

## Production and physiological quality of *Pennisetum glaucum* after zinc (Zn) application

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### Abstract

The purpose of this study was to evaluate the dry biomass production and the physiological quality of millet (*Pennisetum glaucum*) based on the application of zinc (Zn) on the soil. The study was conducted in a completely randomized design consisting of five Zn doses: 0; 3; 6; 9 and 12 kg ha<sup>-1</sup>, with four replications. The following biometrics such as physiological quality, biomass production and nutritional efficiency as for the use of Zn by plants were evaluated. The results showed that doses around 9 kg ha<sup>-1</sup> are the ones promoting the highest observed rates. However, higher Zn supply in the soil negatively affected the physiological quality of the crop, as for the evaluated variables, except for the photochemical efficiency of photosystem II. However, *Pennisetum glaucum* is responsive to the production of dry biomass up to the Zn dose of 9 kg ha<sup>-1</sup>, without reducing the efficiency of the nutrient utilization by the crop.

**Keywords:** Photosynthesis, stomatal conductance, mineral nutrition, transpiration, stress level.

### Introduction

With popularity of direct seeding method on straw, there is a high demand for nutrient use efficient varieties for the cultivation, especially for covering plants (FBPDP, 2014). Hence, the culture of millet (*Pennisetum glaucum*) stands out because of its high capacity of biomass production and its deep and branched root system, capable of acting in the disaggregation, structuring and cycling of nutrients in the soil (Boer et al., 2007). In tropical regions, the cultivation of cereals is quite widespread, since they are used as fodder and soil covering in the no-tillage system (NTS). The cereals are cultivated for animal feed, grazing, hay, grain, and silage production (Guimarães Junior et al., 2009).

However, to obtain the maximum yields of the crop, monitoring its nutritional status is fundamental, mainly to avoid the occurrence of nutritional imbalances that directly affect the yield production. Thus, management of micronutrients has become essential, since albeit they are required in small quantities, they are frequently overlooked, and present a very close range between the limits of deficiency and toxicity to plants (Malavolta, 2006). In tropical regions, there is a predominance of very weathered soils, with a high acidity condition (Lespich, 2010), with recommendations related to mineral fertilization containing zinc (Zn) around 6 kg ha<sup>-1</sup> for most crops grown in these regions (Sousa and Lobato, 2004; Prado, 2008).

Micronutrients play important metabolic roles in plants, such as enzyme activator and constituent, being components of enzymes such as dehydrogenases, proteinases, peptidases and phosphohydrolases (Malavolta, 2006; Prado, 2008). They are also related to the metabolism of carbohydrates, proteins and phosphates, and to the

formation of auxin, RNA and ribosome structures. Zn is a component of RNA polymerase, which leads to the synthesis of RNA through the polymerization of nucleotides, also acting in the maintenance of the membrane structural integrity and the regulation of RNase activity (Malavolta, 2006; Prado, 2008). Phenol metabolism, starch formation, increase in cell size and multiplication, and pollen grain fertility are also related to the functions performed by Zn in plants (Fageria and Moreira, 2015).

In cereals, Zn deficiency reduces the apical growth, as well as promoting short internodes and lower root system development (Nanda and Wissura, 2016). Sarwar et al. 2017 showed a beneficial effect from the application of Zn in proper doses studies, which promotes increases in grain yield and biomass production. On the other hand, high Zn concentrations can lead to toxicity and alter metabolic processes, thus inhibiting growth (Prado, 2008; Silva et al., 2010).

The importance of Zn in plant metabolism, in addition to the scarcity of studies related to the proper management of micronutrients, highlights the need to progress with more detailed studies on the supply of this micronutrient to crops. In addition, the potential of pearl millet cultivation for soil covering in no-tillage systems makes it convenient to develop studies that allow improving crop performance through a proper mineral nutrition for the cultivation in tropical regions. Considering the aforementioned, the purpose of this study was to evaluate the production of dry biomass and the physiological quality of *Pennisetum glaucum* based on the application of zinc (Zn) on the soil.

## Results

### Biometric evaluations

Zinc application on the soil significantly affected the growth of *Pennisetum glaucum*, both in height (Fig. 1) and stem diameter (Fig. 2). The linear adjustments for the evaluations were performed 30 days after germination (DAG) and quadratic adjustments for the evaluations conducted on 65 DAG. At 30 DAG, we observed approximately 60 and 50% increase in height (Fig. 1) and stem diameter (Fig. 2) under highest dose of Zn (12 kg ha<sup>-1</sup>), highlighting the positive effect of the addition of Zn doses on the development of the culture.

In the second evaluation (65 DAG), the best adjustment was the quadratic equation. The maximum height (19.80 cm) and stem diameter (5.16 mm) were obtained with the application of 8 and 7.6 kg ha<sup>-1</sup> of Zn, by which there was an increase of 178 and 51%, respectively, compared to control treatment (without Zn addition).

As for the leaf area, increases with quadratic adjustments were observed for the two performed evaluations, 30 and 65 DAG. The obtained maxima were 145.8 and 340.7 cm<sup>2</sup>, using 12 and 9 kg ha<sup>-1</sup> of Zn, which are increases of 203 and 159%, compared to control treatment, respectively (Fig. 3).

### Physiological evaluations

As for the relative chlorophyll index (RCI), the application of Zn on the soil was increased based on quadratic equations, both at 30 and 65 DAG. The maximum values of 35 and 42 µg cm<sup>-2</sup> were observed with the use of approximately 7 kg ha<sup>-1</sup> of Zn at 30 and 65 DAG, respectively. These increases were about 30 and 24% more compared to the control treatment, respectively (Fig. 4). This resulted in the highest RCI (27.98 µg cm<sup>-2</sup>). Furthermore, at 65 DAG, the best chlorophyll performance (35.07 µg cm<sup>-2</sup>) was observed under dose of 6.84 kg ha<sup>-1</sup>. The interaction between application of Zn doses on the soil and RCI was quadratic; thus, doses above 7.30 and 6.84 kg ha<sup>-1</sup> reduced the RCI in millet at 30 and 65 DAG, respectively (Fig. 4).

The net photosynthetic rate was significantly reduced (50%) compared to the control treatment after application of Zn doses, reaching 10.1 µmol m<sup>2</sup> s<sup>-1</sup> (Fig. 5). We also observed that the behavior of stomatal conductance and the transpiration of millet leaves followed the same trend as net photosynthetic rate. Application of 12 kg ha<sup>-1</sup> reduced the stomatal conductance and transpiration about 60% (Fig. 6a) and 79% (Fig. 6b), respectively, compared to control treatment.

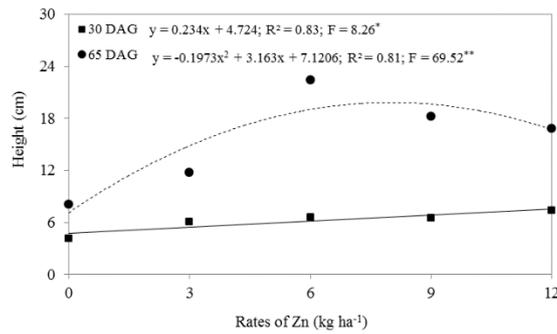
With the reduction of photosynthetic rates, stomatal conductance and transpiration, there were significant linear increases in the internal CO<sub>2</sub> concentration (Fig. 6c), reaching 348.7 µmol m<sup>2</sup> s<sup>-1</sup> with the use of the highest dose applied to the soil, which was 84.2% more than the control treatment. Evaluation of the photosystem II photochemical efficiency (Fv Fm<sup>-1</sup>) was conducted to determine the stress level of the plant according to the culture conditions. Application of Zn on the soil affected this variable in quadratic way. The best result (0.80) was observed with the application of 9.50 kg ha<sup>-1</sup> of Zn in the soil (Fig. 7).

### Production of biomass and nutritional assessment

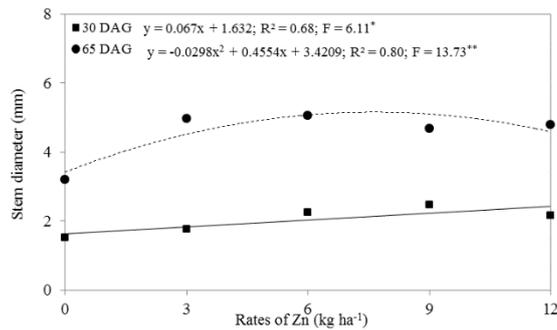
The application of Zn on the soil increased the dry biomass production of *Pennisetum glaucum* shoot and root (Fig. 8). There was a linear relation in the root, where the maximum values of 4.45 to 3.85 g plant<sup>-1</sup> were obtained with application of 12 and 9.6 kg ha<sup>-1</sup> of Zn, respectively. These values suggest about 270% increase compared to the control treatment (Fig. 8). With the increase in dry biomass production of *Pennisetum glaucum*, there was a quadratic increase in the accumulation of Zn in both roots and shoot and also in the whole plant, reaching the maximum points of 0.73, 0.40 and 1.04 mg plant<sup>-1</sup> with the application of 8.3; 11 and 8.3 kg ha<sup>-1</sup> of Zn, respectively (Fig. 9). Evaluation of nutritional efficiency showed that application of Zn in soil significantly affected absorption efficiencies (Fig. 10a), transport (Fig. 10b), and utilization (Fig. 10c). As for the absorption efficiency, there was a quadratic adjustment, where the maximum efficiency of 0.36 mg g<sup>-1</sup> was obtained with the application of 7.2 kg ha<sup>-1</sup> of Zn (Fig. 10a). However, transport efficiency was decreased due to the increased Zn supply into soil, which reached 64.8% with the application of the highest dose (Fig. 10b). Use efficiency was similar to the absorption efficiency, with a quadratic adjustment. The highest efficiency (77 mg g<sup>-1</sup>) was observed with the highest applied dose to the soil (Fig. 10c).

### Discussion

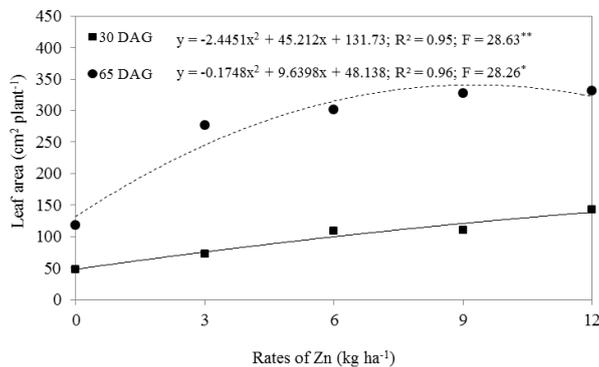
The positive effect of fertilization containing Zn on the growth of *Pennisetum glaucum* is due to the metabolic functions that the nutrient exerts on the plant. Zn is an enzyme activator and constituent. It is related to the synthesis of auxin, a hormone responsible for plant growth. Thus, the proper supply of this micronutrient helps increasing of the growth rates in plant, whereas in nutritional disorder situations, both deficiency and excess cause alterations in the synthesis of auxin within plant tissue, with negative repercussions for its development (Taiz and Zeiger, 2013). The relationship between nitrogen (N) and Zn contents in the plant is due to the fact that Zn is involved in protein synthesis and in the assimilative reduction of nitrate (Prado, 2008). Therefore, the proper supply of Zn into the soil helps the increase in the assimilation of N by the plant. This may be reflected in gains of chlorophyll synthesis, increasing the observed RCI values. This occurs because the leaf content of N is directly related to the chlorophyll content, since for each chlorophyll molecule four N atoms are required (Coelho et al., 2010). As reported in literature, increases in RCI values were observed up to the dose of 7 kg ha<sup>-1</sup> of Zn, mainly 65 days after germination (DAG). This is a Zn dose close to the one recommended by Sousa and Lobato (2004) in the same region. At the higher doses, there were reductions in RCI values, probably associated with the narrow range between micronutrient contents (lower and upper limit), affecting the plant metabolism. Moreover, Zn supply at doses above 7 kg ha<sup>-1</sup> may trigger leaf senescence, promoting the degradation of leaf chlorophyll content (Dordas, 2017).



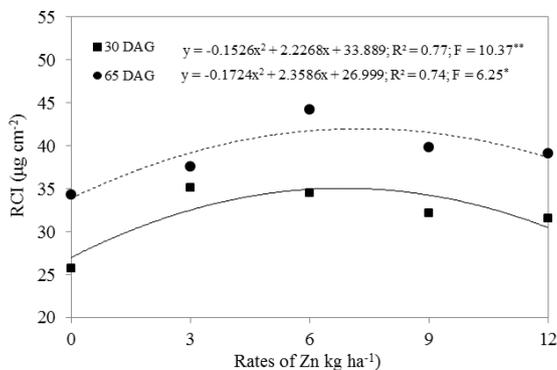
**Fig 1.** Height of millet (*Pennisetum glaucum*) hybrid ADR 300, 30 and 65 days after germination (DAG) based on the application of zinc on the soil. \*\* and \* are significant at 1 and 5% probability by F-test, respectively.



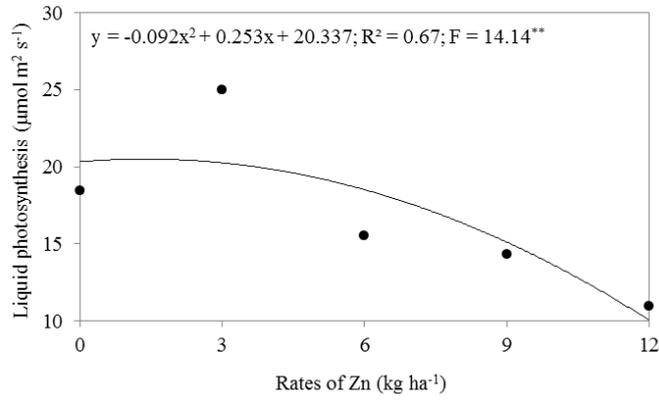
**Fig 2.** Diameter of stem base of millet (*Pennisetum glaucum*) hybrid ADR 300, 30 and 65 days after germination (DAG), based on the application of zinc on the soil. \*\* and \* - significant at 1 and 5% probability by F-test, respectively.



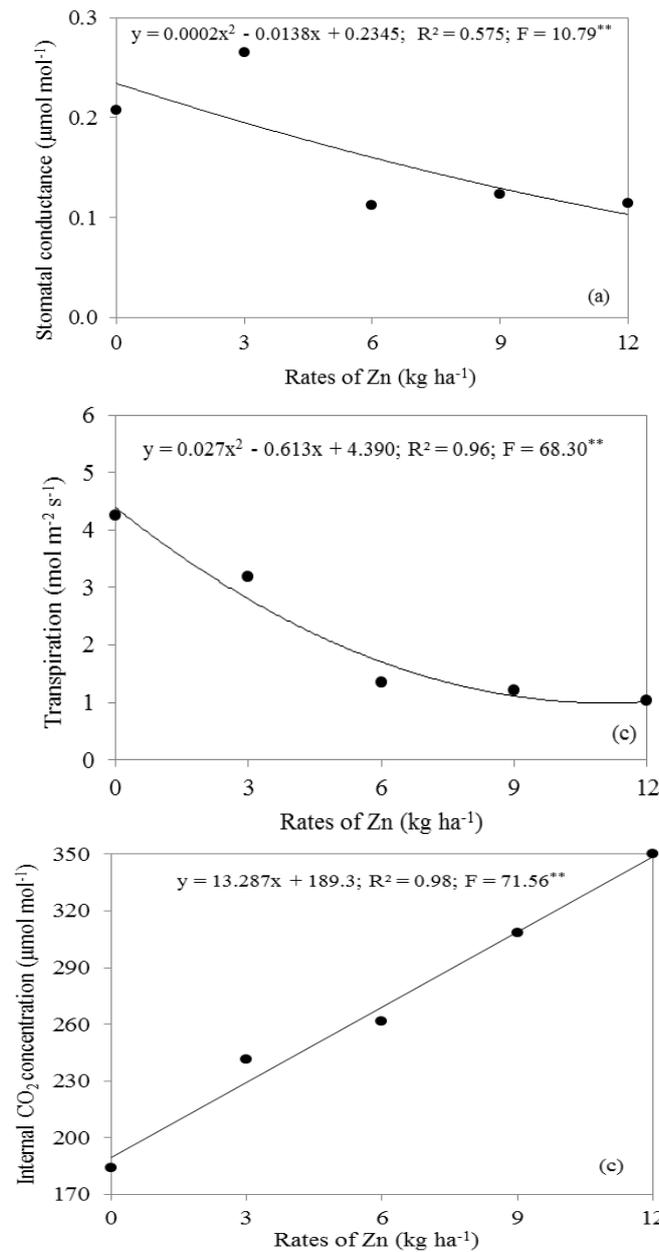
**Fig 3.** Leaf area of millet (*Pennisetum glaucum*) hybrid ADR 300, 30 and 65 days after germination (DAG), based on the application of zinc on the soil. \*\* and \* - significant at 1 and 5% probability by F-test, respectively.



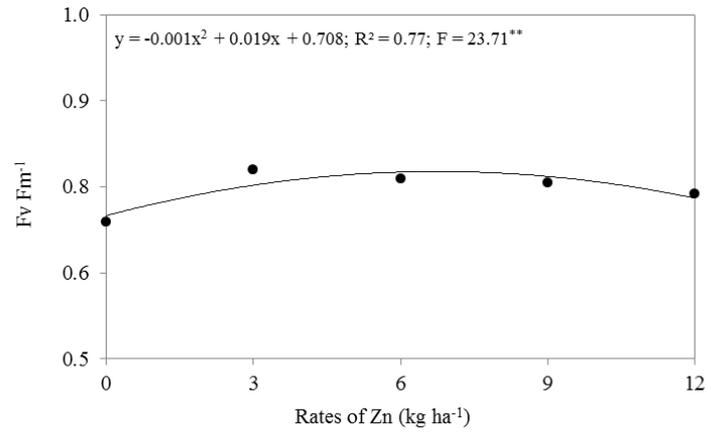
**Fig 4.** Relative chlorophyll index (RCI) of millet (*Pennisetum glaucum*) hybrid ADR 300, 30 and 65 days after germination (DAG), based on the application of zinc on the soil. \*\* and \* - significant at 1 and 5% probability by F-test, respectively.



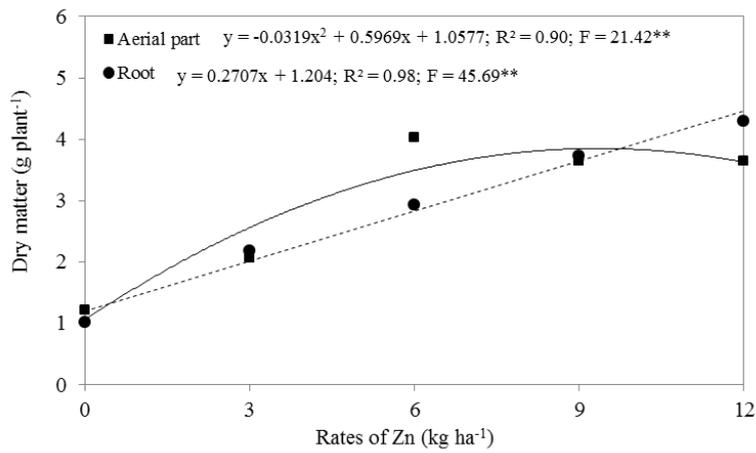
**Fig 5.** Liquid photosynthesis of millet (*Pennisetum glaucum*) hybrid ADR 300 based on the application of zinc on the soil. \*\* - significant at 1% probability by F-test.



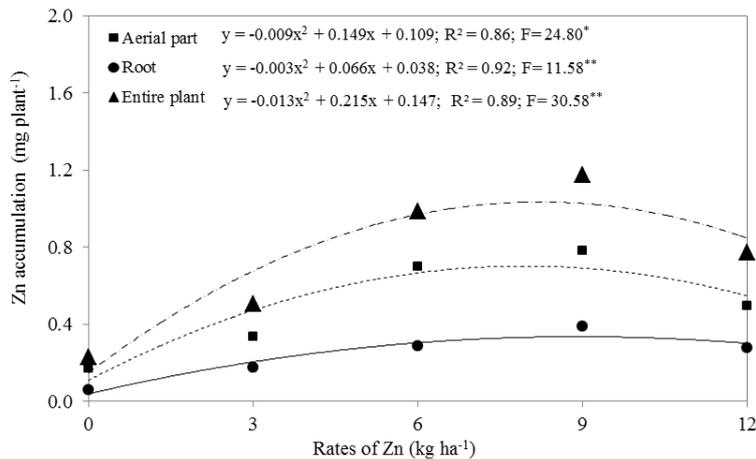
**Fig 6.** Stomatal conductance (a), transpiration (b) and internal carbon concentration (c) of millet (*Pennisetum glaucum*) hybrid ADR 300 based on the application of zinc on the soil. \*\* - significant at 1% probability by F-test.



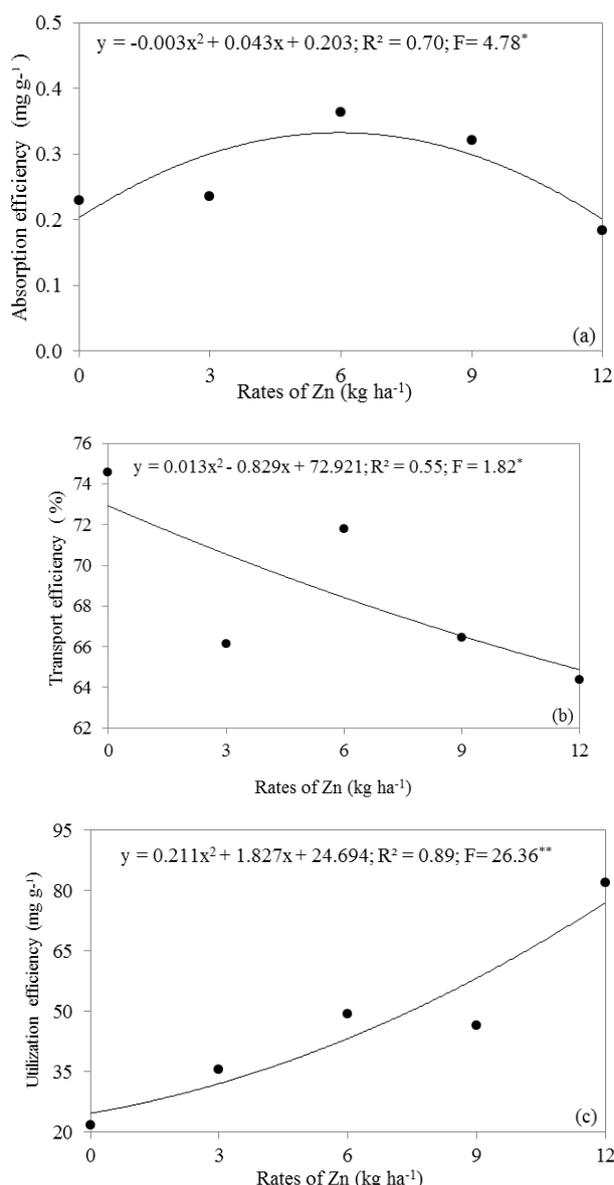
**Fig 7.** Photochemical efficiency of photosystem II (Fv Fm<sup>-1</sup>) in millet (*Pennisetum glaucum*) hybrid ADR 300 based on the application of zinc on the soil. \*\* - significant at 1% probability by F-test.



**Fig 8.** Shoot and root dry biomass of millet (*Pennisetum glaucum*) hybrid ADR 300 based on the application of zinc on the soil. \*\* - significant at 1% probability by F-test.



**Fig 9.** Accumulation of zinc in the shoot, roots and whole plant of millet (*Pennisetum glaucum*) hybrid ADR 300 based on the application of zinc on the soil. \*\* and \* - significant at 1 and 5% probability by F-test, respectively.



**Fig 10.** Absorption (a), transport (b) and use (c) efficiency of millet (*Pennisetum glaucum*) hybrid ADR 300 based on the application of zinc on the soil. \*\* and \* - significant at 1 and 5% probability by F-test, respectively.

High levels of RCI are a desirable feature in plants, since leaf exposure to solar radiation in leaves containing more chlorophyll helps the development of the plant, mainly due to the increase in the efficiency of non-structural carbohydrates, starch and hexoses, which are transformed into structural carbohydrates (Coelho et al., 2010). Zn is also related to the photosynthetic rate of plants through the pyruvic carboxylase enzyme. It is also required for the synthesis of the tryptophan amino acid. It is a precursor of the biosynthesis of indoleacetic acid (IAA), a growth-promoting plant hormone that is directly involved in N metabolism (Malavolta et al., 2006). That way, both N and Zn may interfere, depending on their concentration in leaves, on the proper chlorophyll contents in leaves, and consequently on the capture of the solar energy used in photosynthesis (Taiz and Zeiger, 2013).

In literature, there are some reports about the beneficial effect of Zn-containing nutrition under the net photosynthetic rate of *Pennisetum glaucum*, and the

hypothesis related to this effect is the association of Zn with carbonic anhydrase (CA) (Tavallali, 2017). During the photosynthetic process, CA is a Zn-containing enzyme that is capable of catalyzing the reversible conversion of CO<sub>2</sub> into bicarbonates (HCO<sup>-3</sup> requires Zn for its catalytic activity). Thus, the efficient activity of Zn - CA becomes beneficial to plants, since it helps the supply of CO<sub>2</sub>, where it consequently provides greater stomatal opening instead of CO<sub>2</sub> fixation.

On the other hand, the reduction in increase of stomatal conductance and transpiration, due to the increasing application of Zn on the soil, promotes the increase in CO<sub>2</sub> concentration, which was observed in this study. It occurred because Zn participates as a component of dehydrogenase enzymes, proteinases, peptidases and phosphohydrolases, which is indirectly related to the photosynthetic mechanism of plants, pertinent to CO<sub>2</sub> fixation by RuBisCO (Marschner, 1995).

Demmig and Björkman (1987), studied a large number of vascular species. They determined the values of the  $F_v F_m^{-1}$  ratio, which was around  $0.832 \pm 0.004$ . According to Campostrini (1997), the decline of the  $F_v F_m^{-1}$  ratio is a good indicator of photoinhibitory damage when plants are subject to environmental stresses. In this study, plants presented excellent conditions for their development, and although no visual symptoms of stress were observed, the applied treatments did not promote the presence of values below those indicated in literature. To a certain extent, these results are important indications about the tolerance of *Pennisetum glaucum* to the supposedly adverse conditions of this study and to achieve high production rates.

The increase in biomass production can be justified by the enzymatic functions in several metabolic processes performed by Zn in the plant (Hernandes et al., 2009). Malik et al. (2011) observed increases in the production of dry biomass with the application of Zn doses up to  $300 \text{ mg dm}^{-3}$  on rice cultivated at controlled greenhouse conditions. Similarly, Silva et al. (2010) observed that with the application of  $253 \text{ mg dm}^{-3}$  of Zn in a dystrophic Red Latosol of medium texture, the dry biomass production of millet was decreased by 50%. The authors attributed this result to the excess of Zn, which may have induced oxidative damages in plants, initiating lipid peroxidation and the degradation of other compounds in the plant, promoting the reduction of dry biomass production.

High doses of Zn in the soil can promote phytotoxicity, which reduces the productive yield of plants. Moreover, the excess of Zn in the xylem results in plugs that hinder the rise of the crude sap, as well as affecting the absorption of other cations such as Ca and Mg, nutrients that affect plant growth (Malavolta et al., 1997).

In a similar way to this study, Silva et al. (2010) observed, increases in the Zn leaf content in millet up to the dose of  $253 \text{ mg dm}^{-3}$ , which reached  $451 \text{ mg kg}^{-1}$  of Zn. The authors highlighted that from this dose on, there were reductions in plant growth and dry biomass production. Muner et al. (2011), in studies with increasing Zn doses in a maize crop in a medium-textured Red-Yellow Argisol, observed linear increases in the Zn contents of leaves at doses of up to  $16 \text{ kg ha}^{-1}$ , which reached  $60 \text{ mg kg}^{-1}$ .

Sousa and Lobato (2004) recommend the application of  $6 \text{ kg ha}^{-1}$  of Zn for the cultivation of millet in soils of Cerrado region, when its content is less than  $1.1 \text{ mg dm}^{-3}$ . In this study, the Zn content was increased up to the  $6.61 \text{ kg ha}^{-1}$  dose, where the dry biomass production showed the same behavior. At higher doses, the production of dry biomass decreases, with no visible symptoms of phytotoxicity in millet leaves.

With the increase in leaf Zn content and consequent gains in biomass production, the accumulation of Zn in the plant presented the same behavior. Silva et al. (2010) also observed these results on millet and rice crops, respectively, based on the application of Zn on the soil. Santos et al. (2009) studied fodder grasses in a greenhouse and observed increases in Zn accumulation in both the first and second cuttings, with Zn doses close to  $200 \text{ mg dm}^{-3}$  in the soil.

Therefore, the application of Zn doses ranged from  $7$  to  $9 \text{ kg ha}^{-1}$  provided the highest yields in vegetative growth. This dose is close to the recommended one in national literature. However, it is also possible to notice that the increase of Zn supply in the soil reduces the physiological quality of the

crop, thus reducing transport efficiency. Nonetheless, a more detailed evaluation of the economic viability regarding Zn-containing fertilization management is necessary, since the vegetative growth increase may be economically unviable if the gains in productive yield do not obtain positive differential profits (Silva et al., 2010).

## Materials and methods

### Experimental area

The study was conducted in greenhouse at the School of Agronomy of the Federal University of Goiás ( $16^{\circ}35'12'' \text{ S}$ ,  $49^{\circ}21'14'' \text{ W}$ ,  $730 \text{ m}$ ), State of Goiás, Brazil. The climate of the region, is Aw-type (Tropical Climate with summer rains) or savannah tropical with dry winters and rainy summers according to the Köppen-Geiger classification. The average annual air temperature is  $22.9^{\circ}\text{C}$ . The dry season is concentrated between May and September, and the rainy season lasts from October to April, with an annual average rainfall of  $1,520 \text{ mm}$ .

The chemical analysis of soil before planting presented the following properties: pH: 4.4 ( $\text{CaCl}_2$ );  $\text{Al}^{3+}$ :  $0.5 \text{ cmol}_c \text{ dm}^{-3}$ ; Organic Matter:  $2.3 \text{ g dm}^{-3}$ ; P:  $0.8 \text{ mg dm}^{-3}$ ;  $\text{K}^+$ :  $25 \text{ mg dm}^{-3}$ ;  $\text{Ca}^{2+}$ :  $0.4 \text{ cmol}_c \text{ dm}^{-3}$ ;  $\text{Mg}^{2+}$ :  $0.3 \text{ cmol}_c \text{ dm}^{-3}$ ;  $\text{SO}_4^{2-}$ :  $5.6 \text{ mg dm}^{-3}$ ; Zn:  $1.4 \text{ mg dm}^{-3}$ ; B:  $0.14 \text{ mg dm}^{-3}$ ;  $(\text{H}+\text{Al}^{3+})$ :  $1.27 \text{ cmol}_c \text{ dm}^{-3}$ ; cation exchange capacity (CEC):  $2.03 \text{ cmol}_c \text{ dm}^{-3}$ ; base saturation (V%): 37.4%. The granulometric analysis showed 530, 180 and  $135 \text{ g kg}^{-1}$  of clay, silt, and sand, respectively.

### Experimental design

Treatments were arranged in a completely randomized experimental design (CRD), consisting of five Zn doses: 0 (control treatment); 3; 6; 9 and  $12 \text{ kg ha}^{-1}$ , applied as Zn oxide, and four replications. Each experimental unit consisted of a planter with a  $10 \text{ dm}^3$  capacity, filled with 5  $\text{dm}^3$  samples of a superficial layer at 0.0-0.20 m depth from a typical Red Latosol, with clayey-sandy texture, moderate A horizon, semideciduous forest phase, mildly undular relief, according to the Embrapa classification (2013).

### Plant materials

Millet (*Pennisetum glaucum*) seeds were used (ADR 300 hybrid), with a 92 days life cycle, able to reach  $22 \text{ t green mass ha}^{-1}$ . The hybrid presents good disease resistance and good adaptation to Brazilian edaphoclimatic conditions (Embrapa, 2016).

### Experiment development

After chemical analysis, the soil was corrected to increase its base saturation to 60%, as recommended by Sousa and Lobato (2004). To do so, liming was performed 90 days before millet planting, using dolomitic limestone ( $\text{CaO}=36\%$ ;  $\text{MgO}=15\%$ ;  $\text{PN}=98\%$ ;  $\text{PRNT}=92.54\%$ ), maintaining the soil mass dampened (60% of retention capacity) and incubated for 30 days.

Mineral fertilization of soil was performed by applying  $80 \text{ kg ha}^{-1}$  of  $\text{P}_2\text{O}_5$  triple superphosphate as a nutrient solution,  $80 \text{ kg ha}^{-1}$  of N as urea, applied partly during sowing ( $20 \text{ kg ha}^{-1}$  of N) and the rest ( $60 \text{ kg ha}^{-1}$  of N) divided into two

applications, 15 and 25 days after sowing, respectively, and 60 kg ha<sup>-1</sup> of K<sub>2</sub>O as potassium chloride (Sousa and Lobato 2004).

Planting was performed on 04/04/2017, using the millet hybrid (*Pennisetum glaucum*) ADR 300, recommended as groundcover, sowing 5 seeds per pot. Seven days after emergence (DAE), thinning was performed, maintaining 2 plants per pot. Irrigation was performed by planter weighing method, maintaining the soil moisture content equal to field capacity (θ<sub>cc</sub>).

### Biometric evaluations

Biometric evaluations were performed at two different moments, 30 and 65 days after emergence (DAE), corresponding to the phenological phases ED5 (booting stage, from 28 to 35 days after emergence) and ED9 (physiological maturity stage, 56 to 63 days after emergence), and may vary according to environmental, local and variety conditions (Durões et al., 2003).

For the biometric evaluation, plant height (starting from the base of the stem to the insertion of the last completely visible sheath), tiller/stem diameter (with the help of a digital caliper, measured at the base of the plant, near the soil surface), and leaf area were determined. In order to determine the leaf area, the procedures proposed by Schiavuzzo et al. (1998) were used, through the following equation 1:

$$LA = L \cdot W \cdot 0.835 \cdot N \quad (1)$$

Where; LA is the leaf area (cm<sup>2</sup>), L is the length (cm) and W is the width (cm) of the diagnostic leaf (first fully developed leaf with visible sheath; readings performed in the middle portion of the leaf); 0.835 is the leaf area correction factor of the crop, and N is the number of open leaves with at least 20% of green area.

### Physiological evaluations

The relative chlorophyll index (RCI) was evaluated at two different moments, 30 and 65 days after emergence (DAE), on the diagnostic leaf, with the help of a FALKER® chlorophyll meter, model ClorofiLOG CFL1030.

For the physiological quality evaluation of millet plants, gas exchange measurements were performed on fully expanded leaves with a good phytosanitary appearance, obtained through the mean of three readings per planter (treatment). The analyzed variables were: liquid photosynthesis rate (A), stomatal conductance (Gs), transpiration (E) and internal CO<sub>2</sub> concentration (Ci), and PSII maximum quantum yield (φ PSII or Fv Fm<sup>-1</sup>). To do so, a photosynthesis analyzer system was used, IRGA - Infrared Gas Analyzer connected to a fluorometer, model iFL - Integrated Fluorometer and Gas Exchange System, with a 6.25 cm<sup>2</sup> leaf chamber.

Chlorophyll α fluorescence (F) was determined by applying a saturating light pulse of 1500 μmol m<sup>-2</sup> s<sup>-1</sup>, lasting for 1 second. Fluorescence data were used to calculate φPSII (PSII maximum quantum yield) according to equation 2 (Maxwell and Johnson, 2000).

$$\phi\text{PSII} = (Fm' - Fs)/Fm' \quad (2)$$

Where; Fs, indicates the fluorescence in the dynamic equilibrium state (illuminated leaf) and Fm', the maximum fluorescence of the leaf in an illuminated environment.

A photosynthetic response curve of the plant to light (A-RFA) under ambient [CO<sub>2</sub>] conditions (~ 473 μmol mol<sup>-1</sup>) was previously created to determine the saturating light pulse (1500 μmol m<sup>-2</sup> s<sup>-1</sup>). Photosynthesis values (A) were recorded in saturating light pulses of 800, 1000, 1200, 1400, 1600, 1800, 2000 μmol m<sup>-2</sup> s<sup>-1</sup>. From the generated curve, it was possible to determine the maximum luminous intensity causing the highest photosynthetic rate for the millet culture. It is worth mentioning that this calibration took place under the same edaphoclimatic conditions inside the greenhouse where the experiment was conducted.

### Production of biomass and nutritional assessment

With the end of the evaluations, millet was cut to determine biomass production, separating the shoot from the roots. Then, samples of plant tissue (shoot and root) were properly washed in 0.1% detergent and distilled water solution, for later drying in a forced air circulation oven at 65 °C for 72 hours, until dry biomass stabilization. After this procedure, Zn contents in the plant tissues (shoot and root) were determined, according to procedures proposed by Prado (2008). With the obtained results, it was possible to calculate the Zn accumulation in the shoot, root and whole plant, associated with increase in millet dry biomass.

Starting from the dry biomass (shoot and root) and the Zn content in plant tissues, nutrient index calculations were performed according to Prado (2008). This included nutrient absorption efficiency by the plant (AB<sub>ef</sub>), efficiency of nutrient transport between root and shoot (TR<sub>ef</sub>) and nutrient utilization efficiency in the conversion into dry biomass. For this, calculations occurred according to the following equations:

Equation Swiader et al. (1994):

$$AB_{ef} = \frac{\text{total nutrient content in plant}}{\text{root dry matter}} \quad (4)$$

Equation Li et al. (1991):

$$TR_{ef} = \frac{(\text{total nutrient content in aerial part})}{(\text{total nutrient content in plant})} \times 100 \quad (5)$$

Equation Siddiqi and Glass (1981):

$$UT_{ef} = \frac{(\text{total dry matter produced})^2}{\text{total nutrient content in the plant}} \quad (6)$$

### Statistical analysis

The obtained results were submitted to analysis of variance by F-test, then the polynomial regression analysis was applied. Linear and quadratic mathematical models were tested, applying the models that obtained the best data adjustments. For this, the magnitude of the regression coefficients that were significant at 5% probability by t-test was adopted as the model selection criterion. When significant, the maximum and minimum points were obtained by deriving the equations.

## Conclusion

The application of Zn on the soil affected development and the relative chlorophyll index of *Pennisetum glaucum*. The doses close to 9 kg ha<sup>-1</sup> promoted the highest observed rates. However, a higher Zn supply in the soil negatively affected the physiological quality of the crop, reducing the net photosynthetic rate, stomatal conductance, and transpiration. Consequently, there was an increase in the internal concentration of CO<sub>2</sub>, with no apparent effects on the photochemical efficiency of photosystem II. However, *Pennisetum glaucum* is responsive to application of up to 9 kg ha<sup>-1</sup> Zn for production of dry biomass, even in the soils with average levels of Zn, without reduction of efficiency in crop.

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