UNIVERSIDADE ESTADUAL PAULISTA
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Campus de Ilha Solteira

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CÂMPUS DE ILHA SOLTEIRA

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PHYSIOLOGICAL ROLES OF MAGNESIUM ON UREIDES METABOLISM OF SOYBEAN PLANTS CULTIVATED IN HIGH ALTITUDE CERRADO

Ilha Solteira
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PROGRAMA DE PÓS-GRADUAÇÃO EM AGRONOMIA

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PHYSIOLOGICAL ROLES OF MAGNESIUM ON UREIDES METABOLISM OF SOYBEAN PLANTS CULTIVATED IN HIGH ALTITUDE CERRADO

Thesis presented to the Engineering College of Ilha Solteira – UNESP as part of the requirements to obtain the title of Doctor in Agronomy. Specialization: Production Systems

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Ilha Solteira, 22 de dezembro de 2021

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“Que possamos transmitir a todos em nosso caminho,
a bondade de Deus que habita em nosso coração”
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ABSTRACT

Magnesium (Mg) is often overlooked in fertilization practices and agricultural research. However, its vital role is being increasingly recognized, considering its essentiality in several physiological processes in plants, such as photosynthesis, production and transport of photoassimilates, enzymatic antioxidant metabolism, nitrogen (N) metabolism and biological nitrogen fixation (BNF). The aim of this study was to provide an overview of how these processes are regulated by Mg. For this, chapter 1 of this study composes a systematic literature review, gathering information from 50 articles published in the last two decades about Mg. In chapters 2, 3 and 4, the objective was to evaluate the effect of doses (0 to 90 kg ha^{-1}) and sources of magnesium oxide (MgO) and magnesium sulfate ($MgSO_4$) on the photosynthetic process, production of photoassimilates, N and BNF metabolism in soybean varieties, since the role of Mg in BNF and ureide synthesis is still poorly understood. In chapter 1, the results of the literature demonstrate that the application of Mg increased photosynthetic pigments, liquid photosynthesis and production of photoassimilates. Higher sucrose concentrations in plant roots compared to shoots, together with increased activity of ATP-ases enzymes, indicate greater transport of photoassimilates by the phloem when Mg is applied. Mg increased the resistance of plants to abiotic stresses, reducing the need for expression of the antioxidant enzymes SOD, GPX, APX, CAT and POD, when compared to Mg deficient situations. Mg improved N and FBN metabolism, increasing the activity of nitrate reductase, nitrite reductase, glutamine synthase, GOGAT and nitrogenase enzymes. In chapter 2, the results showed that MgO increased the leaf Mg content of soybeans and did not change the levels of calcium (Ca) and potassium (K) in the leaf tissue, providing an increase in photosynthetic pigments and production of photoassimilates. There was an increase in the concentrations of nitrogen compounds and greater conversion of atmospheric N_2 into ureides in soybean plants. Regarding the results of chapter 3, the use of $MgSO_4$ increased the Mg content in soybean leaves, did not change the K content and reduced the Ca content. There was an increase in photosynthetic and photoassimilated pigments. Higher rates of photoassimilates provided greater conversion of atmospheric N_2 into ureides for NA 5909 RG RR (allantoin and allantoic acid) and P98Y30 (allantoic acid), indicating higher FBN. In chapter 4, MgO and $MgSO_4$ sources increased leaf Mg content and did

not change leaf Ca content in most varieties. With the application of Mg, the photosynthetic and photoassimilated pigments were increased. With greater production of photoassimilates, there was greater activity of N metabolism, due to greater production of amino acids and greater conversion of atmospheric N_2 and synthesis of ureides in most varieties, resulting in greater grain yield. The MgO source promoted the greatest increases in the concentrations of photosynthetic pigments, photoassimilates, ureides and grain yield for most varieties. This thesis contains up-to-date and valuable information on the influence of Mg on important physiological processes in plants. Incorporating these findings in future work can contribute to the optimization of fertilization programs and management strategies through magnesium fertilization in plants.

Keywords: Mg. *Glycine max* (L.) Merrill. biological nitrogen fixation. antioxidant metabolism. genotipic variation.

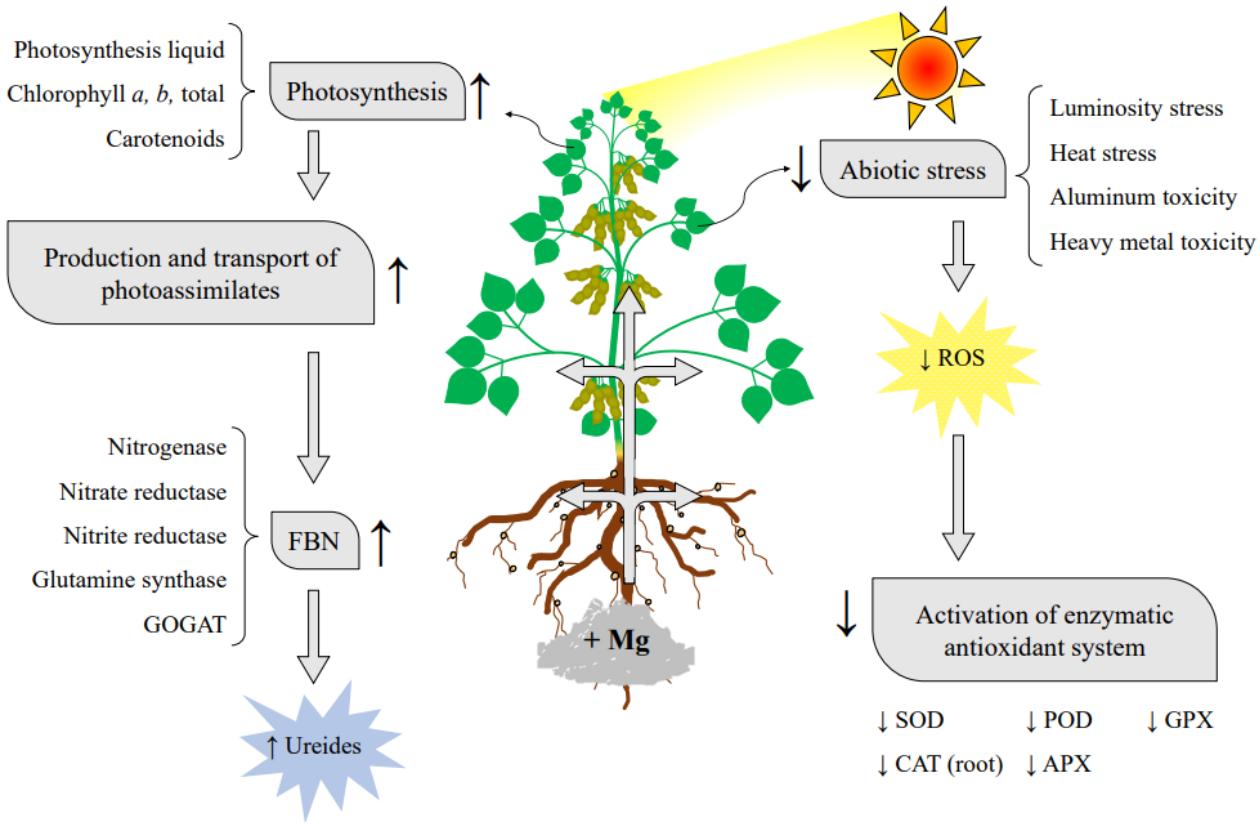
RESUMO

O magnésio (Mg) é frequentemente esquecido nas práticas de fertilização e pesquisas agrícolas. No entanto, seu papel vital está sendo cada vez mais reconhecido, considerando sua essencialidade em diversos processos fisiológicos nas plantas, como fotossíntese, produção e transporte de fotoassimilados, metabolismo antioxidante enzimático, metabolismo de nitrogênio (N) e fixação biológica de nitrogênio (FBN). O objetivo deste estudo foi fornecer uma visão geral de como esses processos são regulados pelo Mg. Para isso, o capítulo 1 deste estudo compõe uma revisão sistemática da literatura, reunindo informações de 50 artigos publicados nas últimas duas décadas sobre o Mg. Nos capítulos 2, 3 e 4, objetivou-se avaliar o efeito de doses (0 a 90 kg ha^{-1}) e fontes de óxido de magnésio (MgO) e sulfato de magnésio ($MgSO_4$) sobre o processo fotossintético, produção de fotoassimilados, metabolismo de N e FBN em variedades de soja, uma vez que o papel do Mg na FBN e síntese de ureídeos ainda é pouco conhecido. No capítulo 1, os resultados da literatura demonstram que a aplicação de Mg aumentou os pigmentos fotossintéticos, a fotossíntese líquida e a produção de fotoassimilados. As maiores concentrações de sacarose nas raízes das plantas em comparação com a parte aérea, juntamente com o aumento da atividade das enzimas ATP-ases, indicam maior transporte de fotoassimilados pelo floema quando o Mg é aplicado. O Mg aumentou a resistência das plantas a estresses abióticos, reduzindo a necessidade de expressão das enzimas antioxidantes SOD, GPX, APX, CAT e POD, quando comparado a situações de deficiência de Mg. O Mg melhorou o metabolismo do N e do FBN, aumentando a atividade das enzimas nitrato redutase, nitrito redutase, glutamina sintase, GOGAT e nitrogenase. No capítulo 2, os resultados mostraram que o MgO aumentou o teor foliar de Mg da soja e não alterou os teores de cálcio (Ca) e potássio (K) no tecido foliar, proporcionando aumento dos pigmentos fotossintéticos e produção de fotoassimilados. Houve aumento nas concentrações de compostos nitrogenados e maior conversão de N_2 atmosférico em ureídeos nas plantas de soja. Em relação aos resultados do capítulo 3, o uso de $MgSO_4$ aumentou o teor de Mg nas folhas de soja, não alterou o teor de K e reduziu o teor de Ca. Houve aumento de pigmentos fotossintéticos e fotoassimilados. Maiores taxas de fotoassimilados proporcionaram maior conversão de N_2 atmosférico em ureídeos para NA 5909 RG RR (alantoína e ácido alantóico) e P98Y30 (ácido alantóico), indicando maior FBN. No capítulo 4, as

fontes de MgO e $MgSO_4$ aumentaram o teor de Mg foliar e não alteraram o teor de Ca foliar na maioria das variedades. Com a aplicação do Mg, os pigmentos fotossintéticos e fotoassimilados foram aumentados. Com maior produção de fotoassimilados, houve maior atividade do metabolismo de N, devido à maior produção de aminoácidos e maior conversão de N_2 atmosférico e síntese de ureídeos na maioria das variedades, resultando em maior rendimento de grãos. A fonte de MgO promoveu os maiores aumentos nas concentrações de pigmentos fotossintéticos, fotoassimilados, ureídeos e rendimento de grãos para a maioria das variedades. Esta tese contém informações atualizadas e valiosas sobre a influência do Mg em importantes processos fisiológicos em plantas. A incorporação desses achados em trabalhos futuros pode contribuir para a otimização dos programas de adubação e estratégias de manejo por meio da adubação com magnésio nas plantas.

Palavras-chave: Mg. *Glycine max* (L.) Merrill; fixação biológica de nitrogênio; metabolismo antioxidante; variação genotípica.

Graphycal abstract: The application of Mg increases the concentrations of Mg in plant tissues, promoting higher concentrations of photosynthetic pigments and liquid photosynthesis. This effect contributes to greater production and transport of photoassimilates in plants, positively influencing the activity of important enzymes belonging to the metabolism of N and FBN, with ureides as the final product. At the same time, the highest concentrations of intracellular Mg can alleviate the inhibitory action of different abiotic stressors in plants, reducing the production of ROS and, consequently, the activity of enzymatic antioxidant enzymes, when compared to situations deficient in Mg.



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SUMARY

1. LITERATURE REVIEW - ROLES OF MAGNESIUM ON CROP YIELD: BIOLOGICAL NITROGEN FIXATION, SUGAR AND ANTIOXIDANT METABOLISM AS ATTENUATORS OF ABIOTIC STRESSES

1.1. ABSTRACT

Magnesium (Mg) has a physiological role in the plant photosynthetic process, production, and transport of photoassimilates, antioxidant metabolism, and nitrogen (N) metabolism. In this study, 50 articles that evaluated the influence of the application of Mg on the physiological and biochemical responses of plants were assessed. Regarding the nutritional status of the plants, 44% of the results presented the range of 1.5 to 3.5 g kg^{-1} of Mg. There was an increase in the concentrations of photosynthetic pigments and the net photosynthetic rate in leaves of plants fertilized with Mg. The concentrations of total sugars in the aerial part and roots of the plants increased by 56 and 75% in response to the application of Mg. Considering the high-energy demand necessary for the transport of photoassimilates, an increase in the activity of ATPase enzymes were also observed. Mg affects the expression of enzymatic antioxidant metabolism by regulating the activity of superoxide dismutase (SOD), guaiacol peroxidase (GPX), peroxidase (POD), and ascorbate peroxidase (APX) enzymes in plant leave to mitigate the toxic effect of reactive oxygen species. In addition Mg increases nodulation in leguminous plants, which reflects the increased activity of nitrogenase, ureides improving N metabolism. There is a significant increase in the activity of nitrate reductase, nitrite reductase, glutamine synthase, and GOGAT in the leaves of plants fertilized with Mg, which reflects in the higher productivity of the plants. The present review reports important information about the effect of Mg in the photosynthetic process, production and transport of photoassimilates (sugars), antioxidant metabolism, and N metabolism in plants, which can contribute to the optimization of fertilization programs with Mg in plants.

Keywords: Nitrogen metabolism. leguminous plants. magnesium. ROS. photosynthesis. antioxidant enzymes.

1.2. INTRODUCTION

The growth and development of plants depend on the ability to uptake the necessary amounts of nutrients, including magnesium (Mg). Although Mg is a “forgotten nutrient” due to the scarcity of research related to the physiological and nutritional role in plants, its vital role is increasingly recognized (CAKMAK; YAZICI, 2010; PENG *et al.,* 2018). Possibly the supply of Mg by liming is the easiest and most economical way to supply Mg to plants (MALAVOLTA, 2006), contributed to the growing disuse of this nutrient. Several studies have addressed the effects of Mg deficiency on the formation of chlorophyll (TRÄNKNER *et al.,* 2016, LI *et al.,* 2017), photosynthesis (YUGUAN *et al.,* 2009, LI *et al.,* 2017), carbohydrate allocation (VERBRUGGEN; HERMANS, 2013; YANG *et al.,* 2017), enzymatic antioxidant system (SILVA *et al.,* 2017; CAI *et al.,* 2019) and nitrogen (N) metabolism enzymes (YIN *et al.,* 2009; YIN *et al.,* 2009; LI *et al.,* 2017). However, despite advances in recent decades, the role of Mg in these important processes has not yet been addressed in a systemic review.

When Mg is present in low concentrations, plant growth and development can be severely affected (VERBRUGGEN; HERMANS, 2013). Natural processes like leaching (GRANSEE; FÜHRS, 2013), soil acidity (KOBAYASHI *et al*., 2013), Al (BOSE *et al*., 2013; CHEN; MA 2013), and Mn (CHEN *et al*., 2017) are among those responsible for reducing Mg concentrations in soils. Anthropic action can also be a limiting factor for Mg in agricultural soils. The unbalanced intake of fertilizers, with high levels of antagonistic ions, such as calcium (Ca), potassium (K) (GRANSEE; FÜHRS, 2013), and ammonium (NH_4^+) (KOBAYASHI *et al.*, 2013; CHEN *et al*., 2017) are among those responsible for interacting antagonistically and reducing Mg concentrations for plants. This unbalanced fertilization, common in modern agricultural systems, has contributed to the Mg deficiency in crops in recent decades (CAKMAK; YAZICI, 2010).

The supply of Mg to the soil is what produces the best results and increases the concentrations of Mg in the tissues of the plants (WHITE; BROADLEY, 2009). When available to plants, Mg is in the form of a divalent cation (Mg^{2+}). Its function as the central atom of the chlorophyll molecule is the

best-studied, with a fundamental role for the realization of photosynthesis (CAKMAK; YAZICI, 2010; CHEN *et al.*, 2017), where it is a key component in the energy transfer process (CAKMAK; YAZICI, 2010). At the same time, it is indispensable for the functioning of more than 300 enzymes (VERBRUGGEN; HERMANS, 2013), including RuBisCO, an enzyme responsible for the assimilation of photosynthetic carbon (BHAT *et al.*, 2017). Such capacities involve Mg in many physiological and biochemical processes, thus acting on carbohydrate metabolism (YANG *et al.*, 2017), detoxification of reactive oxygen species (ROSs) (CAKMAK; KIRKBY 2008; YANG *et al.*, 2013), and in the metabolism of N (YANG *et al.*, 2017).

Mg has a great influence on growth and vigor (HUBER; JONES, 2013). Although little studied, there are reports that Mg may have a positive effect on N metabolism and the BNF (PENG *et al.*, 2018). Many enzymes act by catalyzing the chemical reactions involved in this process and the requirement for Mg to activate some of these enzymes is considerable (JAHNEN-DECHENT; KETTELER, 2012). Mg increases the nodulation of soybean plants and consequently ureide metabolism, increasing the efficiency of BNF and the productivity of soybeans (PENG *et al*., 2018). BNF consists of the activity of soil *Rhizobiaceae* bacteria in symbiosis with plants of the Fabaceae family (*Leguminosae*), enabling these plants to obtain the appropriate amounts of atmospheric N_2 for their metabolism.

Mg plays important role in photosynthesis, production of photoassimilates (sugars), antioxidant metabolism, and N metabolism. Therefore, this study aims to compile the results of the research and provide an overview of the physiological and biochemical responses regulated by Mg. In this bibliographic review, a systematic literature search was carried out, gathering studies on the effects of Mg published in the last two decades. Initially, we reviewed the availability of Mg in agricultural soils, factors that limit its availability and uptake by plants. Then, we highlight the importance of Mg for plants, with an emphasis on the photosynthetic process and its influence on the production of photoassimilates (sucrose). Antioxidant metabolism and BNF metabolism were also reviewed.

1.3. RELATIONSHIP AMONG MAGNESIUM IN SOIL, AND PLANT PROCESSES

Mg in agricultural soils

Mg is the eighth-most abundant mineral element in the earth's crust and the second most abundant cation in plants (SENBAYRAM *et al*., 2015). It is a common constituent of many minerals, such as biotite, dolomite, montmorillonite, and vermiculite. However, most of the Mg in the soil (90-98%) is incorporated into the crystalline structure of these minerals and is not directly available for the absorption of plants (SENBAYRAM *et al*., 2015).

Plant growth and development are highly dependent on its ability to uptake the necessary amounts of nutrients (including Mg) from the soil to meet metabolic demands. Soil texture is a key variable that affects the Mg available to plants since Mg is located in clay minerals and associated with cation exchange sites on clay surfaces. Thus, it is expected that clayey soils contain higher concentrations of Mg available for plant needs, while sandy soils have lower concentrations of Mg (MAYLAND; WILKINSON, 1989). To become soluble, Mg adsorbed on a clay particle needs to be replaced by other cations, such as potassium (K) and hydrogen (H), from the soil solution (SENBAYRAM *et al.,* 2015).

In the world, crops face multiple stressors that can limit the uptake of Mg. Natural processes like leaching (GRANSEE; FÜHRS, 2013), soil acidity, and aluminum toxicity (Al) (CHEN; MA, 2013), in addition to anthropic action, such as inbalanced fertilization with high levels of antagonistic ions, such as calcium (Ca) and potassium (K) (GRANSEE; FÜHRS, 2013) are among those responsible for reducing Mg concentrations for plants.

Mg is a cation that has high mobility in soils compared to other cations (CHEN *et al*., 2017). As Mg has a large hydrated radius, it binds weakly to colloids from negatively charged soils and root cell walls (GRANSEE; FÜHRS, 2013). This can result in a high concentration of this nutrient in the soil solution, as well as an increased risk of leaching, according to the water balance, soil acidity, and concentration of other cations in the soil (CHEN *et al*., 2017). This factor can lead

to symptoms of Mg deficiency for plants growing in leachate soils (SENBAYRAM *et al.,* 2015).

The supply of Mg to the soil and fertilization is what produces the best results and increases the concentrations of Mg in plant tissues (WHITE; BROADLEY, 2009). Sources of Mg such as Mg oxide (MgO) and Mg sulfate ($MgSO_4$) are alternatives to supply the crop's need for this nutrient. However, the solubility of the fertilizer containing Mg to be used must be considered. It is commonly accepted that slow-release Mg fertilizers (dolomite or fertilizers that contain Mg in the form of Mg oxide) offer almost no risk of Mg leaching (SENBAYRAM *et al.,* 2015). On the other hand, the application of Mg-soluble fertilizers can lead to Mg losses by leaching in sandy soils, especially in rainy seasons (LOGANATHAN *et al.*, 2005).

Soil acidity is a common problem in tropical soils. In acidic soils, the uptake of Mg by plants is affected, due to the antagonistic action of H (KOBAYASHI *et al.*, 2013), Al (BOSE *et al.,* 2013), and Mn (CHEN *et al.*, 2017). In this case, the soluble ionic aluminum (Al^{+3}) can quickly inhibit the root growth of plants, negatively influencing the nutrient absorption process. This is considered the main limiting factor for agricultural production in acidic soils (DELHAIZE *et al.*, 2012; KOCHIAN *et al.*, 2015).

In soils with a pH below 4.5, the absorption of Mg is inhibited (KOBAYASHI *et al.*, 2013). As a result, serious consequences are expected for agricultural production. This is a consequence of the growing inability of plants to create and maintain a sufficient electrochemical gradient in the plasma membrane of root cells (GRANSEE; FÜHRS, 2013). In this case, an intense Mg leaching is expected.

The inbalanced entry of fertilizers in agricultural areas can also favor the reduction of Mg in the soils. In this case, fertilization of crops has generally focused only on the application of N, P, and K. Mg can interact antagonistically with other ions in the soil, and this can result in lower availability of Mg for plant roots. This unbalanced fertilization, common in modern agricultural systems, has contributed to the Mg deficiency in crops in recent decades (CAKMAK; YAZICI, 2010). In such cases, high levels of ions K (KOBAYASHI *et al.*, 2013; CHEN *et al.*, 2017), can interact antagonistically with Mg.

The competition between Mg and K ions has been extensively studied. Excess K affects the movement of Mg towards the roots (KARLEY; WHITE, 2009), in addition to reducing the bioavailability of Mg in plants (VAN DER HEIJDEN *et al*., 2013). Thus, significantly increasing the available K concentrations can cause an imbalance in the K/Mg ratio. Studies carried out by Li *et al.* (2018) indicated a significant decrease in Mg absorption in tomatoes, when the K/Mg ratio was increased from 4:1 to 8:1. As a result, Li *et al.* (2018) observed a significant reduction in the total tomato biomass. However, it should be noted that such competition is unidirectional, governed only by K (OMAR; EL-KOBBIA, 1966).

The practice of liming, in turn, is considered the easiest and most economical way to supply Mg to plants (MALAVOLTA, 2006), it can also influence the availability of Mg for plants. This facility contributed and made the growing disuse of this nutrient in routine soil fertilization practices justified. However, high levels of Ca supplied to the soil can also interact antagonistically with Mg. Excess Ca result in less Mg availability for plant roots (CHOU *et al*., 2011; SUN *et al*., 2013). In sandy soils with high water conductivity, it can aggravate the depletion of Mg by the excess of Ca, leaching it (GRANSEE; FÜHRS, 2013). On the other hand, excess Mg can also negatively influence Ca concentrations in the plant. In this case, Mg competes with Ca for membrane binding sites and, as such, has been described as a “calcium channel blocker” (WANG *et al*., 2004). Thus, some reports increasing the concentration of Mg in the culture medium may decrease the absorption of Ca in both the aerial part and the roots of plants (YAMANAKA *et al.,* 2010; NIU *et al.,* 2014).

Mg uptake by plants

When available to plants, Mg is in the form of a divalent cation (Mg^{+2}). Mg is essential for plant growth and development. It is widely accepted that the acquisition of Mg from the soil solution by plants is dominated by two main processes: Mass flow (1), a passive movement of Mg^{+2}, driven by the transpiration current; and Diffusion (2), in which Mg moves from areas of higher concentration to areas of lower concentration. Thus, the amounts of Mg to be

captured by the roots of the plants depend on their concentration in the soil solution, as well as on the capacity of the soil to replenish the soil solution with Mg.

Mg ions are known to reach the root surface to the endoderm through the apoplast pathways and then enter plant cells with the aid of transmembrane transporters (FARHAT *et al*., 2016). Seeking to keep the Mg concentrations in the tissues high, the plants developed specific highly efficient transport and translocation systems. These Mg transporters have been discovered and well described in studies using *Arabidopsis* (GUO *et al*., 2010; YUEXI *et al*., 2012; MAO *et al.*, 2014).

After being uptaken by the roots, the Mg is transferred to the aerial part of the plants. The distribution of Mg is carried out with the aid of the transpiration flow by the xylem. This process occurs preferably in the tissues in development (YAMAJI; MA, 2014). With adequate amounts of Mg in the leaves, the plants can perform photosynthesis, producing carbohydrates, and transport the photoassimilates from the sources to the sinks, via the phloem, ensuring the realization of several physiological processes of interest to the plant (VERBRUGGEN; HERMANS, 2013).

Mg is considered a mobile element in plants. Thus, it is easily translocated via the phloem to fruits, seeds, and tubers (WHITE; BROADLEY, 2009). According to CHEN *et al*. (2017), the majority of Mg transported at the cellular level occurs mainly in the forms of Mg-ATP, Mg enzymes, and Mg-chlorophylls, except for the 5-10% Mg used to stabilize the cell wall.

1.4. THE PHYSIOLOGICAL ROLE OF Mg IN THE PLANT

In this review, after the screening of titles and studies, 50 published articles were compiled, being mandatorily dated between these last two decades, as shown in Table 1 (Appendage A). The data collected included the influence of the application of Mg (leaving the deficient state and/or adding in an appropriate situation) in 17 plant species, separated into 9 families.

Comparisons were made by species in this review, totaling 354 effects, stratified into "Aerial part", "Leaves", "Stems", "Roots" and "Others (including

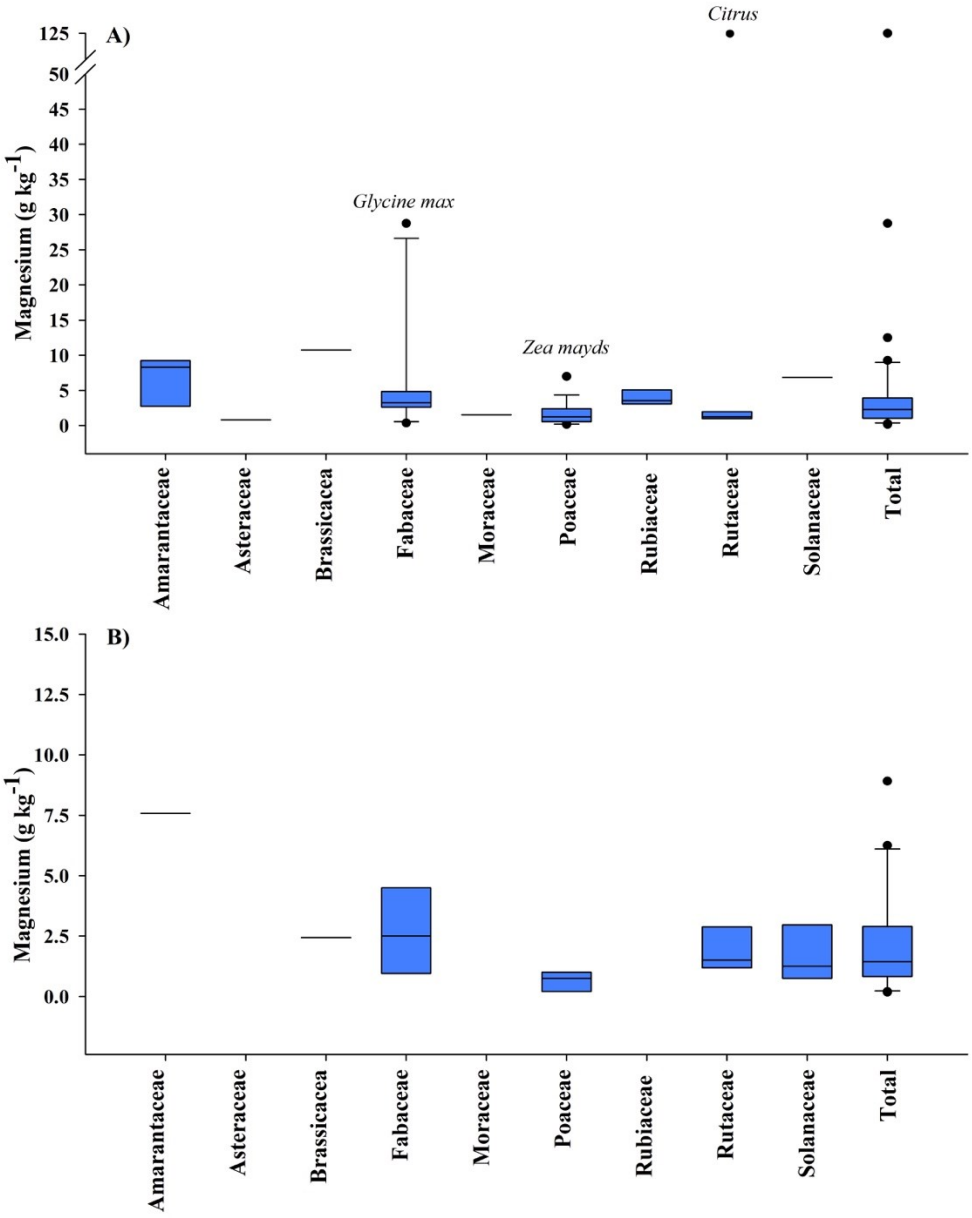
tubers, petiole, fruits or ears, etc.)" (Supplementary material, Table S1). The observed effects contain information on nutritional analyzes (Supplementary material, Tables 2 and 3), photosynthetic parameters (Supplementary material, Table4), sucrose production, total sugars, and ATPase enzyme activity (Supplementary material, Table5). The activity of antioxidant enzymes (Supplementary material, Table S6, and S7) and N metabolism (Supplementary material, Table S8, and S9) were also compared when Mg was applied. The references of the articles used in this study are presented in the Supplementary material, Table S10.

Considering the essentiality of Mg for plant growth, its deficiency affects the productivity and quality of crops worldwide (GERENDÁS; FÜHRS, 2013; CHEN *et al.*, 2018). Possibly, such an effect justifies the fact that the effects of their deficiency on plants characterize the majority of studies published focusing on Mg. Among the 50 studies gathered in this review, 29 addressed the effects of Mg deficiency on plants. The negative effects caused to plants by Mg deficiency have been pointed out in several crops, such as rice (CHOU *et al*., 2011), coffee (SILVA *et al*., 2014; SILVA *et al*., 2017), citrus (YANG *et al*., 2012; TANG *et al*., 2012; LI *et al*., 2017; CAI *et al.,* 2019), spinach (ZE *et al*., 2009; YIN *et al*., 2009; YUGUAN *et al.,* 2009), beans (NEUHAUS *et al*., 2014), corn (ZHOU *et al.,* 2010; ZHOU *et a*l., 2011; ZHAO *et al*., 2012), soybean (TEWARI *et a*l., 2006), among others. Considering that the proper management of all nutrients must be attended to aiming at a successful agricultural practice, these results emphasize an increasing concern with the use of this nutrient in the last two decades.

Mg and the nutritional status of plants

It is accepted that sufficient Mg concentrations for plant growth range from 125 μmol L^{-1} to 8.5 mmol L^{-1} in the soil solution and from 1.5 and 3.5 g kg^{-1} in vegetative parts (KARLEY; WHITE, 2009). In this review, 44% of the data are in this range (1.5 to 3.5 g kg^{-1}), while 19% are above, ranging from 3.7 g kg^{-1} to 28.75 g kg^{-1}, and a maximum value of 125 g kg^{-1}, found in citrus leaves (Figure 1). The other values were observed below this range.

Figure 1. Distribution of Mg concentration (g kg^{-1}) in the shoots (A) and roots (B) of different plant families. In the aerial part (A), concentrations of Mg obtained from leaves, flag leaf, petiole, stem, fruits, and ear are included. The roots (B) include concentrations of Mg obtained from roots and tubers. Data obtained through the average between maximum and minimum values observed from the studies compiled in this review.



Total number of samples: 71; Number of PA samples (shoot, stem, petiole, leaves): 49; Number of R samples (roots and tubers): 22.

Source: The author himself

Concerning the Mg concentrations in the aerial part of the plants, the Poaceae and Fabaceae families showed the highest number of observed effects, 10 and 17 respectively. Considering the Poaceae family, the median quartile was observed at a concentration of 1.25 g kg^{-1}, while the third quartile was observed at 2.4 g kg^{-1}. This fact is justified by the sample composition made with plants of different species, such as rice (*Oryza sativa*), sugar - cane (*Saccharum officinarum*), corn (*Zea mays*), barley (*Hordeum vulgare*), and wheat (*Triticum aestivum*). The maximum value observed in this family was 7 g kg^{-1} in corn. In the roots, Mg concentrations ranged from 0.18 to 5.8 g kg^{-1}. The Fabaceae family, in turn, was composed of soybean (*Glycine max*), in addition to fava beans (2) (*Vicia faba*) and alfalfa (1) (*Medicago sativa*). Even so, there was a high dispersion of data. This is justified by the Mg concentrations in the aerial part vary from 0.38 to 7.5 g kg^{-1}, and a maximum value of 28.75 g kg^{-1} in soybeans. In this case, the median quartile was observed at concentrations of 3.24g kg^{-1}. In roots, the concentrations of Mg in *Fabaceae* ranged from 0.95 to 4.5 g kg^{-1}. These results suggest that plants such as citrus and soybean are more demanding in nutrition with Mg.

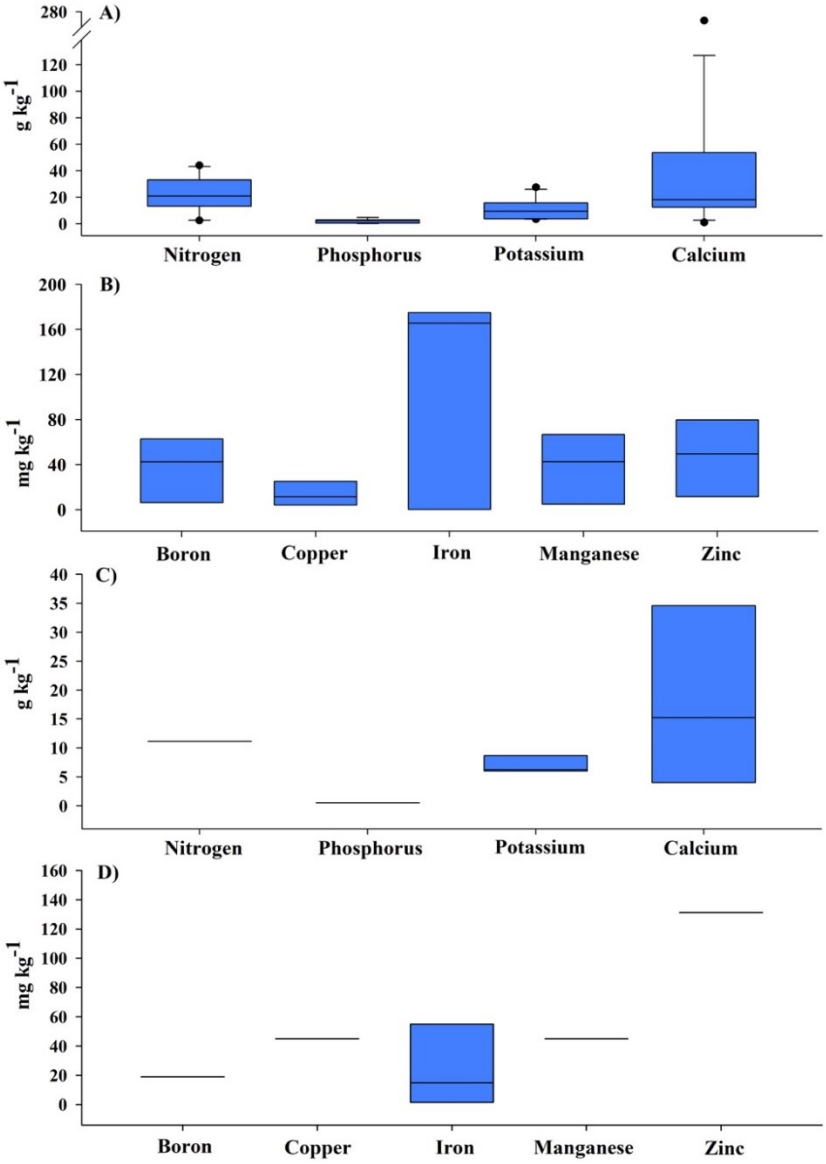
Considering all samples containing Mg, 49 effects were observed on the aerial part and 22 on the roots, totaling 71 effects (Table S2). In the aerial part, the median quartile observed was 2.3 g kg^{-1}, while in the roots it was 1.43 g kg^{-1} (Figure 1). The third quartiles in turn were 4 and 2.9 g kg^{-1} in the aerial part and roots. It should be noted that the addition of Mg can improve plant growth and development, as reported in studies with soybeans and corn (CHEN *et al*., 2017). At the same time, there is strong evidence that the application of Mg can increase the flow of carbohydrates to the roots (CAKMAK; KIRKBY, 2008; VERBRUGGEN; HERMANS, 2013). In this case, root growth is also favored by the application of Mg (FARHAT *et al.*, 2016). Also, the preferential partition of photosynthetic carbon to the roots can increase the limited absorption of elements has also been reported (CAKMAK; HENGELER, 1994; LEMOINE *et al*., 2013). Thus, it is presumed that plants well supplied with Mg can increase the absorption of other nutrients by the plant's roots.

It should be noted that few studies were observed in this review in which they assessed the nutritional status in its entirety or only some nutrients in addition to Mg in the plants in the aerial part and roots of the plants (Table S2 and S3). This was reflected in the few effects observed under the concentrations of macro and micronutrients in the aerial part and roots under the application of Mg.

Considering the aerial part of the plants, the median quartiles observed in the macronutrients N, P, K, and Ca were 21.02, 2.25, 9.38, and 18.15 g kg^{-1} (Figure 2). The third quartiles, in turn (most of the data set), were 33.13, 2.98, 15.8, and 53.71 g kg^{-1} for N, P, K, and Ca respectively. For micronutrients, B, Cu, Fe, Mn, and Zn the median quartiles observed were 42.5, 11.55, 165.55, 42.625, and 49.58 mg kg^{-1}. In the roots of the plants, few effects were also observed taking into account the concentrations of macro and micronutrients when applied Mg. For the macronutrients N, P, K, and Ca the median quartiles observed were 11.13, 0.5, 15.25, and 6.25 g kg^{-1}. For micronutrients, B, Cu, Fe, Mn, and Zn the median quartiles observed were 19, 45, 15, 45 and 131 mg kg^{-1}.

Figure 2. Influence of the application of Mg on the distribution of the concentrations of macronutrients (N, P, K, Ca) and micronutrients (B, Cu, Fe, Mn, Zn) in the aerial part of the plants (A and B) and also under the distribution of the concentrations of macronutrients (N, P, K, Ca) and micronutrients (B, Cu, Fe, Mn, Zn) in the roots of plants (B and C). The aerial part includes concentrations of Mg obtained from leaves, flag leaf, petiole, stem, fruits, and ears. The roots include concentrations of Mg obtained from roots and tubers. Data obtained through the

average between the maximum and minimum values observed in the studies compiled in this review.



Number of PA samples (shoot, stem, petiole, leaves): 74; Number of R samples (roots and tubers): 20.
Source: The author himself

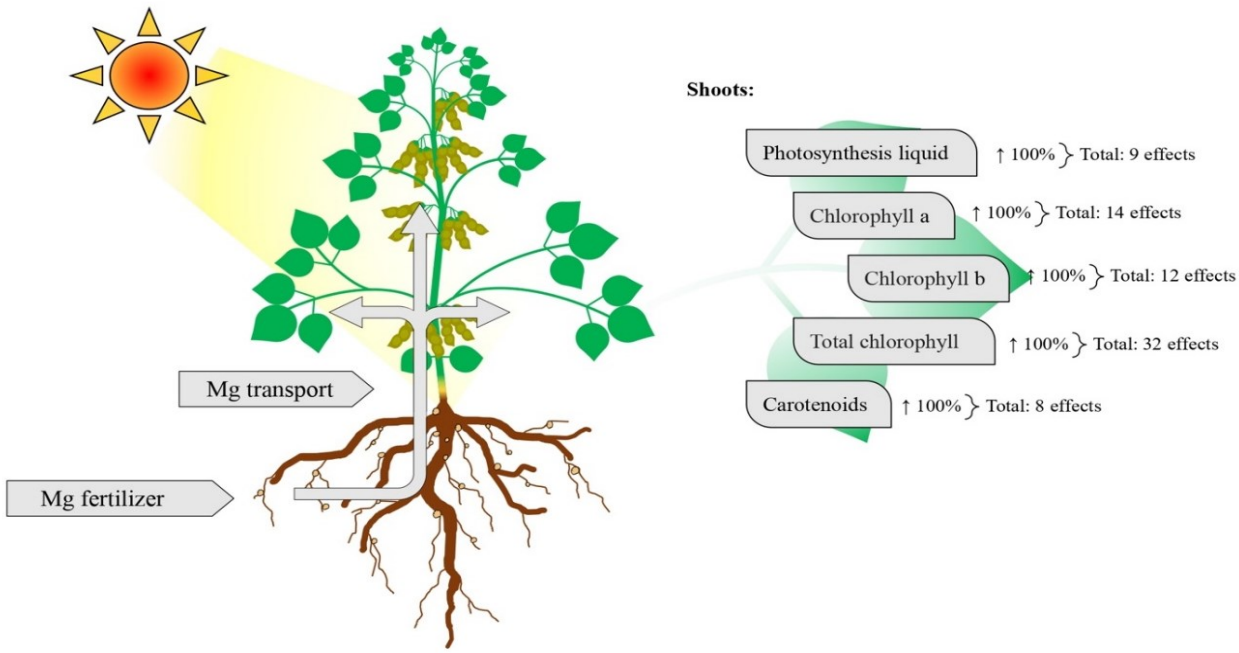
When Mg becomes limiting, plant growth and development can be severely affected (VERBRUGGEN; HERMANS, 2013). This is due to the important function that Mg has in various biochemical and physiological processes. Of these, its function as the central atom of the chlorophyll molecule is included, with a fundamental role for the realization of photosynthesis. (CAKMAK; YAZICI, 2010), act on carbohydrate metabolism (YANG *et al.*, 2017),

detoxification of reactive oxygen species (ROS) (CAKMAK; KIRKBY, 2008; YANG *et al*., 2013), and also in the metabolism of N (YANG *et al.*, 2017).

Mg and photosynthetic pigments

Chlorophylls (chlorophylls a, b, total) and their derivatives (pheophytins a, b, total, carotenoids) are the pigments that comprise the photosynthetic apparatus. These play essential roles in photosynthesis, collecting light energy and transferring them to the reaction centers (CAKMAK; YAZICI, 2010). Mg is considered the most important ion for the photosynthetic process. This is because about 15 to 35% of the plant's total Mg is linked to chloroplasts, mainly as a constituent of chlorophyll (CHEN *et al*., 2017), where it is a key component in the energy transfer process (CAKMAK; YAZICI, 2010). Such information supports the effects observed in this study. The application of Mg increased the concentrations of chlorophyll a and chlorophyll b by 100% of the effects observed in this review, with 14 and 12 effects respectively (Table S4, Figure 3). Concomitantly, there was an increase in total chlorophyll concentrations in 100% of the observed effects.

Figure 3. Influence of the Mg application on the concentrations of photosynthetic pigments (chlorophyll a, b, total, and carotenoids) and liquid photosynthesis of plants. Data presented as a percentage, considering all plant families.



↑: Indicates that there was an increase in pigment concentration or liquid photosynthetic rate with the application of Mg

Source: The author himself

A common response to Mg deficiency is damage to the formation of chlorophyll (MENGUTAY *et al*., 2013; FAUST; SCHUBERT, 2016; TRÄNKNER *et al*., 2016; LI *et al*., 2017). It is known that the typical visual symptoms of Mg deficiency appear initially on the lower leaves. In this condition, the leaf tissues present with chlorosis between the ribs (MENGUTAY *et al*., 2013), can also be tanned, yellow-orange, or reddish between the veins (HARIADI *et al*., 2004). The development of chlorosis occurs due to the degradation of chlorophyll. These symptoms can be severely worsened under high light intensity and high-temperature stresses (CAKMAK; KIRKBY, 2008; MENGUTAY *et al*., 2013; SILVA *et al*., 2017). In arabica coffee, Silva *et al*. (2017) observed that the oxidative damage of the temperature rise is more pronounced when the seedlings are simultaneously exposed to Mg deficiency.

Bearing in mind that in plants well supplied with Mg, about 15 to 35% of the total Mg is linked to chloroplasts (CHEN *et al*., 2017), while the remaining Mg is used as a bridge element for the aggregation of ribosome subunits (MELNIKOV *et al*., 2016), plus activator or cofactor in more than 300 enzymes (GOUT *et al*., 2014). Chlorosis can present itself as a late response to Mg deficiency. In this case, Mg deficiency can present itself latently. Therefore, the symptoms of Mg deficiency may not be visible, making the diagnosis costly. Latent Mg deficiency generally tends to occur during the period of grain filling or fruit expansion. According to Cakmak and Yazici (2010), latent Mg deficiency negatively affects the yield of crops.

In studies with wheat, Ceylan *et al*. (2016) observed that Mg deficiency remained latent until the grain was filled. According to these authors, Mg deficiency affected the production of this crop much more than vegetative biomass. These authors concluded that Mg deficiency affected the production of this crop mainly by limiting the supply of carbohydrates for the development of grains.

Pheophytins, in turn, are organic molecules that resemble chlorophylls. These pigments have the main function of receiving the excited electrons transferred by photosystem II (PSII). Pheophytin a is synthesized by extracting Mg from chlorophyll a (CRIST; HÖRTENSTEINER, 2014; SHIMODA *et al*., 2016). The activity of the enzyme called Mg-chelatase helps in this process, being of great importance for the formation of photosystem II (PSII) (NICKELSEN; RENGSTL, 2013). It is worth mentioning that the enzyme Mg-dechelatase has an important physiological function during plant senescence, acting on the degradation of chlorophyll (SAKURABA *et al*., 2012; CRIST; HÖRTENSTEINER, 2014). This is considered an essential step for the recycling of leaf N, as well as to avoid cell damage and/or induced cell death (HÖRTENSTEINER; KRÄUTLER, 2011). According to Chen *et al.* (2017), since plants with Mg deficiency need to recycle leaf Mg and support the growth of young tissues, leaf senescence induced by Mg deficiency can be potentiated due to chlorophyll degradation.

Carotenoid concentrations also increased in 100% of the cases seen in this review (Table S4). Considering that the light spectrum is a potentially dangerous substrate, chlorophylls when photoactivated (excited state) can

produce ROS. This occurs when electron transfer is limited to $NADP^+$ (KRIEGER-LISZKAY, 2005). In this case, carotenoids contribute to the capture of excess light and absorb excess energy. Thus, the carotenoid pigments maintain the functional stability of the photosynthetic apparatus during fluctuations in the light spectrum (KUSAMA *et al*., 2015). The transfer of this energy to the carotenoids leads to its dissipation in the form of heat. Therefore, carotenoids act as efficient antioxidants, protecting the photosynthetic apparatus.

Mg and photosynthetic activity

The adequate supply of Mg aims to go far beyond supplying the structural function of chlorophyll in the photosynthetic process (MOYNIER; FUJII, 2017). Mg acts to control the stacking of thylakoid (CEPPI *et al*., 2012), being also cofactor and modulator of important enzymes included in this physiological process (enzymes included in the Calvin cycle and ATPases) (VERBRUGGEN; HERMANS, 2013). The activity of RuBisCO, the main enzyme responsible for the assimilation of photosynthetic carbon, must also undergo an activation process that involves the binding of an Mg ion (BHAT *et al*., 2017). According to Portis and Heldt (1976), pumping Mg from thylakoids to the stroma in the presence of light serves to activate the RuBisCO enzyme. Thus, with high concentrations of Mg, greater activity of several enzymes is expected, such as RuBisCO.

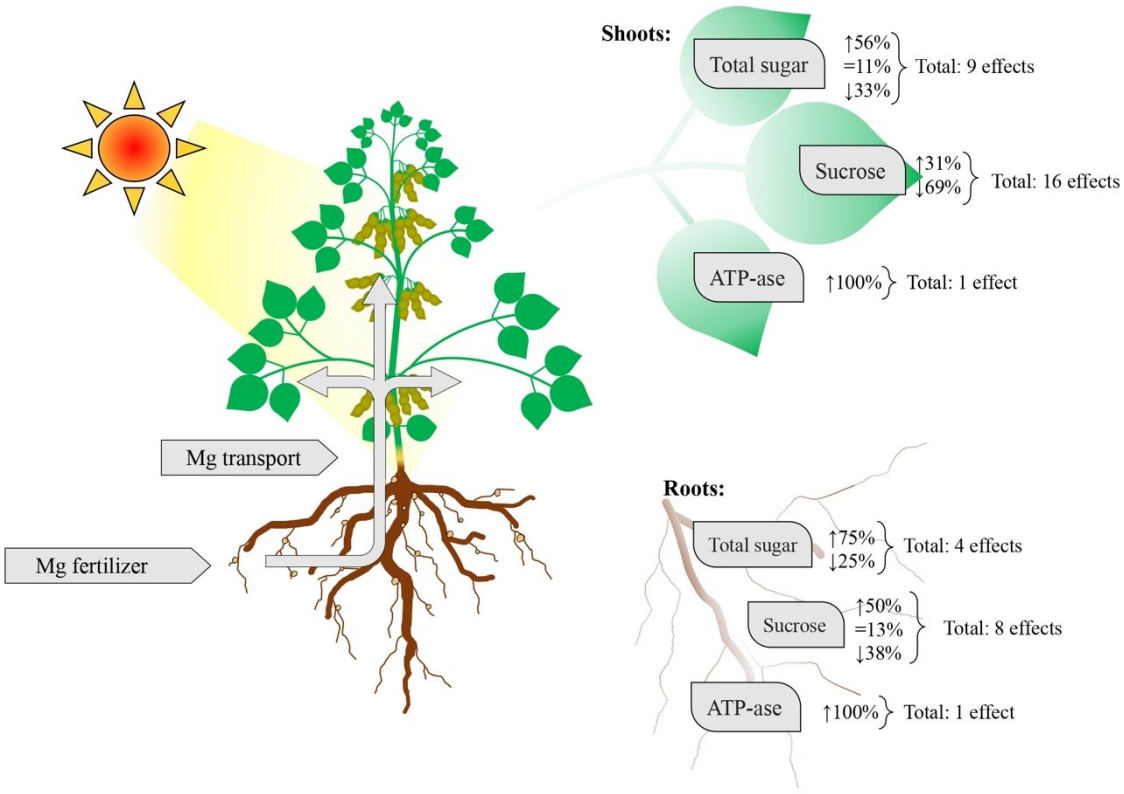
Photosynthetic activity can also be dramatically affected under Mg deficiency. Damage to the fixation of photosynthetic carbon can occur by inhibiting RuBisCO activity (YUGUAN *et al*., 2009; LI *et al.*, 2017) are responses often reported under Mg deficiency. Decreases in the sweating process have also been reported in citrus (PENG *et al.*, 2015) and corn (JEZEK *et al.*, 2014), as well as in gas exchange parameters assimilation of CO_2 and stomatal conductance in citrus (LI *et al*., 2017).

Mg and the production of photoassimilates

The contribution of Mg to photosynthetic activity and photosynthetic carbon dioxide fixation has been well reported in other studies (CAKMAK;

YAZICI, 2010; GERENDÁS; FÜHRS, 2013; DIAS *et al*., 2017). Such information corroborates the findings in this review, where liquid photosynthesis increased by 100% of the observed effects (Table S4, Figure 3). These results promote the importance of Mg in sustaining the growth and the adequate development of the plants. Thus, with a better activity of the photosynthetic apparatus, greater production of photoassimilates is expected. In this review, the concentrations of total sugars in the aerial part of the plants increased in 56% of the observed cases, while they increased by 75% in the roots (Table S5, Figure 4). Among the photoassimilates (sugars), sucrose is the main product generated by plants during the photosynthetic process. This product not only provides energy for other important physiological processes, such as FBN, but it also serves to form carbon skeletons during plant growth.

Figure 4. Influence of the Mg application on the concentrations of total sugars and sucrose, as well as ATPase enzyme activity in the aerial part and roots of plants. Data presented as a percentage, considering all plant families.



↑ indicates "increase", = indicates "has not changed" and ↓ indicates "reduction" in the concentration of compounds or enzyme activity when Mg is applied.

Source: The author himself

It is important to note that the activity of important enzymes involved in carbon metabolism is highly associated with plant responses to stress (NISHIZAWA *et al.*, 2008). The stress caused by Mg deficiency can also negatively influence key enzymes in the synthesis of starch present in chloroplasts that require Mg as a cofactor, such as the enzymes DP-glucose pyrophosphorylase (BALLICORA *et al.*, 2004) and phosphoglucomutase (CASPAR *et al.*, 1985). In this case, with the accumulation of sugars in the chloroplasts, declines in the photosynthetic process and a decrease in the rate of CO_2 diffusion are expected (KEENAN *et al*., 2010).

In deficient situations, the accumulation of sugars in the leaves is also a typical symptom of Mg deficiency. LI *et al.* (2017) have observed an increase in leaf concentrations of glucose, sucrose, fructose, and total non-structural carbohydrates in studies with citrus under Mg deficiency. Several studies support the impairment of the allocation of carbohydrates in the leaves to sinks due to Mg deficiency (CAKMAK; KIRKBY, 2008; VERBRUGGEN; HERMANS, 2013; YANG *et al*., 2017). Such information justifies the data observed in this review under sucrose concentrations. In this review, leaf sucrose concentrations were reduced in 69% of the cases observed (Table S5, Figure 4). These effects may be related to the accumulation of this product in situations of Mg deficiency being high to the production of sucrose in situations after the Mg supply.

According to Tanoi and Kobayashi (2015), the accumulation of sugars in the leaves under Mg deficiency is related to losses in the export of sucrose by phloem. Thus, Mg also plays a crucial role in the partition of carbohydrates from sources to sinks, governing the loading of sucrose by the phloem (CAKMAK; KIRKBY, 2008; VERBRUGGEN; HERMANS, 2013). This is due to the great importance of Mg for the proper functioning of the cellular energy metabolism of plants. ATP is the main source of energy in cells and must be attached to an Mg ion to be biologically active, and what is generally called ATP is Mg-ATP (IGAMBERDIEV; KLECZKOWSKI, 2015). Both ATP synthesis and hydrolysis are highly dependent on the MgATP and Mg-ADP complexes (GOUT *et al*., 2014).

According to Cakmak and Kirkby (2008), a decline in Mg-ATP concentrations at phloem, loading sites may be the main reason for inhibiting sucrose transport in Mg deficient leaves. Therefore, the application of Mg may promote an increase in the export of sucrose by the phloem of the plants. This possibility can be supported by the studies observed in this review, considering that there was an increase in the concentrations of sucrose present in the roots of the plants in 50% of the cases observed (Table S5, Figure 4).

Studies carried out by Peng *et al.* (2018) observed that in soybean plants treated with a low concentration of Mg there was an increase in the concentration of sucrose in the leaves, while it decreased in the stems and roots. This situation leads to a decrease in carbon imports to the nodule infection zone. On the other hand, Peng *et al*. (2018) also observed that the high supply of Mg in the soybean decreased the accumulation and sucrose in the leaves, while it increased its concentration in the roots when compared to the deficient situation of Mg, and consequently, there was an increase in the importation of carbohydrates in the soybean nodules.

It is suggested that Mg deficiency reduces the activity of H-ATPases enzymes located in plasmatic membranes (VERBRUGGEN; HERMANS, 2013). However, the effects of the application of Mg on the energy metabolism of plants are still poorly understood. Among the studies observed in this review, only two addressed the effect of Mg on the activity of the enzyme ATPase. Although the information on the behavior of the ATPase enzyme when the plant is well supplied with Mg is so scarce, the findings in this review support the ability of Mg to increase the activity of the ATPase enzyme, both in the aerial part and in the roots of the plants (Table S5, Figure 4).

Studies carried out by Chen *et al*. (2015) demonstrated that Mg can increase the activity of the enzyme H-ATPase in broad beans (*Vicia faba. L*). According to these authors, such a condition can increase the exudation of organic acids by the roots, relieving the rhizotoxicity caused by Al. Yang *et al*. (2007) also associated the increase in the activity of the enzyme H-ATPase with the application of Mg in roots of rice beans (*Vigna umbellata*).

1.5. ROLE OF Mg IN REDUCING ABIOTIC STRESSES AND ENZYMATIC ANTIOXIDANT ACTIVITY

Mg, aluminum and heavy metal toxicity

Mg can relieve the inhibitory action of plant growth and development by toxic elements, such as aluminum (Al). Plants can increase the absorption of Mg to overcome the toxicity of Al (CHEN *et al.*, 2012; BOSE *et al.,* 2013). According to Watanabe and Okada (2005), millimolar or micromolar concentrations of Mg are sufficient to relieve the toxicity of Al in several plant species. In this case, it seems that there is not a single effect of Mg responsible for reducing the expression of Al toxicity, but an interaction between several factors. There are reports that Mg decreases the activity of Al under the plasma membranes of the superficial cells of the roots (KINRAIDE *et al*., 2004); increases the oxidation of organic acids induced by Al by plant roots, as with soybean (CHEN; LIAO, 2016); better carbohydrate partition (MA *et al*., 2001), between others.

At the cellular level, the mechanisms proposed for reducing the toxicity of Al by Mg have been the existence of competition between Mg and Al ions at apoplastic exchange sites, including cell wall and plasma membrane surfaces (KINRAIDE *et al*., 2004). Competition for membrane transporters and enzyme binding sites has also been observed (KINRAIDE *et al*., 2004, PANDEY *et al*., 2013). In the cytosol, Mg competes with Al for binding to cellular components, such as inorganic phosphate, ATP, RNA, and DNA (MARTIN, 1998). These processes alleviate Al toxicity. Accordingly, studies using Al tolerant Arabidopsis genotypes maintain higher concentrations of Mg in the cytosol than Al sensitive genotypes (BOSE *et al.*, 2013).

Studies carried out by Kong *et al*. (2020) showed that the application of Mg alleviated Al-induced inhibition of root elongation. The accumulation of Al and oxidative stress in corn plants were also reduced with the application of Mg.

The toxicity caused by high concentrations of heavy metals can also be relieved by the application of Mg in some species of plants. Studies using barley (LOCK *et al*., 2007), wheat (LUO *et al*., 2008) and cowpeas (KOPITTKE *et al*., 2011) demonstrated that the application of Mg tends to relieve copper toxicity.

Tolerance to excess manganese was also observed with the application of Mg in some crops, such as wheat and tomatoes (LE BOT *et al*., 1990).

Mg and light and thermal stress

Plants can also experience oxidative stress when exposed to other abiotic factors, such as heat stress and excessive light. Despite the effects that Mg can promote under these conditions are so poorly understood, it is known that under these conditions, serious changes in plant cell metabolism can occur. The photosynthetic process is highly affected since plants close their stomata and limit CO_2 uptake and fixation. The interruption of CO_2 fixation can saturate the electron transport chain, due to the accumulation of NADPH (SILVA *et al*., 2014). Such a situation creates a favorable environment for the generation of ROS. Thus, adequate mineral nutrition has been proposed as essential to mitigate cell damage dependent on these situations. Several studies report the importance of adequate plant nutrition under the damages caused by environmental stresses, such as thermal stress (WARAICH *et al*., 2012; MENGUTAY *et al*., 2013).

It is known that light is an unlimited energy source for the photosynthetic process of plants. Therefore, it is a passive abiotic stress factor that oscillates widely in nature and its use must be carefully managed by plants. Exposure of chlorophylls to excess excitation of light energy can provide a favorable environment for the production of ROS. Thus, changes in cellular metabolism are expected when the capacity of the regulatory mechanisms of this energy is exceeded, of which they have been weakened by the deficiency of nutrients, such as Mg. Mg deficiency can reduce the concentrations of chlorophyll pigments that comprise the photosynthetic apparatus (TRÄNKNER *et al.*, 2016; LI *et al*., 2017). In this case, O_2 presents itself as a potential acceptor of unused electrons in the photochemical step of photosynthesis (FOYER, 2018). Upon accepting electrons from photosystems, O_2 becomes the superoxide radical (O_2^-) and in turn, it can form other ROS, like hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH^-) through a series of reactions (GILL; TUTEJA, 2010). Therefore, adequate Mg

nutrition appears to be critical in agricultural production, especially under conditions of environmental stress (VERBRUGGEN; HERMANS, 2013).

Thermal stress also limits plant growth and productivity by causing changes in cell metabolism and providing a favorable environment for the production of ROS. In this case, high temperature can also influence the final destination of electron transport products in the photosynthetic process, causing oxidative damage in chloroplasts. In particular, photosystem II is an important photosynthetic target due to heat stress (MARUTANI *et al.*, 2012). Substantial decreases in RuBisCO enzyme activity are also seen in plants exposed to heat stress (SHARKEY 2005; CARMO-SILVA *et al*., 2012). These effects can be more pronounced when combined with Mg deficiency when considering its importance for the activation of RuBisCO (BHAT *et al*., 2017). As a consequence, there is an increase in the activity of antioxidant enzymes (MENGUTAY *et al*., 2013). It should be noted that oxidative stress induced by high temperature also affects mitochondria, intensifying breathing and interrupting the electron transport chain. (FAROOQ *et al*., 2011). Thus, plants close their stomata, severely limiting the use of light energy and CO_2 fixation.

The combination of Mg deficiency and high temperature resulted in greater activities of SOD, CAT, and APX, in coffee (SILVA *et al.,* 2017). On the other hand, Silva *et al.* (2017) also observed that plants well supplied with Mg show less H_2O_2 production and, consequently, less lipid peroxidation and protein denaturation than those deficient in Mg under high temperature. These effects support the possibility of Mg positively influencing the protection of plants from stressful situations.

Mg and antioxidant enzyme activity

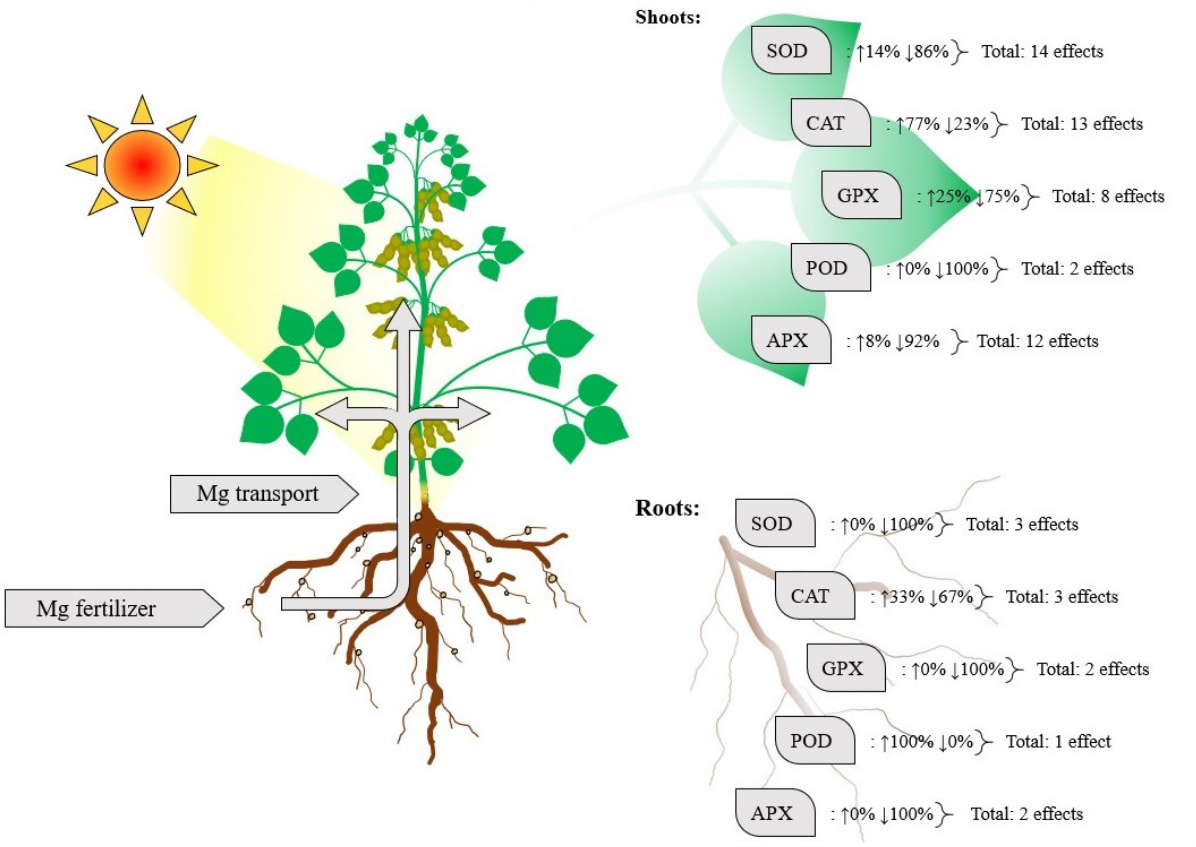
ROS are known to be reactive forms of oxygen (O_2), of which they have at least one unpaired electron. The transfer of electrons to an O_2 molecule leads to the formation of O_2^-, H_2O_2 or OH^-, respectively (FOYER, 2018). ROS can interact with various cellular constituents and oxidize them, providing lipid peroxidation within cytoplasmic membranes and organelles. In general, ROS provide a chain

of auto-catalytic reactions and promote extensive damage to the cytoplasmic membrane and organelles. Normally, the accumulation of ROS in plant cells is low, but under various abiotic stresses, their production and extinction are not maintained in a balanced state. Therefore, oxidative stress comes from an imbalance between the formation of oxidizing compounds and the performance of the antioxidant defense system (BARBOSA *et al.,* 2010).

The enzyme antioxidant system is a protection mechanism that encompasses several enzymes, of which they play an important role in protecting against ROS toxicity (PANDY *et al*., 2016; GUO *et al*., 2018). Among the enzymes that comprise the enzymatic antioxidant defense mechanism, we will highlight the superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), peroxidase (POD), and ascorbate peroxidase (APX). These enzymes act synchronously, converting ROS into non-toxic forms and the adequate supply of Mg can contribute to this process (TEWARI *et al*., 2006; DING *et al.*, 2008). There are reports that the enhanced activity of antioxidant enzymes tested with leaf applied Mg has been associated with enhanced photosynthetic activity and the increasing supply of carbohydrates to sinks (CAKMAK; KIRKBY, 2008; TRÄNKNER *et al*., 2016; CEYLAN *et al.*, 2016).

Among the enzymes that comprise the enzymatic antioxidant defense mechanism, SOD is the first enzyme active in the line of defense against ROS (GUO *et al*., 2018). It is classified according to its metal cofactor: copper/zinc (Cu / Zn-SOD), manganese (Mn-SOD), and iron (Fe-SOD) (BERWAL *et al.*, 2018). It is known that this enzyme is present in the cytosol, mitochondria, and chloroplast of plant cells. Its function is the dismutation of O_2-, with the subsequent formation of H_2O_2 and molecular oxygen (SHARMA *et al.*, 2012). Its high activity has already been well performed in deficient situations of Mg, as in barley plants (TRÄNKNER *et al*., 2016), coffee (SILVA *et al*., 2017), citrus (CAI *et al.,* 2019), corn, and wheat (MENGUTAY et al., 2013). Studies carried out by Silva *et al.* (2017) observed a high correlation between the levels of SOD and H_2O_2, showing that the increase in SOD activity is related to a higher production of H_2O_2. Thus, this situation is shown to be opposite to the data collected in this review. A reduction in the activity of the SOD enzyme was observed in 86% of the cases observed when the Mg was applied to the plants (Table S6, Figure 5).

Figure 5. Influence of the Mg application on the activity of enzymes that comprise the antioxidant enzyme system: SOD, CAT, GPX, POD, and APX present in the aerial part and roots of plants. Data presented as a percentage, considering all plant families.



↑ indicates "increase" and ↓ indicates "reduction" in the concentration of compounds or enzymatic activity when applied Mg

Source: The author himself

The ROS can be produced continuously by plants, as a by-product of various metabolic pathways in different cell compartments (SHARMA *et al.*, 2012) or by the dismutation of other ROSs. H_2O_2 can come from both photorespiration and dismutation of O_2- (SILVA *et al.,* 2017). H_2O_2 can be eliminated and converted to H_2O by various antioxidant enzymes, such as catalase (CAT) (FENG *et al.,* 2013; SILVA *et al.,* 2017), peroxidase (POD) (DE GARA *et al.,*

2003) and ascorbate peroxidase (APX) (SHARMA *et al.,* 2012; FENG *et al*., 2013; SILVA *et al.,* 2017).

CAT is one of the enzymes involved in the H_2O_2 removal process. There are reports of its presence in the cytosol, peroxisomes (SILVA *et al.,* 2017), chloroplast, and mitochondria (MHAMDI *et al.,* 2010). According to Feng *et al*. (2013), its affinity with the H_2O_2 substrate is weaker than that of other antioxidant enzymes. In this case, considering only the aerial part of the plants, the application of Mg promoted an increase in the activity of the CAT enzyme in 77% of the cases observed (Table S6, Figure 5). It should be noted that with the accumulation of H_2O_2 at levels close to 10 µM, important enzymes can reduce up to 50% of their activity, such as those present in the Calvin cycle (fructose-1,6-bisphosphatase, sedoheptulose-1,7-bisphosphatase and phosphoribulokinase) (LEEGOOD; WALKER, 1982).

Studies carried out by Tang *et al.* (2012) demonstrated that CAT was the only enzyme belonging to the enzymatic antioxidant metabolism that showed decreased activity in leaves with Mg deficiency. According to these authors, this may be related to the fact that CAT is sensitive to photo inactivation. On the other hand, Silva *et al.* (2017) also observed a reduction in the activity of the CAT enzyme in citrus plants subjected to Mg deficiency, of which they attributed this effect to the reduction of RuBisCO activity, reflecting a lower rate of photorespiration. Therefore, it is likely that the highest concentrations of intracellular Mg may promote an environment conducive to the proper functioning of this enzyme.

The enzymes APX, GPX, and POD also act in the process of removing H_2O_2 in plant cells, converting it to H_2O (DE GARA *et al*., 2003; FOYER; NOCTOR, 2011; SILVA *et al*., 2017). It is known that in Mg deficient situations, a significant increase in the activity of these enzymes is expected to occur (DING *et al*., 2008; MENGUTAY *et al*., 2013; TRÄNKNER et al., 2016; SILVA et al., 2017). In this case, activation of the antioxidant machinery would be necessary, indicating a greater effort to detoxify plant cells and eliminate ROS. In this review, both enzymes showed reduced activities when Mg was applied to plants. A reduction in the activity of APX, GPX, and POD was observed in 92, 75, and 100% of the cases observed (Table S7, Figure 5). This information corroborates

with studies carried out with barley plants by Tränkner *et al.* (2016). These authors observed that H_2O_2 concentrations are higher in Mg deficient situations when compared to adequate and high Mg situations.

Considering the roots of the plants, few effects were observed on the influence of Mg on the enzymatic antioxidant activity in the review carried out. The activity of most antioxidant enzymes has been reduced. The enzymes SOD, CAT, GPX, and APX reduced by 100, 67, 100, and 100% of the observed effects (Total: 3, 3, 2, 2 effects) (Tables 7 and 8, Figure 5). These findings indicate a gap to be filled in future studies.

Recent discoveries about the role of Mg in increasing plant tolerance to abiotic stresses, as mentioned earlier, may support possible changes in the supply of this nutrient to plants in routine soil fertilization practices. The results in this review support this possibility, considering that the absence or addition of Mg to plants can influence the expression of enzymes involved in the detoxification of ROS. In this case, with the use of Mg, a lesser need for action of the enzymatic antioxidant metabolism was required, when compared to deficient situations. It is worth mentioning that Mg deficient situations tend to require a greater activity of enzymes belonging to the antioxidant metabolism, when compared to deficient situations of other nutrients, such as K (DING *et al*., 2008).

The activity of antioxidant enzymes can also differ between the upper and lower leaves when considering Mg deficient situations. Considering the remobilization process, Mg leaves old leaves for young tissues (new leaves) (BILLARD *et al*., 2016; LI *et al*., 2017). As previously reported, Mg deficiency affects photosynthetic pigments, photosynthetic activity, and carbohydrate transport. These conditions create an enabling environment for the formation of ROS. Previous studies have shown that changes induced by Mg deficiency were more pronounced in the lower leaves than in the upper leaves, which tend to be aggravated by the intensification of Mg deficiency (CAI *et al*., 2019).

1.6. ROLE OF Mg IN BIOLOGICAL NITROGEN FIXATION (BNF)

Mg is essential as an integral part of the abiotic environment. In plants, Mg has a great influence on growth and vigor (HUBER; JONES, 2013). Although little studied, there are reports that its availability may have a predominant effect on the biological activity of the soil, such as BFN. Many enzymes act by catalyzing the chemical reactions involved in this process and the requirement for Mg to activate some of these enzymes is considerable (JAHNEN-DECHENT; KETTELER, 2012).

BFN is one of the most important biological processes on this planet. This process consists of the activity of *Rhizobiaceae* bacteria in the soil in symbiosis with Fabaceae (*Leguminosae*), enabling these plants to achieve the appropriate amounts of N for their metabolism. These bacteria absorb atmospheric N_2 gas and fix it to NH_3 (BARAL *et al.*, 2014), followed by export as ureides (allantoic acid and allantoin) (DUNN, 2014). Therefore, it is an alternative to replace the use of conventional nitrogen fertilizer sources, an important step towards the implementation of systems aimed at sustainable agriculture. Each year, such a symbiotic interaction provides approximately 40 million tons of N_2 in agricultural systems (HERRIDGE *et al.*, 2008). Therefore, BFN is an important source of N in agricultural systems (LI *et al*., 2015).

Role of Mg in nodulation and nitrogenase

Initially, the nodules must develop in the roots of the plants. For this, the appropriate supply of photoassimilates by the host plant is essential. In this process, Mg demonstrates great importance, considering its essentiality in photosynthesis and carbohydrate production, as previously reported. Sucrose is the primary source of carbon distributed by the phloem to the nodules, facilitated by the proteins of the SWEET family (KRYVORUCHKO *et al*., 2016; SUGIYAMA *et al.*, 2017). Thus, it is evident that Mg deficiency impairs the BNF process. With adequate amounts of Mg, plants can perform the photosynthetic process, with the transport of photoassimilates from the sources to the sinks, making BNF viable in the root nodules of the plants. All the functionality of the nodule and the respective BNF are highly dependent on the adequate supply of photoassimilates

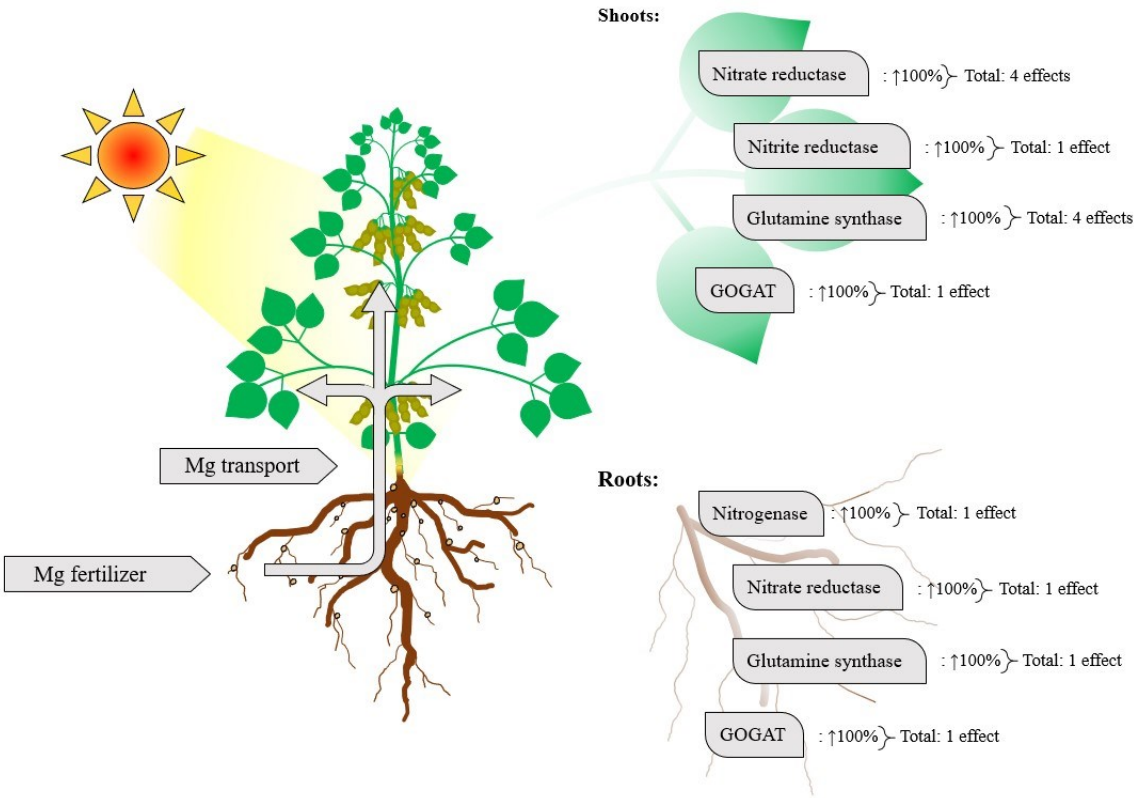
by the leaves since the nodule does not have ample storage capacity for these products (CÂMARA, 2014).

In studies by Peng *et al*. (2018), the external supply of Mg increased nodule growth under limited N conditions, increasing the number of large nodules. Subsequently, these authors also observed that Mg improved N_2 fixation and soybean growth. Accordingly, studies by Hood *et al*. (2015) observed that a direct harmful effect of Mg deficiency is the growth of rhizobial cells and the development of nodules, in peas. Such information has been proven with the mutant strain mgtE, which fails to transport Mg to rhizobium cells.

N_2 fixation is carried out through the enzyme nitrogenase. According to Peng *et al*. (2018), Mg application improves nodule growth and also results in increased nitrogenase activity. It is known that the breakdown of N_2, a triple covalent bond between the two nitrogen atoms (N≡N), is an energetically expensive process and the nitrogenase enzyme requires large amounts of energy (Mg-ATP) (SEEFELDT *et al*., 2009). As previously reported, ATP must be attached to an Mg ion to be biologically active (IGAMBERDIEV; KLECZKOWSKI, 2015). Therefore, Mg demonstrates great importance in providing the necessary energy for the adequate activity of enzymes responsible for the transformation of atmospheric N into nitrogenous compounds. Therefore, Mg homeostasis must be strictly regulated in plants. Such information corroborates the findings in this review, in which an increase in the activity of the enzyme nitrogenase was observed (Table S8, Figure 6). This makes it even more evident the need for plants to be well supplied with Mg, to avoid harmful effects to the metabolic processes that surround the BNF.

Figure 6. Influence of Mg application on the activity of enzymes present in nitrogen metabolism: nitrate reductase, nitrite reductase, glutamine synthase, and GOGAT present in the shoot and nitrogenase, nitrate reductase, glutamine

synthase, and GOGAT enzymes present in the roots of plants. Data presented as a percentage, considering all plant families.



↑ indicates "increase", = indicates "has not changed" and ↓ indicates "reduction" the concentration of compounds or enzymatic activity when Mg is applied.

Source: The author himself

Mg and the conversion of nitrate to ammonia

The roots of the plants actively absorb nitrate from the soil solution. Nitrate is initially reduced in the cytosol of plant cells to nitrite. Nitrate reductase is the first enzyme in the nitrate reduction process (YIN *et al*., 2009). Nitrite, in turn, is highly reactive and potentially toxic. Subsequently, the nitrite is converted to ammonia by the enzyme nitrite reductase. This process is essential for the assimilation of nitrate into organic compounds. In this review, when considering the aerial part of the plants, an increase in the activity of the enzyme nitrate reductase and nitrite reductase was observed in 100% of the cases observed with the application of Mg (Table S8, Figure 6).

Nitrate reductase activity is highly dependent on the availability of nitrate and light (CAMPBELL, 1999), the reducing power of NADH or NADPH, from which they come from photosynthesis (PIWPUAN *et al.*, 2013), in addition to the ATP produced, considering the high energy demand required (SUNIL *et al.,* 2013). In this case, the adequate activity of the photosynthetic apparatus seems to be an essential step for the activity of this enzyme. Thus, the application of Mg can contribute to this process, according to the great influence of this nutrient previously reported. Studies by Ding *et al.* (2006) observed that the activity of the enzyme nitrate reductase can be negatively influenced when Mg concentrations are below 1.5 mg g^{-1} DW in rice. At the same time, these authors observed that concentrations of Mg up to 3 mg g^{-1} DW were adequate for the activity of the enzyme nitrate reductase in rice. Such information supports the possibility of Mg positively influencing the increase in nitrate reductase activity.

Several studies report that Mg deficiency can negatively influence nitrate reductase activity (DING *et al.*, 2006; YIN *et al*., 2009; LI *et al.,* 2017). Yin *et al*. (2009) measured the activity of the nitrite reductase enzyme in spinach plants grown in a nutrient solution. These authors observed that the deprivation of Mg can significantly inhibit the activity of nitrate reductase, suggesting this effect to the reduction of carbon fixation or the low absorption and translocation of nitrate by the roots of spinach.

Mg and the conversion of ammonia to amino acids or ureides

Ammonia is considered the first stable product in the natural process of BFN. In this process, N_2 combines with hydrogen to form ammonia. After synthesized in BFN or by reducing nitrate, ammonia must be quickly converted to ureides (allantoin and allantoic acid) (DUNN, 2014) or incorporated into amino acids by the combined activity of the enzymes glutamate and glutamine synthase by plants (MARTÍNEZ-ANDÚJAR *et al* 2013). If it is accumulated in high levels in plant tissues, ammonia can dissipate the transmembrane proton gradients, necessary for electron transport during the photosynthetic process and in the respiratory chain, in the breathing process. Thus, this process avoids toxicity caused by excess ammonia in plants.

In this review, an increase in the activity of the enzyme glutamine synthase and GOGAT was observed with the application of Mg in the aerial part of the plants in 100% of the cases observed (Table S9, Figure 6). These results indicate the possibility of Mg positively influencing the activity of these enzymes. It is known that the enzyme glutamine synthase is responsible for combining ammonium with glutamate, to form glutamine. High concentrations of glutamine, in turn, stimulate the activity of the GOGAT enzyme. Therefore, optimal levels of carbohydrates are necessary, considering the high energy requirement. In this case, the application of Mg can contribute to the adequate supply of energy for the activity of these enzymes, considering its great importance for the synthesis of Mg-ATP (GOUT *et al.,* 2014).

Ding *et al.* (2006) observed that the maximum activity of the enzyme glutamine synthase was observed in rice sprouts containing 3.5 mg g^{-1} DW Mg. Yin *et al*. (2009) in turn, observed that Mg deprivation inhibits the activity of the enzyme glutamine synthase and GOGAT in spinach. These effects demonstrate the importance of Mg in reducing the plant's organic N.

Under Mg deficiency, free amino acids can accumulate in the original leaves. According to Fischer *et al*. (1998), this is the result of the inhibition of protein synthesis or the delayed export of assimilated by phloem. In this case, this effect is related to the essentiality of Mg as a bridge element in the aggregation of subunits of ribosomes, thus playing a central role in protein synthesis (MELNIKOV *et al*., 2016). Ribosomes are macromolecular structures responsible for protein synthesis. Its active form requires Mg to form bridges between it and two other subunits. Therefore, Mg deficiency can inhibit ribosomal assembly and activity, causing a strong accumulation of free amino acids in the leaves, as reported in other studies (MASCLAUX-DAUBRESSE *et al.*, 2010; YANG *et al*., 2017).

The ammonia synthesized by FBN is quickly converted into ureides (Allantoin and allantoic acid), the end product of this symbiosis. Ureides are derived from the oxidative degradation of purines (CARTER; TEGEDER, 2016). Allantoin (the largest fraction of ureides) is synthesized in peroxisomes from uric acid, while allantoic acid is synthesized from allantoin (LAMBERTO *et al.*, 2010; WERNER; WITTE, 2011). According to Jahnen-Dechent and Ketteler (2012),

many enzymes are involved in FBN, and the requirement for Mg to activate some of these enzymes is considerable. Therefore, these compounds can be used to estimate the FBN process (KING; PURCELL, 2005).

In the FBN process, legumes receive the fixed ureides from the rhizobia and in return, provide carbohydrates and essential elements for the growth and metabolism of the rhizobia (UDVARDI; POOLE, 2013). Thus, variations in the availability of nutrients in the soil affect the growth and development of not only plants but also rhizobia (PENG *et al*., 2018). Studies by Peng *et al.* (2018) observed that the supply of Mg can improve the growth of nodules, also resulting in an increase in the enhanced translocation of ureides (mainly fixed N form) in soybeans.

Few studies on the roots of plants evaluated the influence of Mg on the activity of the enzymes nitrate reductase, glutamine synthase, and GOGAT. In this review, an increase in enzyme activity was observed in 100% of the cases of plants fertilized with Mg (Table S9, Figure 6). Despite the few effects observed, there are reports that Mg deficiency can reduce the activity of these enzymes. Studies by Li *et al*. (2017) observed that Mg deficiency decreased the biosynthesis of amino acids in the roots, as well as the activity of the enzymes nitrate reductase, glutamate synthase, and GOGAT in citrus. Thus, it is considered that the influence of Mg on the activity of enzymes belonging to N metabolism in plant roots is still poorly understood. However, such information is indicative of a possible positive influence of Mg on the activity of these enzymes in the roots of plants.

1.7. SOYBEAN: LEGUMINOUS PLANT MODEL OF AGRICULTURAL INTEREST

Soybean (*Glycine max* (L.) Merrill) is one of the most important cultivated plant species for the world economy. Since 1940, it has become one of America's most important economic plant crop (HE *et al*., 2013). It has great commercial interest due to its high oil content (HE *et al*., 2013). According to the USDA nutritional database (2013), ripe, raw, and dried soybean normally contains 8.5%

moisture, 36.5% protein, 19.9% lipids, and 9.3% dietary fiber. Soybean oil contains 15.6% of total saturated fatty acids, 22.8% of total monounsaturated fatty acids, and 57.7% of total polyunsaturated fatty acids (USDA, 2013). Thus, when considering the high protein content in soybeans, these plants required large amounts of N (RODRÍGUES-NAVARRO *et al.,* 2011; KINUGASA *et al.*, 2012).

Soybean can associate with bacteria of the species *Bradyrhizobium japonicum*, which promotes BFN by nodulating the roots of these plants. Ureides (allantoin and allantoic acid) are the end products of this symbiosis. The main forms of N transport and storage of N by soybean are considered (CHIASSON *et al*., 2014). In this process, legumes receive N fixed mainly in the form of ureides and return, provide the carbohydrates and essential elements for the growth and metabolism of rhizobia (UDVARDI; POOLE, 2013). It should be noted that the supply of Mg can influence the process of photosynthetic fixation of carbon dioxide, as well as the transport of these photoassimilates by plants, as previously reported. The reduction of these processes has already been well reported under deficient situations of Mg (CAKMAK; KIRKBY, 2008; TRÄNKNER *et al*., 2018). Similarly, there are reports that Mg can result in a drastic increase in the flow of carbohydrates to the roots (HERMANS *et al*., 2005; CAKMAK; KIRKBY, 2008; VERBRUGGEN; HERMANS, 2013).

Regarding the nutritional status, there are reports that the application of Mg increased the concentrations of N and P in soybeans (CHEN *et al*., 2017). According to Chen *et al*. (2017), this increase is a result of the nutrients provided by the symbiotic microorganisms. This explanation was supported by positive correlations observed by these authors between plant stoichiometry and increased growth of symbiotic microorganisms from plants with added Mg. At the same time, the application of Mg can also result in greater root growth and a greater area of root contact with the soil (FARHAT *et al*., 2016). Consequently, greater absorption of water and nutrients by the roots of plants fertilized with Mg is expected.

Mg also had a promoting effect on the nodulation process in soybeans. This effect is mainly due to the easier regulation of the transport of carbohydrates from the leaves to the nodules (PENG *et al*., 2018). Thus, there are reports that

soybean plants present larger and more numerous nodules when applied Mg (CHEN *et al*., 2017; PENG *et al*., 2018). Therefore, an increase in the translocation process of ureides (mainly fixed N form) in soybeans is expected with the application of Mg (PENG *et al*., 2018). Therefore, it is expected that with the application of Mg, higher concentrations of N will occur in the soybean leaves, increasing the concentrations of proteins in the grains and, consequently, higher grain productivity.

1.8. CONCLUSION

There was an improvement in the photosynthetic process in plants fertilized with Mg due to an increase in the concentrations of pigments that comprise the photosynthetic apparatus and in the liquid photosynthetic rate, as well as higher production of photoassimilates (sugars) by plants. Simultaneously, an increase in the sucrose concentrations present in the roots of the plants was observed with the application of Mg, suggesting greater exportation by phloem, and considering the high energy demand required, an increase in the activity of ATPase enzymes was also observed.

Regarding the enzymatic antioxidant metabolism, Mg can influence the expression of the enzymes SOD, CAT, POD, GPX, and APX. With the use of Mg, a lesser need for action of the enzymes SOD, GPX, and APX was required in the aerial part and roots of plants when compared to deficient situations, while the CAT enzyme increased when applied Mg in the aerial part, and the enzyme POD increased in the roots. Plants fertilized with Mg showed a very efficient enzyme antioxidant metabolism to combat ROS and attenuation of abiotic stresses such as tolerance to high temperature, luminosity and Al toxicity in the plant root system.

Mg can positively influence the metabolism of plant-bacterial symbiosis in the FBN process in plants. Mg fertilization provides greater nodulation and production of ureides in soybean plants. This results in greater production of photosynthetic pigments, liquid photosynthesis, greater growth and grain productivity of soybeans. Despite few reports, there was an increase in the activity of the enzyme nitrogenase, nitrate reductase, nitrite reductase, glutamine

synthase, and GOGAT present in the aerial part of the plants with the application of Mg. Similarly, the enzymes nitrate reductase, glutamine synthase, and GOGAT also increased in plant roots with the application of Mg.

This study resulted in important information about the influence of Mg in several physiological processes in plants. Incorporating these findings into future research can assist and contribute to the optimization of fertilization programs and management strategies through the addition of Mg in plants.

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APPENDAGE A

Table S1. Overview of the number of studies observed by plant species and separated according to the location of experimental conduction.

Family	Especies	Number of studies/ species	Trial		References
			Greenhouse	Field	
Amaranthaceae	**Beet**	2	2	-	HERMANS *et al*., 2004; HERMANS *et al*., 2005;
Amaranthaceae	**Spinach**	3	3	-	ZE *et al*., 2009; YIN *et al*., 2009; YUGUAN *et al*., 2009;
Asteraceae	**Sunflower**	2[1]	2	-	REHMAN *et al*., 2018; TRANKNER; JAGHDANI, 2019;
Brassicaceae	**Arabidopsi**	3	3	-	HERMANS *et al*., 2010; NIU *et al*., 2014; OGURA *et al*., 2020;
Fabaceae	**Soybean**	12[1]	9	3	CASTRO *et al*., 2016; KWANO *et al*., 2016; CHEN *et al*., 2017; YANG *et al*., 2017;
Fabaceae	**Fava Beans**	2	2	-	NEUHAUS *et al*., 2014; CHEN *et al*., 2015;
Fabaceae	**Alfafa**	1	1	-	MOREIRA *et al*., 2000;
Moraceae	**Blackberry**	1	1	-	TEWARI *et al*., 2006;
Poaceae	**Rice**	4	4	-	DING *et al*., 2006; DING *et al*., 2011; DING *et al*., 2008; CHOU *et al*., 2011;
Poaceae	**Sugar -**	2	1	1	ZAMBROSI *et al*., 2012; GARCIA *et al*., 2019;
Poaceae	**Corn**	8[1]	6	2	ZHOU *et al*., 2010; ZHOU *et al*., 2011; ZHAO *et al*., 2012; MENGUTAY *et al*., 2013;
Poaceae	**Wheat**	3[1]	3	-	MENGUTAY *et al*., 2013; CEYLAN *et al.,* 2016; TRANKNER; JAGHDANI, 2019;
Poaceae	**Barley**	1	1	-	TRÄNKNER *et al.,* 2016;
Rubiaceae	**Coffe**	4	3	1	LAVIOLA *et al*., 2007; SILVA *et al*., 2014; DIAS *et al*., 2017; SILVA *et al*., 2017;
Rutaceae	**Citrus**	5	4	1	YANG *et al*., 2012; TANG *et al*., 2012; LI *et al*., 2017; HUANG *et al*., 2018; CAI *et al*., 2019;
Solanaceae	**Tomato**	1	1	-	LI *et al*., 2018;
Solanaceae	**Potato**	1	1	-	KOCH *et al*., 2019;
Total		**55**	**47**	**8**	**50 estudos**

[1]Contains studies that evaluated two Species (MENGUTAY *et al*., 2013; CASTRO *et al*., 2016 CHEN *et al*., 2017; ALTARUGIO *et al*., 2017; TRANKNER; JAGHDANI, 2019).

Source: The author himself

Table S2. Overview of the total number of studies verified in this review by Species, including how many addressed the deficiency, the genotypes evaluated, and the number of effects investigated by parts of the plant.

Family	Especie	Number of studies / Species	Number of studies / Disability	Evaluated genotypes	Number of observed effects					
					Aerial Part	Leaves	Stems	Roots	Others	Total
Amarantaceae	**Beet**	2	2	1 (Adonis)	-	10	-	6	4[4]	**20**
Amarantaceae	**Spinach**	3	3	1[2]	-	13	-	-	-	**14**
Asteraceae	**Sunflower**	2[1]	-	2 (Delfie, Hysun 336)	-	12	-	-	-	**12**
Brassicacea	**Arabidopsis**	3	2	1[2]	5	2	-	4	-	**11**
Fabaceae	**Soybean**	12[1]	2	19[2] (AN 5909 RGN, YC03-3, Yue Chun 03-3,	2	36	1	7	5[6]	**51**
Fabaceae	**Fava Beans**	2	1	2 (Fuego, Yunnan Candou 8363)	-	2	-	1	-	**3**
Fabaceae	**Alfafa**	1	-	1[2]	5	-	-	-	-	**5**
Moraceae	**Blackberry**	1	1	1 (kanva-2)	-	11	-	-	-	**11**
Poaceae	**Rice**	4	3	2 (Taichung Native 1, Wuyunjing 7)	6	18	1	5	-	**30**
Poaceae	**Sugar - Cane**	2	1	22	-	10	3	3	-	**16**
Poaceae	**Corn**	8[1]	3	8[2] (YunRui-8, híbrido 2B570, P30F53 HX	5	21	-	5	-	**31**
Poaceae	**Wheat**	3[1]	1	2 (Cornetto e Adana 99)	5	3	1	2	1[5]	**12**
Poaceae	**Barley**	1	1	1[2]	-	5	-	-	-	**5**
Rubiaceae	**Coffe**	4	2	5 (Mundo Novo IAC 379/19, Catuaí 144,	-	18	-	5	2[5]	**25**
Rutaceae	**Citrus**	5	5	2 (Sour pummelo, Xuegan)	-	58	9	27	-	**94**
Solanaceae	**Tomato**	1	1	3 (Zhongza 9, Gailiangmaofen, e	4	-	-	2	-	**6**
Solanaceae	**Potato**	1	1	1 (Laura)	-	5	-	2	2[3]	**9**
Total		**55**	**29**	**54**	**32**	**224**	**15**	**69**	**14**	**354**

[1] Contains studies that evaluated two species (MENGUTAY *et al*., 2013; CASTRO *et al*., 2016 CHEN *et al*., 2017; ALTARUGIO *et al*., 2017; TRANKNER; JAGHDANI, 2019); [2] Contains materials without genotypic specification; [3] Tubers; [4] Petiole; [5] Fruits or ears; [6] seedlings grown from seeds incubated in Mg Solutions.

Source: The author himself

Table S3. Overview of the total number of effects verified in this review by species concerning macronutrients: magnesium (Mg), nitrogen (N), calcium (Ca), potassium (K), and phosphorus (P).

Family	Especies	Number of effects															Total of effects
		Mg			N			Ca			K			P			
		Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	
Amarantaceae	**Beet**	-	3	2	-	-	-	-	2	1	-	2	1	-	-	-	**11**
Amarantaceae	**Spinach**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Asteraceae	**Sunflower**	-	2	-	-	1	-	-	-	-	-	1	-	-	1	-	**5**
Brassicacea	**Arabidopsis**	-	2	2	-	-	-	-	1	1	-	-	-	-	-	-	**6**
Fabaceae	**Soybean**	-	10	3	-	4	1	-	3	-	-	3	-	-	3	-	**27**
Fabaceae	**Fava Beans**	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**
Fabaceae	**Alfafa**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Moraceae	**Blackberry**	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**
Poaceae	**Rice**	-	4	2	-	-	-	-	-	-	-	1	-	-	-	-	**7**
Poaceae	**Sugar - Cane**	-	2	1	-	1	-	-	1	-	-	3	1	-	1	-	**10**
Poaceae	**Corn**	-	4	2	-	2	-	-	1	-	-	1	-	-	2	-	**12**
Poaceae	**Wheat**	1	5	2	-	-	-	-	-	-	-	-	-	-	-	-	**8**
Poaceae	**Barley**	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**
Rubiaceae	**Coffe**	1	3	-	-	-	-	1	1	-	-	1	-	-	-	-	**7**
Rutaceae	**Citrus**	-	7	5	-	2	1	-	2	1	-	2	1	-	2	1	**24**
Solanaceae	**Tomato**	-	1	1	-	-	-	-	-	-	-	1	1	-	-	-	**4**
Solanaceae	**Potato**	-	1	2	-	-	-	-	-	-	-	1	2	-	-	-	**6**
Total		**2**	**47**	**22**	**-**	**10**	**2**	**1**	**11**	**3**	**-**	**16**	**6**	**-**	**9**	**1**	**130**

Fr: Fruiting (Fruits and ears); PA: aerial part (leaves, flag leaf, petiole, and stem); R: Root (roots and tubers);

Source: The author himself.

Table S4. Overview of the Total number of effects verified in this review by Species concerning micronutrients: boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn).

Family	Especies	Number of effects															Total of effects
		B			Cu			Fe			Mn			Zn			
		Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	
Amarantaceae	**Beet**	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1	**3**
Amarantaceae	**Spinach**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Asteraceae	**Sunflower**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Brassicacea	**Arabidopsis**	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	**2**
Fabaceae	**Soybean**	-	-	-	-	1	-	-	1	-	-	2	-	-	1	-	**5**
Fabaceae	**Fava Beans**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Fabaceae	**Alfafa**	-	1	-	-	1	-	-	1	-	-	1	-	-	1	-	**5**
Moraceae	**Blackberry**	-	-	-	-	1	-	-	1	-	-	1	-	-	1	-	**4**
Poaceae	**Rice**	-	-	-	-	-	-	-	1	1	-	-	-	-	1	1	**4**
Poaceae	**Sugar - Cane**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Poaceae	**Corn**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Poaceae	**Wheat**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Poaceae	**Barley**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Rubiaceae	**Coffe**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Rutaceae	**Citrus**	-	2	1	-	2	1	-	2	1	-	2	1	-	-	-	**12**
Solanaceae	**Tomato**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Solanaceae	**Potato**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Total		**-**	**3**	**1**	**-**	**5**	**1**	**-**	**7**	**3**	**-**	**6**	**1**	**-**	**6**	**2**	**35**

Fr: Fruiting (Fruits and ears); PA: aerial part (leaves, flag leaf, petiole, and stem); R: Root (roots and tubers);

Source: The author himself

Table S5. Overview of the number of Total effects verified in this review on the influence of Mg on photosynthetic pigments: chlorophyll a, b, total and carotenoids and liquid photosynthesis.

Family	Especies	Photosynthetic pigments										Total of effects
		Chlorophyll a		Chlorophyll b		Chlorophyll total		Carotenoids		Liquid photosynthesis		
		↑	↓	↑	↓	↑	↓	↑	↓	↑	↓	
Amarantaceae	**Beet**	1	-	1	-	1	-	-	-	-	-	**3**
Amarantaceae	**Spinach**	2	-	1	-	2	-	1	-	-	-	**6**
Asteraceae	**Sunflower**	-	-	-	-	1	-	1	-	1	-	**3**
Brassicacea	**Arabidopsis**	-	-	-	-	2	-	-	-	-	-	**2**
Fabaceae	**Soybean**	2	-	2	-	7*	-	-	-	-	-	**11**
Fabaceae	**Fava Beans**	-	-	-	-	1*	-	-	-	-	-	**1**
Fabaceae	**Alfafa**	-	-	-	-	-	-	-	-	-	-	**-**
Moraceae	**Blackberry**	-	-	-	-	1	-	1	-	-	-	**2**
Poaceae	**Rice**	1	-	1	-	2	-	-	-	2	-	**6**
Poaceae	**Sugar - Cane**	1	-	1	-	-	-	1	-	-	-	**3**
Poaceae	**Corn**	3	-	2	-	5*	-	1	-	-	-	**11**
Poaceae	**Wheat**	-	-	-	-	1*	-	-	-	-	-	**1**
Poaceae	**Barley**	-	-	-	-	1*	-	-	-	1	-	**2**
Rubiaceae	**Coffe**	1	-	1	-	1	-	1	-	1	-	**5**
Rutaceae	**Citrus**	3	-	3	-	5	-	2	-	2	-	**15**
Solanaceae	**Tomato**	-	-	-	-	1	-	-	-	1	-	**2**
Solanaceae	**Potato**	-	-	-	-	1	-	-	-	1	-	**2**
Total of effects		**14**	**-**	**12**	**-**	**32**	**-**	**8**	**-**	**9**	**-**	**75**

Fr: Fruiting (Fruits and ears); PA: aerial part (leaves, flag leaf, petiole, and stem); R: Root (roots and tubers);
↑ indicates "increase", ↓ indicates "reduction"

Source: The author himself

Table S6. Overview of the number of Total effects verified in this review on the influence of Mg on the production of sucrose, total sugars, and activity of the enzyme ATPase.

Family	Especies	Number of effects																									Total of effects
		Sucrose									Total Sugars									ATP-ase Enzyme								
		↑			=			↓			↑			=			↓			↑			=			↓			
		Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	
Amarantaceae	**Beet**	-	-	-	-	-	-	-	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**3**
Amarantaceae	**Spinach**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Asteraceae	**Sunflower**	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**
Brassicacea	**Arabidopsis**	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**
Fabaceae	**Soybean**	-	2	1	-	-	-	-	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**7**
Fabaceae	**Fava Beans**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	**1**
Fabaceae	**Alfafa**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Moraceae	**Blackberry**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Poaceae	**Rice**	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	**5**
Poaceae	**Sugar - Cane**	-	2	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**3**
Poaceae	**Corn**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	**1**
Poaceae	**Wheat**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Poaceae	**Barley**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Rubiaceae	**Coffe**	-	-	1	-	-	-	-	1	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	**4**
Rutaceae	**Citrus**	-	1	2	-	-	-	-	3	1	-	1	2	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	**12**
Solanaceae	**Tomato**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Solanaceae	**Potato**	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**
Total of effects		**-**	**5**	**4**	**-**	**-**	**1**	**-**	**11**	**3**	**-**	**5**	**3**	**-**	**1**	**-**	**-**	**3**	**1**	**-**	**1**	**1**	**-**	**-**	**-**	**-**	**-**	**-**	**39**

Fr: Fruiting (Fruits and ears); PA: aerial part (leaves, flag leaf, petiole, and stem); R: Root (roots and tubers);
↑ indicates "increase", = indicates "has not changed" and ↓ indicates "reduction"

Source: The author himself

Table S7. Overview of the Total number of effects verified in this review on the influence of Mg on the activity of the antioxidant enzymes Superoxide dismutase (SOD) and Catalase (CAT).

Family	Especies	Antioxidant Enzymes																		Total of effects
		SOD									CAT									
		↑			=			↓			↑			=			↓			
		Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	
Amarantaceae	**Beet**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Amarantaceae	**Spinach**	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	**2**
Asteraceae	**Sunflower**	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	**2**
Brassicacea	**Arabidopsis**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Fabaceae	**Soybean**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Fabaceae	**Fava Beans**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Fabaceae	**Alfafa**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Moraceae	**Blackberry**	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	**2**
Poaceae	**Rice**	-	-	-	-	-	-	-	2	-	-	1	-	-	-	-	-	1	-	**4**
Poaceae	**Sugar - Cane**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Poaceae	**Corn**	-	-	-	-	-	-	-	1	1	-	1	-	-	-	-	-	-	1	**4**
Poaceae	**Wheat**	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	**2**
Poaceae	**Barley**	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	**1**
Rubiaceae	**Coffe**	-	-	-	-	-	-	-	2	1	-	-	-	-	-	-	-	2	1	**6**
Rutaceae	**Citrus**	-	-	-	-	-	-	-	4	1	-	4	1	-	-	-	-	-	-	**10**
Solanaceae	**Tomato**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Solanaceae	**Potato**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Total of effects		**-**	**2**	**-**	**-**	**-**	**-**	**-**	**12**	**3**	**-**	**10**	**1**	**-**	**-**	**-**	**-**	**3**	**2**	**33**

Fr: Fruiting (Fruits and ears); PA: aerial part (leaves, flag leaf, petiole, and stem); R: Root (roots and tubers);
↑ indicates "increase", = indicates "has not changed" and ↓ indicates "reduction"

Source: The author himself

Table S8. Overview of the Total number of effects verified in this review on the influence of Mg on the activity of the antioxidant enzymes Guaiacol peroxidase (GPX), Peroxidase (POD), and Ascorbate peroxidase (APX).

Family	Especie	Antioxidant Enzymes																									Total of effects
		GPX									POD									APX							
		↑			=			↓			↑			=			↓			↑			=			↓			
		Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	
Amarantaceae	**Beet**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Amarantaceae	**Spinach**	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	**2**
Asteraceae	**Sunflower**	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**
Brassicacea	**Arabidopsis**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Fabaceae	**Soybean**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Fabaceae	**Fava Beans**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Fabaceae	**Alfafa**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Moraceae	**Blackberry**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	**2**
Poaceae	**Rice**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	**2**
Poaceae	**Sugar - Cane**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Poaceae	**Corn**	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	**2**
Poaceae	**Wheat**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	**1**
Poaceae	**Barley**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	**1**
Rubiaceae	**Coffe**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1	**3**
Rutaceae	**Citrus**	-	-	-	-	-	-	-	6	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	1	**13**
Solanaceae	**Tomato**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Solanaceae	**Potato**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Total of effects		**-**	**2**	**-**	**-**	**-**	**-**	**-**	**6**	**2**	**-**	**-**	**1**	**-**	**-**	**-**	**-**	**2**	**-**	**-**	**1**	**-**	**-**	**-**	**-**	**-**	**11**	**2**	**27**

Fr: Fruiting (Fruits and ears); PA: aerial part (leaves, flag leaf, petiole, and stem); R: Root (roots and tubers);

↑ indicates "increase", = indicates "has not changed" and ↓ indicates "reduction"

Source: The author himself

Table S9. Overview of the number of Total effects verified in this review on the influence of Mg on the activity of enzymes belonging to N metabolism: nitrogenase, nitrate reductase, and nitrite reductase.

Family	Especie	Nitrogenase						Nitrate reductase						Nitrite reductase						Total of effects
		↑			↓			↑			↓			↑			↓			
		Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	
Amarantaceae	**Beet**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Amarantaceae	**Spinach**	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	**2**
Asteraceae	**Sunflower**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Brassicacea	**Arabidopsis**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Fabaceae	**Soybean**	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**
Fabaceae	**Fava Beans**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Fabaceae	**Alfafa**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Moraceae	**Blackberry**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Poaceae	**Rice**	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	**1**
Poaceae	**Sugar - Cane**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Poaceae	**Corn**	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	**1**
Poaceae	**Wheat**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Poaceae	**Barley**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Rubiaceae	**Coffe**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Rutaceae	**Citrus**	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	**2**
Solanaceae	**Tomato**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Solanaceae	**Potato**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Total of effects		**-**	**-**	**1**	**-**	**-**	**-**	**-**	**4**	**1**	**-**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**-**	**7**

Fr: Fruiting (Fruits and ears); PA: aerial part (leaves, flag leaf, petiole, and stem); R: Root (roots and tubers);
↑ indicates "increase", ↓ indicates "reduction".

Source: The author himself

Table S10. Overview of the number of Total effects verified in this review on the influence of Mg on the activity of enzymes belonging to N metabolism: glutamine synthase and GOGAT.

Family	Especie	Glutamina sintase						GOGAT						Total of effects
		↑			↓			↑			↓			
		Fr	PA	R	Fr	PA	R	Fr	PA	R	Fr	PA	R	
Amarantaceae	**Beet**	-	-	-	-	-	-	-	-	-	-	-	-	-
Amarantaceae	**Spinach**	-	1	-	-	-	-	-	-	-	-	-	-	1
Asteraceae	**Sunflower**	-	-	-	-	-	-	-	-	-	-	-	-	-
Brassicacea	**Arabidopsis**	-	-	-	-	-	-	-	-	-	-	-	-	-
Fabaceae	**Soybean**	-	-	-	-	-	-	-	-	-	-	-	-	-
Fabaceae	**Fava Beans**	-	-	-	-	-	-	-	-	-	-	-	-	-
Fabaceae	**Alfafa**	-	-	-	-	-	-	-	-	-	-	-	-	-
Moraceae	**Blackberry**	-	-	-	-	-	-	-	-	-	-	-	-	-
Poaceae	**Rice**	-	1	-	-	-	-	-	-	-	-	-	-	1
Poaceae	**Sugar - Cane**	-	-	-	-	-	-	-	-	-	-	-	-	-
Poaceae	**Corn**	-	-	-	-	-	-	-	-	-	-	-	-	-
Poaceae	**Wheat**	-	-	-	-	-	-	-	-	-	-	-	-	-
Poaceae	**Barley**	-	-	-	-	-	-	-	-	-	-	-	-	-
Rubiaceae	**Coffe**	-	-	-	-	-	-	-	-	-	-	-	-	-
Rutaceae	**Citrus**	-	2	1	-	-	-	-	1	1	-	-	-	6
Solanaceae	**Tomato**	-	-	-	-	-	-	-	-	-	-	-	-	-
Solanaceae	**Potato**	-	-	-	-	-	-	-	-	-	-	-	-	-
Total		**-**	**4**	**1**	**-**	**-**	**-**	**-**	**1**	**1**	**-**	**-**	**-**	**8***

Fr: Fruiting (Fruits and ears); PA: aerial part (leaves, flag leaf, petiole, and stem); R: Root (roots and tubers);
↑ indicates "increase"; ↓ indicates "reduction"
* In a study by CAI *et al*. (2019), Mg did not alter the activity of the enzyme Glutamine synthase in Citrus roots (Total: 1 effect), an effect not counted.
Source: The author himself

Table S11. References of studies compiled in this bibliographic review

REFERENCES	DOI
Altarugio *et al.* (2017)	10.1590/S0100-204X2017001200007
Cai *et al.* (2019)	10.1186/s12870-019-1683-4
Castro *et al.* (2016)	10.4067/S0718-95162016005000034
Ceylan *et al.* (2016)	10.1007/s11104-016-2871-8
Chen *et al.* (2015)	10.1093/pcp/pcv038
Chen *et al.* (2017)	10.1021/acs.jafc.6b05764
Chmielowska-Bak *et al.* (2018)	10.1515/eces-2018-0042
Chou *et al.* (2011)	10.1016/j.jplph.2010.12.004
Dias *et al.* (2017)	10.3390/agriculture7100085
Ding *et al.* (2006)	10.1111/j.1744-7348.2006.00080.x
Ding *et al.* (2008)	10.1016/S1002-0160(08)60021-1
Ding *et al.* (2011)	10.4236/ajps.2011.24071
Egamberdieva *et al.* (2018)	10.3389/fmicb.2018.01000
Garcia *et al.* (2019)	10.1007/s42729-019-00096-x
Hasanah *et al.* (2020)	10.1088/1755-1315/454/1/012158
Hermans *et al.* (2004)	10.1007/s00425-004-1340-4
Hermans *et al.* (2005)	10.1007/s00425-004-1376-5
Hermans *et al.* (2010)	10.1111/j.1469-8137.2010.03257.x-
Huang *et al.* (2018)	10.1007/s00468-018-1766-0
Khaitov, (2018)	10.1080/01904167.2018.1485164
Koch *et al.* (2019)	doi.org/10.1111/ppl.12846
Kong *et al.* (2020)	10.1007/s11104-020-04605-1
Kwano *et al.* (2016)	10.1080/01904167.2016.1240198
Laviola *et al.* (2007)	R. Bras. Ci. Solo, 31:319-329,
Li *et al.* (2017)	10.1093/treephys/tpx067
Li *et al.* (2018)	10.1016/S2095-3119(18)61949-5
Mengutay *et al.* (2013)	10.1007/s11104-013-1761-6
Moretti *et al.* (2019)	10.1021/acs.jafc.6b05764
Neuhaus *et al.* (2014)	10.1002/jpln.201300127
Niu *et al.* (2014)	10.1111/pce.12362
Ogura *et al.* (2020)	10.3389/fpls.2020.00563
Peng *et al.* (2018)	10.1111/ppl.12730
Qin *et al.* (2020)	10.1007/s00572-020-00955-x
Rehman *et al.* (2018)	10.1016/j.plaphy.2018.02.031
Silva *et al.* (2014)	10.1007/s11104-014-2150-5
Silva *et al.* (2017)	10.1016/j.scienta.2017.04.029
Tang *et al.* (2012)	10.1002/jpln.201100329
Tewari *et al.* (2006)	10.1016/j.scienta.2005.12.006
Trankner e Jaghdani (2019)	10.1016/j.plaphy.2019.09.040
Tränkner *et al.* (2016)	10.1007/s11104-016-2886-1
Yang *et al.* (2012)	10.1007/s00468-012-0699-2
Yang *et al.* (2017)	10.3389/fpls.2017.02091
Yin *et al.* (2009)	10.1007/s12011-009-8392-z
Yuguan *et al.* (2009)	10.1007/s12011-009-8354-5
Zambrosi *et al.* (2012)	Bragantia, Campinas, v. 71, n. 3, p.400-405, 2011
Ze *et al.* (2009)	10.1007/s10534-009-9246-z
Zhao *et al.* (2012)	10.1007/s12011-012-9340-x
Zhou *et al.* (2010)	10.1007/s12011-010-8769-z
Zhou *et al.* (2011)	10.1007/s12011-010-8830-y

2 MAGNESIUM OXIDE APPLICATION INCREASE PHOTOSYNTHETIC PIGMENTS AND UREIDES IN SOYBEAN PLANTS

2.1. ABSTRACT

The physiological role of magnesium (Mg) in biological nitrogen fixation (BNF) and ureides concentration in legume plants is still poorly understood. The aim of this study was to evaluate possible increases in the photosynthetic process and ureides in soybean plants in response to soil application of Mg oxide. Improvements in the BNF process were determined by evaluating allantoin and allantoic acid in the leaves of soybean varieties. In this study, three soybean varieties (NA 5909 RG RR, BMX Desafio RR and P98Y30 RR) and 6 doses of Mg (0, 7.5, 15, 30, 60 and 90 kg ha^{-1}) were tested. Application of Mg oxide via soil increased the concentrations of Mg in the soybean leaves, but did not change the concentrations of potassium and calcium. Application of Mg oxide did not influence the photosynthetic parameters, however, increased the photosynthetic pigments and sucrose concentration in soybean leaves. There was an increase in the concentrations of ammonia, total amino acids and ureides in response to Mg supply, indicating greater activity of N metabolism of soybean plants. The results indicate that soybean varieties showed higher production of photoassimilates (sugars) when submitted to Mg, allowing greater conversion of N_2 atmospheric into ureides. The present study reports relevant information about the role of Mg in BNF for further studies on Mg fertilization in soybean plants.

Key-words: biological nitrogen fixation; mg doses. allantoin. allantoic acid. *Glycine max* (L.) Merrill.

2.2. INTRODUCTION

In the last decades, farmers and researchers have neglected to use magnesium (Mg), which is part of the chlorophyll molecule (ZHANG *et al.,* 2020). This is justified due the ease Mg supply available in liming as $MgCO_3$. In general, moderate deficiencies of Mg are found in several plant species, and reduced Mg concentration in grains have been reported (CAKMAK; YAZICI, 2010). Numerous studies have already addressed the Mg deficiency effect on the plant growth, antioxidant defense mechanisms and on the photosynthetic process (HUANG *et al.,* 2016; SILVA *et al.,* 2017; TRÄNKNER *et al.,* 2018). The physiological role of Mg in plants is not yet fully understood.

Reductions in Mg concentrations in soils have been widely reported by natural processes such as leaching (GRANSEE; FUHRS, 2013) and by aluminum toxicity (CHEN; MA, 2013), in addition to high levels of antagonistic ions such as calcium and potassium (GRANSEE; FUHRS, 2013). Under low concentrations of Mg, plant development is impaired, particularly affecting the photosynthetic process. It should be noted that chlorophylls, formed by Mg ion, allow plants to perform photosynthesis (MOYNIER; FUJII, 2017). In photosynthesis, RuBisCO is the main enzyme responsible for assimilation of photosynthetic carbon which must be subjected to an activation process, binding to an Mg ion (BHAT *et al.,* 2017). Sucrose export via phloem is also severely impaired, occasioning carbohydrates accumulation in the leaves. This is indicative of a serious insufficiency in the photoassimilates export by the source, via phloem, caused by the absence of Mg (CAKMAK; KIRBY, 2008).

The optimal Mg concentrations for plant growth range from 125 μmol L^{-1} to 8.5 mmol L^{-1} in the soil solution and 1.5 and 3.5 g kg^{-1} in the leaves (KARLEY; WHITE, 2009). Thus, plants can perform photosynthesis, produce carbohydrates and transport them via phloem, ensuring the performance of several physiological processes (VERBRUGGEN; HERMANS, 2013). BNF is a highly relevant process in Fabacea (Leguminosae), which establishes symbiosis with *Rhizobiaceae* bacteria. In this process, N_2 is fixed in NH_3 (BARAL *et al.,* 2014) and later exported as ureides (allantoic and allantoin acid) (DUNN, 2014). It is an energetically expensive process, requiring 16 molecules of adenosine triphosphate (ATP) to reduce N_2. ATP must join to Mg to be biologically active, and what is generally called ATP is actually Mg-ATP

(IGAMBERDIEV; KLECZKOWSKI, 2015). Therefore, Mg homeostasis must be strictly regulated in order to avoid harmful effects to important metabolic processes such as BNF in leguminous plants.

The cultivation of soybean *(Glycine max* L.) has an economic and social prominence worldwide, being the main source of vegetable oil (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, 2020). It is a highly efficient legume in BNF, showing the ability to associate with bacteria *Bradyrhizobium japonicum*, which nodulates the roots of plants. Photoassimilates translocation from shoot to root is necessary for proper nodule development. Nodule functionality and respective BNF depend on the adequate supply of carbohydrates (CÂMARA, 2014). Magnesium fertilization enhance photosynthetic process transporting sugars and carbohydrates from the sources to the drains, making BNF viable in the nodules of soybean plants (CAKMAK; YAZICI, 2010).

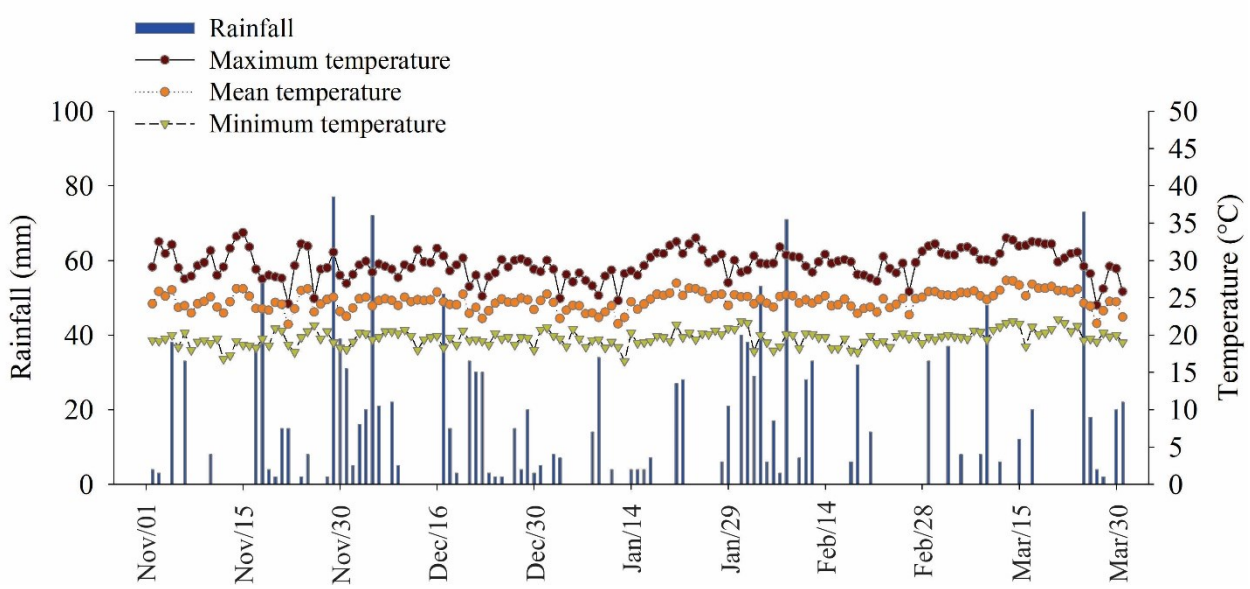
This study hypothesize that Mg supply might enhance photosynthetic pigments and ureide metabolism in soybean plants boosting BNF through the adequate supply of carbohydrates produced in the leaves. The aim of this study was to evaluate the possible increments in the photosynthetic process and BNF resulting from the adequate Mg supply to soybean plants. Improvements in the BNF process were determined by the evaluation of ureides in soybean plants.

2.3. MATERIAL AND METHODS

2.3.1. Growth conditions and experimental design

The study was performed in 2017/2018 at an experimental area at the Research Farm of Fundação Chapadão, located in the municipality of Chapadão do Sul, State of Mato Grosso do Sul, Brazil. Geographical coordinate is 18 ° 46 '48.49 "S and 52 ° 38' 42.59" O at 840 m above sea level. Monthly values of Rainfall, maximum and minimum temperatures are presented in the Figure 1.

Figure 1. Minimum, average and maximum daily rainfall and temperatures during the experimental driving period.



Rainfall: data obtained from a manual data acquisition station, installed at the experiment site. Temperature: data obtained through INMET (Instituto Nacional de Meteorologia), Chapadão do Sul, MS, Highway MS 306 - km 96. Source: www.inmet.gov.br

The soil was classified as Oxisol according to Soil Survey Staff (2014), with clay content in 0,00 – 0,20 m layer of 607.5 g kg^{-1}, silt 62,5 g kg^{-1} and sand 330 g kg^{-1}, determined by the pipette method (EMBRAPA, 2017) and chemical analyses: phosphorus (resin) 29.7 mg dm^{-3}; organic matter 28.8 g dm^{-3}; pH calcium chloride ($CaCl_2$) 4.9; potassium 4.7 $mmol_c$ dm^{-3}; calcium 27.0 $mmol_c$ dm^{-3}; magnesium 10.5 $mmol_c$ dm^{-3}; H + Al 63 $mmol_c$ dm^{-3}; aluminum 0.5 $mmol_c$ dm^{-3}; sulfur 3.8 mg dm^{-3}; cation

exchange capacity 105.2 $mmol_c$ dm^{-3}; and base saturation 40%. The methods of determining the elements followed the standards of Van Raij *et al.* (1996). The area has been cultivated with soybean under no-tillage system under cotton residue.

The experimental design was in randomized blocks, distributed in a 3 × 6 factorial scheme, with 3 replications, where: three soybean varieties (NA 5909 RG RR - early cycle of 100 days; BMX Desafio RR - medium cycle of 115 days; P98Y30 RR - 130 days late cycle) and six Mg doses (0, 7.5, 15, 30, 60 e 90 kg ha^{-1}) applied as Mg oxide (30% Mg).

The soybean varieties were sown on November 29, 2017. Sowing was carried out mechanically in the experimental plots containing six rows of 5.5 m spaced at 0.45 m. Fertilization was performed using 150 kg ha^{-1} of MAP (11-52-00), 200 kg ha^{-1} of agricultural gypsum (12% S) and 3 kg ha^{-1} of ulexite (8% B) in the sowing and 100 kg ha^{-1} of KCl (60% K_2O) after emerging. Seed inoculation was carried out close to soybean sowing through N_2 fixing bacteria (strains: SEMIA 5019 - *Bradyrhizobium elkanii* and SEMIA 5079 - *Bradyrhizobium japonicum*, both with a guarantee of $6x10^9$ CFU mL^{-1}) at the dose of 100 mL ha^{-1}. Magnesium oxide application occurred after 15 days after sowing, on surface. The phytosanitary management practices followed the technical cultivation recommendations and were carried out homogeneously throughout the experimental area.

During anthesis, the fourth fully developed leaves (diagnostic leaves) were collected. The collection of leaves was performed during anthesis because is the period of maximum metabolic activity in soybean plants. The same leaves were collected and the material was prepared to evaluate the contents of Mg, potassium (K), calcium (Ca), nitrogen compounds (nitrate, ammonia, amino acids, allantoin and allantoic acid), total sugars and sucrose. The harvest was carried out on March 20 for NA5909, March 29 for BMX Desafio RR and on April 18 for P98Y30 RR.

2.3.2. Chemical analysis of plant tissue

The nutritional plant status was evaluated by collecting the fourth fully expanded leaf in each plot at the beginning of flowering. The collected leaves were washed with running water, then in a 0,1% neutral detergent solution, after in 0.3% HCl solution and

to rinsed in distilled water, and then dried in an oven at 65 ± 5ºC until constant weight, following the methodology proposed by Bataglia *et al.* (1983). Subsequently, samples were ground in a Wiley-type mill and digested in nitric-perchloric solution. Leaf Mg, K and Ca concentration were determined by atomic absorption according methodology described by Malavolta *et al.* (1997).

2.3.3. Physiological and biochemical analyzes

Gas exchange parameters and photosynthetic pigments

The assessment of gas exchange consisted of non-destructive analysis using the fourth leaf fully developed during the anthesis of each variety between 8 and 11 am. An infrared gas analyzer (Infrared Gas Analyzer - IRGA) model Li-6400XT (LiCor Inc. Lincoln, Nebraska, USA) was used as described by Reis *et al.* (2015). Photosynthetic pigments were extracted using 80% cold acetone and measured using the methodology proposed by Lichtenthaler (1987).

Determination of nitrogen compounds

To determine nitrogenous compounds, 0.3 g of dry leaf samples were extracted in 10 mL of MCW (60% methanol, 25% chloroform and 15% H_2O). The material was centrifuged at 10000 RPM for 10 minutes at 4 ºC. Subsequently, the material was kept at rest for 48 hours. In another tube, 4 mL of supernatant of the MCW extract, 1 mL of chloroform and 1.5 ml of water were added. After the separation of the phases, the water-soluble phase was used to determine sucrose, total sugars, nitrate, ammonia, total free amino acids, allantoin and allantoic acid concentration in soybean leaves.

Sucrose, total sugars, nitrate and ammonia were determined following the methodology of Van Handel (1967) and Dubois *et al.* (1956), Cataldo *et al.* (1975), McCullough (1967) respectively. Total free amino acids were measured according Yemm and Cocking (1955) and ureides (allantoin and allantoic acids) according Vogels and Van Der Drift (1970).

2.4 Statistical analysis

Statistical analysis was performed using R software (R DEVELOPMENT CORE TEAM, 2019). Normality test was analyzed by Shapiro and Wilk (1965). F test (p <0.05) was applied and averages were compared by Tukey test at 5% of error probability. Heatmap was performed as described by Reis *et al.* (2020).

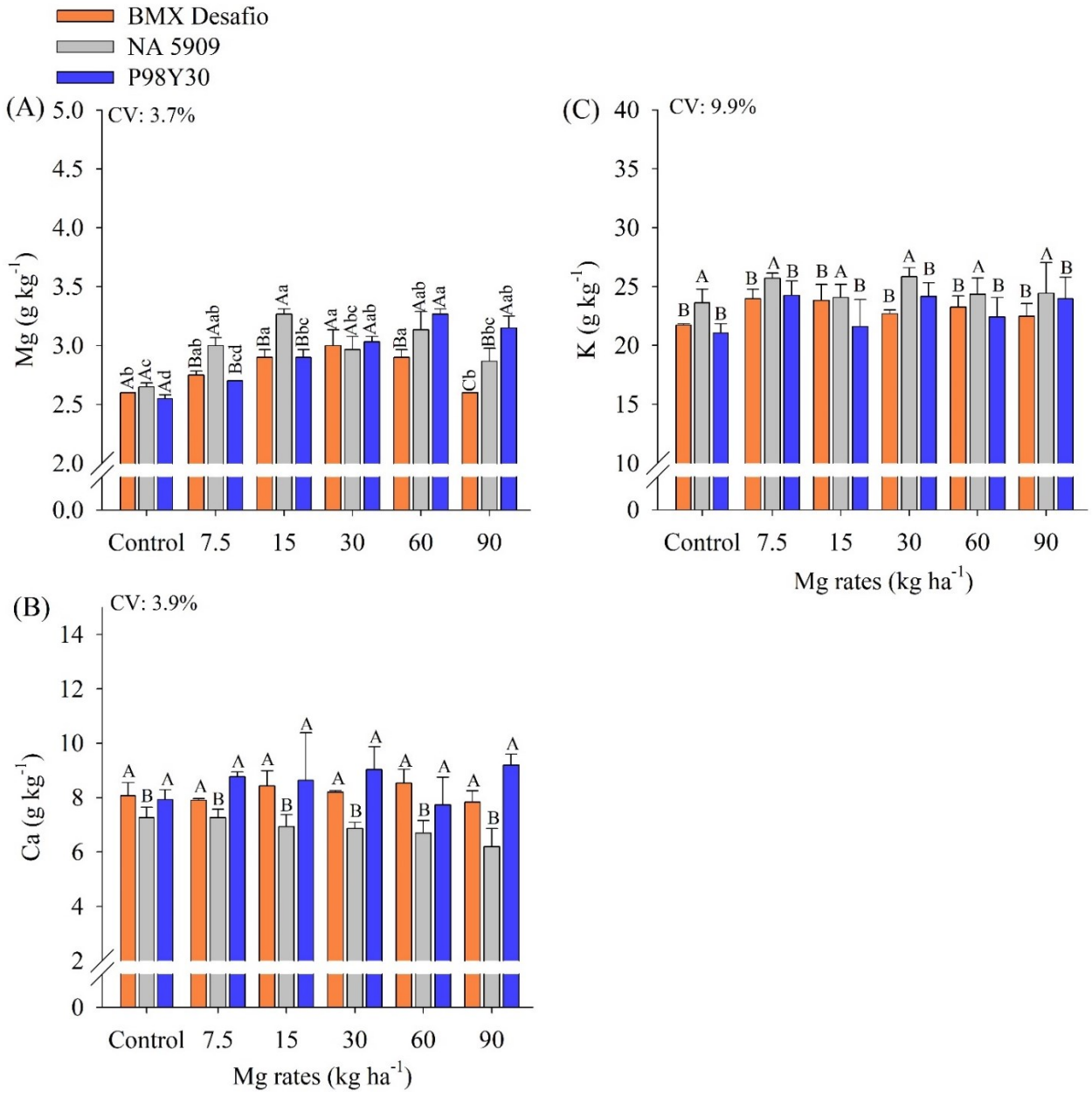
2.4. RESULTS

2.4.1. Chemical analysis of plant tissue

Magnesium oxide application increased the leaf Mg concentration of all soybean varieties (Figure 2A). For BMX Desafio RR, applied Mg doses showed an increase of 12 to 15% in relation to control treatment. For NA 5909 RG RR, the highest Mg concentration in leaves was observed at the dose 15 kg ha^{-1}, 23% higher than control treatment. Fertilization with 60 kg ha^{-1} of Mg showed the highest concentrations in leaf of P98Y30 RR, being 28% higher than untreated plants. Among varieties, NA 5909 RG RR presented the highest Mg concentration due to 7,5, 15 and 60 kg ha^{-1} of Mg supply. P98Y30 RR presented the highest leaf Mg concentration with 90 kg ha^{-1} supply.

Fertilization with Mg did not change leaf Ca and K compared to untreated plants (Figures 2B and 2C). Among varieties, the highest concentration of K in leaf was observed for NA 5909 RG RR, followed by BMX Desafio RR and P98Y30 RR. P98Y30 RR and BMX Desafio RR presented the highest leaf Ca concentration, not differing from each other, followed by NA 5909 RG RR. These results suggest that the access and use of soybean varieties with different cycles to K and Ca were not negatively affected by Mg fertilization.

Figure 2. Effects of Mg rates on leaf Mg (A), Ca (B) and K (C) concentration of soybean varieties. CV means coefficient of variation. The averages followed by the same capital letters are not significantly different and compare soybean varieties in response to Mg supply. The averages followed by the same lower case letters compare each variety in response to Mg doses, according to the Tukey test ($p \leq 0.05$). Bars represent the standard error of the means (n=4).



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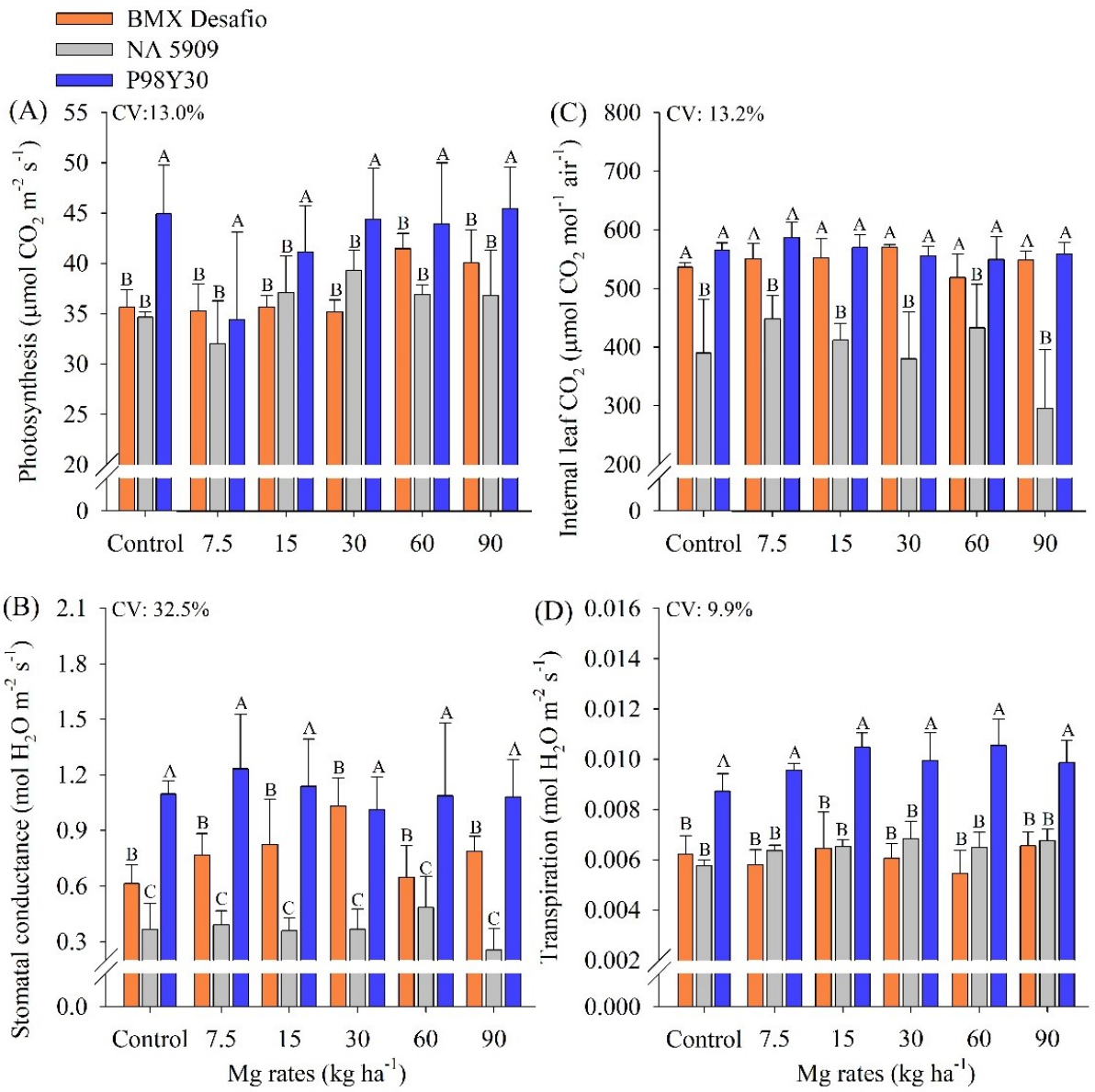
2.4.2. Physiological and biochemical analyzes

Gas exchange parameters

Photosynthetic parameters indicate no difference on net photosynthetic rate (A) fertilized with Mg (Figure 3A). Among varieties, the highest A was observed for P98Y30 RR, followed by BMX Desafio RR and NA 5909 RG RR, respectively. Mg fertilization also did not promote differences in stomatal conductance (gs), internal concentration (substomatal) of CO_2 (Ci) or the transpiration rate (E) in soybean varieties (Figure 3B, C and D). Among varieties, the largest A was observed for P98Y30 RR. The highest Ci were observed for BMX Desafio RR and P98Y30 RR, with values 518 and 572 µmol CO_2 mol air^{-1}, respectively. Similarly, BMX Desafio RR and P98Y30 RR had higher E compared to the other varieties. NA 5909 RG RR presented lower gas, Ci and E. The results obtained indicated that application of Mg oxide did not influence the photosynthetic process of the analyzed soybean varieties.

Figure 3. Effects of Mg rates on photosynthesis (A), stomatal conductance (B), internal leaf CO_2 (C) and transpiration (D) of soybean varieties. CV means coefficient of variation. The averages followed by the same capital letters are not significantly different and compare soybean varieties in response to Mg supply. The averages followed by the same lower case letters compare each variety in response to Mg doses, according to the Tukey test ($p \leq 0,05$). Bars represent the standard error of the means ($n = 4$).

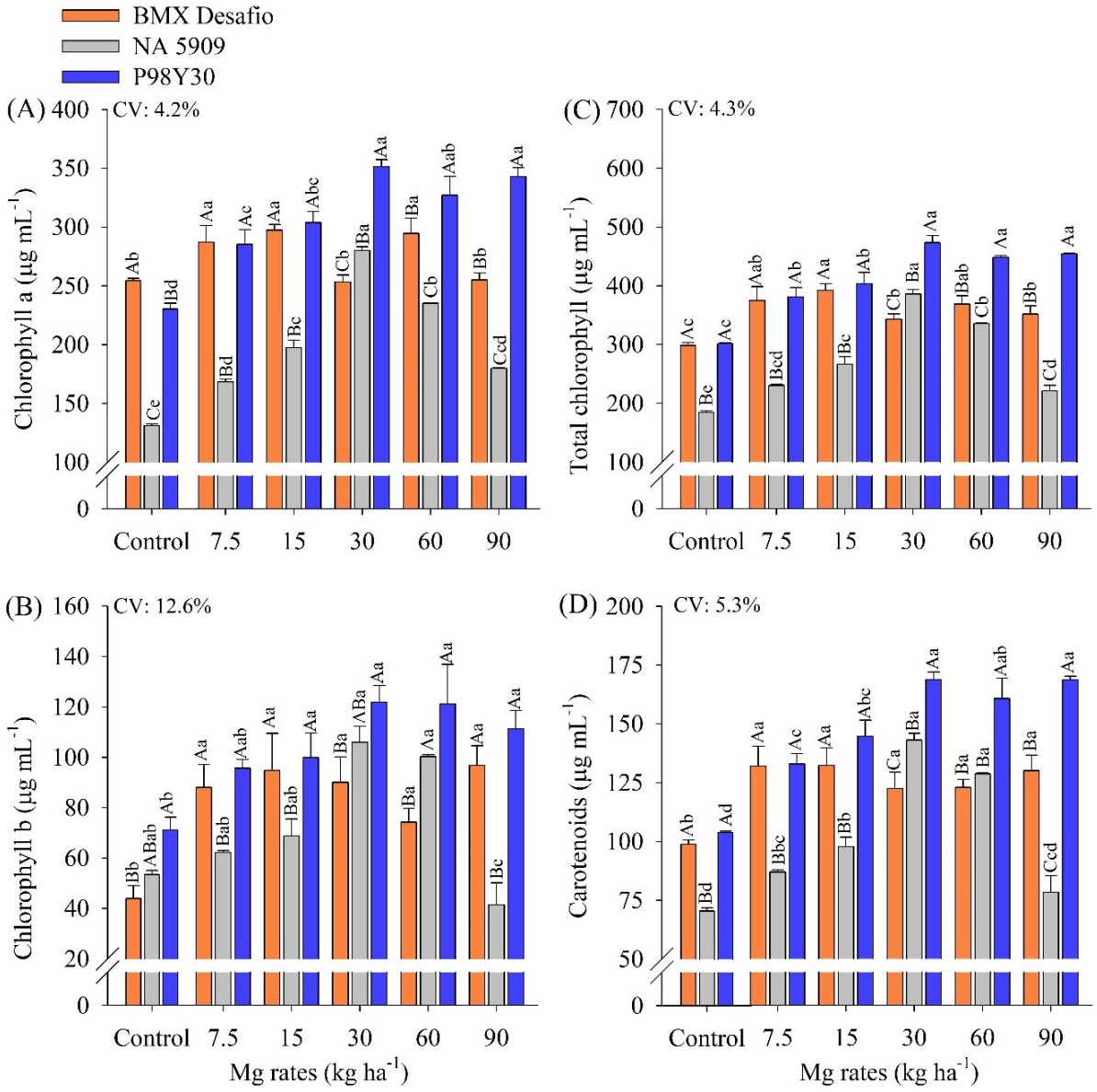


Source: The author himself

Photosynthetic pigments

Photosynthetic pigments increased in response to Mg supply (Figure 4). For BMX Desafio RR, the highest chlorophyll *a* concentration was observed with application of 7,5, 15 and 60 kg ha^{-1} of Mg, which represents an increase of 13 to 17% in relation to untreated plants (Figure 4A). Chlorophyll *a* concentration increased in NA 5909 RG RR and P98Y30 RR with the application of 30 kg ha^{-1}, 113% and 53% higher than untreated plants. There was an increase in chlorophyll *b* concentration with all Mg doses used in the BMX Desafio RR and P98Y30 RR varieties when compared to untreated plants (Figure 4B). For NA 5909 RG RR, the highest concentrations of chlorophyll *b* were observed with application of 30 and 60 kg ha^{-1}. The highest total chlorophyll concentration was observed with 15 kg ha^{-1} of Mg for BMX Desafio RR, which represents concentrations 31% higher than untreated plants (Figure 4C). NA 5909 RG RR presented the highest total chlorophyll concentration with 30 kg ha^{-1}, which represent 109% higher than untreated plants. For P98Y30 RR, the highest concentrations of total chlorophyll were observed with the application of 30, 60 and 90 kg ha^{-1}, representing 57, 49 and 51% higher than the control treatment.

Figure 4. Effects of Mg rates on chlorophyll *a* (A), chlorophyll *b* (B), total chlorophyll (C) and carotenoids (D) concentration in soybean leaves. CV means coefficient of variation. The averages followed by the same capital letters are not significantly different and compare soybean varieties in response to Mg. The averages followed by the same lower case letters compare each variety in response to Mg doses, according to the Tukey test ($p \leq 0,05$). Bars represent the standard error of the means ($n = 4$).

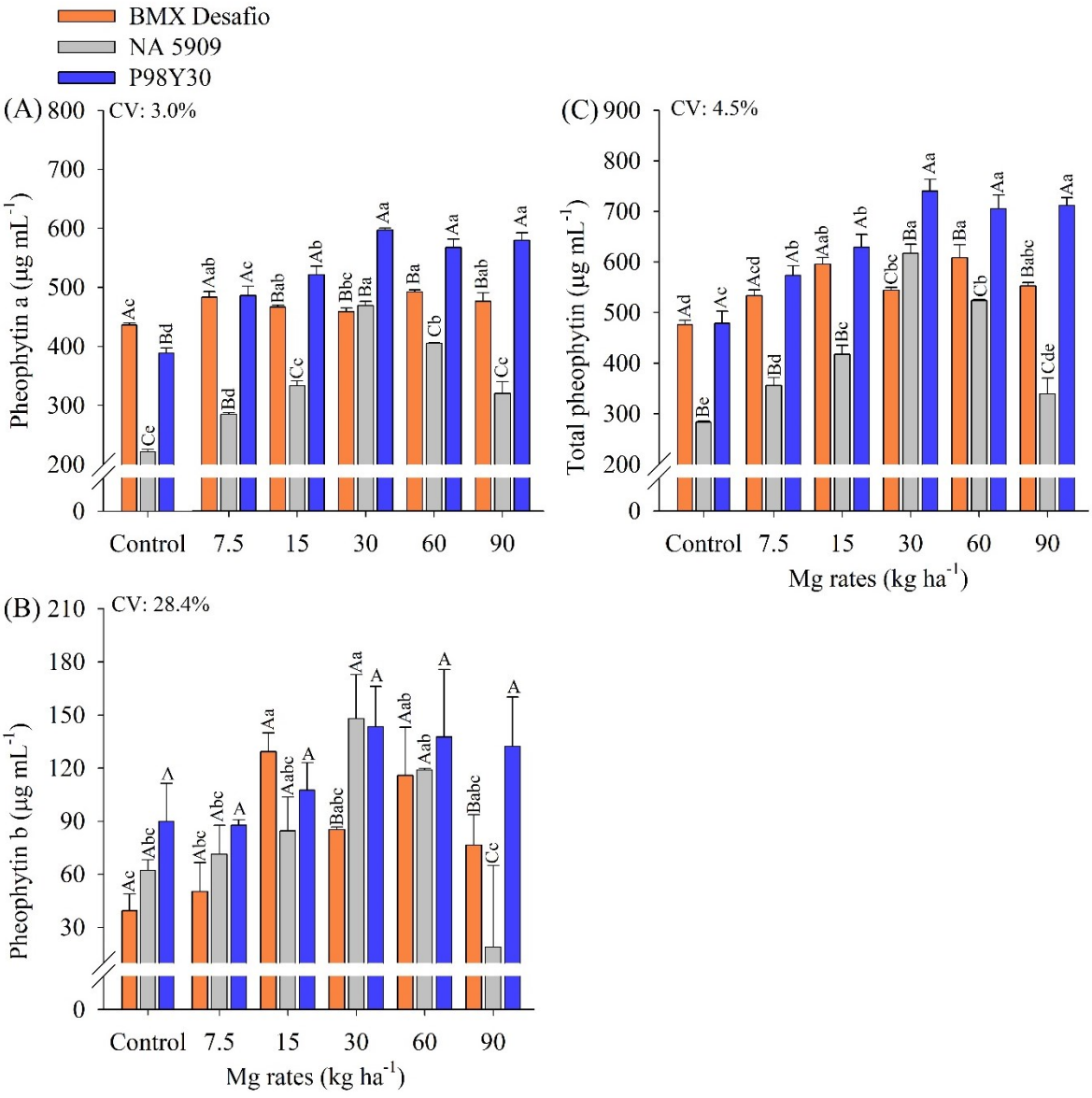


Source: The author himself

Carotenoid concentration increased in BMX Desafio RR plants in response to all doses of Mg (Figure 4D). For the other soybean varieties, carotenoids increased gradually with Mg application. In P98Y30 RR the highest carotenoids concentration was observed with the application of 30 and 90 kg ha^{-1}. For NA 5909 RG RR, the highest carotenoid concentration was observed with 30 and 60 kg ha^{-1}, being 103 and 83% higher than untreated plants.

Pheophytin concentration increased with Mg supply (Figure 4). In BMX Desafio RR, the highest pheophytin *a* concentration was observed with 60 kg ha^{-1} of Mg supply (Figure 5A). For NA 5909 RG RR, the highest concentration of pheophytin *a* was recorded with 30 kg ha^{-1} supply, which is 112% higher than the control treatment. There was an increase in pheophytin *b* for BMX Desafio RR plants treated with 15 kg ha^{-1} of Mg (Figure 5B). For NA 5909 RG RR the highest concentrations of pheophytin *b* were observed at the dose 30 kg ha^{-1}. The highest concentration of total pheophytin was observed for BMX Desafio RR treated with 60 kg ha^{-1}, which is 28% higher than untreated plants (Figure 5C). For NA 5909 RG RR, the highest concentration of total pheophytin was recorded with 30 kg ha^{-1}, 118% higher than untreated plants. The results show a positive influence of Mg application to increase photosynthetic pigments in soybean leaves, which indicates greater protection to the photosynthetic apparatus against high luminosity.

Figure 5. Effects of Mg rates on pheophytin *a* (A), pheophytin *b* (B) and total pheophytin (C) concentration in soybean leaves. CV means coefficient of variation. The averages followed by the same capital letters are not significantly different and compare soybean varieties in response to Mg supply. The averages followed by the same lower case letters compare each variety in response to Mg doses, according to the Tukey test ($p \leq 0,05$). Bars represent the standard error of the means ($n = 4$).



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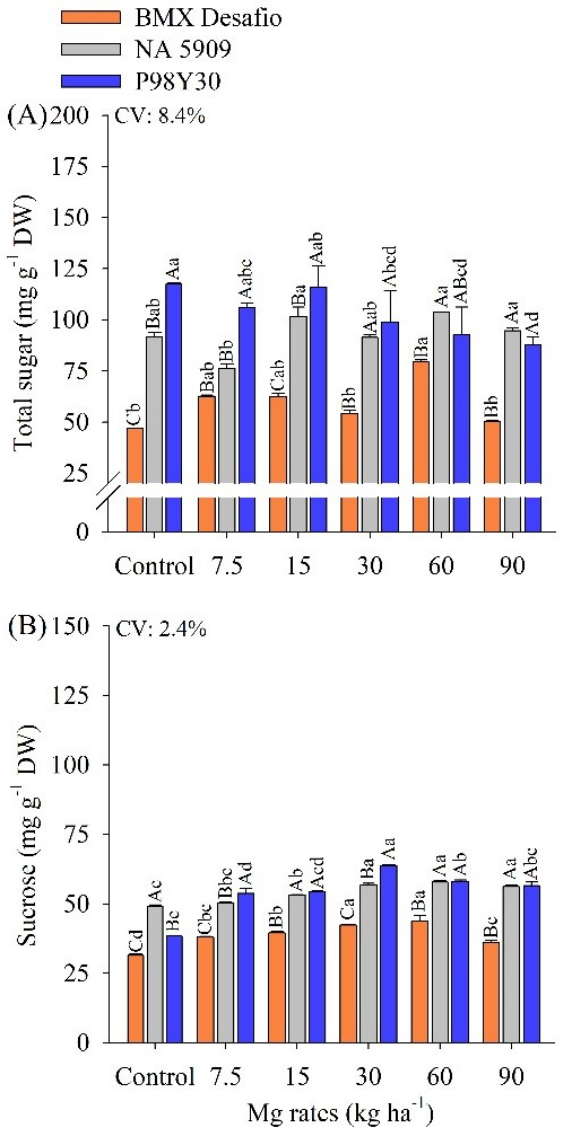
Concentration of sucrose and total sugars

Total sugar concentration increased by 69% for BMX Desafio RR in response to 60 kg ha^{-1} in comparison to untreated plants (Figure 6A). For NA 5909 RG RR, the highest total sugars concentration was observed with 15, 60 and 90 kg ha^{-1}. Among varieties, the highest total sugars concentration was observed for P98Y30 RR not differing from NA 5909 RG RR with of 30, 60 and 90 kg ha^{-1} of Mg supply.

Magnesium supply increased the production of sucrose for the variety BMX Desafio RR with 30 and 60 kg ha^{-1} by 35 and 39%, respectively (Figure 6B). Similarly, NA 5909 RG RR increased sucrose concentration by applying 30, 60 and 90 kg ha^{-1}, which represents 16, 18 and 15% higher than control treatment. P98Y30 RR also increased sucrose in response to 30 kg ha^{-1}, being 66% higher than control treatment.

Figure 6. Effects of Mg rates on total sugar (A) and sucrose (B) concentration in soybean leaves. CV means coefficient of variation. The averages followed by the same capital letters are not significantly different and compare soybean varieties in response to Mg supply. The averages followed by the same lower case letters compare each variety in response to Mg doses, according to the Tukey test ($p \leq 0,05$). Bars represent the standard error of the means ($n = 4$).



Source: The author himself

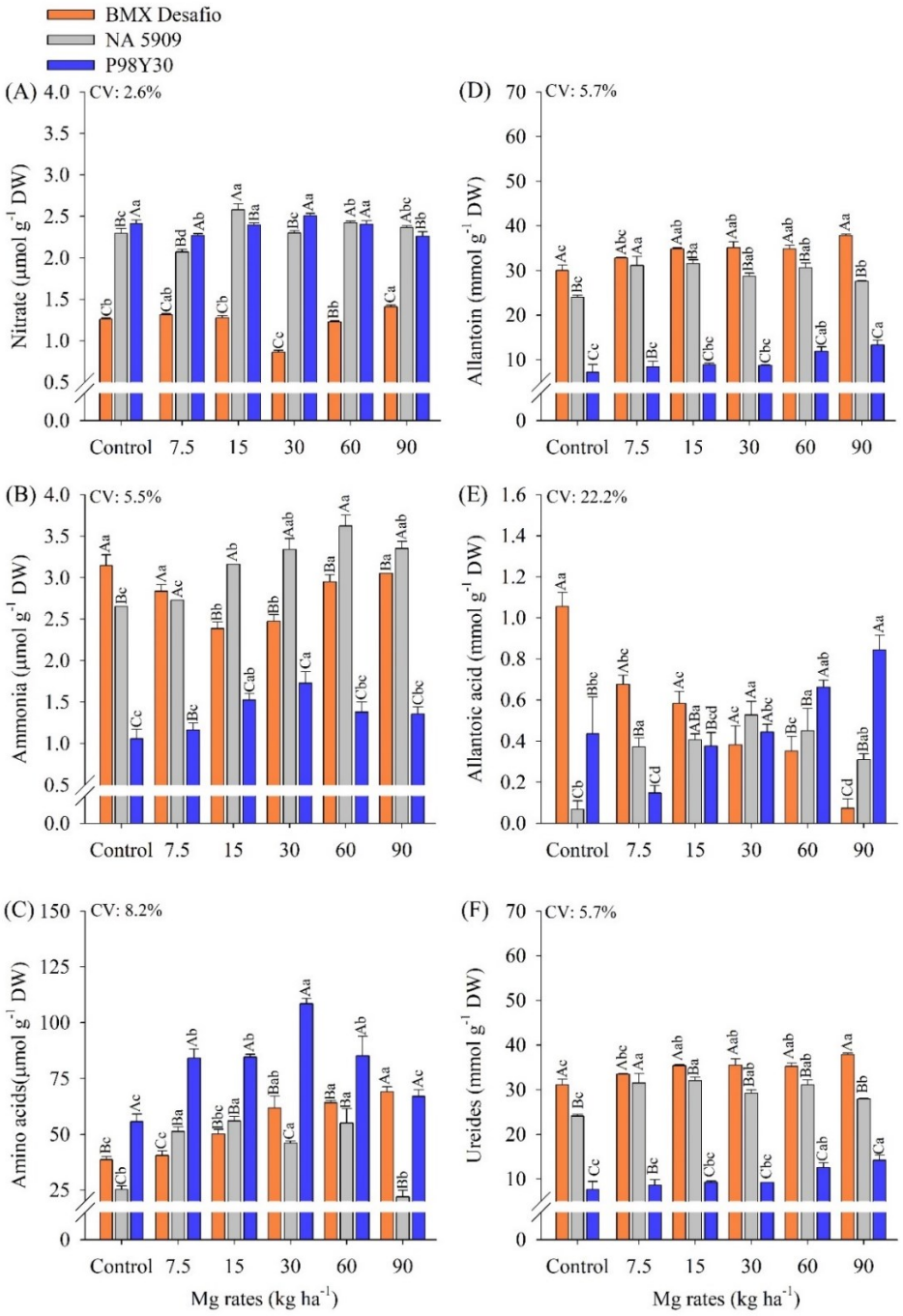
Nitrogen compounds

Nitrate concentration in the BMX Desafio RR decreased with 30 kg ha^{-1} of Mg supply by 46% compared to untreated plants (Figure 7A). For NA 5909 RG RR, fertilization with 7.5 kg ha^{-1} reduced nitrate by 11% compared to the control. Ammonia concentration decreased in the BMX Desafio RR variety with the application of 15 and 30 kg ha^{-1} by 32 and 27%, respectively (Figure 7B). In NA 5909 RG RR there was an increase of 26, 37 and 26% with the application of 30, 60 and 90 kg ha^{-1} compared to untreated plants. For the P98Y30 RR variety there was an increase in ammonia concentration with 15 and 30 kg ha^{-1} in 44 and 63% compared to untreated plants.

Total free amino acids increased with Mg supply for all soybean varieties (Figure 7C). The highest amino acids concentration for BMX Desafio RR was observed with 30, 60 and 90 kg ha^{-1}, which is 60, 66 and 78% higher compared to untreated plants. For P98Y30 RR the highest amino acid was recorded with 30 kg ha^{-1}, 95% compared to untreated plants. Similarly, Mg fertilization promoted an increase in amino acids concentration for NA 5909 RG RR in almost all treatments.

Allantoin concentration increased in response to Mg supply (Figure 7D). For BMX Desafio RR, the highest allantoin was observed with 90 kg ha^{-1} of Mg supply. For NA 5909 RG RR there was an increase of 30 and 32% with application of 7,5 and 15 kg ha^{-1} compared to untreated plants. For P98Y30 RR, fertilization with 60 and 90 kg ha^{-1} of Mg provided an increase of 65 and 85% compared to untreated plants. Magnesium fertilization increased allantoic acid concentration in NA 5909 RG RR and P98Y30 RR (Figure 7E). Mg supply also increased ureide concentration in soybean leaves (Figure 7F). Nitrogen metabolism compounds in the soybean varieties were enhance in response to Mg oxide application, which is assumed to be a beneficial indicator of BNF process.

Figure 7. Effects of Mg rates on nitrate (A), ammonia (B), amino acids (C), allantoin (D), allantoic acid (E) and ureides (F) concentration in soybean leaves. CV means coefficient of variation. The averages followed by the same capital letters are not significantly different and compare soybean varieties in response to Mg. The averages followed by the same lower case letters compare each variety in response to Mg doses, according to the Tukey test ($p \leq 0,05$). Bars represent the standard error of the means ($n = 4$).



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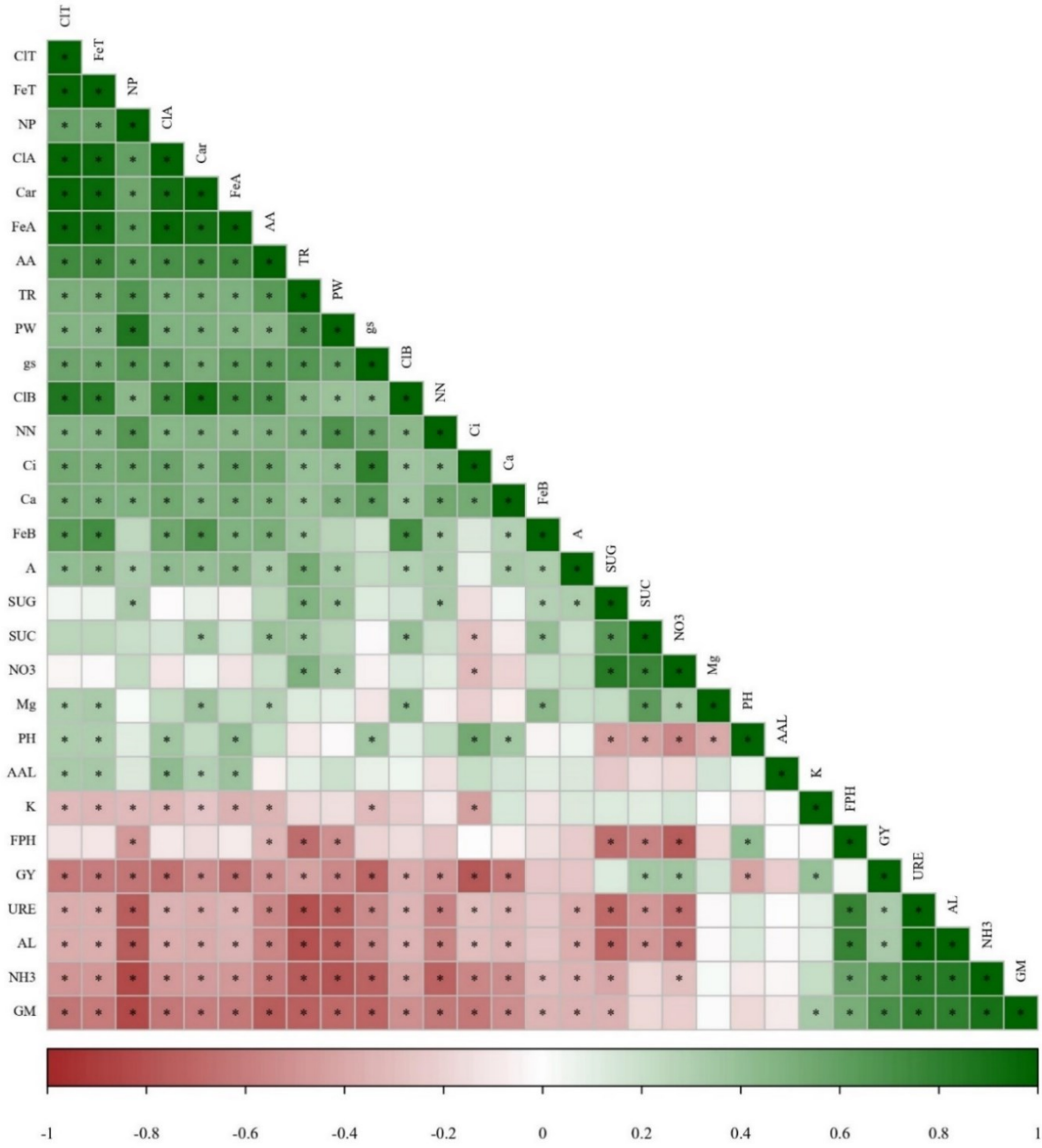
2.5. DISCUSSION

Magnesium oxide application increased leaf Mg concentration of all analyzed soybean varieties. According to White and Broadley (2009), Mg supply to the soil through fertilization is what produces the best results and increases Mg concentration in plant tissues. Considering the essentiality of Mg for plants, it is expected that higher concentration in plants will contribute to greater photosynthetic carbon dioxide fixation, as well as the greater photoassimilates transport, processes that would normally be impaired by Mg deficiency (CAKMAK; KIRKBY, 2008; TRÄNKNER *et al.,* 2018).

Magnesium fertilization did not affect K uptake by soybean plants. Considering that K content in the soil is generally less than Mg, plants developed specific K transport systems in order to guarantee enough K absorption (HORIE *et al.,* 2011). These K transporters cannot be blocked by other nutrients such as Mg. On the other hand, competitive inhibition between nutrients by the same site as the Mg carrier might occur (MALAVOLTA *et al.,* 1989). Although Mg has greater mobility in the soil than Ca (VAN DER HEIJDEN *et al.,* 2013), the high concentration of Ca may influence the uptake of Mg by plants (GRANSEE; FÜHRS, 2013). This would result in variations in Ca leaf concentration or losses in uptake and consequent Mg leaf concentration. However, these processes were not observed in this study. In addition, Mg concentration was not negatively correlated with K and Ca, supporting the results obtained (Figure 8). Leaf concentrations of Mg, K and Ca are within the range considered suitable for soybean cultivation (GUIMARÃES *et al.,* 1999; EMBRAPA, 2013).

Figure 8. Heatmap of Pearson correlation coefficients obtained from variables extracted from soybean.



* indicates significant correlation ($p < 0,05$).

Abbreviation: PH: Plant height; FPH: First pod height; NN: Number of nodes; NP: Number of pods; PW: Pods weight; GM: Grain mass; GY: Grain yield; A: Photosynthesis; gs: Stomatal conductance; Ci: Internal leaf CO_2; Tr: Transpiration; ClA: chlorophyll a; ClB: chlorophyll b; CIT: total chlorophyll; CAR: carotenoids; FeA: pheophytin a, FeB: pheophytin b; FeT: total pheophytin; NO_3^-: Nitrate; NH_3: Ammonia; AL: Allantoin; AAL: Allantoic acid; URE: Ureides; AA: Amino acids; SUC: Sucrose; SUG: Total Sugar; K: Potassium; Ca: Calcium; Mg: Magnesium

Source: The author himself

Magnesium supply did not influence photosynthetic parameters Ci, gs, E and A in soybean varieties. Dias *et al.* (2017) observed contrasting results when studying the development of coffee in different concentrations of Mg. On the other hand, the concentrations of photosynthetic pigments increased in response to Mg. It is known that chlorophylls have Mg as the central ion (MOYNIER; FUJII, 2017) and about 15 to 20% of Mg in plants is associated with these pigments (WHITE; BROADLEY, 2009). Positive correlation coefficients between Mg × total chlorophyll and total pheophytin, as well as the lack of correlation of Mg × photosynthetic parameters support the ability of Mg to increase the concentrations of photosynthetic pigments (Figure 8). In this case, it is possible that higher chlorophyll concentration promotes greater efficiency in the absorption of light energy and electron flow in the photochemical process, increasing the production of ATP and NADPH, which will be used for the synthesis of sugars.

Carotenoid concentrations also increased in soybean leaves in response to Mg application. These pigments are key metabolites that maintain the functional stability of the photosynthetic apparatus during fluctuations in the light spectrum (KUSAMA *et al.,* 2015). With the increase in the production of carotenoids, greater contributions to the photoprotection process of plants against possible cell damage resulting from the excess of light incidence are expected, as well as the rapid ability to extinguish the excited states of chlorophyll, avoiding possible photo-cellular oxidation which would result in the formation of reactive oxygen species (ROS) (FOYER *et al.,* 2018).

Magnesium supply increased sucrose in soybean plants. Mg promoted adequate activity of the photosynthetic apparatus, since sucrose is the main product generated by photosynthesis (SALERNO; CURATTI, 2003). Particularly in the photosynthetic process, Mg has a performance that goes far beyond its structural function of chlorophyll (MOYNIER; FUJII, 2017). Many enzymes that act in this process require or are promoted by Mg, such as RuBisCO (BHAT *et al.,* 2017), RNA polymerases, ATPases and several other enzymes included in the Calvin cycle (VERBRUGGEN; HERMANS, 2013). Possibly, the positive Mg effect on the activity of these enzymes has also contributed to the higher production of sucrose by soybean plants. Positive correlation coefficients between Mg × sucrose concentrations support the results obtained (Figure 8).

The increase in sucrose production in response to Mg supply could also be considered as an inhibition of Mg via phloem which normally occurs under Mg deprivation. In this case, the inhibition of important enzymes related to carbohydrate metabolism would occur, such as fructose-1,6-bisphosphatase, glutamate synthetase and GDPD-glucose pyrophosphorylase (HERMANS; VERBRUGGEN, 2005). Excessive sugar accumulation in leaves might impair photosystems I and II, affecting the photosynthetic process (HERMANS *et al.,* 2004).

Total sugar production increased with the application of Mg in NA 5909 RG RR and BMX Desafio RR. Soluble sugars are involved in ROS combat, as well as can channel NADPH-producing metabolism, such as the oxidative pathway of pentose-phosphate, contributing to the elimination of ROS in plant cell (BOLOURI-MOGHADDAM *et al.,* 2010; KEUNEN *et al.,* 2013). Thus, these changes are associated with some type of abiotic stress, this increase has been driven by the higher sucrose fraction. The positive correlation observed between the production of total sugars × sucrose, compared to the positive correlation with the net photosynthetic rate supports this possibility (Figure 8).

Regarding N metabolism, BMX Desafio RR reduced nitrate concentration with 30 kg ha^{-1}, and in NA 5909 RG RR with 7,5 kg ha^{-1}, as well as P98Y30 RR with 7,5 and 90 kg ha^{-1}. Nitrate reduction in plant cell is catalyzed by the enzyme nitrate reductase (CAMPBELL, 1999). For this reduction to occur, the reducing power of NADH or NADPH from photosynthesis (PIWPUAN *et al.,* 2013) is used, in addition to the ATP given the high energy demand required (SUNIL *et al.,* 2013). ATP must be linked to an Mg ion to be biologically active (MgATP) (IGAMBERDIEV; KLECZKOWSKI, 2015), which makes the need for plants to be well supplied with Mg even more evident. Thus, it is possible that the use of Mg may positively influence the increase in the nitrate reductase activity contributing to the rapid conversion of nitrate to ammonia. Negative correlation coefficients between nitrate × ammonia concentrations support this possibility (Figure 8).

It is important to emphasize that Mg fertilization with 7,5 and 90 kg ha^{-1} increased nitrate concentration for BMX Desafio RR, as well as 15 kg ha^{-1} for NA 5909 RG RR. These results raise the possibility that the application of Mg favors greater absorption of nitrate by plant roots. The positive correlation between Mg × nitrate concentration corroborates this possibility, indicating that Mg supply increases nitrate

concentration in soybean varieties (Figure 8). However, more studies are needed to prove this effect.

There was an increase in ammonia concentration in response to Mg at the rates of 30, 60 and 90 kg ha^{-1} for NA 5909 RG RR and also for P98Y30 RR in response to 15 and 30 kg ha^{-1}. After being synthesized inside the nodule by the symbiotic association, ammonia is converted into ureides (DUNN, 2014) or incorporated into amino acids by the combined activity of enzymes glutamate and glutamine synthase (MARTÍNEZ-ANDÚJAR *et al.,* 2013). In this study, ammonia concentration was antagonistic to photosynthetic parameters, photosynthetic pigments, sucrose production and biometric parameters (Figure 8).

A greater activity of N metabolism induced by Mg supply was observed by the increase of amino acid concentration in BMX Desafio RR (30, 60 and 90 kg ha^{-1}), NA 5909 RG RR (7,5, 15, 30 and 60 kg ha^{-1} of Mg) and P98Y30 RR (30 kg ha^{-1} of Mg). These results indicate that Mg fertilization promotes a greater production of amino acids by soybean plants. It is known that amino acids are produced by the action of enzymes glutamate and glutamine synthase (MARTÍNEZ-ANDÚJAR *et al.,* 2013). These enzymes are affected by Mg according to studies by Yin *et al.* (2009), evaluating the Mg effect on the nitrogen metabolism enzymes in spinach plants. Positive correlation coefficients between Mg × amino acid concentration support the results obtained in this study (Figure 8).

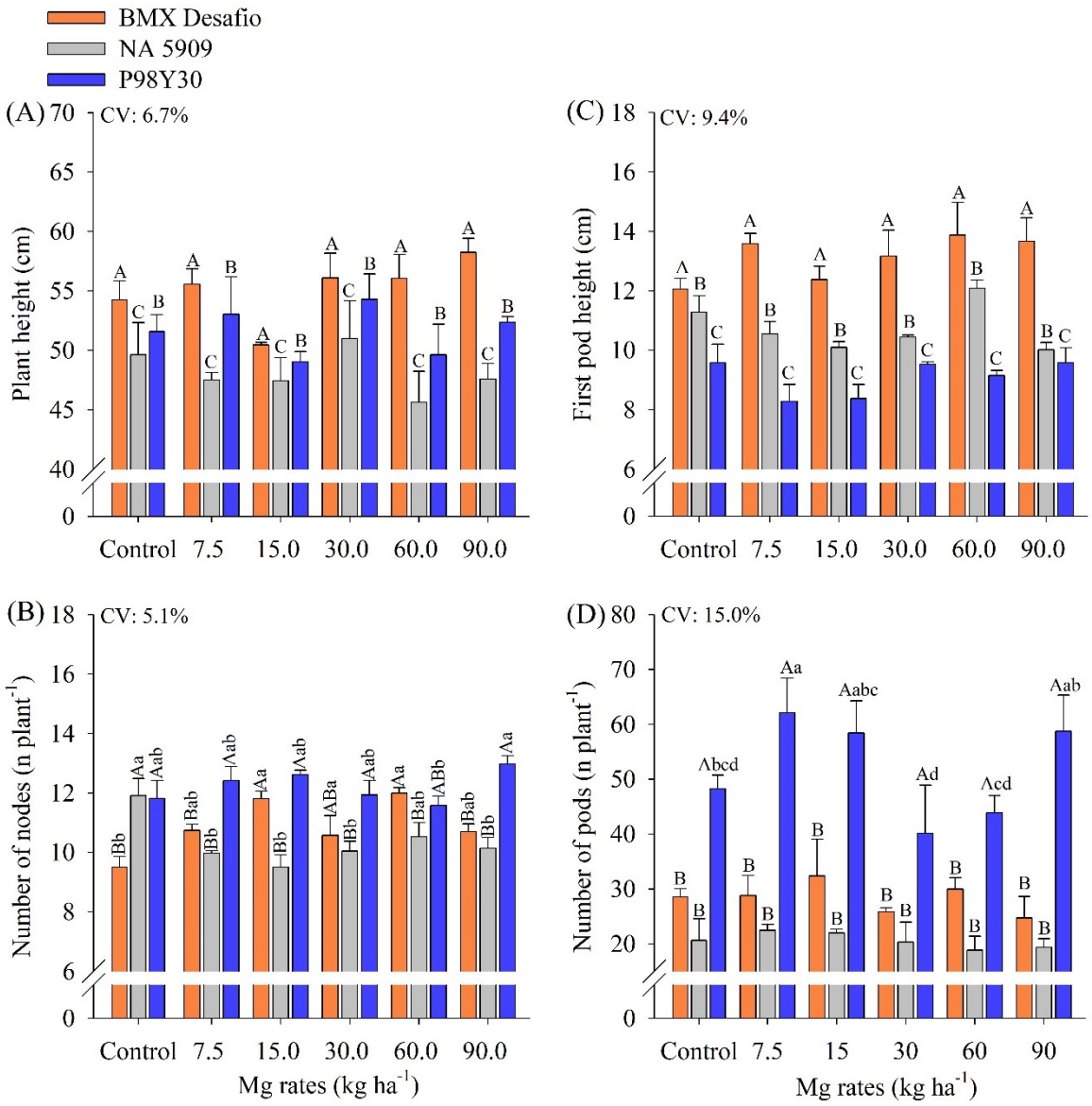
Magnesium fertilization increases allantoin and allantoic acid concentration for NA 5909 RG RR (7,5, 15, 30 and 60 kg ha^{-1}) and P98Y30 RR (60 and 90 kg ha^{-1}). For BMX Desafio RR there was an increase in allantoin concentration (15, 30, 60 and 90 kg ha^{-1}). Ureides are derived from oxidative degradation of purines (CARTER; TEGEDER, 2016). Allantoin is synthesized in peroxisomes from uric acid, while allantoic acid is synthesized from allantoin (LAMBERTO *et al.,* 2010; WERNER; WITTE, 2011). Thus, these results indicate that Mg induced beneficial effects on these assimilation routes, contributing to greater conversion of nitrogen compounds metabolism into ureides in soybean plants.

Ureides from BNF are considered the main form of nitrogen transported in the xylem of soybean plants (CHIASSON *et al.,* 2014). According to Jahnen-Dechent and Ketteler (2012), many enzymes act by catalyzing the chemical reactions involved in BNF, with a considerable requirement for Mg to activate some of these enzymes

(BOMFIM *et al.,* 2017). The results of this study suggest that soybean plants fertilized with Mg showed higher production of photoassimilates (sugars). Plants treated with Mg showed a greater conversion of N_2 atmospheric into ureides, which is the main form of nitrogen transported in soybean plants. The negative correlation coefficients indicate that sucrose and total sugars were inversely proportional to the production of allantoin (Figure 8).

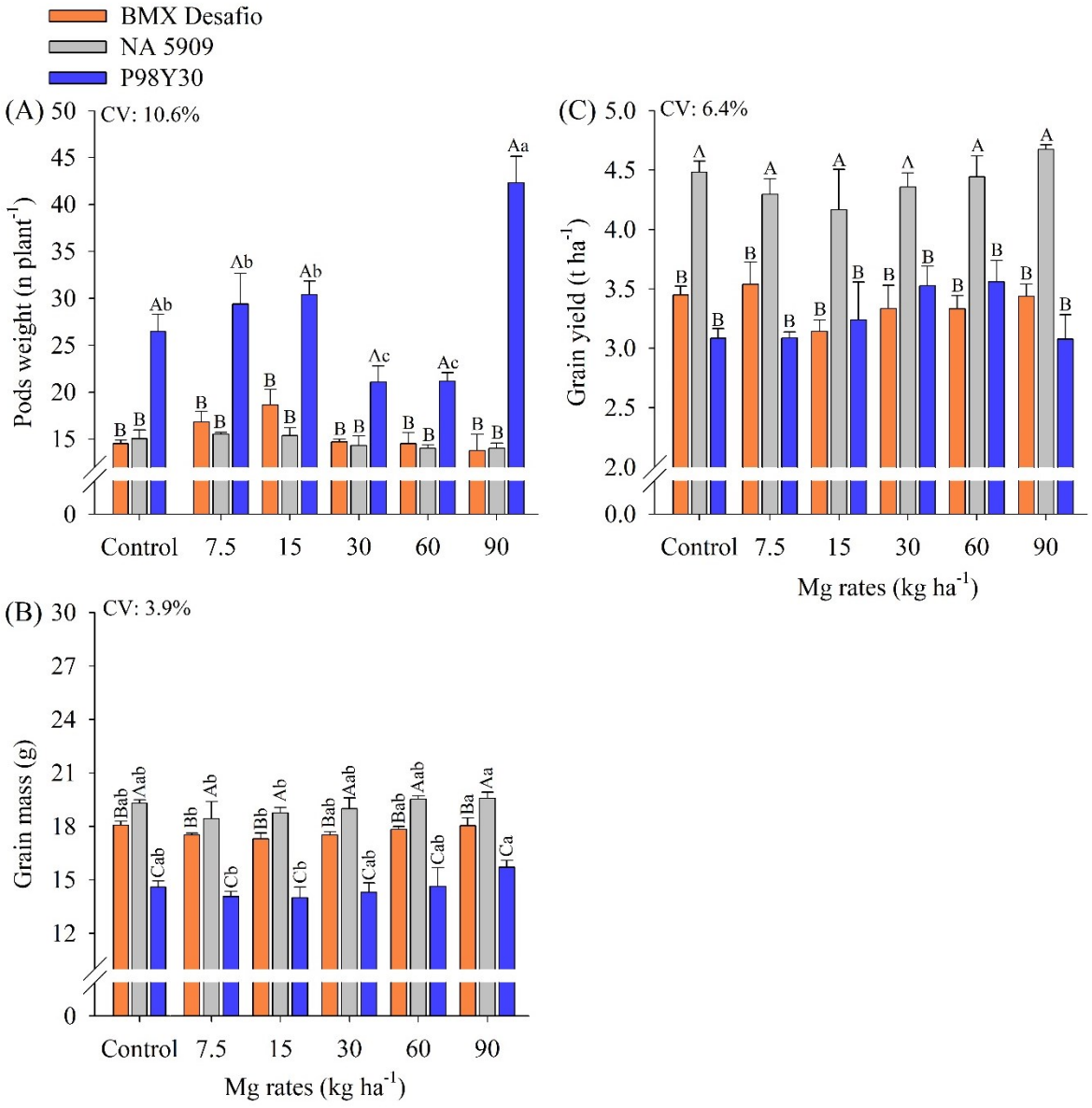
Higher biosynthesis of ureides positively influenced important biometric parameters, such as number of nodes (BMX Desafio RR and P98Y30 RR), number and weight of pods (P98Y30 RR) and grain weight (BMX Desafio RR, NA 5909 RG RR and P98Y30 RR). However, application of Mg oxide did not affect the plant growth or productivity (Figures 9 and 10). Possibly, the absence of this effect is related to the limiting action of some type of abiotic factor, for example, soil with high Mg content. Thus, considering that one of the main challenges of agriculture will be to increase crop yields (REYNOLDS *et al.,* 2012), the results observed in this study are consistent as innovative techniques for handling Mg in the soil and enhance BNF process in soybean plants.

Figure 9. Effects of Mg rates on plant height (A), number of nodes (B), first pod height (C) and number of pods (D) of soybean plants. CV means coefficient of variation. The averages followed by the same capital letters are not significantly different and compare soybean varieties in response to Mg supply. The averages followed by the same lower case letters compare each variety in response to Mg doses, according to the Tukey test ($p \leq 0,05$). Bars represent the standard error of the means ($n = 4$).



Source: The author himself

Figure 10. Effects of Mg rates on pods weight (A), grain mass (B) and grain yield (C) of BMX Desafio RR, NA 5909 RG RR and P98Y30 RR varieties. CV means coefficient of variation. The averages followed by the same capital letters are not significantly different and compare soybean varieties in response to Mg supply. The averages followed by the same lower case letters compare each varieties in response to Mg doses, according to the Tukey test ($p \leq 0,05$). Bars represent the standard error of the means ($n = 4$).



Source: The author himself

2.6. CONCLUSION

Application of Mg oxide doses increased Mg leaf concentrations but did not alter leaf Ca and K concentration in soybean plants. Mg fertilization increased photosynthetic pigments, sucrose, total sugars but did not enhanced photosynthesis of soybean plants. Furthermore, the application of Mg doses did not affect the plant growth and productivity of soybean, in soil with high Mg content.

Magnesium fertilization increased ureides, amino acids and ammonia concentration in soybean leaves, indicating greater activity of BFN in soybean plants. This study resulted in new highlights on ureide metabolism and BNF process in commercial soybean varieties fertilized with Mg. This study shows important information tools for future work aiming to increase the efficiency of Mg fertilization in soybean plants.

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3 SOIL APPLICATION OF MAGNESIUM SULPHATE ENHANCE CHLOROPHYLLS AND PHOTOASSIMILATES IN SOYBEAN PLANTS

3.1. ABSTRACT

Magnesium (Mg) play several physiological roles in higher plants especially in photosynthetic process. However, Mg roles on biological nitrogen fixation (BNF) in legume plants is barely understood. This study aimed to evaluate the effect of Mg rates applied as Mg sulphate on photosynthetic pigments, sugars and relations with ureides as indicator of BNF of soybean plants. Three soybean varieties (NA 5909 RG RR, BMX Desafio RR and P98Y30 RR) and six Mg rates (0, 7.5, 15, 30, 60 and 90 kg ha^{-1}) were tested. Application of 30 kg ha^{-1} as Mg sulphate provided higher concentrations of Mg in leaves. Mg sulphate did not affect leaf potassium (K) concentration but reduced Ca in leaves with 30, 60 and 90 kg ha^{-1}. The concentration of photosynthetic pigments (chlorophyll, carotenoids and pheophytins) increased with Mg supply, but did not affect net photosynthetic rates, stomatal conductance, internal CO_2 concentration and transpiration rates of soybean plants. Higher sucrose and total sugars concentration in NA 5909 RG RR and P98Y30 RR indicate that Mg promoted an adequate carboxylation resulting in greater sugar production. The higher rates of photoassimilates provided greater conversion of atmospheric N_2 into ureides for NA 5909 RG RR (allantoin and allantoic acid) and P98Y30 RR (allantoic acid), indicating greater activity of BNF. The results indicate that soybean plants showed higher concentration of photosynthetic pigments and sugar production, resulting in enhanced ureides metabolism. The present study reports important information regarding management of Mg fertilization on nitrogen metabolism in commercial soybean varieties.

Key-words: Biological nitrogen fixation. photoassimilates. allantoin. allantoic acid. *Glycine max* (L.) Merrill. Mg rates.

3.2. INTRODUCTION

The easiest and most economical way to supply Mg to plants is through the practice of liming (MALAVOLTA, 2006). This ease way in supplying Mg justify the growing disuse of this nutrient by rural producers and researchers in routine soil fertilization practices. As a result, declines in Mg concentrations have been reporting in grains through nutritional status of plants (CAKMAK; YAZICI, 2010). Thus, the proper management of all nutrients for plants must be considered, aiming at a successful agricultural practice. However, the role of Mg in plants is not yet fully understood. Mg function is well documented on chlorophyll and photosynthetic processes in plants and further studies are needed especially on biological nitrogen fixation (BNF) (PENG *et al.,* 2018).

Plant growth and yield can be severely affected under Mg limitation (VERBRUGGEN; HERMANS, 2013). Mg concentrations below 125 μmol L^{-1} in the soil solution and 1,5 g kg^{-1} in vegetative parts affect plant growth (KARLEY; WHITE, 2009). Natural processes such as leaching (GRANSEE; FUHRS, 2013), aluminum toxicity (CHEN; MA, 2013), in addition to high levels of antagonistic ions such as calcium and potassium (GRANSEE; FUHRS, 2013) are responsible to decrease Mg availability to plants. Under Mg deficiency, damage to chlorophyll biosynthesis and fixation of photosynthetic carbon is observed (YUGUAN *et al.,* 2009). The export of sucrose in phloem is also severely impaired (TANOI; KOBAYASHI, 2015). As a result, plant growth is reduced since chlorophyll synthesis and RuBisCO activity are significantly inhibited (YUGUAN *et al.,* 2009).

Mg supply to the soil through fertilization is a good strategy to increase the concentrations of Mg in plant tissues (WHITE; BROADLEY, 2009). Mg sulphate have appeared as an alternative to supply the requirements of Mg nutrition for several crops. Adequate levels of Mg in plant cell go far beyond as structural function in chlorophyll (MOYNIER; FUJII, 2017). Mg acts in the export of photoassimilates (sucrose) via phloem (TANOI; KOBAYASHI, 2015), and is also a cofactor and modulator in more than 300 enzymes (enzymes included in the Calvin cycle, kinases, RNA polymerases and ATPases) (SHAUL, 2002; VERBRUGGEN; HERMANS, 2013). RuBisCO is the main enzyme responsible for assimilation of photosynthetic carbon in plants. For

activation process is necessary the binding of Mg ion into RuBisCO structure in order to carboxylate CO_2 into 3-phosphoglycerate in plant leaves (BHAT *et al.,* 2017).

Soybean (*Glycine max* L.) has an economic prominence worldwide as the main source of vegetable oil (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, 2020). Soybean plants are efficient in biological nitrogen fixation (BNF), a highly relevant and complex process in which Fabaceae (*Leguminosae*) establish symbiosis with Rhizobia bacteria. In the nodules, N_2 is reduced to ammonia (BARAL *et al.,* 2014) and later exported as ureides (allantoin and allantoic acid) (DUNN, 2014). Functionality of nodules and the respective BNF in legume plants depend on the adequate supply of photoassimilates (sugars) by leaves. In order to reduce atmospheric N_2 into ureides, 16 molecules of adenosine triphosphate (ATP) are needed, and ATP must bind to Mg to be biologically active (IGAMBERDIEV; KLECZKOWSKI, 2015). With adequate Mg nutrition, plants can properly perform photosynthetic process, transporting photoassimilates from sources to drains, making BNF viable in the root nodules of soybean plants (PENG *et al.,* 2018).

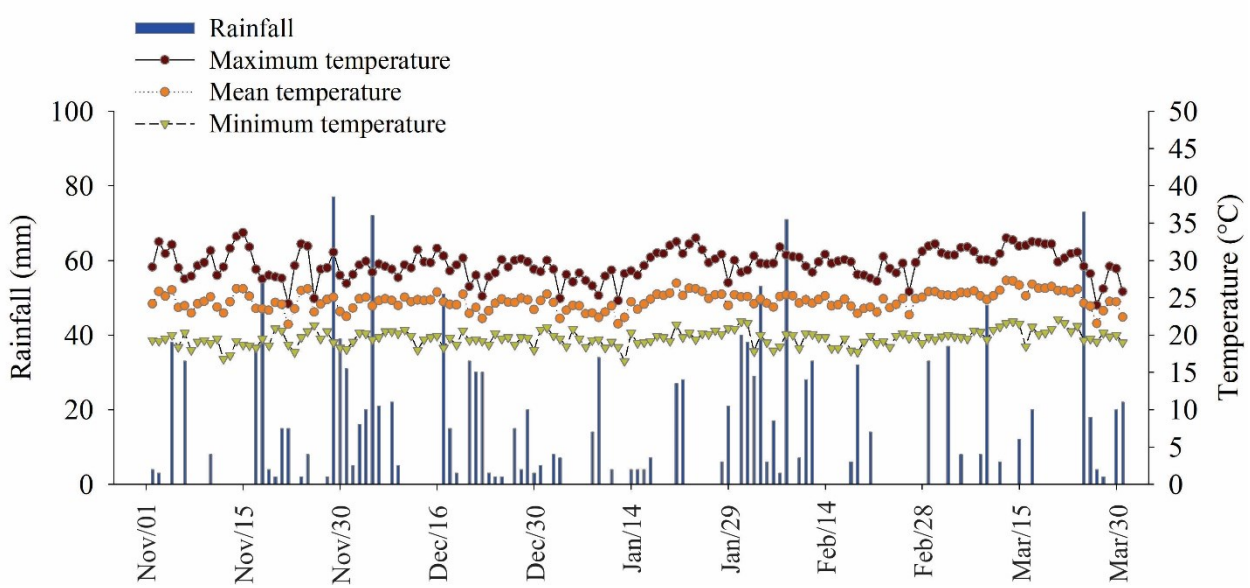
This study hypothesizes that $MgSO_4$ as a source of Mg may present physiological responses in soybean plants, with direct effects on photosynthesis and consequently in BNF through the adequate supply of carbohydrates synthetized in the leaves. This study aimed to evaluate the production of photoassimilates and ureides of soybean varieties as indicator of BNF in response to Mg fertilization applied as $MgSO_4$.

3.3. MATERIAL AND METHODS

3.3.1. Growth conditions

The study was performed in 2017/2018 at an experimental area at the Research Farm of Fundação Chapadão, located in the municipality of Chapadão do Sul-MS, Brazil. Geographical coordinate is 18 ° 46 '48.49 "S and 52 ° 38' 42.59" O at 840 m of sea level. Monthly values of Rainfall, maximum and minimum temperatures are presented in the Figure 1.

Figure 1. Minimum, average and maximum daily rainfall and temperatures during the experimental driving period.



Rainfall: data obtained from a manual data acquisition station, installed at the experiment site.Temperature: data obtained through INMET (Instituto Nacional de Meteorologia), Chapadão do Sul, MS, Highway MS 306 - km 96. Source: www.inmet.gov.br

The soil was classified as Oxisol according to Soil Survey Staff (2014), with clay content in 0.00 – 0.20 m layer of 607.5 g kg^{-1}, silt 62.5 g kg^{-1} and sand 330 g kg^{-1}, determined by the pipette method (EMBRAPA, 2017) and chemical analyses: phosphorus (resin) 29,7 mg dm^{-3}; organic matter 28,85 g dm^{-3}; pH calcium chloride ($CaCl_2$) 4,9; potassium 4,7 $mmol_c$ dm^{-3}; calcium 27,0 $mmol_c$ dm^{-3}; magnesium 10,5 $mmol_c$ dm^{-3}; H+Al 63 $mmol_c$ dm^{-3}; aluminum 0,5 $mmol_c$ dm^{-3}; sulfur 3,85 mg dm^{-3}; cation exchange capacity 105,2 $mmol_c$ dm^{-3}; and base saturation 40%. The methods of determining the elements followed the standards of Van Raij *et al.* (1996). The area has been cultivated with soybean under no-tillage system under cotton residue.

The experimental design was in randomized blocks, distributed in a 3 × 6 factorial scheme, with 3 replications, where: three soybean varieties (NA 5909 RG RR - early cycle of 100 days; BMX Desafio RR - medium cycle of 115 days; P98Y30 RR - 130 days late cycle) and six Mg doses (0, 7,5, 15, 30, 60 and 90 kg ha^{-1}) applied as Mg sulphate (9% Mg).

The soybean varieties were sown on November 29, 2017. Sowing was carried out mechanically in the experimental plots containing six rows of 5,5 m spaced at 0,45

m. Fertilization was performed using 150 kg ha^{-1} of MAP (11-52-00), 200 kg ha^{-1} of agricultural gypsum (12% S) and 3 kg ha^{-1} of ulexite (8% B) in the sowing and 100 kg ha^{-1} of KCl (60% K_2O) after emerging. Seed inoculation was carried out close to soybean sowing through N_2 fixing bacteria (strains: SEMIA 5019 - *Bradyrhizobium elkanii* and SEMIA 5079 - *Bradyrhizobium japonicum*, both with a guarantee of $6x10^9$ CFU mL^{-1}) at the dose of 100 mL ha^{-1}. Magnesium oxide application occurred after 15 days after sowing, on surface.

During anthesis, the fourth fully developed leaves (diagnostic leaves) were collected to evaluate photosynthetic pigments and nitrogen metabolism. The collection of leaves was performed during anthesis because is the period of maximum metabolic activity in soybean plants. The material was prepared to evaluate the contents of Mg, potassium (K), calcium (Ca), nitrogen compounds (nitrate, ammonia, amino acids, allantoin and allantoic acid), total sugars and sucrose. The harvest was carried out on March 20 for NA5909, March 29 for BMX Desafio RR and on April 18 for P98Y30 RR. The phytosanitary management practices followed the technical cultivation recommendations and were carried out homogeneously throughout the experimental area.

3.3.2. Chemical analysis of Plant tissue

The nutritional plant status was evaluated by collecting the fourth fully expanded leaf in each plot at the beginning of flowering. The collected leaves were washed with running water, then in a 0.1% neutral detergent solution, after in 0.3% HCl solution and to rinsed in distilled water, and then dried in an oven at 65 ± 5ºC until constant weight, following the methodology proposed by Bataglia *et al.* (1983). Subsequently, samples were ground in a Wiley-type mill and digested in nitric-perchloric solution. Leaf Mg, K and Ca concentration were determined by atomic absorption according methodology described by Malavolta *et al.* (1997).

3.3.3. Physiological and biochemical analyzes

Photosynthetic pigments and gas exchange parameters

The assessment of gas exchange consisted of non-destructive analysis using the fourth leaf fully developed during the anthesis of each variety between 8 and 11 am. An infrared gas analyzer (Infrared Gas Analyzer - IRGA) model Li-6400XT (LiCor Inc. Lincoln, Nebraska, USA) was used as described by Reis *et al.* (2015). Photosynthetic pigments were extracted using 80% cold acetone and measured using the methodology proposed by Lichtenthaler (1987).

Determination of nitrogen compounds

To determine nitrogenous compounds, 0.3 g of dry leaf samples were extracted in 10 mL of MCW (60% methanol, 25% chloroform and 15% H_2O). The material was centrifuged at 10285 *g* for 10 minutes at 4 ºC. Subsequently, the material was kept at rest for 48 hours. In another tube, 4 mL of supernatant of the MCW extract, 1 mL of chloroform and 1,5 ml of water were added. After the separation of the phases, the water-soluble phase was used to determine sucrose, total sugars, nitrate, ammonia, total free amino acids, allantoin and allantoic acid concentration in soybean leaves. Sucrose, total sugars, nitrate and ammonia were determined following the methodology of Van Handel (1967) and Dubois *et al.* (1956), Cataldo *et al.* (1975), McCullough (1967) respectively. Total free amino acids were measured according Yemm and Cocking (1955) and ureides (allantoin and allantoic acids) according Vogels and Van Der Drift (1970).

3.3.4. Statistical analysis

Statistical analysis was performed using R software (R DEVELOPMENT CORE TEAM, 2019). Normality test was analyzed by Shapiro and Wilk (1965). F test ($p < 0,05$) was applied and averages were compared by Tukey test at 5% of error probability. Heatmap was performed as described by Reis *et al.* (2020).

3.4. RESULTS

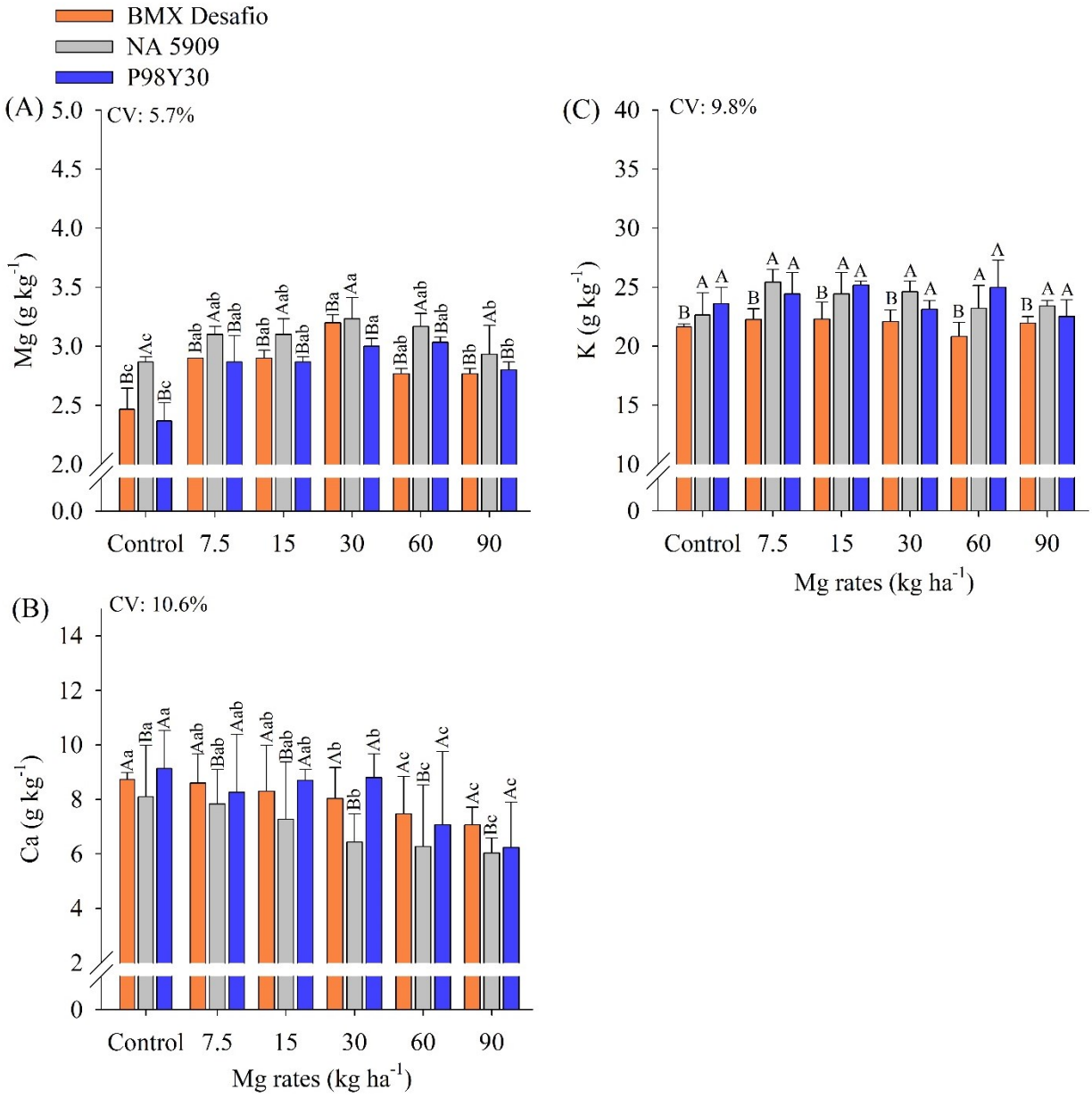
3.4.1. Nutritional analysis

Application of $MgSO_4$ via soil increased leaf Mg concentration of soybean plants (Figure 2A). Soil application of 30 kg ha^{-1} provided the greatest increase of leaf Mg in all soybean varieties. This increase was 30, 13 and 27% for BMX Desafio RR, NA 5909 RG RR and P98Y30 RR compared to untreated plants.

Magnesium fertilization impair leaf Ca concentration of soybean plants (Figure 2B). Soil application of 30, 60 and 90 kg ha^{-1} of Mg reduced leaf Ca concentration for all varieties when compared to untreated plants. The application of 90 kg ha^{-1} reduced Ca concentrations by 12, 12 and 47% in comparison with control treatment for BMX Desafio RR, NA 5909 RG RR and P98Y30 RR.

Leaf K concentration was not impaired by Mg fertilization (Figure 2C). Among varieties, the highest leaf K concentration was observed for NA 5909 RG RR and P98Y30 RR. The results point to a positive effect of $MgSO_4$ application to the increase Mg in soybean leaves. Among varieties, the early cycle (NA 5909 RG RR) showed greatest leaf Mg concentration.

Figure 2. Effects of Mg rates on leaf Mg (A), Ca (B) and K (C) concentration in soybean plants. CV means coefficient of variation. The standard error of the mean (n = 4) is indicated by error bars. Different letters indicate difference between means according to a Tukey test ($p \leq 0,05$). Uppercase letters correspond to Mg rates, and lowercase letters correspond to soybean varieties.



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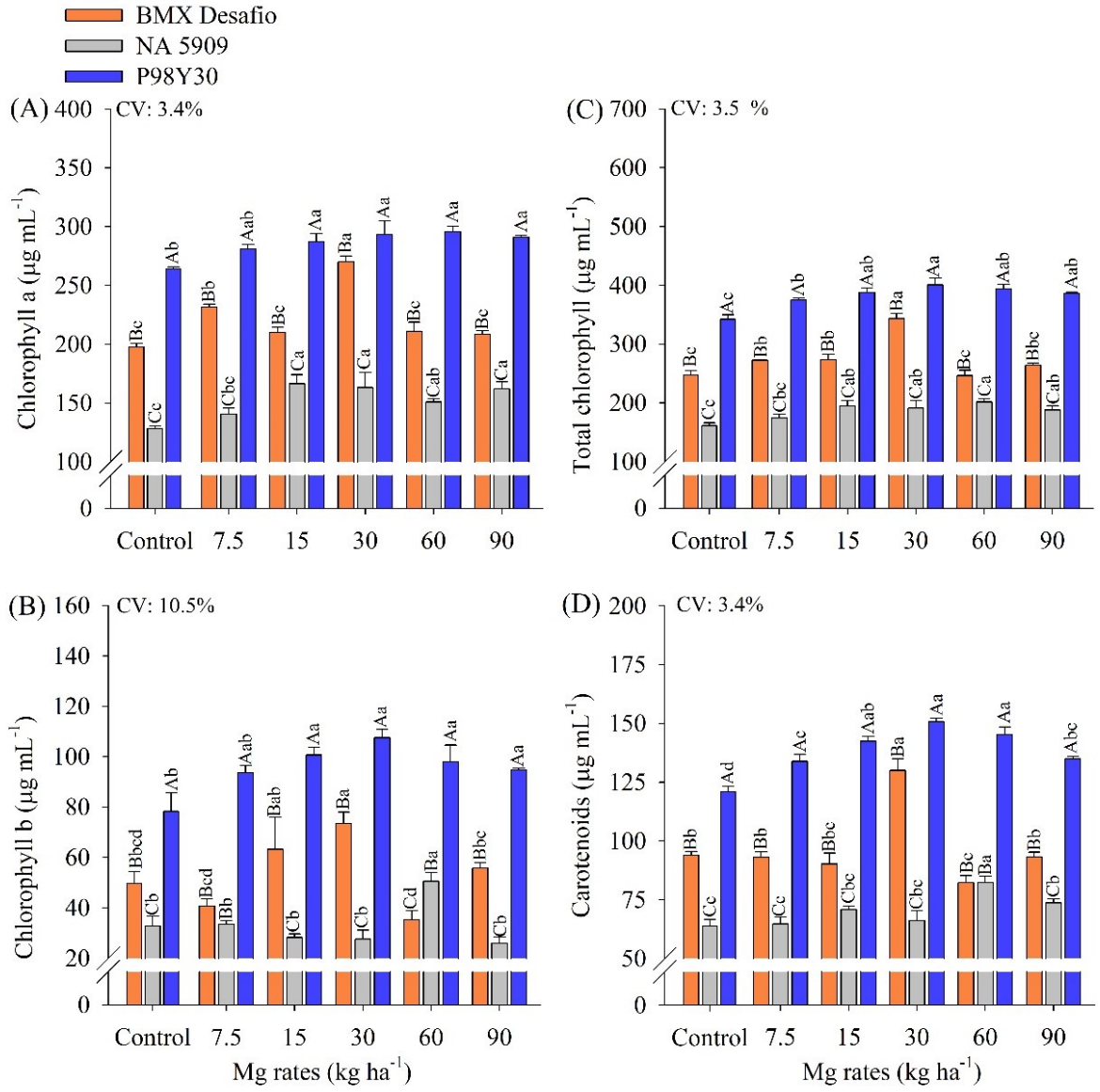
3.4.2. Physiological and biochemical analyzes

Photosynthetic pigments

Photosynthetic pigments (chlorophyll *a*, *b* and total chlorophyll) increased with Mg supply (Figure 3). The highest concentration of chlorophyll *a* for BMX Desafio RR was observed at the rate 30 kg ha^{-1}, 37% higher in comparison to control treatment (Figure 3A). For NA 5909 RG RR, fertilization with 15, 30, 60 and 90 kg ha^{-1} showed the highest concentration of chlorophyll *a*, which are 30, 27, 18 and 26% higher than untreated plants. Similarly, Mg fertilization increased chlorophyll *a* for P98Y30 RR by 7 to 12% compared to untreated plants. There was an increase in chlorophyll *b* concentration for P98Y30 RR in response to Mg supply (Figure 3B). For BMX Desafio RR, 15 and 30 kg ha^{-1} increased chlorophyll *b*, while NA 5909 RG RR increased chlorophyll *b* with 60 kg ha^{-1}. Total chlorophyll concentrations increased in BMX Desafio RR in response to 30 kg ha^{-1} of Mg (Figure 3C). For NA 5909 RG RR, total chlorophyll concentration increased by 21, 19, 25 and 17% in response to 15, 30, 60 and 90 kg ha^{-1} of Mg supply. Similarly, the same rates of Mg increased total chlorophyll concentrations of P98Y30 RR by 13, 17, 15 and 13%.

Carotenoid concentration increased for BMX Desafio RR in response to 30 kg ha^{-1}, 38% higher than untreated plants (Figure 3D). For NA 5909 RG RR, application of 60 kg ha^{-1} showed the highest carotenoid concentrations, 29% higher than control treatment. Among varieties, P99Y30 had the highest carotenoids concentration due to Mg supply.

Figure 3. Effects of Mg rates on chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) and carotenoids (D) in soybean plants. CV means coefficient of variation. The standard error of the mean (n = 4) is indicated by error bars. Different letters indicate difference between means according to a Tukey test ($p \leq 0,05$). Uppercase letters correspond to Mg rates, and lowercase letters correspond to soybean varieties.



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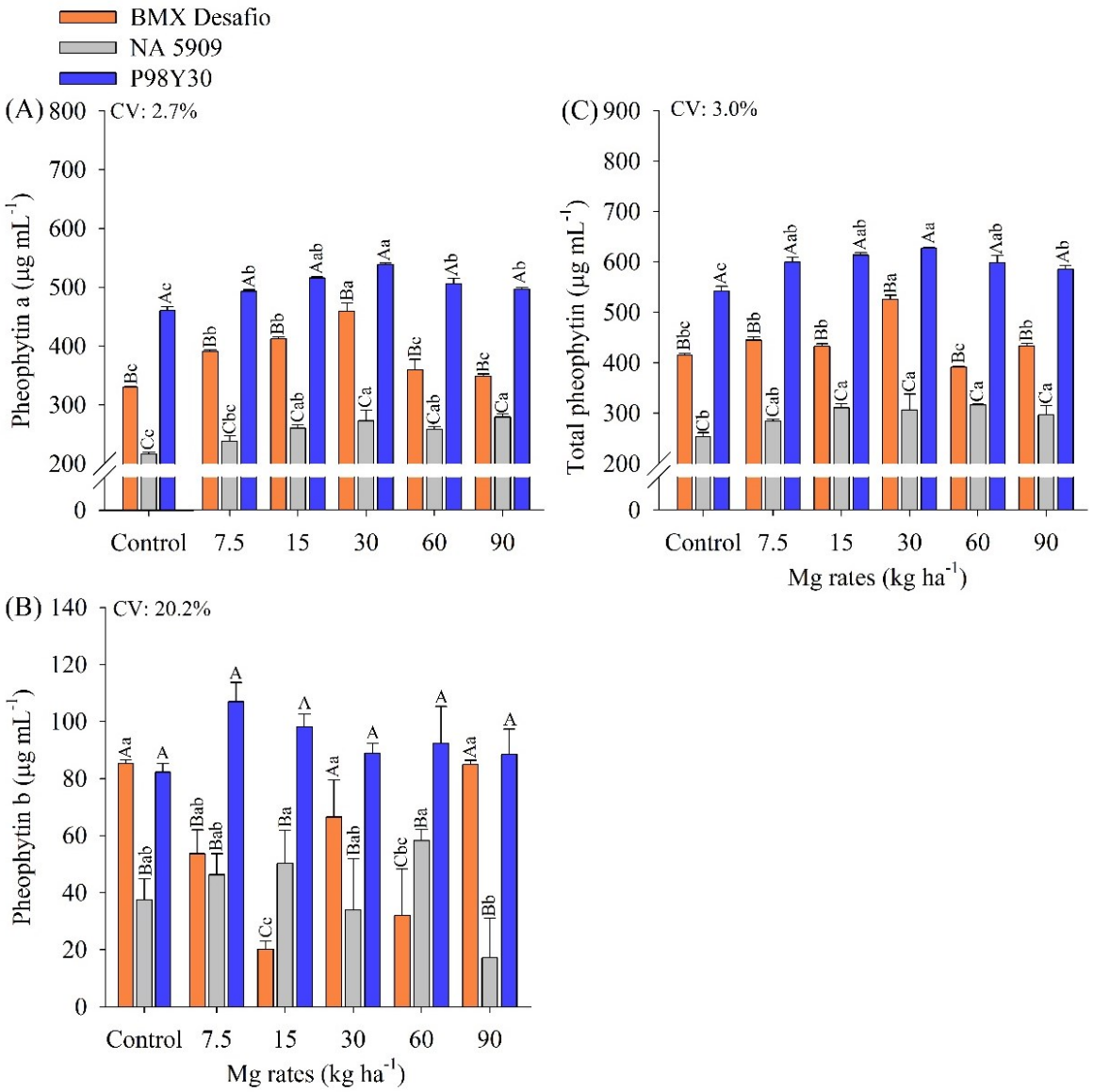
The highest pheophytin *a* concentration was observed in BMX Desafio RR in response to 30 kg ha^{-1}, 39% higher than untreated plants (Figure 4A). For NA 5909 RG RR, the highest pheophytin *a* concentration was observed with the application of 15, 30, 60 and 90 kg ha^{-1}, 20, 26, 19 and 29% higher than control treatment. Mg fertilization with 15 and 60 kg ha^{-1} reduced pheophytin b concentration for BMX Desafio RR by 321% and 167% in relation to the control treatment (Figure 4B). Similarly, application of 60 kg ha^{-1} showed the highest concentrations of pheophytin *b* concentration for NA 5909 RG RR. The highest total pheophytin concentration was observed for BMX Desafio RR with 30 kg ha^{-1}, 27% higher than untreated plants (Figure 4C).

Figure 4. Effects of Mg rates on pheophytin *a* (A), pheophytin *b* (B) and total pheophytin (C) in soybean plants. CV means coefficient of variation. The standard error of the mean (n = 4) is indicated by error bars. Different letters indicate difference between means according to a Tukey test ($p \leq 0.05$). Uppercase letters correspond to Mg rates, and lowercase letters correspond to soybean varieties.



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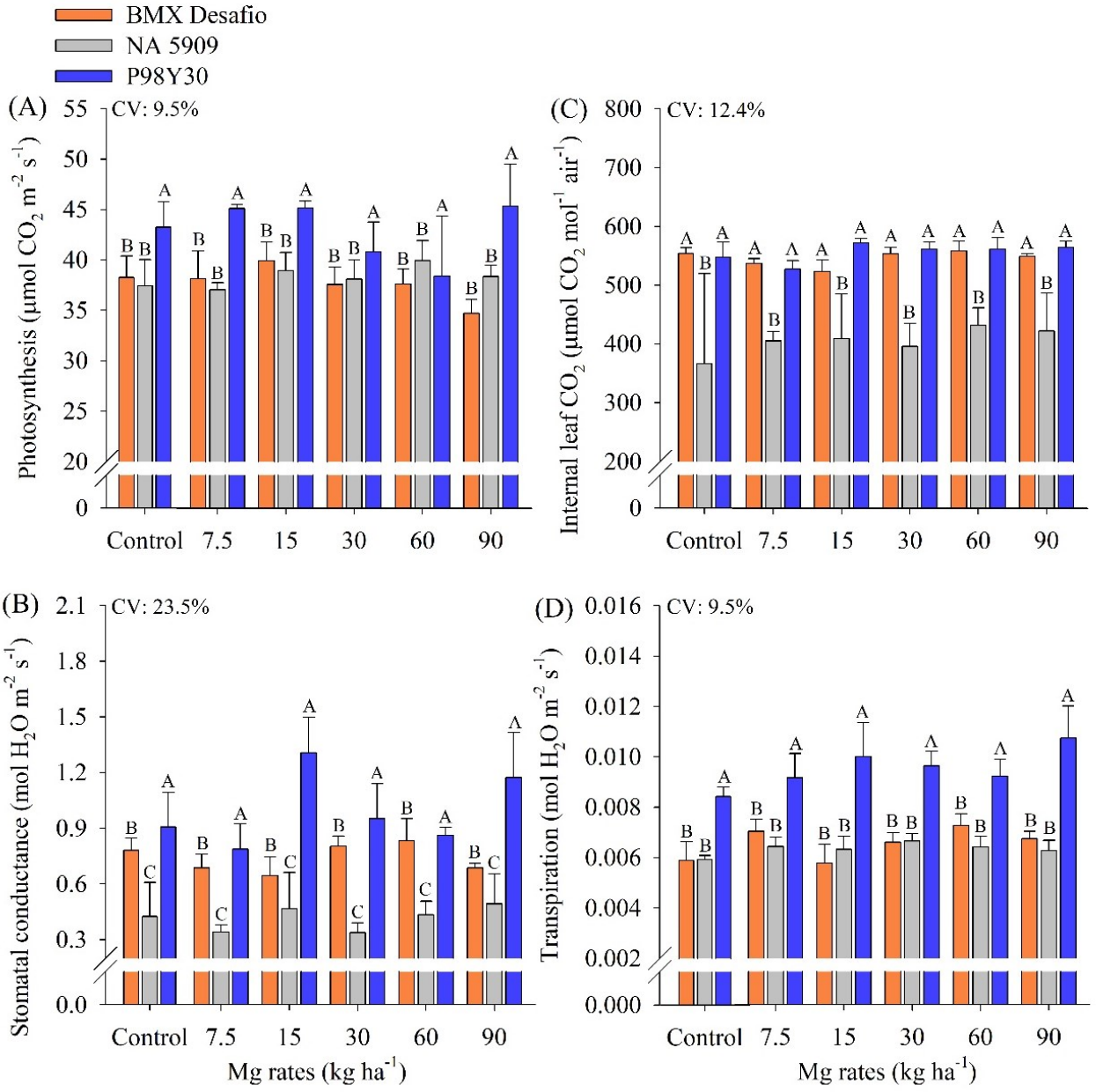
Gas exchange parameters and sugar concentration

The Mg fertilization showed no effect on photosynthetic parameters net photosynthetic rate (A), stomatal conductance (gs), internal concentration of CO_2 (Ci) and transpiration (TR) of soybean plants (Figure 5). On the other hand, there was an increase in total sugar concentrations for BMX Desafio RR with 7.5 kg ha^{-1} of Mg supply (Figure 6A). For the NA 5909 RG RR, there was an increase in total sugar concentrations with 60 kg ha^{-1} in 12% compared to untreated plants. P98Y30 RR showed the highest total sugars concentration with 30, 60 and 90 kg ha^{-1} of Mg supply.

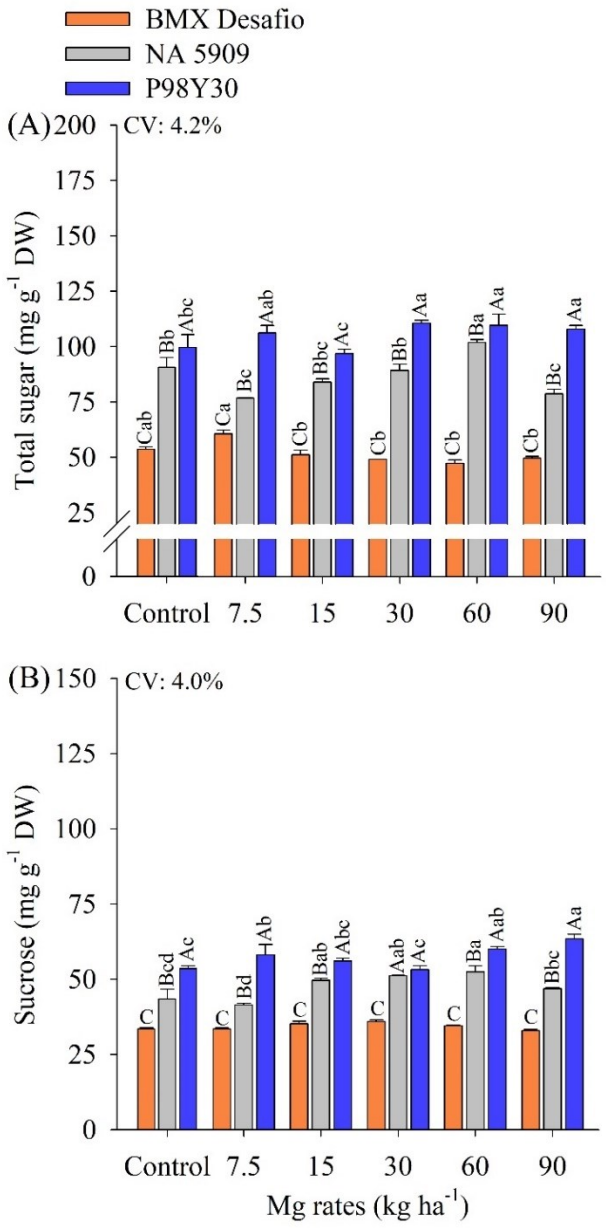
Mg supply increased sucrose concentration for NA 5909 RG RR with 15, 30 and 60 kg ha^{-1}, which is 14, 18 and 21% higher than untreated plants (Figure 5B). There was also an increase in sucrose concentration for P98Y30 RR with 60 and 90 kg ha^{-1}, 12 and 18% higher than the control treatment. These results point to a positive effect of Mg on sugar metabolism of soybean plants.

Figure 5. Effects of Mg rates on photosynthesis (A), stomatal conductance (B), internal leaf CO2 (C) and transpiration (D) in soybean plants. CV means coefficient of variation. The standard error of the mean (n = 4) is indicated by error bars. Different letters indicate difference between means according to a Tukey test ($p \leq 0,05$). Uppercase letters correspond to Mg rates, and lowercase letters correspond to soybean varieties.



Source: The author himself

Figure 6. Effects of Mg rates on total sugar (A) and sucrose (B) in soybean plants. CV means coefficient of variation. The standard error of the mean (n = 4) is indicated by error bars. Different letters indicate difference between means according to a Tukey test ($p \leq 0,05$). Uppercase letters correspond to Mg rates, and lowercase letters correspond to soybean varieties.



Source: The author himself

Nitrogen compounds

The highest nitrate concentration was observed for BMX Desafio RR with 30 kg ha^{-1} of Mg supply (Figure 7A). On the other hand, for NA 5909 RG RR, fertilization with 7,5, 15, 30, 60 and 90 kg ha^{-1} of Mg reduced nitrate concentration by 17, 23, 16, 10 and 23% in comparison to untreated plants. Similarly, there was a reduction in nitrate concentration for P98Y30 RR with 7,5, 15, 30 and 60 kg ha^{-1} of Mg in 13, 18, 13 and 8% compared to untreated plants.

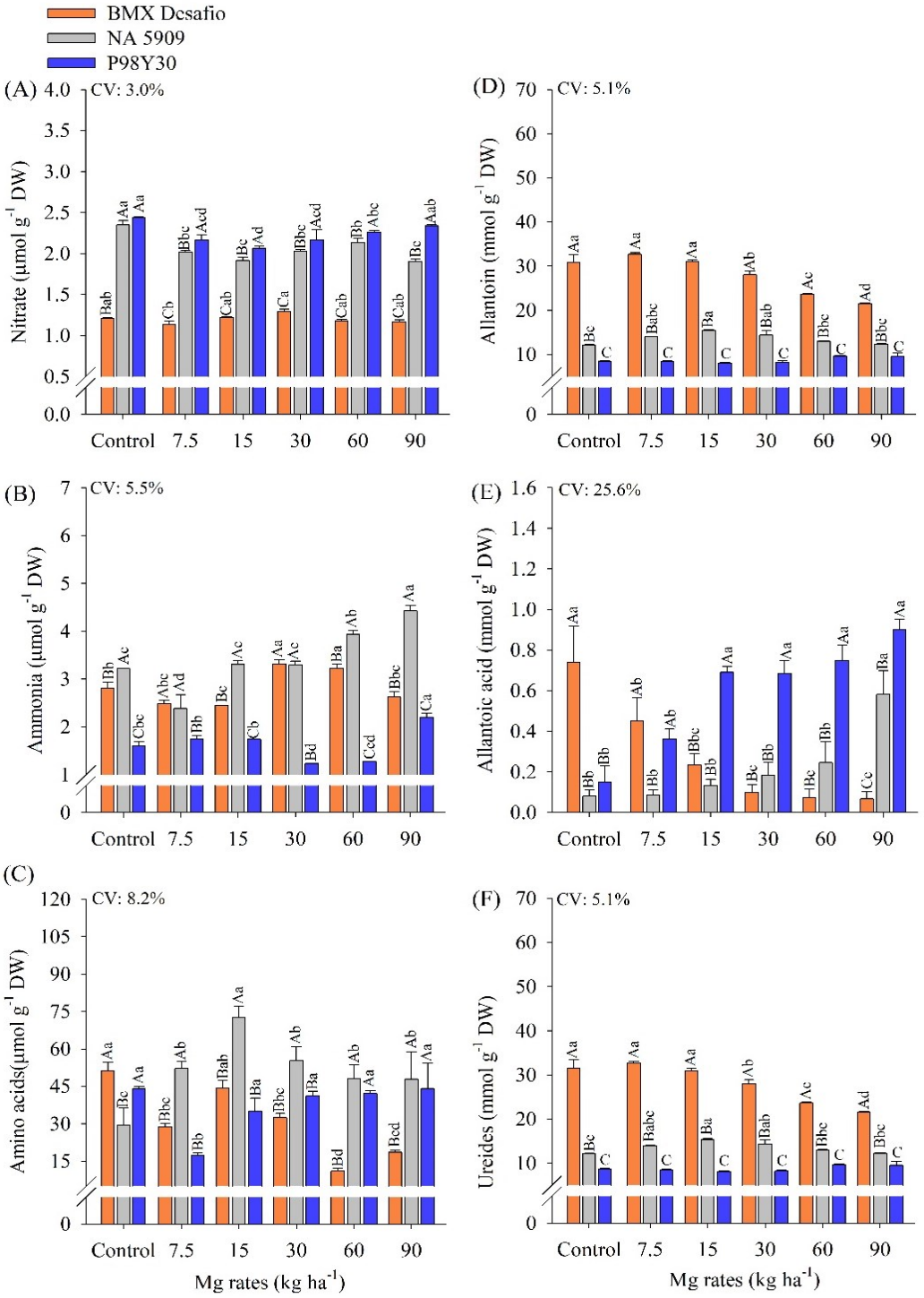
Ammonia concentration increased for BMX Desafio RR by 18 and 15% with 30 and 60 kg ha^{-1} of Mg supply (Figure 7B). For NA 5909 RG RR, there was an increase in ammonia concentration by 22 and 37% in comparison to untreated plants with 60 and 90 kg ha^{-1}. Similarly, P98Y30 RR increased ammonia concentration with 90 kg ha^{-1} of Mg by 37%.

Mg fertilization reduced total free amino acids in soybean leaves of BMX Desafio RR and P98Y30 RR (Figure 7C). On the other hand, Mg promoted an increase in amino acids concentration for NA 5909 RG RR. The highest amino acids concentration was observed with 15 kg ha^{-1} of Mg supply, 147% higher than untreated plants. Among varieties, the highest amino acids concentration was observed for NA 5909 RG RR with Mg fertilization.

Mg fertilization with 30, 60 and 90 kg ha^{-1} reduced allantoin concentration in 10, 30 and 43% for BMX Desafio RR (Figure 7D). On the other hand, there was an increase in allantoin concentration for NA 5909 RG RR of 27 and 19% with 15 and 30 kg ha^{-1} of Mg supply. P98Y30 RR showed no response of allantoin concentration to Mg supply. Among varieties, the highest allantoin concentration was observed for BMX Desafio RR, followed by NA 5909 RG RR and P98Y30 RR.

There was a gradual reduction in allantoic acid concentration for BMX Desafio RR with 7,5, 15, 30, 60 and 90 kg ha^{-1} of Mg supply (Figure 7E). On the other hand, Mg fertilization increased allantoic acid concentration for NA 5909 RG RR with 90 kg ha^{-1}, and for P98Y30 RR with 15, 30, 60 and 90 kg ha^{-1}. Fertilization with Mg showed no effect on the concentration of ureides in P98Y30 RR (Figure 7F). The concentrations of ureides decreased in BMX Desafio RR of 12, 33 and 46% when applied 30, 60 and 90 kg ha^{-1} of Mg. On the other hand, ureides increased 27 and 19% in NA 5909 RG RR with the application of 15 and 30 kg ha^{-1} of Mg. Among varieties, the highest ureides concentrations was observed for BMX Desafio RR.

Figure 7. Effects of Mg rates on nitrate (A), ammonia (B), amino acids (C), allantoin (D) and allantoic acid (E) in soybean plants. CV means coefficient of variation. The standard error of the mean (n = 4) is indicated by error bars. Different letters indicate difference between means according to a Tukey test ($p \leq 0,05$). Uppercase letters correspond to Mg rates, and lowercase letters correspond to soybean varieties.



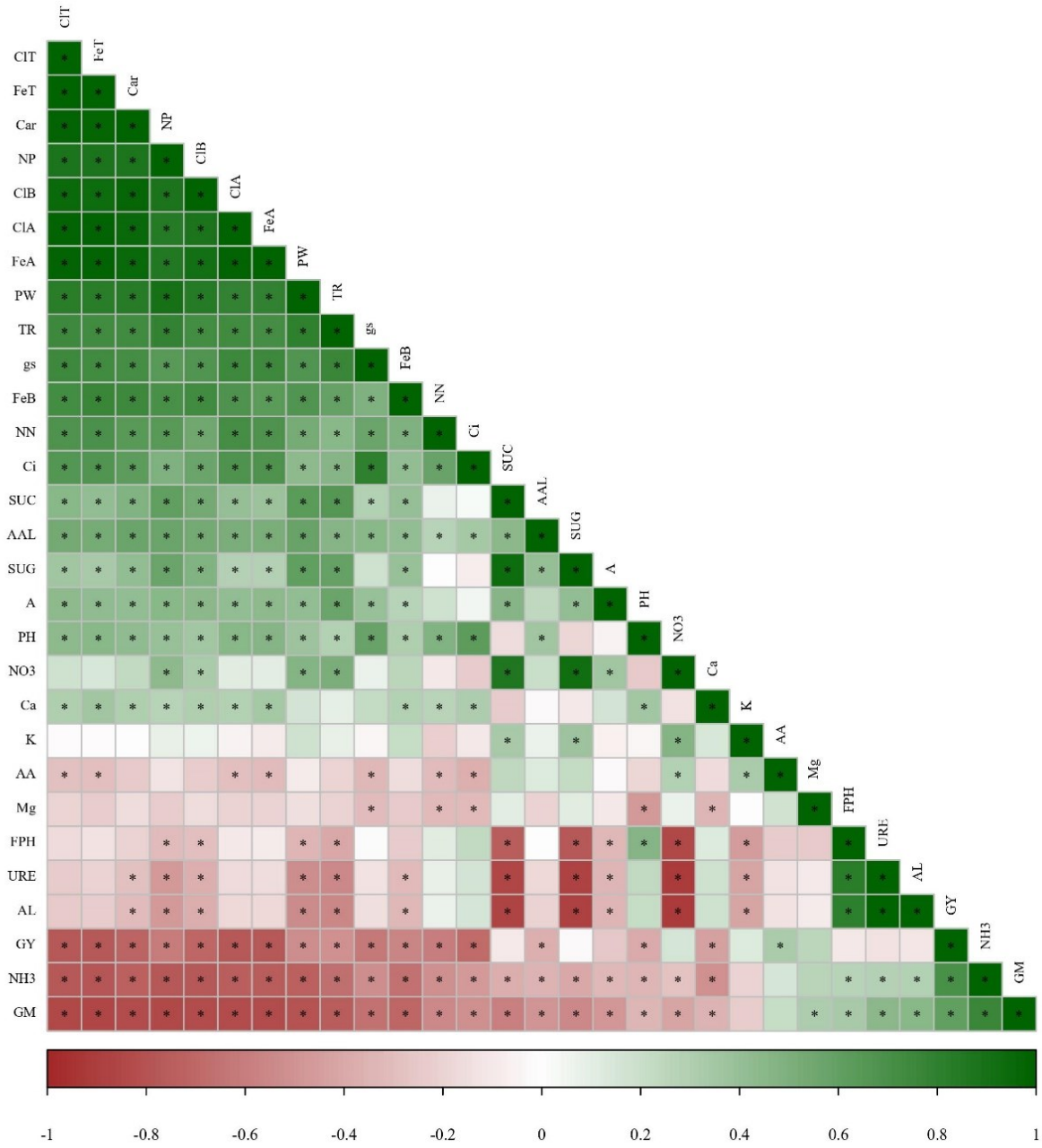
Source: The author himself

3.5. DISCUSSION

Application of $MgSO_4$ increased leaf Mg concentration in all tested soybean varieties. Fertilization with 30 kg ha^{-1} provided the greatest increase of Mg in soybean leaves. These results indicate that $MgSO_4$ is an optimal alternative to supply Mg requirements of soybean plants. Mg is widely involved in photosynthesis process, production and transport of photoassimilates by plants (CAKMAK; KIRKBY, 2008; GRZEBISZ, 2013), as well as for ATP synthesis and ATPase enzyme activity (VERBRUGGEN; HERMANS, 2013; IGAMBERDIEV; KLECZKOWSKI, 2015). Thus, it is expected that higher Mg concentration in soybean leaves will contribute positively to these metabolic processes.

Fertilization with Mg did not affect leaf K concentration in soybean plants. Generally, the amount of K in the soil solution is much less than that of other nutrients. In order to ensure a sufficient absorption of K even at low concentrations, plants have developed specific transport systems that cannot be blocked by other nutrients, such as Mg (HORIE *et al.,* 2011). In addition, Mg concentrations were not correlated negatively with K, supporting that Mg even applied at high concentrations does not impar K uptake by soybean plants (Figure 8). On the other hand, 30, 60 and 90 kg ha^{-1} of Mg supply reduced Ca concentration in leaves. These results indicate that the high concentration of Mg in the soil might affect Ca uptake by soybean plants. In this case, competitive inhibition by the same site can occur, affecting Ca uptake by plants (MALAVOLTA *et al.,* 1989).

Figure 8. Heatmap of Pearson correlation coefficients obtained from variables extracted from soybean.



* indicates significant correlation ($p < 0,05$).

Abbreviation: PH: plant height; FPH: first pod height; NN: number of nodes; NP: number of pods; PW: pods weight; GM: grain mass; GY: grain yield; A: photosynthesis; gs: stomatal conductance; Ci: internal leaf CO2; Tr: transpiration; ClA: chlorophyll *a*; ClB: chlorophyll *b*; ClT: total chlorophyll; CAR: carotenoids; FeA: pheophytin *a*, FeB: pheophytin *b*; FeT: total pheophytin; NO3: nitrate; NH3: ammonia; AL: allantoin; AAL: allantoic acid; URE: ureides; AA: amino acids; SUC: sucrose; SUG: total Sugar; K: potassium; Ca: calcium; Mg: magnesium.

Source: The author himself

The concentration of pigments that comprise photosynthetic apparatus increased in soybean plants fertilized with Mg. There was an increase in total chlorophyll and total pheophytin in soybean leaves with Mg supply. Chlorophyll structure contain Mg and is responsible to capture solar energy for photochemical process of photosynthesis, producing sugars as final product (CAKMAK; YAZICI, 2010). In this study, Mg fertilization with $MgSO_4$ showed no effect on photosynthetic parameters (Ci, gs, TR and A) in soybean plants. The contribution of Mg to photosynthetic activity and photosynthetic carbon dioxide fixation has been well reported in other studies under hydroponics condition (CAKMAK; YAZICI, 2010; GERENDÁS; FÜHRS, 2013; DIAS *et al.,* 2017). Possibly, Mg enhanced enzymatic activity of RuBisCO (BHAT *et al.,* 2017) and ATPases (VERBRUGGEN; HERMANS, 2013) in order to boost sugar metabolism in plants.

Carotenoid concentrations also increased with Mg supply. These are key pigments that maintain the functional stability of photosynthetic apparatus during fluctuations in the light spectrum (KUSAMA *et al.,* 2015). Solar light is a potentially dangerous substrate because chlorophylls can produce O_2^- when photoactivated (excited state) (KRIEGER-LISZKAY, 2005). The transfer of this energy to carotenoids in the light-collecting complex of photosystem II, leads to its dissipation in the form of heat (PANDEY *et al.,* 2009). In this way, carotenoids act as effective antioxidants. These results indicate a greater contribution to the photoprotection process of plants against damage to the photosynthetic apparatus resulting from the incidence of light.

Application of $MgSO_4$ increased the production of sucrose for P98Y30 RR (60 and 90 kg ha^{-1}) and NA 5909 RG RR (15, 30 and 60 kg ha^{-1}). Sucrose is the main product generated by photosynthesis (SALERNO; CURATTI, 2003). These results indicate that Mg promoted an adequate activity of the photosynthetic apparatus.

Total sugar concentration also increased with Mg supply for BMX Desafio RR (7.5 kg ha^{-1}), NA 5909 RG RR (60 kg ha^{-1}) and P98Y30 RR (7.5, 30, 60 and 90 kg ha^{-1} of Mg). The activity of important enzymes involved in carbon metabolism is highly associated with plant responses to stress (NISHIZAWA *et al.,* 2008). In this case, sugars accumulation in the chloroplasts, declines in the photosynthetic process and a decrease in the CO_2 diffusion rate are expected (KEENAN *et al.,* 2010). Therefore, these results indicate intimate involvement of Mg in plant growth and development, by boosting the sucrose fraction.

Regarding nitrogen metabolism, Mg showed little influence on nitrate concentration for BMX Desafio RR, while reduced for the other varieties. These results indicate no accumulation of nitrate in the leaves, ruling out a negative effect on important enzymes involved in their assimilation. Although the effects of Mg on soybeans are not fully known, studies by Yin *et al.* (2009) observed that nitrate reductase activity can be inhibited under Mg deficiency in spinach plants. The activity of nitrate reductase is dependent on the availability of nitrate and light (CAMPBELL, 1999; SUNIL *et al.,* 2013). This makes the need for plants to be well supplied with Mg, considering that ATP must be linked to Mg ion to be biologically active (MgATP) (IGAMBERDIEV; KLECZKOWSKI, 2015). It is possible that Mg might increase nitrate reductase activity, contributing to the rapid conversion of nitrate to nitrite and ammonia.

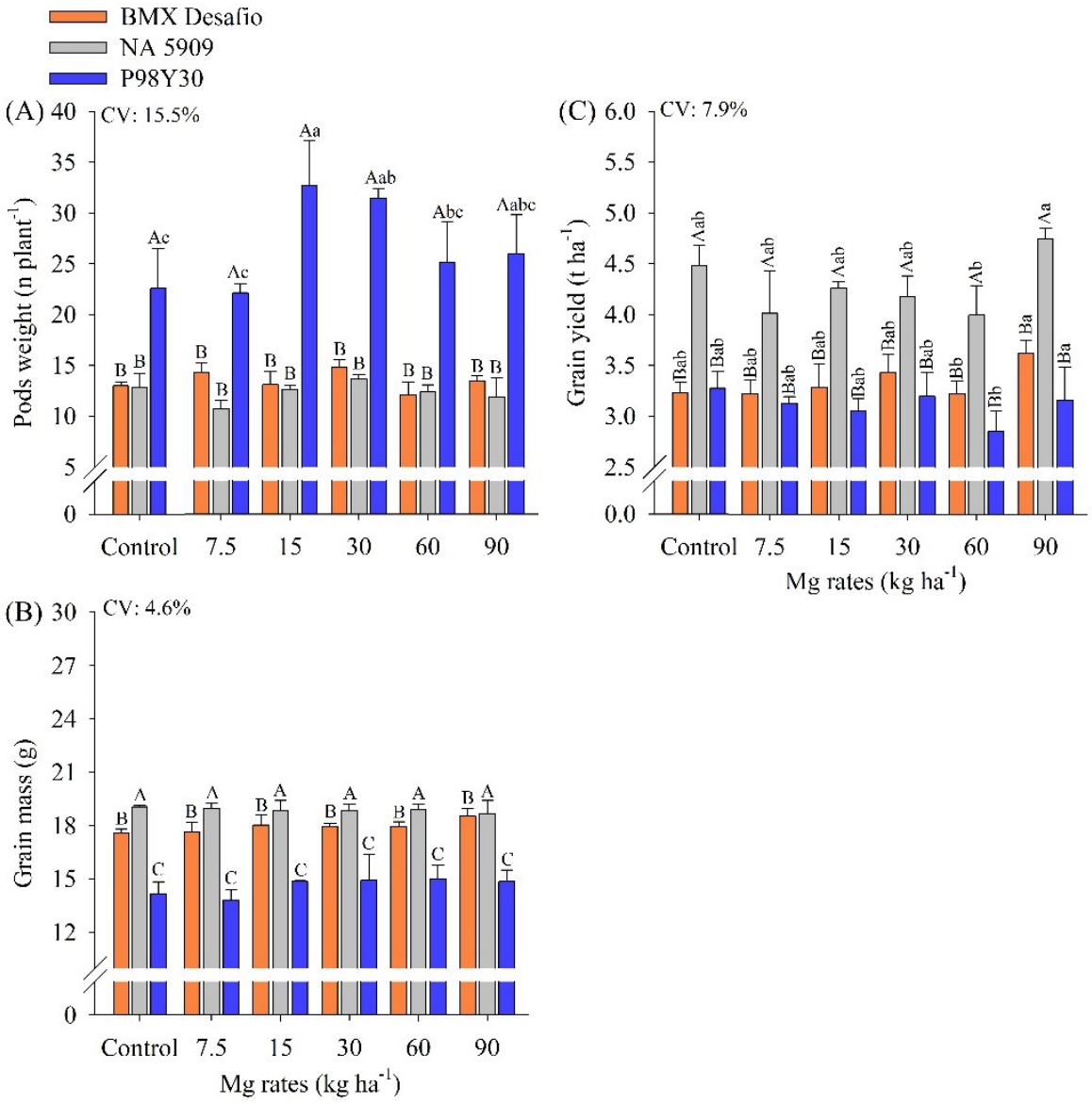
Ammonia concentration increased in BMX Desafio RR (30 and 60 kg ha^{-1}), NA 5909 RG RR and P98Y30 RR (90 kg ha^{-1}) in response to Mg supply. Ammonia from nitrate assimilation or synthesized by symbiotic association, must be quickly converted into ureides (allantoin and allantoic acid) (DUNN, 2014) or incorporated into amino acids by enzymes glutamate and glutamine synthase (MARTÍNEZ-ANDÚJAR *et al.,* 2013). According to Chiasson *et al.* (2014), ureides are the main form of transport and storage of nitrogen in soybean plants.

There was a reduction in leaf amino acid concentration for BMX Desafio RR and P98Y30 RR in response to Mg supply. It is possible that application of $MgSO_4$ affected the activity of nitrogen enzymes in these soybean varieties. Glutamate and glutamine synthase are important enzymes responsible for ammonia assimilation (MARTÍNEZ-ANDÚJAR *et al.,* 2013). Fertilization with Mg had little influence on BNF process for BMX Desafio RR, by reducing the concentrations of allantoic acid (30, 60 and 90 kg ha^{-1}) and allantoin (90 kg ha^{-1}). On the other hand, there was an increase in allantoic acid in P98Y30 RR (15,30,60 and 90 kg ha^{-1}). Ureides are derived from oxidative degradation of purines (CARTER; TEGEDER, 2016), while allantoic acid is synthesized in the endoplasmic reticulum from allantoin, which is synthesized in the peroxisomes from uric acid (LAMBERTO *et al.,* 2010). It should be noted that more than 20 enzymes act in the BNF process, with a considerable requirement for Mg to activate some of these enzymes (JAHNEN-DECHENT; KETTELER, 2012). In addition, ureides are the main products transported from nodules to shoot of soybean plants.

Among varieties, NA 5909 RG RR (early cycle) showed greater leaf Mg concentration in response to $MgSO_4$ supply. $MgSO_4$ is known to be a fertilizer with high solubility (KAWAMURA; RAO, 2007). Possibly, Mg released has favored this variety to a greater Mg uptake. These factors may have influenced the adequate metabolic activity of this variety, providing an increase in the concentrations of amino acids, allantoic acid (90 kg ha^{-1}) and allantoin (7,5, 15 and 30 kg ha^{-1}) and, consequently, greater grain mass and productivity among the studied varieties (Figure 9). Positive correlation coefficients between ureides × grain weight indicate that they are the main nitrogen sources responsible for protein synthesis and the consequent formation of soybean seeds.

The P98Y30 RR variety showed the highest concentration of photosynthetic pigments and the highest A, gs, Ci and E among the soybean varieties. Consequently, this variety showed the highest photoassimilate production. The assimilation and transport of these sugars from the sources to drains via phloem is highly influenced by Mg. This is a physiological process mediated by the action of ATPase enzyme (HERMANS; VERBRUGGEN, 2005), which is dependent on Mg (VERBRUGGEN; HERMANS, 2013). Several studies attribute the reduction of sugar transport in plant via phloem due to Mg deficiency (HERMANS; VERBRUGGEN, 2005; CAKMAK; KIRKBY, 2008; MENGUTAY *et al.,* 2013). On the other hand, allantoic acid increased with Mg supply, assuming beneficial effects on allantoic acid biosynthesis, carried out from allantoin (LAMBERTO *et al.,* 2010).

Figure 9. Effects of Mg rates on pods weight (B), grain mass (C) and grain yield (D) in soybean plants. CV means coefficient of variation. The standard error of the mean (*n* = 4) is indicated by error bars. Different letters indicate difference between means according to a Tukey test ($p \leq 0,05$). Uppercase letters correspond to Mg rates, and lowercase letters correspond to soybean varieties.



Source: The author himself

3.6. CONCLUSION

Application of $MgSO_4$ increased leaf Mg and reduced Ca concentration at higher rates. Leaf K concentration was not impaired by Mg fertilization. Application of Mg rates increased photosynthetic pigments resulting in higher concentration of photossimilates (sucrose and total sugars) for the varieties NA 5909 RG RR and P98Y30 RR.

The higher rate of photoassimilates provided greater conversion of atmospheric N_2 into ureides for NA 5909 RG RR (allantoin and allantoic acid) and P98Y30 RR (allantoic acid), indicating greater activity of N metabolism. This study resulted in important information regarding Mg fertilization and its effects on BNF metabolism in commercial soybean varieties.

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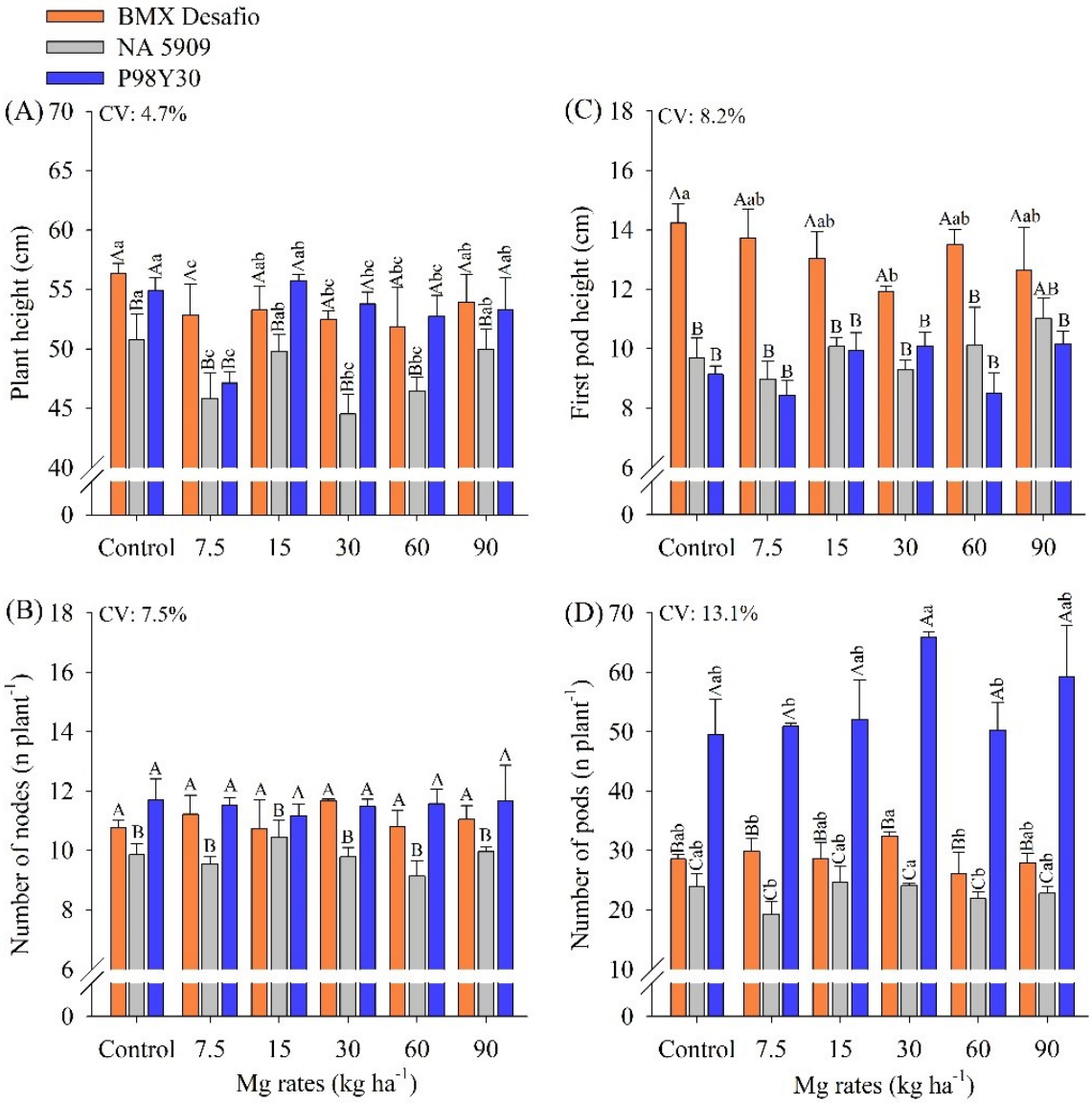
APPENDAGE A

Figure S10. Effects of Mg rates on plant height (A), number of nodes (B), first pod height (C) and number of pods (D) of soybean plants. CV means coefficient of variation. The averages followed by the same capital letters are not significantly different and compare soybean varieties in response to Mg supply. The averages followed by the same lower case letters compare each variety in response to Mg doses, according to the Tukey test ($p \leq 0.05$). Bars represent the standard error of the means ($n = 4$).



Source: The author himself

4 GENOTYPIC VARIATION OF SOYBEAN PLANTS IN RESPONSE TO MAGNESIUM FERTILIZATION: PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES

4.1. ABSTRACT

Magnesium (Mg) plays a plethora of important roles in plants. This study aimed to verify the genotypic variation of soybean on the photosynthetic process and biological nitrogen fixation (BNF) under Mg applications, by evaluating photosynthetic pigments concentration, sucrose, and total sugar synthesis, and N compounds concentration. The experiment was carried out in the field, with 30 soybean varieties, two sources of Mg (Mg oxide – MgO, and Mg sulfate – $MgSO_4$), two application rates (0 and 30 kg ha^{-1}) and four replications. The application of MgO and $MgSO_4$ provided increased leaf Mg concentration without impairing leaf Ca concentration in most soybean varieties. Under Mg application, most soybean varieties presented indicative of higher photosynthetic activities due to an increase in photosynthetic pigments and total sugar and sucrose concentration. Under the higher photoassimilates production, a higher atmospheric N_2 conversion, as well as a higher ureides synthesis, was observed, indicating higher BNF efficiency in plants fertilized with Mg. The total free amino acids concentration also increased in most soybean varieties under Mg application, indicating higher nitrogen (N) metabolism activity. Due to N fixation and incorporation observed, an increase in soybean yield was observed in 22 varieties. The source MgO provided higher increases than $MgSO_4$ on total chlorophyll, total pheophytin, total sugars, sucrose, and grain yield in most varieties. The present study reports valuable information regarding the Mg effect on BNF metabolism in soybean varieties, which might be the basis for future studies regarding Mg fertilization on soybean.

Keywords: Biological nitrogen fixation. photoassimilates. Mg fertilization. allantoin. *Glycine max* (L.) Merrill.

4.2. INTRODUCTION

The soybean (*Glycine max* (L.) Merrill) is known worldwide as the main source of vegetable oil (FOOD AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, 2020). Soybean plants can maintain a symbiotic association with *Bradyrhizobium* bacteria. Leading to benefits from this bacteria's biological nitrogen fixation (BNF). The ureides (allantoin and allantoic acid) are the final products from this symbiosis, provided to soybean from the rhizobia, in exchange for photoassimilates provided from soybean to rhizobia. These photoassimilates are essential for the bacteria's metabolism and development. According to previous reports, the Mg application can improve this symbiotic process (CHEN *et al*., 2017; PENG *et al.,* 2018). As an essential nutrient for plants, Mg deficiency impairs crops yield and quality around the world (CHEN *et al.,* 2018). However, the role of Mg in BNF is still not fully understood. Thus, studies regarding Mg supply to soybean varieties and its potential consequences to BNF might help to maximize the total nitrogen (N) availability to plants, leading the crop to new yield standards.

The hydrated radius of Mg is large, leading to weak bonds with negatively charged colloids in soil, as well as root cell walls (GRANSEE; FÜHRS, 2013). This trait might result in high levels of Mg in soils solution, or a higher chance of nutrients leaching (CHEN *et al.,* 2017). Other stressful factors can also limit Mg availability for plants, as an inbalanced supply of fertilizers with high levels of K and Ca (GRANSEE; FÜHRS, 2013), ammonium (NH_4^+) (KOBAYASHI *et al.,* 2013; CHEN *et al.*, 2017), soil acidity (KOBAYASHI *et al.*, 2013), hydrogen antagonistic action (KOBAYASHI *et al.*, 2013), aluminum (Al_3^+) in soil solution (CHEN; MA, 2013), and others. Thus, plenty of studies already had been performed regarding Mg deficiency on plants and its effect on photosynthesis (YUGUAN *et al.*, 2009, LI *et al.*, 2017), carbohydrates loading (VERBRUGGEN; HERMANS, 2013), and N metabolism enzymes (YIN *et al.*, 2009; LI *et al.*, 2017).

According to White and Broadley (2009), the Mg supply by soil results in better leaf Mg concentration. Among Mg sources for crops, Mg oxide (MgO) and Mg sulfate ($MgSO_4$) are alternatives. The effects of Mg application on photoassimilates production are well documented (CAKMAK; YAZICI, 2010; GERENDÁS; FÜHRS, 2013; DIAS *et al.*, 2017). However, since Mg is essential for the functioning of more than 300

enzymes (VERBRUGGEN; HERMANS, 2013), other processes might be improved by foliar Mg application. Some pieces of evidence are pointing to the potential of Mg fertilization to increase carbohydrates flow to plant roots (CAKMAK; KIRKBY, 2008; VERBRUGGEN; HERMANS, 2013), consequently, to enhance the loading of these compounds to soybean nodules (PENG *et al.*, 2018). Thus, with Mg application, photosynthesis is enhanced as well as production and transport of photoassimilates to the sink organs, which might lead to the improvement in a plethora of physiological processes, including BNF (VERBRUGGEN; HERMANS, 2013).

The Mg application can improve soybean development due to the enhancement of BNF (PENG *et al.*, 2018). The BNF enables *Fabaceae* plants to accumulate adequate amounts of N to their metabolism. In this process, the rhizobia absorb atmospheric N_2 and convert it into NH_3 (BARAL *et al,* 2014), this NH_3 is then exported to plants as ureides (DUN, 2014). According to some reports, Mg application can improve soybean BNF, providing plants with more and bigger nodules, as well as better ureides translocation (PENG *et al.*, 2018). According to Peng *et al.* (2018), this occurs due to the enhanced photoassimilates (sugars) loading from leaves to nodules. Thus, under Mg application is expected higher ureides levels in soybean leaves. Since ureides are the main N-compounds from nodules that are transported and stored in soybean (CHIASSON *et al.*, 2014), these compounds can be used to estimate BNF (KING; PURCELL, 2005).

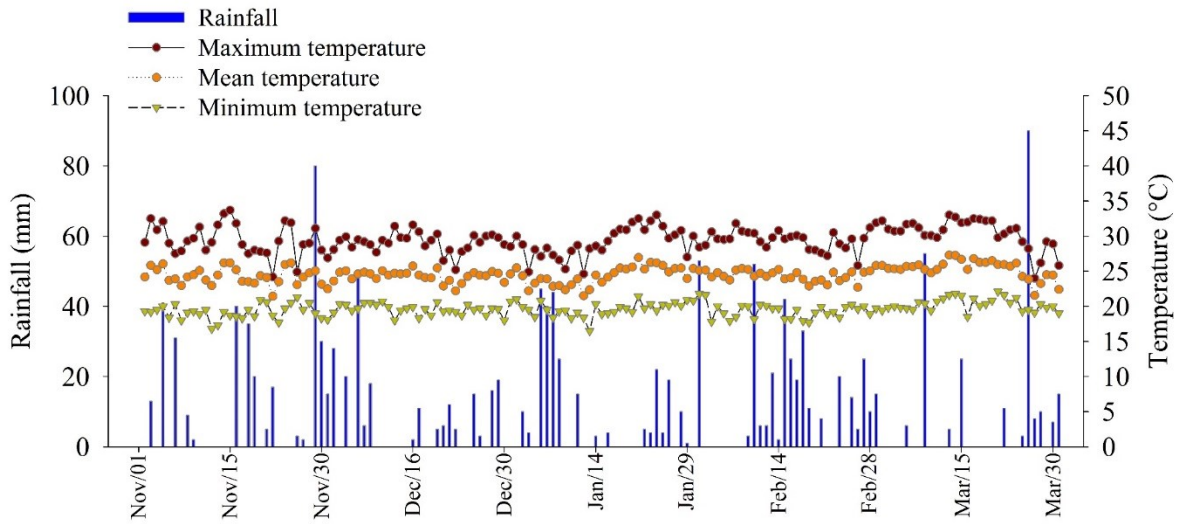
The hypothesis of this study is that soybean genotypes can present different metabolic responses to Mg supply, with direct effects observed on photosynthesis and photoassimilates (sugars) production, which are needed to nodules perform BNF, that will be evaluated by quantification of atmospheric N fixed in the final product: ureides (allantoic acid and allantoin). Thus, this study aimed to evaluate the genotypic variation of soybean under Mg application regarding the photosynthetic process of plants by pigments and photoassimilates (sugars), as well as the BNF by ureides evaluation in leaves.

4.3. MATERIAL AND METHODS

4.3.1. Growth conditions and experimental design

The study was carried out at “Fundação Chapadão” Research Farm located in the municipality of Chapadão do Sul, Mato Grosso do Sul state, Brazil, in the following geographic coordinates 18° 46' 48.49" S e 52° 38' 42.59" O, at 840 m altitudes, during the agricultural year of 2017/2018. According to Köppen, the region was classified as Aw, tropical with two well-defined seasons: a rainy summer and a dry winter. The daily rainfall, minimum, mean, and maximum temperature throughout the experiment duration is presented in Supplementary Figure 1. The annual rainfall was approximately 1418 mm, and the maximum and minimum mean temperatures were respectively 32.5 e 17.5 ° C.

Figure 1. Minimum, average, and maximum daily rainfall and temperatures during the experimental driving period.



Rainfall: data obtained from a manual data acquisition station, installed at the experiment site. Temperature: data obtained through INMET (Instituto Nacional de Meteorologia), Chapadão do Sul, MS, Highway MS 306 - km 96. Source: www.inmet.gov.br.

The initial soil sampling was performed during 2017, the soil was characterized as follows: clay, silt and sand concentration in the 0.00 – 0.20 m layer: 90 g kg^{-1}, 60g kg^{-1} and 850 g kg^{-1} respectively, according to pipette method (EMBRAPA. 2017). The chemical characteristics were determined according to Van Raij *et al*. (1997) and the values are: for 0.00 to 0.20m layer - phosphorus (resin) 16.2 mg dm^{-3}; organic matter 12.7 g dm^{-3}; pH ($CaCl_2$) 5.5; potassium 27.9 mg dm^{-3}; calcium 16.7 $mmol_c$ dm^{-3};

magnesium 4.7 $mmol_c\ dm^{-3}$; H + Al 18.9 $mmol_c\ dm^{-3}$; aluminium 0.4 $mmol_c\ dm^{-3}$; sulfur 1.2 mg dm^{-3}; cation exchange capacity 41.1 $mmol_c\ dm^{-3}$; and base saturation 53.9%. For 0.20-0.40 m layer - phosphorus (resin) 11.20 mg dm^{-3}; organic matter 10.68 g dm^{-3}; pH ($CaCl_2$) 5.44; potassium 17 mg dm^{-3}; calcium 1.44 $cmol_c\ dm^{-3}$; magnesium 0.40 $cmol_c\ dm^{-3}$; H + Al 2.10 $cmol_c\ dm^{-3}$; aluminium 0.03 $cmol_c\ dm^{-3}$; sulfur 1.46 mg dm^{-3}; cation exchange capacity 3.98 $cmol_c\ dm^{-3}$; and base saturation de 47.28%.

The experimental design was a complete randomized block in a 30 x 2 x 2 factorial scheme composed of 30 soybean varieties (Table 1S), two Mg application rates (0 and 30 kg ha^{-1}), and two Mg sources (Mg - 30% Mg; $MgSO_4$ - 9% Mg), in four replications totaling 480 plots. The experimental unit was composed of five soybean rows of 5.2m each, with 0,45m space between rows, totaling 11.70 m² for each experimental unit. The application rate of 30 was established according to previous experiments with various cultures performed by the present research group.

The sowing was performed mechanically for all varieties on November 11, 2017. The sowing fertilization was realized using 150 kg ha^{-1} of MAP (11-52-00), 200 kg ha^{-1} of agricultural gypsum (12% S) and 3 kg ha^{-1} of ulexite (8% B) in the sowing furrow, in addition to top dressing application of 100 kg ha^{-1} of KCl (60% K_2O). The inoculation was performed near the soybean sowing, using the following inoculum strains: SEMIA 5019 - *Bradyrhizobium elkanii* and SEMIA 5079 - *Bradyrhizobium japonicum*, both with a minimum of 6 x 10^9 colony-forming units g^{-1} mL^{-1}. The inoculation was performed at the rate of 100 mL ha^{-1}, using a clean concrete mixer.

Plants emergency occurred on November 17, 2017. The application of MgO and $MgSO_4$ was performed as a side dressing, 15 days after sowing when the cotyledon reserves start to decrease and the photosynthetic pigments, and water and nutrients uptake is enough to the seedling survive on its own. The phytosanitary practices followed the technical cultivation recommendation and were performed homogeneously in the experimental area.

During the anthesis (stage R1 and R2), the moment of maximum metabolically intensity in soybean characterized by opened flowers one or two uppermost nodes in the main stem (FEHR; CAVINESS, 1977), was performed the sampling of diagnosis leaves to nutrient analysis, physiological and, biochemical characteristics. The harvest of 2 m of plants in the central rows of each plot was performed to evaluate soybean yield.

4.3.2. Chemical analysis of plant tissue

The collected samples were properly identified then washed using tap water, then washed using a solution of neutral detergent 0.1 %, then HCl 0.3%, and finally deionized water. In sequence, the material was dried in an oven at 65± until a constant dry mass was achieved and later grounded in a Wiley mill for further digestion in nitric-per-chloric solution, following the methodology proposed by Bataglia *et al.* (1983). The leaf Mg and Ca concentrations were determined in atomic absorption, according to Malavolta *et al.* (1997). The results were expressed in g kg^{-1}

4.3.3. Physiological and biochemical analysis

Photosynthetic pigments

The concentration of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, pheophytin a, pheophytin b, and total pheophytin were evaluated in spectrophotometer according to Lichtenthaler (1987). The samples used in this analysis were collected in acetone 80%.

Determination of nitrogen compounds and photoassimilates evaluation

To evaluate the effect of Mg on BNF, N-compounds were determined by extraction of 0.3 g of dry leaves (diagnostic leaves) into 10 mL of MCW (60% methanol. 25% chloroform, and 15% H_2O v/v), in 15 mL conical tubes, the material was centrifuged at 10.000 rpm for 10 min at 4 ºC. Then, the material was stored for 48 hours. After the waiting, 4 mL of the supernatant were collected from MCW extract and mixed with 1 mL chloroform and 1.5 mL deionized water to phase separation. After this, from the hydrophilic portion, the determinations of sucrose, total sugar, nitrate, ammonia, total free amino acids, and ureides (allantoic acid and allantoin) were performed.

Total sugars and sucrose concentration

To evaluate the effect of Mg on photoassimilates in soybean leaves during the anthesis, in 0.1 mL of extract were added 0.5 mL of KOH 30% and 2 mL of H_2SO_4 using glass tubes, to quantify sucrose (VAN HANDEL, 1967). The mixture was homogenized by vortex and heated at 100ºC for 10 minutes. After cooling to room temperature, the mixture was read in a spectrophotometer at λ = 490 nm (absorbance). To quantify total sugars (DUBOIS *et al.*, 1956). In 0.1 mL of extract were added 0.5 mL of phenol 5% and 2 mL of H_2SO_4 using glass tubes. The mixture was homogenized by vortex and let to cool to room temperature. After cooling, the mixture was read in a spectrophotometer at λ = 490 nm (absorbance). The results of total sugar and sucrose concentration were expressed in mg g^{-1} DW. Both evaluations were estimated using a sucrose standard curve.

Nitrate concentration

The nitrate concentration was performed according to Cataldo *et al*. (1975). In 0.05 mL of extract were added 0.04 mL of salicylic acid 5% prepared in H_2SO_4 (w/v) using 2 ml microtubes. After 20 minutes at room temperature, 1 ml NaOH 2N was slowly added to the mixture. After cooling at room temperature, reading was performed at λ = 410 nm (absorbance). The nitrate concentration was determined according to a sodium nitrate standard curve. The results were expressed in µmol g^{-1} DW.

Ammonia concentration

The ammonia concentration was performed according to McCullough (1967). In 0.2 mL of extract were added 0.5 mL of phenol solution (2.5 g phenol + 12.5 mg sodium nitroprusside in 250 mL final volume) and 0.5 mL of phosphate solution (1.25 g NaOH + 13.4g $Na_2HPO_4.7H_2O$ + 2.5 mL de NaOCl in 250 mL final volume) using 2 ml microtubes. The mixture was incubated for 1 hour at 37º C. The reading was performed

at λ = 630 nm (absorbance). The ammonia concentration was determined according to an ammonium sulfate standard curve. The results were expressed in μmol g^{-1} DW.

Ureides concentration (allantoic acid and allantoin)

The ureides concentration was performed according to Vogels and Van Der Drift (1970). To evaluate the allantoin, the analysis consists of two phases. Phase 1: In 0.25 mL of extract were added 0.25 mL of NaOH 0.5 M and 0.02 mL of phenylhydrazine using glass tubes. The mixture was heated at 100 °C for 8 minutes and then, cooled to room temperature. Phase 2: In the previously prepared mixture were added 0.25 mL of HCl 0.65 N. The mixture was heated at 100 °C for 4 minutes and then, cooled to room temperature. Then it was added 0.25 mL phosphate buffer pH 7.0 0.4 M and 0.25 mL of phenylhydrazine 0.33%. After five minutes at room temperature, the material was incubated in ice by five minutes. In sequence, it was added 1.37 mL of cold HCl and 0.25 mL of potassium ferrocyanide. The tubes were homogenized by vortex and after 15 minutes at room temperature, the reading was performed at λ = 535 nm (absorbance). The evaluation of allantoic acid was performed by skipping phase 1 and starting the analysis directly in phase 2. The reading was performed at λ = 535 nm (absorbance). The allantoic acid and allantoin concentrations were determined according to the allantoin standard curve. The results were expressed in μmol g^{-1} DW.

Total free amino acids concentration

The Total free amino acids concentration was performed according to Yemm and Cocking (1955). In 0.3 mL of extract were added 0.7 mL of deionized water, 0.5 mL of sodium citrate (0.2M), 0.2 mL of ninhydrin solution 5% prepared in ethylene glycol monomethyl ether and 1mL KCN (0.0002 M). The material was heated at 100º C for 15 minutes, then cooled to room temperature. Then it was added 1 mL of ethanol. The reading was performed at λ = 570 nm (absorbance). The total free amino acids concentrations were determined according to the methionine standard curve. The results were expressed in μmol g^{-1} DW.

4.4. Statistical analysis

Using the software R (R Development Core Team 2019), the pack “nortest” was accessed and the data was submitted to normality test (Shapiro & Wilk 1965) Levene’s homoscedasticity test at 0.05 probability ($p < 0.05$). The data was submitted an analysis of variance (ANOVA) by F test ($p < 0.05$), and when the statistical difference was pointed, the means were compared by the Skott-Knott test at 5% probability, using the pack “ExpDes”.

The Pearson’s correlation analysis ($p < 0.05$) was also performed, in order to verify dependent variables correlated regarding the proposed treatments. The pack “corrplot” was accessed to generate a heatmap, using the functions “cor” and “cor.mtes” to createof coefficient and *p-value* matrices, respectively. In significate correlations, an asterisk was inserted in the heatmap cell to improve visualization.

4.5. RESULTS

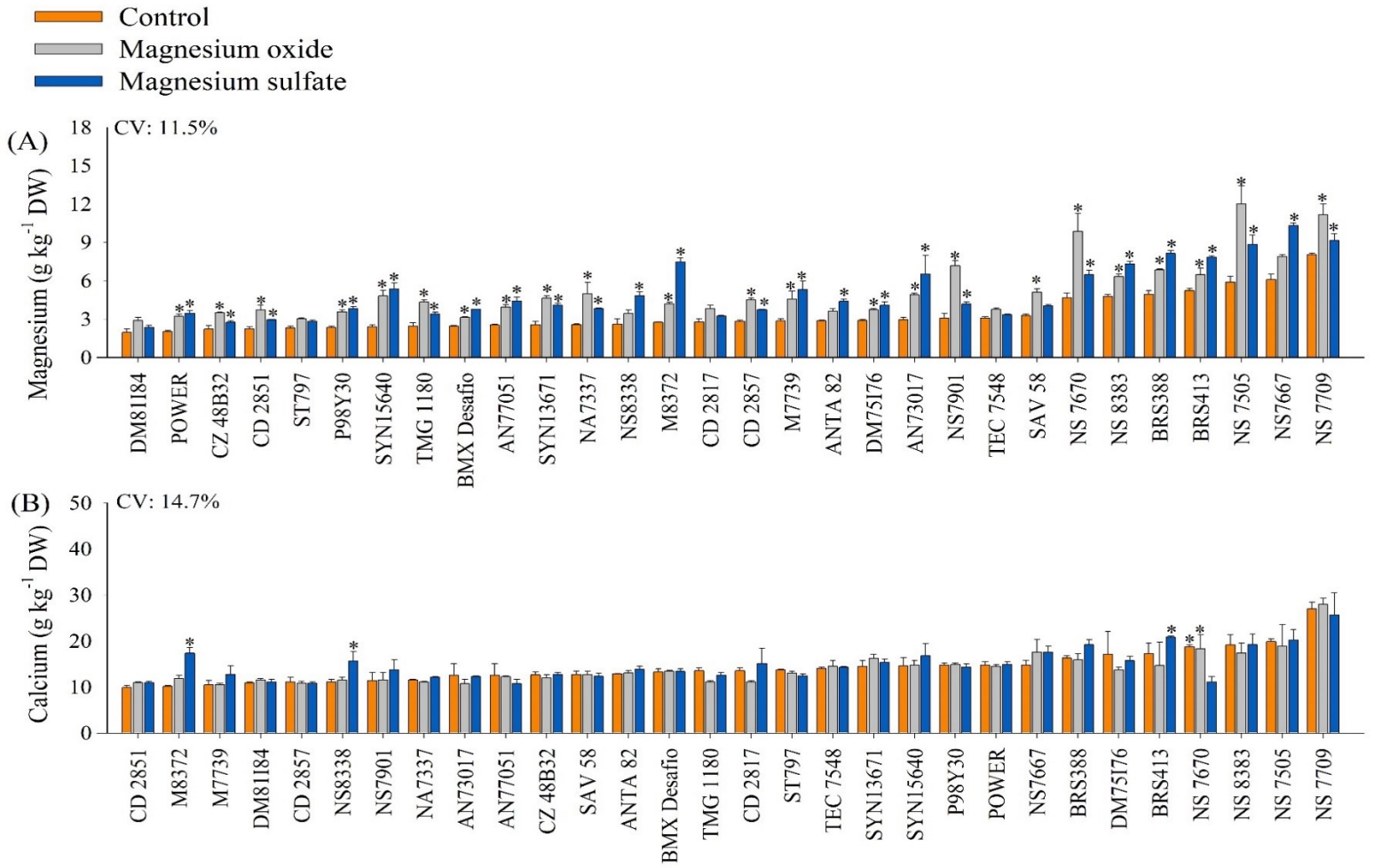
4.5.1. Nutrient analysis

An interaction between Mg application and varieties was observed in Mg and Ca concentration in leaves (*P* <0,018 and *P* <0, 0001; Table 2S). The Mg application resulted in increase Mg concentration in leaves of most varieties, with a mean Mg concentration increase from 34 to 172% compared to control (Figure 2A; Table 3S). Among the 26 varieties that presented higher Mg leaf concentration under Mg application, nine of them (POWER, BMX Desafio, P98Y30, DM75I76, CD 2857, AN77051, SYN13671, M7739, and SYN15640) did not present a difference in leaf Mg concentration between the Mg sources (MgO or $MgSO_4$), with mean Mg concentration between 3.4 and 5.08 g kg^{-1} DW. In the other 9 varieties (CZ 48B32, CD 2851, TMG 1180, NA7337, SAV 58, NS7901, NS 7670, NS 7709, and NS 7505), the highest leaf Mg concentration was observed under application of MgO, presenting a mean concentration of Mg in leaves between 3.5 and 12 g kg^{-1} DW. And finally, 8 varieties (ANTA 82, NS8338, AN73017, NS 8383, M8372, BRS413, BRS388, and NS7667), presented the highest Mg concentration under $MgSO_4$ application, with a mean

concentration of Mg in leaves between 4.38 and 10.32 g kg^{-1} DW. In 4 varieties, no differences in leaf Mg concentration were observed regarding the proposed treatments.

Considering the leaf Ca concentration, 26 soybean varieties did not present a significate difference among them (Figure 2B, Table 4S). However, the application of $MgSO_4$ provided an increase in leaf Ca concentration in the varieties M8372, NS8338 e BRS413, while $MgSO_4$ application led to a decrease in leaf Ca concentration in variety 7670. This result suggests that for most soybean varieties, Mg application did not impair Ca uptake and use in plants.

Figure 2. Effects of Mg rates on leaf Mg (A) and Ca (B) in *Glycine max* varieties. CV: coefficient of variation. "*" compares each variety under Mg rates, indicating the best rate compared to the others according to the Scott Knott test ($p \leq 0.05$). Bars indicate the standard error of the means (n=3)



Source: The author himself.

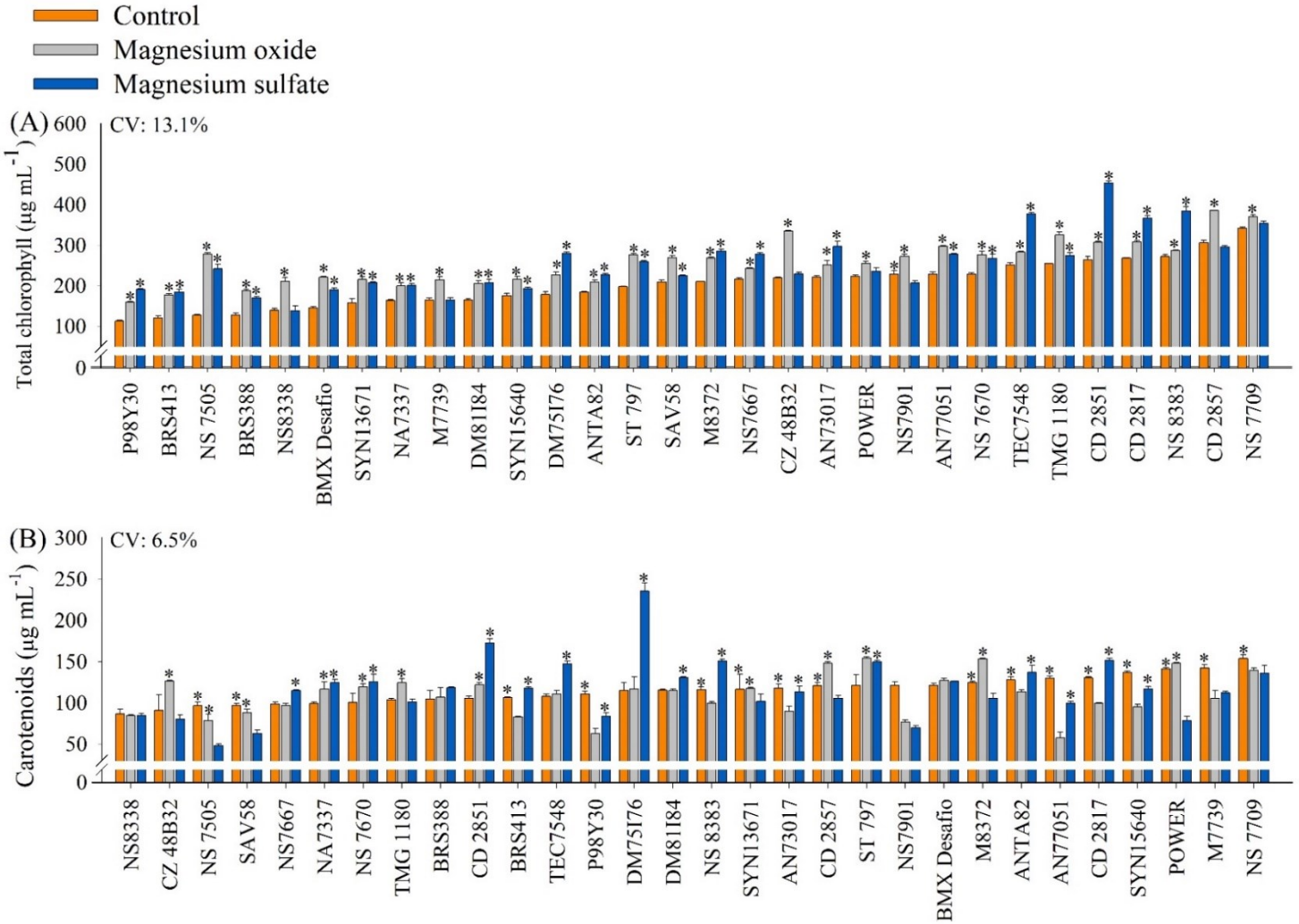
4.5.2. Physiological and biochemical analyzes

Photosynthetic pigments

An interaction between Mg application and varieties was observed regarding the photosynthetic pigments (*P* <0.0001; Table 5S). Increased concentration of total chlorophyll was observed in every soybean variety under Mg application (Figure 3A; Table 8S). Most varieties (CD 2857, NS 7709, CZ 48B32, TMG 1180, AN77051, NS 7505, ST 797, NS7901, SAV58, POWER, BMX Desafio, SYN15640, M7739, NS8338, and BRS388) presented higher total chlorophyll content under MgO application, with an increase between 8 and 119% compared to control. On the other hand, under $MgSO_4$ application, 11 varieties presented higher total chlorophyll concentration (CD 2851, NS 8383, TEC7548, CD 2817, AN73017, M8372, DM75I76, NS7667, ANTA82, P98Y30, and BRS413) with an increase between 29 and 72% compared to control. The varieties did not present differences in leaf total chlorophyll concentration between the two Mg sources used.

In 14 varieties, the Mg application provided an increase in carotenoid concentration in relation to control (without Mg), between 16 and 104% higher than control (Figure 3B; Table 9S). Among these 14 varieties, 7 (DM75I76, CD 2851, CD 2817, NS 8383, TEC7548, DM81I84, NS7667) presented higher carotenoid concentration under $MgSO_4$ application, while 4 varieties (M8372, CD 2857, CZ 48B32, TMG 1180) presented higher carotenoid concentration under MgO application, and for the other 3 varieties (ST 797, NS 7670 and NA7337) both sources increased carotenoids concentration equally. On the other hand, in 13 varieties Mg application decreased carotenoid concentration, for most of them, both sources decreased carotenoid content equally (Figure 2B; Table 9S). For the remaining varieties, the Mg application did not alter the carotenoid concentration. These results indicate a general improvement in photosynthetic pigments provided by Mg application in soybean.

Figure 3. Effects of Mg rates on total chlorophyll (A) and carotenoids (B) in *Glycine max* varieties (January 9, 2018). CV: coefficient of variation. "*" compares each variety under Mg rates, indicating the best rate compared to the others according to the Scott Knott test ($p \leq 0.05$). Bars indicate the standard error of the means (n=3).



Source: The author himself

Total sugar and sucrose concentration

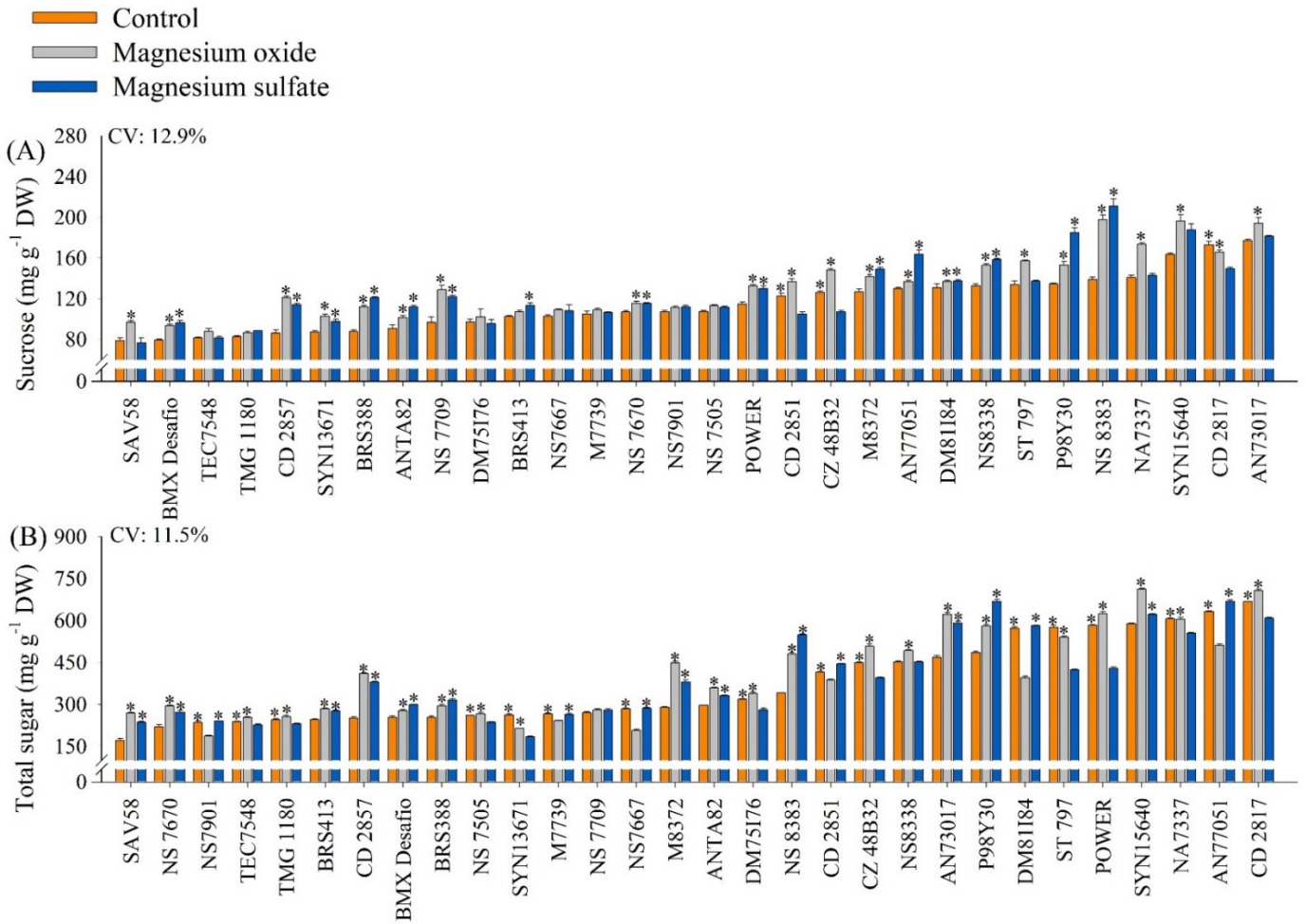
For sucrose and total sugar concentration, an interaction between varieties and Mg sources was observed (*P* <0,0001; Table 13S).

Sucrose concentration increased in 22 out of the 30 soybean varieties under Mg application (Figure 4A; Table 14S). Among the 22 varieties, 9 of them (AN73017, SYN15640, NA7337, ST 797, CZ 48B32, CD 2851, SAV58, NS 7709, and CD 2857) presented higher sucrose concentration under MgO application (between 10 and 40% higher than control). In 7 varieties (NS 8383, P98Y30, AN77051, M8372, BRS388, BRS413, and ANTA82) the highest sucrose concentration was observed under $MgSO_4$, and for the remaining 6 varieties (NS8338, DM81I84, POWER, NS 7670, SYN13671, and BMX Desafio) both sources affected sucrose concentration equally.

For total sugar concentration, Mg application also increased the content of 22 out of the 30 soybean varieties (Figure 4B; Table 15S). Among the 22 varieties, in 14 (SYN15640, CD 2817, POWER, AN73017, CZ 48B32, NS8338, M8372, CD 2857, ANTA82, DM75I76, NS 7670, SAV58, TMG 1180, and TEC7548), the total sugar concentration was higher under MgO application, between 6 and 64% higher than the control. For 7 varieties (AN77051, P98Y30, DM81I84, NS 8383, CD 2851, BRS388, and BMX Desafio) the application of $MgSO_4$ provided the highest increase, between 2 and 38% higher than the control. While both Mg sources increased total sugar concentration equally in the variety BRS413. Regarding the remaining 8 varieties, 7 of them presented a decrease in total sugar concentration under Mg application, between 6 and 27% compared to control, while for one variety (NS 7709), total sugar concentration was not affected by Mg application.

For most of the varieties, Mg application increased total sugar and sucrose concentration. This is indicative of Mg application improving photosynthesis in soybean plants.

Figure 4. Effects of Mg rates on Sucrose (A) and Total Sugar (B) in *Glycine max* varieties. CV means coefficient of variation. "*" compares each variety under Mg rates, indicating the best rate compared to the others according to the Scott Knott test ($p \leq 0.05$). Bars indicate the standard error of the means (n=3).



Source: The author himself

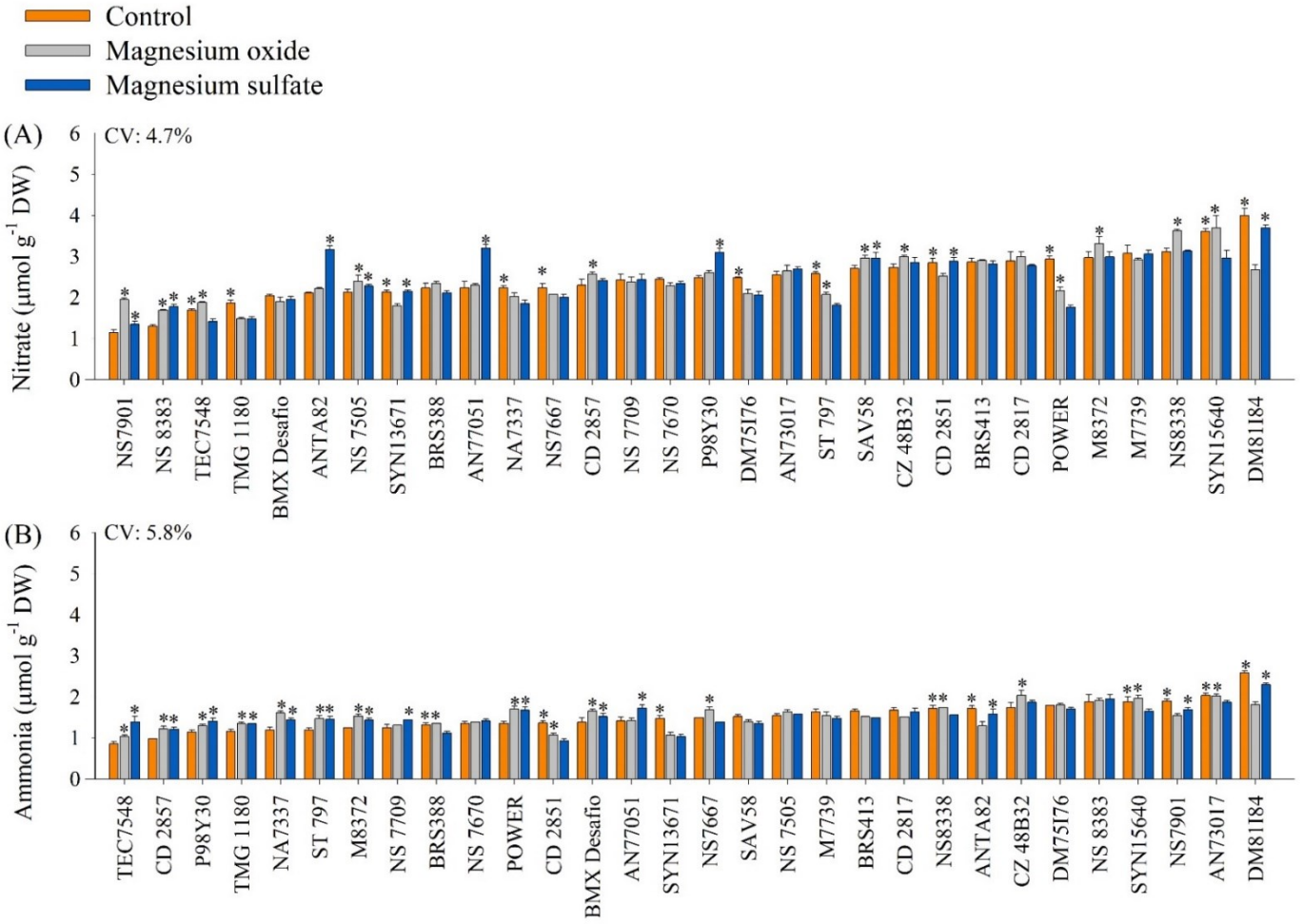
Nitrogen compounds

Regarding the N compounds metabolism, a significate interaction between Mg sources and soybean varieties was observed for nitrate, ammonia, allantoin, allantoic acid, ureides, and total free amino acids (*P* <0,5 to *P* <0, 0001; Table 16S).

Nitrate content decreased in 11 varieties under Mg application (Figure 4A; Table 17S). The decrease of nitrate in 7 varieties (DM81I84, POWER, DM75I76, ST 797, NA7337, NS7667, and TMG 1180) was observed under both MgO and $MgSO_4$ applications. While varieties SYN15640 and TEC7548 presented decreased nitrate concentration under $MgSO_4$ application, and CD 2851 and SYN13671 presented a decrease in nitrate concentration under MgO application. On the other hand, other 11 soybean varieties presented increased nitrate concentration under Mg application (Figure 5A). Among these varieties, 5 of them (NS8338, M8372, CZ 48B32, CD 2857, and NS7901) presented higher nitrate concentration under MgO application, while in 3 varieties (AN77051, ANTA82, and P98Y30) $MgSO_4$ application provided the increased concentration of nitrate in the soybean leaf. And in 3 varieties (SAV58, NS 7505, and NS 8383) both mg sources increased nitrate concentration equally.

Under mg application, 9 out of 30 soybean varieties presented a decrease in ammonia concentration (Figure 5B; Table 18S). Both Mg sources decreased ammonia concentration in 3 varieties (NS7901, SYN13671, and CD 2851). In 4 varieties (SYN15640, AN73017, NS8338, and BRS388) ammonia concentration decreased under $MgSO_4$ application. And in 2 varieties (DM81I84, ANTA82) ammonia concentration decreased under MgO application. On the other hand, in 8 varieties (NS 8383, DM75I76, NS 7505, CD 2817, M7739, BRS413, SAV58, and NS 7670) Mg application did not affect ammonia concentration, while in the remaining varieties ammonia concentration increased under mg application.

Figure 5. Effects of Mg rates on nitrate (A) and ammonia (B) concentration in *Glycine max* varieties CV means coefficient of variation. "*" compares each variety under Mg rates, indicating the best rate compared to the others according to the Scott Knott test ($p \leq 0.05$). Bars indicate the standard error of the means (n=3).

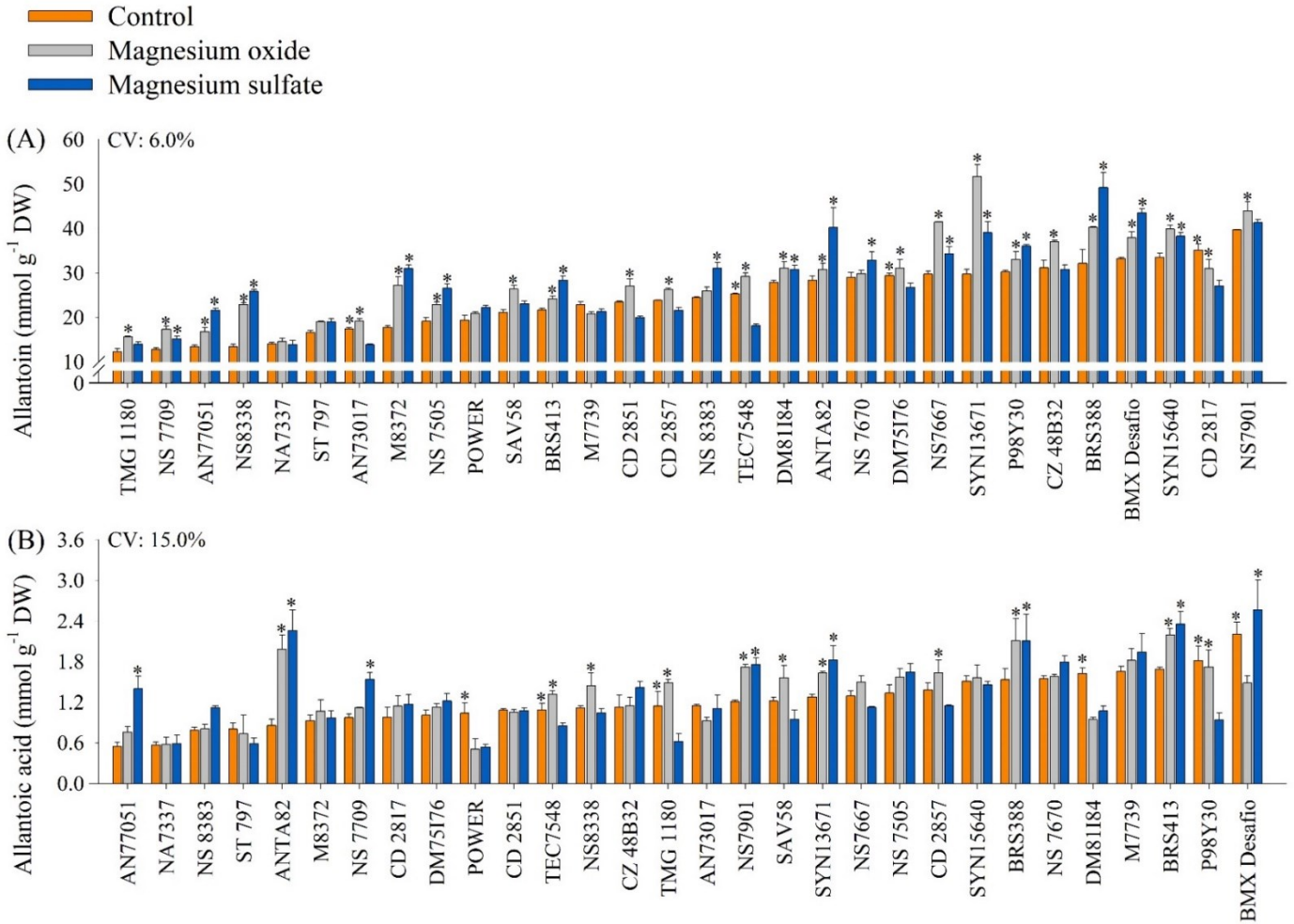


Source: The author himself

The allantoin concentration in soybean plants increased in most varieties under mg application (Figure 6A; Table 19S). Among the varieties, 23 presented increased in allantoin concentration under Mg application, of which 9 varieties (SYN13671, NS7901, NS7667, CZ 48B32, TEC7548, CD 2851, SAV58, CD 2857, TMG 1180) presented the highest concentrations under MgO application, and other 9 varieties (BRS388, BMX Desafio, P98Y30, NS 7670, NS 8383, M8372, NS 7505, NS8338 and AN77051) presented the highest concentrations under $MgSO_4$ application. And for 5 varieties (SYN15640, ANTA82, DM81I84, BRS413, NS 7709) both Mg sources increased allantoin concentration equally. However, 4 varieties (M7739, POWER, ST 797, and NA7337) were not affected by Mg application, and the remaining varieties presented a decrease in allantoin content under Mg application.

Under Mg application allantoic acid concentration was not affected in most varieties (M7739, NS 7505, NS 7670, SYN15640, CZ 48B32, P98Y30, NS7667, DM81I84, M8372, CD 2817, DM75I76, CD 2851, AN73017, NS 8383, TEC7548, POWER, NA7337 and ST 797), while in the remaining 12 varieties, mean allantoic acid concentration observed was between 16 and 156% higher than in the control (Figure 6B; Table 20S). Among these 12 varieties, in 5 (BRS413, ANTA82, BRS388, SYN13671, and NS7901) both Mg sources provided the same increase in allantoic acid concentration, while in 4 varieties (CD 2857, SAV58, TMG 1180, and NS8338), MgO application provided the highest increase, while $MgSO_4$ application provided the highest increase in the remaining 3 varieties (BMX Desafio, NS 7709, AN77051.

Figure 6. Effects of Mg rates on allantoin (A) and allantoic acid (B) concentration in *Glycine max* varieties. CV means coefficient of variation. "*" comparam cada variedade em resposta as doses de Mg, indicando diferença superior sob as demais, de acordo com o teste de Scott Knott ($p \leq 0.05$). Barras representam o erro padrão das médias (n=3).

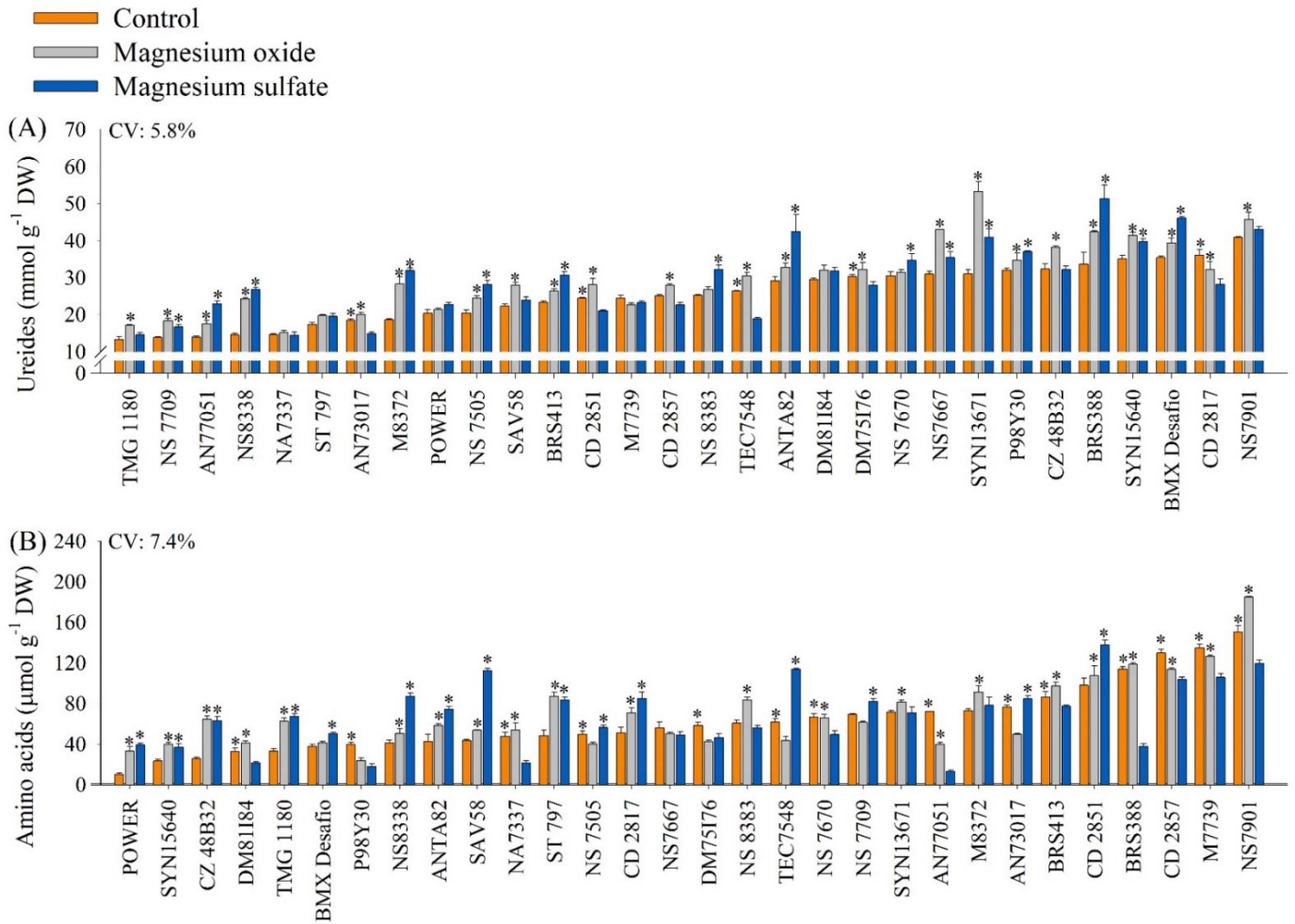


Source: The author himself

The application of Mg increased ureides concentration in 22 soybean varieties, indicating the improvement of Mg to BNF (Figure 7A; Table 21S). Among these 22 varieties, 4 of them (SYN15640, P98Y30, NS8338, and NS 7709) both MgO and $MgSO_4$ equally increased ureides concentration, with a mean increase between 12 and 76% compared to control. In 10 varieties (NS7901, SYN13671, NS7667, CZ 48B32, TEC7548, CD 2851, SAV58, CD 2857, AN77051, and TMG 1180) the highest ureides concentration were found under MgO application, with a mean increase between 11 and 72% higher than the control. While in 8 varieties (BRS388, BMX Desafio, ANTA82, NS 7670, NS 8383, M8372, BRS413, and NS 7505), $MgSO_4$ provided the highest increase in ureides (13 to 71% higher than the control). On the other hand, some varieties presented a decrease under MgO application: ureides concentration in CD 2817 decrease under Both MgO and $MgSO_4$ applications, while DM75I76 and AN73017 presented lower ureides content under $MgSO_4$ application. For the remaining varieties, ureides concentration was not affected by Mg application.

Regarding amino acids concentration, on 19 soybean varieties, the Mg application increased this compound concentration (Figure 7B; Table 22S). Among these 19 varieties, in 5(ST 797, TMG 1180, CZ 48B32, SYN15640, and POWER) both Mg sources increased amino acids concentration equally, with a mean increase between 64 and 259% compared to control. Other 6 varieties (NS7901, BRS413, M8372, NS 8383, SYN13671, and DM81I84) presented increased amino acid concentration (between 13 to 38% higher than control) under MgO application, while $MgSO_4$ application provided an increase (between 33 and 158% compared to control) on 8 varieties (CD 2851, TEC7548, SAV58, NS8338, CD 2817, NS 7709, ANTA82 and BMX Desafio). The variety NS7667 was not affected by Mg application, while the remaining varieties presented decreased amino acid concentration under Mg application.

Figure 7. Effects of Mg rates on ureides (A) and amino acids (B) concentration in *Glycine max* varieties. CV means coefficient of variation. "*" compares each variety under Mg rates, indicating the best rate compared to the others according to the Scott Knott test ($p \leq 0.05$). Bars indicate the standard error of the means (n=3).



Source: The author himself

4.6. DISCUSSION

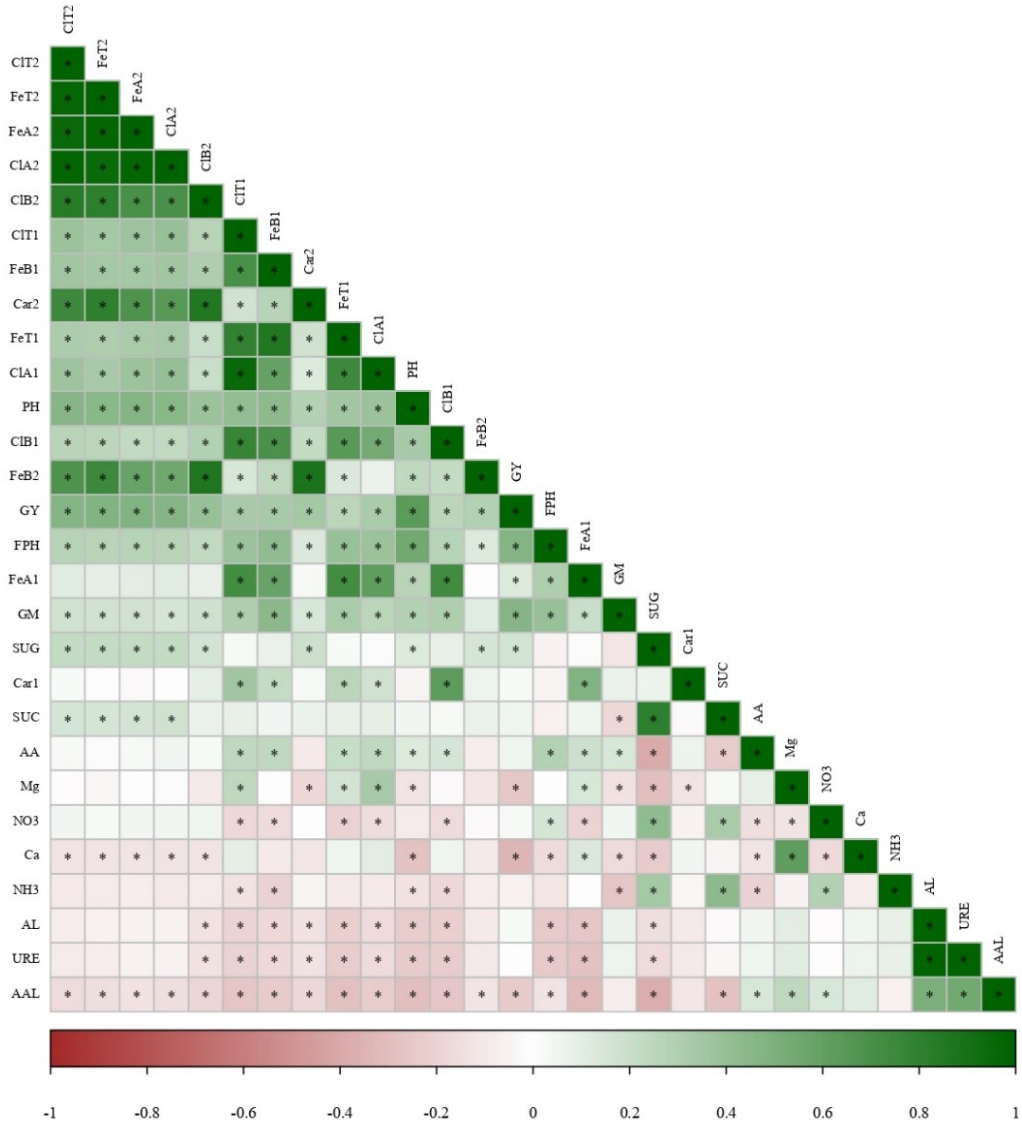
The application of MgO and $MgSO_4$ can increase Mg concentration in soybean leaves. According to Malavolta (2006), the sufficient range of Mg content in soybean leaves is 3.5-4 g kg^{-1} DW. In the present study, under Mg application all varieties except BMX desafio (3.44 g kg^{-1}) presented Mg concentration in leaves above the 3.5 g kg^{-1} threshold, indicating that the application performed was adequate to supply soybean plants. The Mg is essential to photosynthesis (LI *et al.,* 2017), photoassimilates production (GRZEBISZ, 2013), and N metabolism (YIN *et al.,* 2009; LI *et al.,* 2017), thus the physiological enhancements observed in this process, as well as the increased grain yield, corroborates with the data observed (Supplementary Figure S1). In 20 varieties the leaf Mg concentration was above the sufficient range. Particularly varieties NS 7505, NS7667, and NS 7709 presented leaf Mg content between 10 and 12 mg Mg kg^{-1} DW, and despite that, Mg application enhanced important parameters in these varieties, such as total chlorophyll, sucrose, ureides, and grain yield.

In most varieties, leaf Ca concentration was not affected by Mg application. Plenty of previous studies reported a negative influence of high levels of Mg in the soil in leaf Ca concentration (YAMANAKA *et al.,* 2010; NIU *et al.,* 2014). In this case, a competitive inhibition due to the same uptake site might occur, leading to impairment of one nutrient absorption due to the high levels of other nutrients (MALAVOLTA *et al*., 1989). However, this phenomenon was observed only in variety NS 7670, thus the Mg application rate of 30 kg ha^{-1} did not impair the Ca uptake of most soybean varieties. This is also reinforced by the positive correlation coefficient between Mg leaf concentration × Ca leaf concentration observed (Figure 8).

The Mg application increases chlorophyll pigments concentration in soybean plants. These pigments are the light-harvesting compounds in the photochemical process of photosynthesis, thus, pigments play essential roles in photosynthesis (CAKMAK; YAZICI, 2010). This occurs due to Mg being part of chlorophylls. Is estimated that 15 to 35% of all Mg in plants is bound to chloroplasts (CHEN *et al.,* 2017). The positive correlation coefficient between Mg leaf concentration × Total chlorophyll observed, reinforces the previous reports and the present study results (Figure 8).

Carotenoid content in soybean leaf also increased in most varieties under Mg application, while decreasing in 13 varieties. Carotenoids are light-protecting compounds, absorbing the excessive light, thus are essential to keep the balance of light in the photosynthetic apparatus (KUSAMA *et al.*, 2015). In previous studies was observed that Mg alleviates the abiotic stress in pants such as high temperature (SILVA *et al.* 2017) and Al toxicity (CHEN *et al.,* 2012; BOSE *et al.,* 2013). Thus, the present study results indicate an Mg contribution to soybean photoprotection caused by excessive light in the photosynthetic apparatus, as well as signs of acclimation of these varieties to abiotic stress.

Figure 8. Heatmap of Pearson correlation coefficients obtained from variables extracted from soybean.



* indicates significant correlation ($p < 0.05$).

Abbreviation: Mg: Magnesium; Ca: Calcium; PH: Plant height; FPH: First pod height; GM: Grain mass; GY: Grain yield; NO_3: Nitrate; NH_3: Ammonia; AL: Allantoin; AAL: Allantoic acid; URE: Ureides; AA: Amino acids; ClA1: chlorophyll a; ClB1: chlorophyll b; ClT1: total chlorophyll; CAR1: carotenoids; FeA1: pheophytin a, FeB1: pheophytin b; FeT1: total pheophytin; ClA2: chlorophyll a; ClB2: chlorophyll b; ClT2: total chlorophyll; CAR2: carotenoids; FeA2: pheophytin a, FeB2: pheophytin b; FeT2: total pheophytin; SUC: Sucrose; SUG: Total Sugar.

ClA1, ClB1, ClT1, CAR1, FeA1, FeB1, FeT1 analysis was performed on January 9, 2018.

ClA2, ClB2, ClT2, CAR2, FeA2, FeB2, FeT2 analysis was performed on February 8, 2018.

Source: The author himself

Sucrose is the main product of photosynthesis in plants. In the present study, most soybean varieties showed increase leaf sucrose concentration after Mg application. It is known that Mg acts as a cofactor and modulator of important enzymes belonging to the photosynthetic process, such as Calvin cycle enzymes and also ATPases (VERBRUGGEN; HERMAN, 2013). Also, RuBisCO (Ribulose-1,5-bisphosphate carboxylase / oxygenase), the main enzyme in CO_2 assimilation in photosynthesis, must be activated in a process in which a Mg ion is needed (BHAT *et al*., 2017). Thus, the results of these studies reinforce the contribution of Mg to the activity and photosynthetic products observed in most soybean varieties.

Sucrose concentration in soybean leaf was not affected by Mg application in 7 soybean varieties and decreased in only one. According to Kryvoruchko *et al.* (2016) and Sugiyama *et al.* (2017), sucrose is the main source of Carbon distributed throughout phloem to nodules. Is possible that the effect observed might be related to a better sucrose loading from leaves to roots in the 8 aforementioned varieties, aiming to supply nodules metabolism and development, thus, enhancing ureides production. Plenty of studies reported the effects of Mg application in sucrose loading throughout the phloem (CAKMAK; KIRBY, 2008; VERBRUGGEN; HERMANS, 2013; PENG *et al*. 2018). In most of these 8 varieties, ureides concentration increased under Mg application, reinforcing this possibility. Peng *et al.* (2018) also observed Mg application increases carbohydrates transport to nodules, resulting in increased translocation of ureides to soybean plants.

Total sugar concentration increased in most soybean varieties under Mg application. These findings reinforce Mg's importance in photoassimilates productions (sugars) by plants. These results are corroborating with a plethora of previous studies which report Mg contribution to photosynthetic activity and CO_2 fixation into carbon skeleton in plants (CAKMAK; YAZICI, 2010; GERENDÁS; FÜHRS, 2013; DIAS *et al.*, 2017). Is also noteworthy that, in this study, a positive correlation between chlorophyll pigments x sucrose and total sugar content were observed (Figure 8). The carbon-based products from photosynthesis are required for adequate plant growth and development since they are essential for carbon-skeleton synthesis along with plant growth.

Regarding N metabolism, nitrate content decreased in 11 soybean varieties under Mg application. Thus, indicating that no nitrate accumulation occurred under Mg

application in these varieties, ruling out potential negative effects of Mg in enzymes related to nitrate assimilation, such as nitrate reductase (YIN *et al.*, 2009). This enzyme activity is highly dependent on photosynthesis products, such as photoassimilates (sugars), NADP and NADPH reducing power (PIWPUAN *et al.*, 2013), as well as ATP, due to the high energy demand of this process (SUNIL *et al.*, 2013). The APT requires the Mg presence to be biologically activated as Mg-ATP (GUOT *et al.*, 2014; IGAMBERDIEV; KLECZKOWSKI, 2015). Thus, is expected that under high photosynthetic activities and photoassimilate production (in the present study enhanced by Mg application), is an essential step to increase nitrate reductase activity. Thus, these pieces of evidence suggest that Mg can enhance nitrate reductase activity, an essential step to nitrate assimilation in organic compounds.

Plant roots can actively absorb nitrate from the soil solution. Is possible that this effect is not related to the increased nitrate concentration observed in the other 11 varieties under Mg application. Since the negative correlation observed between Mg ×nitrate concentration rejects this possibility (Figure 8). Although Mg effects on N metabolism are not fully elucidated yet, the present results could indicate a potential accumulation of Nitrate in these specific soybean varieties. However, a decrease in nitrate reductase activity was previously reported only under Mg deficiency (DING *et al*., 2006; YIN *et al.*, 2009; LI *et al.*, 2017), which was not evaluated nor occurred in the present study. Thus, the effect of Mg on leaf nitrate concentration in soybean varieties might be regulated by specific characteristics of each variety.

Ammonia is the first stable compound in the BNF process. Under Mg application, 9 soybean varieties presented decreased leaf ammonia concentration while in 8 varieties the Mg application did not alter ammonia content. These results indicate a fast and efficient N assimilation by plants under Mg application, leading to a rapid conversion of ammonia into ureides (allantoic acid and allantoin) (DUNN, 2014), or ammonia assimilation into amino acids by the combined activity of glutamate and glutamine synthetase in plants (MARTÍNEZ-ANDÚKAR *et al.*, 2013). However, on 13 soybean varieties, ammonia concentration increased under Mg application, the elevated levels might lead to phytotoxicity. The negative correlation coefficient between ammonia concentration × growth parameters reinforce the ammonia capacity to impair plants development due to phytotoxicity (Figure 8).

The ammonia from FBN is usually converted into the final product of plant-bacteria symbiosis: the ureides (allantoin and allantoic acid). Under Mg application, most soybean varieties presented increased allantoin concentration, while 12 varieties presented increased allantoic acid concentration. Allantoin is synthesized from uric acid within the peroxisomes, while allantoic acid is synthesized from allantoin (LAMBERTO *et al.,* 2010; WERNER; WITTE, 2011). In this uric acid – allantoin – allantoic acid pathway, more than 20 enzymes are expressed (O'ROURKE *et al.,* 2014), and Mg is required to activate some of these enzymes (JAHNEN-DECHENT; KETTELER, 2012). Thus, the present results indicate that Mg can induce beneficial effects on N assimilation into ureides in soybean. The positive correlation coefficient between leaf Mg concentration × allantoic acid reinforces this possibility (Figure 8).

The more efficient BNF in soybean plants under Mg application is corroborated by the higher ureides concentration in soybean plants under mg application. In the BNF process, *Fabaceae* plants provide photoassimilates to the growth and development of rhizobia, and in exchange, rhizobia provide ureides for plants (UDVARDI; POOLE, 2013). The negative correlation coefficient between ureides concentrations × total sugar concentrations reinforce the symbiotic process between plants and bacteria (Figure 8). This compound might be considered a key factor to yield increases in soybean since it is reported as one of the main N-compound stored and transported (CHIASSON *et al.*, 2014). Thus, this information reinforces the effect of Mg on increased ureides production in soybean plants, resulting in more efficient BNF due to elevated photosynthetic activity and photoassimilates production.

Under Mg application, most soybean varieties also presented increased amino acid concentration. To assimilate ammonia into amino acids, the enzymes glutamate and glutamine synthetase require great cellular effort and Mg can positively influence this process. Previous studies also corroborate this possibility, due to some works reporting Mg positive influence glutamine synthetase (DING *et al.,* 2006), while the lack of Mg might inhibit the activity of glutamate synthase (GOGAT) and glutamine synthetase (YIN *et al.,* 2009). In the present study, the concentration of amino acids presented a positive correlation coefficient with the grain mass (Table 8). Considering the high amount of protein present in soybeans, it is possible that Mg favors greater protein synthesis by soybean, considering its essentiality as a bridge element in the

aggregation of ribosome subunits responsible for protein synthesis (MELNIKOV *et al.,* 2016).

Soybean is well known for its high level of storage protein in grains, requiring high levels of N in its development (RODRÍGUES-NAVARRO *et al.*, 2011; KINUGASA *et al.*, 2012). The present study data indicate that Mg application results in soybean yield increase due to the enhanced BNF and increased activities of N-metabolism enzymes. The increased grain yield observed in 22 soybean varieties under Mg application reinforces these results (Supplementary Figure 5SA). The Mg application provides gain yield increase ranging from 7 up to 54% compared to control (P <0,0001; Table 23S e 25S).

Comparing both Mg sources used in the present study, application of MgO provided more relevant increases in total chlorophyll, pheophytin b, total pheophytin, total sugar, and sucrose in most soybean varieties. Due to the increased photoassimilates provided by the MgO application, this source also led to a higher N_2 conversion into ureides in most soybean varieties, resulting in a higher yield to most soybean varieties. The highest yield means under MgO application surpassed 5 t ha^{-1} (CZ 48B32 and CD 2857), as well as the higher grain, mean (TEC 7548, 24g for every 100 grains) (Supplementary Figure S1; Table 24S). Regarding $MgSO_4$ application, this source provided more relevant enhancements only to chlorophyll b, pheophytin a, carotenoids, and amino acids. The $MgSO_4$ is a high soluble fertilizer (KAWAMURA; RAO, 2007), while MgO presents a slow-release, thus presenting less leaching risk (SENBAYRAM *et al.*, 2015). Is possible that the Mg sources solubility, related to some limiting biotic factor, might result in these differences between MgO and $MgSO_4$ effects. Considering that soluble fertilizer can lead to losses by leaching in sandy soils, especially in rainy seasons (LOGANATHAN *et al*., 2005), is important to consider the fertilizer solubility of the Mg source.

The verified results indicates that Mg application increases photosynthetic pigments concentration and consequently, enhances photosynthesis efficiency due to increased photoassimilates production. Possibly, under Mg application, specially MgO, an increase in photoassimilates loading to soybean nodules occurred, resulting in increased N_2 assimilation into ureides. Finally, leading to an increase in grains yield in most soybean varieties under Mg application in low mg soil. The present information endorses the influence of Mg on BNF and N metabolism in soybean plants, which are

consistent with innovative techniques aiming to increase soybean yield in field conditions.

4.7. CONCLUSION

The MgO and $MgSO_4$ application increased Mg concentration in soybean leaves without impairing leaf Ca concentration in most varieties evaluated.

The application of Mg improved photoassimilates (sucrose and total sugar) production in most varieties evaluated, indicating an enhancement in the photosynthetic process due to increased concentration of pigments. Under higher levels of photoassimilates, higher N_2 conversion occurred and higher ureides synthesis, indicating improvement in BNF efficiency.

Amino acids concentration increased under Mg application in most varieties, indicating higher N metabolism activity.

Between both Mg sources, MgO provided the highest increases to pigments (total chlorophyll, pheophytin b, and total pheophytin), total sugars, sucrose, and ureides in most soybean plants. While $MgSO_4$ application provided higher increases in chlorophyll b, pheophytin a, carotenoids, and amino acids.

This study provides valuable information about the Mg effect on BNF metabolism in soybean varieties. The presented results are important tools to further studies aiming to increase soybean grain yield and BNF by supplying Mg to the plants.

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APPENDAGE A

Table S1. Soybean varieties used in the study.

Varieties	Technology	Sowing date	Starting plant stand	Final plant stand	Harvest date	Cycle
			(PL Ha^{-1})			(Days)
POWER	IPRO	11/11/2017	318.519	303.704	3/6/2018	115
TEC7548	IPRO		288.889	251.852		115
AN73017	RR		259.259	251.852		115
DM75I76	IPRO		281.481	207.407		115
ANTA82	RR		362.963	362.963		115
SYN15640	IPRO		288.889	281.481		115
SYN13671	IPRO		311.111	303.704		115
BMX Desafio	RR		385.185	303.704		115
SAV58	RR		296.296	288.889		115
M7739	IPRO		214.815	207.407		115
BRS413	RR		259.259	251.852		115
BRS388	RR		422.222	400.000		115
NS7709	IPRO		318.518	266.667		115
ST797	IPRO		303.704	259.259	3/22/2018	131
TMG1180	RR		288.889	244.444		131
NA7337	RR		244.444	229.630		131
AN77051	RR		288.889	237.037		131
P98Y30	RR		281.481	251.852		131
CZ 48B32	IPRO		274.074	259.259		131
DM81I84	IPRO		281.481	229.630		131
CD 2857	IPRO		281.481	259.259		131
CD 2851	IPRO		296.296	274.074		131
CD 2817	IPRO		214.815	204.815		131
M8372	IPRO		288.889	259.259		131
NS8338	IPRO		296.296	288.889		131
NS 8383	RR		281.481	266.667		131
NS7901	RR		259.259	251.852		131
NS 7670	RR		274.074	259.259		131
NS7667	IPRO		288.889	192.593		131
NS 7505	IPRO		222.222	214.815		131

PL ha^{-1} - Plants per hectare.

Table S2. Fc and P values of analysis of variance (ANOVA) for leaf Mg and Ca concentrations in soybean varieties.

	Source of variation	Fc	Pr >Fc	
MAGNESIUM	Varieties (A)	117.79	0.00000	*
	Mg sources (B)	361.09	0.00000	*
	A X B	9.96	0.00000	*
	Source of variation	Fc	Pr >Fc	
CALCIUM	Varieties (A)	22.55	0.00000	*
	Mg sources (B)	4.11	0.01795	*
	A X B	1.60	0.01024	*

Sources of variation: Soybean varieties (A), Mg sources (B), and the interaction between the previously mentioned factors (A X B).

Source: The author himself

Table S3. Comparison of means of leaf Mg concentration among soybean varieties according to Scott Knott test ($p \leq 0.05$).

	MAGNESIUM (g kg^{-1} DW)		
VARIETIES	**CONTROL**	**MAGNESIUM OXIDE**	**MAGNESIUM SULFATE**
DM81I84	1,95 ± 0,27 D a	2,90 ± 0,23 H a	2,35 ± 0,17 H a
POWER	2,02 ± 0,09 D b	3,22 ± 0,19 H a	3,47 ± 0,18 G a
CZ48B32	2,22 ± 0,26 D b	3,50 ± 0,03 H a	2,75 ± 0,13 H b
CD2851	2,25 ± 0,17 D b	3,72 ± 0,36 H a	2,95 ± 0,03 H b
ST797	2,32 ± 0,14 D a	3,02 ± 0,09 H a	2,83 ± 0,08 H a
P98Y30	2,33 ± 0,11 D b	3,58 ± 0,14 H a	3,83 ± 0,14 G a
SYN15640	2,38 ± 0,16 D b	4,80 ± 0,43 G a	5,35 ± 0,50 E a
TMG1180	2,43 ± 0,28 D c	4,33 ± 0,16 H a	3,42 ± 0,12 G b
BMXDesafio	2,43 ± 0,06 D b	3,13 ± 0,06 H a	3,75 ± 0,03 G a
AN77051	2,53 ± 0,06 D b	3,95 ± 0,17 H a	4,42 ± 0,32 F a
SYN13671	2,57 ± 0,24 D b	4,63 ± 0,18 G a	4,07 ± 0,18 F a
NA7337	2,58 ± 0,04 D c	4,98 ± 0,88 G a	3,82 ± 0,06 G b
NS8338	2,60 ± 0,43 D a	3,43 ± 0,29 H b	4,83 ± 0,32 E b
M8372	2,75 ± 0,03 D c	4,18 ± 0,14 H b	7,48 ± 0,31 C a
CD2817	2,78 ± 0,26 D a	3,80 ± 0,27 H a	3,25 ± 0,07 G a
CD2857	2,83 ± 0,09 D b	4,50 ± 0,13 G a	3,73 ± 0,06 G a
M7739	2,85 ± 0,20 D b	4,57 ± 0,62 G a	5,32 ± 0,66 E a
ANTA 82	2,88 ± 0,02 D b	3,60 ± 0,20 H b	4,38 ± 0,18 F a
DM75I76	2,90 ± 0,07 D b	3,73 ± 0,09 H a	4,07 ± 0,29 F a
AN73017	2,95 ± 0,17 D c	4,90 ± 0,13 G b	6,53 ± 1,44 D a
NS7901	3,07 ± 0,39 D c	7,18 ± 0,41 E a	4,17 ± 0,18 F b
TEC7548	3,08 ± 0,11 D a	3,77 ± 0,09 H a	3,35 ± 0,07 G a
SAV 58	3,27 ± 0,12 C b	5,12 ± 0,26 G a	4,03 ± 0,08 F b
NS7670	4,67 ± 0,36 C c	9,87 ± 1,42 C a	6,48 ± 0,34 D b
NS8383	4,77 ± 0,18 C c	6,33 ± 0,18 F b	7,30 ± 0,20 C a
BRS388	4,93 ± 0,29 C c	6,87 ± 0,04 E b	8,13 ± 0,24 C a
BRS413	5,22 ± 0,18 C c	6,48 ± 0,49 F b	7,83 ± 0,09 C a
NS7505	5,90 ± 0,47 B c	12,03 ± 1,39 A a	8,83 ± 0,74 B b
NS7667	6,08 ± 0,41 B b	7,88 ± 0,16 D b	10,32 ± 0,22 A a
NS 7709	8,05 ± 0,10 A c	11,15 ± 0,87 B a	9,15 ± 0,53 B b

Means followed by the same letter are not significantly different according to the Scott Knott test ($p \leq 0.05$). Uppercase letters compare varieties in each Mg source, and lowercase letters compare the Mg sources in each variety.

Source: The author himself

Table S4. Comparison of means among leaf Ca concentration in soybean varieties according to Scott Knott test ($p \leq 0.05$).

	CALCIUM (g kg¹ DW)		
VARIETIES	**CONTROL**	**MAGNESIUM OXIDE**	**MAGNESIUM SULFATE**
CD2851	9,87 ± 0,48 D a	10,93 ± 0,21 D a	10,90 ± 0,33 D a
M8372	10,12 ± 0,26 D b	11,85 ± 0,73 D b	17,33 ± 1,24 B a
M7739	10,45 ± 1,00 D a	10,52 ± 0,31 D a	12,73 ± 1,91 D a
DM81I84	10,87 ± 0,18 D a	11,57 ± 0,29 D a	11,08 ± 0,61 D a
CD2857	11,10 ± 1,03 D a	10,82 ± 0,39 D a	10,85 ± 0,27 D a
NS8338	11,15 ± 0,57 D b	11,50 ± 0,60 D b	15,63 ± 2,09 C a
NS7901	11,42 ± 1,71 D a	11,55 ± 1,63 D a	13,75 ± 2,17 C a
NA7337	11,55 ± 0,17 D a	11,12 ± 0,06 D a	12,08 ± 0,11 D a
AN73017	12,52 ± 2,59 C a	10,70 ± 1,00 D a	12,20 ± 0,20 D a
AN77051	12,52 ± 2,59 D a	12,20 ± 0,20 D a	10,70 ± 1,00 D a
CZ48B32	12,65 ± 0,67 C a	11,93 ± 0,69 D a	12,75 ± 0,43 D a
SAV58	12,72 ± 0,76 C a	12,70 ± 0,73 C a	12,35 ± 0,60 D a
ANTA82	12,88 ± 0,04 C a	13,08 ± 0,51 C a	13,83 ± 0,71 C a
BMXDesafio	13,23 ± 0,72 C a	13,45 ± 0,20 C a	13,38 ± 0,56 C a
TMG1180	13,55 ± 0,60 C a	11,13 ± 0,28 D a	12,60 ± 0,60 D a
CD2817	13,55 ± 0,60 C a	11,13 ± 0,28 D a	15,12 ± 3,29 C a
ST797	13,72 ± 0,19 C a	13,03 ± 0,36 C a	12,43 ± 0,36 D a
TEC7548	14,07 ± 0,28 C a	14,53 ± 1,18 C a	14,27 ± 0,19 C a
SYN13671	14,45 ± 1,30 C a	16,22 ± 0,89 B a	15,32 ± 0,71 C a
SYN15640	14,58 ± 1,81 C a	14,77 ± 0,96 C a	16,77 ± 2,66 B a
P98Y30	14,73 ± 0,41 C a	14,85 ± 0,37 C a	14,30 ± 0,70 C a
POWER	14,78 ± 0,71 C a	14,48 ± 0,36 C a	14,95 ± 0,53 C a
NS7667	14,80 ± 1,00 C a	17,57 ± 2,71 B a	17,58 ± 1,34 B a
BRS388	16,32 ± 0,52 B a	15,92 ± 1,29 B a	19,22 ± 1,04 B a
DM75I76	17,13 ± 4,88 B a	13,73 ± 0,59 C a	15,73 ± 0,96 C a
BRS413	17,32 ± 2,21 B b	14,70 ± 5,00 C b	20,83 ± 0,26 B a
NS7670	18,73 ± 0,41 B a	18,35 ± 3,03 B a	11,10 ± 1,17 D b
NS8383	19,13 ± 2,24 B a	17,35 ± 2,17 B a	19,23 ± 2,24 B a
NS7505	19,85 ± 0,57 B a	18,87 ± 4,71 B a	20,20 ± 2,27 B a
NS7709	27,00 ± 1,47 A a	27,90 ± 1,37 A a	25,65 ± 4,80 A a

Means followed by the same letter are not significantly different according to the Scott Knott test ($p \leq 0.05$). Uppercase letters compare varieties in each Mg source, and lowercase letters compare the Mg sources in each variety.

Source: The author himself

Table S5. Fc and P values of analysis of variance (ANOVA) for chlorophyll a, b, total chlorophyll, carotenoids, pheophytin a, b, and total pheophytin in soybean varieties.

	Source of variation	Fc	Pr >Fc	
CHLOROPHYLL A	Genotypes(A)	381.71	0.00000	*
	Mg sources (B)	2586.31	0.00000	*
	A X B	48.34	0.00000	*
	Source of variation	Fc	Pr >Fc	
CHLOROPHYLL B	Genotypes(A)	265.37	0.00000	*
	Mg sources (B)	16.67	0.00000	*
	A X B	46.31	0.00000	*
	Source of variation	Fc	Pr >Fc	
TOTAL CHLOROPHYLL	Genotypes(A)	526.42	0.00000	*
	Mg sources (B)	1535.03	0.00000	*
	A X B	55.12	0.00000	*
	Source of variation	Fc	Pr >Fc	
CAROTENOIDS	Genotypes(A)	58.05	0.00000	*
	Mg sources (B)	25.93	0.00000	*
	A X B	31.16	0.00000	*
	Source of variation	Fc	Pr >Fc	
PHEOPHYTIN A	Genotypes(A)	828.11	0.00000	*
	Mg sources (B)	204.40	0.00000	*
	A X B	282.60	0.00000	*
	Source of variation	Fc	Pr >Fc	
PHEOPHYTIN B	Genotypes(A)	375.33	0.00000	*
	Mg sources (B)	160.25	0.00000	*
	A X B	55.70	0.00000	*
	Source of variation	Fc	Pr >Fc	
TOTAL PHEOPHYTIN	Genotypes(A)	1006.52	0.00000	*
	Mg sources (B)	1332.21	0.00000	*
	A X B	215.13	0.00000	*

Sources of variation: Soybean varieties (A), Mg sources (B), and the interaction between the previously mentioned factors (A X B).

Source: The author himself.

Table S6. Comparison of means of chlorophyll-a concentration among soybean varieties according to Scott Knott test ($p \leq 0.05$).

	CHLOROPHYLL A (µg mL^{-1})		
VARIETIES	**CONTROL**	**MAGNESIUM OXIDE**	**MAGNESIUM SULFATE**
P98Y30	63,70 ± 1,99 K c	132,72 ± 1,30 L b	155,35 ± 1,47 I a
BRS413	95,07 ± 4,68 J b	140,84 ± 3,16 K a	135,92 ± 5,41 K a
NS7505	100,99 ± 1,24 J c	229,93 ± 3,13 D a	212,65 ± 7,73 E b
M7739	101,78 ± 3,62 J c	180,94 ± 7,10 H a	130,63 ± 5,09 K b
NS8338	106,28 ± 3,61 I c	193,31 ± 7,97 G a	129,54 ± 10,21 K b
SYN13671	111,38 ± 5,47 I b	160,39 ± 5,83 J a	161,13 ± 2,74 H a
SYN15640	113,02 ± 5,36 I c	172,27 ± 6,47 I a	141,79 ± 1,53 J b
BRS388	115,38 ± 3,29 H c	155,51 ± 1,96 J a	139,70 ± 4,13 J b
NA7337	119,62 ± 1,03 H b	145,55 ± 7,26 K a	146,00 ± 3,79 J a
BMXDesafio	120,42 ± 3,12 H c	168,56 ± 1,72 I a	138,58 ± 4,80 J b
DM81I84	124,07 ± 0,97 G b	158,51 ± 5,27 J a	151,00 ± 5,96 I a
ANTA82	126,73 ± 1,94 G b	157,85 ± 3,08 J a	164,94 ± 1,78 H a
DM75I76	131,63 ± 3,17 G c	178,55 ± 1,13 H b	214,02 ± 3,12 E a
ST797	144,41 ± 0,41 F c	207,91 ± 1,61 F a	189,58 ± 7,39 F b
AN73017	145,49 ± 3,09 F c	199,74 ± 8,35 F b	239,84 ± 7,09 C a
CZ48B32	147,43 ± 4,89 F c	263,75 ± 2,43 B a	178,16 ± 3,64 G b
AN77051	151,39 ± 6,92 E c	270,15 ± 0,88 B a	229,00 ± 1,93 D b
M8372	153,76 ± 1,62 E c	194,48 ± 2,32 G b	217,14 ± 5,19 E a
NS7667	156,35 ± 0,83 E c	185,04 ± 1,17 H b	212,11 ± 0,67 E a
NS7901	156,96 ± 4,89 E c	241,63 ± 2,14 C a	185,91 ± 4,55 G b
NS7670	163,64 ± 2,94 D c	207,03 ± 6,16 F a	196,91 ± 4,41 F b
POWER	165,25 ± 2,46 D b	190,67 ± 3,65 G a	193,46 ± 8,61 F a
SAV58	167,20 ± 5,56 D c	228,98 ± 5,29 D a	211,37 ± 1,50 E b
TEC7548	172,17 ± 1,91 C c	207,15 ± 3,98 F b	275,23 ± 2,93 B a
CD2817	176,04 ± 3,58 C b	241,14 ± 7,37 C a	248,78 ± 6,34 C a
TMG1180	179,47 ± 3,88 C c	231,52 ± 3,46 D a	205,35 ± 1,38 E b
CD2851	180,42 ± 5,16 C c	218,46 ± 1,59 E b	323,05 ± 2,81 A a
NS8383	204,79 ± 4,18 C c	224,50 ± 2,49 E b	281,34 ± 7,75 B a
CD2857	207,54 ± 5,81 B c	277,70 ± 1,84 A a	230,97 ± 2,68 D b
NS7709	222,83 ± 0,35 A b	284,25 ± 3,84 A a	275,32 ± 4,99 B a

Means followed by the same letter are not significantly different according to the Scott Knott test ($p \leq 0.05$). Uppercase letters compare varieties in each Mg source, and lowercase letters compare the Mg sources in each variety.

Source: The author himself

Table S7. Comparison of means of chlorophyll b concentration among soybean varieties according to Scott Knott test ($p \leq 0.05$).

	CHLOROPHYLL B (µg mL^{-1})		
VARIETIES	**CONTROL**	**MAGNESIUM OXIDE**	**MAGNESIUM SULFATE**
BRS388	12,64 ± 1,59 K b	31,83 ± 1,53 I a	30,05 ± 2,61 I a
BMXDesafio	24,64 ± 0,32 J b	52,40 ± 0,22 F a	51,61 ± 0,66 G a
BRS413	25,88 ± 1,09 J c	36,00 ± 1,44 H b	49,10 ± 0,78 G a
NS7505	26,10 ± 1,92 J b	47,79 ± 1,56 G a	29,45 ± 3,29 I b
NS8338	33,03 ± 1,82 I a	17,86 ± 1,17 K b	8,97 ± 1,22 K c
DM81I84	41,42 ± 1,33 H b	46,84 ± 1,70 G b	56,56 ± 1,77 F a
SAV58	41,94 ± 0,26 H a	39,89 ± 0,69 H a	13,85 ± 1,00 K b
NA7337	44,53 ± 0,64 H b	54,19 ± 2,38 F a	54,69 ± 2,30 F a
DM75I76	46,59 ± 4,16 G b	48,22 ± 6,37 G b	66,14 ± 0,00 E a
SYN13671	46,93 ± 4,51 G b	55,02 ± 2,13 F a	47,03 ± 1,25 G b
P98Y30	48,99 ± 0,99 G a	27,00 ± 0,78 J c	35,33 ± 3,05 I b
ST797	53,31 ± 1,73 G b	68,87 ± 5,02 D a	69,68 ± 3,87 E a
M8372	56,51 ± 0,80 F b	73,73 ± 1,22 D a	68,69 ± 1,10 E a
ANTA82	58,24 ± 1,64 F a	51,39 ± 3,39 F b	62,42 ± 3,97 E a
POWER	58,75 ± 1,77 F a	64,11 ± 1,28 E a	41,73 ± 5,61 H b
NS7667	60,43 ± 2,88 F b	57,87 ± 1,43 F b	66,68 ± 2,18 E a
M7739	62,66 ± 4,17 E a	34,12 ± 0,87 I b	34,88 ± 0,03 I b
SYN15640	62,68 ± 1,99 E a	43,61 ± 1,33 G c	51,14 ± 2,38 G b
NS7670	65,10 ± 1,07 E a	69,52 ± 5,67 D a	70,85 ± 5,59 E a
NS8383	67,33 ± 2,02 E b	62,62 ± 3,33 E b	103,00 ± 6,80 C a
NS7901	71,42 ± 3,86 D a	30,96 ± 4,28 I b	20,59 ± 1,42 J c
CZ48B32	71,59 ± 2,61 D a	71,68 ± 0,58 D a	50,93 ± 0,01 G b
TMG1180	74,82 ± 3,63 D b	93,97 ± 3,89 B a	69,32 ± 6,16 E b
AN73017	75,66 ± 1,66 D a	51,63 ± 5,28 F b	57,44 ± 5,74 F b
AN77051	77,34 ± 2,13 C a	27,51 ± 0,97 J c	49,32 ± 3,32 G b
TEC7548	78,73 ± 5,25 C b	75,74 ± 2,11 D b	102,52 ± 5,69 C a
CD2851	83,57 ± 2,86 C b	88,62 ± 1,44 C b	130,06 ± 2,40 A a
CD2817	92,43 ± 3,01 B b	66,51 ± 2,94 E c	117,39 ± 1,41 B a
CD2857	98,43 ± 2,95 B b	107,57 ± 2,03 A a	65,40 ± 2,62 E c
NS7709	119,16 ± 2,31 A a	86,20 ± 5,55 C b	78,31 ± 1,11 D c

Means followed by the same letter are not significantly different according to the Scott Knott test ($p \leq 0.05$). Uppercase letters compare varieties in each Mg source, and lowercase letters compare the Mg sources in each variety.

Source: The author himself

Table S8. Comparison of means of total chlorophyll concentration among soybean varieties according to Scott Knott test ($p \leq 0.05$).

	TOTAL CHLOROPHYLL (µg mL^{-1})		
VARIETIES	**CONTROL**	**MAGNESIUM OXIDE**	**MAGNESIUM SULFATE**
P98Y30	112,69 ± 2,49 L c	159,72 ± 1,25 M b	190,69 ± 2,84 K a
BRS413	120,95 ± 4,98 K c	176,84 ± 4,25 L b	185,02 ± 5,81 K a
NS7505	127,09 ± 2,05 K c	277,72 ± 3,46 F a	242,10 ± 11,03 H b
BRS388	128,02 ± 4,88 K c	187,35 ± 3,41 K a	169,75 ± 3,14 L b
NS8338	139,31 ± 5,29 J b	211,17 ± 9,14 I a	138,51 ± 11,43 M b
BMXDesafio	145,06 ± 3,34 J c	220,96 ± 1,73 H a	190,19 ± 5,45 K b
SYN13671	158,30 ± 9,97 I b	215,41 ± 6,82 I a	208,16 ± 1,49 J a
NA7337	164,14 ± 1,48 I b	199,74 ± 8,14 J a	200,68 ± 5,54 J a
M7739	164,44 ± 5,95 I b	215,05 ± 7,10 I a	165,51 ± 5,09 L b
DM81I84	165,49 ± 1,93 I b	205,35 ± 6,97 I a	207,56 ± 7,73 J a
SYN15640	175,69 ± 5,73 H c	215,88 ± 7,76 I a	192,93 ± 3,30 K b
DM75I76	178,23 ± 7,04 H c	226,78 ± 7,50 H b	280,16 ± 3,12 F a
ANTA82	184,97 ± 1,27 H c	209,24 ± 5,62 I b	227,36 ± 2,55 I a
ST797	197,73 ± 1,47 G c	276,78 ± 3,41 F a	259,27 ± 3,52 G b
SAV58	209,14 ± 5,30 F c	268,87 ± 5,29 F a	225,22 ± 1,77 I b
M8372	210,26 ± 1,11 F c	268,21 ± 2,44 F b	285,83 ± 4,09 F a
NS7667	216,78 ± 2,90 F c	242,91 ± 1,62 G b	278,78 ± 2,76 F a
CZ48B32	219,01 ± 2,28 F b	335,43 ± 1,86 C a	229,08 ± 3,64 I b
AN73017	221,15 ± 4,01 E c	251,37 ± 10,30 G b	297,29 ± 12,83 E a
POWER	224,00 ± 2,40 E b	254,77 ± 4,94 G a	235,19 ± 9,60 I b
NS7901	228,38 ± 8,75 E b	272,59 ± 5,97 F a	206,50 ± 5,40 J c
AN77051	228,73 ± 5,56 E c	297,66 ± 1,66 D a	278,32 ± 1,61 F b
NS7670	228,74 ± 3,45 E b	276,54 ± 9,21 F a	267,76 ± 9,93 G a
TEC7548	250,90 ± 5,73 D c	282,89 ± 1,86 E b	377,75 ± 3,38 B a
TMG1180	254,29 ± 0,56 D c	325,50 ± 7,34 C a	274,67 ± 7,33 F b
CD2851	263,99 ± 8,02 C c	307,08 ± 3,03 D b	453,11 ± 5,21 A a
CD2817	268,47 ± 0,57 C c	307,65 ± 4,43 D b	366,17 ± 6,77 C a
NS8383	272,12 ± 4,94 C c	287,12 ± 0,83 E b	384,34 ± 11,17 B a
CD2857	305,97 ± 6,49 B b	385,27 ± 0,94 A a	296,37 ± 3,11 E b
NS7709	341,99 ± 2,40 A b	370,46 ± 4,21 B a	353,64 ± 6,11 D b

Means followed by the same letter are not significantly different according to the Scott Knott test ($p \leq 0.05$). Uppercase letters compare varieties in each Mg source, and lowercase letters compare the Mg sources in each variety.

Source: The author himself

Table S9. Comparison of means of carotenoid concentration among soybean varieties according to Scott Knott test ($p \leq 0.05$).

	CAROTENOIDS (µg mL^{-1})		
VARIETIES	**CONTROL**	**MAGNESIUM OXIDE**	**MAGNESIUM SULFATE**
NS8338	86,90 ± 5,80 E a	84,59 ± 1,40 E a	85,14 ± 2,09 G a
CZ48B32	91,35 ± 18,77 E b	126,50 ± 1,16 B a	80,20 ± 5,08 G b
NS7505	96,75 ± 4,51 E a	78,93 ± 7,20 E b	48,35 ± 2,01 I c
SAV58	97,00 ± 2,13 E a	88,34 ± 4,26 D a	63,13 ± 4,14 H b
NS7667	98,66 ± 2,63 E b	97,19 ± 2,53 D b	114,91 ± 1,43 E a
NA7337	99,07 ± 2,14 E b	116,79 ± 8,91 B a	124,49 ± 4,01 D a
NS7670	100,75 ± 10,77 E b	119,47 ± 3,95 B a	125,61 ± 9,16 D a
TMG1180	103,80 ± 1,42 D b	124,33 ± 5,10 B a	101,38 ± 3,38 F b
BRS388	104,98 ± 10,06 D a	106,85 ± 11,88 C a	118,38 ± 1,10 E a
CD2851	105,58 ± 3,00 D c	122,05 ± 2,61 B b	172,54 ± 5,58 B a
BRS413	106,64 ± 0,95 D a	83,03 ± 0,75 E b	118,11 ± 1,20 E a
TEC7548	107,90 ± 2,97 D b	110,89 ± 3,94 B b	147,32 ± 3,34 C a
P98Y30	110,93 ± 3,30 D a	63,01 ± 6,16 F c	84,22 ± 3,81 G b
DM75I76	115,30 ± 9,25 C b	117,20 ± 14,38 B b	235,36 ± 9,86 A a
DM81I84	115,60 ± 0,81 C b	114,85 ± 2,26 B b	130,95 ± 0,97 D a
NS8383	116,29 ± 3,28 C b	99,73 ± 1,99 C c	150,95 ± 1,96 C a
SYN13671	116,64 ± 18,09 C a	117,38 ± 1,63 B a	101,85 ± 8,84 F b
AN73017	118,09 ± 4,53 C a	90,14 ± 5,63 D b	113,61 ± 7,12 E a
CD2857	120,86 ± 3,77 C b	148,06 ± 2,24 A a	105,77 ± 3,15 F c
ST797	120,98 ± 12,95 C b	154,33 ± 1,87 A a	149,75 ± 2,09 C a
NS7901	121,12 ± 4,18 C a	76,96 ± 2,65 E b	69,70 ± 2,54 G b
BMX Desafio	121,37 ± 2,46 C a	127,18 ± 2,91 B a	126,18 ± 0,62 D a
M8372	124,52 ± 1,68 C b	153,33 ± 1,18 A a	105,78 ± 5,59 F c
ANTA82	127,83 ± 3,98 C a	113,48 ± 2,30 B b	137,20 ± 8,29 D a
AN77051	129,94 ± 2,82 C a	57,68 ± 7,18 F c	99,86 ± 2,52 F b
CD2817	130,40 ± 1,68 C b	99,83 ± 0,62 C c	151,50 ± 2,52 C a
SYN15640	137,17 ± 1,68 B a	95,52 ± 2,81 D c	117,13 ± 2,77 E b
POWER	141,42 ± 1,41 B a	148,11 ± 0,84 A a	78,52 ± 5,26 G b
M7739	142,45 ± 3,86 B a	105,32 ± 9,46 C b	112,04 ± 2,25 E b
NS7709	153,79 ± 4,31 A a	139,33 ± 3,28 A b	135,99 ± 9,22 D b

Means followed by the same letter are not significantly different according to the Scott Knott test ($p \leq 0.05$). Uppercase letters compare varieties in each Mg source, and lowercase letters compare the Mg sources in each variety.

Source: The author himself

Table S10. Comparison of means of pheophytin a concentration among soybean varieties according to Scott Knott test ($p \leq 0.05$).

	PHEOPHYTIN A (µg mL^{-1})		
VARIETIES	**CONTROL**	**MAGNESIUM OXIDE**	**MAGNESIUM SULFATE**
NA7337	212,14 ± 1,27 N b	260,32 ± 3,21 K a	261,67 ± 3,35 O a
BRS388	238,25 ± 4,66 M b	257,22 ± 3,88 K a	256,36 ± 5,03 O a
DM81I84	247,85 ± 3,78 L c	287,84 ± 7,41 I a	275,47 ± 0,40 N b
ST797	252,89 ± 3,28 L c	368,46 ± 3,25 E a	358,05 ± 2,80 J b
P98Y30	254,68 ± 2,75 L b	132,33 ± 11,78 N c	271,64 ± 7,14 N a
DM75I76	261,47 ± 5,39 K b	221,98 ± 1,76 L c	369,22 ± 5,79 I a
BRS413	264,16 ± 5,51 K a	185,45 ± 3,67 M b	256,87 ± 3,10 O a
ANTA82	266,94 ± 4,65 K b	249,01 ± 1,26 K c	311,35 ± 5,95 M a
M8372	279,82 ± 6,32 J c	352,28 ± 1,02 F b	378,86 ± 5,00 H a
NS7670	285,53 ± 2,75 J c	367,77 ± 2,00 E a	346,80 ± 4,36 K b
CZ48B32	295,66 ± 2,99 I c	438,46 ± 5,45 B a	334,22 ± 10,37 L b
CD2851	301,77 ± 6,31 I c	363,11 ± 1,38 E b	516,57 ± 1,54 A a
SYN13671	311,54 ± 3,67 H a	271,81 ± 3,79 J b	229,92 ± 1,47 Q c
BMXDesafio	314,25 ± 1,35 H a	253,99 ± 0,55 K b	260,27 ± 7,37 O b
SYN15640	315,05 ± 2,43 H a	214,45 ± 1,38 L c	264,78 ± 4,66 O b
TMG1180	315,19 ± 3,35 H c	390,83 ± 6,96 D a	341,89 ± 4,51 K b
POWER	322,50 ± 2,31 G c	346,89 ± 0,76 F a	335,75 ± 2,82 L b
M7739	324,92 ± 1,67 G a	211,00 ± 2,40 L c	241,05 ± 3,60 P b
TEC7548	325,31 ± 2,44 G c	347,56 ± 2,13 F b	458,13 ± 1,10 C a
AN73017	328,35 ± 1,42 G b	414,47 ± 2,71 C a	405,58 ± 2,97 F a
NS7667	332,37 ± 10,78 G b	328,79 ± 8,18 G b	366,48 ± 3,98 I a
NS8338	348,41 ± 1,70 F a	211,40 ± 3,93 L b	220,38 ± 5,56 Q b
CD2857	381,13 ± 0,40 E b	456,00 ± 7,90 A a	344,20 ± 2,73 K c
SAV58	383,93 ± 4,65 E a	355,56 ± 8,30 E b	279,47 ± 4,78 N c
NS7505	386,55 ± 6,57 E a	343,16 ± 5,33 F b	174,81 ± 2,67 R c
NS8383	397,36 ± 9,05 D b	362,79 ± 5,75 E c	490,24 ± 0,09 B a
CD2817	412,16 ± 1,98 C a	299,09 ± 5,60 H b	421,80 ± 1,92 E a
NS7901	414,91 ± 1,40 C a	320,66 ± 3,83 G b	272,02 ± 9,86 N c
AN77051	462,40 ± 2,29 B a	251,15 ± 3,25 K c	387,85 ± 5,16 G b
NS7709	492,17 ± 1,40 A a	458,58 ± 3,48 A b	439,54 ± 4,45 D c

Means followed by the same letter are not significantly different according to the Scott Knott test ($p \leq 0.05$). Uppercase letters compare varieties in each Mg source, and lowercase letters compare the Mg sources in each variety.

Source: The author himself

Table S11. Comparison of means of pheophytin b concentration among soybean varieties according to Scott Knott test ($p \leq 0.05$).

	PHEOPHYTIN b (µg mL^{-1})		
VARIETIES	**CONTROL**	**MAGNESIUM OXIDE**	**MAGNESIUM SULFATE**
M7739	8,85 ± 0,58 I b	29,99 ± 0,34 K a	12,87 ± 0,59 I b
DM81I84	9,20 ± 0,49 I c	32,54 ± 2,28 K b	43,99 ± 3,25 G a
P98Y30	13,60 ± 1,21 I b	21,20 ± 0,36 L b	45,19 ± 3,20 G a
POWER	15,44 ± 0,83 I b	28,40 ± 0,33 K a	27,12 ± 1,12 H a
SYN13671	16,78 ± 0,81 I b	19,23 ± 0,43 L b	27,87 ± 0,36 H a
SYN15640	19,25 ± 0,61 I b	30,53 ± 0,64 K a	21,54 ± 0,52 I b
SAV58	22,12 ± 0,34 H a	26,67 ± 1,75 K a	26,46 ± 0,63 H a
BMX Desafio	22,71 ± 1,60 H b	54,73 ± 0,42 I a	50,08 ± 0,81 G a
DM75I76	22,91 ± 0,35 H a	25,96 ± 2,00 K a	23,05 ± 0,50 H a
NS 7505	24,45 ± 1,42 H b	45,38 ± 2,64 J a	30,26 ± 5,91 H b
NA7337	26,29 ± 1,98 H b	46,94 ± 3,20 J a	51,88 ± 1,39 G a
ANTA82	26,66 ± 0,64 H a	32,28 ± 0,72 K a	28,21 ± 0,50 H a
NS8338	28,25 ± 1,65 H a	12,37 ± 1,53 L b	18,88 ± 1,01 I b
ST797	32,71 ± 2,20 H c	63,49 ± 3,25 H a	50,01 ± 1,62 G b
BRS413	35,71 ± 2,14 G a	16,83 ± 1,37 L b	21,00 ± 0,39 I b
BRS388	37,41 ± 1,65 G a	31,05 ± 1,40 K a	23,81 ± 2,23 H b
AN77051	40,57 ± 0,55 G c	66,99 ± 2,70 H a	51,92 ± 0,39 G b
CZ48B32	43,38 ± 3,25 G c	108,14 ± 4,45 F a	94,09 ± 0,45 C b
NS8383	46,30 ± 1,55 G b	39,72 ± 1,26 K b	85,40 ± 1,85 D a
M8372	53,23 ± 0,29 F b	69,65 ± 2,87 H a	44,08 ± 0,80 G c
AN73017	53,42 ± 3,20 F c	131,72 ± 2,47 D a	65,23 ± 1,29 F b
NS7670	54,66 ± 2,95 F a	43,81 ± 0,36 J b	60,68 ± 0,12 F a
NS7667	64,10 ± 0,29 E b	34,75 ± 0,52 K c	84,52 ± 2,21 D a
TMG1180	75,23 ± 2,24 D b	99,09 ± 2,56 G a	92,40 ± 5,13 C a
CD2817	79,27 ± 0,60 D c	150,29 ± 1,66 C a	135,06 ± 2,59 A b
TEC7548	90,49 ± 2,82 C b	122,78 ± 1,86 E a	114,50 ± 1,65 B a
NS7901	93,73 ± 2,89 C a	35,41 ± 3,16 K b	25,24 ± 1,79 H c
CD2851	96,06 ± 1,70 C c	170,83 ± 5,19 B a	106,63 ± 2,55 B b
CD2857	123,96 ± 1,24 B b	182,61 ± 3,07 A a	72,70 ± 1,64 E c
NS7709	141,66 ± 32,26 A a	64,40 ± 0,63 H b	64,30 ± 2,01 F b

Means followed by the same letter are not significantly different according to the Scott Knott test ($p \leq 0.05$). Uppercase letters compare varieties in each Mg source, and lowercase letters compare the Mg sources in each variety.

Source: The author himself

Table S12. Comparison of means of total pheophytin concentration among soybean varieties according to Scott Knott test ($p \leq 0.05$).

	TOTAL PHEOPHYTIN (µg mL^{-1})		
VARIETIES	**CONTROL**	**MAGNESIUM OXIDE**	**MAGNESIUM SULFATE**
P98Y30	145,93 ± 12,99 M c	275,89 ± 3,11 R b	316,83 ± 9,01 L a
M7739	219,86 ± 2,98 L c	354,91 ± 1,95 M a	253,92 ± 4,19 O b
SYN15640	233,69 ± 1,95 K c	345,58 ± 1,79 N a	286,32 ± 4,13 N b
NA7337	238,43 ± 2,65 K b	307,26 ± 5,65 Q a	313,55 ± 4,74 L a
DM75I76	244,88 ± 1,79 J c	287,43 ± 4,96 R b	392,27 ± 6,27 I a
SYN13671	246,70 ± 1,49 J c	330,76 ± 4,10 O a	299,68 ± 4,12 M b
DM81I84	257,05 ± 3,66 J b	320,37 ± 8,33 P a	319,46 ± 3,30 L a
BRS388	275,66 ± 3,81 I a	288,27 ± 5,28 R a	280,17 ± 5,23 N a
ANTA82	275,67 ± 0,63 I c	299,22 ± 3,93 Q b	339,56 ± 6,45 K a
BMXDesafio	282,99 ± 8,96 I c	368,98 ± 1,38 L a	304,08 ± 0,48 M b
ST797	285,60 ± 5,07 I c	431,95 ± 2,06 I a	408,06 ± 1,45 H b
AN77051	291,72 ± 2,71 H c	529,40 ± 4,66 E a	439,77 ± 4,77 G b
BRS413	299,87 ± 5,00 H a	202,28 ± 3,58 T c	277,87 ± 3,44 N b
SAV58	301,59 ± 4,44 H c	410,60 ± 6,40 J a	382,01 ± 8,92 I b
M8372	333,05 ± 6,04 G c	448,51 ± 4,09 H a	396,35 ± 0,61 I b
POWER	337,94 ± 3,14 G b	375,29 ± 1,09 L a	362,87 ± 2,32 J a
CZ48B32	339,04 ± 1,73 G c	442,36 ± 9,24 H b	532,54 ± 5,72 C a
NS7670	340,19 ± 5,71 G b	411,58 ± 2,36 J a	407,48 ± 4,30 H a
NS8338	376,66 ± 2,78 F a	223,77 ± 4,43 S c	239,26 ± 6,57 P b
CD2817	378,36 ± 5,63 F c	572,09 ± 3,57 C a	547,22 ± 4,57 B b
AN73017	381,77 ± 2,33 F c	546,19 ± 0,35 D a	470,81 ± 2,85 E b
TMG1180	390,41 ± 5,58 E c	489,91 ± 9,52 F a	434,29 ± 0,81 G b
NS7667	396,47 ± 10,67 E b	363,54 ± 8,46 M c	451,01 ± 1,86 F a
CD2851	397,83 ± 8,00 E c	687,40 ± 6,26 A a	469,74 ± 3,93 E b
NS7505	411,01 ± 5,60 D a	388,54 ± 3,97 K b	205,07 ± 5,28 Q c
TEC7548	415,81 ± 4,97 D c	470,34 ± 1,66 G b	572,63 ± 2,75 A a
NS8383	443,66 ± 10,23 C b	402,51 ± 7,01 J c	575,64 ± 1,79 A a
CD2857	505,10 ± 1,50 B b	638,61 ± 10,97 B a	416,90 ± 3,69 H c
NS7901	508,64 ± 3,95 B a	356,08 ± 0,83 M b	297,26 ± 11,65 M c
NS7709	633,83 ± 33,66 A a	522,98 ± 2,86 E b	503,84 ± 4,40 D c

Means followed by the same letter are not significantly different according to the Scott Knott test ($p \leq 0.05$). Uppercase letters compare varieties in each Mg source, and lowercase letters compare the Mg sources in each variety.

Source: The author himself

Table S13. Fc and P values of analysis of variance (ANOVA) for sucrose and total sugars concentration in soybean varieties.

	Source of variation	Fc	Pr >Fc	
SUCROSE	Genotypes(A)	602.34000	0.00000	*
	Mg sources (B)	475.78000	0.00000	*
	A X B	30.61	0.00000	*
	Source of variation	Fc	Pr >Fc	
TOTAL SUGARS	Genotypes(A)	5744.10000	0.00000	*
	Mg sources (B)	544.60000	0.00000	*
	A X B	290.10000	0.00000	*

Sources of variation: Soybean varieties (A), Mg sources (B), and the interaction between the previously mentioned factors (A X B).

Source: The author himself

Table S14. Comparison of means of sucrose concentration among soybean varieties according to Scott Knott test ($p \leq 0.05$).

	SUCROSE (mg g^{-1} DW)		
VARIETIES	**CONTROL**	**MAGNESIUM OXIDE**	**MAGNESIUM SULFATE**
SAV58	79,04 ± 2,32 L b	96,67 ± 1,98 L a	76,55 ± 4,76 L b
BMXDesafio	79,60 ± 0,87 L b	93,86 ± 1,08 L a	96,11 ± 2,69 J a
TEC7548	81,52 ± 0,83 L a	87,67 ± 2,88 M a	81,57 ± 1,57 L a
TMG1180	82,72 ± 0,58 L a	86,42 ± 1,16 M a	88,29 ± 0,40 K a
CD2857	86,24 ± 3,09 K c	120,89 ± 1,46 I a	114,08 ± 1,49 H b
SYN13671	87,28 ± 1,54 K b	102,84 ± 2,07 K a	97,53 ± 2,53 J a
BRS388	87,55 ± 1,67 K c	112,06 ± 1,53 J b	120,83 ± 1,14 G a
ANTA82	90,82 ± 3,30 K c	101,55 ± 1,91 K b	111,87 ± 1,47 H a
NS7709	96,86 ± 5,06 J c	128,88 ± 4,38 H a	121,53 ± 1,63 G b
DM75I76	97,37 ± 2,53 J a	102,26 ± 7,55 K a	95,41 ± 4,16 J a
BRS413	102,46 ± 0,72 I b	107,16 ± 0,99 J b	113,40 ± 2,43 H a
NS7667	102,84 ± 1,46 I a	109,34 ± 0,63 J a	108,07 ± 5,88 I a
M7739	105,19 ± 2,37 H a	109,04 ± 1,59 J a	106,59 ± 0,40 I a
NS7670	106,76 ± 1,32 H b	115,43 ± 1,82 J a	115,37 ± 1,07 H a
NS7901	107,07 ± 1,53 H a	111,17 ± 1,84 J a	111,78 ± 1,85 H a
NS7505	107,31 ± 1,24 H a	113,06 ± 1,18 J a	111,54 ± 1,17 H a
POWER	114,81 ± 2,17 G b	132,32 ± 1,70 G a	130,05 ± 2,32 F a
CD2851	122,77 ± 2,74 F b	136,94 ± 2,45 G a	104,97 ± 2,03 I c
CZ48B32	126,26 ± 1,15 F b	147,97 ± 1,77 E a	107,21 ± 1,27 I c
M8372	126,50 ± 3,31 F c	141,92 ± 1,72 F b	149,06 ± 1,67 D a
AN77051	130,04 ± 1,14 E c	136,83 ± 1,13 G b	163,67 ± 3,96 C a
DM81I84	130,82 ± 3,65 E b	137,03 ± 1,22 G a	137,57 ± 0,91 E a
NS8338	132,80 ± 1,64 D b	152,68 ± 1,62 D a	158,32 ± 1,29 C a
ST797	133,70 ± 3,71 D b	157,29 ± 0,59 D a	137,19 ± 0,76 E b
P98Y30	134,43 ± 0,85 D c	153,22 ± 3,03 D b	185,09 ± 4,67 B a
NS8383	139,09 ± 2,26 C c	197,70 ± 4,50 A b	211,21 ± 6,94 A a
NA7337	140,83 ± 2,27 C b	173,62 ± 1,03 B a	142,79 ± 2,02 E b
SYN15640	163,64 ± 1,15 B b	196,53 ± 5,91 A a	187,52 ± 6,05 B b
CD2817	173,14 ± 3,38 A a	165,85 ± 1,95 C b	149,28 ± 1,42 D c
AN73017	177,15 ± 1,57 A b	194,31 ± 5,36 A a	181,62 ± 0,31 B b

Means followed by the same letter are not significantly different according to the Scott Knott test ($p \leq 0.05$). Uppercase letters compare varieties in each Mg source, and lowercase letters compare the Mg sources in each variety.

Source: The author himself

Table S15. Comparison of means of total sugars concentration among soybean varieties according to Scott Knott test ($p \leq 0.05$).

	TOTAL SUGAR (mg g^{-1} DW)		
VARIETIES	**CONTROL**	**MAGNESIUM OXIDE**	**MAGNESIUM SULFATE**
SAV58	172,00 ± 7,38 R c	269,91 ± 2,12 P a	236,26 ± 3,20 O b
NS7670	219,75 ± 7,43 Q c	295,52 ± 2,88 N a	272,56 ± 6,04 N b
NS7901	234,90 ± 5,04 P a	187,40 ± 2,88 T b	240,20 ± 1,38 O a
TEC7548	238,84 ± 0,72 P b	254,35 ± 2,28 Q a	226,78 ± 4,22 P c
TMG1180	244,28 ± 5,10 P b	255,71 ± 5,25 Q a	231,20 ± 2,10 P c
BRS413	246,06 ± 1,10 P b	284,78 ± 1,58 O a	275,99 ± 2,88 M a
CD2857	250,81 ± 5,80 O c	411,03 ± 2,33 J a	380,31 ± 2,00 I b
BMXDesafio	253,51 ± 4,76 O c	277,72 ± 3,83 O b	299,26 ± 2,04 L a
BRS388	254,53 ± 3,06 O c	294,97 ± 5,41 N b	314,52 ± 6,68 K a
NS7505	260,22 ± 1,68 N a	265,59 ± 5,96 P a	236,30 ± 2,36 O b
SYN13671	262,20 ± 3,07 N a	214,47 ± 1,10 S b	183,97 ± 1,96 Q c
M7739	265,90 ± 3,17 N a	242,09 ± 1,53 R b	264,34 ± 3,23 N a
NS7709	270,61 ± 3,78 N a	280,99 ± 4,31 O a	280,01 ± 4,16 M a
NS7667	284,29 ± 2,39 M a	206,87 ± 4,22 S b	285,81 ± 3,60 M a
M8372	290,19 ± 2,82 M c	448,03 ± 6,48 I a	380,37 ± 6,53 I b
ANTA82	297,25 ± 1,12 L c	359,75 ± 1,90 L a	332,08 ± 1,35 J b
DM75I76	318,97 ± 1,32 K b	339,71 ± 3,39 M a	280,73 ± 5,56 M c
NS8383	340,49 ± 1,66 J c	480,46 ± 3,60 H b	549,58 ± 2,36 E a
CD2851	415,06 ± 1,94 I b	386,74 ± 2,97 K c	445,35 ± 0,89 F a
CZ48B32	448,94 ± 1,78 H b	507,68 ± 7,96 F a	395,73 ± 1,07 H c
NS8338	451,22 ± 4,29 H b	492,66 ± 2,42 G a	452,00 ± 2,55 F b
AN73017	468,00 ± 7,71 G c	622,52 ± 5,48 B a	590,53 ± 6,57 D b
P98Y30	485,94 ± 3,55 F c	580,30 ± 4,73 D b	668,17 ± 6,79 A a
DM81I84	571,50 ± 6,75 E b	395,75 ± 6,01 K c	581,86 ± 2,03 D a
ST797	575,20 ± 7,43 E a	540,56 ± 4,01 E b	423,05 ± 3,26 G c
POWER	583,85 ± 0,72 D b	623,70 ± 7,34 B a	428,69 ± 5,37 G c
SYN15640	588,41 ± 2,46 D c	711,19 ± 4,11 A a	621,89 ± 3,29 B b
NA7337	605,73 ± 2,46 C a	604,14 ± 7,26 C a	554,92 ± 2,02 E b
AN77051	632,15 ± 3,34 B b	511,10 ± 5,91 F c	668,42 ± 5,78 A a
CD2817	667,89 ± 0,51 A b	706,14 ± 5,83 A a	607,90 ± 2,79 C c

Means followed by the same letter are not significantly different according to the Scott Knott test ($p \leq 0.05$). Uppercase letters compare varieties in each Mg source, and lowercase letters compare the Mg sources in each variety.

Source: The author himself

Table S16. Fc and P values of analysis of variance (ANOVA) for nitrate, ammonia, allantoin, allantoic acid, ureides, and total free amino acids concentration in soybean varieties.

	Source of variation	Fc	Pr >Fc	
NITRATE	Genotypes(A)	191.78	0.00000	*
	Mg sources (B)	1.90	0.15244	*
	A X B	20.50	0.00000	*
	Source of variation	Fc	Pr >Fc	
AMMONIA	Genotypes(A)	84.22	0.00000	*
	Mg sources (B)	0.61	0.54235	*
	A X B	10.48	0.00000	*
	Source of variation	Fc	Pr >Fc	
ALLANTOIN	Genotypes(A)	233.65	0.00000	*
	Mg sources (B)	183.62	0.00000	*
	A X B	16.86	0.00000	*
	Source of variation	Fc	Pr >Fc	
ALLANTOIC ACID	Genotypes(A)	35.83	0.00000	*
	Mg sources (B)	12.12	0.00001	*
	A X B	6.81	0.00000	*
	Source of variation	Fc	Pr >Fc	
UREIDES	Genotypes(A)	239.38	0.00000	*
	Mg sources (B)	188.78	0.00000	*
	A X B	17.13	0.00000	*
	Source of variation	Fc	Pr >Fc	
TOTAL FREE AMINO ACIDS	Genotypes(A)	312.50	0.00000	*
	Mg sources (B)	33.10	0.00000	*
	A X B	48.61	0.00000	*

Sources of variation: Soybean varieties (A), Mg sources (B), and the interaction between the previously mentioned factors (A X B).

Source: The author himself

Table S17. Comparison of means of nitrate concentration among soybean varieties according to Scott Knott test ($p \leq 0.05$).

	NITRATE (µmol g^{-1} DW)		
VARIETIES	**CONTROL**	**MAGNESIUM OXIDE**	**MAGNESIUM SULFATE**
NS7901	1,14 ± 0,07 H c	1,95 ± 0,05 G a	1,35 0,07 H b
NS8383	1,29 ± 0,04 H b	1,69 ± 0,02 H a	1,77 0,06 G a
TEC7548	1,69 ± 0,04 G a	1,88 ± 0,03 G a	1,42 0,06 H b
TMG1180	1,87 ± 0,07 G a	1,49 ± 0,03 I b	1,48 0,05 H b
BMXDesafio	2,04 ± 0,04 F a	1,90 ± 0,11 G a	1,96 0,07 F a
ANTA82	2,11 ± 0,02 F b	2,21 ± 0,03 E b	3,17 0,10 B a
NS7505	2,13 ± 0,07 F b	2,40 ± 0,15 E a	2,28 0,04 E a
SYN13671	2,13 ± 0,04 F a	1,79 ± 0,05 G b	2,15 0,03 F a
BRS388	2,23 ± 0,12 F a	2,34 ± 0,05 E a	2,11 0,05 F a
AN77051	2,23 ± 0,16 F b	2,30 ± 0,05 E b	3,21 0,09 B a
NA7337	2,23 ± 0,06 F a	2,02 ± 0,10 F b	1,86 0,08 G b
NS7667	2,24 ± 0,10 F a	2,07 ± 0,00 F b	2,01 0,06 F b
CD2857	2,30 ± 0,15 F b	2,58 ± 0,04 D a	2,41 0,04 E b
NS7709	2,43 ± 0,15 E a	2,37 ± 0,13 E a	2,43 0,14 E a
NS7670	2,44 ± 0,05 E a	2,28 ± 0,07 E a	2,34 0,05 E a
P98Y30	2,48 ± 0,06 E b	2,60 ± 0,05 D b	3,10 0,11 B a
DM75I76	2,49 ± 0,01 E a	2,10 ± 0,09 F b	2,06 0,09 F b
AN73017	2,55 ± 0,10 E a	2,65 ± 0,14 D a	2,69 0,05 D a
ST797	2,58 ± 0,05 E a	2,07 ± 0,05 F b	1,81 0,05 G c
SAV58	2,70 ± 0,08 D b	2,96 ± 0,07 C a	2,95 0,14 C a
CZ48B32	2,73 ± 0,08 D b	2,99 ± 0,03 C a	2,85 0,12 D b
CD2851	2,85 ± 0,10 D a	2,53 ± 0,06 D b	2,88 0,10 D a
BRS413	2,87 ± 0,09 D a	2,91 ± 0,02 C a	2,82 0,07 D a
CD2817	2,89 ± 0,22 D a	2,99 ± 0,13 C a	2,77 0,03 D a
POWER	2,93 ± 0,08 C a	2,16 ± 0,09 F b	1,76 0,06 G c
M8372	2,97 ± 0,14 C b	3,31 ± 0,17 B a	2,99 0,12 C b
M7739	3,08 ± 0,20 C a	2,92 ± 0,04 C a	3,06 0,09 B a
NS8338	3,12 ± 0,09 C b	3,63 ± 0,04 A a	3,13 0,03 B b
SYN15640	3,61 ± 0,08 B a	3,70 ± 0,30 A a	2,96 0,18 C b
DM81I84	3,99 ± 0,18 A a	2,68 ± 0,12 D c	3,70 0,07 A b

Means followed by the same letter are not significantly different according to the Scott Knott test ($p \leq 0.05$). Uppercase letters compare varieties in each Mg source, and lowercase letters compare the Mg sources in each variety.

Source: The author himself

Table S18. Comparison of means of ammonia concentration among soybean varieties according to Scott Knott test (p≤0.05).

	AMMONIA (µmol g^{-1} DW)		
VARIETIES	**CONTROL**	**MAGNESIUM OXIDE**	**MAGNESIUM SULFATE**
TEC7548	0,85 ± 0,05 G c	1,03 ± 0,05 G b	1,39 ± 0,14 D a
CD2857	0,98 ± 0,00 G b	1,22 ± 0,07 F a	1,21 ± 0,05 E a
P98Y30	1,14 ± 0,05 F b	1,29 ± 0,05 E a	1,41 ± 0,07 D a
TMG1180	1,15 ± 0,05 F b	1,34 ± 0,04 E a	1,34 ± 0,00 D a
NA7337	1,18 ± 0,07 F c	1,61 ± 0,05 C a	1,43 ± 0,05 D b
ST797	1,19 ± 0,05 F b	1,47 ± 0,07 D a	1,45 ± 0,07 D a
M8372	1,25 ± 0,00 F b	1,53 ± 0,04 D a	1,43 ± 0,05 D a
NS7709	1,25 ± 0,07 F b	1,31 ± 0,00 E b	1,44 ± 0,00 D a
BRS388	1,32 ± 0,05 E a	1,36 ± 0,00 E a	1,11 ± 0,05 E b
NS7670	1,35 ± 0,05 E a	1,38 ± 0,00 E a	1,41 ± 0,05 D a
POWER	1,35 ± 0,05 E b	1,70 ± 0,07 C a	1,68 ± 0,07 C a
CD2851	1,36 ± 0,05 E a	1,07 ± 0,05 G b	0,93 ± 0,05 F c
BMXDesafio	1,38 ± 0,11 E b	1,65 ± 0,05 C a	1,52 ± 0,07 D a
AN77051	1,41 ± 0,10 E b	1,42 ± 0,07 E b	1,73 ± 0,08 C a
SYN13671	1,47 ± 0,07 D a	1,06 ± 0,07 G b	1,03 ± 0,05 F b
NS7667	1,49 ± 0,00 D b	1,68 ± 0,07 C a	1,38 ± 0,00 D b
SAV58	1,52 ± 0,05 D a	1,40 ± 0,05 E a	1,35 ± 0,05 D a
NS7505	1,54 ± 0,05 D a	1,64 ± 0,05 C a	1,58 ± 0,00 C a
M7739	1,63 ± 0,08 C a	1,54 ± 0,09 D a	1,47 ± 0,05 D a
BRS413	1,65 ± 0,05 C a	1,52 ± 0,00 D a	1,50 ± 0,00 D a
CD2817	1,68 ± 0,05 C a	1,51 ± 0,00 D a	1,63 ± 0,10 C a
NS8338	1,71 ± 0,08 C a	1,74 ± 0,00 B a	1,56 ± 0,00 C b
ANTA82	1,71 ± 0,07 C a	1,29 ± 0,10 E b	1,58 ± 0,12 C a
CZ48B32	1,74 ± 0,12 C b	2,04 ± 0,12 A a	1,87 ± 0,04 B b
DM75I76	1,79 ± 0,00 C a	1,80 ± 0,05 B a	1,70 ± 0,05 C a
NS8383	1,88 ± 0,17 B a	1,91 ± 0,05 A a	1,95 ± 0,10 B a
SYN15640	1,89 ± 0,12 B a	1,96 ± 0,08 A a	1,65 ± 0,05 C b
NS7901	1,90 ± 0,05 B a	1,54 ± 0,05 D c	1,69 ± 0,05 C b
AN73017	2,03 ± 0,05 B a	2,02 ± 0,05 A a	1,87 ± 0,05 B b
DM81I84	2,59 ± 0,05 A a	1,82 ± 0,06 B b	2,29 ± 0,05 A a

Means followed by the same letter are not significantly different according to the Scott Knott test (p≤0.05). Uppercase letters compare varieties in each Mg source, and lowercase letters compare the Mg sources in each variety.

Source: The author himself

Table S19. Comparison of means of allantoin concentration among soybean varieties according to Scott Knott test ($p \leq 0.05$).

	ALLANTOIN (mmol g^{-1} DW)		
VARIETIES	**CONTROL**	**MAGNESIUM OXIDE**	**MAGNESIUM SULFATE**
TMG1180	12,21 ± 0,77 H b	15,68 ± 0,15 J a	13,96 ± 0,59 I b
NS7709	12,81 ± 0,31 H b	17,31 ± 0,70 J a	15,13 ± 0,71 I a
AN77051	13,38 ± 0,37 H c	16,79 ± 0,88 J b	21,60 ± 0,49 G a
NS8338	13,45 ± 0,52 H c	22,90 ± 0,37 H b	25,84 ± 0,48 F a
NA7337	14,06 ± 0,32 H a	14,59 ± 0,70 J a	13,90 ± 0,93 I a
ST797	16,55 ± 0,53 G a	19,05 ± 0,17 I a	18,98 ± 0,73 H a
AN73017	17,42 ± 0,31 G a	19,15 ± 0,58 I a	13,85 ± 0,19 I b
M8372	17,73 ± 0,39 G c	27,23 ± 1,93 G b	30,98 ± 0,82 E a
NS7505	19,14 ± 0,83 F c	22,89 ± 0,54 H b	26,52 ± 0,97 F a
POWER	19,37 ± 1,08 F a	20,86 ± 0,44 I a	22,21 ± 0,56 G a
SAV58	21,10 ± 0,65 F b	26,35 ± 0,92 G a	23,03 ± 0,66 G b
BRS413	21,66 ± 0,37 E b	24,16 ± 0,67 G a	28,37 ± 0,90 F a
M7739	22,86 ± 0,69 E a	20,83 ± 0,44 I a	21,32 ± 0,61 G a
CD2851	23,43 ± 0,21 E b	27,03 ± 1,66 G a	20,00 ± 0,26 H c
CD2857	23,77 ± 0,15 E b	26,27 ± 0,32 G a	21,53 ± 0,64 G b
NS8383	24,48 ± 0,09 D b	25,99 ± 0,82 G b	31,03 ± 1,33 E a
TEC7548	25,26 ± 0,11 D b	29,19 ± 0,84 F a	18,13 ± 0,35 H c
DM81I84	27,88 ± 0,42 C b	31,09 ± 1,38 F a	30,74 ± 0,91 E a
ANTA82	28,33 ± 0,92 C b	30,79 ± 1,36 F a	40,18 ± 4,48 C a
NS7670	28,98 ± 1,15 C b	29,86 ± 0,67 F b	32,85 ± 1,87 E a
DM75I76	29,37 ± 0,59 C a	31,13 ± 1,86 F a	26,73 ± 1,04 F b
NS7667	29,70 ± 0,66 C c	41,47 ± 0,14 C a	34,29 ± 1,62 D b
SYN13671	29,73 ± 1,11 C c	51,68 ± 2,65 A a	39,07 ± 2,52 C b
P98Y30	30,22 ± 0,37 C c	33,03 ± 1,82 E b	36,04 ± 0,36 D a
CZ48B32	31,17 ± 1,68 C b	37,05 ± 0,25 D a	30,77 ± 1,01 E b
BRS388	32,12 ± 3,14 B c	40,26 ± 0,14 C b	49,21 ± 3,34 A a
BMXDesafio	33,23 ± 0,25 B c	37,91 ± 1,41 D b	43,53 ± 0,89 B a
SYN15640	33,54 ± 0,96 B b	39,85 ± 0,89 C a	38,25 ± 0,79 C a
CD2817	35,07 ± 1,50 B a	31,01 ± 2,03 F b	27,05 ± 1,33 F c
NS7901	39,69 ± 0,15 A b	43,99 ± 1,98 B a	41,32 ± 0,73 B b

Means followed by the same letter are not significantly different according to the Scott Knott test ($p \leq 0.05$). Uppercase letters compare varieties in each Mg source, and lowercase letters compare the Mg sources in each variety.

Source: The author himself

Table S20. Comparison of means of allantoic acid concentration among soybean varieties according to Scott Knott test ($p \leq 0.05$).

	ALLANTOIC ACID (mmol g^{-1} DW)		
VARIETIES	**CONTROL**	**MAGNESIUM OXIDE**	**MAGNESIUM SULFATE**
AN77051	0,55 ± 0,06 D b	0,76 ± 0,08 D b	1,40 ± 0,19 C a
NA7337	0,57 ± 0,04 D a	0,58 ± 0,10 D a	0,59 ± 0,13 E a
NS8383	0,79 ± 0,05 D a	0,81 ± 0,07 D a	1,12 ± 0,03 D a
ST797	0,81 ± 0,09 D a	0,73 ± 0,28 D a	0,59 ± 0,09 E a
ANTA82	0,86 ± 0,09 D b	1,98 ± 0,22 A a	2,26 ± 0,30 A a
M8372	0,93 ± 0,08 C a	1,07 ± 0,17 C a	0,97 ± 0,11 D a
NS7709	0,98 ± 0,05 C b	1,12 ± 0,01 C b	1,54 ± 0,10 B a
CD2817	0,98 ± 0,14 C a	1,14 ± 0,15 C a	1,17 ± 0,14 D a
DM75I76	1,01 ± 0,08 C a	1,13 ± 0,05 C a	1,22 ± 0,11 D a
POWER	1,04 ± 0,15 C a	0,51 ± 0,15 D b	0,54 ± 0,05 E b
CD2851	1,08 ± 0,02 C a	1,05 ± 0,04 C a	1,08 ± 0,04 D a
TEC7548	1,09 ± 0,10 C a	1,32 ± 0,05 B a	0,85 ± 0,04 E b
NS8338	1,12 ± 0,04 C b	1,44 ± 0,19 B a	1,04 ± 0,06 D b
CZ48B32	1,13 ± 0,17 C a	1,15 ± 0,12 C a	1,42 ± 0,09 C a
TMG1180	1,15 ± 0,22 C b	1,49 ± 0,05 B a	0,62 ± 0,11 E c
AN73017	1,15 ± 0,02 C a	0,93 ± 0,05 C a	1,11 ± 0,20 D a
NS7901	1,21 ± 0,03 C b	1,72 ± 0,04 B a	1,76 ± 0,10 B a
SAV58	1,22 ± 0,05 C b	1,56 ± 0,18 B a	0,95 ± 0,14 D b
SYN13671	1,28 ± 0,04 C b	1,63 ± 0,02 B a	1,82 ± 0,22 B a
NS7667	1,29 ± 0,08 C a	1,50 ± 0,09 B a	1,12 ± 0,02 D a
NS7505	1,34 ± 0,12 B a	1,57 ± 0,13 B a	1,65 ± 0,12 B a
CD2857	1,38 ± 0,10 B b	1,63 ± 0,19 B a	1,15 ± 0,01 D b
SYN15640	1,51 ± 0,08 B a	1,57 ± 0,18 B a	1,46 ± 0,05 C a
BRS388	1,54 ± 0,17 B b	2,11 ± 0,33 A a	2,11 ± 0,40 A a
NS7670	1,55 ± 0,05 B a	1,58 ± 0,03 B a	1,80 ± 0,09 B a
DM81I84	1,63 ± 0,09 B a	0,94 ± 0,04 C b	1,07 ± 0,07 D b
M7739	1,66 ± 0,08 B a	1,82 ± 0,18 A a	1,94 ± 0,27 B a
BRS413	1,69 ± 0,03 B b	2,20 ± 0,09 A a	2,36 ± 0,18 A a
P98Y30	1,82 ± 0,21 B a	1,72 ± 0,25 B a	0,94 ± 0,11 D b
BMXDesafio	2,21 ± 0,18 A b	1,49 ± 0,10 B c	2,57 ± 0,44 A a

Means followed by the same letter are not significantly different according to the Scott Knott test ($p \leq 0.05$). Uppercase letters compare varieties in each Mg source, and lowercase letters compare the Mg sources in each variety.

Source: The author himself

Table S21. Comparison of means of ureides concentration among soybean varieties according to Scott Knott test ($p \leq 0.05$).

	UREIDES (mmol g^{-1} DW)		
VARIETIES	**CONTROL**	**MAGNESIUM OXIDE**	**MAGNESIUM SULFATE**
TMG1180	13,35 ± 0,68 H b	17,17 ± 0,19 K a	14,58 ± 0,63 J b
NS7709	13,78 ± 0,33 H b	18,44 ± 0,69 J a	16,67 ± 0,63 J a
AN77051	13,93 ± 0,34 H c	17,56 ± 0,95 K a	23,00 ± 0,65 H b
NS8338	14,57 ± 0,48 H b	24,35 ± 0,22 H a	26,88 ± 0,44 G a
NA7337	14,63 ± 0,28 H a	15,17 ± 0,60 K a	14,49 ± 0,85 J a
ST797	17,35 ± 0,62 G a	19,78 ± 0,28 J a	19,56 ± 0,75 I a
AN73017	18,57 ± 0,31 G a	20,08 ± 0,63 J a	14,95 ± 0,38 J b
M8372	18,66 ± 0,31 G c	28,30 ± 1,87 G b	31,95 ± 0,71 F a
POWER	20,41 ± 0,96 F a	21,37 ± 0,46 I a	22,74 ± 0,61 H a
NS7505	20,47 ± 0,81 F c	24,46 ± 0,67 H b	28,17 ± 1,09 G a
SAV58	22,32 ± 0,60 F b	27,91 ± 0,77 G a	23,98 ± 0,80 H b
BRS413	23,35 ± 0,34 E c	26,36 ± 0,60 G b	30,72 ± 0,94 F a
CD2851	24,51 ± 0,19 E b	28,09 ± 1,70 G a	21,08 ± 0,23 H c
M7739	24,52 ± 0,76 E a	22,65 ± 0,59 I a	23,27 ± 0,42 H a
CD2857	25,15 ± 0,26 E b	27,90 ± 0,49 G a	22,69 ± 0,64 H b
NS8383	25,27 ± 0,13 E b	26,80 ± 0,81 G b	32,15 ± 1,32 F a
TEC7548	26,35 ± 0,17 D b	30,51 ± 0,86 F a	18,98 ± 0,34 I c
ANTA82	29,19 ± 1,02 C c	32,77 ± 1,14 F b	42,44 ± 4,71 C a
DM81I84	29,51 ± 0,47 C a	32,03 ± 1,34 F a	31,81 ± 0,94 F a
DM75I76	30,38 ± 0,54 C a	32,26 ± 1,81 F a	27,95 ± 1,04 G b
NS7670	30,52 ± 1,11 C b	31,45 ± 0,69 F b	34,64 ± 1,88 E a
NS7667	30,99 ± 0,74 C c	42,97 ± 0,09 C a	35,42 ± 1,60 E b
SYN13671	31,01 ± 1,12 C c	53,31 ± 2,67 A a	40,90 ± 2,30 D b
P98Y30	32,04 ± 0,52 C b	34,76 ± 1,89 E a	36,99 ± 0,32 E a
CZ48B32	32,30 ± 1,51 C b	38,19 ± 0,37 D a	32,19 ± 0,98 F b
BRS388	33,66 ± 3,25 B c	42,37 ± 0,37 C b	51,32 ± 3,74 A a
SYN15640	35,05 ± 1,05 B b	41,42 ± 0,82 C a	39,71 ± 0,84 D a
BMXDesafio	35,44 ± 0,43 B c	39,39 ± 1,30 D b	46,09 ± 0,44 B a
CD2817	36,06 ± 1,57 B a	32,15 ± 2,14 F b	28,22 ± 1,47 G c
NS7901	40,90 ± 0,18 A b	45,72 ± 2,03 B a	43,07 ± 0,78 C b

Means followed by the same letter are not significantly different according to the Scott Knott test ($p \leq 0.05$). Uppercase letters compare varieties in each Mg source, and lowercase letters compare the Mg sources in each variety.

Source: The author himself

Table S22. Comparison of means of total free amino acids concentration among soybean varieties according to Scott Knott test ($p \leq 0.05$).

	TOTAL FREE AMINO ACIDS (µmol g^{-1} DW)		
VARIETIES	**CONTROL**	**MAGNESIUM OXIDE**	**MAGNESIUM SULFATE**
POWER	10,12 ± 1,33 L b	33,11 ± 4,78 I a	39,45 ± 1,41 I a
SYN15640	23,36 ± 1,58 K b	39,88 ± 1,77 H a	36,88 ± 3,57 I a
CZ48B32	25,81 ± 1,11 K b	64,64 ± 3,07 F a	63,24 ± 3,87 F a
DM81I84	32,54 ± 3,84 J b	41,14 ± 2,15 H a	21,53 ± 1,18 J c
TMG1180	33,18 ± 2,31 J b	62,71 ± 3,36 F a	67,09 ± 2,87 F a
BMXDesafio	37,97 ± 1,81 I b	41,57 ± 1,30 H b	50,52 ± 1,37 H a
P98Y30	39,65 ± 2,27 I a	23,89 ± 2,60 J b	17,95 ± 3,08 J b
NS8338	41,34 ± 2,56 I c	50,64 ± 4,82 G b	87,14 ± 3,16 D a
ANTA82	42,59 ± 7,34 I c	58,33 ± 1,99 G b	74,26 ± 2,72 E a
SAV58	43,57 ± 1,18 I c	53,57 ± 0,74 G b	112,44 ± 2,33 B a
NA7337	47,74 ± 4,10 H a	53,99 ± 6,99 G a	21,69 ± 2,18 J b
ST797	48,44 ± 5,15 H b	86,82 ± 4,52 E a	83,58 ± 2,62 D a
NS7505	49,77 ± 3,02 H a	40,18 ± 2,00 H b	56,36 ± 2,60 G a
CD2817	51,22 ± 5,84 H c	70,90 ± 4,77 F b	85,04 ± 6,52 D a
NS7667	56,10 ± 5,76 G a	50,16 ± 1,66 G a	48,92 ± 3,21 H a
DM75I76	58,44 ± 3,12 G a	42,42 ± 1,72 H b	46,52 ± 3,70 H b
NS8383	60,59 ± 3,28 G b	83,49 ± 2,65 E a	56,10 ± 2,23 G b
TEC7548	62,02 ± 2,95 G b	43,62 ± 3,64 H c	113,88 ± 0,99 B a
NS7670	66,53 ± 3,63 F a	65,75 ± 3,72 F a	49,41 ± 3,96 H b
NS7709	69,43 ± 0,87 F b	61,31 ± 1,49 F b	82,00 ± 3,03 D a
SYN13671	71,68 ± 1,61 F b	81,44 ± 1,79 E a	70,77 ± 5,98 E b
AN77051	72,22 ± 0,00 F a	39,99 ± 1,66 H b	13,13 ± 1,39 J c
M8372	72,89 ± 1,82 F b	91,26 ± 6,47 D a	78,27 ± 8,05 E b
AN73017	76,51 ± 1,96 F a	49,92 ± 0,80 G b	84,60 ± 3,01 D a
BRS413	86,35 ± 5,73 E b	97,31 ± 4,09 D a	77,51 ± 0,94 E c
CD2851	98,07 ± 7,00 D c	107,55 ± 9,41 C b	137,77 ± 5,22 A a
BRS388	114,05 ± 2,29 C a	118,54 ± 1,79 B a	37,87 ± 2,63 I b
CD2857	129,88 ± 3,77 B a	113,54 ± 1,77 C b	103,68 ± 2,62 C c
M7739	134,78 ± 3,77 B a	126,46 ± 1,03 B b	105,82 ± 3,51 C c
NS7901	150,44 ± 6,25 A b	185,14 ± 0,69 A a	119,55 ± 3,33 B c

Means followed by the same letter are not significantly different according to the Scott Knott test ($p \leq 0.05$). Uppercase letters compare varieties in each Mg source, and lowercase letters compare the Mg sources in each variety.

Source: The author himself

Table S23. Fc and P values of analysis of variance (ANOVA) for plant height, first pod height, grain mass, and grain yield in soybean varieties.

	Source of variation	Fc	Pr >Fc	
PLANT HEIGHT	Genotypes(A)	55.68	0.00000	*
	Mg sources (B)	5.65	0.00418	*
	A X B	1.03	0.43046	NS
	Source of variation	Fc	Pr >Fc	
FIRST POD HEIGHT	Genotypes(A)	33.42	0.00000	*
	Mg sources (B)	0.57	0.56645	*
	A X B	0.76	0.89285	NS
	Source of variation	Fc	Pr >Fc	
GRAIN MASS	Genotypes(A)	53.48	0.00000	*
	Mg sources (B)	10.58	0.00005	*
	A X B	1.73	0.00351	*
	Source of variation	Fc	Pr >Fc	
GRAIN YIELD	Genotypes(A)	163.30	0.00000	*
	Mg sources (B)	97.20	0.00000	*
	A X B	3.63	0.00000	*

Sources of variation: Soybean varieties (A), Mg sources (B), and the interaction between the previously mentioned factors (A X B).

Source: The author himself

Table S24. Comparison of means of grain mass among soybean varieties according to Scott Knott test ($p \leq 0.05$).

	GRAIN MASS (g)														
VARIETIES	**CONTROL**					**MAGNESIUM OXIDE**					**MAGNESIUM SULFATE**				
BRS413	11,73	±	0,69	G	a	12,53	±	0,18	E	a	12,80	±	0,20	E	a
NS8383	12,03	±	0,42	G	a	12,63	±	0,16	E	a	13,27	±	0,62	E	a
POWER	12,47	±	0,36	G	b	13,60	±	0,20	E	a	14,23	±	0,42	E	a
ANTA82	13,30	±	0,73	F	a	13,77	±	0,51	E	a	12,90	±	0,73	E	a
NS7505	13,43	±	0,44	F	a	14,27	±	0,29	D	a	14,23	±	0,42	E	a
AN77051	13,70	±	0,13	F	a	14,17	±	0,29	D	a	14,00	±	0,93	E	a
TMG1180	13,73	±	0,36	F	a	15,33	±	0,38	C	a	14,00	±	0,27	E	a
AN73017	13,77	±	0,16	F	a	13,57	±	0,44	E	a	14,07	±	0,71	E	a
NA7337	13,77	±	0,51	F	a	14,07	±	0,31	D	a	13,40	±	0,13	E	a
P98Y30	13,83	±	0,11	F	a	14,13	±	0,62	D	a	14,73	±	0,64	D	a
ST797	13,93	±	0,51	F	a	14,10	±	0,20	D	a	13,60	±	0,13	E	a
BRS388	13,97	±	0,62	F	a	14,87	±	0,38	D	a	14,03	±	0,51	E	a
NS7901	14,13	±	0,16	F	b	16,03	±	0,42	D	a	14,97	±	0,29	D	b
M7739	14,23	±	0,18	F	a	14,17	±	0,24	D	a	13,87	±	0,31	E	a
NS7670	14,27	±	0,42	F	b	16,23	±	0,51	D	a	14,70	±	0,73	D	b
SYN15640	14,30	±	0,47	F	a	14,97	±	0,58	D	a	16,00	±	0,13	D	a
NS7667	14,90	±	0,20	E	a	16,70	±	0,47	C	a	15,17	±	0,84	D	a
DM75I76	15,10	±	0,67	E	a	16,57	±	0,56	C	a	15,73	±	0,49	D	a
SYN13671	15,23	±	0,91	E	a	15,87	±	0,49	C	a	14,97	±	1,18	D	a
DM81I84	15,33	±	0,38	E	a	16,27	±	0,42	C	a	15,40	±	0,40	D	a
BMXDesafio	15,70	±	0,07	E	a	14,80	±	0,80	C	a	15,57	±	0,11	D	a
SAV58	16,23	±	0,71	D	a	14,80	±	1,33	C	b	14,43	±	1,24	E	b
NS7709	16,57	±	1,56	D	a	15,93	±	2,38	C	a	13,40	±	0,80	E	b
CD2817	16,87	±	0,44	D	a	16,93	±	0,38	C	a	16,47	±	0,71	C	a
CZ48B32	17,00	±	0,13	D	a	16,87	±	0,24	C	a	17,70	±	0,53	B	a
CD2851	17,47	±	0,36	C	a	17,37	±	0,22	C	a	16,43	±	1,29	C	a
M8372	18,10	±	0,47	C	a	18,80	±	0,33	B	a	17,63	±	0,42	B	a
NS8338	18,23	±	0,36	C	a	17,63	±	0,82	C	a	17,50	±	0,67	B	a
CD2857	19,50	±	0,13	B	a	19,47	±	0,49	B	a	18,83	±	0,56	B	a
TEC7548	21,67	±	0,31	A	b	24,17	±	0,78	A	a	21,80	±	0,13	A	b

Means followed by the same letter are not significantly different according to the Scott Knott test ($p \leq 0.05$). Uppercase letters compare varieties in each Mg source, and lowercase letters compare the Mg sources in each variety.

Source: The author himself

Table S25. Comparison of means of grain mass among soybean varieties according to Scott Knott test ($p \leq 0.05$).

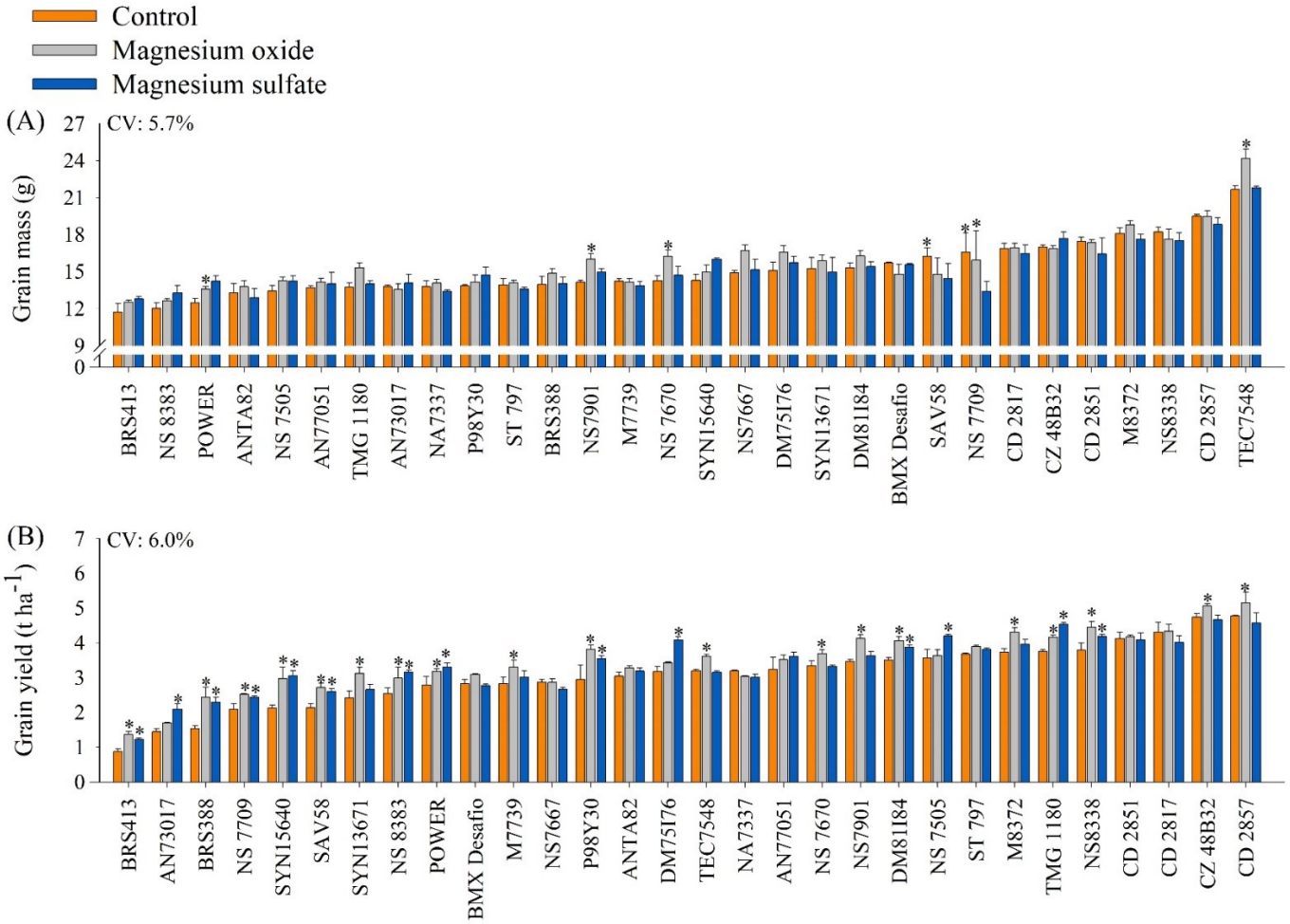
	GRAIN YIELD (t ha^{-1})		
VARIETIES	**CONTROL**	**MAGNESIUM OXIDE**	**MAGNESIUM SULFATE**
BRS413	0,87 ± 0,09 J b	1,36 ± 0,09 H a	1,22 ± 0,04 H a
AN73017	1,44 ± 0,10 I b	1,70 ± 0,01 G b	2,09 ± 0,16 G a
BRS388	1,54 ± 0,07 I b	2,43 ± 0,29 F a	2,30 ± 0,14 G a
NS7709	2,08 ± 0,16 H b	2,53 ± 0,02 F a	2,44 ± 0,05 G a
SYN15640	2,12 ± 0,09 H b	2,98 ± 0,33 D a	3,05 ± 0,16 E a
SAV58	2,14 ± 0,11 H b	2,72 ± 0,13 E a	2,60 ± 0,09 F a
SYN13671	2,42 ± 0,19 G b	3,12 ± 0,17 D a	2,65 ± 0,16 F b
NS8383	2,54 ± 0,17 G b	2,99 ± 0,31 D a	3,16 ± 0,05 E a
POWER	2,79 ± 0,24 F b	3,18 ± 0,08 D a	3,30 ± 0,13 E a
BMXDesafio	2,83 ± 0,13 F a	3,09 ± 0,03 D a	2,77 ± 0,04 F a
M7739	2,83 ± 0,18 F b	3,30 ± 0,20 D a	3,01 ± 0,18 E b
NS7667	2,87 ± 0,07 F a	2,86 ± 0,11 E a	2,67 ± 0,05 F a
P98Y30	2,94 ± 0,41 F b	3,82 ± 0,12 C a	3,55 ± 0,07 D a
ANTA82	3,04 ± 0,12 E a	3,28 ± 0,06 D a	3,19 ± 0,09 E a
DM75I76	3,17 ± 0,15 E b	3,43 ± 0,02 D b	4,08 ± 0,10 C a
TEC7548	3,19 ± 0,04 E b	3,61 ± 0,05 C a	3,15 ± 0,04 E b
NA7337	3,19 ± 0,02 E a	3,03 ± 0,02 D a	3,01 ± 0,09 E a
AN77051	3,23 ± 0,35 E a	3,52 ± 0,13 C a	3,62 ± 0,12 D a
NS7670	3,34 ± 0,15 D b	3,70 ± 0,11 C a	3,32 ± 0,04 E b
NS7901	3,47 ± 0,06 D b	4,13 ± 0,11 B a	3,63 ± 0,11 D b
DM81I84	3,50 ± 0,07 D b	4,06 ± 0,12 B a	3,87 ± 0,06 C a
NS7505	3,57 ± 0,24 D b	3,63 ± 0,17 C b	4,20 ± 0,05 B a
ST797	3,68 ± 0,04 C a	3,90 ± 0,03 C a	3,81 ± 0,04 D a
M8372	3,73 ± 0,11 C b	4,31 ± 0,13 B a	3,96 ± 0,13 C b
TMG1180	3,75 ± 0,05 C c	4,16 ± 0,06 B b	4,54 ± 0,05 A a
NS8338	3,79 ± 0,22 C b	4,45 ± 0,17 B a	4,19 ± 0,05 B a
CD2851	4,13 ± 0,18 B a	4,18 ± 0,05 B a	4,09 ± 0,20 C a
CD2817	4,31 ± 0,29 B a	4,34 ± 0,20 B a	4,02 ± 0,19 C a
CZ48B32	4,75 ± 0,10 A b	5,06 ± 0,08 A a	4,67 ± 0,13 A b
CD2857	4,78 ± 0,01 A b	5,15 ± 0,30 A a	4,57 ± 0,29 A b

Means followed by the same letter are not significantly different according to the Scott Knott test ($p \leq 0.05$). Uppercase letters compare varieties in each Mg source, and lowercase letters compare the Mg sources in each variety.

Source: The author himself

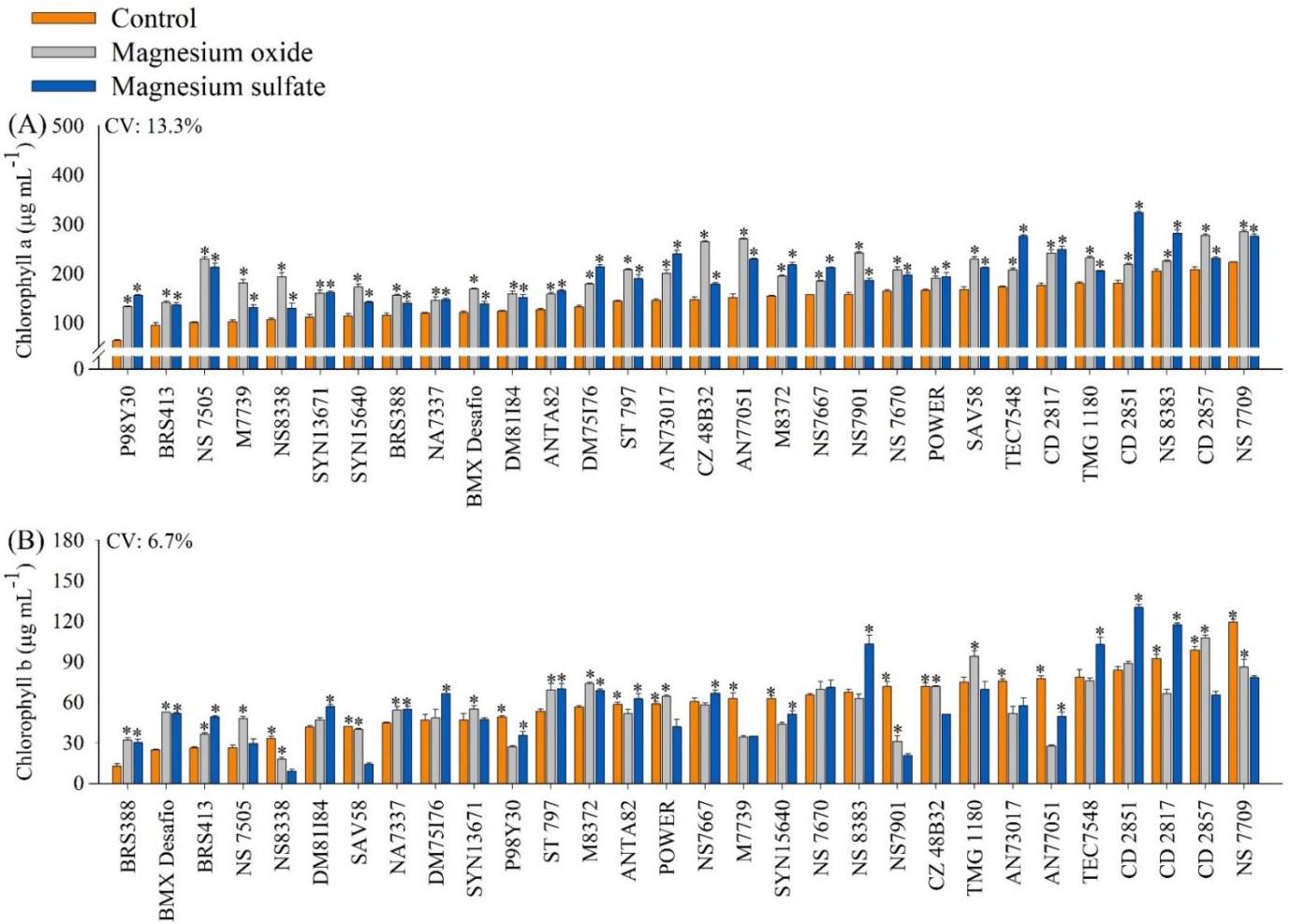
APPENDAGE B

Figure S9. Effects of Mg rates on Grain mass (A) and Grain yield (B) in *Glycine max* varieties. CV means coefficient of variation. "*" compares each variety under Mg rates, indicating the best rate compared to the others according to the Scott Knott test ($p \leq 0.05$). Bars indicate the standard error of the means (n=3).



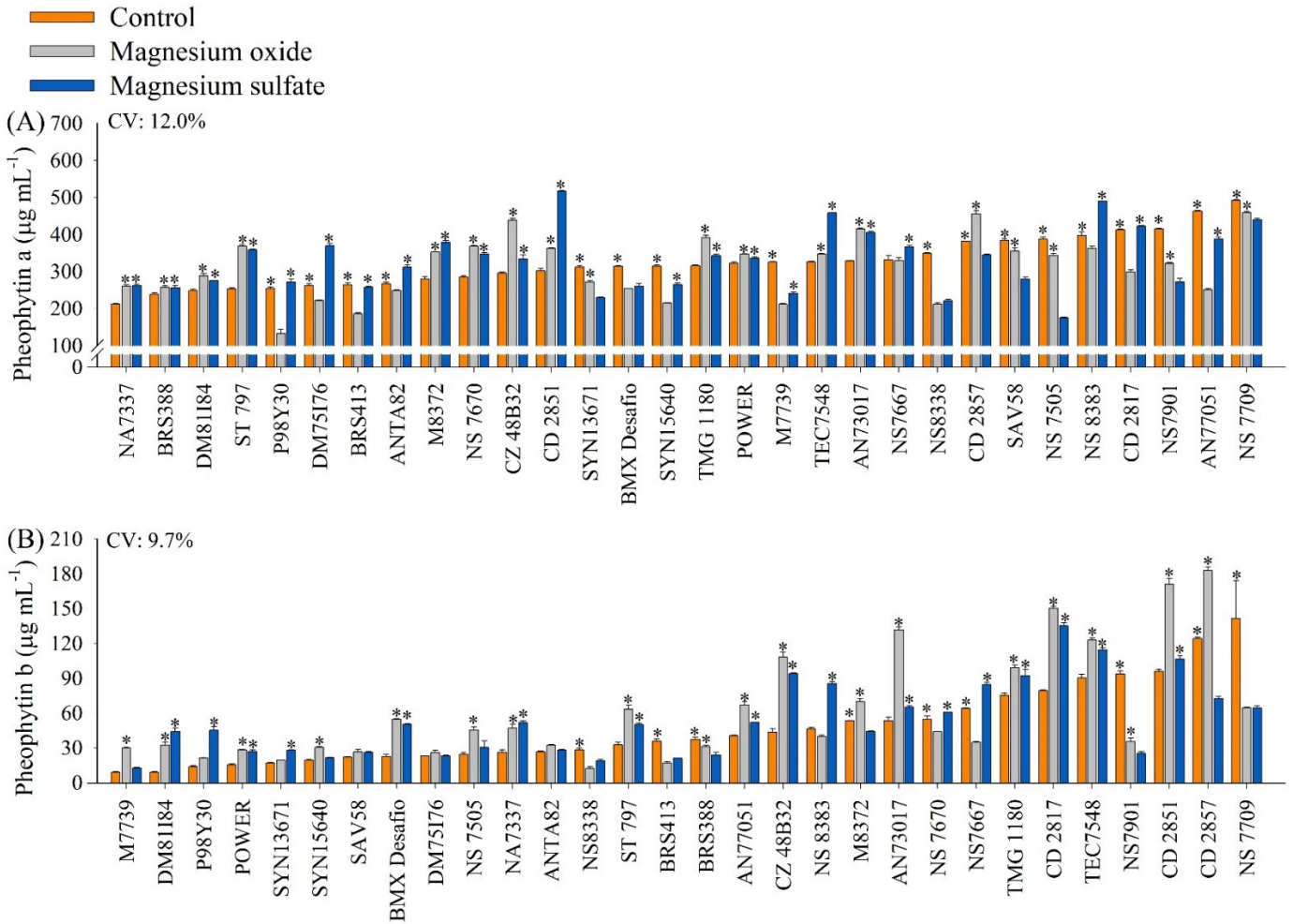
Source: The author himself

Figure S10. Effects of Mg rates on chlorophyll an (A) and chlorophyll b (B) in *Glycine max* varieties (January 9, 2018). CV means coefficient of variation. "*" compares each variety under Mg rates, indicating the best rate compared to the others according to the Scott Knott test ($p \leq 0.05$). Bars indicate the standard error of the means (n=3).



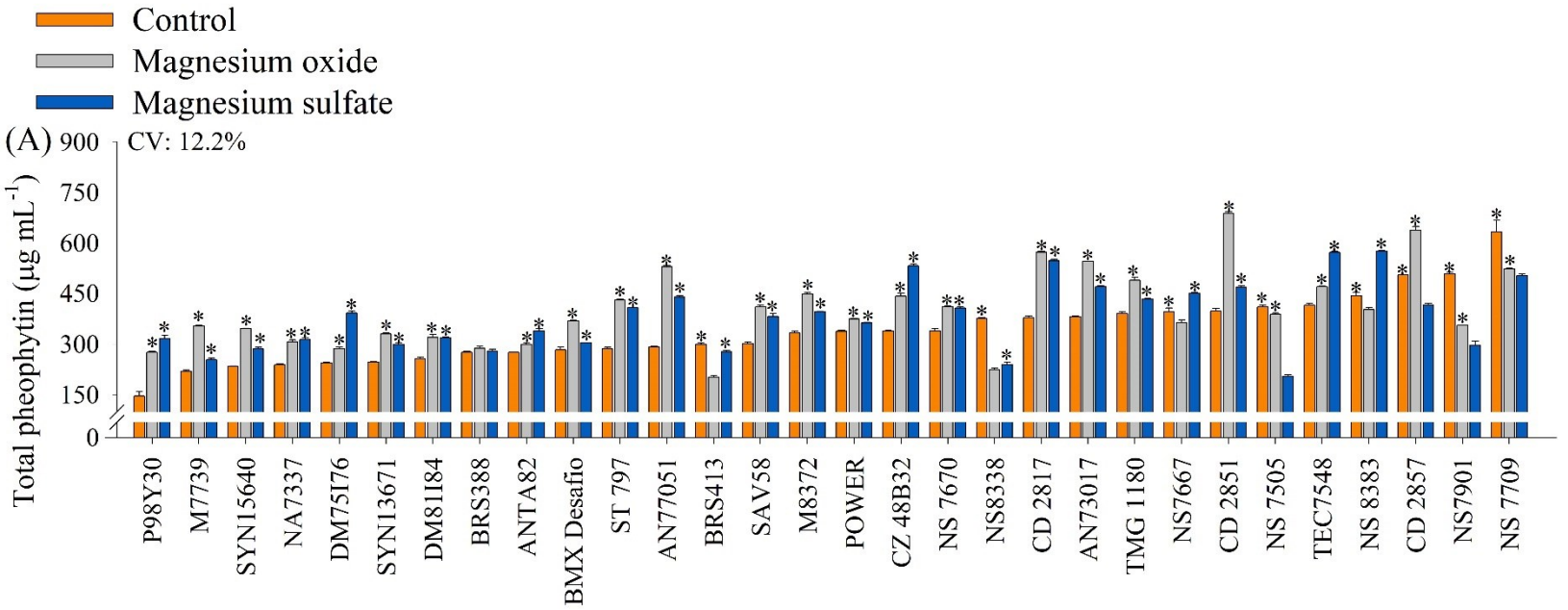
Source: The author himself

Figure S11. Effects of Mg rates on pheophytin a (A) and pheophytin b (B) in *Glycine max* varieties (january, 9, 2018). CV means coefficient of variation. "*" compares each variety under Mg rates, indicating the best rate compared to the others according to the Scott Knott test ($p \leq 0.05$). Bars indicate the standard error of the means (n=3).



Source: The author himself

Figure S12. Effects of Mg rates on total pheophytin (A) in *Glycine max* varieties (January 9, 2018). CV means coefficient of variation. "*" compares each variety under Mg rates, indicating the best rate compared to the others according to the Scott Knott test ($p \leq 0.05$). Bars indicate the standard error of the means (n=3).



Source: The author himself