf biomass, yellow and wilted leaves) and in some structural traits (high SLA and LWC with *P. purpureum*). Apart from transpiration, these alterations might result in either a higher ability for osmotic adjustment or a specific resource use strategy that requires further investigation. Silica concentration also varied with species. Part of this variation may be related to variation in leaf anatomy. In all pooled treatments, soluble ash and silica concentrations in leaf blades largely differed between species, but these traits were significantly and positively correlated. *Panicum* accessions proved less sensitive to moisture stress in terms of silica accumulation than *P. purpureum*. While high leaf biomass and high ash concentration are desirable in fodder grasses for production and nutritive value, high silica concentration is undesirable through reduced palatability. To obtain a better understanding of the inter-specific variation in silica accumulation in tropical grasses, much larger species samples will need to be assessed, with emphasis on variations in silica and: (i) leaf anatomy on one hand; and (ii) the various components of pasture nutritive value on the other hand.

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