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Proportions of nitrate and ammonium and their impact in two species of *Poaceae*: a study with nanoparticles and ionic nutrients in soil and nutrient solution

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Thesis presented to obtain the degree of Doctor in Science. Area: Soil and Plant Nutrition

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RESUMO

Proporções de nitrato e amônio e seus impactos em duas espécies de *Poaceae*: um estudo com nanopartículas e nutrientes iônicos em solo e solução nutritiva

O nitrogênio (N) é o principal modulador do crescimento de gramíneas forrageiras. Estudos mostraram que o fornecimento de proporções de nitrato e amônio (NO_3^-/NH_4^+) melhoraram a performance do capim tanzânia [*Panicum maximum* Jacq. cv. Tanzania (syn. *Megathyrsus maximus* (Jacq.) B.K.Simon & S.W.L. Jacobs)] em situação de toxidez por excesso de cobre (Cu). No entanto, há poucos relatos indicando o efeito positivo de proporções de NO_3^-/NH_4^+ em gramíneas que receberam Cu via nanopartículas, excesso de manganês (Mn) ou sofreram limitação nutricional de S. Por isso, planejaram-se um experimento com milho (*Zea mays* L.) e dois com capim tanzânia com o objetivo de confirmar a hipótese que proporções de NO_3^-/NH_4^+ alteram a performance de gramíneas em condição de excesso e limitação de nutriente: i) um fatorial 2 × 4 em blocos completos ao acaso com quatro repetições, sendo os fatores: duas proporções de NO_3^-/NH_4^+ (100/0 e 50/50) combinada com quatro doses de nanopartículas de cobre (Cu) (0, 25, 50 e 100 mg kg^{-1}) em um Espodossolo da Florida, Estados Unidos, cultivado com milho; ii) um fatorial 2 × 4 em blocos completos ao acaso com quatro repetições, sendo os fatores: duas proporções de NO_3^-/NH_4^+ (100/0 e 70/30) combinadas com quatro doses de Mn (10, 500, 1.500 e 3.000 μmol L^{-1}) em solução nutritiva cultivada com capim tanzânia e; iii) um fatorial 2 × 3 em blocos completos ao acaso com quatro repetições, sendo os fatores: duas proporções de NO_3^-/NH_4^+ (100/0 e 70/30) combinadas com três doses de enxofre (S) (0,1, 1,0 e 2,0 mmol L^{-1}) em solução nutritiva cultivada com capim tanzânia. No experimento i) a produção de massa seca foi mais alta nas plantas de milho que receberam N na proporção 100/0 NO_3^-/NH_4^+, a qual propiciou maior disponibilidade de Cu e acumulação desse nutriente tanto na parte aérea quanto nas raízes, melhores crescimento, fotossíntese e absorção de nutrientes, aumento do pH do solo, especialmente na rizosfera, melhora na disponibilidade de nutrientes importantes, bem como a diminuição de outros tóxicos. No experimento ii) o excesso de Mn 3.000 μmol L^{-1} não afetou o crescimento do capim tanzânia. A máxima concentração de Mn foi obtida nas plantas que receberam todo o N na forma de NO_3^- e Mn 3.000 μmol L^{-1}, com valores acima do reportado para hiper acumuladoras. Esse efeito induziu o estresse oxidativo no capim, afetando negativamente a atividade da glutamina sintetase e fotossíntese. No entanto, nessa condição, ocorreu aumento da concentração de prolina, a qual foi relacionada à melhor sobrevivência da planta em situação de alto Mn. No experimento iii) em situação de suficiência de S, plantas crescidas com N na forma de NO_3^- e NH_4^+ cresceram melhor e acumularam mais S do que plantas crescidas com N somente como NO_3^-. A aplicação de NH_4^+ em plantas sob limitação de S induziu maior acumulação de NO_3^- nas raízes do que nas plantas crescidas somente com NO_3^-. Apesar disso, a limitação de S diminuiu a atividade fotossintética das plantas que receberam N como NO_3^-, mas não nas plantas que receberam conjuntamente NO_3^- e NH_4^+. A limitação de S causou estresse oxidativo, o qual foi mitigado pela ação da guaiacol peroxidase estimulada pelo efeito positivo da aplicação conjunta de NO_3^- e NH_4^+. Proporções de NO_3^-/NH_4^+ alteram a performance de duas espécies de *Poaceae* em condições de excesso e limitação de nutriente, mesmo na forma de nanopartículas, evidenciando que na fertilização nitrogenada deve-se levar em conta as proporções entre os íons de N e as condições abióticas impostas às plantas.

Palavras-chave: Assimilação de nitrogênio, Enzimas antioxidantes, Estresse oxidativo, Fotossíntese, Limitação de enxofre, *Megathyrsus maximum*, Nanopartículas de cobre, Prolina, Toxidez de manganês, *Zea mays*

ABSTRACT

Proportions of nitrate and ammonium and their impact in two species of *Poaceae*: a study with nanoparticles and ionic nutrients in soil and nutrient solution

Nitrogen (N) plays a key role for forage grass growth. Studies have been shown that the proportions of nitrate and ammonium (NO_3^-/NH_4^+) improved the growth of Tanzania guinea grass [*Panicum maximum* Jacq. cv. Tanzania (syn. *Megathyrsus maximus* (Jacq.) B.K.Simon & S.W.L. Jacobs)] in situations of copper (Cu) excess. However, no reports are indicating the positive effects of NO_3^-/NH_4^+ proportions for grasses that received Cu via nanoparticles (CuNP), excess manganese (Mn), or submitted to S limitation. For this reason, one experiment with maize (*Zea mays* L.) and two with Tanzania guinea grass were carried out to confirm the hypothesis that the proportions of NO_3^-/NH_4^+ affect the performance of grasses under conditions of excess nutrient and limitation. The experiments were: i) a factorial 2 × 4 in complete randomized block design with four replications. Factors were: two proportions of NO_3^-/NH_4^+ (100/0 and 50/50) combined with four rates of Cu nanoparticles (0, 25, 50, and 100 mg kg^{-1}) in a Spodosol soil from Florida, United States, grown with corn; ii) a factorial 2 × 4 in complete randomized block design with four replications. Factors were: two proportions of NO_3^-/NH_4^+ (100/0 and 70/30) combined with four rates of Mn (10, 500, 1,500, and 3,000 μmol L^{-1}) in nutrient solution grown with Tanzania guinea grass and; iii) a factorial 2 × 3 in complete randomized block design with four replications. Factors were: two proportions of NO_3^-/NH_4^+ (100/0 and 70/30) combined with three rates of sulfur (S) (0.1, 1.0, and 2.0 mmol L^{-1}) in nutrient solution grown with Tanzania guinea grass. Experiment i) the dry biomass of the maize plants was high at 100/0 NO_3^-/NH_4^+, which provided greater concentration and accumulation of Cu in the shoots and roots, as well as better growth, photosynthesis, and nutrient accumulation, increasing soil pH, especially in the rhizosphere soil, which improved the availability of important nutrients, as well as mitigating toxic effects of CuNP. Experiment ii) excess Mn at 3,000 μmol L^{-1} did not affect the dry biomass of Tanzania guinea grass. The maximum concentration of Mn was obtained in the plants that received a combination of all N as NO_3^- and Mn 3,000 μmol L^{-1}, with the averages higher than that reported for hyperaccumulators plants. This effect induced oxidative stress in the grass, negatively affecting the activity of glutamine synthetase and photosynthesis. However, in that condition, there was an increase in the proline concentration, which was related to the better plant survival in a situation of excess Mn. Experiment iii) in a situation of sufficient S, plants grown at 70/30 NO_3^-/NH_4^+ grown better and accumulated more S than those plants grown with NO_3^- solely. The application of NH_4^+ for plants under S limitation showed a high accumulation of NO_3^- in the roots. However, S limitation decreased the photosynthetic activity in the plants grown at N as NO_3^- solely, but not in the plants that received both NO_3^- and NH_4^+. The S limitation caused oxidative stress, which was mitigated by guaiacol peroxidase stimulated by the positive effects of NO_3^- and NH_4^+. Proportions of NO_3^-/NH_4^+ change the performance of two species of *Poaceae* in conditions of excess and nutrient limitation, even in the form of nanoparticles, indicating that the N fertilization must be carefully applied, considering proportions between N ions and abiotic conditions imposed for plants.

Keywords: Antioxidant enzymes, Copper nanoparticles, Manganese toxicity, *Megathyrsus maximum*, Nitrogen assimilation, Oxidative stress, Photosynthesis, Proline, Sulfur limitation, *Zea mays*

1. INTRODUCTION

Proportions of NO_3^-/NH_4^+ can modulate the N assimilation, biomass production, and the tolerance of Tanzania guinea grass [*Panicum maximum* Jacq. cv. Tanzania (syn. *Megathyrsus maximus* (Jacq.) B.K.Simon & S.W.L. Jacobs)] to abiotic stress under copper (Cu) toxicity (Souza Junior et al., 2018; Souza Junior et al., 2019). This effect aroused interest to verify interactions with other important nutrients, both in ionic and non-ionic forms, such as nanoparticles that can also be uptake, assimilated, and used by plants. It has been known that proportions of NO_3^-/NH_4^+ can mitigate the oxidative stress caused by heat (Hu et al., 2017; Liu et al., 2017), excess cadmium (Cd) (Leite and Monteiro, 2019), and salt (Meng et al., 2016) in plants. However, it has not yet been reported the effects of N forms associated with non-ionic nutrients, such as Cu nanoparticles, as well as this effects on stress mitigation caused by excess Mn or S limitation. Abiotic factors were selected as the following justification:

1. Cu nanoparticles: a) in recent years, several studies have shown a positive effect of Cu nanoparticles in agriculture. Copper nanoparticles are on average 10 times less toxic than ionic Cu (Ameh and Sayes, 2019) and more efficient than Cu sulfate for plant uptake (Palit and Goswami, 2015); b) copper is an essential element for life, but very toxic for humans at the concentrations above-recommended limits, as it can cause Alzheimer, reduction in cognition, and neurodegeneration (Brewer, 2010); c) no reports are showing the relationship between proportions of $NO_3^-/NH4^+$ and Cu nanoparticles, especially by analyzing the rhizosphere soil since NO_3^- can affect the chemical reactions in this zone.

2. Excess Mn: a) industrial and urban residues containing heavy metals are deposited on agricultural areas (Nogueirol et al., 2013), which causes contaminations of agricultural soil and water with Mn (Millaleo et al., 2010); b) manganese is a heavy metal essential for life, but its excess is very toxic. When entering in the food chain, Mn can intoxicate humans and cause neurodegeneration, alteration of motor functions, reduced response to speed, problems with smell, mood changes, memory lack, and cognitive impairment (Santos et al., 2014); c) just a few plants can live in soils highly contaminated with Mn, such as areas within and next to mining (Boojar and Goodarzi, 2008), which encourages researchers to explore the potential of nutrients to increase the plant tolerance against heavy metals, improving Mn phytoextraction and phytostabilization (Souza Junior et al., 2018; Souza Junior et al., 2019).

3. Limitation of S: a) sulfur is a nutrient very important for grasses growth when N fertilization is carried out (De Bona et al., 2013). However, the preferences for more concentrated fertilizers that do not contain S and its exportation by crops due to intensive

cultivation can affect the plant growth (Gao et al., 2016); b) in recent years, global agreements have been signed to reduce the emissions of S gases (Stern and Rydge, 2012), which led to a low deposition of S from the atmosphere to the soil, increasing the frequency of S deficiency in plants (Gao et al., 2016); c) sulfur is directly related to the tolerance of plants to abiotic stress and its deficiency can affect the plant growth and production in a global stressful context caused by climate and environmental changes (Bagheri et al., 2017; Rabêlo et al., 2016).

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2. NITRATE SUPPLY ENHANCES AVAILABILITY OF NUTRIENTS IN RHIZOSPHERE AND BULK SOIL AND COPPER NANOPARTICLES ACCUMULATION BY MAIZE PLANT

ABSTRACT

Maize (*Zea mays* L.) is one of the most responsive crops as a function of nitrogen (N) fertilization, which is required in large amounts and can be supplied with a combination of NO_3^- and NH_4^+. Copper (Cu) is a micronutrient for plants and Cu nanoparticles (CuNP) is one of the sources of Cu fertilizers used. However, there are no studies associating proportions of NO_3^-/NH_4^+ and CuNP for maize plants. The objective was to evaluate the effects of NO_3^-/NH_4^+ proportions combined with Cu nanoparticles on soil properties, nutrient availabilities, chlorophyll fluorescence, nutrient accumulations, and growth of maize plants. The experiment was performed in a greenhouse with a 4 × 2 factorial in a randomized complete block design, with four replications. The treatments included proportions of NO_3^-/NH_4^+ (100/0 and 50/50) combined with applications of CuNP (0, 25, 50, and 100 mg kg^{-1}) in a Spodosol soil. A two-way analysis of variance and the SNK test at 5 % was performed. The dry biomass production was the highest when the maize plants received a combination of 100/0 NO_3^-/NH_4^+ and CuNP 25 mg kg^{-1}. Applications of CuNP did not increase the dry biomass production when maize plants received 50/50 NO_3^-/NH_4^+. The maximum efficiency of photosystem II was high when the plants were grown under 100/0 NO_3^-/NH_4^+. Applications of CuNP decreased the quantum efficiency of photosystem II and photochemical quenching, as well as increased the non-photochemical quenching, but only when the plants received 50/50 NO_3^-/NH_4^+. The accumulations of C, N, P, K, Ca, Mg, Cu, Fe, Mn, and Zn were higher at the combination between 100/0 NO_3^-/NH_4^+ and CuNP 25 mg kg^{-1} than at the other combinations, which were related to the lowest availability of those nutrients, pH, and electric conductivity in the rhizosphere soil. There are synergetic effects of CuNP when the maize plants received all N as NO_3^-. However, the positive effects of CuNP became non-significant when the plants received 50/50 NO_3^-/NH_4^+. For a better uptake of Cu by maize plants, it is suggested an application of all N as NO_3^-.

Keywords: Chlorophyll fluorescence; Copper nanoparticles; Nitrogen; Nutrient accumulation; *Zea mays*

2.1. INTRODUCTION

Maize (*Zea mays* L.) is one of the most important agricultural products and nutrient-responsive crops (Maresma et al., 2017), which is often studied to understand the role of fertilizers in growth and production. The United States of America lead the ranking of the world's largest maize producers with 370 million tons in 2017, ahead of China and Brazil with 215 and 97 million tons, respectively (Faostat, 2019). Maize plays a central role in global food security as a source of carbohydrate in the diet for humans and animals, as well as oil and sugar for industry (Adhikari et al., 2016; Zhang and Zhao, 2019).

Nitrogen (N) is required in a large amount by maize (Zhang and Zhao, 2019) since N is a constituent of all amino acids and proteins, nitrogenous bases, membranes, and plant hormones

(Heldt, 2005; Taiz et al., 2017). Nitrogen ions such as NO_3^- and NH_4^+ are the major N forms applied as fertilizers for maize as they can be directly absorbed by plant roots (Zhang and Zhao, 2019). The proper proportion of NO_3^-/NH_4^+ for plant development depends on the crop species and environmental conditions (Liu et al., 2017; Nogueirol et al., 2018; Souza Junior et al., 2018). Pedersen et al. (2019) studied five combinations of NO_3^-/NH_4^+ for maize cultivated in sandy soil without application of micronutrients and they observed that the best proportion for plant development and nutrient accumulation was 50/50 NO_3^-/NH_4^+. Going further, Souza Junior et al. (2019) showed that the use of NO_3^- solely improved the plant survival under the excess Cu in the growth media.

Copper is an essential element for biota. In plants, Cu is related to electron transport in photosynthesis as a constituent of plastocyanin, the first electron donor from photosystem II to photosystem I. Copper also plays an essential role in the constitution of enzymes, respiration, and N metabolism (Epstein and Bloom, 2006; Mahanty et al., 2012). Since Cu is a micronutrient, it is required in a very small amount by most crops and there is a fine line between essentiality and toxicity (Liu et al., 2015). Therefore, Cu must be applied carefully and it is desirable to optimize other nutrients in the soil, which can raise the Cu accumulation and mitigate its possible toxicity, as demonstrated by Souza Junior et al. (2019).

Several studies have been shown the positives effects of Cu nanoparticles (CuNP) in agriculture (Palit and Goswami, 2015; Rajput et al., 2018; Ameh and Sayes, 2019). Many techniques have been presented to produce nanoparticles, such as grinding, chemical reactions, and laser attacks (Rajput et al., 2018). Copper nanoparticles are different from regular Cu fertilizers in terms of physical, chemical, and biological characteristics (Ameh and Sayes, 2019). It was reported that CuNP are on average 10 times less toxic than ionic Cu (Ameh and Sayes, 2019) and more efficient than Cu sulfate for plant uptake (Palit and Goswami, 2015). This is due to their extremely small particle sizes, which allow greater reactivity and easy transport across cell membranes even though at low rates (Ameh and Sayes, 2019).

Copper nanoparticles can be a potential xenobiotic due to their high cell reactivity (Melegari et al., 2013; Song et al., 2015; Costa and Sharma, 2016) and excess Cu can inhibit various plant functions such as enzymatic activity, photosynthesis, chlorophyll synthesis, and plasma membrane integrity, culminating in low plant development (Nogueirol et al., 2018; Palit and Goswami, 2015; Souza Junior et al., 2019). The toxicity of CuNP depends on plant species, growth conditions, element exposure timing, metal concentration, and particle size (Rajput et al., 2018). However, an important point that is rarely mentioned in literature is their relationship between N and their ionic forms.

Copper availability in soils has been known to be directly influenced by soil pH. Lower soil pH can increase Cu mobility and availability (Fan et al., 2011b, 2011a). However, rhizosphere pH has been also known to be particularly important for Cu uptake and one of the ways to modify the rhizosphere soil pH is an adjustment in proportions of NO_3^-/NH_4^+ (Zhang and Zhao, 2019). Plant roots expose to a high amount of NO_3^- exude HCO_3^- to rhizosphere soil to maintain a plant anionic balance, causing an increase in rhizosphere pH. Conversely, when plant roots uptake NH_4^+ they exude H^+ to maintain a plant cation balance, thus lowering rhizosphere pH (Marschner, 2012).

The hypothesis is that the proper proportion of NO_3^-/NH_4^+ enhances the growth and production of maize grown under rates of CuNP. The objective was to evaluate the effects of the proportions of NO_3^-/NH_4^+ combined with rates of CuNP on chlorophyll fluorescence, soil properties, nutrient availabilities, nutrient accumulations, and growth of maize plants.

2.2. CONCLUSION

There are many positive effects of CuNP when the maize plants are applied with all N as NO_3^-. The plant growth, photosynthesis, and nutrient accumulations are high when the plants receive 100/0 NO_3^-/NH_4^+ and the growth potential of maize plants can be improved by applying a specific rate of CuNP, which positively affects all response variables. No beneficial effects from the application of CuNP are noticed when the plants are applied with 50/50 NO_3^-/NH_4^+. This is particularly important for the calibration of N fertilization in the next studies with CuNP, opting to choose all N as NO_3^- to ensure that all beneficial effects from CuNP will be achieved.

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3. NITRATE FERTILIZATION ENHANCES MANGANESE PHYTOEXTRACTION BY TANZANIA GUINEA GRASS – A NOVEL HYPERACCUMULATOR PLANT?

ABSTRACT

Manganese (Mn) is essential for plants, but very toxic in high concentrations since it can cause oxidative stress and negatively affects plant growth. However, the use of NO_3^- may improve Mn phytoextraction and also the phytoremediation potential of Tanzania guinea grass [*Panicum maximum* Jacq. cv. Tanzania (syn. *Megathyrsus maximus* (Jacq.) B.K.Simon & S.W.L. Jacobs)]. The objective was to evaluate the effect of excess Mn toxicity on physiological, metabolic, nutritional, and productive indicators of Tanzania guinea grass grown with proportions of NO_3^-/NH_4^+. The experiment involved a 2 × 4 factorial in randomized complete block design with four replications carried out in a growth chamber using Hoagland and Arnon's nutrient solution. The factors were proportions of NO_3^-/NH_4^+ (100/0 and 70/30) combined with rates of Mn (10, 500, 1,500, and 3,000 μmol L^{-1}). Statistical analysis was performed by using the analysis of variance, comparing the averages by the Tukey test at 5%. Excess Mn did not affect the plant growth, relative chlorophyll index (RCI), nitrate reductase, and the accumulation of N and its ions in the plant tissue. Nonetheless, the highest concentrations and accumulations of Mn were verified when the plants were grown with all N as NO_3^-, reaching up to 5,500 and 21,187 mg kg^{-1} (concentrations) in the shoots and roots, respectively, or 76.2 and 31.1 mg per pot (accumulations) in the shoots and roots, respectively (total Mn phytoextraction of 107.3 mg per pot). The high concentrations of Mn in the plant tissue increased H_2O_2 concentration, causing oxidative stress, which decreased glutamine synthetase activity, net photosynthesis, stomatal conductance, electron transport rate, photochemical quenching, as well as increased non-photochemical quenching. In that stress condition, proline synthesis seems to be more important to mitigate oxidative stress than the antioxidant enzymes, buffering excess H_2O_2 caused by excess Mn. Tanzania guinea grass is very tolerant to excess Mn, accumulating high amounts of this metal in the plant tissue, and NO_3^- supply can increase even more its concentration, enhancing Mn accumulation as much as a hyperaccumulator plant. It is suggested to indicate Tanzania guinea grass as a Mn hyperaccumulator.

Keywords: Glutamine synthetase, *Megathyrsus maximus*, Nutrient accumulation, Photosynthesis, Phytoremediation

3.1. INTRODUCTION

Pastures occupy around 196 million hectares in Brazil, equivalent to 71% of the agricultural area or 23% of the territory (FAO, 2016). High productive pastures are compounded especially by Tanzania guinea grass [*Panicum maximum* Jacq. cv. Tanzania (syn. *Megathyrsus maximus* (Jacq.) B.K.Simon & S.W.L. Jacobs)] due to its high potential for nutrient extraction and biomass production (Silveira and Monteiro, 2011). Tanzania guinea grass has been also indicated as an important phytoremediation plant (Souza Junior et al., 2019; Leite and Monteiro, 2019; Cardoso and Monteiro, 2021). The tolerance of Tanzania guinea grass to the toxicities of copper (Cu), cadmium (Cd), and barium (Ba) and its high phytoextraction potential was previously confirmed (Gilabel et al., 2014; Souza Junior et al., 2019; Leite and Monteiro, 2019; Cardoso and Monteiro, 2021). Recently, the importance of proportions of nitrate/ammonium (NO_3^-/NH_4^+) to improve the tolerance of Tanzania guinea grass to Cu and Cd stress was indicated, which could modulate its phytoextraction potential (Souza Junior et al., 2017; Souza Junior et al., 2018; Leite and Monteiro, 2019).

The major nutrient for the growth of forage grasses is nitrogen (N), which is uptaken by plant roots in the ionic forms of NO_3^- and NH_4^+. Nitrogen participates in the biosynthesis of all amino acids and proteins, as well as compounds related to plant development and tolerance to heavy metal excess in the growth media (Heldt, 2005; Taiz et al., 2017). Applications of proportions of NO_3^-/NH_4^+ led to a greater growth of *Megathyrsus maximus* cv. Aruana compared to plants grown with N as NO_3^- (Santos et al., 2013). The proper proportion of NO_3^-/NH_4^+ for Tanzania guinea grass growing without heavy metal toxicity was 70/30 (Souza Junior et al., 2018).

Proper proportions of NO_3^-/NH_4^+ increased N use efficiency and the production of forage grasses without increasing the rates of N (Santos et al., 2013). The positive effect of moderate concentrations of NH_4^+ for affinity-plants occurs because the uptake of NO_3^- and its reduction in the plant cell by nitrate reductase (NR) requires metabolic energy and reducing power. Thus, free energy can be saved by plants when N assimilation is performed directly in the form of NH_4^+ since glutamine synthetase (GS) requires less reducing power and chemical energy than NR and the free energy can be used for other purposes like plant growth or stress mitigation (Epstein and Bloom, 2006; Taiz et al., 2017). However, the effects of proportions of NO_3^-/NH_4^+ in forage grasses under heavy metal toxicity are poorly reported.

Excesses of heavy metals induce an excessive synthesis of reactive oxygen species (ROS) by plant cells, which are buffered through the action of dismutases, peroxidases, and osmoregulatory amino acids (Myouga et al., 2008). The enzymes are mainly: superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and guaiacol peroxidase (GPX) (Gratão et al., 2008; Myouga et al., 2008); as well as the amino acid proline (Singh et al., 2010; Hayat et al., 2012).

Manganese is essential for plants with an important function in photosynthesis, mediating the electron transfer from water to pheophytin in a series of redox reactions named water photolysis or Hill's reaction (Taiz et al., 2017). In addition, Mn has a relevant role as an enzyme activator (Malavolta, 2006; Taiz et al., 2017). One of the classic effects of excess Mn is its influence on increasing oxidative stress, affecting N metabolism and photosynthesis (You et al., 2017; Pan et al., 2018; Rajpoot et al., 2020). However, no studies are reporting the effects of Mn excess for Tanzania guinea grass indicated as tolerant to metals in a controlled situation.

Many factors can influence stress intensity such as plant species and type of stress (Schulze et al., 2002). So far, only one research has been found regarding the effect of N exclusively as NO_3^- or NH_4^+ (3.75 mmol L^{-1}) associated with rates of Mn, which studied the sexual dimorphism of *Populus cathayana* L. under Mn toxicity, without assessing the increases in the phytoextraction potential (Zhao et al., 2018). The results of that study indicated that proportions of NO_3^-/NH_4^+ might have the potential to increase the tolerance of plants to excess Mn.

The hypothesis is Tanzania guinea grass is highly tolerant to Mn contamination, but a proper proportion of NO_3^-/NH_4^+ can modulate its tolerance and improve even more its phytoextractor potential. The objective was to evaluate the effect of excess Mn on physiological, metabolic, and nutritional parameters, revealing the role of the proportions of NO_3^-/NH_4^+ as stress mitigators for Tanzania guinea grass.

3.2. CONCLUSION

Tanzania guinea grass is very tolerant to excess Mn. This plant can concentrate Mn in its biomass as much as a hyperaccumulator plant and even excess Mn its growth is not affected. However, to maximize the phytoextraction and the heavy metal tolerance, the grass should be fertilized with all N as NO_3^-. The excess Mn negatively affects photosynthesis and glutamine synthetase activity due to oxidative stress caused by H_2O_2. In this context, proline synthesis seems to plays a key role as a ROS mitigator, protecting plants from metabolic impairment caused by excess Mn. Tanzania guinea grass has the potential to be recognized as a Mn hyperaccumulator and this recognition will improve its utilization in phytoremediation programs.

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4. AMMONIUM ADDITION MITIGATES OXIDATIVE STRESS CAUSED BY SULFUR LIMITATION IN TANZANIA GUINEA GRASS

ABSTRACT

Nitrogen is the major stimulator of forage grass and a proper proportion of NO_3^-/NH_4^+ can improve the uptake of N and S and plant production. However, no reports show the role of N forms to mitigate S limitation for Tanzania guinea grass [*Panicum maximum* Jacq. cv. Tanzania (syn. *Megathyrsus maximus* (Jacq.) B.K.Simon & S.W.L. Jacobs)]. The objective was to evaluate the effect of S limitation and sufficiency on metabolic, physiological, nutritional, and productive parameters, revealing the role of N forms as a stress mitigator for Tanzania guinea grass. A 2 × 3 factorial experiment in a growth chamber and randomized complete block design with four replications was carried out. Factors were proportions of 100/0 and 70/30 NO_3^-/NH_4^+ combined with rates of S 0.1 (limitation), 1 (intermediate), and 2 mmol L^{-1} (sufficient). The Hoagland and Arnon's nutrient solution in a hydroponic system was used. The analysis of variance and Tukey test at 5% to compare averages were applied. In a situation of S sufficiency, the shoots and roots dry biomass were higher in the plants grown at 70/30 NO_3^-/NH_4^+ than at NO_3^- solely, showing a high relative chlorophyll index under 70/30 NO_3^-/NH_4^+. The supply of S decreased the glutamine synthetase activity in the plants grown at NO_3^- solely, but did not affect the glutamine synthetase activity in the plants that received 70/30 NO_3^-/NH_4^+. The plants cultivated with NO_3^- solely and S limitation showed the highest NH_4^+ concentration in the shoots. The application of 100/0 NO_3^-/NH_4^+ on the plants grown under S limitation showed a higher accumulation of NO_3^- in the shoots than at 70/30 NO_3^-/NH_4^+, but under S sufficiency it was verified the opposite effect. Under S sufficiency, the accumulation of S in the shoots and roots was higher in the plants grown at 70/30 NO_3^-/NH_4^+ than at NO_3^- solely. The rates of S improved the accumulations of N and NH_4^+ in the shoots and roots. Under S limitation, the quantum efficiency of photosystem II and electron transport rate were higher in the plants grown at 70/30 NO_3^-/NH_4^+ than NO_3^- solely. Overall, the highest net photosynthesis was verified in the plants cultivated with 70/30 NO_3^-/NH_4^+ or S 1 and 2 mmol L^{-1}. The malondialdehyde concentration was lower in the root plants grown at 70/30 NO_3^-/NH_4^+ than at NO_3^- solely, regardless of the rates of S, and similar effects were found for H_2O_2 concentration in plants grown under S 1 mmol L^{-1}. These results are consistent with guaiacol peroxidase activity in the shoots since its activity was higher at S limitation or sufficiency combined with NO_3^-/NH_4^+ than at NO_3^- solely. The rates of S decreased the ascorbate peroxidase activity in the plants grown at 100/0 NO_3^-/NH_4^+, but the opposite effect was verified in the plants grown at 70/30 NO_3^-/NH_4^+. Tanzania guinea grass has a great tolerance to S limitation. However, the combination of NO_3^- and NH_4^+ further improves plant tolerance. A combination of 70/30 NO_3^-/NH_4^+ mitigates S starvation, ensuring proper development of Tanzania guinea grass.

Keywords: Antioxidant enzymes, Glutamine synthetase, *Megathyrsus maximus*, Nitrate, Photosynthesis

4.1. INTRODUCTION

Brazil is one of the most important countries for world beef production with 196 million hectares of pastures for animal production (FAO, 2016). Pastures with high production are represented by plants such as Tanzania guinea grass [*Panicum maximum* Jacq. cv. Tanzania (syn. *Megathyrsus maximus* (Jacq.) B.K.Simon & S.W.L. Jacobs)] because of its high growth potential and forage quality (Silveira and Monteiro, 2011). The growth of forage grasses can be affected by many abiotic stressors such as nutrient deficiency (Schulze et al., 2002). However, few studies have investigated the effects of abiotic stressors for Tanzania guinea grass, one of the greatest species of tropical forage grass.

A topic with great importance is related to sulfur (S) limitation since this nutrient has an interesting correlation with nitrogen (N), the major nutrient for forage grass production (De Bona et al., 2013, Schmidt et al, 2013). Nitrogen is the major stimulator of Tanzania guinea grass production (Silveira and Monteiro, 2011). This is because N is an integral part of all amino acids and proteins like nitrogenous bases, membranes, hormones, and other compounds (Heldt, 2005; Marschner, 2012; Taiz et al., 2017). Nitrogen is assimilated in the form of NO_3^- and NH_4^+ and adjustments on the proportions of NO_3^-/NH_4^+ can also improve Tanzania guinea grass development (Souza Junior et al., 2018; Leite and Monteiro, 2019).

Proper proportions of NO_3^-/NH_4^+ can improve N uptake and then the forage grass production without any increase of N rate, as previously reported (Santos et al., 2013; Souza Junior et al., 2018; Leite and Monteiro, 2019). Positive effects of NH_4^+ addition occurs because the assimilation of N via NO_3^- and its chemical reduction in the plant cell requires a high investment of ATP and NADPH, majors metabolic energy used for plants. When N assimilation occurs via NH_4^+ then less amount of ATP and NADPH are required (Epstein and Bloom, 2006; Taiz et al., 2017), saving free energy that can be used to mitigates stress from nutrient starvation or limitation (Schulze et al., 2002; Meng et al., 2016). The best proportion of NO_3^-/NH_4^+ for Tanzania guinea grass was 70% NO_3^- combined with 30% NH_4^+ (Souza Junior et al., 2018; Leite and Monteiro, 2019), which raised nutrient accumulation and plant production and improved the antioxidant defense system against abiotic stress.

Sulfur is an essential element for plants and very important when N is optimized in growth media (De Bona et al., 2013; Schmidt et al., 2013; Schmidt and Monteiro, 2014) since S has great importance in protein synthesis and plays a unique role as an integral constituent of amino acids like cysteine and methionine, as well as coenzyme A, thiamine pyrophosphate, glutathione, biotin, vitamin B, and pantothenic acid, essentials for plant metabolism (Taiz et al., 2017). Preferences of using fertilizers without S, sulfur exportation by crops, and international agreements aiming to decrease emissions of S gases also decreases S deposition and might induce S limitation in plants, which decrease the efficiency of N assimilation and induces oxidative stress, affecting photosynthesis (Stern and Rydge, 2012; Khan et al., 2015; Sorin et al., 2012). So far, there is no report regarding the role of N ionic forms to mitigate S limitation.

Sulfur limitation can induce oxidative stress by unbalancing reactive oxygen species (ROS) (Schulze et al., 2002; Khan et al., 2015). Hydrogen peroxide (H_2O_2) is one of the major ROS induced by abiotic stress (Gratão et al., 2008; Myouga et al., 2008) that damages cellular membranes, causing lipid peroxidation. This process is also indicated by high concentrations of malondialdehyde (MDA), which might decrease plant growth and development (Sing et al., 2010). In response to this, plants developed an efficient antioxidant defense system, acting from enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and guaiacol peroxidase (GPX), turning H_2O_2 into H_2O by a redox reaction named Fenton and Harber-Weiss reaction (Liu et al., 2015). A non-enzymatic defense system was also developed for plants to fight abiotic stress like the amino acid proline, an important compound that can eliminate ROS directly and act as an osmoregulator in the plant cells (Sing et al., 2010).

The hypothesis is the combination of NO_3^- and NH_4^+ mitigates oxidative stress from S limitation more than plants receiving NO_3^- solely. The objective was to compare the effect of S limitation and sufficiency on metabolic, physiological, nutritional, and productive indicators, revealing the role of NO_3^- and NH_4^+ as a stress reliever and growth-enhancing for Tanzania guinea grass.

4.2. CONCLUSION

The S limitation is an adverse condition that causes oxidative stress, negatively affecting photosynthesis. However, Tanzania guinea grass has a great tolerance to S limitation. The tolerance of Tanzania guinea grass to S limitation is better manifested when the plants grow at a proper proportion of NO_3^- and NH_4^+ since a combination of these ions decreases oxidative stress by stimulating mainly guaiacol peroxidase, improving the photosynthesis. In this context, a combination of 70/30 NO_3^-/NH_4^+ for Tanzania guinea grass mitigates the oxidative stress caused by S limitation. It is suggested a combination of 70/30 NO_3^-/NH_4^+ to ensure the proper development of Tanzania guinea grass even under S limitation.

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