



Potassium-magnesium imbalance causes detrimental effects on growth, starch allocation and Rubisco activity in sugarcane plants

Ariani Garcia¹ · Carlos Alexandre Costa Crusciol¹ · Ciro Antonio Rosolem¹ · João William Bossolani¹ · Carlos Antonio Costa Nascimento¹ · James Mabry McCray² · André Rodrigues dos Reis¹ · Ismail Cakmak³

Received: 28 June 2021 / Accepted: 8 November 2021 / Published online: 16 January 2022
© The Author(s), under exclusive licence to Springer Nature Switzerland AG 2021

Abstract

Background and aims While there is abundant literature on the antagonistic interaction between potassium (K) and magnesium (Mg) during root uptake and transport, there is, however, little published data on the interaction between these nutrients within tissue, especially for sugarcane plants having high demand for K and Mg. This study aimed to investigate the effects of the interactions between K and Mg.

Methods Plants were grown in nutrient solution with increasing K application doses at low and adequate Mg treatments under controlled greenhouse conditions. Leaves, stalks and roots have been analyzed for growth, nutrient concentrations, starch partitioning, and activity of Rubisco.

Results There were significant decreases in Mg concentration in all plant parts by increasing K supply, especially in roots. Leaf symptoms of Mg deficiency appeared not only in low Mg, but also in Mg adequate plants when plants treated with very high K. Magnesium adequate plants contained much higher amount of starch in roots, stalks and young leaves than the low Mg plants when K applications were at low

Responsible Editor: Miroslav Nikolic.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11104-021-05222-2>.

A. Garcia · C. A. C. Crusciol (✉) · C. A. Rosolem · J. W. Bossolani
Department of Crop Science/UNESP, College of Agricultural Sciences, José Barbosa de Barros Street, n° 1780, Botucatu, SP, Brazil
e-mail: carlos.crusciol@unesp.br

A. Garcia
e-mail: ariani_garcia@hotmail.com

C. A. Rosolem
e-mail: ciro.rosolem@unesp.br

J. W. Bossolani
e-mail: bossolani.agro@gmail.com

C. A. C. Nascimento
Department of Soil Science, Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo (USP), Piracicaba, SP, Brazil
e-mail: cacasnascimento@usp.br

J. M. McCray
Everglades Research & Education Center, Wedgworth Lab. Bldg. Rm. #113., 3200 East Palm Beach Road, Belle Glade, FL 33430, USA
e-mail: jmmccray@ufl.edu

A. R. dos Reis
Department of Sciences and Engineering, São Paulo State University, Tupã, SP 17602-496, Brazil
e-mail: andre.reis@unesp.br

I. Cakmak
Faculty of Engineering & Natural Sciences, College of Agricultural, Sabanci University, 34956 Tuzla, Istanbul, Turkey
e-mail: cakmak@sabanciuniv.edu

levels. By contrast, there was a high accumulation of starch in the older leaves of the Mg deficient plants. Magnesium deficiency was also associated with significant decreases in Rubisco activity in leaves.

Conclusion Our results show clearly that high K doses interfere significantly with the positive effects of Mg on plant growth, Rubisco activity and starch accumulation in sink organs such as roots and stalks. It is obvious that the imbalance between K and Mg nutrition in sugarcane results in adverse consequences in sugar yield capacity.

Keywords Sugarcane · *Saccharum spp* · Starch allocation · Shoot and root growth · Potassium · Magnesium and Rubisco

Introduction

Most of magnesium (Mg) existing in the Earth's crust is not readily available to plants (Senbayram et al. 2016). Clayey soils generally provide sufficient amounts of Mg for plant nutrition, while sandy soils are usually deficient in Mg and require applications of Mg-containing fertilizers (Gransee and Fuehrs 2013). In addition, Mg is usually less-tightly adsorbed to soil constituents, and therefore, prone to leaching, especially in sandy acidic soils. In low pH soils, fertilizer recommendations are mainly focused on nitrogen (N), phosphorus (P) and potassium (K) fertilizers (Guo et al. 2016; Maguire and Cowan 2002), while Mg supply to plants usually depends on liming (Gransee and Fuehrs 2013). However, lime typically has a high concentration of calcium (Ca) (30–40%) but low Mg (1–13%) (Cazotti et al. 2019; Stevens et al. 2005; Soratto et al. 2019), which often causes a nutrient imbalance in the rhizosphere. There are number of published reports showing antagonistic effects of high K on root uptake of Mg and impairments in Mg nutritional status of plants (Dechen et al. 2016; Ding et al. 2006; Li et al. 2018; Morton et al. 2008; Koch et al. 2019). Most probably, high K concentrations in growth medium impairs root Mg uptake by blocking the unspecific Mg transporters localized on root cell membranes (Senbayram et al. 2016; Xie et al. 2020). Generally, root Mg uptake is maintained through a non-selective ion channel that also controls root K and Ca uptake (Shabala and Hariadi 2005; Senbayram et al. 2016). It is, therefore, very common to

observe an inhibitory effect of high K or Ca supplies on root Mg uptake. By contrast, high Mg concentrations have no or limited effect on root K uptake, probably because the specific K transporters controlling root K uptake are not affected by high Mg treatments (Horie et al. 2011; Senbayram et al. 2016).

Despite large number of published data on antagonistic effects of K on root Mg uptake, little is known on how the physiological functions of Mg and K within plant tissues are affected by the antagonistic interactions between K and Mg. Magnesium is known to be the most abundant divalent cation in cytosol and plays key roles in many important physiological processes (Cakmak and Kirkby 2008; Guo et al. 2016; Tränkner et al. 2018; Geng et al. 2021; Jaghdani et al. 2021). For example, Mg has a direct effect on the H⁺-ATPase-driven phloem loading and transport of sucrose (Cakmak and Kirkby 2008; Hermans et al. 2005). Similarly, K is also required for phloem loading of sucrose by exerting direct effect on H⁺-ATPase-driven phloem loading process (Cakmak 2005; Dreyer et al. 2017; Zörb et al. 2014). Consequently, low Mg or K nutrition is associated with substantial accumulation of starch in source leaves (Cakmak et al. 1994a, b; Tränkner et al. 2018; Koch et al. 2019) as well as rapid decline in growth of sink organs such as roots and youngest shoot parts, seeds and fruits (Cakmak et al. 1994a; Ceylan et al. 2016; Hermans and Verbruggen 2005; Zhang et al. 2020; Geng et al. 2021). Very recently, Garcia et al. (2020) showed that Mg deficiency reduced sucrose concentration in the stalks of sugarcane plants by 87% compared to the plants with adequate Mg supply. It is known that accumulation of sugars in leaves has a negative feedback effect on Calvin cycle activity by downregulating the Rubisco activity (Araya et al. 2006). A reduced activity of Rubisco can be expected in low Mg and K plants due to excessive accumulation of photoassimilates in leaves. Additionally, Mg enhances the activity of the Rubisco enzyme by binding the active site of the enzyme (Hazra et al. 2015; Tränkner et al. 2018).

It would be important to know how excessive applications of K affects the physiological functions of Mg within plant tissue. Ding et al. (2006) highlighted importance of the imbalance between K and Mg concentrations in leaf tissue and showed that Mg deficiency was not compensated by high K supplies. By contrast, Mg deficiency was enhanced by high

K. The imbalance between K and Mg concentrations in the leaf tissue was also studied regarding to sugar concentrations and photosynthetic activity in rice plants. However, in the study, sink and source leaves were not investigated separately (Ding et al. 2006). According to Gerendás and Führes (2013) imbalance in composition of K and Mg in plant tissues associated with high levels of K may induce Mg deficiency and impairments in quality parameters such as fruit acidity. Therefore, it was suggested that leaf K/Mg ratio might be more relevant than the leaf Mg concentration under such high imbalance between leaf concentrations of K and Mg. Research on sugarcane in respect to interactions between K and Mg is very limited. In one experiment it has been shown that sugarcane is sensitive to low Mg supply, especially in case of high K applications. According to Rhodes et al. (2018), high K applications represents an important agronomic factor affecting the occurrence of Mg deficiency in sugarcane growing fields. Sugarcane is a suitable crop to study sugar transport and accumulation. Sugarcane has been shown to be exceptional crop in terms of transportation and accumulation of very high amounts of sugars in stalk tissue, even up to 50% of the stem dry weight (Lingle et al. 2010; Patrick et al. 2013). Around 70% of the sugar produced globally is derived from sugarcane (Contreras et al. 2009).

In the present study, we aimed to understand how increasing K applications from low up to very high level under low and adequate Mg treatments can alter the growth of leaves, stalks and roots, K and Mg nutritional balance, and Rubisco activity, as well as starch accumulation in sink and source organs of sugarcane plants.

Material and methods

Seedling establishment

The experiment was conducted in a climate-controlled greenhouse with 12 h light ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$) and 12 h darkness, located at São Paulo State University, Botucatu, Brazil ($48^\circ 23' \text{ W}$, $22^\circ 51' \text{ S}$). The heating and evaporative cooling systems of the greenhouse maintained the temperature at 32°C in the daytime, at 22°C at

night, and relative humidity at 75%. The sugarcane variety used in the experiments was RB867515, which is the most commonly cultivated variety in Brazil.

The sugarcane for seedling preparation was harvested from a commercial nursery area. Cane cuttings with approximately 3 cm in length and one vegetative bud were obtained. The cuttings received were placed in plastic trays filled with washed sand and irrigated by using deionized water under greenhouse conditions for the germination. At 20 days after emergence, four seedlings were selected according to their sprouting uniformity and transferred to plastic pot containing 4 l of aerated nutrient solution, for each treatment, starting from that moment the trial. Sixty days after this first transplant, the plants about 60 cm height were transferred to larger pots with 14 L of nutrient solution volume.

Nutrient solution and treatments establishment

In the experiments, the nutrient solution composition described by Furlani and Furlani (1988) and Garcia et al. (2020) was used after some modifications for the sugarcane studies. The composition of the nutrient solution was as following: $160 \text{ mg L}^{-1} \text{ N-NO}_3^-$; $32 \text{ mg L}^{-1} \text{ N-NH}_4^+$; $16 \text{ mg L}^{-1} \text{ P}$; $178 \text{ mg L}^{-1} \text{ Ca}$; $0.05 \text{ mg L}^{-1} \text{ Cu}$; $3.6 \text{ mg L}^{-1} \text{ Fe}$; $0.5 \text{ mg L}^{-1} \text{ Mn}$; $0.09 \text{ mg L}^{-1} \text{ Mo}$; $0.15 \text{ mg L}^{-1} \text{ Zn}$ and $0.27 \text{ mg L}^{-1} \text{ B}$. Potassium was added to the nutrient solution in the forms of K_2SO_4 , KNO_3 and KCl to create the following 4 K treatments: K1 = 103, K2 = 206, K3 = 309 and K4 = 412 mg L^{-1} . The K dose of 206 mg L^{-1} has been considered as the adequate K dose for sugarcane in nutrient solution experiments by considering the previous studies (Furlani and Furlani 1988; Garcia et al. 2020). The experimental design was completely randomized and consisted of four K levels and two Mg levels with 4 independent replications. Magnesium has been applied as $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ at low (2 mg Mg L^{-1}) and adequate (17 mg Mg L^{-1}) levels, and the levels of low and adequate Mg were based on the previous experiments (Furlani and Furlani 1988; Garcia et al. 2020). In all treatments, the S concentration of the nutrient solution was 56 mg L^{-1} and balanced by using Na_2SO_4 . The Na

concentrations of the solutions were 17.5 mg L^{-1} and 46 mg L^{-1} in case of adequate and low Mg treatments (see Supplemental Table S1 for further details of the nutrient solutions used).

All chemicals were obtained from Sigma-Aldrich®. The sugarcane seedlings were transferred to a plastic pot with a nutrient solution corresponding to each treatment since the beginning of the experiment. The nutrient solution was diluted to one-third ionic strength during the first week and half ionic strength during the second week of plant growth. Thereafter, full-ionic-strength solution was used. The water lost by evapotranspiration was replaced with deionized water. The pH of the nutrient solution was monitored daily and maintained within a range of 5.5 to 6.0 by using 0.1 M HCl or 0.1 M NaOH solutions. The nutrient solutions were replaced weekly. The plants were grown for 110 days after the plants were transplanted into the nutrient solutions. Experimental plants harvested were then used for measurement of several parameters described below.

Growth data

At harvest, plant height was measured from the root-shoot junction to the top visible dewlap leaf. For determination of the dry matter production, experimental plants were divided into roots, stalks, fully expanded (old) leaves and young leaves and dried in a forced-air oven at 65°C until they reached a constant weight. At the harvest, plants had 7 leaves and the leaves 6 and especially 7 (from the top) started to show signs of senescence. The leaves 1 and 5 (from the top) were used as older (fully expanded) and younger leaves for the analysis described below. Part of fully expanded older leaves was collected and frozen in liquid nitrogen until use for the measurement of the enzymes and protein content. Plants were also analyzed for the root length by using WinRhizo software version 3.8-b (Regent Instruments Inc., Quebec, Canada) as described by Tennant (1975).

The plant samples for nutrient analysis were kept at room temperature after drying and milling, whereas the leaf samples for Rubisco activity analysis were placed in liquid nitrogen and stored at -80°C until further analysis.

Determination of the concentrations of potassium, magnesium and chloride

The plant parts samples were ground to pass through a 1 mm screen in a Willey-type mill, after which the samples were digested by nitroperchloric digestion (AOAC 1995). The macronutrients K and Mg were determined by atomic absorption spectrophotometry Perkin Elmer Analyst 200 (Norwalk, CT, USA). As the main source of K applied was KCl, the Cl concentrations were also determined in order to understand if the Cl concentrations could interfere with the results obtained. Chloride was extracted with a solution of calcium nitrate and titrated with standardized silver nitrate solution in the presence of potassium chromate as an indicator.

Determination of the concentration and content (total amount) of starch

The starch was extracted considering methods described by Somogyi (1952) and Nelson (1994). Briefly, the samples of about 200 mg were homogenized in 42 mL of deionized water and 100 μL of termamyl (50%), and the pH of the extraction obtained was subsequently adjusted to 4.8 by using sodium acetate buffer. Then, 10 μL of the enzyme α -amylase was added to the sample, followed by stirring in a water bath at 90°C for 2.5 h. After cooling, 100 μL of amyloglucosidase was added, and the samples were stirred in a water bath for an additional 2.5 h. Then, 4 M NaOH solution was added to the extract to neutralize the acidity. The volume of each sample was adjusted to 250 mL, from which 5 mL was sampled, transferred to a 100-mL flask, and adjusted to the final volume with water of 100 mL. For readings, 1 mL of each sample was mixed with 1 mL of alkaline Cu reagent and placed in a water bath for 10 min. After cooling and adding arsenomolybdate reagent, the color intensity has been measured at 535 nm spectrophotometrically (Shimadzu UV-2700, Kyoto, Japan) and glucose used as reagent in the assay. The total amount of starch accumulated in the plant organs studied was calculated by multiplying the dry weight by the starch concentrations of the corresponding plant organ.

Ribulose-1,5-bisphosphate carboxylase activity (rubisco, EC. 4.1.1.39)

Fresh leaf material frozen (0.3 g) in liquid nitrogen was homogenized for 2 min with 1.5 mL of 42 mM KH_2PO_4 , 58 mM K_2HPO_4 and 1 mM EDTA (Reid et al. 1997). Total Rubisco activity in the supernatant was measured by 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 supernatant were incubated at 30 °C for 5 min with 900 μL of the assay mixture in the absence of ribulose-1,5-bisphosphate (RuBP) to enable carbamylation of the enzyme. The oxidation of NADP occurred after the addition of 16.66 mM RuBP to the cuvette (adapted from Reid et al. 1997), and the absorbance changes were recorded at 340 nm using a spectrophotometer (Shimadzu UV-2700, Kyoto, Japan). Rubisco activity was calculated from the difference in the absorbance readings at 0 and 1 min (without removing the cuvette from the spectrophotometer).

Statistical analysis

All data were analyzed for normality and homoscedasticity by Shapiro and Wilk (1965) and Levene's (1960) test, respectively. Data were subjected to analysis of variance (ANOVA) at $p \leq 0.05$, using the software R (R Development Core Team 2015). For plant height, root length, K:Mg ratio, starch partitioning, and Rubisco data, the means were compared using the least significant difference (LSD, $p \leq 0.05$) for both K and Mg factors. Dry matter production and nutritional status data were submitted to regression analysis ($p \leq 0.05$) for K factor and LSD test for Mg factor ($p \leq 0.05$), and the equations were fit as a function of K response using SigmaPlot® 12.5 (Systat Software, Inc. SigmaPlot for Windows).

Results

Plant growth

At a given K treatment, adequate Mg supply improved root and shoot growth of the plants (Fig. 1).

Consequently, whole plant growth was also improved at adequate Mg supply. The stalk growth was also very sensitive to low Mg supply. Irrespective of the Mg treatment, increasing K applications firstly increased dry matter production of the plants and then resulted in a clear impairment, except the root growth under low Mg treatment. The root growth under low Mg was first not affected by K supply, but then showed similar pattern like the Mg adequate plants (Fig. 1). It appeared that the K application of 206 mg L^{-1} under adequate Mg supply is the best K application dose in terms of increasing plant growth under given experimental conditions. The changes in plant height depending on K and Mg treatments were similar to the changes in plant growth (Table 1). The highest K supply under low Mg caused largest decrease in plant height. The root length was also affected by the treatments and showed a decline by increasing K doses under low Mg. As shown in Table 1, the root length of the Mg adequate plants was sharply reduced by the highest K supply (Table 1).

Nutrient concentrations

As expected, increasing K application enhanced K concentrations in roots, stalks and older leaves under both Mg treatments (Fig. 2). The increases in plant K concentrations were more distinct in case of adequate Mg supply. When K supply was low, adequate Mg application additionally reduced plant K concentration, especially in roots (Fig. 2). By contrast, at higher K applications, varied Mg treatment did not affect plant K concentration. There were particular decreases in plant Mg concentrations by increasing K application, and these decreases were pronounced in roots at adequate Mg supply. For example, increasing K supply from 103 mg L^{-1} to 412 mg L^{-1} reduced root Mg concentration from 3.9 g kg^{-1} to 1.8 g kg^{-1} in roots (Fig. 2). Under low Mg supply, decreases in plant Mg concentrations by increasing K supply were either minimal or absent. Table 2 shows the K:Mg ratios in all plant organs depending on the K and Mg treatments. At a given Mg treatment, increasing K applications resulted in marked increases in K:Mg ratios (Table 2), particularly in the Mg-adequate plants. Since most of K treatments has been realized in form of KCl (Table S1), we interested to know the changes in Cl concentrations of plants following K applications.

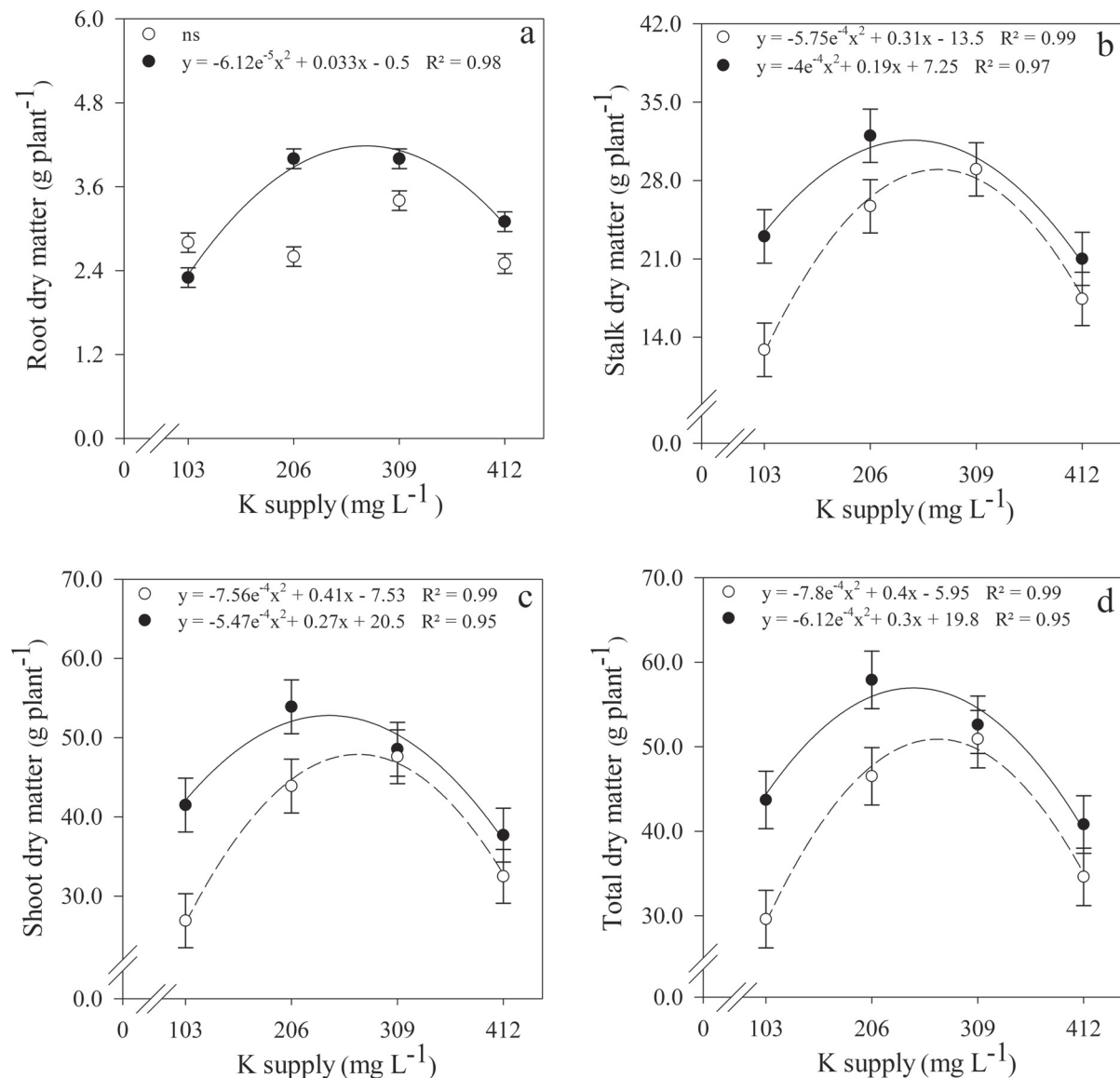


Fig. 1 Root (a), stalk (b), shoot (c) and total dry matter (d) per plant of sugarcane plants grown under increasing K treatments (i.e., 103, 206, 309 and 412 mg K L⁻¹) at low

(2 mg L⁻¹; ---○---) and adequate (17 mg L⁻¹; —●—) Mg supplies in nutrient solution for 110 days. Vertical bars indicate the LSD test ($p \leq 0.05$)

The results obtained showed that in all sugarcane organs analyzed (i.e., root, stalk, younger and fully expanded leaves) the Cl concentrations showed an increasing trend with the increase of K applications (Table S2). However, the increasing trend in Cl concentration by K application was not statistically different.

Concentration and accumulation of starch

Differences in the Mg treatments differently affected the concentration and accumulation of starch in the plant organs under varied K treatments (Fig. 3). Under low Mg treatment, starch concentrations were reduced at lower K doses in the young leaves, but showed

Table 1 Root length and plant height of sugarcane plants grown under increasing K treatments (i.e., 103, 206, 309 and 412 mg K L⁻¹) at low (2 mg L⁻¹) and adequate (17 mg L⁻¹) Mg supplies in nutrient solution for 110 days. †Lowercase let-

ter compare the K levels within the same Mg condition, whilst uppercase letters compare the Mg levels within the same K level by LSD test ($p \leq 0.05$)

K Treatments (mg L ⁻¹)	Root length		Plant height	
	Mg-deficient	Mg-adequate	Mg-deficient	Mg-adequate
	(m plant ⁻¹)			
103	384aB†	438bA	1.00cB	1.06bA
206	359bB	562aA	1.10aA	1.12aA
309	346bB	580aA	1.03bB	1.12aA
412	315cB	355cA	0.90dA	0.92cA

†Lowercase letter compare the K levels within the same Mg condition, whilst uppercase letters compare the Mg levels within the same K level by LSD test ($p \leq 0.05$)

substantial increases in the old leaves. In case of higher K treatments (309 and 412 mg K L⁻¹), young leaves of the low Mg plants had more starch, while in the old leaves, the differences in starch concentration between low and adequate Mg treatments were minimal. As found in young leaves, also the stalk and root organs of the Mg adequate plants contained significantly higher starch concentrations under low K treatments. By contrast, low Mg plants had more starch under high K treatments when compared to Mg adequate plants, especially in stalk at the highest K supply (Fig. 3).

The changes in total amount of starch per plant were generally similar to the starch accumulation (Fig. 3). The young leaves and roots of Mg-adequate plants accumulated much more starch per plant than the low Mg plants at each K treatment, except the highest K application. In contrast to the roots and young leaves, the old leaves of the low Mg plants exhibited more starch accumulation at all K treatments when compared to the Mg adequate plants (Fig. 3). The changes in total amount of starch in stalk were different than in the leaves and roots. When plants grown at lower K applications, adequate Mg supply resulted in more starch accumulation in stalk than the low Mg plants (more than 4-fold). However, at higher K treatments, low Mg plants contained higher amount of starch than the Mg adequate plants (Fig. 3).

Rubisco activity

The adequate Mg supply combined with the K dose of 206 mg L⁻¹ provided the highest Rubisco activity in sugarcane plants (Fig. 4). Additionally, under low

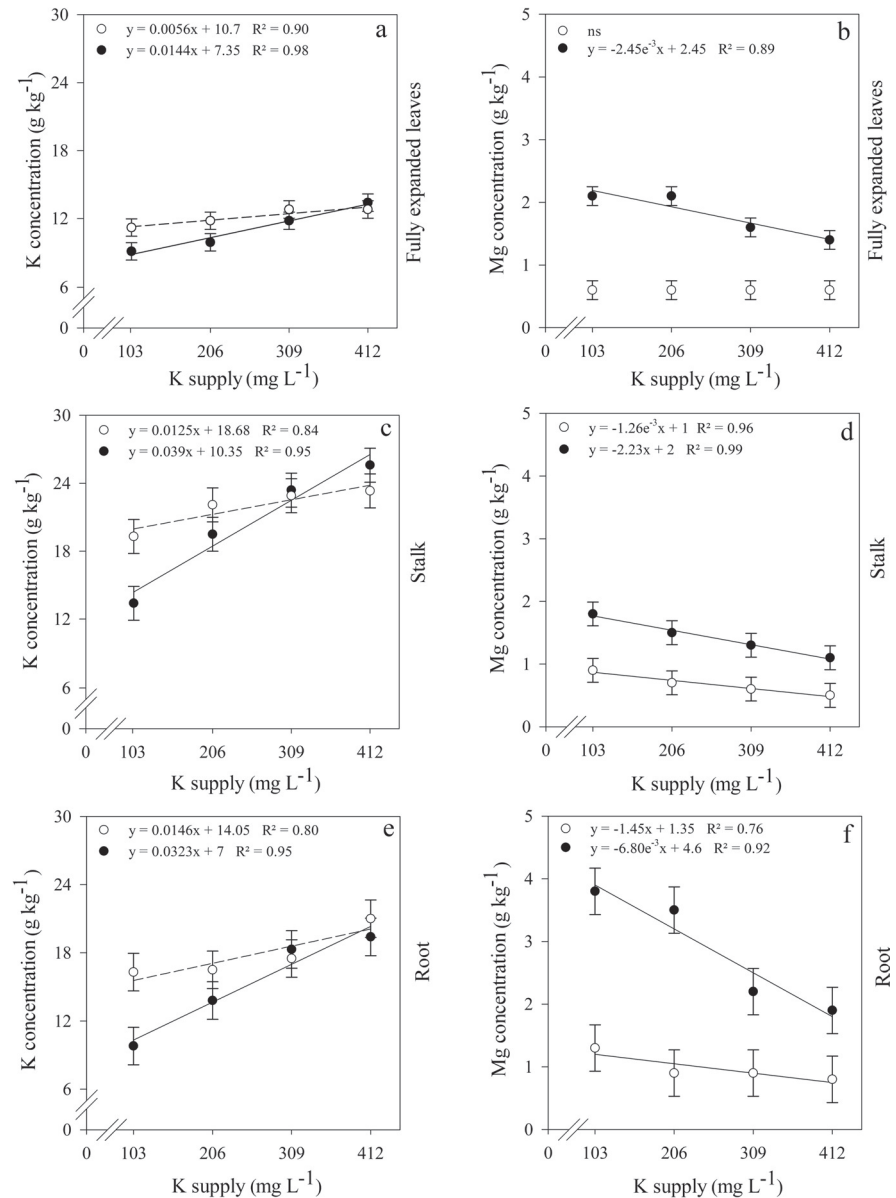
Mg supply, the highest K dose significantly increased Rubisco activity, whereas at adequate Mg supply, the two highest doses of K reduced the activity of the enzyme. Interestingly, under low Mg supply, the highest K dose increased Rubisco activity up to 3-fold.

Discussion

The shoot and root dry matter production were always better with adequate Mg supply at each K treatment (Fig. 1). The K dose with 206 mg L⁻¹ was found to be most suitable K dose resulting in the best dry matter production under given experimental conditions. The lowest and highest K treatments reduced dry matter production of the plants at both Mg treatments. The decrease in growth of Mg-adequate plants at the highest K dose indicates that higher K treatments very likely resulted in impairments in Mg nutritional status of plants. In accordance with this suggestion, the plants grown under adequate Mg but with the highest K dose developed leaf chlorosis along the veins like the Mg deficient plants, although it was not so severe as expressed in the plants with low Mg. High K supply usually leads to inhibitory effect on root Mg uptake. In addition, it is known that Mg deficiency in plants generally results in expression of an interveinal chlorosis on older leaves (Marschner 2012).

The root length and plant height were also very susceptible to the highest K dose under low Mg supply (Table 1). In case of the most suitable K application dose (i.e., 206 mg K L⁻¹), it appeared that root growth was more sensitive to low Mg supply than the shoot growth (Fig. 1). This result obtained

Fig. 2 Potassium (K) and magnesium (Mg) concentration in the fully expanded leaves (**a, b**), stalks (**c, d**) and root (**e, f**) of sugarcane plants grown under increasing K treatments (i.e., 103, 206, 309 and 412 mg K L⁻¹) at low (2 mg L⁻¹; ---○---) and adequate (17 mg L⁻¹; —●—) Mg supplies in nutrient solution for 110 days. Vertical bars indicate the LSD test ($p \leq 0.05$)



in sugarcane plants is in a good agreement with the results published previously for several crop plants (Ericsson and Kähr 1995; Mengutay et al. 2013; Koch et al. 2019; Tränkner and Jaghdani 2019; Jaghdani et al. 2021). Beside the root dry matter production, also the root length was found to be sensitive to low Mg supply, especially under higher K treatments (Table 1). Significant decreases in root length under low Mg supply were also reported for *Arabidopsis* (Gruber et al. 2013) and potato (Koch et al. 2019) plants. Mg-deficient plants accumulate

photoassimilates in the source leaves before photosynthesis is affected by the deficiency of this nutrient (Cakmak and Kirkby 2008). Under these conditions, low amounts of carbohydrates are transferred to the roots (sink organs), implying a significant reduction in root growth under Mg deficiency (Cakmak et al. 1994a; Cakmak and Kirkby 2008; Bang et al. 2021).

The commonly accepted critical deficiency Mg concentration in leaves is below 1 g Mg kg⁻¹ for most of crop plants (Hauer-Jákli and Tränkner 2019; Marschner 2012). As shown in Fig. 2, the

Table 2 K:Mg ratios in the root, stalk, fully expanded old leaves and young leaves of sugarcane plants grown under increasing K treatments (i.e., 103, 206, 309 and 412 mg K L⁻¹)at low (2 mg L⁻¹) and adequate (17 mg L⁻¹) Mg supplies in nutrient solution for 110 days

K Treatments (mg L ⁻¹)	K:Mg ratio							
	Root		Stalk		Fully expanded leaves		Young leaves	
	Mg-def.	Mg-adq	Mg-def.	Mg-adq.	Mg-def.	Mg-adq.	Mg-def.	Mg-adq.
103	12.2 cA†	2.7 bB	24.5 cA	7.4 cB	18.4 aA	4.4 bB	15.2 abA	5.9 cB
206	19.1 bA	4.1 bB	33.6 bA	13.1 bcB	21.3 aA	5.1 bB	14.8 bA	6.6 bcB
309	20.2 bA	8.2 aB	40.8 bA	18.5 abB	21.3 aA	7.5 abB	17.0 aA	8.3 bB
412	25.6 aA	10 aB	51.2 aA	25.8 aB	20.8 aA	10 aB	17.0 aA	10.4 aB

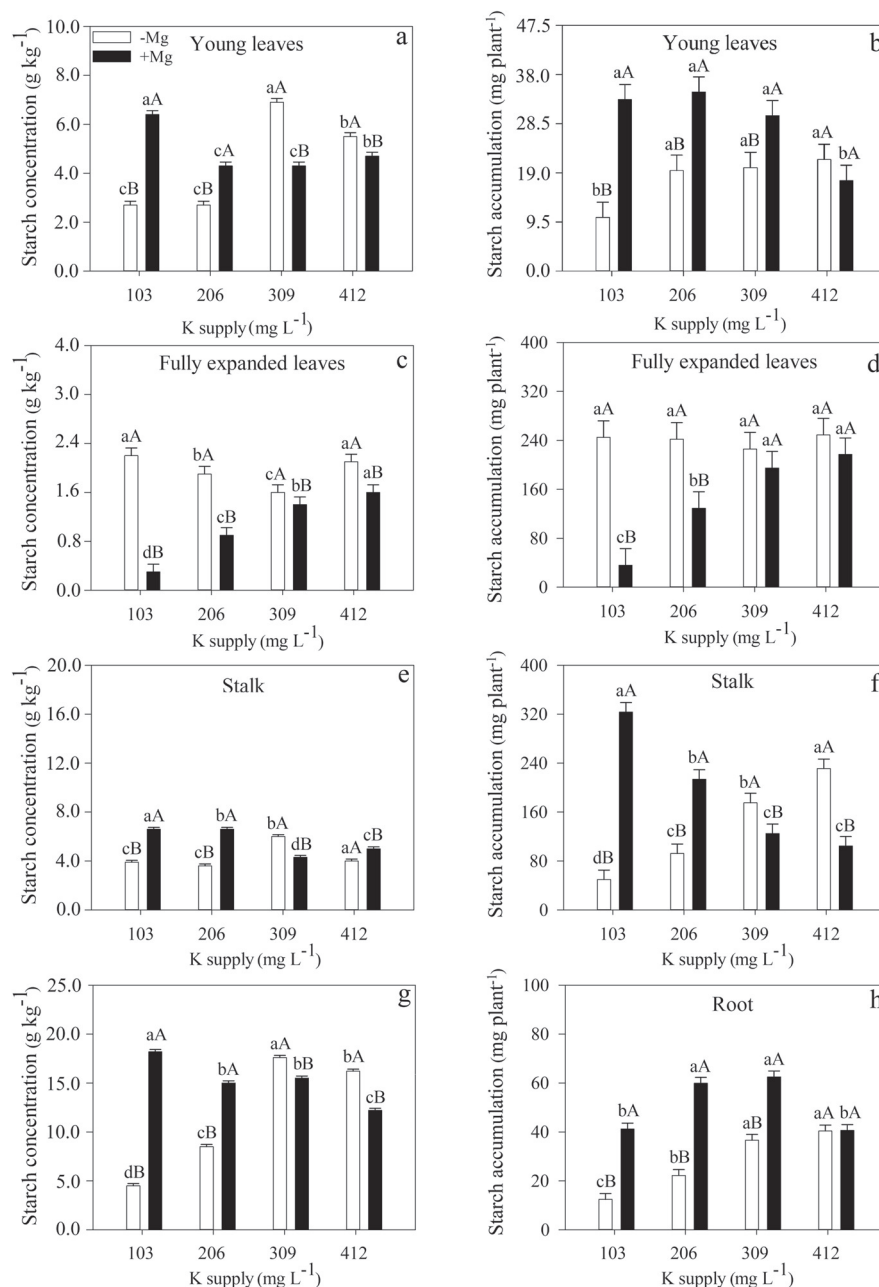
†Lowercase letter compare K levels within the same Mg condition, whilst uppercase letters compare the Mg levels within the same K level by LSD test ($p \leq 0.05$). Mg-def and Mg-adq show the plants with low and adequate Mg supply, respectively

Mg concentration of fully expanded leaves and also the stalks is below 1.0 g kg⁻¹ for the Mg-deficient plants, regardless of the K dose applied. In case of K-induced Mg deficiency despite adequate Mg supply, leaf Mg concentration was 1.4 g kg⁻¹ suggesting that the Mg concentration of the leaves may not be a good indicator for the Mg nutritional status of plants when grown under conditions of high K applications. Very high concentration of K within leaf tissue might be a competitive antagonist of Mg which may then cause imbalance and deficiency with Mg in leaf tissue (Gerendás and Führes 2013; Marschner 2012). Therefore, leaf K:Mg ratios might be useful under growth conditions where K fertilizers are commonly applied at high doses. In the present study, the plants affected by Mg deficiency due to low Mg supply, leaf K:Mg ratio is above 15 (Table 2). However, in case of the K-induced Mg deficiency despite adequate Mg supply, K:Mg ratio was about 10 (Table 2). Since the growth of plants with adequate Mg treatment showed a decline even at the dose of 309 mg K L⁻¹, it can be suggested that the leaf K:Mg ratios ≥ 7 might be indicative of Mg deficiency stress caused by the high K accumulation in leaf tissue under given experimental conditions. The problem with impaired Mg nutrition due to high levels of K is increasing in cropping systems (Morton et al. 2008; Römhild and Kirkby 2010) as well as in animal nutrition (Zelal 2017). In addition, it is important to mention that the Cl concentrations of the experimental plants were within the normal range found in 670 plant species (Watanabe et al. 2007) and minimally affected by the treatments. Therefore, the effects associated with the K supply (in form of KCl) are not related to any possible Cl effect.

Impairment in Mg nutritional status of plants by high K applications is well-documented for several crops including rice (Ding et al. 2006), citrus (Morton et al. 2008), tomato (Li et al. 2018) and potato (Koch et al. 2019). Rhodes et al. (2018) also showed a clear depressive effect of increasing K application on leaf Mg concentration in sugarcane plants grown in different soils of South Africa. It is obvious that high K application has an inhibitory effect on root uptake and shoot accumulation of Mg in sugarcane. A similar result was also found in our study (Fig. 2). However, the present study showed that decreases in Mg concentration of sugarcane plants by high K treatments was pronounced more in root (Fig. 2). This result indicates that K also adversely affects the root-to-shoot transport of Mg in sugarcane plants. Similar findings were also reported by Ding et al. (2006) for rice and Li et al. (2018) for tomato. Probably, the Mg transporters responsible for mediating Mg transport from roots into shoots (Sun et al. 2019; Tanoi et al. 2014) are sensitive to high K concentrations in roots. Additional research is required to study the effects of high K doses on the activity of Mg transporters involved in loading and transport of Mg in xylem of the roots.

In contrast to xylem transport of Mg, the effects of high K on root Mg uptake have been well-studied. The transporters involved in root Mg uptake are non-specific and maintain also uptake of other cations like K (Xie et al. 2020). Therefore, root Mg uptake through these unspecific transporters is impaired at high K application doses (Senbayram et al. 2016). By contrast, the transporters responsible specifically for root K uptake are not sensitive to Mg. However,

Fig. 3 Concentration and accumulation of starch in the young leaves (**a**, **b**), fully expanded leaves (**c**, **d**), stalk (**e**, **f**) and root (**g**, **h**) of sugarcane plants grown under increasing K treatments (i.e., 103, 206, 309 and 412 mg K L⁻¹) at low (2 mg L⁻¹; ---○---) and adequate (17 mg L⁻¹; —●—) Mg supplies in nutrient solution for 110 days. Vertical bars indicate the LSD test ($p \leq 0.05$)



recently it has been reported that root Mg uptake in *Arabidopsis* plants measured by using radioisotope ²⁸Mg was sensitive to Ca but not to K (Ogura et al. 2018). It is clear that further physiological and molecular research is needed investigating simultaneously root uptake and xylem loading of Mg at different K doses.

Majority of the publications in literature shows no effect of Mg on root K uptake, even at low K

concentrations in growth medium (Gransee and Fuehrs 2013; Senbayram et al. 2016; Xie et al. 2020). In the experiments conducted with young lemon trees, Troløve and Reid (2012) showed that increasing Mg treatments from very low to very high doses did not affect leaf K concentrations, even at the excessive Mg application dose that depressed plant growth. The lack of the effect of Mg on root K uptake has been described to less sensitivity of high K transport

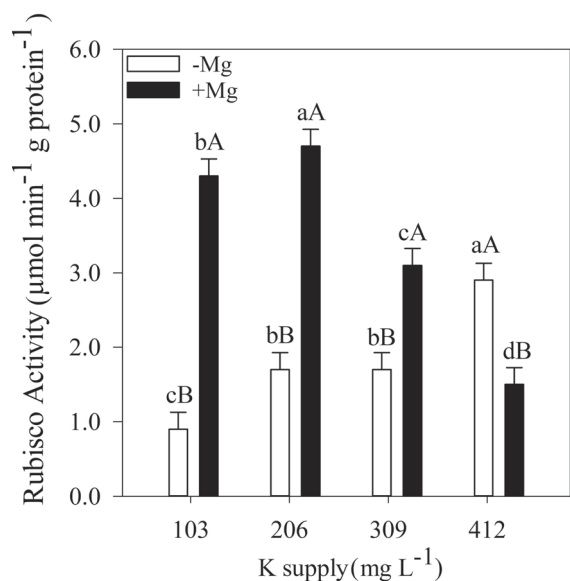


Fig. 4 Ribulose-1,5-biphosphate carboxylase (Rubisco) activity in the fully expanded leaves of sugarcane plants grown under increasing K treatments (i.e., 103, 206, 309 and 412 mg K L⁻¹) at low (2 mg L⁻¹; ---○---) and adequate (17 mg L⁻¹; —●—) Mg supplies in nutrient solution for 110 days. Lowercase letter compare K levels within the same Mg condition, whilst uppercase letters compare Mg levels within the same K level by the LSD test ($p \leq 0.05$)

systems to Mg (Horie et al. 2011; Lan et al. 2010). The results in Fig. 2 showed that the plant K concentrations are either not or only minimally affected by higher Mg application. Only at the lowest K application, a reducing effect of Mg has been found on K concentration of plants. There are published reports showing possible antagonistic effects of high Mg on K uptake (Hauer-Jákli and Tränkner 2019). It can be generalized and suggested that the antagonistic effect of Mg on root K uptake is, obviously, not as strong as the antagonistic effect of K on Mg uptake.

Accumulation of starch in Mg deficient source organs such as old leaves is well-known and caused mainly by the impairments of the phloem export of sucrose from source leaves (Cakmak et al. 1994a, b; Hermans et al. 2005). This finding has been also shown recently in plants grown under field conditions (Zhang et al. 2020). In Mg-deficient sugarcane plants there is an extensive accumulation of starch in the source leaves, especially at lower K applications. By contrast, the sink organs such as young leaves and

roots contained lower amounts of starch when compared to the Mg adequate plants. Differential starch accumulation in source and sink organs as found in sugarcane plants was also reported for common bean (Cakmak et al. 1994a), wheat (Ceylan et al. 2016) and soybean (Peng et al. 2018). It is clear that in sugarcane plants, Mg deficiency has a significant adverse effect on phloem export of sucrose from source organs. It was interesting to note that in case of the highest K application, the plants under low and adequate Mg treatments were almost same in their effects on starch accumulation in roots, young and old leaves (Fig. 3). This is probably because the Mg-adequate plants behaved like Mg-deficient plants due to high K-induced Mg deficiency.

Rubisco enzyme is a well-known Mg dependent enzyme (Marschner 2012; Tränkner et al. 2018; Tränkner and Jaghdani 2019) and its activity is significantly affected by the Mg treatments (Fig. 4). Reduced Rubisco activity can be expected in low Mg and K plants due to excessive accumulation of photoassimilates in leaves. Accordingly, Araya et al. (2006) showed that high accumulation of carbohydrates in leaves causes a negative feedback effect on the Rubisco activity. Our results showed that adequate Mg supply increased leaf Rubisco activity at each K treatments, except the highest K treatment, most likely due to K-induced Mg deficiency. There is no clear consistent effect of varied K nutrition on Rubisco activity in literature (Jákli et al. 2017; Tränkner et al. 2018); but, majority of the results show no direct effect of K on Rubisco activity (Tränkner et al. 2018). On the other hand, our results suggested that high K levels resulted in an increase in Rubisco activity in Mg-deficient plants. Although there is no evidence that K is involved in Rubisco activation, it is possible that this element can change the Rubisco activity by affecting the cell pH-optimum for its activation and the amount of substrate for its reaction (CO₂ and possibly RuBP) (Tränkner et al. 2018). Therefore, the increase in the K concentration in the nutrient solution can stimulate Rubisco activity in Mg-deficient plants. In addition, the highest Rubisco activity (at adequate Mg supply plus 206 mg L⁻¹ K) coincided with the increase in shoot and root growth, indicating a possible positive correlation between these parameters. Interestingly, increasing K application under low Mg increased the activity of Rubisco which might be

related to a partial replacement of the positive effects of Mg on Rubisco activity by K, especially in case of K-induced Mg deficiency. This issue, however, needs a further investigation.

Conclusions

Our results clearly showed that the detrimental effects of Mg deficiency on sugarcane plant growth were pronounced when plants are grown under very high K doses. Even, the leaf symptoms of Mg deficiency also occurred in the sugarcane plants grown in good Mg medium but treated with very high K. At sufficient Mg supply, high K supply interfered with the positive effects of Mg on Rubisco activity and starch allocation. In plants grown with adequate Mg but also with very high K, starch concentrations of the sink organs such as roots and stalks are reduced. It is obvious that the imbalance between K and Mg nutrition in sugarcane plants caused by excess K applications may result in serious consequences in sugar yield with important economic impacts. Finally, it is important to mention that vinasse as a high K source is being extensively applied in sugarcane production (Ortegon et al. 2016; Garcia et al. 2020), which may induce Mg deficiency in plants due to unbalanced K: Mg in this product. Therefore, a particular attention should be paid to Mg nutritional status of plants treated commonly by vinasse.

Acknowledgements AG would like to thank the National Council for Scientific and Technological Development (CNPq) for financial support (Grant number 140435/2015-8), CACC and CAR for the award for excellence in research, and ARR for the research fellowship (Grant number 309380/2017-0).

Author contributions A.G and C.A.C.C. worked on the research designing and conduction, data analysis, writing and formatting the manuscript. C.A.R, J.W.B., C.A.C.N., J.M.M, A.R.d.R. revised this draft by rewriting, discussing and commenting. I.C. helped with writing, results interpretation and revision of the manuscript. All authors contributed significantly on this manuscript.

Funding National Council for Scientific and Technological Development (CNPq) - Grant number 140435/2015-8.

Data availability Data can be provided upon request.

Code availability Not Applicable.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

- Araya T, Noguchi K, Terashima I (2006) Effects of carbohydrate accumulation on photosynthesis differ between sink and source leaves of *Phaseolus vulgaris* L. *Plant Cell Physiol* 47:644–652
- Association Official Analytical Chemistry - AOAC (1995) Official methods of analysis. 13th ed. Washington, 1015 p
- Bang TC, Husted S, Laursen KH, Persson DP, Schjoerring JK (2021) The molecular–physiological functions of mineral macronutrients and their consequences for deficiency symptoms in plants. *New Phytol* 229(5):2446–2469
- Cakmak I (2005) The role of potassium in alleviating detrimental effects of abiotic stresses in plants. *J Plant Nutr Soil Sci* 168:521–530
- Cakmak I, Kirkby EA (2008) Role of magnesium in carbon partitioning and alleviating photooxidative damage. *Physiol Plant* 133:692–704
- Cakmak I, Hengeler C, Marschner H (1994a) Partitioning of shoot and root dry matter and carbohydrates in bean plants suffering from phosphorus, potassium and magnesium deficiency. *J Exp Botany* 45:1245–1250
- Cakmak I, Hengeler C, Marschner H (1994b) Changes in phloem export of sucrose in leaves in response to phosphorus, potassium and magnesium deficiency in bean plants. *J Exp Botany* 45:1251–1257
- Cazotti MM, Costa LMD, Cecon PR (2019) Biogenic, sedimentary, and metamorphic limestone: a comparative characterization of soil amendment. *Rev Ceres* 66:63–71
- Ceylan Y, Kutman UB, Mengutay M, Cakmak I (2016) Magnesium applications to growth medium and foliage affect the starch distribution, increase the grain size and improve the seed germination in wheat. *Plant Soil* 406:145–156
- Contreras AM, Elena R, Maylier P, Langenhove HV, Dewulf J (2009) Comparative life cycle assessment of four alternatives for using by-products of cane sugar production. *J Clean Prod* 17:772–779
- Dechen AR, Carmello QADC, Monteiro FA, Nogueirol RC (2016) Role of magnesium in food production: an overview. *Crop Pasture Sci* 66:1213–1218
- Ding Y, Luo W, Xu G (2006) Characterisation of magnesium nutrition and interaction of magnesium and potassium in rice. *Ann Appl Biol* 149:111–123
- Dreyer I, Gomez-Porras JL, Riedelsberger J (2017) The potassium battery: a mobile energy source for transport processes in plant vascular tissues. *New Phytol* 216:1049–1053
- Ericsson T, Kähr M (1995) Growth and nutrition of birch seedlings at varied relative addition rates of magnesium. *Tree Physiol* 15:85–93
- Furlani AMC, Furlani PR (1988) Composição e pH de soluções nutritivas para estudos fisiológicos e seleção de plantas

- em condições nutricionais adversas. Technical Report 121, Agronomic Institute Campinas 1–34 (in Portuguese)
- Garcia A, Crusciol CAC, McCray JM, Nascimento CAC, Martello JM, Siqueira GF, Tarumoto MB (2020) Magnesium as a promoter of technological quality in sugarcane. *J Plant Nutr Soil Sci* 20:19–30
- Geng GT, Cakmak I, Ren T, Lu ZF, Lu JW (2021) Effect of magnesium fertilization on seed yield, seed quality, carbon assimilation and nutrient uptake of rapeseed plants. *Field Crop Res* 264:108082. <https://doi.org/10.1016/j.fcr.2021.108082>
- Gerendás J, Führes H (2013) The significance of magnesium for crop quality. *Plant Soil* 368:101–128
- Granssee A, Führes H (2013) Magnesium mobility in soils as a challenge for soil and plant analysis, magnesium fertilization and root uptake under adverse growth conditions. *Plant Soil* 368:5–21
- Gruber BD, Giehl RF, Friedel S, von Wörén N (2013) Plasticity of the Arabidopsis root system under nutrient deficiencies. *Plant Physiol* 163:161–179
- Guo W, Nazim H, Liang Z, Yang D (2016) Magnesium deficiency in plants: an urgent problem. *Crop J* 4:83–91
- Hauer-Jákli M, Tränkner M (2019) Critical leaf magnesium thresholds and the impact of magnesium on plant growth and photo-oxidative defense: a systematic review and meta-analysis on 70 years of research. *Front Plant Sci* 10:766. <https://doi.org/10.3389/fpls.2019.00766>
- Hazra S, Henderson JN, Liles K, Hilton MT, Wachter RM (2015) Regulation of ribulose-1, 5-bisphosphate carboxylase/oxygenase (rubisco) activase product inhibition, cooperativity, and magnesium activation. *J Biol Chem* 290:24222–24236
- Hermans C, Verbruggen N (2005) Physiological characterization of mg deficiency in *Arabidopsis thaliana*. *J Exp Botany* 56:2153–2161
- Hermans C, Bourgis F, Faucher M, Delrot S, Strasser RJ, Verbruggen N (2005) Magnesium deficiency in sugar beet alters sugar partitioning and phloem loading in young mature leaves. *Planta* 220:541–549
- Horie T, Brodsky DE, Costa A, Kaneko T, Schiavo FL, Katsuhara M, Schroeder JI (2011) K⁺ transport by the *OsHKT2*; 4 transporter from rice with atypical Na⁺ transport properties and competition in permeation of K⁺ over Mg²⁺ and Ca²⁺ ions. *Plant Physiol* 156:1493–1507
- Jaghdani SJ, Jahns P, Tränkner M (2021) Mg deficiency induces photo-oxidative stress primarily by limiting CO₂ assimilation and not by limiting photosynthetic light utilization. *Plant Science*, in press. <https://doi.org/10.1016/j.plantsci.2020.110751>
- Jákli B, Tavakol E, Tränkner M, Senbayram M, Dittert K (2017) Quantitative limitations to photosynthesis in K deficient sunflower and their implications on water-use efficiency. *J Plant Phys* 209:20–30
- Koch M, Busse M, Naumann M, Jákli B, Smit I, Cakmak I, Hermans C, Pawelzik E (2019) Differential effects of varied potassium and magnesium nutrition on production and partitioning of photoassimilates in potato plants. *Physiol Plant* 166:921–935
- Lan WZ, Wang W, Wang SM, Li LG, Buchanan BB, Lin HX, Luan S (2010) A rice high-affinity potassium transporter (*HKT*) conceals a calcium-permeable cation channel. *Proc Natl Acad Sci* 107:7089–7094
- Levene H (1960) Robust tests for equality of variances. In Ingram Olkin; Harold Hotelling; et al. (eds.). Contributions to probability and statistics: essays in honor of Harold Hotelling. Stanford University Press, pp. 278–292
- Li HX, Chen ZJ, Zhou T, Liu Y, Zhou JB (2018) High potassium to magnesium ratio affected the growth and magnesium uptake of three tomato (*Solanum lycopersicum* L.) cultivars. *J Integr Agric* 17:2813–2821
- Lingle SE, Johnson RM, Tew TL, Viator RP (2010) Changes in juice quality and sugarcane yield with recurrent selection for sucrose. *Field Crops Res* 118:152–157
- Maguire ME, Cowan JA (2002) Magnesium chemistry and biochemistry. *Biometals* 15(3):203–210
- Marschner H (2012) Mineral nutrition of higher plants, 3rd edn. Elsevier, London, p 643p
- Mengutay M, Ceylan Y, Kutman UB, Cakmak I (2013) Magnesium nutrition mitigates adverse effects of heat stress on maize and wheat. *Plant Soil* 368:57–72
- Morton AR, Trolove SN, Kerckhoffs LHJ (2008) Magnesium deficiency in citrus grown in the Gisborne district of New Zealand. *N Z J Crop Hortic Sci* 36:199–213
- Nelson N (1994) A photometric adaptation of the Somogyi method for the determination of glucose. *J Biol Chem* 153:375–390
- Ogura T, Kobayashi NI, Suzuki H, Iwata R, Nakanishi TM, Tanoi K (2018) Magnesium uptake characteristics in *Arabidopsis* revealed by 28 mg tracer studies. *Planta* 248:745–750
- Ortegon GP, Arboleda FM, Candela L, Tamoh K, Valdes-Abellan J (2016) Vinasse application to sugar cane fields. Effect on the unsaturated zone and groundwater at Valle del Cauca (Colombia). *Sci Total Environ* 539:410–419
- Patrick JW, Botha FC, Birch RG (2013) Metabolic engineering of sugars and simple sugar derivatives in plants. *Plant Biotech J* 11:142–156
- Peng WT, Zhang LD, Zhou Z, Fu C, Chen ZC, Liao H (2018) Magnesium promotes root nodulation through facilitation of carbohydrate allocation in soybean. *Physiol Plant* 163:372–385
- Reid CD, Tissue DT, Fiscus E, Strain BR (1997) Comparison of spectrophotometric and radioisotopic methods for the assay of rubisco in ozone-treated plants. *Physiol Plant* 101:398–404
- Rhodes R, Miles N, Hughes JC (2018) Interactions between potassium, calcium and magnesium in sugarcane grown on two contrasting soils in South Africa. *Field Crops Res* 223:1–11
- Römheld V, Kirkby EA (2010) Research on potassium in agriculture: needs and prospects. *Plant Soil* 335:155–180
- Senbayram M, Granssee A, Wahle V, Thiel H (2016) Role of magnesium fertilisers in agriculture: plant–soil continuum. *Crop Pasture Sci* 66:1219–1229
- Shabala S, Hariadi Y (2005) Effects of magnesium availability on the activity of plasma membrane ion transporters and light-induced responses from broad bean leaf mesophyll. *Planta* 221:56–65
- Shapiro SS, Wilk MB (1965) An analysis of variance test for normality (complete samples). *Biometrika* 52:591–611

- Somogyi M (1952) Notes on sugar determination. *J Biol Chem* 195:19–23
- Soratto RP, Crusciol CAC, Fernandes AM, Cantarella H, Quaggio JA, Vitti GC (2019) Reactivity of sedimentary and metamorphic limestones of different particle sizes under controlled conditions. *Commun Soil Sci Plant Anal* 50:464–473
- Stevens G, Gladbach T, Motavalli P, Dunn D (2005) Soil calcium: magnesium ratios and lime recommendations for cotton. *J Cotton Sci* 9:65–71
- Sun J, Li S, Wang S, Xiao Z, Zhang Z, Li J, Li N (2019) Characterization of the main magnesium transporters mediating different mg translocation from root to shoot between mg-tolerant and mg-sensitive *Brassica napus* cultivars under magnesium deficiency stress. *Plant Soil* 445:453–468
- Tanoi K, Kobayashi NI, Saito T, Iwata N, Kamada R, Iwata R, Nakanishi TM (2014) Effects of magnesium deficiency on magnesium uptake activity of rice root, evaluated using ²⁸mg as a tracer. *Plant Soil* 384:69–77
- Team R C (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.[Internet]. Vienna, Austria
- Tennant D (1975) A test of a modified line intercept method of estimating root length. *The Journal of Ecology* 63:995–1001
- Tränkner M, Jaghdani SJ (2019) Minimum magnesium concentrations for photosynthetic efficiency in wheat and sunflower seedlings. *J Plant Physiol Biochem* 144(234):243
- Tränkner M, Tavakol E, Jákl B (2018) Functioning of potassium and magnesium in photosynthesis, photosynthate translocation and photoprotection. *Physiol Plant* 163:414–431
- Trolove SN, Reid JB (2012) What is the critical leaf magnesium concentration to identify magnesium deficiency in Meyer lemon? *N Z J Crop Hortic Sci* 40:139–146
- Watanabe T, Broadley MR, Jansen S, White PJ, Takada J, Satake K, Takamatsu T, Tuah SJ, Osaki M (2007) Evolutionary control of leaf element composition in plants. *New Phytol* 174:516–523
- Xie K, Cakmak I, Wang S, Zhang F, Guo S (2020) Synergistic and antagonistic interactions between potassium and magnesium in higher plants. *Crop J* 2020
- Zelal A (2017) Hypomagnesemia tetany in cattle. *Adv Dairy Res* 5:2–9
- Zhang B, Cakmak I, Feng J, Yu C, Chen X, Xie D, Wu L, Song Z, Cao J, He Y (2020) Magnesium deficiency reduced the yield and seed germination in wax gourd by affecting the carbohydrate translocation. *Front Plant Sci* 11:797
- Zörb C, Senbayram M, Peiter E (2014) Potassium in agriculture-status and perspectives. *Plant Physiol* 171:656–669

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.