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**ARIANI GARCIA**

# INTERACTION OF MAGNESIUM WITH POTASSIUM IN SUGARCANE

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Thesis presented to São Paulo State University, School of Agriculture, to obtain Doctor degree in Agronomy (Energy in Agriculture).

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Co-advisor: James Mabry McCray

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## CERTIFICADO DE APROVAÇÃO

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I dedicate this thesis to my parents José and Osmarina,

my brother Alessandro and my niece Raissa,

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“One, remember to look up at the stars and not down at your feet. Two, never give up work. Work gives you meaning and purpose and life is empty without it. Three, if you are lucky enough to find love, remember it is there and don't throw it away.” —
Stephen Hawking

## RESUMO

Objetivou-se avaliar o efeito do fornecimento de concentrações de $Mg^{2+}$e $K^{+}$, e da aplicação foliar de $Mg^{2+}$ no desenvolvimento da raiz e parte aérea, bem como possíveis alterações no estado nutricional e na partição de carboidratos em plantas de cana-de-açúcar. Para tanto, três estudos foram conduzidos: i) avaliação do efeito de concentrações de $Mg^{2+}$ (controle e deficiente) no desenvolvimento de plantas de cana-de-açúcar; ii) alterações metabólicas e nutricionais na cana-de-açúcar decorrentes do desequilíbrio de $Mg^{2+}$ causado pelo alto nível de $K^{+}$; iii) eficiência da aplicação foliar de $Mg^{2+}$ em plantas de cana-de-açúcar cultivadas em nutrição variada de $K^{+}$ e $Mg^{2+}$. Os experimentos foram conduzidos em casa de vegetação e as plantas cultivadas em solução nutritiva. Foram avaliados: o teor de clorofila nas folhas, parâmetros morfológicos das raízes (comprimento e diâmetro radicular), produção de matéria seca das partes da planta, composição nutricional e partição de amido, sacarose e açúcares redutores nas diferentes partes da planta. No geral, o adequado fornecimento de $Mg^{2+}$ levou a maior produção de matéria seca, concentração de sacarose nos colmos e relação raiz/parte aérea. Clorofila *a*, *b* e carotenoides foram menores em plantas deficientes em $Mg^{2+}$. Ademais, foi observado maior concentração de amido e açúcares solúveis nas folhas e menor concentração de sacarose nos colmos destas plantas. Além disso, plantas deficientes em $Mg^{2+}$ tiveram menor comprimento radicular e maior diâmetro, do que as quais apresentaram maior concentração de $K^{+}$ na raiz e maior translocação deste cátion para a parte aérea. O aumento da concentração de $K^{+}$ na solução nutritiva reduziu a concentração de $Mg^{2+}$ nas raízes, folhas novas, folhas velhas e colmos da cana-de-açúcar, mesmo nas plantas controle de $Mg^{2+}$. Independente da nutrição com $Mg^{2+}$, no maior nível de $K^{+}$, todos os parâmetros biométricos avaliados neste estudo foram reduzidos. Plantas cultivadas sob deficiência de $Mg^{2+}$ obtiveram maior concentração de amido nos colmos em altas concentrações de $K^{+}$. A aplicação foliar de $Mg^{2+}$ aumentou o conteúdo de sacarose nos colmos e reduziu o de amido, independentemente do nível de $K^{+}$ avaliado. Além disso, foi observado plantas maiores com a aplicação foliar e alto nível de K, em ambos níveis de $Mg^{2+}$ avaliados. Para os parâmetros biométricos, com ou sem aplicação foliar, a deficiência de $Mg^{2+}$ pareceu afetar mais as plantas de cana-de-açúcar do que os altos níveis de $K^{+}$.

**Palavras-chave:** *Saccharum* spp.. Partição de carboidratos. Produção de clorofila. Estado nutricional. Nutrição de plantas. Potássio.

## ABSTRACT

The aim of this study was to evaluate the effect of $Mg^{2+}$ and $K^{+}$ concentrations, and $Mg^{2+}$ foliar application on the root and shoot growth, as well as to check the alterations on the nutritional status and carbohydrates partitioning in sugarcane plants. For this purpose, three studies were conducted: i) evaluation of the effect of $Mg^{2+}$ concentrations (adequate and deficient) on the sugarcane plants development; ii) metabolic and nutritional alterations in sugarcane due to $Mg^{2+}$ imbalance caused by $K^{+}$ high level; iii) efficiency of the $Mg^{2+}$ foliar application in sugarcane plants grown under varied levels of $K^{+}$ and $Mg^{2+}$. The experiments were estabilished in greenhouse and the plants grown in nutrition solution. The evalutions were: leaf chlorophyll content, root morphological parameters (length and root diameter), dry matter production of plant parts, nutritional composition and starch, sucrose and reducing sugars partitioning in the different plant parts. In general, the adequate supply of $Mg^{2+}$ resulted in higher dry matter production, sucrose concentration in the stalks and root/shoot ratio. Chlorophyll *a, b* and carotenoids were lower in $Mg^{2+}$ deficient plants. Moreover, higher concentration of starch and soluble sugars were observed in the leaves and lower starch concentration in the stalks of these plants. Besides, Mg-deficient plants had shorter root length and larger diameter, which presented higher $K^{+}$ concentrations in the root and higher translocation of this cation to the shoot. Increase in $K^{+}$ concentration in the nutrition solution reduced $Mg^{2+}$ concentration in the roots, young leaves, old leaves and stalks of sugarcane, even in $Mg^{2+}$ control plants. Regardless $Mg^{2+}$ nutrition, at the highest level of $K^{+}$, all the plant growth parameters evaluated in this study were reduced. Plants grown under $Mg^{2+}$ deficiency obtained higher starch concentration in the stalks at higher $K^{+}$ level. Magnesium foliar application raised the sucrose concentration in the stalks and reduced the starch, regardless the $K^{+}$ level evaluated. In addition, bigger plants were observed with foliar application and high $K^{+}$ level, at both $Mg^{2+}$ levels. For the plant growth parameters, with or without foliar application, $Mg^{2+}$ deficiency seemed to greater affect the sugarcane plants than the high levels of $K^{+}$.



**Keywords:** *Saccharum* spp.. Carbohydrate partitioning, Chlorophyll production, Nutritional status. Potassium

**SUMMARY**

## GENERAL INTRODUCTION

The worldwide demand for ethanol from renewable sources combined with large cropping areas and favorable soil and climate conditions for sugarcane has made Brazil the largest producer of sugarcane and an important country for the export of sugar as a commodity (CONAB, 2018). The sugarcane production estimate for the 2018/19 cropping season is 615.84 million tons, which is 2.8% lower than that of the previous cropping season. With respect to ethanol, the expectation is an increase of 18.6% during this season due to the increased need for total recoverable sugar production for anhydrous ethanol, with a production of 32.3 billion liters (CONAB, 2018).

Vinasse is a residue derived from the alcohol production process. This residue is generated in amounts ranging from 10 to 18 L $L^{-1}$ ethanol and is rich in potassium ($K^+$) and organic matter. When applied at the correct ratio and at the correct timing, this subproduct offers great rational and economical possibilities with respect to incorporation into the soil (NICOCHELLI et al., 2012; DEL NERY et al., 2018).

As regard to sugarcane, in addition to crop yield, the quality of the raw material is highly important for industrial purposes. The use of vinasse in sugarcane fields has undoubted benefits from agronomic and economic perspectives, since vinasse-treated fields yield the most tons of sugarcane per hectare (SILVA; BONO; PEREIRA, 2014). However, these fields can present low economic returns due to low sucrose concentrations within the stalks.

Potassium is directly correlated with the ash content in sugarcane juice (RODELLA; FERRARI, 1977; SILVA et al., 1978; FREIRE; CORTEZ, 2000). This ash content is an important factor in the industrialization process due to the negative effects of sugar crystallization. In plants, high $K^+$ level can induce the production of potassium saccharate instead of sucrose, reducing the amount of total recoverable sugar per ton of sugarcane produced (RODRIGUES, 1995; FREIRE; CORTEZ, 2000). Furthermore, high concentrations of $K^+$ in sugarcane juice seems to be strongly correlated with trans-aconitic acid contents in the stalks. Clarke and Brannan (1983) reported that this acid can be used as an indicator of the maturity level of sugarcane, since as the plant matures, the content of trans-aconitic acid decreases. Therefore, this acid might be another important indicator of the relatively lower sugar concentrations in sugarcane stalks grown in vinasse-treated fields.

According to Oliveira et al. (2014), the inclusion of vinasse during fertigation supplies the nutritional needs of sugarcane beginning from the second harvest; however, vinasse inclusion results in lower agroindustry quality parameters such as Brix value, sucrose and total recoverable sugar due to an increase in mineral fertilization from fertigation.

Compared with magnesium ($Mg^{2+}$) concentrations, $K^+$ concentrations are relatively high in vinasse (NUNES; VELLOSO; LEAL, 1981), suggesting that a deficiency in this divalent cation can be induced by continual applications of large volumes of vinasse, especially in Mg-deficient soils (NUNES; LEAL; VELLOSO, 1982).

Although the ionic radius of Mg is smaller than that of K, the radius of the hydrated ion of Mg is larger, indicating the need for ion-specific transport proteins. Moreover, the easy mobility of $K^+$ throughout the xylem is associated with the strong interaction of $Mg^{2+}$ with the negative charges of the xylem vessels, and the apoplast can slow $Mg^{2+}$ translocation to shoots (MARSCHNER, 1996; CAKMAK; YAZICI, 2010; GRANSEE; FÜHRS, 2013). At a very early stage of deficiency, photoassimilate phloem loading is severely impaired and occurs before any visible changes in shoot growth, chlorophyll concentrations or photosynthetic activity (CAKMAK et al., 1994a, b; HERMANS et al., 2005; CAKMAK; KIRKBY, 2008).

Reduced photosynthate transport from source to sink organs caused by $Mg^{2+}$ deficiencies in plants may affect the amount of sucrose in stalks (GERENDÁS; FÜHRS, 2013), mainly because adequate $Mg^{2+}$ nutrition is needed during the preripening stage to maintain and maximize source-to-sink transport (CAKMAK; KIRKBY, 2008).

Although antagonism between $K^+$ and $Mg^{2+}$ is well documented for other crop species, such as barley (JAKOBSEN, 1993), maize (MEURER; FONSECA, 1997), common bean (TUMA et al., 2004), rice (DING et al., 2008), safflower (FARHAT et al. 2013) and tomato (LI et al., 2018), this phenomenon as well as its impact on plant metabolism has not been widely recognized in sugarcane.

The present study presents the following hypotheses: a) $Mg^{2+}$ deficiency causes changes in root morphological parameters (diameter and length) as well as in carbohydrate partitioning in the sugarcane roots and shoots due to impaired sucrose export in the phloem and reduced carbohydrate supply to the roots, causes accumulations of starch and soluble sugars in the leaves, with lower amounts of these

carbohydrates allocated to the stalks, and reduces chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoid contents due to reduced Mg concentrations and increased accumulation of starch in the leaves; b) High $K^+$ levels decrease $Mg^{2+}$ uptake by the roots by reducing the affinity of this ion for plasma membrane transporters, decrease $Mg^{2+}$ translocation from the roots to the shoots, and affect sugarcane photosynthetic metabolism as well as the partitioning and translocation of starch and sucrose, resulting in reduced accumulations of this polysaccharide in the stalks; and c) $Mg^{2+}$ foliar applications may rescue the effects caused by high $K^+$ levels on the $Mg^{2+}$ uptake by roots.

Therefore, we aimed to evaluate the effects of $K^+$ and $Mg^{2+}$ concentrations in nutrient solutions and the foliar applications of $Mg^{2+}$ on root and shoot development of sugarcane as well as on the possible nutritional and metabolic alterations in the plants.

# CHAPTER 1 - MAGNESIUM AS A PROMOTER OF TECHNOLOGICAL QUALITY IN SUGARCANE *

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## ABSTRACT

**Purpose:** The aim of the present study was to evaluate the effect of magnesium ($Mg^{2+}$) supply on the nutritional status and carbohydrate partitioning of sugarcane plants. **Methods:** The treatments consisted of two $Mg^{2+}$ levels in nutrient solution (2 mg $L^{-1}$ and 10 mg $L^{-1}$). The following parameters were evaluated: leaf chlorophyll content, root morphological parameters, dry matter partition, nutritional composition, partition of carbohydrates and soluble sugars. **Results:** All plant growth parameters were influenced by $Mg^{2+}$ concentrations, except stalk dry matter. Regarding the dry matter apportioning, Mg-control plants presented higher dry matter in leaves than those under Mg deficiency. Plants malnourished with Mg presented higher concentration of $K^+$ but not total dry matter accumulation. In addition, photosynthetic pigments production was lowered by Mg deficiency. The concentration of reducing sugars in leaves or roots were not affected by magnesium levels. However, in the stalks, the sucrose concentration was higher in $Mg^{2+}$ well-nourished plants. **Conclusions**: Although the dry matter in stalks was not affected by Mg supply, Mg-control plants had higher sucrose accumulation in stalks compared to Mg-deficient. Therefore, Mg fertilization seems to be a key to increase sugar yield in sugarcane fields, mainly in those with high K input.

**Keywords:** *Saccharum spp*, carbohydrate partitioning, photosynthetic activity, root development, nutrient concentration.

## 1.1 INTRODUCTION

The role of magnesium ($Mg^{2+}$) is well understood in several vital functions in plants. However, there are only very few reports about its effect on sugarcane quality. Among the sugarcane quality indexes, sucrose juice content is one of most important for economic returns of sugarcane crop (XIAO et al., 2017; YANG et al., 2013).

Magnesium is closely linked to the partition between the synthesis of starch, transport of triose phosphates in the cytosol and the formation of sucrose, so that low availability of $Mg^{2+}$ leads to reduction in cytosolic glucose-fructose bonds (MARSCHNER, 2012). Thereby, the triose phosphate is retained within the chloroplast and the starch is synthesized instead of the sucrose, leading to high stalk yield, but with low sucrose concentration in the stalks.

The deficiency of $Mg^{2+}$ in plants may be caused by soil reserves depletion, but also can be induced by low $Mg^{2+}$ assimilation by the roots due to competitive inhibition caused by imbalance of some cations such as calcium (Ca) and potassium (K), especially in sugarcane fields where vinasse is frequently used (MENGEL AND KIRKBY, 2001; SHAUL, 2002). Vinasse has a high concentration of $K^+$ (0.04 - 11 kg $m^{-3}$) and can replace the total K mineral fertilizer (PARSAEE et al., 2019; SALOMON et al., 2011; SILVA et al., 2019). In addition, the high water content in vinasse increases the soil moisture content, consequently improving the amount of K moving toward plant roots (COSTA et al., 2009; SILVA et al., 2018). The easy $K^+$ mobility throughout xylem associated with the high interaction of the $Mg^{2+}$ with the negative charges on cell walls of both xylem and apoplast can slow down $Mg^{2+}$ translocation to shoots leading to a imbalance on the Mg:K ratio in plant tissues (CAKMAK AND YAZICI, 2010; GRANSEE AND FÜHRS, 2013; MARSCHNER, 1986). Even with this competition between $K^+$ and $Mg^{2+}$, the $Mg^{2+}$ concentration is not lowered to the point of reducing crop yield, but this may affects carbohydrates partitioning in the plant (GERENDÁS AND FÜHRS, 2013). As a result, sugarcane fields with vinasse application reach the highest sugarcane yields (SALOMON et al., 2011; SILVA et al., 2014), but in most cases these fields provide low economic returns due to low sucrose concentration in the sugarcane stalks (STR) (ABEYSINGHA et al., 2017; SINGH et al., 2007).

At very early stage of deficiency the phloem loading of photo-assimilate is severely impaired, occurring before any visible changes in shoot growth, chlorophyll concentration or photosynthetic activity (CAKMAK et al., 1994a, b; CAKMAK AND KIRKBY, 2008; HERMANS et al., 2005). Reduced photosynthate transport from source to sink organs caused by plant $Mg^{2+}$ deficiency may affect the amount of sucrose in stalks (GERENDÁS AND FÜHRS, 2013), mainly because adequate $Mg^{2+}$ nutrition is needed at the beginning ripening stage in order to maintain and maximize the source to sink transport (CAKMAK AND KIRKBY, 2008). Reduced transport and accumulation of carbohydrates in Mg-deficient leaves cause alterations in photosynthetic carbon

metabolism and restrict the $CO_2$ fixation (CAKMAK et al., 1994a, b; CAKMAK AND KIRKBY, 2008; HERMANS, et al. 2005). The electrons and excitation energy not used in photosynthetic $CO_2$ fixation are channeled to molecular $O_2$, which generates highly reactive $O_2$ species (ROS) that damage the chloroplasts constituents such as chlorophyll a and b (ASADA, 2006; CAKMAK AND KIRKBY, 2008; MENGUTAY et al., 2013; MITTLER 2002;).

However, the accumulation of carbohydrates in leaves is an evident response, which is part of a signaling system that still needs further studies to understand the role of $Mg^{2+}$ on photoassimilates phloem-loading and the amassing of metabolites in deficient leaves (GUO et al., 2015, 2016) especially in sugarcane.

Although antagonism between $K^+$ and $Mg^{2+}$ is well documented for other crop species, such as barley (JAKOBSEN, 1993), maize (MEURER AND FONSECA, 1997), common bean (TUMA et al., 2004), rice (DING et al., 2008), safflower (FARHAT et al., 2013) and tomato (LI et al., 2018), this phenomenon as well as its impact on the quality parameters of sugarcane has not been widely recognized. This is the first study presenting the sugarcane response due to $K^+$ and $Mg^{2+}$ antagonism in this culture. Thus, we aimed to evaluate the effect of $Mg^{2+}$ supply on the nutrition and partitioning of carbohydrates in sugarcane.

## 1.2 MATERIALS AND METHODS

### 1.2.1 Experimental design

This experiment was conducted in a greenhouse that had a climate-controlled environment. The greenhouse had an internal heating/cooling air circulatory system to maintain the temperature between 22 °C and 32 °C.

The experimental design was completely randomized and consisted of two $Mg^{2+}$ levels (2 mg $L^{-1}$ and 10 mg $L^{-1}$, deficient and control plants, respectively, based on previous experiments), each replicated 8 times. The variety used was RB867515 due this is the most cultivated variety in Brazil, country that is the largest sugarcane producer in the world.

### 1.2.2 Seedlings obtainment and trial set-up

The cane cuttings for seedling preparation were harvested from a commercial nursery area. Cane cuttings that were approximately 3 cm in length and that contained

one vegetative bud were obtained. The buds were extracted from the upper third of each cane cutting so that improved sprouting uniformity was achieved.

Afterwards, for germination, the cane cuttings were placed in plastic trays that contained course sand and were irrigated every three days with deionized water. The trays were placed on benches inside the greenhouse with controlled air humidity. At 20 days after emergence, seedlings were selected according to their sprouting uniformity and then were transplanted into the nutrient solutions.

The selected seedlings were fixed in styrofoam plates that were subsequently transferred to plastic vases so that only the root system was in contact with the nutrient solution. Each vase received four sugarcane seedlings and was properly equipped so that four liters of nutrient solution was constantly aerated.

### 1.2.3 Nutrient solution

The nutrient solution (Table 1) was diluted to one-third ionic strength during the first week and half ionic strength during the second week, after which it was replaced with a full-ionic-strength solution during the third week to avoid interference of any possible saline effects of the nutrient solution on the initial growth of the seedling roots.

Table 1. Composition of the nutrient solution of Furlani and Furlani (1988), modified.

| | Stock Solution Components | Concentration Mg Def. | Concentration Mg Cont. | Relation Stock Solution/ Nutrient Solution | Nutrient solution Concentration | |
|---|---|---|---|---|---|---|
| Nº | | g $L^{-1}$ | g $L^{-1}$ | ml $L^{-1}$ | Nutrient | mg $L^{-1}$ |
| 1 | $Ca(NO_3)_2.4H_2O$ | 350 | 350 | 3 | $N\text{-}NO_3^-$ | 157 |
| 2 | $NH_4NO_3$ | 49 | 49 | 1 | $N\text{-}NH_4^+$ | 23 |
| 3 | KCl | 4.31 | 54 | 1 | Ca | 178 |
| | $K_2SO_4$ | 103 | 83 | 3 | K | 206 |
| | $KNO_3$ | 57.4 | 57.4 | 3 | S | 59 |
| 4 | $MgSO_4.7H_2O$ | 20.5 | 103 | 1 | P | 16 |
| 5 | $(NH_4)_2HPO_4$ | 68 | 67 | 1 | Fe | 3.6 |
| 6 | Rexolim® | 55.4 | 55.4 | 1 | B | 0.27 |
| 7 | $H_3BO_3$ | 2.0 | 2.0 | 1 | Mn | 0.5 |
| | $MnCl_2.4H_2O$ | 1.75 | 1.75 | | Zn | 0.15 |
| | $ZnSO_4.7H_2O$ | 0.66 | 0.66 | | Cu | 0.05 |
| | $CuSO_4.5H_2O$ | 0.20 | 0.20 | | Mo | 0.09 |
| | | | | | Cl | 43 |
| | | | | | Magnesium | mg $L^{-1}$ |
| | | | | | Deficient | 2 |
| | | | | | Control | 10 |

The maximum variation of the solution volume was 5%, with water lost by evapotranspiration replaced with deionized water. The pH was monitored daily and maintained within a range of 5.5 to 6.0; when necessary, 0.1 M HCl or 0.1 M NaOH solution was used for adjustments. The nutrient solution was exchanged weekly.

The evaluations were conducted 65 days days after the plants were transplanted into the nutrient solution. Thus, the following were evaluated:

### 1.2.4 Morphological, physiological, and chemical analysis

#### 1.2.4.1 Leaf chlorophyll content

To determine the amount of photosynthetic pigments (Chl *a*, Chl *b* and carotenoids) present in the leaf tissues, three discs were cut between the edge and the leaf central vein using a paper punch (0.5 cm in diameter) from the leaf +1 (fresh plant material). The discs were kept for 24 hours in 12 mL glass vials (with lids) that contained 2 mL of N, N-dimethylformamide (DMF) and that were covered with aluminum foil, as proposed by Lichtenthaler (1987).

The absorbance of Chl *a*, Chl *b* and carotenoids was determined by a spectrophotometer at wavelengths of 664, 647 and 480 nm, respectively. The absorbance readings were determined with 1 mL of the extract and 1 mL of deionized water; a mixture of 1 mL of DMF solution plus 1 mL of deionized water was used as a “blank”. The calculations for the pigment determinations were in accordance with the methods of Wellburn (1994).

#### 1.2.4.2 Root morphological parameters

The plants were divided into roots, stalks and leaves. Three subsamples of the roots of each experimental unit were subsequently cut lengthwise, i.e., from the root-shoot junction to the end of the root system, to obtain the different types of roots. Afterwards, the samples were stored in a 100 mL universal collector that contained an alcohol solution (30%) and that was conditioned in a refrigerated environment (2 °C) for further analysis (TENNANT, 1975). The length (RL) and mean root diameter (RDI) were measured by a digitizer coupled to a computer with the WinRhizo software version 3.8-b (REGENT INSTRUMENTS INC., QUEBEC, CANADÁ), as described by Tennant (1975).

After these variables were determined, the samples used were dried in a forced-

air oven at 65 °C until they reached constant weight to determine the dry matter (DM) yield. Using the DM data obtained from the samples and from the remaining root system, the length of the entire plant root system was estimated (NEWMAN 1966).

1.2.4.3 Dry matter production of plant parts

The different parts of the plants from each experimental unit were conditioned in paper bags and subsequently subjected to drying in a forced-air oven at 65 °C until they reached a constant weight in order to determine the root dry matter (RDM), stalk dry matter (SDM) and leaves dry matter (LDM).

1.2.4.4 Determination of the nutritional composition of the plant parts

The DM of the plant parts was ground in a Willey type mill with a 1 mm screen, and subsequently, the $K^+$ and $Mg^{2+}$ concentrations were determined (AOAC 1995).

1.2.4.5 Quantification of soluble sugar and starch concentrations of the plant parts

The partitioning of carbohydrates between the plant parts was determined by measuring the reducing sugars (RS), sucrose (Suc) and starch (ST) concentrations in the DM according to the methods of *Somogyi-Nelson* (NELSON, 1994; SOMOGYI, 1952). The readings were performed by a spectrophotometer at 535 nm.

1.2.5 Statistical analysis

The results of the statistical analysis were obtained by the statistical program Sisvar (FERREIRA, 2011) and the treatment means were compared by the F-test ($p<0.05$). Correlation analysis was used to correlate all measured parameters with each other. Stepwise regression, forward and backward, was used to correlate starch, sucrose and reducing sugars concentrations in the stalks with all measured parameters. After performed the stepwise regression, the model was selected using the Akaike information criterion (AIC). Both correlation and regression analyses were carried out in JMP statistical software version 10.0.0 (*SAS* INSTITUTE INC., CARY, NC*, USA*).

## 1.3 RESULTS

### 1.3.1 Biometric and nutritional parameters

All biometric variables were influenced by $Mg^{2+}$ concentrations, except stalk dry matter (SDM). The low $Mg^{2+}$ availability compromised the root system development in 22% of dry matter (RDM) yield and 37% of the length (RL), although the diameter (RDi) was 20% larger (Table 2).

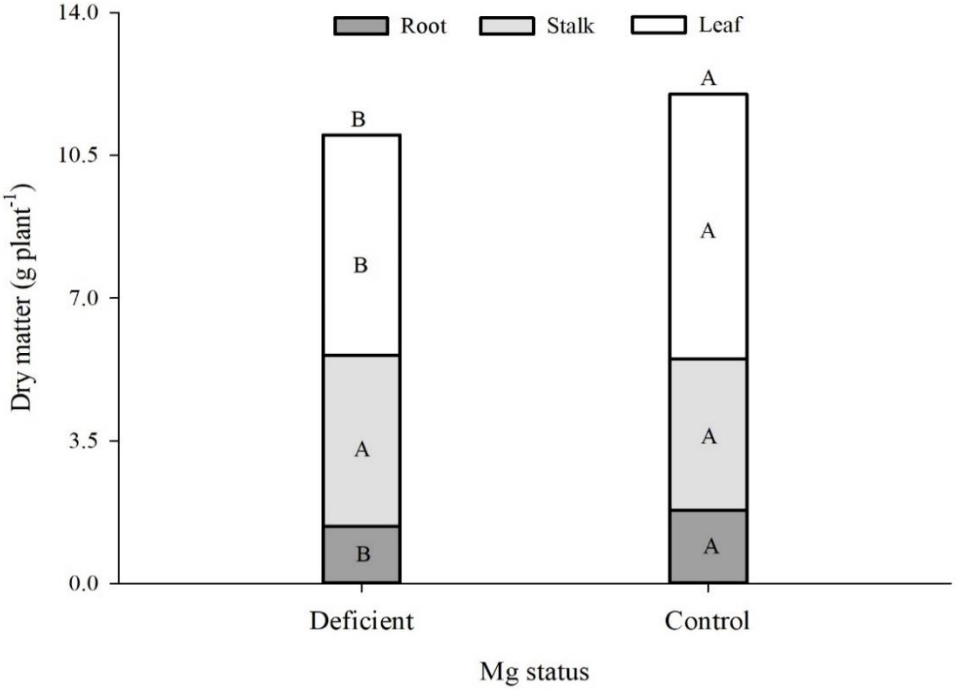
Table 2. Root lenght (RL), diameter (RDi), dry matter (RDM) and root/shoot ratio per sugarcane plant submitted to magnesium levels in nutrient solution for 65 days.

| | RL | RDi | RDM | R/S |
|---|---|---|---|---|
| Treatment | (m) | (mm) | (g) | |
| Deficient | 184b* | 0.3263a | 1.4b | 0.15b |
| Control | 292a | 0.2722b | 1.8a | 0.17a |
| F probability | | | | |
| Treatment | <0.001 | <0.001 | <0.001 | 0.02 |

* Means followed by the same letter do not differ by the LSD Test at 5% probability.

Regarding the partitioning of dry matter yield in leaves (figure 1), Mg-control plants presented higher mass (6.5 g $plant^{-1}$) than Mg-deficient plants (5.4 g $plant^{-1}$). However, the root/shoot ratio was lower in Mg-deficient plants (Table 2).

**Figure 1:** Root, stalk, leaf and dry matter apportioning in sugarcane plants as affected by Mg levels in nutrient solution. Means followed by the same letter do not differ by the F-Test at 5% probability.



Plants malnourished with $Mg^{2+}$ presented higher concentration of $K^{+}$ in the root, leaf and stalk (table 3). The concentration of $Mg^{2+}$ in the root, leaf and stalks was higher in Mg well-nourished plants.

Table 3. Potassium (K) and magnesium (Mg) concentration in the root (R), leaf (L) and stalk (S) of sugarcane submitted to magnesium levels in nutrient solution for 65 days.

| | K | | | Mg | | |
|---|---|---|---|---|---|---|
| | | | (g $Kg^{-1}$) | | | |
| Treatment | R | L | S | R | L | S |
| Deficient | 55a* | 35a | 67a | 0.4b | 0.6b | 0.8b |
| Control | 33b | 26b | 43b | 1.3a | 0.9a | 1.4a |
| F probability | | | | | | |
| Treatment | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

* Means followed by the same letter do not differ by the LSD Test at 5% probability.

### 1.3.2 Photosynthetic pigments and carbohydrates

Photosynthetic pigments production was affected by $Mg^{2+}$ availability. Lower concentration of Chl *a*, Chl *b* and carotenoids was observed in plants under low $Mg^{2+}$ concentration (Figure 2). Plants with $Mg^{2+}$ limitation synthesized 23, 40 and 29% less Chl a, Chl b and carotenoids, respectively.

The $Mg^{2+}$ nutrition did not influence the concentration of reducing sugars in leaves or roots (Table 4). However, in the stalks, the sugar concentration was higher in Mg-control plants. When the plant was insufficiently supplied with $Mg^{2+}$ the accumulation of starch increased by 15 and 28%, respectively in the roots and leaf. Oppositely, Mg-control plants showed increased in the starch accumulation in the stalk by 13%. Although the accumulation of sucrose in leaves of Mg-control plants was 13.5% higher, sucrose export to stalks of these plants was 87% higher compared to plants with deficient supply in this element.

Figure 2. Chlorophyll a, b and carotenoids concentration of sugarcane submitted to magnesium levels in nutrient solution for 65 days. Means followed by the same letter do not differ by the F-Test at 5% probability. Error bars express the standard error of the mean (n = 8).

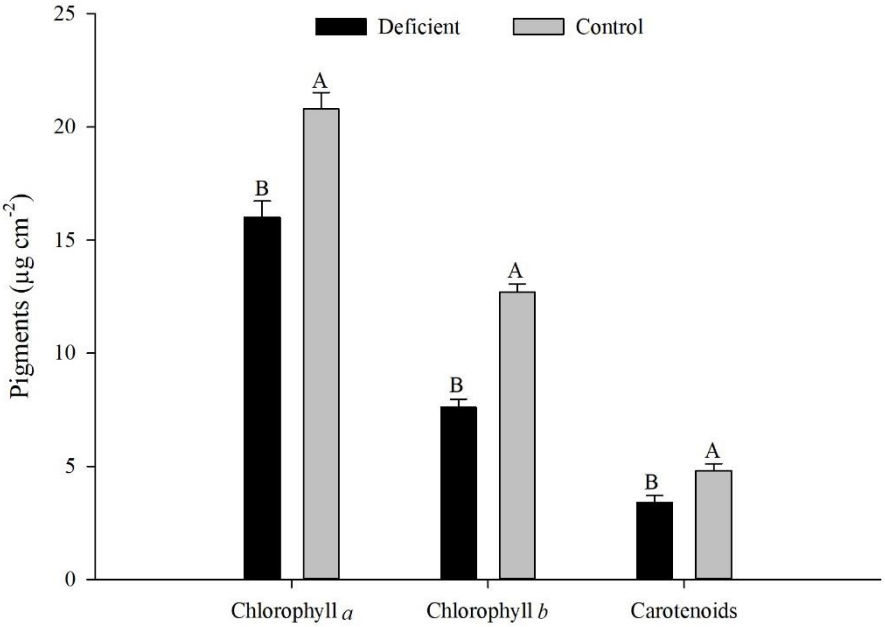



Table 4. Reducing sugars, sucrose and starch in the roots (R), leaves (L) and stalks (S) of sugarcane submitted to magnesium levels in nutrient solution for 65 days.

| | Reducing Sugars | | | Sucrose | | | Starch | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | (g kg$^{-1}$) | | | | | |
| Treatment | R | L | S | R | L | S | R | L | S |
| Deficient | 8.6a* | 16.4a | 25.0b | 7.3a | 32.0b | 16.0b | 210a | 142a | 117b |
| Control | 8.6a | 16.6a | 30.0a | 7.3a | 37.0a | 30.0a | 183b | 111b | 132a |
| F probability | | | | | | | | | |
| Treatment | 0.873 | 0.76 | <0.001 | 0.956 | 0.027 | <0.001 | <0.001 | <0.001 | 0.035 |

* Means followed by the same letter not differ by the LSD Test at 5% probability.

### 1.3.3 Correlation analysis

Several stalk properties were significantly correlated with the nutritional and biometric parameters (Table 5). For the sucrose concentration in the stalks (SucS), the highest positive correlations were observed for Cla (r = 0,80), Clb (r = 0,81), RDM (r = 0.77), RL (r = 0,85) and LDM (r = 0,77), and MgR, MgS and MgL (r=0,89). Oppositely, the highest negative correlations were observed for KR (r=-0,76), KS (r=-0,83), KL (r=-0,88) and RDi (r=-0,81). Similarly, the sucrose concentration in the leaves (SucL) was highly correlated with the properties found for SucS concentration. Contrasting with the

correlation observed for the SucS, all the proprieties correlated in the positive way were oppositely correlated with the StR and StL concentration.

Stepwise regression was also used to explain the variability in the stalk's carbohydrates found (Table 6). The properties that had the greatest influence on the SucS was Caro. For the StS, was observed high influence of the RSL, KL, MgR, MgS, as well as, StR and KR in the RSS value.

Table 5. Summary of the correlation analysis with the respective correlation P-values.

| *Variables* [a] | RDM[¥] | SDM | LDM | Cla | Clb | Caro | RL | Rdi | StR | RSR | SucR | StL | RSL | SucL | StS | RSS | SucS | KR | KS | KL | MgR | MgS | MgL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RDM | - | | | | | | | | | | | | | | | | | | | | | | |
| SDM | **-0.70** | - | | | | | | | | | | | | | | | | | | | | | |
| LDM | **0.91** | -0.65 | - | | | | | | | | | | | | | | | | | | | | |
| Cla | **0.76** | -0.44 | **0.74** | - | | | | | | | | | | | | | | | | | | | |
| Clb | **0.93** | -0.55 | **0.94** | **0.87** | - | | | | | | | | | | | | | | | | | | |
| Caro | **0.71** | -0.35 | **0.82** | **0.72** | **0.73** | - | | | | | | | | | | | | | | | | | |
| RL | **0.97** | -0.65 | **0.90** | **0.87** | **0.97** | **0.71** | - | | | | | | | | | | | | | | | | |
| Rdi | **-0.91** | 0.51 | **-0.87** | **-0.92** | **-0.94** | **-0.82** | **-0.96** | - | | | | | | | | | | | | | | | |
| StR | **-0.88** | 0.62 | **-0.85** | **-0.94** | **-0.95** | -0.65 | **-0.96** | **0.93** | - | | | | | | | | | | | | | | |
| RSR | -0.25 | 0.02 | -0.14 | 0.20 | 0.00 | -0.25 | -0.07 | 0.03 | -0.20 | - | | | | | | | | | | | | | |
| SucR | 0.05 | 0.12 | 0.11 | -0.32 | -0.06 | 0.09 | -0.06 | 0.07 | 0.30 | -0.67 | - | | | | | | | | | | | | |
| StL | **-0.94** | 0.58 | **-0.95** | **-0.83** | **-0.95** | **-0.82** | **-0.96** | **0.96** | **0.89** | 0.15 | -0.15 | - | | | | | | | | | | | |
| RSL | 0.15 | -0.13 | -0.08 | 0.06 | 0.08 | -0.25 | 0.18 | -0.15 | -0.22 | 0.36 | -0.41 | -0.01 | - | | | | | | | | | | |
| SucL | **0.83** | -0.65 | **0.79** | **0.49** | **0.66** | **0.74** | **0.74** | **-0.71** | -0.55 | -0.56 | 0.47 | **-0.83** | -0.18 | - | | | | | | | | | |
| StS | -0.34 | 0.13 | -0.65 | -0.54 | -0.62 | -0.57 | -0.44 | 0.51 | 0.55 | -0.39 | -0.01 | 0.58 | 0.25 | **-0.22** | - | | | | | | | | |
| RSS | 0.69 | -0.24 | 0.41 | 0.47 | 0.50 | 0.48 | 0.64 | -0.66 | -0.48 | -0.48 | 0.04 | -0.56 | 0.40 | **0.58** | 0.23 | - | | | | | | | |
| SucS | **0.77** | **-0.75** | **0.77** | **0.80** | **0.81** | 0.51 | **0.85** | **-0.78** | **-0.86** | 0.17 | -0.03 | **-0.83** | -0.01 | **0.64** | -0.52 | 0.23 | - | | | | | | |
| KR | **-0.94** | 0.59 | **-0.84** | **-0.89** | **-0.93** | **-0.72** | **-0.98** | **0.97** | **0.95** | 0.02 | 0.17 | **0.91** | -0.31 | **-0.68** | 0.38 | **-0.72** | **-0.76** | - | | | | | |
| kS | **-0.96** | 0.63 | **-0.88** | **-0.89** | **-0.95** | **-0.71** | **-1.00** | **0.98** | **0.96** | 0.05 | 0.08 | **0.95** | -0.23 | -0.73 | 0.42 | -0.67 | **-0.83** | **0.99** | - | | | | |
| KL | **-0.95** | 0.66 | **-0.84** | **-0.80** | **-0.90** | -0.61 | **-0.97** | **0.91** | **0.88** | 0.16 | -0.12 | **0.94** | -0.15 | -0.80 | 0.35 | -0.64 | **-0.88** | **0.92** | **0.96** | - | | | |
| MgR | **0.95** | -0.68 | **0.94** | **0.87** | **0.96** | **0.77** | **0.98** | **-0.96** | **-0.94** | -0.05 | 0.01 | **-0.98** | 0.07 | 0.79 | -0.55 | 0.53 | **0.89** | **-0.94** | **-0.97** | **-0.95** | - | | |
| MgS | **0.90** | -0.62 | **0.96** | **0.85** | **0.96** | **0.79** | **0.94** | **-0.93** | **-0.91** | -0.01 | 0.07 | **-0.98** | -0.02 | 0.76 | -0.67 | 0.42 | **0.89** | **-0.89** | **-0.93** | **-0.91** | **0.99** | - | |
| MgL | **0.95** | -0.67 | **0.94** | **0.89** | **0.97** | **0.76** | **0.98** | **-0.96** | **-0.96** | 0.00 | -0.04 | **-0.98** | 0.10 | 0.75 | -0.57 | 0.52 | **0.89** | **-0.95** | **-0.98** | **-0.95** | **1.00** | **0.98** | - |

Root dry matter (RDM), stalk dry matter (SDM), leaves dry matter (LDM), chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoids (Caro), root lenght (RL), root diameter (RDi), starch in the roots (StR), sucrose in the root (SucR), reducing sugars n the root (RSR), starch in the leaves (StL), sucrose in the leaves (SucL), reducing sugars n the leaves (RSL), starch in the stalks (StS), sucrose in the stalks (SucS), reducing sugars n the stalks (RSS), potassium in the root (KR), potassium in the stalk (KS), potassium in the leaves (KL), magnesium in the root (MgR), magnesium in the stalk (MgS), and magnesium in the leaves (MgL)

Table 6. Summary of stepwise regression used to correlate the metabolic and nutricional parameters with the values of sucrose (Suc), starch (St) and reducing sugars (RS) in the stalks (S).

| Parameter | Metabolic and Nutritional properties indicated by the stepwise regression * | $R^2$ |
|---|---|---|
| SucS | Caro | 0.94 |
| StS | RSL, KL , MgR, MgS | 0.99 |
| RSS | StR, KR | 0.96 |

* Carotenoids (Caro), Reducing sugars in the Leaf (RSL), Potassium concentration in the leaf (KL) and root (KR), Magnesium concentration in the root (MgR) and stalk (MgS) and Starch concentration in the root (StR).

## 1.4 DISCUSSION

### 1.4.1 Biometrics and nutritional parameters

Although no difference was observed in the shoot dry matter of plants grown with control or deficient $Mg^{2+}$ level, the root system of plants grown under low $Mg^{2+}$ concentration presented lower dry matter. One of the first plant reactions to Mg-deficiency seems to be its impact on the dry matter partitioning between roots and shoot. There are several evidences indicating that $Mg^{2+}$ plays a key role in the phloem loading of photoassimilates as its deficiency results in a drastic increase in the accumulation of carbohydrates in the leaves (CAKMAK et al., 1994a, b; HERMANS et al., 2004; MARSCHNER et al., 1996). Thusly, it is possible that restriction of the carbohydrates exportation from leaves to root had occurred, compromising the carbohydrate partitioning between root and shoot. According to Fischer and Bussler (1988) and Cakmak et al. (1994a), one of the well-proclaimed early reaction of plants to $Mg^{2+}$ deficiency is a decrease in root/shoot ratio, which is associated with a massive accumulation of carbohydrates in the source leaves, especially sucrose and starch. However, in our study, sucrose accumulation in leaves was higher in Mg-control plants (Table 4). The discrepancy found between our results and other reports may be due to duration of the experiment. In our experiment, the plants were grown for 65 days, while most studies reporting Mg deficiency had evaluated younger plants (up to 35 days) (CAKMAK et al., 1994a; GUO et al., 2015; HERMANS et al., 2005; HERMANS et al., 2010a; DIAS et al., 2018). Thus, due to $Mg^{2+}$ deficiency first affecting phloem loading, leading to long-term accumulation of sucrose and starch in the leaves, lack of $Mg^{2+}$ also affects photosynthesis by reducing sucrose production (CAKMAK AND KIRKBY, 2008). Thus, the sucrose content in this organ will be reduced due to: (i) the sucrose replenishment to the leaf; (ii) consumption for leaf maintenance (GRAHAM

AND MARTIN, 2000); and (iii) phloem loading, what continues to occur under $Mg^{2+}$ deficiency, but in a very low rate (HANSTEIN et al., 2011).

In sugar beet, Mg-deficiency caused a high accumulation of sucrose and starch in the shoot leaves, which were obtained at the lowest $Mg^{2+}$ concentrations according to study conducted by Hermans et al. (2005). The sucrose accumulation, according to the same authors, occurred due to a severe inhibition on sucrose exportation from those leaves to the roots. Depending on the severity of $Mg^{2+}$ deficiency, plants under low supply of this element may show 3 to 12 times higher concentrations of sucrose in the source leaves than plants with adequately nourished with $Mg^{2+}$ (CAKMAK et al., 1994a).

Another tempting prospect for investigating the determinant factors of $Mg^{2+}$ impact on root development is the hormonal measurement. According to Hermans et al. (2010a), the abscisic acid (ABA) may have an important role signaling $Mg^{2+}$ scarcity, since ABA signaling (RUBIO et al., 2009) is massively triggered in the roots, so that, in study, half of the genes regulated by $Mg^{2+}$ (cluster LII) were also responsive to ABA. According to the same authors, no difference was observed in ABA concentration of $Mg^{2+}$ deficient leaves compared to the controls after 28 hours of treatment, indicating the exclusive occurrence of this marker in the roots. In addition, ABA is an important activator of ethylene in roots, so that, high concentrations cause root development interruption (GUO et al., 2015; HERMANS et al., 2010a), which is important to enlighten the lower root development under low $Mg^{2+}$ concentration (Table 2). According to Hermans et al. (2010b), Mg-deficient plants produced twice as much gas as control plants. Thereby, the authors have shown that, although ethylene is mentioned as an inducer of foliar senescence, ethylene-regulated responses also appear to be essential for stress tolerance.

There was higher uptake and translocation of $K^+$ to the shoot of plants grown under the lowest $Mg^{2+}$ concentration in the nutrient solution (Table 3). In a study conducted by Epstein et al. (1963), the authors demonstrated that the $K^+$ uptake by the plant roots is controlled by two mechanisms, which occur at different $K^+$ external concentrations. When the external $K^+$ concentration is low, its absorption occurs by transporters (integral membrane proteins). At high $K^+$ external concentration, the absorption of this cation is mediated through channels, both highly selective to $K^+$. For $Mg^{2+}$, Ogura et al. (2018), studying the $^{28}Mg$ uptake in Arabidopsis plants found

evidences of a high and low affinity transport system for this ion, so that Arabidopsis grown under low supply of $Mg^{2+}$ expressed the genes *AtMRS2-4* and *AtMRS2-7*, responsible for coding the proteins that modulate the absorption of this divalent cation. In moderate conditions of $Mg^{2+}$ supply and higher concentration of other cations, the antagonism in $Mg^{2+}$ seemed to occur through non-selective ion channels, closely related to the $Ca^{2+}$ uptake system (OGURA et al., 2018; WHITE, 1998). Despite this knowledge, the root area and the uptake mechanism that undergoes antagonistic interference of $Mg^{2+}$ in presence of high $K^+$ levels is still not yet fully elucidated (KOCH et al., 2019). Some authors claim that the competition for the active sites on the cellular plasma membrane, where the ion transport protein is located, seems to be the main reason for the antagonism between $K^+$ and $Mg^{2+}$ (DING et al., 2008; KAMIYA et al., 2012; SHEWMAKER et al., 2008). Narwal et al. (1985) observed synergistic effect on the uptake of $K^+$ at low $Mg^{2+}$ concentrations and an antagonistic effect in the presence of higher concentrations of $Mg^{2+}$. Hannaways (1982) observed no effect of increasing $K^+$ concentrations in the nutrient solution on the $Mg^{2+}$ uptake by the roots. However, the accumulation of $Mg^{2+}$ in the shoot of the plant tall fescue (*Festuca arundinacea*) was significantly inhibited. Thereby, according to these authors, the antagonistic and synergic ionic effects on the $Mg^{2+}$ uptake as function of $K^+$ concentrations is likely related to the translocation of these cations to the plant shoot, and not to specific competitive effects for the radicular plasma membrane transporters (HANNAWAYS, 1982; MENGEL AND KIRKBY, 1982).

### 1.4.2 Photosynthetic pigments and carbohydrates

The key role played by $Mg^{2+}$ in the biosynthesis of Chl is already known, which are pigments responsible for light absorption by the leaves, closely linked to its formation and present as central atom of this molecule. The first step of Chl biosynthesis occurs with the insertion of $Mg^{2+}$ into the porphyrin structure, catalyzed by $Mg^{2+}$ chelatase (WALKER AND WEINSTEIN, 1991). ATP is essential for this enzyme functioning, which plays a role in the activation of the enzyme as well as in the inclusion of $Mg^{2+}$ into protoporphyrin IX (WALKER AND WEINSTEIN, 1991), thus, requiring additional $Mg^{2+}$. An efficient carbohydrate formation requires high light interception in the receptors complex bond to the photosystem I and II (PSI and PSII). Because $Mg^{2+}$ is an essential constituent of Chl *a* and *b*, internal low concentration of

$Mg^{2+}$ may lead to a lower concentration of these pigments, directly influencing the complex receptors efficiency (GERENDÁS AND FÜHRS, 2013). Therefore, $Mg^{2+}$ deficiency in the leaves results in significant reduction in the photosynthetic rate (FISCHER AND BREMER, 1993; HERMANS et al., 2004; LAING et al., 2000; PEASLEE AND MOSS, 1966; SUN AND PAYN, 1999).

Several studies have demonstrated that one of the early well-pronounced plant reactions to $Mg^{2+}$ deficiency is a massive carbohydrate accumulation in the source leaves (CAKMAK et al., 1994a; FISCHER AND BUSSLER, 1988) as was determined in this study (Table 4). These results suggest that the carbohydrate accumulation in $Mg^{2+}$ deficient leaves is directly caused by this nutrient deficiency, and not as a consequence of the reduced photosynthetic activity (CAKMAK AND KIRKBY, 2008) and it is possible to be detected well before the visual symptoms appearance of $Mg^{2+}$ deficiency (HERMANS et al., 2004; HERMANS AND VERBRUGGEN, 2005).

Early sugar accumulations along with low levels of $Mg^{2+}$ in the leaves seems to result in a down-regulation of genes involved in photosynthesis, such as with the one encoding chlorophyll *a/b* bond to the protein (*Cab2*) (HERMANS AND VERBRUGGEN, 2005). The carbohydrate accumulation causes changes in the carbon photosynthetic metabolism, restriction of $CO_2$ fixation and, consequently, decrease in the transport of electrons. Thus, the accumulation of unused electrons and non-absorbed energy are channeled to molecular $O_2$, leading to the generation of highly reactive oxygen species (ROS), which, in consequence, causes damage to chloroplast constituent such as chlorophylls and membrane lipids, a possible reason for the lower concentration of Chl a, b and carotenoids obtained in plants with insufficient supply of $Mg^{2+}$ in this study (Figure 2) (ASADA, 2006; MARSCHNER AND CAKMAK, 1989; MITTLER, 2002).

The sucrose transport in the phloem is an active process catalyzed by a proton gradient and a co-transporter $H^+$/sucrose, established by one $H^+$-ATPase localized in the plasma membrane (BOUCHE'-PILLON et al., 1994; WARD et al., 1998). For its proper functioning, a Mg-ATP is essential (BUSH, 1989; GETZ AND KLEIN, 1995; IGAMBERDIEV AND KLECZKOWSKI, 2003).

Plants grown under low $Mg^{2+}$ supply acquired higher starch concentration in the leaves (Table 4), suggesting a drastic inhibition of sucrose discharging in the phloem (CAKMAK et al., 1994a, b; HERMANS et al., 2004; MARSCHNER et al., 1996). In $C_4$ plants, such as sugarcane, $CO_2$ is fixed in the mesophyll cells by phosphoenolpyruvate

(PEPCase) to form oxaloacetate (a four-carbon organic acid). Then, the oxaloacetate is converted to malate and transported into the bundle sheath cells, which is decarboxylated to release $CO_2$. The released carbon is then fixed again by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) to synthetize triose phosphates, which is transported in the cytoplasm to synthesize sugar through Calvin Cycle (MARSCHNER, 2012). These sugars, mainly as sucrose form, are immediately loaded into the phloem in order to export to the sink organs, such as stalks, roots and young leaves.

When sugars in cytoplasm are excessive, due to some interruption in the phloem loading of sucrose, they are transported into vacuoles for transient storage and triose phosphates are used to synthesize starch in chloroplasts, since larger assimilates such as sucrose induce high turgid pressure (WANG et al., 2017). Thus, it is possible that the lower $Mg^{2+}$ concentration in the leaves (Table 3) provided a decrease in the $H^+$-ATPase activity in the phloem, reducing sucrose exportation to the stalk (Table 4) (ZHAO et al., 2000), promoting the accumulation of this sugar in $Mg^{2+}$ deficient leaves, which posteriorly caused the triose-phosphate to synthesize starch, justifying the high concentration of this carbohydrate in the leaves with $Mg^{2+}$ limitation.

In the stalks, properly nourished plants with $Mg^{2+}$ exported 87% more sucrose than plants under insufficient supply (Table 4), reinforcing the function of this nutrient in the sucrose exportation from the source leaves to the stalks. Further, it was observed that stalks of plants well supplied with $Mg^{2+}$ accumulated more starch, which also might be explained by the triose phosphate action on this carbohydrate synthesis, thus being able to store a greater quantity with lower cell turgescence pressure, since these plants were still at vegetative stage (65 days), and require photoassimilates for growth (WANG et al., 2017). In the study developed by McCormick et al. (2009), it was observed that there was a gradual decrease in photosynthetic rate with sugarcane maturation, demonstrating existence of a feedback regulation on source leaves as a function of the sucrose concentration present in the stalks. In this way, the sucrose accumulation in the stalks is determined by the supply besides the leaves-sources and the stalks demand. In order for sucrose to be continuously metabolized in this phase (tillering), it is necessary to have a low sucrose concentration in the stalks, which justifies, once again, the high starch concentration in the stalks of $Mg^{2+}$ well-nourished plants. Thereby, it is possible to store more carbohydrates keeping turgor pressure low and maintaining a high photosynthetic rate.

### 1.4.3 Correlation analysis and Stepwise regression

Correlation analysis showed that RDi had negative correlation with SucS concentration. Although root growth restriction is one of the pronunciation symptoms of Mg-deficiency, the biochemical functions of $Mg^{2+}$ in the root growth process are still not well defined (CAKMAK et al. 1994a, b; HERMANS et al., 2005; HERMANS AND VERBRUGGEN, 2005). The roots thickening of Mg-deficiency plants is an unfavorable aspect to the growth and development of them, since fine roots are the main roots responsible for the absorption of water and nutrients. (FITTER, 2002; VILELA AND ANGHINONI, 1984). The nutrient uptake efficiency by plants varies directly with both root length and thickness because these attributes influence in the absorption surface (FITTER, 2002; HORN et al., 2006; VILELA AND ANGHINONI, 1984; ZONTA et al., 2006). In this way, it was observed that Mg-deficient plants presented smaller RL and larger RDi. Thus, the negative correlation found for the SucS concentration can be explained by the greater difficulty in Mg uptake. In addition to the low $Mg^{2+}$ concentration in this treatment, the roots morphological changes of these plants may have aggravated this nutrient uptake. Consequently, there were lower number of nutrient uptake sites in contact with the nutrient solution, resulting in a lower concentration of $Mg^{2+}$ in the plants shoots. So, the low $Mg^{2+}$ concentration in the leaves may have impaired its function in the sucrose loading from the leaves to the phloem and, later, compromised its translocation to the stalks. Inverse correlation was also found for the $K^+$ concentrations in the roots, leaves and stalks in relation to the SucS accumulation. It is known that there is also the possibility of interactions between nutrients. In higher plants, these interactions occur when the supply of a nutrient affects the absorption, redistribution or function of another nutrient, inducing deficiency or toxicity, and modifying plant development. (LOUÉ, 1993; MALAVOLTA et al., 1997; TARIQ AND MOTT, 2007). Thus, it is also possible that the high $K^+$ uptake found in Mg-deficient plants caused a slight nutritional imbalance within the plants, further affecting the $Mg^{2+}$ uptake and its translocation to the shoot (CORRÊA et al., 2006; TARIQ AND MOTT, 2007). By contrast, $Mg^{2+}$ nutrition provided plants with higher RL, which in a positive way due to their higher $Mg^{2+}$ uptake and translocation correlated with the SucS concentration.

The positive correlation found for SucS concentration and the values of Cl a, Cl b, MgR, MgS and MgL may be explained by the key role that Mg performs in the

chlorophyll biosynthesis. Due to a tendency of $Mg^{2+}$ to form complex octahedrons, it is capable to occupy the central position in the chlorophyll molecule *a* and *b*, which are pigments responsible for light absorption by the leaves (BEALE, 1999). Plants require $Mg^{2+}$ to capture solar energy and conduce it to the photochemical process. The proportion of $Mg^{2+}$ bond to chlorophyll depends on the plant species, nutrient supply and intensity of received light (IGAMBERDIEV AND KLECZKOWSKI, 2001; WU et al., 1991). However, it is estimated that the quantity of structural $Mg^{2+}$ associated with chlorophyll represents 15 to 20% of leaf content. In general, in leaf cells, at least 25% of the total protein is located in chloroplasts. It explains why a $Mg^{2+}$ deficiency affects particularly the size, structure and function of chloroplasts, including the electrons transference to photosystem II (MARSCHNER, 2012). Thereby, since $Mg^{2+}$ is an essential component of Chl *a* and *b*, low internal concentration of $Mg^{2+}$ may lead to lower concentration of these pigments, directly influencing the efficiency of the light receptor's complexes (GERENDÁS AND FÜHRS, 2013) and, later, in the production and accumulation of sucrose in stalks.

Due to the complex role of $Mg^{2+}$ in chlorophyll and protein biosynthesis, negative impacts on the primary metabolism (energy) impaired by $Mg^{2+}$ deficiency are expected not only on the final crop yield but also on the sugarcane qualitative parameters. Since several plant organs are not able to satisfy their demand for photoassimilates, their demands are compensated by a direct transport from the source leaves to the sink organs mediated by the phloem. Although yet not entirely clarified, $Mg^{2+}$ seems to play a key role in the phloem-loading of sucrose from source leaves. Surprisingly, no study on the importance of $Mg^{2+}$ to the quality of sugarcane was published. Therefore, this study represents an important component that demonstrates the real contribution of $Mg^{2+}$ in the carbohydrate's accumulation in the stalks, which probably gains more prominence during the maturation stage of sugarcane.

## 1.5 CONCLUSION

Magnesium-deficient plants provide the highest starch accumulation in leaves and have lower concentration of chlorophyll *a*, *b* and carotenoids. While, stalks of well-nourished plants have less starch but more sucrose content as compared with those from deficient plants.

Both the uptake and the translocation of $K^+$ to the leaves are raised by $Mg^{2+}$ deficiency, what lead to nutritional imbalance in sugarcane plants.

In addition, the results of this study underscore the importance of monitoring $Mg^{2+}$ status in sugarcane leaves, which can avoid loss of sugarcane fields profit, mainly due to $Mg^{2+}$ deficiency often does not visually noticeable and does not affect the stalk biomass.

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# CHAPTER 2 - ALTERATIONS TO SUGARCANE METABOLISM DUE TO MAGNESIUM IMBALANCE CAUSED BY EXCESS POTASSIUM*

* – This chapter will be submitted in the "*Journal of Plant and Soil*"


## ABSTRACT

*Aims*

Evaluate the effects of Mg and potassium (K) supplies on the partitioning of carbohydrates, plant growth, nutrient status and Rubisco and antioxidant enzyme activity of sugarcane in response to a Mg imbalance caused by high K levels.

*Methods*

This experiment was conducted in a greenhouse that had a climate-controlled environment, and consisted of four K levels (103, 206, 309 and 412 mg $L^{-1}$) and two Mg levels (2 and 17 mg $L^{-1}$). The variety used was RB867515.

*Results*

The K concentration in all plant parts analyzed increased as the K concentration increased in the nutrient solution. Decreases in Mg concentrations were detected in all plant parts of Mg-adequate plants as a function of increasing K concentrations. Magnesium adequate plants presented more sucrose content in the stalks at all K levels. Plants Mg-deficient presented high starch concentrations in the fully expanded leaves and increased starch contents in the stalks as the K levels increased. Greater growth parameters were observed in Magnesium adequate plants

Compared to deficient plants.

*Conclusion*

High K level reduces Mg concentrations in all plant parts in both Mg-adequate and Mg-deficient plants. The highest K concentration compromised all of the root and shoot growth parameters analyzed. Magnesium adequate conditions provide plants with two times more sucrose, reducing sugars and starch in the stalks, all of which decreased at the highest K level. Magnesium deficient leaves presented much lower Rubisco activity with higher antioxidant enzymes.


**Key words:** *Saccharum spp*, carbohydrate partitioning, rubisco activity, root development, nutrient concentration, antioxidant enzymes.

## 2.1 INTRODUCTION

Although magnesium (Mg) is abundant in the earth's crust, much of it exists within the structure of crystalline minerals and therefore is not readily available to plants (SAYBARIAM et al., 2015). Clayey soils generally provide sufficient amounts of Mg for plant nutrition, while sandy soils are deficient in this nutrient and require applications of fertilizer that contain Mg (MAYLAND AND WILKINSON, 1989). Fertilizer recommendations are mainly focused on nitrogen (N), phosphorus (P) and potassium (K) supplies (MAGUIRE AND COWAN, 2002; GUO et al., 2016), while Mg supplies depend on liming (GRANSEE AND FÜHRS, 2013). However, lime typically has a high concentration of calcium (Ca) (30–40%) but a low Mg concentration (1–13%) (STEVENS et al., 2005; CAZOTTI et al., 2018; SORATTO et al., 2019), which may cause a nutrient imbalance in the rhizosphere.

Magnesium deficiency has become a concern in sugarcane fields. First, sugarcane cultivation has expanded to sandy soils (FRANCO et al., 2015; CHERUBIN et al., 2015). Second, liming is generally performed every 2 years, and Mg supplies are not considered when calculating liming rates (RAIJ et al., 1997; CRUSCIOL et al., 2017). In addition, applications of high rates of vinasse, a K-rich sugarcane byproduct (FUESS et al., 2018), as fertilizer increase the formation of trans-aconitic acid. This phenomenon leads to negative effects on both sugarcane ripening and the components of sugarcane juice, which could leads to reductions in sucrose concentrations and Brix values (STUPIELLO et al., 1977), apart from providing high K rates and from increasing the K/Mg ratio. Thus, Mg deficiency in plants may be due to depletion of soil reserves but can also be induced by low Mg assimilation by roots, as competitive inhibition is caused by an imbalance of some cations, such as K.

The antagonistic effect of K and Mg is well documented for some crop species, such as barley (JAKOBSEN, 1993), maize (MEURER AND FONSECA, 1997), common bean (TUMA et al., 2004), rice (DING et al., 2008), safflower (FARHAT et al., 2013), tomato (LI et al., 2018) and potato (KOCH et al., 2018). However, this phenomenon has not been widely recognized in sugarcane because no articles describing the impact of low Mg or high K rates on sugarcane metabolism and carbohydrate partitioning have been published.

Magnesium is the most abundant divalent cation in the cytosol and plays an essential role in many important processes within plants (LI et al., 2001). Magnesium

seems to be closely linked to the phloem loading of sucrose. Therefore, Mg deficiency affects the proton-motive force generated by $H^+$/ATPase, which requires Mg-ATP for its operation (MAGUIRE AND COWAN, 2002; HERMANS et al., 2005), and the translocation of photoassimilates to sink organs; thus, in the case of sugarcane, Mg deficiency may affect the amount of sucrose in the stalks and, consequently, sugarcane profitability (GERENDÁS AND FÜHRS, 2013).

Owing to the complex role of Mg in protein biosynthesis and enzyme activation, negative effects on the primary metabolism (energy) in response to Mg deficiency are expected not only on final crop yields but also on sugarcane qualitative parameters. Therefore, this study represents an important component that demonstrates the true contribution of Mg and K to carbohydrate accumulation in sugarcane stalks.

Thus, we aimed to evaluate the effects of Mg and K supplies on the partitioning of carbohydrates, plant growth, nutrient status and Rubisco and antioxidant enzyme activity of sugarcane in response to a Mg imbalance caused by high K levels.

## 2.2 MATERIALS AND METHODS

### 2.2.1 Experimental design

This experiment was conducted in a greenhouse that had a climate-controlled environment. The greenhouse was equipped with an internal heating/cooling air circulator system for maintaining the temperature between 22 and 32 °C.

The experimental design was completely randomized and consisted of four K levels (103, 206, 309 and 412 mg $L^{-1}$) and two Mg levels (2 and 17 mg $L^{-1}$) each replicated 4 times; the two Mg levels correspond to Mg-deficient plants and Mg-adequate plants (on the basis of previous experiments), respectively. The variety used was RB867515.

### 2.2.2 Seedlings obtainment and trial set-up

The cane cuttings for seedling preparation were harvested from a commercial nursery area. Cane cuttings that were approximately 3 cm in length and that contained one vegetative bud were obtained. The buds were extracted from the upper third of each cane cutting so that improved sprouting uniformity was achieved.

Afterwards, for germination, the cane cuttings were placed in plastic trays that

contained course sand and were irrigated every three days with deionized water. The trays were placed on benches inside the greenhouse, whose air humidity was controlled. At 20 days after emergence, seedlings were selected according to their sprouting uniformity and then were transplanted into the nutrient solutions.

The selected seedlings were fixed in styrofoam plates that were subsequently transferred to plastic pot so that only the root system was in contact with the nutrient solution. Each pot received four sugarcane seedlings and was properly equipped so that four liters of nutrient solution was constantly aerated. After 60 days, the plants were transferred to larger pots with 14 liters of nutrient solution capacity.

### 2.2.3 Nutrient solution

The nutrient solution (Table 1a and 1b) was diluted to one-third ionic strength during the first week and half ionic strength during the second week, after which it was replaced with a full-ionic-strength solution during the third week to avoid interference of any possible saline effects of the nutrient solution on the initial growth of the seedling roots.

The maximum variation of the solution volume was 5%, with water lost by evapotranspiration replaced with deionized water. The pH was monitored daily and maintained within a range of 5.5 to 6.0; when necessary, 0.1 M HCl or 0.1 M NaOH solutions were used for adjustments. The nutrient solutions were exchanged weekly.

Table 1a. Composition of the nutrient solution of Furlani and Furlani (1988), modified.

| *Dificient Mg level* | | | | | |
|---|---|---|---|---|---|
| | Stock Solution Components | Concentration | Relation Stock Solution/ Nutrient Solution | Nutrient solution Concentration | |
| Nº | | g $L^{-1}$ | ml $L^{-1}$ | Nutrient | mg $L^{-1}$ |
| 1 | $Ca(NO_3)_2.4H_2O$ | 350 | 3 | $N\text{-}NO_3^-$ | 156 |
| | $NH_4NO_3$ | 31.53 | 3 | $N\text{-}NH_4^+$ | 31 |
| | $Na_2SO_4$ | 124.64 | 1 | Ca | 178 |
| 2 | $K_2SO_4$ | 45.9 | 3 | S | 56 |
| | $KNO_3$ | 35.76 | 3 | P | 16 |
| 3 | $MgSO_4.7H_2O$ | 20.54 | 1 | Fe | 3.6 |
| 4 | $(NH_4)_2HPO_4$ | 68 | 1 | B | 0.27 |
| 5 | Rexolim® | 55.4 | 1 | Mn | 0.5 |
| 6 | $H_3BO_3$ | 2.0 | 1 | Zn | 0.15 |
| | $MnCl_2.4H_2O$ | 1.75 | | Cu | 0.05 |
| | $ZnSO_4.7H_2O$ | 0.66 | | Mo | 0.09 |
| | $CuSO_4.5H_2O$ | 0.20 | | Mg | 2 |
| | Components | g $L^{-1}$ | | K | mg $L^{-1}$ |
| | KCl | 0.0 | 3 | 0.5 | 103 |
| | | 65.56 | 3 | 1.0 | 206 |
| | | 131.83 | 3 | 1.5 | 309 |
| | | 196.76 | 3 | 2.0 | 412 |

Table 1b. Composition of the nutrient solution of Furlani and Furlani (1988), modified.

| *Adequate Mg level* | | | | | |
|---|---|---|---|---|---|
| | Stock Solution Components | Concentration | Relation Stock Solution/ Nutrient Solution | Nutrient solution Concentration | |
| Nº | | g $L^{-1}$ | ml $L^{-1}$ | Nutrient | mg $L^{-1}$ |
| 1 | $Ca(NO_3)_2.4H_2O$ | 350 | 3 | $N\text{-}NO_3^-$ | 156 |
| | $NH_4NO_3$ | 31.53 | 3 | $N\text{-}NH_4^+$ | 31 |
| | $NaNO_3$ | 42.80 | 1 | Ca | 178 |
| 2 | $K_2SO_4$ | 60.5 | 3 | S | 56 |
| | $KNO_3$ | 18.79 | 3 | P | 16 |
| 3 | $MgSO_4.7H_2O$ | 174.6 | 1 | Fe | 3.6 |
| 4 | $(NH_4)_2HPO_4$ | 68 | 1 | B | 0.27 |
| 5 | Rexolim® | 55.4 | 1 | Mn | 0.5 |
| 6 | $H_3BO_3$ | 2.0 | 1 | Zn | 0.15 |
| | $MnCl_2.4H_2O$ | 1.75 | | Cu | 0.05 |
| | $ZnSO_4.7H_2O$ | 0.66 | | Mo | 0.09 |
| | $CuSO_4.5H_2O$ | 0.20 | | Mg | 17 |
| | Components | g $L^{-1}$ | | K | mg $L^{-1}$ |
| | KCl | 0.0 | 3 | 0.5 | 103 |
| | | 67.5 | 3 | 1.0 | 206 |
| | | 131.17 | 3 | 1.5 | 309 |
| | | 196.76 | 3 | 2.0 | 412 |

Evaluations were conducted 110 days after the plants were transplanted into the nutrient solutions. The parameters described below were evaluated.

### 2.2.4 Plant height

A graduated ruler was used to measure plant height. The measurements were made from the root-shoot junction to the auricular region of leaf +1 or to top visible dewlap (TVD) leaf, according to the numbering suggested by Kuijper (DILLEWIJN, 1952).

### 2.2.5 Root length

The plants were divided into roots, stalks, fully expanded leaves and young leaves. The root length was measured by a digitizer coupled to a computer with WinRhizo software version 3.8-b (REGENT INSTRUMENTS INC., QUEBEC, CANADA), as described by Tennant (1975).

### 2.2.6 Dry matter production of plant parts

The different parts of the plants from each experimental unit were conditioned in paper bags and subsequently subjected to drying in a forced-air oven at 65 °C until they reached a constant weight, after which they weighed for determining the root dry matter, stalk dry matter, shoot dry matter (fully expanded leaves, young leaves and stalks) and total dry matter.

### 2.2.7 Determination of the K and Mg concentration of the plant parts

The dry matter of the plant parts was ground in a Willey-type mill with a 1 mm screen, after which the K and Mg concentrations were determined (AOAC 1995).

### 2.2.8 Carbohydrates

The partitioning of carbohydrates between the plant parts was determined by measuring the reducing sugar, sucrose and starch concentrations in the dry matter according to the methods of Somogyi-Nelson (SOMOGYI, 1952; NELSON, 1994). The readings were collected via a spectrophotometer at 535 nm.

### 2.2.9 Analysis of antioxidant enzymes

Approximately 1.5 g of frozen plant material was weighed and then macerated in liquid N in a porcelain mortar. Four milliliters of 100 mM potassium phosphate buffer (containing 1 mM ethylenediaminetetraacetic acid (EDTA), 3 mM dithiothreitol (DTT), and 4% (w/v) polyvinylpolypyrrolidone (PVPP), pH 7.5) was added to the macerated material according to the methods of Santos et al. (2017), with modifications. The homogenized material was subsequently vortexed and then centrifuged in a refrigerated centrifuge at 9.000 rpm for 20 minutes at 4 °C.

#### 2.2.9.1 Soluble proteins

The concentrations of the total soluble proteins were determined in accordance with the Bradford (1976) method to calculate the specific activity of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR). The measurements were performed by the addition of 5 µL of supernatant to 1 mL of Bradford reagent. After 10 minutes, the absorbance at 595 nm was measured via a spectrophotometer. The protein concentration in the leaf tissue was subsequently calculated on the basis of a standard curve for bovine serum albumin (BSA).

#### 2.2.9.2 Superoxide dismutase (SOD, EC:1.15.1.1)

A reaction chamber under fluorescent lamp-based illumination (15 W) at 25 °C was used. In accordance with the methods of Giannopolitis and Ries (1977), 50 µL of the sample was added to 2 mL of potassium phosphate buffer (pH 7.8) (50 mmol $L^{-1}$) supplemented with 250 µL of methionine (13 mmol $L^{-1}$), 200 µL of nitroblue tetrazolium (NBT) (75 mmol $L^{-1}$), 200 µL of EDTA (0.1 mmol $L^{-1}$) and 250 µL of riboflavin (2 µmol $L^{-1}$). The samples were then vortexed, stored with exterior light-protected, and kept under illumination for 25 minutes. The samples were again vortexed and then subjected to absorbance readings at 560 nm via a spectrophotometer.

### 2.2.9.3 Catalase (CAT, 1.11.1.6)

In a test tube, 1 mL of 100 mM potassium phosphate buffer (pH 7.5) was added to 2 µL of 30% $H_2O_2$, followed by the addition 150 µL of protein extract. Immediately afterward, the tube was quickly vortexed. Enzyme activity was determined by the decomposition of $H_2O_2$ during a 2 minute interval in a spectrophotometer at a wavelength of 240 nm at 25ºC, according to the methods of Azevedo et al. (1998).

### 2.2.9.4 Ascorbate peroxidase (APX, EC:1.11.1.11)

Six hundred microliters of 80 mM potassium phosphate buffer (pH 7.0), 100 µL of 5 mM ascorbate, and 100 µL of 1 mM EDTA were mixed together, to which 100 µL of the plant extract was subsequently added. The mixture was then incubated in a 30 °C water bath for 5 minutes in the dark. One hundred microliters of 1 mM $H_2O_2$ was then added, after which the absorbance at 290 nm was immediately read for 2 minutes, in accordance with the methods proposed by Moldes et al. (2008).

### 2.2.9.5 Glutathione reductase (GR, EC 1.6.4.2)

Glutathione reductase activity was determined according to the methodology described by Gomes-Junior et al. (2006). One milliliter of 100 mM potassium phosphate buffer (pH 7.5), 500 µL of 1 mM nitrobenzoic acid (DTNB), 100 µL of 1 mM oxidized glutathione (GSSG) and 100 µL of 0.1 mM NADPH were added to an Eppendorf tube and mixed together. Fifty microliters of the supernatant was then added, after which the tube was vortexed. Afterward, the solution was transferred to a cuvette, and the absorbance at 412 nm was immediately recorded for 1 minute.

### 2.2.9.6 Rubisco Activity

Via a porcelain mortar, 0.3 g of material (fresh weight) was homogenized for 2 minutes with 1.5 mL of 42 mM $KH_2PO_4$, 58 mM $K_2HPO_4$ and 1 mM EDTA. The homogenate was then centrifuged at 14,000 g for 25 minutes at 4 °C, and the supernatant was maintained at 4 °C (adapted from Sage, Sharkey and Seemann 1988; Reid et al. 1997). Total Rubisco activity was measured via an assay mixture containing 100 mM Bicine-NaOH (pH 8.0), 25 mM $KHCO_3$, 20 mM $MgCl_2$, 3.5 mM ATP, 5 mM phosphocreatine, 0.25 mM NADH, 80 nkat glyceraldehyde-3-phosphate

dehydrogenase, 80 nkat 3-phosphoglyceric phosphokinase and 80 nkat creatine phosphokinase. Seventy microliters of plant extract was incubated at 30 °C for 5 minutes with the assay mixture in the absence of ribulose-1,5-bisphosphate (RuBP) to enable carbamylation of the enzyme. The oxidation of NADP occurred by the addition of 16.66 **mM** RuBP to the cuvette (adapted from REID et al., 1997).

### 2.2.10 Statistical analysis

The data were subjected to ANOVA. The means were separated by the least significant difference (LSD) test ($p \leq 0.05$), and regression was used to determine the K levels. The statistical analysis was performed by the statistical program Sisvar (FERREIRA, 2011), and the figures were created via SigmaPlot® 12.5 software.

## 2.3 RESULTS

### 2.3.1 Nutritional aspects

The K concentration in all plant parts analyzed increased linearly as the K concentration increased in the nutrient solution (Table 2). In addition, Mg-deficient plants presented higher concentrations of K than did Mg-adequate plants.

Table 2. Concentration of K in the root, fully expanded leaves, young leaves and stalk of sugarcane plants submitted to K (103, 206, 309 and 412 mg $L^{-1}$) and Mg (deficient and control) levels in nutrient solution for 110 days.

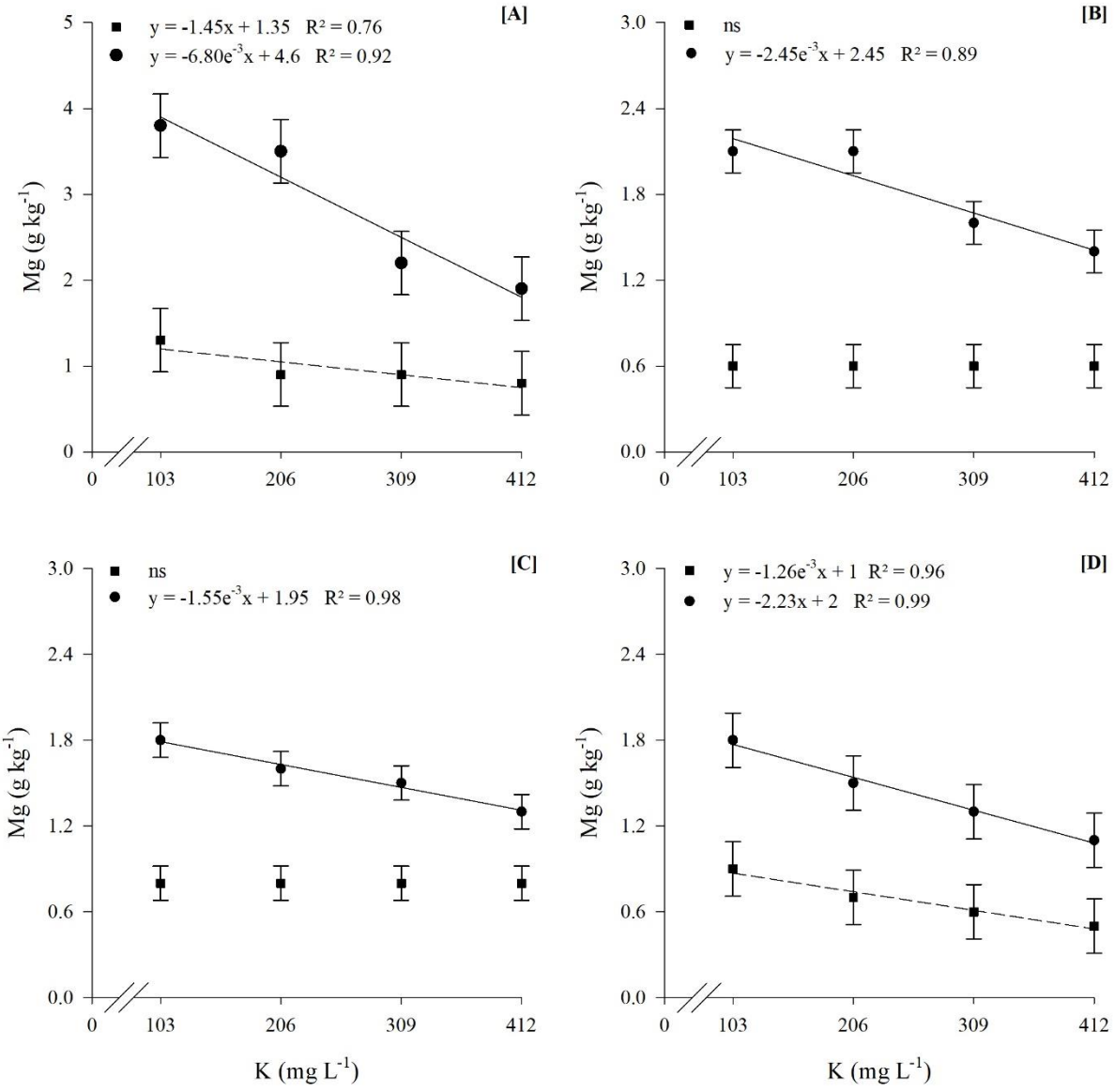
| Factors | Root | Fully expanded leaves | Young leaves | Stalks |
|---|---|---|---|---|
| Potassium mg $L^{-1}$ (K) | | K (mg $kg^{-1}$) | | |
| 106 | 13.1[(1)] | 10.2[(2)] | 10.9[(3)] | 16.3[(4)] |
| 206 | 15.1 | 10.8 | 11.3 | 20.8 |
| 309 | 17.9 | 12.3 | 12.9 | 23.2 |
| 412 | 20.2 | 13.1 | 13.5 | 24.4 |
| Magnesium (Mg) | | | | |
| Deficient | 17.8a | 12.2a | 12.8a | 21.9a |
| Adequate | 15.3b | 11.0b | 11.4b | 20.4b |

Means followed by the same letter in the column do not differ by the LSD Test at 5% probability. $y^{(1)}= 0.02x+10.5$ ($R^2=0.99$); $y^{(2)}= 0.01x+9$ ($R^2=0.98$); $y^{(3)}= 0.01x+9.8$ ($R^2=0.93$); $y^{(4)}= 0.03x+14.5$ ($R^2=0.93$)

Significant interaction effects of the factors were detected with respect to Mg concentrations within the plants analyzed (Figure 1). Under an adequate Mg supply, decreases in Mg concentrations were detected in the roots, fully expanded leaves,

young leaves and stalks as a function of increasing K concentrations (Figure 1A, B, C and D, respectively); however, these concentrations were greater than those in the Mg-deficient plants in all parts evaluated.

Figure 1. Interaction effect in the Mg concentration in the root [A], fully expanded leaves [B], young leaves [C] and stalk [D] of sugarcane plants submitted to K (103, 206, 309 and 412 mg $L^{-1}$) and Mg [Mg-deficient **(---■---)** and Mg-adequate **(—●—)**] levels in nutrient solution for 110 days. Vertical bars indicate the LSD value by the t test (LSD) at 5 %.

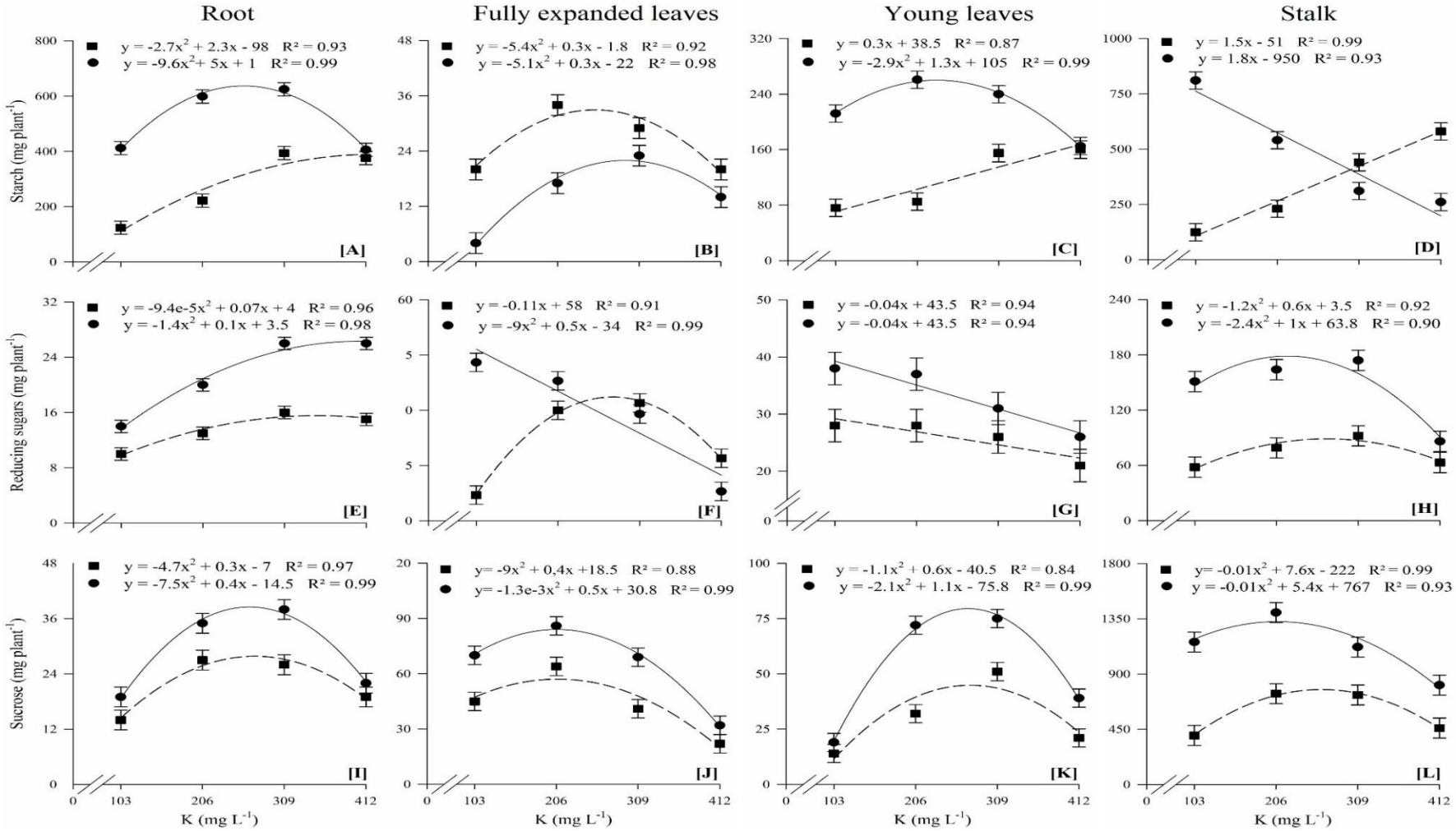


### 2.3.2 Carbohydrates

An adequate supply of Mg provides plants with relatively high sucrose contents in the roots, fully expanded leaves, young leaves and stalks (Figure 2). Compared with Mg-deficient plants, Mg-adequate plants presented approximately 2 times more sucrose content in the stalks at all K levels (Figure 2L). The maximum technical efficiency level (MTEL) for Mg-adequate plants occurred at approximately 270 mg $L^{-1}$, with 1,5 mg $plant^{-1}$ of sucrose. In addition, compared with the Mg-deficient plants, the Mg-adequate plants presented greater contents of reducing sugars in the stalks (Figure 2H). However, at 412 mg $L^{-1}$ K, both Mg-deficient and Mg-adequate plants presented relatively low contents of reducing sugars in the young leaves and stalks (Figure 2G and H).

Plants deficient in Mg presented relatively high starch concentrations in the fully expanded leaves (Figure 2B) and increased starch contents in the stalks (Figure 2D) as the K levels increased. In contrast, the Mg-adequate plants presented a linear decrease in starch content in the stalks as the K level increased (Figure 2D).

Figure 2. Interaction effect in the sucrose, reducing sugars and starch content in the root, fully expanded leaves, young leaves and stalk of sugarcane plants submitted to K (103, 206, 309 and 412 mg $L^{-1}$) and Mg [Mg-deficient **(---■---)** and Mg-adequate **(—●—)**] levels in nutrient solution for 110 days. Vertical bars indicate the LSD value by the t test (LSD) at 5 %.

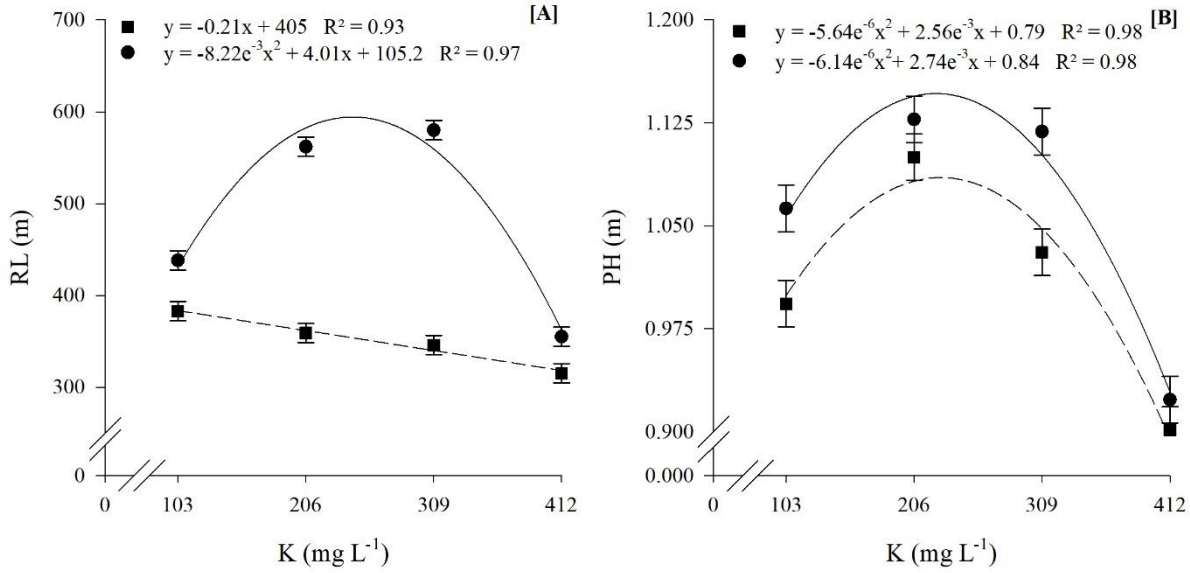


### 2.3.3 Root morphological parameters, plant growth and biomass production

Root system development was markedly affected by K and Mg concentrations. The highest root length (594 m) (Figure 3A) and root dry matter (4.0 g plant$^{-1}$) (Figure 4A) for the Mg-adequate plants occurred at K concentrations of 244.5 and 270 mg L$^{-1}$, respectively.

The root length of the Mg-adequate plants was greater than that of the Mg-deficient plants at all K concentrations (Figure 3A), whereas the plants presented greater root dry matter than did the Mg-deficient plants at 206 and 309 mg L$^{-1}$ K (Figure 4A).

Figure 3. Interaction effect in the root length (RL) [A] and plant height (PH) [B] of sugarcane submitted to K [103, 206, 309 and 412 mg L$^{-1}$) and Mg [Mg-deficient **(---■---)** and Mg-adequate **(—●—)**] levels in nutrient solution for 110 days. Vertical bars indicate the LSD value by the t test (LSD) at 5 %.



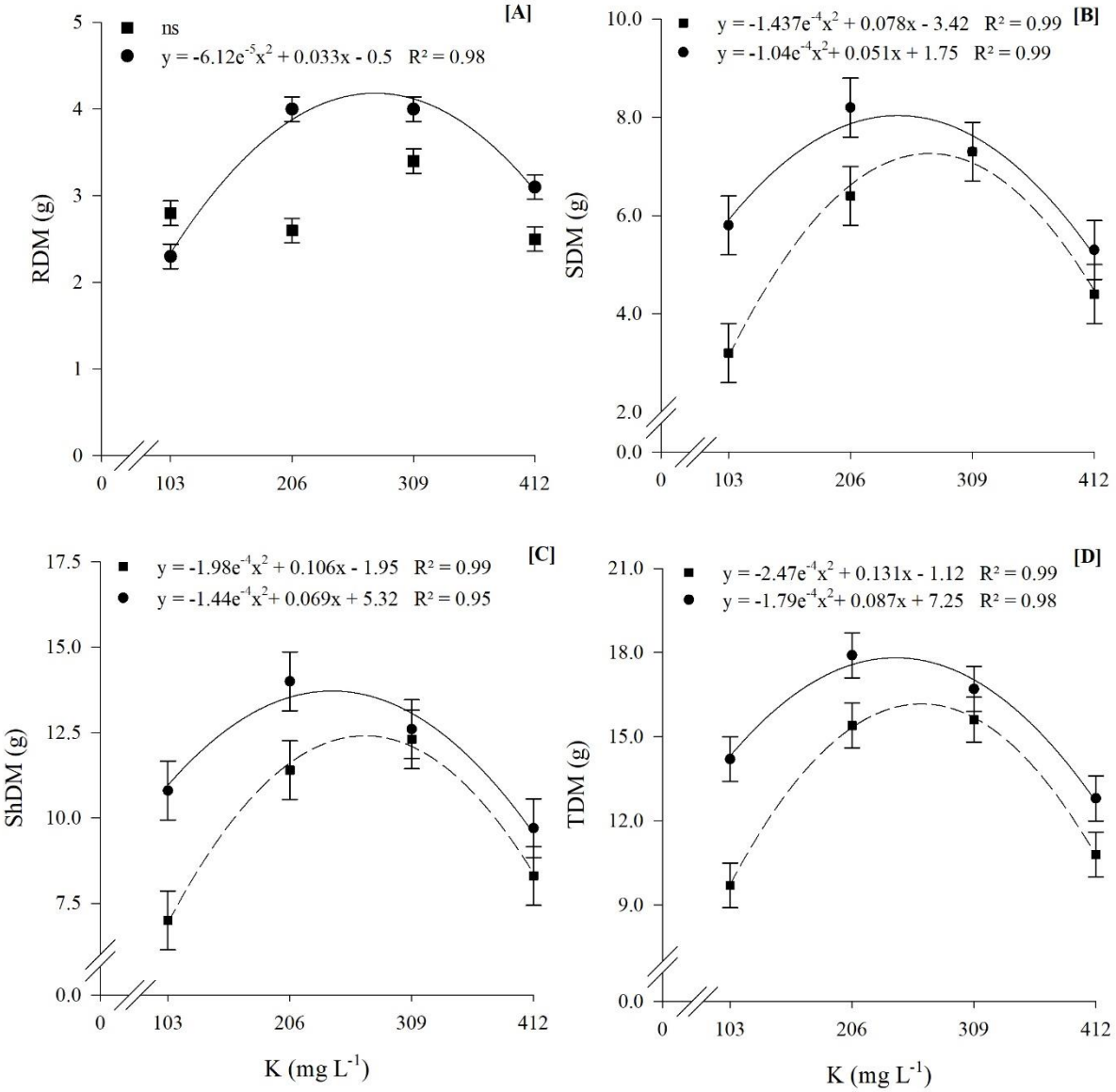

With respect to biometric variables, there were interaction effects on plant height, stalk dry matter, shoot dry matter and total dry matter.

Compared with the Mg-deficient conditions, the adequate Mg supply promoted greater plant height at 103 and 309 mg L$^{-1}$ K (Figure 3B). The greatest plant height (1.15 m) of the Mg-adequate plants occurred at 223.1 mg L$^{-1}$ K, which was 6.1% greater than that of the Mg-deficient plants. However, even in plants that were supplied with relatively high amounts of Mg, a high K concentration (412.0 mg L$^{-1}$) reduced the

plant height by 19.7%, although there was no difference in height between these plants and those growing under low Mg supply.

The Mg-adequate plants presented the greatest values of stalk dry matter (8.0 g plant$^{-1}$), shoot dry matter (13.6 g plant$^{-1}$) and total dry matter (17.8 g plant$^{-1}$) at 245, 239.6 and 243 mg L$^{-1}$ K, respectively, but these parameters were not significantly different from those of the Mg-deficient plants at 309 or 412 mg L$^{-1}$ K for all variables analyzed (Figure 4B, C and D). With respect to the Mg-deficient plants, the MTEL occurred at approximately 271.4, 267.7 and 265.2 mg L$^{-1}$ K for stalk dry matter, shoot dry matter and total dry matter, respectively; the values were 7.2, 12.2 and 16.2 (g plant$^{-1}$) and were 10.5, 9.9 and 8.8% lower than the greatest value of the Mg-adequate plants, respectively.

Figure 4. Interaction effect in the root dry matter (RDM) [A], stalk dry matter (SDM) [B], shoot dry matter (ShDM) [C] and total dry matter (TDM) [D] per plant of sugarcane plants submitted to K [103, 206, 309 and 412 mg $L^{-1}$) and Mg [Mg-deficient **(---■---)** and Mg-adequate **(—●—)**] levels in nutrient solution for 110 days, and number of internode (NI) [C] in function of K levels. Vertical bars indicate the LSD value by the t test (LSD) at 5 %.




## 2.3.4 Rubisco and antioxidant enzyme activity

Superoxide dismutase activity increased as the K levels increased in both fully expanded leaves and young leaves (Table 3). With respect to Mg nutrition, the fully expanded leaves of Mg-deficient plants presented greater SOD activity than did those of Mg-adequate plants. However, in the young leaves, Mg nutrition did not influence SOD activity.

Table 3. Superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) and rubisco activity in the fully expanded leaves and young leaves of sugarcane plants submitted to K and Mg levels in nutrient solution for 110 days.

| | Fully Expanded Leaves | | | | |
|---|---|---|---|---|---|
| Factors | *SOD* | *APX* | CAT | GR | Rubisco |
| Potassium mg $L^{-1}$ (K) | U $mg^{-1}$ protein$^{-1}$ | | µmol $min^{-1}$ g protein$^{-1}$ | | |
| 106 | 88(1) | 73(2) | 101(3) | 6.1(4) | 2,6(5) |
| 206 | 86 | 63 | 98 | 6.0 | 3,2 |
| 309 | 92 | 74 | 109 | 5.6 | 2,4 |
| 412 | 101 | 76 | 79 | 4.4 | 2,2 |
| Magnesium (Mg) | | | | | |
| Deficient | 108a | 86a | 149a | 4.0b | 1,8b |
| Adequate | 76b | 56b | 44b | 7.0a | 3,4a |
| | Young Leaves | | | | |
| Factors | *SOD* | *APX* | CAT | GR | Rubisco |
| Potassium mg $L^{-1}$ (K) | U $mg^{-1}$ protein$^{-1}$ | | µmol $min^{-1}$ mg protein$^{-1}$ | | |
| 106 | 45(6) | 35(7) | 85(8) | 4.9(9) | 0,8(10) |
| 206 | 45 | 32 | 60 | 3.8 | 1,4 |
| 309 | 53 | 36 | 67 | 4.2 | 1,0 |
| 412 | 63 | 41 | 43 | 6.3 | 0,9 |
| Magnesium (Mg) | | | | | |
| Deficient | 51a | 37a | 85a | 5.0a | 0,7b |
| Adequate | 52a | 36a | 43b | 4.6a | 1,4a |

Means followed by the same letter in the column do not differ by the LSD Test at 5% probability. $y^{(1)}= 10,5x^2-17,2x+94$ ($R^2=0,99$), $y^{(2)}=$ ns, $y^{(3)}= -271x^2+566x+769$ ($R^2=0,68$), $y^{(4)}=$ ns, $y^{(5)}= -0,77x^2+1,54x+2$ ($R^2=0,60$), $y^{(6)}= 9,38x^2-12,4x+48,6$ ($R^2=0,99$); $y^{(7)}= 7,2x^2-13x+38,9$ ($R^2=0,97$), $y^{(8)}= -239x+936$ ($R^2=0,78$), $y^{(9)}= 0,32x^2-0,70x+0,76$ ($R^2=0,99$), $y^{(10)}= 0,82x^2+2x+0,03$ ($R^2=0,68$)

Ascorbate peroxidase activity was relatively high at 103 and 412 mg $L^{-1}$ K in the fully expanded leaves, but it was not influenced by K levels in the fully expanded leaves or young leaves. In addition, the fully expanded leaves of Mg-deficient plants presented greater APX activity than did those of Mg-adequate plants, but the young leaves were not influenced by the Mg treatments.

Catalase activity decreased as K levels increased in the young leaves, but in Mg-deficient plants, relatively high CAT activity was detected in both the fully expanded leaves and young leaves.

Glutathione reductase activity decreased in the fully expanded leaves and increased in the young leaves as K levels increased, but only in the fully expanded leaves did the Mg-adequate plants present increased GR activity.

Rubisco activity was relatively high at 206 mg $L^{-1}$ K in the fully expanded leaves and young leaves; however, Mg-adequate plants presented almost 2 times more Rubisco activity than did Mg-deficient plants in the fully expanded leaves and young leaves (Table 3).

## 2.4 DISCUSSION

### 2.4.1 Nutritional aspects

One of the typical Mg-deficiency symptoms in crop plants is fully expanded leaf chlorosis (MARSCHNER, 2012), which was the same symptom observed in all Mg-deficient plants and in the Mg-adequate plants treated with high K concentrations (309 and 412 mg $L^{-1}$). However, plants grown under Mg deficiency were able to maintain their growth and establishment until the end of the experiment. It appears that plants are able to conserve their concentration of cellular Mg within a proper range to maintain minimum plant development (ODA et al., 2016). According to several studies (MAO et al., 2014; ODA, 2016; OGURA et al., 2018), genes of the *MRS2* family are essential for plant survival under low-Mg conditions via a high-affinity uptake system.

According to Marschner et al. (2012), the Mg requirement for optimal plant growth is 1.5 to 3.0 g $kg^{-1}$ within vegetative parts. In Mg-deficient plants in the present study, the Mg concentration in fully expanded leaves and young leaves was less than 1.0 g $kg^{-1}$, regardless of the K level applied (Figure 1B and C). Thus, losses in protein synthesis and photosynthetic activity of plants under such conditions are expected (MAGUIRE AND COWAN, 2002; IGAMBERDIEV AND KLECZKOWSKI, 2003; HERMANS et al., 2004).

The K concentration at the 412 mg $L^{-1}$ level in fully expanded leaves and young leaves was 13.1 and 13.5 g $kg^{-1}$, respectively (Table 2), which is considered adequate for sugarcane plant development (10 to 16 g $kg^{-1}$) (RAIJ AND CANTARELA, 1997). Moreover, relatively high K concentrations in the leaves reduced the Mg concentration to values below the appropriate range in the Mg-adequate plants. Several studies have reported a strong antagonistic relationship between K and Mg (JAKOBSEN, 1993; TUMA et al., 2004; DING et al., 2008; FARHAT et al., 2013; LI et al., 2018).

Apparently, under moderate Mg conditions, this element enters into the root cells through low-selectivity channels, similar to how Ca enters (WHITE, 1998). This channel (*rca* channel homolog), despite being defined as a Ca channel, is also permeable to a wide variety of cations, including Mg and K (PINEROS AND TESTER, 1995;1997; WHITE, 1998). As such, although the ionic radius of Mg is smaller than that of K, the hydrated radius of Mg is larger, such that K is preferred in terms of the order of root uptake (MAGUIRE AND COWAN, 2002). In addition, K uptake by plant roots is controlled by two capture mechanisms that occur at different external K

concentrations (EPSTEIN et al., 1963). When the external K concentration is high, channels mediate the absorption of this element. At low external K concentrations, its absorption occurs by transporters (integral membrane proteins), both of which are highly selective for K (GRANSEE AND FÜHRS, 2013). Thus, high K concentrations can significantly reduce Mg uptake by roots and its translocation to the shoots when the concentration of Mg is adequate (MARSCHNER, 2012). According to the same authors, the competition among these cations appears be associated with the translocation of these elements to the shoots; no specific competitive effects among the transporters of the radicle membrane were detected (MENGEL AND KIRKBY, 1982; HANNAWAYS, 1982). Hannaways (1982) did not observe an effect of increased K concentration in the nutrient solution on the Mg uptake by roots; however, the Mg accumulation in the shoots of the Kenhy tall fescue (*Festuca arundinacea*) plants was significantly inhibited. Nevertheless, the root area and the absorption mechanism that undergoes antagonistic interference of Mg in the presence of high K concentrations have not yet been fully elucidated (SHABALA AND HARIADI, 2005; KOCH et al., 2018).

### 2.4.2 Carbohydrates

Compared with Mg-adequate plants, Mg-deficient plants presented lower starch concentrations in the young leaves (Figure 2C) at 103, 206 and 309 mg $L^{-1}$ K. However, a significant increase in the accumulation of this carbohydrate in the young leaves of Mg-deficient plants with increasing K levels was detected. In addition, more starch accumulated in the fully expanded leaves of Mg-deficient plants than in those of Mg-adequate plants at all K levels (Figure 2B).

Relatively high sucrose concentrations in the stalks (Figure 2L) and fully expanded leaves (Figure 2J) were detected in Mg-adequate plants. Sugars are synthesized in the Calvin-Benson cycle, and while primarily in the form of sucrose, they are immediately loaded into the phloem for export to sink organs such as roots, young leaves and stalks. Sucrose loading in the phloem is an active process catalyzed by $H^+$/Suc cotransporters and a proton gradient established by $H^+$-ATPases localized in the plasma membrane (BOUCHE'-PILLON et al., 1994, WARD et al., 1998). For proper gradient function, Mg-ATP is essential (IGAMBERDIEV AND KLECZKOWSKI, 2003; BUSH, 1989; GETZ AND KLEIN, 1995). Thus, compared with plants with relatively low

Mg concentrations in their source leaves (fully expanded leaves), those with relatively high Mg concentrations in their source leaves have better-functioning $H^+$/Suc cotransporters and transport more sucrose from their leaves to the roots, young leaves and stalks (Figure 2I, K and L).

One of the key points for the maintenance of the photosynthetic process is the coordination between photoassimilate production and the use of these products for the maintenance of high metabolic and growth-related activity (GRAHAM AND MARTIN, 2000). Thus, coordination of the source-sink relationship of plants is needed. Consumption of a high proportion of carbohydrates results in an increase in sink strength and possibly a signal for photosynthesis, generating positive feedback and thereby stimulating the production of sugars in leaves. In the fully expanded leaves of Mg-adequate plants in the present study (Figure 2B), the reduced starch concentration is possibly related to the increase in sucrose synthesis for export, which consumes triose phosphates, preventing their accumulation in the chloroplasts of the leaves. However, high K levels in Mg-deficient plants reduced the Mg concentrations in the leaves of those plants (Figure 1C), compromising the phloem loading of sucrose, the production of sugars in the leaves (2J), and the accumulation of sucrose in those leaves, favoring starch accumulation in those organs later (Figure 2B).

An accumulation of sucrose in the leaves leads to the deactivation of sucrose phosphate synthase (SPS) enzymes and an increase in fructose 2,6-biphosphate. As a result, triose phosphate is not exported from the chloroplasts to the cytosol, and starch accumulates in the chloroplasts (STITT, 1999). However, in the present study, it is possible that the relatively low Mg concentrations in the leaves (Figure 1C) led to a decrease in or altered $H^+$-ATPase (Mg-ATP) activity in the phloem cells, reducing sucrose export to the stalk (Figure 2L) (ZHAO et al., 2000) and promoting sucrose accumulation in the leaves, which subsequently led to the accumulation of starch in the chloroplasts; these phenomena would justify the abundance of this carbohydrate in the fully expanded leaves of Mg-deficient plants (Figure 2B).

Phloem loading of sucrose can occur via an entirely symplastic pathway, where sucrose flows passively through plasmodesmata without the expenditure of energy, or by the apoplastic pathway, in which sucrose is carried through specific transporters of the plasma membrane (Lambers et al., 2008). In sugarcane, the phloem cells are not connected to the cells of the leaves by plasmodesmata (ROBINSON-BEERS AND

EVERT, 1991), which suggests that the phloem loading in this species is carried out exclusively by the apoplastic pathway and depends on mechanisms that expend energy (RAE et al., 2005). Therefore, any changes in Mg concentration and Mg-ATP function, such as high K levels and reduced Mg uptake by the roots and translocation to the shoots, may drastically impair the continuous function of $H^+$-ATPases and sucrose loading from source leaves into the phloem and sucrose accumulation in the stalks (BUSH, 1989; GETZ AND KLEIN, 1995; IGAMBERDIEV AND KLECZKOWSKI, 2003).

Moreover, a high concentration of K in the stalks (Table 1) may lead to the production of potassium saccharate instead of sucrose, reducing the amount of sugar accumulation in those organs under both Mg conditions (Figure 2L) (RODRIGUES, 1995; FREIRE AND CORTEZ, 2000). Also important is the strong correlation in the literature of high concentrations of K in the stalks and the production of trans-aconitic acid by plants (GUTIERREZ et al., 1988; STUPIELLO et al., 1997). This acid is found in relatively high proportions in grass species (STOUT et al., 1967) and could be used in sugarcane as an indicator of maturation, because as the plants mature, the acid content decreases (CLARKE AND BRANNAN, 1983). Thus, a high K concentration in the stalk (Table 1) may promote increased production of this acid there, thereby reducing the concentration of sugar in that organ (Figure 2L). This phenomenon corroborates the results reported by Gutierrez et al. (1988), where the application of vinasse as a fertilizer caused a significant increase in K concentration in the stalks, which was highly correlated with the high concentration of trans-aconitic acid in the sugarcane juice and with reduced maturity.

### 2.4.3 Root morphological parameters, plant growth and biomass production

Magnesium deficiency drastically compromised the development of the root system of sugarcane (root length and root dry matter), which was aggravated by increased levels of K (Figures 3A and 4A). According to Fischer and Bussler (1988) and Cakmak et al. (1994a), one of the early reactions of plants to Mg deficiency is a decrease in the root/shoot ratio, which is associated with a massive accumulation of carbohydrates, especially sucrose and starch, in the source leaves and reduced translocation of these carbohydrates to the roots.

Cakmak and Kirkby (2008) reported that Mg plays a key role in the phloem loading of photoassimilates. Thus, in the early stage of Mg deficiency, photoassimilate export in the phloem is severely impaired. The same authors stated that this effect seems to be very specific and unrelated to any side effects. Magnesium-deficient plants showed lower sugar, reducing sugar and starch contents in the roots than did Mg-adequate plants (Figure 2A, E and I). The loading of carbohydrates and soluble sugars in the phloem is an active process catalyzed by $H^+$/Suc cotransporters and involves a proton gradient across the plasma membrane of phloem cells. Therefore, it seems likely that a decrease in the Mg concentration in the leaves may promote a decrease in the concentration of Mg-ATP at the phloem loading sites; this phenomenon could be mainly responsible for both the inhibited transport of photoassimilates from Mg-deficient source leaves and the reduced supply of photoassimilates for root development. Thus, the inhibition of phloem loading of photoassimilates from Mg-deficient source leaves is probably responsible for the altered carbohydrate partitioning in those plants (WILLIAMS AND HALL, 1987), whose root development was also reduced (Figures 3A and 4A).

However, observations of the effects of Mg scarcity on the shoot/root ratio vary according to the species and age of plants studied. The first studies reported a severe decrease in the root biomass of spinach (FISHER AND BREMER, 1993) and common bean plants (CAKMAK et al., 1994a, b). Similarly, Silva et al. (2005) reported increased root lengths with increasing Mg supplies in soybean (*Glycine max*. L. Merr.), and Kellermeier et al. (2013) documented a strong reduction in root elongation in K-deficient *Arabidopsis thaliana*. In addition, Mengutay et al. (2013) noted stronger impairment of root growth compared with shoot growth in Mg-deficient wheat (*Triticum aestivum* cv. Adana 99) and maize (*Zea mays* cv. Shemal); similar phenomena were reported by Neuhaus et al. (2014) in faba bean plants (*Vicia faba* L.) and Koch et al. (2018) in potato plants. Nevertheless, in contrast to these findings, studies with *A. thaliana* (HERMANS AND VERBRUGGEN, 2005) and sugar beet (HERMANS et al., 2005) showed controversial results by which Mg deficiency had a greater effect on shoot growth than on root growth and consequently resulted in a decrease in the shoot/root ratio. Explanations for this difference in Mg deficiency in terms of dry matter between the shoots and roots are still unknown, but according to the authors, the cause

may be related to differences in plant species, phenological growth stage evaluated and severity of Mg deficiency.

Moreover, the impact of Mg deficiency on assimilate partitioning can strongly influence the ability of plants to deal with other unfavorable environmental conditions, such as water deficit and low availability of other nutrients. In the field, Mg-deficient plants presenting relatively high shoot/root ratios would be less resilient to a decrease in water or nutrient availability in the soil, since there would be greater demand of the shoots for water and nutrients per unit root length (DELL AND HUANG, 1997).

In the present study, the development of Mg-deficient plants was compromised, with relatively low plant height (Figure 3B), stalk dry matter, shoot dry matter and total dry matter (Figure 4B, C and D), especially at 412 mg $L^{-1}$ K. The highest level of K also compromised the development of plants supplied with proper amounts of Mg, such that these plants and the Mg-deficient plants grew similarly.

During the initial vegetative stages of sugarcane, the production of new cells represents the main demand for carbohydrates essential for root expansion, stalks (nodes and internodes) and leaves. During this period, coordination of photoassimilate production and the use of these products for plant metabolism and growth are necessary (GRAHAM AND MARTIN, 2000). Plants regulate their use of sucrose via specific enzymes as needed. During the growth period, sucrose breakdown occurs primarily by invertase and sucrose synthase (SuSy) (SALERNO AND CURATTI, 2003; KOCH, 2004). The performance of these enzymes is important for breaking down the sucrose molecule into fast-use simple monosaccharides, which are required for the formation and maintenance of the cell wall and membrane, allowing the elongation of internodes. In the Mg-deficient plants in the present study, the massive accumulation of carbohydrates in the fully expanded leaves is believed to have occurred because of a reduction in the fixation of $CO_2$ during photosynthesis, resulting in changes in photosynthetic carbon metabolism, restriction of $CO_2$ fixation and reductions in both the electron transport chain and the photosynthetic rate (CAKMAK AND KIRKBY, 2008). Thus, the electrons and excitation energy not used in photosynthetic $CO_2$ fixation are channeled to molecular $O_2$, which generates highly ROS that damage chloroplast constituents such as chlorophyll molecules and membrane lipids (MITTLER, 2002; ASADA, 2006; CAKMAK AND KIRKBY, 2008; MENGUTAY et al., 2013).

Similarly, any alterations to the photosynthetic activity and carbohydrate flow from source leaves to the phloem can increase the amount of carbohydrates in the leaves, drastically compromising the activity of these enzymes (invertase and SuSy) and reducing the usage of monosaccharides for the development of these plants, which would lead to impaired plant height, stalk dry matter, shoot dry matter and, consequently, total dry matter (Figures 3B and 4B, C and D).

### 2.4.4 Rubisco and antioxidant enzymes activity

Both fully expanded leaves and young leaves presented increased SOD activity as increased K levels (Table 3). Similarly, fully expanded leaves of Mg deficient plants showed greater activity of this enzyme. Cakmak and Marschner (1992) also reported that Mg-deficient primary leaves of *Phaseolus vulgaris* had increased SOD activity. This result corroborates those found by Riga et al. (2005) in pepper, Tewari et al. (2006) in mulberry plants and by Ding et al. (2008) in rice, suggesting that SOD activity seems to be an important mechanism to avoid oxidative stress caused by Mg deficiency.

Magnesium deficiency also negatively influenced APX activity in fully expanded leaves, however, its effect was not pronounced in young leaves. In addition, both K deficiency (103 mg $L^{-1}$) and the highest levels of this element (309 and 412 mg $L^{-1}$) increased APX activity (Table 3).

According to Marschner (2012), the reduction in the photosynthetic $CO_2$ fixation and in the transport and use of photoassimilates can result in excess of electrons and unused energy, leading to the generation of reactive oxygen species (ROS). Such effects can be attributed either to the deficiency of K and Mg, or to the Mg deficiency caused by K excess. In Mg-deficient plants, antioxidant defense enzyme activities levels including SOD, APX, CAT and GR are increased to mitigate oxidative damage in cells. In Mg scarcity, photosynthetic carbon fixation is compromised (attributed to the lower Rubisco activity) (FISCHER AND BREMER, 1993; HERMANS et al., 2004), as a result of carbohydrate accumulation in fully expanded leaves (Figure 2) and interrupted phloem loading of carbohydrates (CAKMAK et al., 1994b; HERMANS et al., 2005). Thereby, limited conversion of absorbed light energy to chemical energy (ATP) and unused excitation energy can be transferred to $O_2$, resulting in highly toxic singlet oxygen ($^1O_2$) generation in the PSII reaction center (ASADA, 2006). Thus, the

antioxidant efforts of these defense enzymes under Mg deficiency are justified (FOYER AND NOCTOR, 2003).

Catalase activity was reduced in both fully expanded leaves and young leaves as increased K level. Moreover, both leaves had higher CAT activity in Mg deficient plants. The reduction in CAT activity at higher K level can be attributed to severe destruction of the antioxidant system in plants and, thus, plants lose adequate stress responses (DING et al., 2008).

Glutathione reductase activity was not influenced by Mg nutrition in young leaves, however, the increase in K levels enhanced this enzyme activity. Regarding the fully expanded leaves, Mg-adequate plants had greater GR activity compared to leaves of Mg-deficient plants. By contrast, increased GR activity in leaves of Mg-deficient plants was found in bean (CAKMAK AND MARSCHNER, 1992), in *Mentha pulegium* (CANDAN AND TARHAN, 2003), in pepper (RIGA et al., 2005) and in mulberry plants (TEWARI et al., 2006). Differences in responses can be attributed to the age that the plants were evaluated. In general, in the mentioned studies, the glutathione reductase was determined in the early stages of plant development. For sugarcane plants, after 110 days under Mg deficiency the lack of GR response can be attributed also to severe destruction of the antioxidant system in plants and, thus, plants lose adequate stress responses (DING et al., 2008).

Both in fully expanded and young leaves, Mg deficiency drastically influenced Rubisco activity, decreasing approximately 50% of its activity. Magnesium is known to be crucial in protein synthesis (DING et al., 2008; FAUST AND SCHUBERT, 2016; MENGUTAY et al., 2013), thus, any change in optimal Mg concentration can significantly affect protein synthesis and Rubisco activity. Low protein concentrations due to Mg deficiency may be caused to Mg involvement in stacking and maintenance of ribosomal integrity (YAMAMOTO et al., 2010; PETROV et al., 2012). Peng et al. (2015), by proteomic analysis, showed that Rubisco protein abundance decreased under Mg deficiency in *Citrus sinensis*, indicating that Rubisco amount and activity were affected, which coincided with 88% reduction in $CO_2$ assimilation rates in Mg-deficient leaves.

Regarding K level, both the lowest K level (103 mg $L^{-1}$) and the highest levels (309 and 412 mg $L^{-1}$) negatively influenced rubisco activity. An adequate K concentration in chloroplasts induces a broader pH range optimal for Rubisco functioning (which should be close to 8, since, the binding of Mg to the enzyme

increases its affinity for $CO_2$) (SUGIYAMA et al., 1968; MARSCHNER, 1995; TAIZ AND ZAIGER, 2004; MENGEL AND KIRKBY, 2001). In addtion, negative regulation of Rubisco activity in K-deficient leaves may occur due to low $CO_2$ fixation (JÁKLI et al., 2017). Similarly to Mg deficiency, reduced phloem export rates due to K deficiency result in high sucrose concentrations in cell cytosol (CAKMAK et al., 1994a) and decrease in electron transport in PSs (EREL et al., 2015), which may cause Rubisco activity inhibition (GOLDSCHMIDT AND HUBER, 1992).

The maximum electron transfer rate is associated with RuPP regeneration that consumes ATP and NADPH (FARQUHAR et al., 1980), thus, the lower the electron transfer, the less is the number of receptor molecules for $CO_2$ fixation. In addition, there is no evidence that K is involved in Rubisco activating, but this element indirectly regulates Rubisco activity affecting its optimal pH and the amount of substrate ($CO_2$ and possibly RuBP) (TRÄNKNER et al., 2018). Regarding the highest K levels, rubisco activity must have been decreased by the lower Mg concentration found in the leaves.

## 2.5 CONCLUSION

Regardless Mg nutrition, a high K level reduces Mg concentration in the roots in the roots, fully expanded leaves, young leaves and stalks. Deficient and Adequate Mg nutrition seems to be uptake by two different capture mechanisms.

Compared with plants that grown under moderate K and Mg conditions, the root length of Mg-deficient plants was 40% lower. However, regardless of Mg nutrition, the highest K level compromised all of the root and shoot growth parameters analyzed.

Adequate Mg nutrition provide plants with 50% more sugar, reducing sugars and starch in the stalk compared to Mg-deficient plants. However, all of which decreased at the highest K level.

Magnesium deficient leaves presented much lower Rubisco activity with higher antioxidant enzymes activity such as SOD, APX and CAT, which effects were more pronounced in fully expanded leaves.

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# CHAPTER 3 - MAGNESIUM FOLIAR FERTILIZATION EFFICIENCY IN SUGARCANE PLANTS GROWN IN VARIED POTASSIUM AND MAGNESIUM NUTRITION

## 3.1 INTRODUCTION

Magnesium ($Mg^{2+}$) is the most abundant divalent cation in the cytosol of plant cells and plays an essential role in many important processes inside plants (LI et al., 2001). Its most known physiological functions are: as the central atom of the chlorophyll molecule, essential in harvesting solar energy (BEALE, 1999), in the thylakoid grana stacking (KAFTAN et al., 2002), and in the activation of enzymes in metabolic biochemistry, including carboxylases, phosphatases, kinases, RNA polymerases and ATPases (MAGUIRE; COWAN, 2002).

In sugarcane, where the stalk is the organ of agronomic interest, $Mg^{2+}$ becomes even more important, since it is an essential component in the photoassimilate partitioning between source and sink organs. Magnesium seems to be closely linked to the phloem loading of sucrose. Its deficiency affects the proton-motive force generated by $H^+$/ATPase, which requires Mg-ATP for its operation (MAGUIRE; COWAN 2002, HERMANS et al. 2005), the translocation of photoassimilates to the sink organs, and thus, in the case of sugarcane, it may affect the amount of sucrose in the stalks and consequently the final commercial yield (GERENDÁS; FÜHRS, 2013).

Magnesium-deficiency in plants may be caused by soil reserves depletion, but also can be induced by low $Mg^{2+}$ assimilation by the roots due to competitive inhibition caused by imbalance of some cations such as calcium ($Ca^{2+}$) (MENGEL; KIRKBY, 2001; SHAUL, 2002) and $K^+$, especially in sugarcane fields where vinasse is frequently used.

The antagonism effect of $K^+$ and $Mg^{2+}$ is well documented for some crops, such as barley (JAKOBSEN, 1993), maize (MEURER; FONSECA, 1997), common bean (TUMA et al., 2004), rice (DING et al., 2008), safflower (FARHAT at al 2013) and tomato (LI et al, 2018). However, this phenomenon has not been widely recognized in sugarcane, since no articles was published for this crop describing the impact of low $Mg^{2+}$ and high $K^+$ supplies on the plants metabolism.

A possible transport system of high and low affinity also for $Mg^{2+}$ has recently been demonstrated (OGURA et al., 2018), besides reports regarding the synergistic effect between $K^+$ and $Mg^{2+}$, where Ding et al. (2006) showed a synergistic mechanism

between $K^+$ uptake and translocation in rice and subsequent raise in $Mg^{2+}$ supply. Therefore, the mode of interaction between $K^+$ and $Mg^{2+}$ in plants remains uncertain, making the current efforts in understanding the interactions between these two cations indispensable.

Thereby, magnesium foliar application (MgF) may represent an excellent fertilization strategy to improve $Mg^{2+}$ concentration in sugarcane leaves from vinasse areas, where $K^+$ and $Mg^{2+}$ antagonism can occur, and thus improve crop yield or avoid quality losses. According to Römheld and Kirkby (2007), the MgF would be effective during the reproductive stages by reducing the effect of $K^+$ antagonism on $Mg^{2+}$ uptake at this moment when the transport of assimilates to the harvested organs is raised.

Improvements on the nutritional status or quality parameters were reported with the MgF in apples and carrots. (WEBSTER; CROWE, 1968; REAY et al., 1998; WSZELACZYNSKA; POBEREZNY, 2011), sugar beet (BARLOG; GRZEBISZ, 2001), soybean (VRATARIC et al., 2006; TEKLIC et al., 2009), and oregano (DORDAS, 2009). In contrast, the effect of MgF was less apparent or not evident on sugar beet (KRISTEK et al., 2000) and soybean (REINBOTT; BLEVINS, 1995). Althoutgh these differences are not yet known, it can attributed to the different experimental conditions or the solution concentration foliar sprayed. Gerendás and Führs (2013) conducted a comprehensive review of the $Mg^{2+}$ impact on crop yield and quality parameters and indicate that there is still a limited number of studies available.

Therefore, the present study has the following hypothesis: the high $K^+$ level reduces the $Mg^{2+}$ uptake by the root and decreases its translocation from the root to shoot; the high $K^+$ level causes changes in the root morphological parameters (length, and diameter) as well as the partition of carbohydrates deposited in the root and shoot because of the $Mg^{2+}$ uptake impairment by the roots; The high $K^+$ level can reduce the Chl *a*, *b*, and carotenoids content in the leaves due to lower $Mg^{2+}$ availability and cause starch and soluble sugars accumulation in the leaves, due to the impaired discharging of these photoassimilates in the phloem, and consequently, lower deposition in the shoots; Magnesium foliar application mitigates the effects from high $K^+$ levels in the $Mg^{2+}$ uptake by the root and its use by the plants.

Thereby, we aimed to evaluate the effect the $Mg^{2+}$ foliar application on the root and shoot development, as well as on the possible nutritional alterations and in the photoassimilation production in sugarcane. This is the first study presenting the

efficiency of $Mg^{2+}$ foliar application in respect to varied $K^+$ and $Mg^{2+}$ nutrition in this culture.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Experimental design

This experiment was conducted in a greenhouse that had a climate-controlled environment. The greenhouse had an internal heating/cooling air circulatory system to maintain the temperature between 22 °C and 32 °C.

The experimental design was completely randomized, composed of the following treatments: 1), 206 mg $L^{-1}$ of $K^+$ and 2 mg $L^{-1}$ of $Mg^{2+}$ (K-Mg), 2) 412 mg $L^{-1}$ of $K^+$ and 2 mg $L^{-1}$ of $Mg^{2+}$ (KH-Mg), 3) 206 mg $L^{-1}$ of $K^+$ and 17 mg $L^{-1}$ of $Mg^{2+}$ (KM+Mg), 4) 412 mg $L^{-1}$ of $K^+$ and 17 mg $L^{-1}$ of $Mg^{2+}$ (KH+Mg), without and with Mg foliar application, with 4 replicates. The concentrations used were considered as 206 and 412 mg L-1 for K-moderado (KM) and K-high (KH) and 2 and 17 mg L-1 for Mg-deficient (-Mg) and Mg-moderado (+Mg), respectively, which were determinate based on previous experiments. The variety used was RB867515.

### 3.2.2 Seedlings obtainment and trial set-up

The cane cuttings for seedling preparation were harvested from a commercial nursery area. Cane cuttings that were approximately 3 cm in length and that contained one vegetative bud were obtained. The buds were extracted from the upper third of each cane cutting so that improved sprouting uniformity was achieved.

Afterwards, for germination, the cane cuttings were placed in plastic trays that contained course sand and were irrigated every three days with deionized water. The trays were placed on benches inside the greenhouse, whose air humidity was controlled. At 20 days after emergence, seedlings were selected according to their sprouting uniformity and then were transplanted into the nutrient solutions.

The selected seedlings were fixed in styrofoam plates that were subsequently transferred to plastic vases so that only the root system was in contact with the nutrient solution. Each vase received four sugarcane seedlings and was properly equipped so that four liters of nutrient solution was constantly aerated. After 60 days, the plants were transferred to larger vases that held 14 liters of nutrient solution.

### 3.2.3 Nutrient solution

The nutrient solution (Table 1a and 1b) was diluted to one-third ionic strength during the first week and half ionic strength during the second week, after which it was replaced with a full-ionic-strength solution during the third week to avoid interference of any possible saline effects of the nutrient solution on the initial growth of the seedling roots.

The maximum variation of the solution volume was 5%, with water lost by evapotranspiration replaced with deionized water. The pH was monitored daily and maintained within a range of 5.5 to 6.0, when necessary, 0.1 M HCl or 0.1 M NaOH solution was used for adjustments. The nutrient solution was exchanged weekly.

The evaluations were conducted 110 days after the plants were transplanted into the nutrient solution. Thus, the following were evaluated:

Table 1a – Composition of the nutrient solution of Furlani and Furlani (1988), modified.

| | | **Deficient Mg level** | | | |
|---|---|---|---|---|---|
| | Stock Solution Components | Concentration | Relation Stock Solution/ Nutrient Solution | Nutrient solution Concentration | |
| Nº | | g $L^{-1}$ | ml $L^{-1}$ | Nutrient | mg $L^{-1}$ |
| 1 | $Ca(NO_3)_2.4H_2O$ | 350 | 3 | $N\text{-}NO_3^-$ | 156 |
| | $NH_4NO_3$ | 31.53 | 3 | $N\text{-}NH_4^+$ | 31 |
| | $Na_2SO_4$ | 124.64 | 1 | Ca | 178 |
| 2 | $K_2SO_4$ | 45.9 | 3 | S | 56 |
| | $KNO_3$ | 35.76 | 3 | P | 16 |
| 3 | $MgSO_4.7H_2O$ | 20.54 | 1 | Fe | 3.6 |
| 4 | $(NH_4)_2HPO_4$ | 68 | 1 | B | 0.27 |
| 5 | Rexolim® | 55.4 | 1 | Mn | 0.5 |
| 6 | $H_3BO_3$ | 2.0 | 1 | Zn | 0.15 |
| | $MnCl_2.4H_2O$ | 1.75 | | Cu | 0.05 |
| | $ZnSO_4.7H_2O$ | 0.66 | | Mo | 0.09 |
| | $CuSO_4.5H_2O$ | 0.20 | | Mg | 2 |
| | Components | g $L^{-1}$ | | K | mg $L^{-1}$ |
| | KCl | 65.56 | 3 | Moderate | 206 |
| | | 196.76 | 3 | High | 412 |

Table 1b – Composition of the nutrient solution of Furlani and Furlani (1988), modified.

| Moderate Mg level | | | | | |
|---|---|---|---|---|---|
| | Stock Solution Components | Concentration | Relation Stock Solution/ Nutrient Solution | Nutrient solution Concentration | |
| Nº | | g $L^{-1}$ | ml $L^{-1}$ | Nutrient | mg $L^{-1}$ |
| 1 | $Ca(NO_3)_2.4H_2O$ | 350 | 3 | $N\text{-}NO_3^-$ | 156 |
| | $NH_4NO_3$ | 31.53 | 3 | $N\text{-}NH_4^+$ | 31 |
| | $NaNO_3$ | 42.80 | 1 | Ca | 178 |
| 2 | $K_2SO_4$ | 60.5 | 3 | S | 56 |
| | $KNO_3$ | 18.79 | 3 | P | 16 |
| 3 | $MgSO_4.7H_2O$ | 174.6 | 1 | Fe | 3.6 |
| 4 | $(NH_4)_2HPO_4$ | 68 | 1 | B | 0.27 |
| 5 | Rexolim® | 55.4 | 1 | Mn | 0.5 |
| 6 | $H_3BO_3$ | 2.0 | 1 | Zn | 0.15 |
| | $MnCl_2.4H_2O$ | 1.75 | | Cu | 0.05 |
| | $ZnSO_4.7H_2O$ | 0.66 | | Mo | 0.09 |
| | $CuSO_4.5H_2O$ | 0.20 | | Mg | 17 |
| | Components | g $L^{-1}$ | | K | mg $L^{-1}$ |
| | KCl | 67.5 | 3 | Moderate | 206 |
| | | 196.76 | 3 | High | 412 |

### 3.2.4 Foliar application

The MgF was conducted 100 days after transplanting to the nutrient solution, at 7:00 am, using a $CO_2$-pressurized backpack sprayer (Herbicat, Catanduva, SP, Brazil), equipped with four Jacto AVI 11002 nozzles (Jacto, Pompeia, Brazil) spaced at 0.5 m. Due to the plants height at the time of spraying, the application was conducted by holding the 2-meter long spray bar at shoulder height at an angle of 60º and spray nozzle at an angle of 30º, so that the spray was performed at 90º from the target and at a fixed distance of 0.5 m from the leaves. The Mg fertilizer was sprayed at a volume rate of 200 L $ha^{-1}$ and operating pressure of 60 psi, producing coarse droplets. The product's dose used was 400 g of Mg $ha^{-1}$ (product composed by 8% of Mg and 5% of nitrogen). Distilled water was used in the spray mixture.

In order to isolate the $Mg^{2+}$ factor, urea was sprayed in the treatments that did not receive the product with $Mg^{2+}$ in the same nitrogen amount found in the product used.

The evaluations were conducted at 10 days after the foliar application. Thereby, it was evaluated using the follows:

### 3.2.5 Growth parameters

To measure plant height, a graduated ruler was used. The measurements were made from the root-shoot junction to the auricular region of leaf +1 or to top visible dewlap (TVD) leaf, according to the numbering suggested by Kuijper (DILLEWIJN, 1952). The stalk diameter was measured with the aid of a digital caliper in the middle third of the stalk. The internode number was also counted.

### 3.2.6 Leaf chlorophyll content

To determine the amount of photosynthetic pigments (Chl *a*, Chl *b* and carotenoids) present in the leaf tissues, three discs were cut between the edge and the leaf central vein using a paper punch (0.5 cm in diameter) from the leaf +1 (fresh plant material) and the leaf +5 (the last leaf that was not at the senescence stage), which were considered a young and fully expanded leaf of the plant, respectively. The discs were kept for 24 hours in 12 mL glass vials (with lids) that contained 2 mL of N,N-dimethylformamide (DMF) and that were covered with aluminum foil, as proposed by Lichtenthaler (1987).

The absorbance of Chl *a*, Chl *b* and carotenoids was determined by a spectrophotometer at wavelengths of 664, 647 and 480 nm, respectively. The absorbance readings were determined with 1 mL of the extract and 1 mL of deionized water; a mixture of 1 mL of DMF solution plus 1 mL of deionized water was used as a “blank”. The calculations for the pigment determinations were in accordance the methods of Wellburn (1994).

### 3.2.7 Root morphological parameters

The plants were divided into roots, stalks and leaves. Three subsamples of the roots of each experimental unit were subsequently cut lengthwise, i.e., from the root-shoot junction to the end of the root system, to obtain the different types of roots. Afterwards, the samples were stored in a 100 mL universal collector that contained an alcohol solution (30%) and that was conditioned in a refrigerated environment (2 °C) for further analysis (TENNANT, 1975). The length and mean root diameter were

measured by a digitizer coupled to a computer with the WinRhizo software version 3.8-b (Regent Instruments Inc., Quebec, Canadá), as described by Tennant (1975).

After these variables were determined, the samples used were dried in a forced-air oven at 65 °C until they reached constant weight to determine their dry matter. Using the dry matter data obtained from the samples and from the remaining root system, the length of the entire plant root system was estimated (NEWMAN, 1966).

### 3.2.8 Dry matter production of plant parts

The different parts of the plants from each experimental unit were conditioned in paper bags and subsequently subjected to drying in a forced-air oven at 65 °C until they reached a constant weight, after which they weighed to determine the root dry matter, stalk dry matter, young leaves dry matter and fully expanded leaves dry matter.

### 3.2.9 Determination of the nutritional composition of the plant parts

The DM of the plant parts was ground in a Willey type mill with a 1 mm screen, and subsequently, the $K^+$ and $Mg^{2+}$ concentrations were determined (AOAC, 1995).

### 3.2.10 Carbohydrates

The partitioning of carbohydrates between the plant parts was determined by measuring the reducing sugars, sucrose and starch concentrations in the dry matter according to the methods of Somogyi-Nelson (SOMOGYI, 1952; NELSON, 1994). The readings were performed by a spectrophotometer at 535 nm.

### 3.2.11 Statistical analysis

The data were subjected to ANOVA. The means were separated by the least significant difference (LSD) test ($p \leq 0.05$). The statistical analysis was performed by the statistical program Sisvar (FERREIRA, 2011), and the figures were made using SigmaPlot® 12.5 software.

## 3.3 RESULTS AND DISCUSSION

There was no significant difference on the nitrogen (N), phosphorus (P), calcium (Ca) and sulfur (S) concentration due to Mg foliar application (MgF) or interaction between the treatments for the plant parts analyzed. Therefore, data will be not shown.

The treatments influenced $K^+$ concentration in the fully expanded leaves, stalk and root, as well as $Mg^{2+}$ concentration in fully expanded leaves (Table 2). Potassium and $Mg^{2+}$ concentration in young leaves and $Mg^{2+}$ concentration in the stalks and roots were significantly affected by the interaction of the factors.

Potassium concentration was significantly increased in plants supplied with KH, regardless the $Mg^{2+}$ level (KH+Mg and KH-Mg) in the fully expanded leaves, stalk and roots, when compared to KM+Mg and KM-Mg plants (Table 2). Under $K^+$ moderate condition, the low $Mg^{2+}$ supply (KM-Mg) provided higher $K^+$ concentration in theses organs compared to plants moderately supplied with $Mg^{2+}$ (KM+Mg). In the fully expanded leaves, plants fed with moderate K and Mg supplies (KM+Mg) showed the highest significant $Mg^{2+}$ concentration, whereas plants grown under low $Mg^{2+}$ concentration, on both levels of $K^+$ supply, showed the lowest values (KM-Mg and KH-Mg).

Table 2 – Concentration of potassium (K) and magnesium (Mg) in the young, fully expanded leaves, stalk and root of sugarcane plants submitted to potassium and magnesium treatment in the nutrient solution for 110 days and magnesium foliar application (MgF).

| | Young leaves | | Fully expanded leaves | | Stalk | | Root | |
|---|---|---|---|---|---|---|---|---|
| Factors | K | Mg | K | Mg | K | Mg | K | Mg |
| Treatments (T) | (g $kg^{-1}$) | | | | | | | |
| KM-Mg | 12.3b | 0.8c | 12.0b | 0.7c | 22.8b | 0.7c | 17.6b | 0.1c |
| KH-Mg | 13.3a | 0.8c | 13.0a | 0.7c | 24.4a | 0.5c | 20.6a | 0.7d |
| KM+Mg | 11.5c | 1.8a | 10.5c | 2.0a | 21.1c | 1.6a | 14.0c | 3.3a |
| KH+Mg | 13.0a | 1.3b | 13.2a | 1.4b | 25.1a | 1.0b | 19.5a | 2.1b |
| Mg Foliar (MgF) | | | | | | | | |
| Without | 12.3a | 1.1b | 12.0a | 1.1a | 22.6a | 0.9b | 17.7a | 1.8a |
| With | 12.7a | 1.3a | 12.5a | 1.2a | 23.6a | 1.0a | 18.1a | 1.7a |
| ANOVA *(F Probability)* | | | | | | | | |
| T | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| MgF | 0.1377 | <0.001 | 0.1518 | 0.1993 | 0.9757 | 0.2423 | 0.3700 | 0.1183 |
| T x MgF | <0.001 | 0.0161 | 0.4253 | 0.5186 | 0.0873 | <0.001 | 0.1751 | <0.001 |

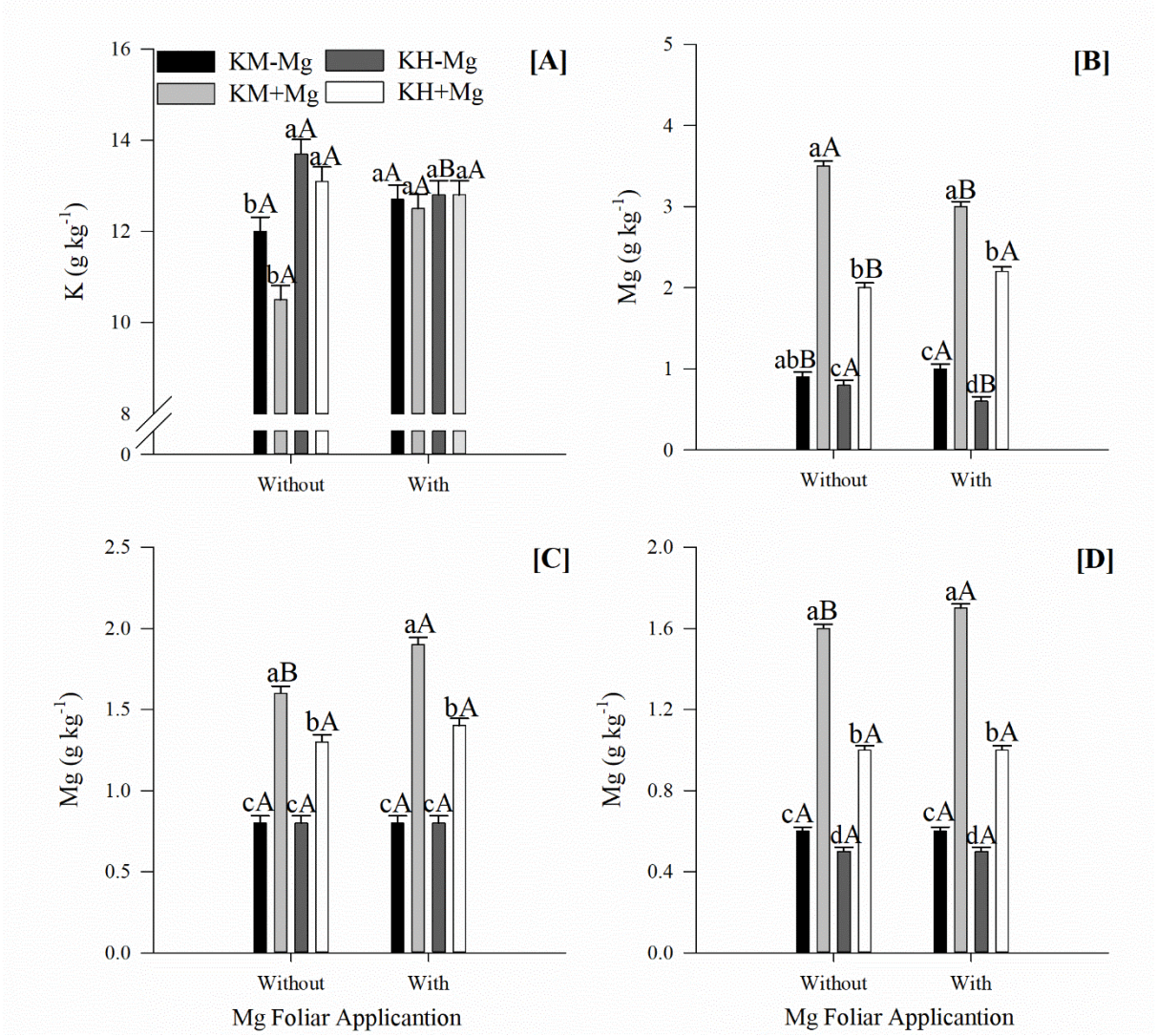
Means followed by the same letter in the column do not differ by the LSD Test at 5% probability. KM-Mg: 206 mg $L^{-1}$ of $K^+$ and 2 mg $L^{-1}$ of $Mg^{2+}$, KH-Mg: 412 mg $L^{-1}$ of $K^+$ and 2 mg $L^{-1}$ of $Mg^{2+}$, KM+Mg: 206 mg $L^{-1}$ of $K^+$ and 17 mg $L^{-1}$ of $Mg^{2+}$, KH+Mg: 412 mg $L^{-1}$ of $K^+$ and 17 mg $L^{-1}$ of $Mg^{2+}$.

Plants treated with KM-Mg and KM+Mg plants (Figure 1A) showed the lowest significant $K^+$ concentrations compared to young leaves of plants fed with high $K^+$ supplies (KH-Mg and KH+Mg). On the other hand, plants that received MgF did not differ between the treatments on the $K^+$ concentration in the young leaves. Despite this, KH-Mg plants without MgF presented the highest value compared to those that received foliar spray. Oppositely, KH-Mg plants exhibited the lowest $K^+$ concentration in young leaves without MgF.

The highest $Mg^{2+}$ concentration in the young leaves, stalk and root (figure 1B, C and D, respectively) were obtained in the KM+Mg plants without and with MgF. However, although the $Mg^{2+}$ concentration in the young leaves was greater in these plants without MgF, an inverse pattern was obtained for its concentration in the stalk and root (KM+Mg and MgF). Moreover, the lowest $Mg^{2+}$ concentration in young leaves, stalk and root were shown in plants grown under KM-Mg and KH-Mg, regardless the MgF.

The MgF was not effective in correcting Mg-deficiency in plants grown under low supply of this nutrient, in none of the analyzed parts (KM-Mg and KH-Mg) (Table 2 and Figure 1B, C and D). This result may have occurred due to the low $Mg^{2+}$ concentration applied, since these plants were under severe deficiency of this nutrient over a long period of time (100 days). Plants moderately-nourished with $Mg^{2+}$ and under high $K^+$ level had $Mg^{2+}$ concentration improvements in the young leaves after the MgF (Figure 1B).

Figure 1 – Interaction effect in the potassium (K) concentration in the young leaves [A] and magnesium (Mg) concentration in the young leaves [D], stalk [E] and root [F] of sugarcane plants submitted to potassium (206 and 412 mg $L^{-1}$) and magnesium levels (2 and 17 mg $L^{-1}$) in the nutrient solution for 110 days and Mg foliar application.




Means followed by the same uppercase letters are not significantly different and compare MgF. Means followed by the same lowercase letters compare each treatment, according to the LSD test ($p \leq 0.05$). Error bars express the standard error of the mean (n = 4).

The low supply of $Mg^{2+}$ in the nutrient solution (KM-Mg) provided a similar concentration of $Mg^{2+}$ in the different plant parts, even with increased $K^{+}$ concentration (KH-Mg) and independently of the MgF. It seems likely that the $Mg^{2+}$ uptake by the roots involves a high transport system (OGURA et al., 2018). These authors showed that the roots of Arabidopsis plants were able to absorb $^{28}Mg$ even under very low $Mg^{2+}$ supply condition, revealing a high affinity uptake system. In addition, at environmental

temperature of 4º C, the $^{28}Mg$ uptake rate did not increase, discarding diffusion influence in this element absorption.

Reducing sugars, sucrose and starch in young and fully expanded leaves, stalk and roots were significantly affected by the interaction of the factors (Table 3).

The reducing sugars concentration in young and fully expanded leaves was the greatest in KM+Mg plants without MgF (Figure 2A and 3A). For KH+Mg plants, the use of MgF improved reducing sugars concentration in young leaves in 10%. However, except for KH+Mg plants, the MgF decreased reducing sugars concentration in fully expanded leaves in all treatments.

Sucrose and starch concentration in young leaves were greatly increased in KM+Mg plants with MgF (Figure 2B and C). In addition, the MgF improved sucrose concentration in young leaves by 73, 11, 4.5 and 41% in KM-Mg, KM+Mg, KH-Mg and KH+Mg, respectively (Figure 2B). By contrast, starch concentration in young leaves were decreased in the treatments KM-Mg, KH-Mg and KH+Mg using the MgF. In fully expanded leaves, greater sucrose concentration was exhibited by the plants with MgF, although KM+Mg plants did not differ from those without foliar spraying (Figure 3B). Except for KH+Mg, starch concentration was massively decreased with the MgF, especially in KM-Mg and KH-Mg plants (Figure 3C).

Although the highest reducing sugars concentration in the stalk was obtained in KM+Mg plants, the use of the MgF significantly decreased its concentration in these plants (Figure 4A). Despite this, it increased the concentration of reducing sugars in KH+Mg plants. The highest sucrose concentration in the stalk was obtained in KM+Mg plants with MgF (Figure 4B), while the highest starch value was observed in KH-Mg plants without MgF (Figure 4C). In all the treatments, the MgF drastically decreased the starch in the stalk.

Magnesium foliar application significantly decreased the reducing sugars concentration in the root of KH+Mg plants (Figure 5A). Oppositely, in KM-Mg plants, the use of MgF increased its concentration in the root by 39%. The same pattern was observed for KM+Mg plants with MgF in sucrose concentration, increasing 58% (Figure 5B) and obtaining the highest value in this regard. The highest starch concentration in the root was detected in KH-Mg plants without MgF and in KM+Mg plants with MgF (Figure 5C).

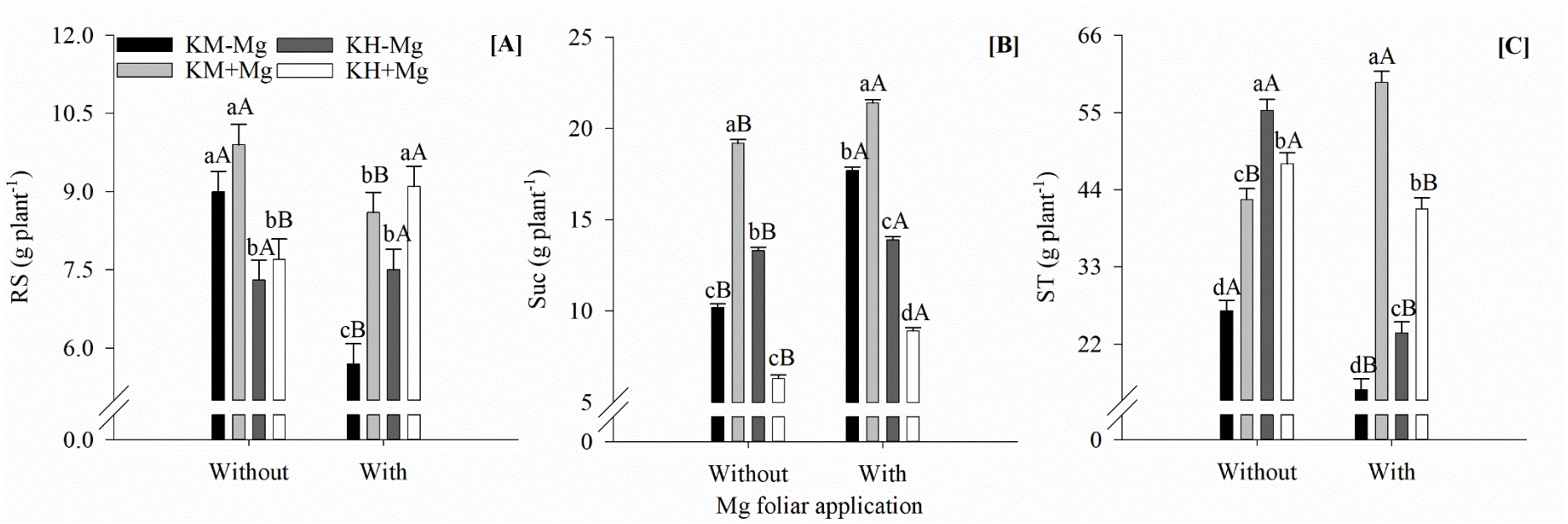
Table 3 – Concentration of reducing sugars (RS), sucrose (Suc) and starch (ST) in the young, fully expanded leaves and root, and concentration of reducing sugars (RS), sucrose (Suc), starch (St) and fiber (Fb) in the stalk of sugarcane plants submitted to potassium and magnesium treatment in the nutrient solution for 110 days and magnesium foliar application

| | Young Leaves | | | Fully expanded leaves | | |
|---|---|---|---|---|---|---|
| Factors | RS | Suc | ST | RS | Suc | St |
| Treatments (T) | | | (g kg $^{-1}$) | | | |
| KM-Mg | 7.4c | 14.0b | 21.0d | 13.2b | 40.2ab | 14.5c |
| KH-Mg | 7.4c | 13.6b | 39.5c | 14.3a | 29.5c | 15.9b |
| KM+Mg | 9.2a | 20.3a | 51.0a | 14.4a | 42.4a | 7.63d |
| KH+Mg | 8.4b | 7.6c | 44.8b | 8.9c | 37.4b | 22.0a |
| Mg Foliar (MgF) | | | | | | |
| Without | 8.5a | 12.2b | 43.0a | 15.6a | 34.3b | 16.2a |
| With | 7.7b | 15.5a | 35.1b | 9.7b | 40.5a | 13.8b |
| ANOVA *(F Probability)* | | | | | | |
| T | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| MgF | 0.0113 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| T x MgF | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

| | Stalk | | | | Root | | |
|---|---|---|---|---|---|---|---|
| Factors | RS | Suc | ST | Fb | RS | Suc | ST |
| Treatments (T) | | (g kg $^{-1}$) | | (%) | | (g kg $^{-1}$) | |
| KM-Mg | 12.6d | 101d | 26.9d | 307a | 4.4d | 9.5b | 106c |
| KH-Mg | 14.5c | 134c | 98.3a | 288 | 5.5b | 7.6c | 152a |
| KM+Mg | 17.0b | 179a | 54.2b | 287 | 5.0c | 11.0a | 149a |
| KH+Mg | 18.0a | 150b | 47.3c | 275 | 6.9a | 6.2d | 121b |
| Mg Foliar (MgF) | | | | | | | |
| Without | 15.8a | 136b | 70.7a | 290a | 5.6a | 8.5a | 130b |
| With | 15.2a | 146a | 42.6b | 289a | 5.2b | 8.7a | 134a |
| ANOVA *(F Probability)* | | | | | | | |
| T | <0.001 | <0.001 | <0.001 | 0.0029 | <0.001 | <0.001 | <0.001 |
| MgF | 0.3031 | <0.001 | <0.001 | 0.8411 | <0.001 | 0.1114 | <0.001 |
| T x MgF | <0.001 | <0.001 | <0.001 | 0.4814 | <0.001 | <0.001 | <0.001 |

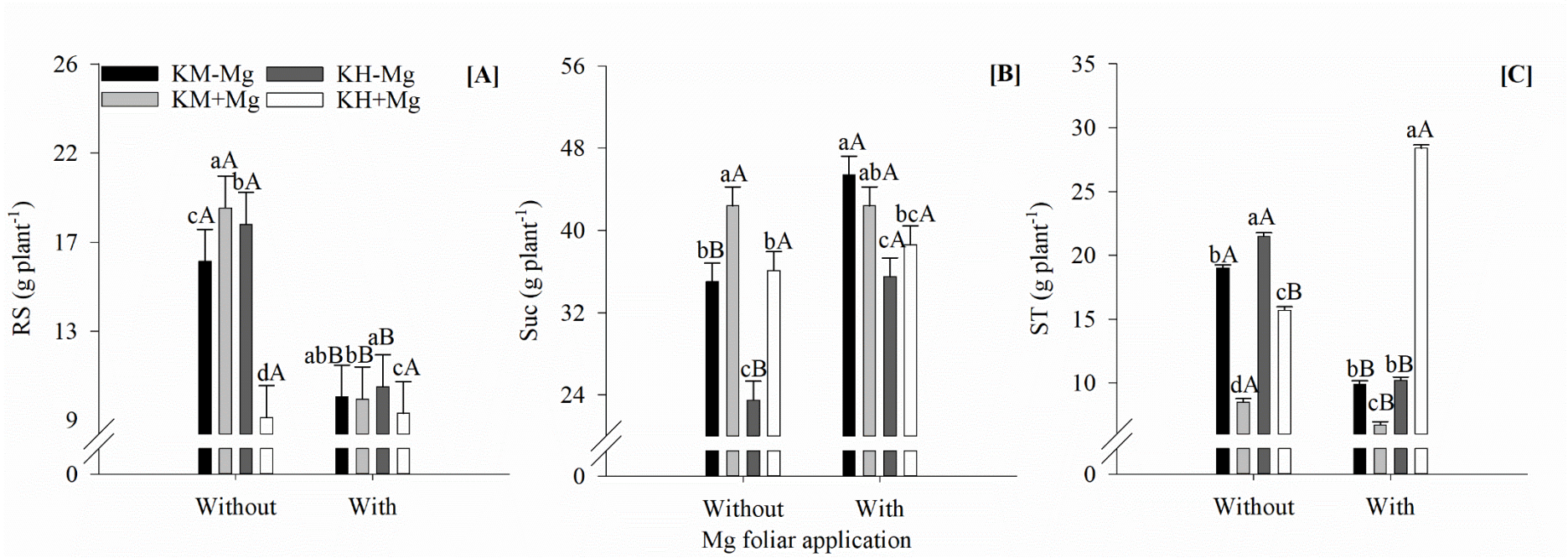
Means followed by the same letter in the column do not differ by the LSD Test at 5% probability. KM-Mg: 206 mg $L^{-1}$ of $K^+$ and 2 mg $L^{-1}$ of $Mg^{2+}$, KH-Mg: 412 mg $L^{-1}$ of $K^+$ and 2 mg $L^{-1}$ of $Mg^{2+}$, KM+Mg: 206 mg $L^{-1}$ of $K^+$ and 17 mg $L^{-1}$ of $Mg^{2+}$, KH+Mg: 412 mg $L^{-1}$ of $K^+$ and 17 mg $L^{-1}$ of $Mg^{2+}$.

Figure 2 – Interaction effect in the reducing sugar (RS) [A], sucrose (Suc) [B] and starch (ST) [C] concentration in the young leaves of sugarcane plants submitted to potassium (206 and 412 mg $L^{-1}$) and magnesium levels (2 and 17 mg $L^{-1}$) in the nutrient solution for 110 days and Mg foliar application.



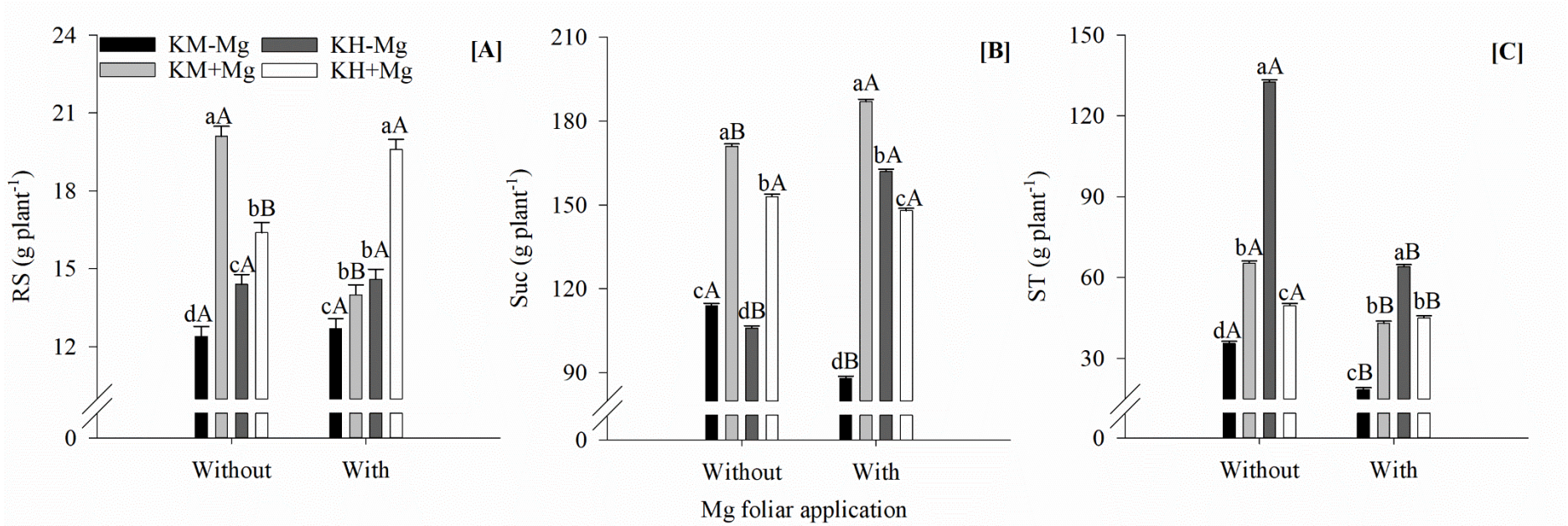

Means followed by the same uppercase letters are not significantly different and compare MgF. Means followed by the same lowercase letters compare each treatment, according to the LSD test ($p \leq 0.05$). Error bars express the standard error of the mean ($n = 4$).

Figure 3 – Interaction effect in the reducing sugar (RS) [A], sucrose (Suc) [B] and starch (ST) [C] concentration in the fully expanded leaves of sugarcane plants submitted to potassium (206 and 412 mg $L^{-1}$) and magnesium levels (2 and 17 mg $L^{-1}$) in the nutrient solution for 110 days and Mg foliar application.



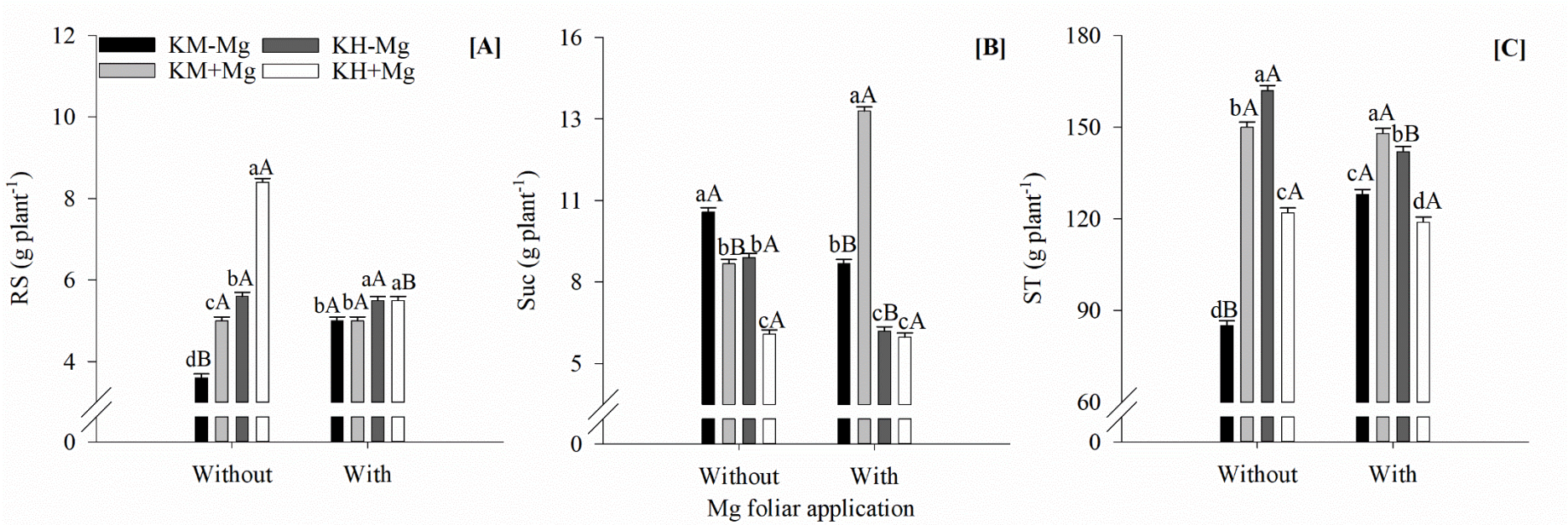

Means followed by the same uppercase letters are not significantly different and compare MgF. Means followed by the same lowercase letters compare each treatment, according to the LSD test ($p \leq 0.05$). Error bars express the standard error of the mean ($n = 4$).

Figure 4 – Interaction effect in the reducing sugar (RS) [A], sucrose (Suc) [B] and starch (ST) [C] concentration in the stalk of sugarcane plants submitted to potassium (206 and 412 mg $L^{-1}$) and magnesium levels (2 and 17 mg $L^{-1}$) in the nutrient solution for 110 days and Mg foliar application.




Means followed by the same uppercase letters are not significantly different and compare MgF. Means followed by the same lowercase letters compare each treatment, according to the LSD test ($p \leq 0.05$). Error bars express the standard error of the mean ($n = 4$).

Figure 5 – Interaction effect in the reducing sugar (RS) [A], sucrose (Suc) [B] and starch (ST) [C] concentration in the root of sugarcane plants submitted to potassium (206 and 412 mg $L^{-1}$) and magnesium levels (2 and 17 mg $L^{-1}$) in the nutrient solution for 110 days and Mg foliar application.




Means followed by the same uppercase letters are not significantly different and compare MgF. Means followed by the same lowercase letters compare each treatment, according to the LSD test ($p \leq 0.05$). Error bars express the standard error of the mean ($n = 4$).

Chlorophyll *a (*Chl *a), (*Chl *b)* and carotenoids were significantly affected by the interaction of the factors (Table 4).

Plants treated with moderate K and Mg supply (KM+Mg) without MgF exhibited the highest Chl *a* concentration in the young leaves (Figure 6A). The Chl *a* concentration in young leaves of KH+Mg plants increased 41% with MgF. However, in KH-Mg plants a different behavior was observed. In fully expanded leaves, the highest Chl *a* concentration was detected in KH-Mg plants without MgF (Figure 6B). By contrast, MgF increased significantly the Chl *a* concentration in fully expanded leaves of all plants, except for KH-Mg. The highest Chl *b* production was observed in plants moderately fed with $K^+$ and $Mg^{2+}$, both in young and fully expanded leaves (Figure 6C and D, respectively). In addition, MgF did not improve the Chl *b* concentration in young leaves in any of the treatments. However, in fully expanded leaves, all treatments were improved in this regard by the MgF, except for KM+Mg (Figure 6D).

Lower carotenoids concentrations in young leaves were detected in the KH-Mg and KH+Mg plants (Figure 6E), regardless the MgF. However, these differences were not significant. In fully expanded leaves, the greatest carotenoids production was obtained in KM+Mg plants, both without and with MgF. Besides, MgF improved 10% of the carotenoids concentration in fully expanded leaves (Figure 6F) of plants cultivated in high $K^+$ and adequate $Mg^{2+}$ concentrations (KH+Mg).

The concentration of Chl *a* and *b* in fully expanded leaves was raised by the MgF in all the treatments, except for Chl *a* production in KH-Mg plants and Chl *b* in KM+Mg plants (Figure 6B and D). The increase in chlorophyll concentration in fully expanded leaves through MgF was also found by Neuhaus et al. (2013) in *Vicia faba* plants, as well as by Teklic et al. (2009) and Dordas (2009) in oregano and soybean, respectively. According to Neuhaus et al. (2013), MgF can mitigate the negative effects of Mg-deficiency on the transcription reduction of the subunit $H^+$ of Mg-chelatase, an enzyme catalysing the insertion of $Mg^{2+}$ into the chlorophyll precursor molecule. Thereby, the chlorophyll molecule degradation known in Mg-deficient plants may be reduced (MERHAUT, 2007). This was also reported by Verbruggen and Hermans (2013), who observed a down-regulation of the chlorophyll *a* / *b* synthesizing protein under Mg-deficiency, which could be a reason for the chlorophyll loss.

Therefore, the factors leading to chlorophyll destruction, such as $Mg^{2+}$ loss to protein synthesis or export assimilation, may be attenuated in response to the MgF. It was observed that the MgF influenced more the old leaves rather than the young ones.

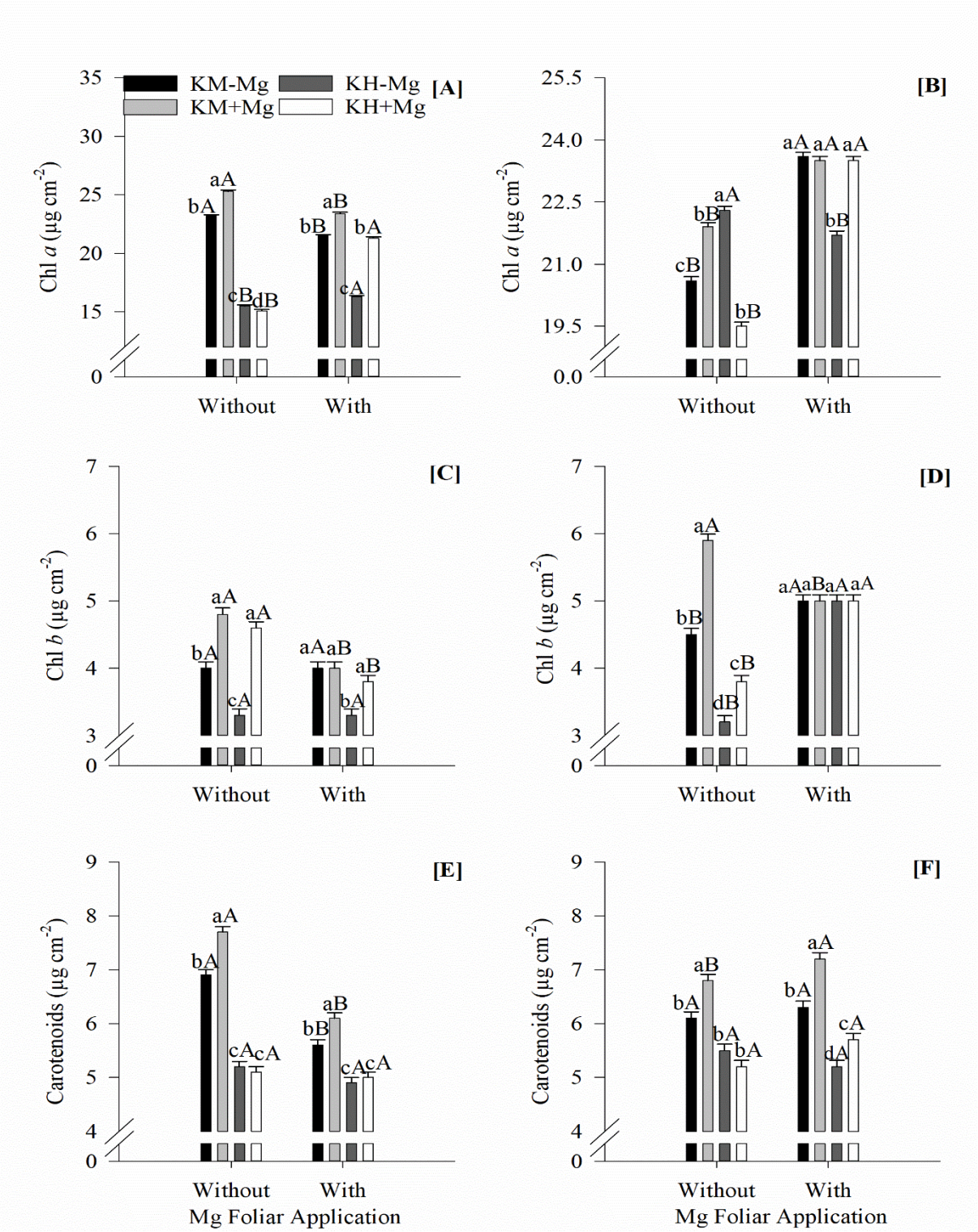
This fact occurs since Mg-deficiency usually becomes more pronounced in older leaves, since this element is mobile in the phloem and translocated to developing organs (MARSCHNER, 2012).

Table 4 – Chlorophyll *a (Chl a), b (Chl b)* and carotenoids (Caro) in the young and fully expanded leaves of sugarcane plants submitted to potassium and magnesium treatment in the nutrient solution for 110 days and magnesium foliar application

| | Young leaves | | | Fuly expanded leaves | | |
|---|---|---|---|---|---|---|
| Factors | *Chl a* | *Chl b* | Caro | *Chl a* | *Chl b* | Caro |
| Treatments (T) | | | (ug $cm^{-2}$) | | | |
| KM-Mg | 22.3b | 4.0b | 6.2b | 22.1b | 4.7b | 6.2b |
| KH-Mg | 15.9d | 3.3c | 5.0c | 22.0b | 4.1c | 5.4c |
| KM+Mg | 24.3a | 4.4a | 6.9a | 22.7a | 5.4a | 7.0a |
| KH+Mg | 18.1c | 4.2ab | 5.0c | 21.5c | 4.4bc | 5.5c |
| Mg Foliar (MgF) | | | | | | |
| Without | 19.7b | 4.1a | 6.2a | 21.1b | 4.3b | 5.9b |
| With | 20.6a | 3.8b | 5.4b | 23.0a | 5.0a | 6.1a |
| ANOVA *(F Probability)* | | | | | | |
| T | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| MgF | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.0335 |
| T x MgF | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.0254 |

Means followed by the same letter in the column do not differ by the LSD Test at 5% probability. KM-Mg: 206 mg $L^{-1}$ of $K^{+}$ and 2 mg $L^{-1}$ of $Mg^{2+}$, KH-Mg: 412 mg $L^{-1}$ of $K^{+}$ and 2 mg $L^{-1}$ of $Mg^{2+}$, KM+Mg: 206 mg $L^{-1}$ of $K^{+}$ and 17 mg $L^{-1}$ of $Mg^{2+}$, KH+Mg: 412 mg $L^{-1}$ of $K^{+}$ and 17 mg $L^{-1}$ of $Mg^{2+}$.

Figure 6 – Interaction effect in the chlorophyll *a (Chl a*, chlorophyll *b (Chl b)* and carotenoids (*Caro*) concentration in the young [A, C and E] and fully expanded leaves [B, D and F], respectively, of sugarcane plants submitted to potassium (206 and 412 mg $L^{-1}$) and magnesium levels (2 and 17 mg $L^{-1}$) in the nutrient solution for 110 days and Mg foliar application.




Means followed by the same uppercase letters are not significantly different and compare MgF. Means followed by the same lowercase letters compare each treatment, according to the LSD test ($p \leq 0.05$). Error bars express the standard error of the mean (n = 4).

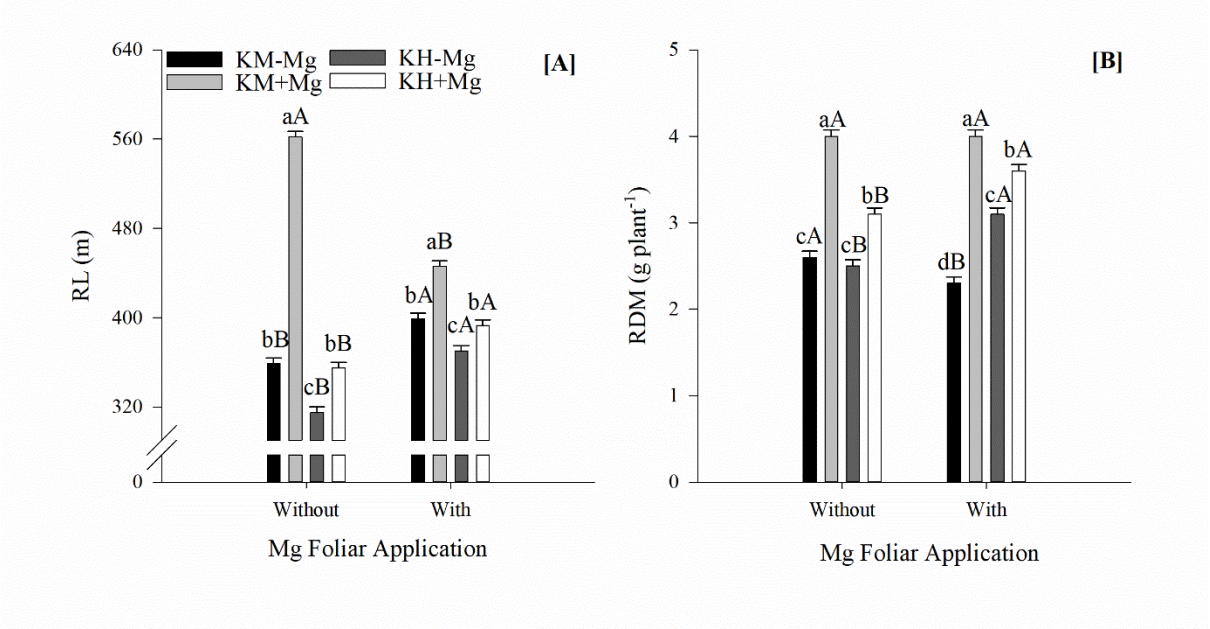
Root diameter was not affected by the treatments, MgF or interaction between them (Table 5). The highest root length and root dry matter was exhibited in the KM+Mg plants, both without and with MgF (Figure 7A and B). However, those plants presented greater root lenght when did not receive MgF, while for root dry matter, no difference was observed with this treatment. For KH-Mg and KH+Mg plants, improvement of root dry matter was detected with MgF, which were 24% and 16% heavier than those plants without MgF, respectively (Figure 7B).

Table 5 – Root length (RL), diameter (Rdi) and dry matter (RDM) per plant of sugarcane plants submitted to potassium and magnesium treatment in the nutrient solution for 110 days and magnesium foliar application

| Factors | RL | Rdi | RDM |
|---|---|---|---|
| Treatments (T) | (m) | (mm) | (g) |
| KM-Mg | 379b | 0.3275b | 2.5d |
| KH-Mg | 342c | 0.3337b | 2.8c |
| KM+Mg | 504a | 0.3450a | 4.0a |
| KH+Mg | 374b | 0.3450a | 3.3b |
| Mg Foliar (MgF) | | | |
| Without | 398a | 0.3406a | 3.0a |
| With | 402a | 0.3350a | 3.2a |
| ANOVA *(F Probability)* | | | |
| T | <0.001 | <0.001 | <0.001 |
| MgF | 0.2013 | 0.0536 | 0,2568 |
| T x MgF | <0.001 | 0.3546 | <0.001 |

Means followed by the same letter in the column do not differ by the LSD Test at 5% probability. KM-Mg: 206 mg $L^{-1}$ of $K^{+}$ and 2 mg $L^{-1}$ of $Mg^{2+}$, KH-Mg: 412 mg $L^{-1}$ of $K^{+}$ and 2 mg $L^{-1}$ of $Mg^{2+}$, KM+Mg: 206 mg $L^{-1}$ of $K^{+}$ and 17 mg $L^{-1}$ of $Mg^{2+}$, KH+Mg: 412 mg $L^{-1}$ of $K^{+}$ and 17 mg $L^{-1}$ of $Mg^{2+}$.

Figure 7 – Interaction effect in the root length (RL) [A] and dry matter (RDM) [B] per plant of sugarcane of sugarcane plants submitted to potassium (206 and 412 mg $L^{-1}$) and magnesium levels (2 and 17 mg $L^{-1}$) in the nutrient solution for 110 days and Mg foliar application.




Means followed by the same uppercase letters are not significantly different and compare MgF. Means followed by the same lowercase letters compare each treatment, according to the LSD test ($p \leq 0.05$). Error bars express the standard error of the mean (n = 4).

The number of internode in the stalk was not influenced by the treatments, MgF or interaction between them (Table 6). Meanwhile, plant height, stalk diameter, stalk dry matter, young leaves dry matter, fully expanded leaves dry matter, shoot dry matter and total dry matter were significantly influenced by the interaction of the factors.

Taller plants were obtained under adequate K conditions (KM-Mg and KM+Mg), compared to those grown under high $K^+$ level (KH-Mg and KH+Mg). However, MgF improved the plant height of KH-Mg and KH+Mg plants by 11 and 45%, respectively (Figure 8A). The highest stalk diameter was observed in KM+Mg plants without MgF, not differing from KM-Mg plants. For those plants that received MgF, the highest value of stalk diameter was observed for KH+Mg, 17% thicker than the same plants without MgF (Figure 8B).

The greatest young leaves dry matter was detected in KM+Mg plants without MgF (Figure 9A). On the other hand, for the plants with MgF, the parameter mentioned above was the highest in KH+Mg plants, which did not differ from those plants with the same treatment but without MgF. Fully expanded leaves dry matter of plants cultivated

under moderate (KM+Mg and KH+Mg) and deficient-Mg conditions (KH-Mg) were increased by 30, 111 and 40% with MgF, respectively (Figure 9B). However, the same pattern was not observed for the KM-Mg plants. Similarly, the stalk dry matter of KM+Mg, KH+Mg and KH-Mg plants was increased by the MgF in 11, 53 and 16.3%, respectively (Figure 9C).

The highest shoot dry matter and total dry matter was exhibited by the plants with the treatment KM+Mg with MgF, which was increased by 7 and 6% due to the foliar spraying treatment (Figure D and E). Following the same behavior, KH-Mg and KH+Mg increased 12 and 40% the ShDM and 14 and 34% the total dry matter, respectively. Even though KM-Mg plants showed reduction in the shoot dry matter with the MgF, the total dry matter was 16% higher due to the MgF (figure 9E).

During the night period, metabolism and growth are completely dependent on plant reserves (WALTER; SCHURR, 2005; NOZUE; MALOOF, 2006), generated in the photosynthetic process during the day (GRAF et al., 2010). These compounds are then directed to the plant growth, which can be determined by the dry matter, volume and length, resulting from division, expansion and cell differentiation (LAMBERS et al., 2008). In most plants, the carbon for overnight supply is accumulated in the form of starch (LAMBERS et al., 2008). Besides, some grasses such as *Hordeum vulgare* and *Poe annua* produce sucrose for both export to the sink organs and to accumulation in the leaf cell vacuoles to provide carbon for nocturnal growth (GORDON et al., 1980; BORLAND; FARRAR 1988).

Table 6 – Plant height (PH), stalk diameter (SDi), number of internode (NI), young leaf dry matter (YLDM), old leaf dry matter (OLDM), stalk dry matter (SDM), shoot dry matter (ShDM) and total dry matter per plant of sugarcane plants submitted to potassium and magnesium treatment in the nutrient solution for 110 days and magnesium foliar application

| Factors | PH | SDi | NI | YLDM |
|---|---|---|---|---|
| Treatments (T) | (m) | (mm) | | (g) |
| KM-Mg | 1.07ab | 17.1b | 7.6ab | 2.9b |
| KH-Mg | 0.94c | 15.4c | 7.0b | 2.9b |
| KM+Mg | 1.10a | 18.0a | 7.8a | 3.5a |
| KH+Mg | 1.04bc | 17.2ab | 7.4ab | 3.5a |
| Mg Foliar (MgF) | | | | |
| Without | 1.01b | 16.8a | 7.4a | 3.3a |
| With | 1.07a | 17.1a | 7.5a | 3.0b |
| ANOVA *(F Probability)* | | | | |
| T | <0.001 | <0.001 | 0.0452 | <0.001 |
| MgF | <0.001 | 0.4217 | 0.7318 | <0.001 |
| T x MgF | <0.001 | <0.001 | 0.2060 | <0.001 |
| Factors | OLDM | SDM | ShDM | TDM |
| Treatments (T) | | (g) | | |
| KM-Mg | 1.7b | 6.3c | 10.9c | 13.4c |
| KH-Mg | 1.2b | 4.7d | 8.8d | 13.4c |
| KM+Mg | 2.3a | 8.6a | 14.4a | 18.5a |
| KH+Mg | 1.4b | 6.7b | 11.6b | 15.0b |
| Mg Foliar (MgF) | | | | |
| Without | 1.4b | 6.0b | 10.8b | 13.9b |
| With | 1.9a | 7.1a | 12.0a | 15.3a |
| ANOVA *(F Probability)* | | | | |
| T | <0.001 | <0.001 | <0.001 | <0.001 |
| MgF | <0.001 | <0.001 | <0.001 | <0.001 |
| T x MgF | <0.001 | <0.001 | <0.001 | <0.001 |

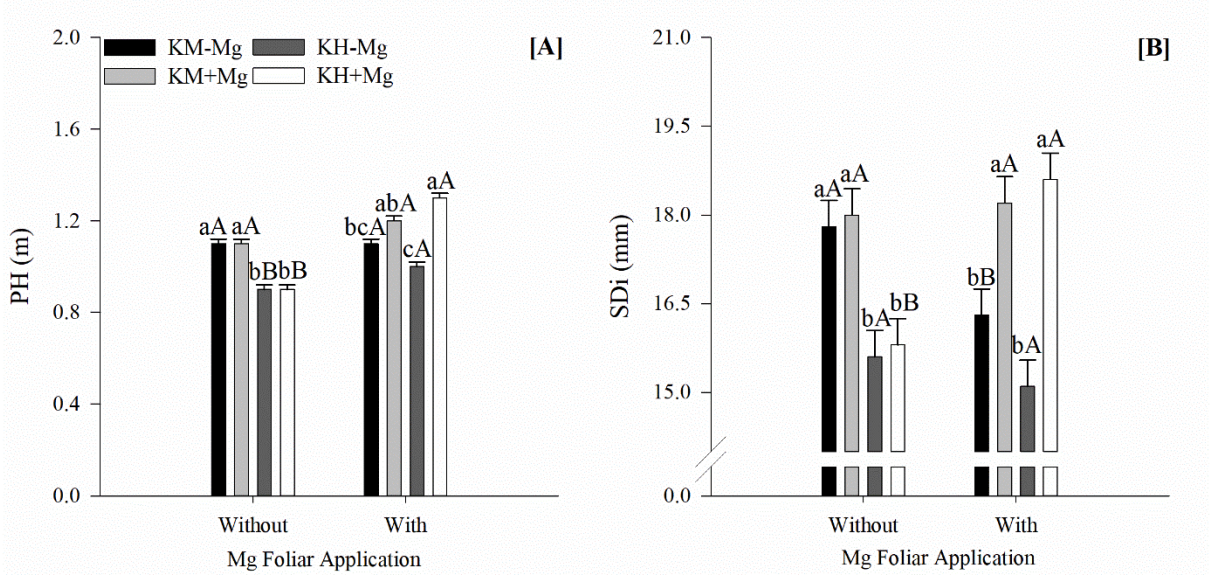
Means followed by the same letter in the column do not differ by the LSD Test at 5% probability. KM-Mg: 206 mg $L^{-1}$ of $K^+$ and 2 mg $L^{-1}$ of $Mg^{2+}$, KH-Mg: 412 mg $L^{-1}$ of $K^+$ and 2 mg $L^{-1}$ of $Mg^{2+}$, KM+Mg: 206 mg $L^{-1}$ of $K^+$ and 17 mg $L^{-1}$ of $Mg^{2+}$, KH+Mg: 412 mg $L^{-1}$ of $K^+$ and 17 mg $L^{-1}$ of $Mg^{2+}$.

Magnesium seems to play a key role in the root development of some plants (SILVA et al., 2005; KELLERMEIER et al., 2013; MENGUTAY et al., 2013, NEUHAUS et al., 2014). Because of that, it is observed that the Mg-deficiency drastically affected the root development of plants grown under low Mg supply (KM-Mg and KH-Mg) (Figure 7). Despite it, $Mg^{2+}$ supply through foliar application improved the root development of these plants. This fact can be attributed to the better supply of carbohydrates supplied by the leaves, with increase of sucrose produced in these organs (Figure 2B and Figure 3B). Later, the sucrose would be translocated to the

roots and stored as starch (Figure 5C), since these bigger compounds like sucrose induce high turgid pressure in the cells (WANG et al., 2017), thus providing maintenance of the photosynthetic process and coordination between the production of photoassimilates (GRAHAM; MARTIN, 2000).

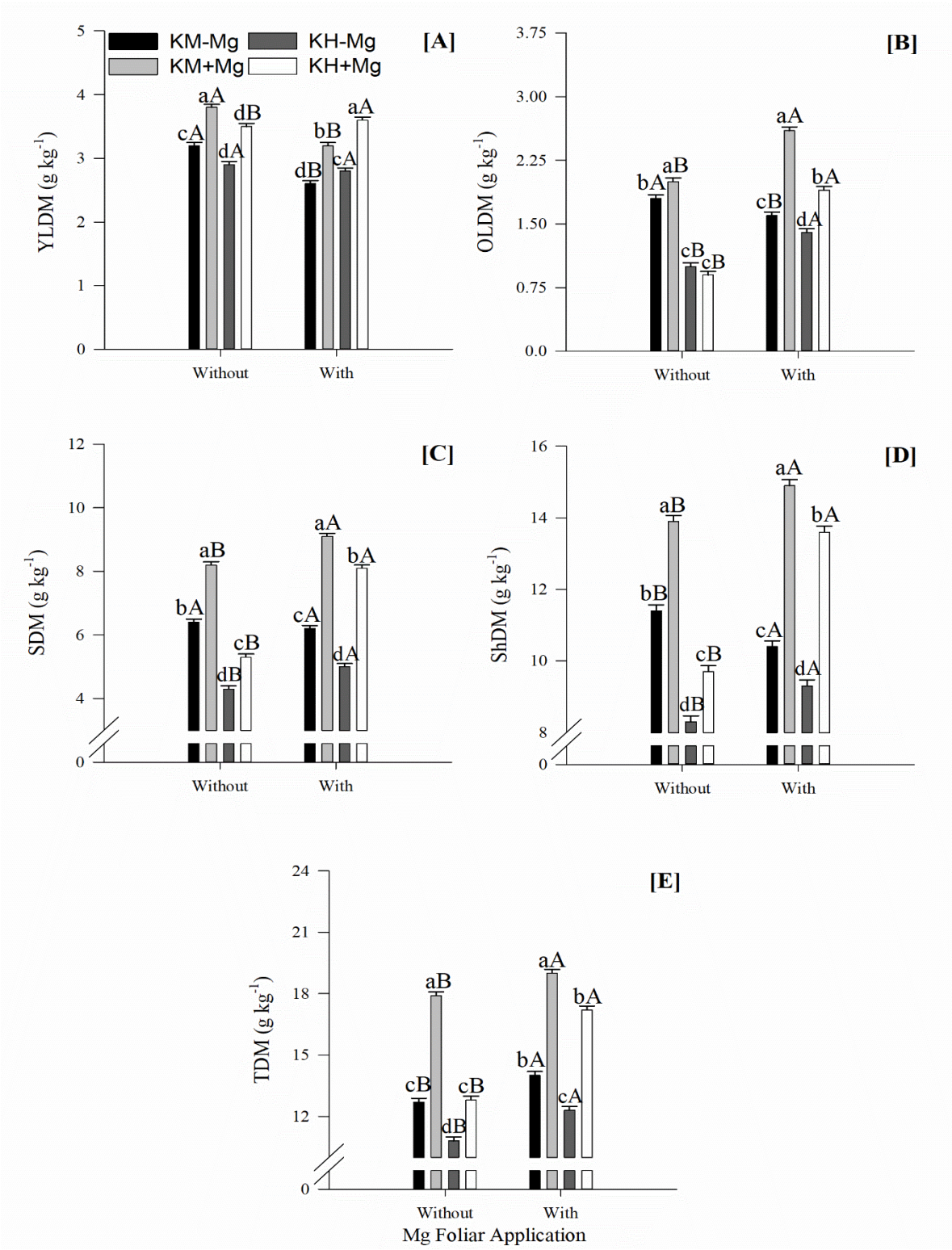
The reduction on starch concentration in the fully expanded leaves (Figure 3C) of these plants may also be attributed to the increase in sucrose by this organ (Figure 3B), which are then exported to the roots through the phloem, not allowing sucrose to be accumulated in the leaves. Consequently, there is no activation of the ADP-glucose pyrophosphorylase (AGPase) enzyme nor the favoring for starch synthesis (SONNEWALD, 2001).

Figure 8 – Interaction effect in the plant height (PH) [A], stalk diameter (SDi) [B] per plant of sugarcane submitted to potassium (206 and 412 mg $L^{-1}$) and magnesium levels (2 and 17 mg $L^{-1}$) in the nutrient solution for 110 days and Mg foliar application. Means followed by the same uppercase letters are not significantly different and compare MgF.




Means followed by the same lowercase letters compare each treatment, according to the LSD test ($p \leq 0.05$). Error bars express the standard error of the mean (n = 4).

Figure 9 – Interaction effect in the young leaves dry matter (YLDM) [A], fully expanded leaves dry matter [B], stalk dry matter (SDM) [C], shoot dry matter (ShDM) [D] and total dry matter (TDM) [E] per plant of sugarcane submitted to potassium (206 and 412 mg $L^{-1}$) and magnesium levels (2 and 17 mg $L^{-1}$) in the nutrient solution for 110 days and Mg foliar application.




Means followed by the same uppercase letters are not significantly different and compare MgF. Means followed by the same lowercase letters compare each treatment, according to the LSD test ($p \leq 0.05$). Error bars express the standard error of the mean (n = 4).

## 3.4 CONCLUSIONS

High level of $K^+$ reduces $Mg^{2+}$ uptake by the root and decreases its translocation from the root to shoot. Moreover, Mg foliar application did not improve its concentration in any part of these plants.

Low $Mg^{2+}$ availability, as well as high $K^+$ content, reduces root length, which were attenuated by the foliar Mg application.

Magnesium foliar application improves Chl *a* and *b* production in fully expanded leaves of plants grown under high $K^+$ level, as well as sucrose concentration in young and fully expanded leaves. In addition, Mg foliar application influences differently the carbohydrate partitioning of the plants root and shoot.

Magnesium foliar application improved the total dry matter of the plants in all treatments.

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## GENERAL CONSIDERATIONS

Sugarcane is a crop specie with great socioeconomic importance in Brazil. Vinasse has been used in an increasingly intense way as an organic fertilizer. With respect to mills, vinasse is seen as a product of economic value in the production of sugar and alcohol because applications of this natural product through fertigation results in increased agricultural productivity and reduced fertilization and irrigation costs.

However, the sugarcane productivity of vinasse-treated fields is still below the genetic potential of the varieties. This fact can be attributed to the internal Mg concentration imbalance in the plants caused by the high K concentration in the vinasse, making acquisition of this nutrient by plant roots difficult.

Due to the complex role of Mg in chlorophyll and protein biosynthesis, negative impacts are expected not only on final crop yields but also on the qualitative parameters of sugarcane under Mg deficiency. Since several plant organs (roots, stalks and gems) are not able to satisfy their demand for photoassimilates (sugars, amino acids, etc.), their demands are compensated by the direct transport from the source leaves to the sink organs, mediated by phloem. Although not entirely clarified, Mg seems to play a key role in the phloem loading of sucrose from source leaves, emphasizing the importance of an adequate supply of this nutrient to crops. In addition, due to the ability of the leaves to absorb nutrients, Mg foliar applications represent an excellent fertilization strategy to improve the internal concentration of this nutrient in plants. Thus, additional studies should be carried out to elucidate the mechanisms of K and Mg interaction as well as alternatives to Mg supply in combination with vinasse use, since several benefits occur in response to the use of this product.

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