EFFECTS OF VARIED MAGNESIUM AND POTASSIUM NUTRITION ON WHEAT GROWN UNDER AMBIENT AND ELEVATED ATMOSPHERIC CARBON DIOXIDE CONDITIONS

by

KADRIYE KAHRAMAN

Submitted to the Graduate School of Engineering and Natural Sciences

in partial fulfillment of the requirements for the degree of

Master of Science in Biological Sciences and Bioengineering

Sabanci University

December 2014

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APPROVED BY:

Assoc. Prof. Dr. Levent ÖZTÜRK.....
(Thesis Supervisor)

Prof. Dr. Ersin GÖĞÜŞ.....

Prof. Dr. Hikmet BUDAK.....

DATE OF APPROVAL: 26/12/2014

ABSTRACT

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Kadriye Kahraman

Biological Sciences and Bioengineering, Master Thesis, 2014

Supervised by: Assoc. Prof. Dr. Levent Öztürk

Keywords: Wheat, Elevated Carbon Dioxide, Magnesium, Potassium, Photosynthesis Parameters

Atmospheric carbon dioxide (CO_2) has been continuously increasing from 280 μmol mol^{-1} in 1800's up to 395 μmol mol^{-1} as of today and projected to elevate to some point in between 530 and 970 μmol mol^{-1} by the end of the 21^{st} century. This study aimed to understand how low magnesium (Mg) and potassium (K) supply affects plant growth and physiology in an elevating CO_2 environment using two major wheat species (*Triticum aestivum* cv. Adana 99 and *Triticum durum* cv. Sarıçanak 98) as model plants. As expected low Mg and K treatments resulted in retarded biomass production and occurrence of severe leaf deficiency symptoms. Photosynthesis rate was significantly induced by elevated CO_2 treatments, however this induction was hampered by low Mg and K supply. Elevation of CO_2 resulted in accumulation of carbohydrates in source leaves particularly in low-Mg and low-K plants. In plants grown with adequate Mg and K, shoot and root biomass, root length and volume were significantly increased with elevated CO_2. However, growth enhancement resulting from elevated CO_2 was less pronounced in low-Mg and low-K plants. Total antioxidant capacity, lipid peroxidation and membrane stability were altered by low Mg and K supply irrespective of the [CO_2] treatments. Due to the detrimental effects of low Mg and K supply on phloem export of carbohydrates, photosynthesis rate, root properties linked to nutrient uptake from soil, antioxidative system and membrane structure, nutritional status of plants with Mg and K has crucial importance to take advantage of an atmosphere with elevating CO_2 levels.

ÖZET

FARKLI MAGNEZYUM VE POTASYUM BESLENME DÜZEYLERİNİN YÜKSELTİLMİŞ ATMOSFERİK KARBONDİOKSİT KOŞULLARINDA BÜYÜTÜLMÜŞ BUĞDAY ÜZERİNE ETKİSİNİN ARAŞTIRILMASI

Kadriye Kahraman

Biyoloji Bilimleri ve Biyomühendislik, Yüksek Lisans Tezi, 2014

Tez Danışmanı: Assoc. Prof. Dr. Levent Öztürk

Anahtar sözcükler: Buğday, Yükseltilmiş Karbondioksit, Magnezyum, Potasyum, Fotosentez Parametreleri

Sürekli artmakta olan atmosferik karbondioksit konsantrasyonu (CO_2) 1800'lü yıllarda 280 µmol mol^{-1} olup bu gün 395 µmol mol^{-1} düzeyine yükselmiştir ve içinde bulunduğumuz yüzyılın sonunda 530-970 µmol mol^{-1} aralığında bir noktaya çıkacağı öngörülmektedir.Bu çalışmada bitki modeli olarak iki temel buğday türü (*Triticum aestivum* cv. Adana 99 and *Triticum durum* cv. Sarıçanak 98) kullanılarak düşük magnezyum (Mg) ve potasyum (K) beslenmesinin yükseltilmiş karbondioksit koşullarında bitki büyümesini ve fizyolojisini nasıl etkileyeceğinin anlaşılması amaçlanmıştır. Beklenildiği gibi düşük Mg ve K uygulamalarında biyokütle üretiminin geçikmesi ve yaprakta şiddetli eksiklik semptomları gözlemlenmiştir. Yükseltilmiş CO_2 uygulamalarında fotosentez hızı anlamlı bir şekilde artmıştır, ancak bu artış düşük Mg ve K beslenmesiyle engellenmiştir. Yükseltilmiş CO_2 konsantrasyonu özellikle düşük Mg ve K bitkilerinde olmak üzere gelişimini tamamlamış yapraklarda karbonhidrat birikimini yol açmıştır. Yeterli Mg ve K koşullarında yetiştirilmiş bitkilerde, gövde ve kök biyokütlesi, kök uzunluk ve hacmi yükseltilmiş CO_2 ile anlamlı bir şekilde artmıştır. Ancak bu yükseltilmiş CO_2 ile büyüme artışı düşük Mg ve K beslenmesinde daha az görülmüştür. Toplam antioksidan kapasitesi, lipid peroksidasyonu ve membran stabilitesi karbondioksit uygulamarından bağımsız olarak düşük Mg ve K beslenmesi ile değişmiştir. Düşük Mg ve K beslenmesinin floem karbonhidrat taşınımı, fotosentez hızı, topraktan besin alınımı ile bağlantılı olan kök özellikleri, antioksidan sistemi ve membran yapısındaki kötü etkilerinden dolayı, bitkilerin CO_2 artışından yararlanabilmesi için bitkilerde Mg ve K beslenme düzeyi kritik önem taşımaktadır.

This work is dedicated

To my family, **Mehmetali**, **Habibe** and **Fahri**

Who always put their weight behind me and share their endless love.

ACKNOWLEDGEMENT

Every project big or small is successful largely due to the effort of a number of wonderful people and I would like to express my gratitude to people who have always given their valuable advice and helped me in every respect.

In first place, I would like to express the deepest appreciation to my advisor Assoc. Prof. Dr. Levent Ozturk who has lend a helping hand to me in regard to research and scholarship, and prime me about every part of project with his extensive knowledge.

I would like to thank my committee members, Prof. Dr. Hikmet Budak, Prof. Dr. Ersin Gogus and Asst. Prof. Husnu Yenigun for their precious time, advice and contributions to my education in Sabanci University.

I express sincerely gratitude to Ozlem Yilmaz who is precious partner in our laboratory and always support me. We share lots of things during project, and she is an unforgettable person for me.

I would like to express my gratitude to Prof. Dr. Ismail Cakmak who provides me the opportunity to work with him and his great research team. I really appreciate to know him and benefit from his brilliant knowledge.

I would like to thank to all members of Plant Physiology Lab, especially Atilla Yazici for his precious assistance and guidance, Muhammad Asif, Yasemin Ceylan, Ozge Cevizcioglu, Yusuf Tutus and Umit Baris Kutman for their friendly companies and endless helping to improve my knowledge.

Last but not the least I place a deep sense of gratitude to my family and my friends who have been constant source of inspiration and encouraged me during the preparation of this work.

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ADP ..... adenosine diphosphate

ATP ..... adenosine triphosphate

B ..... boron

C ..... carbon

C1 ..... conductivity 1

C2 ..... conductivity 2

C_3. ..... three-carbon organic acids

C_4. ..... four-carbon organic acids

Ca ..... calcium

$CaCl_2$ ..... calcium chloride

$Ca(NO_3)_2$ ..... calcium nitrate

$Ca(H_2PO_4)_2$ ..... calcium dihydrogen phosphate

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

CER ..... CO_2 exchange rate

Chl a ..... chlorophyll a

Chl b ..... chlorophyll b

Chl a/b ..... chlorophyll a/b ratio

Co ..... cobalt

CO_2 ..... carbon dioxide

$[CO_2]$ ..... carbon dioxide concentration

Cu ..... copper

$CuSO_4$ ..... copper sulfate

cv. ..... cultivar

DNA ..... deoxyribonucleic acid

EDTA ..... ethylenediamine tetraacetic acid

FACE ..... free air CO_2 enrichment

Fe ..... iron

Fe-EDTA ..... iron ethylenediamine tetraacetic acid

G_s ..... stomatal conductance

H^+ ..... hydrogen ion

H^+-ATPase ..... proton-exporting ATPase

H_2O_2 ..... hydrogen peroxide

H_2SO_4 ..... sulfuric acid

H_3BO_3 ..... boric acid

HNO_3 ..... nitric acid

ICP-OES ..... inductively coupled plasma optical emission spectrometry

IPCC ..... international panel on climate change

K ..... potassium

K^+ ..... potassium ion

KCl ..... potassium chloride

KH_2PO_4 ..... potassium dihydrogen phosphate

K_2SO_4 ..... potassium sulfate

MDA ..... malondialdehyde

Mg ..... magnesium

Mg^{2+} ..... magnesium ion

$MgSO_4$ ..... magnesium sulfate

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

Mn ..... manganase

$MnSO_4$ ..... manganese sulfate

Mo ..... molybdenum

MSI ..... membrane stability index

N ..... nitrogen

Na ..... sodium

Na-phosphate ..... sodium phosphate

NADPH ..... nicotinamide adenine dinucleotide

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

$NiCl_2$ ..... nickel(II) chloride

O_2 ..... oxygen

P ..... phosphorus

Pb ..... lead

PSI ..... photosystem I

PSII ..... photosystem II

RNA ..... ribonucleic acid

ROS ..... reactive oxygen species

RPM ..... revolutions per minute

RuBP ribulose-1,5-biphosphate

Rubisco ..... ribulose-1,5-biphosphate carboxylase-oxygenase

S ..... sulfur

SPAD ..... special products analysis division

TBA ..... thiobarbituric acid

TCA ..... trichloroacetic acid

T_r ..... transpiration rate

Zn ..... zinc

$ZnSO_4$ ..... zinc sulfate

(A) INTRODUCTION

A.1. Climate Change

Human and natural drivers have a big role in climate change. The energy balance of climate system is affected by the changes in abundance of greenhouse gases, in solar radiation and land surface properties. The climate system is altered by human and natural factors as warming or cooling influences on global climate. Carbon dioxide, methane and nitrous oxide are the main gases that increase due to fossil fuel use, and hence cause the change of climate system.

One of the most important greenhouse gases is known as the carbon dioxide (CO_2). The atmospheric CO_2 concentration has increased by 40% since pre-industrial time, and reached around 391 ppm in 2011 while pre-industrial value was about 280 ppm (IPCC 2007, 2013, 2014, Co2now.org 2014). Although there is year-to-year variability in growth rate of CO_2 concentration, the increase of CO_2 concentration was larger in the last years than in many years ago. Moreover, the global atmospheric CO_2 concentration still continues to increase by human and natural factors.

The unbalanced climate system has an impact on environment, such as arctic temperatures and ice, widespread changes in precipitation amounts, ocean salinity, wind patterns and aspects of extreme weather including droughts, heat waves, heavy precipitation and the intensity of tropical cyclones. Warming of the climate system causes increment of temperature of global average air and ocean. The increase of temperature is directly related with the melting of snow and ice, and hence rising global average sea level (IPCC 2007, 2014). The average atmospheric water vapour content is another impacted part that has been increased year-to-year. When today's mountain glaciers and snow cover are compared with the past years, in both hemispheres they have declined on average; and the decline of glaciers and snow cover ends up with the sea level rise.

According to the United Nations, the world population increases to 9.6 billion in 2050 and 10.9 billion by 2100, and the population increase will be directly related with food demand. Bruinsma (2009) indicated that 70% more food should be produced by 2050 to feed the increasing world population, and climate change will be main difficulty to face

up to provide same fertility level for world agriculture. There is also another limited parameter of agricultural production, known as environmental stresses which limit the agricultural production and yield with an increase of food demand. Climate change has an impact on food production in a negative way. According to IPCC (2007), the average global temperature is expected to rise by 1-6ºC in the 21st century. Increase of global temperature comes with high light intensity and extreme temperatures that will affect on productivity of crops. Different growth-limiting problems such as mineral nutrient deficiencies will likely to increase with changes in climate. For example, Mg deficiency is one of the most observed deficiencies with heat because of inhibition of Mg uptake by high temperature (Gransee and Führs 2012) and plants deficient in Mg become more susceptible to heat stress (Mengutay et al. 2013).

A.2. Effects of Elevated CO_2

Today's atmospheric CO_2 concentration is at its highest recorded level since the beginning of accurate measurements about 54 years ago and continues to increase rapidly. Especially after industrial revolution, CO_2 concentration entered a rapid growth period, and it increased from 280 μmol mol^{-1} to 395 μmol mol^{-1} (IPCC 2007, CO2now.org 2013). According to projections, atmospheric CO_2 concentration will be at a level of 530 to 970 μmol mol^{-1} at the end of 21st century (IPCC 2007). While scientific researches show that increase of atmospheric CO_2 is converted to carbohydrate and other organic matters with photosyntetic ways by plants, it is not exactly understood how it affects the trend of atmospheric CO_2 increase. The increase of atmospheric CO_2 concentration results in increase of photosynthesis rate particularly for C_3 plants, and consequently can affect the capacity of growth and yield in a positive way (Ainsworth and Rogers 2007, Taiz ve Zeiger 2010). However, plants should be in favorable conditions in terms of nutrition with other essential elements to utilize and benefit from an elevated CO_2 environment. For instance, Reddy and Zhao (2005) reported that distribution of dry matter was unbalanced in K deficient cotton plants and more K was needed for an effective phloem transport under elevated CO_2 conditions.

The main reason of the persistent increase of atmospheric CO_2 concentration in the past 200-years is the anthropogenic use of fossil fuels. From an optimistic point of view, the increment of CO_2 concentration can be defined as beneficial for autotrophic plants due

to the increase in abundance of CO_2 which is the sole carbon source for the formation of high-energy organic compounds through photosynthesis. This assumption is proved by many studies conducted with C_3 plants grown under optimum mineral nutritional conditions. Moreover, some researchers pointed out that the yield increase due to elevated CO_2 is only a little, mostly because of the bottleneck in phloem transport of photo-assimilates (Korner et al. 1995, Komor 2000). In many plant species grown under elevated CO_2 conditions, it is known that phloem export of carbohydrates is reduced and carbohydrates are accumulated in photosynthetically active mature leaves. On the other side, long-term field studies indicate that plants should be grown under optimum nutrition and environmental conditions to efficiently benefit from elevated CO_2 conditions.

In general most of the studies about the relation between nutritional status of plants and elevated CO_2 were focused on nitrogen (N) nutrition, and other important macro nutrients such as Mg and K were barely studies. However, previous studies clearly showed that Mg and K nutrition play a key role in phloem loading and export of photo-assimilates from source (mature leaves) to sink (young leaves, roots, fruits) tissues. For instance, it was reported that one of the most important reaction of plants grown under low Mg and K is the accumulation of carbohydrates in mature leaves (Cakmak et al. 1994b, Cakmak 2005). It was also indicated that under elevated [CO_2] conditions distribution of shoot to root biomass were unbalanced, and this destabilization could be related with the role of K nutrition on phloem transportation (Jordan-Meille and Pellerin 2008, Hafsi et al. 2014). Therefore, carbohydrate accumulation in leaves as a result of either elevated [CO_2] conditions or low Mg and K nutrition constitutes an important problem in terms of plant metabolism and dry matter distribution.

Teng et al. (2006) showed that elevated CO_2 reduced stomatal density, transpiration rate (*T*r) and stomatal conductance (*G*s). They indicated that decrease of *T*r and *G*s was caused by the reduction of stomatal aperture. It was also noted that elevated CO_2 treatment increased soluble sugar and starch contents. This study also showed a decrease in nutrient concentration of leaves as CO_2 concentration increased. Starch accumulation could be one of the reasons for the decrease of mineral nutrients at the elevated CO_2 treatments resulting a dilution of nutrients. The decrease in mineral nutrients can also be associated with the reduction in stomatal conductance and transpiration rate due to elevated CO_2 environment in which plants tend to transpire

less. However, nutrients which are mainly taken up by mass flow in the rhizosphere (e.g. Mg and K) can be particulary affected negatively in such an environment.

It is thought that C_3 plants benefit from elevated CO_2 with increasing photosynthesis rates by using CO_2 converted to carbohydrate, and enhancing of productivity and growth (Ainsworth and Long 2005, Ainsworth and Rogers 2007, Leakey et al. 2009). However, other studies indicated that photosynthetic capacity may decrease in time and end up in an acclimation phase which may be caused by reduced by sink activity and nutritional limitations (Drake et al. 1997, Ellsworth et al. 2012, Komatsu et al. 2013, Xu et. al. 2013).

Reddy et al. (2010) emphasized the effects of elevated CO_2 on source-sink balance, plant productivity and growth. It is suggested that C_3 plants will be affected positively by increase of carbon assimilation, growth and yield due to their specific photosynthesis pathway. They also notified that root surface and root volume increased by rising CO_2 because of enhancing allocation of carbon to root growth. Elevated CO_2 also affected the stomatal conductance, carboxylation capacity and accumulation of photoassimilates. With increase of CO_2, reduction of stomatal conductance was observed. Decreased stomatal conductance might cause to increase leaf surface temperature, and increment of leaf surface temperature could be related with the increase of transpiration rate (Bernacchi et al. 2007, Reddy et al. 2010).

According to a study by Pritchard and Amthor (2005), biomass and grain yield of wheat will rise about 7-11% per 100 μmol mol^{-1} increase of CO_2 concentration. They also concluded that the maximum increase will be around 30% at about 750 μmol mol^{-1} CO_2 concentration under controlled conditions.

Mahdu and Hatfield (2013) worked on effects of rising CO_2 on root growth. According to their study, roots become more numerous, longer and thicker under the elevated CO_2. Branching and extention of roots was linked to changes of water and nutrient availability with the increase of CO_2 concentration. Several other studies also reported that with CO_2 enrichment, root length was increased in many plant species with becoming more numerous, longer, and thicker (Norby 1994, Prior et al. 1995, Rogers et al. 1999, Bernacchi et al. 2000, Pritchard and Rogers 2000).

With the steady increase of atmospheric CO_2 concentration, root biomass also increases and this reflects to whole plant biomass (Rogers et al. 1994, Obrist and Arnone 2003).

Free air CO_2 enrichment studies (FACE) with spring wheat also showed about 37% increase in total root dry mass in the elevated CO_2 (Wechsung et al. 1999).

Kimball (2011) and Kimball et al. (2002) worked on both sufficient and deficient level of water and N for 300 μmol mol^{-1} increase in atmospheric CO_2 concentration. They reported an increase of root biomass in all conditions, but this was less pronounced in plants grown under N deficiency levels.

Elevated atmospheric CO_2 concentration has also impact on protein and elemental concentration of plants. Manderscheid et al. (1995) indicated how elevated atmospheric CO_2 concentrations affected nutrient concentrations and grain quality. They determined the concentrations of macro and micronutrients and amino acid composition of the grain protein. They reported that concentrations of all amino acids in grain were decreased in wheat cultivars grown under elevating [CO_2]. They also concluded concentration of nearly all macro and micronutrients were also reduced by elevated atmospheric CO_2 concentrations.

According to study of Högy et al. (2009), wheat aboveground biomass, leaf ear biomass, number of ears and grains per unit ground area were increased with encrichment of CO_2 concentration. However, concentrations of total grain protein and amino acids per unit of flour were significantly reduced because of elevated atmospheric CO_2 concentration. Högy et al. (2013) also reported that total protein concentration was decreased due to elevating [CO_2]. However, the increment of C/N ratio was observed under elevated atmospheric CO_2 concentrations. It could be linked with increased C and lower amount of total N which was related with decrement of protein under CO_2 enrichment. They also indicated that concentrations of Ca, Fe, Co and Mg were decreased while B, K, Pb and Mo were increased by elevated atmospheric CO_2 concentrations.

Fernando et al. (2012) reported similar results about nutrient concentrations in grains. They concluded that nutritive value of grain including protein concentration, flour protein concentration and most of the macro and micronutrients was decreased due to elevated atmospheric CO_2 concentrations. According to their study, concentrations of Zn, Na S and P were also decreased by elevating [CO_2] while no differences were observed in K, Cu and Mn concentrations. They also indicated total grain uptake of Fe Mn B, Cu, Zn, Ca, Mg, K, P and S on an area basis was increased while the

concentrations of most macro and micronutrients were reduced due to elevated atmospheric CO_2 concentrations. This could be related with the increment of grain yield with elevating [CO_2].

Myers et al. (2014) reported that C_3 grains and legumes grown under elevated atmospheric CO_2 concentrations have lower concentration of zinc and iron. According to their study, C_3 crops are more sensitive to elevating [CO_2] and lower concentrations of protein have been observed in C_3 crops grown under elevated atmospheric CO_2 concentrations than in legumes and C_4 crops.

A.3. Roles of Magnesium in Plants

Magnesium is one of the most important nutrients for plant growth. It has several structural and physiological roles, and is the most abundant cation in the cytosol of plants. Magnesium has also an impact on activity of enzymes in chloroplasts (Shaul 2002, Epstein and Bloom 2004, Cakmak and Kirkby 2008). Magnesium is known as the central atom in the structure of chlorophyll molecule. Therefore, leaf chlorosis is primarily observed in the Mg deficient plants. It is also related with increase of ROS generation and oxidative damage because of Mg deficiency (Cakmak 1994, Sun and Payn 1999, Marschner 2012, Waraich et al. 2012). Photosynthesis process is also adversely affected from low level of Mg due to the role of Mg on photosynthetic enzymes, reduction of stomatal conductance and increase of carbohydrate accumulation in leaves (Fischer and Bremer 1993, Sun and Payn 1999, Laing et al. 2000, Hermans et al. 2004, Cakmak and Kirkby 2008).

As it is known, one of the physiological roles of magnesium is to harvest solar enegry by occupying central position in the chlorophyll structure as a cofactor and promoter for many enzymes (i.e. carboxylases, kinases, phosphatases, RNA polymerases and ATPases) (Cowan 2002, Shaul 2002). Therefore, magnesium has crucial roles in chlorophyll synthesis, photochemical reactions, carbon fixation and stomata functioning. Under Mg deficiency, increment of chlorophyll a/b (Chl a/b) ratio was reported in several studies (Lavon et al. 1999, Hermans et al. 2004, Hermans and Verbruggen 2005, Verbruggen and Hermans 2013). Chl b is related with the light harvesting complex connected to photosystem II (PSII), and the increased Chl a/b ratio

by Mg deficiency causes the loss of PSII peripheral antenna or change in photosystem stoichiometry in favour of photosystem I (PSI). Under Mg deficiency, photosynthesis rate is altered and an excess of absorbed light is evident. As a result of excess light absorption, D1 protein which has important role in activation of PSII is disrupted, and excess electrons are produced by PSI. It ends up with light dependent generation of ROS in chloroplast which causes the oxidative cell damage as a result of disruption of chloroplast pigments and membranes (Richter et al. 1990a, b, Hermans et al. 2004, Gill and Tuteja 2010).

Magnesium is also important for phloem transport and carbon fixation. Magnesium deficiency causes the impairment of photosynthetic carbon fixation, accumulation of carbohydrates in source leaves because of altered phloem transport, and enhancement of antioxidative damage (Fischer and Bremer 1993, Cakmak et al. 1994b, Hermans et al. 2004, Hermans et al. 2005, Hermans and Verbruggen 2008). Magnesium deficient plants become very susceptible to high light because of decline of CO_2 fixation that causes less absorbed light energy captured by chlorophyll molecule and consequently over-reduction of the photosynthetic electron transport induces activation of O_2 to ROS (Cakmak and Kirkby 2008, Yang et al. 2012, Verbruggen and Hermans 2013).

Another crucial role of Mg is about phloem loading of sucrose, carbohydrate partitioning between source and sink tissues and transport of photoassimilates into sink organs (Cakmak et al. 1994a, b, Marschner et al. 1996, Hermans et al. 2005, Cakmak and Kirkby 2008, Cakmak 2013). In magnesium deficient plants, accumulation of carbohydrates is generally observed in source tissues. Due to accumulation of sugar and amino acids under Mg deficiency, growth of sink organs was inhibited, and photosynthesis rate decreased (Verbruggen and Hermans 2013). Accumulation of sugars under Mg deficiency may pave the way for pathogen invasion and infection (Cakmak 2013). There are also several other studies that link nutrient deficiencies to increase susceptibility of crops to infection (Schroth et al. 2000, McMahon 2012). Marschner (2002) also indicated that adequate nutrition helps to reduce pest/disease damage.

Magnesium has role on ATPase enzyme that drives the phloem loading of sucrose, hence Mg deficiency directly alters the phloem assimilates. Phloem loading is known as an energy requiring process and involves formation of electrochemical gradients by H^+-

ATPase and H^+-sucrose cotransport into the phloem. At this stage Mg-ATP is the source of energy utilized and formed from Mg^{2+} and ATP (Bush 1989, Cakmak and Kirkby 2008, Gerendás and Führs 2013).

Magnesium is also one of the most important nutrients for growth and development of plants. Magnesium status plays role in the transport and utilization of photosynthates, and has influence on carbohydrate partitioning between sourse and sink organs. Mg deficiency alters root to shoot dry weight ratio due to impaired phloem export of photoassimilates from source to sink tissue, and decrease of root:shoot ratio was observed in several studies. Reduction of root growth may also be related with the disrupted photosynthate supply and reduction of dry matter partitioning to roots. There is convincing evidence in the literature that Mg deficiency induces the reduction of carbohydrate transport towards the roots, and thereby causes the accumulation of sucrose and starch in leaves that is in accordance with inhibition of sucrose export from leaves into roots. Magnesium deficiency also reduces root uptake of nutrient from soil, and thereby causes the reduction of root growth and surface (Cakmak et al. 1994b, McDonald et al. 1996, Hermans et al. 2005, Cakmak and Kirkby 2008, Cakmak 2013). Magnesium has also impact on specific leaf weight which is calculated as the leaf dry weight per unit leaf area. According to study of Verbruggen and Hermans (2013), specific weight was increased in Mg deficient plant. Chen and Black (1983) indicated that specific leaf weight was related to net photosynthesis, respiration and translocation. Under Mg deficiency, accumulation of sucrose and starch became evident as a result of disruption of phloem export from source to sink organs as well as impairment of photosynthesis process, and thereby increment of specific leaf weight was observed in plants grown with low Mg supply. Marschner (2002) also indicated that insufficient root supply under Mg deficiency provided the remobilization of magnesium from mature leaves, hence leaf area was reduced.

Reduction in protein biosynthesis was observed under Mg deficiency in several studies (Fischer et al. 1998, Marschner 2012, Gerendás and Führs 2013). Magnesium is essential for consistance and stability of ribosomes and their aggregation to polysomes. Another crucial role of Mg nutrient is the bridge formation between two subunits of ribosomes that are responsible for protein biosynthesis. Adequate Mg^{2+} concentration is necessary for amino acid incorporation, gene transcription and translation. Magnesium also plays role in activation of enzymes concerned with energy metabolism, and thereby

is required for amino acid activation as well as for the release of the peptide chain from the ribosome (Amberger 1975, Maathuis 2009). Moreover, Harper and Paulsen (1969) indicated that nitrate reductase activity and nitrate content were decreased under nutrient deficiencies (i.e. nitrogen, magnesium, phosphorus and calcium), and nitrate reductase activity requires ability of the tissue to synthesize protein.

Increase in levels of antioxidants and activities of antioxidative defence enzymes were other observations in Mg deficient plants. Enhancement of antioxidative capacity was observed at an early stage of Mg deficiency, hence it is thought as one of the first physiological responses of plants to Mg deficiency as well as reduction of partitioning of dry matter to roots (Cakmak and Kirkby 2008).

In brief, functions of magnesium include chlorophyll formation, phloem loading, and photophosphorylation as ATP formation, CO_2 fixation, protein synthesis and partitioning of photoassimilates. Generation of ROS, photooxidation in leaf tissues, inhibition of phloem exports, carbohydrate accumulation, and reduction of root growth and surface are main responses of plants to Mg deficiency (Cakmak and Kirkby 2008, Cakmak and Yazici 2010).

A.4. Roles of Potassium in Plants

Potassium is among the essential mineral nutrients for growth and development of plants. It is important for survival of crop plants under environmental stress conditions (Waraich et al. 2012). There are several roles of K such as photosynthesis, translocation of photosynthates, activation of enzymes, and turgidity of plants (Marschner 2002, Kirkby 2011, Mäkelä et al. 2012). As in magnesium deficiency, reduction in CO_2 fixation and disruption in partitioning of photosynthates were observed under K deficient plants (Waraich et al. 2012). Generation of ROS was also induced with K deficiency by excess of photosynthetically produced electrons and enhancement of activity of NADPH oxidases. This may also be linked to membrane damage and chlorophyll degradation in plants grown under K deficiency (Waraich et al. 2011, Hafsi et al. 2014).

Increase of antioxidative enzyme activity, chlorosis and necrosis at high light intensity were other responses of K deficient plants (Waraich et al. 2012). Phloem export was

also inhibited by K deficiency, and linked to restriction of sucrose transportation into roots and accumulation of photoassimilates in leaves (Hafsi et al. 2014). Kanai et al. (2007) reported that photosynthesis was impaired by K deficiency. There were several studies that corroborate the reduction of photosynthesis activity in K deficient plants (Bednarz et al. 1998, Zhao et al. 2001). Because of growth inhibition, translocation of photosynthates was also reduced by K deficiency (Kanai et al. 2007). Cakmak (2005) also indicated that K deficiency caused to decrease in photosynthetic C metabolism and utilization of fixed carbon. Potassium deficiency also induced accumulation of carbohydrates in source leaves because of inhibition of photosynthetic C reduction. With alteration of photosynthetic C metabolism, an excess of light energy and photoelectrons which leads to photoactivation of molecular O_2 and occurence of photo-oxidative damage was existed in K deficient plants.

Potassium ion is important for various metabolic reactions because of the roles on activation of many enzymes such as vacuolar Ppase isoforms, pyruvate kinase, ADP-glucose starch synthase, and phosphofructokinase (Maathuis 2009). It is also a fundamental nutrient for crop yield, and related with sugar allocation, amino acid levels and nitrogen assimilation. Hence, potassium deficiency causes downregulation of nitrate uptake and synthesis of nitrogen-rich amino acids (Amtmann and Rubio 2012).

Potassium deficiency has an impact on plants likewise Mg deficiency about shoot to root dry weight ratio. Enhancement of shoot:root ratio was observed in K deficient plants with the accumulation of sugars in source leaves (Cakmak and Kirkby 2008).

Another crucial role of potassium is about ion diffusion whose efficiency depends on the availability of K. According to study of Mäser et al. (2002), K^+ is essential for phloem solute transport and maintenance of cation:anion balance in the cystol. Mäkelä et al. (2012) indicated that the cuticle and walls of epidermis thickened in barley straw with increasing K fertilizer treatment, and thought that thickening of cuticle is linked to guard cell movement in which availability of K plays a crucial role.

Potassium plays an important role in the regulation of osmotic potential and turgor (Hafsi et al. 2014). Cell turgor recovery in osmotically-generated stress was regulated by elevating K ion by root cells, which was mediated by voltage-gated K^+ transporters at the cellular plasma membrane. Potassium also induces solute accumulation, thereby

plays role in lowering osmotic potential and maintaining plant cell turgor under osmotic stress (Wang et al. 2013).

Stomata have a vital role as to control plant water loss via transpiration. Potassium maintains turgor regulation within the guard cells during stomatal movement. Under stress conditions, rapid release of K^+ from the guard cells into the leaf apoplast helps the stomatal closure (Marschner 2012, Wang et al. 2013). Based on the effects of K ion on guard cells, K deficiency induce stomatal closure and stomata would be difficult to remain open (Jin et al. 2011). According to study of Benlloch-Gonzalez et al. (2008), K deficiency provided stomatal opening and induced transpiration under drought stress.

In brief, functions of K nutrient include photosynthesis, enzymes activation, protein synthesis, osmoregulation, maintenance of cation-anion balances, and regulation of stomatal movement (Hafsi et al. 2014).

(B) MATERIALS AND METHODS

B.1. Plant Growth and Experimental Design

B.1.1. Experiments on Effects of Varied Mg Nutrition under Ambient and Elevated Carbon Dioxide Environments

In this experiment the aim was to study the effects of varied Mg nutrition under different atmospheric CO_2 concentrations. Bread wheat (*Triticum aestivum* cv. Adana 99) and durum wheat (*Triticum durum* cv. Sarıçanak 98) were grown hydroponically in growth chambers under controlled climatic conditions. Plants were grown with 16 hours day and 8 hours dark cycles. The light-period temperature was set to 24^{o}C and the dark-period temperature to 20^{o}C. The photosynthetic photon flux density was 400 μmol m^{-2} s^{-1} at the canopy level. The elevated CO_2 conditions were 600 μmol mol^{-1} and 900 μmol mol^{-1}. The relative humidity was kept at 65% and 75% during the light and dark periods, respectively.

Seeds were germinated in perlite wetted with saturated $CaSO_4.2H_2O$ solution. Following 6 days of germination at room temperature in dark, the resulting seedlings were then transferred to nutrient solution culture pots filled with 2.7 L of the following nutrient solution: 4 mM $Ca(NO_3)_2$, 0.75 mM K_2SO_4, 1 mM $MgSO_4$, 0.25 mM KH_2PO_4, 0.1 mM KCl, 1 μM $ZnSO_4$, 1 μM $MnSO_4$, 1 μM H_3BO_3, 0.2 μM $CuSO_4$, 0.01 μM $(NH_4)_6Mo_7O_{24}$, 0.01 μM $NiCl_2$ and 100 μM Fe-EDTA. Magnesium was added in the form of $MgSO_4.7H_2O$ at three different concentrations to achieve low (i.e 75 μM), marginal (i.e. 150 μM) and adequate (i.e. 1000 μM) nutrition with Mg. Nutrient solutions were continuously aerated and renewed every three days throughout the experiment.

The experiment was designed as a three pot-replicate with six plants in each pot. Twenty days after sowing, all plants were harvested. Three plants were used for analysis of soluble carbohydrate, and three plants for Mg analysis.

Same experiment was designed for analysis of total antioxidant capacity, lipid peroxidation, membrane stability, root properties (i.e. volume, surface area, length and tips) and phloem export of carbohydrates. Four plants were used for analysis of total

antioxidant capacity and lipid peroxidation, four plants for membrane stability, and three plants for collection of phloem exudate.

B.1.2. Experiments on Effects of Varied K Nutrition under Ambient and Elevated Carbon Dioxide Environments

In this experiment the aim was to study the effects of varied K nutrition under different atmospheric CO_2 concentrations.

All plant growth conditions were identical with the previous experiment (see B.1.1.) except for K and Mg supplies in nutrient solution. Magnesium was supplied at adequate level (i.e. 1000 μM) whereas K was added in the form of K_2SO_4 at three different concentrations to achieve low (i.e. 10 μM), marginal (i.e. 30 μM) and adequate (i.e. 750 μM) nutrition with K. 0.1 mM $CaCl_2$ and 0.25 mM $Ca(H_2PO_4)_2$ was supplied at deficient level instead of KH_2PO_4 and KCl.

B.2. Digestion and Elemental Analysis

All shoot and root samples were ground to fine powder in an agate vibrating cup mill (Pulverisette 9; Fritsch GmbH; Germany) for elemental analysis. Around 0.3 g of ground shoot and root samples was weighed respectively for acid digestion in a closed-vessel microwave system (MarsExpress; CEM Corp., Matthews, NC, USA) with 2 ml of 30% H_2O_2 and 5 ml of 65% HNO_3. After digestion, each sample was diluted to 20 ml with ultra deionized water and then the samples were filtered by quantitative filter papers. Elemental analysis was performed with inductively coupled plasma optical emission spectrometry (ICP-OES; Vista-Pro Axial; Varian Pty Ltd, Mulgrave, Australia) to determine Mg and K concentrations in shoot and root samples.

B.3. Determination of Leaf Specific Weight and Shoot and Root Dry Matter Production

For determination of leaf specific weight, second oldest leaf was used from three plants per pot. Following determination of fresh weight, leaf sample were quickly scanned and

dried until a constant weight in a forced oven set to 50^{o}C. Area of scanned leaf images were determined by the ImageJ software. Specific leaf weight was calculated by the ratio of leaf dry weight to leaf area and expressed as mg cm^{2}.

Whole plant shoot and root samples were harvested separately and dried at 70^{o}C in a forced oven until constant weight. Dried samples were weighed for determination of shoot and root dry matter. Whole biomass production was calculated by summing shoot and root dry matter per plant.

B.4. Detection of Photosynthetic Parameters

A portable photosynthesis measurement system (LiCor-6400; LiCor Inc. Lincoln, NE, USA) was used to determine photosynthesis rate (μmol $m^{-2}s^{-1}$), stomatal conductance (mol $m^{-2}s^{-1}$), and transpiration rate (μmol mol^{-1}). All measurements were performed in the second oldest leaf prior to harvest of plants. During measurement of gas exchange, light intensity and temperature were set to 500 μmol $m^{-2}\cdot s^{-1}$ and 24^{o}C, respectively. Carbon dioxide concentration was set according to experimental CO_2 levels (i.e. 400, 600 or 900 μmol mol^{-1}).

Chlorophyll concentration was measured by a portable chlorophyll-meter instrument (Minolta SPAD-502). Readings of SPAD values were obtained from middle of leaf blade of each fully expanded second oldest leaf.

B.5. Soluble Carbohydrate Analysis

Yemm and Wills' (1954) anthrone method was used for soluble carbohydrate analysis with slight modifications. The anthrone reagent was prepared by dissolving 0.15 g of anthrone in 75 ml of 98% H_2SO_4 and 25 ml of 20% ethanol. Soluble carbohydrates in dried and ground leaf and root samples were extracted with 10 ml of 80% ethanol and vortexed for 10 min at room temperature. The suspensions were centrifuged at 4600 *g* for 10 min, and 1 ml supernatant was collected in 2 ml centrifuge tubes. The ethanol extraction was repeated twice and finally 2 ml supernatant was collected. In 250 μl of sample extract, 4 ml of anthrone reagent was added and the mixture was incubated in a water bath set to 95ºC for 11 min. The samples were then quickly cooled down to room

temperature in ice bath. The absorbance of samples was read at 620 nm against D-glucose standards by a spectrophotometer.

B.6. Analysis of Antioxidative Systems

B.6.1. Measurement of Membrane Stability Index

Second oldest leaf was used to determine membrane stability index (MSI). The leaf was cut into three parts, rinsed in distilled water and then placed in 50 ml falcon tubes containing 25 ml of ultra deionized water. Tubes were horizontally placed on a rotating shaker set to 50 RPM for 18 hours. Electrical conductivity of incubation solution was measured after the incubation period and recorded as C1. All tubes were then incubated in a water bath set to 100ºC for 20 min and cooled down to room temperature. Electrical conductivity was measured for the second time and recorded as C2. MSI was calculated by using the equation below:

$$MSI= [1-(C1/C2)] \times 100$$

B.6.2. Measurement of Lipid Peroxidation

Lipid peroxidation was signified as malondialdehyde (MDA) content and performed according to the spectroscopic method described by Heath and Packer (1968). First and second oldest leaves were used for the measurement of lipid peroxidation. Weighed and frozen leaf samples were homogenized by 10 ml 0.1% trichloroacetic acid (TCA), and then centrifuged at 4600 *g* for 5 min. 1 ml of supernatant was collected to 15 ml falcon tubes and added with 4 ml of 20% TCA and 5% thiobarbituric acid (TBA) solution. Tubes were incubated in a water bath set to 95ºC for 30 min and cooled down immediately in ice bath. The samples were centrifuged at 4600 *g* for 10 min and absorbance was read at 532 and 620 nm. MDA was calculated by using the equation below and expressed per unit fresh weight:

$$MDA\ (nmol\ ml^{-1}) = [(A532-A600)/155,000] \times 10^{6}$$

B.6.3. Measurement of Total Antioxidant Activity

Total antioxidant activity was determined according to the spectroscopic method described by Prieto et al. (1999). Third and fourth leaves were used for the measurement of total antioxidant activity. Leaf samples were extracted by 5 ml ethanol, centrifuged for 10 min at 4600 *g* and 0.1 ml of supernatant was collected into 15 ml falcon tubes. 3 ml of reaction solution consisted of 0.6 M H_2SO_4, 28 mM Na-phosphate and 4 mM ammonium molibdate was added to samples and the mixture was incubated in a water bath set to 95ºC for 90 min. Following cool down, absorbance of samples was read at 695 nm. L-ascorbic acid was used to prepare standard solutions for generation of a calibration curve. Results were expressed as mg g^{-1} per unit fresh weight.

B.7. Determination of Root Properties

Roots of plants were weighed to determine fresh weight and then spread on a transparent plastic tub filled with water at a depth of 1 cm. Roots were scanned with a calibrated scanner dedicated to the WinRHIZO digital imaging software. Total root length, number of root tips, root surface area and root volume of each plant were analyzed. Root samples were finally dried at 70ºC until constant weight to determine root dry matter production.

B.8. Collection and Analysis of Phloem Exudates

Collection of phloem exudates was performed according to the EDTA method described by King and Zeevaart (1974) with slight modifications. Second, third and forth leaves of two plants were placed in eppendorf tubes filled with 2 ml of 20 mM EDTA. The part collected in first 15 min was discarded, and leaves were placed in a new tube. Then leaves were incubated at dark for three hours, and photoassimilates collected in the eppendof tubes were frozen at 80ºC for analysis. Leaf samples were weighed to determine fresh weight and then dried at 70ºC until constant weight to determine dry weight.

Yemm and Wills' (1954) anthrone method was used for soluble carbohydrate analysis with slight modifications. The anthrone reagent was prepared by dissolving 0.2 g of

anthrone in 75 ml of 98% H_2SO_4 and 25 ml of 20% ethanol. To 250 µl of phloem exudate, 4 ml of the anthrone reagent was added, and the mixture was incubated in a water bath set to 95ºC for 11 min. When the samples were cooled down to room temperature in ice bath, the absorbance was read at 620 nm against D-glucose standards. Results were expressed as D-glucose equivalent soluble carbohydrates per unit dry weight.

B.9. Statistical analysis

Statistical analyses were performed by using the JMP software. Student t-test was used to indicate the differences between cultivars and treatments and p-values were indicated as * (p<0.05), ** (p<0.01) or *** (p<0.001) where the effects were found significant.

(C) RESULTS

C.1. Growth of Experimental Plants

As expected, low-and also marginal-Mg supplied plants showed typical interveinal chlorosis and reduced shoot biomass resulting from Mg deficiency. The development of leaf chlorosis caused by Mg deficiency became more severe with elevating [CO_2] in both cultivars. Severe K deficiency symptoms of brown scorching, necrosis and leaf tip burns were observed in K deficient plants mostly in the older leaves. Adequate Mg and K supplied plants produced more biomass with elevating [CO_2], however elevating [CO_2] had no such effect in low and marginal Mg or K supplied plants (Figure 1.1 and Figure 1.2).

Saricanak 98

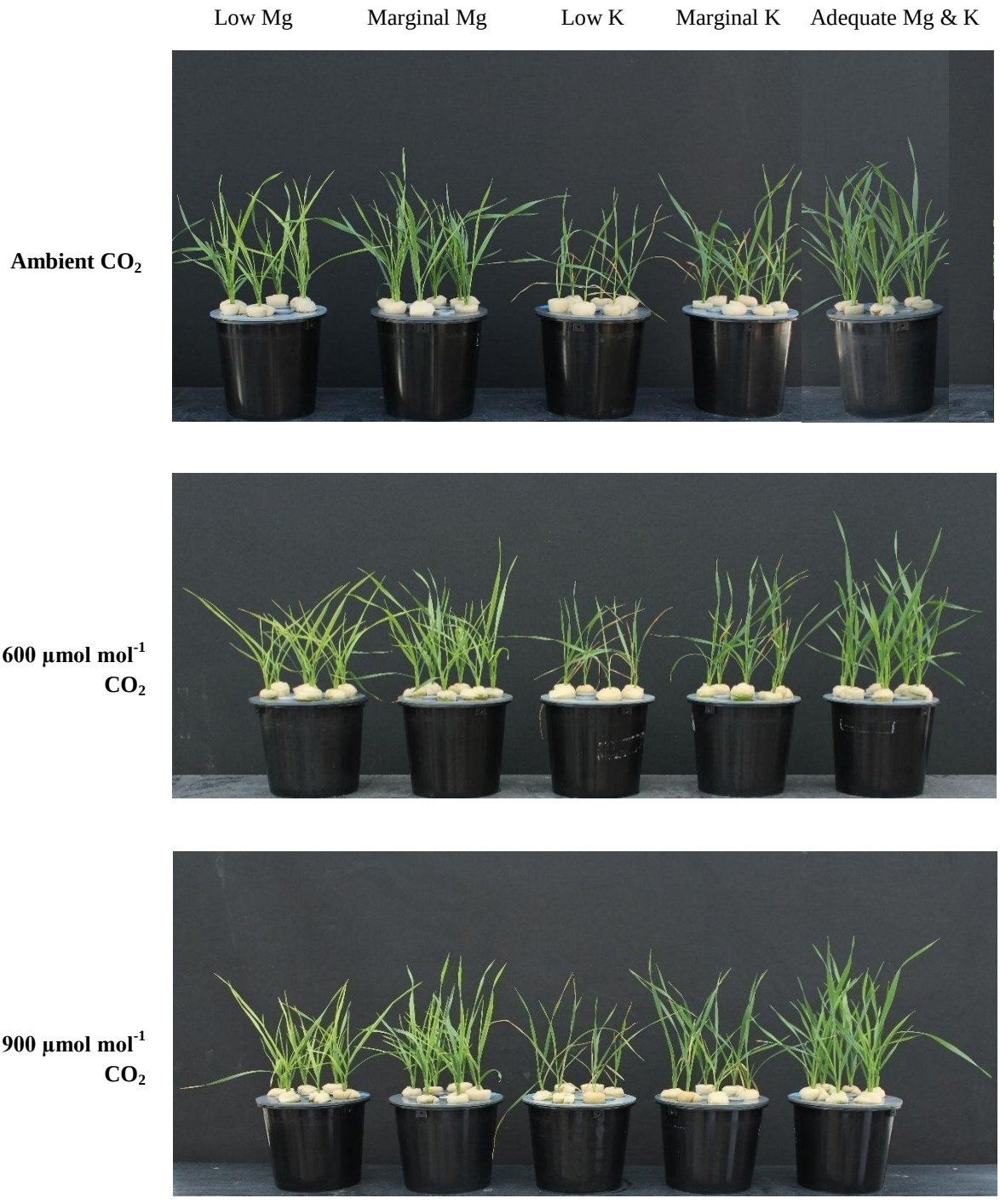

Figure 1.1: Growth of Saricanak 98 (*T. durum*) plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM), marginal Mg (150 µM), low K (10µM) and marginal K (30 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

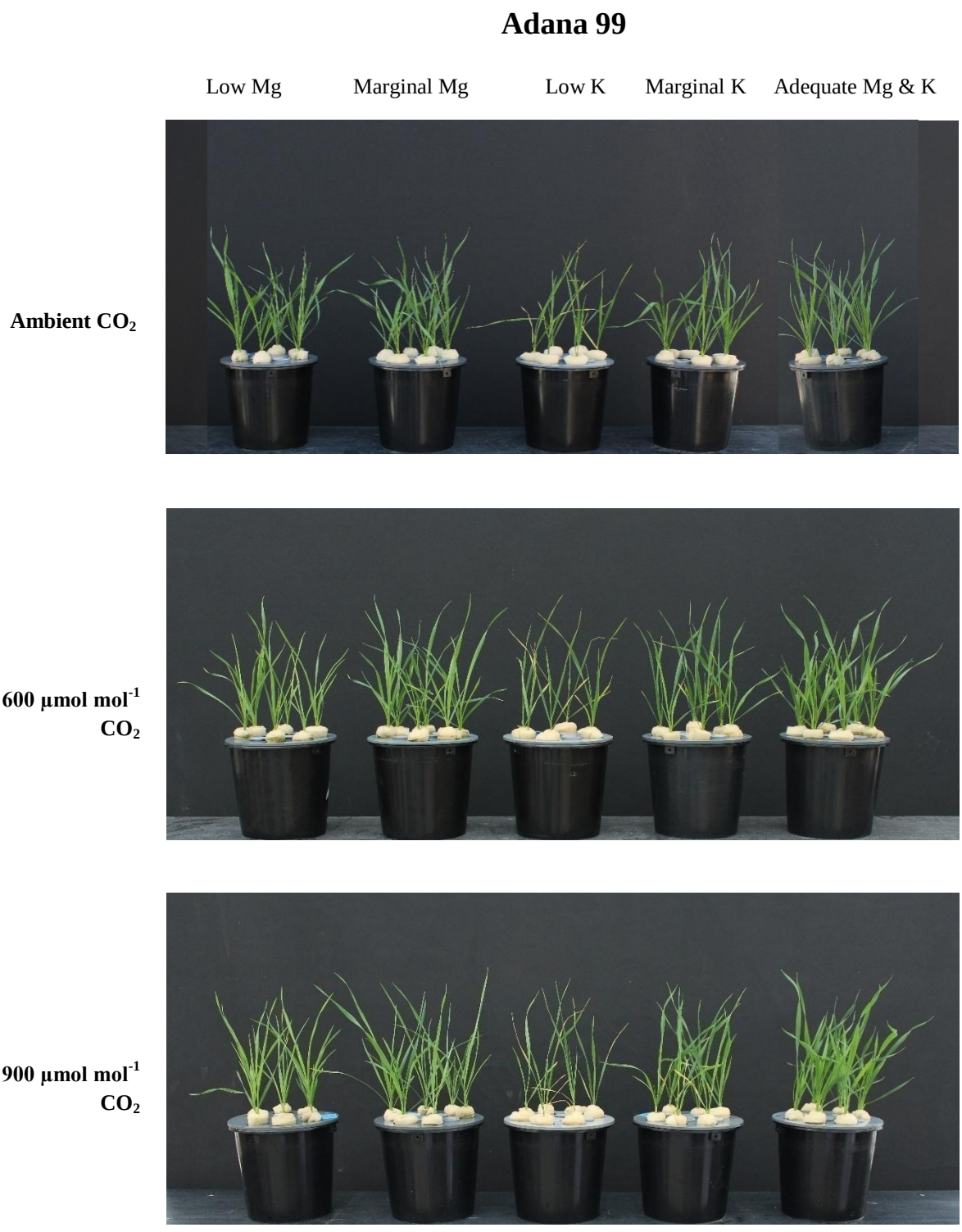

Figure 1.2: Growth of Adana 99 (*T. aestivum*) plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM), marginal Mg (150 µM), low K (10µM) and marginal K (30 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

C.2. Experiments on Mg Nutrition under Ambient and Elevated Carbon Dioxide Environments

Dry Matter:

Magnesium deficiency caused decrease of shoot dry matter production of plants, whereas shoot dry weight significantly increased with elevating [CO_2] in both Saricanak 98 and Adana 99 cultivars supplied with adequate Mg supply. However, according to statistical analysis, this increase was more in Adana 99 cultivar than in Saricanak 98 cultivar. Shoot dry matter of adequate Mg plants of Saricanak 98 showed significant increase in 600 µmol mol^{-1} [CO_2] compare to ambient [CO_2], whereas there was no such difference between plants in 600 µmol mol^{-1} [CO_2] and 900 µmol mol^{-1} [CO_2] conditions. While shoot dry weight was increasing with elevating [CO_2] in adequate Mg plants, there was a decrement or a very slight increment in low- and marginal-Mg plants. Moreover, while shoot dry matter of both cultivars was differently affected by Mg deficiency, they were affected by CO_2 treatment in a similar way (Figure 2.1 and Table 2.1).

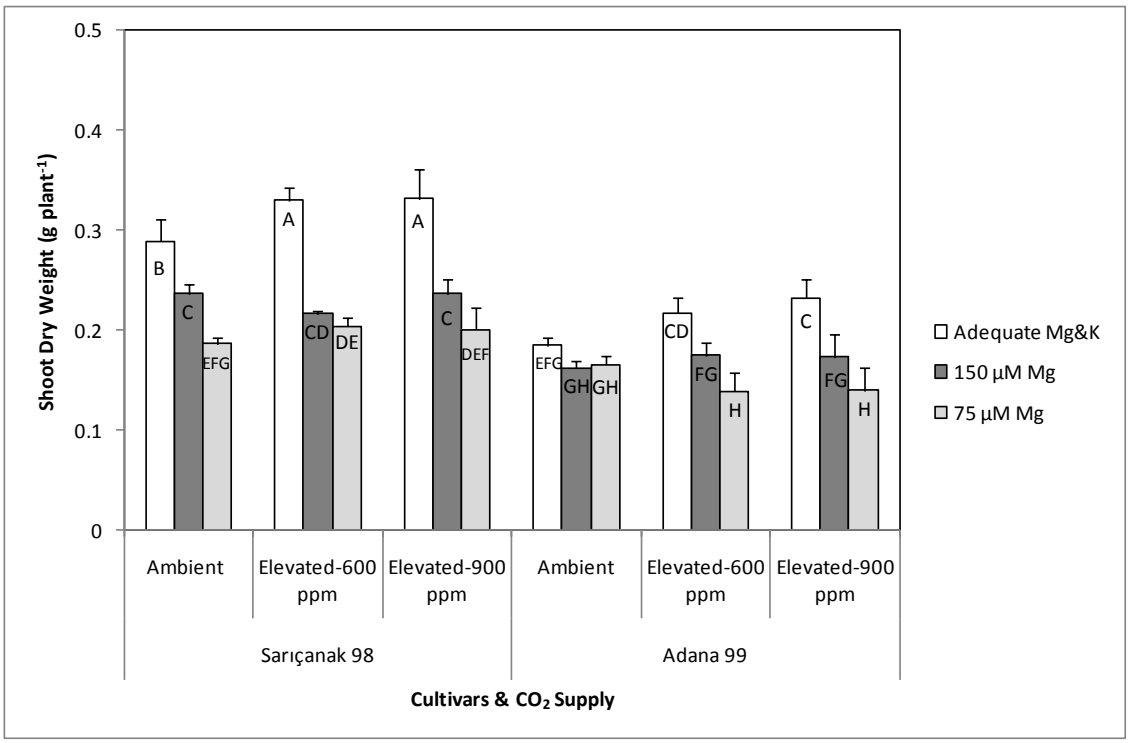

Figure 2.1: Shoot dry weight of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM) and marginal Mg (150 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

Mg deficiency also reduced root growth. Root dry matter results showed similar trend with shoot dry matter results. A statistically significant increment of root dry weight was observed in Saricanak 98 plants grown with adequate Mg supply and elevated 600 µmol mol^{-1} [CO_2]. Interestingly, a decrease was observed when 600 and 900 µmol mol^{-1} CO_2 concentrations were compared. There was a slight increase in plants of Adana 99 with enhancement of CO_2 concentration. While root dry weight of both cultivars was affected by CO_2 treatment in a similar way, they were affected differently by Mg deficiency (Figure 2.2 and Table 2.1).

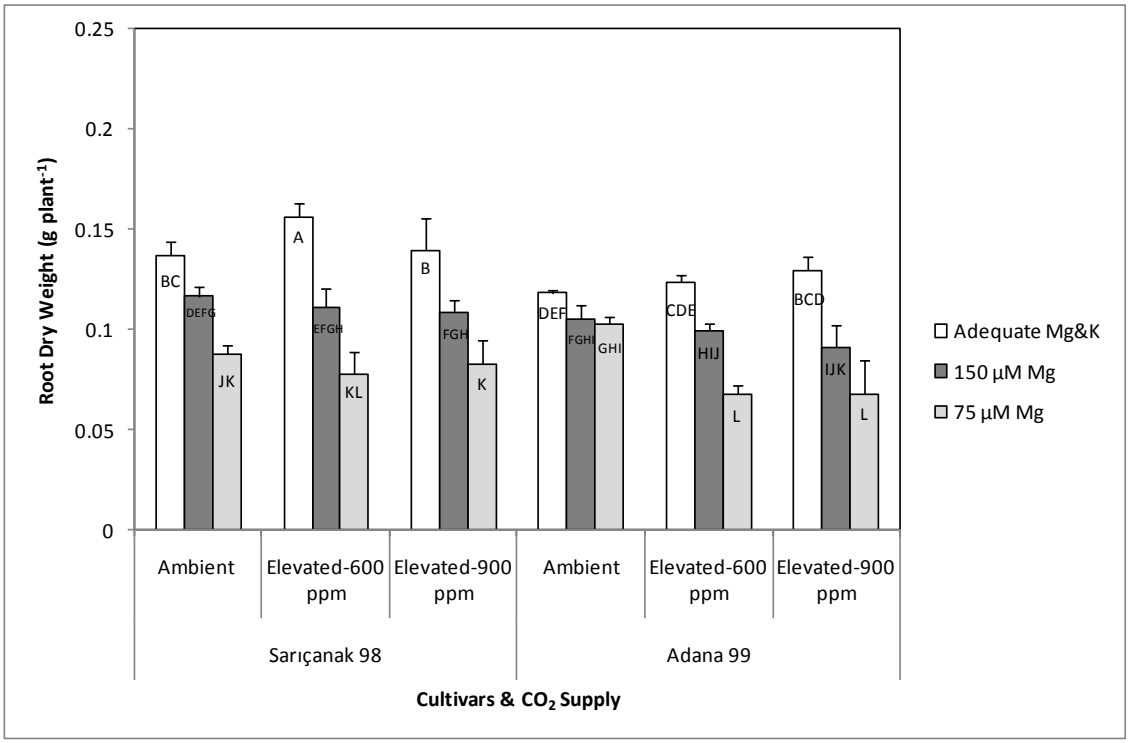

Figure 2.2: Root dry weight of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM) and marginal Mg (150 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

A significant decrease in whole biomass dry weight (i.e. shoot+root) was observed in Mg deficient plants. Biomass of adequate Mg plants significantly increased with the elevated [CO_2] in both cultivars. However, there was a very slight increase or no change in Mg deficient plants' biomass with elevated [CO_2] (Figure 2.3).

Under ambient [CO_2] conditions, almost no differences of shoot-to-root ratio were observed among different Mg treatments. However with elevating [CO_2], highest change of shoot-to-root ratio was observed in plants grown with lowest Mg supply (Figure 2.4).

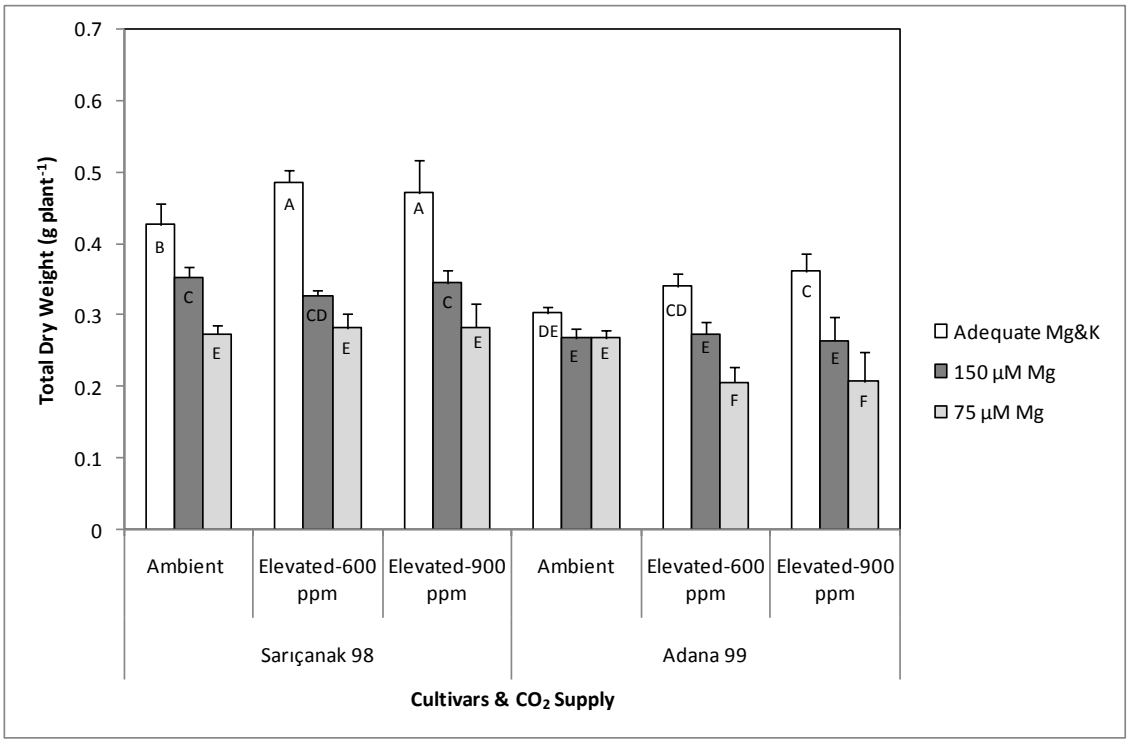

Figure 2.3: Total dry weight of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM) and marginal Mg (150 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

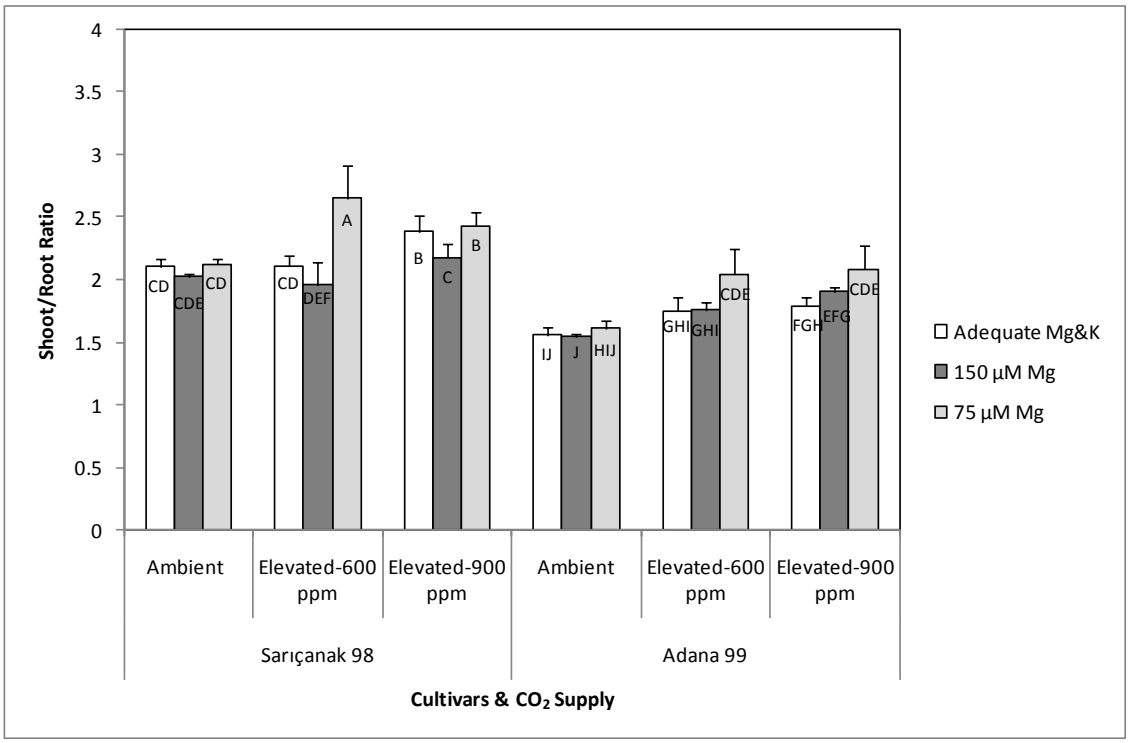

Figure 2.4: Shoot-to-root ratio of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM) and marginal Mg (150 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

In summary shoot, root and total (shoot+root) dry matter productions were very significantly affected by Mg supply and cultivar treatments ($p < 0.0001$, $p < 0.0001$). Consequently, Mg*cultivar interaction was also found significant for shoot, root and total dry weight. Carbon dioxide treatments alone positively affected shoot dry weight, however root dry weight was affected negatively. As a consequence, total dry weight was not statistically affected by elevating CO_2. Also there was no CO_2*cultivar interaction in shoot and root dry matter production, meaning that cultivars did not differ in dry matter production upon CO_2 treatments. Moreover there was statistically significant interaction of CO_2 with Mg supply, suggesting that Mg supply responded differently upon CO_2 treatments in terms of shoot, root and total dry weight (Table 2.1).

According to statistical analysis of shoot-to-root ratio, p-values of CO_2, Mg and cultivar treatments were found less than 0.0001, meaning that significant differences were observed among CO_2, Mg and cultivar treatments, respectively. However, the cross analysis of CO_2 treatment with cultivar was not found statistically significant. There were significant differences in Mg treatments upon cultivars (Table 2.1).

Table 2.1: p-values of shoot, root and total dry weight, and shoot-to-root ratio according to statistical analysis

	p-value			
Treatments	**Shoot Dry Weight**	**Root Dry Weight**	**Total Dry Weight**	**Shoot-to-Root Ratio**
CO_2	0.0385	0.0261	0.7183	<.0001
Mg	<.0001	<.0001	<.0001	<.0001
Cultivar	<.0001	<.0001	<.0001	<.0001
CO_2*Mg	0.0042	0.0004	0.0018	0.0017
CO_2*Cultivar	0.7336	0.0857	0.4183	0.2498
Mg*Cultivar	<.0001	0.0207	0.0002	0.0548
CO_2*Mg*Cultivar	0.0752	0.0532	0.0676	0.1119

Specific Weight:

In Saricanak 98 plants, a slight increase of specific weight was observed with enhancement of CO_2 concentration. However, there was no change among Adana 99 plants. Highest value of specific weight was observed in plants grown with lowest Mg supply under elevated 900 μmol mol^{-1} [CO_2] (Figure 2.5).

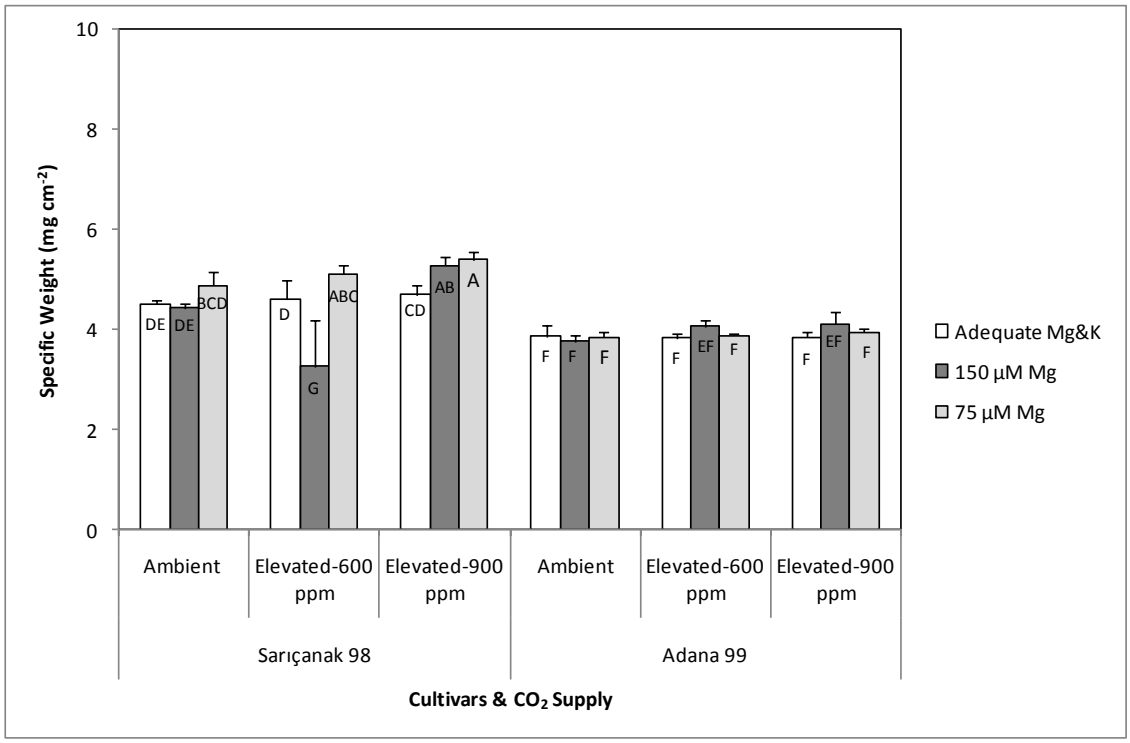

Figure 2.5: Specific weight of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM) and marginal Mg (150 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

Cultivars responded differently at a given CO_2 and Mg supply revealing significant interactions of CO_2*cultivar (p = 0.0011) and Mg*cultivar (p = 0.0001). CO_2 treatments, Mg supply and cultivars also showed statistically significant differences, respectively (p = 0.0001, p = 0.0011, p < 0.0001) (Table 2.2).

Table 2.2: p-values of specific weight according to statistical analysis

	p-value
Treatments	**Specific Weight**
CO_2	0.0001
Mg	0.0011
Cultivar	<.0001
CO_2*Mg	0.0009
CO_2*Cultivar	0.0011
Mg*Cultivar	0.0001
CO_2*Mg*Cultivar	0.0004

Carbohydrate Concentration:

There were statistically significant differences in the leaf carbohydrate concentration of plants. Highest level of leaf carbohydrate concentration was observed in plants grown with the lowest Mg supply. Enhancement of CO_2 concentration caused increase in leaf carbohydrate concentration in all treatments, especially in 75 µM Mg supply (Table 2.3).

Table 2.3: Leaf carbohydrate concentration of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM) and marginal Mg (150 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

Treatments			Leaf Carbohydrate Concentration
Cultivar	**CO_2 Supply**	**Mg Level**	**(mg g^{-1})**
Sarıçanak 98	Ambient-400 µmol mol^{-1}	75 µM Mg	65 ± 12 BCD
		150 µM Mg	43 ± 11 EFGH
		Adequate Mg&K	25 ± 4 H
	Elevated-600 µmol mol^{-1}	75 µM Mg	109 ± 13 A
		150 µM Mg	78 ± 5 B
		Adequate Mg&K	35 ± 7 FGH
	Elevated-900 µmol mol^{-1}	75 µM Mg	110 ± 3 A
		150 µM Mg	74 ± 4 BC
		Adequate Mg&K	33 ± 3 GH
Adana 99	Ambient-400 µmol mol^{-1}	75 µM Mg	58 ± 22 BCDE
		150 µM Mg	77 ± 32 B
		Adequate Mg&K	42 ± 11 EFGH
	Elevated-600 µmol mol^{-1}	75 µM Mg	58 ± 3 BCDE
		150 µM Mg	46 ± 16 DEFGH
		Adequate Mg&K	31 ± 7 H
	Elevated-900 µmol mol^{-1}	75 µM Mg	54 ± 13 CDEFG
		150 µM Mg	54 ± 8 CDEF
		Adequate Mg&K	55 ± 12 CDEF
LSD (0.05)			21.5
CV(%)			22.3
F Test			***

*Values given mean±standart deviation. Mean values compared by Student's t-test.
*Levels not connected by same letter are significantly different.
*If $p < 0.001$, F-test=***; if $p < 0.01$, F-test=**; if $p < 0.05$, F-test=*

Root carbohydrate concentration was affected from both Mg and CO_2 treatments. Significant increase was observed in Mg-deficient plants. However, root carbohydrate concentration was found higher in adequate-Mg plants than in Mg-deficient plants in 900 µmol mol^{-1} CO_2 treatments. There was no meaningful change in root carbohydrate

concentration upon different Mg treated plants. A slight decrease was observed in root carbohydrate concentration of Saricanak 98 plants with elevated [CO_2] (between ambient and 600 µmol mol^{-1} [CO_2] treatments). However, this trend was not observed when ambient condition results were compared with results of 900 µmol mol^{-1} [CO_2] treatments. Adana 99 cultivar responded differently to CO_2 treatments, when compared with Saricanak 98. While significant increase was observed in adequate Mg supplied plants with elevated [CO_2], slight increase was observed in Mg-deficient plants (Figure 2.6).

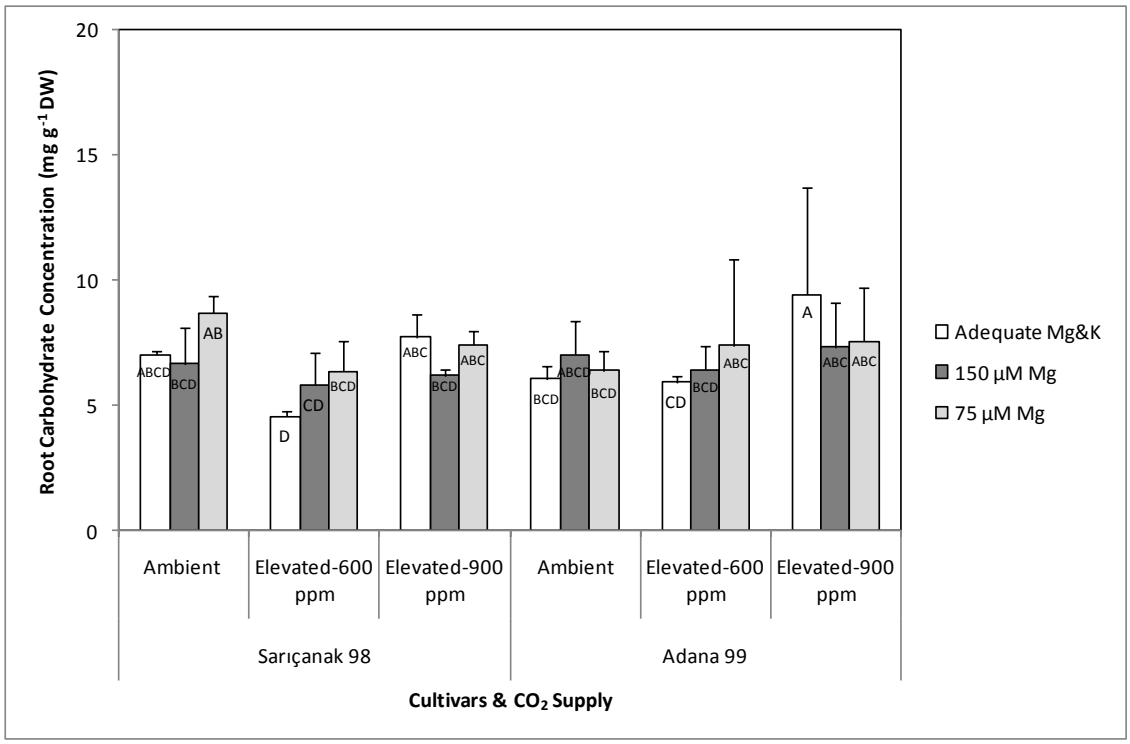

Figure 2.6: Root carbohydrate concentration of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM) and marginal Mg (150 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

According to results of leaf carbohydrate concentration, statistically significant interactions were observed in CO_2*cultivar and Mg*cultivar. Although different CO_2, Mg and cultivar treatments resulted significant differences respectively, CO_2*Mg interaction was not found statistically significant. The only statistically significant difference in the results of root carbohydrate concentration was found in the CO_2 treatments (Table 2.4).

Table 2.4: p-values of both leaf and root carbohydrate concentrations according to statistical analysis

	p-value	
Treatments	**Leaf Carbohydrate Concentration**	**Root Carbohydrate Concentration**
CO_2	0.0398	0.0305
Mg	<.0001	0.4101
Cultivar	0.0039	0.4579
CO_2*Mg	0.2242	0.2313
CO_2*Cultivar	<.0001	0.1411
Mg*Cultivar	<.0001	0.5373
CO_2*Mg*Cultivar	0.0490	0.8193

Photosynthetic Parameters:

Photosynthesis rate was altered among CO_2 treatments. Statistically significant increase was observed in Saricanak 98 plants grown under elevated [CO_2]. Similar trend was observed in Adana 99 plants, however the increase was less pronounced in Adana 99 plants than in Saricanak 98 plants. Mg deficiency clearly induced the reduction of photosynthesis rate irrespective of CO_2 and cultivar treatments (Figure 2.7).

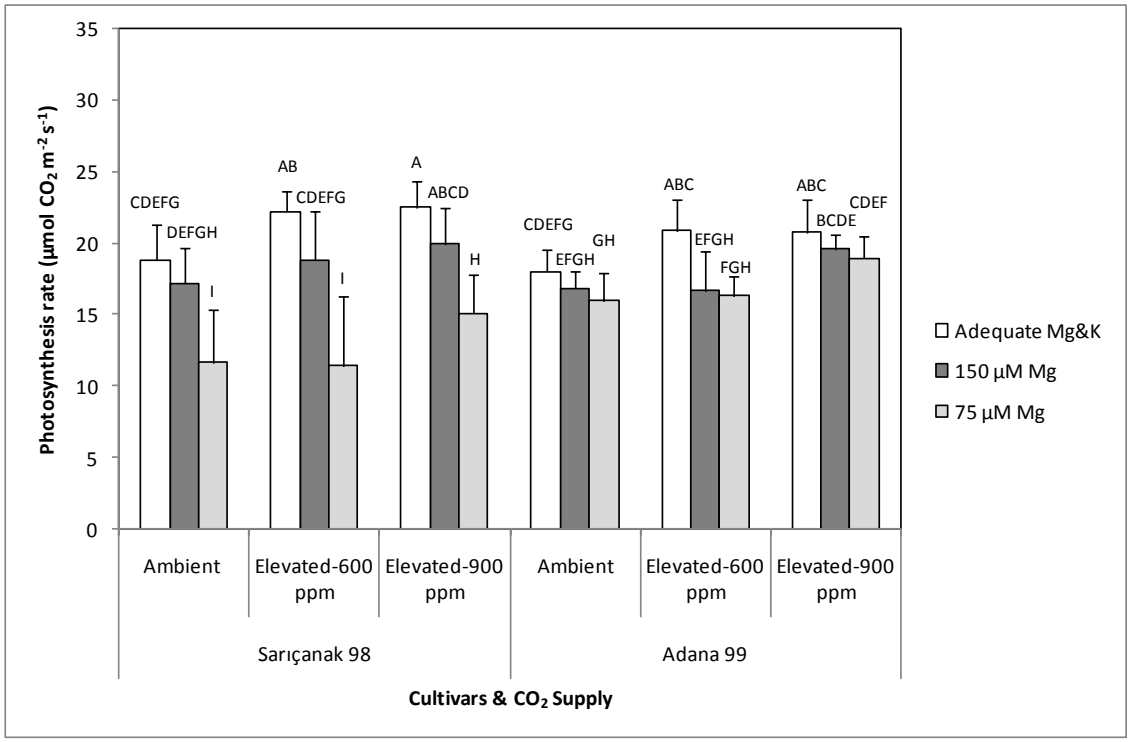

Figure 2.7: Photosynthesis rate of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM) and marginal Mg (150 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

Stomatal conductance and transpiration rate showed similar trends with respect to different CO_2 conditions. Elevated [CO_2] caused decrease in both stomatal conductance and transpiration rate. While Saricanak 98 plants grown with low Mg supply were more affected by elevating CO_2 than Adana 99 cultivar, Saricanak 98 plants grown with adequate Mg supply were less affected than Adana 99 plants. Interestingly, the downward trend between ambient and 600 µmol mol^{-1} [CO_2] was not observed between 600 and 900 µmol mol^{-1} [CO_2], especially plants grown with low Mg supply (Figure 2.8 and Figure 2.9).

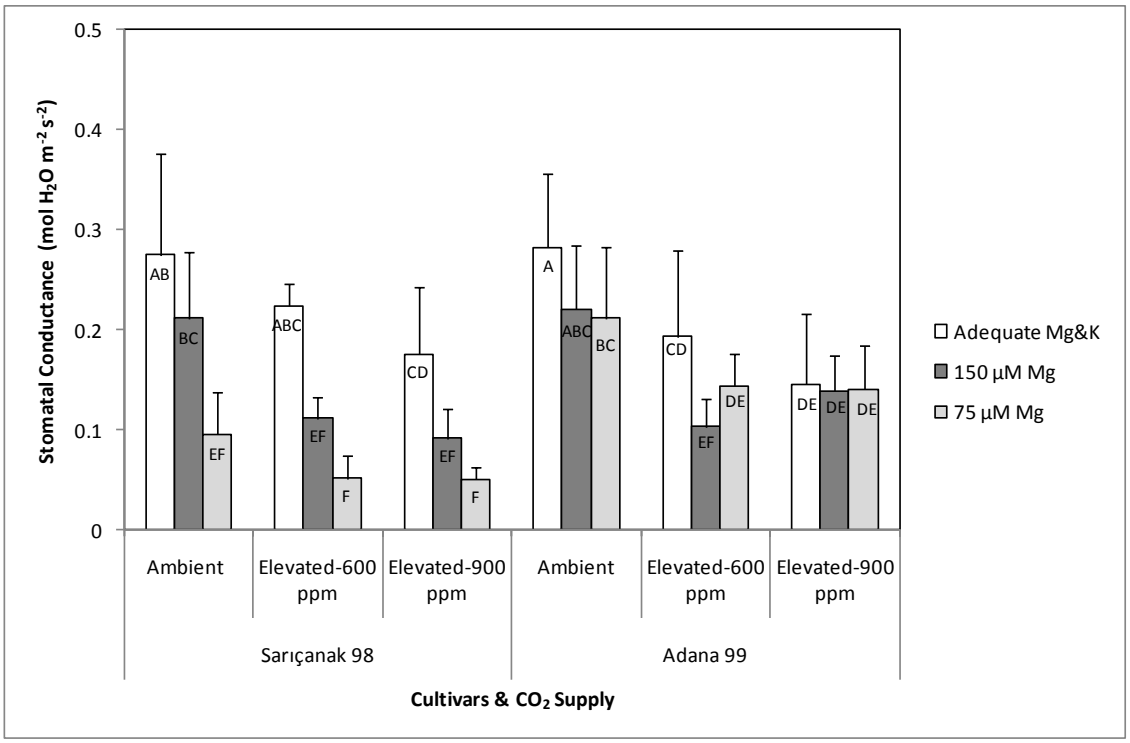

Figure 2.8: Stomatal conductance of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM) and marginal Mg (150 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

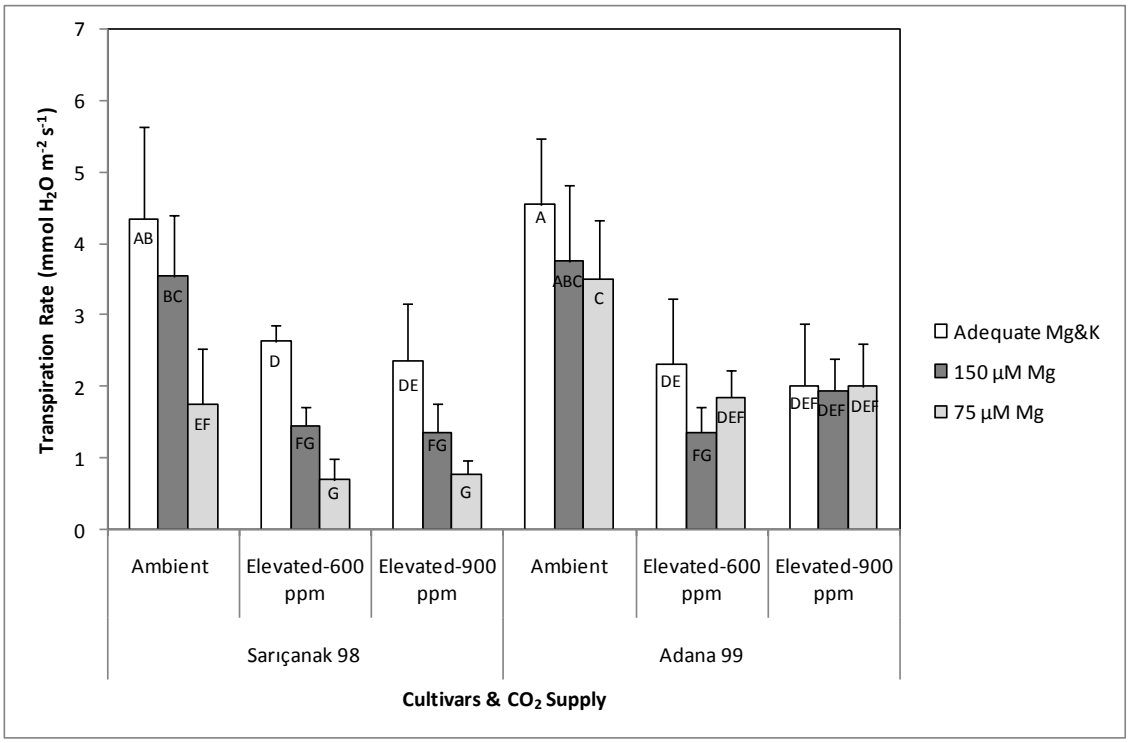

Figure 2.9: Transpiration rate of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM) and marginal Mg (150 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

Photosynthetic parameters (i.e. photosynthesis rate, stomatal conductance and transpiration rate) showed statistically significant differences with CO_2 ($p < 0.0001$) and Mg ($p < 0.0001$) treatments, respectively. There were statistically significant differences in cultivar upon stomatal conductance and transpiration rate. The interaction of cultivar with Mg supply was also found statistically significant in all three parameters (i.e photosynthesis rate, stomatal conductance and transpiration rate). However, no significant results were observed when CO_2, Mg and cultivar treatments were taken into account together (Table 2.5).

Table 2.5: p-values of photosynthetic parameters according to statistical analysis

	p-value		
Treatments	**Photosynthesis Rate**	**Stomatal Conductance**	**Transpiration Rate**
CO_2	<.0001	<.0001	<.0001
Mg	<.0001	<.0001	<.0001
Cultivar	0.1561	0.0032	0.0008
CO_2*Mg	0.1930	0.1645	0.0781
CO_2*Cultivar	0.8708	0.5972	0.3889
Mg*Cultivar	<.0001	<.0001	<.0001
CO_2*Mg*Cultivar	0.8798	0.7760	0.7822

Chlorophyll Concentration:

As expected, Mg deficiency caused statistically significant decrease in chlorophyll concentration. The lowest chlorophyll concentration was observed in plants of Adana 99 cultivar grown with the lowest Mg supply under elevated CO_2 conditions. Chlorophyll level did not change significantly upon increasing CO_2 at the adequate Mg supply. However, with elevated CO_2 concentrations, significant decrement was observed in plants grown with deficient Mg supplies (Figure 2.10).

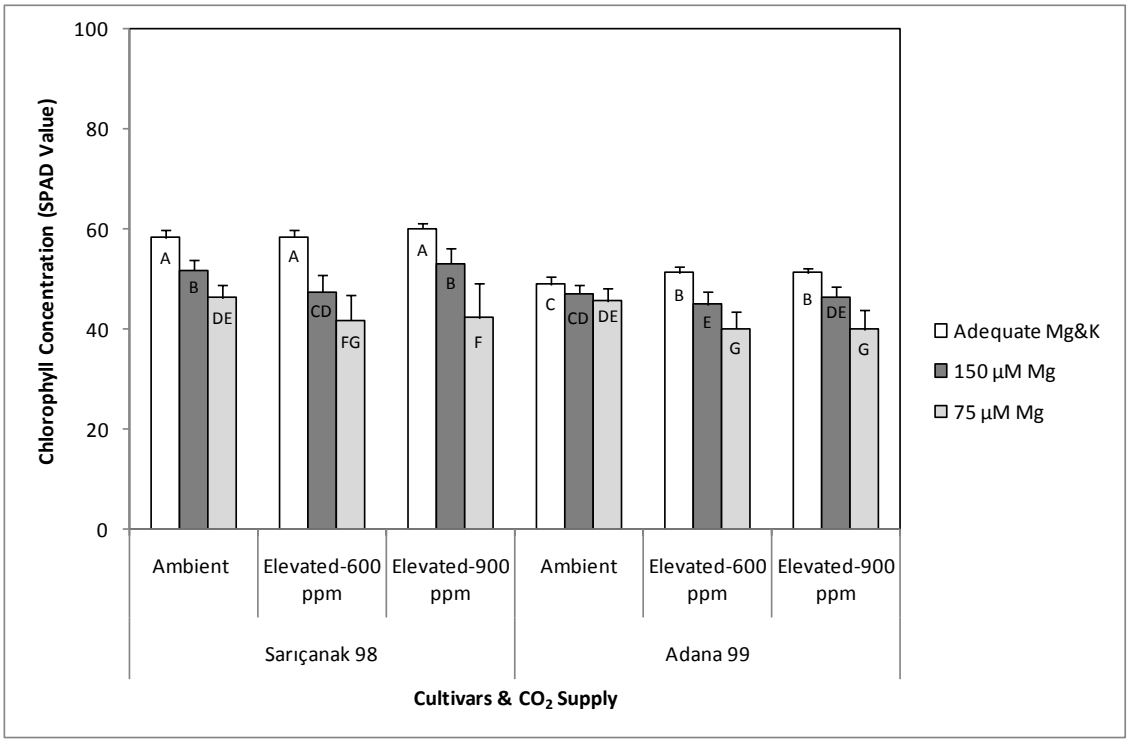

Figure 2.10: Chlorophyll concentration of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM) and marginal Mg (150 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

According to chlorophyll concentration results, the interactions between CO_2*cultivar and Mg*cultivar were found statistically significant. CO_2 treatments, Mg supply and cultivars also caused statistically significant differences, respectively ($p < 0.0001$, $p < 0.0001$, $p < 0.0001$). Mg supply also showed significant differences with alteration of CO_2 concentration (Table 2.6).

Table 2.6: p-values of chlorophyll concentration according to statistical analysis

	p-value
Treatments	**Chlorophyll Concentration**
CO_2	<.0001
Mg	<.0001
Cultivar	<.0001
CO_2*Mg	<.0001
CO_2*Cultivar	0.040
Mg*Cultivar	<.0001
CO_2*Mg*Cultivar	0.292

Shoot and Root Mg Concentrations:

Shoot Mg analysis results proved that low and marginal Mg levels were achieved in the test plants. Adequate and low Mg supply resulted statistically significant differences of Mg concentration in both shoot and root of plants (Figure 2.11 and Figure 2.12).

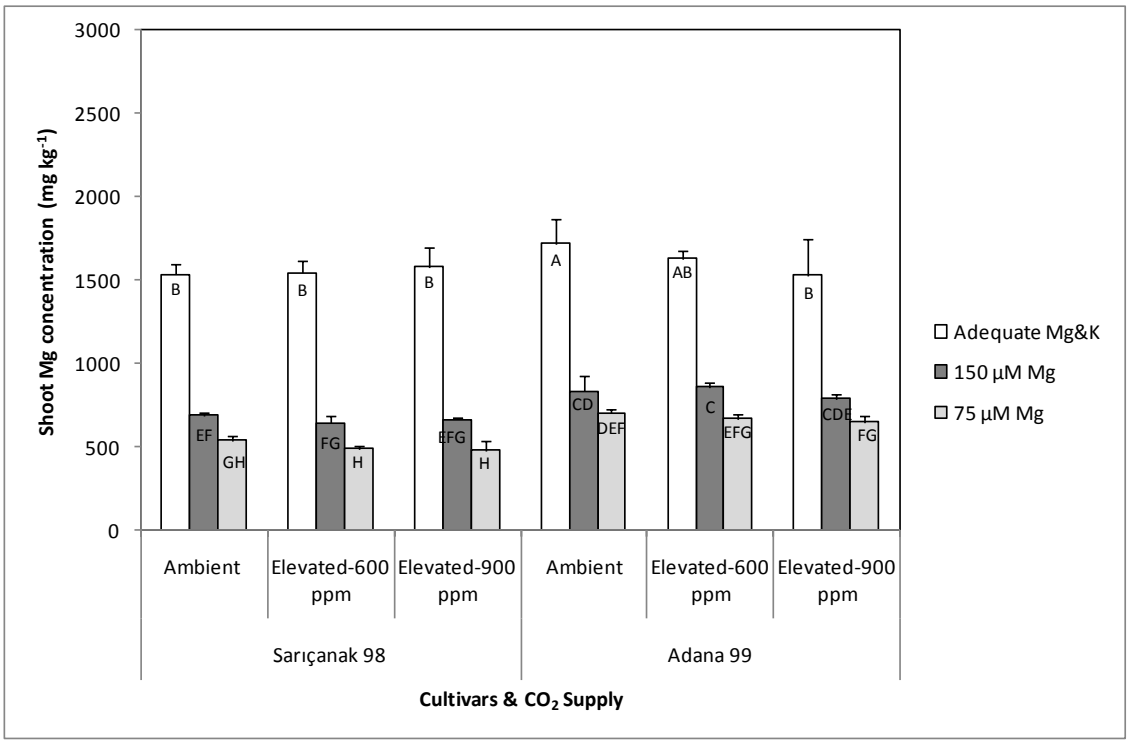

Figure 2.11: Shoot Mg concentration of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM) and marginal Mg (150 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

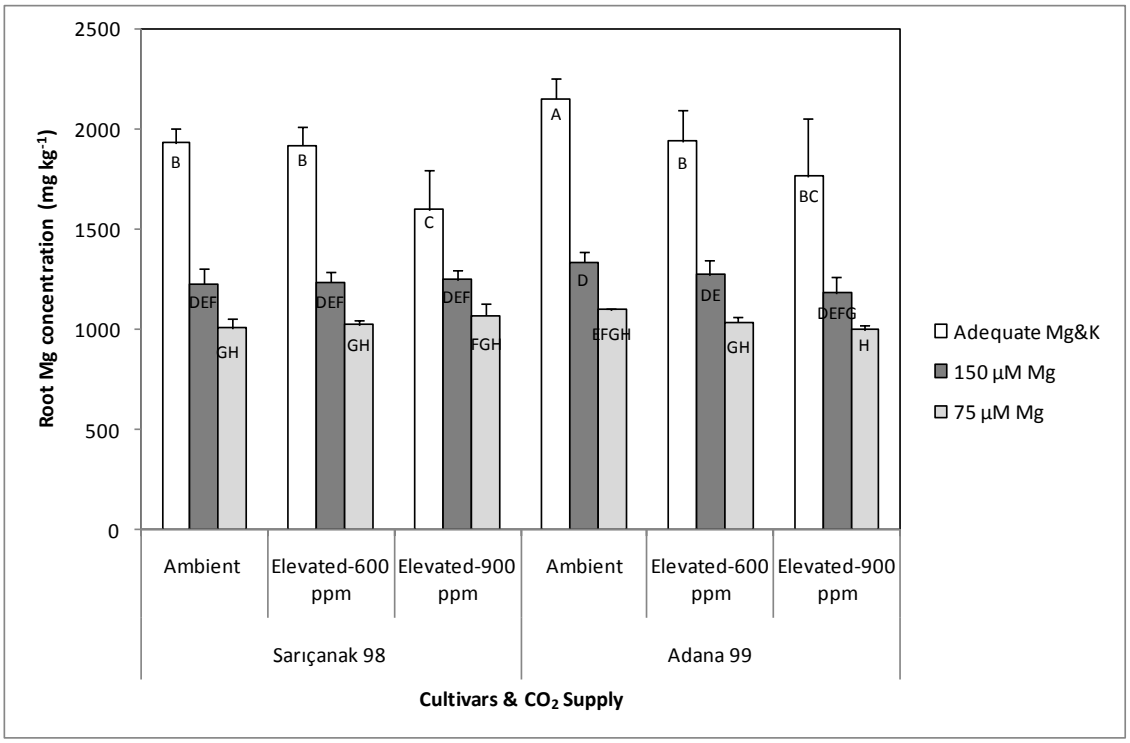

Figure 2.12: Root Mg concentration of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM) and marginal Mg (150 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

Different Mg supplies caused statistically significant differences in Mg concentration of shoot ($p < 0.0001$). Cultivars also showed significant differences with respect to Mg concentration of shoot. However, there were no significant differences when CO_2, Mg and cultivar treatments were taken into account together (Table 2.7).

According to root Mg concentration, CO_2 and Mg treatments showed statistically significant differences, respectively ($p = 0.001$, $p < 0.0001$). Moreover, interaction between CO_2 and Mg treatments was found statistically significant ($p = 0.0044$) (Table 2.7).

Table 2.7: p-values of magnesium concentration of both shoot and root according to statistical analysis

	p-value	
	Magnesium Concentration	
Treatments	**Shoot**	**Root**
CO_2	0.1338	0.0010
Mg	<.0001	<.0001
Cultivar	<.0001	0.0661
CO_2*Mg	0.9744	0.0044
CO_2*Cultivar	0.2245	0.1510
Mg*Cultivar	0.1466	0.1962
CO_2*Mg*Cultivar	0.3694	0.6638

Root Properties:

Root length was altered with both Mg and CO_2 concentration. According to statistical analysis, significant increase in root length was observed with elavating [CO_2] in all treatments. When the effects of Mg concentration on root length were considered, it was clear that Mg deficiency caused decrease in root length, especially in Saricanak 98 cultivar (Figure 2.13).

Similar trend was observed in root surface area. Statistically significant decrease was found in low Mg supplied plants compare to adequate Mg plants. The highest area of root surface was observed in Saricanak 98 cultivar treated with the adequate Mg supply and highest [CO_2] treatment. Enhancement of CO_2 concentration caused significant increase in root surface area in all treatments. However, Saricanak 98 cultivar was seemed to be more affected by CO_2 concentration than Adana 99 cultivar (Figure 2.14).

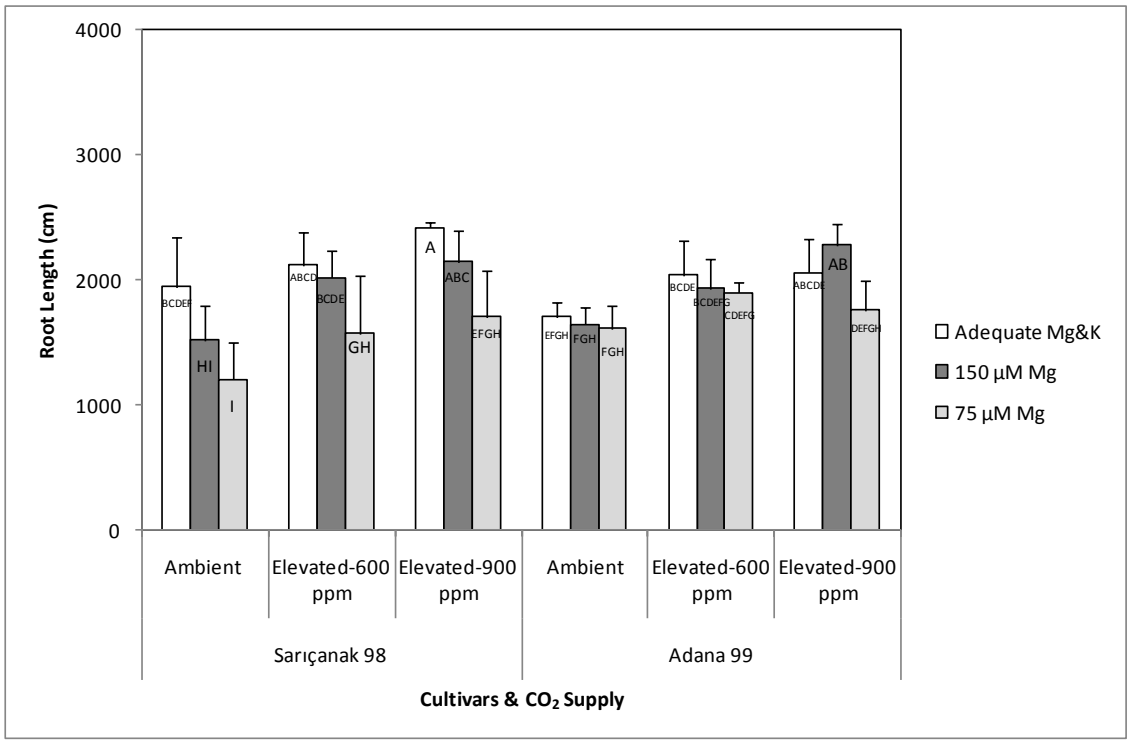

Figure 2.13: Root length of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM) and marginal Mg (150 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

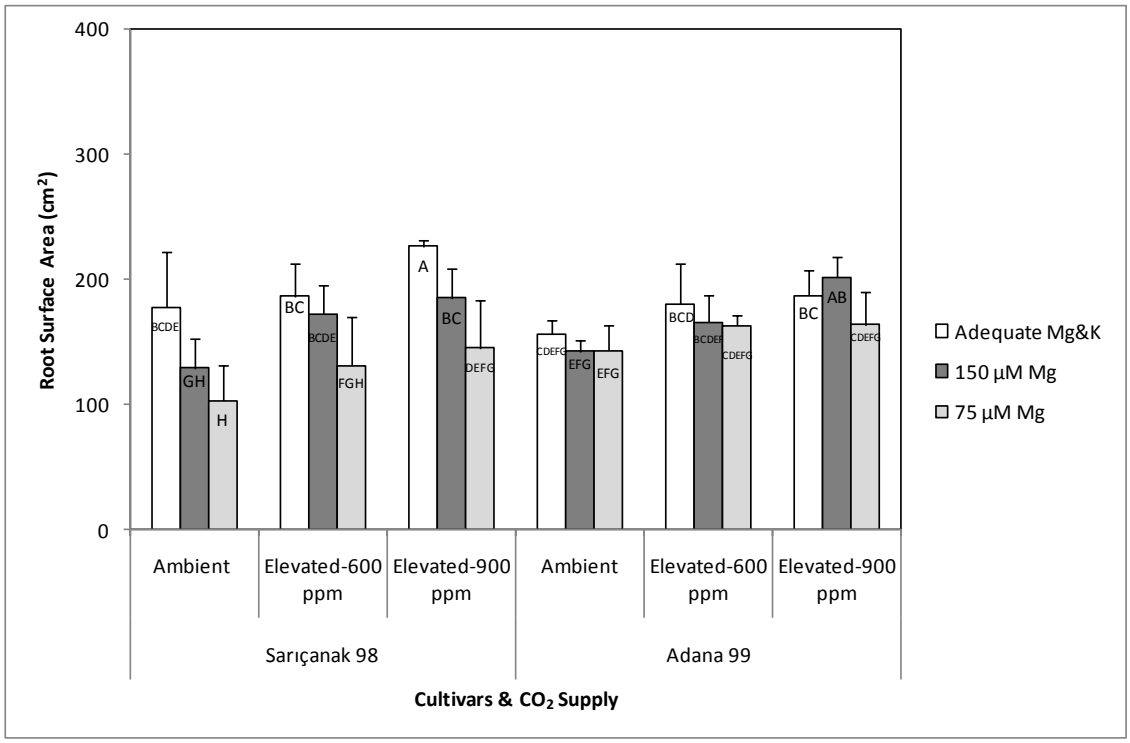

Figure 2.14: Root surface area of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM) and marginal Mg (150 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

According to root volume results, both Mg and CO_2 treatments had significant effects. Magnesium deficiency significantly decreased root volume, whereas elevating [CO_2] induced increase of root volume and these effects were more evident in Saricanak 98 (Figure 2.15).

Same trend was observed in root tips with the alteration of Mg concentration. Mg deficiency caused decrease in root tips in Saricanak 98 cultivar. However, Adana 99 cultivar did not show similar results. With elevating CO_2 concentration, there were slight increases in root tips (Figure 2.16).

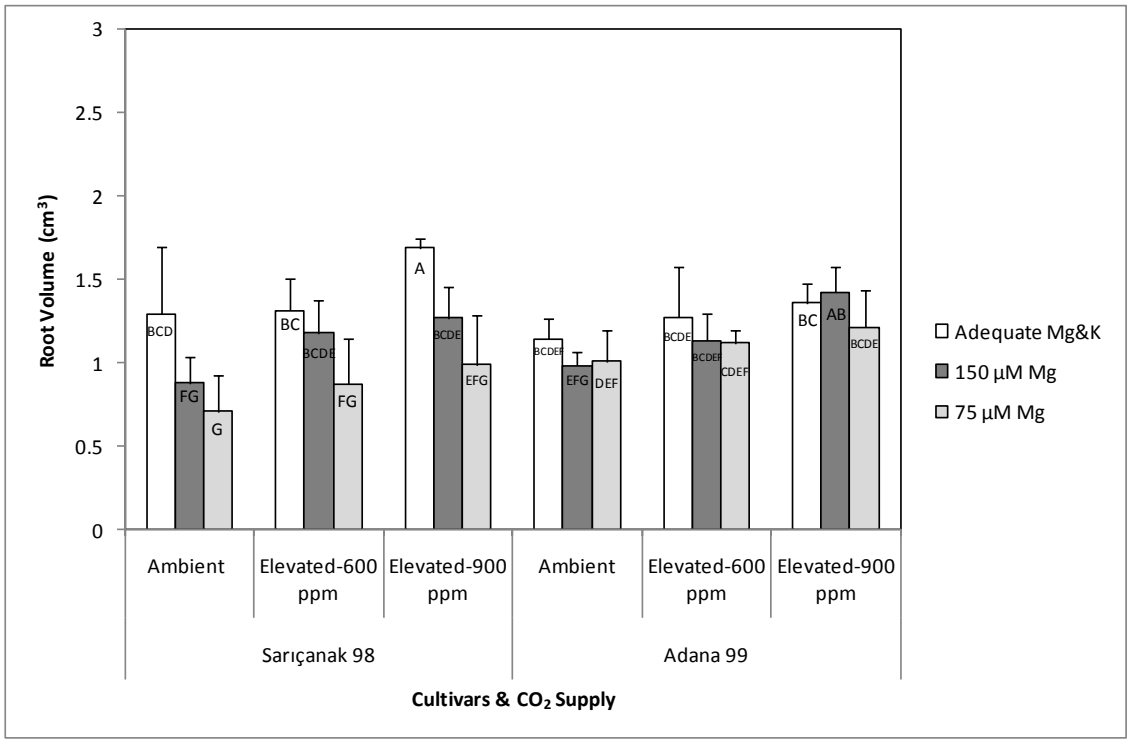

Figure 2.15: Root volume of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM) and marginal Mg (150 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

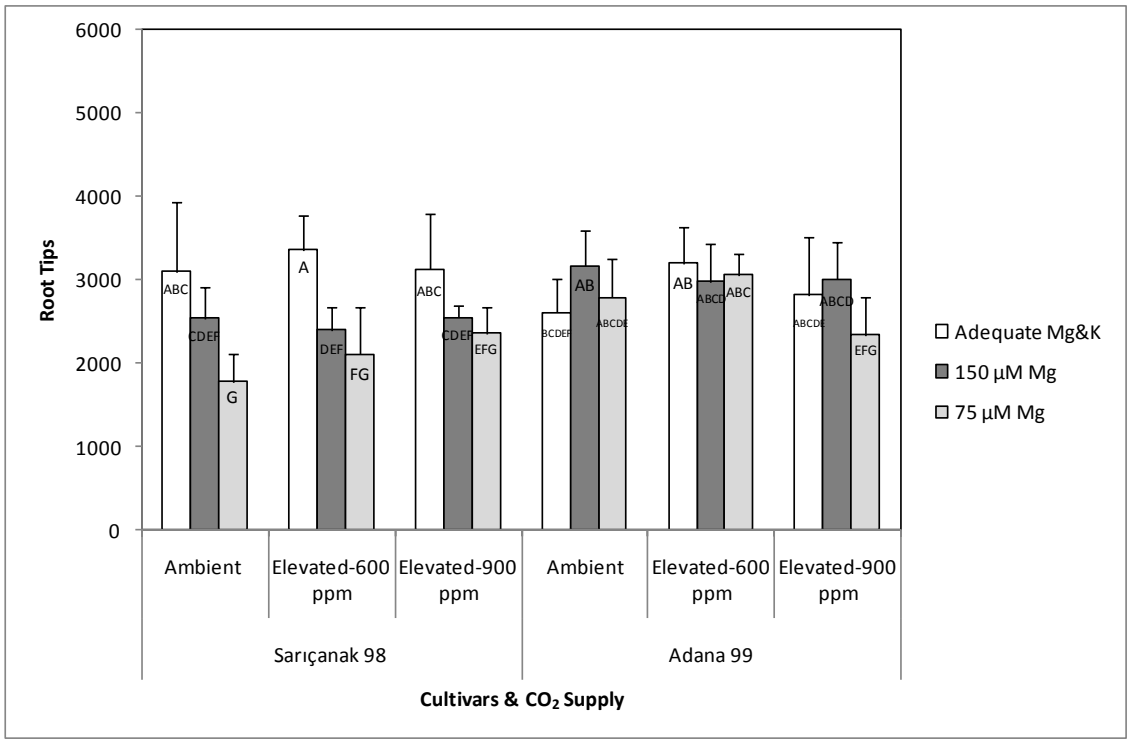

Figure 2.16: Root tips of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM) and marginal Mg (150 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

According to root length values, different Mg supplies caused statistically significant differences ($p < 0.0001$). CO_2 treatments also caused significant differences in root length values. The interaction of Mg supply with cultivar was also found statistically significant ($p = 0.0067$). However, there was no significant difference when CO_2, Mg and cultivar treatments were taken into account together (Table 2.8).

According to statistical analysis, both CO_2 treatment and Mg supply showed significant differences with respect to root surface area ($p < 0.0001$, $p < 0.0001$). Mg supply also caused significant differences upon cultivars ($p = 0.0034$) (Table 2.8).

Similar results were observed in root volume. CO_2 and Mg treatments showed significant differences, respectively ($p < 0.0001$, $p < 0.0001$). The interaction of Mg supply with cultivar was also found statistically significant ($p = 0.0034$). However, there was no significant difference when CO_2, Mg and cultivar treatments were taken into account together (Table 2.8).

Although CO_2 treatments did not show significant results with respect to root tips, statistically significant differences were observed in both Mg supply and cultivar, respectively ($p < 0.0001$, $p = 0.0058$). There was also significant difference found in the interaction of Mg with cultivar ($p = 0.0004$). However, there was no significant difference when CO_2, Mg and cultivar treatments were taken into account together (Table 2.8).

Table 2.8: p-values of length, surface area, volume and tips of root according to statistical analysis

	p-value			
Treatments	**Root Length**	**Root Surface Area**	**Root Volume**	**Root Tips**
CO_2	<.0001	<.0001	<.0001	0.2926
Mg	<.0001	<.0001	<.0001	<.0001
Cultivar	0.5949	0.3897	0.2941	0.0058
CO_2*Mg	0.4665	0.5999	0.7115	0.3834
CO_2*Cultivar	0.5669	0.7258	0.8518	0.2299
Mg*Cultivar	0.0067	0.0034	0.0034	0.0004
CO_2*Mg*Cultivar	0.5521	0.6177	0.6081	0.3352

Total Antioxidant Capacity:

Total antioxidant concentration was altered with both Mg and CO_2 concentrations. According to statistical analysis, significant increase was observed among low Mg supply with elavated [CO_2]. Different trend was observed in plants grown under ambient condition (Figure 2.17).

The highest level of total antioxidant concentration was observed in Adana 99 plants grown with low Mg supply and 900 µmol mol^{-1} [CO_2] treatment. Enhancement of CO_2 concentration also induced significant increment of total antioxidant capacity (Figure 2.17).

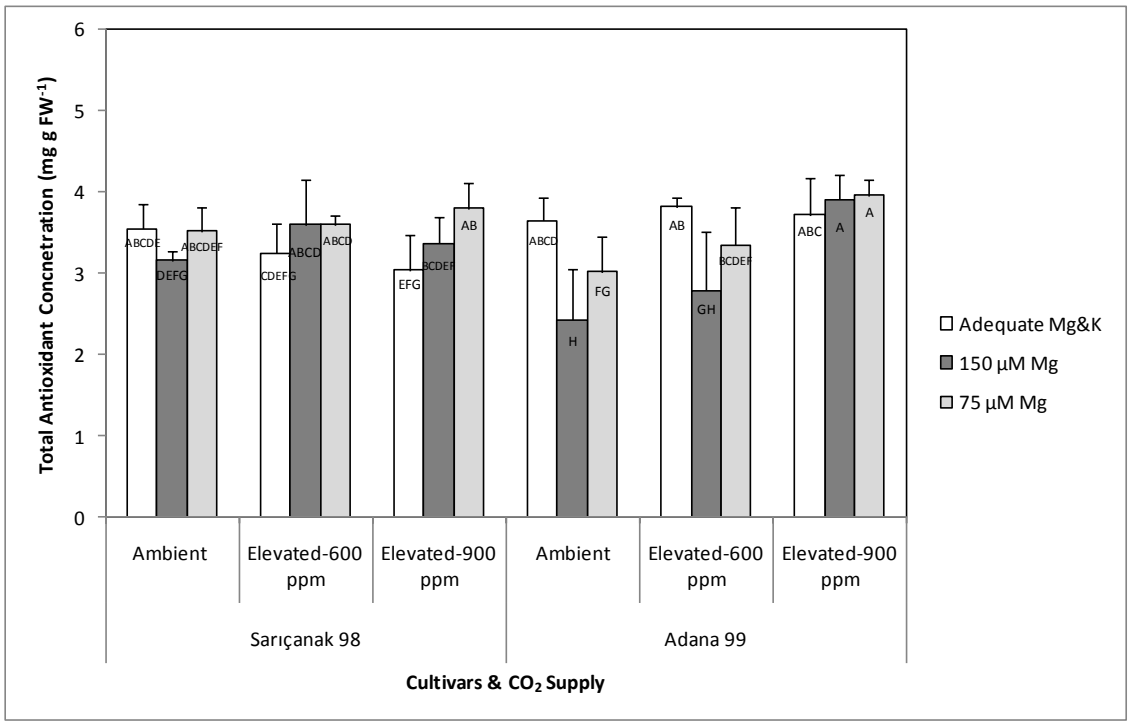

Figure 2.17: Total antioxidant capacity of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM) and marginal Mg (150 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

According to total antioxidant capacity, different Mg supply and CO_2 treatments were found statistically significant (p = 0.0009, p = 0.0038). There were also significant differences in interactions of cultivar with both Mg and CO_2 treatments (p = 0.0008, p = 0.0005). However, there was no significant difference when CO_2, Mg and cultivar treatments were taken into account together (Table 2.9).

Table 2.9: p-values of total antioxidant capacity according to statistical analysis

	p-value
Treatments	**Total Antioxidant Capacity**
CO_2	0.0009
Mg	0.0038
Cultivar	0.7520
CO_2*Mg	0.0018
CO_2*Cultivar	0.0005
Mg*Cultivar	0.0008
CO_2*Mg*Cultivar	0.1722

Lipid Peroxidation:

Lipid peroxidation was altered with different Mg supplies. Mg deficiency caused statistically significant increase in lipid peroxidation levels and the highest level of lipid peroxidation was observed in the lowest Mg supplied plants. However, enhancement of CO_2 concentration did not affect lipid peroxidation levels in a similar way (Figure 2.18).

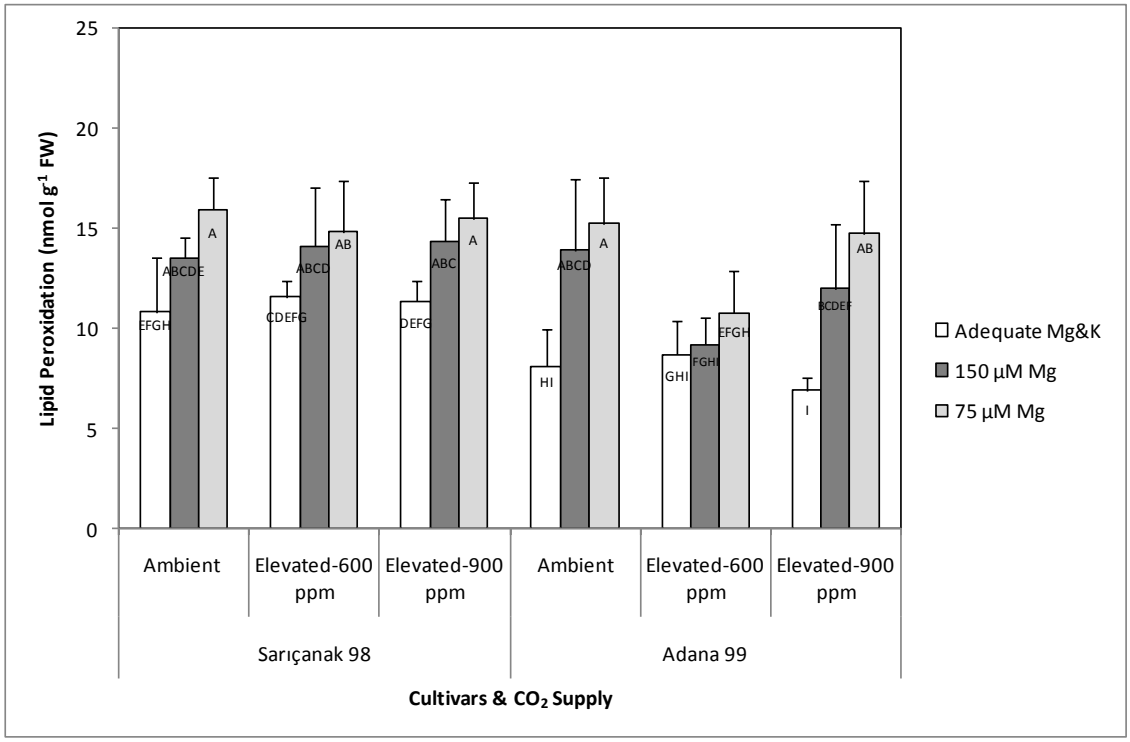

Figure 2.18: Lipid peroxidation of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM) and marginal Mg (150 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

Different Mg supplies caused significantly different lipid peroxidation levels ($p < 0.0001$). Cultivar also responded statistically different with respect to lipid peroxidation levels ($p < 0.0001$). However, there was no significant difference when CO_2, Mg and cultivar treatments were taken into account together (Table 2.10).

Table 2.10: p-values of lipid peroxidation according to statistical analysis

	p-value
Treatments	**Lipid Peroxidation**
CO_2	0.0627
Mg	<.0001
Cultivar	<.0001
CO_2*Mg	0.1055
CO_2*Cultivar	0.0520
Mg*Cultivar	0.4256
CO_2*Mg*Cultivar	0.2921

Carbohydrate Concentration in Phloem Exudate:

Mg supply altered the phloem carbohydrate concentration and significant increment was observed in Mg-deficient plants. While elevating [CO_2] caused increase in phloem carbohydrate concentration in plants grown with adequate Mg supply, same trend was not observed in low Mg supplied plants (Figure 2.19).

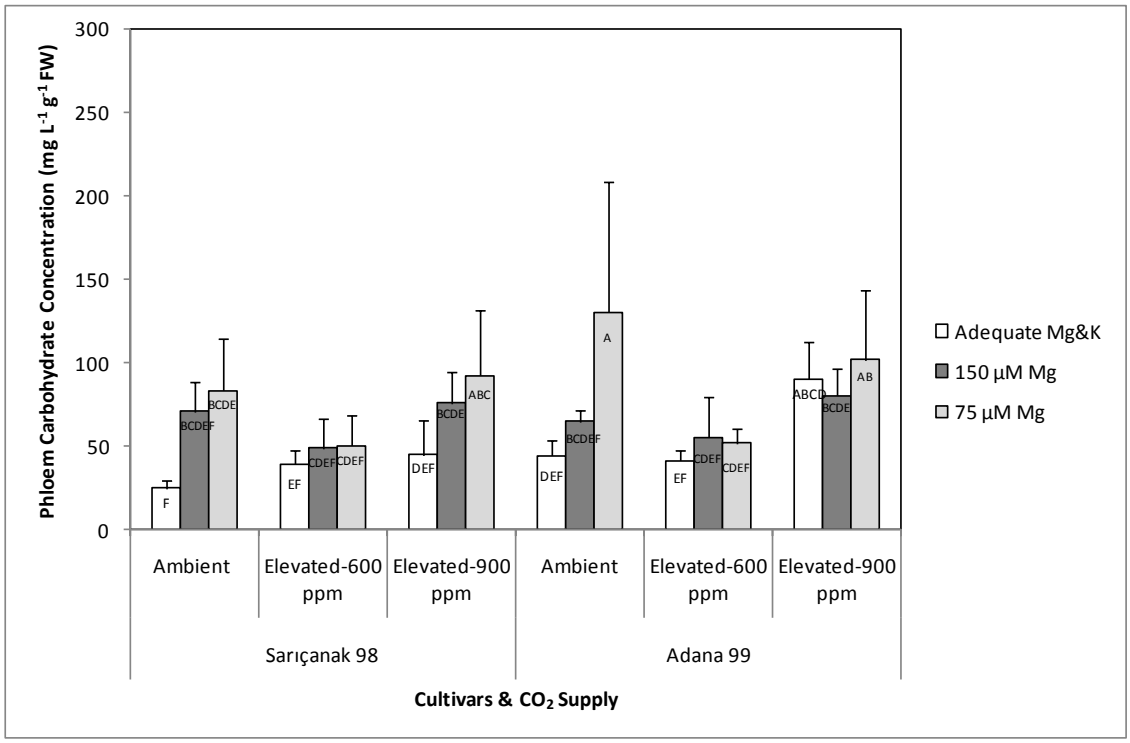

Figure 2.19: Phloem carbohydrate concentration of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM) and marginal Mg (150 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

According to statistical analysis, there were significant differences in both CO_2 and Mg treatments, respectively (p = 0.0042, p = 0.0014). However, there was no significant difference when CO_2, Mg and cultivar treatments were taken into account together (Table 2.11).

Table 2.11: p-values of phloem carbohydrate concentration according to statistical analysis

	p-value
Treatments	**Phloem Carbohydrate Concentration**
CO_2	0.0042
Mg	0.0014
Cultivar	0.0677
CO_2*Mg	0.1216
CO_2*Cultivar	0.5903
Mg*Cultivar	0.4955
CO_2*Mg*Cultivar	0.5388

Membrane Stability Index (MSI):

Slight decreases were observed in plants grown with low Mg supply. The highest level of MSI was observed in adequate Mg supplied plants of Saricanak 98 cultivar under elevated CO_2 treatments. There was no significant effect of CO_2 treatments upon membrane stability index (Figure 2.20).

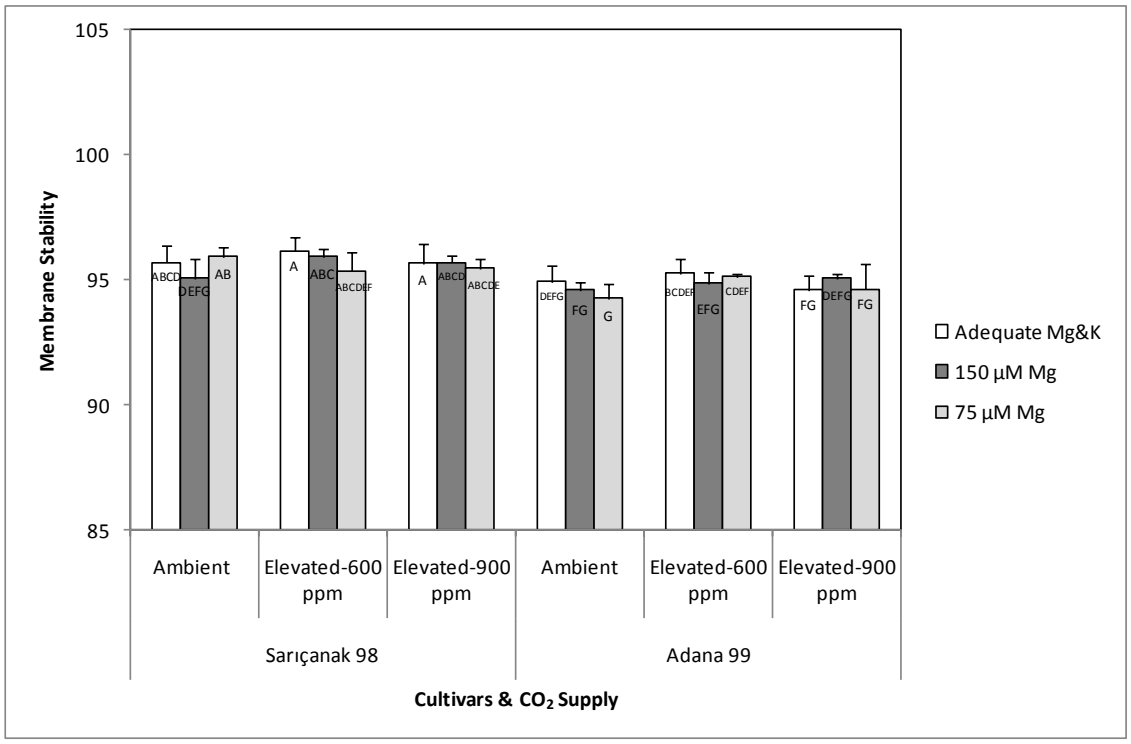

Figure 2.20: Membrane stability index of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low Mg (75 µM) and marginal Mg (150 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

According to statistical analysis of MSI results, p-value of cultivar was found smaller than 0.0001, meaning that significant differences were observed upon cultivars. However, the cross analyses between all treatments were not found statistically significant (Table 2.12).

Table 2.12: p-values of membrane stability index according to statistical analysis

	p-value
Treatments	**Membrane Stability**
CO_2	0.0777
Mg	0.2944
Cultivar	<.0001
CO_2*Mg	0.3395
CO_2*Cultivar	0.7489
Mg*Cultivar	0.8105
CO_2*Mg*Cultivar	0.1463

C.3. Experiments on K Nutrition under Ambient and Elevated Carbon Dioxide Environments

Dry Matter:

Potassium deficiency triggered reduction of shoot dry weight of plants. Shoot dry weight was significantly increasing with elevated [CO_2] in adequate K plants of both Saricanak 98 and Adana 99 cultivars. Shoot dry matter of adequate K plants of Saricanak 98 cultivar showed significant increase in 600 μmol mol^{-1} [CO_2] compare to ambient [CO_2], whereas there was no such difference between plants in 600 μmol mol^{-1} [CO_2] and 900 μmol mol^{-1} [CO_2] conditions. Statistical analysis demonstrated that the change of shoot dry matter of Adana 99 cultivar was similar with Saricanak 98 cultivar.

While shoot dry weight was increasing with elevated [CO_2] in adequate K plants, there was a continuous decrement in K-deficient plants of both cultivars. However, there was no statistically different result between 600 and 900 μmol mol^{-1} [CO_2] treated plants of Adana 99 cultivar. Moreover, while shoot dry matter of both cultivars was differently affected by K deficiency, they were affected by CO_2 treatment in a similar way (Figure 3.1).

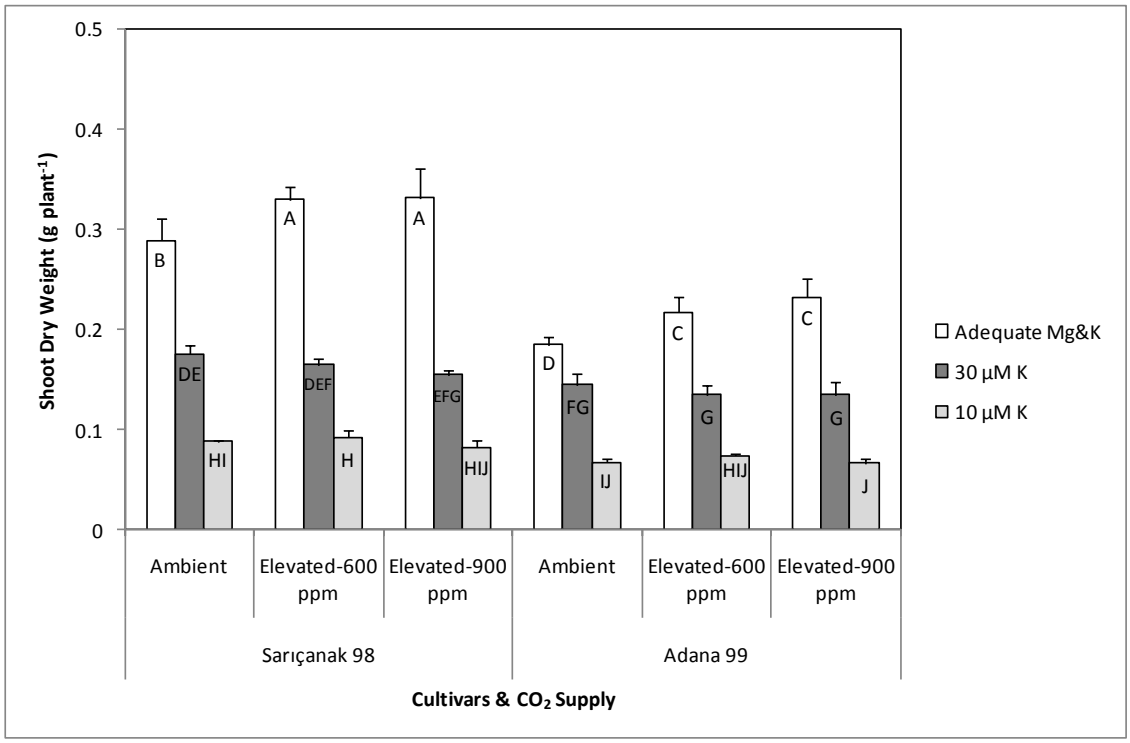

Figure 3.1: Shoot dry weight of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low K (10 µM) and marginal K (30 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

Potassium deficiency also reduced root growth. Root dry matter results showed similar trend with shoot dry matter results. A statistically significant increment of root dry weight was observed in Saricanak 98 plants grown with adequate K supply and elevated 600 µmol mol^{-1} [CO_2]. Interestingly, a decrease was observed when 600 and 900 µmol mol^{-1} CO_2 concentrations were compared. There was a slight increase in plants of Adana 99 cultivar when adequate K and 600 µmol mol^{-1} [CO_2] treated plants were compared. However, a statistically significant difference was observed between adequate K and 900 ppm [CO_2] treated plants of Adana 99 cultivar. There was a slight decrease in K deficient plants with elevated [CO_2]. Moreover, no statistically significant difference was observed in the low K supplied plants with alteration of [CO_2] (Figure 3.2).

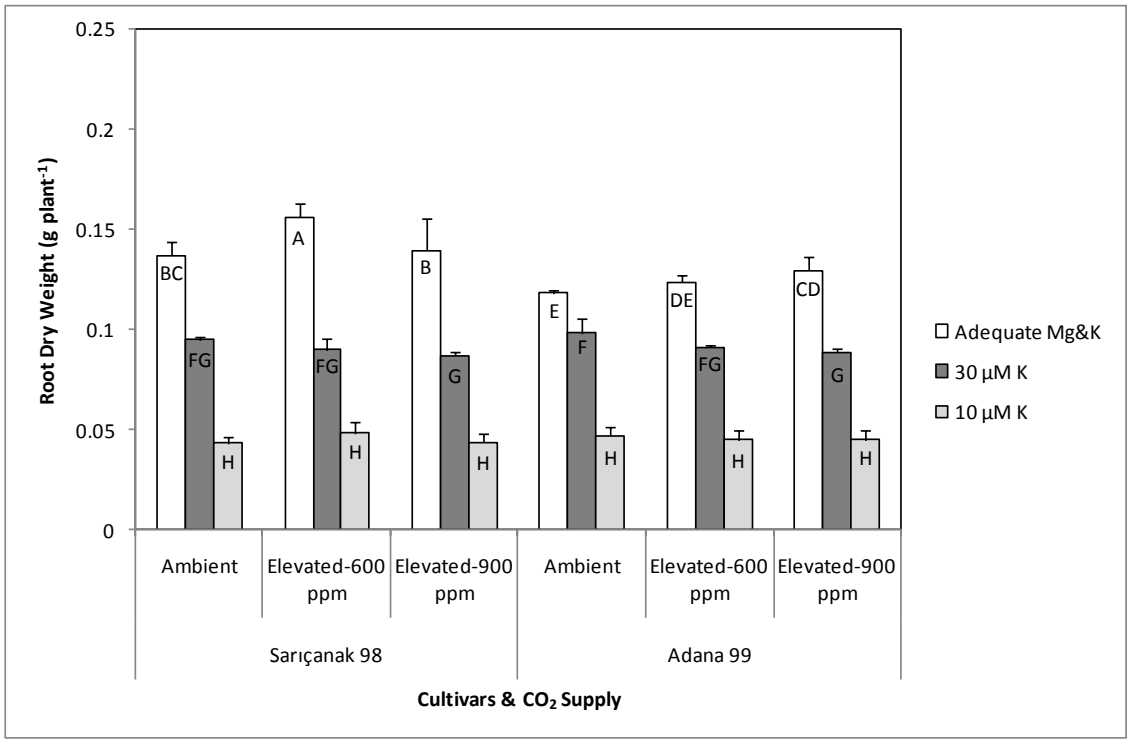

Figure 3.2: Root dry weight of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low K (10 µM) and marginal K (30 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

Significant decrease in total dry weight was observed in K deficient plants. Total dry weight of adequate K plants significantly increased with the elevated CO_2 concentration in both cultivars. However, there was a slight decrease or no change in deficient plants with elevated [CO_2] (Figure 3.3).

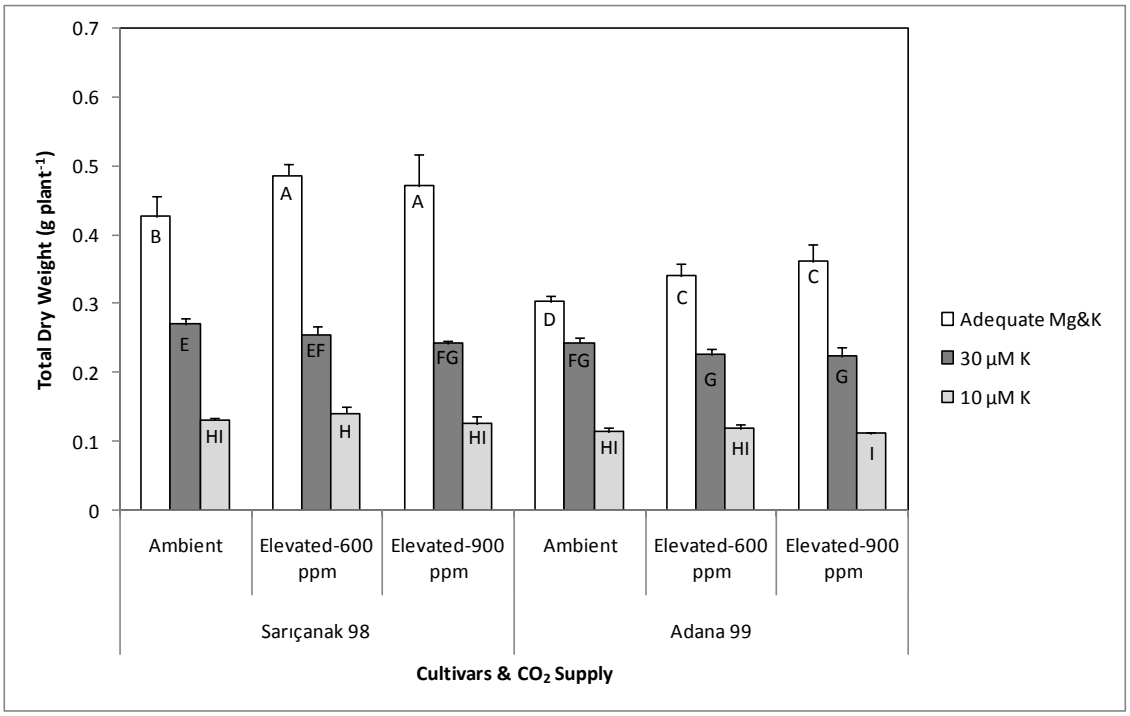

Figure 3.3: Total dry weight of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low K (10 µM) and marginal K (30 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

Under ambient [CO_2] conditions, very slight difference of shoot-to-root ratio was observed upon different K supplies. Different shoot-to-root ratio was observed among two cultivars. While the lowest shoot-to-root ratio was found in 30 µM K supplied and 900 µmol mol^{-1} [CO_2] treated plants of Saricanak 98 cultivar, statistically similar results were observed in 10 and 30 µM K supplied plants of Adana 99 cultivar with elevated [CO_2] (Figure 3.4).

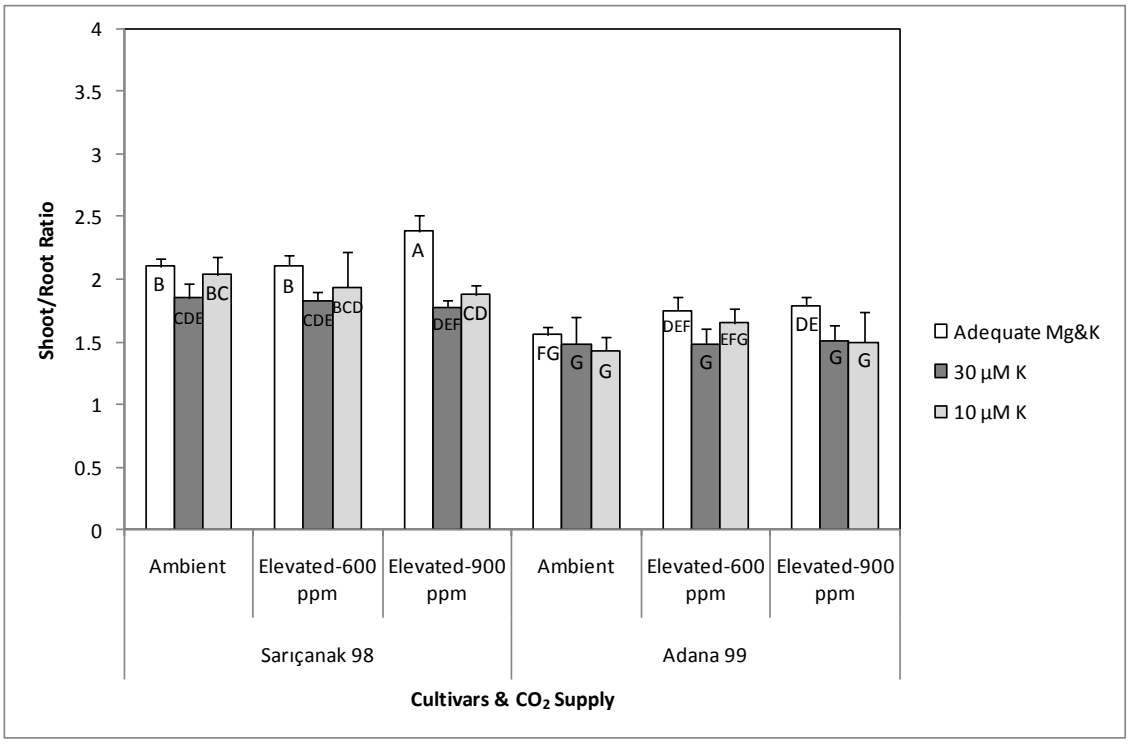

Figure 3.4: Shoot-to-root ratio of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low K (10 µM) and marginal K (30 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

Different K supplies caused statistically significant differences in shoot dry weight ($p < 0.0001$). There was also significant difference among cultivars when considered shoot dry weight. Significant difference was found in the interaction of K supply with cultivar, meaning that cultivars responded differently to K treatments ($p < 0.0001$). However, there was no significant difference in interaction between CO_2 and cultivar treatments.

Similar statistical results were found in root dry weight. Potassium supply and cultivar caused statistically significant differences, respectively ($p < 0.0001$, $p = 0.0003$). Cultivars also responded differently to both CO_2 and K treatments ($p = 0.0339$, $p < 0.0001$). Interaction of CO_2 with K supply was also found statistically significant (Table 3.1).

Different K treatments and cultivars caused significant different total dry weight results, respectively. Plants supplied with different K concentrations showed statistically significant differences in total dry weight with respect to both CO_2 and cultivar treatments, meaning that interactions of K supply with both CO_2 and cultivar were

found statistically significant ($p < 0.0001$, $p < 0.0001$). However, no significant results were observed in interaction between CO_2 and cultivar treatments (Table 3.1).

According to statistical analysis of shoot-to-root ratio, p-values of K supply and cultivar were found smaller than 0.0001, meaning that significant differences were observed upon different K supplies and cultivars, respectively. The interaction of CO_2 treatment with K supply was also found statistically significant ($p = 0.0444$). However, the cross analysis of CO_2 treatment with cultivar was not found statistically significant (Table 3.1).

Table 3.1: p-values of shoot, root and total dry weight, and shoot-to-root ratio according to statistical analysis

	p-value			
Treatments	**Shoot Dry Weight**	**Root Dry Weight**	**Total Dry Weight**	**Shoot-to-Root Ratio**
CO_2	0.0393	0.1530	0.0693	0.3347
K	<.0001	<.0001	<.0001	<.0001
Cultivar	<.0001	0.0003	<.0001	<.0001
CO_2*K	<.0001	0.0024	<.0001	0.0444
CO_2*Cultivar	0.5573	0.0339	0.2776	0.1628
K*Cultivar	<.0001	<.0001	<.0001	0.1700
CO_2*K*Cultivar	0.9811	0.1503	0.8254	0.4558

Specific Weight:

Plants of Saricanak 98 cultivar grown under ambient CO_2 conditions did not show any significant differences with alteration of K concentrations. There was a slight or no difference in Adana 99 plants with elevated [CO_2]. Both different K supplies and enhancement of CO_2 concentrations had not significant impact on both cultivars with respect to specific weight (Figure 3.5).

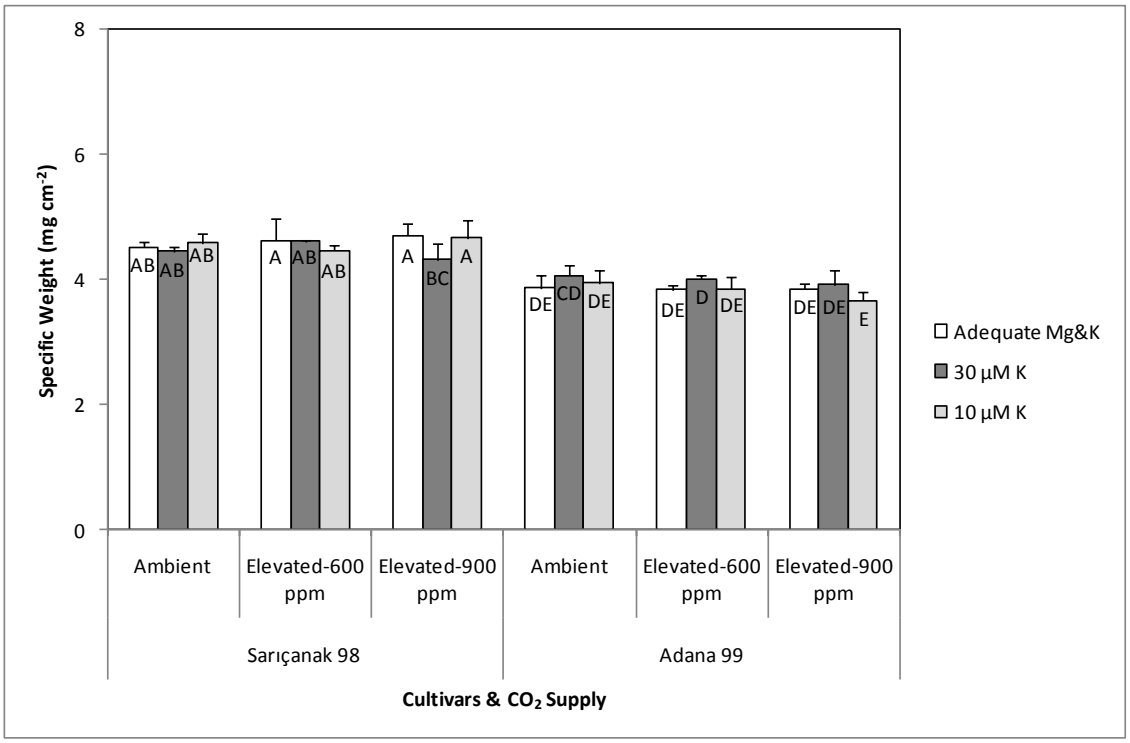

Figure 3.5: Specific weight of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low K (10 µM) and marginal K (30 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

According to specific weight, statistically significant difference was observed upon cultivar ($p < 0.0001$). Different K supplies also showed statistically significant differences with cultivars, meaning that interaction between K and cultivar treatments was found statistically significant ($p = 0.0226$). However, no significant result was observed when CO_2, K and cultivar treatments were taken into account together (Table 3.2).

Table 3.2: p-values of specific weight according to statistical analysis

	p-value
Treatments	**Specific Weight**
CO_2	0.6617
K	0.8222
Cultivar	<.0001
CO_2*K	0.3392
CO_2*Cultivar	0.2735
K*Cultivar	0.0226
CO_2*K*Cultivar	0.4115

Carbohydrate Concentration:

There were statistically significant differences in different K supplied plants with respect to leaf carbohydrate concentration. Highest level of leaf carbohydrate concentration was observed in plants grown with low K supply for ambient and 900 μmol mol^{-1} [CO_2] treatments. In 600 μmol mol^{-1} [CO_2], 30 μM K supplied plants showed the highest level of leaf carbohydrate concentration in both cultivars. Enhancement of CO_2 concentration caused increase in leaf carbohydrate concentration in all treatments, except 600 μmol mol^{-1} [CO_2] treatment (Table 3.3).

Table 3.3: Leaf carbohydrate concentration of plants grown with adequate Mg and K (1000 μM Mg and 750 μM K), low K (10 μM) and marginal K (30 μM) supply under three different CO_2 environments (ambient: 400 μmol mol^{-1}, elevated: 600 and 900 μmol mol^{-1})

Treatments			Leaf Carbohydrate Concentration	
Cultivar	**CO_2 Supply**	**K Level**	**(mg g^{-1})**	
Sarıçanak 98	Ambient-400 μmol mol^{-1}	10 μM K	62 ± 18	ABCD
		30 μM K	45 ± 6	DEFGH
		Adequate Mg&K	25 ± 4	H
	Elevated-600 μmol mol^{-1}	10 μM K	43 ± 9	DEFGH
		30 μM K	51 ± 6	CDEFG
		Adequate Mg&K	35 ± 7	EFGH
	Elevated-900 μmol mol^{-1}	10 μM K	78 ± 19	A
		30 μM K	57 ± 17	ABCDE
		Adequate Mg&K	33 ± 3	FGH
Adana 99	Ambient-400 μmol mol^{-1}	10 μM K	51 ± 28	CDEFG
		30 μM K	53 ± 8	CDEFG
		Adequate Mg&K	42 ± 11	DEFGH
	Elevated-600 μmol mol^{-1}	10 μM K	35 ± 6	EFGH
		30 μM K	43 ± 21	DEFGH
		Adequate Mg&K	31 ± 7	GH
	Elevated-900 μmol mol^{-1}	10 μM K	75 ± 9	AB
		30 μM K	68 ± 15	ABC
		Adequate Mg&K	55 ± 12	BCDEF
	LSD (0.05)		22.28	
	CV(%)		27.42	
	F Test		***	

*Values given mean±standart deviation. Mean values compared by Student's t-test.
*Levels not connected by same letter are significantly different.
*If $p < 0.001$, F-test=***; if $p < 0.01$, F-test=**; if $p < 0.05$, F-test=*

Root carbohydrate concentration was affected from both K and CO_2 treatments. Significant differences were observed among different K supplies, and statistically significant increment was observed in plants grown with low K supply. Lowest root

carbohydrate concentration was observed in adequate K supplied plants. However, slight or no change was observed in plants grown with adequate condition with elevating [CO_2]. While slight decrement was observed in K-deficient plants of Saricanak 98 cultivar grown under 600 µmol mol^{-1} [CO_2], there was slight increment in 30 µM K supplied plants of Saricanak 98 cultivar grown under 900 µmol mol^{-1} [CO_2]. However, statistically significant increase was observed in K-deficient plants of Adana 99 cultivar with elevating [CO_2], especially in plants grown under 900 µmol mol^{-1} [CO_2] (Figure 3.6).

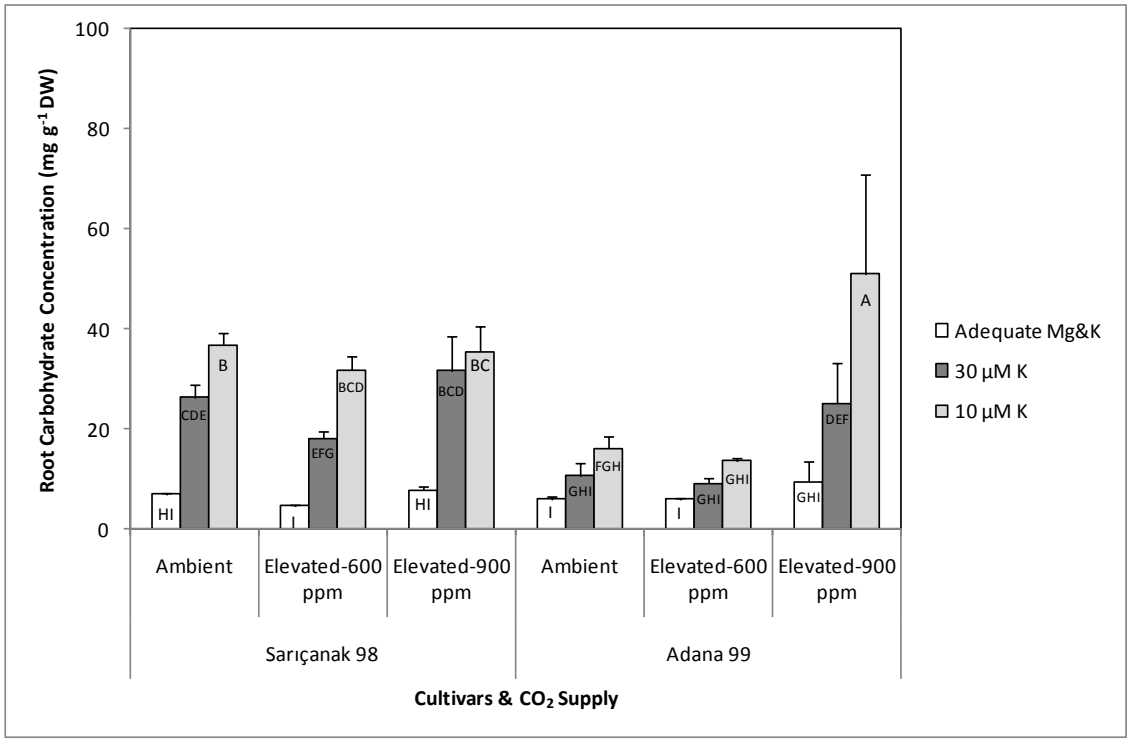

Figure 3.6: Root carbohydrate concentration of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low K (10 µM) and marginal K (30 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

According to results of leaf carbohydrate concentration, statistically significant differences were observed in different CO_2 and K treatments, respectively (p = 0.0001, p = 0.0001). However, no statistically significant differences were found with the cross analysis among CO_2 treatment, K supply and cultivars (Table 3.4).

According to statistical analysis of root carbohydrate analysis, significant differences were observed in CO_2 treatments, different K supplies and cultivars, respectively

($p < 0.0001$, $p < 0.0001$, $p = 0.0006$). According to cross analysis, cultivars responded differently to both CO_2 treatments and different K supplies, respectively ($p = 0.0004$, $p = 0.0154$). K supply also responded differently to CO_2 treatments, meaning that interaction of CO_2 treatment with K supply was found statistically significant ($p = 0.0076$). When CO_2, K and cultivar treatments were taken into account together, significant difference was also observed (Table 3.4).

Table 3.4: p-values of both leaf and root carbohydrate concentrations according to statistical analysis

	p-value	
Treatments	**Leaf Carbohydrate Concentration**	**Root Carbohydrate Concentration**
CO_2	0.0001	<.0001
K	0.0001	<.0001
Cultivar	0.4561	0.0006
CO_2*K	0.1536	0.0076
CO_2*Cultivar	0.1694	0.0004
K*Cultivar	0.1338	0.0154
CO_2*K*Cultivar	0.7989	0.0021

Photosynthetic Parameters:

Photosynthesis rate was altered with different K supplies. Statistically significant increase was observed in Saricanak 98 plants grown under elevated [CO_2]. Similar trend was observed in Adana 99 plants, however the increase was less pronounced in Adana 99 plants than in Saricanak 98 plants. The low K supply clearly caused reduction of photosynthesis rate. Different CO_2 treatments significantly affected the photosynthesis rate. Enhancement of CO_2 concentrations caused increase in photosynthesis rate in all treatments. As expected, the highest photosynthesis rate was observed in plants grown under elevated [CO_2]. However, slight increase was observed in plants grown under 900 μmol mol^{-1} [CO_2] compare to plants grown under 600 μmol mol^{-1} [CO_2] (Figure 3.7).

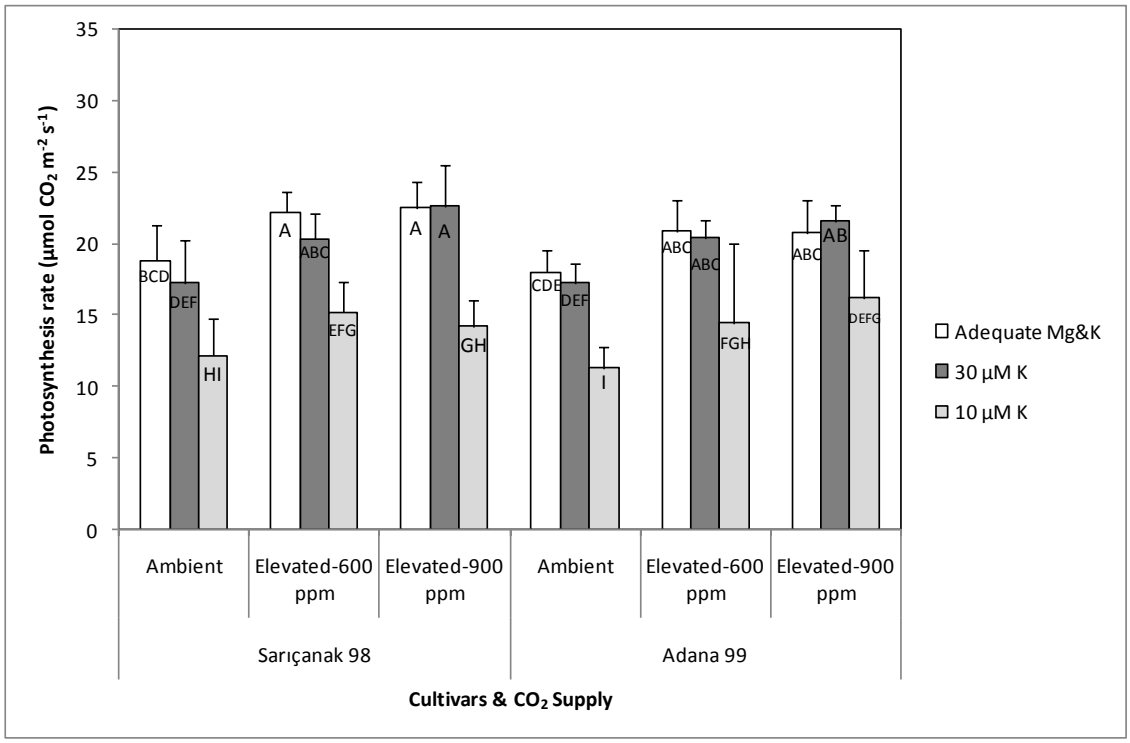

Figure 3.7: Photosynthesis rate of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low K (10 µM) and marginal K (30 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

Significant decrement was observed in both stomatal conductance and transpiration rate in plants grown with low K supplies. Stomatal conductance and transpiration rate showed similar trend with respect to different CO_2 conditions. Elevating [CO_2] caused decrease in both stomatal conductance and transpiration rate. Interestingly, the downward trend between ambient and 600 µmol mol^{-1} [CO_2] was not observed between 600 and 900 µmol mol^{-1} [CO_2], especially plants grown with low K supplies (Figure 3.8 and Figure 3.9).

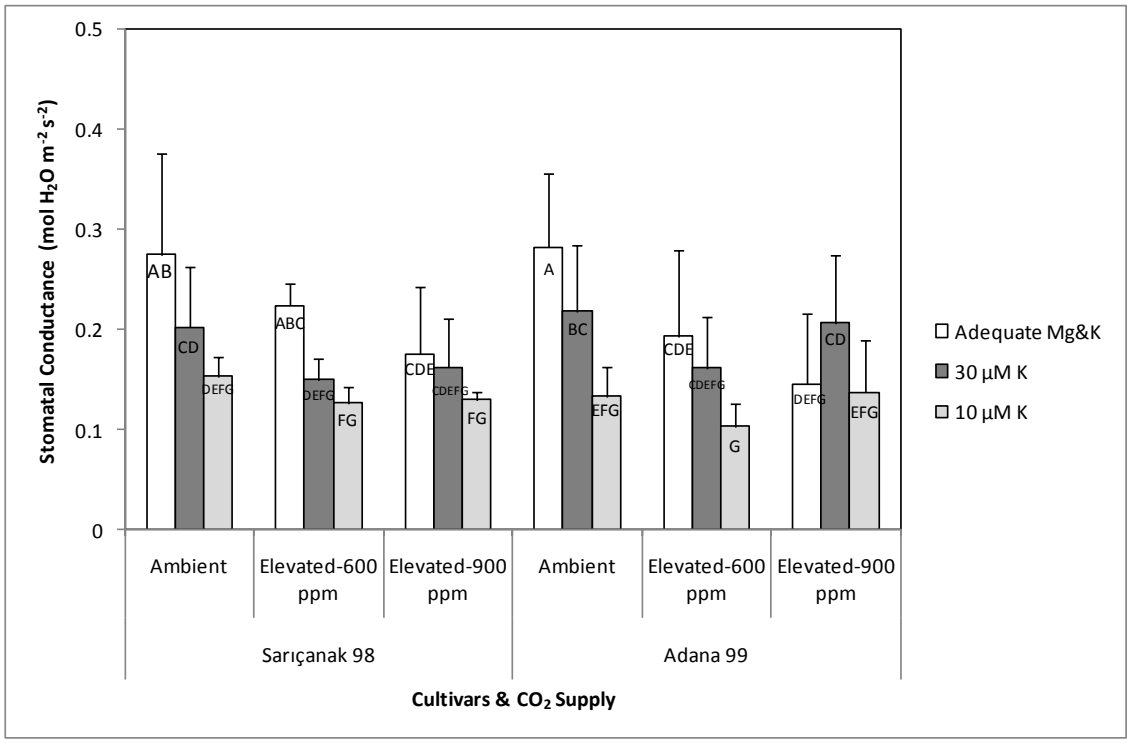

Figure 3.8: Stomatal conductance of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low K (10 µM) and marginal K (30 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

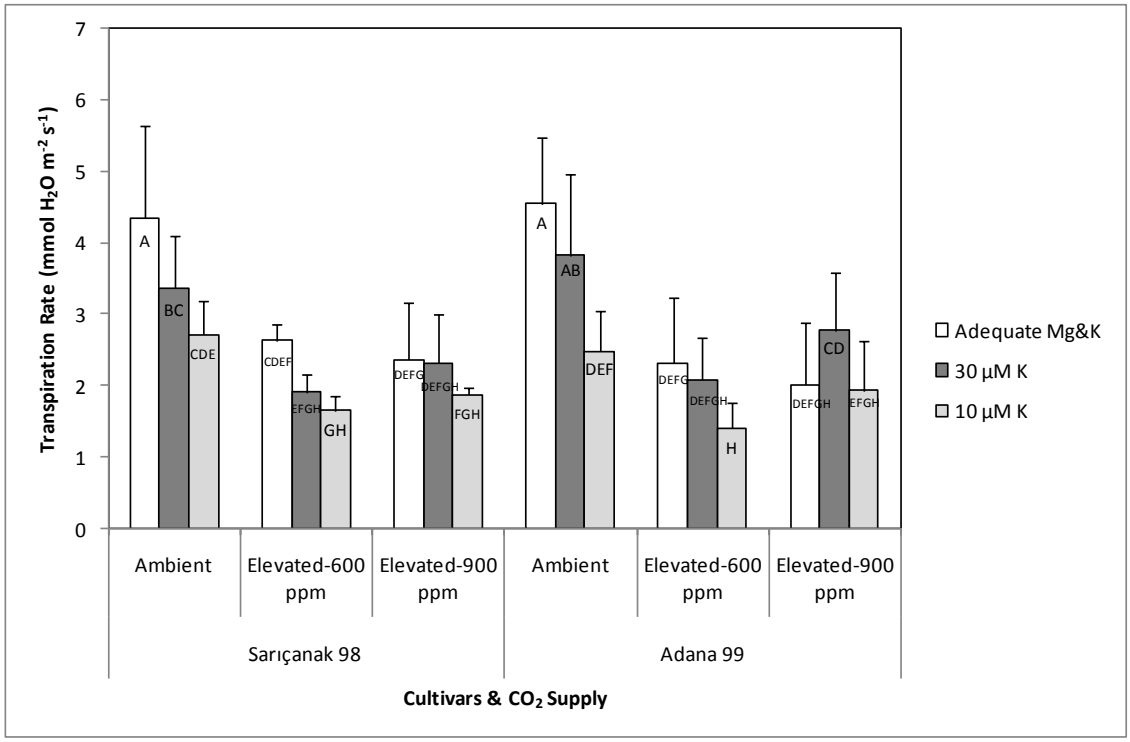

Figure 3.9: Transpiration rate of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low K (10 µM) and marginal K (30 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

Photosynthetic parameters (i.e. photosynthesis rate, stomatal conductance and transpiration rate) showed statistically significant differences with CO_2 treatments and different K supplies, respectively. CO_2 treatments also showed significant differences with different K supplies with respect to both stomatal conductance and transpiration rate, meaning that interaction of CO_2 with K treatment was found statistically significant upon stomatal conductance and transpiration rate (p = 0.0077, p = 0.0034). However, no significant results were observed when CO_2, K and cultivar treatments were taken into account together (Table 3.5).

Table 3.5: p-values of photosynthetic parameters according to statistical analysis

	p-value		
Treatments	**Photosynthesis Rate**	**Stomatal Conductance**	**Transpiration Rate**
CO_2	<.0001	<.0001	<.0001
K	<.0001	<.0001	<.0001
Cultivar	0.2904	0.8570	0.8683
CO_2*K	0.7457	0.0077	0.0034
CO_2*Cultivar	0.9414	0.7007	0.7229
K*Cultivar	0.4540	0.2295	0.2162
CO_2*K*Cultivar	0.5529	0.8358	0.8924

Chlorophyll Concentration:

As expected, K deficiency caused statistically significant reduction in chlorophyll concentration. Cultivars responded differently to both K and CO_2 treatments. The lowest chlorophyll concentration was observed in Adana 99 plants grown with low K supply under elevated CO_2 conditions. Similar results were observed in adequate K plants with enhancement of CO_2 concentrations. The highest chlorophyll concentration was observed in Saricanak 98 plants grown with adequate K supply under 900 μmol mol^{-1} CO_2 concentration. While adequate K supplied plants showed upward trend with elevated [CO_2], low K supplied plants behaved contrarily (Figure 3.10).

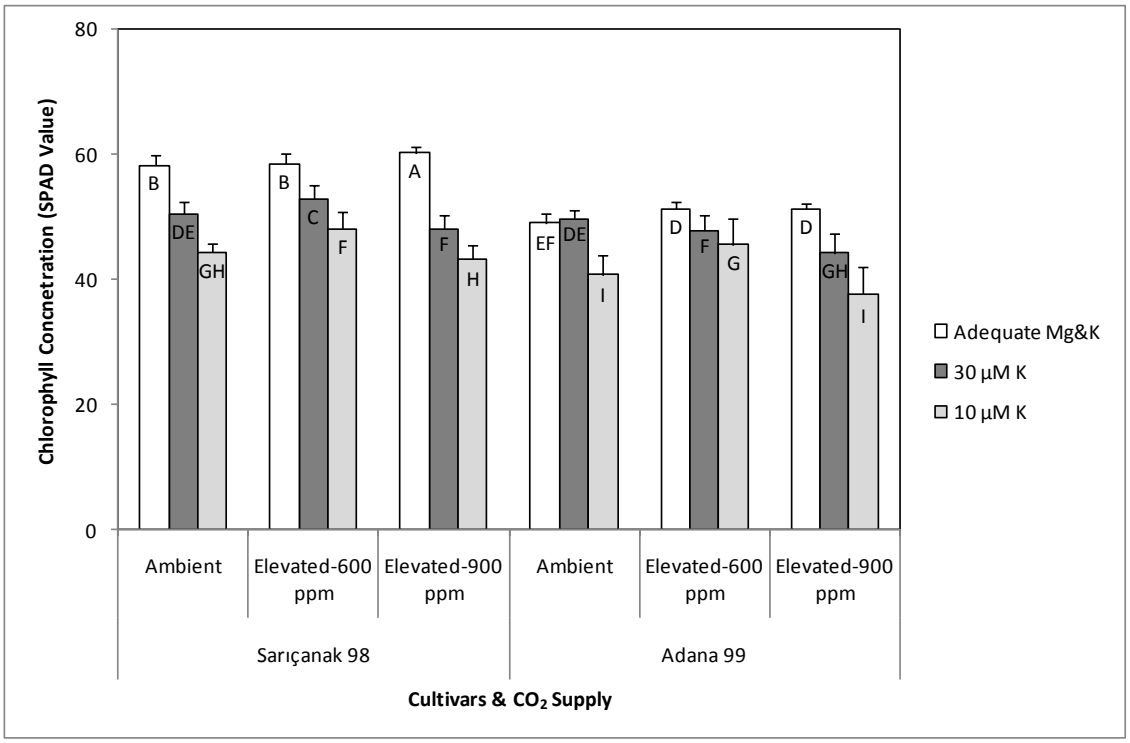

Figure 3.10: Chlorophyll concentration of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low K (10 µM) and marginal K (30 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

According to chlorophyll concentration results, CO_2 treatment, K supply and cultivar showed statistically significant differences, respectively ($p < 0.0001$, $p < 0.0001$, $p < 0.0001$). Interactions of cultivar with both CO_2 and K treatments were found statistically significant ($p = 0.0412$, $p < 0.0001$). K supplies also showed significant differences with alteration of CO_2 concentration, meaning that interaction of CO_2 with K treatment was found statistically significant. Moreover, statistically significant result was observed when CO_2, K and cultivar treatments were taken into account together (Table 3.6).

Table 3.6: p-values of chlorophyll concentration according to statistical analysis

	p-value
Treatments	**Chlorophyll Concentration**
CO_2	<.0001
K	<.0001
Cultivar	<.0001
CO_2*K	<.0001
CO_2*Cultivar	0.0412
K*Cultivar	<.0001
CO_2*K*Cultivar	0.0008

Shoot and Root K Concentrations:

Results of both shoot and root K analyses proved that low and marginal K levels were achieved in the test plants. Adequate and low K supply resulted statistically significant differences of K concentration in both shoot and root of plants (Figure 3.11 and Figure 3.12). Alteration of CO_2 concentrations caused slight differences in ambient K supplied plants. However, no or very slight differences were observed in deficient plants in both shoot and root K concentration. The highest K concentration of shoot was observed in ambient K supplied and 600 µmol mol^{-1} CO_2 treated plants in both cultivars (Figure 3.11). However, the highest K concentration of root was observed in ambient K supplied and 900 µmol mol^{-1} CO_2 treated plants of Adana 99 cultivar (Figure 3.12).

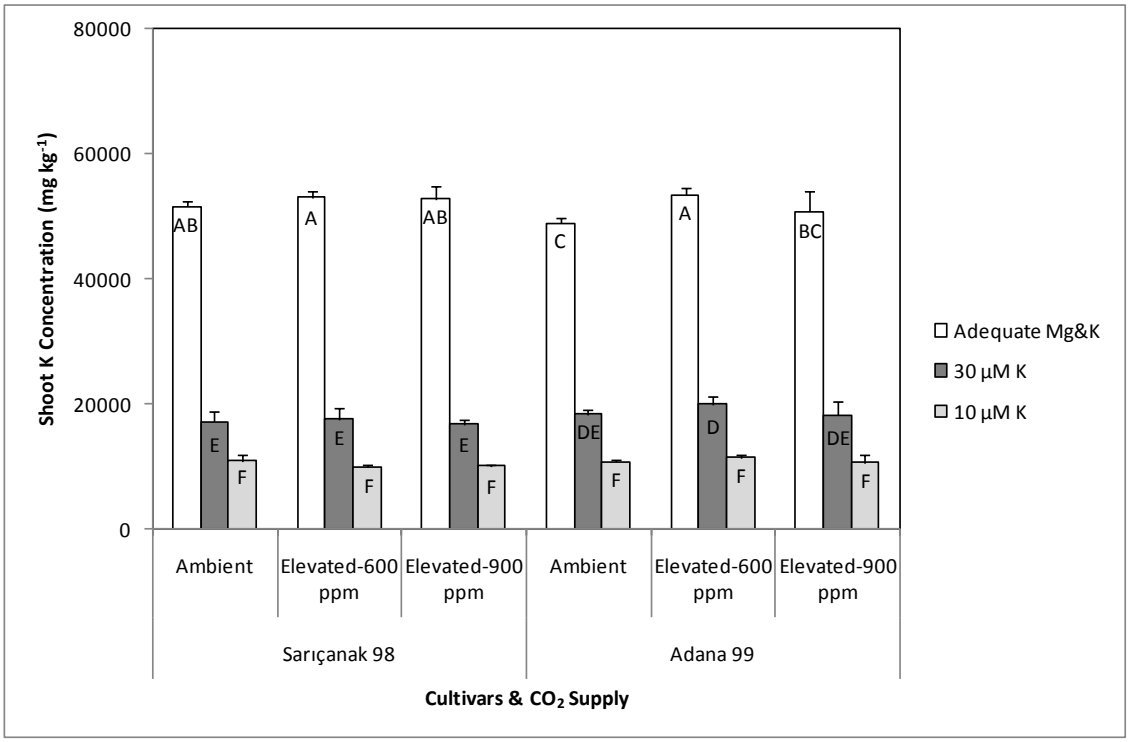

Figure 3.11: Shoot K concentration of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low K (10 µM) and marginal K (30 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

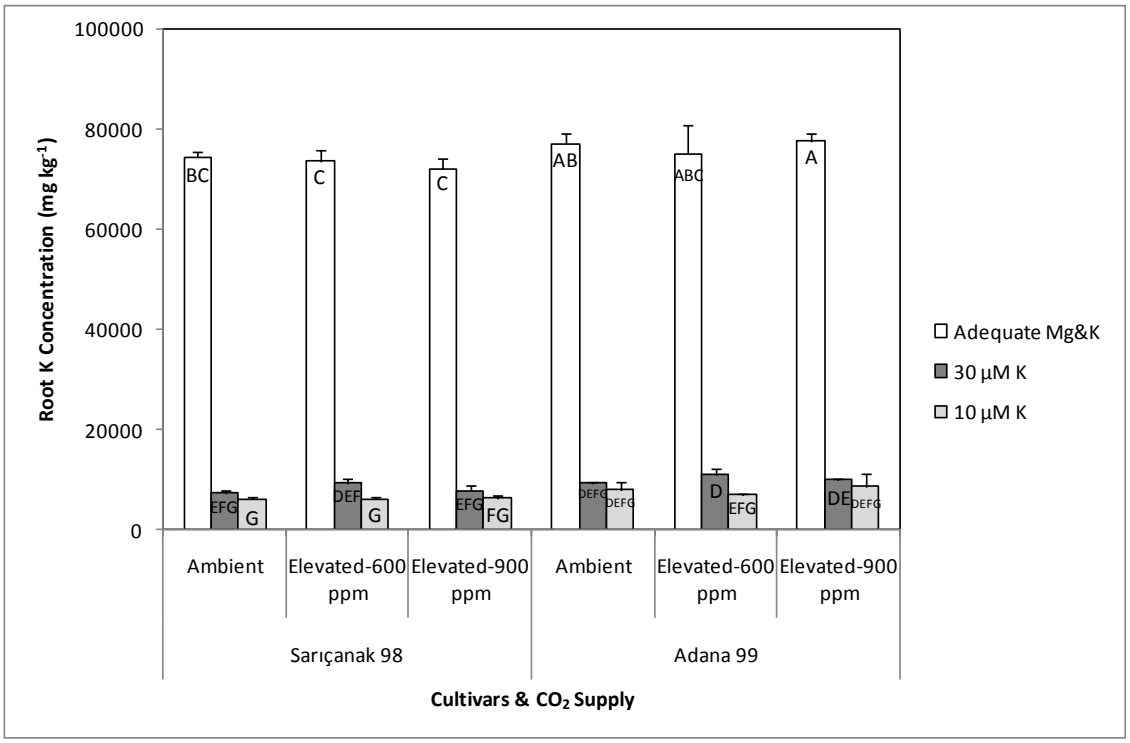

Figure 3.12: Root K concentration of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low K (10 µM) and marginal K (30 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

Different K supplies caused statistically significant differences in both shoot and root K concentration. Although cultivars did not show statistical significance with respect to shoot K concentration, significant difference was found in cultivars with respect to root K concentration. Moreover, interaction between K and cultivar treatments was found statistically significant upon shoot K concentration. However, there was no significant difference in both shoot and root K concentration when CO_2, K and cultivar treatments were taken into account together (Table 3.7).

Table 3.7: p-values of potassium concentration of both shoot and root according to statistical analysis

	p-value	
	Potassium Concentration	
Treatments	Shoot	Root
CO_2	0.0157	0.9965
K	<.0001	<.0001
Cultivar	0.4018	<.0001
CO_2*K	0.0736	0.2703
CO_2*Cultivar	0.0934	0.2495
K*Cultivar	0.0038	0.4466
CO_2*K*Cultivar	0.9154	0.8359

Root Properties:

Root length was altered with both different K and CO_2 concentrations. When effects of K concentration on root length were considered, it is clear that K deficiency caused significant decrease in root length in both cultivars. According to statistical analysis, significant increase was observed with elavated [CO_2] in all treatments. However, slight decrement was observed in low K supplied and 900 µmol mol^{-1} [CO_2] treated plants compare to 600 µmol mol^{-1} [CO_2] treated plants (Figure 3.13).

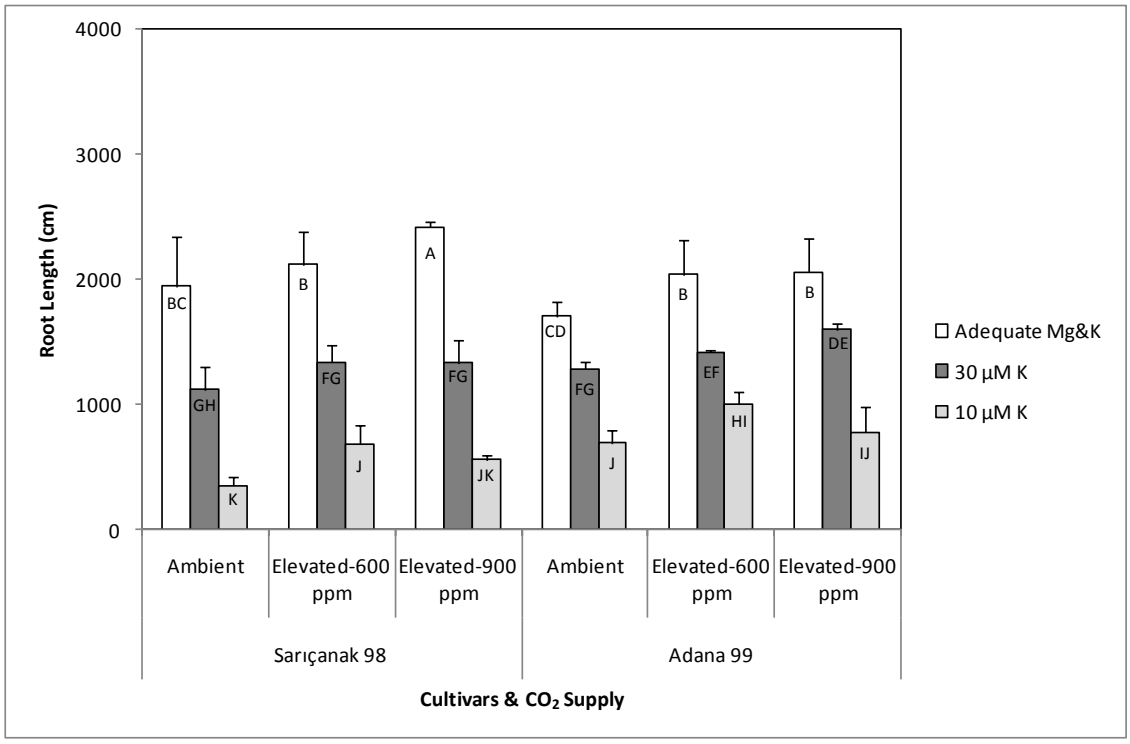

Figure 3.13: Root length of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low K (10 µM) and marginal K (30 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

Similar trend was observed in root surface area. Statistically significant decrease was found in low and marginal K supplied plants compare to adequate supplied plants. The highest area of root surface was observed in Saricanak 98 cultivar treated with adequate K supply and highest [CO_2] treatment. Enhancement of CO_2 concentration caused significant increase in all treatments. However, when low K supplied plants treated with 600 µmol mol^{-1} and 900 µmol mol^{-1} [CO_2] treatments were compared, there was a slight decrease in 900 µmol mol^{-1} [CO_2] treated plants in both cultivars (Figure 3.14).

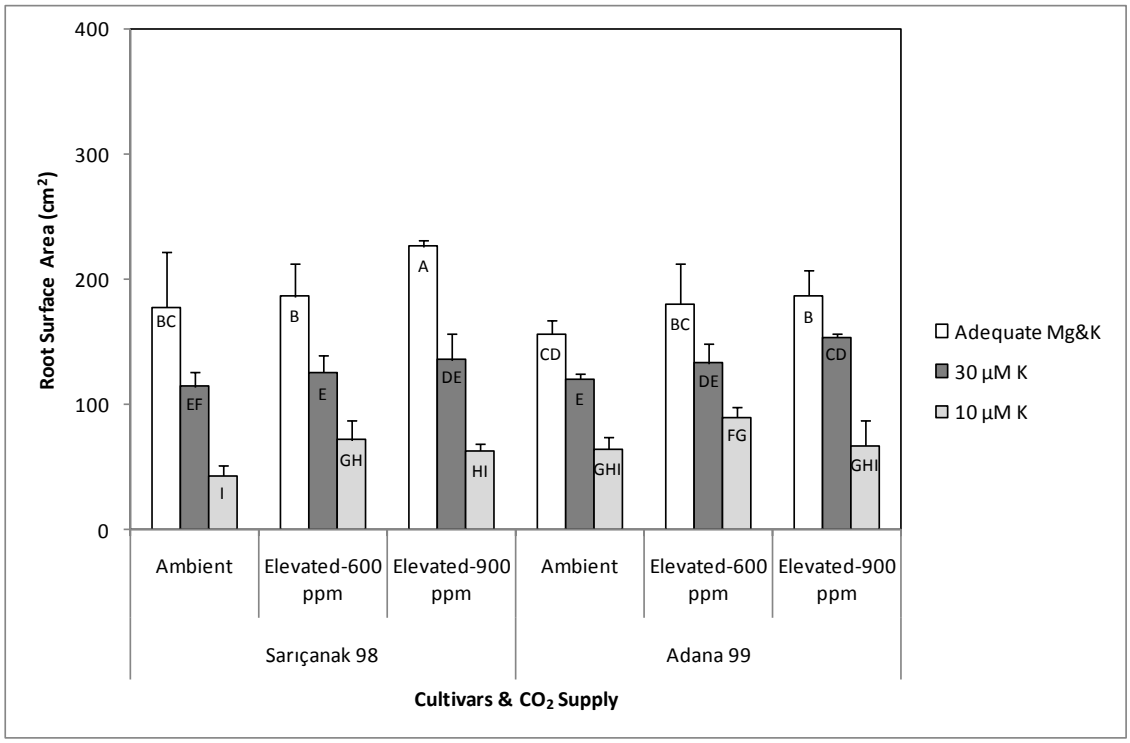

Figure 3.14: Root surface area of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low K (10 µM) and marginal K (30 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

According to root volume, K deficiency caused significant decrease in root volume in both cultivars. Whereas elavated [CO_2] caused significant increase in root volume in adequate K supplied plants, no significant differences were observed in K-deficient plants with enhancement of CO_2 concentration (Figure 3.15).

Same trend was observed in root tips with alteration of K concentration. K deficiency caused significant decrease in root tips in both cultivars. With enhancement of CO_2 concentration, slight increase was observed between ambient and 600 µmol mol^{-1} [CO_2] treatments. However, there was slight or no difference between 600 µmol mol^{-1} and 900 µmol mol^{-1} [CO_2] treatments with respect to root tips (Figure 3.16).

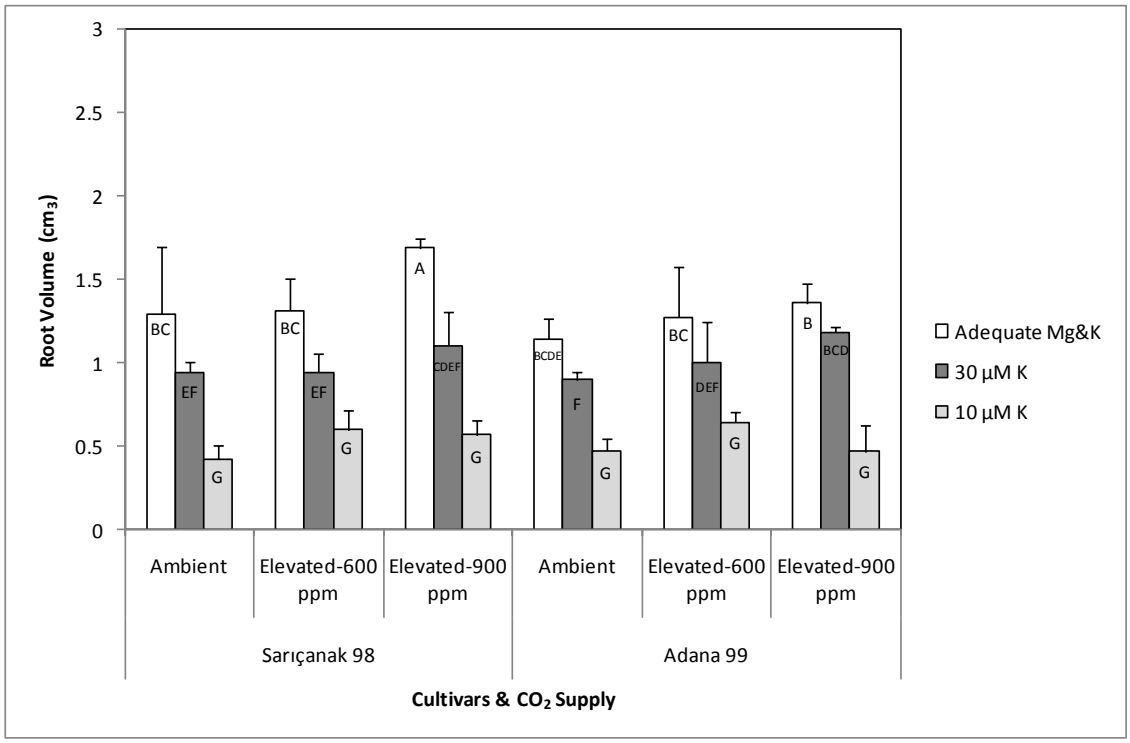

Figure 3.15: Root volume of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low K (10 µM) and marginal K (30 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

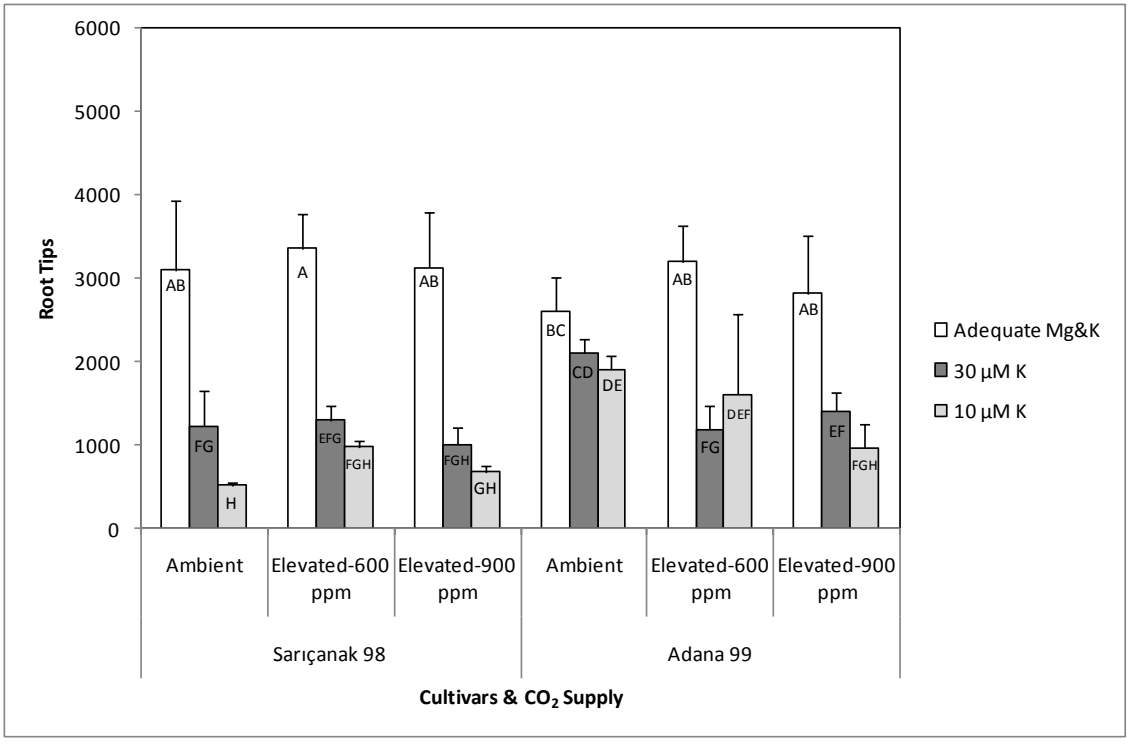

Figure 3.16: Root tips of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low K (10 µM) and marginal K (30 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

According to root length values, different K supplies caused statistically significant differences ($p < 0.0001$). CO_2 treatments also caused significant differences in root length. The interaction between K and cultivar treatments was found statistically significant ($p < 0.0001$). However, there was no significant difference when CO_2, K and cultivar treatments were taken into account together (Table 3.8).

According to statistical analysis, both CO_2 treatments and different K supplies showed significant differences with respect to root surface area ($p < 0.0001$, $p < 0.0001$). Interactions of K with both CO_2 and cultivar treatments were also found statistically significant, respectively ($p = 0.0436$, $p = 0.0021$). However, there was no significant difference when CO_2, K and cultivar treatments were taken into account together (Table 3.8).

Similar results were observed in root volume. CO_2 treatments and K supplies showed significant differences, respectively ($p = 0.0006$, $p < 0.0001$). However, there was no significant difference when CO_2, K and cultivar treatments were taken into account together (Table 3.8).

Although CO_2 treatments did not show significant difference with respect to root tips, statistically significant differences were observed in both K supplies and cultivars, respectively ($p < 0.0001$, $p = 0.0109$). K supplies responded differently to cultivars, meaning that K*cultivar interaction was found statistically significant ($p = 0.0004$). However, there was no significant difference when CO_2, K and cultivar treatments were taken into account together (Table 3.8).

Table 3.8: p-values of length, surface area, volume and tips of root according to statistical analysis

	p-value			
Treatments	**Root Length**	**Root Surface Area**	**Root Volume**	**Root Tips**
CO_2	<.0001	<.0001	0.0006	0.0796
K	<.0001	<.0001	<.0001	<.0001
Cultivar	0.0668	0.8384	0.2256	0.0109
CO_2*K	0.0765	0.0436	0.0624	0.0830
CO_2*Cultivar	0.7872	0.5465	0.3716	0.1251
K*Cultivar	<.0001	0.0021	0.0858	0.0004
CO_2*K*Cultivar	0.4785	0.5176	0.6156	0.1439

Total Antioxidant Capacity:

Significant differences were observed in different K supplies. K deficiency caused increase in total antioxidant capacity. Although significant increase was observed between ambient and 600 μmol mol^{-1} [CO_2] treatment, no continuous increase was observed in 900 μmol mol^{-1} [CO_2] treatment (Figure 3.17).

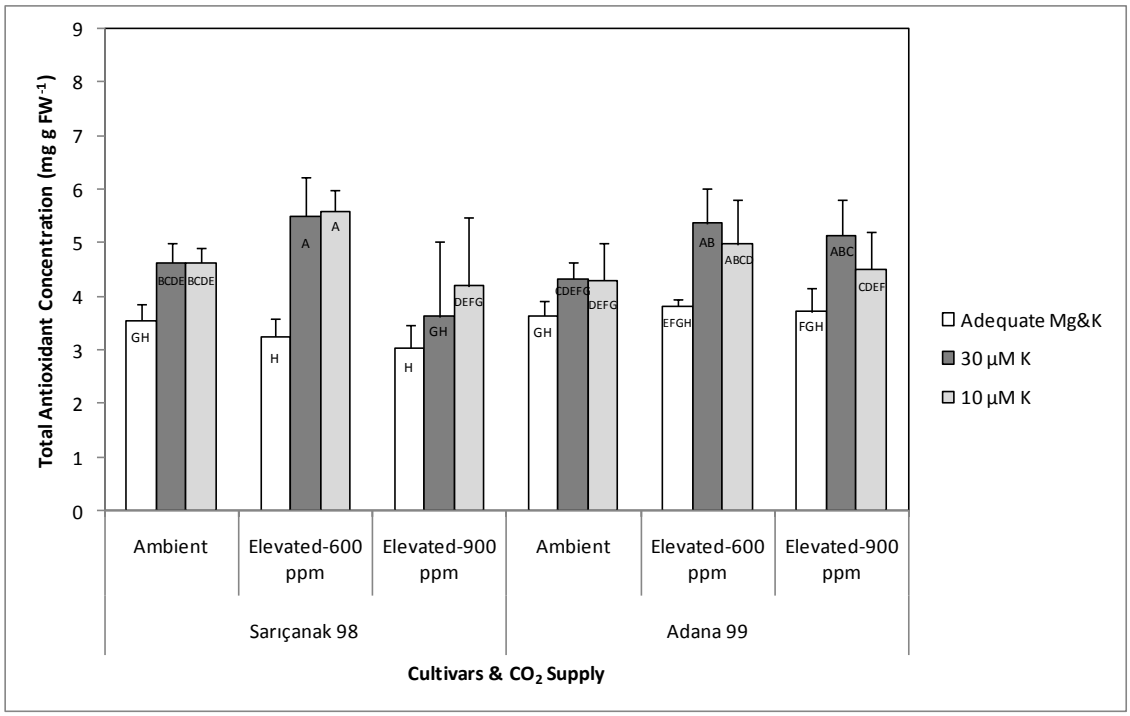

Figure 3.17: Total antioxidant capacity of plants grown with adequate Mg and K (1000 μM Mg and 750 μM K), low K (10 μM) and marginal K (30 μM) supply under three different CO_2 environments (ambient: 400 μmol mol^{-1}, elevated: 600 and 900 μmol mol^{-1})

According to statistical analysis, both CO_2 treatments and K supplies caused significant differences with respect to total antioxidant capacity (p = 0.0003, p < 0.0001). Interaction of CO_2 with cultivar was also found statistically significant (p = 0.0084). However, there was no significant difference when CO_2, K and cultivar treatments were taken into account together (Table 3.9).

Table 3.9: p-values of total antioxidant capacity according to statistical analysis

	p-value
Treatments	**Total Antioxidant Capacity**
CO_2	0.0003
K	<.0001
Cultivar	0.1550
CO_2*K	0.0975
CO_2*Cultivar	0.0084
K*Cultivar	0.1247
CO_2*K*Cultivar	0.3366

Lipid Peroxidation:

Different K supplies altered the level of lipid peroxidation and there was significant difference between adequate and low K supplied plants. Significant increment was observed in K-deficient plants. However, there was no significant difference with enhancement of CO_2 concentration (Figure 3.18).

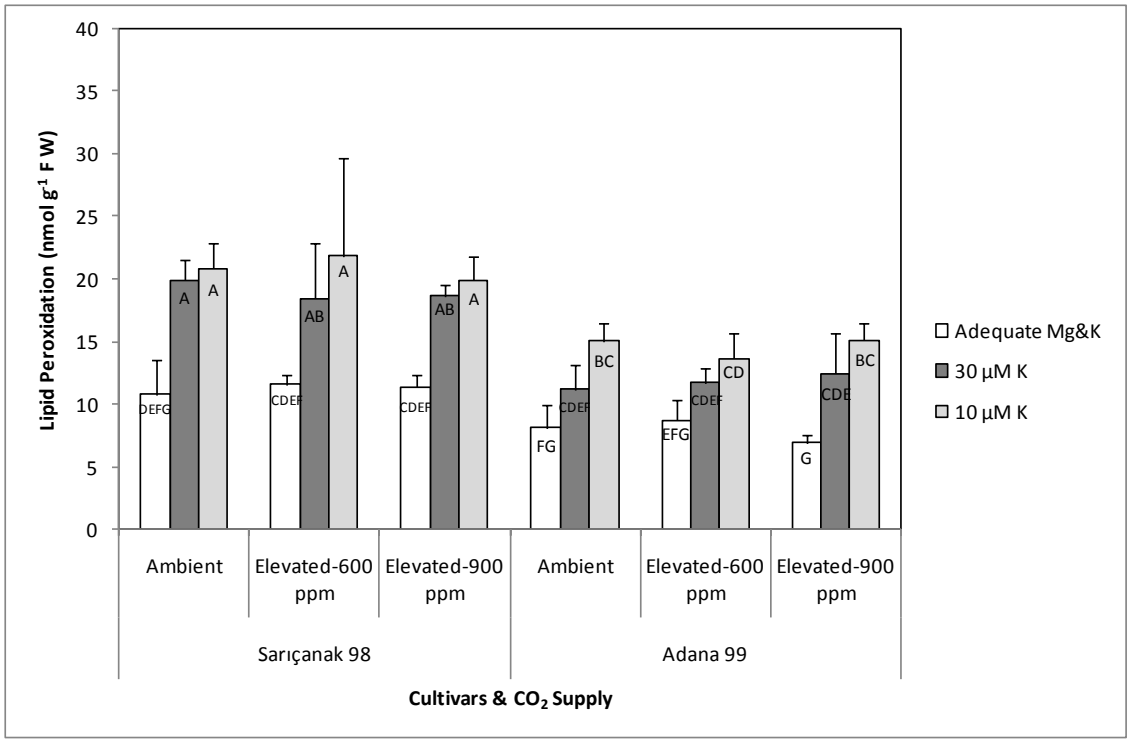

Figure 3.18: Lipid peroxidation of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low K (10 µM) and marginal K (30 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

According to statistical analysis, significant differences were observed in both K and cultivar treatments, respectively ($p < 0.0001$, $p < 0.0001$). Cultivars also responded differently to different K supplies, meaning that the interaction of K with cultivar was found statistically significant ($p = 0.0483$) (Table 3.10).

Table 3.10: p-values of lipid peroxidation according to statistical analysis

	p-value
Treatments	**Lipid Peroxidation**
CO_2	0.9366
K	<.0001
Cultivar	<.0001
CO_2*K	0.9597
CO_2*Cultivar	0.8848
K*Cultivar	0.0483
CO_2*K*Cultivar	0.6178

Pholem Carbohydrate Concentration:

According to phloem carbohydrate concentration, both different K and CO_2 concentrations altered phloem carbohydrate concentration. Whereas slight increase was observed in Saricanak 98 plants grown with low and marginal K supply, significant increment was observed in K-deficient plants of Adana 99 cultivar. However, slight or no differences were observed with enhancement of CO_2 concentrations. The highest level of phloem carbohydrate concentration was observed in the lowest K supplied plants of Adana 99 cultivar grown under 900 μmol mol^{-1} [CO_2] (Figure 3.19).

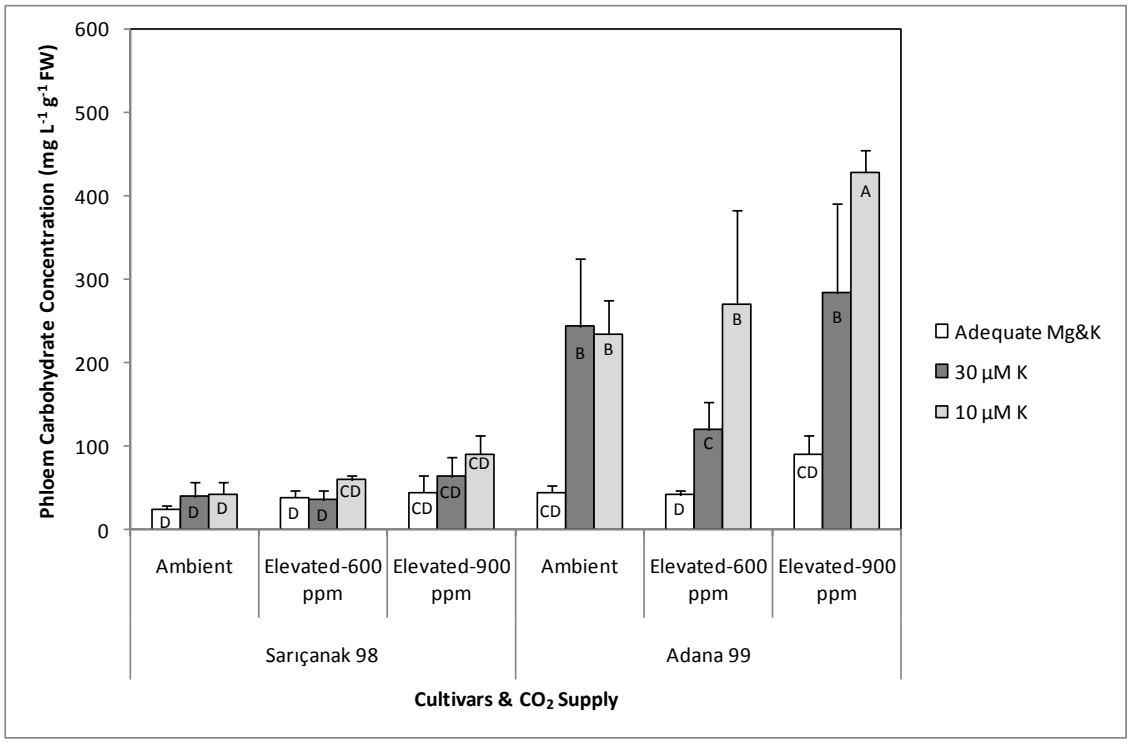

Figure 3.19: Phloem carbohydrate concentration of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low K (10 µM) and marginal K (30 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

According to statistical analysis, significant differences were observed in CO_2 treatments, K supplies and cultivars, respectively ($p < 0.0001$, $p < 0.0001$, $p < 0.0001$). When cross analysis were considered, CO_2 treatment responded differently to both K supply and cultivar, meaning that interactions of CO_2 with K and cultivar treatments were found statistically significant ($p = 0.0294$, $p = 0.0071$). The interaction of K with cultivar was also found statistically significant ($p < 0.0001$) (Table 3.11).

Table 3.11: p-values of phloem carbohydrate concentration according to statistical analysis

	p-value
Treatments	**Phloem Carbohydrate Concentration**
CO_2	<.0001
K	<.0001
Cultivar	<.0001
CO_2*K	0.0294
CO_2*Cultivar	0.0071
K*Cultivar	<.0001
CO_2*K*Cultivar	0.2047

Membrane Stability Index:

Membrane stability was altered by K supplies and significant decrement was observed in K-deficient plants, especially in Saricanak 98 cultivar. While elevated [CO_2] had almost no effect on plants grown under ambient CO_2 condition, significant increase in membrane stability index was observed in deficient plants with enhancement of CO_2 concentration (Figure 3.20).

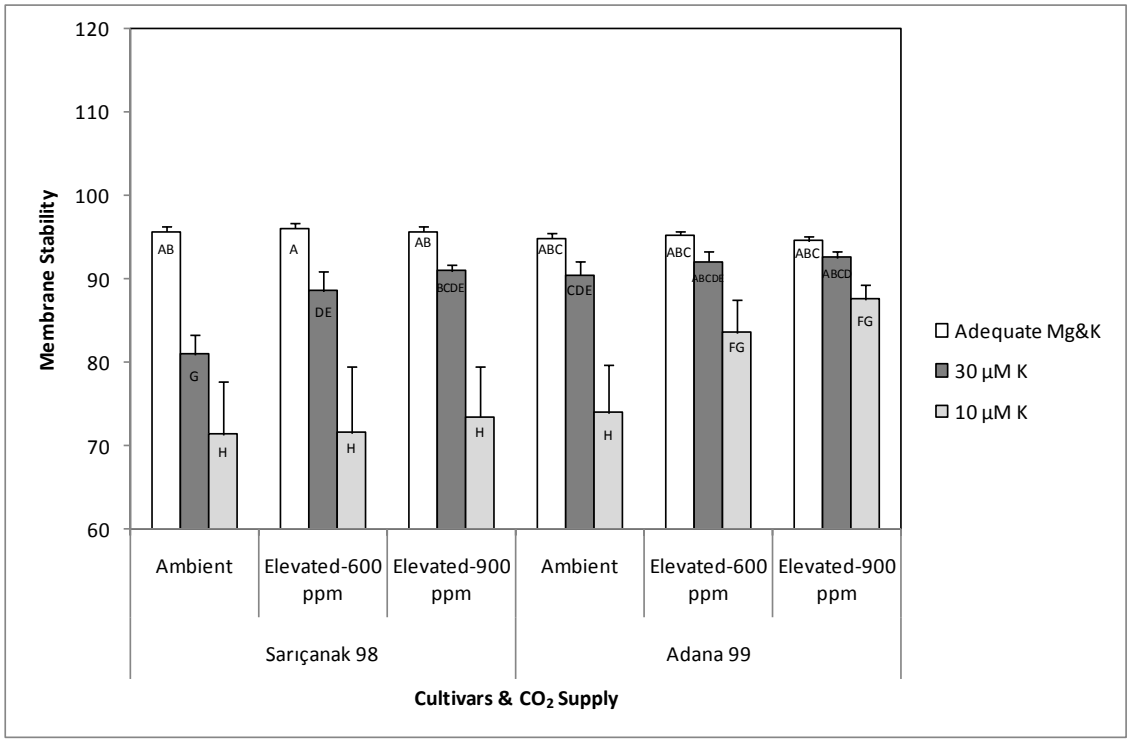

Figure 3.20: Membrane stability index of plants grown with adequate Mg and K (1000 µM Mg and 750 µM K), low K (10 µM) and marginal K (30 µM) supply under three different CO_2 environments (ambient: 400 µmol mol^{-1}, elevated: 600 and 900 µmol mol^{-1})

According to statistical analysis, there were significant differences in all treatments, and p-value was found smaller than 0.0001 in CO_2 treatment, K supply and cultivar. According to cross analysis, different K supplies responded differently to CO_2 treatments, meaning that CO_2*K interaction was found statistically significant (p = 0.0242). The interaction of CO_2 with cultivar was also found statistically significant (p < 0.0001). Moreover, there was also significant difference when CO_2, K and cultivar treatments were taken into account together (p = 0.0036) (Table 3.12).

Table 3.12: p-values of membrane stability index according to statistical analysis

	p-value
Treatments	**Membrane Stability**
CO_2	<.0001
K	<.0001
Cultivar	<.0001
CO_2*K	0.0242
CO_2*Cultivar	0.8139
K*Cultivar	<.0001
CO_2*K*Cultivar	0.0036

(D) DISCUSSION & CONCLUSIONS

D.1. Discussion

The main leaf symptom of Mg deficiency is known as interveinal chlorosis and it is observed in older leaves firstly since Mg is a highly mobile nutrient (Marschner and Cakmak 1989, Hermans et al. 2005, Tewari et al. 2006, Marschner 2012). Wheat plants particularly supplied with low Mg (i.e. 75 μM) supply in this study also exhibited severe interveinal chlorosis in their older leaves while younger leaves remained green (Figure 1.1, 1.2).

Magnesium has a vital role in growth and development of plants. Consequently significant decreases were observed in both shoot and root dry weight because of Mg deficiency (Figure 2.1, 2.2, 2.4). In studies conducted with bean, silver birch and wheat plants an increment of shoot:root ratio was observed with Mg deficiency (Cakmak et al. 1994b, McDonald et al. 1996, Cakmak and Kirkby 2008, Cakmak and Yazici 2010, Cakmak 2013). However, there was only a slight increase or no change in shoot:root ratio of wheat cultivars used in this experiment. This could be caused by the same proportion of decrease in both shoot and root of plants. However, a significant increase in shoot:root ratio was observed with respect to elevated CO_2 concentration, especially under low Mg supply. When the effects of CO_2 concentration on biomass are considered, several studies indicated that increase of plant biomass was observed with rising [CO_2] (Rogers et al. 1994, Kimball et al. 2002, Obrist and Arnone 2003, Kimball 2011). In the present study, significant biomass increase was also observed in both shoot and root of wheat plants grown under elevated CO_2 conditions. However, induction of Mg deficiency severely reduced the biomass gain brought about by the elevated CO_2 environment, due to its negative effects on photosynthesis and carbohydrate transport as well as production of reactive O_2 species under Mg deficiency.

Specific leaf weight of wheat cultivars was increased with low Mg availability which is consistent with previous reports in pea (Verbruggen and Hermans 2013), and bean (Cakmak 1994a). Among the cultivars, Saricanak 98 had particularly accumulated more specific weight, and elevation of CO_2 concentration induced build up of leaf specific weight. Obviously, carbohydrate accumulation interferes with plant productivity and

both Mg deficiency and elevated CO_2 exacerbate utilization of carbohydrates by the sink tissues.

As expected, photosynthesis rate was significantly reduced in plants supplied with low or even marginal Mg nutrition. This could be a consequence of altered chlorophyll biosynthesis and photosynthetic enzyme activity under the given conditions (Fischer and Bremer 1993, Sun and Payn 1999, Laing et al. 2000, Hermans et al. 2004, Cakmak and Kirkby 2008). One of the roles of magnesium is to harvest solar enegry by occupying central position in the chlorophyll structure as a cofactor and promoter for many enzymes (Cowan 2002, Shaul 2002). Therefore, magnesium is a crucial element for chlorophyll synthesis, photochemical reactions, carbon fixation. Because of alteration of Chl a/b ratio by Mg deficiency, photosystem stoichiometry is changed. The increase of photosynthesis rate and decrase of stomatal conductance is a well established physiological response in plants subjected to elevating [CO_2] (Devakumar et al. 1998, Bernacchi et al. 2006, Ainsworth and Rogers 2007, Reddy et al. 2010). The reason of increment of photosynthesis rate could be related with the increase of substrate availability with rising [CO_2] for C_3 plants which are influenced by RuBP carboxylase-oxygenase (Rubisco), and enhancement of CO_2 concentration provided the increase of activity of Rubisco and thus a more efficient carboxylation process. The findings of present study supported the literature in accordance with photosynthetic parameters, and significant reduction was observed in both stomatal conductance and transpiration rate with rising [CO_2] (Teng et al. 2006).

In literature, several studies showed an increase in soluble sugar and starch concentration with elevating CO_2 concentration (Long and Drake 1992, Moore et al. 1997, Rogers et al. 2004, Teng et al. 2006). In the present study, statistically significant increase of carbohydrate concentration was observed, especially in Saricanak 98 cultivar. However, Adana 99 cultivar was less affected compared to Saricanak 98 cultivar in terms of carbohydrate accumulation in source leaves. The reason of carbohydrate accumulation in source leaves could be the structural damage of phloem tissues, and hence impairment of phloem loading (Fischer and Bremer 1993, Cakmak et al. 1994b, Hermans et al. 2004, Hermans et al. 2005, Hermans and Verbruggen 2008). However, results derived from wheat species used in this study have revealed that carbohydrates can also accumulate in the phloem stream (Figure 2.19 and Table 2.12), suggesting that Mg deficiency-incuced accumulation of carbohydrates in source tissue

can be a consequence of impaired unloading of carbohydrates at the sink sites rather than the loading at the source sites. This study also showed the existence of a substantial difference in Mg-deficiency induced carbohydrate accumulation among wheat species. It is proposed that fast growing cultivars such as Saricanak 98 in this study can suffer more from the impaired phloem carbohydrate unloading probably due to lack of immediate Mg reserves at the unloading sites.

As expected, Mg deficiency caused significant reductions in chlorophyll concentration (Figure 2.10). Mg element is the central atom of the chlorophyll molecule, thus chlorophyll concentration is directly linked to Mg availability in the shoot tissue. The changes in chlorophyll concentration detected in this study was in agreement with the study of McGrath and Lobell (2013) who have shown a decrease in chlorophyll due to reduced Mg and elevated CO_2 levels.

Root properties were directly affected by Mg supply and low Mg resulted in significant decreases in root length, surface area and volume which could be attributed to inhibition of root cell proliferation and thus growth in general. On the contrary, elevation of CO_2 concentration increased root length, number of root tips, surface area and volume per plant. Increase in root length upon elevating [CO_2] has been previously shown in many studies (Norby 1994, Rogers et al. 1999, Pritchard et al. 2000, Bernacchi et al. 2000, Reedy et al. 2010, Mahdu and Hatfield 2013). Increment of root properties could be related with increase in the C allocation towards the roots by elevated [CO_2]. These results are also in accordance with Mahdu and Hatfield (2013) who concluded that roots became more numerous, longer and thicker under elevated CO_2 conditions.

Significant decreases were observed with elevating [CO_2] in both shoot and particularly root Mg concentrations. Teng et al. (2006) showed similar effects of elevated CO_2 on Mg in *Arabidopsis thaliana*. Reduced Mg concentrations can result from accumulation of soluble sugars, and/or dilution in tissue. It may also be related with the alteration of stomatal conductance and transpiration rate by elevating [CO_2]. Decrease in both stomatal conductance and transpiration rate probably interfere with uptake of Mg which is mostly taken up by mass flow from the rhizosphere.

Both low Mg supply and elevated CO_2 concentration induced total antioxidant capacity in leaves. The increment of total antioxidant capacity could be related with the increase of ROS generation in plants grown with low Mg supply. It is well-known that plants

supplied with low Mg enhances generation of ROS that causes direct damage to membrane lipids, proteins and DNA, and consequently cell death. At adequate Mg supply, ROS formation and detoxification are balanced by the plant antioxidative defence system (Hameed et al. 2011). However, in Mg deficient plants, it has been reported that synthesis of antioxidant metabolites and activity of antioxidant enzymes are upregulated (Tewari et al. 2006, Cakmak and Kirkby 2008, Yang et al. 2012, Verbruggen and Hermans 2013).

Measurement of malondialdehyde (MDA) concentration in stressed tissues is a widely used method to analyse the extent of lipid peroxidation, a stress indicator in cellular membranes (Taulavuori et al. 2001). In the present study, Mg-deficient plants showed a significant increase in lipid peroxidation. Mg deficiency induces the generation of ROS that causes directly to membrane damage, thereby it is expected to see increase in lipid peroxidation under stress conditions. In concert with the increased lipid peroxidation, Mg deficiency also caused slight decrease in membrane stability, which can be attributed to Mg deficiency-induced ROS generation (Cakmak 1994, Sun and Payn 1999, Marschner 2012, Waraich et al. 2012).

The main leaf symptom of K deficiency is known as brown scorching and chlorosis between leaf veins. The experiment results supported the literature with observation of chlorosis and reddish brown necrosis in leaf tips (Figure 1.1, 1.2). Potassium deficiency was observed firstly in older leaves because of the mobility property of K (Hopkins and Huner 2009).

Potassium is one of the essential mineral nutrients for growth and development of plants because of its major effects on cell metabolism. In particular, K is important for survival of crop plants under environmental stress conditions (Waraich et al. 2012). In agreement with literature, statistical analysis showed that significant decreases were observed in both shoot and root dry weight of K deficient plants (Figure 3.1, 3.2, 3.4). According to Cakmak and Kirkby (2008), enhancement of shoot:root ratio is a major growth response in K deficient plants in relation to accumulation of sugars in source leaves. However, shoot:root ratio in the present study showed no significant change upon low K treatments. However, elevating [CO_2] caused significant increases in shoot:root ratio, especially under low K supply. Several studies indicated that increase of plant biomass had been observed with rising [CO_2] (Rogers et al. 1994, Kimball et al.

2002, Obrist and Arnone 2003, Kimball 2011). In the present study, significant increase was observed in both shoot and root biomass of plants grown under optimum conditions. However, a similar trend was not observed when plants were grown with low K.

Potassium had also impact on photosynthetic parameters. Significant decrease was observed in photosynthesis rate with K deficiency. This could be related with the influence of K on CO_2 exchange rates (CERs) and also stomatal closure (Huber 1984). Potassium deficiency caused the decrease of CERs, and hence accumulation of carbohydrates. Therefore, the photosynthesis rate decreased as a result of K deficiency (Hermans et al. 2006). There were also several studies that supported the findings of the present study about photosynthesis rate in K-deficient plants (Bednarz et al. 1998, Zhao et al. 2001, Kanai et al. 2007). Other studies showed increase of photosynthesis rate and decrase of stomatal conductance by elevating [CO_2] (Devakumar et al. 1998, Bernacchi et al. 2006, Ainsworth and Rogers 2007, Reddy et al. 2010). In accordance with literature, significant increase in photosynthesis rate was observed with elevated [CO_2] irrespective of the K supply. The reason of increment of photosynthesis rate can be related with the increase of substrate availability with rising [CO_2] for C_3 plants which are influenced by RuBP carboxylase-oxygenase (Rubisco), and enhancement of CO_2 concentration provided the increase of activity of Rubisco which means increase of carboxylation process. There were also significant decreases in both stomatal conductance and traspiration rate because of K deficiency, as in Mg deficiency which is also supported by other studies (Teng et al. 2006).

In mature leaf tissue, carbohydrate concentration was significantly increased upon low supply of K. The reason of low-K induced impairment of soluble sugar export from mature leaves is not fully understood. Many researchers suggest involvement of a structural damage of phloem tissues due to K deficiency, and hence impairment of phloem loading (Fischer and Bremer 1993, Cakmak et al. 1994b, Hermans et al. 2004, Hermans et al. 2005, Hermans and Verbruggen 2008). Significant increase in carbohydrates was also observed with enhancement of CO_2 concentration. This finding is also further supported by literature showing increment of soluble sugar and starch content with elevating CO_2 concentration in various plant species (Long and Drake 1992, Moore et al. 1997, Rogers et al. 2004, Teng et al. 2006).

Significant reduction in chlorophyll concentration was another observation in plants grown with low K supply (Figure 3.10). Most probably K deficiency caused excess production of ROS which in turn cause membrane damage and chlorophyll degradation in K-deficient plants (Waraich et al. 2011, Hafsi et al. 2014).

Similar to Mg, K deficiency also had significant effects on root properties. Root length was significantly decreased with low K supply. Reduction of root length could be caused by restriction of sucrose transportation into roots (Hafsi et al. 2014) in plants with low K supply. On the other hand, elevated CO_2 concentration significantly increased root length irrespective of K supply. Increment of root length with elevating [CO_2] has been shown by many studies (Norby 1994, Rogers et al. 1999, Pritchard et al. 2000, Bernacchi et al. 2000, Reedy et al. 2010, Mahdu and Hatfield 2013). When root volume and surface area are considered, elevated CO_2 also had an additive effect. In agreement with the literature, significant increase was observed in both surface area and volume of roots with rising [CO_2], especially in plants grown with adequate K supply. Increase of root surface area and volume could be related with increase of C allocation to root growth by elevating [CO_2]. However, K deficiency caused significant decrease in both surface area and volume of the roots. The present study also showed a significant reduction in number of root tips by K deficiency.

Potassium also significantly affected total antioxidant capacity. Unfavorable environmental conditions such as nutrient deficiency, drought or pathogenic infections are known to enhance ROS production. Antioxidant defense systems take part in the protection of plants against to these toxic oxygen intermediates. There are several studies that showed the increase of antioxidant capacity against several environmental stresses (i.e. salinity, drought) (Sudhakar et al. 2001, Cakmak 2005, Gill and Tuteja 2010, Pi et al. 2014). In the present study, K deficiency induced total antioxidant capacity and this result was consistent at all [CO_2] levels studied.

Under environmental stresses, production of ROS is induced causing oxidative damage to nucleic acids, lipids and proteins. Membrane damage is generally used as a parameter to determine the level of lipid destruction (Gill and Tuteja 2010, Simova-Stoilova et al. 2010, Pi et al. 2014). In the present study, K deficient plants showed more lipid peroxidation levels comparing to adequate K plants suggesting an enhanced.

Phloem transport is a major route for nutrient and photoassimilate transport, and can be altered by nutritional status of plants. As similar in low Mg plants, low K plants also exhibited an increased phloem carbohydrate concentration particularly in Adana 99 cultivar.

Potassium has a crucial role in cell membrane stability and maintenance of membrane integrity. There are several studies that showed the role of K on cell membrane properties and alteration of cell membranes under environmental stresses (i.e. drought, water stress) (Premachandra et al. 1991, Wang et al. 2013). In the present study membrane stability was significantly reduced in plants with low K supply suggesting that cellular membranes were damaged most probably due to ROS attack induced by K deficiency stress. In summary results presented here indicate the importance of an adequate nutrition with Mg and K to benefit from the positive effects of an elevated CO_2 environment.

D.2. Conclusions

Atmospheric carbon dioxide concentration ($[CO_2]$) has been continuously increasing due to fossil fuel consumption. The $[CO_2]$ was increased from 280 μmol mol^{-1} in 1800's up to 395 μmol mol^{-1} as of today and today's atmospheric CO_2 concentration is the highest recorded level and continues to rapidly increase (Prentice et al. 2001). Especially after industrial revolution, CO_2 concentration entered a rapid growth period. According to modeling data, it is indicated that atmospheric CO_2 concentration will be at a level of 530 and 970 μmol mol^{-1} at the end of 21^{st} century (IPCC 2007, 2013). The understanding of how elevating CO_2 concentration affect the plant kingdom is important to overcome the detrimental effects of climate change. The increase of atmospheric CO_2 concentration provides increase in photosynthesis rate of especially C_3 plants, and consequently it could affect the capacity of growth and yield in a positive way (Ainsworth and Rogers 2007, Taiz ve Zeiger 2010). However, long-term studies pointed out that other essential nutrients should be in ample quantities in order to get higher yields and quality from crop plants cultivated under elevated $[CO_2]$. This study supported this idea, and proved that plants grown with adequate Mg and K can maximize benefits from elevated CO_2 environments, whereas supplied with low Mg and K could not perform better in elevated CO_2 environments compared to control plants

with adequate Mg and K nutritional status. Both shoot and root growths are directly affected by low Mg and K supply. While enhancement of CO_2 concentration positively affected shoot and root biomass, root length and volume of adequate Mg and K plants, no positive effect was detected in Mg and K-deficient plants with respect to enhancement of CO_2. As expected, photosynthesis rate was significantly enhanced by elevated CO_2 treatments. However, induction of photosynthesis was hampered by low Mg and K treatments particularly in the durum wheat cultivar. Elevation of [CO_2] induced accumulation of carbohydrates in source leaves particularly in the case of low-Mg and low-K plants. Total antioxidant capacity, lipid peroxidation and membrane stability were altered by low Mg and K supply irrespective of the [CO_2] treatments. It is concluded that an adequate nutrition with Mg and K is crucial for an effective phloem transport of photo-assimilates particularly under elevated CO_2 conditions to provide a balance dry matter distribution and thus higher yields. In several studies, elevating CO_2 concentration positively affects plants in accordance with photosynthesis rate, and provides increase of carbon assimilation, growth and yield. The present study aggrees with the previous findings in the literature in regard to these plant traits however it is clearly shown that nutritional status of plants with Mg and K is of crucial importance in taking advantage of an atmosphere with elevating [CO_2] levels.

(E) REFERENCES

Ainsworth EA, Long SP (2005). What have we learned from 15 years of free-air CO2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytol. 165(2):351-71. PubMed PMID: 15720649

Ainsworth EA and Rogers A (2007). The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. Plant, Cell and Environment. 30, 258–270

Amberger A (1975). Protein biosynthesis and effect of plant nutrients on the process of protein formation. Proceedings of the 11th Colloquium of the International Potash Institute held in Ronne -Bornholm, Denmark

Amtmann A and Rubio F (2012). Potassium in Plants. Wiley Online Library. doi: 10.1002/9780470015902.a0023737

Bednarz CW, Oosterhuis DM, Evans RD (1998) Leaf photosynthesis and carbon isotope discrimination of cotton in response to potassium deficiency. Environ Exp Bot 39:131-139

Benlloch-Gonzalez M, Arquero O, Fournier JM, Barranco D, Benlloch M (2008). K^+ starvation inhibits water-stress-induced stomatal closure. J. Plant Physiol. 165: 623-630

Bernacchi CJ., Coleman JS, Bazzaz FA, McConnaughay KDM. (2000). Biomass allocation in old-field annual species grown in elevated CO2 environments: No evidence for optimal partitioning. Glob. Change Biol. 6:855-863. doi:10.1046/j.1365-2486.2000.00370.x

Bernacchi CJ, Leakey ADB, Heady LE, Morgan PB, Dohleman FG, McGrath JM, Gillespie KM, Wittig VE, Rogers A, Long SP, Ort DR (2006). Hourly and seasonal variation in photosynthesis and stomatal conductance of soybean grown at future CO_2 and ozone concentrations for 3 years under fully open-air field conditions. Plant, Cell and Environment. 29, 2077–2090. doi: 10.1111/j.1365-3040.2006.01581.x

Bernacchi CJ, Kimball BA, Quarles DR, Long SP, Ort DR (2007). Decreases in stomatal conductance of soybean under open-air elevation of [CO_2] are closely coupled with decreases in ecosystem evapotranspiration. Plant Physiology. 143: 134-144

Bruinsma J (2009). The resource outlook to 2050. By how much do land, water use and crop yields need to increase by 2050?FAO Expert Meeting on How to Feed the World in 2050. 24-26

Bush DR (1989) Proton-coupled sucrose transport in plasmalemma vesicles isolated from sugar beet (*Beta vulgaris* L. cv. Great Western) leaves. Plant Physiol 89:1318-1323. doi: 10.1104/pp.89.4.1318

Cakmak I (1994) Activity of ascorbate-dependent H_2O_2-scavenging enzymes and leaf chlorosis are enhanced in magnesium and potassium-deficient leaves, but not in phosphorus-deficient leaves. J Exp Bot 45: 1259-1266. doi: 10.1093/jxb/45.9.1259

Cakmak I, Hengeler C, Marschner H (1994a) Partitioning of shoot and root dry matter and carbohydrates in bean plants suffering from phosphorus, potassium and magnesium deficiency. J Exp Bot 45:1245-1250. doi: 10.1093/jxb/45.9.1245

Cakmak I, Hengeler C, Marschner H (1994b) Changes in phloem export of sucrose in leaves in response to phosphorus, potassium and magnesium deficiency in bean plants. J Exp Bot 45:1251-1257. doi: 10.1093/jxb/45.9.1251

Cakmak I (2005). The role of potassium in alleviating detrimental effects of abiotic stresses in plants. J. Plant Nutr. Soil Sci. 168: 521-530. doi: 10.1002/jpln.200420485 521

Cakmak I (2005). Role of mineral nutrients in tolerance of crop plants to environmental stress factors. In: Fertigation: Optimizing the utilization of water and nutrients. Fertigation Proceedings: Selected papers of the IPI-NATESC-CAU-CAAS. International Symposium on Fertigation, Beijing, China, 20-24 September 2005: 35-48

Cakmak I and Kirkby EA (2008) Role of magnesium in carbon partitioning and alleviating photooxidative damage. Physiol Plantarum 133:692-704. doi: 10.1111/j.1399-3054.2007.01042.x

Cakmak I and Yazici A (2010) Magnesium: A forgotten element in crop production. Better Crops 94:23-25

Cakmak I (2013). Magnesium in crop production, food quality and human health. Plant Soil. 368:1–4. doi: 10.1007/s11104-013-1781-2

Chen SS, Black Jr CC (1983). Diurnal changes in volume and specific tissue weight of Crassulacean acid metabolism plants. Plant Physiol. 71: 373-378

Cowan JA (2002). Structural and catalytic chemistry of magnesium dependent enzymes. Biometals. 15: 225-235

Devakumar AS, Shesha Shayee MS, Udayakumar M, Prasad TG (1998) Effect of elevated CO_2 concentration on seedling growth rate and photosynthesis in *Hevea brasiliensis*. J.Biosci. 23(1):33-36

Drake BG, Gonzalez-Meler MA, Long SP (1997). More efficient plants: a consequence of elevated carbon dioxide?. Annu Rev Plant Physiol Plant Mol Biol 48:607–640

Ellsworth DS, Thomas R, Crous KY, Palmroth S, Ward E, Maier C, DeLucia E (2012). Elevated CO2 affects photosynthetic responses in canopy pine and subcanopy deciduous trees over 10 years: a synthesis from Duke FACE. Global Change Biol 18:223–242

Epstein E, Bloom AJ (2004) Mineral nutrition of plants: principles and perspectives. 2nd edn. Sinauer Associates, Sunderland, MA

Fernando N, Panozzo J, Tausz M, Norton RM, Fitzgerald GJ, Myers S, Walker C, Stangoulis J, Seneweera S (2012). Wheat grain quality under increasing atmospheric CO_2 concentrations in a semi-arid cropping system. Journal of Cereal Science 56: 684-690

Fischer ES, Bremer E (1993) Influence of magnesium deficiency on rates of leaf expansion, starch and sucrose accumulation and net assimilation in Phaseolus vulgaris. Physiol Plant. 89:271–276

Fischer ES, Lohaus G, Heineke D, Heldt HW (1998) Magnesium deficiency results in accumulation of carbohydrates and amino acids in source and sink leaves of spinach. Physiol Plant. 102:16–20

Gerendás J and Führs H (2013). The significance of magnesium for crop quality. Plant Soil (2013) 368:101–128. doi: 10.1007/s11104-012-1555-2

Gill SS, Tuteja N (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry. 48: 909-930

Gransee A, Führs H (2012). Magnesium mobility in soils as a challenge for soil and plant analysis, magnesium fertilization and root uptake under adverse growth conditions. Plant Soil. doi: 10.1007/s11104-012-1567-y

Hafsi C, Debez A, Abdelly C (2014). Potassium deficiency in plants: effects and signaling cascades. Acta Physiol Plant. 36:1055–1070

Hameed A, Bibi N, Akhter J, Iqbal N (2011). Differential changes in antioxidants, proteases, and lipid peroxidation in flag leaves of wheat genotypes under different levels of water deficit conditions. Plant Physiology and Biochemistry. 49: 178-185

Harper JE, Paulsen GM (1969). Nitrogen assimilation and protein synthesis in wheat seedlings as affected by mineral nutrition. I. Macronutrients. Plant Physiol. 44: 69-74

Hermans C, Johnson GN, Strasser RJ, Verbruggen N (2004) Physiological characterization of magnesium deficiency in sugar beet: acclimation to low magnesium differentially affects photosystems I and II. Planta 220:344-355. doi: 10.1007/s00425-004-1340-4

Hermans C, Bourgis F, Faucher M, Strasser RJ, Delrot S, Verbruggen N (2005) Magnesium deficiency in sugar beets alters sugar partitioning and phloem loading in young mature leaves. Planta 220:541-549. doi: 10.1007/s00425-004-1376-5

Hermans C and Verbruggen N (2005). Physiological characterization of magnesium deficiency in Arabidopsis thaliana. J Exp Bot. 56: 2153-2161

Hermans C, Hammond JP, White PJ, Verbruggen N (2006). How do plants respond to nutrient shortage by biomass allocation?. TRENDS in Plant Science, 11; 12, 1360-1385

Hermans C and Verbruggen N (2008) Enhancement of magnesium content in plants by exploiting ionomics and transcriptomics. In: Yardley AW (ed) Dietary magnesium: new research. Nova Science Publishers, pp 159–175

Hopkins WG and Huner NPA (2009) Introduction to Plant Physiology (Fourth Edition). The American Phytopathological Society. APS Press

Högy P, Wieser H, Köhler P, Schwadorf K, Breuer J, Erbs M, Weber S, Fangmeier A (2009). Does elevated atmospheric CO2 allow for sufficient wheat grain quality in the future? Journal of Applied Botany and Food Quality. 82: 114 – 121

Högy P, Brunnbauer M, Koehler P, Schwadorf K, Breuer J, Franzaring J, Zhunusbayeva D, Fangmeier A (2013). Grain quality characteristics of spring wheat (*Triticum aestivum*) as affected by free-air CO_2 enrichment. Environmental and Experimental Botany 88: 11– 18

Huber SC, (1984). Biochemical Basis for Effects of K-Deficiency on Assimilate Export Rate and Accumulation of Soluble Sugars in Soybean Leaves. Plant Physiol., 76: 424-430

IPCC (2007). Technical summary. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) Climate Change 2007: The Physical Science Basis. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

IPCC (2013). Stocker TF, Qin D, Plattner GK, Tignor M, Allen S, Boschung J, Nauels A, Xia Y, Bex W, Midgley P. Climate Change 2013: The Physical Science Basis. Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

IPCC (2014). Core writing team, Pachauri RK, Meyer L Climate Change 2014: Synthesis Report. IPCC Fifth Assessment Synthesis Report. Yann Arthus-Bertrand / Altitude, David J. Wilson, Ocean/Corbis

Jin SH, Huang JQ, Li XQ, Zheng BS, Wu JS, Wang ZJ, Liu GH, Chen M (2011). Effects of potassium supply on limitations of photosynthesis by mesophyll diffusion conductance in *Carya cathayensis*. Tree Physiol.31: 1142-1151

Kanai S, Ohkura K, Adu-Gyamfi JJ, Mohapatra PK, Nguyen NT, Saneoka H, Fujita K (2007). Depression of sink activity precedes the inhibition of biomass production in tomato plants subjected to potassium deficiency stress. Journal of Experimental Botany. 58 (11):2917–2928

Kimball BA, Kobayashi K, Bindi M (2002). Responses of agricultural crops to free air CO_2 enrichment. Advances in Agronomy. 77, 293–368

Kimball BA. (2011). Lessons from FACE: CO2 effects and interactions with water, nitrogen and temperature. In: D. Hillel and C. Rosenzweig, editors, Handbook of climate change and agroecosystems: Impacts, adaptation, and mitigation. Imperial College Press, Hackensack, NJ. p. 87-107

Komatsu M, Tobita H, Watanabe M, Yazaki K, Koike T, Kitao M (2013). Photosynthetic downregulation in leaves of the Japanese white birch grown under elevated CO2 concentration does not change their temperature-dependent susceptibility to photoinhibition. Physiol Plant. 147:159–168

Komor E (2000). Source physiology and assimilate transport: the interaction of sucrose metabolism, starch storage and phloem export in source leaves and the effects on sugar status in phloem. Aust. J. Plant Physiol. 27: 497-505. doi: 10.1071/PP99127

Korner C, Pelaezriedl S, Van-bel AJE (1995). CO_2 responsiveness of plants – a possible link to phloem loading. Plant Cell Environ. 18: 595-600. doi:10.1111/j.1365-3040.1995.tb00560.x

Laing W, Greer D, Sun O, Beets P, Lowe A, Payn T (2000) Pysiological impacts of Mg deficiency in Pinus radiata: growth and photosynthesis. New Phytol 146:47-57

Lavon R, Salomon R, Goldschmidt EE (1999) Effect of potassium, magnesium, and calcium deficiencies on nitrogen constituents and chloroplast components in Citrus leaves. J Am Soc Hort Sci 124:158-162

Leakey AD, Ainsworth EA, Bernacchi CJ, Rogers A, Long SP, Ort DR (2009). Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. Journal Experimental Botany. 60(10):2859-76. doi: 10.1093/jxb/erp096PubMed PMID: 19401412

Long SP, Drake BG (1992). Photosynthetic CO2 assimilation and rising atmospheric CO2 concentrations. In: Baker NR, Thomas H, eds. Crop photosynthesis: spatial and temporal determinants. Amsterdam, the Netherlands: Elsevier, 69–95

Maathuis FJM (2009). Physiological functions of mineral macronutrients. Current Opinion in Plant Biology. 12:250–258. doi: 10.1016/j.pbi.2009.04.003

Madhu M and Hatfield JL (2013). Dynamics of Plant Root Growth under Increased Atmospheric Carbon Dioxide. Agron. J. 105:657–669. doi:10.2134/agronj2013.0018

Mäkelä PSA, Manninen-Egilmez P, Santanen A, Kleemola J (2012). Role of potassium in barley plant stand architecture and yield formation. Communications in Soil Science and Plant Analysis. 43: 2603-2614

Mäser P, Gierth M, Schroeder JI (2002). Molecular mechanisms of potassium and sodium uptake in plants. Plant and Soil. 247: 43-54

Manderscheid R, Bender J, Jager HJ, Weigel HJ (1995). Effects of season long CO_2 enrichment on cereals. II. Nutrient concentrations and grain quality. Agriculture, Ecosystems and Environment. 54: 175-185

Marschner H, Cakmak I (1989) High light intensity enhances chlorosis and necrosis in leaves of zinc, potassium, and magnesium deficient bean (*Phaseolus vulgaris*) plants. J Plant Physiol 134:308-315. doi: 10.1016/S0176-1617(89)80248-2

Marschner H (2002). Mineral Nutrition in Higher Plants. 2nd edition. London: Academic Press

Marschner H, Kirkby EA, Cakmak I (1996) Effect of mineral nutritional status on shoot root partitioning of photoassimilates and cycling of mineral nutrients. J Exp Bot 47:1255-1263

Marschner P (2012). Marschner's Mineral Nutrition of Higher Plants (Third Edition). Elsevier Ltd

McMahon P (2012). Effect of nutrition and soil function on pathogens of tropical tree crops. Plant Pathology. Dr. Christian Joseph Cumagun (Ed.). ISBN: 978-953-51-0489-6

McDonald AJ, Ericsson T, Larsson CM (1996) Plant nutrition, dry matter gain and partitioning at the whole-plant level. J Exp Bot 47:1245–1253

McGrath JM, Lobell DB (2013). Reduction of transpiration and altered nutrient allocation contribute to nutrient decline of crops grown in elevated CO2 concentrations. Plant, Cell and Environment. 36, 697–705

Mengutay M, Ceylan Y, Kutman UB, Cakmak I (2013). Adequate magnesium nutrition mitigates adverse effects of heat stress on maize and wheat. Plant Soil. 368: 57–72

Moore BD, Palmquist DE, Seemann JR (1997). Influence of plant growth at high CO_2 concentrations on leaf content of ribulose-1,5-bisphosphate carboxylase/oxygenase and intracellular distribution of soluble carbohydrates in tobacco, snapdragon, and parsley. Plant Physiology. 115: 241–248

Myers SS, Zanobetti A, Kloog I, Huybers P, Leakey AD, Bloom AJ, Carlisle E, Dietterich LH, Fitzgerald G, Hasegawa T, Holbrook NM, Nelson RL, Ottman MJ, Raboy V, Sakai H, Sartor KA, Schwartz J, Seneweera S, Tausz M, Usui Y (2014). Increasing CO_2 threatens human nutrition. Nature. 5;510(7503):139-42. doi: 10.1038/nature13179

Norby RJ. (1994). Issues and perspectives for investigating responses to elevated atmospheric carbon dioxide. Plant Soil 165:9–20. doi:10.1007/BF00009958

Obrist D. and Arnone JA. (2003). Increasing CO2 accelerates root growth and enhances water acquisition during early stages of development in Larrea tridentate. New Phytol. 159:175-184. doi:10.1046/j.1469-8137.2003.00791.x

Palmer LJ, Dias DA, Boughton B, Roessner U, Graham RD, Stangoulis JCR (2014). Metabolite profiling of wheat (*Triticum aestivum* L.*)* phloem exudate. Plant Methods. 10:27

Pi Z, Stevanato P, Yv LH, Geng G, Guo XL, Yang Y, Peng CX, Kong S (2014). Effects of potassium deficiency and replacement of potassium by sodium on sugar beet plants. Russian Journal of Plant Physiology. 61(2): 224-230

Premachandra GS, Saneoka H, Ogata S (1991). Cell membrane stability and leaf water relations asaffected by potassium nutrition of water-stressed maize. J. Exp. Bot. 42: 739-745

Prentice IC, Farquhar GD, Fasham MJR, et al. (2001). The carbon cycle and atmospheric carbon dioxide. In: Houghton JT, Ding Y, Griggs DJ, et al. (Eds). Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change, p 183–237. Cambridge, UK: Cambridge University Press

Prior SA, Rogers HH, Runion GB, Kimball BA, Mauney JR, Lewin KF, Nagy J, Hendrey GR. (1995). Free-air CO2 enrichment of cotton: Root morphological characteristics. J. Environ. Qual. 24:678–683. doi:10.2134/jeq1995.00472425002400040019x

Pritchard SG and Rogers HH. (2000). Spatial and temporal deployment of crop roots in CO2-enriched environments.New Phytol.147:55–71.doi:10.1046/j.1469-8137.2000.00678.x

Pritchard SG, Amthor JS (2005) Crops and Environmental Change: an Introduction to Effects of Global Warming, Increasing Atmospheric CO_2 and O_3 Concentrations, and Soil Salinization on Crop Physiology and Yield. Food Products Press, New York, 421 p

Reddy KR and Zhao D (2005). Interactive effects of elevated CO_2 and potassium deficiency on photosynthesis, growth, and biomass partitioning of cotton. Field Crop Res. 94, 201–213.

Reddy AR, Rasineni GK, Raghavendra A S (2010). The impact of global elevated CO_2 concentration on photosynthesis and plant productivity. Current Science.Vol.99, No.1

Richter M, Rühle W, Wild A (1990a). Studies on the mechanism pf photosystme II photoinhibition. I. A two-step degradation of D1-protein. Photos Res. 24: 229-235 doi:10.1007/BF00032310

Richter M, Rühle W, Wild A (1990b). Studies on the mechanism pf photosystme II photoinhibition. II. The involvement of toxic oxygen species. Photos Res. 24: 237-243 doi:10.1007/BF00032311

Rogers HH, Runion GB, Krupa SV. (1994). Plants' responses to atmospheric enrichment with emphasis on roots and the rhizosphere. Environ. Pollut. 83:155-189. doi:10.1016/0269-7491(94)90034-5

Rogers HH, Runion GB, Prior SA, Torbert HA (1999). Responses of plants to elevated atmospheric CO_2: Root growth, mineral nutrition, and soil carbon. In Y. Luo and H. A. Mooney (eds.), Carbon dioxide and environmental stress, pp. 215–244. Academic Press, San Diego

Rogers A, Allen DJ, Davey PA, Morgan PB, Ainsworth EA, Bernacchi CJ, Cornic G, Dermody O, Heaton EA,Mahoney J, Zhu X-G, DeLucia EH, Ort DR, Long SP (2004) Leaf photosynthesis and carbohydrate dynamics of soybeans grown throughout their lifecycle under free-air carbon dioxide enrichment. Plant Cell Environ 27:449–458

Schroth G, Krauss U, Gasparotto L, Duarte Aguilar JA ,Vohland K (2000) Pests and diseases in agroforestry systems of the humid tropics. Agroforestry Systems. 50: (3) 199-241

Shaul O (2002) Magnesium transport and function in plants: the tip of the iceberg. Biometals 15:307–321. doi: 10.1023/A:1016091118585

Simova-Stoilova L, Vaseva I, Grigorova B, Demirevska K, Feller U (2010). Proteolytic activity and cysteine protease expression in wheat leaves under severe soil drought and recovery. Plant Physiol. Biochem. 48: 200-206

Sudhakar C, Lakshmi A, Griridarakumar S (2001). Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba*L.) under NaCl salinity. Plant Sci. 161: 613-619

Sun OJ and Payn TW (1999) Magnesium nutrition and photosynthesis in Pinus radiata: clonal variation and influence of potassium. Tree Physiol 19:535–540

Taiz L, Zeiger E. (2010). Plant physiology, 5th edn. Sunderland, MA: Sinauer Associates, Inc., Publishers.

Taulavuori E, Hellström E-K, Taulavuori K, Laine K (2001). Comparison of two methods used to analyse lipid peroxidation from Vaccinium myrtillus (L.) during snow removal, reacclimation and cold acclimation. Journal of Experimental Botany. 52(365): 2375-2380

Teng N, Wang J, Chen T, Wu X, Wang Y, Lin J (2006). Elevated CO_2 induces physiological, biochemical and structural changes in leaves of *Arabidopsis thaliana*. New Phytologist. 172 (1): 92–103. doi:10.1111/j.1469-8137.2006.01818.x. PubMed PMID: 16945092

Tewari RK, Kumar P, Sharma PN (2006) Magnesium deficiency induced oxidative stress and antioxidant responses in mulberry plants. Sci Hortic-Amsterdam 108:7-14. doi: 10.1016/j.scienta.2005.12.006

Verbruggen N and Hermans C (2013). Physiological and molecular responses to magnesium nutritional imbalance in plants. Plant Soil. 368:87–99.doi:10.1007/s11104-013-1589-0

Wang M, Zheng Q, Shen Q, Guo S (2013). The critical role of potassium in plant stress response. Int. J. Mol. Sci.14: 7370-7390. doi:10.3390/ijms14047370

Waraich EA, Ahmad R, Saifullah, Ashraf MY, Ehsanullah (2011) Role of mineral nutrition in alleviation of drought stress in plants. Aust J Crop Sci 5: 764–777

Waraich EA, Ahmad R, Halim A, Aziz T (2012). Alleviation of temperature stress by nutrient management in crop plants: a review. Journal of Soil Science and Plant Nutrition. 12 (2), 221-244

Wechsung G., Wechsung F, Wall GW, Adamsen FJ, Kimball BA, Pinter Jr. PJ, Kartschall T, Garcia RL, LaMorte RL. (1999). The effects of free-air CO2 enrichment and soil water availability on spacial and seasonal patterns of wheat root growth. Glob. Change Biol. 5:519–529. doi:10.1046/j.1365-2486.1999.00243.x

Xu Z, Shimizu H, Yagasaki Y, Ito S, Zheng Y, Zhou G (2013). Interactive Effects of Elevated CO2, Drought, and Warming on Plants. J Plant Growth Regul. 32:692–707. doi: 10.1007/s00344-013-9337-5

Yang GH, Yang LT, Jiang HX, Li Y, Wang P, Chen LS (2012). Physiological impacts of magnesium-deficiency in Citrus seedlings: photosynthesis, antioxidant system and carbohydrates. Trees. 26(4):1237-1250. doi:10.1007/s00468-012-0699-2

Zhao D, Oosterhuis DM, Bednarz CW (2001) Influence of potassium deficiency on photosynthesis, chlorophyll content, and chloroplast ultrastructure of cotton plants. Photosynthetica. 39(1):103-109