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REGULAR ARTICLE

Adequate magnesium nutrition mitigates adverse effects of heat stress on maize and wheat

Melis Mengutay · Yasemin Ceylan · Umit Baris Kutman · Ismail Cakmak

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Abstract
Aims Heat stress is a growing concern in crop production because of global warming. In many cropping systems heat stress often occurs simultaneously with other environmental stress factors such as mineral nutrient deficiencies. This study aimed to investigate the role of adequate magnesium (Mg) nutrition in mitigating the detrimental effects of heat stress on wheat (*Triticum aestivum*) and maize (*Zea mays*).
Methods Wheat and maize plants were grown in solution culture with low or adequate Mg supply at 25/22 °C (light/dark). Half of the plants were, then, exposed to heat stress at 35/28 °C (light/dark). Development of leaf chlorosis and changes in root and shoot growth, chlorophyll and Mg concentrations as well as the activities of major antioxidative enzymes were quantified in the experimental plants. Additionally, maize plants were analyzed for the specific weights (e.g., dry or fresh weight per a given leaf surface area) and soluble carbohydrate concentrations of sink and source leaves.
Results Visual leaf symptoms of Mg deficiency were aggravated in wheat and maize when exposed to heat stress. In both species, root growth was more sensitive to Mg deficiency than shoot growth, and the shoot-to-root ratios peaked when heat stress was combined with Mg deficiency. Magnesium deficiency markedly reduced soluble carbohydrate concentrations in young leaf; but resulted in substantial increase in source leaves. Magnesium deficiency also increased activities of antioxidative enzymes, especially when combined with heat stress. The highest activities of superoxide dismutase (up to 80 % above the control), glutathione reductase (up to 250 % above the control) and ascorbate peroxidase (up to 300 % above the control) were measured when Mg-deficient plants were subjected to heat, indicating stimulated formation of reactive oxygen species (ROS) in Mg deficient leaves under heat stress.
Conclusions Magnesium deficiency increases susceptibility of wheat and maize plants to heat stress, probably by increasing oxidative cellular damage caused by ROS. Ensuring a sufficiently high Mg supply for crop plants through Mg fertilization is a critical factor for minimizing heat-related losses in crop production.

Keywords Heat Stress · Leaf chlorosis · Magnesium · Maize · Oxidative stress · Wheat

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M. Mengutay · Y. Ceylan · U. B. Kutman · I. Cakmak (✉)
Faculty of Engineering and Natural Sciences,
Sabancı University, 34956 Istanbul, Turkey
e-mail: cakmak@sabanciuniv.edu

Introduction

Abiotic stress factors, including drought, extreme temperatures, excess light, salinity, and mineral nutrient deficiencies among others, result in significant losses

in global crop production. Heat stress, often co-occurring with drought (Braun et al. 1996; Carmo-Silva et al. 2012) and/or high light intensity (Larkindale and Knight 2002), is of particular concern because of global warming. Increasing number of reports is available indicating that the average global temperature is expected to rise by 1–6 °C in the 21st century (De Costa 2011). Moreover, spells of extremely high temperatures are expected to become more and more frequent.

Photosynthetic electron transport and CO_2 fixation processes are among the most heat-sensitive processes in plants (Berry and Björkman 1980). The photosystem II with its oxygen-evolving complex is known to be a major photosynthetic target of heat stress (Allakhverdiev et al. 2008; Marutani et al. 2012), particularly under combined stress of heat and high light (Yamamoto et al. 2008). Substantial decreases in Rubisco activity and photosynthetic performance of plants were shown in plants exposed to heat stress (Sharkey 2005; Carmo-Silva et al. 2012). As expected, such marked disturbances in activities of photosystems and photosynthetic enzymes in heat-stressed plants severely limit utilization of absorbed light energy in photosynthesis process which leads to exposure of chloroplasts to excess excitation energy and thus generation of reactive oxygen species (ROS) such as superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$) and singlet oxygen ($^1O_2$) (Yamashita et al. 2008; Suzuki et al. 2012; Marutani et al. 2012). Therefore, oxidative cell damage is a common phenomenon in heat-stressed plants which results from the attack of ROS on chloroplast pigments and membranes (Suzuki and Mittler 2006; Gill and Tuteja 2010).

Increases in generation of ROS and associated oxidative damage to chloroplasts are also very common in plants under mineral nutrient deficiencies, especially under magnesium (Mg) deficiency (Marschner and Cakmak 1989; Cakmak 1994; Yang et al. 2012; Waraich et al. 2012). In Mg-deficient plants, impairment of the photosynthetic carbon fixation (Fischer and Bremer 1993; Hermans et al. 2004) and excessive accumulation of carbohydrates in source leaves due to disrupted phloem transport (Cakmak et al. 1994b; Hermans et al. 2005) lead to over-reduction of the photosynthetic electron transport and thus activation of O_2 to ROS (Kiyoshi et al. 1999; Cakmak and Kirkby 2008).

Due to the central role of Mg in the utilization of light energy in photosynthesis and photoassimilates in growth of plants, adequate Mg nutrition is critical in crop production, especially under environmental stress conditions (Cakmak and Kirkby 2008; Verbruggen and Hermans 2013). Magnesium deficiency occurs mainly in highly weathered, acidic and sandy soils with a low cation exchange capacity and also in intensive cropping systems with high Mg depletion problem in soil profile (Cakmak and Yazici 2010; Gransee and Führs 2012). Since Mg is mainly transported by mass flow, abiotic stress conditions like heat and drought can severely inhibit Mg uptake and thus aggravate Mg deficiency (Gransee and Führs 2012). Development of leaf chlorosis under Mg deficiency is stimulated when plants are exposed to high light intensity and a partial shading of leaves greatly delays occurrence of leaf chlorosis (Marschner and Cakmak 1989). These observations indicate that photooxidative damage contributes to the leaf symptoms associated with Mg deficiency. In Mg-deficient plants, the levels of antioxidants (e.g. ascorbic acid, glutathione) and the activities of antioxidative defense enzymes including superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) are elevated to mitigate oxidative damage (Cakmak and Marschner 1992; Tewari et al. 2004, 2006; Riga et al. 2005; Yang et al. 2012). Higher expression of genes involved in antioxidative defense and increased oxidation state of total glutathione and ascorbate pools were also reported in *Arabidopsis thaliana* upon Mg starvation (Hermans et al. 2010).

Similar to Mg deficiency, heat stress also causes peroxidative damage in chloroplasts and plants respond to it by increasing the levels of antioxidative metabolites and the activities of antioxidative defense enzymes, as shown in wheat, maize and turfgrass plants (Gong et al. 1997; Jiang and Huang 2001; Dash and Mohanty 2002). Alleviation of oxidative cell damage in heat-stressed plants after application of ROS-scavenging antioxidants represents a further evidence for the role of ROS in heat-induced cellular damage (Larkindale and Knight 2002; Suzuki and Mittler 2006; Ma et al. 2006).

The observations made in plants with Mg deficiency or heat stress indicate existence of very similar physiological alterations in chloroplasts in terms of ROS generation and oxidative damage to chlorophyll. It is, therefore, plausible to suggest that oxidative

damage in leaf tissue induced by Mg deficiency may be more pronounced when Mg-deficient plants are simultaneously exposed to heat stress. As indicated above, heat stress is a very common environmental stress factor seriously affecting plant growth and yield under different cropping systems. Adequate mineral nutrition has been proposed to be essential to mitigate heat stress-dependent cellular damage in plants (Cakmak 2005; Römheld and Kirkby 2010; Waraich et al. 2012). Calcium (Ca), for example, was shown to be protective against heat stress in several studies (Gong et al. 1997; Jiang and Huang 2001; Larkindale and Knight 2002; Tan et al. 2011).

To our knowledge, there has been no published report on the interaction of Mg deficiency and heat stress in crop plants. In order to investigate how Mg nutritional status of plants affects plant growth under heat stress, wheat (*Triticum aestivum*) and maize (*Zea mays*) plants supplied with low or adequate Mg in solution culture were grown under two different temperature regimes (25 °C vs. 35 °C during the day period). Various growth parameters and the activities of major antioxidative enzymes were measured for studying the responses of these two cereal species to Mg deficiency, heat stress and their combination.

Materials and methods

Plant growth conditions

Bread wheat (*Triticum aestivum* cv. Adana 99) and maize plants (*Zea mays* cv. Shemal) were grown hydroponically in growth chambers under controlled climatic conditions. The photosynthetic photon flux density in growth chamber was about 400 μmol m^{-2} s^{-1} at the level where plants were grown. The control condition with respect to temperature was 25 °C during the light period and 22 °C during the dark period. For the exposure of plants to higher temperature, the light-period temperature was set to 35 °C and the dark-period temperature to 28 °C, and this condition was referred to as heat stress treatment in the present study. The relative humidity was kept at 60 % and 70 % during the light and dark periods, respectively.

Perlite wetted with saturated $CaSO_4.2H_2O$ solution was used as germination medium. Seeds were germinated for 5 days at room temperature, and then transferred to solution culture. For both wheat and maize experiments, seedlings were grown in 3-L plastic pots. The nutrient solution was composed of 2 mM $Ca(NO_3)_2.4H_2O$, 0.7 mM K_2SO_4, 0.2 mM KH_2PO_4, 0.1 mM KCl, 100 μM Fe-EDTA, 1 μM $ZnSO_4.7H_2O$, 1 μM H_3BO_3, 1 μM $MnSO_4.H_2O$, 0.2 μM $CuSO_4.5H_2O$ and 0.14 μM $(NH_4)_6Mo_7O_{24}.4H_2O$. Magnesium was added in the form of $MgSO_4.7H_2O$ at two different levels: Low Mg pots were supplied with 15 μM and 20 μM for wheat and maize, respectively. Adequate Mg pots were supplemented with 450 μM Mg for both species. Nutrient solutions were continuously aerated and refreshed 2 or 3 times a week throughout the growing period.

All experiments had completely randomized and full factorial designs with four pot replicates. In each pot, 24 seedlings were grown in the wheat experiment and six seedlings in the maize experiments. One half of the pots were subjected to heat for a period of time, whereas the other half were kept at control temperature throughout the experimental period. Heat treatment started 15 days after sowing (DAS) and continued until the harvest 22 DAS in the case of wheat and 23 DAS in the case of maize. Additionally, a parallel maize experiment with the same experimental conditions was performed for the measurement of protein concentration and antioxidative enzyme activities as described below.

For the determination of specific weights and soluble carbohydrate concentrations, leaf disc samples of known surface area were taken from the 2nd (referred to as old) and 3rd (referred to as middle) leaves from the bottom as well as the youngest fully expanded leaves (referred to as young) of maize plants. The fresh leaf discs were weighed immediately and then dried at 50 °C for 3 days. The specific fresh and dry weights of these discs were calculated as mg cm^{-2}. The soluble carbohydrate analysis was performed on these discs as described below. Samples for the determination of protein concentration and antioxidative enzyme activities were taken from the older leaves (the 2nd and 3rd leaves from the bottom). These were frozen in liquid nitrogen and stored at −80 °C.

In order to measure shoot and root dry matter and analyze Mg concentrations, plant roots were, first, washed in 1 mM $CaCl_2$ solution for 3 min, 1 mM EDTA solution for 3 min and finally deionized water. Whole shoot and root samples were dried at 70 °C for 2 days. Dried samples were weighed and then ground to fine powders in an agate vibrating cup mill (Pulverisette 9; Fritsch GmbH; Germany). They were,

then, used for the determination of Mg concentration as described below.

Digestion and Mg analysis

Ground shoot and root samples (ca. 0.3 g) were acid-digested in a closed-vessel microwave system, (MarsExpress; CEM Corp., Matthews, NC, USA) with 2 ml of 30 % H_2O_2 and 5 ml of 65 % HNO_3. After the digestion, the total volume of each sample was brought up to 20 ml with double-deionized water. Inductively coupled plasma optical emission spectrometry (ICP-OES; Vista-Pro Axial; Varian Pty Ltd, Mulgrave, Australia) was used to determine the Mg concentrations of the samples. Measurements were checked by using certified standard reference materials obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA). The Mg contents of shoot and roots were calculated by multiplying the Mg concentrations by their dry weights.

Protein and antioxidative enzyme assays

Frozen wheat and maize leaf samples (ca. 0.5 g) were homogenized in 5 ml of 50 mM potassium phosphate (K-P) buffer. The K-P buffer was prepared by mixing 50 mM KH_2PO_4 and 50 mM K_2HPO_4 and the pH was adjusted to 7.6. Then, 0.1 mM EDTA Titriplex-III was added to this mixture for the homogenization step. The homogenates were then centrifuged at 15000*g* for 30 min, and the supernatants were used for protein and enzyme analysis.

Protein concentrations in the crude extracts were measured by using the Bradford assay as described by Bradford (1976).

Superoxide dismutase (SOD) activity was measured by a slightly modified version of the photochemical method described by Giannopolitis and Ries (1977). This assay is based on the inhibition of the photochemical reduction of *p*-nitro blue tetrazolium chloride (NBT) by SOD and its spectroscopic measurement at 560 nm. One tube of reaction mixture contains 500 μl 50 mM Na_2CO_3, 500 μl 12 mM L-methionine, 500 μl 75 μM NBT and 500 μl 2 μM riboflavin as well as enzyme extracts (50–150 μl). The total volume was brought up to 5 ml with K-P (pH 7.6) containing 0.1 mM Na-EDTA. The reaction was started by adding the riboflavin to the mixture and placing the vials under the lights in growth chamber for about 8 min. One unit of SOD activity is defined as the SOD activity that results in a 50 % decrease in the NBT reduction.

Glutathione reductase (GR) activity was determined by recording the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm according to Foyer and Halliwell (1976) with a few modifications. The 1-ml reaction mixture consisted of 100 μl of 0.5 mM oxidized glutathione (GSSG), 100 μl of 0.12 mM NADPH, 50–150 μl of the enzyme extract and 650–750 μl of 50 mM K-P buffer (pH 7.6) with 0.1 mM Na-EDTA. Results were adjusted for the non-enzymatic oxidation of NADPH by observing the decrease of absorbance at 340 nm in the absence of GSSG.

Ascorbate peroxidase (APX) activity was measured according to Nakano and Asada (1981) by monitoring the decrease in absorbance of ascorbic acid at 290 nm. The 1-ml reaction mixture contained, 100 μl of 12 mM H_2O_2, 100 μl of 0.25 mM ascorbic acid, 50–150 μl of the enzyme extract in addition to 650–750 μl of 50 mM K-P buffer (pH 7.6) containing 0.1 mM Na-EDTA.

Catalase (CAT) activity was determined by monitoring the decrease in the absorbance of H_2O_2 at 240 nm. The reaction mixture contained 100 μl of 100 mM H_2O_2 dissolved in K-P buffer, 50–150 μl of the enzyme extract and sufficient 50 mM K-P buffer (pH 7.6) containing 0.1 mM Na-EDTA to bring up the total volume to 1 ml.

Soluble carbohydrate analysis

Soluble carbohydrate analysis was performed according to the spectroscopic method described by Yemm and Wills (1954) with slight modifications. D-glucose was used to prepare standard solutions for the calibration of spectrophotometer. The anthrone reagent was prepared by dissolving 0.6 g of anthrone in 300 ml of 98 % H_2SO_4 and 100 ml of 20 % ethanol. Soluble carbohydrates of dried and ground leaf samples were extracted with 80 % ethanol (1:100 w:v). The suspensions were centrifuged at 15000*g* for 20 min, and the supernatants were collected. To 250 μl of sample extract, 4 ml of the anthrone reagent was added, and the mixture was incubated in a water bath set to 90 °C for 20 min. When the samples cooled down, the absorbance was read at 620 nm.

Statistical analysis

Statistical analyses were performed by using the JMP software. Analysis of variance (ANOVA) was used to determine the significance of the effects of the treatments and their interactions on the addressed traits. Significant differences between means were determined by Tukey's honestly significant difference (HSD) test ($p \leq 0.05$) where ANOVA indicated a significant effect.

Results

Three-week-old wheat and maize plants grown in nutrient solution with low Mg supply exhibited interveinal chlorosis on their older leaves (Figs. 1 and 2). In addition, maize plants developed a reddish-purple coloration under Mg deficiency, especially on the leaf tips. Heat treatment aggravated the severity of these visual symptoms in Mg-deficient plants. As expected, the chlorophyll concentrations (SPAD values) of old leaves were significantly reduced in both maize and wheat under Mg deficiency (Fig. 3). The decreases in chlorophyll concentration caused by low Mg supply were further aggravated when plants were exposed to heat stress, while in plants with adequate Mg supply, the chlorophyll amount was not affected or slightly increased by heat (Figs. 2 and 3).

In wheat, low Mg reduced the shoot dry matter yield on average by about 15 % and the root yield by over 30 % (Table 1). Although the shoot dry weight of wheat was unaffected by heat under given conditions, its root dry weight was, however, significantly reduced by heat. Consequently, the shoot-to-root ratio of wheat was markedly higher at low Mg supply, particularly under heat treatment. In the case of maize, low Mg supply reduced the shoot biomass by over 50 % at control temperature and by over 75 % under heat treatment. Maize plants with adequate Mg produced significantly more shoot biomass at higher temperature. The root dry weight of low Mg maize was, on average, only 20 % of the root dry weight of adequate Mg plants. Thus, the shoot-to-root ratio of maize increased dramatically in response to both low Mg and heat treatments.

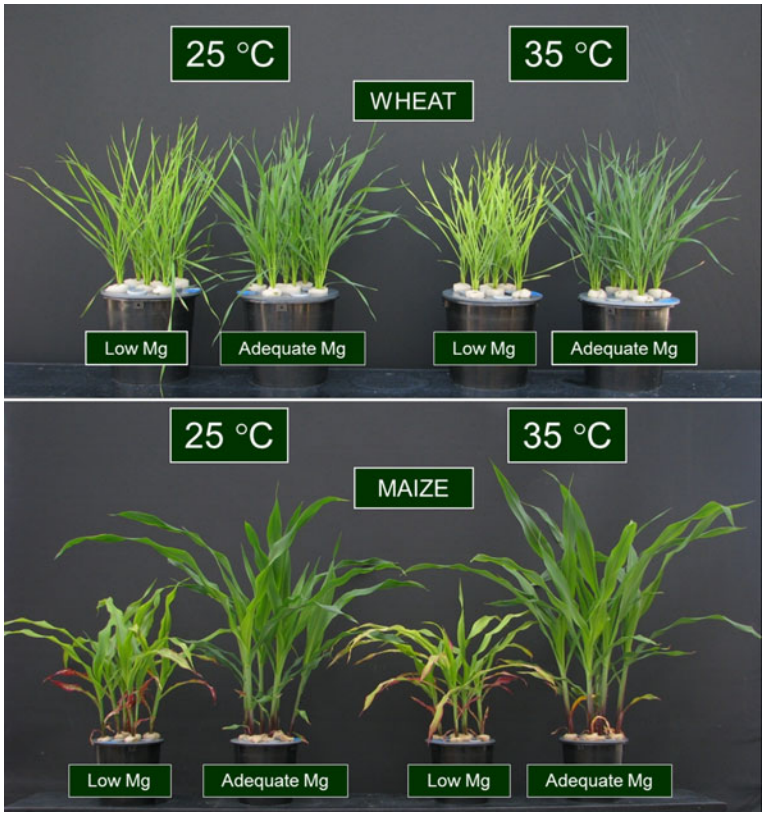


Fig. 1 22-d-old wheat (*Triticum aestivum* cv. Adana 99) and 23-d-old maize (*Zea mays* cv. Shemal) plants grown in nutrient solutions with low and adequate Mg supply at different temperatures. Plants were first grown at 25 °C until day 15, then half of the plants exposed to 35 °C until the harvesting time, and the remaining plants continued to grow at 25 °C

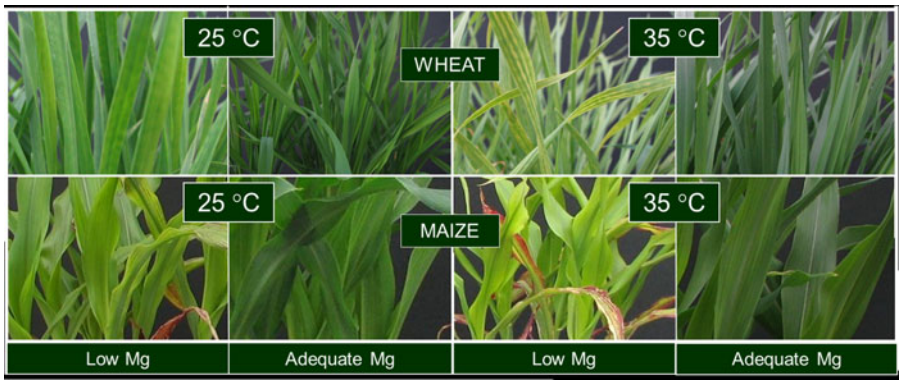
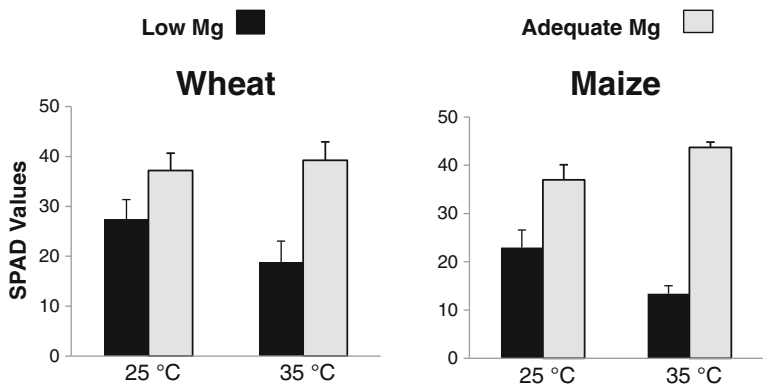


Fig. 2 Leaves of 22-d-old wheat (*Triticum aestivum* cv. Adana 99) and 23-d-old maize (*Zea mays* cv. Shemal) plants grown in nutrient solutions with low and adequate Mg supply at different temperatures. Plants were first grown at 25 °C until day 15, then half of the plants exposed to 35 °C until the harvesting time, and the remaining plants continued to grow at 25 °C

The shoot Mg concentrations and contents of wheat plants supplied with adequate Mg were about 3–4 times higher than those of Mg-deficient plants (Table 2). Similarly, adequate Mg more than doubled the Mg concentration and content of wheat roots. Heat treatment lowered the shoot Mg concentration and thus the shoot Mg content considerably, but it did not have a significant effect on the root Mg concentration or content of wheat. Maize plants with low Mg had much lower Mg concentrations and content in their shoot and roots than adequate Mg plants. Heat treatment did not affect the root Mg concentration or content of maize. In plants with adequate Mg, higher temperature resulted in lower shoot Mg concentration but higher shoot Mg content (Table 2).

The specific fresh and dry weights (mg cm^{-2}) of discs taken from old, middle and young leaves of maize plants are shown in Fig. 4. Under the adequate Mg condition, the specific fresh and dry weights did not differ significantly depending on leaf age. In contrast, a decreasing trend in specific weights was observed from old to young leaves under Mg deficiency. Notably, the specific dry weights of leaf discs taken from the low Mg plants exhibited more distinct differences depending on leaf age when compared to their specific fresh weights. Old leaves of low Mg plants had higher specific dry weights than those of the plants with adequate Mg, whereas young leaves of Mg-deficient plants showed lower specific dry weights than those of adequate Mg plants (Fig. 4). Per unit area, dry old leaves of low Mg plants



Wheat: $HSD_{0.05}$ (Mg; Heat; MgxHeat) = (1; 1; 3)

Maize: $HSD_{0.05}$ (Mg; Heat; MgxHeat) = (2; *n.s*; 4)

Fig. 3 SPAD (chlorophyll) values of the 22-d-old wheat (*Triticum aestivum* cv. Adana 99) and 23-d-old maize (*Zea mays* cv. Shemal) plants grown in nutrient solution with low (15 μM for wheat; 20 μM for maize) or adequate (450 μM) Mg supply under different temperatures. Plants were first grown at 25 °C until day 15, then half of the plants exposed to 35 °C until the harvesting time, and the remaining plants continued to grow at 25 °C

Table 1 Shoot and root dry weights (DW) and shoot-to-root ratios of 22-d-old wheat (*Triticum aestivum* cv. Adana 99) and 23-d-old maize (*Zea mays* cv. Shemal) plants grown in nutrient solution with low (15 μM for wheat; 20 μM for maize) or adequate (450 μM) Mg supply under different temperatures. Plants were first grown at 25 °C until day 15, then half of the plants exposed to 35 °C until the harvesting time, and the remaining plants continued to grow at 25 °C. Data points represent the means and standard deviations of four replicates

Temperature	Mg supply	Shoot DW (mg plant^{-1})	Root DW	Shoot-root ratio
Wheat				
25 °C	Low	136±15	43±3	3.1±0.2
	Adequate	163±13	61±7	2.7±0.5
35 °C	Low	146±17	34±1	4.3±0.5
	Adequate	165±17	54±3	3.0±0.2
Maize				
25 °C	Low	1301±483	177±29	7.4±2.4
	Adequate	3019±747	864±122	3.5±1.3
35 °C	Low	1352±370	134±20	10.1±2.9
	Adequate	5692±960	902±202	6.3±1.4

Wheat:

Shoot DW $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(17; *n.s; n.s*)

Root DW $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(4; 4; *n.s*)

Shoot-Root Ratio $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(0.4; 0.4; *n.s*)

Maize:

Shoot DW $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(644; 644; 1230)

Root DW $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(113; *n.s; n.s*)

Shoot-Root Ratio $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(2.0; 2.0; *n.s*)

were more than twice as heavy as their dry young leaves. Heat treatment tended to increase the specific dry weights of all leaves of adequate Mg plants. In low Mg plants, only the middle leaves had higher specific dry weights upon heat treatment.

Soluble carbohydrates were analyzed in leaf discs, which were used for the determination of specific weights. When the Mg supply to maize plants was adequate, similar levels of soluble carbohydrates were measured in old, middle and young leaves (Fig. 5). However, in the case of low Mg supply, leaf age had a substantial effect on soluble carbohydrates. In young leaves, the concentration of soluble carbohydrates decreased markedly due to Mg deficiency, whereas old leaves exhibited significant accumulation of soluble carbohydrates. Similar trends were observed at both 25 °C and 35 °C.

Low supply of Mg reduced the protein concentration of leaves by about 30–40 % (Table 3). Heat treatment did not affect the protein concentration of wheat leaves significantly, whereas it resulted in about 25 % lower concentration of protein in maize leaves.

Table 4 shows the effects of Mg and heat treatments on the activities of selected antioxidative enzymes of wheat plants on both fresh weight and protein basis. The SOD activity per g fresh sample was elevated by Mg deficiency in heat-treated wheat, though it was unaffected by Mg supply in the non-treated plants. Stronger responses to Mg and heat treatments were observed in the specific SOD activity. In response to Mg deficiency, wheat grown at control temperature showed an increase in specific SOD activity by 35 %, in contrast to heat-stressed wheat, which exhibited an increase by 80 %. Low Mg and heat stress conditions also enhanced the GR and APX activities per both g fresh weight and mg protein. The effects of Mg deficiency on the specific activities of these antioxidative enzymes in wheat were potentiated by heat treatment (Table 4). In the case of APX, low Mg supply almost doubled the specific activity at 25 °C, but more than tripled it when plants were subjected to heat stress. Heat treatment also caused the CAT activity of wheat to increase significantly.

Table 2 Shoot and root Mg concentrations and contents of 22-d-old wheat (*Triticum aestivum* cv. Adana 99) and 23-d-old maize (*Zea mays* cv. Shemal) plants grown in nutrient solutions with low (15 μM for wheat; 20 μM for maize) or adequate (450 μM) Mg supply under different temperatures. Plants were first grown at 25 °C until day 15, then half of the plants exposed to 35 °C until the harvesting time, and the remaining plants continued to grow at 25 °C. Data points represent the means and standard deviations of four replicates

Temperature	Mg supply	Mg concentrations		Mg contents	
		Shoot (mg kg^{-1})	Root	Shoot (μg $plant^{-1}$)	Root
Wheat					
25 °C	Low	465±27	587±98	63±11	25±3
	Adequate	1627±89	1248±295	266±26	78±24
35 °C	Low	358±5	612±50	52±7	21±1
	Adequate	1366±37	1410±88	225±28	76±5
Maize					
25 °C	Low	548±64	1732±292	688±193	300±20
	Adequate	2028±159	9829±426	6044±1070	8480±1127
35 °C	Low	485±39	2292±121	649±145	306±32
	Adequate	1437±164	9242±961	8118±1158	8203±1215

Wheat:

Shoot Mg Conc. $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(55; 55; 105)

Root Mg Conc. $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(178; *n.s; n.s*)

Shoot Mg Cont. $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(22; 22; *n.s*)

Root Mg Cont. $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(13; *n.s; n.s*)

Maize:

Shoot Mg Conc. $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(114; 114; 217)

Root Mg Conc. $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(521; *n.s*; *n.s*)

Shoot Mg Cont. $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(756; 756; *1443*)

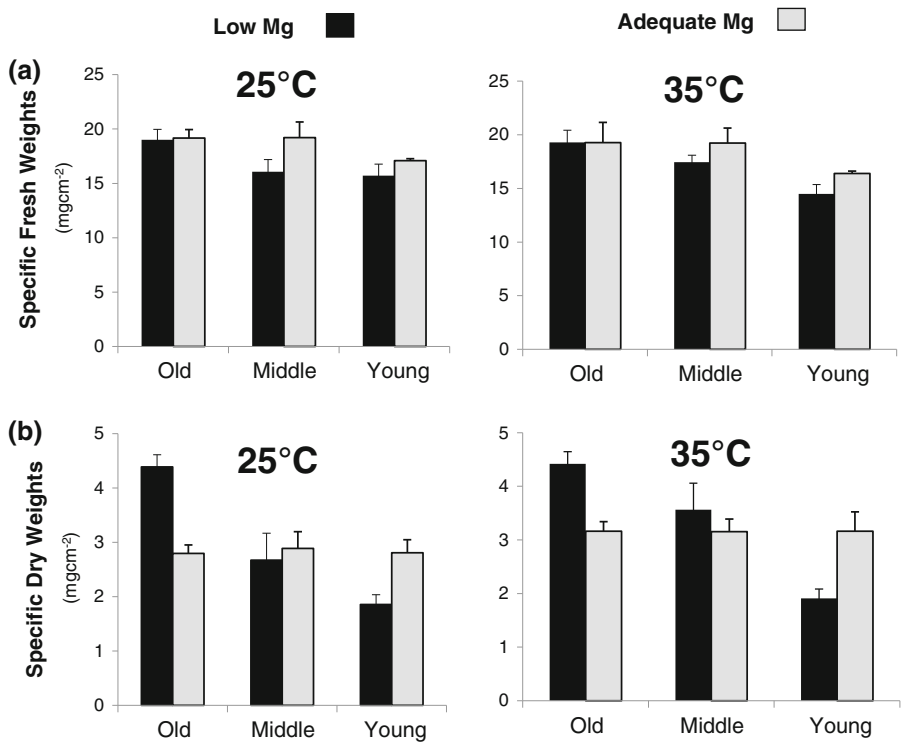
Root Mg Cont. $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(786; *n.s; n.s*)

However, CAT was the only antioxidative enzyme, which appeared to have a lower activity in plants with low Mg supply compared to adequate Mg plants.

In maize plants, low Mg supply enhanced the SOD activity per g fresh weight by 10 % and the specific SOD activity by over 60 % (Table 5). Heat treatment seemed to decrease the SOD activity measured in maize leaves, but tended to increase the specific SOD activity, although the increase was insignificant. Among the enzymes of interest, GR exhibited the most impressive increases in response to Mg deficiency in maize. Irrespective of the temperature regimes, the GR activity of maize per g fresh sample was doubled and the specific GR activity was tripled by Mg deficiency. The fresh weight-based GR activity appeared lower at higher temperature, whereas the specific GR activity was enhanced by heat treatment, as in the case of SOD. APX activity per fresh weight was less affected from the temperature and Mg treatments. In case of specific activity, low Mg treatment significantly increased APX activity under normal temperature, while Mg treatments were not effective on APX activity under heat stress. The changes in CAT activity in response to low Mg supply were remarkably different than the responses of other antioxidative enzymes. CAT had lower activity in low Mg plants than in adequate Mg plants. In heat-treated maize plants, this negative effect of Mg deficiency on the CAT activity was particularly pronounced.

Discussion

Interveinal chlorosis in leaves is a typical symptom of Mg deficiency in crop plants (Marschner and Cakmak 1989; Hermans et al. 2005; Tewari et al. 2006). Usually, leaf chlorosis due to Mg deficiency appears



Specific fresh weights:

$HSD_{0.05}$ (Leaf; Mg; Heat; LeafxMg; LeafxHeat; MgxHeat; LeafxMgxHeat) = (0.8; 0.6; *n.s*; 1.4; *n.s* ; *n.s; n.s*)

Specific dry weights:

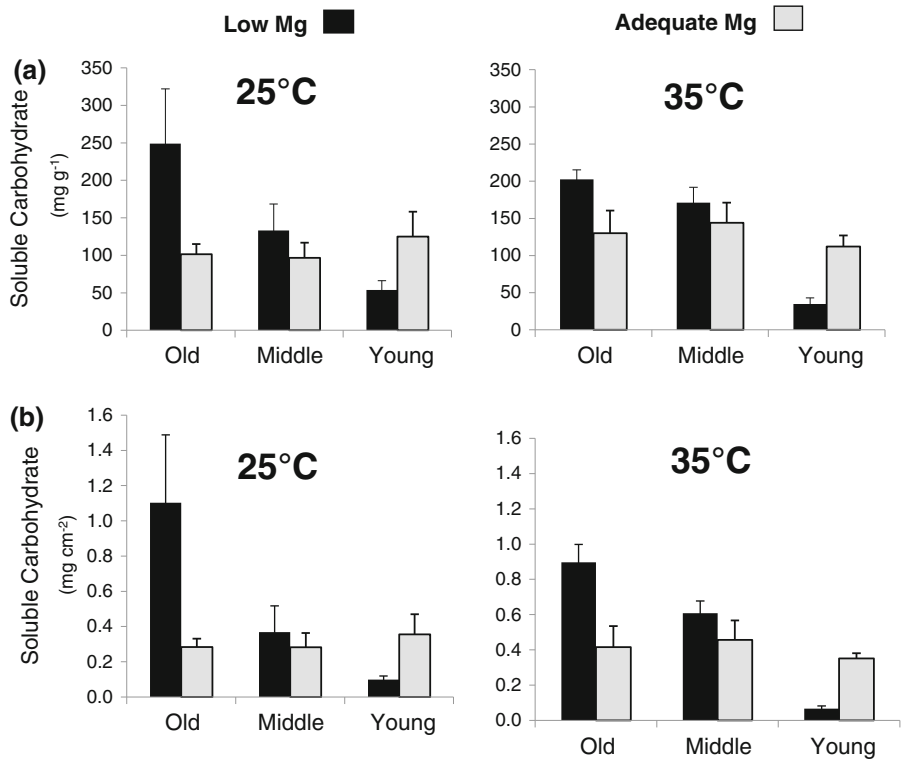
$HSD_{0.05}$ (Leaf; Mg; Heat; LeafxMg; LeafxHeat; MgxHeat; LeafxMgxHeat) = (0.2; 0.1; 0.1; 0.4; 0.4; *n.s*; 0.6)

Fig. 4 Specific fresh weights (**a**) and dry weights (**b**) of old, middle and young leaves of 23-d-old maize (*Zea mays* cv. Shemal) plants grown in nutrient solutions with low (20 μM) or adequate (450 μM) Mg supply under different temperatures. Plants were first grown at 25 °C until day 15, then half of the plants exposed to 35 °C until the harvesting time, and the remaining plants continued to grow at 25 °C

first in older leaves, which is commonly explained by the relatively high phloem mobility of Mg (Bergmann 1992). In agreement with the literature, old leaves of wheat and maize plants turned chlorotic under Mg-deficient conditions (Figs. 1 and 2).

At early vegetative growth, the critical Mg deficiency concentration in whole shoot was reported as 0.10 % for wheat and 0.15 % for maize (Jones et al. 1991). According to Table 2, the Mg concentrations in the shoots of both wheat and maize plants were sufficiently high when Mg supply was adequate, but below the critical deficiency levels when Mg supply was low. It is notable that the shoot Mg concentration of wheat and maize decreased upon heat treatment, especially under adequate Mg conditions (Table 2). However, the shoot Mg content (total amount of Mg per shoot) of maize grown with adequate Mg supply was actually elevated by heat treatment, indicating that the observed reduction in shoot Mg concentration is due to dilution.

As shown in Figs. 2 and 3, leaf symptoms of Mg deficiency in maize and wheat were aggravated when plants were exposed to heat stress. Plants under low Mg supply were visually more damaged under higher temperature, while plants with adequate Mg supply were not affected. The growth of maize was even stimulated by higher temperature under adequate Mg supply. This observation can be explained by the fact that maize is a C_4 species while wheat represents a C_3 plant species. It is well-known that C_4 plants respond positively to increases in air temperature in terms of plant growth due to their higher optimum growth temperature (Berry and Björkman 1980; Edwards and Walker 1983). However, Mg-deficient maize plants did not respond positively to temperature increase (Table 1) and showed more severe stress symptoms by increasing temperature



Soluble carbohydrates (mg g^{-1}):

$HSD_{0.05}$ (Leaf; Mg; Heat; LeafxMg; LeafxHeat; MgxHeat; LeafxMgxHeat) = (24; 16; 16; 42; 42; *n.s; n.s*)

Soluble carbohydrates (mg cm^{-2}):

$HSD_{0.05}$ (Leaf; Mg; Heat; LeafxMg; LeafxHeat; MgxHeat; LeafxMgxHeat) = (0.1; 0.1; *n.s*; 0.2; 0.2; *n.s; n.s*)

Fig. 5 Soluble carbohydrate concentrations per mg g-1 (**a**) and mg cm-2 (**b**) of old, middle and young leaves of 23-d-old maize (*Zea mays* cv. Shemal) plants grown in nutrient solutions with low (20 μM) or adequate (450 μM) Mg supply under different temperatures. Plants were first grown at 25 °C until day 15, then half of the plants exposed to 35 °C until the harvesting time, and the remaining plants continued to grow at 25 °C

(Figs. 1b and 2b). These observations clearly indicate that the susceptibility of plants to heat stress increases under low mg conditions, and the positive response of C_4 plants to higher temperatures depends very much on the Mg nutritional status of plants. Similarly, Mg deficiency was also found to make the plants highly susceptible to high light intensity (Marschner and Cakmak 1989; Cakmak and Marschner 1992). Recently, it was reported that transpiration was defective in Mg-deficient rice (Kobayashi et al. 2012). Defects in transpiration could, in theory, also increase the susceptibility of well-watered plants to heat stress by impairing evaporative cooling.

As expected, dry matter production of plants was affected by Mg deficiency. Root growth was, however, more sensitive to Mg deficiency than shoot growth in both wheat and maize, which resulted in significantly higher shoot-to-root ratios under Mg deficiency (Table 1). More severe depression of root than shoot growth and increase in shoot-to-root dry weight ratio due to Mg deficiency were documented for various species such as common bean (*Phaseolus vulgaris*) (Cakmak et al. 1994a), birch (*Betula pendula* Roth) (Ericsson and Kähr 1995), spinach (*Spinacia oleracea*) (Fischer et al. 1998), pepper (*Capsicum annuum* L) (Riga and Anza 2003) and citrus (Yang et al. 2012). However, some apparently contradictory studies in the literature showed a more adverse effect of Mg deficiency on shoot than root growth in *Arabidopsis thaliana* (Hermans and Verbruggen 2005) and sugar beet (*Beta vulgaris*) (Hermans et al. 2004, 2005) and rice (Ding et al. 2006; Kobayashi et al. 2012). Probably, the reported differences in the relative responses of root and shoot growth to Mg deficiency are related to species and/or

Table 3 Leaf protein concentrations of 22-d-old wheat (*Triticum aestivum* cv. Adana 99) and 23-d-old maize (*Zea mays* cv. Shemal) plants grown in nutrient solutions with low (15 μM for wheat, 20 μM for maize) or adequate (450 μM) Mg suppl under different temperatures. Plants were first grown at 25 °C until day 15, then half of the plants exposed to 35 °C until the harvesting time, and the remaining plants continued to grow at 25 °C. Data points represent the means and standard deviations of four replicates

Temperature	Mg supply	Protein concentration	
		Wheat (mg g^{-1} FW)	Maize
25 °C	Low	15±1	9±1
	Adequate	21±3	13±1
35 °C	Low	14±1	6±0
	Adequate	22±3	10±2

Wheat $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(2; *n.s; n.s*)

Maize $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(1.1; 1.1*; n.s*)

experimental conditions. For example, in the study conducted by Hermans et al. (2004), the experimental plants were grown first under sufficient Mg supply and then exposed to varied Mg supply, whereas in the present study, plants were grown from the beginning of the experiment under low or adequate Mg supply.

Heat treatment of wheat or maize plants under low Mg supply did not reduce their shoot biomass at the time of harvest (Table 1), although these plants were visually more affected than the non-treated ones (Fig. 1). In contrast, the root growth of low Mg plants was reduced by heat treatment. Higher susceptibility of the root growth to heat than the shoot growth has been also shown in creeping bentgrass (*Agrostis palustris)* (Huang and Gao 2000), black spruce seedlings (*Picea mariana*) (Way and Sage 2008) and wheat plants (Tahir et al. 2008). Published data show that carbohydrate translocation from shoots into roots is reduced under high temperature conditions (Timlin et al. 2006; Huang et al. 2012), which could be an explanation for the higher sensitivity of root growth to heat stress. Increased respiratory carbon loss in root tissue is known to be enhanced under high temperature which probably also contributes to higher heat sensitivity of root growth (Wang et al. 2009; Huang et al. 2012). Lower levels of carbohydrates found in roots of the heat-treated plants might be, therefore, associated with both reduced transport of carbohydrates from shoot and increased respiratory losses (Huang et al. 2012). Since Mg deficiency also reduces carbohydrate concentration in roots due to inhibited phloem export of photoassimilates from shoot into roots (Cakmak et al. 1994b), it can be suggested that the adverse impact of heat stress on root growth might be more pronounced when plants are simultaneously exposed to Mg deficiency. The results presented in Table 1 are in well agreement with this suggestion, especially in the case of maize plants.

The well-documented inhibitory role of Mg deficiency in the phloem export of photosynthates has been also found in the present study. Source leaves of maize plants with low Mg supply had higher specific leaf dry weights (e.g., weight per area of leaves) than those of adequate Mg plants, probably due to high accumulation of starch in source leaves (Fig. 4). An intensive accumulation of starch in Mg-deficient source leaves is a well-known phenomenon (Cakmak et al. 1994a; Hermans and Verbruggen 2005). By contrast, young sink leaves had reduced specific dry weights under Mg-deficient conditions, indicating impaired transport of photosynthates to sink organs. In the literature, Mg deficiency in common bean (Cakmak et al. 1994a) and K deficiency in cotton (*Gossypium hirsutum* L.) (Pettigrew 1999) were reported to increase specific leaf dry weight, and these results were discussed in relation to impaired photoassimilate export from source tissues under Mg or K deficiency. Sucrose loading from source tissues into the phloem channel is known to be specifically inhibited in Mg-deficient common bean (Cakmak et al. 1994b), sugar beet (Hermans et al. 2005) and *Arabidopsis thaliana* (Hermans and Verbruggen 2005), probably due to the impairment of H^+/ATPase activity in phloem companion cells, which mediates phloem loading of sucrose (Bush 1989; Zhao et al. 2000). Since Mg is believed to be primarily required by plasma membrane-bound ATPases in form of Mg-ATP (Hanstein et al. 2011; White 2012), a reduced level of Mg-ATP in Mg-deficient leaf tissue might be one plausible reason for the impaired phloem transport of photoassimilates under Mg deficiency. Accordingly, there was a large increase in concentration of soluble carbohydrates in source leaves but a distinct decline in sink leaves of Mg-deficient plants (Fig. 5a). When the soluble carbohydrate concentrations are calculated per unit of dry leaf area, the differences in soluble carbohydrates in source and sink leaves mentioned between low and adequate Mg plants become more pronounced (Fig. 5b). It is obvious that there is a strong inhibition in translocation of photosynthates from source into young leaf tissue.

Table 4 Total activities and specific activities of superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), and catalase (CAT) in leaves of 22-d-old wheat (*Triticum aestivum* cv. Adana 99) plants grown in nutrient solutions with low (15 μM) or adequate (450 μM) Mg supply under different temperatures. Plants were first grown at 25 °C until day 15, then half of the plants exposed to 35 °C until the harvesting time, and the remaining plants continued to grow at 25 °C. Data points represent the means and standard deviations of four replicates

Total Activity					
Temperature	Mg Supply	SOD	GR	APX	CAT
		(U g^{-1} FW)	(μmol [NADPH] g^{-1} FW min^{-1})	(μmol H_2O_2 g^{-1} FW min^{-1})	
25 °C	Low	110±4	25±6	45.4±9.7	1832±227
	Adequate	110±8	21±2	31.7±4.6	2712±181
35 °C	Low	119±4	36±1	87.3±6.8	2297±197
	Adequate	101±11	25±2	40.6±1.7	3868±641
Specific Activity					
Temperature	Mg Supply	SOD	GR	APX	CAT
		(U mg^{-1} Prt.)	(μmol [NADPH] mg^{-1} prt. min^{-1})	(μmol H_2O_2 mg^{-1} prt. min^{-1})	
25 °C	Low	7.2±0.5	1.64±0.31	2.9±0.4	120±6
	Adequate	5.4±0.4	1.01±0.10	1.5±0.2	134±19
35 °C	Low	8.4±0.2	2.56±0.16	6.1±0.2	162±11
	Adequate	4.6±0.1	1.16±0.05	1.9±0.3	177±13

Total Activity

SOD $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(*n.s; n.s*; 15)

GR $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(4; 4; *n.s*)

APX $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(6.9; 6.9; 13.4)

CAT $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(398; 398; *n.s*)

Specific Activity

SOD $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(0.74; *n.s*; 0.7)

GR $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(0.21; 0.21; 0.42)

APX $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(0.3; *n.s*; 0.6)

CAT $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(15; 15; *n.s*)

Reduction in soluble carbohydrate concentrations in young sink tissue might be also a consequence of the lower sink activity in Mg-deficient shoot tips. Since plasma membrane-bound ATPases are also involved in cell elongation and expansion in meristem tissues (Hager 2003; Pitann et al. 2009), a reduced ATPase activity might be expected in Mg-deficiency sink tissues due to lack of Mg-ATP, which may result in lower sink demand for carbohydrates (Schubert et al. 2012). This is an interesting topic, which needs to be studied in future in detail. However, rapid restoration of phloem export of sucrose in Mg-deficient leaves after 12-h Mg resupply to Mg deficient plants (Cakmak et al. 1994b) and substantial accumulation of carbohydrates in Mg-deficient source leaves before any change in shoot growth and chlorophyll concentration (Cakmak et al. 1994a; Hermans and Verbruggen 2005) indicate a primary role of Mg nutrition in phloem export of photoassimilates into the sink organs.

Both Mg deficiency and heat stress are known to induce the production of ROS and oxidative cellular damage (Suzuki and Mittler 2006; Cakmak and Kirkby 2008). Under both stresses, the level of oxidative damage depends on light intensity (Cakmak and Marschner 1992; Larkindale and Knight 2002; Yamamoto et al. 2008), indicating that they target mainly chloroplasts where they cause photooxidative stress. Heat-induced oxidative stress also targets the mitochondria, where the respiration is enhanced and the electron transport chain is disrupted, and the peroxisomes where high H_2O_2 production is expected due to increased photorespiration (Suzuki and Mittler 2006; Wahid et al. 2007; Farooq et al. 2011). In the present study, Mg deficiency enhanced the specific SOD, APX and GR activities in both wheat and maize

Table 5 Total activities and specific activities of superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), and catalase (CAT) in leaves of 23-d-old maize (*Zea mays* cv. Shemal) plants grown in nutrient solutions with low (20 μM) or adequate (450 μM) Mg supply under different temperatures. Plants were first grown at 25 °C until day 15, then half of the plants exposed to 35 °C until the harvesting time, and the remaining plants continued to grow at 25 °C. Data points represent the means and standard deviations of four replicates

Total Activity					
Temperature	Mg Supply	SOD	GR	APX	CAT
		(U g^{-1} FW)	(μmol [NADPH] g^{-1} FW min^{-1})	(μmol H_2O_2 g^{-1} FW min^{-1})	
25 °C	Low	114±2	10.4±0.9	21±5	516±61
	Adequate	102±5	5.0±0.3	19±1	768±47
35 °C	Low	88±4	8.5±1.1	15±2	158±47
	Adequate	82±14	4.4±0.5	23±5	721±162
Specific Activity					
Temperature	Mg Supply	SOD	GR	APX	CAT
		(U mg^{-1} Prt.)	(μmol [NADPH] mg^{-1} prt. min^{-1})	(μmol H_2O_2 mg^{-1} prt. min^{-1})	
25 °C	Low	12.8±1.5	1.16±0.11	2.4±0.3	57±6
	Adequate	7.8±0.6	0.39±0.03	1.5±0.2	59±5
35 °C	Low	14.1±1.2	1.36±0.12	2.5±0.3	25±8
	Adequate	8.4±1.7	0.45±0.05	2.4±0.7	73±17

Total Activity

SOD $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(9; 9*; n.s*)

GR $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(0.8; 0.8*; n.s*)

APX $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(*n.s*; *n.s;* 8)

CAT $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(101; 101; 195)

Specific Activity

SOD $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(1.4; 1.4*; n.s*)

GR $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(0.10; 0.10*; n.s*)

APX $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(0.5; 0.5*; n.s*)

CAT $HSD_{0.05}$ (Mg; Heat; MgxHeat)=(11; 11*; 22*)

(Tables 4 and 5), in agreement with previous reports on common bean (Cakmak 1994), maize (Tewari et al. 2004), pepper (Riga et al. 2005) and citrus (Tang et al. 2012). Increases in specific SOD activities were pronounced under heat-stress treatment, suggesting an increased requirement for superoxide scavenging in chloroplasts and possibly also other compartments where SOD isozymes are found including mitochondria and the cytosol (Scandalios 1993). Similarly, the specific activities of APX and GR, two critical enzymes of the Halliwell-Asada pathway in chloroplasts, also reached the highest levels when Mg-deficient plants were heat-stressed (Table 4 and 5). These results indicate that generation of ROS in Mg-deficient leaf tissue is probably promoted under heat stress treatment, which might be the reason why plants with low Mg supply appeared more damaged with heat treatment (Figs. 1 and 2).

The higher CAT activities measured in heat-stressed wheat plants can be explained by increased rates of photorespiration as photorespiratory H_2O_2 production accounts for most of the total H_2O_2 formation in C_3 species (Noctor et al. 2002). Interestingly, several studies documented that the peroxisome enzyme CAT, in contrast to SOD, APX and GR, had lower activities in Mg-deficient plants (Tewari et al. 2006; Esfandiari et al. 2010; Tang et al. 2012). The reason behind may be the similarity of CAT to the D1 protein of photosystem II with respect to its sensitivity to photooxidative damage: It can be easily photo-inactivated by conditions, which do not affect the activities of other antioxidative enzymes (Feierabend et al. 1992). Reduced CAT activity under Mg deficiency was also observed in the present study (Tables 4 and 5), especially in heat-treated maize plants. This may be an indicator of aggravated photooxidative damage in Mg-deficient and heat-treated maize.

Conclusions

Heat stress is a growing concern in crop production because of global warming (Asseng et al. 2011; Gourdji et al. 2013). Currently, several agronomic and genetic strategies are being discussed to mitigate heat-related impairments in plant growth and developments (Asseng et al. 2011; Zheng et al. 2012; Chapman et al. 2012). It is known that Mg deficiency is becoming an important mineral nutrient deficiency in agricultural soils, particularly in acidic sandy soils and under intensive crop production systems leading to Mg depletion in the soil profile. Detrimental effects of heat stress on wheat and maize plants are pronounced when plants are simultaneously exposed to low Mg supply. The root growth is particularly impaired, and markedly increased shoot-to-root ratios are observed. The results presented in this study indicate that plants suffering from Mg deficiency have a higher susceptibility to heat stress, and adequate Mg nutrition is critical in optimally preparing the plants for heat stress events. The maintenance of a sufficient Mg supply can be an effective nutritional strategy to minimize heat stress-related losses in crop production.

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