RESEARCH ARTICLE

Antagonistic K/Mg ratios: is foliar application of MgSO$_4$ a superior alternative to root resupply?

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Abstract

Backround and aims The main cause of magnesium (Mg) deficiency is its competition with potassium (K). Maize, as the world's most widely grown crop, has a high risk of interplay with poorly balanced soils. Since foliar fertilization is applied when availability and distribution of nutrients is limited, this study aimed to determine whether Mg foliar application (FA) is to be favored over root resupply (RS) under such conditions.

Methods Plants of *Zea mays* L. were grown hydroponically with low and sufficient Mg supply under adequate to excess [K]. These ratios were combined with FA or RS of Mg, and plants were analyzed for Mg uptake, translocation and distribution. The primary physiological functions of Mg were quantified by chlorophyll content, photosynthetic rate and starch accumulation.

Results Maize showed a restriction in the uptake antagonism of Mg by K, synergism in translocation, but no effect at distribution. Whilst tissue [Mg] remained unaffected by K, the increased K/Mg ratio impaired the physiological functions of Mg. The FA significantly reduced this K/Mg ratio, but neither the decline in chlorophyll levels and photosynthesis nor starch accumulation was reduced any faster or more markedly than by RS via nutrient solution (NS).

Conclusions Foliar Mg application is an effective alternative under high K/Mg ratios, but due to the restricted antagonism and the unknown suppressive mechanism of K on the physiological functions of Mg it is not superior to a RS via NS. Under field conditions when compared to soil fertilization, however, it may offer a decisive advantage.

Introduction

Magnesium (Mg) was labelled the forgotten element in crop production by Cakmak and Yazici in 2010. Accounting for 1.9% of the earth's crust, it is the fourth most abundant essential element (Fleischer 1954) and also one of the most abundant cations in plant tissue (Shaul 2002). Although its various critical functions in plants have been known for a long time, comparatively little research has been conducted (Cakmak and Yazici 2010). Among the numerous critical functions the involvement in photosynthesis,

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carbohydrate partitioning and protein synthesis are the most notable (Hawkesford et al. 2012). Of key importance is the activation of over 300 enzymes by Mg, more than any other element in the plant. In particular, the activation of Ribulose-1,5-bisphosphate Carboxylase-Oxygenase (RubisCO) and the activation of Mg chelatase, which catalyzes the biosynthesis of chlorophyll, are of decisive importance for photosynthesis (Masuda 2008). Further, the binding of magnesium to ATP is of central importance for the transport of carbohydrates, as this serves as a substrate for the H^+-ATPase located in the plasma membrane. This indirectly ensures phloem loading and thus the export of sucrose from the source leaves which can lead to an impeded shoot-root partitioning and thus to reduced root growth (Cakmak et al. 1994b; Hanstein et al. 2011; Hermans and Verbruggen 2005). Magnesium is also required in protein synthesis for the binding of ribosomal subunits, glutamine or glutathione synthase, and the stabilization of RNA (Hawkesford et al. 2012; Maathuis 2009). Hence, the consequences of Mg deficiency are reflected by reduced chlorophyll content, a collapse in the rate of photosynthesis, an accumulation of carbohydrates in older leaves and an increase of amino acids, which are visually evident as interveinal leaf chlorosis (Ceylan et al. 2016; Jezek et al. 2015; Neuhaus et al. 2014).

Due to the essential involvement of Mg in energy metabolism and protein synthesis, insufficient Mg supply puts both yield and quality, and therefore the most important parameters of food security, at risk (Gerendás and Führs 2013). Magnesium deficiency can occur through two mechanisms: (1) an absolute deficiency in the soil and (2) poor availability, whereby the interaction with potassium (K) is of the greatest importance here (Li et al. 2018; Xie et al. 2020). The latter occurs particularly on tropical soils (Dechen et al. 2015) but can also be induced on other soils in intensive cropping systems by one-sided K fertilization with simultaneous high Mg removal by the crop (Cakmak and Yazici 2010). In general, this cation interaction is mainly responsible for the worldwide occurrence of Mg deficiency in the field (Xie et al. 2020). High concentrations of K in the soil solution can suppress Mg uptake, but due to an additional specific K transport system, this uptake antagonism does not work vice versa (Senbayram et al. 2016). While this uptake antagonism is already well documented, there is further evidence of antagonistic suppression of K on Mg during shoot translocation and during distribution from older to younger tissue (Xie et al. 2020). In addition, Garcia et al. (2022) found an impairment of the physiological effectiveness of Mg even at sufficient concentrations in the tissue when the K/Mg ratio was increased. Accordingly, K appears to have a suppressive effect on Mg at various sites within the plant.

Foliar fertilization (FA) plays an alternative or complementary role in impaired root nutrient supply (Fernández and Brown 2013). Such impairments can often be the result of abiotic stress factors such as drought, nutrient competition, unadapted pH values, among others (Bergmann 1993; White 2012). However, FA is also used in cases of poor distribution characteristics within the plant due to the chemical properties of nutrients such as calcium (Ca), sulphur (S) or iron (Fe) (Mengel 2002). Excessive K concentrations represent an abiotic stress factor and, as described above, might lead to poor uptake, distribution and physiological efficacy of Mg. It has already been demonstrated in the case of maize that under Mg deficiency without the influence of excessive K, FA can alleviate physiological Mg deficiency to the same extent as root RS (Jezek et al. 2015). However, the efficacy of FA under K-induced Mg deficiency is unclear to date.

In the last two decades, maize cultivation has been greatly expanded and now shows the highest production among cereals with an increase in harvest volume of 104% since 2000 (FAO 2021).

It is grown both on marginal soils with the risk of Mg deficiency and, this is the majority, primarily as part of intensive cropping systems in America and China (Mi et al. 2016), which can carry the risk of poorly balanced K/Mg concentrations (Cakmak and Yazici 2010). Due to the immense importance of cultivation and the probable interplay with wide K/Mg ratios, the following hypotheses were investigated for maize in this study: (1) A wide K/Mg ratio suppresses the root-to-shoot translocation of Mg. (2) Under high [K], a Mg-FA increases [Mg] of later developing leaves (sinks) significantly higher than a resupply (RS) via NS to the root. (3) For K-induced Mg deficiency, the leaves recover significantly faster from deficiency symptoms by Mg-FA than by a RS via NS.

Materials and methods

Experimental setup

The experiment was conducted at the experimental station of the Institute of Plant Nutrition and Soil Science at Kiel University (54°20′50″ N, 10°6′55″ E). Maize plants (*Zea mays* L. cv. DKC 3096, Bayer AG, Leverkusen, Germany) were grown hydroponically under controlled conditions in the greenhouse from February-March with an additional light regime of 16 h (7 a.m. – 11 p.m.) with 250 μmol photons m^{-2} s^{-1}. Relative humidity was set at $50 \pm 15\%$ at a temperature of 20/15°C. Seeds were germinated in perlite and, after 7 days, transferred to 9-L plastic pot (two plants pot^{-1}) containing 25% of full-strength nutrient solution (NS). Nutrient concentration was increased by 25% every second day until 100% were reached. The full-strength NS was: 1.3 mM Ca(NO$_3$)$_2$, 0.7 mM NH$_4$NO$_3$, 4.0 mM K$_2$SO$_4$, 0.2 mM KH$_2$PO$_4$, 2.0 mM CaCl$_2$ for the excess K amounts (variant 8+, 8-, 8-/FA, 8-/FA). To balance sulfur, for the moderate/high K supply (variant 2+, 2-, 2-/FA, 2-/RS, 5+, 5-, 5-/FA, 5-/RS) the NS composition was adjusted to 1.65 mM Ca(NO$_3$)$_2$, 1.0 or 2.5 mM K$_2$SO$_4$, 0.2 mM KH$_2$PO$_4$, 0.625 mM CaCl$_2$, 0.35 mM (NH$_4$)$_2$SO$_4$ and 1.025 mM CaSO$_4$. All variants also received 200 μM Fe-EDTA, 5.0 μM H$_3$BO$_3$, 2.0 μM MnSO$_4$, 0.5 μM ZnSO$_4$, 0.3 μM CuSO$_4$ and 0.01 μM (NH$_4$)$_6$Mo$_7$O$_{24}$. The moderate, high and excessive levels of K (2, 5, 8 mM) were combined with 0.05 mM MgSO$_4$ as moderate deficiency (-) and with 0.5 mM as sufficiency (+) in the NS. Each K level with moderate Mg deficiency was also combined with a foliar application (FA) and a resupply (RS) of MgSO$_4$ to the root via the NS. A total of twelve variants, each with four independent biological replicates, were investigated. Pots were arranged in a block design, according to the replicates, and completely randomized within blocks on a weekly basis.

Foliar treatment and resupply

Foliar fertilization took place in the morning at 05:00 a.m. in darkness with a relative humidity of 60%. 30 days after germination (DAG), the first of three FA and simultaneous resupply was carried out. Fertilization two and three followed four days apart. Foliar application was performed using a sponge tong using a 200 mM MgSO$_4 \cdot$ 7 H$_2$O solution containing 0.1% Silwet® Gold as a wetting agent. The sponge tong enabled minimally inversive simultaneous abaxial and adaxial wetting of the leaves without drip loss. At all three time points, the FA was only applied to leaves more than 25% unfolded. To avoid wetting the rolled-up sink leaves by runoff, the final FA was only applied up to leaf 8 (leaf 9 less than 25% unfolded). The exact amount applied could be determined by weighing the solution and tong before and after application. This weight-determined amount was provided in equal amounts to the RS variants via the root/NS.

Consequently, a total of 21.8 g was applied via the leaf (FA variants) and 21.38 mL via the root (RS variants) per pot, giving a total of 104 mg Mg. This applied amount thus corresponded ($\pm 5\%$) to the NS concentration of the variants with 0.5 mM Mg (+ variants).

SPAD values and photosynthesis

Relative chlorophyll changes over time were measured with a non-destructive portable chlorophyll meter (SPAD-502, Minolta, Japan). The fifth and sixth leaves of both plants per pot were examined with four measurements each from 22 DAG and 32 DAG to 40 DAG, respectively, and mean values were calculated.

The measurement of net photosynthesis rate started two days before the first FA was conducted. Each measurement from 26 DAG to 40 DAG was carried out on light-adapted plants between 10:00 a.m. and 01:00 p.m. using a portable gas exchange system (LI-6400, LI-COR Biosciences, Lincoln, NE, USA). In the center of the fifth leaf, close to the middle rip, a 6 cm^2 segment was marked to ensure measurements were taken on the exact same spot over time. Irradiation of this segment during measurements was 2500 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) and was provided by a red/blue LED light source (6400-02B, LI-COR Biosciences, Lincoln, NE, USA). At a controlled temperature of 25 °C of the cuvette, air was introduced at a flow rate of 500 μmol s^{-1} with a CO$_2$ concentration of 405 μmol mol^{-1} regulated by external CO$_2$ injection (LI-6400-01, LI-COR Biosciences, Lincoln, NE, USA). The humidity level corresponded to the ambient air. All settings followed the protocol by Henningsen et al. (2022).

Determination of starch

Starch concentration and content were determined following the protocol of Brandt et al. (1987). For this procedure, the second, third, fourth, and fifth leaves of one plant per pot were harvested 34 DAG, then dried at 40 °C, weighed, and ground for homogenization (Cyclotec 1093, Foss Tecator, Höganäs, Sweden). In summary, 300 mg of plant material were mixed with 0.1 mL of termamyl and 15 mL deionized water and then heated in a shaking bath at ≥ 95 °C for 45 min (min). To 1 mL of this sample solution, 0.05 mL of amyloglucosidase and 5 mL of sodium acetate buffer were added and heated at 60 °C for an additional 30 min. 0.2 mL of this filtrate were mixed with 4 mL of enzyme reagent (Glucose GOD FS, DIaSys Diagnostic Systems GmbH, Holzheim, Germany), and the extinction was measured at 37 °C after 10 min in a spectrophotometer (Helios Omega UV-VIS, Thermo Scientific, Waltham, MA, USA) at 500 nm wavelength. The starch concentration was calculated using standards run in parallel.

Partitioning and analysis of plant tissue

At DAG 41 all plant parts were harvested, washed with deionized water and then divided into the following six parts: Root, stem, lower source leaves (leaf 4, 5, 6), upper source leaves (leaf 7, 8, 9), $\leq$ 60% unfolded lower sink leaves (leaf 10) and $\leq 30\%$ unfolded upper sink leaves (leaf 11, 12). Leaves were then dried at 65 °C, weighed, and ground for homogenization. For the subsequent nutrient analysis, 200 mg of the ground samples were dissolved by acid digestion method with 10 mL of 69% HNO_3 (ROTIPURAN Supra) in a microwave oven (MARS 6 Xpress, CEM, Matthews, MC, USA). Elemental analysis was done by inductively coupled plasma optical emission spectroscopy (ICP-OES, Optima 3000, PerkinElma, Waltham, MA, USA) as previously described by Bahamonde et al. (2023). Shoot yield and mineral concentration were calculated from the sum of the stem and the four leaf fractions.

Statistics

The statistical software R (R v4.2.2; R Core Team 2022) was used to analyze the data. An appropriate statistical mixed model was defined. The model included the factors potassium, magnesium, and time-point and all their interaction terms (two- and three-fold). The residuals were assumed to be normally distributed and, depending on the parameter under study, to be homo- or heteroscedastic. These assumptions are based on a graphical residual analysis. Based on this model, an analysis of variance (ANOVA) was performed, followed by multiple contrast tests (Hothorn et al. 2008) to compare the different levels of the influencing factors.

Results

Uptake, translocation and distribution of mg under K and vice versa

The [K] of the different plant parts showed an increase with increasing exposure to K in the NS, independent of the Mg supply (Fig. 1). In the root, the [K] at 8 mM K in NS was up to 2.7 times higher than at 2 mM K in NS. The differences between the K levels in the shoot were much smaller but still significant. In contrast to the shoot, however, the root showed differences depending on the Mg supply within the same K level. The FA variants achieved significantly higher [K] in all three K levels compared to the sufficient Mg supply via the NS.

When comparing the Mg variants across the three K treatments, it is striking that in the sufficient positive control (+) the [Mg] in the root was about fivefold lower at 5 and 8 *mM* compared to 2 *mM* K. A similar pattern was recorded by RS, which reached the level of positive control in each case. The deficient negative control (-) showed a 37% reduction with increasing K from 2 to 8 *mM* in the NS. Foliar fertilization did not affect root concentration and was at the level of negative control (Fig. 2A). In contrast to the root, the shoot [Mg] of the positive control decreased only by 32% upon exposure to additional K (Fig. 2B). Correspondingly, no reduction was observed in the negative control. Hence, the root showed a clear suppression in [Mg] that was hardly reflected in the shoot. Like the positive control, [Mg] in the shoot of the RS declined moderately but significantly with increasing K dose. This was found for all leaf tiers studied. Similarly, [Mg] of the FA variants remained unaffected by K (Fig. 2C, D, E & F). In shoot, FA only reached the Mg-level of RS at 5 *mM* K, while the level of the
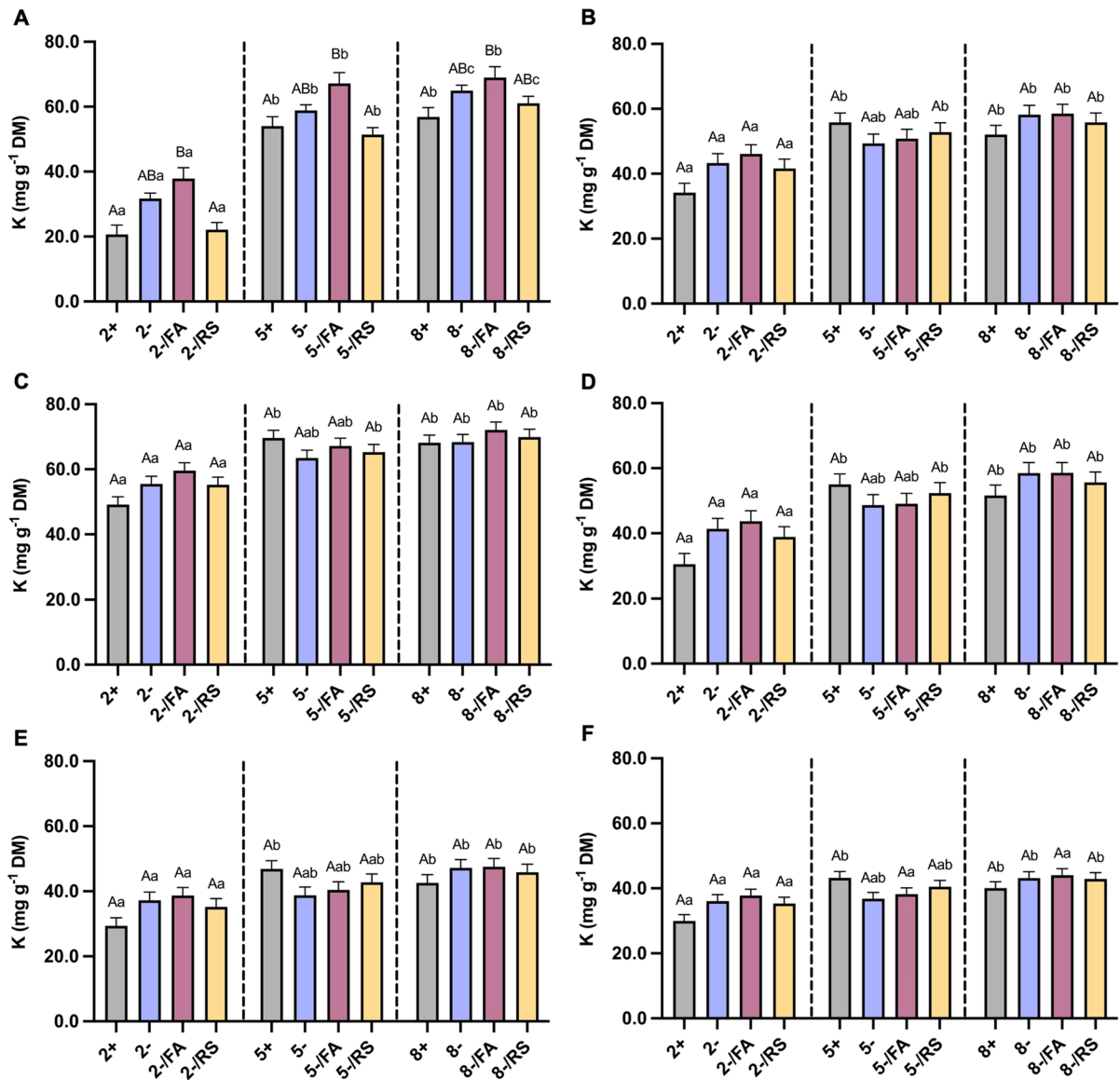
Fig. 1 Potassium (K) concentration in the dry matter of different maize plant tissues at harvest (41 days after germination) with (A) root (B) shoot (C) lower source leaves (D) upper source leaves (E) lower sink leaves (F) upper sink leaves. Potassium (K) levels with 2, 5 and 8*mM* K in NS were combined with the following Mg treatments: +, 0.5 *mM* MgSO$_4$ in NS; -, 0.05 *mM* MgSO$_4$ in NS; -/FA, 0.05 *mM* MgSO$_4$ in NS and foliar application of 200 *mM* MgSO$_4$ for three times;

-/RS, 0.05 *mM* MgSO$_4$ in NS and resupply via NS for three times in the amount of foliar application. ($n=4$ biological replicates ± SE; capital letters indicate significant differences between Mg treatments at the same K level according to Tukey's HSD test ($p \leq 0.05$); small letters indicate differences between K levels with the same Mg treatments according to Tukey's HSD test ($p \leq 0.05$)

positive control was obtained at 8 but not 5 *mM* K. The lower source leaves (lSo) were the only components of the plant where RS could not match the level of the positive control (Fig. 2C). Starting on the same level at 2 *mM* K, FA compared to RS in this leaf tier

was already significantly higher at 5 *mM* and approximately twice as high at 8 *mM* K. In the upper source (uSo) leaves, unlike the lSo, FA no longer reached [Mg] of RS at 2 *mM* K. Due to the decrease of the [Mg] of positive control and RS under the rise of K in
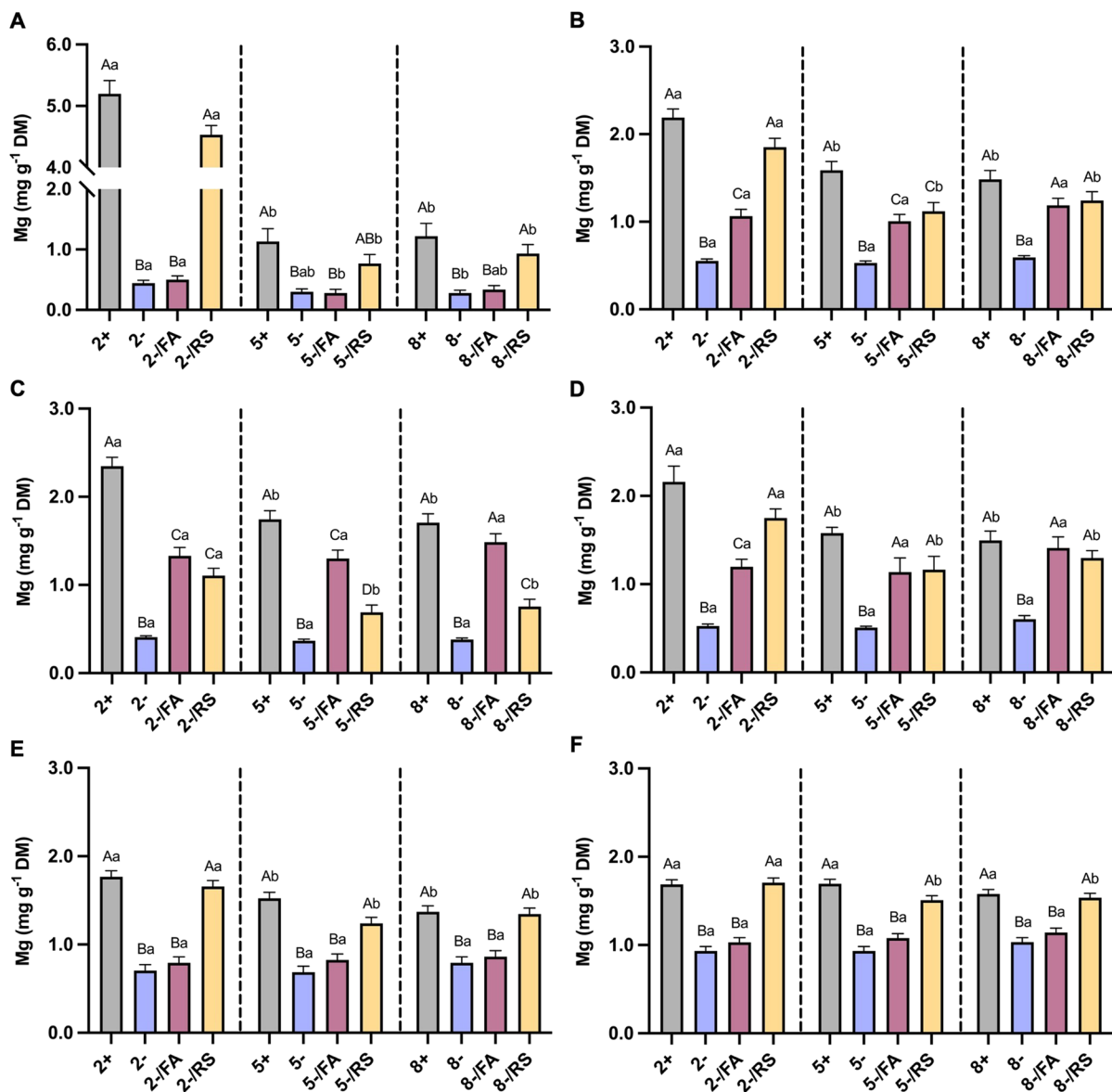
Fig. 2 Magnesium (Mg) concentration in the dry matter of different maize plant tissues at harvest (41 days after germination) with (**A**) root (**B**) shoot (**C**) lower source leaves (**D**) upper source leaves (**E**) lower sink leaves (**F**) upper sink leaves. Potassium (K) levels with **2**, **5** and **8** *mM* K in NS were combined with the following Mg treatments: **+**, 0.5 *mM* MgSO$_4$ in NS; **-**, 0.05 *mM* MgSO$_4$ in NS; **-/FA**, 0.05 *mM* MgSO$_4$ in NS and foliar application of 200 *mM* MgSO$_4$ for three times; **-/RS**, 0.05 *mM* MgSO$_4$ in NS and resupply via NS for three times in the amount of foliar application. ($n = 4$ biological replicates $\pm$ SE; capital letters indicate significant differences between Mg treatments at the same K level according to Tukey's HSD test ($p \le 0.05$); small letters indicate differences between K levels with the same Mg treatments according to Tukey's HSD test ($p \le 0.05$)

NS, both were in line with the FA at 5 and 8 *mM* K. In the lower (lSi) but not in the upper sink leaves (uSi), a K effect on the positive control was observed, while RS continued to show suppression in both leaf tiers.

The FA already from the lSi no longer reached significantly higher [Mg] than the negative control and thus remained considerably behind the RS. Therefore, FA strongly increased [Mg] in the lower leaves under

high and excess K but did not arrive in the sinks, while the RS via the NS behaved rather vice versa.

SPAD measurements

The SPAD values on the fifth leaf revealed significant differences with regard to the influence of K on the low Mg-nourished plants. Figure 2A shows that variant 8- had significantly lower relative chlorophyll values from 27 DAG onwards than the variant supplied with 2 *mM* K. Chlorophyll values of 5- lay clearly in between and dropped significantly to the level of 8- from 37 DAG on. The SPAD values of the 6th leaf showed a highly comparable pattern from 32 DAG onwards (Fig. 3A).

The negative trend of increasing K on SPAD values was also evident when comparing the well-Mg-nourished to the deficient plants. At 2 *mM* K in the NS at 40 DAG, the negative to positive control showed 25% lower relative chlorophyll values. In contrast, the difference was nearly twice as high at 5 *mM* K with 42% and at 8 *mM* with 48% (Fig. 3B, C, D). This also influenced the SPAD-values of FA and RS at these [K]: at moderate K exposure, the SPAD under FA remained at the level of the positive control from the beginning on, while RS reached this level one day later. At 5 and 8 *mM* K, both application types no longer matched the level of the positive control and could only be significantly differentiated from the negative control from 37 to 40 DAG onwards (see also visualization of symptoms in Fig. 4).

The sixth leaf showed a similar trend across all K levels, with the difference that RS reached the positive control in all variants at the time of harvest and the FA showed a clear positive trend towards it. With increasing [K] in the NS, the relative chlorophyll values of the negative control, the FA as well as the RS decreased.

Net photosynthesis

Similar to the SPAD values, the net photosynthetic rate (Fig. 5) showed a 34% reduction by increasing [K] in the NS, however, no further significant reduction was observed by a further increase of the K/Mg ratio (8 *mM* K). Under adequate K supply, neither the level nor the type of Mg supply led to a change in net photosynthetic rate except for one day between the 2nd and 3rd FA. On the contrary, the variants under high and excess K showed continuously distinct trends as a result of Mg supply from the same time point onwards (34 DAG). Both FA and RS, except for 34 DAG with FA, remained at the level of the positive control at 5 and 8 *mM* K, while the negative control performed significantly lower.

Under moderate Mg deficiency, the net photosynthetic rate was only significantly impaired by simultaneous high K exposure, but not further depressed by excessive K. Correspondingly, FA and RS were only necessary at high to excess K supply to maintain the net photosynthetic rate, whereby both were equally successful.

Starch accumulation

The [starch] showed a clear response to an expansion of the K/Mg ratio: with moderate Mg deficiency, [starch] was increased by 51% at 5 *mM* K compared to 122% at 8 *mM* K. In comparison, a non-significant 2.4-fold increase in [starch] between Mg-sufficient and -deficient plants was observed at 2 *mM* K. Hence [starch] showed no significant change in dependence on Mg supply when K supply was adequate. When K supply was in excess, leaves exhibited a significant 5.9-fold increase (Fig. 6). The FA and RS only showed a significant reduction in [starch] of 23–32% at excessive [K] compared to the negative control. Consequently, FA and RS were still 4 to 4.5 times higher than the adequate Mg-supplied plants. An effect of FA and RS was therefore only detectable under excess K, with both application types being on a similar level in terms of their effectiveness and only slightly reducing the starch accumulation.

Discussion

The suppressive effect of high K concentrations in the soil is considered to be the main cause of Mg deficiency in arable crops (Cakmak and Yazici 2010). In such poorly balanced soils, sustaining an adequate K/Mg ratio can often only be achieved over several years and is associated with high costs. Thus, foliar fertilization can be an alternative or complementary treatment to ensure an optimal supply of Mg to the plant (Mengel 2002). Since, in addition to the well-documented uptake antagonism, there are also indications of a suppressive effect of K on translocation,

Fig. 3 Relative chlorophyll concentrations over time as SPAD values of the fifth and sixth leaf of maize plants. **A** comparison of potassium (K) levels under moderate magnesium (Mg) deficiency, **B** Mg treatments at 2 *mM* K in NS, **C** Mg treatments at 5 *mM* K in NS, and (**D**) Mg treatments at 8 *mM* K in NS. **+**, 0.5 *mM* MgSO$_4$ in NS; **-**, 0.05 *mM* MgSO$_4$ in NS; **-/FA**, 0.05 *mM* MgSO$_4$ in NS and foliar application of 200 *mM* MgSO$_4$ for three times; **-/RS**, 0.05 *mM* MgSO$_4$ in NS and resupply via NS for three times in the amount of foliar application. Arrows show date of foliar application and resupply. Mean (*n* = 4 biological replicates; SE ranged between 1.28 and 1.42; small letters indicate differences between treatments according to Tukey's HSD test (*p* ≤ 0.05)
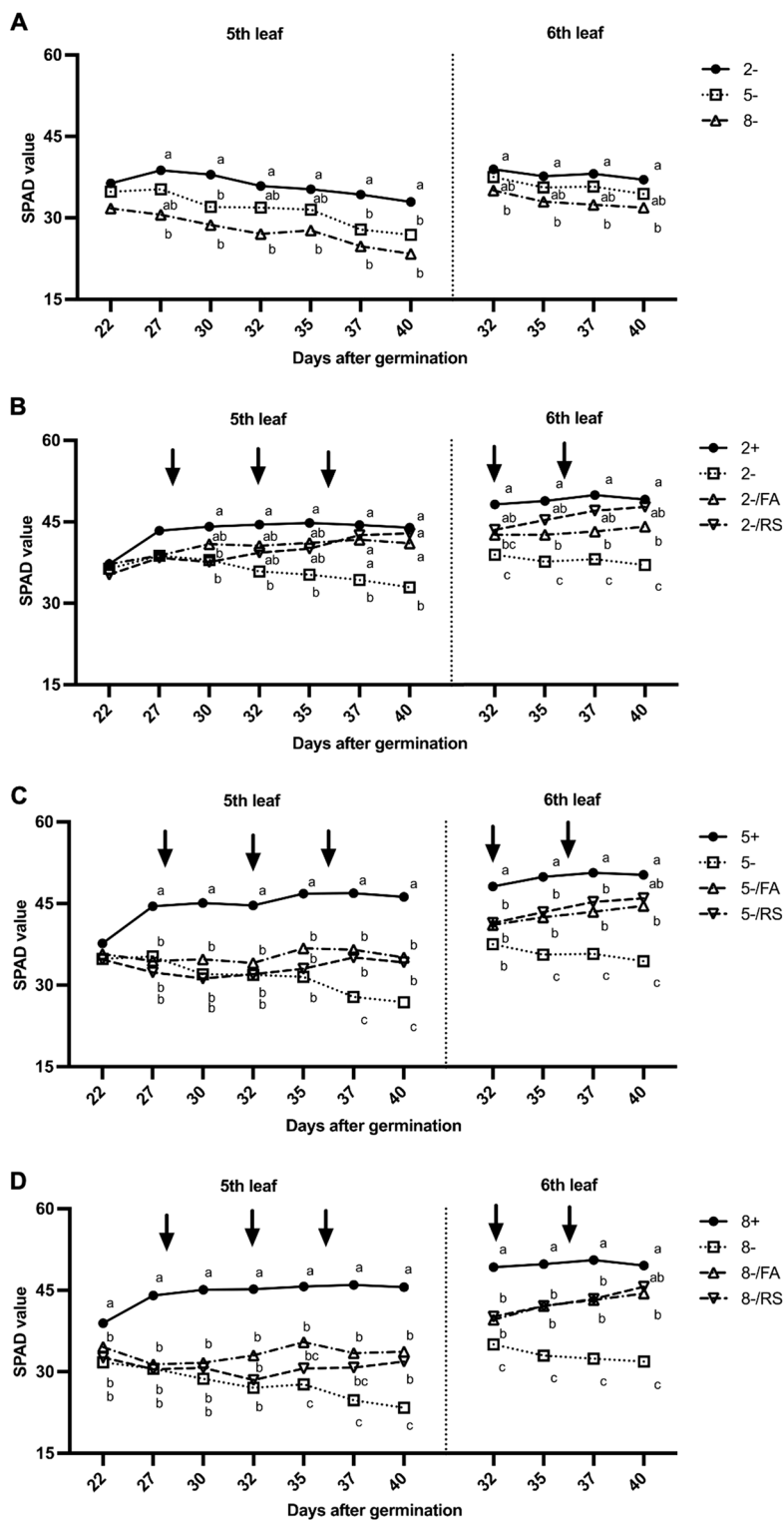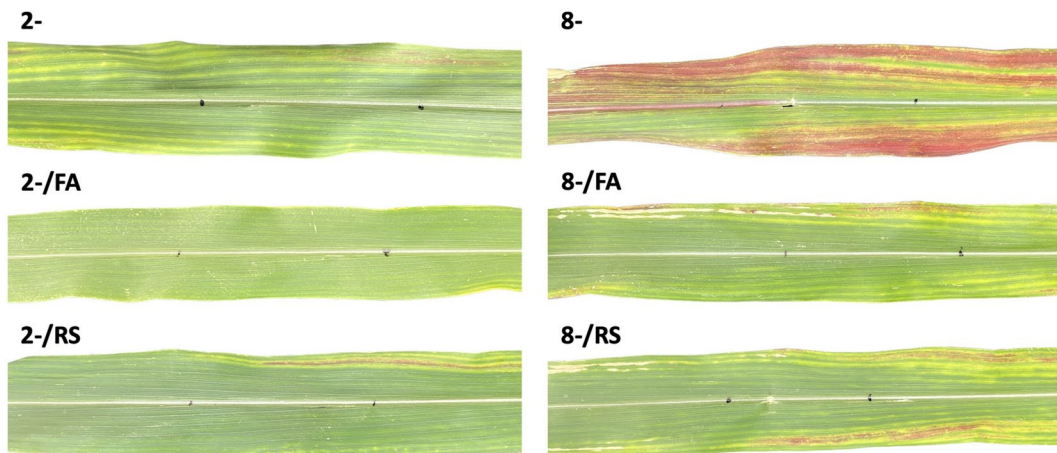
Fig. 4 Visual phenological appearance of representative 5th leaves of maize plants 41 days after germination at harvest. Potassium (K) levels with **2** and **8***mM* K in NS were combined with the following magnesium (Mg) treatments: **-**, 0.05 *mM* MgSO$_4$ in NS; **-/FA**, 0.05 *mM* MgSO$_4$ in NS and foliar application of 200 *mM* MgSO$_4$ for three times; **-/RS**, 0.05 *mM* MgSO$_4$ in NS and resupply via NS for three times in the amount of foliar application

distribution, and physiological efficacy of Mg (Garcia et al. 2022; Xie et al. 2020), the question has been raised whether foliar Mg fertilization can better meet the needs of the plant under excessive K conditions in NS than a RS. According to current knowledge, Mg uptake is a function of the K/Mg ratio in the rhizosphere (Senbayram et al. 2016). Since soil fertilization and the physical properties of the soil have a major influence on the K/Mg ratio at the root (Grimme and Németh 1975), a hydroponic setup was deliberately chosen to regulate this ratio precisely. To examine the raised hypotheses, the elemental composition and the main physiological responses to Mg deficiency were investigated under different K/Mg ratios in combination with treatments of Mg via FA and as a RS via the NS.

Uptake, translocation and distribution

The nutrient element concentrations in the root showed a clear pattern with regard to the interaction of the three cations K, Ca and Mg. Potassium was more strongly absorbed in the relative absence of Mg. These elevated [K] in the root under Mg deficiency in the NS (Fig. 1A) was also described by Jezek et al. (2015). The authors attributed this to previously shown correlations with impaired Mg storage in the vacuole, impaired K release from the vacuole due to reduced ATPase activity, and the upregulation of

K-transporter genes under Mg deficiency. In the case of Ca, this was particularly suppressed by the sum of K + Mg (Online Resource 2A). This observation has been described extensively in different cultures (Bergmann 1993). In the shoot the [Ca] did not differ significantly between adequate and excessive K supply nor between the Mg variants (Online Resource 2B). As a result, the [Ca] was in the physiologically sufficient range for all variants (Bergmann 1993).

Analyzing [Mg] of the root as a function of [K] in the NS, there was a clear reduction, i.e. antagonism, in the plants with an adequate supply of Mg, but only minor antagonism in the plants with an insufficient Mg supply (Fig. 2A) even though the [K] increased equally in both Mg levels (Fig. 1A). In the former, however, the antagonism was limited, as there was no further reduction in [Mg] beyond a K supply of 5 *mM*. A potential explanation is provided by Mao et al. (2014), who identified specific Mg transporters (MGT6) that are particularly expressed at sub millimolar concentrations. These transporters are said to be primarily responsible for the Mg supply in the insufficiently fertilized plants (0.05 *mM* Mg in NS) and will remain unaffected by K due to their specificity. However, it is noticeable that the antagonism in the shoot was much less pronounced in the case of sufficient Mg supply and was not found at all when Mg supply was insufficient (Fig. 2B). Thus, with increasing K exposure and the associated
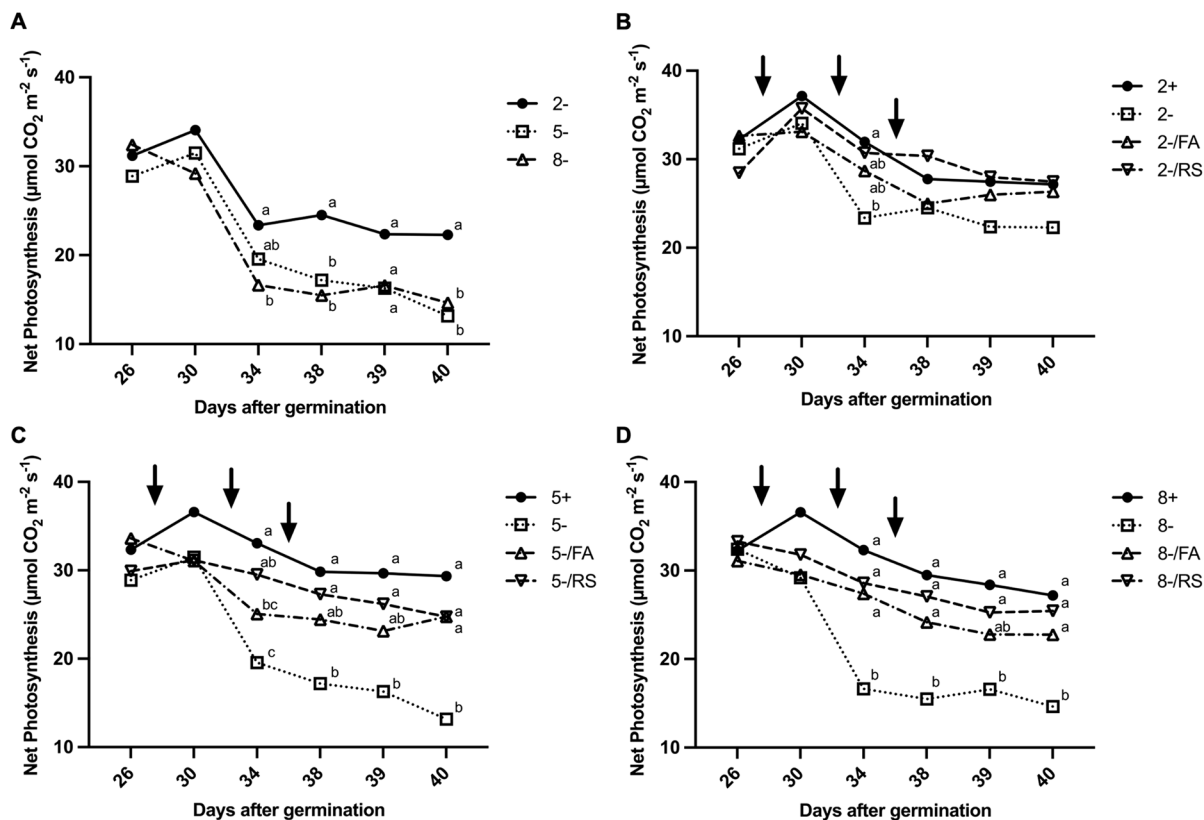
Fig. 5 Net photosynthesis rate over time of the fifth leaf of maize plants. **A** comparison of potassium (K) levels under moderate magnesium (Mg) deficiency, **B** Mg treatments at 2 *mM* K in NS, **C** Mg treatments at 5 *mM* K in NS, and (**D**) Mg treatments at 8 *mM* K in NS. **+**, 0.5 *mM* MgSO$_4$ in NS; **-**, 0.05 *mM* MgSO$_4$ in NS; **-/FA**, 0.05 *mM* MgSO$_4$ in NS and foliar application of 200 *mM* MgSO$_4$ for three times; **-/RS**, 0.05 *mM* MgSO$_4$ in NS and resupply via NS for three times in the amount of foliar application. Arrows show date of foliar application and resupply. Mean ($n = 4$ biological replicates; SE ranged between 1.60 and 1.83; small letters indicate differences between treatments according to Tukey's HSD test ($p \leq 0.05$)

higher [K] in the root (Fig. 1A), relatively more Mg is translocated from the root, which favors synergism rather than antagonism. A possible explanation is the recent discovery of Mg transporters (MGR) which are responsible for xylem loading of Mg and are expressed in higher levels when root [Mg] is low (Meng et al. 2022). Consequently, the hypothesized translocation antagonism of K to Mg in maize must be rejected. As the Mg-RS via the NS was only inhibited in uptake but not in translocation under excessive K, Mg was provided to the shoot as effectively as supply by FA (Fig. 2B). In contrast, at 8 *mM* K, [Mg] of the oldest, lower leaves, which are the source leaves for Mg distribution (Kirkby and Mengel 1976), was increased twofold by FA relative to RS (Fig. 2C). In contrast to the root transport system described above,

the uptake of nutrients via the leaf takes place without transporter proteins by diffusion along a concentration gradient. Here, uptake is subject to the physico-chemical properties of the applied nutrient salt, the environmental conditions and those of the plant (Henningsen et al. 2023; Fernández et al. 2020). Interactions with other nutrients such as K are thus bypassed with the application of pure MgSO$_4$ fertilizer. Nevertheless these higher [Mg] concentrations of the older leaves at 8 *mM* K were not found in the elongating sink leaves (Fig. 2E, F), consistent with the findings of Neuhaus et al. (2014). This contradicts the high phloem mobility of Mg and the redistribution processes between old and young leaves under deficiency (Bergmann 1993; White and Broadley 2009). Nevertheless, the data of Jezek et al. (2015)
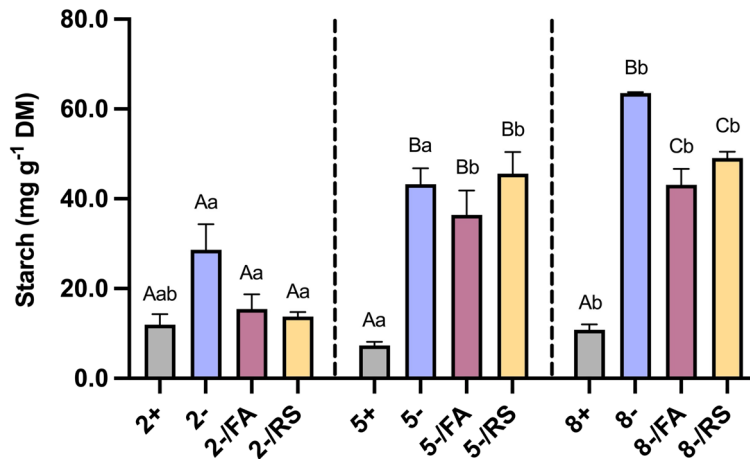
Fig. 6 Starch concentration of source leaves (second, third, fourth and fifth leaf combined) 34 days after germination after the second foliar application and resupply was added. Potassium (K) levels with **2**, **5** and **8***mM* K in NS were combined with the following Mg treatments: **+**, 0.5*mM* MgSO$_4$ in NS; **-,** 0.05 *mM* MgSO$_4$ in NS; **-/FA**, 0.05 *mM* MgSO$_4$ in NS and foliar application of 200 *mM* MgSO$_4$ for three times; **-/RS**, 0.05 *mM* MgSO$_4$ in NS and resupply via NS for three times in the amount of foliar application. ($n = 4$ biological replicates $\pm$ SE; capital letters indicate significant differences between Mg treatments at the same K level ($p \leq 0.05$); small letters indicate differences between K levels with the same Mg treatments according to Tukey's HSD test ($p \leq 0.05$)

also showed no significant increase in [Mg] in the root or in the youngest leaf after FA despite severe Mg deficiency. However, the chlorophyll content of the latter was at the level of well supplied plants, which leads to the conclusion that even under severe deficiency, the youngest leaves are sufficiently supplied with Mg and their sink strength is limited concerning the additional Mg from FA. In contrast to other nutrients, there is no remobilization of Mg during senescence (Garz 1966; White 2018), which also indicates a comparatively low Mg requirement of the sink organs. Although [K] of the lower source leaves of the negative control was a significant 36% higher when exposed to excess K compared to adequate K (Fig. 1C), there was no difference in [Mg] of the sink leaves (Fig. 2E, F). Thus, neither the FA nor the negative control shows an effect of K on the distribution of Mg within the shoot. Therefore, not only a limited uptake antagonism but also a synergism in the translocation must be assumed as explained above. The distribution antagonism postulated by Xie et al. (2020) and also hypothesized here must be rejected.

Physiological deficiency

Regardless of the amount and application form of Mg, no significant differences in root and shoot growth were observed across K levels (Online Resource 1). Similarly, no differences in DM were found between the Mg variants at moderate and high K exposure. Nevertheless, at 8 mM, Mg- and FA were found to form significantly lower root and shoot dry masses compared to Mg+. The reasons why Mg deficiency under excess K has more detrimental effects on the formation of TM are discussed in the following part on the measured key functions of Mg.

As discussed above, there were no differences in [Mg] in the negative control for all leaf layers. However, clear differences in chlorophyll can be observed visually (Fig. 4) and are also confirmed quantitatively in the SPAD values (Fig. 3). Remarkably, the relative chlorophyll levels of FA and RS both are as high as the positive control at 2 *mM* K, but no longer from 5 *mM* K (Fig. 3B, C, D) whereas the [Mg] of the FA behaved in exactly the opposite manner reaching the level of the positive control at 8 *mM* K but not at 2 *mM* K (Fig. 2C). Apparently, the collapse of chlorophyll levels under high and excess K was not fully reversible by FA, whereas in Jezek et al. (2015) the chlorophyll levels recovered almost completely under single and more severe Mg deficiency, despite the prior more severe decline. Both Garcia et al. (2022) and Gerendás and Führs (2013) suggested that the tissue K/Mg ratio is a more suitable indicator of

physiological deficiency than [Mg] alone. Here, however, FA almost halved the K/Mg ratio compared to the RS, while the SPAD values remained identically over the entire period. This indication is therefore not sufficiently reliable, at least in the case of FA, since the additional Mg apparently had no physiological effect. On the other hand, the latter could also indicate that senescence has already started. In other studies, the primary functions of Mg generally associated with chlorophyll degradation, namely biosynthesis by Mg-chelatase and the chloroplast ultrastructure (Tränkner et al. 2018), responded very rapidly to FA (Neuhaus et al. 2014). Kobayashi et al. (2013) demonstrated that in Mg-deficient rice plants the initial initiation of senescence starts by the fifth leaf, which was also investigated here. This senescence is the result of various impaired functions as a consequence of Mg deficiency, such as a reduction in transpiration, a decrease in the rate of photosynthesis, the development of starch accumulation and the formation of reactive oxygen species (ROS), although the order in the cascade is still unclear (Tanoi and Kobayashi 2015). Finally, as a fundamental component of senescence, ROS are also generated due to nutrient imbalances in the tissue (Hermans et al. 2010), such as wide K/Mg ratios. This explanation of already initiated senescence is supported by the pattern in SPAD values of the sixth leaf (Fig. 3), which received the first FA at the earliest possible time point, i.e. during unrolling in the sink organ phase. Despite the suppressive effect of [K] on the SPAD values when Mg was low, which was still consistent with the fifth leaf, FA of the sixth leaf showed an unaltered trend across all K levels, indicating almost complete recovery, as in single Mg deficiency described in Jezek et al. (2015). However, since it did not differ from RS here either, FA is apparently not able to increase the chlorophyll levels on already developed or still developing leaves more effectively than a RS via the NS to the root.

In contrast to the SPAD values, CO_2 assimilation, measured on the fifth leaf, showed a rapid and complete recovery as a consequence of FA and RS regardless of K supply (Fig. 5B, C, D). The former corresponds to the results of Jezek et al. (2015). However, it is striking that a sustained decline in the photosynthesis rate only occurred in combination with high [K], even at low Mg (0.05 mM) (Fig. 5). Since [Mg] of the lower leaves remained unaffected by K, high

K must have an inhibitory effect on the physiological functions of Mg with regard to the photosynthetic rate. Only a few studies have investigated the reciprocal effects of K and Mg on photosynthesis and mostly under deficiency of one of the two, so the mechanisms have not yet been identified (Xie et al. 2020). Magnesium is directly or indirectly involved in photosynthesis through various functions, such as in CO_2 fixation, as a co-factor in activating RubisCO (Hazra et al. 2015) and in photophosphorylation via Mg-ATP (Lin and Noble 1971). In C4 plants such as maize, Mg is also involved in the activation of PEP carboxylase (Mukerji 1974; Wedding and Black 1988). Potassium deficiency, such as Mg deficiency, can either reduce the formation of RubisCO through its contribution to protein biosynthesis or be involved in the accumulation of carbohydrates through its key role in phloem loading, which inhibits the rate of photosynthesis and gene expression by a negative feedback regulation (Hermans and Verbruggen 2005; Tränkner et al. 2018). However, it remains questionable whether this suppressive effect of high [K] on the key physiological functions of Mg requires direct involvement in these functions. Taken together, Mg-FA can counteract the suppression of photosynthesis, but not more effectively than a RS via NS.

Despite the reduced rate of photosynthesis, the increase in K resulted in a twofold rise in [starch] (Fig. 6). According to Cakmak et al. (1994a), the phloem export of sucrose is much more sensitive, i.e. more rapidly impaired, to Mg deficiency than photosynthesis. Garcia et al. (2022) also described an increase in assimilate accumulation in the fully expanded leaves of sugar cane as a result of higher K/Mg ratios. In contrast to our study, however, [Mg] decreased at the same time as a consequence of high K. Starch accumulation occurs under Mg deficiency due to the reduced amount of MgATP, which is required as a substrate for the proton (H^+) pumps to provide the H^+ gradient for H^+/sucrose cotransport into the phloem (Cakmak and Kirkby 2008). The FA investigated here was able to reduce [starch], but only to the same moderate extent as the RS. This in turn is consistent with the low chlorophyll levels, as these correlate negatively with [starch] (Hermans and Verbruggen 2005). The fact that FA under high [K] hardly reduced starch accumulation, although Cakmak and Kirkby (2008) were able to show a very rapid recovery through RS, might either be due to the

already discussed unknown effect of high K levels on Mg-specific functions or to the above hypothesized induced senescence as a result of the ROS associated with the high [starch] (Tränkner et al. 2018).

As a result of the limited antagonism of K on [Mg] and despite lower K/Mg ratios, the hypothesized advantage of FA over RS via the NS could not be confirmed for the physiological functions either. It should be mentioned that the RS via NS was immediately available to the root for uptake due to the hydroponic experimental setup. Fertilization to the soil is subject to completely different mechanisms and thus different transport rates and times to the root. The main reasons for this are the cation exchange complex, which strongly influences the composition of the soil solution, and the water potential of the soil, which determines the absorption of the cations by the plants (Tavakkoli et al. 2010). In addition, soil fertilization generally influences the concentration of non-fertilized cations in the soil solution by displacing the cations at the cation exchange complex. Mg fertilization via the soil therefore not only leads to different uptake rates compared to resupply via NS, it also leads to a different K/Mg ratios in the rhizosphere (Grimme and Németh 1975). It can therefore be assumed that Mg foliar fertilization under field conditions might offer the hypothesized potential advantages in terms of speed and bypassing nutrient interactions.

Conclusions

In this study, maize showed very limited antagonism in uptake and even synergism in translocation according to high K/Mg ratios. Despite this lack of suppression of [Mg] in leaves, the physiological primary functions of Mg were found to be impaired by the high tissue [K]. Additional $MgSO_4$ foliar application markedly reduced the K/Mg ratio, but, as with the resupply to the nutrient solution, only partially alleviated the inhibitory effects of K on the physiological functions of Mg. The study therefore concludes that foliar application could serve as an alternative to root application for maize under poorly balanced conditions, although further studies comparing soil fertilization and foliar fertilization will be required. In addition, future research needs to focus on K/Mg interactions in physiological functions to improve the effectiveness of Mg fertilization via the leaves or the roots.

Declarations

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