Silicon and tanzania guinea grass tolerance to stress by copper toxicity

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Dissertation presented to obtain the degree of Master in Science. Area: Soil and Plant Nutrition

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1. Elemento benéfico 2. Estresse oxidativo 3. Fitorrremediação 4. Forrageiras 5. Interação Cu × Si 6. Toxidez por metal I. Título
RESUMO

Silício e a tolerância do capim-tanzânia ao estresse pela toxidez por cobre

O cobre (Cu) é um elemento essencial para as plantas, porém, quando em excesso, pode causar danos irreversíveis às plantas. Este metal induz a produção excessiva de espécies reativas de oxigênio (ERO), que danificam organelas causando a disfunção delas. Uma possível maneira de aumentar a tolerância de plantas aos metais é o fornecimento de silício (Si). Um experimento foi conduzido com o objetivo de avaliar o papel do Si (0, 1 e 3 mmol L\(^{-1}\)) nas respostas morfológicas, nutricionais, metabólicas e fisiológicas do Panicum maximum cv. Tanzânia sob doses de Cu (0,3, 250, 500 e 750 µmol L\(^{-1}\)). Esse capim foi cultivado hidroponicamente em casa de vegetação por dois períodos de crescimento (33 e 30 dias). Treze dias após a semeadura, plântulas foram transplantadas para solução nutritiva, fornecendo-se apenas as doses de Si por 25 dias. A exposição ao cobre foi realizada apenas no primeiro crescimento das plantas e durou sete dias. O segundo corte ocorreu 31 dias após o primeiro corte. O experimento consistia de seis blocos completos ao acaso: três para avaliações de produção, morfologia e análises nutricionais e três para análises metabólicas e fisiológicas. A produção, a morfologia e o metabolismo das plantas foram quantificados na parte aérea e nas raízes. O índice de conteúdo de clorofila (valores SPAD) e as análises fisiológicas foram determinados nas lâminas diagnósticas (LD), e as concentrações de Cu e Si nas LD e nas raízes. Para o cálculo dos acúmulos de Cu e Si levou-se em consideração toda a biomassa da planta. Plantas expostas a doses de Cu acima de 0,3 µmol L\(^{-1}\) apresentaram menores valores de produtividade, parâmetros morfológicos e de SPAD. Plantas supridas com Si apresentaram menor concentração e acúmulo de Cu, e maiores valores de produtividade, parâmetros morfológicos e SPAD do que aquelas que não receberam o fornecimento de Si. A concentração e o acúmulo de silício foram maiores nas plantas expostas ao excesso de Cu do que nas expostas à dose controle de Cu (0,3 µmol L\(^{-1}\)). Os parâmetros de trocas gasosas das plantas no primeiro crescimento foram afetados positivamente pelo Si e negativamente pelo incremento nas doses de Cu. No segundo crescimento, observou-se evento de eustresse em que plantas expostas à dose de Cu residual apresentaram os valores mais altos de parâmetros de troca gasosa e os valores mais baixos de indicadores de estresse. As atividades de enzimas antioxidantes foram reduzidas com o incremento nas doses de Cu. O suprimento de silício resultou em incremento na atividade da superóxido dismutase (SOD). O capim tanzânia suplementado com Si foi capaz de suportar melhor a toxicidade do Cu, mostrando um aumento na produção de biomassa da planta, e em parâmetros morfológicos e de trocas gasosas. As plantas suplementadas com Si reduziram a absorção de Cu e, consequentemente, plantas expostas a altas taxas de Cu e suplementadas com Si ainda foram capazes de produzir uma biomassa apreciável na rebrota.

Palavras-chave: Elemento benéfico; Estresse oxidativo; Fitorremediação; Forrageiras; Interação Cu × Si; Toxidez por metal
**ABSTRACT**

**Silicon and tanzania guinea grass tolerance to stress by copper toxicity**

Whist copper (Cu) is an essential element for plants, when this element is present in excess quantities it can cause irreversible damage. This metal induces excessive production of reactive oxygen species (ROS), which damages organelles causing dysfunction. A possible means for the promotion of metal tolerance in plants is the addition of the element silicon (Si). The current study was conducted with the aim of evaluating the role of Si (0, 1 and 3 mmol L⁻¹) on the morphologic, nutritional, metabolic and physiological responses of *Panicum maximum* cv. Tanzania under different Cu rates (0.3, 250, 500 and 750 µmol L⁻¹). The grass was grown in a greenhouse under hydroponic conditions for two growth periods (33 and 30 days). Thirteen days after sowing, the seedlings were transplanted to a nutrient solution and supplied just with the Cu rate of 0.3 µmol L⁻¹ and the set Si rates for 25 days. The remaining Cu rates were only added for a seven day period during the first growth stage. The second harvest took place 31 days after the first harvest. The experiment had six randomized blocks: three for yield, morphology and nutritional analyses and three for metabolic and physiological analyses. Plant yield, morphology and metabolic parameters were quantified in shoots and roots. Chlorophyll content index (SPAD values) and gas exchange parameters were determined in diagnostic leaves (DL), and Cu and Si concentrations were analysed from the DL and roots. The calculation of Cu and Si contents took into account the whole plant biomass. Plants exposed to Cu rates above 0.3 µmol L⁻¹ showed low values of plant yield, morphologic parameters and SPAD, in both growth periods. Silicon supplied plants showed lower Cu concentration and content, and higher values of plant yield, morphologic parameters and SPAD than the ones with no Si application. Silicon concentration and content were higher in plants exposed to excess Cu compared to those exposed to the control rate (0.3 µmol L⁻¹). Gas exchange parameters in plants of the first growth were positively affected by Si supply and negatively affected by Cu rates. In the second growth, an eustress event was observed, in which plants exposed to stressing rates of residual Cu showed the highest values of gas exchange parameters and the lowest values of stress indicators. The activities of antioxidant enzymes were reduced with the increment in Cu rates. Silicon supply resulted in an increment in superoxide dismutase (SOD) activity. Tanzania guinea grass supplied with Si was able to better deal with Cu toxicity, showing increases in plant yield, morphologic and gas exchange parameters. Silicon supplied plants reduced their absorption of Cu and consequently, plants exposed to high Cu rates were still able to produce considerable biomass in the regrowth.

**Keywords:** Beneficial element; Cu × Si interaction; Forage grass; Metal toxicity; Oxidative stress; Phytoremediation
1. INTRODUCTION

The total area of permanent pasture in Brazil is about 197 million hectares, corresponding to 71% of the country's agricultural area and 23% of total land area (FAO 2012). Due to the fact that most of Brazil's land area is located within tropical climate zones, tropical pastures are responsible for feeding the majority of the national herd. *Panicum maximum* is a tropical forage grass species that is grown throughout ~20% of the 100 million hectares of cultivated pastures in Brazil (Torres et al. 2016). This species has a number of cultivars, including tanzania grass, which is characterized by its rusticity, high leaf/stem ratio, high seed production, high regrowth rate and low seasonality (Jank et al. 2010, Jank et al. 2013). Besides the use of this grass for animal feeding and ground cover, its potential for phytoextraction of contaminant metals has been investigated in a number of studies (Monteiro et al. 2011, Gilabel et al. 2014, Rabêlo et al. 2016).

Plant species that are used for recovery of contaminated soils have the ability to absorb metals from within the environment. However, the efficiency of such plants can be limited by the presence of these chemicals within the substrate. A benefit of tanzania guinea grass in phytoremediation is that it is relatively tolerant to excess metals such as copper (Cu), cadmium (Cd) and barium (Ba), and has the ability to maintain high productivity in highly contaminated environments (Monteiro et al. 2011, Gilabel et al. 2014, Rabêlo et al. 2016). Additionally, the regrowth of the forage grasses results in a greater phytoextraction of the metal as the plant does not require resowing.

Heavy metals have adverse effects on human health and therefore the contamination of the food chain by these metals deserves special attention (Ali et al. 2013, Zeng et al. 2015). Some metals such as Cu, are essential for plants, classified as a micronutrient due to the low concentration in plant tissue, which reflects the low demand in most cultivated plants. Copper acts in many functions in the plant, mainly related to photosynthesis, respiration, metabolism of reactive oxygen species (ROS), remodelling of the cell wall and stacking of thylakoids (Burkhead et al. 2009). The excess of this metal in plants induces excessive production of reactive oxygen species (ROS), which damage mitochondria, chloroplasts and peroxisomes of the cells, causing dysfunction (Chandna et al. 2012). Plant processes are affected by excess Cu because these organelles are responsible for the main metabolic processes, including the production of chemical energy, photosynthesis, photorespiration, oxidative phosphorylation, \( \beta \)-oxidation and tricarboxylic acid cycle. Copper is naturally present in soils as rocks, sediments and minerals (Baker 1990). In addition to weathering, other additions of anthropic Cu to the soil can occur via
fertilizers, sewage sludge, pesticides (sulfate, oxychloride, Bordeaux syrup, among others) and atmospheric deposition by dust, rain and industrial smoke (Malavolta 2006).

Research aiming to enhance the phytoremediation potential of the plants and to modulate the phytoextraction of the metal has been conducted by adding nutrients and beneficial elements such as silicon (Si) to the growth medium. The beneficial effects of Si in the relief of abiotic stress, especially those caused by heavy metals, are attributed to its deposition mainly in the cell walls of roots, leaves and stems, which creates binding sites for metals. In addition, Si can reduce apoplastic flow and consequently, the translocation of toxic metals (Ma & Yamaji 2006). Silicon may also contribute to the compartmentalization of heavy metals within the cell vacuole, as it can attach to the metal and carry it further into the compartment (Wang et al. 2015). Although Si has already been shown to be effective in reducing toxicity effects of excess Cu in certain species, this effect has not yet been proven for tropical grass, such as Tanzania guinea grass.

In this study, the main objective was to evaluate the effect of Si application on the metabolic, physiological, nutritional and productive attributes of Panicum maximum cv. Tanzania exposed to differing Cu rates in nutrient solution in greenhouse conditions.

REFERENCES

Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals-Concepts and applications. Chemosphere 91:869-881. https://doi.org/10.1016/j.chemosphere.2013.01.075

Baker DE (1990) Copper. In: Alloway BJ (ed) Heavy metals in soils. John Wiley & Sons Inc., Somerset, pp 151-176

Burkhead JL, Reynolds KAG, Abdel-Ghany SE, Cohu CM, Pilon M (2009) Copper homeostasis. New Phytol 182:799-816. https://doi.org/10.1111/j.1469-8137.2009.02846.x

Chandna R, Hakeem KU, Ahmad P (2012) Proteomic markers for oxidative stress: New tools for reactive oxygen species and photosynthesis research. In: Ahmad P , Prasad MNV (ed) Abiotic stress responses in plants: Metabolism, productivity and sustainability. Springer, New York, pp 181-196

FAO (2012) Food and Agriculture Organization of the United Nations - statistics division. http://faostat3.fao.org/. Accessed 26 April 2018

Gilabel AP, Nogueirol RC, Garbo AI, Monteiro FA (2014) The role of sulfur in increasing guinea grass tolerance of copper phytotoxicity. Water Air and Soil Poll 225:1806-1816. https://doi.org/10.1007/s11270-013-1806-8

Jank L, Martuscello JA, Euclides VPB, Valle CB, Resende RMS (2010) Panicum maximum In: Fonseca DM , Martuscello JA (ed) Forage plants. UFV, Viçosa, pp 166-196 (in Portuguese)

Jank L, Braz TGS, Martuscello JA (2013) Warm-season grasses. In: Reis RA, Bernardes TF, Siqueira GR (ed) Forage farming: science, technology and management of forage resources. Multipress, Jaboticabal, pp. 109-125 (in Portuguese)

Ma JF, Yamaji N (2006) Silicon uptake and accumulation in higher plants. Trends in Plant Sci 11:392-397. https://doi.org/10.1016/j.tplants.2006.06.007
Malavolta E (2006) Mineral plant nutrition guide. Agronômica Ceres Ltda., São Paulo, p 638 (in Portuguese)

Monteiro FA, Nogueiro RC, Melo LCA, Artur AG, Rocha F (2011) Effect of barium on growth and macronutrient nutrition in tanzania guineagrass grown in nutrient solution. Commun Soil Sci Plan 42:1510-1521. https://doi.org/10.1080/00103624.2011.581725

Rabêlo FHS, Azevedo RA, Monteiro FA (2016) Proper supply of S increases GSH synthesis in the establishment and reduces tiller mortality during the regrowth of Tanzania guinea grass used for Cd phytoextraction. J Soils Sediments 1:1-10 https://doi.org/10.1007/s11368-016-1429-y

Torres FE, Teodoro PE, Benites SB, Oliveira EF, Correa CCG, da Silva FA (2016) Number of cuts for estimating forage productivity in Panicum maximum. Biosci J 32:172-178 http://dx.doi.org/10.14393/BJ-v32n1a2016-29307

Wang SH, Wang FY, Gao SC (2015) Foliar application with nano-silicon alleviates Cd toxicity in rice seedlings. Environ Sci Pollut R 22:2837-2845 https://doi.org/10.1007/s11356-014-3525-0

Zeng XF, Wang ZW, Wang J, Guo JT, Chen XJ, Zhuang J (2015) Health risk assessment of heavy metals via dietary intake of wheat grown in Tianjin sewage irrigation area. Ecotoxicology 24:2115-2124 https://doi.org/10.1007/s10646-015-1547-0
2. SILICON MODULATES COPPER ABSORPTION AND INCREASES YIELD OF TANZANIA GUINEA GRASS UNDER COPPER TOXICITY

Conclusion

Excess Cu negatively affected the biomass production and chlorophyll contents of *Panicum maximum* cv. Tanzania, and increased Cu concentration. Silicon supply improved biomass production and chlorophyll content by decreasing Cu concentration. Besides reducing Cu absorption, the most important role of Si was to reduce the transport of Cu from roots to shoots. Although Si supply did not increase Cu phytoextraction, it allowed successive harvesting of the aboveground biomass, which could be an interesting approach in phytoremediation programs.

REFERENCES

Abdula SE, Lee HJ, Ryu H, Kang KK, Nou I, Sorrells ME, Cho YG (2016) Overexpression of BrCIPK1 gene enhances abiotic stress tolerance by increasing proline biosynthesis in rice. Plant Mol Biol Rep 34:501-511. https://doi.org/10.1007/s11105-015-0939-x

Adrees M, Ali S, Rizwan M, Zia-ur-Rehman M, Ibrahim M, Abbas F, Farid M, Qayyum MF, Irshad MK (2015) Mechanisms of silicon-mediated alleviation of heavy metal toxicity in plants: A review. Ecotox Environ Safe 119:186-197. https://doi.org/10.1016/j.ecoenv.2015.05.011

Ali S, Rizwan M, Ullah N, Bharwana SA, Waseem M, Farooq MA, Abbasi GH, Farid M (2016) Physiological and biochemical mechanisms of silicon-induced copper stress tolerance in cotton (*Gossypium hirsutum* L.). Acta Physiol Plant 38:262. https://doi.org/10.1007/s11738-016-2279-3

Chandna R, Hakeem KU, Ahmad P (2012) Proteomic markers for oxidative stress: New tools for reactive oxygen species and photosynthesis research. In: Ahmad P , Prasad MNV (ed) Abiotic stress responses in plants: Metabolism, productivity and sustainability. Springer, New York, pp. 181-196

Collin B, Doelsch E, Keller C, Cazeveille P, Tella M, Chaourand P, Panfili F, Hazemann JL, Meunier JD (2014) Evidence of sulfur-bound reduced copper in bamboo exposed to high silicon and copper concentrations. Environ Pollut 187:22-30. https://doi.org/10.1016/j.envpol.2013.12.024

Cooke J, Leishman MR (2016) Consistent alleviation of abiotic stress with silicon addition: a meta-analysis. Funct Ecol 30:1340-1357. https://doi.org/10.1111/1365-2435.12713

Crestana S, Guimarães F, Jorge LAC, Ralish R, Tozzi CL, Torre A, Vaz CMP (1994) Evaluation of root distribution in soil by digital image processing. Rev Bras Cienc Solo 18:365-37 (in portuguese)

Elliott CL, Snyder GH (1991) Autoclave-induced digestion for the colorimetric determination of silicon in rice straw. J Agr Food Chem 39:1118-1119. https://doi.org/10.1021/jf00006a024
Epstein E (1994) The anomaly of silicon in plant biology. P Natl Acad Sci USA 91:11-17. https://doi.org/10.1073/pnas.91.1.11
Epstein E, Bloom A (2004) Mineral nutrition of plants: Principles and perspectives. Sinauer Associates, Sunderland, p 380
Frantz JM, Khandekar S, Leisner S (2011) Silicon differentially influences copper toxicity response in silicon-accumulator and non-accumulator species. J Am Soc Hortic Sci 136:329-338
Gilabel AP, Nogueirol RC, Garbo AI, Monteiro FA (2014) The role of sulfur in increasing guinea grass tolerance of copper phytotoxicity. Water Air Soil Poll 225:1806-1816. https://doi.org/10.1007/s11270-013-1806-8
Hoagland D, Arnon DI (1950) The water culture method for growing plants without soil. California Agricultural Experiment Station, Berkeley, p 32
Iwasaki K, Maier P, Fecht M, Horst WJ (2002) Leaf apoplastic silicon enhances manganese tolerance of cowpea (Vigna unguiculata). J Plant Physiol 159:167-173. https://doi.org/10.1016/S0176-1617(01)00691-X
Kim YH, Khan AL, Kim DH, Lee SY, Kim KM, Waqas M, Jung HY, Shin JH, Kim JG, Lee IJ (2014) Silicon mitigates heavy metal stress by regulating P-type heavy metal ATPases, Oryza sativa low silicon genes, and endogenous phytohormones. BMC Plant Biol 14:13. https://doi.org/10.1186/1471-2229-14-13
Langer RHM (1979) How grasses grow. Eduard Arnold Ltd., London, p 66
Lavres Júnior J, Santos JDG, Monteiro FA (2010) Nitrate reductase activity and SPAD readings in leaf tissues of guinea grass submitted to nitrogen and potassium rates. Rev Bras Cienc Solo 34:801-809. http://dx.doi.org/10.1590/S0100-06832010000300022
Li J, Leisner SM, Frantz J (2008) Alleviation of copper toxicity in Arabidopsis thaliana by silicon addition to hydroponic solutions. J Am Soc Hortic Sci 133:670-677
Ma JF, Yamaji N (2006) Silicon uptake and accumulation in higher plants. Trends Plant Sci 11:392-397. https://doi.org/10.1016/j.tplants.2006.06.007
Maksymiec W (1997) Effect of copper on cellular processes in higher plants. Photosynthetica 34:321-342. https://doi.org/10.1007/A1006818815528
Mateos-Naranjo E, Galle A, Florez-Sarasa I, Perdomo JA, Galmes J, Ribas-Carbo M, Flexas J (2015) Assessment of the role of silicon in the Cu-tolerance of the C-4 grass Spartina densiflora. J Plant Physiol 178:74-83. https://doi.org/10.1016/j.jplph.2015.03.001
Megatelli S, Dosnon-Olette R, Trotel-Aziz P, Geffard A, Semsari S, Couderchet M (2013) Simultaneous effects of two fungicides (copper and dimethomorph) on their phytoremediation using Lemna minor. Ecotoxicology 22:683-692. https://doi.org/10.1007/s10646-013-1060-2
Monteiro FA, Nogueirol RC, Melo LCA, Artur AG, da Rocha F (2011) Effect of barium on growth and macronutrient nutrition in tanzania guineagrass grown in nutrient solution. Commun Soil Sci Plan 42:1510-1521. https://doi.org/10.1080/00103624.2011.581725
Nogueirol RC, Alleoni LRF, Nachtigall GR, de Melo GW (2010) Sequential extraction and availability of copper in Cu fungicide-amended vineyard soils from Southern Brazil. J Hazard Mater 181:931-937. https://doi.org/10.1016/j.jhazmat.2010.05.102
Nowakowski W, Nowakowska J (1997) Silicon and copper interaction in the growth of spring wheat seedlings. Biol Plantarum 39:463-466. https://doi.org/10.1023/A:1001009100026
Rabelo FHS, Azevedo RA, Monteiro FA (2016) Proper supply of S increases GSH synthesis in the establishment and reduces tiller mortality during the regrowth of tanzania guinea grass used for Cd phytoextraction. J Soils Sediments 1:1-10 https://doi.org/10.1007/s11368-016-1429-y
Rogalla H, Romheld V (2002) Role of leaf apoplast in silicon-mediated manganese tolerance of Cucumis sativus L. Plant Cell Environ 25:549-555. https://doi.org/10.1046/j.1365-3040.2002.00835.x
Sarruge JR, Haag HP (1974) Chemical analysis in plants. ESALQ, Piracicaba, p 56 (in Portuguese)

SAS Institute (2008): SAS/STAT: Qualification tools user's guide, version 9.2, Cary

Souza Junior JC, Nogueiro RC, Monteiro FA (2018) NO₃⁻/NH₄⁺ ratios affect nutritional homeostasis and production of Tanzania guinea grass under Cu toxicity. Environ Sci Pollut R 25: 14083–14096. https://doi.org/10.1007/s11356-018-1541-1

Van Bockhaven J, De Vleesschauwer D, Hofte M (2013) Towards establishing broad-spectrum disease resistance in plants: silicon leads the way. J Exp Bot 64:1281-1293. https://doi.org/10.1093/jxb/ers329

Vulavala VKR, Elbaum R, Yermiyahu U, Fogelman E, Kumar A, Ginzberg I (2016) Silicon fertilization of potato: expression of putative transporters and tuber skin quality. Planta 243:217-229. https://doi.org/10.1007/s00425-015-2401-6

Wang SH, Wang FY, Gao SC (2015) Foliar application with nano-silicon alleviates Cd toxicity in rice seedlings. Environ Sci Pollut R 22:2837-2845. https://doi.org/10.1007/s11356-014-3525-0

Werner JC, Paulino VT, Cantarella H, Andrade NO, Quaggio JA (1996) Forages. In: Van Raij B, Cantarella H, Quaggio JA, Furlani AMC (ed) Fertilization and liming recommendations for São Paulo State. IAC, Campinas, pp. 263-273 (in portuguese)
Conclusion

Although copper is an essential element to plant life, when present in excessive rates, it can cause irreversible damage, even in plants with metal tolerance such as Panicum maximum cv. Tanzania. Silicon supply improved photosynthetic parameters in the first growth period. As a consequence of this, stress indicators were considerably lower in plants supplied with Si. In the second growth, Cu stress increased gas exchange parameters and decreased stress indicators, suggesting an eustress event. Besides the fact that the activities of antioxidant system enzymes were reduced by Cu stress, SOD activity was increased by Si supply.

REFERENCES

Ali S, Rizwan M, Ullah N, Bharwana SA, Waseem M, Farooq MA, Abbasi GH, Farid M (2016) Physiological and biochemical mechanisms of silicon-induced copper stress tolerance in cotton (Gossypium hirsutum L.). Acta Physiol Plant 38:262. https://doi.org/10.1007/s11738-016-2279-3

Azevedo RA, Alas RM, Smith RJ, Lea PJ (1998) Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and roots of wild-type and a catalase-deficient mutant of barley. Physiol Plantarum 104:280-292. https://doi.org/10.1034/j.1399-3054.1998.1040217.x

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205-207. https://doi.org/10.1007/BF00018060

Bradford MM (1976) Rapid and sensitive method for quantification of microgram quantities of protein utilizing principle of protein-dye binding. Anal Biochem 72:248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Burkhead JL, Reynolds KAG, Abdel-Ghany SE, Cebu CM, Pilon M (2009) Copper homeostasis. New Phytol 182:799-816. https://doi.org/10.1111/j.1469-8137.2009.02846.x

Burzynski M, Klobus G (2004) Changes of photosynthetic parameters in cucumber leaves under Cu, Cd, and Pb stress. Photosynthetica 42:505-510. https://doi.org/10.1007/S11099-005-0005-2

Cakmak I, Horst WJ (1991) Effect of aluminum on lipid-peroxidation, superoxide-dismutase, catalase, and peroxidase-activities in root-tips of soybean (Glycine max). Physiol Plantarum 83:463-468. https://doi.org/10.1111/j.1399-3054.1991.tb00121.x

Chandna R, Hakeem KU, Ahmad P (2012) Proteomic markers for oxidative stress: New tools for reactive oxygen species and photosynthesis research. In: Ahmad P, Prasad MNV (ed) Abiotic stress responses in plants: Metabolism, productivity and sustainability. Springer, New York, pp. 181-196
Cooke J, Leishman MR (2016) Consistent alleviation of abiotic stress with silicon addition: a meta-analysis. Funct Ecol 30:1340-1357. https://doi.org/10.1111/1365-2435.12713

Deng Z, Zhao MM, Liu H, Wang YK, Li DJ (2015) Molecular cloning, expression profiles and characterization of a glutathione reductase in *Hevea brasiliensis*. Plant Physiol Bioch 96:53-63. https://doi.org/10.1016/j.plaphy.2015.07.022

Du F, Shi HJ, Zhang XC, Xu XX (2014) Responses of reactive oxygen scavenging enzymes, proline and malondialdehyde to water deficits among six secondary successional serral species in Loess Plateau. Plos One 9: e98872. https://doi.org/10.1371/journal.pone.0098872

Epstein E, Bloom A (2004) Mineral nutrition of plants: Principles and perspectives. Sinauer Associates, Sunderland, p 380

Fidalgo F, Azenha M, Silva AF, de Sousa A, Santiago A, Teixeira J (2013) Copper-induced stress in *Solanum nigrum* L. and antioxidant defense system responses. Food Energy Secur 2:70-80. https://doi.org/10.1002/fes3.20

Foyer CH, Noctor G (2000) Oxygen processing in photosynthesis: regulation and signalling. New Phytol 146:359-388. https://doi.org/10.1046/j.1469-8137.2000.00667.x

Gay C, Collins J, Gebicki JM (1999) Hydroperoxide assay with the ferric-xylene orange complex. Anal Biochem 273:149-155. https://doi.org/10.1006/abio.1999.4208

Gilabel AP, Nogueirol RC, Garbo AI, Monteiro FA (2014) The role of sulfur in increasing guinea grass tolerance of copper phytotoxicity. Water Air Soil Poll 225:1806-1816. https://doi.org/10.1007/s11270-013-1806-8

Gratao PL, Polle A, Lea PJ, Azevedo RA (2005) Making the life of heavy metal-stressed plants a little easier. Funct Plant Biol 32:481-494. https://doi.org/10.1071/FP05016

Gratao PL, Monteiro CC, Antunes AM, Peres LEP, Azevedo RA (2008) Acquired tolerance of tomato (*Lycopersicon esculentum* cv. Micro-Tom) plants to cadmium-induced stress. Ann Appl Biol 153:321-333. https://doi.org/10.1111/j.1744-7348.2008.00299.x

Heldt HW, Piechulla B (2005) Plant biochemistry. 3rd edn. Academic Press, Cambridge, p 656

Hideg E, Jansen MAK, Strid A (2013) UV-B exposure, ROS, and stress: inseparable companions or loosely linked associates? Trends Plant Sci 18:107-115. https://doi.org/10.1016/j.tplants.2012.09.003

Hoaghland D, Arnon DI (1950) The water culture method for growing plants without soil. California Agricultural Experiment Station, Berkeley, p 32

Jank L, Martuscello JA, Euclides VPB, Valle CB, Resende RMS (2010) *Panicum maximum* In: Fonseca DM , Martuscello JA (ed) Forage plants. UFV, Viçosa, pp 166-196 (in Portuguese)

Jank L, Braz TGS, Martuscello JA (2013) Warm-season grasses. In: Reis RA, Bernardes TF, Siqueira, GR (ed) Forage farming: science, technology and management of forage resources. Multipress, Jaboticabal, pp. 109-125 (in Portuguese)

Khandekar S, Leisner S (2011) Soluble silicon modulates expression of *Arabidopsis thaliana* genes involved in copper stress. J Plant Physiol 168:699-705. https://doi.org/10.1016/j.jplph.2010.09.009

Kim YH, Khan AL, Kim DH, Lee SY, Kim KM, Waqas M, Jung HY, Shin JH, Kim JG, Lee IJ (2014) Silicon mitigates heavy metal stress by regulating P-type heavy metal ATPases, *Oryza sativa* low silicon genes, and endogenous phytohormones. BMC Plant Biol 14:13. https://doi.org/10.1186/1471-2229-14-13

Ma JF, Yamaji N (2006) Silicon uptake and accumulation in higher plants. Trends Plant Sci 11:392-397. https://doi.org/10.1016/j.tplants.2006.06.007

Malavolta E (2006) Mineral plant nutrition guide. Agronômica Ceres Ltda., São Paulo, p 638 (in Portuguese)
Mateos-Naranjo E, Galle A, Florez-Sarasa I, Perdomo JA, Galmes J, Ribas-Carbo M, Flexas J (2015) Assessment of the role of silicon in the Cu-tolerance of the C-4 grass *Spartina densiflora*. J Plant Physiol 178:74-83. https://doi.org/10.1016/j.jplph.2015.03.001

Matsuno H, Uritani I (1972) Physiological behavior of peroxidase isoenzymes in sweet potato root tissue injured by cutting or with black rot. Plant Cell Physiol 13:1091-1101. https://doi.org/10.1093/oxfordjournals.pcp.a074815

Megateli S, Dosnon-Olette R, Trotel-Aziz P, Geffard A, Semsari S, Couderchet M (2013) Simultaneous effects of two fungicides (copper and dimethomorph) on their phytoremediation using *Lemna minor*. Ecotoxicology 22, 683-692. https://doi.org/10.1007/s10646-013-1060-2

Mihara M, Uchiyama M, Fukuzawa K (1980) Thiobarbituric acid value on fresh homogenate of rat as a parameter of lipid-peroxidation in aging, CCL4 intoxication, and vitamin-E deficiency. Biochem Med Metab B 23:302-311. https://doi.org/10.1016/0006-2944(80)90040-X

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405-410. https://doi.org/10.1016/S1360-1385(02)02312-9

Monteiro CC, Carvalho RF, Gratao PL, Carvalho G, Tezotto T, Medici LO, Peres LEP, Azevedo RA (2011a) Biochemical responses of the ethylene-insensitive never ripe tomato mutant subjected to cadmium and sodium stresses. Environ Exp Bot 71:306-320. https://doi.org/10.1016/j.envexpbot.2010.12.020

Monteiro FA, Nogueiro RC, Melo LCA, Artur AG, da Rocha F (2011b) Effect of barium on growth and macronutrient nutrition in tanzania guineagrass grown in nutrient solution. Commun Soil Sci Plan 42:1510-1521. https://doi.org/10.1080/00103624.2011.581725

Noreen S, Akhter MS, Yaamin T, Arfan M (2018) The ameliorative effects of exogenously applied proline on physiological and biochemical parameters of wheat (*Triticum aestivum* L.) crop under copper stress condition. J Plant Interact 13:221-230. https://doi.org/10.1080/17429145.2018.1437480

Paulose B, Chhikara S, Coomey J, Jung HI, Vatamaniuk O, Dhankher OP (2013) A gamma-glutamyl cyclotransferase protects Arabidopsis plants from heavy metal toxicity by recycling glutamate to maintain glutathione homeostasis. Plant Cell 25:4580-4595. https://doi.org/10.1105/tpc.113.111815

Rabêlo FHS, Azevedo RA, Monteiro FA (2016): Proper supply of S increases GSH synthesis in the establishment and reduces tiller mortality during the regrowth of tanzania guinea grass used for Cd phytoextraction. J Soils Sediments 1:1-10 https://doi.org/10.1007/s11368-016-1429-y

SAS Institute (2008) SAS/STAT: Qualification tools user’s guide, version 9.2, Cary

Schutzendubel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J Exp Bot 53:1351-1365. https://doi.org/10.1093/jxb/53.372.1351

Souza Junior JC, Nogueirol RC, Monteiro FA (2018): NO3⁻/NH4⁺ ratios affect nutritional homeostasis and production of Tanzania guinea grass under Cu toxicity. Environ Sci Pollut R 25: 14083–14096. https://doi.org/10.1007/s11356-018-1541-1

Stadtman ER, Oliver CN (1991) Metal-catalyzed oxidation of proteins - Physiological consequences. J Biol Chem 266:2005-2008

Szabados L, Savoure A (2010) Proline: a multifunctional amino acid. Trends Plant Sci 15:89-97. https://doi.org/10.1016/j.tplants.2009.11.009