CHANGES IN ROOT MORPHOLOGY AND NUTRIENT UPTAKE IN WHEAT PLANTS WITH VARIED POTASSIUM AND MAGNESIUM SUPPLY

by

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ABSTRACT

CHANGES IN ROOT MORPHOLOGY AND NUTRIENT UPTAKE IN WHEAT PLANTS WITH VARIED POTASSIUM AND MAGNESIUM SUPPLY

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Keywords: potassium, magnesium, root morphology, nutrient use efficiency, wheat

Mineral nutrient deficiencies on agricultural soils is a widespread problem affecting crop productivity worldwide. This study was conducted to investigate the effects of potassium (K) and magnesium (Mg) supply on biomass production, root morphology and uptake of other mineral nutrients in wheat (*Triticum aestivum* cv. Ceyhan-99). Changes in root morphology as well as nutrient uptake by roots were monitored under various K and Mg treatments. Results showed that K and Mg deficiency significantly reduced shoot and root growth and induced changes in nutrient uptake by roots. K deficiency reduced nitrate (NO_3^-) and phosphorus (P), but increased Mg uptake by roots. In general, all root morphological attributes analyzed were significantly affected by low K and Mg supply. However, root length, root area, root volume and number of tips were the most affected attributes which lead to severe reductions in nutrient acquisition and use efficiency. Moreover, K deficiency resulted in impaired use of absorbed nitrogen (N) in protein biosynthesis. Total free amino acid concentration increased sharply in response to K starvation and resulted in severe inhibition of N uptake by roots due to the negative feedback effect. It is concluded that ensuring adequate K and Mg nutrition is required to maximize agricultural production and to improve use efficiency of nutrients applied to agricultural lands.

ÖZET

FARKLI POTASYUM VE MAGNEZYUM KONSANTRASYONLARINDA YETİŞTİRİLEN BUĞDAY BİTKİLERİNİN KÖK MORFOLOJİSİ VE BESİN ABSORPSİYONUNDA MEYDANA GELEN DEĞİŞİKLİKLER

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Tarım topraklarında besin elementi eksikliği, verimi olumsuz yönde etkileyen yaygın bir problem haline gelmiştir. Bu çalışma, potasyum (K) ve magnezyum (Mg) eksikliğinin buğday (*Triticum aestivum* cv. Ceyhan-99) bitkilerinin biyokütle, kök morfolojisi ve diğer besin elementlerinin absorpsiyonu üzerindeki etkilerini araştırmak amacıyla yürütülmüştür. Sonuçlar, K ve Mg eksikliğinin yeşil aksam ve kök büyümesini önemli derecede azalttığını ve besin elementlerinin absorpsiyonlarında değişikliklere yol açtığını göstermektedir. Potasyum eksikliği, kök nitrat (NO_3) ve fosfor (P) alımını azaltırken, Mg alımını artırmıştır. İncelenen tüm kök parametreleri K ve Mg eksikliğinden ciddi derecede etkilenmiştir. Ancak, kök uzunluğu, kök alanı, kök hacmi ve kök ucu sayısı en çok etkilenen parametreler arasındadır ve besin elementlerinin absorpsiyonu ve kullanım verimliliğini önemli derecede azaltmıştır. Ayrıca, K eksikliğinde yetiştirilen bitkiler, kökten alınan azotu (N) protein biyosentezinde başarılı bir şekilde kullanamamıştır. Bu bitkilerde serbest amino asit konsantrasyonu artmıştır ve bu, negatif geri bildirim etkisiyle köklerden N alımını ciddi derecede azaltmıştır. Sonuçlar, yeterli K ve Mg beslenmesinin hem tarımsal verimi hem de besin elementlerinin bitkiler tarafından kullanılabilirliliğini artırmak için gerekli olduğunu göstermektedir.

This work is dedicated

to my family, **Kaan**, **İclal** and **Sinan**.
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ADP.....adenosine diphosphate

Al.....aluminium

ANOVA.....analysis of variance

ATP.....adenosine triphosphate

C.....carbon

Ca.....calcium

ca.....circa

$Ca(H_2PO_4)_2$.....calcium dihydrogenphosphate monohydrate

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

$CaCl_2$.....calcium chloride

$CaCl_2.2H_2O$.....calcium chloride dihydrate

$CaSO_4$.....calcium sulphate

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

cm.....centimeter

cm^2.....square centimeter

cm^3.....cubic centimeter

Cu.....copper

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

cv.....cultivar

DAS.....days after sowing

DAT.....days after transfer

DW.....dry weight

FAO.....food and agriculture organization

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

g.....gram

h.....hour

H^+/ATPase.....proton ATPase

H_2O_2.....hydrogen peroxide

H_2SO_4.....sulfuric acid

H_3BO_3.....boric acid

HNO_3.....nitric acid

HSD.....honestly significant test

i.e.....id est

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

K.....potassium

K_2SO_4.....potassium sulfate

KCl.....potassium chloride

kg.....kilogram

KH_2PO_4.....potassium dihydrogen phosphate

L.....liter

m.....meter

Mg-ATP.....magnesium bound ATP

mg.....miligram

Mg.....magnesium

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

ml.....mililiter

mM.....milimolar

$MnSO_4.4H_2O$.....manganase sulfate tetrahydrate

MΩ.....mega-ohm

NaOH.....sodium hydroxide

NH_4.....ammonium

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

$NiCl_2.6H_2O$.....nickel chloride hexahydrate

nm.....nanometer

NO_3.....nitrate

NR.....nitrate reductase

NRA.....nitrate reductase activity

NUE.....nitrogen use efficiency

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

P.....phosphorus

PEP.....phesphoenolpyruvate

ROS.....reactive oxygen species

RuBP.....ribulose bisphosphate

s.....second

S.....sulfur

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

µg.....microgram

µl.....microliter

µM.....micromolar

µmol.....micro mol

°C.....degrees celcius

(A) INTRODUCTION

A.1. General Introduction

A great concern of today is the rapidly expanding world population. It is projected that the world population will exceed nine billion by the year 2050. It is therefore of vital importance to provide enough food for the expanding human population. In order to meet the increasing food demand, a massive increase in agricultural crop production is necessary. However, many factors constrain the agricultural productivity in terms of quality and quantity. Mineral element deficiencies in agricultural soils is one of these major limiting factors and this MSc thesis will mainly focus on the physiological consequences of potassium (K) and magnesium (Mg) deficiency in wheat as a model crop.

Both K and Mg are of great significance due to their key roles in physiological and biochemical processes that affect plant growth and development. The depletion of these nutrients is a growing concern. A considerable area of agricultural land has been reported to be K-deficient and soil K balance declines dramatically with time (Dobermann et al., 1999; Hoa et al., 2006; Andrist-Rangel et al., 2007; Krauss, 2003). The major sources of K and Mg depletion are removal by crop plants, leaching losses and soil erosion (Fageria, 2009). Unfavorable soil structure (sandy soil) with low cation exchange capacity (CEC) and depletion zones around the rhizosphere may induce K deficiency (Kayser and Isselstein, 2005; Moody and Bell, 2006; Andrist-Rangel et al., 2007). Mg deficiency is of great concern on soils fertilized only with nitrogen (N), phosphorus (P) and K, as well as on acidic soils due to its potential for leaching and interaction with Al_{3+} (Cakmak and Yazici, 2010). In addition, increased application or high levels of soil K or Ca can also lead to Mg deficiency (Fageria, 2009).

A.2. Potassium: Physiological Roles and Deficiency-Related Problems in Plants

K is an essential macronutrient that is required for the plant metabolism, growth and development. K is a univalent cation and found most abundantly in the cytosol. Next to nitrogen (N), K is the element that is required in the largest amounts by plants: about 2%-5% of total plant dry matter (Marschner, 1995). K plays key roles in numerous physiological functions, including enzyme activation, photosynthesis, osmoregulation, protein synthesis, cation-anion balance, stress resistance and phloem transport. K deficiency results in reduced shoot and root growth and yield.

K balances the charge of soluble and insoluble anions in the cytosol and chloroplasts, and thus maintains the pH in these compartments between 7 and 8 (Marschner, 2012). This range is the optimum for most enzyme reactions. According to Suelter (1970), most of the enzymes are either activated or stimulated by K^+. K^+ induces conformational changes in proteins, thus activating them. These K^+-induced changes increase the rate of catalytic reactions and also the substrate-affinity (Evans and Wildes, 1971). Under K deficiency, the enzyme activation may be inhibited and this phenomenon is attributed to the inability to maintain the optimum pH in the cytosol. Pyruvate kinase, phosphofructokinase, starch synthase, proton-pumping ATPases and vacuolar pyrophosphatase isoforms are enzymes that are sensitive to K deprivation (Laeuchli and Pflüger, 1978; Nitsons and Evans, 1969; Gibrat et al., 1990; Darley et al., 1998).

K-deficiency-induced changes in enzyme activities mostly lead to imbalances in the carbon and nitrogen metabolism. The concentration of soluble carbohydrates and soluble organic N compounds, especially N-rich amino acids, increase under K deprivation, whereas the concentrations of nitrate tend to increase in K-deficient plant tissues (Armengaud et al., 2009). These impairments are also related to the role of K in protein synthesis.

Photosynthesis is also affected by K nutritional status. It is well documented that K plays a crucial role in the maintenance of turgor pressure and thus regulating the stomatal function. Apart from these, K is known to regulate ribulose bisphophate (RuBP) carboxylase activation (Peoples and Koch, 1979). K deficiency leads to significant reductions in photosynthesis rate, RuBP-

carboxylase activity and photorespiration, whereas stomatal resistance and dark respiration rates increase under K deprivation. The reduction in photosynthesis is mostly attributed to stomatal limitations (Oosterhuis et al., 2013), however researchers have also reported the inhibition of photosynthesis may also ocur due to accumulation of photoassimilates and reduced translocation into sink organs (Pflüger and Cassier, 1977; Pier and Berkowitz, 1987; Kanai et al., 2007).

Translocation of carbon and nitrogen compounds from source to sink organs are highly dependent on transpiration rates, which is also regulated by K nutritional status. Adequate K nutrition is essential for optimum translocation of photoassimilates, amino acids and nitrate. K is known to influence the rate of phloem loading and assimilate partitioning. K deprivation leads to reduced assimilate transport to roots and eventually root growth of K-deficient plants is inhibited (Cakmak, 1994; Cakmak et al., 1994b).

K^+ plays a crucial role in cation-anion balance in the cytoplasm, chloroplasts, vacuoles, xylem and also phloem. K^+ serves as the dominant cation for counterbalancing immobile or mobile anions (Marschner, 2012). For example, K^+ serves as the accompanying counterion for NO_3^- in long-distance transport in the xylem.

Apart from its physiological roles, K is also known to increase biotic (Prabhu et al., 2007) and abiotic stress tolerance in plants (Cakmak, 2005). K-deficient plants are more susceptible to high-light intensity (Marschner and Cakmak, 1989), low temperature (Grewal and Singh, 1980), drought (Sen Gupta et al., 1989) and also pest invasion (Amtmann et al., 2008). Therefore, adequate K nutrition is essential to withstand such stress factors.

A.3. Magnesium: Physiological Roles and Deficiency-Related Problems

Mg is a macronutrient that is essential for normal plant growth and development and plays key roles in physiological and biochemical processes. Mg^{2+} is a divalent cation and has an undispensable role in enzyme activation, phosphorylation, protein and chlorophyll biosynhtesis, photosynthesis and carbohydrate partitioning (Marschner, 2012). Along with K, Mg is also involved

in cation-anion balance in cells as well as in maintaining cell turgor (Marschner, 2012; Gerendas and Führs, 2013).

The most obvious visible symptom of Mg deficiency is interveinal chlorosis of older leaves due to impairments in the chlorophyll biosynthesis. Mg serves as the central atom in the chlorophyll and its biosynthesis requires the presence of Mg (Walker and Weinstein, 1991; Kobayashi et al., 2008). Protein biosynthesis is also terminated under Mg deficiency due to its key role in the aggregation of ribosome subunits (Cammarano et al., 1972). Likewise, nucleic acid biosynthesis and functions have been reported to be affected by Mg status of the plant (Galling, 1963; Sreedhara and Cowan, 2012).

The activity of many enzymes such as glutathione synthase, phesphoenolpyruvate (PEP) carboxylase, RuBP carboxylase, glutamine synthase and fructose-1,6-bisphosphatase either require Mg or is enhanced by its presence (Marschner, 2012; Gerhardt et al., 1987; O'Neal and Joy, 1974; Pierce, 1986). The phosphorylation of adenosine-diphosphate (ADP) and the synthesis of adenosine triphosphate (ATP) are also absolutely Mg-dependent processes.

The presence of Mg affects carbohydrate metabolism within the plant. Mg-deficient leaves typically accumulate carbohydrates as a result of inhibited phloem export and low rates of phloem export into sink organs lead to reduced root growth (Cakmak et al., 1994a). Impairments in phloem-loading from source to sink organs under Mg-deficiency are mostly attributed to the critical role of Mg^{2+} for the activity of proton-pumping ATPase (H^{+}-ATPase) (Williams and Hall, 1987). As a result of accumulation of photoassimilates in the source leaves, RuBP oxygenase activity, and thus the generation of reactive oxygen species are favoured (Cakmak and Kirkby, 2008). Increased activity of superoxide radical (O_2^{-}) and hydrogen peroxide (H_2O_2) scavenging enzymes (i.e., superoxide dismutase, ascorbate peroxidase and glutathione reductase) and increased concentrations of antioxidants have been reported in the Mg-deficient leaves (Cakmak and Marschner, 1992). Due to this oxidative stress, Mg-deficient leaves are more susceptible to high light and increasing light intensity contributes to the severity of chlorosis and/or necrosis.

Both the dependency of photosynthethic enzymes on the presence of Mg and the accumulation sugars in the leaves lead to the inhibition of photosynthesis

under Mg deficiency (Laing et al., 2000; Hermans et al., 2004; Wingler and Roitsch, 2008). Peaslee and Moss (1966) reported that inhibition of chlorophyll biosynthesis under Mg deficiency may be another reason for the reduced photosynthesis rate.

Along with the carbon metabolism, N metabolism is also affected by Mg status. Due to its role in protein synthesis, Mg deficiency leads to accumulation of non-protein N, mainly amino acids, and lower concentrations of protein N (Marschner, 2012). In addition, some enzymes of N metabolism (nitrogen reductase, glutamate synthase, glutamate dehydrogenase, urease) have been reported to be inhibited in spinach under Mg deficiency (Yin et al., 2009).

Mg has also a crucial role in mitigating heavy metal toxicities. For example, Cu^{2+} phytotoxicity has been reported to be alleviated by high Mg^{2+} treatment in wheat (Luo et al., 2008), barley (Lock et al., 2007), cowpea (Kopittke et al., 2011) and grapevine (Chen et al., 2013). Likewise, adequate Mg nutrition was found to be able to mitigate Al^{3+} toxicity by a number of different pathways in soybean (Silva et al., 2001) and wheat plants (Kinraide et al., 2004).

A.4. Nutrient Use Efficiency

Food production increases annually due to expanding World population and demand. Increasing food production requires higher energy inputs. Fertilizers are one of the means of increasing grain yield of crop plants. However, both the production and the use of commercially available fertilizers are expensive due to high costs of energy and raw materials (White and Brown, 2010).

In many agricultural systems, a huge proportion of the applied fertilizer is lost from the soil due to various factors such as soil leaching, erosion, denitrification and volatilization (Xu et al., 2012) and consequently, cannot be used by crop plants. For example, only 40% of the applied N fertilizer is taken up and utilized by plants. In addition, the use of inorganic fertilizers also threatens the sustainability of the environment. Synthesis of N fertilizers has

been reported to contribute to the production of greenhouse gases (Galloway et al., 2008; Smith et al., 2008). It was also reported that the use of N and P fertilizers is one of the major contributors to eutrophication process in waters (Conley et al., 2009; White and Hammond, 2009). In order to reduce fertilizer costs and preserve the environment, the use efficiency of applied fertilizers has to be maximized due to above-mentioned commercial and environmental reasons.

Nutrient use efficiency refers to the ability of a plant to acquire nutrients and successfully utilize them within itself (Blair, 1993). Studying and understanding nutrient use efficiency is of great importance since it can contribute to a sustanaible and productive agriculture (Masclaux-Daubresse, 2010) by reducing fertilizer input costs, enhancing crop yields and decreasing the rate of nutrient losses (Baligar et al., 2001).

Plant genetic, morphological and physiological traits and many external factors affect nutrient use efficiency in plants. Nutrient use efficiency may vary withing different species, cultivars and genotypes (Baligar and Duncan, 1990; Baligar et al., 2001; Clark, 1984; Gerloff and Gabelman, 1983) Physiological features such as shoot yield, harvest index and root architecture also control nutrient use efficiency. External factors include soil temperature, soil pH, soil moisture, climatic conditions, the source, rate and time of fertilizers (Baligar and Bennett, 1986a, 1986b; Baligar and Fageria, 1997; Duncan, 1994; Fageria, 1992).

There are numerous approaches to improve nutrient use efficiency of plants including breeding for root systems that are more efficient in nutrient acquisition (Coque et al., 2008), overexpression of transporters that facilitate the acquisiton and translocation of nutrients, enhancing cellular pH balance, manipulating key genes of nutrient metabolism by molecular breeding (Xu et al., 2012). In addition to these approaches, a balanced mineral fertilization supplied at the right time and rate for the crop in practice. Mineral elements can affect root uptake of other nutrients and their utilization within the plant (Marschner, 2012). The phenomena of antagonism and synergism have been reported between mineral nutrients. Some ions may compete for transport into root cells, whereas some may promote the uptake of another.

K may affect the uptake, assimilation and utilization of other nutrients. Antagonistic interactions of K^+ with Mg^{2+} amd Ca^{2+} have been reported (Johnson et al., 1968; Dibb and Thompson, 1985). Excess applications of K often induces Mg or Ca deficiency by depressing their root uptake and accumulation in shoots. K^+ is a monovalent cation and competes with other cations for their binding sites (Marschner, 2012). Resultingly, the uptake of other cations may be inhibited.

Positive interaction of K with N and P has also been reported. Studies showed that efficient use of N and P fertilizers requires high soil K (Dibb and Thompson, 1985; Fageria et al., 1997a, 1997b). The form of N (ammonium: $NH4^+$ or nitrate: NO_3^-) determines its interaction with K. High concentrations of $NH4^+$ inhibits the uptake of K^+ (Marschner, 2012), but the rate of K applied does not affect NH4+-uptake (Mengel et al., 1976; Rufty et al, 1982a; Shaviv et al., 1987). In case of NO_3^-, N root uptake and shoot transport are enhanced by the presence of K^+ (Minotti et al., 1968; Blevins et al., 1978; Ivashikina and Feyziev, 1998) and studies have proved the existence of a close relationship between K^+ and NO_3^- uptake by roots (Rufty et al., 1981; Ashley and Goodson, 1972).

The root uptake of K^+ and NO_3^- is facilitated by the synergism of these two counter-ions. K^+ also plays a key role in the distribution of NO_3^- between shoot and root (Ruiz and Romero, 2002) by serving as an accompanying cation in the xylem (Blevins et al., 1978a, 1978b; Dong et al., 2004). K^+ is the most abundant cation in plant cells and contributes to anion-balance. Siebrecht and Tischner (1999) have shown that the withdrawal of K supply from the environment directly decreases the nitrate concentration in the xylem.

Apart from its role in acquisition and translocation of N, K^+ is also required for efficient N assimilation (Drosdoff et al., 1947; Wang et al., 2012). Armengaud et al. (2009) reported impairments in NO_3^- assimilation and protein synthesis under K deprivation. Nitrate reductase (NR) catalyzes the reduction of nitrate to nitrite and this reaction is the rate-limiting step in the NO_3^- assimilation pathway (Beevers and Hageman, 1969). The activity of NR (NRA) is enhanced with increasing K supply (Armengaud et al., 2004; Beevers and Hageman, 1969; Blevins et al., 1978; Li et al., 2011) and K starvation significantly reduces NRA. Proteins are principle products of NO_3^- assimilation. K deficiency is correlated

with high protease and peptidase activity and protein degradation (Hu et al., 2016) and as a result, a higher ratio of free amino acids to protein is observed. Amino acid export in phloem was also decreased under K starvation. Impairments in the protein metabolism directly affects the uptake and utilization of N.

Due to its important role in acquisition, transport and assimilation of N, mineral fertilization with K can increase N use efficiency (NUE) of crop plants. Previous research has showed that increased K supply is required for a better response to increased N fertilization (Better Crops, 1998; Webb, 2009).

Interactions of Mg with other nutrients have also been reported by researchers. As mentioned above, high concentrations of K^+ inhibit Mg uptake from roots and its translocation to shoots (Ohno and Grunes, 1985; Huang et al., 1990). For example, excess application of K resulted in decreased shoot Mg concentration in wheat (Ohno and Grunes, 1985), sorghum (Ologunde and Sorensen, 1982) and tall fescue (Hannaway et al., 1982). However, there is no effect of Mg supply on K^+ uptake.

Interactions of Mg^{2+} with Ca^{2+} were studied in tomato (Schwartz and Bar-Yosef, 1983), rice (Fageria et al., 1983), cassava, sunflower and maize (Spear et al., 1978) and in all of the studies it was concluded that Ca^{2+} suppresses Mg^{2+} uptake by decreasing Mg^{2+} transport capacity of roots or by competing for Mg^{2+}-absorption sites.

Positive interactions of N and P with Mg were reported (Wilkinson et al., 2000). NO_3^- fertilization promotes Mg uptake due to cation-anion balance. In addition, due to its role in RNA and protein synthesis (Marschner, 2012), NO_3^- uptake may be down-regulated in the absence of Mg. Aluminium-tolerance is attributed to greater uptake of Mg in potato, corn and wheat (Foy, 1984; Ali, 1973). Mg can either compete with Al^{3+} for absorption sites, thus reducing the Al^{3+}-root contact, or decrease the Al^{3+}-activity (Foy, 1984).

A.5. Morphology and Functions of Plant Roots as Affected by Potassium and Magnesium Deficiency

Nutrients are taken up from the environment via roots and the ability of a plant to acquire nutrients is determined by root system achitecture. Therefore, roots have the most important role in resource capture (Fitter, 1988b; Lynch, 1995; Lynch and Brown, 2001). The acquisition of nutrients by plant roots plays the most crucial role in nutrient acquisition (Gutschick, 1993).

Root size and morphology directly affects nutrient acquisition efficiency (Baligar and Duncan, 1990; Barber, 1995; Marschner, 1998). Root morphology parameters such as length, area, volume, diameter, density, number of roots are good indicators of nutrient uptake capactiy (Bechmann et al., 2014; Jia et al., 2010) and can be affected by deficiencies or toxicities of mineral elements (Bennet, 1993; Hagemeyer and Breckle, 1996; Hodge et al., 1999a, 2000c; Marschner, 1995; Robinson et al., 1999).

In order to adapt the environmental conditions, plants may alter their root architecture. The effect of N and P on root growth have been has been extensively studied, however, K has a different mechanism on root growth and requires more attention. It is well-known that K starvation inhibits root growth and development. Root elongation and lateral root formation and thus the capacity of nutrient use from soil are significantly reduced under K deficiency (Drew, 1975; Shin and Schachtmann, 2004; Armengaud et al., 2004; Zhi-Yong et al., 2008). Root morphogoly was found to be affected by different K levels in many species including pea, red clover, lucerne, rye, perennial ryegrass, barley, oilseed rape, cotton and *Arabidopsis* (Hogh-Jensen and Podersen, 2003; Sanchez-Calderon et al., 2005). Disruptions in root morphology and growth are mostly attributed to the impaired photosynthate supply into roots because photosynthetic rate as well as carbon-partitioning between shoots and roots highly depend on the presence of K (Cakmak et al., 1994; Bernarz et al., 1998; Pettigrew, 1999; Zhao et al., 2001). Therefore, the negative effects of K deficiency on root growth may highly restrict nutrient acquisition from the rhizosphere.

Levels of Mg have also profound effects on the root growth of plants. Reduced root growth is defined as a good indicator of Mg deficiency and as in

the case of K, it is most likely to be the consequence of impaired carbohydrate transport from source leaves (Gransee and Führs, 2012). Additionally, a recent transcriptomic study showed that the highest number of regulated genes in response to Mg stavation was found in roots (Hermans et al., 2010b) suggesting that Mg could affect root development (Niu et al., 2014). However, there is very little research and published data on the effect of Mg deficiency on root morphological parameters. In a recent research, it was reported that Mg deficiency significantly decreased lateral root outgrowth and length in *Arabidopsis thaliana* (Xiao et al., 2015).

A.6. Scope

Mineral element deficiencies of essential nutrients are a widely occurring problem on world's agricultural lands and associated with numerous reasons. Lack of an essential nutrient can significanlty limit plant growth and yield. In order to maximize crop production to meet the increasing demand, fertilizers are commonly used by farmers. Fertilizer use efficiency is directly related to nutrient use and utilization efficiency of crop plants. Major constraints of nutrient use efficiency include root architecture/plasticity and nutritional status of plants. The aim of this thesis is to reveal the effects of K and Mg deficiency on root morphology of wheat plants as well as the interactions of these nutrients with other elements in terms of uptake and utilization.

(B) MATERIALS AND METHODS

B.1 Seed Material & Germination

A standard spring-type bread wheat cultivar adapted to Mediterranean climate and widely grown in the Cukurova plain of Turkey (*Triticum aestivum* cv. Ceyhan-99) was used in all experiments conducted throughout this thesis. For germination, seeds were sown in perlite wetted with saturated $CaSO_4$ solution and placed in a dark growth chamber for 2-3 days set to constant temperature of 24^oC. When seeds were germinated and the emerging coleoptiles were visible, the light/dark cycle in growth chamber was started for further plant development. Following six days after sowing, young wheat seedlings were transferred to nutrient solution culture.

B.2 Experimental Design

B.2.1 Potassium Nutrition and Root Morphology

This experiment was conducted in a growth chamber with 16 / 8 h light/dark periods. The temperature was maintained at 24°C and 18°C during light/dark periods respectively. During the light period, the photosynthetic flux density was 400 μmol m^{-2} s^{-1}. Relative humidity was kept at 60% during light and 70% during dark periods.

This experiment was designed to monitor the effect of K nutrition on the root morphological parameters. Wheat seedlings were transferred to 3-L plastic pots and grown in nutrient solution culture with different K treatments. The nutrient solution was composed of 2 mM $Ca(NO_3)_2.4H_2O$, 1 mM $MgSO_4.7H_2O$, 0.03 mM Fe-EDTA, 1 μM $ZnSO_4.7H_2O$, 1 μM $MnSO_4.4H_2O$, 1μM H_3BO_3, 0.2

μM $CuSO_4.5H_2O$, 0.1 μM $(NH_4)_6Mo_7O_{24}.4H_2O$ and 0.2 μM $NiCl_2.6H_2O$. K was supplied at four different levels (i.e., very low, low, medium and adequate). Adequate K pots was supplied 0.2 mM KH_2PO_4, 0.85 mM K_2SO_4 and 0.1 mM KCl, whereas all of the deficiency pots received 0.05 mM $CaCl_2.2H_2O$ and 0.85 mM $CaSO_4.2H_2O$. K was supplied in the form of KH_2PO_4 and its concentration was 0.01, 0.03 and 0.05 mM in very low, low and medium-K pots respectively. $Ca(H_2PO_4)_2$ was added to K-deficiency pots at a level of 0.1, 0.09 and 0.08 mM depending on the K-treatment (i.e., very low, low and medium-K, respectively). The nutrient solution was renewed every three days.

The experiment had a completely randomized and full factorial design with three replicate pots for each treatment. 10 seedlings were transferred in each pot. Five days following the transfer to solution culture, seedlings were thinned to nine in each pot. On the 8th day after transfer to solution culture, four plants from each pot were harvested. On the 10th and 12th day after transfer to solution culture, three and two plants were harvested, respectively. The same harvest procedure was followed in all of three harvests: Harvested plants were first separated into shoot and root fractions. Shoots were rinsed in distilled water and placed in 20 mL volume glass vials whereas roots were first analyzed for morphological features using an image analysis system as described in Section B.4. Following the image analysis, roots were incubated in 1 mM $CaCl_2$ and then distilled water for 2 min each and placed in 20 mL volume glass vials. All vials with harvested shoot and root samples were placed in a forced oven set to 60ºC to dry the samples until a constant weight.

B.2.2 Magnesium Nutrition and Root Morphology

This experiment was conducted in a growth chamber under controlled climatic conditions in order to study the effect of Mg nutrition on root growth and morphology. The growth chamber was set to 16 / 8 h light/dark period. The temperature was kept at 24/20°C and the humidity at 60/70% during light/dark periods, respectively. The photosynthetic flux density was 400 μmol m^{-2} s^{-1} in the growth chamber.

Young wheat seedling were transferred to 3-L solution culture pots. The nutrient solution was composed of 2 mM $Ca(NO_3)_2.4H_2O$, 0.2 mM KH_2PO_4, 0.85 mM K_2SO_4, 0.1 mM KCl, 0.03 mM Fe-EDTA, 1 μM $ZnSO_4.7H_2O$, 1 μM $MnSO_4.4H_2O$, 1 μM H_3BO_3, 0.2 μM $CuSO_4.5H_2O$, 0.1 μM $(NH_4)_6Mo_7O_{24}.4H_2O$ and 0.2 μM $NiCl_2.6H_2O$. Mg was supplied in the form of $MgSO_4.7H_2O$ and at 4 different rates (i.e., very-low, low, medium and adequate). Adequate Mg pots were supplied with 1 mM, very-low, low and medium Mg pots were supplied with 0.05, 0.01 and 0.025 mM Mg, respectively. All of the deficiency pots were additionally supplied with 1 mM $CaSO_4.2H_2O$. The nutrient solution was renewed every 3 days throughout the experiment.

The experimental design was completely randomized full-factorial. There were four different Mg treatments with 3 pot replicates. At first, 10 plants were planted in each pot. Following 5 days after transfer, seedlings were thinned to nine in each pot. First 4, then 3 and lastly 2 plants were harvested on 8th, 10th and 12th day after transfer to solution culture, respectively. Using an image analysis software described in Section B.4, roots were analyzed on the same day of harvest. Shoots were washed in distilled water. Roots were first soaked in 1 mM CaCl2 solution, then washed with distilled water. Washed shoots and roots were put into small glass tubes and oven-dried at 60°C until a constant weight.

B.2.3 Potassium Resupply to Deficient Plants

An additional experiment was conducted to monitor the changes in the root morphology of K-deficient plants as affected by a short term K resupply. The experiment was carried out in a growth chamber with 16/8 h light/dark periods. The photon flux density in the growth chamber was 400 μmol m^{-2} s^{-1} and the temperature was set to 24/18°C and relative humidity to 60/70 % during light/dark periods. The experiment had a completely randomized full factorial design with 4 replications.

Wheat seedlings transferred to solution culture were supplied with 2 mM $Ca(NO_3)_2.4H_2O$, 1 mM $MgSO_4.7H_2O$, 1 μM $ZnSO_4.7H_2O$, 1 μM $MnSO_4.4H_2O$, 0.03 mM Fe-EDTA, 1 μM H_3BO_3, 0.2 μM $CuSO_4.5H_2O$, 0.1 μM $(NH_4)_6Mo_7O_{24}.4H_2O$ and 0.2 μM $NiCl_2.6H_2O$. There were different different K

application rates (i.e. low and adeqaute). Low K pots were supplied with 0.015 KH_2PO_4, 0.09 mM $Ca(H_2PO4)_2$ and 0.85 mM $CaSO_4.2H_2O$, whereas adequate K pots received 0.2 mM KH_2PO_4, 0.85 mM K_2SO_4 and 0.1 mM KCl. Following 12 days after sowing, half of the low-K pots were resupplied with the adequate-K-nutrient solution. To monitor the changes during the resupply period, 4 pots from each treatment were harvested (i) at the time of resupply, (ii) 24 hours after resupply, (iii) 48 hours after resupply and (iv) 72 hours after resupply. Harvested roots were analyzed using an image analysis system as described in Section B.4. Roots were washed first with 1 mM $CaCl_2$ solution and then with distilled water. Washed shoots and roots were put into paper bags and oven-dried at 60°C for 3 days.

B.2.4 Effect of varied K Nutrition on Uptake of Other Mineral Nutrients

This experiment was conducted in a computer-controlled greenhouse located in Sabanci University, Istanbul, Turkey (40°53'25''N, 29°22'47''E). The temperature was maintained at 22°C (±2) during the experiment. The design of the experiment was completely randomized with 7 replications for each treatment.

Wheat seedlings were grown in 3-L solution culture pots. The nutrient solution culture composed of 2 mM $Ca(NO_3)_2.4H_2O$, 1 mM $MgSO_4.7H_2O$, 1 µM $ZnSO_4.7H_2O$, 0.03 mM Fe-EDTA, 1 µM $MnSO_4.4H_2O$, 1 µM H_3BO_3, 0.2 µM $CuSO_4.5H_2O$, 0.1 µM $(NH_4)_6Mo_7O_{24}.4H_2O$ and 0.2 µM $NiCl_2.6H_2O$. K was supplied at three different concentrations (i.e., low, medium and adequate). Adequate K pots were supplied with 0.2 mM KH_2PO_4, 0.85 mM K_2SO_4 and 0.1 mM KCl, whereas deficiency pots received 0.85 mM $CaSO_4.2H_2O$, 0.05 mM $CaCl_2.2H_2O$, 0.025 or 0.05 mM KH_2PO_4 and 0.09 or 0.08 mM $Ca(H_2PO_4)_2$ as additional nutrients. The nutrient solutions were refreshed every 3 days. At 10 days after transfer of plants (10 DAT) nutrient solution was renewed for a final time and then sampled at 0 h and 72 h (i.e. at 13 DAT) to calculate changes in uptake of nutrients as affected by different K application rates. At 13 DAT plants were harvested in the following fractions. Out of 25 plants in each pot, 15 plant shoots were harvested seperately for mineral element analysis, whereas roots of

all 25 plants were harvested together. Roots were washed first in 1 mM $CaCl_2$ solution, then with distilled water. Shoots of the remaining 10 plants were divided into two fractions: the two oldest leaves and the remaining shoot parts. Harvested samples were put into paper bags and oven-dried at 60°C until a constant weight.

B.3. Digestion and Element Analysis

B.3.1 Closed-vessel digestion

Oven-dried shoot and root samples were ground into fine powder using an agate vibrating cup mill (Pulverisette 9; Fritsch GmbH; Germany). These powder samples were then weighed (ca. 0.2 g) and digested in a closed-vessel microwave system (MarsExpress, CEM Corp., Matthews, NC, USA) with 2 ml of 30 % H_2O_2 (w/v) and 5 ml of 65 % HNO_3 (w/v). Following the digestion, the sample volume was brought up to 20 mL with ultra-pure water (18.2 MΩ). After filtration, mineral element concentrations were determined with an inductively coupled plasma optical emission spectrometer (ICP-OES) (Vista-Pro Axial, Varian Pty Ltd., Mulgrave, Australia).

B.3.2 Open-vessel digestion

Shoots and roots were harvested into 20 mL glass vials and oven-dried. The dry weight of the samples ranged between 50 and 370 mg. All vials were added 1.5 ml of 30% H_2O_2 (w/v) and then 3 ml of 65 % HNO_3 (w/v) including blank samples. Samples were incubated overnight and then wet-digested on a hot plate set to 130°C. Digestate was disolved in 20 ml of 5 % HNO_3, filtred and analyzed for ICP-range mineral elements as described above.

B.4 Analysis of Plant Root Systems

Whole roots of single plants were immersed in a transparent plastic tray filled with ultra-pure water and scanned with a calibrated scanner (Epson Perfection V700 Photo, Epson, Japan). Root length, root surface area, root volume, number of root tips and number of root forks were determined using the WinRHIZO image analysis software (Regent Instruments Inc., Quebec, Canada).

B.5 Determination of Nitrate Concentration

Nitrate concentration in leaves and nutrient solution was determined according to the colorimetric method described by Cataldo et al. (1975). 50 mg (±1) of fine powder sample was weighed and extracted in 5 ml distilled water in a water bath set to 45°C for one hour. Samples were then centrifuged and the supernatans were collected. To 100 µL of sample extract, 0.4 mL sulfuric acid containing 5% salicylic acid was added. After 20 minutes, 9.5 mL of 2 N NaOH solution was added. Samples were cooled down to room temperature and the intensity of the yellow color was read at 410 nm against nitrate standards.

B.6 Determination of Total Free Amino Acids

Total free amino acid concentration in leaves was determined according to the spectroscopic method described by Sadasivam and Manickam (1996). 50 mg (±1) of fine powdered leaf samples were extracted in 5 mL 80% Ethanol (v/v). Following the centrifugation, the supernatants were collected. To 100 µl sample extract, 1 mL ninhydrin reagent was added and the total volume was brought up to 2 mL by adding distilled water. The mixture was incubated in a water bath set to 95°C for 20 minutes. To that mixture, 5 mL of diluent solvent (1:1 n-propanol:distilled water) was added. After 15 minutes, the intensity of the purple color was read at 570 nm against leucine standards.

B.7 Determination of Water-soluble Carbohydrates

The procedure described by Yemm and Willis (1954) was used to determine water-soluble carbohydrate concentrations in leaves and roots, but with slight modifications. Fine-powdered leaf and root samples (ca. 50 mg (±1)) were extracted in 5 ml 80 % ethanol (v/v). The extracts were centrifuged at 5000 *g* and the supernatants were collected. For the preparation of anthrone reagent, 0.6 g anthrone was weighed in a glass beaker and 100 ml 20 % ethanol (v/v) was added. To this solution, 300 mL 98 % H_2SO_4 was added very slowly. The glass beaker was kept in a container full of ice and the anthrone solution was allowed to cool down to room temperature before using. To 250 µl sample extract, 4 mL cold anthrone reagent was added. The mixture was incubated in a water bath set to 95°C for 11 minutes. The samples were allowed to cool down to room temperature and the color intensity was read at 620 nm against D-glucose standards.

B.8 Statistical Analysis

All statistical analyses were carried out using JMP (13.0.0) (SAS Institute Inc., Cary, NC, USA) software. The data were subjected to analysis of variance (ANOVA) to evaluate the significance of treatment effects. Tukey's honestly significant difference (HSD) test at the 5 % level ($p < 0.05$) was applied to determine the significant differences between treatment means.

(C) RESULTS

C.1. Experiments on K and Mg Nutrition on Root Morphology

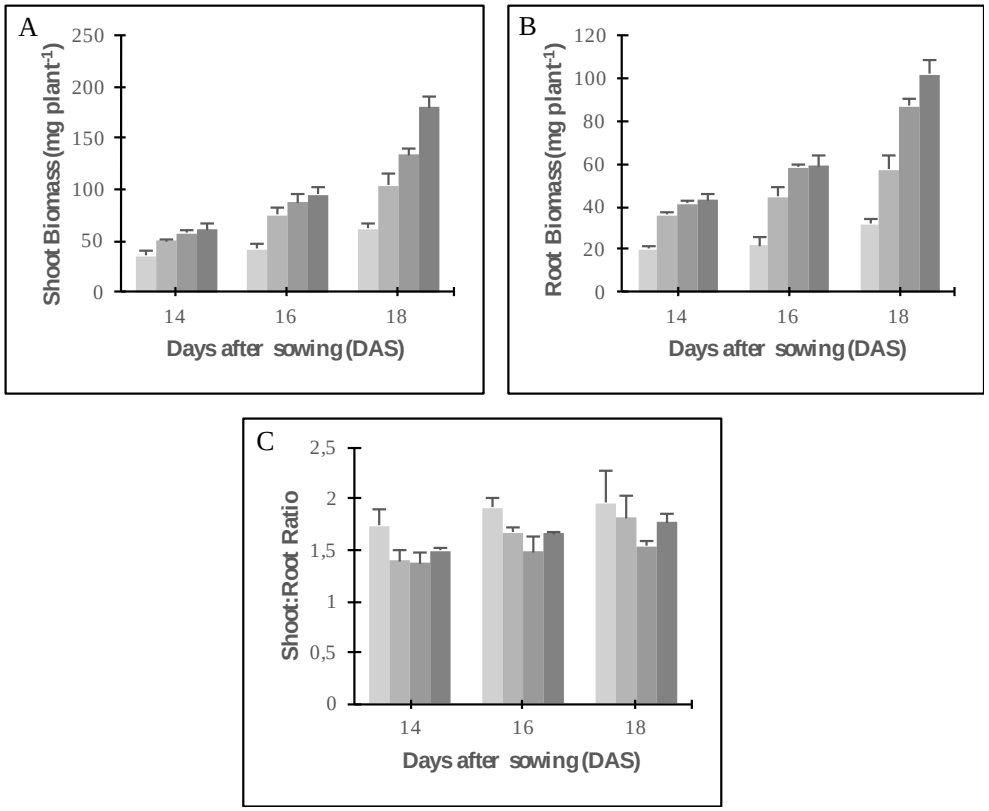
C.1.1. Potassium Nutrition on Root Morphology

K-deficient plants showed leaf tip burns and necrotic lesions in the older leaves, whereas these symptoms were not present in plants supplied with adequate K. K deficiency greatly restricted both root and shoot growth and resulted in stunted plants. Root growth was appeared to be more affected by K deficiency than the shoot growth (Figure 1.1.1).

Shoot and root dry matter production and shoot-to-root ratios of 14-, 16- and 18-day-old wheat plants grown under varied K nutrition are shown in Figure 1.1.2. K deficiency dramatically reduced shoot and root dry matter production in all of the deficiency treatments when compared to K-adequate plants. The difference between the shoot dry matter of K-deficient plants and K-adequate plants increased with time. Compared to control plants, lowest K (10 μM) treatment reduced shoot biomass by 42%, 56% and 65% in 14-, 16-, and 18-day-old wheat plants respectively. The reduction in root dry matter in the same treatment was higher (i.e. 52%, 63% and 68% in 14-, 16-, and 18-day-old wheat plants respectively), leading to a greater shoot-to-root ratio in K-deficient plants. Interaction of KxTime was found as significant ($p<0.05$) for all shoot, root and shoot:root ratio due to increasing effect of K-deficiency stress with duration of time (Figure 1.1.2.).

Figure 1.1.1: Shoot and root growth of 18-day-old wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown in nutrient solution with very low (10 µM), low (30 µM), medium (50 µM) and adequate (2000 µM) K supply.

Figure 1.1.3 shows scanned images of shoots and roots of the 14-, 16-, and 18-day-old wheat plants grown hydroponically under various K treatments. K-deficient plants had long and slender leaves, whereas K-adequate plants had thicker leaves. Both time and increasing K supply enhanced shoot and root growth. The shoot and root growth rate of K-deficient plants were much slower than K-adequate plants. Root growth was even more affected than the shoot growth in all deficiency treatments. Lateral root and root hair formation were severely reduced as a result of K deficiency. Root density was increased by increasing K supply.



Shoot Biomass: $HSD_{0.05}$ (K, Time, KxTime) = (9.15, 7.18, 20.72)
Root Biomass: $HSD_{0.05}$ (K, Time, KxTime) = (5.06, 3.97, 11.46)
Shoot:Root Ratio: $HSD_{0.05}$ (K, Time, KxTime) = (0.18, 0.14, 0.4)

Figure 1.1.2: Shoot (A) and root (B) biomass production and shoot-to-root ratio (C) of 14-, 16-, and 18-day-old wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown in nutrient solution with very low (10 µM), low (30 µM), medium (50 µM) and adequate (2000 µM) K supply.

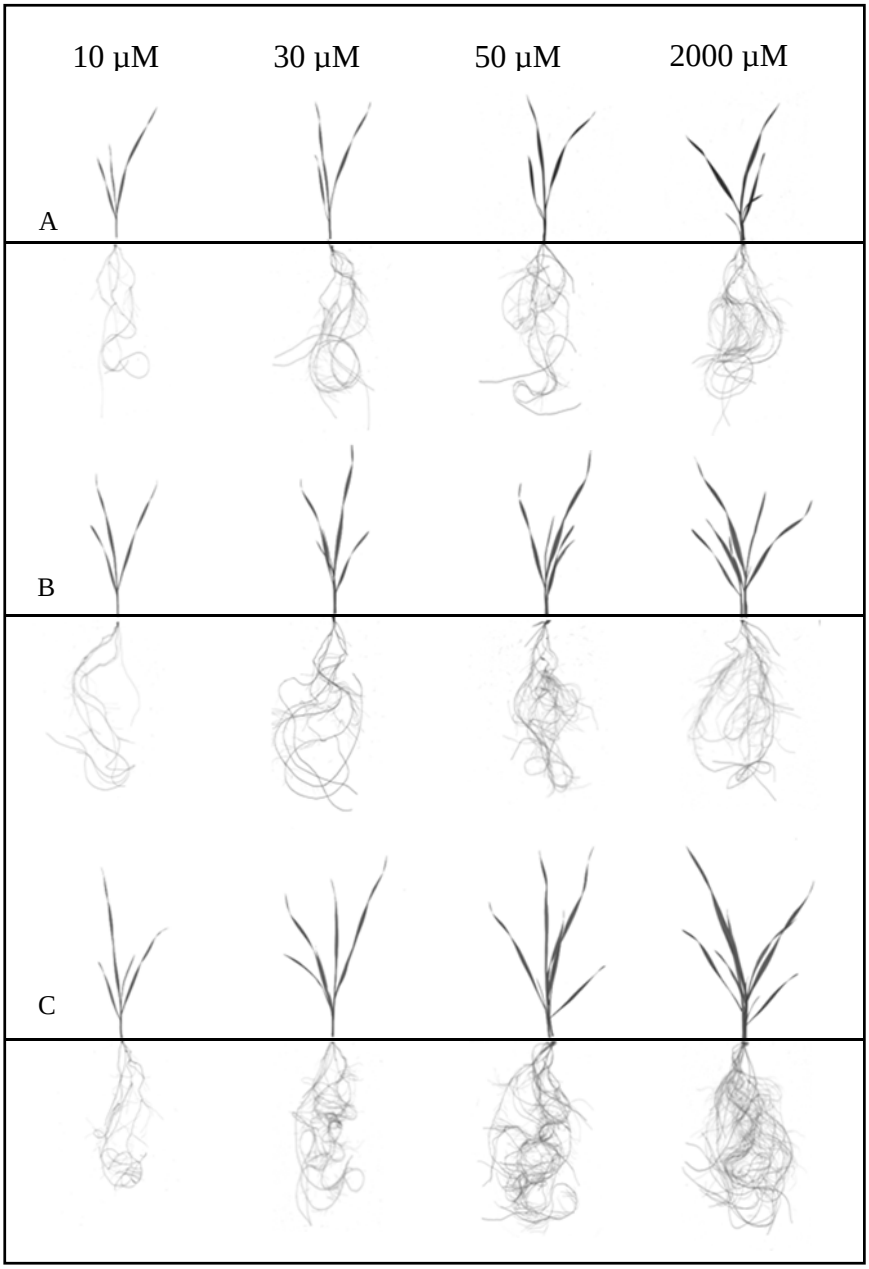
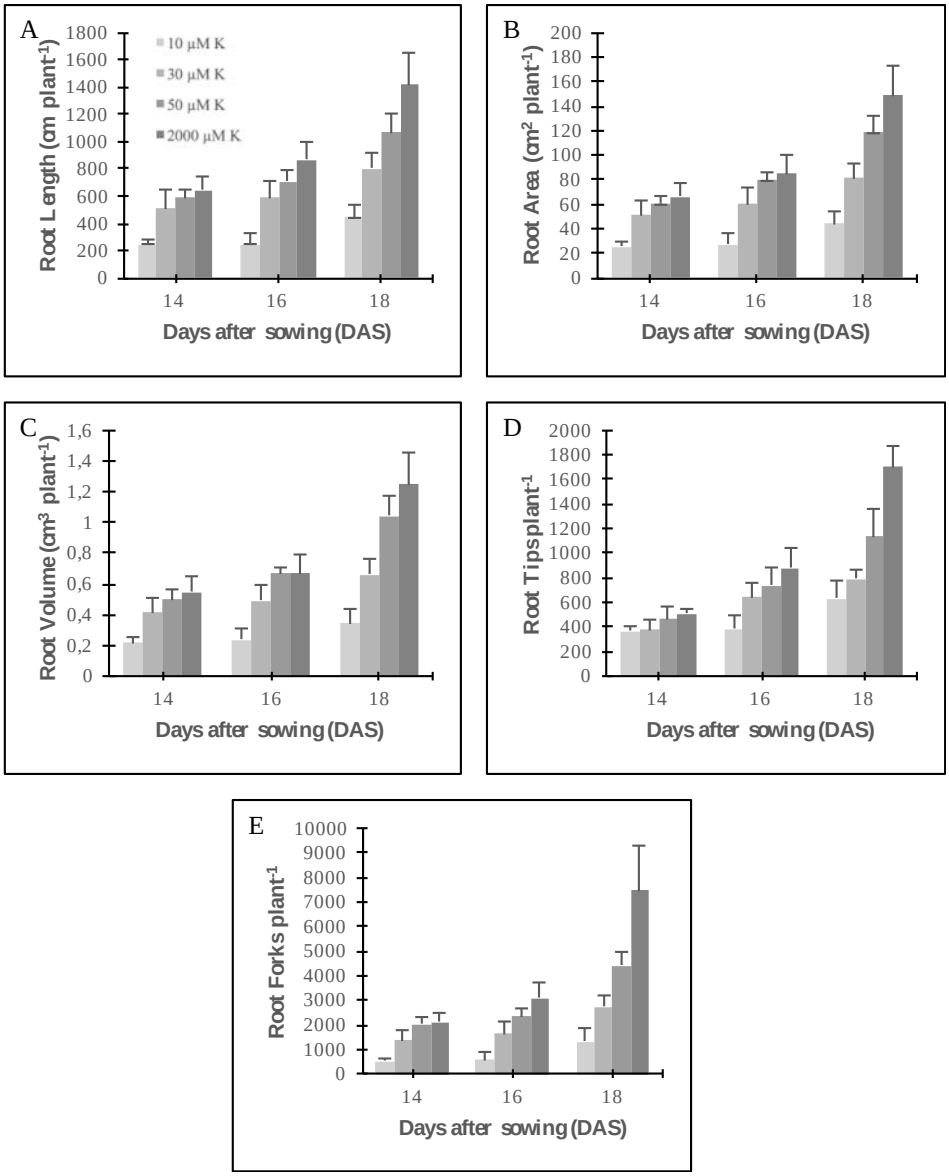


Figure 1.1.3: Shoot and root images of 14- (A), 16- (B) and 18-day-old (C) wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown in nutrient solution with very low (10 µM), low (30 µM), medium (50 µM) and adequate (2000 µM) K supply.

Root morphological parameters (i.e., root length, root area, root volume, number of root tips and root forks) were significantly affected by the absence of adeqaute K supply. Among the root parameters studied, the most sensitive parameter was found to be the number of root forks (Fig 1.1.4). Compared to adequate-K plants, lowest K treatment reduced number of root forks by 82% in 18-day-old wheat plants. This reduction was 63% and 40% in low-K and medium-K treatments, respectively. On 14 days after germination, the overall reduction in root morphological parameters was over 26%, 21% and 7% in very low, low and medium-K treatments. On 18 days after germination, the overall reduction was over 63%, 43% and 16%, respectively.



Root Length: $HSD_{0.05}$ (K, Time, KxTime) = (67, 53, 149)
Root Area: $HSD_{0.05}$ (K, Time, KxTime) = (6.9, 5.5, 15.3)
Root Volume: $HSD_{0.05}$ (K, Time, KxTime) = (0.06, 0.04, 0.13)
Root Tips: $HSD_{0.05}$ (K, Time, KxTime) = (72, 57, 158)
Root Forks: $HSD_{0.05}$ (K, Time, KxTime) = (384, 304, 846)

Figure 1.1.4: Root length (A), root area (B), root volume (C), number of root tips (D) and forks (E) of 14-, 16- and 18-day-old wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown in nutrient solution with very low (10 µM), low (30 µM), medium (50 µM) and adequate (2000 µM) K supply.

As expected, both shoot and root K concentrations and contents (Table 1.1.1) of wheat plants increased with increasing K supply. Compared to adequate K treatment, lowest K treatment reduced the shoot and root K concentration of 18-day-old wheat plants by over 6- and 11-fold, respectively. Increasing K supply reduced this difference between deficient and adequate plants. Shoot K contents of 18-day-old wheat plants ranged from 0.53 and 9.5 mg plant^{-1} and it decreased by 18-fold in very-low-K treatment in comparison to adequate-K treatment. Similarly, root K content of adequate-K plants was over 36-, 20- and 10-fold higher than of very-low-, low- and medium-K plants, respectively.

Table 1.1.1: Shoot and root K concentrations (A) and contents (B) of 14-, 16- and 18-day-old wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown in nutrient solution with very low (10 µM), low (30 µM), medium (50 µM) and adequate (2000 µM) K supply.

A	K Concentration (%)					
K Supply	**14 DAS**		**16 DAS**		**18 DAS**	
	Shoot	Root	Shoot	Root	Shoot	Root
Very Low	0.92 ± 0.04	0.68 ± 0.01	1.14 ± 0.06	0.70 ± 0.03	0.85 ± 0.05	0.60 ± 0.02
Low	1.66 ± 0.12	0.82 ± 0.05	1.79 ± 0.04	0.77 ± 0.03	1.27 ± 0.13	0.57 ± 0.05
Medium	2.36 ± 0.11	1.05 ± 0.10	2.41 ± 0.20	1.07 ± 0.12	1.50 ± 0.03	0.77 ± 0.02
Adequate	5.65 ± 0.23	7.11 ± 0.14	5.37 ± 0.14	6.90 ± 0.10	5.28 ± 0.10	6.79 ± 0.24
B	**K Content (mg plant^{-1})**					
K Supply	**14 DAS**		**16 DAS**		**18 DAS**	
	Shoot	Root	Shoot	Root	Shoot	Root
Very Low	0.32 ± 0.04	0.14 ± 0.01	0.47 ± 0.04	0.15 ± 0.03	0.53 ± 0.02	0.19 ± 0.02
Low	0.82 ± 0.04	0.30 ± 0.03	1.33 ± 0.13	0.34 ± 0.04	1.32 ± 0.09	0.33 ± 0.04
Medium	1.35 ± 0.01	0.44 ± 0.05	2.08 ± 0.11	0.63 ± 0.07	2.01 ± 0.13	0.67 ± 0.02
Adequate	3.44 ± 0.24	2.91 ± 0.24	5.10 ± 0.44	3.95 ± 0.26	9.50 ± 0.52	6.91 ± 0.42

Shoot K Concentration: $HSD_{0.05}$ (K, Time, KxTime) = (0.11, 0.09, 0.26)
Root K Concentration: $HSD_{0.05}$ (K, Time, KxTime) = (0.1, 0.086, 0.24)
Shoot K Content: $HSD_{0.05}$ (K, Time, KxTime) = (0.2, 0.16, 0.45)
Root K Content: $HSD_{0.05}$ (K, Time, KxTime) = (0.01, 0.01, 0.03)

In the absence of adequate K supply, shoot and root Mg concentrations of 14-, 16- and 18-day old wheat plants were found to be increased (Table 1.1.2). Following 18 days after sowing, shoot Mg concentration of adequate-K plants was only one third of very-low-K plants. Low- and medium-K treatments also increased the shoot Mg concentration, but in lower rates. In comparison to adequate-K treatment, root Mg concentration in very-low-, low- and medium was 5-, 5.6- and 6-fold higher, respectively. The similar trend was also observed in shoot and root Mg contents of 14-, 16- and 18-day-old wheat plants.

K-deficient plant shoots and roots were found to be richer in Mg content than adequate-K plants.

Table 1.1.2: Shoot and root Mg concentrations (A) and contents (B) of 14-, 16- and 18-day-old wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown in nutrient solution with very low (10 µM), low (30 µM), medium (50 µM) and adequate (2000 µM) K supply.

Mg Concentration (mg kg^{-1})						
K Supply	**14 DAS**		**16 DAS**		**18 DAS**	
	Shoot	Root	Shoot	Root	Shoot	Root
Very Low	5911 ± 565	10320 ± 391	6388 ± 287	9403 ± 141	6793 ± 741	8377 ± 446
Low	5422 ± 117	12375 ± 238	5396 ± 156	10637 ± 278	5844 ± 299	9440 ± 538
Medium	4584 ± 62	10778 ± 42	4840 ± 355	9733 ± 411	5315 ± 59	10265 ± 227
Adequate	2377 ± 187	1965 ± 208	2301 ± 89	1733 ± 184	2243 ± 285	1683 ± 186

Mg Content (μg plant^{-1})						
K Supply	**14 DAS**		**16 DAS**		**18 DAS**	
	Shoot	Root	Shoot	Root	Shoot	Root
Very Low	208 ± 13	211 ± 16	266 ± 41	206 ± 36	421 ± 25	269 ± 35
Low	270 ± 3	443 ± 27	403 ± 48	474 ± 34	607 ± 38	545 ± 87
Medium	262 ± 15	448 ± 12	419 ± 36	570 ± 24	709 ± 43	891 ± 38
Adequate	144 ± 3	81 ± 17	219 ± 17	100 ± 19	405 ± 70	172 ± 26

Shoot Mg Concentration: $HSD_{0.05}$ (K, Time, KxTime) = (434, 340, 982)
Root Mg Concentration: $HSD_{0.05}$ (K, Time, KxTime) = (399, 313, 903)
Shoot Mg Content: $HSD_{0.05}$ (K, Time, KxTime) = (45.5, 38, 103)
Root Mg Content: $HSD_{0.05}$ (K, Time, KxTime) = (47, 37, 107)

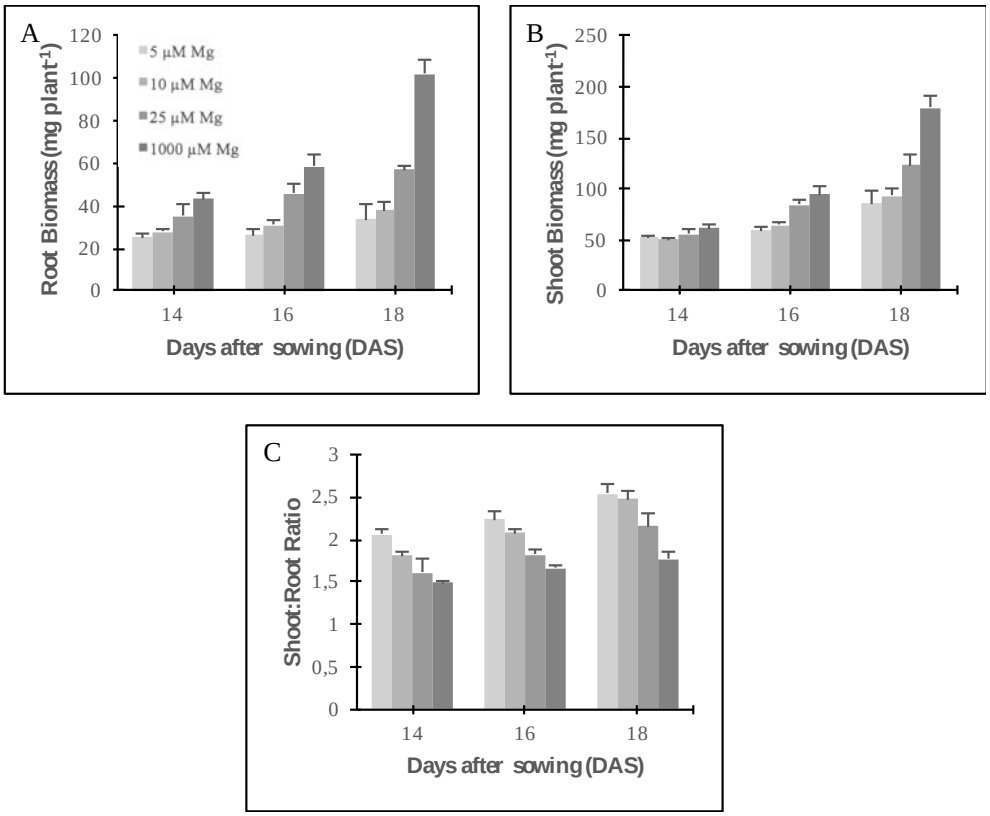
C.1.2 Magnesium Nutrition and Root Morphology

Magnesium deficient plants showed typical interveinal chlorosis in the older leaves. Chlorotic symptoms were pronounced with decreasing Mg supply. Magnesium deficiency clearly reduced both shoot and root growth (Fig 1.2.1). Newly emerging leaves of deficient plants were very thin and not fully developed, whereas the young leaves of adequate-Mg plants grew very healthy. Increasing Mg supply enhanced shoot elongation. Very-low-Mg plants were about half the size of adequate-Mg-plants in terms of shoot elongation.

Figure 1.2.1: Shoot and root growth of 18-day-old wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown in nutrient solution with very low (5 µM), low (10 µM), medium (25 µM) and adequate (1000 µM) Mg supply.

In parallel with the visual observations, shoot and root dry matter production was significantly restricted in the absence of adequate Mg nutrition (Fig 1.2.2). Root dry mass production of 18-day-old wheat plants ranged from 34 to 102 mg plant-1 under varied Mg nutrition. Very-low-, low- and medium-Mg treatments reduced root biomass by 3-, 2.6- and 1.7-fold, respectively. In comparison to roots, shoot dry matter production was less affected by Mg deficiency. Following 18 days after sowing, shoot biomass ranged from 86 to 180 mg plant-1 and in lowest-Mg treatment, shoot dry weight was reduced by 50% in comparison to adequate-Mg treatment. Root growth being much more affected resulted in higher shoot-to-root ratios under deficiency treatments.

Shoot and root images of 14-, 16- and 18-day-old wheat plants grown hydroponically under various Mg supply are presented in Figure 1.2.3. All of the deficiency treatments resulted in limited shoot and root growth, whereas higher Mg supply enhanced shoot and root growth rate. Root architecture was disrupted in the absence of adequate Mg supply. Root hair formation was severly restricted or even completely inhibited under deficiency treatments. Increasing Mg supply resulted in denser root systems (i.e., increased root surface area, root volume).



Shoot Biomass: $HSD_{0.05}$ (Mg, Time, MgxTime) = (9,17, 7.18, 20.04)
Root Biomass: $HSD_{0.05}$ (Mg, Time, MgxTime) = (5.52, 4.33, 12.5)
Shoot:Root Ratio: $HSD_{0.05}$ (Mg, Time, MgxTime) = (0.12, 0.09, 0.27)

Figure 1.2.2: Shoot (A) and root biomass (B) production and shoot:root ratio (C) of 14-, 16-, and 18-day-old wheat (Triticum aestivum cv. Ceyhan-99) plants grown in nutrient solution with very low (5 µM), low (10 µM), medium (25 µM) and adequate (1000 µM) Mg supply.

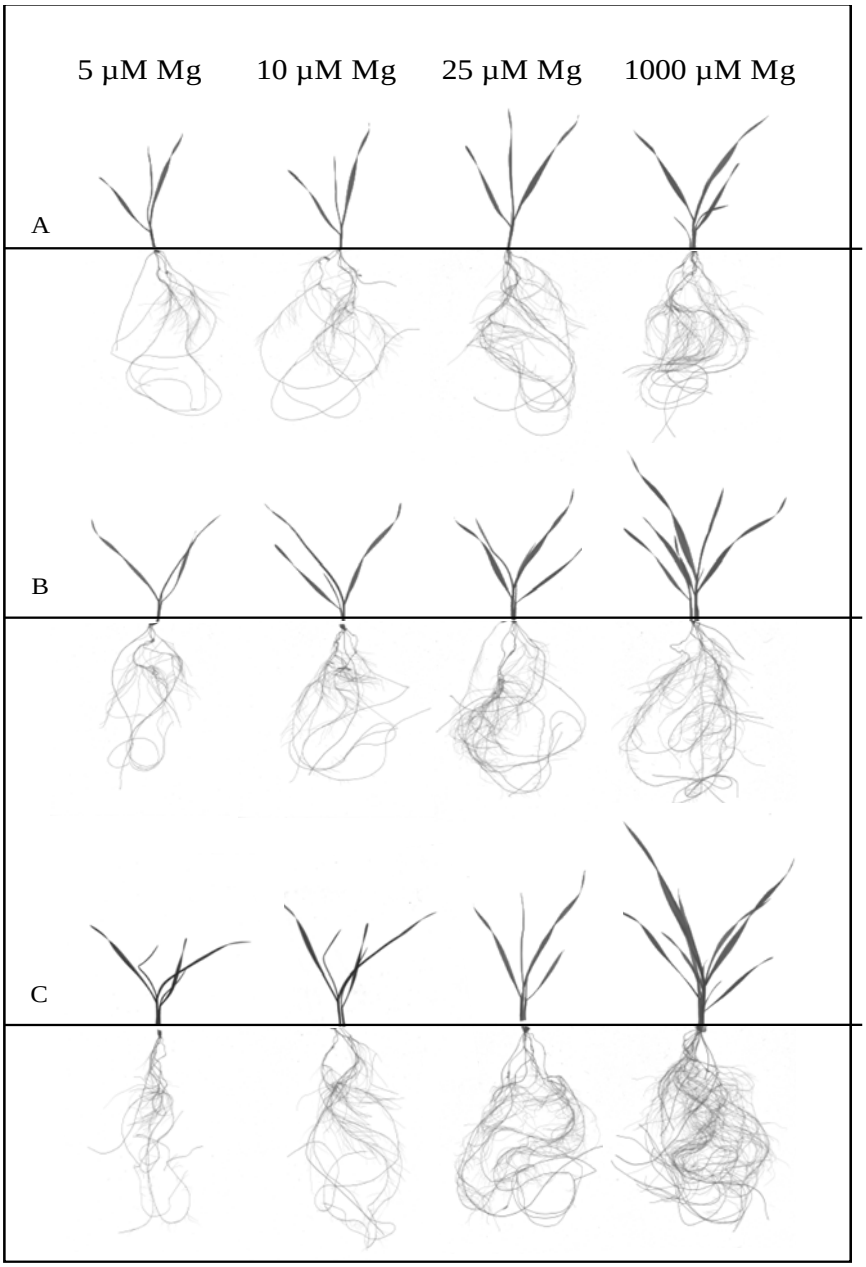
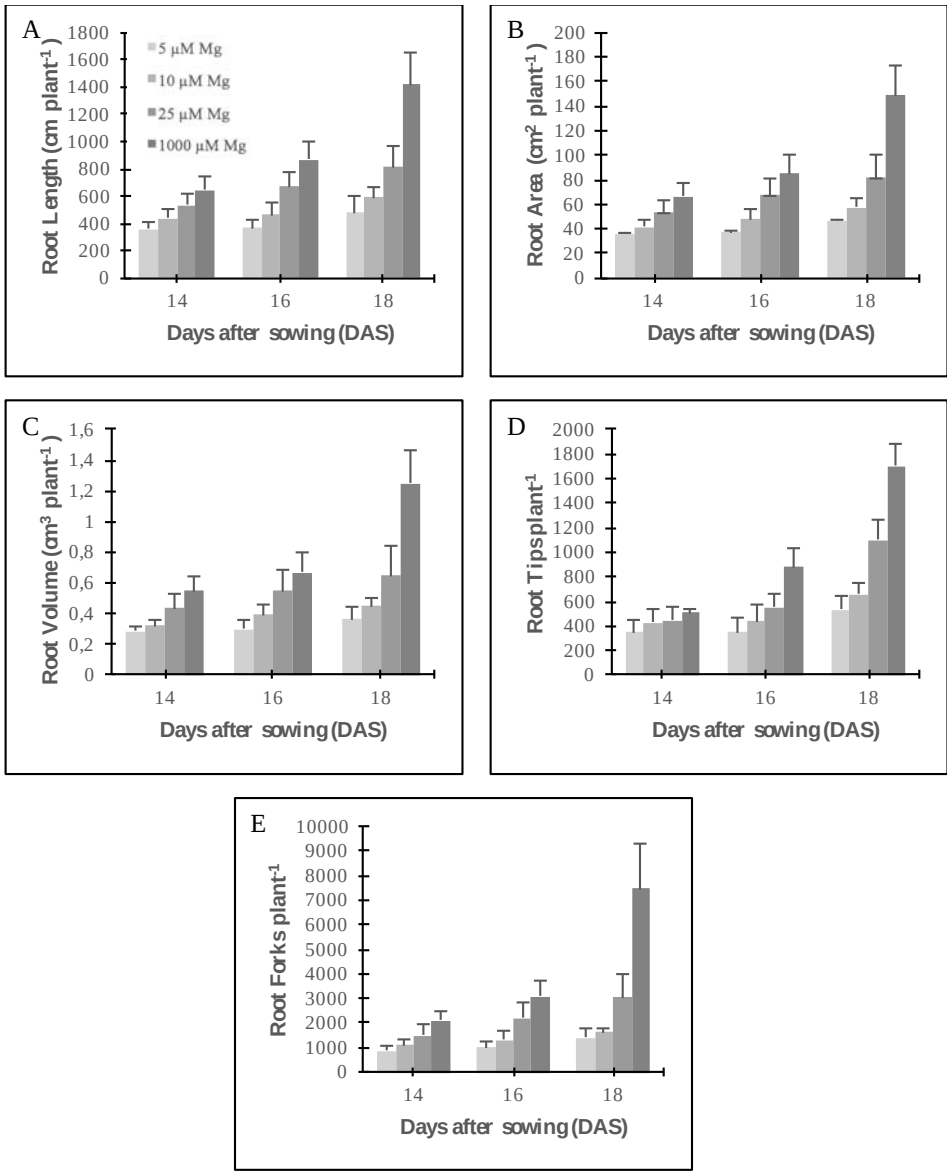


Figure 1.2.3: Shoot and root images of 14- (A), 16- (B) and 18-day-old (C) wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown in nutrient solution with very low (5 µM), low (10 µM), medium (25 µM) and adequate (1000 µM) Mg supply.

The effect of Mg nutrition on root morphological parameters are shown in Figure 1.2.4. As expected, both time and increasing Mg supply promoted root length, root area, root volume, number of root tips as well as number of root forks. All of these parameters were found to be very sensitive to Mg nutrition. Very-low-Mg treatment resulted in an overall reduction by over 65% in the studied parameters on the 18. day after sowing. This reduction was over 58% and 35% in low- and medium-Mg treatments, respectively. Among the parameters studied, the most sensitive root morphological parameter to Mg nutrition was found to be the number of root forks and root volume. Root volume was increased upto 70% under adequate Mg supply in comparison to deficiency treatments. Root forks were even more sensitive to Mg deficiency. Lowest-Mg treatment reduced the number of root forks by over 80% compared to adequate-Mg treatment.



Root Length: $HSD_{0.05}$ (Mg, Time, MgxTime) = (92, 72, 205)
Root Area: $HSD_{0.05}$ (Mg, Time, MgxTime) = (10, 8, 22)
Root Volume: $HSD_{0.05}$ (Mg, Time, MgxTime) = (0.09, 0.07, 0.2)
Root Tips: $HSD_{0.05}$ (Mg, Time, MgxTime) = (113, 89, 252)
Root Forks: $HSD_{0.05}$ (Mg, Time, MgxTime) = (574, 452, 1279)

Figure 1.2.4: Root length (A), root area (B), root volume (C), number of root tips (D) and forks (E) of 14-, 16- and 18-day-old wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown in nutrient solution with very low (5 µM), low (10 µM), medium (25 µM) and adequate (1000 µM) Mg supply.

The impact of various Mg supply on shoot and root Mg concentration and content of experimental plants is given in Table 1.2.1. As expected, increasing Mg supply had a positive effect on both on shoot and root Mg concentrations and contents. In all 14-, 16- and 18-day-old wheat plants highest shoot and root Mg concentrations were observed at adequate Mg supply. Similarly, Mg content of shoots and roots increased significantly with increasing Mg treatments. Mg content in shoots varied between 28 and 405 µg plant^{-1} and shoot Mg content of adequate-Mg plants was over 14-fold higher than of very-low-Mg plants. Similar results were obtained in root Mg contents as well.

Table 1.2.1: Shoot and root Mg concentrations (A) and contents (B) of 14-, 16- and 18-day-old wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown in nutrient solution with very low (5 µM), low (10 µM), medium (25 µM) and adequate (1000 µM) Mg supply.

Mg Concentration (mg kg^{-1})						
Mg Supply	**14 DAS**		**16 DAS**		**18 DAS**	
	Shoot	Root	Shoot	Root	Shoot	Root
Very Low	459 ± 25	637 ± 18	368 ± 12	586 ± 18	332 ± 15	551 ± 26
Low	501 ± 8	728 ± 28	429 ± 6	651 ± 19	383 ± 44	640 ± 17
Medium	659 ± 24	862 ± 102	543 ± 25	762 ± 87	478 ± 18	805 ± 127
Adequate	2378 ± 187	1966 ± 208	2302 ± 89	1733 ± 184	2244 ± 285	1683 ± 186

Mg Content (µg plant^{-1})						
Mg Supply	**14 DAS**		**16 DAS**		**18 DAS**	
	Shoot	Root	Shoot	Root	Shoot	Root
Very Low	24 ± 2	16 ± 1	22 ± 2	15 ± 1	28 ± 4	19 ± 4
Low	25 ± 1	20 ± 1	28 ± 2	20 ± 1	36 ± 5	24 ± 3
Medium	37 ± 4	30 ± 8	46 ± 3	35 ± 1	59 ± 6	46 ± 6
Adequate	144 ± 3	81 ± 17	219 ± 17	100 ± 19	405 ± 70	172 ± 26

Shoot Mg Concentration: $HSD_{0.05}$ (Mg, Time, MgxTime) = (135, 106, 305)
Root Mg Concentration: $HSD_{0.05}$ (Mg, Time, MgxTime) = (145, 114, 328)
Shoot Mg Content: $HSD_{0.05}$ (Mg, Time, MgxTime) = (27, 21, 62)
Root Mg Content: $HSD_{0.05}$ (Mg, Time, MgxTime) = (14, 12, 32)

Previous results showed that Mg concentration and content both in shoots and roots tend to be increased in the absence of adequate K supply (Table 1.1.2). However, there was no such remarkable changes in K concentration or content of Mg-deficient plants (Table 1.2.2). Mg deficiency only slightly increased the shoot and root K concentration and this effect was not significant. K content of shoots and roots increased both with time and increasing Mg supply, whereas low K supply significantly enhanced shoot and root Mg content (Table 1.1.2).

Table 1.2.2:. Shoot and root K concentrations (A) and contents (B) of 14-, 16- and 18-day-old wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown in nutrient solution with very low (5 µM), low (10 µM), medium (25 µM) and adequate (1000 µM) Mg supply.

K Concentration (%)						
Mg Supply	**14 DAS**		**16 DAS**		**18 DAS**	
	Shoot	Root	Shoot	Root	Shoot	Root
Very Low	6.41 ± 0.44	6.79 ± 0.23	6.41 ± 0.23	7.15 ± 0.34	6.22 ± 0.20	7.08 ± 0.33
Low	6.18 ± 0.07	7.22 ± 0.19	6.17 ± 0.33	7.29 ± 0.11	6.19 ± 0.19	7.27 ± 0.19
Medium	5.96 ± 0.17	7.12 ± 0.17	5.37 ± 0.31	6.92 ± 0.13	5.13 ± 0.15	7.54 ± 1.38
Adequate	5.65 ± 0.23	7.11 ± 0.14	5.37 ± 0.14	6.90 ± 0.10	5.28 ± 0.10	6.79 ± 0.24

K Content (mg plant^{-1})						
Mg Supply	**14 DAS**		**16 DAS**		**18 DAS**	
	Shoot	Root	Shoot	Root	Shoot	Root
Very Low	3.35 ± 0.22	1.72 ± 0.04	3.78 ± 0.09	1.89 ± 0.13	5.31 ± 0.68	2.41 ± 0.54
Low	3.11 ± 0.12	2.01 ± 0.12	3.98 ± 0.39	2.27 ± 0.13	5.79 ± 0.39	2.77 ± 0.32
Medium	3.33 ± 0.22	2.49 ± 0.37	4.51 ± 0.06	3.20 ± 0.32	6.32 ± 0.63	4.29 ± 0.67
Adequate	3.44 ± 0.24	2.91 ± 0.24	5.10 ± 0.44	3.95 ± 0.26	9.50 ± 0.52	6.91 ± 0.42

Shoot K Concentration: $HSD_{0.05}$ (Mg, Time, MgxTime) = (0.305, 0.23, 0.64)
Root K Concentration: $HSD_{0.05}$ (Mg, Time, MgxTime) = (0.58, 0.45, 1.32)
Shoot K Content: $HSD_{0.05}$ (Mg, Time, MgxTime) = (2.02, 1.58, 4.56)
Root K Content: $HSD_{0.05}$ (Mg, Time, MgxTime) = (0.24, 0.19, 0.55)

C.1.3 Potassium Resupply to Deficient Plants

Wheat plants grown under low K supply exhibited deficiency symptoms (Figure 1.3.1). Older leaves of the deficient plants were dry on the leaf tip and had a yellowish-brown color. In comparison to adequate-K plants, the leaves and stems of the low-K plants were thinner and tillering was reduced by K-deficiency.

Figure 1.3.1: Shoot growth of 15-day-old wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown in nutrient solution. K was supplied to plants at low (25 μM) and adequate (2000 μM) concentration or resupplied to 12-day-old wheat plants at adequate concentration for 72 hours.

In paralell with the findings of previous experiment, root and shoot growth of wheat plants grown in the absence of adeqaute K nutrition were significantly reduced (Fig 1.3.2). On 12 days after sowing, half of the low-K-treated plants were resupplied with K at adequate concentration for 72 hours. A short term K-resupply enhanced root and shoot growth.

Figure 1.3.2: Effect of K resupply on shoot and root growth of 15-day-old wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown in nutrient solution. K was supplied to plants at low (25 μM) and adequate (2000 μM) concentration or resupplied to 12-day-old wheat plants at adequate concentration for 72 hours.

As expected, low K treatment significantly reduced shoot and root dry matter production (Table 1.3.1). The effect of K resupply on shoot biomass production was significant. 72 hours of K resupply increased shoot dry matter, however its effect on root biomass could not be observed. The effect of K deficiency and K resupply on shoot-to-root ratio was not significant or not an effect at all.

Table 1.3.1: Effect of K resupply on shoot (A) and root (B) biomass production and shoot-to-root ratio (C) of 12-day-old wheat (*Triticum aestivum* cv. Ceyhan-99) plants. K was supplied to plants at low (25 μM) and adequate (2000 μM) concentration or resupplied to 12-day-old wheat plants at adequate concentration for 72 hours.

A	Shoot Biomass (mg plant^{-1})			
K Supply	**0 h**	**24 h**	**48 h**	**72 h**
Low	24.2 ± 0.8	28.3 ± 1.3	34.6 ± 1.6	34.2 ± 2.6
Low + Resupply	24.2 ± 0.8	29.9 ± 1.3	32.7 ± 1.7	37.6 ± 0.9
Adequate	31.0 ± 0.4	39.4 ± 1.7	48.3 ± 1.0	52.9 ± 0.8
B	**Root Biomass (mg plant^{-1})**			
K Supply	**0 h**	**24 h**	**48 h**	**72 h**
Low	14.9 ± 0.9	17.1 ± 0.1	20.3 ± 1.3	20.1 ± 1.7
Low + Resupply	14.9 ± 0.9	16.4 ± 0.9	16.0 ± 2.0	20.5 ± 1.1
Adequate	19.1 ± 1.1	25.1 ± 1.6	30.9 ± 1.2	30.8 ± 2.0
C	**Shoot:Root Ratio**			
K Supply	**0 h**	**24 h**	**48 h**	**72 h**
Low	1.62 ± 0.08	1.66 ± 0.08	1.70 ± 0.04	1.71 ± 0.08
Low + Resupply	1.62 ± 0.08	1.82 ± 0.06	2.05 ± 0.19	1.84 ± 0.07
Adequate	1.62 ± 0.11	1.57 ± 0.04	1.56 ± 0.07	1.72 ± 0.11

Shoot Biomass: $HSD_{0.05}$ (K, Time, KxTime) = (1.18, 1.5, 3.35)
Root Biomass: $HSD_{0.05}$ (K, Time, KxTime) = (1.16, 1.47, 3.31)
Shoot:Root Ratio: $HSD_{0.05}$ (K, Time, KxTime) = (0.08, 0.1, 0.22)

K deficiency and a short term K resupply induced changes in shoot and root morphology of wheat plants (Figure 1.3.2). Stems and leaves of K-deficient plants were remarkably thinner. K resupply accelerated tillering and enhanced shoot growth. Although root dry matter production was not affected, resupplied K induced root hair formation and increased root density as compared to low-K plants.

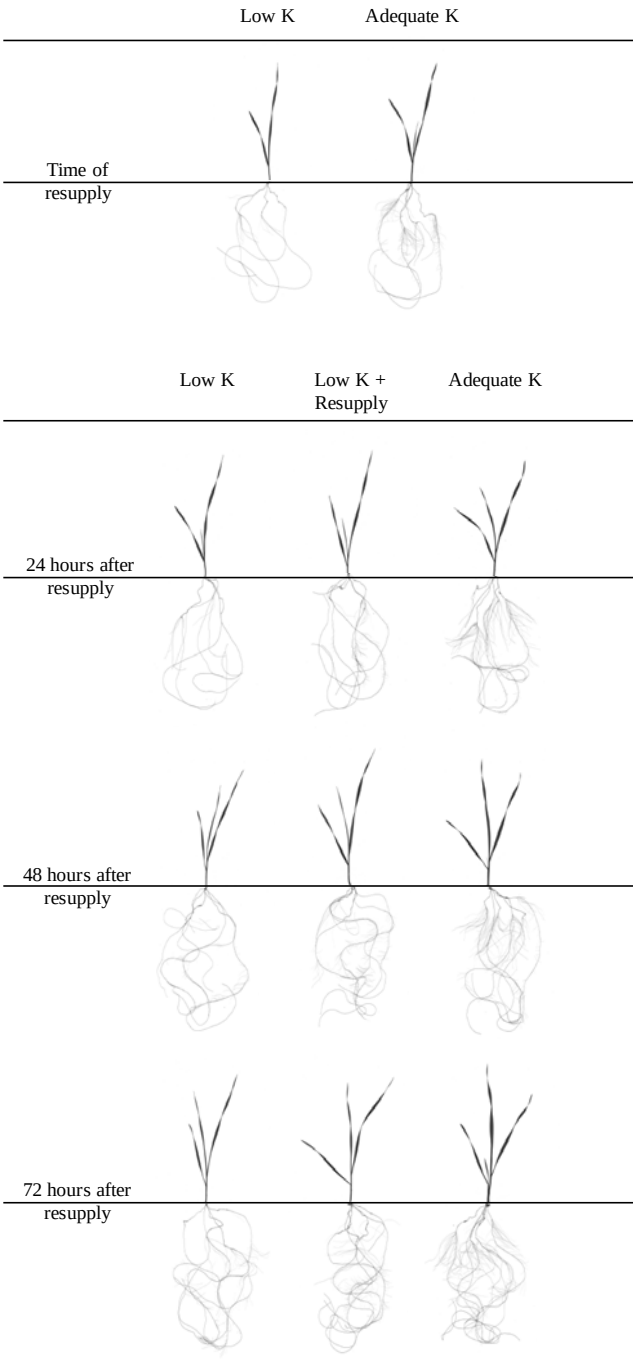
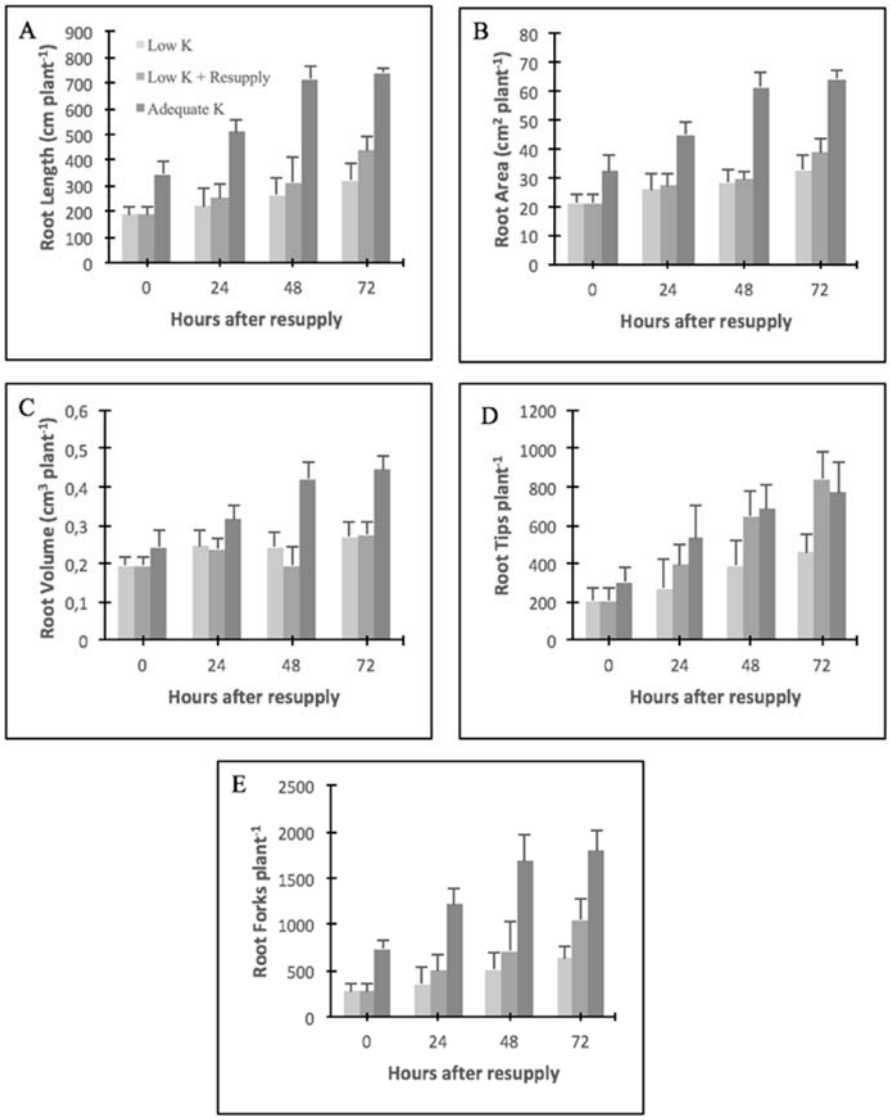


Figure 1.3.3: Effect of K resupply on shoot and root morphology of 12-day-old wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown in nutrient solution. K was supplied to plants at low (25 μM) and adequate (2000 μM) concentration or resupplied to 12-day-old wheat plants at adequate concentration for 72 hours.

As expected, there were significant alterations in the root morpholgy under K depletion (Figure 1.3.3). Most sensitive parameter to K deficiency was found to be root length and number of root forks, whereas root volume was the least sensitive parameter. K resupply on deficient plants markedly increased number of root tips and forks, by 83% and 65%, respectively, after 72 hours. Root length was another parameter that responded to K resupply. In comparison to deficient plants, root length was increased by 36% after 72 hours of K resupply. Such effects were not observed in root area and root volume.



Root Length: $HSD_{0.05}$ (K, Time, KxTime) = (33, 42, 94)
Root Area: $HSD_{0.05}$ (K, Time, KxTime) = (2.5, 3.17, 7.05)
Root Volume: $HSD_{0.05}$ (K, Time, KxTime) = (0.01, 0.02, 0.05)
Root Tips: $HSD_{0.05}$ (K, Time, KxTime) = (68, 86, 191)
Root Forks: $HSD_{0.05}$ (K, Time, KxTime) = (108, 137, 304)

Figure 1.3.3: Effect of K resupply on root length **(A)**, root area **(B)**, root volume **(C)**, number of root tips **(D)** and forks **(E)** of 12-day-old wheat (*Triticum aestivum* cv. Ceyhan-99) plants. K was supplied to plants at low (25 μM) and adequate (2000 μM) concentration or resupplied to 12-day-old wheat plants at adequate concentration for 72 hours.

A short term resupply of K significantly increased shoot and root K concentrations of deficient plants (Table 1.3.2). Very soon after resupply treatment (i.e. 24 hours following K-resupply), the shoot K concentration increased by 2.6 fold in comparison to low-K plants and there was no significant difference in the shoot K concentration of K-resupplied and K-adequate plants. Similarly, the root K concentration increased by 4.1 fold. The positive effect of K-resupply was also observed in shoot and root K contents. In comparison to low-K treatment, 72 hours of K-resupply increased shoot and root K content by 4.7 and 6.2 fold, respectively.

Table 1.3.2: Effect of K resupply on shoot and root K concentration **(A)** and contents **(B)** 12-day-old wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown in nutrient solution. K was supplied to plants at low (25 μM) and adequate (2000 μM) concentration or resupplied to 12-day-old wheat plants at adequate concentration for 72 hours.

		K Concentration (%)			
	K Supply	**0 h**	**24 h**	**48 h**	**72 h**
Shoot	**Low**	1.5 ± 0	1.4 ± 0	1.3 ± 0.1	1.3 ± 0.1
	Low + Resupply	1.5 ± 0	5.1 ± 0.1	5.4 ± 0.1	5.4 ± 0.1
	Adequate	5.7 ± 0.3	5.3 ± 0	5.4 ± 0.2	5.5 ± 0.1
Root	**Low**	0.9 ± 0	0.8 ± 0	0.7 ± 0	0.7 ± 0
	Low + Resupply	0.9 ± 0	3.6 ± 0.1	4.1 ± 0.2	4.1 ± 0
	Adequate	5.9 ± 0.1	6.1 ± 0.2	6.0 ± 0.1	5.5 ± 0.1

		K Content (μg plant^{-1})			
	K Supply	**0 h**	**24 h**	**48 h**	**72 h**
Shoot	**Low**	360 ± 14	400 ± 13	438 ± 28	431 ± 23
	Low + Resupply	360 ± 14	1532 ± 74	1772 ± 96	2037 ± 66
	Adequate	1777 ± 113	2094 ± 81	2622 ± 108	2915 ± 79
Root	**Low**	140 ± 8	135 ± 7	143 ± 2	134 ± 11
	Low + Resupply	140 ± 8	592 ± 26	667 ± 111	832 ± 47
	Adequate	1120 ± 72	1525 ± 85	1871 ± 125	1684 ± 111

Shoot K Concentration: $HSD_{0.05}$ (K, Time, KxTime) = (0.09, 0.12, 0.28)
Root K Concentration: $HSD_{0.05}$ (K, Time, KxTime) = (0.09, 0.125, 0.2804)
Shoot K Content: $HSD_{0.05}$ (K, Time, KxTime) = (60, 77, 172)
Root K Content: $HSD_{0.05}$ (K, Time, KxTime) = (59, 75, 169)

C.2. Effect of varied K Nutrition on Uptake of Other Mineral Nutrients

K deficiency resulted in yellowish-brown leaves with necrotic lesions (Figure 2.1). Leaves of K-deficient plants were also narrower and stems were thinner than of adequate-K plants. Adequate-K plants had larger leaf area as well. Along with the leaf symptoms, K-deficient plants also reduced shoot and root growth (Figure 2.2). Tillering was significantly restricted in K-deficient plants. Root growth was also severely affected by low and medium K treatments. The roots of these plants were thinner and shorter.

Figure 2.1: Leaves of 18-day-old wheat wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown hydroponically with low (25 μM) or adequate (2000 μM) K supply.

In parallel with visual observations, shoot and root dry mass production were significantly reduced by low and medium K treatments (Table 2.1). Compared to adequate-K plants, shoot biomass was reduced by about 46% and 24% and root biomass was reduced by 44% and 20% under low and medium K supply, respectively. There was no significant difference in the shoot-to-root ratio between treatments.

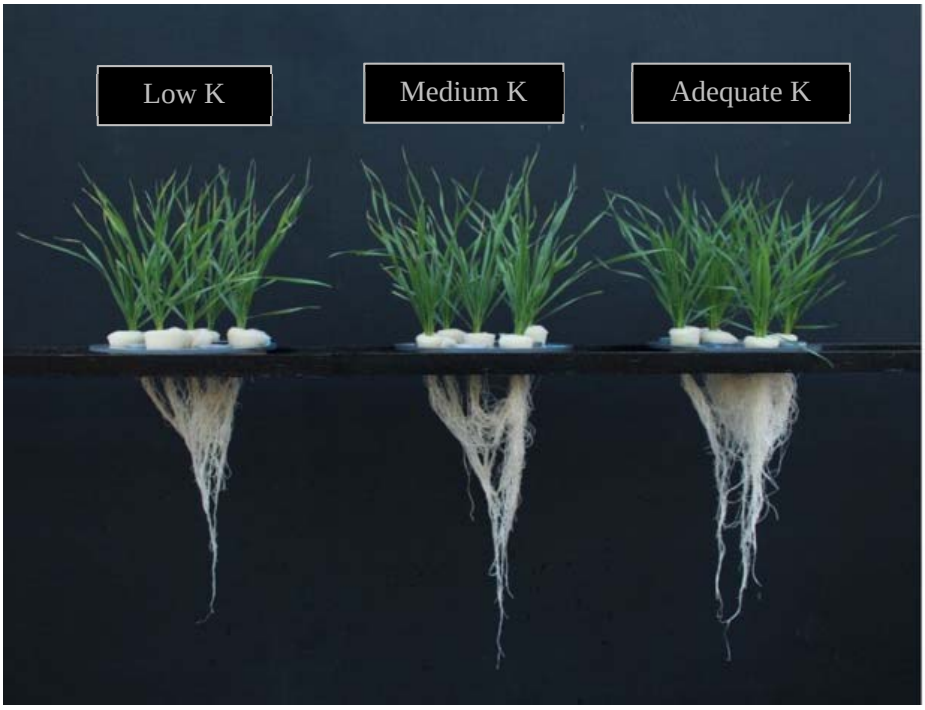


Figure 2.2: Shoot and root growth of 18-day-old wheat wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown hydroponically with low (25 μM), medium (50 μM) and adequate (2000 μM) K supply.

Table 2.1: Shoot and root biomass production and shoot-to-root ratio of 18-day-old wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown hydroponically with low (25 μM), medium (50 μM) and adequate (2000 μM) K supply.

	Shoot Biomass	Root Biomass	Shoot:Root Ratio
K Supply	(mg plant^{-1})		
Low	42 ± 1 c	16 ± 0 c	2.6 ± 0.1 a
Medium	59 ± 1 b	23 ± 1 b	2.5 ± 0.1 a
Adequate	78 ± 4 a	29 ± 2 a	2.7 ± 0.2 a

** Values with different letters within each column differ significantly at P = 0.05 probability level. Each data represents the mean of seven replications.*

The uptake of nutrients was found to be altered by varied K supply (Table 2.2). As expected, there was a linear increase in the K uptake μmol plant^{-1} with increasing K supply. Along with K, the uptake of P and NO_3 per plant also enhanced significantly with increasing K supply. Low K supply reduced P uptake by 46% and NO_3 uptake by 71%. Under medium K supply, the reduction in uptake was 30% and 42% for P and N uptake, respectively. On the other hand, Mg, S and Ca uptake per dry weight unit of root was increased significantly in the absence of adequate K supply. For example, Mg uptake under low K supply was increased by about 160% in comparison to adequate K supply. Similarly, Ca uptake was increased by 276% and 133% under low and medium K supply, respectively.

Table 2.2: Cumulative K, P, S, Mg, Ca and NO_3 uptake of 18-day-old wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown hydroponically with low (25 μM), medium (50 μM) and adequate (2000 μM) K supply.

	Cumulative uptake in 48 hours					
K Supply	**K**	**P**	**S**	**Mg**	**Ca**	**NO_3**
			(μmol plant^{-1})			(mg plant^{-1})
Low	3± 0 b	7± 1 c	22± 5 a	14± 2 a	46± 6 a	2± 1 c
Medium	6± 0 b	9± 1 b	23± 6 a	16± 2 a	41± 6 a	4± 1 b
Adequate	61± 4 a	13± 0 a	20± 6 a	10± 3 b	22± 6 b	7± 1 a

	Cumulative uptake in 48 hours (mg g^{-1} root)					
K Supply	**K**	**P**	**S**	**Mg**	**Ca**	**NO_3**
Low	7 ± 1 b	14 ± 1 a	43 ± 10 a	21 ± 3 a	113 ± 16 a	131 ± 30 b
Medium	10 ± 0 b	12 ± 1 b	32 ± 8 b	16 ± 2 b	70 ± 13 b	152 ± 29 b
Adequate	82 ± 5 a	14 ± 1 a	22 ± 6 b	8 ± 2 c	30 ± 8 c	254 ± 36 a

** Values with different letters within each column differ significantly at P = 0.05 probability level. Each data represents the mean of seven replications.*

Mineral element concentration and content in shoots and roots of 18-day-old wheat plants (Table 2.3) also reflect the effect of K nutrition on the uptake of nutrients. As per the uptake results, K concentration and content in shoots and roots increased with increasing K supply. Unlike the increasing trend in P uptake (Table 2.2), P was found to be more concentrated in K-deficient shoots. Both Mg concentration and content in shoots and roots were significantly affected by various K applications. In the absence of adequate K supply, Mg concentration peaked in shoots and roots, however adequate K supply significantly suppressed shoot and root Mg concentration. Similarly, Mg content of shoots and roots were enhanced particularly at the medium-K level.

Table 2.3: K, Mg, P and S concentrations **(A)** and contents **(B)** in shoots and roots of 18-day-old wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown hydroponically with low (25 μM), medium (50 μM) and adequate (2000 μM) K supply.

A		Mineral Concentrations (mg kg^{-1})			
	K Supply	K	Mg	P	S
Shoot	Low	9859 ± 256 c	6061 ± 54 a	9243 ± 363 a	4200 ± 85 b
	Medium	12482 ± 273 b	5912 ± 117 b	8959 ± 281 a	4587 ± 136 a
	Adequate	53188 ± 691 a	1932 ± 39 c	8559 ± 209 b	4158 ± 73 b
Root	Low	5605 ± 412 b	4853 ± 491 b	5399 ± 263 c	2482 ± 148 b
	Medium	7178 ± 356 b	6217 ± 224 a	6014 ± 90 b	2676 ± 115 a
	Adequate	53830 ± 3395 a	1556 ± 133 c	6924 ± 221 a	2354 ± 86 b

B		Mineral Contents (μg plant^{-1})			
	K Supply	K	Mg	P	S
Shoot	Low	415 ± 9 c	255 ± 5 b	389 ± 9 c	177 ± 4 c
	Medium	738 ± 18 b	350 ± 10 a	530 ± 17 b	271 ± 7 b
	Adequate	4174 ± 252 a	152 ± 8 c	672 ± 44 a	326 ± 13 a
Root	Low	91 ± 9 b	79 ± 10 b	88 ± 6 c	40 ± 3 b
	Medium	168 ± 13 b	145 ± 9 a	141 ± 6 b	63 ± 4 a
	Adequate	1567 ± 186 a	45 ± 6 c	201 ± 16 a	68 ± 6 a

** Values with different letters within each column differ significantly at P = 0.05 probability level. Each data represents the mean of seven replications.*

There were significant alterations in shoot nitrate concentration between treatments (Table 2.4). In all treatments, shoot nitrate was found to be more concentrated in younger leaves than in older leaves. Medium K treatment reduced nitrate concentration in old and young leaves by 72% and 42.5%, respectively. In contrast to nitrate, free amino acid concentration in old leaves, young leaves and shoots was significantly increased under K deficiency (Table 2.5). The highest concentration of free amino acids was observed in the low-K treatment.

Table 2.4: Shoot nitrate concentration of 18-day-old wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown hydroponically with low (25 μM), medium (50 μM) and adequate (2000 μM) K supply.

	Nitrate Concentration (mg g^{-1} DW)		
K Supply	Old Leaves	Young Leaves	Shoot
Low	9 ± 2 b	16 ± 6 b	14 ± 2 b
Medium	7 ± 2 b	16 ± 2 b	17 ± 3 b
Adequate	25 ± 6 a	28 ± 4 a	33 ± 3 a

** Values with different letters within each column differ significantly at P = 0.05 probability level. Each data represents the mean of seven replications.*

Along with free amino acids, concentration of soluble carbohydrates was also affected by K rate (Table 2.6). Soluble carbohydrate concentration was significantly increased by K deficiency, especially in the old leaves. For example, soluble carbohydrate concentration in old leaves was 78% higher in medium-K treatment than in adequate-K. Similarly in the young leaves, it was increased by 46% in comparison to adequate-K plants.

Table 2.5:. Total free amino acid concentration of 18-day-old wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown hydroponically with low (25 μM), medium (50 μM) and adequate (2000 μM) K supply.

	Free Amino Acid Concentration (mg g^{-1} DW)		
K Supply	Old Leaves	Young Leaves	Shoot
Low	32 ± 5 a	31 ± 3 a	35 ± 5 a
Medium	29 ± 3 a	27 ± 2 a	33 ± 6 a
Adequate	22 ± 2 b	20 ± 2 b	23 ± 1 b

** Values with different letters within each column differ significantly at P = 0.05 probability level. Each data represents the mean of seven replications.*

Table 2.6:. Water-soluble carbohydrate concentration of 18-day-old wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown hydroponically with low (25 μM), medium (50 μM) and adequate (2000 μM) K supply.

	Soluble Carbohydrate Concentration (mg g^{-1} DW)		
K Supply	Old Leaves	Young Leaves	Shoot
Low	150 ± 29 b	83 ± 4 b	90 ± 12 b
Medium	211 ± 24 a	101 ± 8 a	125 ± 10 a
Adequate	118 ± 15 c	69 ± 10 c	83 ± 12 b

** Values with different letters within each column differ significantly at P = 0.05 probability level. Each data represents the mean of seven replications.*

Total N concentration in old leaves, young leaves, shoot and roots is presented in Table 2.7 Total N concentration was found to be affected by K treatments. Total N (%) in young leaves, shoot and root showed a linear response to K fertilization and peaked at adequate K level.

Table 2.7: Total nitrogen concentration of 18-day-old wheat (*Triticum aestivum* cv. Ceyhan-99) plants grown hydroponically with low (25 μM), medium (50 μM) and adequate (2000 μM) K supply.

	Nitrogen %			
K Supply	Old Leaves	Young Leaves	Shoot	Root
Low	4,0 ± 0,1 b	5,5 ± 0,4 b	5,4 ± 0,2 c	3,8 ± 0,1 c
Medium	3,6 ± 0,3 c	5,9 ± 0,2 a	5,8 ± 0,1 b	4,2 ± 0,2 b
Adequate	5,0 ± 0,1 a	6,2 ± 0,2 a	7,0 ± 0,2 a	4,6 ± 0,2 a

** Values with different letters within each column differ significantly at P = 0.05 probability level. Each data represents the mean of seven replications.*

(D) DISCUSSION

Development of interveinal chlorosis on older, fully-expanded leaves is typical symptom of Mg deficiency. Expression of leaf chlorosis is attributed to the role of Mg in chlorophyll biosynthesis, since it serves as the central molecule in the chlorophyll molecule (Gransee and Führs, 2012). In the present study, wheat plants supplied with low concentrations of Mg showed interveinal chlorosis on the older leaves (Figure 1.2.1) due to high phloem-mobility of Mg (Broadley et al., 2008). As expected, severity of these symptoms intensified with decreasing Mg availability in growth medium.

Magnesium is one of the essential macronutrients that is required by plants for growth and development. As expected, Mg deficiency significantly decreased shoot and root dry matter production in wheat plants (Figure 1.2.2). Root growth was found to be more affected by Mg deficiency than shoot growth, leading to a greater shoot-to-root ratio in wheat plants. Similar results have also been reported in a number of plant species including wheat, bean, maize and citrus (Cakmak et al., 1994b; Mengutay et al., 2013; Yang et al., 2012).

Root growth is a growth process that requires delivery photoassimilates from shots. Mg is known to be a key player in phloem loading and transport of sucrose (Cakmak and Kirkby, 2008). Very recently, Farhat et al (2016) showed in ^{14}C labeling experiments that the radioactivity distribution within plants was severely affected by the Mg nutritional status of the plants. In case of Mg deficiency, ^{14}C transportation from leaves to shoots was completely inhibited. Cakmak et al. (1994b) reported that a short term resupply of Mg to Mg-deficient plants immediately regenerated sucrose export; suggesting that Mg is directly involved in sucrose-loading into phloem and transport from source organs (fully-expanded leaves) to sink organs such as roots. H^+/sucrose co-transport catalyzes the phloem-loading of sucrose and the proton gradient required for this process is generated by an H^+/ATPase (Bouche-Pillon et al., 1994; Ward et al., 1997). Mg is required for the proper functioning of this enzyme (Bush, 1989; Getz and Klein, 1995) and any alteration in its activity is highly correlated with decreased sucrose export

from source leaves to sink organs such as roots (Zhao et al., 2000). Mg-ATP is one of the important Mg compounds in plant cells and it is used by the H^+/ATPase enzyme in phloem loading of sucrose (Bush, 1989). Probably, low amount of Mg-ATP in the phloem loading zones is a further reason for the impaired phloem transportation of sucrose in plants.

Accumulation of sugars and starch in leaves is a well-documented phenomenon observed under Mg deficiency, suggesting impaired phloem transport of carbohydrates into roots or other sink organs such as seeds. Alterations in the photoassimilate allocation into sink organs is most likely the main cause of reduced root biomass production and increased shoot-to-root ratio (Cakmak and Kirkby, 2008; Gransee and Führs, 2012).

Potassium is also a highly phloem-mobile element and readily translocated within the plant, thus symptoms of deficiency, typically brown scorching and cholorosis, appear firstly on mature old leaves (Hopkins and Huner, 2009). In agreement with the literature, K deficient plants in all experiments presented in this thesis showed yellowish-brown leaf tips with necrotic spots (Figure 1.1.1, 1.3.1, 2.1).

As expected, K deficiency dramatically reduced shoot and root dry weights of wheat plants (Figure 1.1.2, Table 1.3.1, 2.1). Potassium has diverse of critical cellular functions in plants, as described by Marschner (2012), including protein biosynthesis, photosynthesis, transportation of photoassimilates, increasing disease tolerance and mitigation of abiotic stress factors such drought and salinity. Its deficiency therefore results in significant depressions in growth and development of crop plants. After N, K is taken up by crop plants in the largest amount. Severe reductions in shoot and root dry matter production under low supply of K have been reported in a variety of species including wheat (Hirata et al., 1982) and bean (Cakmak et al., 1994b).

As indicated above, K also has a key role in partitioning of carbohydrates between shoot and root and its functions in this process are similar to those of Mg. K deficient plants also accumulate sucrose in source leaves a consequence of impaired phloem loading (Marschner et al., 1996). This shift in C partitioning restricts root growth and directly related to reduced root dry weight under K deficiency. In well-agreement with the existing literature, water-soluble carbohydrate concentration was increased under K deficiency in the present study (Table 2.6) and was found to be more concentrated in the older source leaves than in the younger sink leaves, indicating an inhibited carbohydrate-export from source to sink.

The well-known enhancement in shoot-to-root ratio due to disrupted C allocation to roots has been observed only in the first experiment (Figure 1.1.2). In the third and fourth experiment, the effect of K-level on shoot-to-root ratio was statistically non-significant (Table 1.3.1, 2.1). In these experiments, wheat plants were subjected to K-deficiency stress for a shorter period of time and most probably suffered rather from a milder deficiency. The extent of K deficiency in these plants did not induce any significant changes (i.e. increase) in shoot-to-root ratio.

The third experiment was conducted to monitor changes in growth of the K-deficient wheat plants over a 72 h K re-supply to deficient plants. 72 hours of K-resupply significantly enhanced shoot dry matter production, however this effect could not be observed in roots, most probably due to selected experimental conditions and severity of the K deficiency stress. Upon K application to deficient plants, 4 different pots from each treatment were harvested every 24 hours. Inconsistent root dry weight data is most likely to be the result of the use of different pots for each harvest.

Potassium is directly involved in photosynthesis due to its role in CO_2 exchange rates and stomatal opening/closing (Huber, 1984) therefore, K deficiency dramatically decreases photosynthesis rate (Bednarz et al., 1998; Hermans et al., 2006). Rapid enhancement of shoot dry weight upon K-resupply is most probably due to high involvement of K in photosynthesis. Increased photosynthesis rates would allow plants to produce more photoassimilates, which eventually add up to shoot biomass.

In order to evaluate the damage exerted on roots by K and Mg deficiency, root morphology parameters were studied under varied deficiency conditions of K and Mg. Previous evidence suggests that root morphology is altered by the absence of K and Mg (Niu et al., 2004; Zhang et al., 2008). Both K and Mg deficiency treatments in this study resulted in significant reductions in root length, area, volume, number of root tips and forks (Figure 1.1.4, 1.2.4, 1.3). As discussed above, K and Mg are two key elements that facilitate photosynthate transport between shoot and root. Many authors have pointed out the importance of the assimilate available for root growth (Pearsall, 1923; White, 1937), because maintenance of a high root growth rate depends transportation of carbohydrates from the shoots. Growth process in root or shoots is an energy dependent process and under direct influence of mitochondrial respiration (Marschner, 2012). In relation to reduced root growth, the reductions in the root morphology parameters are a result of impaired C allocation from source leaves.

Number of root tips and forks are good indicators of root hairs and were reduced dramatically under K and Mg deficiency. Root tips of K-deficient plants increased significantly within 24 hours after K-resupply, indicating that root hair formation was very sensitive to K nutrition and promoted if a sufficient K supply is maintained in the growth medium. Root hairs are crucial in terms of nutrient acquisition efficiency, since they facilitate nutrient and water uptake by increasing the interface for absorption. The importance of root hairs on the exploitation of soil nutrients was underlined by previous studies (Claasen and Jungk, 1984). Inhibition of root hair formation, therefore would lead to decreased uptake of nutrients due to less explored soil volume and less access to resources (Cakmak and Kirkby, 2008).

There was an increasing trend in root length, area and number of root forks upon K-resupply, but not in root volume. The reason of this unexpected effect could not be understood. It seems that the most sensitive parameter to K-resupply is the number of root tips and root length. This positive impact of K-resupply is presumably due to increased allocation of assimilates into roots and this suggestion is consistent with the findings reported by Muller et al. (1998), who have shown that carbohydrate availability controls root elongation.

According to Jones et al. (1991), critical deficiency concentrations during early vegetative growth are around 4% for K and 0.1% for Mg. Shoot Mg concentrations were significantly decreased below the critical deficiency threshold by growing the plants under low Mg-(Table 1.2.1). By contrast, plants with adequate Mg supply had sufficiently high Mg in shoots. Similarly, K concentrations of shoots and roots were fairly high at adequate K-level, and were below the critical deficiency threshold under K deficiency treatments (Table 1.1.1, 1.3.2, 2.3). Shoot and root K concentrations of deficient plants increased remarkably upon K-resupply. Only within 24 hours, shoot K concentration increased above critical deficiency threshold and reached sufficient levels. This increment was also observed in roots, but in slower rates. These findings indicate clearly that the plants under low K supply respond very rapidly to re-supply of K and show high root uptake and root-to-shoot transport of K.

The antagonism between K^+ and Mg^{2+} has been widely studied and reported previously (Johnson et al., 1968; Fageria, 1983). High concentrations of K^+ in growth medium inhibit Mg^{2+} uptake from roots as well as its translocation from roots to shoots. In the present study, similar results were observed. Under K-deficiency, Mg uptake per dry weight unit of root was increased by almost 4-fold (Table 2.2) and this promoted root

uptake were reflected well in the shoot and root Mg concentrations (Table 1.1.2, 2.3). These results were consistent with previous studies in wheat (Ohno and Grunes, 1985), tomato (Schwartz and Bar-Yosef, 1983) and rice (Ding et al., 2006). Inhibition of Mg uptake is a consequence of the negative effect of K on (i) net Mg^{2+} translocation from roots to shoots (Huang et al.,1990) and (ii) Mg^{2+}-transport capacity of root (Moore et al., 1961). Probably, there are channel proteins and Mg transporter proteins on root cell membranes which mediate both K and Mg uptake and transport, and in case of high K concentrations in growth medium a competitive inhibition take places in Mg uptake through high K treatments (Guo et al., 2010; Senbayram et al., 2015).

On the contrary, the effect of Mg on K uptake was found to be less effective (Table 1.2.2), although there was an inverse relationship between tissue K concentration and Mg supply. Similar results have been reported by Ologunde and Sorensen (1982) and Ding et al. (2006) in wheat and rice, respectively. These findings support the evidence that the antagonistic effect of K on Mg was more significant than that of Mg on K.

A varied K nutrition had also effects on root uptake and tissue concentrations of other nutrients. The effect of K on Ca uptake was similar to that on Mg. Highest Ca uptake was observed under severe K starvation and increasing K supply dramatically suppressed Ca uptake by roots (Table 2.2). These results were also consistent with shoot and root Ca concentrations (data not shown). The decrease in Ca uptake by roots is closely associated with the increase in K uptake, indicating that there is a competitive interaction between K and Ca, most likely due to well-known antagonism between the cationic ions during root uptake (Marschner, 2012). Similar results have been also reported in literature (Johnson et al., 1968; Fageria, 1983). By contrast, it was shown that there is a positive interaction of K and P (Dibb and Thompson, 1985). In the present study, increasing K supply promoted P uptake from nutrient solution. Lastly, NO_3^- uptake was also affected by K status. Previous evidence suggests that K affects NO_3^- uptake and translocation directly or indirectly (Blevins et al., 1978; Marschner, 2012). K deficiency significantly reduced NO_3^- uptake in the present study. These results are in well-agreement with the findings of Minotti et al. (1969) who have reported impairments in uptake and translocation of NO_3^- in the absence K.

Decreased NO_3^- uptake under K deficiency is also supported by the nitrate contents of young wheat plants (Table 2.4). K deficiency significantly reduced NO_3^- content both in old and young leaves. The decrease in NO_3^- content is due to inhibition of NO_3^- uptake and transport (Armengaud et al.,2009; Gajdanowicz et al., 2011). In

addition, NO_3^- accumulated mainly in young leaves than in old leaves, indicating that N (as amino acids) is phloem-mobile and readily translocated to growing parts from mature source leaves.

Inhibition of NO_3^- uptake under K deprivation is mainly caused by several reasons and these include (i) reduced root growth and size, (ii) synergistic effect of K^+ on uptake and translocation and (iii) changes in concentrations of N-containing compounds. As mentioned above, nutrient acquisition capacity of roots greatly depends on root size, since larger root surface area corresponds to greater interface for nutrient uptake in the rhizosphere, therefore, larger roots are capable of absorb more nutrients from the environment. In relation to this, smaller roots grown under K deficiency can take up less NO_3^- than of those grown under adequate K supply.

K^+ and NO_3^- are two counter-ions, and the root uptake and shoot translocation of these elements greatly depends on their simultaneous presence in growth medium. The synergism of these two ions is also related to their opposite charges which facilitates root uptake. Moreover, root- NO_3^- is translocated to shoot via the xylem, accompanied with K^+ as counter-ion (Coskun et al., 2016). Some studies have shown that K deficiency may result in an increase in the degree of NO_3^- reduction in roots relative to shoots, most probably because NO_3^- cannot be transported in the xylem in the absence of the accompanying cation (Rufty et al., 1981; Förster and Jeschke, 1993; Hu et al., 2016b).

Another aspect that may lead to inhibition of NO_3^- uptake is changes in concentrations of the nitrogenous (N)- compounds in K deficient plants. Accumulation of free amino acids in K deficient leaves has been shown in tobacco (Koch and Mengel, 1974), barley (Helal and Mengel, 1979), maize (Hsiao et al., 1970) and cotton (Hu et al., 2016b). Increase in free amino acid content can be a result of (i) K deficiency-related high peptidase and protease activity, indicating protein degradation, (ii) inhibited protein biosynthesis and (ii) low amino acid export in phloem. It is known that K deficiency severely reduces protein synthesis leading to accumulation of amino acids because of less usage of amino acids in protein synthesis. This suggestion can also be supported with higher free amino acid-to-soluble protein ratio observed in cotton under K deficiency (Hut et al., 2017). In accordance with previous studies, K deficient plants accumulated more amino acid in shoots (Table 2.5) and total N content found to be decreased in low K plants (Table 2.7). These findings indicate that K plays a key role in the distribution of N-compounds and between amino acid and protein. Such changes in amino acid and

protein concentrations may affect regulation of N metabolism and thus depress N assimilation (Wang et al., 2012).

(E) CONCLUSION

Potassium and Mg deficiencies occur commonly in crop plants with severe impacts on growth and yield capacity, especially under environmental stress conditions. Due to their diverse physiological and biochemical functions at cellular level, occurrence of K and Mg deficiencies on agricultural soils affects seriously productivity and also nutrient use efficiency. In this present study, it has been shown that one of the fundamental problems caused by Mg and K deficiencies is the substantial alteration in root morphological parameters. Potassium and Mg deficient plants have failed to develop an efficient root system. Root length, root surface area and number of root tips are most critical root parameters affecting nutrient uptake by roots. These parameters were significantly affected in plants under low supply of K and Mg. Therefore, a reduced nutrient acquisition and nutrient use efficiency can be expected in plants with low K and Mg supply.

It is known that maintenance of an adequate root N uptake depends on the utilization of the root-absorbed N in protein biosynthesis. Protein biosynthesis is very sensitive to low K supply. If the use of absorbed N in protein synthesis is impaired (for example due to K deficiency), this will have a negative feed-back effect on root N uptake, with severe inhibition in N uptake by roots (Marschner, 2012). Therefore, it is of great importance to keep sufficient amounts of K and $N\text{-}NO_3$ in growth medium in order to maintain synergistic uptake between $N\text{-}NO_3$ and K.

Hereby we conclude that ensuring a good K and Mg nutrition is of great importance in terms of both maximizing production as well as the use efficiency of mineral nutrients applied into soils.

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