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"Luiz de Queiroz" College of Agriculture

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. Fitorremediação 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 adition 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, morphlogic 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 invetigated 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, β-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.

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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.

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3. SILICON IMPROVES PHOTOSYNTHETIC ACTIVITY AND INDUCES SOD ACTIVITY IN TANZANIA GUINEA GRASS UNDER COPPER TOXICITY

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, sugesting 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.

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