CHANGES IN GROWTH AND MAGNESIUM CONCENTRATION OF WHEAT
AND COFFEE PLANTS GROWN UNDER VARIOUS MAGNESIUM AND WATER
STRESS TREATMENTS

by

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COFFEE PLANTS GROWN UNDER VARIOUS MAGNESIUM AND WATER STRESS
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ABSTRACT

CHANGES IN GROWTH AND MAGNESIUM CONCENTRATION OF WHEAT AND COFFEE PLANTS GROWN UNDER VARIOUS MAGNESIUM AND WATER STRESS TREATMENTS

YASEMİN CEYLAN

Magnesium (Mg) deficiency has become a widespread problem in acidic and sandy agricultural soils, and it is often associated with marginal soil conditions such as drought stress. Impairment in growth and development of sink organs is a common consequence of Mg deficiency. However, mode of action of these impairments is not well understood. This study was conducted to investigate the changes in growth and Mg concentrations of wheat (*Triticum aestivum* cv. Adana99) and coffee (*Coffea arabica* cv. Murta) plants that were grown under controlled greenhouse conditions with different Mg supplies and water stress treatments. Growing wheat plants under varied Mg supply showed that foliar application of Mg to low Mg plants improved grain yield by increasing seed weight without affecting seed number per spike. Starch content and Mg concentration of the seeds were increased under foliar application of Mg to Mg-deficient plants. Growth and grain yield of low Mg plants were further reduced when grown under drought stress. An adequate Mg supply was needed to maintain better yield and higher grain Mg concentrations under drought. In experiment with coffee plants, Mg transport within plants was studied after the immersion of the fully expanded young leaves in a solution containing stable Mg isotope (^{26}Mg). Transport of ^{26}Mg from treated leaves was greater in plants with adequate Mg supply than the plants with low Mg. In addition, under low Mg supply ^{26}Mg concentration of roots was found higher when compared to Mg-adequate roots. The results obtained highlighted the importance of Mg in growth and seed formation and accumulation of Mg in sink organs such as seed and young leaves after foliar treatment of Mg.

ÖZET

ÇEŞİTLİ MAGNEZYUM VE SU STRESİ UYGULAMALARI ALTINDA YETİŞTİRİLEN BUĞDAY VE KAHVE BİTKİLERİNİN BÜYÜME VE MAGNEZYUM KONSANTRASYONLARININ DEĞİŞİMİ

YASEMİN CEYLAN

Magnesium (Mg) eksikliği asidik ve kumlu bünyeye sahip topraklarda yaygın bir problem olarak ortaya çıkmaktadır ve özellikle kuraklık stresi gibi marjinal toprak koşullarında daha sık görülmektedir. Gelişmekte olan organların büyümesinin ve gelişiminin bozulması Mg eksikliğinde sıklıkla görülen bir problemdir. Anılan problemlerin ortaya çıkış mekanizması iyi anlaşılamamıştır. Bu tez çalışması kontrollü sera koşulları altında değişik Mg uygulamaları ve su stresi koşullarında yetiştirilen buğday (*Triticum aestivum* cv. Adana99) ve kahve (*Coffea arabica* cv. Murta) bitkilerinin büyüme ve Mg konsantrasyonlarını araştırmak amacıyla yürütülmüştür. Düşük Mg ile beslenen bitkilere püskürtme yoluyla yapraktan uygulanan Mg, buğday başağındaki dane sayısını etkilememiş ancak bireysel dane ağırlığını arttırmıştır. Magnezyum eksikliği altındaki bitkilere püskürtülerek uygulanan Mg, tohumların nişasta içeriğini ve Mg konsantrasyonunu yükseltmiştir. Kuraklık koşulları altında yetersiz Mg ile yetiştirilen bitkilerin büyümesi ve tane verimi azalmıştır. Sonuçlar, yeterli düzeyde Mg beslenmesinin kuraklık koşulları altında daha iyi verim ve yüksek Mg konsantrasyonu elde etmek için gerekli olduğunu göstermektedir. Kahve bitkilerinde Mg taşınımı, gelişimini tamamlamış genç yapraklara daldırma yöntemi ile stabil Mg izotop (^{26}Mg) çözeltisi uygulanarak araştırılmıştır. ^{26}Mg taşınımı, yeterli Mg içeren bitkilerde düşük Mg içeren bitkilere kıyasla daha yüksek bulunmuştur. Ancak, köklerde Mg noksanlığı durumunda daha fazla ^{26}Mg bulunmuştur. Elde edilen sonuçlar yapraktan veya topraktan yapılan Mg beslenmesinin bitkilerin büyümesi ve tohum oluşumunda ve generative organlarda (örneğin tohumda) Mg birikimi üzerinde öenmli olduğunu göstermektedir.

Bu çalışmayı sevgili annem ve babam, **Hülya** ve **İbrahim Ceylan'a** ithaf ediyorum…
Her şey onların sonsuz sevgisi ve desteği sayesindedir.

…ve hayat arkadaşım, eşim, **Emre'ye**
Aşkına ve sabrına hayranım. İyi ki varsın.

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Al .... aluminium

Adeq. .... adequate

ADP .... adenosine diphosphate

ANOVA .... analysis of variance

At .... atom percent

ATP .... adenosine triphosphate

B .... boron

°C .... degrees celcius

C_3. .... three-carbon organic acids

C_4. .... four-carbon organic acids

ca .... circa (approximaltely)

Ca .... calcium

$CaCl_2$ .... calcium chloride

$CaCO_3$ .... calcium carbonate

$CaH_4O_8P_2.H_2O$ .... calcium tetrahydrogenbisphosphate monohydrate

CAM .... crassulacean acid metabolism

$CaMg(CO_3)_2$ .... calcium magnesium carbonate (dolomite)

$Ca(NO_3)_2.4H_2O$ .... calcium nitrate tetrahydrate

$CaSO_4.2H_2O$ .... calcium sulfate dihydrate

Cl .... chloride

CO_2 .... carbon dioxide

Cu .... copper

$CuSO_4.5H_2O$ .... copper sulfate pentahydrate

cv .... cultivar

DAS .... days after sowing

dH_2O .... distilled water

ddH_2O .... double distilled water

DNA .... deoxyribonucleic acid

DW .... dry weight

ECF. .... European coffee federation

ml .... milliliter

µmol .... micro mol

mM .... millimolar

µM .... micromolar

Mn .... manganase

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

$MnSO_4.4H_2O$ .... manganese sulfate tetrahydrate

Mo .... molybdenum

N .... nitrogen

n.d .... no date

NH_4Ac .... ammonium acetate

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

$(NH_4)_2SO_4.$ .... ammonium sulfate

n.s .... non significant

$^1O_2$ .... singlet oxygen

O_2 .... oxygen

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

$OH^.$ .... hydroxyl radicals

P .... phosphorus

PEP .... phosphophenol pyruvate

ppm .... parts per million

ROS .... reactive oxygen species

Rubisco .... ribulose-1,5-bisphosphate carboxylase/oxygenase

RuBP .... ribulose bisphosphate

s .... second

S .... sulfur

WHO .... world health organization

Zn .... zinc

$ZnSO_4.7H_2O$ .... zinc sulfate heptahydrate

A) GENERAL INTRODUCTION

A.1 Roles of Magnesium in plants

Magnesium (Mg) is one of the essential macronutrients which is taken up in large amounts by plants to sustain their growth and development (Williams and Salt, 2009). Magnesium is a divalent cation and it is the most abundant free cation in the cytosol of plants (Shaul, 2002). As the central atom of the chlorophyll molecule (Marschner, 2012), Mg greatly contributes to the absorption of light energy and its utilization in the photosynthesis (Cowan, 2002). Mg is exceptional in terms of its effect on the enzymes; it activates a greater number of enzymes than any other mineral nutrient element (Epstein and Bloom, 2004). Phosphoenolpyruvate (PEP) carboxylase which is in charge for the initial fixation of CO_2 in C_4 and CAM plants, and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) which is the key enzyme in the carboxylation step in the Calvin cycle are examples of crucial enzymes activated by Mg in the photosynthetic machinery (Wedding and Black, 1988; Portis, 1992). Low photosynthetic activity of Mg-deficient leaves is widely ascribed to reduced activity of the Rubisco enzyme (Cakmak and Kirkby, 2008).

According to Karley and White (2009), most of the Mg in leaves of plants is associated with protein biosynthesis, remaining portions of it found in chlorophyll pigments or stored in vacuole. Sufficient activity of nucleic acid synthesizing polymerases and nucleases are dependent on adequate Mg supply (Sreedhara and Cowan, 2002). Magnesium is also needed for protein synthesis because of its bridging role in aggregation of subunits of ribosomes (Marschner, 2012; Fischer et al., 1998).

Magnesium is a necessary element in both synthesis and function of ATP, and therefore ATP requiring mechanisms in plants are also dependent on Mg (Ko et al., 1999; Igamberdiev and Kleczkowski, 2001). The proton pump, H^+-ATPase that is

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located in the plasma membrane of sieve tube cells is dependent on Mg-ATP complex to generate the electrochemical proton gradient to drive the phloem loading of sucrose (Bush, 1989). In other words, Mg plays a critical role in phloem loading of sucrose.

In addition to its other physiological functions, Mg has a crucial role in mitigating stress factors such as aluminum (Al) toxicity (Tan et al., 1992; Silva et al., 2001; Ryan et al., 1994; Yang et al., 2007). According to Bose et al (2011), an adequate Mg nutrition mitigates Al toxicity in plants in various ways including i) better transport of photoassimilates from shoots to roots, ii) increasing H^+-ATPase activity that is needed for release of organic acids from roots to inactivate Al, and iii) improving antioxidative defense system against Al-toxicity-induced free radical generation. Also, supplying sufficient amount of Mg to plants reduces the oxidative stress, especially under conditions of high light or heat stress, by maintaining the phloem loading of sucrose and preventing the carbohydrate accumulation in leaves (Cakmak and Kirkby, 2008; Mengütay et al., 2013).

A.2 Magnesium deficiency-related problems in plants

Numerous physiological impairments occur in plants exposed to Mg deficiency. The most typical visual symptom of Mg deficiency is leaf chlorosis (Marschner, 2012). Because Mg is the central atom in chlorophyll structure, lack of it damages the chlorophyll molecule and leads to the creation of chlorosis and even necrosis. Magnesium deficient plants are also highly sensitive to high light intensity (Marschner and Cakmak, 1989); therefore development of leaf chlorosis and necrosis is also affected from the light intensity under low Mg supply.

Since Mg acts as the cofactor or activator of many photosynthetic enzymes, various studies have shown that under low Mg conditions, the rate of photosynthesis is dramatically reduced (Fischer and Bremer, 1993; Laing et al., 2000; Hermans et al., 2004). Magnesium deficiency-related loss of chlorophyll also contributes to reduced photosynthetic activity (Peaslee and Moss, 1966). Low activity of photosynthesis could be also a consequence of increased mesophyll resistance to CO_2 flux into chloroplasts from atmosphere as shown in pine seedlings (Laing et al., 2000).

Under Mg-deficient conditions, due to disrupted photosynthetic capacity and rate, plants obtain more light energy than required for photosynthesis and other related processes. So high-energy electrons accumulate and enhance the generation of reactive

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oxygen species (ROS) in forms of superoxide ($O_2.-$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) (Asada, 1994). Small concentrations of ROS can be detoxified by the plant itself, but when it is produced in high concentrations it cannot be scavenged properly. This uncontrolled production of ROS damages chlorophyll, phospholipids, proteins and DNA, causes severe alterations in chloroplast structure, impairs the functional stability of biological membranes, and disrupts photosynthetic enzymes (Asada, 2006; Cakmak and Kirkby, 2008).

Due to its fundamental role in phloem loading of sucrose, Mg is critical for carbohydrate partitioning between source and sink organs (Cakmak et al., 1994a,b; Marschner et al., 1996; Hermans et al., 2005). When Mg is deficient, the carbohydrate transportation process is impaired, leading to accumulation of carbohydrates begins in source tissues. Consequently, newly growing parts of the plant cannot get sufficient amount of photoassimilates and eventually this situation leads to a reduction in growth and development of sink organs such as roots, tubers, shoot tips and seeds (Hermans et al., 2004; Hermans and Verbruggen, 2005; Mengütay et al., 2013). Under low supply of Mg, reduction in the root growth is often more pronounced than the reduction in shoot growth, resulting in higher shoot-to-root ratio (Cakmak et al., 1994a; Fischer et al., 1998; Yang et al., 2012).

A.3) Drought Stress

Crop production is greatly limited due to various abiotic and biotic stress factors worldwide. Drought stress, limits the agricultural production and food security more than any other environmental stress factors globally (Cattivelli et al., 2008). In many agricultural regions, drought stress in crop plants often occurs in combination with heat stress. The recent increases in global mean surface temperature are thought to be caused by increased the atmospheric CO_2 concentrations due to human activities. Thus the temperature is expected to rise about 1.4 to 5°C by the year 2100 (Intergovernmental Panel on Climate Change, 2001, 2007). According to Schiermeier (2008) the annual precipitation rate may decrease about 20% per year and reductions in soil moisture will intensify impairments in productivity due to increase in global annual temperatures. Drought stress together with heat stress can alter too many processes including growth, development, physiology, yield and quality crops (Prasad et al., 2008). For example combination of heat and drought stress altered the quality, leaf relative water content

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and chlorophyll content in turfgrass (Jiang and Huang, 2001). Under simultaneous drought and heat stress, enhanced respiration, suppressed photosynthesis and accumulation of high levels of sucrose were observed in Arabidopsis plants while the average leaf temperature increased in tobacco plants due to closed stomata (Rizhsky et al., 2004; Rizhsky et al., 2002). In addition, nitrogen anabolism was weakened, protein catabolism was strengthened and lipid peroxidation was incited under combination of drought and heat conditions in perennial grass *Leymus chinensis* (Xu and Zhou, 2006). Also the duration of grain filling period in wheat plants was shortened under both drought and heat stressed conditions more than either treatment alone (Nicolas et al., 1985a; Altenbach et al. 2003; Shah and Paulsen 2003).

Drought conditions can affect the photosynthesis through affecting stomatal closure and reduced flow of CO_2 into mesophyll tissue (Chaves et al., 2003; Flexas et al., 2004). Drought can also affect photosynthesis adversely by direct impairments in metabolic activities such as by causing alterations in photosynthetic enzyme activities (Farquhar et al., 1989). The initial cause for the reduced photosynthesis under limited water supply is the decreased stomatal conductance (Cornic, 2000). Decreased levels in ribulose bisphosphate (RuBP) and Rubisco protein content (Bota et al., 2004), decline in the Rubisco activity (Parry et al., 2002) and impaired ATP synthesis can be listed as the main metabolic changes under drought stress. In addition to these impairments, according to Alves and Setter (2004), the most sensitive growth process to drought stress is the leaf expansion. Cell division and cell growth are also susceptible to drought stress.

Among the impairments in metabolic and developmental processes, reproductive organs of the plants are also highly affected under drought stress. For example, seed size and seed number per plant can be adversely affected by water shortage. If drought stress condition starts before the pollination, seed number can be dramatically reduced due to abortion of seeds or lack of pollination. However, seed size is mainly dependent on the currently available photosynthates or those that can be transported from source organs to grains (Prasad et al., 2008). According to Zhang et al. (1998) completing grain filling period as fast as possible and enhancement of mobilization of stored carbohydrates can reduce the effects of drought stress on yield. There is increasing evidence indicating that under drought stress conditions, pools and remobilization of soluble carbohydrates from stem tissues play a critical role and affect yield capacity of the plants up to 60 to 70% (Reynolds et al., 2007; Xue et al., 2008). As

discussed before, Mg is important both for production and transportation of carbohydrates from source organs (such as from stem tissue during the reproductive growth stage) into seeds. Therefore, an adequate Mg nutrition could be particularly important for better productivity under low water supply.

Considering that Mg is mainly transported in soil by mass flow, under drought stress conditions, delivery of Mg to plants might be reduced (Gransee and Führs, 2012). Similarly, especially during generative growth stages, top soil is often dry (see below for further discussion). Under such conditions plant uptake of Mg could be seriously affected leading to inadequate nutrition with Mg. Since Mg is a crucial element for phloem loading of sucrose and it affects the transportation of carbohydrates from source organs to sink organs, grain filling period can be dramatically affected, leading to severe losses in crop yield under both drought and Mg-deficient conditions. This topic is one of the main tasks of the PhD project.

A.4) Magnesium deficiency and drought stress in world soils

Increasing world population is correlated with the increase in food demand. According to Bruinsma (2009) 70% more food will needed to be produced to feed the increasing world population by the year of 2050. Since the arable land area in world is limited, and environmental stress factors such as drought, extreme temperatures, salinity and mineral nutrient deficiencies are frequently observed, the production of qualified food is become a critical issue.

Agricultural areas and forested ecosystems are being destroyed by increasing human activities and the expanding human population (Allen et al., 2010). In addition, with increased emissions of greenhouse gasses, global mean temperature is also rising (IPCC, 2007). Even though humans develop plans and programs to conserve the nature and minimize the detrimental facts, estimates are showing that in near future global mean temperature will rise about 2-4°C. This increase in temperature will eventually lead to a serious drying in specific regions (IPCC, 2007; Seager et al., 2007), increased frequency and severity of drought stress and heat waves (IPCC, 2007; Sterl et al., 2008). Together with these factors, in many regions of the world, mineral nutrient element deficiency problems are rising. In acidic and sandy soils deficiency of Mg is a commonly observed problem, especially in tropical regions of the world. Up to 30 to

40% of world soils that have low pH and Al toxicity problem (see Figure A.1) in which Mg deficiency is very common (Gransee and Führs, 2012).

Soil pH

Data taken from: IGBP-DIS Global Soils Dataset (1998)

Atlas of the Biosphere
Center for Sustainability and the Global Environment
University of Wisconsin - Madison
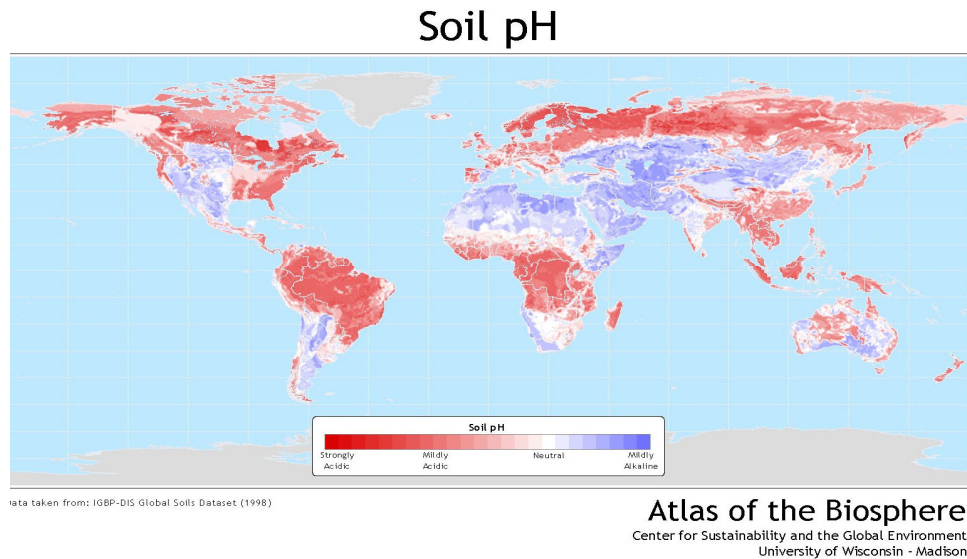
Figure A.1: Soil pH map showing the pH distribution (strongly acidic, mildly acidic, neutral and mildly alkaline soil pH is shown with dark red, pink, white and blue color respectively) of the world soils (retrieved from the Atlas of Biosphere, http://nelson. wisc.edu/sage/data-and-models/atlas/maps/soilph/atl_soilph.jpg, 31.10.2015).

It is known that high soluble Al in acidic soils interact with root Mg uptake due to antagonistic (competitive) reactions during root uptake (Bose et al., 2011; Gransee and Führs, 2012). In such soils, Mg is also always under leaching risk with high amounts contributing to poor Mg nutrition of plants (Gransee and Führs, 2012). Thus, soil deficiency of Mg is very characteristic in such acidic soils in combination with tropical climates where heat and drought stress can be observed simultaneously. Figure A.2 shows the forest mortality areas (in white dots) due to climatic stress from drought and high temperature. According to these facts, in near future probably too many agricultural soils will encounter with Mg deficiency problem together in combination with drought stress.
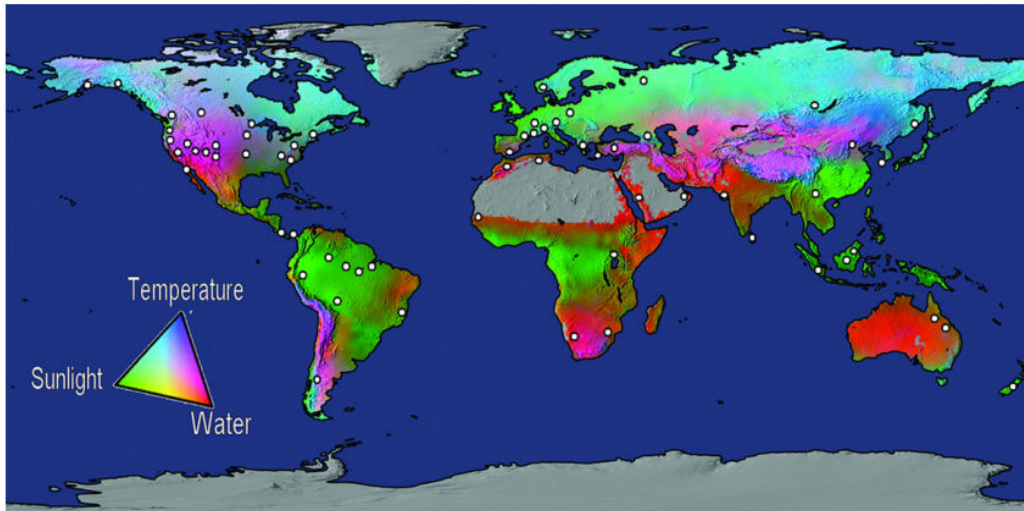
Figure A.2: Forest mortality locations (white dots) due to climatic stress factors such as drought and high temperatures (Allen et al., 2010). Colored map shows potential environmental limits to vegetation net primary production (Boisvenue and Running, 2006).

A.5) Roles of Magnesium in human and animal health

Magnesium is a crucial element for maintaining a healthy life. It is required for sufficient physiological functioning of heart, brain and skeletal muscles (de Baaij et al., 2015). Magnesium content in fruit and vegetables was decreased about 20-30% over the 60 years (Worthington, 2001). In addition, de Baaij et al. (2015) stated that western diet contains more refined grains and processed food and according to estimations 80-90% of the Mg is lost during the food processing. Correspondingly, human population started to show Mg deficiency. For example, survey studies in USA and England show that about 50% of the adult population has limited Mg intake (Rosanoff, 2013). Similar reduced Mg intake has been also reported for the developing countries (Joy et al., 2014). In western countries, high daily intake of Ca represents an important problem in terms of Mg nutrition of human populations. According to Rosanoff (2013), high Ca intake results in increased Ca/Mg ratio in body which then impairs Mg nutritional status of human body.

Patients with a magnesium deficiency often have cardiovascular diseases especially hypertension disorders (Dyckner and Wester, 1983; Gremmler et al., 2008; Gröber, 2009; Hunger, 2008). Magnesium deficiency (hypomagnesaemia) plays a role in the development of diabetes mellitus (Guerrero-Romero et al., 2004, 2011) and 13.5-47.7% of patients diagnosed with type II diabetes have hypomagnesaemia (Swaminathan, 2003; Kisters and Gröber, 2013).

A sufficient supply of Mg in the diets is also important for animals, especially for their productivity and better physiological status (Shaul, 2002). When ruminants graze on grass fields which have low Mg concentration or bioavailability, a serious disorder "grass tetany" can occur, that induces diverse of physiological disorders in body (e.g., overactive neurological reflexes) and even cause loss of animals (Swaminathan, 2003). Therefore this situation can be an important source of economic loss (Harris et al., 1983).

Concentration of Mg in human diet is became a critical issue that affecting adversely the nutrition and health of human population world-wide (Broadley and White, 2010). To overcome the negative course of events which can caused by Mg-deficient food products, plant biotechnologists, breeders and nutritionists have to work together and try to increase the content and bioavailability of Mg in food and feed.

This PhD thesis has been conducted to generate new information and deepen the knowledge known on the role of Mg in plant growth. Special attention has been given to how Mg nutrition influences sees formation by affecting production and deposition of starch. Additionally, it was important to know how plant growth is affected from Mg nutiriton when they suffer from drought stress, because both Mg deficiency and drought stress affect photosynthetic performance of plants and generation of reactive oxygen species in chloroplasts in a similar way. Finally, leaf absorbtion and translocation within plants of the foliarly-sprayed Mg has been studied. This is an area where very limited published evidence is available in literature.

B) GENERAL MATERIALS AND METHODS

B.1 Plant Growth Facilities

Experiments explained in Chapter 1 and Chapter 2 were conducted in a computer-controlled, Venlo-type greenhouse with supplemental lighting at Sabanci University, Istanbul, Turkey (40°53′25″ N, 29°22′47″ E). During the experiment, the heating and evaporative cooling systems of the greenhouse kept the temperature at 24±3°C in the daytime and at 18±3°C at night.

The experiment performed in Chapter 3 was established in the greenhouse located in Institute of Applied Plant Nutrition (IAPN), Göttingen, Germany (51°32'49.9"N, 9°56'40.5"E). This greenhouse had its own controlled heating system.

B.2. Soil Culture

All of the soil culture experiments that were conducted for this thesis established under greenhouse conditions located in Sabanci University.

The soil used in first experiment of Chapter 2 was transported from Tuzlukçu, Konya, Turkey location. This experimental soil was calcareous (23.5% $CaCO_3$), alkaline (pH 8.2), low in organic matter (0.23%) with sandy-loam texture. The ammonium acetate (NH_4Ac)-extractable Mg concentration was found for this soil as 46 mg kg^{-1} soil. To deplete the Mg in this soil, 4 maize plants (*Zea mays* cv.Shemal) per pot for 2.5 kg of soil were planted and grown for 3 months. After maize plants were harvested, roots of the maize plants were separated from the soil, and after all of the soil was mixed homogenously, the NH_4Ac-extractable Mg was found as 33 mg.kg^{-1}.

The experimental soil that used for the second experiment of Chapter 2 was transported from Ordu, Turkey location. This soil's $CaCO_3$ concentration was 0.52%, pH was measured as 4.9, organic matter content was 6.2% and texture class was sandy-loam. No additional depletion methods were used for this soil. The (NH_4Ac)-extractable Mg concentration was found for this soil as 39 mg kg^{-1} soil.

Before sowing the seeds, required mineral nutrients (explained in specific chapter's material and methods part) were homogenously mixed with the experimental soil. Watering of the plants was made daily with deionized water, once or twice a day depending on the season, plant stage and demand. To avoid the uncontrolled loss of nutrients dissolved in water, an independent source plate for each pot was used.

B.3 Nutrient Solution Culture

The experiment explained in Chapter 1, which was conducted for the solution culture, seeds first soaked in $CaSO_4$ containing dH_2O for half an hour. After the soaking step was completed, wheat seeds were sown in wetted perlite and placed in the greenhouse under dark conditions. Water status of the perlite was checked daily and if necessary deionized water was added to wet the perlite. When the coleoptile emerged on the perlite, seedlings were taken up under the light to complete the successful germination step (completed development of coleoptiles and radicula). Germination process of the seeds was usually last around 5-7 days.

When the seedlings reached the appropriate length (about 3-5 cm shoot length), they were transferred to 3L or 5L plastic pots that equipped with an aeration system. Nutrient solution of the plants usually contained: $Ca(NO_3)_2.4H_2O$, KH_2PO_4, $MgSO_4.7H_2O$, K_2SO_4, KCl, Fe-EDTA, $ZnSO_4.7H_2O$, $MnSO_4.4H_2O$, $CuSO_2.5H_2O$, H_3BO_3, and $(NH_4)_6Mo_7O_{24}.4H_2O$ at different rates depending on the experiment mentioned in the corresponding chapters. Nutrient solution of the plants was changed 2-3 times a week depending of the age of plant and it was continuously aerated.

B.4 Harvest

Harvesting stage of the plants differed according to the age of the plant tissues. Matured plant samples were cut directly and placed in paper boxes to dry for 3-4 days at 70°C in the oven. Fresh plant samples were washed with dH_2O first, and then placed in the oven for the drying stage. Root samples were washed with dH_2O first, then washed in 1mM $CaCl_2$ and 1 mM EDTA solution for 3 minutes separately and thereafter washed in dH_2O again and dried in the oven at 70°C.

All the grains obtained in the experiments were separated from their husk with the help of a trashing machine. For seed yield and biomass determination, all the plant samples were weighed at room temperature.

B.5. Mineral Element Analysis

For the analysis of mineral nutrients and the starch determination, dried plant samples were ground into fine powders by using an agate vibrating cup mill (Pulverisette 9; Fritsch GmbH; Germany). To measure the mineral element concentrations in the plant samples, fine ground powder of the samples were undergone in acid digestion step. For the digestion process, dried and milled sample powder was weighed (ca. 0.2 g) and put 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. When the acid digestion step was completed, sample volume was adjusted to 20 ml by adding ddH_2O and the digests were filtered through ashless quantitative filter papers. To each set of 40 samples, 1 blank sample was added to check for contamination and 1 certified standard reference material obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA) was added to check for accuracy.

Mineral nutrient concentrations, except nitrogen (N), were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) (Vista-Pro Axial, Varian Pty Ltd, Mulgrave, Australia). Grain N concentrations were measured with a LECO TruSpec C/N analyzer (LECO Corp., St. Joseph, MI, USA). Certified standard reference materials that were obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA) were used to check the accuracy of the measurements.

B.6 Starch measurements

Starch concentrations in plant samples were determined by using Megazyme Total Starch HK Assay kit (Megazyme International, Total Starch HK kit, K-TSHK, Ireland). All the measurements were done according to the instruction manual following these principles: i) Thermostable α-amylase was used to hydrolyze the starch in the sample into soluble maltodextrins, ii) Maltodextrins were hydrolyzed quantitatively by amyloglucosidase to D-glucose, iii) D-glucose was phosphorylated by the enzyme hexokinase (HK) and adenosine-5'-triphosphate (ATP) to glucose-6-phosphate (G-6-P) with the simultaneous formation of adenosine-5'-diphosphate (ADP), iv) Then the presence of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH), G-6-P was oxidized by nicotinamide-adenine dinucleotide phosphate (NADP$^+$) to gluconate-6-phosphate with the formation of reduced nicotinamide-adenine dinuclotide phosphate (NADPH), v) At last, the amount of NADPH formed in this reaction was stoichiometric with the amount of D-glucose, consequently the increased absorbance at 340 nm measured the NADPH amount to calculate the starch concentration in the samples.

B.7 Calculations

Mineral element concentration data were taken from the ICP-OES software as values of ppm. To find the actual concentration value for the sample, this data was multiplied with the dilution factor. Dilution factor was obtained by dividing the total sample volume (ml) to digested sample weight (g).

For the mineral element and starch content calculations, which were measured as the mg or μg of specific element or starch matter found in the plant tissue, calculated as the multiplication of concentration data with the dry weight data of interested plant part.

B.8 Statistical Analysis

Statistical analysis of the data was conducted by using JMP (12.0.1) (SAS Institute Inc., Cary, NC, USA). The significance of treatment effects was evaluated by analysis of variance (ANOVA). Then, Tukey's honestly significant difference (HSD) test ($p < 0.05$) was used as a post-hoc test to determine significant differences between means.

CHAPTER 1

ADEQUATE MAGNESIUM NUTRITION IS REQUIRED FOR BETTER SEED YIELD THROUGH ITS POSITIVE EFFECT ON STARCH ACCUMULATION

1.1 Introduction

For plants, magnesium (Mg) is an essential cationic macronutrient with structural and regulatory functions related to its interaction with nucleophilic ligands (Shaul, 2002; Cakmak and Kirkby, 2008). It is the most abundant free cation in the cytosol of plants (Shaul, 2002) and activates more enzymes than any other mineral nutrient (Epstein and Bloom, 2004). As the central atom in the chlorophyll molecule and the activator of critical photosynthetic enzymes including ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and phosphoenolpyruvate (PEP) carboxylase, Mg is a key element in photosynthesis (Wedding and Black, 1988; Portis, 1992; Marschner, 2012). Protein synthesis ultimately depends on Mg because Mg is essential for the aggregation of ribosome subunits. Magnesium is also required for the synthesis and function of nucleic acids and adenosine triphosphate (ATP) (Sreedhara and Cowan, 2002; Igamberdiev and Kleczkowski, 2015). Up to 90% of cytoplasmic ATP is complexed to Mg^{2+} in Mg-sufficient plant cells (Yazaki et al., 1988).

Magnesium is critically involved in the phloem loading of sucrose and thus carbohydrate partitioning between source and sink tissues (Cakmak et al., 1994a, b; Hermans et al., 2005). The proton-motive force generated by an H^+-pumping ATPase energizes H^+-sucrose symporters loading sucrose into sieve tube cells (Bush, 1989; Hermans et al., 2005). About 2 mM Mg^{2+} is needed for maximizing the activity of the

H^+-pumping ATPase (Williams and Hall, 1987). The cytoplasmic Mg^{2+} concentration falls below this level in Mg-deficient plants (Marschner, 2012), and carbohydrates start accumulating in source leaves before other physiological processes such as photosynthesis are affected by Mg deficiency (Laing et al., 2000; Hermans et al., 2004; Hermans and Verbruggen, 2005). While excess carbohydrates enhance the production of reactive oxygen species (ROS) in source tissues and limit photosynthesis by negative feedback effect, sink organs such as roots, seeds and tubers are deprived of carbohydrates (Cakmak and Kirkby, 2008). Depending on the species and experimental conditions, alterations in carbohydrate partitioning result in altered root-to-shoot ratios under Mg deficiency (Cakmak et al., 1994a, b; Hermans et al., 2005; Ding and Xu, 2011; Mengutay et al., 2013). Impaired sugar transport into seeds may affect grain size and thus quality in cereals (Cakmak, 2013; Gerendas and Fuhrs, 2013).

When compared to other major cations such as calcium (Ca^{2+}) and potassium (K^+), Mg^{2+} ion has a distinctly larger hydrated radius (Bose et al. 2011; Marschner 2012). Therefore, Mg^{2+} binds only weakly to negatively-charged soil particles, which makes it highly prone to leaching (Hermans, et al. 2004; Cakmak and Kirkby, 2008). Magnesium deficiency typically occurs in acidic and light-textured soils with low cation exchange capacities when Mg in the root zone is removed to deeper layers by leaching (Bose et al., 2011; Gransee and Fuhrs, 2013). Another common cause of Mg deficiency in the field is ionic antagonism. Competing cations do not only displace Mg^{2+} from the cation exchange sites and thus contribute to its leaching but also strongly inhibit its root uptake (Mengel and Kirkby, 2001). These cations include protons (H^+) and aluminum (Al^{3+}) in acidic soils, Ca^{2+} in calcareous soils, K^+ in over-fertilized soils and sodium (Na^+) in saline/sodic soils (Mengel and Kirkby, 2001; Gransee and Fuhrs, 2013). Also, the risk of Mg deficiency is increasing in intensive cropping systems where the Mg reserves in the root zone are being depleted as high-yielding varieties are grown continuously with heavy applications of nitrogen (N), phosphorus (P) and K fertilizers (Hermans et al., 2005; Cakmak and Yazici, 2010). Since Mg is predominantly supplied to plant roots by mass flow in soil (Lambers et al., 2008), dry soils and low transpiration rates may aggravate Mg deficiency (Jezek et al., 2015).

Magnesium is also an essential mineral for human health (de Baaij et al., 2015). In the human body, Mg^{2+} serves as cofactor for over 600 enzymes and as activator for an additional 200 enzymes (Bairoch, 2000; Caspi et al., 2012). Magnesium appears to be particularly important for heart, brain and skeletal muscle physiology (de Baaij et al.,

2015). Its deficiency has been associated with several chronic diseases including hypertension, type II diabetes, Alzheimer's disease, stroke and migraine (Gröber et al., 2015). In the second half of the 20th century, the Mg concentrations of conventionally grown fruits and vegetables decreased by 20-30% on average (Worthington 2001). The Mg concentrations of cereal grains also declined significantly over the past decades while the grain yields increased (Cakmak, 2013). Substantial Mg losses during food processing and excessive Ca intake further reduce the average daily Mg intake (de Baaij et al., 2015). According to recent surveys, Mg deficiency is widespread in the general population (King et al., 2005; Broadley and White, 2010).

There is limited information on the impact of Mg deficiency on carbohydrate partitioning, yield components and grain quality in wheat. The hypothesis of this study was that Mg deficiency would affect the yield formation of wheat more than its vegetative growth and reduce the grain quality due to its effects on carbohydrate partitioning and that foliar Mg application during generative development would alleviate these problems. The effects of Mg supply on various growth and yield parameters and starch partitioning were studied in bread wheat. In addition, the concentrations of Mg and other mineral nutrients were measured and discussed from both a plant and a human nutrition perspective.

1.2 Materials and Methods

This solution culture experiment was done with *Triticum aestivum* cv. Adana99 seeds and designed as 5 replicates from each treatment and 4 plants per pot in 5L pots. This experiment conducted under greenhouse conditions (See Section B.1) and seeds were germinated according to the instructions explained in Section B.3.

The nutrient solution was composed of the following components: 2 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.2 mM KH_2PO_4, 0.85 mM K_2SO_4, 0.1 mM KCl, 100 μM Fe-EDTA, 1 μM $ZnSO_4 \cdot 7H_2O$, 1 μM $MnSO_4 \cdot H_2O$, 1 μM H_3BO_3, 0.2 μM $CuSO_4 \cdot 5H_2O$ and 0.1 μM $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$. As Mg source, $MgSO_4 \cdot 7H_2O$ was added to the nutrient solution at two different levels: 50 μM for the low Mg treatment and 500 μM for the adequate Mg treatment. In addition to the low Mg and adequate Mg treatments, there was a low + foliar Mg treatment. For this treatment, plants were supplied with low Mg

(50 µM) from the solution throughout the experiment, and starting just after anthesis (82 days after sowing), they were sprayed with 4% (w/v) $MgSO_4.7H_2O$ mixed with 0.01% Tween20 as surfactant once a week for 3 times. For each treatment, there were 5 replicate pots.

When all plants fully senesced 148 days after sowing, they were harvested in 5 fractions: roots, spikes, flag leaves, other leaves (all leaves except flag leaves) and stems. Roots were washed first in dH_2O, then in 1 mM $CaCl_2$, then 1 mM EDTA and finally again in dH_2O. All plant samples were put in paper bags, dried at 60°C for 3 days, and then weighed at room temperature. The harvested spikes were threshed, and grains and husks were bagged separately. Mineral element concentrations and starch measurements were done according to the steps explained in Sections B.5 and B.6.

The term "husk" refers to all vegetative tissues of the spike, the term "shoot" refers to all above-ground parts of the plant including the grains, and the term "straw" refers to all vegetative tissues (stems, leaves and husk) of the shoot. Starch content per grain equals grain starch concentration times thousand grain weight (TGW) divided by 1000 (for further calculation steps see Section B.7).

1.3 Results

Low Mg application resulted in severely chlorotic wheat plants (Figure 1.1). When compared to wheat plants grown with adequate Mg, these Mg-deficient plants senesced earlier. Post-anthesis foliar Mg application mitigated these deficiency symptoms and resulted in a 50% increase in the flag leaf SPAD values of low-Mg plants but it could not fully substitute for adequate Mg supply from the nutrient solution. The flag leaf SPAD values of 115-day-old adequate-Mg plants were about twice as high as those of low-Mg plants. Notably, Mg status did not have any visual effects on the vegetative vigor and final size of wheat plants.

Figure 1.1: 115-day-old bread wheat (*Triticum aestivum* cv. Adana99) plants grown hydroponically with low (50 μM), low + foliar (50 μM + 4% (w/v) MgSO$_4$•7H$_2$O) and adequate (500 μM) Mg under greenhouse conditions. Mean leaf SPAD values are shown at the top of the figure.

In parallel with visual observations, Mg applications had mostly negligible effects of the dry weights of vegetative tissues at maturity (Table 1.1). While Mg applications did not affect the husk, stem and total straw dry weights of mature plants, increasing Mg supply slightly but significantly reduced the leaf (flag and other) dry weights. Roots exhibited a statistically non-significant decrease in biomass upon increasing Mg supply.

Table 1.1: Dry weights of vegetative tissues of mature (148-day-old) bread wheat (*Triticum aestivum* cv. Adana99) plants grown hydroponically with low (50 μM), low + foliar (50 μM + 4% MgSO$_4$) and adequate (500 μM) Mg under greenhouse conditions

	Dry Weight (g.plant^{-1})					
Mg Supply	**Husk**	**Flag Leaves**	**Remaining Leaves**	**Stem**	**Root**	**Straw**
Low	11.4 a	2.4 a	5.7 a	16 a	3.0 a	36 a
Low + Foliar	9.1 a	2.1 ab	5.1 ab	15 a	2.7 a	31 a
Adequate	11.8 a	2.1 b	4.3 b	17 a	2.5 a	34 a

Values are means of five independent replicates. Different letters indicate significant differences between means according to one-way ANOVA and Tukey's HSD test (p≤0.05).

In contrast to vegetative biomass, the grain yield was significantly enhanced by Mg applications (Table 1.2). When compared to low Mg, foliar Mg increased the grain yield by 50% and adequate Mg by nearly 100%. Foliar Mg application did not result in a significant increase in the total shoot (straw + grain) dry weight of the low-Mg plants while adequate Mg supply significantly improved the shoot dry weight. The number of spikes per plant and the number of grains per spike were not significantly affected by Mg supply. The low Mg treatment was associated with a sharp decline in the thousand grain weight (TGW). With foliar Mg application, the TGW of low-Mg plants almost reached the TGW of adequate-Mg plants.

Table 1.2: Grain yield, shoot dry weight (DW), thousand-grain weight (TGW), number (#) of spikes per plant and number (#) of grains per spike of mature (148-day-old) bread wheat (*Triticum aestivum* cv. Adana99) plants grown hydroponically with low (50 μM), low + foliar (50 μM + 4% MgSO$_4$) and adequate (500 μM) Mg under greenhouse conditions

Mg Supply	Grain Yield (g.plant^{-1})	Shoot DW (g.plant^{-1})	# of Spikes (plant^{-1})	# of Grains (spike^{-1})	TGW (g)
Low	19 a	55 a	25 a	31 a	24 a
Low + Foliar	28 b	60 a	21 a	35 a	39 b
Adequate	36 c	71 b	23 a	38 a	41 b

Values are means of five independent replicates. Different letters indicate significant differences between means according to one-way ANOVA and Tukey's HSD test (p≤0.05).

In agreement with the TGW data, grains obtained from the low-Mg plants appeared distinctly smaller, thin and deformed (Figure 1.2). Foliar Mg application clearly improved the grain size and minimized shriveling. The largest grains with the best shapes were produced by plants supplied with adequate Mg from the nutrient solution.
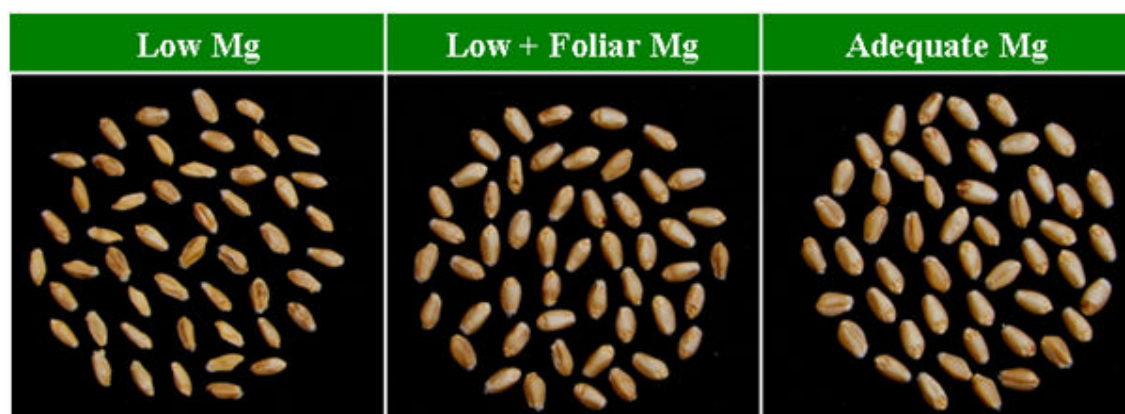
Figure 1.2: Mature seeds of bread wheat (*Triticum aestivum* cv. Adana99) grown hydroponically with low (50 μM), low + foliar (50 μM + 4% MgSO$_4$) and adequate (500 μM) Mg under greenhouse conditions

Mature wheat plants grown with low Mg had explicitly lower Mg concentrations and contents in all their vegetative tissues when compared to those grown with adequate Mg (Table 1.3). At adequate Mg supply, leaves had by far the highest Mg concentrations among all the vegetative tissues. Leaf Mg concentrations of wheat plants at maturity declined by nearly 90% when plants were cultivated with low Mg.

Table 1.3: (A) Mg concentrations and **(B)** Mg contents of vegetative tissues of mature (148-day-old) bread wheat (*Triticum aestivum* cv. Adana99) plants grown hydroponically with low (50 μM) and adequate (500 μM) Mg under greenhouse conditions

(A)	Mg Concentration (mg.kg^{-1})				
Mg Supply	Husk	Flag Leaves	Remaining Leaves	Stem	Root
Low	226 a	299 a	336 a	94 a	231 a
Adequate	647 b	2308 b	3212 b	356 b	391 b

(B)	Mg Content (mg.plant^{-1})				
Mg Supply	Husk	Flag Leaves	Remaining Leaves	Stem	Root
Low	2.59 a	0.71 a	1.9 a	1.53 a	0.69 a
Adequate	7.63 b	4.80 b	13.8 b	6.12 b	0.98 a

Values are means of five independent replicates. Different letters indicate significant differences between means according to one-way ANOVA and Tukey's HSD test ($p \leq 0.05$).

Vegetative tissues of plants supplied with low + foliar Mg were not analyzed for Mg because of surface contamination.

Under low-Mg conditions, the grain Mg concentration fell below 50% of the concentration obtained under adequate-Mg conditions (Table 1.4A). A relatively small but significant improvement in the grain Mg concentration was achieved by foliar Mg application. The N and P concentrations of grains did not show a clear response to Mg applications (Table 1.4A). Low Mg supply without foliar Mg supplementation was associated with enhanced grain K concentrations. Among micronutrients, Fe and Zn responded oppositely to increasing Mg supply. The grain Fe concentration increased significantly with higher Mg supply whereas the grain Zn concentration decreased. For all mineral nutrients except Zn in Table 1.4B, yields were improved significantly by higher Mg supply. In particular, the grain Mg yield increased steeply (Table 1.4B).

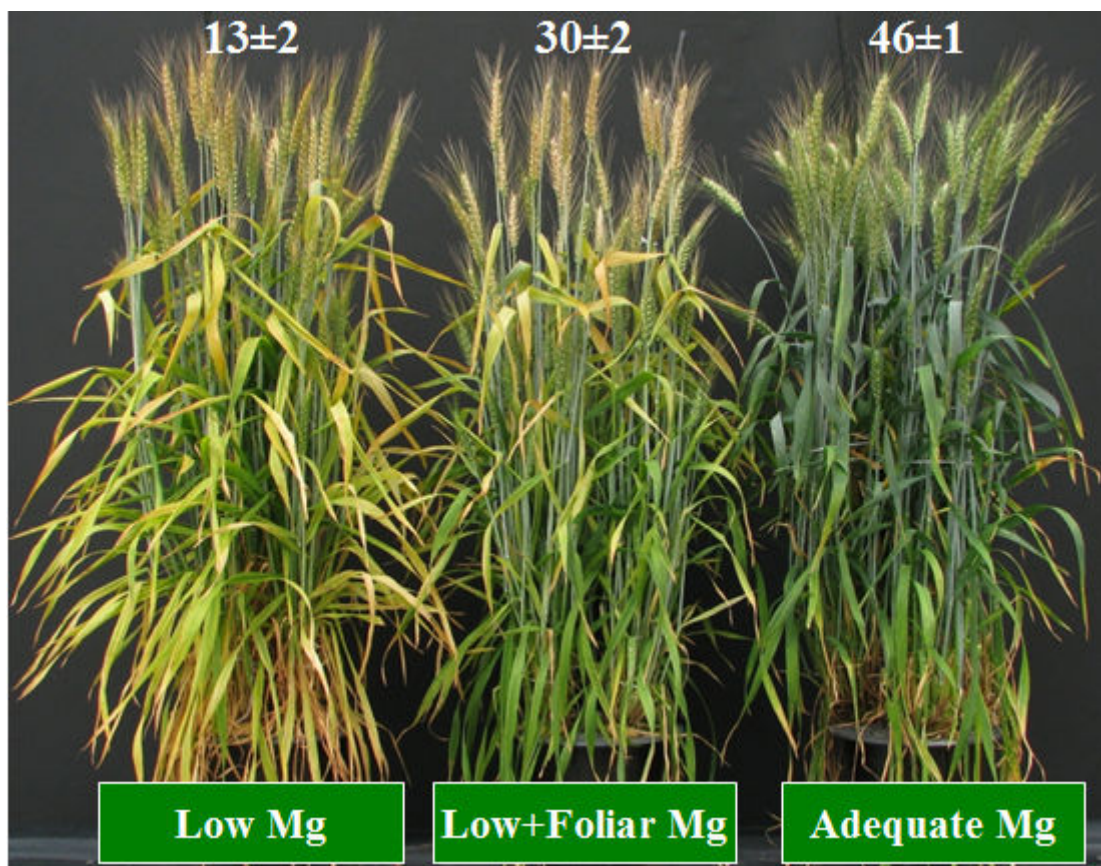**Table 1.4: (A)** Grain mineral concentrations and **(B)** grain mineral yields of mature (148-day-old) bread wheat (*Triticum aestivum* cv. Adana99) plants grown hydroponically with low (50 μM), low + foliar (50 μM + 4% MgSO$_4$) and adequate (500 μM) Mg under greenhouse conditions

(A)	Grain Mineral Concentrations					
	Mg	**N**	**P**	**K**	**Fe**	**Zn**
Mg Supply	**(%)**	**(%)**	**(%)**	**(%)**	**(mg.kg^{-1})**	**(mg.kg^{-1})**
Low	0.06 a	2.98 a	0.48 ab	0.69 a	51 a	65 a
Low + Foliar	0.08 b	2.77 b	0.46 a	0.53 b	63 ab	52 b
Adequate	0.14 c	2.93 ab	0.51 b	0.54 b	69 b	43 b

(B)	Grain Mineral Yields (mg.plant^{-1})					
Mg Supply	**Mg**	**N**	**P**	**K**	**Fe**	**Zn**
Low	11 a	560 a	90 a	129 a	0.95 a	1.21 a
Low + Foliar	22 b	783 b	131 b	149 a	1.77 b	1.45 a
Adequate	51 c	1038 c	182 c	191 b	2.47 c	1.51 a

Values are means of five independent replicates. Different letters indicate significant differences between means according to one-way ANOVA and Tukey's HSD test (p≤0.05).

The starch concentrations measured in the flag and other leaves were highest for low Mg, lower for low + foliar Mg and lowest for adequate Mg (Table 1.5A). Also, the leaf starch contents decreased significantly when Mg supply increased (Table 1.5B). In contrast, the root starch concentrations and contents were lowest for low-Mg plants not treated with foliar Mg. (Table 1.5). The starch concentration and content of stem tissue was unaffected by Mg treatments.

Table 1.5: (A) Starch concentrations and **(B)** starch contents of vegetative tissues of mature (148-day-old) bread wheat (*Triticum aestivum* cv. Adana99) plants grown hydroponically with low (50 μM), low + foliar (50 μM + 4% MgSO$_4$) and adequate (500 μM) Mg under greenhouse conditions

(A)	Starch Concentration (mg.g^{-1})			
Mg Supply	Flag Leaves	Remaining Leaves	Stem	Root
Low	3.3 a	3.6 a	1.2 a	1.0 a
Low + Foliar	2.8 ab	2.5 b	1.1 a	1.7 b
Adequate	2.1 b	2.1 b	1.2 a	1.7 b
(B)	Starch Content (mg.plant^{-1})			
Mg Supply	Flag Leaves	Remaining Leaves	Stem	Root
Low	8.0 a	20.6 a	19 a	2.9 a
Low + Foliar	5.8 b	12.8 b	16 a	4.5 a
Adequate	4.3 b	9.3 b	21 a	4.1 a

Values are means of five independent replicates. Different letters indicate significant differences between means according to one-way ANOVA and Tukey's HSD test (p≤0.05).

When compared to low Mg, adequate Mg enhanced the grain starch concentration by 10%, the average starch content per grain by 85% and the grain starch yield per plant by over 100% (Table 1.6). Foliar Mg application to low-Mg plants provided the same significant improvements of grain starch concentration and content but was significantly less effective than adequate Mg in enhancing the grain starch yield per plant.

**Table 1.6:** Grain starch concentration, starch content and starch yield of mature (148-day-old) bread wheat (*Triticum aestivum* cv. Adana99) plants grown hydroponically with low (50 μM), low + foliar (50 μM + 4% MgSO$_4$) and adequate (500 μM) Mg under greenhouse conditions

	Grain Starch		
Mg Supply	**Concentration (mg.g^{-1})**	**Content (mg.grain^{-1})**	**Yield (g.plant^{-1})**
Low	520 a	12.6 a	9.8 a
Low + Foliar	575 b	22.4 b	16.3 b
Adequate	575 b	23.6 b	20.4 c

Values are means of five independent replicates. Different letters indicate significant differences between means according to one-way ANOVA and Tukey's HSD test (p≤0.05).

1.4 Discussion

In wheat, Mg deficiency typically results in light green beading along the veins of fully extended leaves, which progresses into interveinal chlorosis as deficiency becomes more severe (Scott and Robson, 1991; Craighead and Martin, 2001; Mengutay et al., 2013). Remobilization of Mg from mature leaves causes early senescence (Marschner, 2012). In this study, low-Mg plants appeared senescent while adequate-Mg plants were still dark green (Figure 1.1). The increase in the leaf chlorophyll concentration upon foliar Mg application to low-Mg plants indicates that foliar Mg was effective in increasing the longevity of leaves.

The apparent size of the plants at grain-filling (Figure 1) and the straw dry weight at maturity (Table 1.1) were unaffected by Mg supply, implying that even the low-Mg treatment in this study provided sufficient Mg to the plants to maintain vegetative growth. In previous hydroponic pot studies on Mg deficiency in wheat, significant declines in vegetative biomass production were reported but in those studies, the Mg supply per plant was below the low-Mg level in this study (Scott and Robson, 1991; Mengutay et al., 2013). Here, the purpose was to mimic a latent Mg deficiency which becomes more severe at later stages of the development as the Mg demand increases with increasing sink activity. It is important to note that the results presented

in Table 1.1 are the dry weights of vegetative tissues after senescence. The significant decreases in leaf dry weights of senesced plants upon improved Mg supply suggest enhanced carbon remobilization from source to sink tissues (Table 1.1).

The yield responses to Mg supply were impressive (Table 1.2). Taken together, the dry weight and yield data in Tables 1.1 and 1.2 indicate that a Mg supply which does not impair the vegetative growth of wheat at all may reduce its grain yield by 50%. Moreover, here, the Mg supply from the solution was constant and not discontinued during grain-filling. In practice, wheat is widely grown as a rain-fed crop in Mediterranean-type or semi-arid climates, and the top soil dries out toward the end of the growing season (Elias and Manthey, 2005; Distelfeld et al., 2007), which limits mass flow-driven uptake of minerals including Mg (Jezek et al., 2015). Under such conditions, the yield depression caused by a previously latent Mg deficiency may be even more dramatic. That post-anthesis foliar Mg application could significantly reduce yield losses due to Mg deficiency (Table 1.2) is a very important finding of this study because minerals applied to soil at grain-filling stage may not be efficiently taken up when soil and environmental conditions are not favorable (Gooding and Davies, 1992; Fageria et al., 2009; Jezek et al., 2015). Foliar Mg application was also shown to be effective in correcting Mg deficiency in other crops (Barlog and Grzebisz, 2001; Vrataric et al., 2006; Neuhaus et al., 2014; Jezek et al., 2015).

Grain yield per plant can be expressed as the product of 3 factors: number of spikes per plant, number of grains per spike and single grain weight (TGW / 1000) (Grzebisz, 2013). Hydroponically-grown wheat plants typically produce a very high number of tillers under hydroponic conditions and have a very high yield potential (Kutman et al., 2012). It is noteworthy that each plant produced more than 20 spikes in this study, irrespective of Mg supply (Table 1.2). While the number of grains per spike tended to increase in response to higher Mg supply, suggesting a slight impairment of grain setting by Mg deficiency, it is evident that the impact of Mg supply on grain yield was mainly a result of its impact on TGW (Table 1.2; Figure 1.2). In the literature, increases in TGW upon Mg fertilization were reported for barley (Beringer and Forster, 1981) and wheat (Al'Shevskii and Derebon, 1982). The TGW is a yield component that heavily depends on carbohydrate supply during grain filling (Grzebisz, 2013). So, the effect of Mg supply on the TGW can be explained by the disruption of phloem loading and thus carbohydrate translocation from source tissues to developing grains by Mg deficiency (Hermans et al., 2005; Cakmak and Kirkby, 2008). The TGW is also

considered an important technological quality parameter because the milling efficiency depends on the grain size (Greffeuille et al., 2006; Gerendas and Fuhrs, 2013).

The critical leaf concentration for Mg deficiency is about 1000-1100 mg/kg in wheat (Jones et al., 1991; Scott and Robson, 1991; Reuter and Robinson, 1997). In this study, the leaf Mg concentrations of the adequate-Mg plants were 2-3 times higher than this critical level whereas those of the low-Mg plants were only about 1/3 of it at maturity (Table 1.3). As Mg is remobilized from source tissues during senescence (White and Broadley, 2008), it is safe to assume that the Mg concentrations of vegetative tissues were higher than those reported in Table 1.3 before senescence. Under both low- and adequate-Mg conditions, 60% of the total shoot Mg was allocated to grains at maturity (Tables 1.2 and 1.3). Foliar Mg application to the low-Mg plants was not as effective as adequate Mg supply in enhancing the grain Mg concentration (Table 1.3), which is important for the nutritional quality of wheat grain. In contrast, foliar applications of Zn are more effective than soil applications in increasing the grain Zn concentration of wheat (Yilmaz et al., 1997; Kutman et al., 2011; Zhao et al., 2014).

Both the uptake and the assimilation of N are impaired under Mg-deficient conditions (Ding et al., 2006; Grzebisz, 2013). Magnesium deficiency was also reported to depress the phloem export of amino acids (Cakmak et al., 1994b; Ruan et al., 2012). In this study, the lack of a clear response of the grain N concentration to Mg supply (Table 1.4A) may be attributed to dilution due to significant yield improvement by Mg. The grain N yield, on the other hand, shows clearly that higher Mg supply enhanced the N use (Table 1.4B). The well-documented antagonistic interaction between K and Mg (Zengin et al., 2008; Cai et al., 2012) was also observed in this experiment. The grain K concentration increased significantly at low Mg supply (Table 1.4A). Intriguingly, while the grain Zn concentration decreased in response to higher Mg supply (Table 1.4A), which can be explained by yield dilution considering the opposite trend in the grain Zn content (Table 1.4B), the grain Fe concentration increased significantly when the plant Mg status was improved. There is apparently a specific positive effect of Mg on grain Fe. This finding suggests that Mg-deficient wheat grain may be at the same time Fe-poor, which is important from a human nutritional point of view as Fe is another mineral often lacking in human diet and a major target mineral in biofortification efforts (White and Broadley, 2008; Cakmak et al., 2010).

The typical starch concentration of whole grains of American and Canadian bread wheat varieties grown under field conditions ranges from 63 to 72% (Lineback

and Rasper, 1988; Hucl et al., 1996). In this solution culture study, the grain starch concentrations of the adequate-Mg plants were slightly lower than this range, which might be a varietal effect or caused by the experimental conditions (Table 1.6). Here, the important finding is that the grain starch concentration was depressed significantly by Mg deficiency and enhanced by post-anthesis foliar Mg treatment to the level measured at adequate Mg supply. The average starch content per grain and the starch yield per plant show the impact of Mg on grain starch accumulation even clearer. In agreement with impaired source-to-sink translocation of carbohydrates under Mg deficiency (Hermans and Verbruggen, 2005; Cakmak and Kirkby, 2008), the starch concentrations and contents of leaves increased whereas those of roots decreased significantly at low Mg supply (Table 1.5). Accumulation of carboydrates in source leaves under Mg deficiency was reported for various crop species (Cakmak et al., 1994b; Hermans et al., 2004; Hermans and Verbruggen, 2005; Mengutay et al., 2013). However, it should be noted that irrespective of the Mg treatment, less than 1% of the total starch in the shoot of mature wheat plants was in vegetative tissues (Tables 1.5 and 1.6). So, the huge impact of Mg supply on grain accumulation cannot be explained solely by impaired phloem loading of carbohydrates. It is well-documented that the photosynthetic output of Mg-deficient plants is depressed (Fischer and Bremer, 1993; Laing et al., 2000; Hermans et al., 2004). This is most likely a combined effect of several factors including the negative feedback effect of accumulating carbohydrates on photosynthesis, secondary oxidative stress, loss of chlorophyll, reduced activity of key photosynthetic enzymes and regulatory problems (Portis, 1992; Jezek et al., 2005; Cakmak and Kirkby, 2008).

From the results of this study, it can be concluded that Mg deficiency affects wheat yield mainly by limiting the carbohydrate supply to developing grain and reducing the thousand grain weight, which is at the same time an important technological quality parameter. The mineral nutritional quality of wheat grain is also adversely affected by Mg deficiency as the grain Mg and Fe concentrations are reduced. Since vegetative biomass production of wheat is far less affected than yield formation, Mg deficiency may remain latent until grain-filling. Foliar Mg application is a promising tool to alleviate Mg deficiency during grain-filling and minimize its impact on grain yield and quality parameters.

CHAPTER 2

ADEQUATE MAGNESIUM SUPPLY THROUGH SOIL CONTRIBUTES TO ALLEVIATION OF DROUGHT STRESS AND IMPROVING GRAIN YIELD

2.1 Introduction

A better plant growth and development is under direct influence of environmental factors such as temperature, light and soil moisture. Any unfavorable changes in environmental factors can adversely affect the physiological and biochemical balance of plants, leading to impaired growth (Garg, 2010). It has been predicted that by the end of this century average global surface temperature will increase about 1.1-6.4°C (IPCC, 2007). Estimations based on the model studies indicate that there will be an increase from 1% to 30% in extreme drought land area with global warming which leading to an increase in evaporation and lower water availability (IPCC, 2007). Therefore scarcity of water in growth medium, i.e. drought, represents the main and large-scale limitation to agricultural production (Boyer, 1982; Delmer, 2005). Drought stress leads to a decline in grain yield for most of the crop plants by 50% and it is one of the major source of crop loss worldwide (Wang et al. 2003).

During most of the grain filling period in wheat plants encounter with increasing temperatures and decreasing moisture stress (Blum, 1998), and this period will be exposed to more adverse conditions with temperature and drought stress as a result of the expected changes in global warming in the world. Wheat plants are highly sensitive to drought stress especially during the grain filling period, because during the reproductive stage wheat plants suffer more severely from drought in combination with heat (Zinselmeier et al. 1995, 1999). According to Stratonovitch and Semenov (2015),

breeding new wheat cultivars with high tolerance to heat stress during the reproductive stage is an important breeding strategy that can greatly contribute to higher and stable grain yields in wheat. It is widely belived that in future drought stress will co-occur more severely and frequently together with heat stress and therefore breeding programs should focus on these 2 traits (Lobell et al., 2015).

In early grain filling period, limited water availability in growth medium causes remarkable effect on grain yield capacity of crop plants via decreasing endosperm cell number and strength of sink organs (Ho, 1998). Ciais et al (2005) stated that the number and period of extreme climatic phenomena are increasing in wheat growing areas. According to Barnabas et al (2008) and Farooq et al (2009), drought conditions reduce not only the grain yield but also nutritional quality, especially when occurred during reproductive growth stage.

Developing of caryopses from fertilized ovaries is the last stage of growth in cereals and referred as the grain filling period; the final grain weight is determined by its duration and rate (Yang and Zhang, 2006). In the grain filling stage, assimilates are transferred to grains by two carbon resources: direct translocation of current assimilates and redistribution of the reserve pool of assimilates (Pheloung and Siddique, 1991; Kobata et al., 1992; Schnyder, 1993). Various studies in wheat plants showed that pre-anthesis reserve pool of assimilates contributes 10-40% of the final grain weight under sufficient water supply (Rawson and Evans, 1971; Gallagher et al., 1976; Bidinger et al., 1977; Schnyder, 1993; van Herwaaden et al., 1998; Gebbing and Schnyder, 1999) and 75-100% under dry field conditions (van Herwaaden et al., 1998). Accordingly, remobilization of the stored carbohydrates gains extreme importance under water stress conditions (Nicolas et al., 1985a, b; Palta et al., 1994; Asseng and van Herwaarden, 2003; Plaut et al., 2004).

Starch obtained from grains is considered as the essential end-product of cereals (Thitisaksakul et al., 2012). According to Koehler and Wieser (2013), approximately 66-76% of the cereal grains consist of carbohydrates and the around 55-70% of the carbohydrate is starch. Due to efficient textural properties of starch in making bread, it gains more importance for human nutrition (Koehler and Wieser, 2013). Although diet preferences are not stable in different parts of the world, it is shown that 50% of the calories of the human diet is derived from starch (Galliard, 1987; WHO, 2003), and in impoverished countries this value goes up to 80% (Burrell, 2003). The significance of starch that derived from cereals point towards itself globally; 51% of the harvested area

in the world is occupied by cereals (Bruinsma, 2009). However, with decline in the arable agricultural land and continuously increasing world population, increase in the production of cereals is indispensable.

Decrease in the accumulation of starch in cereal grains often observed under various abiotic stresses during the grain filling period, for example such a trend inspected in barley under drought and heat stress (Savin and Nicolas, 1996), in rice under salinity stress (Siscar-lee et al., 1990) and in wheat under heat stress (Labuschagne et al., 2009). The activity of the enzyme called starch synthase which converts sugars to starch in the maturing grain observed to be decreased under such environmental stress factors leading to a reduced starch amount in cereal grains (Wang and Frei, 2011). The altered activity of this enzyme reported in different crops such as in wheat under drought stress and heat stress (Rijven, 1986; Keeling et al., 1993), in maize under heat stress (Singletary et al., 1993), or in rice under salinity stress (Khan and Abdullah, 2003).

Xu et al (2010) listed the six aspects of plants response to water stress as following:

(1) drought escape mechanism by completing the life cycle earlier than expected to encounter minimal level of water shortage during the reproductive growth stage (Geber and Dawson, 1990);

(2) avoidance of drought by enhancing better root systems or reducing the stomata to use water more effectively (Schulze. 1986; Jackson et al. 2000);

(3) improving tolerance to drought by increasing the ability of osmotic adjustment and improving the elasticity of cell wall to preserve and maintain the tissue turgidity (Morgan, 1984);

(4) resistance to drought by improving the antioxidative defense mechanisms and related metabolism (Bartoli et al. 1999; Penuelas et al. 2004);

(5) abandoning drought via exfoliating older leaves under water deficit (Chavez et al. 2003) and

(6) genetic mutation or modification under long-term drought stress conditions (Hoffmann and Merila, 1999; Sherrard et al. 2009; Maherali et al. 2010).

Mobilization of minerals in soil depends on water availability; thus under water deficient conditions the uptake of minerals by roots can be altered. Oktem (2008) stated that under low water availability, the uptake of Fe, Zn and Cu minerals from soil was reduced leading to decreased concentration of these elements in corn grains. Such a

situation can be observed for Mg. Under deficiency of water, uptake of Mg via roots can be limited and led to Mg deficiency in plants. Under such condition, when drought stress could be observed with Mg deficiency, stress might be doubled for plant and coping mechanisms start to encounter with too many physiological problems. If plants supplied with sufficient amount of Mg, their reaction to drought stress will be narrowed down and became easier to cope with.

Severity of drought stress can be greatly influenced from the mineral nutritional status of plants (Cakmak, 2005; Waraich et al 2011; Marschner, 2012) by affecting stomatal conductance for CO_2 and H_2O (Laing et al, 2000; Cakmak and Kirkby, 2008; Putra et al., 2012), activity of photosynthetic enzymes and pools of stress tolerance proteins (Mengutay et al, 2013; Verbruggen and Hermmans, 2013; Peng et al., 2015) and root formation (Cakmak et al., 1994a; Cakmak and Kirkby, 2008). High accumulation of abscisic acid (ABA) in Mg deficient plants (Chao et al., 2012) could be also associated with limited stomatal conductance to water, and accumulation of ABA is an early change in drought stressed plants (Harb et al., 2010).

One of the well documented mechanisms by which drought stress causes cellular damages to plants is related to generation of reactive oxygen species (ROS) and their oxidative attack to chloroplasts (Selote and Khanna-Chopra, 2006; Suzuki et al., 2012). Since very similar physiological impairments also occur in Mg deficient leaves (Cakmak and Marschner, 1992; Mengutay et al., 2013), it is plausible to suggest that that combination of a drought stress with Mg deficiency stress in plants may accelerate oxidative damage in chloroplasts and decline in yield capacity of plants. These observations and findings indicate importance of an adequate Mg nutrition of plants under drought stress. In a previous study, Mengutay et al (2013) showed that plants with low Mg supply had higher susceptibility to heat stress and showed rapid development of leaf chlorosis and necrosis when they grow under heat stress.

To our knowledge, there is very limited published evidence in relation to the effect of varied Mg nutrition on plant growth and yield under drought stress conditions. The aim of this study was, therefore, to show that an adequate Mg nutrition is required to mitigate cellular damage and contribute to better productivity under water-deficiency conditions. This study was conducted to investigate changes in grain yield under low and adequate Mg supplies at different water regimes. Additionally, changes in starch and Mg content of seeds were studied to understand better the interrelationship between low Mg supply and drought stress.

2.2 Materials and Methods

Two separate experiments were conducted in this part of the thesis. Both experiments designed as factorial experiment were established by using Triticum *aestivum* cv. Adana 99 bread wheat seeds under greenhouse conditions.

In first drought experiment, 5 independent replicates from each treatment were used. 12 seeds per pot were sown in plastic pots with 2.5 kg of experimental soil # 1 (See Section B.2 for soil features). This soil was supplied with 400 ppm N in the form of $Ca(NO_3)_2.4H_2O$, 100 ppm of P in the form of KH_2PO_4, 25 ppm of S in the form of $Ca(SO_4).2H_2O$. Two different levels of Mg supply were added additionally in soil: 0 ppm and 50 ppm of Mg in the form of $MgSO_4.7H_2O$.

Calculation of the field capacity was done by weighing the pot containing the dry soil inside first (non-saturated weight). Then the soil in the pot was saturated with deionized water until the water starts to come from the holes which were located under the pot. To avoid any loss of soil with the water coming from the pot, source plates were used. When the water efflux from the saturated soil stopped completely, the weight of the pot was measured again (saturated weight). Non-saturated weight was subtracted from the saturated weight and result was the net water holding capacity of the soil, i.e. 100% field capacity (FC). To calculate interested field capacity (e.g., severity of drought stress as 30% or 40% FC), 100% FC was multiplied with the desired water supply percentage. Since the drought treatment started after the heading stage of wheat plants, the weight of the plants were not negligible. At the beginning of the drought treatment 3 extra pots from each treatment were taken to calculate average weight of the whole plant per pot and obtained value included in the daily water supply.

Until the heading stage, 12 plants per pot were thinned down, and the experiment continued with 4 plants per pot till the end of the harvest. In this experiment, first, plants were grown at 70% of the FC until 58 days old with low and adequate Mg supply using the experimental soil described in General Material and Method section (see: section B.2.). On day 58, plants completed almost their heading stage. Thereafter, the experimental plants are exposed to 3 levels of water regimes in soil as following: i) 70 % of the FC (control), ii) 40 % of the FC and iii) 30 % of the FC. The Mg treatments were 50 mg Mg per kg soil in form of $MgSO_4$ (Mg-adequate plants) and no Mg application (Mg deficient plants). On day 81, plants were harvested.

At harvest, plants were harvested in 5 fractions: i) husk (separated from grains), ii) flag leaves, iii) remaining leaves, iv) stem and v) grains.

In the second drought experiment, growth conditions in greenhouse were similar to the first experiment described above. This 2nd experiment was conducted due to absence of Mg effect on grain yield at the 70 % of FC. In this experiment, for each treatment 6 independent replicates were used. 15 seeds per pot were sown in plastic pots filled with 1.8 kg of experimental soil # 2 (See Section B.2 for soil features) supplied with 450 ppm N in the form of $Ca(NO_3)_2.4H_2O$, 250 ppm of P in the form of KH_2PO_4, 25 ppm of S in the form of K_2SO_4, 50 ppm P in the form of $CaH_4O_8P.2H_2O$. The rates of Mg treatments were same of the first experiment.

In the 2nd experiment, experimental plants reached to heading stage when they were 68 days old. Then, plants are exposed 2 different water regimes in growth medium: i) 70 % of the FC and ii) 30 % of the FC. On day 122, the plants were harvested in 5 fractions as in the first experiment: i) husk (separated from grains), ii) flag leaves, iii) remaining leaves, iv) stem and v) grains. Straw dry weights mentioned in this chapter is consisted of the sum of dry weights of whole vegetative parts of the plants (husk, flag leaves, remaining leaves and stem)

As described in the General Material and Method section (see: section B), harvested plant materials were subjected to analysis of dry matter production, mineral nutrients by ICP-OES and starch by using Megazyme Total Starch HK Assay kit.

2.3 Results

In the first experiment, experimental plants were grown until flowering stage (58 days old) with adequate water supply (70% of the FC) and two different Mg treatments (0 and 50 ppm). Then, part of the plants has been exposed to 2 different drought stress treatments including 30% and 40% of FC. The remaining plants continued to grow at 70% of FC. At the harvest, following plants parts were separately harvested: i) husk (separated from grain), ii) flag leaves, iii) remaining leaves, iv) stem and v) grains.

The dry weight results are presented in Table 2.1. Increasing water status of the plants greatly improved dry matter production and grain yield. Under given experimental conditions, varied Mg supply did not consistently affect dry matter production of the plant parts. There were clear reductions in shoot dry matter under 50

ppm Mg supply when compared to the low Mg plants, probably due to reduced dry matter allocation from shoot into grain under low Mg. However, grain yield values were not affected from different Mg treatments (Table 2.1). The decrease in grain yield by reducing water supply was higher under low Mg than under adequate Mg supply (e.g., 1.9-fold decrease with low Mg and 1.60-fold with adequate Mg).

Grain yield was enhanced with 50 ppm supply of Mg around 18% and 28% under 30% and 40% of FC respectively. With 70% of the FC (control), there was no increase or decrease in grain yield with respect to Mg supply. Under low Mg supply grain yield increased by 5.8% when water supply increased from 30% to 40% of FC. 80% more grain yield was produced when the water supply was increased from 40% to 70% FC under low Mg supply. Under adequate Mg supply recorded increase in grain yield was 15% and 40% when the water supply increased from 30% to 40% of the FC and 40% to 70% FC respectively.

Table 2.1: Dry weights (mg.plant^{-1}) of 81 days-old bread wheat (*Triticum aestivum* cv. Adana99) plants grown with low (0 ppm) and adequate (50 ppm) Mg applications and with 3 different water supplies (30%, 40% and 70% of FC) under greenhouse conditions.

		Dry Weights (g.plant^{-1})					
Mg Supply	Water Supply	Husk	Flag Leaf	Remaining Leaves	Stem	Grain	Straw
	30% FC	0.57±0.04	0.05±0.01	0.63±0.07	0.69±0.09	0.85±0.27	1.94±0.16
0 ppm	**40% FC**	0.62±0.16	0.05±0.01	0.52±0.12	0.70±0.12	0.90±0.36	1.89±0.40
	70% FC	1.08±0.17	0.10±0.02	0.88±0.19	1.18±0.26	1.62±0.22	3.24±0.60
	30% FC	0.55±0.05	0.05±0.01	0.53±0.05	0.63±0.03	1.01±0.21	1.76±0.08
50 ppm	**40% FC**	0.54±0.02	0.05±0.01	0.53±0.09	0.65±0.05	1.16±0.27	1.77±0.13
	70% FC	0.81±0.09	0.09±0.01	0.58±0.11	0.93±0.11	1.62±0.32	2.41±0.26

Husk: HSD$_{0.05}$ (Mg; Water; MgxWater) = (0.25; 0.11; 0.20)

Flag Leaf: HSD$_{0.05}$ (Mg; Water; MgxWater) = (*n.s*; 0.01; *n.s*)

Rem. Leaves: HSD$_{0.05}$ (Mg; Water; MgxWater) = (0.08; 0.12; 0.22)

Stem: HSD$_{0.05}$ (Mg; Water; MgxWater) = (0.09; 0.14; *n.s*)

Grain: HSD$_{0.05}$ (Mg; Water; MgxWater) = (*n.s*; 0.30; *n.s*)

Straw: HSD$_{0.05}$ (Mg; Water; MgxWater) = (0.25; 0.36; 0.64)

Magnesium concentrations of harvested plant parts are presented in Table 2.2. Increasing Mg application from 0 ppm to 50 ppm resulted in increased Mg concentrations under all water treatments in every plant part. Lowest Mg concentration was found under low Mg treatment, especially at 30% FC water supply. Strongest reaction to increasing Mg supply was observed in remaining leaves under all water regimes. In case of grains, increases in Mg concentration by increasing Mg supply were much less and no clear change was found under low Mg supply (Table 2.2).

Table 2.2: Magnesium concentrations (mg.kg^{-1}) of 81 days-old bread wheat (*Triticum aestivum* cv. Adana99) plants grown with low (0 ppm) and adequate (50 ppm) Mg applications and 3 different water supplies supplies (30%, 40% and 70% of FC) under greenhouse conditions.

		Mg Concentration (mg.kg^{-1})			
Mg Supply	Water Supply	Husk	Remaining Leaves	Stem	Grain
	30% FC	455 ± 158	781 ± 36	122 ± 26	1039 ± 184
0 ppm	**40% FC**	582 ± 416	824 ± 97	170 ± 22	1138 ± 276
	70% FC	496 ± 96	912 ± 161	188 ± 42	1088 ± 88
	30% FC	488 ± 121	2359 ± 269	184 ± 15	1196 ± 103
50 ppm	**40% FC**	598 ± 254	2195 ± 143	224 ± 48	1282 ± 179
	70% FC	729 ± 288	2387 ± 333	242 ± 32	1476 ± 108

Husk: HSD$_{0.05}$ (Mg; Water; MgxWater) = (*n.s*; *n.s*; *n.s*)
Rem. Leaves: HSD$_{0.05}$ (Mg; Water; MgxWater) = (150; *n.s*; *n.s*)
Stem: HSD$_{0.05}$ (Mg; Water; MgxWater) = (24; 36; *n.s*)
Grain: HSD$_{0.05}$ (Mg; Water; MgxWater) = (127; *n.s*; *n.s*)

As expected, the plants with 50 ppm Mg supply had higher Mg content in most of the plant parts analyzed (Table 2.3). Increased Mg supply from the soil significantly increased the Mg content of remaining leaves. Higher supply of water at adequateMg significantly increased the grain Mg content. Under the 30% and 40% of the field capacity treatments, when Mg application increased from 0 to 50 ppm, grain Mg content was increased by 41% and 53%, respectively. Adequate Mg treatment from the soil under sufficient water supply (70% of the FC) did not affect Mg content of grains as much as the drought stressed plants.

Table 2.3: Magnesium contents ($\mu g.kg^{-1}$) of 81 days-old bread wheat (*Triticum aestivum* cv. Adana99) plants grown under low (0 ppm) and adequate (50 ppm) Mg applications with 3 different water supplies (30%, 40% and 70% of FC) under greenhouse conditions.

Mg Supply	Water Supply	Mg Content ($\mu g.plant^{-1}$)			
		Husk	Remaining Leaves	Stem	Grain
0 ppm	30% FC	264 ± 106	495 ± 59	85 ± 25	844 ± 113
	40% FC	340 ± 217	430 ± 113	118 ± 21	945 ± 226
	70% FC	542 ± 168	774 ± 56	219 ± 56	1754 ± 184
50 ppm	30% FC	270 ± 89	1259 ± 191	117 ± 15	1193 ± 217
	40% FC	320 ± 135	1176 ± 271	144 ± 25	1450 ± 171
	70% FC	605 ± 290	1527 ± 334	239 ± 61	2212 ± 464

Husk: $HSD_{0.05}$ (Mg; Water; MgxWater) = (*n.s*; 202; *n.s*)
Rem. Leaves: $HSD_{0.05}$ (Mg; Water; MgxWater) = (150; 223; *n.s*)
Stem: $HSD_{0.05}$ (Mg; Water; MgxWater) = (*n.s*; 42; *n.s*)
Grain: $HSD_{0.05}$ (Mg; Water; MgxWater) = (191; 284; *n.s*)

The grain samples of the experimental plants were analyzed for the starch concentration. As shown in Table 2.4, under both Mg levels, there were little changes in grain starch concentration. Increasing drought stress tended to enhance the grain starch concentration, probably due to lower grain yield (Table 2.4). Therefore, the starch content of grains showed clear increase (Table 2.4) with the increase in grain yield (Table 2.1). Highest starch content values were obtained under 50 ppm Mg and 70% FC water supply.

Table 2.4: Changes in starch concentration (mg.g^{-1}) and content (mg.plant^{-1}) of 81 days-old bread wheat (*Triticum aestivum* cv. Adana99) grains grown with low (0 ppm), and adequate (50 ppm) Mg applications and 3 different water supplies (30%, 40% and 70% of the F.C.) under greenhouse conditions.

		Grain Starch	
Mg Supply	**Water Supply**	**Concentration (mg.g^{-1})**	**Content (mg.plant^{-1})**
0 ppm	**30% FC**	524±35	441±120
	40% FC	521±34	466±183
	70% FC	446±39	721±93
50 ppm	**30% FC**	518±16	519±97
	40% FC	508±20	585±123
	70% FC	457±29	734±115

Concentration: HSD$_{0.05}$ (Mg; Water; MgxWater) = (*n.s*; 32; *n.s*)
Content: HSD$_{0.05}$ (Mg; Water; MgxWater) = (*n.s*; 146; *n.s*)

Due to absence of the effect of varied Mg nutrition in the experiment described above, an additional experiment has been conducted. In this second experiment, plants with low Mg (0 ppm) and adequate Mg (50 ppm) were first grown at 70% of FC until 68 DAS. At this time, plants were around anthesis stage. Then, part of the plants was exposed to low water supply (e.g., to 30% of the field capacity). The remaining plants continued to receive adequate water supply (70% of FC) under two levels of soil Mg treatments (e.g., 0 ppm: low Mg plants and 50 ppm: Mg adequate plants). In contrast to the first experiment, there was a clear effect of low Mg supply on growth of plants (Fig. 2.1). The negative effect of Mg deficiency on growth of wheat plants was evident before the start of the drought stress treatment (Figure 2.1).
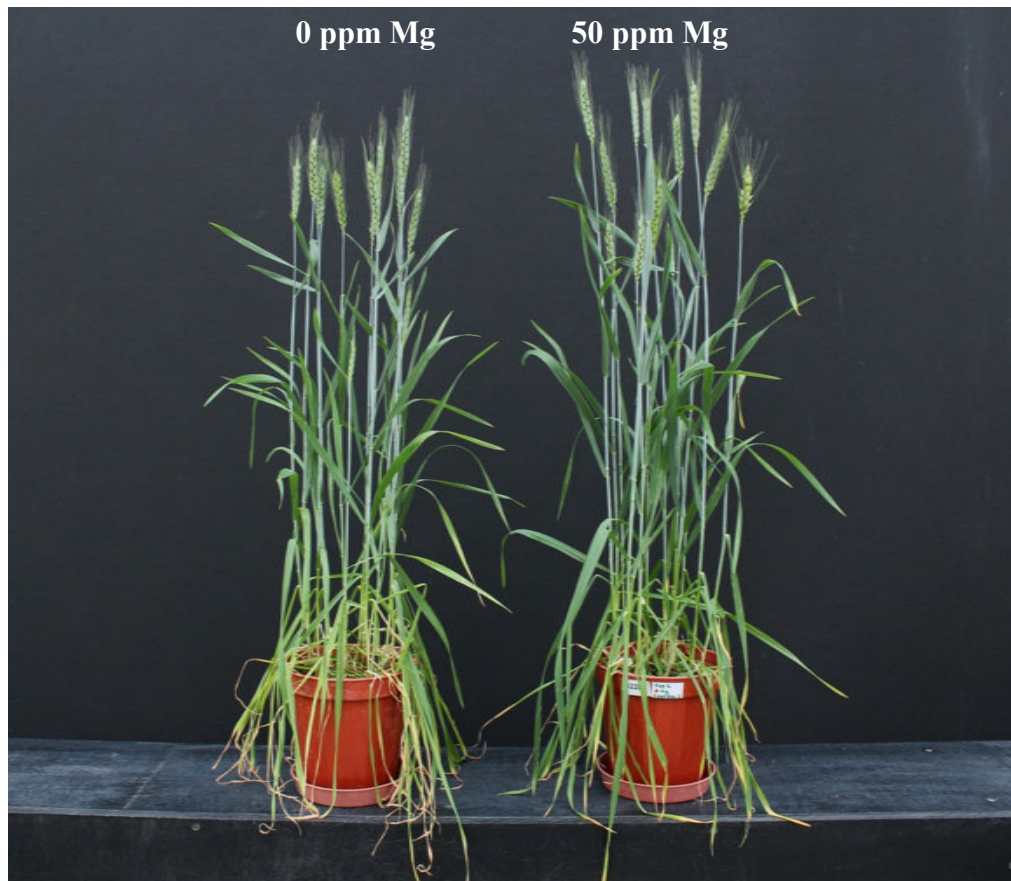
Figure 2.1: 68 days old wheat plants grown under low Mg (0 ppm) and adequate Mg (50 ppm) with 70% of the field capacity.

These visual observations were also reflected in the dry weight results of stem, grain and straw dry weights at the harvest (Table 2.5). With exception of flag leaves, at each water treatment higher Mg treatment improved dry matter production and grain yield. However, in case of husk and remaining leaves, the Mg effects on dry matter were not significant. In contrast, adequate water supply increased the dry weights of these specific plant parts regardless of Mg supply (Table 2.5). Dry weight of stem and grain yield were affected positively from both increasing Mg and water supply. Additionally, grain yield of low Mg (0 ppm Mg) plants decreased dramatically with drought stress treatment regardless of the Mg supply. When the water supply was sufficient, increasing Mg supply increased the grain yield about 20%.

Table 2.5: Effects of low (0 ppm), adequate (50 ppm) Mg applications and two different water supplies (30% and 70% of the field capacity) on dry matter production of 122 days-old bread wheat (*Triticum aestivum* cv. Adana99) plants under greenhouse conditions.

		Dry Weights (g.plant^{-1})					
Mg Supply	**Water Supply**	**Husk**	**Flag leaf**	**Remaining leaves**	**Stem**	**Grain**	**Straw**
0 ppm	**30% FC**	0.34±0.01	0.05±0.01	0.36±0.12	0.49±0.04	0.99±0.11	1.24±0.11
	70% FC	0.45±0.03	0.05±0.01	0.35±0.04	0.59±0.03	1.18±0.12	1.44±0.06
50 ppm	**30% FC**	0.37±0.05	0.05±0.01	0.42±0.09	0.56±0.05	1.06±0.13	1.39±0.05
	70% FC	0.46±0.03	0.05±0.00	0.38±0.01	0.67±0.03	1.42±0.12	1.56±0.06

Husk: $HSD_{0.05}$ (Mg; Drought; MgxDrought) = (*n.s*; 0.02; *n.s*)
Flag Leaf: $HSD_{0.05}$ (Mg; Drought; MgxDrought) = (*n.s*; 0.005; *n.s*)
Rem. Leaves: $HSD_{0.05}$ (Mg; Drought; MgxDrought) = (*n.s*; *n.s*; *n.s*)
Stem: $HSD_{0.05}$ (Mg; Drought; MgxDrought) = (0.03; 0.03; *n.s*)
Grain: $HSD_{0.05}$ (Mg; Drought; MgxDrought) = (0.10; 0.10; *n.s*)
Straw: $HSD_{0.05}$ (Mg; Drought; MgxDrought) = (0.06; 0.06; *n.s*)

At harvest, appearance of the plants with low (0 ppm Mg) and adequate Mg (50 ppm Mg) treatments at two water regimes was presented in Figs. 2.2, 2.3, 2.4 and 2.5.
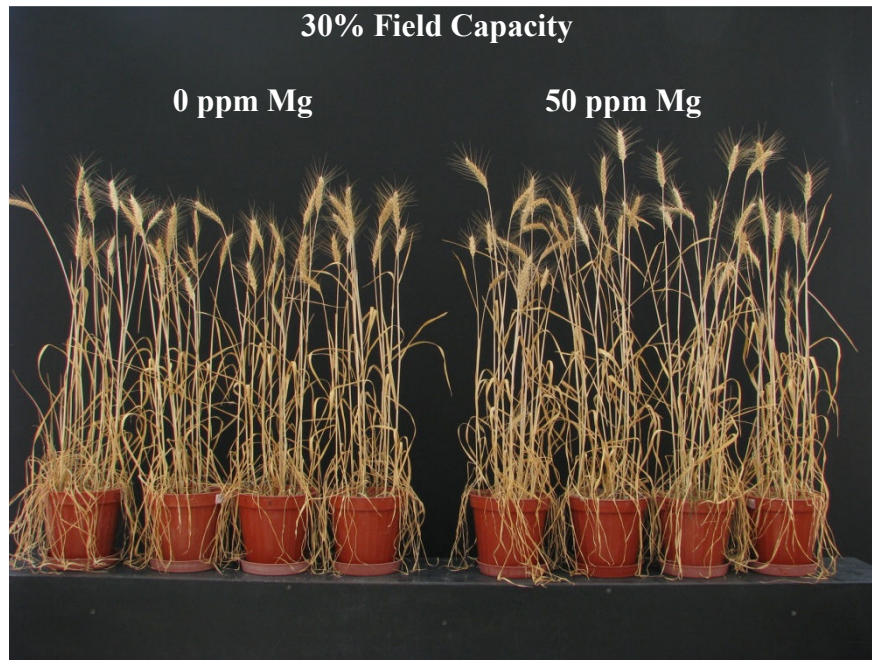
Figure 2.2: Growth of 122 days old wheat plants (*Triticum aestivum* cv. Adana99) under 30% of FC with low (0 ppm) and adequate (50 ppm) Mg treatments.
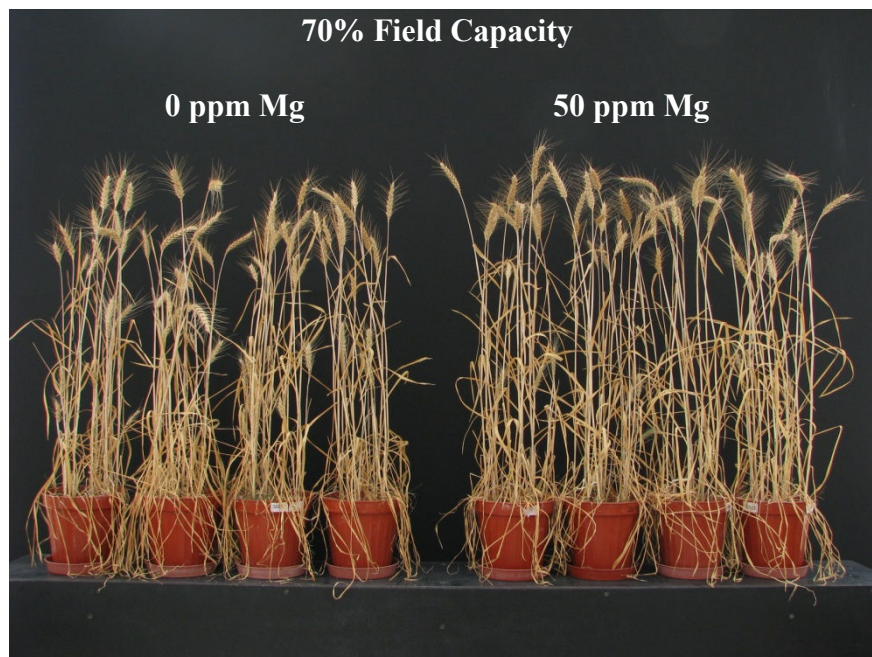
Figure 2.3: Effects of low (0 ppm) and adequate Mg (50 ppm) treatments on growth of 122 days old wheat plants (*Triticum aestivum* cv. Adana99) under sufficient water supply (70% of the field capacity).
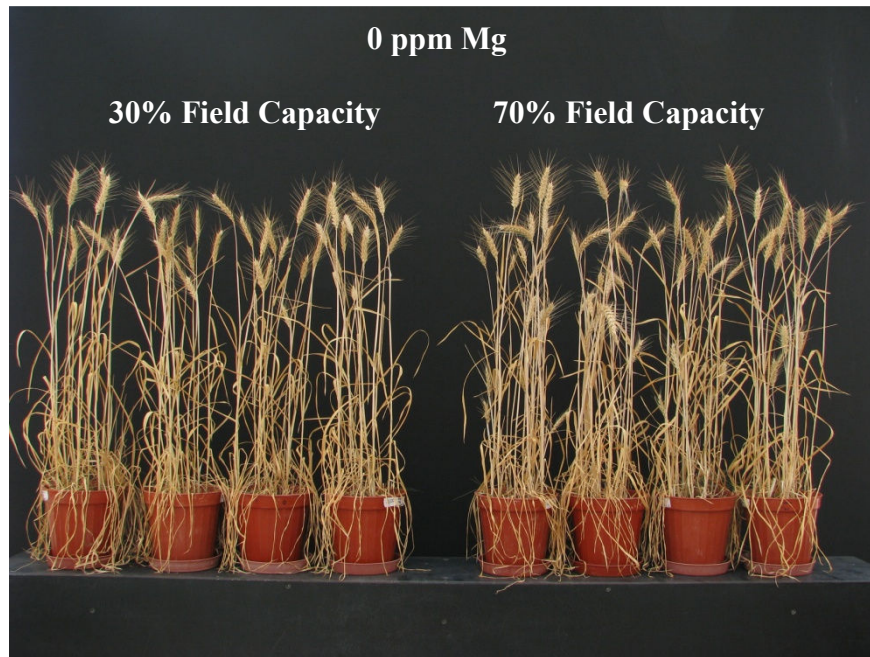
Figure 2.4: Effects of low (30% of FC) and adequate (70% of FC) water supply on growth of 122-days-old wheat plants (*Triticum aestivum* cv. Adana99) at low Mg (0 ppm) supply.
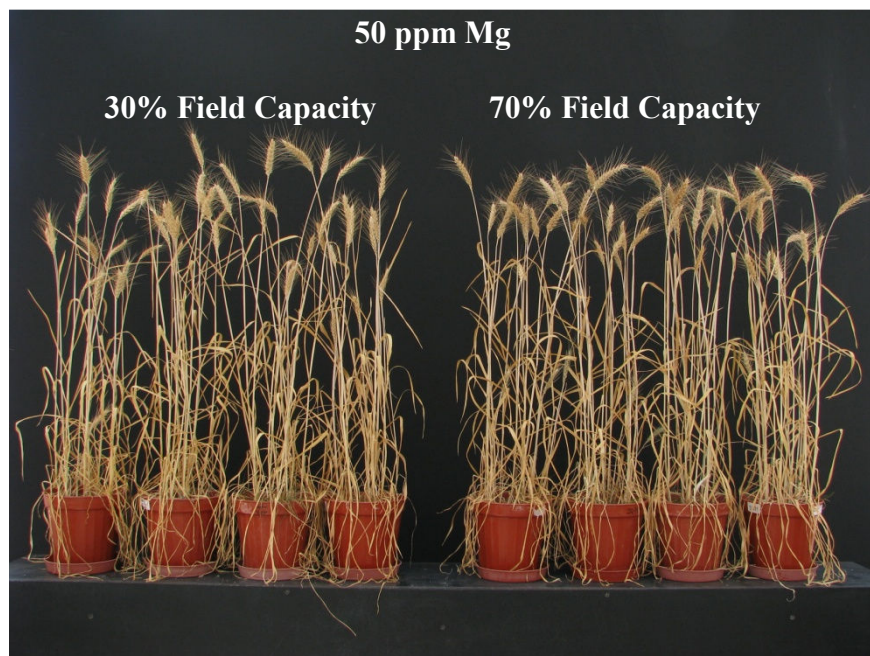
Figure 2.5: Growth of 122 days old wheat plants (*Triticum aestivum* cv. Adana99) with sufficient (50 ppm) Mg supply at 30% and 70% of FC conditions.

Plants which were under drought stress conditions and supplied with 0 ppm Mg remained smaller when compared to plants those were supplied with 50 ppm Mg (Figure 2.2). Under sufficient water supply, height of Mg deficient plants was close to adequate Mg plants (Figure 2.3). Magnesium deficient plants that are under drought stress or sufficient water supplied conditions did not show distinct differences in terms of plant height between them (Figure 2.4).When plants under drought stress and supplied with 50 ppm Mg, there was no distinct differences between the well-watered plants in terms of height (Figure 2.5).

The plants with adequate Mg supply had more Mg than the plants with low Mg at both water regimes (Table 2.6). Low water supply generally reduced Mg concentrations of plants. Even though plants were suffering from low water availability, all the plant fractions were able to show a consistent increase of Mg concentration with supply of 50 ppm Mg (Table 2.6). Plant parts contained Mg under drought condition as much as under adequate water supply conditions.

Table 2.6: Magnesium concentrations ($mg.kg^{-1}$) of 122 days-old bread wheat (*Triticum aestivum* cv. Adana99) plants grown with low (0 ppm), adequate (50 ppm) Mg applications and two different water rates (30% and 70% of the field capacity) under greenhouse conditions.

Mg Supply	Water Supply	Mg Concentration ($mg.kg^{-1}$)				
		Husk	Flag leaf	Remaining leaves	Stem	Grain
0 ppm	30% FC	253 ± 53	587 ± 106	931 ± 96	132 ± 22	1015 ± 71
	70% FC	317 ± 65	602 ± 281	1057 ± 273	189 ± 8	1300 ± 158
50 ppm	30% FC	495 ± 125	1951 ± 430	2418 ± 212	233 ± 58	1250 ± 53
	70% FC	511 ± 113	1646 ± 631	2649 ± 111	231 ± 34	1537 ± 149

Husk: $HSD_{0.05}$ (Mg; Water; MgxWater) = (80; *n.s*; *n.s*)

Flag Leaf: $HSD_{0.05}$ (Mg; Water; MgxWater) = (349; *n.s*; *n.s*)

Rem. Leaves: $HSD_{0.05}$ (Mg; Water; MgxWater) = (159; 159; *n.s*)

Stem: $HSD_{0.05}$ (Mg; Water; MgxWater) = (30; *n.s*; *n.s*)

Grain: $HSD_{0.05}$ (Mg; Water; MgxWater) = (100; 100; *n.s*)

Similar to Mg concentration, also Mg content of the plants (e.g., total Mg uptake) was also increased with the supply of Mg (Table 2.7). The increases in Mg content were particularly high in case of grain.

Table 2.7: Effects of low (0 ppm) and adequate (50 ppm) Mg applications with different water supplies (30% and 70% of the field capacity) on Mg content ($\mu g.plant^{-1}$) of 122 days-old bread wheat (*Triticum aestivum* cv. Adana99) plants under greenhouse conditions.

		Mg Content ($\mu g.plant^{-1}$)				
Mg Supply	Water Supply	Husk	Flag leaf	Remaining leaves	Stem	Grain
0 ppm	30% FC	86 ± 16	27 ± 4	339 ± 115	65 ± 12	1011 ± 138
	70% FC	143 ± 30	32 ± 10	371 ± 131	111 ± 6	1520 ± 104
50 ppm	30% FC	183 ± 53	93 ± 11	1011 ± 246	131 ± 39	1326 ± 168
	70% FC	233 ± 53	85 ± 31	1001 ± 37	155 ± 23	2172 ± 75

Husk: $HSD_{0.05}$ (Mg; Mg; Water; MgxWater) = (35; 35; *n.s*)
Flag Leaf: $HSD_{0.05}$ (Mg; Water; MgxWater) = (*n.s*; 45; *n.s*)
Rem. Leaves: $HSD_{0.05}$ (Mg; Water; MgxWater) = (129; *n.s*; *n.s*)
Stem: $HSD_{0.05}$ (Mg; Water; MgxWater) = (19; 19; *n.s*)
Grain: $HSD_{0.05}$ (Mg; Water; MgxWater) = (107; 107; *n.s*)

Grains and flag leaves of the experimental plants were used for the starch analysis, and the grain results are shown in Table 2.8 and 2.9. Grain starch concentrations were not affected from the Mg and water treatments. All starch concentrations of the treatments looked very similar. Most probably, due to increases in grain yield by increasing water supply, starch content in grains showed increases, especially in case of adequate Mg supply (Table 2.8).

Table 2.8: Changes in the grain starch concentration (mg.g^{-1}) and content (mg.plant^{-1}) of 122 days-old bread wheat (*Triticum aestivum* cv. Adana99) plants grown with low (0 ppm), adequate (50 ppm) Mg applications and two different water regimes (30% and 70% of the field capacity) under greenhouse conditions.

		Grain Starch	
Mg Supply	**Water Supply**	**Concentration (mg.g^{-1})**	**Content (mg.plant^{-1})**
0 ppm	**30% FC**	569±49	566±74
	70% FC	553±15	652±69
50 ppm	**30% FC**	567±23	600±67
	70% FC	564±33	802±85

Concentration: HSD$_{0.05}$ (Mg; Water; MgxWater) = (*n.s*; *n.s*; *n.s*)
Content: HSD$_{0.05}$ (Mg; Water; MgxWater) = (63; 63; 119)

It was clear to notice that low Mg plants had more starch concentration in flag leaves than the Mg-adequate plants (especially in case of sufficient water supply), although the effects were not significant (Table 2.9). There was also no significant change in starch contents of the flag leaves under given treatments. At adequate water supply, low Mg plants showed more starch than the plants with adequate Mg supply.

Table 2.9: Changes in the flag leaf starch concentration (mg.g^{-1}) and starch content (mg.plant^{-1}) of 122 days-old bread wheat (*Triticum aestivum* cv. Adana99) plants grown with low (0 ppm), adequate (50 ppm) Mg applications and two different water supplies (30% and 70% of the field capacity) under greenhouse conditions.

		Flag Leaf Starch	
Mg Supply	**Water Supply**	**Concentration (mg.g^{-1})**	**Content (mg.plant^{-1})**
0 ppm	**30% FC**	4.72±1.69	0.22±0.08
	70% FC	5.56±2.32	0.30±0.13
50 ppm	**30% FC**	4.46±1.35	0.22±0.10
	70% FC	4.36±2.21	0.23±0.13

Concentration: HSD$_{0.05}$ (Mg; Water; MgxWater) = (*n.s*; *n.s*; *n.s*)
Content: HSD$_{0.05}$ (Mg; Water; MgxWater) = (*n.s*; *n.s*; *n.s*)

2.4 Discussion

As expected, the drought stress treatments substantially reduced growth and yield of plants under both low and adequate Mg treatments (Table 2.1). Decrease in plant growth under drought stress is a common phenomenon and shown several times in different crop plants such as in maize (Subramanian and Charest, 1997), soybean (Specht et al., 2001), sunflower (Tahir and Mehid, 2001) and wheat (Zareian et al., 2014; Abdullah et al., 2015). The first reaction of plants to drought stress is to close the stomata (Cornic and Massacci, 1996) which leads to a chain reaction of negative impacts such as decrease of CO_2 accumulation into leaves (Farooq et al., 2009) that reduces photosynthesis and causes high energy electrons to form ROS at cost of inhibited photosynthesis (Fu and Huang, 2001; Reddy et al., 2004). Consequently, structural and functional integrity of chloroplasts is severely affected from the oxidative attack of ROS, leading to impairment in the capacity of plants to yield better (Flexas et al., 2002; Suzuki et al., 2012). In drought stressed plants, photo-oxidative damage occurs typically due to impaired use of absorbed light energy and released electrons in photosynthetic CO_2 fixation (Miller et al., 2010; Noctor et al., 2014).

According to Mengutay et al, (2013), combination of heat stress with Mg deficiency stress further induces photooxidative damage and growth depressions. Similarly, also in the present study it has been shown that plants with low Mg supply and drought stress treatment showed the lowest grain yield. As mentioned before, under Mg deficiency, use of absorbed light energy in photosynthesis is reduced due to accumulation of carbohydrates in leaves as a result of limited transportation of carbohydrates from source organs to sink organs (Cakmak et al. 1994a; Hermans et al. 2005). Such conditions in chloroplasts of low Mg plants result in high risk for photooxidative damage. Therefore, plants under low Mg supply show exceptionally high sensitivity to high light and became rapidly chlorotic (Marschner and Cakmak, 1989). It can be suggested that exposure of low Mg plants to an environmental stress such as drought will intensify cell damage by promoted production of ROS as demonstrated for low Mg plants under heat stress (Mengutay et al., 2013). Consequently, the decrease in growth or yield under low Mg is expected to be pronounced when exposed to drought stress as shown in Tables 2.1 and 2.5.

There were unexpected results in the first experiment with two Mg and three water treatments. Grain yield of the plants was not affected from low and adequate Mg treatments at adequate water supply (Table 2.1). However, at the 40 % and 30 % of the field capacity, Mg adequate plants tended to have more grain yield. In contrast to grain yield, dry matter production of straw was very distinctly increased under low Mg supply when compared to the plants with adequate Mg (Table 2.1). These results indicate that dry matter allocation from vegetative parts into grains is impaired under low Mg plants and therefore straw dry matter is higher under low supply of Mg. Generally, a similar observation was also made for most of the individual vegetative parts of plants. Decreases in transport of photoassimilates from source organs (e.g., leaves) into sink organs (e.g., grains/seeds) are known well for Mg deficient plants (Cakmak and Kirkby, 2008). Probably, because of this impairment in transport of photoassimilates, vegetative parts of low Mg plants had generally higher dry matter than the Mg adequate plants. However, in contrast to the results in Table 2.1 of the first experiment, in the second experiment, low Mg plants did not show greater straw dry matter than the Mg adequate plants. The reason of such differential result between two experiments could not be understood well. In case of the nutrient solution experiment in Chapter 1, where very clear Mg deficiency stress could be induced, most of the vegetative parts of the low Mg plants had more dry matter production than the Mg adequate plants supporting the results of the 1st experiment of this chapter.

Reduced grain yield under Mg deficiency was a common observation in both experiments of this chapter, except the treatment at 70 % of the field capacity of the 1st experiment. The reduction in grain yield under low Mg could be a consequence of several processes. First, an impairment of phloem loading of sucrose from source organs causes accumulation of carbohydrates in the leaves (Cakmak and Kirkby, 2008), and this effect leads to a reduction in photosynthesis rate (Laing et al., 2000; Hariadi and Shabala, 2004; Neuhaus et al., 2013). Photosynthesis rate can be also reduced under low Mg due to reduced activity of photosynthetic enzymes such as Rubisco (Marschner, 2012) and reduced inflow of CO_2 into chloroplasts (Cakmak and Kirkby, 2008). Reduced production of photoassimilates and impaired translocation of photoassimilates into seeds/grains are most plausible explanations for the reduction in grain yield under low Mg. In a good agreement with this explanation, the accumulation of starch in grains (e.g., content) was generally increased by increasing Mg supply and also by improving water status of plants (Table 2.4 and Table 2.8). Accumulation of starch in Mg deficient

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flag leaves (Table 2.9) indicates reduced transportation of carbohydrates from source organs into grains under low Mg supply. The clear decrease in starch content by increasing water stress in plants under both low and adequate Mg supply (Tables 2.4 and 2.8) is probably a reflection of both reduced photosynthetic activity and inhibited transportation of photo-assimilates into grains under drought. In addition, the activity of the enzyme called starch synthase which converts sugars to starch in the maturing grain was found to be decreased under environmental stress factors such as drought, heat or salinity which further leading to a reduced starch amount in cereal grains (Wang and Frei, 2011). Reduction in activity of this enzyme was reported in different crops such as in wheat under droughtand heat stress (Rijven, 1986; Keeling et al., 1993).

In mature wheat plants, the critical Mg deficiency concentration of plants is reported to be around 0.1% (Jones et al. 1991). Magnesium concentration of low Mg plantsin the both Mg concentrations measured in these two experiments were found to be under this critical level (Tables 2.2 and 2.6). In addition, Mg concentration of whole vegetative tissues in 1st experiment was measured when plants were 30 days old and the Mg concentrations were found as 0.07% and 0.13% for 0 ppm and 50 ppm Mg plants respectively. These results also support the deficiency of Mg from the beginning of the experiment.

Especially under drought conditions Mg concentration levels dropped dramatically when compared to the plants at 70% of field capacity. Root uptake of Mg depends on water availability of the soil and successful root growth and activity (Gransee et al., 2013). In good agreement with this, Mg concentrations of almost all plant parts at the lowest water supply were reduced compared to the plants with adequate water supply (Table 2.2; Table 2.6). Usually, Mg concentration of grains increased under all drought treatments when Mg supply was increased from 0 to 50 ppm. In a previous study it has been shown that under drought stress conditions Mg concentration of maize grains increased significantly when compared to adequate water supplied conditions and the mechanism behind this result is explained by the enhanced routes and/or transport mechanisms for Mg under decreased water availability (Ti et al., 2010). However, such increase in grain Mg under drought stress could also be a consequence of "concentration effects" due to reduced grain yield under drought.

In conclusion, the results highlighted that reductions in grain yield of plants under low Mg supply is pronounced when Mg deficiency is combined with drought stress conditions. This observation could be related both to reduced Mg uptake of plants

and increasing risk with photooxidative damage in plants exposed to low Mg in growth medium. Low Mg plants were associated with lower starch content in grains, probably due to reduced synthesis and transportation of carbohydrates in the grains. This finding is a further support for the decreases in grain yield capacity of low Mg plants, especially under drought stress conditions (Table 2.4). It is concluded that under water-limited growth conditions Mg nutritional status of plants is of great importance in respect to maintenance of a proper photosynthetic rate and transportation of carbohydrates into actively growing parts of plants such as seeds and roots.

CHAPTER 3

FOLIAR APPLICATION OF ^{26}Mg ISOTOPE TO COFFEE PLANTS: A TRANSLOCATION EXPERIMENT

3.1 Introduction

Coffee is a dicotyledon woody perennial and evergreen plant belonged to Rubiaceae family. The most commercially important coffee species are Coffea Arabica and Coffea canephore (ECF, 2015) but among these species Coffea Arabica is the mostly produced coffee plant which is reported as 75-80% of the world's population (Griffin, n.d). There are around 70 countries that producing coffee all around the world. Brazil is the biggest producer of coffee and 40% of the world's total supply is attributed to Brazil. In 2014, Brazil produced around 3 billion kilograms of coffee beans (ICO, 2015). There are some factors that limit the production of coffee in Brazil and one of the important limiting factors is the soil acidity and poor calcium and magnesium content of the soil (Matiello, 1985).

According to Malavolta (1993), coffee is not a tolerant crop to mineral deficieny conditions in soils. It is a known fact that some mineral element deficiencies can not be detected for days until a foliar deficiency symptom becomes visible. This situation is known as "hidden deficiency". If the deficiency condition is not detected and corrected at the proper time, growth and development of plants can be severely altered and economic losses will be unavoidable (Fernandes et al., 2012).

There are limited number of published reports about the effects of mineral status of coffee plants on growth and development. For example one of these studies showed that the caffeine content of leaves in coffee plants decreased about 17% under Mg deficient conditions (Mazzafera, 1999). Nine-fold yield reduction was observed in coffee plants that were grown under low nitrogen and potassium fertilization for two

years (Dean and Beaumont, 1958). A recent study showed that coffee plants grown under Mg-deficient conditions showed an increase in the ROS production (da Silva et al., 2014) just like in bean and wheat plants (Cakmak and Marschner, 1992; Mengutay et al., 2013).

Acidic soils are typical soils for Mg deficiency, mainly due to high leaching risk from soil profile and antagonistic effects of Al and Mn ions on root uptake (Marschner, 2012; Gransee et al., 2013). Dolomite ($CaMg(CO_3)_2$) is often applied in such acidic soils in order to correct low pH and contribute to Mg nutrition of plants. However, there is increasing number of debates in literature about agronomic effectiveness of dolomite in providing adequate soluble Mg to plant roots (Gransee et al., 2013; Senbayram et al.2015). Therefore, soluble sources of Mg fertilizers are suggested to apply into acidic soils to ensure a better Mg nutrition of crop plants. In acidic soils, subsoil acidity represents another constraint to root growth. It seems to be difficult to solve subsoil acidity and related nutritional problems such as Mg deficiency through liming and Mg fertilization. These strategies may help to mitigate the mentioned constraints in the topsoil; but not in the subsoil. Since root growth is extremely sensitive to low Mg supply, especially in acidic soils, it is important to provide Mg into subsoil. Foliar application of Mg would be one strategy to deliver Mg from shoot to roots grown in subsoil. To our knowledge, there is, however, no study focused on effect of foliarly applied Mg on Mg concentration of roots of plants growing widely in acidic soils such as coffee plants. In this part of the thesis we investigated role of foliar Mg fertilization on translocation of Mg into roots. In order to distinguish better the foliarly applied Mg from the Mg existing already in the plants we used stable isotope enriched-$MgSO_4$ in foliar application.

Studies conducted with isotopes to monitor the nutrient uptake and remobilization within the plant is increasing both in plant nutrition and plant physiology related studies. There are usually two forms for an element to existing naturally, stable and nonstable (radioactive) forms. Two or more stable isotopes are available for most of the elements and usually one of them has a greater abundance compared to others (Rundel et al., 1989). Stable isotopes are frequently used in plant and human physiological studies and gave a chance to direct measurement and monitoring of the mineral uptake and remobilization (Proe et al., 2000). In studies involving usage of isotopes and in case of unstable isotopes radiotracing and mass spectrometry can be used (Roscher et al., 2000).

There are 3 types of stable isotopes for Mg: i) ^{24}Mg with abundance of 78.70%, ii) ^{25}Mg with abundance of 10.13% and iii) ^{26}Mg with abundance of 11.17%. There are increasing number of studies using stable isotopes of Mg and other elements to investigate potential relationships with other mineral nutrient deficiencies and characterize physiological and biochemical mechanisms such as uptake, translocation and deposition of ^{25}Mg. The stable Mg isotope ^{26}Mg has been used in Norway spruce to study root uptake and accumulation of ^{26}Mg in plants under the influence of pH and Al (Kuhn et al., 1995). In a previous study, Grusak (1997) reviewed studies dealing with use of stable isotope labeling tool in human nutritional studies.

As indicated above, we are not aware of a study investigating transportation of MgSO$_4$ labelled with stable isotope of Mg (^{26}Mg) within coffee plants after its application of coffee plants. Most of the studies in the present thesis are focused on wheat plants. Because of high relevance of coffee plants in terms of studying Mg transportation within plants (especially into roots), this part of the thesis used coffee as experimental material to investigate the transport and uptake of Mg in coffee plant treated foliarly with ^{26}Mg-enriched MgSO$_4$. In the studies conducted here, transportation of ^{26}Mg in coffee plants has been studied by immersing part of the leaves in a solution containing MgSO$_4$ enriched ^{26}Mg isotope, and this work has been realized by using both Mg deficient and sufficient coffee plants.

3.2 Materials and Methods

This experiment was established with coffee plants (*Coffea Arabica* cv.Murta) by using 5 independent replicates for each treatment involving transportation of foliarly-treated ^{26}Mg application within plants. In addition, 3 independent replicates from each treatment were used for the control treatment without ^{26}Mg application (e.g., ddH$_2$O application).

Coffee seedlings used in this study were received from Prof. Dr. Andreas Burkert, Kassel University, Germany. Seedlings received were first grown hydroponically in 5L pots for 224 days; then 1 day before application of ^{26}Mg they were transferred to 1L pots as 1 seedling per pot. Throughout the entire experiment time the plants were supplied with the following nutrients: 1 mM of (NH$_4$)$_2$SO$_4$, 0.5 mM Ca(NO$_3$)$_2$, 0.075 mM of KH$_2$PO$_4$, 0.25 mM of K$_2$SO$_4$, 0.4 mM of CaCl$_2$, 0.03 mM of

Fe-EDTA, 1 µM of $ZnSO_4.7H_2O$, 5 µM of $MnSO_4.H_2O$, 0.2 µM of $CuSO_4$, 10 µM of H_3BO_3 and 0.14 µM of $(NH_4)_6Mo_7O_{24}.4H_2O$. Coffee plants were supplied with low and adequate (0.01 and 0.4 mM) Mg in the form of $MgSO_4.7H_2O$.

[26]Mg isotope was obtained from MaTecK (Ireland) with 99.62% enrichment in the form of MgO. Since the absorption of MgO through leaves is extremely limited, MgO was converted into $MgSO_4.7H_2O$ by following the steps according to the student manual of Preparation of Magnesium Sulfate (n.d): 50 mg MgO isotope was mixed with 1M H_2SO_4 solution, and completed to 20 ml and pH of the solution was adjusted to 5.5. The final concentration ofl [26]$MgSO_4.7H_2O$ in the solution was 1.5%. Considering that coffee leaves had a very waxy structure, [26]Mg solution was mixed with a surfactant Tween 20 in ratio of 0.01% to get a better coverage on the leaves.

Treatment of coffee plants with [26]Mg solution was realized by immersing (dipping) of the selected leaves into a [26]Mg solution as following. Fully expanded leaves that were the 7th leaves from bottom for each plant were used in immersing leaves into [26]Mg solution as shown in Figure 3.1.

Figure 3.1: Dipping of coffee (*Coffea arabica* cv. Murta) plant leaf in [26]Mg solution under greenhouse conditions.

The control plants were immersed into double distilled water (ddH_2O). The immersion process of the leaves has been conducted by using cylindrical flasks and the leaves were dipped (for about 10 seconds) into these solutions 3 times a day for 10

days. To enhance the absorption of the solutions and to avoid the quick drying of them, ddH_2O was sprayed to all plants in a form of mist 3 times a day.

Ten days after applying the first ^{26}Mg solution, experimental plants were harvested. At harvest, following plant fractions were harvested separately: 1st fraction was the Mg^{26}-applied leaves (app. leaves), 2nd fraction was the shoot part that was above the application leaves (youngest leaves), 3rd fraction was the remaining shoot part that is under the Mg^{26}-applied leaves (old leaves) and 4th fraction was the roots. Before drying plant parts, all of the harvested parts of the plants were washed in 1 mM $CaCl_2$, 1mM EDTA and ddH_2O solutions for about 3 minutes to desorb any ^{26}Mg adhered on the leaf surface and existing in leaf apoplast.

The plant parts harvested were dried in the oven for 3 days at 70°C. After the drying stage, dry weights of the fractions were taken. Thereafter, samples were ground, digested in microwave and subjected to ICP-MS analysis for measurement of ^{26}Mg. The measurement of ^{26}Mg in digested samples was realized in collaboration with Prof. Dr. Roland Bol and Dr. Bei Wu at the Institute of Bio- and Geosciences (IBG), Forschungszentrum Jülich in Jülich, Germany. Measurement ^{26}Mg was performed in all of the plants; however there was no additional ^{26}Mg rather than the natural aboundance found in ddH_2O treated plants, because of that ^{26}Mg results of ddH_2O treated plants was not shown. In all tables except ^{26}Mg concentration table (Table 3.2), all of the plants were used for the calculations, because there was no significant difference in dry weights, Mg and K concentrations with respect to ^{26}Mg and ddH_2O applications. Additionally ^{26}Mg concentrations were expressed and calculated as the enrichment of ^{26}Mg in the plants (^{26}Mg concentration in found in the plant parts except the natural abundance of ^{26}Mg).

3.3 Results

Coffee plants supplied with low Mg exhibited chlorosis and necrosis symptoms in their old and middle leaves, whereas the plants supplied adequately with Mg did not show any chlorosis or necrosis symptoms and looked very healthy (Figure 3.2).

Figure 3.2: Shoot growth of coffee (*Coffea arabica* cv. Murta) plants in 5L nutrient solution with low (0.01 mM) and adequate (0.4 mM) Mg supply under greenhouse conditions before starting the foliar treatment experiment with ^{26}Mg solution.

Table 3.1 shows the average dry weights of the experimental plants used in the experiments with and without ^{26}Mg treatment. Compared to shoot growth, root growth was more clearly affected from low Mg supply than the adequate Mg supply (Figure 3.3; Table 3.1). As shown in Figure 3.3, shoot heights of Mg-deficient plants were less affected from low Mg supply. Both dry weight (Table 3.1) and density (Figure 3.3) of roots exhibited a clear difference between low and adequate Mg supply. Because shoot growth was less sentitive to low Mg supply, the shoot to root ratio was distinctly increased under low Mg supply (Table 3.1). These visible shoot and root biomass differences were reflected well in the dry weight results. As average, low Mg supply reduced the total shoot biomass by over 22%. Dry weights of the application (app.) leaves, old leaves and roots were significantly decreased under Mg-deficient conditions. Young leaves of coffee plants were weighed 19% higher under Mg-deficient conditions; however this increase was not significant. As indicated above, root growth was very sensitive to low Mg. Root dry weights of the Mg-adequate plants were on average 2 fold higher than the root dry weight of low Mg plants. Consequently, a significantly higher shoot-to-root ratio was found under Mg-deficient conditions.

Figure 3.3: Growth of 224 days old coffee (*Coffea arabica* cv. Murta) plants in 5L nutrient solutions with low (0.01 mM) and adequate (0.4 mM) Mg supply under greenhouse conditions.

Table 3.1: Dry matter production of different parts of coffee plants (*Coffea Arabica* cv. Murta) used in the experiments with or withour 26Mg treatment and grown hydroponically with low (0.01 mM) and adequate (0.4 mM) Mg under greenhouse conditions.

	Dry Weights (g.plant^{-1})					
Mg Supply	Young Leaves	App. Leaves	Old Leaves	Roots	Total shoot biomass	Shoot/ Root
Low	0.44 a	0.21 a	0.94 a	0.51 a	1.59 a	3.18 a
Adequate	0.37 a	0.33 b	1.37 a	1.09 b	2.06 b	1.92 b

Values are means of eight independent replicates. Different letters indicate significant differences between means according to one-way ANOVA and Tukey's HSD test ($p \leq 0.05$).

The concentrations and contents of the ^{26}Mg that transported from the treated leaves to the rest of the plant parts are presented in Table 3.2 A and B, respectively. The ^{26}Mg concentration in application leaves was not significantly affected by the Mg supply (Table 3.2 A). For example, ^{26}Mg concentrations in the young leaves of the Mg-sufficient plants were found 3.5 times higher than the low Mg plants. In case of old leaves, Mg sufficient plants had 10 fold higher ^{26}Mg than the Mg deficient plants. In contrast, low Mg plants showed higher ^{26}Mg concentrations than the Mg-adequate plants. In the roots there was nearly 2.8-fold higher ^{26}Mg concentration compared to the adequate Mg plants.

Interestingly, old and young leaves of the Mg-adequate plants contained significantly higher amount of enriched ^{26}Mg than the low Mg plants, while roots have shown a significant decrease in low Mg plants (Table 3.2 B). The enriched ^{26}Mg content did not significantly differed between the application leaves of low and adequate Mg supplied plants, There was however slight increase in ^{26}Mg concentration of the treated leaves under adequate Mg supply. Mg-deficient plants translocated nearly the same amount of ^{26}Mg to young and old leaves; in contrast sufficient Mg-supplied plants translocated more amounts of ^{26}Mg to old leaves when compared to young leaves.

Table 3.2: Changes in the enriched concentrations (A) and contents (B) of ^{26}Mg (mg.kg^{-1}) measured by ICP-MS in coffee plants (*Coffea arabica* cv. Murta) grown with low (0.01 mM) and adequate (0.4 mM) Mg and treated with ^{26}Mg by immersing selected leaves into ^{26}Mg-contatining solution under greenhouse conditions.

A)

Mg Supply	^{26}Mg enrichment concentration (mg.kg^{-1})			
	Young Leaves	App. Leaves	Old Leaves	Roots
Low	3.2 a	103 a	1.5 a	2.5 a
Adequate	11.3 b	86 a	15.1 b	0.9 b

Values are means of five independent replicates. Different letters indicate significant differences between means according to one-way ANOVA and Tukey's HSD test (p≤0.05).

B)

Mg Supply	^{26}Mg enriched content (µg.plant^{-1})			
	Young Leaves	App. Leaves	Old Leaves	Roots
Low	1.25 a	21.7 a	1.30 a	1.23 a
Adequate	4.00 b	28.3 a	18.41 b	0.94 b

Values are means of five independent replicates. Different letters indicate significant differences between means according to one-way ANOVA and Tukey's HSD test (p≤0.05).

As expected, Mg concentration of Mg-deficient plants was much lower than the Mg-adequate plants. At least 2 times more Mg was found in plants with adequate Mg supply (Table 3.3). Similarly, also Mg content results were significantly affected from varied Mg supplies (Table 3.4). In good agreement with better growth, plants with adequate Mg supply had much higher content (total uptake) of Mg.

Table 3.3: Concentrations of Mg (mg.kg^{-1}) in different parts of the coffee plants (*Coffea arabica* cv. Murta) supplied with low (0.01 mM) and adequate (0.4 mM) Mg under greenhouse conditions.

	Mg concentration (mg.kg^{-1})			
Mg Supply	Young Leaves	App. Leaves	Old Leaves	Roots
Low	1139 a	534 a	655 a	1371 a
Adequate	2744 b	2440 b	2819 b	3130 b

Values are means of eight independent replicates. Different letters indicate significant differences between means according to one-way ANOVA and Tukey's HSD test ($p \leq 0.05$).

Table 3.4: Contents of Mg (µg.kg^{-1}) in different parts of coffee plants (*Coffea arabica* cv. Murta) supplied with low (0.01 mM) and adequate (0.4 mM) Mg under greenhouse conditions.

	Mg Content (µg.kg^{-1})			
Mg Supply	Young Leaves	App. Leaves	Old Leaves	Roots
Low	498 a	108 a	613 a	710 a
Adequate	933 b	797 b	3863 b	3371 b

Values are means of eight independent replicates. Different letters indicate significant differences between means according to one-way ANOVA and Tukey's HSD test ($p \leq 0.05$).

Potassium concentrations of the plants were also measured and shown in Table 3.5. It is obvious that K concentrations were very much affected by the Mg supply level (Table 3.5). Potassium concentrations of low Mg plants were found higher when compared to Mg sufficient plants. All fractioned plant parts of Mg deficient plants (including roots) had shown increased concentration of K especially in leaves.

Table 3.5: Concentrations of K (%) in different parts of the coffee plants (*Coffea arabica* cv. Murta) supplied with low (0.01 mM) and adequate (0.4 mM) Mg under greenhouse conditions.

	K Concentration (%)			
Mg Supply	**Young Leaves**	**App. Leaves**	**Old Leaves**	**Roots**
Low	3.10 a	3.37 a	3.18 a	2.73 a
Adequate	2.15 b	1.57 b	1.74 b	2.41 b

Values are means of eight independent replicates. Different letters indicate significant differences between means according to one-way ANOVA and Tukey's HSD test (p≤0.05).

Potassium content of the plants is shown in Table 3.6. Similar trend that observed for K concentration results was also observed for K content data. Under Mg deficient conditions, K content of the all plant parts was significantly increased.

Table 3.6: Contents of K (mg.plant^{-1}) in different parts of the coffee plants (*Coffea arabica* cv. Murta) supplied with low (0.01 mM) and adequate (0.4 mM) Mg under greenhouse conditions.

	K Content (mg.plant^{-1})			
Mg Supply	**Young Leaves**	**App. Leaves**	**Old Leaves**	**Roots**
Low	13.6 a	7.0 a	29.9 a	13.9 a
Adequate	7.8 b	5.1 b	23.7 b	26.1 b

Values are means of eight independent replicates. Different letters indicate significant differences between means according to one-way ANOVA and Tukey's HSD test (p≤0.05).

3.4 Discussion

Intervenial chlorosis and necrosis of leaves is a common symptom of Mg deficiency in plants. In the present experiment, coffee plant developed yellowish color and too many necrotic spots when exposed to low Mg supply. Similar observations have been also made previously on 2 different variaties of coffee plants by Silva et al. (2014). According to Nagao et al. (1986), Mg-deficient coffee plants first developed slight chlorosis symptoms on the older leaves; then when the Mg-deficiency conditions prolonged light brown necrotic spots was observed on older leaves while younger leaves were not affected as observed in the present study. The chlorosis symptoms and necrotic spots are attributed to chlorophyll degration under Mg deficient conditions (Gransee and Führs, 2013), most probably by the peroxidative attack of oxygen free radicals which generated in chloroplasts, especially when plants exposed to high light (Cakmak and Kirkby, 2008, Mengutay et al., 2013).

In this study, as expected, low Mg plants produced less shoot and root biomasses when compared to adequate Mg plants (Table 3.1). Root growth of Mg deficient coffee plants was found to be more susceptible to Mg deficiency than shoot growth (Table 3.1; Figure 3.2). Therefore, a higher shoot to root ratio was found under low Mg conditions. Increased shoot to ratios under Mg deficiency was also reported for different plant species such as citrus (Yang et al. 2012), common bean (Cakmak et al. 1994a), spinach (Fisher et al. 1988) and pepper (Riga and Anza, 2003). Susceptibility of root growth to Mg deficiency can be a result of impaired carbohydrate supply from source to sink organs, such as roots (Cakmak et al. 1994b).

The adequate leaf Mg and K concentrations for coffee plants are given as 0.25-0.40% and 2.1-2.6% respectively (FAO, 2015). Also in other studies, following Mg ranges for coffee plants were reported as 0.25-0.40% for sufficient Mg concentration (Lima Filho and Malavolta, 2003), and 0.30-0.35% for sufficient and 0.04-0.11% for deficient Mg conditions (Nagao et al., 1986). In this study Mg concentrations in the shoot parts of coffee plants were in sufficient range when Mg supply was adequate (Table 3.3). Under Mg-deficient conditions, Mg concentration was found below the optimum level.

To our knowledge, there is no published report that studied the translocation and absorption rate of ^{26}Mg from leaves in coffee plants under low and adequate Mg concentrations. The results showed that Mg-adequate plants absorbed and translocated more ^{26}Mg than the low Mg plants (Table 3.2). This result was not expected. Normally, the hypothesis was low Mg plants should exhibit higher uptake and transportation rate due to increased demand for Mg. For this contradictory result: one explanation could be the presence of higher leaf K concentrations in low Mg plants (Table 3.5). Higher tissue K concentrations in the ^{26}Mg-treated leaves may represent an inhibitory factor for leaf uptake and transport of Mg. It is well-known that higher K concentrations in growth medium have an inhibitory effect on root uptake of Mg (Ding and Xu, 2011; Gransee et al., 2013). Previously, also Pettiet (1988) showed that root Mg uptake reduces when there is sufficiently high K in the soil. Antagonistic relationships between Mg and K elements were studied in a variety of plant species such as tomato (*Solanum lycopersicum*) (Hartz et al. 1999), wheat (*Triticum aestivum*) (Ohno and Grunes, 1985), maize (*Zea Mays*) (Pathak and Kalra, 1971; Bertic et al. 1989) and spinach (*Spinacia oleracea*) (Hohlt and Maynard, 1996).

Despite less absorption of ^{26}Mg by low Mg plants, there was, however, higher accumulation of ^{26}Mg in roots of low Mg plants than Mg-adequate plants (Table 3.2 B). Probably, the ^{26}Mg that was observed by low Mg plants is preferentially translocated into roots. Since root growth shows much higher sensitivity to low Mg supply than shoot growth (Table 3.1; Cakmak et al., 1994), probably roots are a very strong sink for the absorbed ^{26}Mg under low Mg supply. Consequently, roots of low Mg plants accumulated higher amounts of ^{26}Mg when compared to Mg sufficient roots. Since adequate Mg roots had already sufficiently high amount of Mg, transportation of ^{26}Mg into roots was restricted in plants with adequate Mg supply.

The results clearly demonstrate that coffee plants can absorb foliarly applied Mg and transport it in other parts of shoots and roots. This result might be important in respect to better root growth and development in acidic soil conditions, especially in subsoil part of soils where soil applied Mg cannot be moved adequately. Since low Mg plants did not show higher foliar absorption of 26Mg in contrast to the expectations, there is a need to repeat such type of foliar spray experiment by using 26Mg also in other crop plants such as wheat and maize.

Collection of information on leaf absorption and transportation of foliarly sprayed Mg is also important in terms of human nutrition. Biofortification of food crops

with Mg is becoming an important topic because of increasing number of people is affected from low dietary intake of Mg, especially in western countries due to low dietary intake (Rosanoff, 2013). Improving Mg concentrations of edible parts of food crops, such seeds/grains and leaves by foliar spray of Mg-containing fertilizers would be an effective way to mitigate Mg deficiency problem in human populations.

(C) GENERAL DISCUSSION AND CONCLUSIONS

Rising of the global mean temperature (IPCC, 2007) is expected to lead to extreme environmental conditions on a large scale for crop plants such as drought and heat stress. It a known fact that the size of agricultural lands is limited and together with increasing risk with more severe environmental stress factors it will become too challenging to feed the increasing world population and produce nutritious food (Schmidhuber and Tubiello, 2007). In addition to heat and drought stress factors, nearly 40% of the agricultural soils in world have acidity and Al toxicity problem which eventually leads to Mg deficiency (Gransee and Fuhrs, 2012). The soils which have low pH and Al toxicity problem are also found in the tropical climate regions where drought stress and high temperature can be observed often and simultaneously. Under such adverse circumstances risk with Mg deficiency in plants and foods become inevitable.

Besides its importance for crop production, Mg also represents a crucial element for human diet and required for a successful and healthy functioning of organs and muscles (de Baaij et al., 2015). However, according to Worthington (2001) Mg content of food crops, especially in fruits, cereal grains and vegetables are decreasing and up to 90% of the Mg content in foods is lost during the food processing (Rosanoff, 2013; de Baaij et al., 2015). The loss of Mg in diet results in diverse of impairments in human health including cardiovascular diseases, stroke, neurodegenerative disease etc. (Bo and Pisu, 2008; Broadley and White, 2010; Rosanoff et al., 2012) and increasing the content of Mg in food and feed became an important challenge of plant scientists.

With its diverse of critical physiological functions Mg greatly contributes to maintenance of successful growth and development process. For example, distribution of Mg in plant leaves gives an important clue about the function of Mg: up to 75% of the Mg is associated with the protein synthesis, approximately 20% of it located in the chloroplasts and the remaining Mg is found to be stored in vacuoles (Karley and White, 2009). Therefore, efficienct functioning of the chlorophyll for light energy capturing

(Cowan, 2002), activation of key enzymes in the photosynthetic pathway (Wedding and Black, 1988; Portis, 1992); synthesis and function of the ATP (Ko et al., 1999); functioning of H^+-ATPase required to drive the phloem loading of assimilates (Bush, 1989, Hermans et al., 2005) depend on adequate Mg nutrition of plants. In addition, Mg-ATP has been shown as the major comple of ATP in cellular systems (Getz and Klein, 1995).

Under drought stress conditions, photosynthesis machinery is seriously affected. As a result of closure of stomata and decreased accumulation CO_2 (Chaves et al., 2003; Flexas et al., 2004); due to reduced flow of CO_2 into mesophyll tissues and thus restriction in CO_2 fixation, high energy light electrons which are normally released for carbon fixation are transferred to O_2 instead of CO_2 leading to generation os highly dangerous ROS (Asada, 1994). Since due to very similar physiological impairments, also Mg deficiency promotes ROS generation in chloroplasts (Cakmak and Kirkby, 2008; Mengutay et al., 2013), it is plausible to suggest that combination of Mg deficiency with drought stress condition will maximize depressions in growth and yield formation. In Chapter 2 (Table 2.1 and 2.5), the results obtained showed that growing low Mg plants under water limited conditions, grain yield was more severely depressed. It is known that grain yield capacity of wheat under drought stress conditions is greatly affected from the total amount of water soluble carbohydrates from stem into seeds (Goggin and Setter, 2004; Ehdai et al., 2006; Rebetzke et al., 2008). It is suggested that up to 70% of grain yield capacity of wheat under drought stress depends on delivery of carbohydrates from stem into grain. Since Mg has fundamental effects on both biosynthesis and transportation of carbohydrates in plants (Cakmak et al., 1994 b; Hermans et al., 2005; Cakmak and Kirkby, 2008), Mg nutritional status of plants under drought stress should be highly important. Probably, low Mg supply caused further decreases in yield capacity of plants because of its adverse effect on transportation of carbohydrates not only from leaves but also stem into grains.

In chapter 2, exposure of plants simultaneously to low Mg and drought stress conditions severely reduced straw dry weights and grain yield. Under low water availability, adequate Mg supply enhanced the grain yield, concentration and content of grain Mg and grain starch contents. These results indicate that even though plants are suffering from low amount of water, supply an adequate Mg can improve grain yield probably due to better transportation of carbon from vegetative tissues into grains and consequently higher starch content in grains as shown in both Chapter 1 and 2.

In Chapter 1, it was shown that starch content of seeds were increased by adequate Mg supply, while starch was accumulated in vegetative parts of low Mg plants. Starch contents and concentrations were found to be higher in flag and remaining leaves under low compared to adequate Mg supply, while concentrations and especially content of grain starch were markedly higher in Mg adequate plants. It was also found that the adverse effect of low Mg supply on grain yield is related to decreases in weight of individual seeds rather than number of seeds per spike. These results highlight importance of Mg in yield formation by affecting carbon allocation into seeds during the reproductive growth stage. Foliar spray of Mg in form of $MgSO_4$ to low Mg plants was very effective to improve grain yield, and this positive effect of foliar Mg spray was closely related to better transportations of photoassimilates into grain because grain starch content and seed weight were strongly improved by Mg spray. Foliar supply of Mg nearly doubled the starch content per grain that emphasizes again particular role of Mg in phloem transportation of photoassimilates into seeds.

In practical agriculture, foliar spray of Mg fertilizers is often used. Foliar Mg fertilization could be of great importance under low water status of soils. It is known that Mg reaches to roots through bulk flow in soils which is directly under influence of soil water content (Granse and Fuhrs, 2013). Transpiration capacity of plants has also a significant effect on root Mg uptake (Jezek et al., 2015). Based on these observations and findings it can be suggested that low soil moisture can impair Mg nutritional status of plants. Cakmak and Kirkby (2008) indicated that during the reproductive growth stage topsoil is usually dry which may reduce root Mg uptake substantially. Therefore, under such growth conditions and growth stages a foliar spray of Mg fertilizers could be very beneficial. As shown in the present study, foliar Mg application was very effective to mitigate deficiency stress and improve grain yield. Since Mg deficiency might be hidden and may reduce yield without appearance of leaf symptoms it is advisable for growers to monitor Mg nutritional status of plants during the reproductive growth stage and examine plant response to foliar Mg spray.

The positive effects of foliar Mg application in Chapter 1 were found with application of 4% $MgSO_4$. Interestingly, there was no significant change in straw and shoot dry weights between the low Mg and low+foliar Mg plants. Also, the number of grains per spike and number of spikes per plant remained unaffected from different Mg supply rates. However, grain yield and thousand grain weight (TGW) of grains were significantly increased with foliar Mg application to Mg-deficient plants, indicating

important role of foliarly sprayed Mg in transportation of photoassimilates into grain. Probably, foliar spray of Mg had also increasing effect on phloem transportation of amino acids and Zn. A positive effect of Mg on phloem transportation of amino acids has been shown previously (Cakmak et al. 1994b), supporting the findings with grain protein in Chapter 1. Grain contents of N were significantly increased under foliar application of Mg than low Mg conditions.

Foliar application of Mg could be also very important in acidic soils, especially in case of subsoil acidity. Correction of pH and overcoming Al toxicity could be realized in topsoil by applying lime or organic substances (Masrschner, 2012). Similarly, other typical nutritional problems of acidic soils such as Ca and Mg deficiencies can be also minimized by applying Mg- and Ca-containing fertilizers to soil. However, such agronomic tools are not helpful to mitigate Al toxicity or Mg deficiency in subsoil. One approach to provide Mg to roots growing in subsoil zone could be foliar spray of Mg fertilizer. In Chapter 3, the effectiveness of foliar Mg spray in increasing root Mg was studied. In this part of the thesis, we used coffee seedlings because coffee is a typical crop in acidic soils and very sensitive to low Mg (Matiello, 1985; Nagao et al., 1986). There is also no published evidence about the role of foliarly sprayed Mg in transportation of Mg in the roots, at least for coffee.

The experiment in Chapter 3 was designed to investigate the uptake and transport of foliarly applied Mg in Mg-deficient and Mg-sufficient plants. To measure the translocation rate of Mg precisely and distinguish the foliarly absorbed Mg from the Mg absorbed from roots or exiting in plants already, a stable isotope of magnesium, ^{26}Mg, was used. The results have shown that coffee plants are very sensitive to low Mg in growth medium, especially the root growth. The results with ^{26}Mg applications showed that leaves absorb and translocate ^{26}Mg under both low and adequate Mg supply. Interestingly, low Mg plants translocated more ^{26}Mg into roots than the Mg-adequate plants; but much less in the leaves. The ^{26}Mg concentrations of young and old leaves of the adequate Mg plants were found significantly higher than the Mg-deficient plants. It is not well understood why foliarly absorbed ^{26}Mg is translocated preferentially in the roots but not in the other sink organs such as young leaves in plants with low Mg supply. Additional experiments are needed to clarify this result. One explanation could be related to root growth. As mentioned several times, root growth is highly sensitive to low Mg. Probably, roots under low Mg are stronger sink for the foliarly absorbed Mg than other plant organs.

Based on the results presented in this thesis, it can be concluded that Mg has critical functions in plants that affect both productivity and nutritional quality of plants. In the Chapter 1 and 2, results are presented showing that by affecting the amounts of protein and micronutrient concentrations (especially Fe), an adequate Mg nutrition may contribute to better nutritional value of the harvested products. Foliar spray of Mg is also important in improving grain Mg and also root Mg. Improving root Mg by foliar spray is of great importance for the marginal soil conditions which reduce root capacity of plants to absorb Mg. Increase in grain-Mg by foliar Mg applications can contribute to higher Mg concentrations in cereal based foods and thus better dietary intake of Mg in human populations.

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