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Nitrogen forms and the mitigation of cadmium toxicity in tanzania guinea grass

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Dissertation presented for the degree of Master in Science. Area: Soils and Plant Nutrition

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ABSTRACT

Nitrogen forms and the mitigation of cadmium toxicity in tanzania guinea grass

Adequate nutrition plays a key role in the phytoextraction of soil contaminants and may influence the response of plants to heavy metal toxicity. Nevertheless, there is no information on the influence of nitrogen (N), particularly in the forms of nitrate (NO_3^-) and ammonium (NH_4^+), on tanzania guinea grass (*Panicum maximum* cv. Tanzania) under cadmium (Cd) stress. Thus, in the present work, it was aimed to study the effect of these ions on the mitigation of Cd toxicity in this grass. Plants were grown in a greenhouse, using plastic pots containing nutrient solution and ground quartz as substrate. The experiment was laid out in a randomized complete block design, in a 3 × 3 factorial arrangement, with six replications. The factors tested were NO_3^-/NH_4^+ ratios (100/0, 70/30 and 50/50) and Cd rates (0.0, 0.5 and 1.0 mmol L^{-1}). Morphological, physiological and biochemical changes in the plants were studied throughout two growth periods. In addition to causing oxidative stress and visual toxicity symptoms, Cd exposure resulted in reduced chlorophyll content and photosynthetic activity, negatively affecting shoot and root growth. Moreover, it altered N metabolism and induced the accumulation of NO_3^- and NH_4^+ mainly in shoots, increasing plant total N concentration. The exclusive use of NO_3^- mitigated toxicity symptoms by favoring Cd accumulation in roots, maintaining normal N metabolism and increasing guaiacol peroxidase activity. On the other hand, although the simultaneous supply of NO_3^- and NH_4^+ increased the uptake, transport and accumulation of this metal by the grass, it also increased the photosynthetic capacity along with the synthesis of proline and protein. The use of these N forms at a 50/50 ratio increased the tolerance of the plants to Cd by inducing high superoxide dismutase and glutathione reductase activities in shoots and roots, respectively, maintaining cellular homeostasis and reducing oxidative stress. Cd uptake and accumulation in tanzania guinea grass are strongly related to the form of N available, and the negative effects of this metal on photosynthesis and on the balance between oxidants and antioxidants are attenuated by the partial replacement of NO_3^- by NH_4^+ in the nutrient solution.

Keywords: Ammonium; Antioxidant enzymes; Nitrate; Nitrogen fertilization; Nitrogen metabolism; *Panicum maximum*; Photosynthetic activity; Phytoextraction

RESUMO

Formas do nitrogênio e a mitigação da toxidez por cádmio no capim tanzânia

A nutrição adequada desempenha papel fundamental na fitoextração de contaminantes do solo e pode influenciar a resposta das plantas à toxidez por metais pesados. No entanto, não há informações sobre a influência do nitrogênio (N), particularmente nas formas de nitrato (NO_3^-) e amônio (NH_4^+), no capim tanzânia (*Panicum maximum* cv. Tanzânia) sob estresse por cádmio (Cd). Assim, no presente trabalho, objetivou-se estudar o efeito desses íons na mitigação da toxidez por Cd nesse capim. As plantas foram cultivadas em casa de vegetação, utilizando vasos plásticos contendo solução nutritiva e quartzo moído como substrato. O experimento foi arranjado em delineamento de blocos completos ao acaso, em esquema fatorial 3 × 3, com seis repetições. Os fatores testados foram proporções de NO_3^-/NH_4^+ (100/0, 70/30 e 50/50) e doses de Cd (0,0, 0,5 e 1,0 mmol L^{-1}). Foram estudadas alterações morfológicas, fisiológicas e bioquímicas nas plantas ao longo de dois períodos de crescimento. Além de causar estresse oxidativo e sintomas visuais de toxidez, a exposição ao Cd resultou em baixos teor de clorofila e atividade fotossintética, afetando negativamente o crescimento da parte aérea e das raízes. Além disso, ela alterou o metabolismo do N e induziu o acúmulo de NO_3^- e NH_4^+ principalmente na parte aérea, aumentando a concentração de N total na planta. O uso exclusivo do NO_3^- mitigou sintomas de toxidez favorecendo o acúmulo de Cd nas raízes, mantendo o metabolismo normal do N e aumentando a atividade da guaiacol peroxidase. Por outro lado, embora o suprimento simultâneo de NO_3^- e NH_4^+ tenha aumentado a absorção, o transporte e o acúmulo desse metal pelo capim, também aumentou a capacidade fotossintética juntamente com a síntese de prolina e proteína. O uso dessas formas de N na proporção de 50/50 aumentou a tolerância das plantas ao Cd induzindo altas atividades da superóxido dismutase e da glutationa redutase na parte aérea e nas raízes, respectivamente, mantendo a homeostase celular e reduzindo o estresse oxidativo. A absorção e o acúmulo de Cd no capim tanzânia estão fortemente relacionados à forma de N disponível, e os efeitos negativos desse metal na fotossíntese e no balanço entre oxidantes e antioxidantes são atenuados pela substituição parcial de NO_3^- por NH_4^+ na solução nutritiva.

Palavras-chave: Adubação nitrogenada; Amônio; Atividade fotossintética; Enzimas antioxidantes; Fitoextração; Metabolismo do nitrogênio; Nitrato; *Panicum maximum*

1. INTRODUCTION

The area devoted to pasture cultivation in Brazil has increased over the last few decades, given that it constitutes the basis of ruminant production in the country [1, 2]. In the genus *Panicum*, the *P. maximum* species has been widely cultivated, in both tropical and subtropical regions, due mainly to its tolerance and adaptability to diverse edaphoclimatic conditions [3]. Tanzania guinea grass (*P. maximum* cv. Tanzania) is one of the most important cultivars, because of its agronomic and nutritional value. In addition to a rapid growth and high biomass production, this forage grass uses its extensive root system to regrow over successive cycles. These are some characteristics that indicate a high potential for phytoextraction of soil contaminants [1, 4].

Cadmium (Cd) is a heavy metal widespread in the environment and highly toxic to animals and plants [5]. This element has no known biological function and enters the root via transmembrane carriers responsible for the uptake of other divalent cations, such as Ca, Mg, Fe, Cu and Zn [6]. One of the factors determining the suitability of plants for phytoextraction purposes is the ability to accumulate a given contaminant in their aboveground biomass. Silva et al. [3] found that tanzania guinea grass effectively transports Cd from roots to shoots, increasing the concentration of this metal in leaves and culms. Consequently, it may induce physiological and biochemical dysfunctions and affect the overall plant growth. However, the response of plants to soil contaminants depends on several factors, including their nutrient status [7].

Apart from carbon, oxygen and hydrogen, nitrogen (N) is the most abundant element in plants. The adequate supply of this nutrient is indispensable for plant growth and development, since it is found in amino acids, proteins, nucleic acids, nucleotides and other organic compounds. In addition, N is required for the formation of new cells and tissues and is also essential for carbon assimilation [8]. Nitrate (NO_3^-) and ammonium (NH_4^+) are the main inorganic forms of N absorbed by plants, which benefit from the simultaneous supply of these ions [9]. Nevertheless, although excess NH_4^+ is rapidly stored in the vacuoles, in large quantities it can cause toxicity by dissipating transmembrane proton gradients and affecting photosynthesis and respiration [8].

Santos et al. [9] studied the growth and productive responses of *P. maximum* cv. Aruana to six NO_3^-/NH_4^+ ratios (100/0, 85/15, 70/30, 55/45, 40/60 and 25/75) and observed that the N use efficiency was improved with the use of ratios ranging from 70/30 to 55/45. There were increased shoot (numbers of tillers and leaves and leaf area) and root (length and surface area) growth in plants supplied with combinations of NO_3^- and NH_4^+, proving the influence of N

forms on the grass yield. The authors found that when N is supplied, absorbed and assimilated as NH_4^+, less energy is spent in the NO_3^- assimilation pathway. The energy saved could then be used in other metabolic processes, enhancing plant growth.

Several studies have addressed the effect of N on the phytoextraction of soil contaminants, and showed that this nutrient can influence their availability and uptake by plants [7, 10, 11]. In this context, the supply of NO_3^- and NH_4^+ can help to mitigate the deleterious effects of Cd on perennial forage grasses. The use of strategies to stimulate the tolerance of tanzania guinea grass to heavy metals is of paramount importance for its agronomic management in contaminated sites [3, 12]. Adequate nutrition plays a key role in the phytoextraction of heavy metals [4, 13]. However, there is no information on the influence of N forms on this grass under Cd stress.

Therefore, morphological, physiological and biochemical responses of tanzania guinea grass grown with NO_3^-/NH_4^+ ratios and exposed to Cd rates were evaluated. The hypothesis was that the supply of N in the form of adequate NO_3^-/NH_4^+ ratios improves the plant nutrient status and enhances Cd phytoextraction. In addition, the simultaneous use of these N forms mitigates Cd toxicity, attenuating oxidative stress and improving the tolerance of the grass to this metal.

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2. NITROGEN FORM REGULATES CADMIUM UPTAKE AND ACCUMULATION IN TANZANIA GUINEA GRASS USED FOR PHYTOEXTRACTION

Conclusion

The development of tanzania guinea grass revealed that cadmium (Cd) uptake and accumulation are strongly related to the form of nitrogen (N) available. Although it increased plant total N concentration, Cd exposure reduced the chlorophyll content and negatively affected shoot and root growth. In addition, it altered N metabolism and induced the accumulation of nitrate (NO_3^-) and ammonium (NH_4^+) mainly in shoots. It is concluded that the exclusive use of NO_3^- mitigates Cd toxicity by favoring the accumulation of this metal in roots and maintaining normal N metabolism in the plant. Conversely, despite disturbing the glutamine synthetase activity and changing leaf and root morphology under Cd toxicity, the supply of NO_3^- and NH_4^+ at a 50/50 ratio increases the uptake, transport and accumulation of this metal. Thus, high Cd phytoextraction by tanzania guinea grass grown with both these N forms is achieved through enhanced Cd uptake rather than increased plant growth.

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3. PARTIAL REPLACEMENT OF NITRATE BY AMMONIUM INCREASES PHOTOSYNTHESIS AND REDUCES OXIDATIVE STRESS IN TANZANIA GUINEA GRASS EXPOSED TO CADMIUM

Conclusion

The negative effects of cadmium (Cd) on photosynthesis and on the balance between oxidants and antioxidants in tanzania guinea grass are attenuated by the partial replacement of nitrate (NO_3^-) by ammonium (NH_4^+) in the nutrient solution. This metal reduced photosynthesis by causing stomatal closure and impairing electron transport. However, the simultaneous supply of NO_3^- and NH_4^+, particularly at a 50/50 ratio, led to a eustress state that restored gas exchange and improved the function of photosystem II, increasing the photosynthetic capacity of the grass. Plants grown with 50/50 showed reduced lipid peroxidation along with increased synthesis of proline and protein. Moreover, despite inhibiting the guaiacol peroxidase activity in the presence of Cd, this NO_3^-/NH_4^+ ratio increased the tolerance of tanzania guinea grass to the metal by inducing high superoxide dismutase and glutathione reductase activities in shoots and roots, respectively, maintaining cellular homeostasis and reducing oxidative stress.

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