# MAGNESIUM NUTRITION MITIGATES ADVERSE EFFECTS OF HEAT AND HIGH LIGHT STRESS ON MAIZE AND WHEAT

by

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## ABSTRACT

ADEQUATE MAGNESIUM NUTRITION MITIGATES ADVERSE EFFECTS OF HEAT AND HIGH LIGHT STRESS ON MAIZE AND WHEAT

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Heat stress and excess light intensity are growing concerns in crop production because of global warming. In many cropping systems these stresses often occur 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 and high light stress on wheat (*Triticum aestivum*) and maize (*Zea mays*). Visual leaf symptoms of Mg deficiency were aggravated in wheat and maize when exposed to heat or high light stress. Magnesium deficiency markedly reduced soluble carbohydrate concentrations in young leaves; but resulted in substantial increase in source leaves, indicating reduced transportation of carbohytrates from older (source) leaves into younger (sink) leaves. Magnesium deficiency also increased activities of antioxidative enzymes, especially when combined with heat and high light 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 or high light stress, suggesting stimulated formation of reactive oxygen species (ROS) in Mg deficient leaves under heat or high light stress. These results indicate that Mg deficiency increases susceptibility of wheat and maize plants to heat or high light 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 or high light-related cellular damage in leaves and losses in crop production.

# ÖZET


YETERLİ MAGNEZYUM BESLENMESİYLE MISIR VE BUĞDAYDA YÜKSEK SICAKLIK VE IŞIK STRESİNİN ZARARLI ETKİLERİNİN AZALMASI

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Anahtar sözcükler: Isı-Işık stresi . Magnezyum . Oksidatif Stres . Mısır . Buğday

Yüksek sıcaklık stresi ve yüksek ışık şiddeti, küresel ısınma ile birlikte gittikçe artan bir kaygı uyandırmaktadır. Birçok tarımsal sistemde bu stresler mineral eksikliği gibi diğer stres faktörleriyle birlikte ortaya çıkabilmektedir. Bu tez çalışması ile buğday ve mısırda yeterli magnezyum (Mg) beslenmesinin yüksek sıcaklık ve ışık şiddetinin tahrip edici etkilerinin hafifletilmesi üzerindeki rolü araştırılmaktadır. Mısırda ve buğdayda Mg eksikliği yaprak semptomları yüksek sıcaklık ve ışığa maruz kalındığında daha şiddetli ortaya çıkmaktadır. Magnezyum eksikliği genç yapraklarda çözünür karbonhidrat miktarını önemli derecede azaltırken yaşlı yapraklarda ise ciddi oranda artışa sebep olmuştur. Bu durum yaşlı yapraklardan genç yapraklara karbonhidrat taşınımının Mg eksikliğinde azaldığını göstermektedir. Magnezyum eksikliği özellikle yüksek sıcaklık ve ışığa maruz kalındığında bazı antioksidatif enzimlerin aktivitesini arttırmıştır. Bu artışlar, en çarpıcı biçimde; süperoksit dismutazda 80% oranında, glutatyon reduktazda %250 oranında, ve askorbat peroksidazda %300 oranında görülmüştür. Anılan artışlar, Mg eksikliğinin buğday ve mısır bitkilerinde yüksek ısı ve ışığa duyarlılığını arttırdığını ve hücrelerde oksidatif zararlanmaya yol açan zararlı/reaktif oksijen türevlerinin artan miktarda ortaya çıktığını göstermektedir. Sonuçlar, kültür bitkilerinde yüksek sıcaklık ve yüksek ışık yoğunluğuna bağlı olarak ortaya çıkan hücre zararlanması ve verim kayıplarını minimize etmede yeterli düzeyde bir Mg gübrelemesinin önemli olduğuna işaret etmektedir.

This work is dedicated

To my family, **Sami**, **Ceyda** and **Mete**
Who always encourage me with their greatest love and support;

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Al ..... aluminium
ANOVA ..... analysis of variance
APX ..... ascorbate peroxidase
AsA ..... ascorbic acid
ATP ..... adenosine triphosphate
B ..... boron
ca ..... circa (approximately)
Ca ..... calcium
CAM ..... crassulacean acid metabolism
$Ca(NO_3)_2.4H_2O$ ..... calcium nitrate tetrahydrate
$CaSO_4.2H_2O$ ..... calcium sulfate dihydrate
$CO_2$ ..... carbon dioxide
CAT. ..... catalase
$CuSO_4.5H_2O$ ..... copper sulfate pentahydrate
cv. ..... cultivar
$C_3$. ..... three-carbon organic acids
$C_4$. ..... four-carbon organic acids
DNA ..... deoxyribonucleic acid
DW ..... dry weight
Fe ..... iron
GR ..... glutathione reductase
GSSG ..... oxidised glutathione
Fe-EDTA ..... iron ethylenediamine tetraacetic acid
FW ..... fresh weight
$H_2O_2$ ..... hydrogen peroxide
$H_3BO_3$ ..... boric acid
$HNO_3$ ..... nitric acid
HSD ..... honestly significant test
ICP-OES ..... inductively coupled plasma optical emission spectrometry
IPCC ..... international panel on climate change

K ..... potassium

KCl ..... potassium chloride

$KH_2PO_4$ ..... potassium dihydrogen phosphate

$K_2SO_4$ ..... potassium sulfate

MDHA ..... monodehydroascorbate

Mg ..... magnesium

$MgSO_4.7H_2O$ ..... magnesium sulfate heptahydrate

Mn ..... manganase

$MnSO_4.H_2O$ ..... manganese sulfate monohydrate

N ..... nitrogen

NADPH ..... nicotinamide adenine dinucleotide

NBT ..... nitroblue tetrazolium chloride

$(NH_4)_6Mo_7O_{24}.4H_2O$ ..... ammonium heptamolybdate (paramolybdate) tetrahydrate

$^1O_2$ ..... singlet oxygen

$O_2$ ..... oxygen

$O_2^{\cdot -}$ ..... superoxide

$OH^{\cdot}$ ..... hydroxyl radicals

P ..... phosphorus

PEP ..... phosphophenol pyruvate

$PP_i$ ..... pyrophosphate

PSI ..... photosystem I

PSII ..... photosystem II

RNA ..... ribonucleic acid

ROS ..... reactive oxygen species

S ..... sulfur

SOD ..... superoxide dismutase

SPAD ..... special products analysis division

Temp. ..... temperature

tRNA ..... transfer ribonucleic acid

UV ..... ultraviolet

Zn ..... zinc

$ZnSO_4 \cdot 7H_2O$ ..... zinc sulfate heptahydra

# (A) INTRODUCTION

## A.1. General

World population increases rapidly every year. According to estimates and projections made by the l United Nations, the world population of 7.2 billion in mid-2013 is anticipated to increase 9.6 billion in 2050 and 10.9 billion by 2100, even considering that fertility levels will continue to decrease. Such increase is also correlated with the increase in food demand. By 2050 it will be needed to produce 70% more food to feed the increasing world population and while doing this, using natural resources more efficiently and adapting to climate changes will be the main challenges world agriculture will face in the future (Bruinsma, 2009).

Together with the increasing food demand, agricultural production is greatly limited by environmental stresses. Plants are frequently exposed to abiotic stress factors, including drought, extreme temperatures, excess light, salinity, soil acidity and mineral nutrient deficiencies which can lead to reduction in yields while the arable land area is continuing to decline. Yield losses caused by these abiotic stress factors vary between 60-82% for corn, wheat and soybean (Bray et al. 2000). Because of global warming, heat stress, often co-occurring with drought (Carmo-Silva et al. 2012) and/or high light intensity (Larkindale and Knight 2002), is of particular concern. Changes in climate like global warming can also have additional remarkable negative effects on food production around the world. According to the 4$^{th}$ assessment report of intergovernmental panel on climate change (IPCC) published in 2007, the average global temperature is expected to rise by 1-6ºC in the 21$^{st}$ century. Moreover, spells of extremely high temperatures and high light intensity are expected to become more and more frequent. These changes may also have significant impact on productivity of crops. In addition to environmental factors, at least 60% of cultivated soils worldwide have growth-limiting problems

arising from mineral nutrient deficiencies such as Mg deficiency (Cakmak, 2002; Cakmak and Yazici, 2010). When such soil nutritional problems occur at the same time with other environmental stress factors, severe losses in crop production worldwide are unavoidable.

Magnesium deficiency occurs under different environmental conditions and has become a widespread problem in agriculture and forestry (Hermans et al.2004). It mainly occurs 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, high light and drought can severely inhibit Mg uptake and thus aggravate Mg deficiency (Gransee and Führs 2012).

## A.2. Heat Stress

Depending on regional scales of warming and cultivars used, cereal crop yields are estimated to be reduced due to increase in air temperature, and the greatest amount of reduction in crop yields will likely occur in temperate and sub-tropical agricultural areas as a result of extreme temperature episodes (Teixeira et al. 2013). In some African countries, products from rain-fed agriculture in hot and drought years can decrease by as much as 50% by 2020. This decline seems to be aggravated by climate change (Boko et al. 2007; Easterling et al. 2007).

High temperature is considered as one of the major stress factor that greatly limits the agricultural production. Heat stress in generally observed together with high light intensity and drought; however in tropical climates, it can be observed independently from drought. Apart from decreasing the reproduction rate and impaired seed viability of plants; seed-filling duration is directly influenced by high temperature causing seed size to be smaller that lowers the crop yields (Prasad et al. 2008).

The most heat-sensitive processes in plants are photosynthesis and $CO_2$ fixation (Berry and Björkman 1980). The photosystem II (PSII) with its oxygen-evolving complex, carbon fixation by Rubisco and the ATP generating system are the main

photosynthetic targets of heat stress (Allakhverdiev et al. 2008; Marutani et al. 2012). Thermal inhibition of Rubisco activase activity may lead to substantial decreases in Rubisco activation state under heat stress (Sharkey 2005; Carmo-Silva et al. 2012). In addition, higher temperatures favor the oxygenase activity of Rubisco over its carboxylase activity by increasing the dissolved $O_2$ to $CO_2$ ratio and the specificity of Rubisco for $O_2$ (Ogren 1984). As a result, in $C_3$ plants, photorespiration increases due to high temperature, which further reduces yield capacity of plants; whereas in $C_4$ plants, photorespiration suppressed by increasing the $CO_2$ concentration by suppressing the oxygenase activity of Rubisco enzyme (Lara and Andreo 2011). Consequently, heat stress decreases the net rate of photosynthesis by both enhancing photorespiration and directly reducing the photosynthetic carbon fixation (Farooq et al. 2011).

Assimilate translocation via the phloem, which is highly sensitive to Mg deficiency, can also be impaired by heat stress, at least indirectly as a result of lower source and/or sink activities (Plaut et al. 2004; Farooq et al. 2011). In wheat, when concurrent carbon assimilation during grain filling is restricted due to heat stress, the relative contribution of pre-anthesis stem reserves to grain filling becomes particularly important (Blum 1998; Fokar et al. 1998; Tahir and Nakata 2005). Efficient mobilization of stem reserves is considered a critical trait for ensuring high yields under heat stress conditions (Blum 1998).

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 ROS (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).

### A.3. High Light Stress

Among other environmental stress factors, high light stress is also responsible for great amount of yield loss, and it is mostly observed together with heat and drought stress. Plants are exposed to higher light intensity than they need to derive

photosynthesis. Therefore, exposure of plants to excess light is a common process and plants are well equipped to deal with excess light (Mittler, 2002; Foyer and Noctor, 2005). Dissipation of excess light through carotenoids (e.g., xantophyll cycle) is a well-documented response of plants to excess light. Dissipation of excess light energy is often accompanied by increased formation of xanthophyll pigment zeaxanthin, which is formed from violaxanthin through light-dependent xanthophyll cycle (Demmig-Adams and Adams 1996).

Excess light absorption occurs as a result of decreased rate of photosynthesis due to environmental stresses such as drought, low temperature or mineral nutrient deficiency (Owens 1996; Suzuki and Mittler 2006; Cakmak and Kirkby 2008). Inhibition of photosynthesis plays a major role in reducing the growth and development of plants. Since plants use light energy to drive photosynthesis; if the absorption of light energy exceeds the capacity of photosynthetic electrons to transport it, then inhibition of photosynthesis by light, called photoinhibition, occurs (Powles 1984). In this case, excess light excitation arriving at the photosytem-II (PSII) reaction center can cause disruption of D1 protein which is the reason for the inactivation of PSII and the excess electrons produced by photosystem-I (PSI) causes light dependent generation of ROS in chloroplast (Richter et al. 1990a, b; Barber and Andersson 1992). The degree of photoinhibition is greatly related to the balance between damage of photons to the PSII complex and its repair but high amount of ROS can prevent PSII repair by inhibiting the translation of mRNAs that encode proteins in the PSII complex (Takahashi and Murata 2008).

It is known that the formation of ROS in the chloroplast is enhanced under high light intensity, especially when plants are simultaneously exposed to an environmental stress factor. Under such situation, high light-driven generation of ROS occurs that induces photooxidative damage to chloroplasts. This is a typical situation for Mg deficient plants, and as a defense against enhanced production of ROS, Mg deficient plants enhances activity of antioxidative enzymes (Cakmak and Marschner 1992; Cakmak and Kirkby, 2008). Since ROS are highly toxic, and their production is promoted under stress, the well-known cell damage and cell death in plants subjected to many environmental stress factors are most likely caused by ROS (Foyer et al. 1997; Foyer and Noctor, 2005).

## A.4. Roles of Magnesium in Plants

Magnesium is known to be the most abundant free cation in the cytosol of plants (Shaul 2002), and has various structural and physiological roles in plant cells (Cakmak and Kirkby 2008). One of the well-known roles of Mg is related to its impact on acitivity of enzymes. Magnesium activates more enzymes than any other mineral nutrient (Epstein and Bloom 2004). Magnesium exists in the central position in the structure of the chlorophyll molecule, and therefore it is not surprising that plants under low Mg supply show leaf chlorosis (Marschner 2012). There are several enzymes in chloroplasts, which are adversely affected from low supply of Mg including photosynthetic enzymes and consequently photosynthesis process is impaired (Shaul 2002; Cakmak and Kirkby 2008). Rubisco, driving the initial carboxylation step in the Calvin cycle, and phosphoenolpyruvate (PEP) carboxylase, responsible for the initial fixation of $CO_2$ in $C_4$ and CAM plants, are among the critical Mg-activated enzymes of photosynthetic machinery (Wedding and Black 1988; Portis 1992). Numerous reports have documented that the rate of photosynthesis is markedly reduced in Mg-deficient plants (Fischer and Bremer 1993; Laing et al. 2000; Hermans et al. 2004). Plants with low Mg are highly responsive to foliar spray of Mg and show rapid increases in photosynthetic rate and chlorophyll concentration when Mg sprayed, as shown in broad bean plants (Neuhaus et al. 2013).

Additionally, nucleic acid-synthesizing polymerases and degrading nucleases require Mg for their sufficient activity (Sreedhara and Cowan 2002). Protein synthesis also requires Mg since the aggregation of two subunits requires Mg to create a bridge among them so to help activation of ribosomes (Marschner 2012; Fischer et al. 1998).

One of the well document positive impacts of Mg nutrition in plants is related to its stress-mitigating roles. Magnesium is able to alleviate Al toxicity as shown in a numerous of plants (Tan et al. 1992; Silva et al. 2001; Ryan et al. 1994; Yang et al. 2007). Magnesium also protects plants from oxidative damage initiated by excess light intensity by contributing to usage of absorbed light energy in photosynthesis (Cakmak and Kirkby, 2008). 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. Increases in generation of ROS and associated oxidative damage to chloroplasts are also very common in plants under mineral nutrient deficiencies, especially under 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). 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; Riga et al. 2005; Tewari et al. 2006; 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).

Magnesium has a pivotal role in phloem loading of sucrose and thus carbohydrate partitioning between source and sink tissues (Cakmak et al. 1994a, b; Marschner et al. 1996; Hermans et al. 2005). Accumulation of carbohydrates in source tissues due to Mg deficiency precedes other symptoms including loss of chlorophyll, reduction of shoot growth and impairment of photosynthesis (Hermans et al. 2004; Hermans and Verbruggen 2005). As root growth depends on carbohydrates synthesized in the shoot, reduced root growth and lower root-to-shoot ratio are typical early symptoms of Mg deficiency (Cakmak et al. 1994a; Fischer et al. 1998; Yang et al. 2012).

Since Mg is essential for the synthesis and function of ATP, all ATP-dependent processes are at the same time Mg-dependent (Ko et al. 1999; Igamberdiev and Kleczkowski 2001). One of the critical enzymes dependent on the Mg-ATP complex is the proton pump ($H^+$-ATPase) located in the plasma membrane of sieve tube cells and generating the electrochemical proton gradient which drives the phloem loading of sucrose via a secondary active $H^+$-sucrose symporter (Bush 1989). It was recently

suggested that the activity of the plasma membrane H+-ATPase decreases under Mg deficiency (Hanstein et al. 2011).

### A.5. Generation and Detoxification of Reactive Oxygen Species (ROS) in Plants

As indicated above, highly reactive forms of oxygen are called ROS and they are produced during the photosynthetic and respiratory electron transport. The most common ROS forms in plant cells are superoxide ($O_2^{\cdot -}$), singlet oxygen ($^1O_2$), hydrogen peroxide ($H_2O_2$) and hydroxyl radicals ($OH^{\cdot}$) (Asada 1994). Generation of ROS in plant cells is unavoidable. When produced at higher levels, plants are oxidatively damaged by the attack of ROS to critical cell components such as cell membranes (especially lipids), proteins and chlorophyll. Generation of ROS in plants is intensified when plants are exposed to environmental stress conditions such as drought, extreme temperatures, high radiation and nutrient deficiencies (Suzuki and Mittler 2006; Cakmak and Kirkby 2008; Sharma et al. 2012). Under nutrient deficiency, the intensity of excess light that plants absorb can increase due to stress-induced reductions in the photosynthesis capacity. As in the case of Mg deficiency, both high light intensity and heat stress induce production of toxic ROS and thus lead to photooxidative damage in chloroplasts (Suzuki and Mittler 2006). When these ROS are not readily detoxified so high in concentration, they damage lipid, chlorophyll, membrane structure, DNA and proteins including photosynthetic enzymes (Asada 2006; Cakmak and Kirkby 2008). If the concentration of ROS is low, they are also well-known second messengers in some of cellular processes including tolerance to abiotic stresses despite their destructive role (Desikan et al. 2001; Yan et al. 2007). Whether ROS will act as destructive or signaling molecule depends on the fragile balance between ROS production and scavenging.

Different forms of ROS are produced in different locations of chloroplast. Electron transport chain in PSI and PSII are the main locations of ROS in chloroplasts. Generation of ROS by these sources is promoted in plants by situations limiting $CO_2$ fixation, such as temperature, salt and drought stresses as well as by the combination of these stresses with high light stress (Sharma et al. 2012).

In order to minimize the oxidative effects of stresses, plants can substantially increase the levels of ROS-detoxifying antioxidants (e.g. vitamin E, ascorbic acid, glutathione) and antioxidative enzymes including superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR) and catalase (CAT) (Cakmak and Marschner 1992; Tewari et al. 2004; Riga et al. 2005; Tewari et al. 2006). SOD catalyses the dismutation of the superoxide anion and it can be found in most of the subcellular compartments that produce activated oxygen. CAT takes part in the detoxification of hydrogen peroxide especially in peroxisomes and it has high affinity for $H_2O_2$ but weak activity towards organic peroxides. GR uses NADPH to maintain a pool of reduced glutathione that can accept an electron from superoxide or $H_2O_2$. APX also reduce $H_2O_2$ in ascorbate-glutathione pathway (Foyer et al. 1997; Asada 1999). It uses two molecules of ascorbic acid (AsA) to reduce $H_2O_2$ to $H_2O$ with accompanying production of two molecules of monodehydroascorbate (MDHA).

## A.6. Objectives

Based on the results published and reviewed above it seems very likely that Mg deficiency, heat stress and high light stress causes very similar physiological alterations in chloroplasts in terms of ROS generation and oxidative damage to chlorophyll. Maintaining high photosynthesis rate represents an important condition to minimize ROS generation under stress situations such as heat and high light. As highlighted above, Mg has number of critical functions in photosynthesis. 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 or high light intensity. It is very clear to suggest that adequate Mg supply is needed to protect plants from high light stress and also heat stress. Adequate mineral nutrition has been proposed to be essential to mitigate high light or 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 (Cakmak and Marschner 1992; Gong et al. 1997; Jiang and Huang 2001; Larkindale and Knight 2002; Tan et al. 2011). In this study, we aimed to study role of varied Mg nutrition in protection of plants from heat and high light stress. To our knowledge, there

is no information in literature about the role of Mg nutrition in mitigating adverse impacts of heat stress in plants. This study is also first testing role of adequate Mg nutrition on high light damage in a $C_3$ (wheat) and $C_4$ (maize) plants in the same work.

## (B) MATERIALS AND METHODS

### B.1. Plant Growth Facilities and Experimental Design

#### B.1.1. Experiments on Heat Stress and Mg Nutrition

In this experiment the aim was to study role of varied Mg nutrition on heat stress in maize and wheat plants. Bread wheat (*Triticum aestivum* cv. Adana 99) and maize plants (*Zea mays* cv. Shemal) were grown hydroponically in growth chambers under controlled climatic conditions. Plants were grown in a growth chamber with 16 hours day and 8 hours dark. The photosynthetic photon flux density in the growth chamber was 400 μmol $m^{-2}$ $s^{-1}$ at the canopy level. The control condition with respect to temperature was 25°C for the light period and 22°C for the dark period. For heat treatment, the light-period temperature was set to 35°C and the dark-period temperature to 28°C. 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 three times a week throughout the growing period.

All experiments had completely randomized and full factorial designs. One half of the pots were subjected to heat for a period of time, whereas the other half was kept at control temperature throughout the experimental period. The wheat experiment was designed as a 4 pot-replicate experiment with 24 seedlings per pot, and the main maize experiment was designed as a 5 pot-replicate experiment with 6 plants in each pot. 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 design (but 4 pot replicates per treatment) was performed just for the measurement of protein concentration and antioxidative enzyme activities as described below.

For the determination of specific weights and soluble carbohydrate concentration, leaf disc samples of known surface area were taken from the 3$^{rd}$ oldest (referred to as oldest), 4$^{th}$ oldest (referred to as middle) and youngest leaves of maize plants in the main maize experiment. (2 plants per pot) The fresh leaf discs were weighed and then dried at 50 °C for 3 days. The specific fresh and dry weights of these discs (mg cm$^{-2}$) were calculated. 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 3$^{rd}$ and 4$^{th}$ oldest leaves of wheat (4 plants per pot) and 4$^{th}$ oldest leaves of maize (2 plants per pot) plants. These were frozen in liquid nitrogen and stored at -80°C.

Whole shoots of wheat and maize plants not used for carbohydrate or enzyme sampling were harvested separately. Plant roots were also harvested, washed in 1mM $CaCl_2$ solution for 3 min, 1mM 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 used for the determination of Mg concentration as described below.

### B.1.2. Experiments on High Light Stress and Mg Nutrition

Additional experiments were established to study effect of varied Mg nutrition on high light stress in maize and wheat plants. Bread wheat (*Triticum aestivum* cv. Adana 99) and maize plants (*Zea mays* cv. Pioneer) were grown hydroponically in growth chambers under controlled climatic conditions. The growth conditions were same as described above, except light intensity (see below). The temperature was 25°C during the light period and 22°C during the dark period. 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  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, as desribed for the heat stress experiment.  Adequate Mg pots were supplemented with 450 µM Mg for both species. Nutrient solutions were continuously aerated and refreshed 3 times a week throughout the growing period.

All experiments had completely randomized and full factorial designs. One half of the pots were subjected to low light for a period of time, whereas the other half was kept at high light throughout the experimental period. Both wheat and maize experiments were designed as a 3 pot-replicate experiment with 20 seedlings per pot for wheat and 4 plants per pot for maize. After growing 15 days at 400 µmol $m^{-2}$ $s^{-1}$ (high light intensity), part of the seedlings were exposed to 175 µmol $m^{-2}$ $s^{-1}$ (low light intensity) by using a white shade, remaining plants continued to grow at 400 µmol $m^{-2}$ $s^{-1}$. Plants were exposed to low (175 µmol $m^{-2}$ $s^{-1}$) and high (400 µmol $m^{-2}$ $s^{-1}$) light intensity for 2 weeks and then were harvested. At harvesting time, plants were 29-days-old.

Following analyses were applied to both heat stress and high light stress experiments.

## B.2. Digestion and Magnesium 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.

## B.3. 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 (pH 7.6). The homogenates were then centrifuged at 15000 g for 30 min, and the supernatants were used for protein and enzyme analysis.

### B.3.1. Measurement of Protein Concentration

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

### B.3.2. Superoxide Dismutase (SOD) Activity

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 nitro blue tetrazolium (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 12mM L-methionine, 500 µl 75 µM *p*-nitro blue tetrazolium chloride 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. Adding the riboflavin to the mixture and placing the vials under the lights in growth chamber started the reaction and samples were kept under light 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.

### B.3.3. Glutathione Reductase (GR) Activity

GR activity was determined by recording the oxidation of 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.

### B.3.4. Ascorbate Peroxidase (APX) Activity

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 2.5 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.

### B.3.5. Catalase (CAT) Activity

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.

### B.4. 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 90ºC for 20 min. When the samples cooled down, the absorbance was read at 620 nm.

### B.5. 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.

## (C) RESULTS

### C.1. Experiments on Heat Stress and Mg Nutrition

When plants grown under low Mg supply, older leaves developed Mg deficiency symptoms. As shown in Figs. 1.1 and 1.2, Mg deficiency leaf symptoms were intensified with the exposure of plants to heat stress.

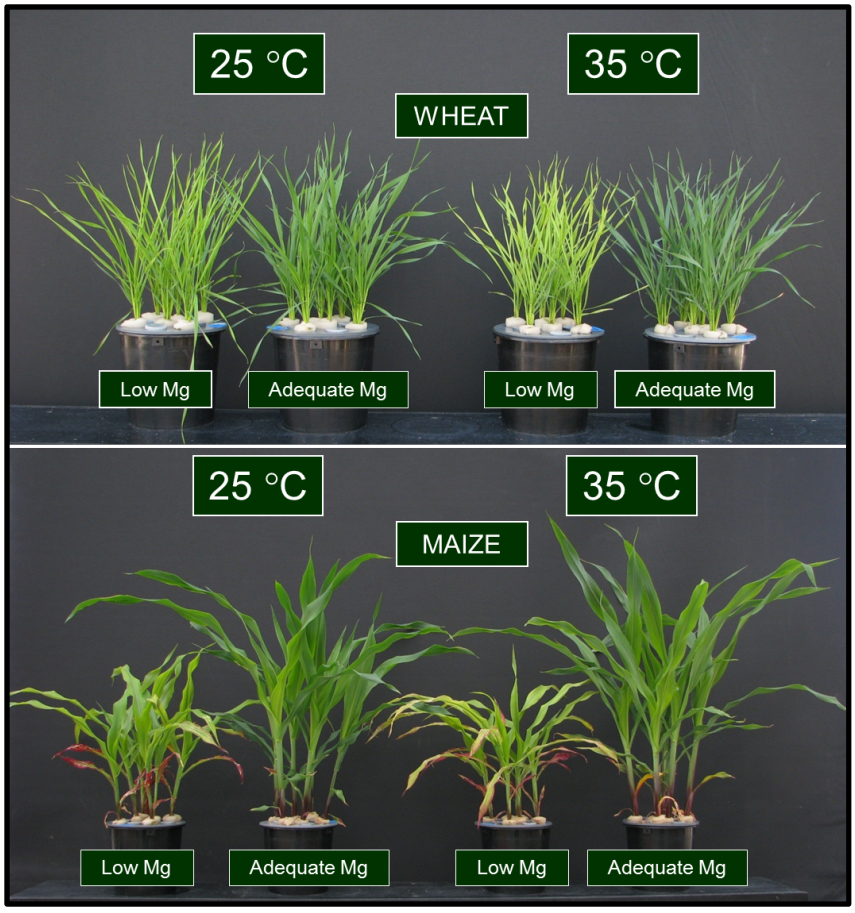



**Figure 1.1:** Growth of 22-day-old wheat (*Triticum aestivum* cv. Adana 99) and 23-day-old maize (*Zea mays* cv. Shemal) plants grown in nutrient solutions with low and adequate Mg supply at different temperatures.

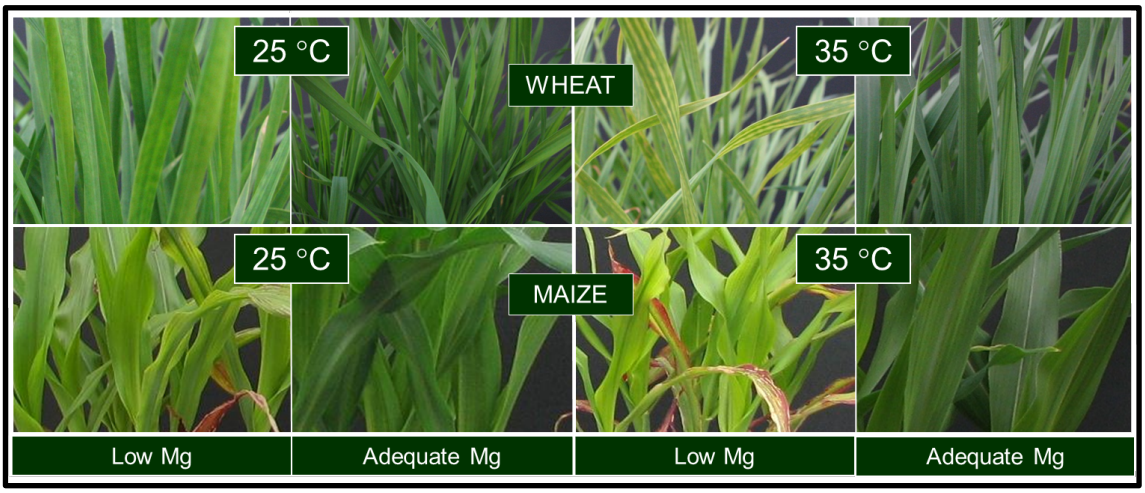
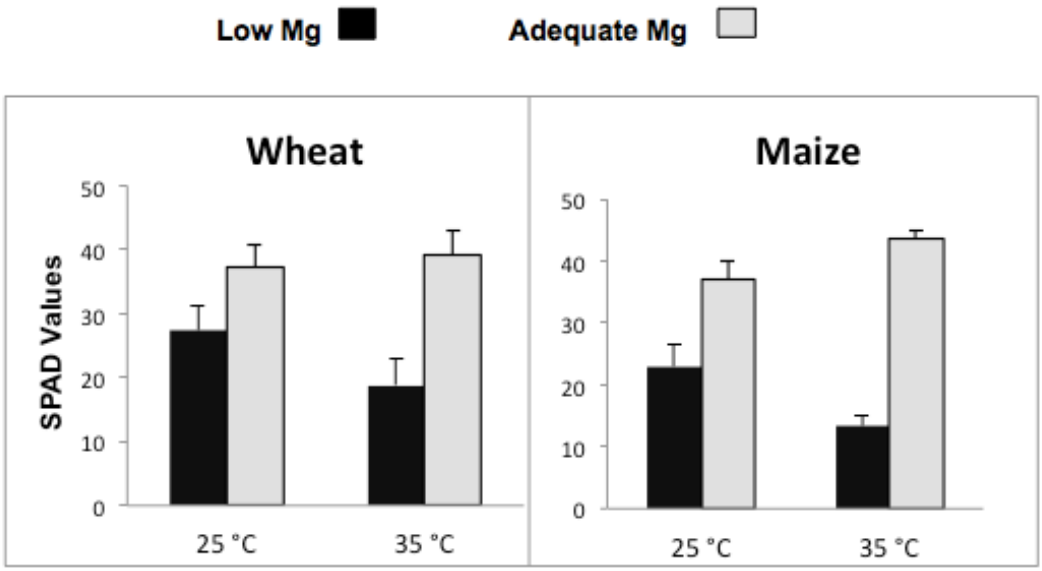



**Figure 1.2:** Leaves of 22-day-old wheat (*Triticum aestivum* cv. Adana 99) and 23-d-old maize (*Zea mays* cv. Shemal) plants grown in nutrient solutions with low and adequate Mg supply at different temperatures.

Heat treatment very distinctly aggravated the visual symptoms of Mg deficiency both in maize and wheat, while in case of adequate Mg supply, heat treatment did not affect leaves (Fig. 1.2). These observations were in good agreement with the measurement of the SPAD values (chlorophyll concentrations) of maize and wheat leaves at different temperatures. As shown in Fig. 1.3, leaf SPAD values showed clear decline in plants under low Mg supply when exposed to heat treatment, whereas in case of adequate Mg supply, heat treatment did not affect leaf SPAD values, even tended to increase SPAD values.




Wheat: $HSD_{0.05}$ (Mg; Heat; MgxHeat) = (1; 1; 3)
Maize: $HSD_{0.05}$ (Mg; Heat; MgxHeat) = (2; *n.s*; 4)

**Figure 1.3:** SPAD (chlorophyll) values of the 22-day-old wheat (*Triticum aestivum* cv. Adana 99) and 23-day-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.

In wheat, low Mg reduced the shoot growth on average by about 15% and the root growth by over 30% (Table 1.1 and Fig. 1.4). Although the shoot dry weight of wheat was unaffected by heat under the conditions of this experiment, its root dry weight was significantly reduced by heat. Consequently, the shoot-to-root ratio of wheat was markedly higher at low Mg supply, particularly in the case of heat-treated plants (Fig. 1.4).

**Table 1.1:** Shoot and root dry weights (DW) and shoot-to-root ratios of 22-day-old wheat (*Triticum aestivum* cv. Adana 99) and 23-day-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.

**WHEAT**

| Temp. | Mg Supply | Shoot DW | Root DW | Shoot-Root Ratio |
|---|---|---|---|---|
| | | (mg plant$^{-1}$) | | |
| 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**

| Temp. | Mg Supply | Shoot DW | Root DW | Shoot-Root Ratio |
|---|---|---|---|---|
| | | (mg plant$^{-1}$) | | |
| 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: HSD0.05 (Mg; Heat; MgxHeat) = (644; 644; 1230)

Root DW: HSD0.05 (Mg; Heat; MgxHeat) = (113; n.s; n.s)

Shoot-Root Ratio: HSD0.05 (Mg; Heat; MgxHeat) = (2.0; 2.0; n.s)

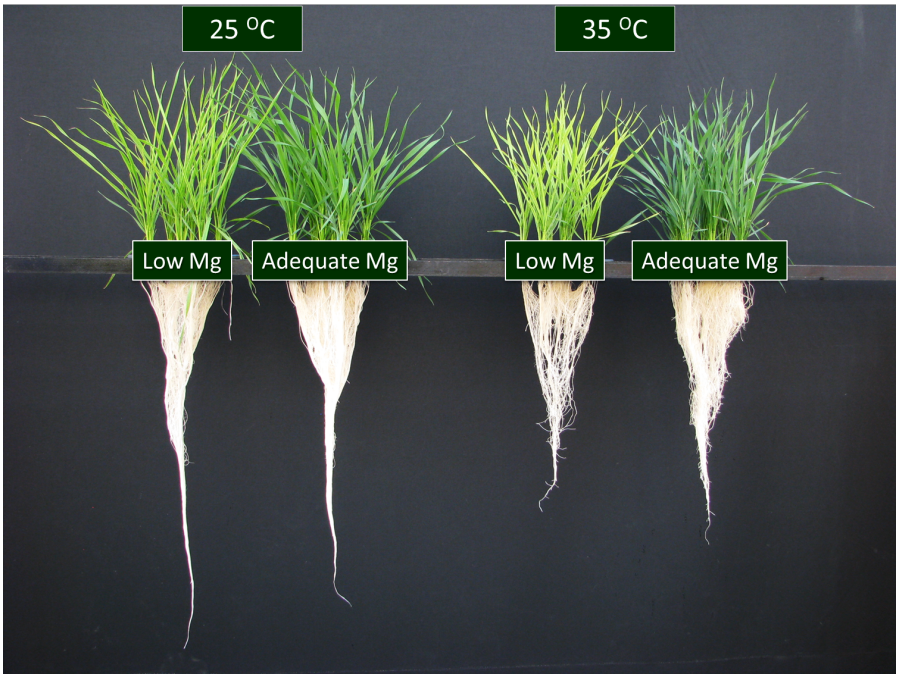



**Figure 1.4:** Growth of 22-day-old wheat (*Triticum aestivum* cv. Adana 99) plants in nutrient solutions with low and adequate Mg supply at different temperatures.

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. Despite the smaller appearance of low Mg maize under heat treatment (Fig. 1.1B and 1.5), their shoot biomass was not significantly different than that of non-treated low Mg maize (Table 1.1). Adequate Mg maize 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.

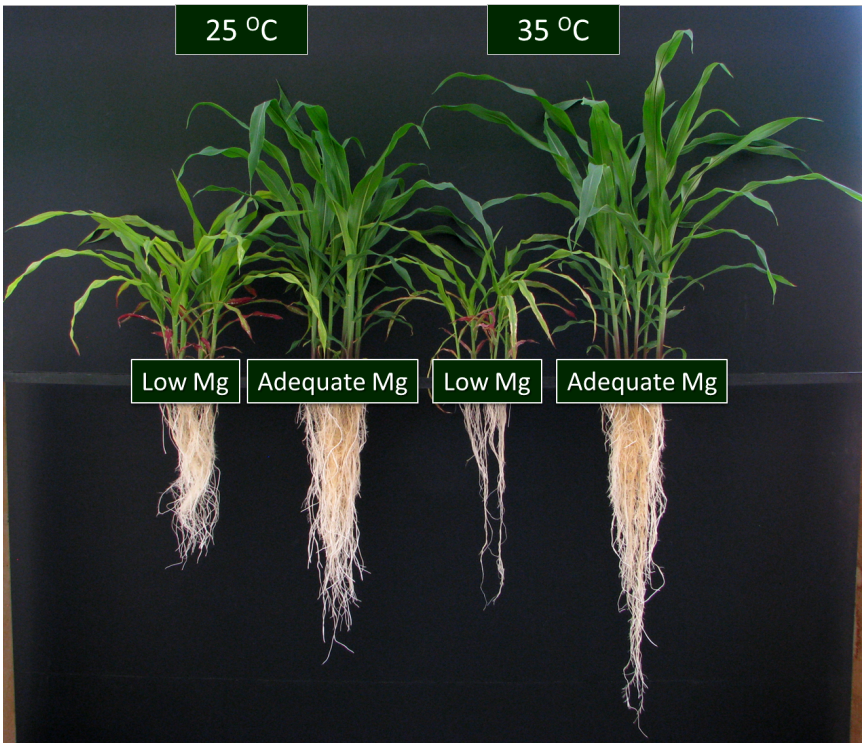



**Figure 1.5:** Growth of 23-day-old maize (*Zea mays* cv. Shemal) plants in nutrient solutions with low and adequate Mg supply at different temperatures.

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 1.2). 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. In maize, low Mg plants had 3-6 times lower Mg concentrations in their shoot and roots than adequate Mg plants. When the shoot and root Mg contents were considered, these differences were even more dramatic. Heat treatment did not affect the root Mg concentration or content of maize. In adequate Mg maize, higher temperature resulted in lower shoot Mg concentration but higher shoot Mg content.

**Table 1.2:** Shoot and root Mg concentrations and contents of 22-day-old wheat (*Triticum aestivum* cv. Adana 99) and 23-day-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.

**WHEAT**

| Temp. | Mg Supply | Mg Concentrations | | Mg Contents | | |
|---|---|---|---|---|---|---|
| | | Shoot | Root | Shoot | Root | Shoot+Root |
| | | (mg kg$^{-1}$) | | (µg plant$^{-1}$) | | |
| 25 °C | Low | 465 ± 27 | 587 ± 98 | 63 ± 11 | 25 ± 3 | 89 |
| | Adeq. | 1627 ± 89 | 1248 ± 295 | 266 ± 26 | 78 ± 24 | 344 |
| 35 °C | Low | 358 ± 5 | 612 ± 50 | 52 ± 7 | 21 ± 1 | 73 |
| | Adeq. | 1366 ± 37 | 1410 ± 88 | 225 ± 28 | 76 ± 5 | 302 |

**MAIZE**

| Temp. | Mg Supply | Mg Concentrations | | Mg Contents | | |
|---|---|---|---|---|---|---|
| | | Shoot | Root | Shoot | Root | Shoot+Root |
| | | (mg kg$^{-1}$) | | (µg plant$^{-1}$) | | |
| 25 °C | Low | 548 ± 64 | 1732 ± 292 | 688 ± 193 | 300 ± 20 | 988 |
| | Adeq. | 2028 ± 159 | 9829 ± 426 | 6044 ± 1070 | 8480 ± 1127 | 14524 |
| 35 °C | Low | 485 ± 39 | 2292 ± 121 | 649 ± 145 | 306 ± 32 | 955 |
| | Adeq. | 1437 ± 164 | 9242 ± 961 | 8118 ± 1158 | 8203 ± 1215 | 16322 |

**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.: HSD0.05 (Mg; Heat; MgxHeat) = (114; 114; 217)

Root Mg Conc.: HSD0.05 (Mg; Heat; MgxHeat) = (521; n.s; n.s)

Shoot Mg Cont.: HSD0.05 (Mg; Heat; MgxHeat) = (756; 756; 1443)

Root Mg Cont.: HSD0.05 (Mg; Heat; MgxHeat) = (786; n.s; n.s)

Table 1.3 shows protein concentration of leaves under given experimental conditions. Low Mg supply reduced the protein concentration by about 30-40% (Table 1.3). Heat treatment did not affect the protein concentration of wheat leaves significantly, whereas it resulted in about 25% lower protein concentration in maize leaves.

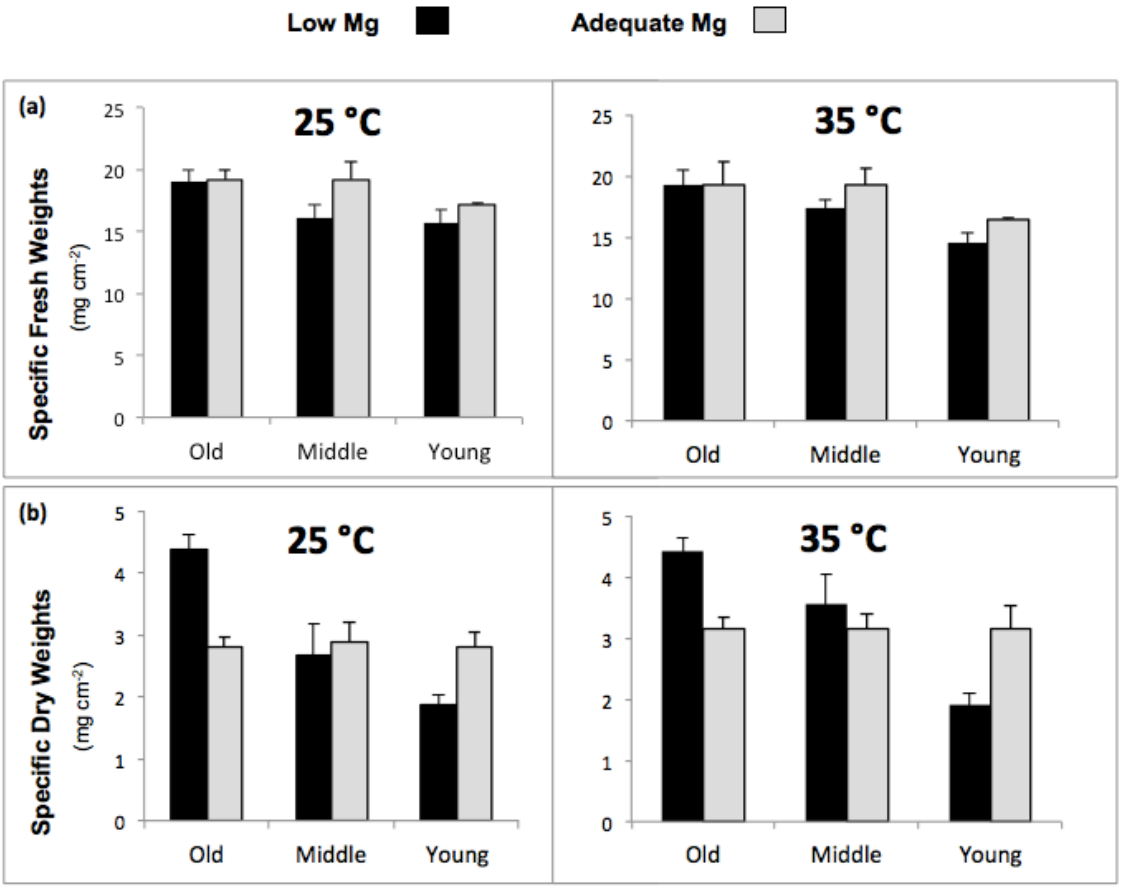
**Table 1.3**: Leaf protein concentrations of 22-day-old wheat (*Triticum aestivum* cv. Adana 99) and 23-day-old maize (*Zea mays* cv. Shemal) plants grown in nutrient solutions with low (15 µM for wheat, 20 µM for maize) or adequate (450 µM) Mg supply under different temperatures.

| Temperature | Mg Supply | Protein Concentration | |
|---|---|---|---|
| | | Wheat | Maize |
| | | (mg $g^{-1}$ FW) | |
| 25 °C | Low | 15 ± 1 | 9.0 ± 1.0 |
| | Adequate | 21 ± 3 | 13.0 ± 1.0 |
| 35 °C | Low | 14 ± 1 | 6.3 ± 0.2 |
| | Adequate | 22 ± 3 | 9.9 ± 1.5 |

**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*

The specific fresh and dry weights (mg $cm^{-2}$) of discs taken from old, middle-aged and young maize leaves are shown in Fig. 1.6. These measurements were made on only maize plants due to better suitability of the maize leaves. Under adequate Mg condition, the specific fresh and dry weights did not differ significantly depending on leaf age. In contrast, decreasing trends in specific weights were observed from oldest to youngest leaves under Mg deficiency. Notably, the specific dry weights of leaf discs taken from low Mg plants exhibited more distinct differences depending on leaf age than their specific fresh weights. Oldest leaves of low Mg plants had higher specific dry weights than those of adequate Mg plants, whereas youngest leaves of Mg-deficient plants had lower specific dry weights than those of adequate Mg plants. Per unit area, dry oldest leaves of low Mg plants were more than twice as heavy as their dry youngest leaves, probably due to higher amount of carbohydrates as discussed below.

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.

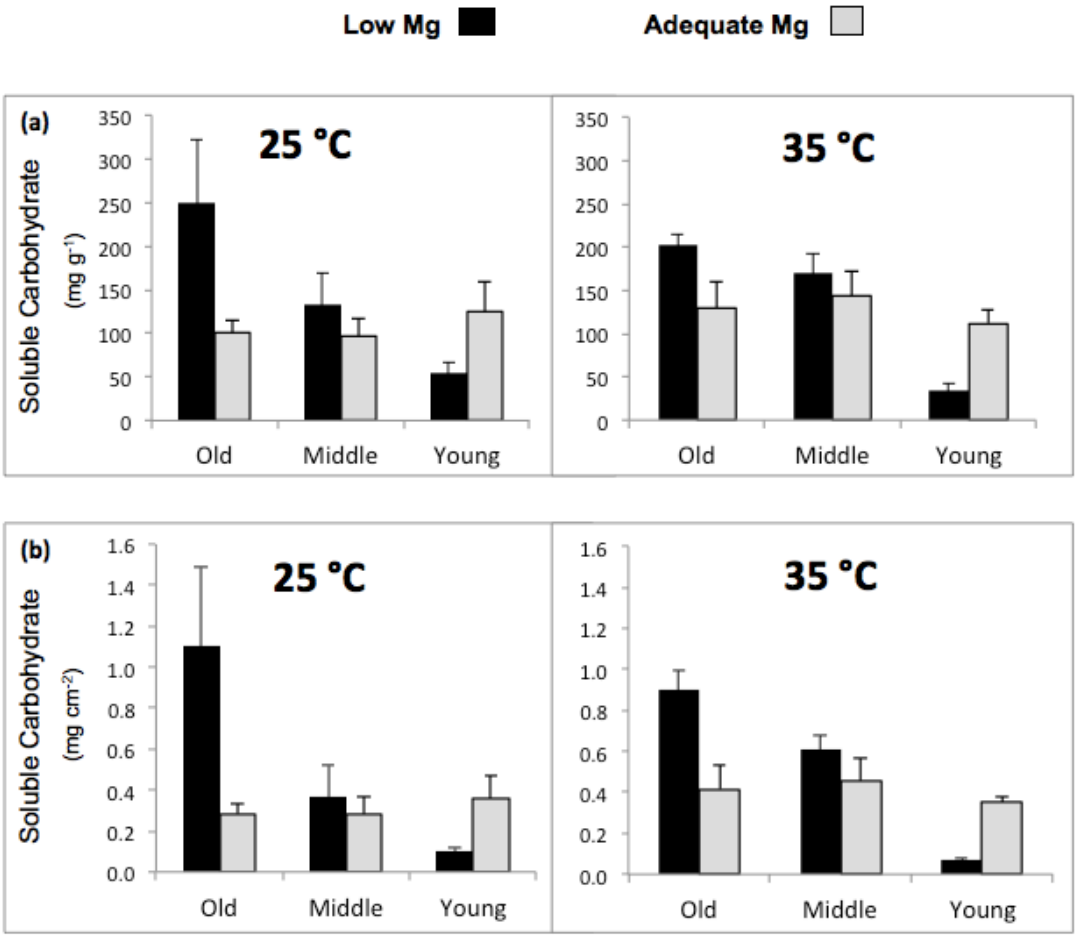



**Specific fresh weights:**
$HSD_{0.05}$ (Leaf; Mg; Heat; LeafxMg; LeafxHeat; MgxHeat; LeafxMgxHeat) = (0.8; 0.6; *n.s*; 1.4; *n.s* ; *n.s; n.s*)

**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)

**Figure 1.6:** Specific fresh weights (a) and dry weights (b) of old, middle and young leaves of 23-day-old maize (*Zea mays* cv. Shemal) plants grown in nutrient solutions with low (20 µM) or adequate (450 µM) Mg supply under different temperatures.

Soluble carbohydrates were analyzed in the 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 oldest, middle-aged and youngest leaves (Fig. 1.7).




**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*)

**Figure 1.7:** Soluble carbohydrate concentrations per mg g-1 (a) and mg cm-2 (b) of old, middle and young leaves of 23-day-old maize (*Zea mays* cv. Shemal) plants grown in nutrient solutions with low (20 µM) or adequate (450 µM) Mg supply under different temperatures.

In the youngest leaves, the concentration of soluble carbohydrates decreased markedly due to Mg deficiency (Figure 1.7). On the contrary, oldest leaves exhibited significant accumulation of soluble carbohydrates under the low Mg condition. Similar trends were observed at both 25ºC and 35ºC.

Table 1.4 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 superoxide dismutase (SOD) activity per g fresh sample was elevated in heat-treated wheat by Mg deficiency, though it was unaffected by Mg supply in 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 glutathione reductase (GR) and ascorbate peroxidase (APX) activities per both g fresh sample and mg protein. The effects of Mg deficiency on the specific activities of these antioxidative enzymes in wheat were potentiated by heat treatment. In the case of APX, low Mg supply almost doubled the specific activity at lower temperature, but more than tripled it when plants were subjected to heat stress. Heat treatment also caused the catalase (CAT) activity of wheat to increase significantly. However, CAT was the only antioxidative enzyme which appeared to have a lower activity in low-Mg wheat than in adequate-Mg wheat.

**Table 1.4**: Total activities and specific activities of superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), and catalase (CAT) in leaves of 22-day-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.

**Total Activity**

| Temp. | 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**

| Temp. | 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 |

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*)

SOD_Sp: $HSD_{0.05}$ (Mg; Heat; MgxHeat) = (0.74; *n.s*; 0.7)
GR_Sp: $HSD_{0.05}$ (Mg; Heat; MgxHeat) = (0.21; 0.21; 0.42)
APX_Sp: $HSD_{0.05}$ (Mg; Heat; MgxHeat) = (0.3; *n.s*; 0.6)
CAT_Sp: $HSD_{0.05}$ (Mg; Heat; MgxHeat) = (15; 15; *n.s*)

Table 1.5 shows that low Mg supply to maize enhanced the SOD activity per g fresh sample by 10% and the specific SOD activity by over 60%. 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. The GR activity of maize per g fresh sample was doubled under the low Mg conditions, and the specific GR activity was tripled. 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. Both low Mg and heat treatments had significant positive effects on the specific APX activity. The response of maize catalase to low Mg supply was remarkably different than the responses of other antioxidative enzymes and similar to the response of wheat catalase: It 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 catalase activity was particularly pronounced.

**Table 1.5**: Total activities and specific activities of superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), and catalase (CAT) in leaves of 23-day-old maize (*Zea mays* cv. Shemal) plants grown in nutrient solutions with low (20 µM) or adequate (450 µM) Mg supply under different temperatures.

**Total Activity**

| Temp. | 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**

| Temp. | 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 |

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)

SOD_Sp: $HSD_{0.05}$ (Mg; Heat; MgxHeat) = (1.4; 1.4*; n.s*)
GR_Sp: $HSD_{0.05}$ (Mg; Heat; MgxHeat) = (0.10; 0.10*; n.s*)
APX_Sp: $HSD_{0.05}$ (Mg; Heat; MgxHeat) = (0.5; 0.5*; n.s*)
CAT_Sp: $HSD_{0.05}$ (Mg; Heat; MgxHeat) = (11; 11*; 22*)

## C.2. Experiments on High Light Stress and Mg Nutrition

As expected, wheat plants under low Mg supply became chlorotic as a result of low Mg supply (Fig. 2.1A). The development of leaf chlorosis under Mg deficiency became stronger and quicker when maize or wheat plants exposed to high light intensity. Under given experimental conditions, Mg deficiency was clearer in case of wheat and therefore high light stress was more effective on wheat (Fig. 2.1).

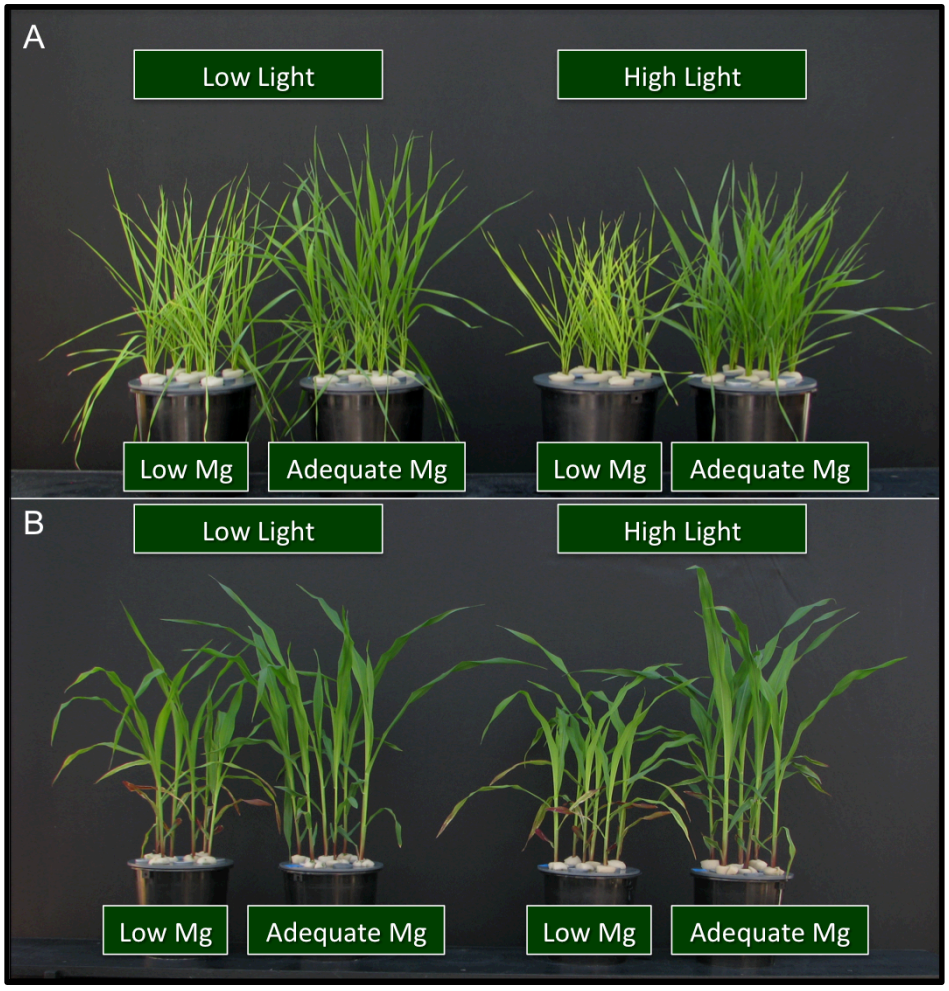
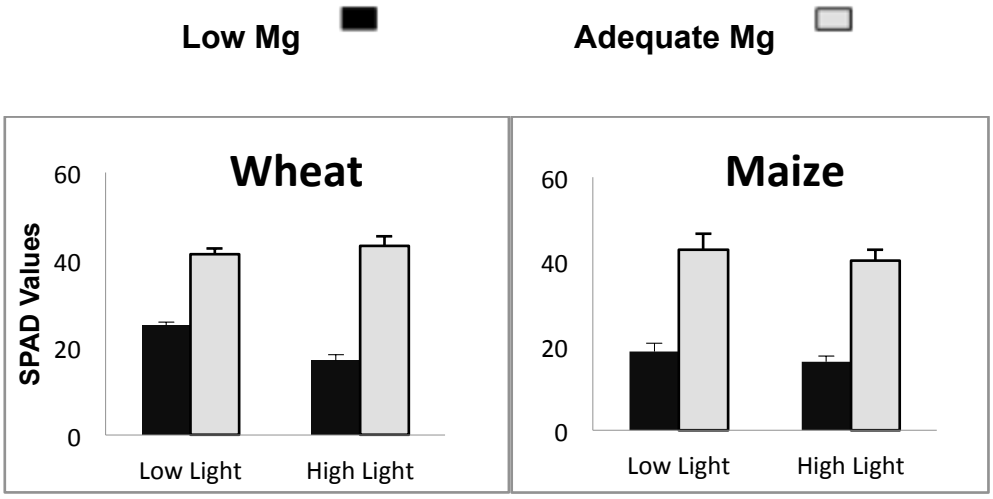



**Figure 2.1:** Growth of 29-day-old wheat (*Triticum aestivum* cv. Adana 99) and maize (*Zea mays* cv. Pioneer) plants in nutrient solutions with low and adequate Mg supply at different light intensities.

Fig. 2.2 shows the SPAD (chlorophyll) values of the wheat and maize leaves. The decrease in chlorophyll levels caused by low Mg supply, further lowered by the high light treatment, while in plants with adequate Mg supply, the chlorophyll values were not affected significantly. Consequently, the difference between the SPAD values of leaves were more pronounced in low Mg plants under high light. Under the adequate Mg condition, the chlorophyll levels were unaffected by high light.




Wheat: $HSD_{0.05}$ (Mg; Heat; MgxHeat) = (4; *n.s*; 7)
Maize: $HSD_{0.05}$ (Mg; Heat; MgxHeat) = (5;*n.s*; *n.s*)

**Figure 2.2:** SPAD (chlorophyll) values of the 29-day-old wheat (*Triticum aestivum* cv. Adana 99) maize (*Zea mays* cv. Pioneer) plants grown in nutrient solution with low (15 µM for wheat; 20 µM for maize) or adequate (450 µM) Mg supply under different light intensities.

In wheat, low Mg reduced the shoot growth on average by about 10% and the root growth by almost 50% (Table 2.1). Shoot dry weight of wheat plants increased by high light intensity irrespective of Mg supply while there was no significant difference in case of root dry weight (Table 2.1 and Fig. 2.3). This situation led to higher shoot-to-root ratio at low Mg supply in wheat.

**Table 2.1:** Effect of of low and high light treatments on the shoot and root dry weights (DW) and shoot-to-root ratios of 29-day-old wheat (*Triticum aestivum* cv. Adana 99) and maize (*Zea mays* cv. Pioneer) plants grown in nutrient solution with low (15 µM for wheat; 20 µM for maize) or adequate (450 µM) Mg supply.

**WHEAT**

| Treatment | Mg Supply | Shoot DW | Root DW | Shoot-Root Ratio |
|---|---|---|---|---|
| | | (mg plant$^{-1}$) | | |
| Low Light | Low | 198 ± 29 | 65 ± 7 | 3.0 ± 0.3 |
| | Adequate | 222 ± 27 | 121 ± 50 | 1.8 ± 0.2 |
| High Light | Low | 204 ± 15 | 68 ± 11 | 3.0 ± 1.2 |
| | Adequate | 352 ± 70 | 136 ± 45 | 2.6 ± 0.9 |

**MAIZE**

| Treatment | Mg Supply | Shoot DW | Root DW | Shoot-Root Ratio |
|---|---|---|---|---|
| | | (mg plant$^{-1}$) | | |
| Low Light | Low | 1804 ± 192 | 234 ± 32 | 7.7 ± 0.5 |
| | Adequate | 3252 ± 353 | 529 ± 25 | 6.2 ± 0.8 |
| High Light | Low | 2380 ± 219 | 360 ± 13 | 6.6 ± 0.6 |
| | Adequate | 4569 ± 724 | 891 ± 109 | 5.1 ± 0.3 |

**Wheat:**

Shoot DW: $HSD_{0.05}$ (Mg; Light; MgxLight) = (54; 54*; n.s*)

Root DW: $HSD_{0.05}$ (Mg; Light; MgxLight) = (46;*n.s;n.s*)

Shoot-Root Ratio: $HSD_{0.05}$ (Mg; Light; MgxLight) = (*n.s; n.s; n.s*)

**Maize:**

Shoot DW: HSD0.05 (Mg; Light; MgxLight) = (570; 570; n.s)

Root DW: HSD0.05 (Mg; Light; MgxLight) = (78; 78; 152)

Shoot-Root Ratio: HSD0.05 (Mg; Light; MgxLight) = (0.8; 0.8; n.s)


**Figure 2.3:** Root and shoot growth of 29-days-old wheat (*Triticum aestivum* cv. Adana 99) plants in nutrient solutions with low and adequate Mg supply and at different light intensities.

In the case of maize, regardless of the Mg supply, increasing light intensity enhanced shoot and root dry matter production; but, these increases were less pronounced in case of low Mg supply (Table 2.1 and Fig. 2.4). Also in this experiment, root growth was affected from low Mg supply at higher amount than the shoot growth, especially under high light intensity. Consequently, low Mg supply resulted in higher shoot-to-root ratio when compared to the plants with adequate Mg supply.

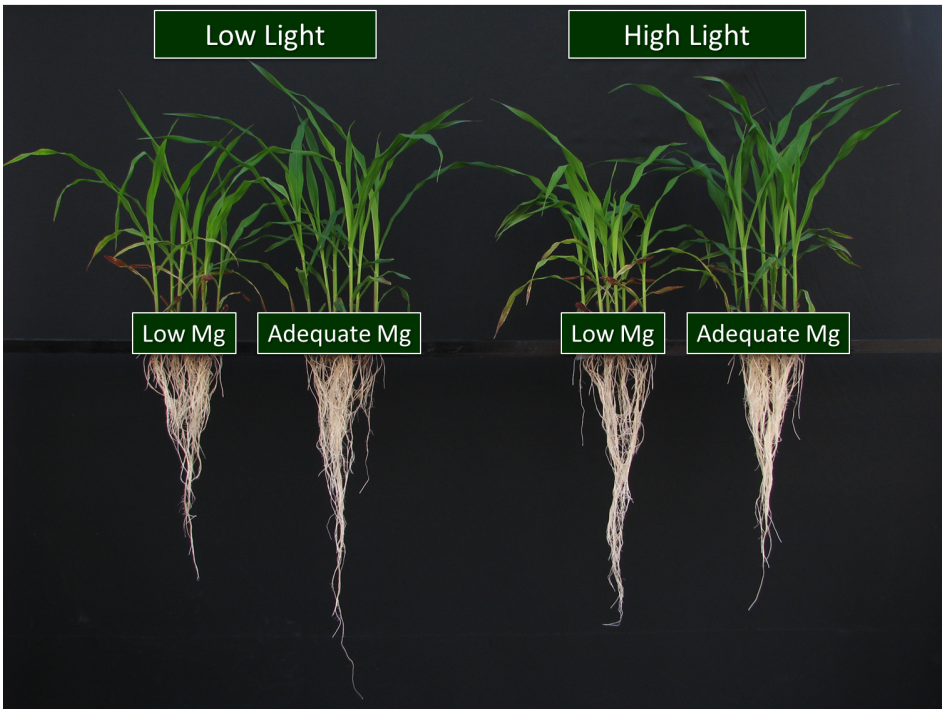



**Figure 2.4:** Root and shoot groth of 29-days-old maize (*Zea mays* cv. Pioneer) plants grown in nutrient solutions with low and adequate Mg supply and at different light intensities.

As expected, plants with adequate Mg supply had more Mg than the plants with low Mg supply under both light intensities (Table 2.2). The differences in shoot and root Mg concentrations between low and high Mg treatments were more obvious at high light intensity. High light intensity lowered the shoot Mg concentration and thus the shoot Mg content considerably in low Mg, but it did not have a significant effect on the root Mg concentration or content of wheat. In maize, low Mg plants had 3-7 times lower Mg concentrations in their shoot and roots than adequate Mg plants. When the shoot and root Mg contents were considered, these differences were even more dramatic. High light intensity resulted in decrease in Mg concentrations of shoot and root in maize. Despite Mg concentration was reduced by high light in low Mg plants, Mg content was increased again by high light intensity.

**Table 2.2:** Effect of low and high light intensity treatments on the shoot and root Mg concentrations and contents of 29-day-old wheat (*Triticum aestivum* cv. Adana 99) and (*Zea mays* cv. Pioneer) plants grown in nutrient solutions with low (15 µM for wheat; 20 µM for maize) or adequate (450 µM) Mg supply.

**WHEAT**

| | | Mg Concentrations | | Mg Contents | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Mg Supply** | **Shoot** | **Root** | **Shoot** | **Root** | **Shoot+Root** |
| | | ($mg\ kg^{-1}$) | | ($\mu g\ plant^{-1}$) | | |
| **Low Light** | **Low** | 470 ± 26 | 597 ± 30 | 93 ± 16 | 39 ± 4 | 132 |
| | **Adequate** | 1594 ± 30 | 1399 ± 245 | 353 ± 41 | 177 ± 106 | 531 |
| **High Light** | **Low** | 356 ± 53 | 565 ± 82 | 73 ± 16 | 38 ± 1 | 111 |
| | **Adequate** | 1428 ± 127 | 1821 ± 478 | 508 ± 138 | 261 ± 147 | 769 |

**MAIZE**

| | | Mg Concentrations | | Mg Contents | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Mg Supply** | **Shoot** | **Root** | **Shoot** | **Root** | **Shoot+Root** |
| | | ($mg\ kg^{-1}$) | | ($\mu g\ plant^{-1}$) | | |
| **Low Light** | **Low** | 683 ± 62 | 1149 ± 156 | 1234 ± 198 | 266.3 ± 15 | 1500 |
| | **Adequate** | 2807 ± 267 | 8180 ± 790 | 9068 ± 337 | 4316 ± 317 | 13384 |
| **High Light** | **Low** | 559 ± 10 | 1001 ± 48 | 1330 ± 125 | 361 ± 28 | 1691 |
| | **Adequate** | 1777 ± 252 | 6856 ± 1292 | 8007 ± 469 | 6070 ± 1159 | 14077 |

**Wheat:**
Shoot Mg Conc.: HSD0.05 (Mg; Light; MgxLight) = (95; 95; ,n.s)
Root Mg Conc.: HSD0.05 (Mg; Light; MgxLight) = (362; n.s; n.s)
Shoot Mg Cont.: HSD0.05 (Mg; Light; MgxLight) = (22; 22; n.s)
Root Mg Cont.: HSD0.05 (Mg; Light; MgxLight) = (13; n.s; n.s)
**Maize:**
Shoot Mg Conc.: HSD0.05 (Mg; Light; MgxLight) = (248; 248; 487)
Root Mg Conc.: HSD0.05 (Mg; Light; MgxLight) = (1014; n.s; n.s)
Shoot Mg Cont.: HSD0.05 (Mg; Light; MgxLight) = (415; 415; 815)
Root Mg Cont.: HSD0.05 (Mg; Light; MgxLight) = (800; 800; 1571)

In the leaves of both wheat and maize, where antioxidative enzyme activities were measured, high light intensity reduced the protein concentration by about 30-50% while Mg treatment did not have a significant effect on protein concentration (Table

2.3). Probably, enhancements in dry matter production by high ligh intensity (Table 2.1) contributed to reduction in protein concentration by causing dilution.

**Table 2.3:** Effect of low and high light treatments on the leaf protein concentrations of 29-day-old wheat (*Triticum aestivum* cv. Adana 99) and (*Zea mays* cv. Pioneer) plants grown in nutrient solutions with low (15 µM for wheat, 20 µM for maize) or adequate (450 µM) Mg supply.

| Treatment | Mg Supply | Protein Concentration | |
|---|---|---|---|
| | | Wheat | Maize |
| | | (mg $g^{-1}$ FW) | |
| Low Light | Low | 26.0 ± 4.9 | 11.2 ± 3.0 |
| | Adequate | 20.8 ± 2.5 | 10.6 ± 1.2 |
| High Light | Low | 14.0 ± 2.3 | 8.7 ± 1.2 |
| | Adequate | 14.3 ± 0.8 | 8.3 ± 0.9 |

Wheat: $HSD_{0.05}$ (Mg; Light; MgxLight) = (*n.s*; *n.s; n.s*)
Maize: $HSD_{0.05}$ (Mg; Light; MgxLight) = (*n.s*; 4.0*; n.s*)

Table 2.4 shows the effects of Mg and light treatments on the activities of selected antioxidative enzymes of wheat plants on both fresh weight and protein basis. The superoxide dismutase (SOD) activity per g fresh sample was elevated by Mg deficiency in wheat under both light intensities, and the highest activity was measured at high light intensity when Mg is deficient. Much stronger responses to high light intensity were observed in the case of specific SOD activity. High light resulted in increase of about 45% in the specific activity of SOD. Low Mg also enhanced the glutathione reductase (GR) activity under high light intensity both per both g fresh sample and mg protein (Table 2.4). Under low light intensity there is no remarkable change in the GR activity per g fresh sample whereas the specific activity of GR decreased about 8% by Mg supply. In the case of APX, low Mg supply did not have a remarkable effect on activity both per g fresh sample and specific activities at low light intensity while at high light intensityt it increased the activities by 30%.

On the contrary, high light intensity caused very distinct decreases in the catalase (CAT) activity per gr fresh sample of wheat. Similarly, also the protein based activity of CAT was lowered by Mg deficiency under both light intensities in wheat. The lowest CAT activities were observed in the Mg deficient wheat and maize under high light intensity (Table 2.4).

**Table 2.4:** Total and specific activities of superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), and catalase (CAT) in leaves of 29-day-old wheat (*Triticum aestivum* cv. Adana 99) plants grown in nutrient solutions with low (15 µM) or adequate (450 µM) Mg supply under different light intensities.

**Total Activity**

| Treatment | Mg Supply | SOD | GR | APX | CAT |
|---|---|---|---|---|---|
| | | (U $g^{-1}$ FW) | (µmol [NADPH] $g^{-1}$ FW $min^{-1}$) | (µmol $H_2O_2$ $g^{-1}$ FW $min^{-1}$) | |
| Low Light | Low | 134 ± 13 | 14.8 ± 0.7 | 38 ± 5 | 3529 ± 177 |
| | Adequate | 121 ± 12 | 13.0 ± 2.0 | 37 ± 5 | 3050 ± 64 |
| High Light | Low | 151 ± 3 | 22.2 ± 2.5 | 86 ± 6 | 1443 ± 246 |
| | Adequate | 148 ± 6 | 15.5 ± 1.1 | 62 ± 4 | 1929 ± 285 |

**Specific Activity**

| Treatment | Mg Supply | SOD | GR | APX | CAT |
|---|---|---|---|---|---|
| | | (U $mg^{-1}$ Prt.) | (µmol [NADPH] $mg^{-1}$ prt. $min^{-1}$) | (µmol $H_2O_2$ $mg^{-1}$ prt. $min^{-1}$) | |
| Low Light | Low | 5.2 ± 0.5 | 0.58 ± 0.09 | 0.8 ± 0.2 | 138 ± 20 |
| | Adequate | 5.8 ± 0.2 | 0.63 ± 0.05 | 0.9 ± 0.0 | 148 ± 14 |
| High Light | Low | 11.0 ± 1.8 | 1.65 ± 0.50 | 3.1 ± 0.4 | 104 ± 10 |
| | Adequate | 10.4 ± 0.8 | 1.09 ± 0.11 | 2.2 ± 0.1 | 135 ± 14 |

SOD: $HSD_{0.05}$ (Mg; Light; MgxLight) = (n.s; 13; *n.s*)
GR: $HSD_{0.05}$ (Mg; Light; MgxLight) = (2.9; 2.9; *n.s*)
APX: $HSD_{0.05}$ (Mg; Light; MgxLight) = (7; 7; 13)
CAT: $HSD_{0.05}$ (Mg; Light; MgxLight) = (*n.s*; 280; 550)

SOD_Sp: $HSD_{0.05}$ (Mg; Light; MgxLight) = (*n.s*; 1.3; *n.s*)
GR_Sp: $HSD_{0.05}$ (Mg; Light; MgxLight) = (*n.s*; 0.35; *n.s*)
APX_Sp: $HSD_{0.05}$ (Mg; Light; MgxLight) = (0.3; 0.3; 0.6)
CAT_Sp: $HSD_{0.05}$ (Mg; Light; MgxLight) = (20; 20; *n.s*)

In case of maize, low Mg supply enhanced the SOD activity per g fresh sample by 10-20% (Table 2.5). The specific SOD activity increased about 5% by Mg deficiency under low light intensity whereas under high light intensity, the activity increased by 20%. The GR activity per g fresh sample was enhanced by Mg deficiency in maize under both light intensity, and the largest activities were measured at high light intensity when Mg is applied at low level. The same trend was also observed in the activities of APX. Again, low Mg supply resulted in significant increase in both g fresh sample- and protein-based acitvities of APX at both light intensities, and the greatest activities were measured in Mg deficient plants under high light intensity. As in the case of wheat, the response of catalase to low Mg supply in maize was remarkably different than the responses of other antioxidative enzymes. Although CAT activity per g fresh sample increased about 5% by low Mg supply at low light, this change was not observed in the protein based activity of CAT. Magnesium deficiency caused both fresh weight- and protein-based activities of CAT to dramatically and reduced activity by about 50% under high light intensity.

**Table 2.5:** Total activities and specific activities of superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), and catalase (CAT) in leaves of 29-day-old maize (*Zea mays* cv. Pioneer) plants grown in nutrient solutions with low (20 µM) or adequate (450 µM) Mg supply under different light intensities.

**Total Activity**

| Treatment | Mg Supply | SOD | GR | APX | CAT |
|---|---|---|---|---|---|
| | | (U $g^{-1}$ FW) | (µmol [NADPH] $g^{-1}$ FW $min^{-1}$) | (µmol $H_2O_2$ $g^{-1}$ FW $min^{-1}$) | |
| Low Light | Low | 93 ± 18 | 4.3 ± 1.2 | 28 ± 4 | 711 ± 172 |
| | Adequate | 87 ± 13 | 3.1 ± 0.5 | 17 ± 3 | 686 ± 139 |
| High Light | Low | 127 ± 19 | 9.7 ± 2.0 | 32 ± 4 | 219 ± 44 |
| | Adequate | 99 ± 14 | 5.2 ± 0.8 | 13 ± 2 | 458 ± 106 |

**Specific Activity**

| Treatment | Mg Supply | SOD | GR | APX | CAT |
|---|---|---|---|---|---|
| | | (U $mg^{-1}$ Prt.) | (µmol [NADPH] $mg^{-1}$ prt. $min^{-1}$) | (µmol $H_2O_2$ $mg^{-1}$ prt. $min^{-1}$) | |
| Low Light | Low | 8.5 ± 0.9 | 0.39 ± 0.06 | 1.3 ± 0.2 | 64 ± 3 |
| | Adequate | 8.2 ± 0.3 | 0.29 ± 0.05 | 0.8 ± 0.2 | 65 ± 9 |
| High Light | Low | 14.6 ± 0.3 | 1.10 ± 0.11 | 1.8 ± 0.1 | 25 ± 3 |
| | Adequate | 11.8 ± 0.9 | 0.62 ± 0.04 | 0.8 ± 0.1 | 55 ± 9 |

SOD: $HSD_{0.05}$ (Mg; Light; MgxLight) = (n.s; 22; *n.s*)
GR: $HSD_{0.05}$ (Mg; Light; MgxLight) = (1.7; 1.7; n.s)
APX: $HSD_{0.05}$ (Mg; Light; MgxLight) = (5; 5; 9)
CAT: $HSD_{0.05}$ (Mg; Light; MgxLight) = (*n.s*; 166; n.s)

SOD_Sp: $HSD_{0.05}$ (Mg; Light; MgxLight) = (0.9; 0.9; 1.8)
GR_Sp: $HSD_{0.05}$ (Mg; Light; MgxLight) = (0.09; 0.09; 0.19)
APX_Sp: $HSD_{0.05}$ (Mg; Light; MgxLight) = (0.21; 0.21; 0.42)
CAT_Sp: $HSD_{0.05}$ (Mg; Light; MgxLight) = (9; 9; 18)

# (D) DISCUSSION AND CONCLUSIONS

## D.1. Discussion

Interveinal chlorosis is a typical symptom of Mg deficiency in crops (Marschner and Cakmak 1989; Hermans et al. 2005; Tewari et al. 2006). Usually, chlorosis due to Mg deficiency appears first in older source leaves, which is commonly explained by the relatively high mobility of Mg (Bergmann 1992; Marschner 2012). In agreement with the literature, in both experiments, leaves of wheat and maize plants turned chlorotic under Mg-deficient conditions (Figs. 1.1, 1.2, 1.3, 2.1 and 2.2). Under Mg deficiency, younger leaves remain green while older leaves show chlorosis, which clearly indicate remobilization of limited Mg resources from source (older leaves) to sink organs (younger leaves).

Numerous reports indicate that nutritional disorders impair the tolerance of plants to environmental stress factors (Cakmak 2005; Marschner 2012; Waraich et al. 2012). High light intensity was shown to enhance chlorosis and necrosis in not only Mg-deficient but also K- or Zn-deficient bean plants by causing increased photooxidative damage (Marschner and Cakmak 1989; Cakmak and Marschner 1992). In the present study, under Mg deficiency, high light intensity also reduced chlorophyll levels of wheat and maize (Figs. 2.1 and 2.2). Plants under low Mg supply became rapidly chlorotic when exposed to high light. Heat treatment also aggravated visual stress symptoms observed in Mg-deficient plants, but it did not result in chlorosis when Mg supply was adequate (Figs. 1.1, 1.2 and 1.3). Plants under low Mg supply were visually more damaged under higher temperature and higher light intensity, while plants with adequate Mg supply were not affected. These observations suggest that Mg deficiency increases sensitivity of both cereal crops to heat and high light stresses.

Generally, growth of plants under low Mg supply remained similar or showed slight increases under high light or heat treatment, while plants under adequate Mg showed very obvious increases in growth, especially maize (Figs. 1.1B, 1.5, 2.1B and 2.4). The particular stimulation of maize growth by higher temperature and higher light intensity under adequate Mg supply 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 and light in terms of plant growth due to their higher optimum growth temperature and light intensity (Berry and Bjorkman 1980; Edwards and Walker 1983). However, as mentioned, Mg-deficient maize plants did not respond positively to temperature increase (Table 1.1) and showed more severe stress symptoms by increasing temperature (Figs. 1.1B and 1.2B). Similarly, positive growth response of maize to high light intensity decreased by 5% when Mg is deficient (Fig. 2.1B and Table 2.1). These observations clearly indicate that the susceptibility of plants to heat and light 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; Tang et al. 2012).

As expected, dry matter production of plants, in both heat and light experiments, was affected by Mg deficiency. Root growth was more sensitive to Mg deficiency than shoot growth in wheat, which resulted in significantly higher shoot-to-root ratios under Mg deficiency (Tables 1.1 and 2.1; Figs. 1.4, 1.5, 2.3 and 2.4). Maize plants also exhibited markedly higher shoot-to-root ratios in response to Mg deficiency. Although some contradictory studies in literature showed a stronger impact of Mg deficiency on shoot than root growth in *Arabidopsis thaliana* (Hermans and Verbruggen 2005) and sugar beet (Hermans et al. 2005), the majority of reports, in agreement with the results presented here, indicated the opposite: 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 (Cakmak et al. 1994a), birch (Ericsson and Kahr 1995), spinach (Fischer et al. 1998), pepper (Riga and Anza 2003) and citrus (Yang et al. 2012). The relatively higher sensitivity of root growth to Mg deficiency is most probably related to restricted carbohydrate supply from source leaves to sink organs including roots, as discussed by Cakmak and Kirkby (2008). Also,

growing plants under low light reduces photosynthesis and plant growth, particularly root growth (Van den Driessche 1987; Grechi et al. 2007) that was also observed in wheat and maize plants in case of root growth.

Heat or light treatment of low Mg wheat or maize plants did not reduce their shoot biomass at the time of harvest (Tables 1.1 and 2.1), although these plants appeared smaller than non-treated ones (Figs. 1.1 and 2.1). In contrast, the root growth of low Mg plants was clearly reduced by heat treatment (Figs. 1.4 and 1.5). 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 and also high light stress on root growth might be more pronounced when plants are simultaneously exposed to Mg deficiency. The results presented in Table 1.1 are in well agreement with this suggestion, especially in the case of maize plants.

When the light intensity is higher, dry matter production of wheat and maize plants increased regardless of Mg supply, most probably because of the high photosynthesis rate (Table 2.1), which was also previously observed by Marschner and Cakmak (1989). Despite the root dry matter was not affected by different light treatments in wheat under given experimental conditions; maize exhibited elevated root dry weights by high light treatment at both low and adequate Mg supply. These observations can be explained by the fact that maize is a $C_4$ species in contrast to wheat, which is a $C_3$ species. The $C_4$ photosynthetic pathway evolved as an adaptation to high temperature, high light intensity and limited water availability (Edwards et al. 2010).

Thus, under high light intensity more amounts of photoassimilates produced in maize could be transported to roots (Table 2.1 and Fig. 2.4). However, Mg-deficient maize did not respond positively to temperature increase (Table 1.1) and showed more severe stress symptoms (Figs. 1.1B, 2.1B and 1.5), again suggesting that Mg deficiency may lower the heat stress threshold of crops.

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 Tables 1.2 and 2.2, in both heat and high light experiments, the Mg concentrations in the shoots of both wheat and maize plants were sufficiently high when Mg supply was adequate, but below the critical 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 1.2). 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 most likely due to dilution. High light lowered the shoot Mg concentration in maize and wheat regardless of the Mg supply (Table 2.2). Magnesium content of wheat increased in adequate Mg conditions by high light intensity while Mg content of maize decreased by high light when Mg is adequate meaning there is a dilution effect.

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. 1.4). An intensive accumulation of starch in Mg-deficient source leaves is a well-known phenomenon (Cakmak et al. 1994a; Hermans and Verbruggen 2005). On the contrary, 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). In agreement with these findings, the soluble carbohydrate concentration increased in source leaves of low Mg maize plants but decreased in sink leaves (Fig. 1.5). 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. 1.5a). When the soluble carbohydrate concentrations are calculated per unit of dry leaf area, the difference in soluble carbohydrates in source and sink leaves mentioned between low and adequate Mg plants becomes more pronounced (Fig. 1.5b). It is obvious that there is a strong inhibition in translocation of photosynthates from source into young leaf tissue. When low Mg plants were heat-treated, the concentration of soluble carbohydrates reached an even higher level in middle leaves and decreased to an even lower level in youngest leaves. Higher soluble carbohydrate concentrations measured in the source leaves of adequate Mg plants at high temperature may be related to increased photosynthetic activity of Mg-sufficient maize at high temperature (Crafts-Brandner and Salvucci 2002).

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.

Magnesium is required to stabilize polyphosphate compounds including ATP, pyrophosphate ($PP_i$) and RNA by neutralizing the negative charges (Cole and Schimmel 1970). The involvement of Mg in structural stability and proper functioning of ribosome (Shenvi et al. 2005), transcription by DNA-dependent RNA polymerase (Sosunov et al. 2003) and amino acid activation by aminoacyl tRNA synthetase (Cole and Schimmel 1970) renders it essential for protein synthesis. Deficiency of Mg is known to impair protein synthesis (Marschner 2012). In agreement with the literature, both wheat and maize leaves had significantly lower levels of protein under Mg-deficient conditions in heat experiment (Table 1.3). Heat stress caused further reductions in protein concentrations of maize leaves. Negative effects of heat stress on total protein concentration were also demonstrated in other cultivated species including strawberry (Gulen and Eris 2004) and bentgrass (He et al. 2005). Although in light experiment the concentrations of protein were not much affected by Mg deficiency (probably due to less severity of Mg deficiency stress in this experiment), high light treatment, however, leads protein concentrations to decrease in both low and adequate Mg supply in maize and wheat which is probably due to higher production of ROS under high light intensity which is known with its oxidizing effect on the proteins in the cell (Cabiscol et al. 2000; Mittler 2002). High light stress inducing photoinhibition and protein degradation of photosystem I in *Brassica rapa* was also found in the study with rapeseed plants by Jiao (2004).

Magnesium deficiency, heat stress and high light intensity are known to induce the production of ROS (Cakmak and Marschner 1992; Suzuki and Mittler 2006; Cakmak and Kirkby 2008). Under these stresses, the level of oxidative damage maintained by ROS depends on light intensity and pronounced in chloroplasts (Cakmak and Marschner 1992; Larkindale and Knight 2002; Yamamoto et al. 2008). Light-dependent cell damage in chloroplasts is called "photooxidative damage". Heat-induced oxidative stress also targets the mitochondria where the respiration is enhanced and the electron transport chain is disrupted leading generation of more ROS in mitochondria (Suzuki and Mittler 2006; Wahid et al. 2007). Similarly also in peroxisomes, heat stress elevated levels of $H_2O_2$ as a consequence of heat-induced photorespiration (Suzuki and Mittler 2006; Wahid et al. 2007; Farooq et al. 2011).

In both heat and light experiments, Mg deficiency enhanced the specific SOD, APX and GR activities in wheat and maize except for the low light condition in wheat

(Tables 1.4, 1.5, 2.4 and 2.5). Increases of the activities of antioxidative enzymes are in agreement with previous reports on common bean (Cakmak and Marschner 1992), maize (Tewari et al. 2004), pepper (Riga et al. 2005) and citrus (Tang et al. 2012). Enhancement in specific SOD activities were pronounced under heat-stress and high light treatment, suggesting an increased requirement for superoxide scavenging in chloroplasts and possibly also other compartments where SOD isozymes are found such as mitochondria and the cytosol (Scandalios 1993). SOD activity has been also reported to be enhanced in plants subjected to different abiotic stress conditions such as high light, drought and metal toxicity (Cakmak and Marschner 1992; Sharma and Dubey 2005; Mishra et al. 2011). This high activity of SOD is an indicator of enhanced oxidative stress tolerance in plants (Gupta et al. 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 under heat or high light stress (Tables 1.4, 1.5, 2.4 and 2.5). Increased activities of APX and GR were also reported as a response to environmental stresses such as high light, drought, salinity, chilling and UV irradiation (Cakmak and Marschner 1992; Boo and Jung 1999; Sharma and Dubey 2005; Han et al. 2009; Hefny and Abdel-Kader 2009). These results indicate that generation of ROS in Mg-deficient leaf tissue is probably promoted under heat and high light treatment, which might be the reason why plants with low Mg supply appeared more damaged with heat and high light treatment (Figs. 1.1, 1.2 and 2.1).

The protective role of CAT in peroxisomes against free radicals is limited due to its low affinity to $H_2O_2$ and high sensitivity to light induced inactivation (Cakmak and Marschner 1992; Foyer et al. 1994). It can be the reason that high light intensity significantly reduced CAT activity in wheat and maize, and the lowest CAT activities were observed under Mg deficiency (Tables 2.4 and 2.5). 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). Enhanced UV-B (30%) was also found to be the triggering reason of high activities of SOD, APX and CAT (Han et al. 2009). 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: CAT 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 1.4, 1.5, 2.4 and 2.5), especially in heat and high light treated maize plants. This may be an indicator of aggravated photooxidative damage in Mg-deficient and heat or high light treated maize.

## D.2. Conclusions

Heat stress and high light intensity are 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 and light-related impairments in plant growth and developments (Asseng et al. 2011; Zheng et al. 2012; Chapman et al. 2012). This study showed that adequate Mg supply is needed to minimize leaf damage and growth loss associated with heat stress and high light intensity. It is known that Mg deficiency is becoming an important mineral nutrient deficiency in agricultural soils globally, particularly in acidic sandy soils and under intensive crop production systems leading to Mg depletion in the soil profile. Detrimental effects of heat and high light stress on wheat and maize plants, particularly decreases in root growth are pronounced when plants are simultaneously exposed to low Mg supply. Very early impairments in root growth and related decline in root surface by Mg deficiency under stress conditions may have serious impacts on the acquisition of mineral nutrients and uptake of water by roots, especially under water-limited and nutrient-deficient soil conditions. Decreases in root growth may also impair root adaptation to problem soils such as Al-toxic soils. Therefore, ensuring better Mg nutrition of plants under stress conditions is an important agronomic practice. Late foliar application of Mg (just before or after flowering) might be also very important to guarantee efficient retranslocation of carbohydrates into harvest products (e.g. grains, fruits, tubers) and thus for maintenance of high yield. The maintenance of a sufficient Mg supply represents an effective nutritional strategy to minimize heat and high light stress-related losses in crop production.

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