

Effect of Nutrient Limitation on Physiological and Morphological Plant Traits Related to Growth and Quality of Tomato

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Dedicated to

The souls of my parents

**The soul of my supervisor Prof. Dr. Fathy Header who
passed away during this PhD work**

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LIST OF ABBREVIATIONS

A	Photosynthetic rate	SRL	Specific root length
A/E	Water use efficiency	SSL	Specific stem length
ABA	Absciscic acid	TA	Titrateable acidity
AsA	Ascorbic acid	TD	Root tissue density
CNB	Carbon nutrient balance		
CYT	Cytokinin		
DAT	Days after start of treatments		
DM	Dry mass		
E	Transpiration rate		
F	Root fineness		
FM	Fresh mass		
GDB	Growth differentiation balance		
LA	Leaf area		
LAR	Leaf area ratio		
LMR	Leaf mass ratio		
NAR	Net assimilation rate		
NSC	Non-structural carbohydrate		
PCM	Protein competition model		
RA	Root surface area		
RD	Root diameter		
RGR	Relative growth rate		
RL	Root length		
RLR	Root length ratio		
RMR	Root mass ratio		
ROS	Reactive oxygen species		
RSA	Radical scavenging activity		
RV	Root volume		
SLA	Specific leaf area		
SMR	Stem mass ratio		

Summary

This thesis aims to increase our understanding about physiological and morphological plant responses to K, Mg and N limitation and their relationship with fruit quality. Tomato plants were cultured in nutrient solution. In the control treatment (100% biomass growth), plants were grown at high nutrient concentration to prevent growth limitation by nutrients. In the nutrient-limitation treatments, either K or Mg or N was supplied at rates which reduced total biomass growth to about 80% (mild deficiency) or 60% (severe deficiency) of the control. This experimental approach allowed comparing the long-term plant responses to deficiency of K, Mg or N at well-defined intensities of nutrient limitation.

In chapter two, focus was on the effects of nutrient limitation on biomass and nutrient allocation. Twenty days after start of treatments (DAT), the effect of nutrient limitation on biomass and nutrient allocation to various plant organs was nutrient-specific, but not much dependent on the intensity of nutrient limitation (severe – mild). In Mg-deficient plants, high proportion of biomass was allocated to leaves and low proportion to stem and roots. In N-deficient plants, in contrast, low proportion of mass was allocated to leaves and high mass was allocated to roots. In K-deficient plants, biomass allocation to both, leaves and roots was increased, whereas biomass allocation to stems was reduced. The effect of nutrient limitation on nutrient allocation was also nutrient-specific, but differed from the effect on biomass allocation. In Mg-deficient plants, low proportion of Mg was allocated to leaves and high proportion to stems. In K- and N-deficient plants, high proportion of K and particularly N was allocated to leaves at the cost of reduced K and N allocation to stems. The differences between biomass and nutrient allocation in response to nutrient deficiency indicate separate mechanisms for the regulation of biomass and nutrient allocation among plant organs.

Twenty DAT, K and N supply had no effect on non-structural carbohydrate (NSC) concentrations in different plant organs, whereas in Mg-deficient plants, NSC concentration was reduced in sink organs (stem, root and blossoms). The effect of low N and Mg supply on carbohydrate concentrations in leaves was dependent on the specific form of carbohydrates. Mg deficiency was associated with higher concentrations of soluble sugars, whereas N deficiency was associated with higher starch concentrations. This indicates specific nutrient effects on carbohydrate metabolism within leaves. N deficiency only slightly affected N concentrations in leaves, whereas K and particularly Mg deficiency resulted in severely reduced concentrations of K and Mg in all organs. This indicates that for N, low rates of supply were closely paralleled by

lower rates of biomass formation, whereas in K- and Mg-deficient plants, biomass formation was less affected than K and Mg uptake and accumulation.

At optimal nutrient supply, biomass allocation to fruits increased after flowering at the expense of reduced biomass allocation to leaves. At maturity (90 DAT), N, K and Mg allocation to fruits was increased. For N, this was at the expense of lower N allocation to leaves, whereas for K and Mg this was at the expense of lower allocation to stems. Mg allocation to fruits was increased by only 10%, whereas K allocation to fruits was increased by 30% and N allocation to fruits by 25%. In K- and Mg-deficient plants, the increase of biomass allocation to fruits was similar as in the control plants. In N-deficient plants, however, biomass allocation to fruits was more increased than in control plants and biomass allocation to leaves was more reduced than in control plants. This indicates a strong preference for biomass allocation to generative organs in association with senescence-induced biomass remobilization from leaves. Nutrient allocation to fruits in deficient plants was generally more increased than in control plants. In K- and N-deficient plants, this was due to net remobilization from leaves, whereas in Mg-deficient plants, this was due to net remobilization from roots.

In chapter three, focus was on the effects of nutrient deficiency on morphological changes of shoot and roots, and their relationship with photosynthetic activity in the vegetative growth phase. Physiological and morphological shoot responses to nutrient deficiency were nutrient-specific. Net assimilation rate (NAR) of N-deficient plants was not affected, while NAR of K-deficient plants was slightly reduced, and NAR of Mg-deficient plants was severely reduced. Maintenance of high NAR in N-deficient plants was associated with strong reduction of leaf area and leaf area ratio (LAR, leaf area per total plant biomass). In K- and Mg-deficient plants, leaf area was less affected, and LAR was enhanced in comparison to control plants. Thus, in N-deficient plants growth reduction was mainly due to lower LAR, whereas in K- and particularly in Mg-deficient plants growth reduction was mainly due to lower NAR.

The root responses to nutrient deficiency also were nutrient-specific. N deficiency slightly reduced specific root length (SRL), but increased biomass allocation to roots. Thus, root length ratio (RLR, root length per total plant biomass) was not affected by N deficiency. In contrast, in Mg deficiency was associated with increased SRL, but reduced biomass allocation to roots. Thus, RLR was also not affected by Mg deficiency. In contrast, K deficiency was associated with higher RLR because both, biomass allocation to roots and SRL were increased.

In chapter four, the effect of nutrient limitation on fruit yield and quality was investigated. In K- and Mg-deficient plants, fruit growth was similarly reduced as shoot growth, whereas in N-deficient plants fruit growth was less reduced than total shoot growth. Thus, the

fruit index (ratio of fruit biomass to total shoot biomass) was little affected by K and Mg limitation, but was strongly increased particularly in severely N-deficient plants. Fruit lycopene concentrations in nutrient-deficient plants were generally higher than in fruits of control plants. This was possibly due to the reduction of fruit volume, and thus the ratio of lycopene-rich fruit peel. In N-deficient plants, fruit sugar concentrations were increased regardless whether the limitation was mild or severe. In Mg- and K-deficient plants, fruit sugar concentrations were only increased under mild limitation. Fruits of N-deficient plants also contained higher concentrations of ascorbic acid and higher percentage of radical scavenging activity, whereas these parameters were not affected by K and Mg limitation. Thus, with regard to fruit quality and concentrations of bioactive compounds, N limitation was superior to K and Mg limitation.

In chapter five, physiological and morphological responses to Mg deficiency were studied in more detail. In one experiment, in addition to the three treatments tested before (optimal supply, mild and severe Mg limitation) as fourth treatment Mg was completely withdrawn from the nutrient solution, and responses to Mg deficiency were measured 6 and 12 DAT. In another experiment, the source/sink ratio of plants was reduced to investigate if the effect of Mg deficiency on sugar accumulation in source leaves and inhibition of photosynthesis can be attenuated.

Six DAT, Mg limitation had no effect on plant growth, despite of severely reduced Mg concentrations in all plant organs. This shows that for Mg deficiency there is no early growth response as it is typical for example for N deficiency. Twelve DAT, the Mg deficiency-induced reduction of leaf area and plant biomass was most severe in the treatment without Mg supply followed by severe and mild limitation. The rates of leaf gas exchange (transpiration rate, net CO₂ assimilation rate) only decreased after severe reduction of leaf Mg concentrations, and the decrease of leaf gas exchange was associated with severe reduction of leaf chlorophyll concentration and increase of soluble sugar concentrations.

In control plants, which were well supplied with Mg, shading of the basal source leaves decreased the concentrations of NSC in the unshaded leaves. In Mg-deficient plants, in contrast, soluble sugars accumulated despite of shading of basal leaves. This is in accordance with the suggestion that sugar export is reduced due to inhibition of phloem loading.

1. General introduction

There is a vast amount of information on plant strategies to cope with limiting resources, in natural environments, where limitation by a single resource is uncommon, and plants must simultaneously optimize the use of multiple resources (Schulze et al. 1991). Among essential resources are inorganic macronutrients, which play vital roles in plant growth and development (Schachtman and Shin 2007). Low availability of one or more types of macronutrients is a key factor limiting crop yields in many agroecosystems (Sanchez 2002). Now the question is how do plants react to nutrient deficiency? Plants may adapt to nutrient deficiency by increasing nutrient efficiency. Two components may contribute to overall nutrient efficiency: uptake efficiency, which is the amount of nutrients absorbed from soil, and utilization efficiency, which characterizes the efficiency with which the absorbed nutrients are utilized to produce biomass or yield (George et al. 2012). If the nutrient deficiency-induced increase of nutrient efficiency is not sufficient to meet the plant demand for nutrients, plants generally respond to suboptimal conditions through reduction in growth rate and changes in biomass allocation to various plant organs to minimize the limitation of growth by any single factor (Lambers et al. 2008). If also this strategy is not sufficient to avoid nutrient deficiency at the cellular level, plants have developed a range of mechanisms to reduce the damage at the cellular level, which is caused by reactive oxygen species which are formed under nutrient deficiency (Marschner 1995).

1.1 Increased uptake efficiency

Plants have developed different mechanisms to acclimate to nutrient limitation:

- Physiological acclimations (e.g., induction of specific ion-uptake systems when nutrients are in short supply, and excretion of phosphate-hydrolyzing enzymes)
- Anatomical acclimations (e.g., formation of longer root hairs when inorganic phosphate is in short supply).
- Morphological acclimations (e.g., increase in root mass ratio when N is limiting for growth).

These anatomical and morphological acclimations also have a physiological basis, and often require induction of specific genes, after a shortage of nutrient has been sensed (Lambers et al. 2008).

1.1.1 Physiological acclimations

Plants exposed to nutrient deficiency activate a range of mechanisms to increase nutrient acquisition. The rate of nutrient uptake depends on both the concentration in the environment

and the demand by the plant as well as on the inherent capacity of a plant to take up certain nutrients (Lambers et al. 2008). Once nutrients arrive at the root surface, they must pass the plasma membrane of the root cells. A common response of plant roots to nutrient starvation is the up-regulation of genes coding for high-affinity transporters (Bucher 2007). However, this may not be effective for nutrients with low concentration in the soil solution and low mobility in the soil (such as K^+ , NH_4^+ and particularly $H_2PO_4^-$) where the transport to the root is the main factor limiting nutrient uptake (George et al. 2012). Plants may exude organic compounds (carboxylates, phenolics, carbohydrates, enzymes, etc.) and protons (Rengel and Marschner 2005). Exuded organic acid anions may have a role both in solubilization of mineral nutrients and as growth substrates for microorganisms. Typical carboxylates (organic acid anions) found in root exudates include citrate, malate, malonate, acetate, fumarate, succinate, lactate and oxalate (Rengel 2002). Sparingly soluble nutrients may be mobilized at the soil/root interface through root exudates. The release of H^+ into the rhizosphere will increase availability of micronutrients (except Mo) and Ca phosphates, whereas the release of OH^- will improve availability of P bound to Fe and Al oxides in acid soils (Gahoonia et al. 1992). Even more effective is the exudation of chelators such as organic acid anions for P, Zn, and of phytosiderophores for Fe and Zn solubilization, particularly if combined with morphological adaptations aimed at concentrating the root exudates in a small soil volume. For this, the formation of cluster roots in response to P deficiency is an excellent example (Lambers et al. 2006).

1.1.2 Anatomical acclimations

Rates of nutrient uptake depend not only on the uptake properties of the root surface (see 1.1.1) but also on the quantity of root surface area. The root surface area is strongly influenced by root hairs. Length and density of root hairs per unit root length is affected by nutrient supply. Nitrogen and phosphorus seem to have the most pronounced effect on root hairs (Marschner 1995). Among the anatomical characteristics, increasing the root length while reducing the root diameter, thus increasing the root surface area per assimilate investment in root growth, appears to be a successful strategy in response to low P supply and a characteristic of P efficient genotypes (Lynch and Ho 2005, Lambers et al. 2006). Not the absolute root growth but rather the root length/shoot weight ratio (root length ratio; RLR) is an indicator of P efficiency (Fohse et al. 1988). Fine roots, and especially root hairs (Gahoonia et al. 2001, Nigussie et al. 2003), are effective in scavenging P from the soil environment because of a large surface area in contact with the soil (Rengel and Marschner 2005).

1.1.3 Morphological acclimation

One of the mechanisms by which plants adjust to deficiency of exogenous resources is allocation of new biomass to the organs that are involved in acquiring the resources that are scarcest (Marschner 1995), i.e. changing allocation to organs and functions most directly related to improving the uptake of the most limiting resource. Plants allocate biomass to the roots for the acquisition of water and nutrients and to the shoot for the acquisition of light and carbon dioxide (Bazzaz 1996). The response to nutrient shortage is also functional. The investment in plant parts that acquire the limiting resource is favored, at the expense of allocation to plant parts that have a high requirement for the limiting resource (Lambers et al. 2008). However, the effect of N shortage on biomass allocation is stronger than that of other nutrients. P may have similar effects; possibly acting through an effect on N acquisition (Kuiper et al. 1989). Plants growing in P-deficient soil allocate a greater proportion of assimilates to root growth (Gahoonia et al. 2001, Nigussie et al. 2003). In most physiological studies, K and particularly Mg-deficient plants allocate high proportion of mass to shoot and low proportion of mass to roots (Cakmak et al. 1994a). This acclimatory response is a consequence of metabolic changes in the shoot and an adjustment of carbohydrate transport to the root (Hermans et al. 2006). N and P deficiency alter carbohydrate allocation to roots; while in K and Mg-deficient plants accumulate carbohydrate in their leaves consequently reduce carbohydrate allocation to roots (Cakmak et al. 1994 b, Hermans et al. 2006).

1.2 Internal nutrient utilization

Plants may respond to low nutrient supply by increasing the efficiency of internal nutrient utilization at the cellular level. For example, low P supply leads to release of vacuolar phosphate to buffer cytoplasmic phosphate concentration under P deficiency (Pratt et al. 2009). Phosphorus starvation may also lead to replacement of phospholipids with lipids devoid of phosphorus (Jouhet et al. 2003). Furthermore, low P supply may induce alternative pathways of glycolysis and mitochondrial electron chain, which are less dependent on phosphate (Theodorou and Plaxton 1993).

At the whole plant level, the efficiency of nutrient utilization can be increased by translocation to plant organs with high metabolic demand or high return in terms of net assimilation. Typical example is deficiency-induced remobilization of nutrients from lower shaded leaves to younger leaves, which are exposed to full sun light (Marschner 1995, Lambers et al. 2008).

Nutrient utilization efficiency becomes equally or even more important than nutrient uptake efficiency under conditions of mild nutrient-deficiency stress (Wang et al. 2010). Nutrient utilization efficiency can be enhanced by reducing the nutrient concentration in the harvested product, e.g., in cereal grains. However, this strategy may lead to lower nutritional quality (protein, P) and seedling emergence under conditions of low soil nutrient supply (P). Breeding crops for high harvest index (HI, biomass of the harvested organ relative to the total plant biomass) has been a very successful strategy in enhancing crop-yielding capacity (George et al. 2012).

1.3 Growth reduction due to hormonal signalling

One of the main adaptation mechanisms of plants in natural vegetation to soils low in available nutrients is a reduction in growth rate (George et al. 2012). Reduction of growth rate before the internal nutrient concentrations markedly decrease can prevent disturbance of cellular metabolism due to nutrient deficiency. Leaf expansion rates are decreased at a low N supply (Gastal et al. 1992). Leaves of plants grown with a limiting N supply are smaller, compared with those of plants grown with an optimum nutrient supply, predominantly due to an effect on meristem size and cell number (Terry 1970). There is evidence that under conditions of nutrient deficiency, growth reduction at an early stage of nutrient limitation is not caused by nutrient deficiency at the cellular level, but that plants sense changes in nutrient availability and transmit the signals by short- and long-range pathways. Sugars, cytokinins (CYT), and auxin have been considered to be possible long-range signals of roots responding to nutrition status (Forde 2002, Jain et al. 2007, Rietz et al. 2010). The role of CYT in the reduction of plant growth at low supply of nutrients became clear, e.g., when plantain plants were grown for a long period at low nutrient supply, and their growth rate and tissue CYT concentration were lower than in control plants grown at high nutrient supply (Kuiper et al. 1988). A reduced nutrient supply to the roots reduces the synthesis of CYT in the root tips and their subsequent export to the leaves (Fetene and Beck 1993, Van der Werf and Nagel 1996). N appears to be the predominant nutrient that leads to this response (Kuiper et al. 1989). Due to the lower CYT import into leaves of plants grown with a limiting N supply, growth of the leaves is reduced (Simpson et al. 1982). Nitrogen affects the growth of leaves and roots in an opposite manner: root growth is either stimulated or unaffected by a low N supply (Lambers et al. 2008). Also, abscisic acid (ABA) plays a role as a signal between roots and leaves of plants exposed to a nutrient supply that is limiting to plant growth (Munns and Cramer 1996). Enhanced synthesis and higher concentration of ABA in roots and shoots are typical of N-deficient plants (Wilkinson and Davies 2002, Jiang and

Hartung 2008). For example, when the N supply to sunflower plants was interrupted, ABA concentration in all parts of the shoot increased strongly within 7 days (Goldbach et al. 1975). Low K^+ supply also resulted in an increased biosynthesis of ABA in the roots. This caused a slightly increased deposition of ABA in the roots and higher root to shoot ABA signal in the xylem or phloem (Peuke et al. 2002).

1.4 Alteration of the rate of plant development

Resource limitation also affects the rate of plant development. This can also be regarded as a kind of acclimation to stressful conditions. Delay of plant development increases the period available for resource acquisition during a specific ontogenetic phase, e.g. the vegetative phase until flowering. This might increase the source capacity of the plant which is available to supply the seeds with assimilates and nutrients. Acceleration of plant development, e.g. after flowering, may reduce the time period in which plants are subjected to stressful environmental conditions, and thus might ensure formation of fully developed seeds.

Nutrient deficiency may delay plant development in the vegetative growing phase. In barley the number of days to reach the booting stage is about twice as high for Mn-deficient than for Mn-sufficient plants (Longnecker et al. 1991). The thermal time elapsing between the visual appearances of two successive leaf tips (phyllochron in degree-days leaf⁻¹) is also influenced by nutrition. In wheat plants, phyllochron was reduced from 124 degree days leaf⁻¹ in P-deficient plants to 94 degree days leaf⁻¹ in highly fertilized plants (Rodriguez et al. 1998). Furthermore, the total number of tillers per plant was also significantly increased by high P supply in a P-deficient soil (Rodriguez et al. 1998). Nitrogen deficiency has also been shown to increase the phyllochron (Adamowicz and Le Bot 2008). It is well known in agricultural and horticultural plant production that tillering and axillary branching of field-grown plants can be stimulated by N fertilization (Engels et al. 2012).

Abiotic stresses could accelerate flowering as a part of a stress-avoidance strategy; instead of improving stress tolerance, plants could shorten their life cycle to ensure at least some seed production (Kolar and Senkova 2008). Several ecological studies have indicated that populations from more stressful conditions (like drier climate) flower earlier (e.g. Rice and Mack 1991, Aronson et al. 1992). The effect of abiotic stresses is often species-specific on flowering in some plants. For example, salt stress accelerated flowering in *Mesembryanthemum crystallinum* (Adams et al. 1998) but delayed it in *A. thaliana* (Achard et al. 2006). Abiotic stresses can promote flowering in a number of plants, and this could be ecologically relevant in their natural habitats. The promoting effect of drought on flowering has been well documented

in the genus *Citrus* (Monselise 1985, cited in Kolar and Senkova 2008). Nutrient deficiency had diverse effects on plant development. Poor mineral nutrition had no effect on bolting (i.e., premature flowering and seed formation) in *A. thaliana* (Luquez et al. 2006). In other studies, plants flowered several days later when grown in low nutrient supply compared with high nutrient supply (Van Tienderen et al. 1996, Pigliucci et al. 1995, Pigliucci and Schlichting, 1995, Zhang and Lechowicz 1994). In contrast, transferring plants to diluted medium caused acceleration of flowering which was by nutrient stress, not by a change in the osmotic potential of the medium, because of addition of mannitol had no effect on the promotion of flowering (Kolar and Senkova 2008). Sugars are also involved in the regulation of developmental phase changes, such as the progression from juvenile to adult phases, flowering and senescence. When the Rubisco small subunit was expressed in an antisense orientation in tobacco, leaf source strength decreased with a concomitant extension in the length of an early phase of shoot development (Gibson 2005). Similarly, sugar levels have been postulated to affect the timing of flowering in at least some plant species. Flowering earlier under long day condition, was found to be correlated with greater export of carbohydrate from the leaves and increased flower induction (Corbesier et al. 1998).

1.5 Avoidance of damage induced by nutrient deficiency

There is evidence that nutrient deficiency at the cellular level is associated with increased production of reactive oxygen species (ROS) in the chloroplasts and mitochondria (Cakmak and Marschner 1992, Engels et al. 2012). Leaves are particularly susceptible to damage induced by ROS when they are exposed to high light intensity in combination with other environmental stress factors such as drought, low temperatures or nutrient deficiencies. Excess excitation energy in the chloroplasts depresses photosynthesis and quantum yield, which although usually reversible (dynamic photoinhibition), may also lead in the long term to irreversible damage of the photosynthetic apparatus which results in decreased maximum photosynthesis (chronic photoinhibition) as well as chlorosis and necrosis of the leaves (photooxidation). These latter symptoms are caused by the formation of ROS (Engels et al. 2012). Plants possess a range of protective adaptations and systems to reduce damage by ROS. These include, for example, light reflecting wax cover of leaf epidermis, changes in leaf angle, leaf rolling and chloroplast movement to reduce light absorption. If excess light energy is absorbed, plants can (i) dissipate the energy in the form of heat (Ort 2001), (ii) activate detoxification mechanisms against damage by ROS (Niyogi 1999), and (iii) repair photo-damaged PSII by fast and efficient turnover of the D1 protein (Nishiyama et al. 2006).

The antioxidative machinery includes a battery of antioxidative enzymes, e.g., superoxide dismutase, catalase, peroxidase, glutathione peroxidase and enzymes of Halliwell-Asada pathway (Alscher et al. 1997, Foyer et al. 1997). Little is known about the antioxidative enzyme responses of plants to S deficiency-stress, limited information in this regard is available in respect of N (Logan et al. 1999), K (Cakmak 1994), and Mg (Cakmak 1994, Hawkesford et al. 2012, Cakmak and Marschner 1992, Candan and Tarhan 2003). In corn plants, activities of superoxide dismutase and ascorbate peroxidase were stimulated by the deficiency of each of these macronutrients. Enhanced activity of catalase was observed in plants deficient in K, and of non-specific peroxidase under S deficiency (Tewari et al. 2004).

Another component of the antioxidative machinery are antioxidative compounds like ascorbates, α -tocopherol, carotenoids, flavonoids and glutathione (Alscher et al. 1997, Foyer et al. 1997). Carotenoids, xanthophylls in particular, play an important role in both, scavenging singlet oxygen and discharging excess photon flux energy as heat, a process called thermal dissipation or non-photochemical quenching (Ort 2001, Johnson et al. 2007). Antioxidative compounds like ascorbate and flavonoids play also an important role as antioxidants in plants scavenging ROS generated during ripening and under excessive radiation and cold and heat stress. According to the so-called “Oxidative Pressure”-hypothesis, it can be expected that biosynthesis of antioxidative compounds is increased by external factors leading to increased formation of ROS (Treutter 2005). It is important to note that antioxidative compounds are thought to be also beneficial to human health, since they reduce the risk of cancer and cardiovascular disease (Dumas et al. 2003).

Tomatoes contain many anti-oxidative compounds such as phenols, vitamin C and lycopene (Dumas et al. 2003). Thus, the consumption of tomatoes and tomato products has been associated with a lower risk of developing digestive tract and prostate cancer (Dumas et al. 2003). Tomato is considered one of the most popular and extensively consumed vegetable crops worldwide (Giuntini et al. 2005). The main carotenoid in tomato is lycopene which constitutes about 80 - 90 % of the total carotenoids content of red-ripe tomatoes (Shi and Maguer 2000).

1.6 Objectives of this study

In the present study, effects of nutrient deficiency on growth, physiological and morphological plant characteristics and chemical plant composition were assessed at the whole plant level. Emphasis was put on comparing plant responses to deficiency of different nutrients. Numerous studies have shown that plants respond to nutrient deficiency with a large set of parallel changes in growth, in morphological and physiological characteristics (Chapin 1991),

and in plant composition (Wiesler 2012). Most of these studies focused on phosphorus (P) and nitrogen (N) deficiency, whereas studies on other nutrients are less numerous. In the present study, plant responses to potassium (K) and magnesium (Mg) deficiency were measured.

In most studies, deficiency effects are measured for one specific nutrient. Thus, the comparison of nutrient-specific plant responses is complicated because experimental conditions vary among different studies. In the present study, different sets of plants were subjected to deficiency of K or Mg or N within one experiment, thus allowing investigating if plant responses are nutrient-specific.

It may be expected that plant responses to nutrient deficiency depend on the intensity of nutrient deficiency, and on the mode how deficiency is induced. In soil-grown plants the intensity of nutrient deficiency can not be exactly regulated because soil nutrient supply strongly depends on chemical, physical and biological processes in the rhizosphere (Marschner 1995) which can not be controlled. In many physiological studies, plants are therefore grown in nutrient solutions in which the nutrient under study is either supplied at optimal concentration, or, after a preculture period with optimal concentration, is completely withdrawn from nutrient solution. In the initial phase after nutrient withdrawal, plants can use the internal nutrient pools which were accumulated during preculture, and thus, continue to grow. Under these conditions nutrient deficiency in plant tissue becomes increasingly severe with increasing time from nutrient withdrawal. The duration of these experiments is confined by the increasing deficiency stress of plants, which eventually leads to plant death. When comparing nutrient-specific responses to nutrient deficiency, this experimental approach has the disadvantage, that for nutrients like N, which are needed by plants in large amounts, the internal reserve pools are more rapidly depleted than for nutrients like Mg which are needed by plants in lower amounts. Thus, there is a tendency that at each specific date after nutrient withdrawal, at the plant tissue level, N deficiency is more severe than Mg deficiency. In consequence, it is hardly possible to assess to which extent differences in plant responses are due to the specific nutrient, or due to the intensity of deficiency.

Therefore, in the present study a different approach to induce nutrient deficiency was used. Plants were cultured in nutrient solution at three different rates of either N, or K or Mg supply. For optimal supply, it was ensured that the nutrient concentration in the nutrient solution did not decrease to concentrations that limit nutrient uptake (Marschner, 1995). Under these conditions the rate of plant growth was limited by other environmental conditions (e.g., light intensity, air temperature), which were identical in all treatments. This rate of plant growth was set as 100%. In a second set of “rate of nutrient supply”-treatments, the nutrient under study (N

or K or Mg) was added to the nutrient solution in regular intervals at a dosage that should result in growth reduction to 80% of optimal growth. Within each interval, the amount of nutrient under study, which was added to the nutrient solution, was completely absorbed by the plants. In these three treatments plant responses to slight nutrient deficiency could be measured. In a third set of “rate of nutrient supply”-treatments, the nutrient under study (N or K or Mg) was added to the nutrient solution in regular intervals at a dosage that should result in growth reduction to 60% of optimal growth. In these three treatments plant responses to severe nutrient deficiency could be measured. With this experimental approach, the intensity of nutrient deficiency as quantified by the % growth reduction relative to optimal growth could be regulated and be maintained over an extended period from the vegetative growing phase to the generative growing phase until maturity.

For our study, we used tomato as experimental plant. Tomato is one of the most widely consumed vegetables in the world. It is the second after potato in the global vegetable production (14 % of total vegetable production worldwide) (FAO, 2006). China, the USA, Turkey, Egypt and India are the most important producing countries. The high consumption rate of tomato is due to year-round availability, relatively low prices, and well – established storage and handling practices. Tomatoes contain many bioactive components, including those that act as antioxidant, such as the vitamins C and E and many carotenoids and the main carotenoid in tomato is lycopene. Sometimes tomatoes assumed to be responsible for the positive health effect seen with increased intake (Nguyen and Schwartz 1999, Canene-Adams et al. 2005). It is assumed that the polyphenols present in food and particularly the flavonoids, have an antioxidant function in vivo in animal cells (Serio et al. 2006). From the experimental point of view, tomato has the advantage that hybrid seed is commercially available, and thus, experiments can be performed with uniform plants.

In chapter 2, effects of nutrient deficiency on allocation of biomass and mineral nutrients to various plant organs are shown. According to “ecological theory”, biomass should preferentially be allocated to the organ, which is responsible for resource acquisition. As all minerals are absorbed from soil by roots, the response with regard to biomass allocation should not be nutrient-specific. Plant physiological studies, in contrast, indicate that responses with regard to allocation are nutrient-specific. In accordance with “ecological theory”, it is generally found that N deficiency is associated with increased biomass allocation to roots (Scheible et al. 2004, Remans et al. 2006). However, in many studies on Mg and K deficiency it has been found that biomass allocation to roots was decreased (Cakmak et al. 1994b). The decrease of biomass allocation to roots has been attributed to impaired phloem loading and transport of

photoassimilates under Mg and K deficiency (Marschner 1995). In most physiological studies on Mg and K deficiency, Mg and K were completely withdrawn from nutrient solution. Thus, it is possible that impaired phloem loading and transport is confined to very severe deficiency which lead to inhibition of cellular metabolism, and thus prevention of adaptive plant responses. Inhibition of phloem transport under these conditions should also reduce allocation of biomass to other sink organs like young leaves and fruits. In our experiment, plants were subjected to different rates of nutrient deficiency. We expected that with moderate deficiency plant metabolism should not be severely disturbed, thus allowing adaptive plant responses, whereas strong deficiency should lead to disturbance of plant metabolism. Thus, our hypothesis was that

- (i) Plant responses are nutrient-specific and dependent on the intensity of nutrient deficiency.**

Sink organs are supplied via phloem not only with photoassimilates but also with mineral nutrients. Thus, our hypothesis was that

- (ii) Plant responses with regard to biomass allocation to various plant organs are similar to plant responses with regard to nutrient allocation.**

In chapter 3 effects of nutrient deficiency on morphological shoot and root traits are described. Nutrient acquisition from soil is dependent on physiological root traits that control nutrient transport across the plasmalemma of root cells and nutrient availability in the rhizosphere, and on morphological root traits that control spatial availability of nutrients in soil (Marschner 1995). It is well documented that physiological root traits are strongly regulated by the plant nutritional status in a nutrient-specific way (Marschner 1995). At low rate of nutrient supply (i.e. nutrient deficiency) nutrients are transported to the root surface mainly by diffusion (Barber 1995). The amount of nutrients which are supplied to the root surface by diffusion is controlled by morphological root traits like root diameter (Claassen, 1990). This is true irrespective of the specific nutrient. Thus, our hypothesis was that

- (iii) The plant responses to nutrient deficiency with regard to morphological root traits are not nutrient-specific.**

It is well documented that the rate of nutrient supply can also modify shoot morphology. For example, typical N deficiency-induced changes of shoot morphology include reduction of stem elongation, decrease of leaf area and increase of leaf thickness (Marschner, 1995). Changes of shoot morphology are often believed to be regulated by hormone action, whereby N plays a

prominent role for the regulation of phytohormones (Kuiper et al. 1989), whereas effects of K and particularly of Mg on phytohormones are less prominent. Thus, our hypothesis was that

- (iv) Plant responses to nutrient deficiency with regard to morphological shoot traits are nutrient-specific.**

In chapter 4 effects of nutrient deficiency on the fruit content of health-promoting minerals and organic compounds are described. Here we were mainly interested in bioactive compounds, which are secondary metabolites. Secondary metabolites are derived from precursors which are also used for primary (central) metabolism, and thus for plant growth. Production of secondary metabolites has been shown to be dependent on carbon and nutrient availability in plants. The carbon nutrient balance (CNB) hypothesis postulates that plant metabolism is directed towards biosynthesis of carbon-rich secondary metabolites in nutrient-deficient plants. It has also been shown in some experiments that secondary metabolite levels are dependent on the rate of resource availability (growth differentiation balance (GDB) hypothesis; Wilkens et al. 1996, Stamp 2003). For example, secondary metabolite levels have been shown to be low at very low rates of nutrient supply, when both, growth and photosynthesis, are severely reduced. At medium rates of nutrient supply, photosynthesis is more strongly increased than growth, leading to high availability of photoassimilates for biosynthesis of secondary metabolites, and thus high secondary metabolite levels. When nutrient supply is further increased, plant growth, and thus, metabolite use for central metabolism is more increased than photosynthesis, leading to a decrease of photoassimilate availability for biosynthesis of secondary metabolites, and thus low secondary metabolite levels. Thus, our hypothesis was that

- (v) The fruit contents of secondary metabolites is dependent on the rate of nutrient supply.**

In chapter 5, Mg deficiency-induced plant responses with regard to photosynthesis and leaf carbohydrate concentrations are described in more detail. In the experiments described in this chapter, we assessed plant responses not only to moderate and strong reduction of Mg supply, but also to complete Mg withdrawal from nutrient solution. Hence, our hypothesis was that.

- (vi) Plant response to low Mg supply differs from response to complete Mg withdrawal**

In short term experiment, photosynthetic rate, carbohydrate, chlorophyll and Mg concentration were measured in young and old leaf of plants to clarify the relationship between the reduction of photosynthetic rate and other parameters. Thus, our hypothesis was that.

(vii) Mg deficiency-induced decrease of photosynthesis is due to accumulation of sugars in source leaves.

It has been suggested that Mg deficiency leads to inhibition of phloem loading, and thus high carbohydrate concentrations in source leaves (Cakmak et al. 1994b). High carbohydrate concentrations in source leaves, in turn, should reduce leaf photosynthesis (Stitt 1990). In one experiment, the source/sink ratio of plants grown with high or no Mg supply was modified through shading of basal leaves. Shading of basal leaves was expected to decrease the ratio of carbohydrate biosynthesis in source organs to carbohydrate utilization in sink organs. Thus, in this experiment we assessed whether Mg deficiency effects on leaf carbohydrate accumulation and photosynthesis in non-shaded source leaves are dependent on the source to sink ratio of plants. Our hypothesis was that

(viii) Shading of basal leaves reduces leaf carbohydrate concentrations in Mg sufficient plants because sugars are exported via the phloem. In Mg-deficient plants, in contrast, carbohydrate concentrations in non-shaded source leaves remain high, because sugar export via phloem is prevented.

2. Biomass and nutrients allocation in tomato plants at different intensities of N, K and Mg deficiency

Abstract

The aim was to investigate if the effect of nutrient deficiency on biomass and nutrient allocation is nutrient-specific and dependent on intensity of deficiency. Tomato plants were cultured in nutrient solution at three different rates of nutrient supply (optimal, or growth reduction to 80 % and 60 % induced either by K, Mg or N deficiency) until fruit maturity. The results showed at 20 DAT that the effect of nutrient deficiency on biomass and allocation was nutrient-specific and independent on the intensity of nutrient deficiency in most cases. High proportion of mass was allocated to roots at the cost of reduced biomass allocation to stem of K-deficient plants and to leaves of N-deficient plants. In contrast, Mg deficiency high proportion of biomass was allocated to leaves and low proportion to stem and roots. The effect of nutrient deficiency on nutrient allocation was also nutrient-specific, but differed from the influence on biomass allocation. In K- and N-deficient plants, high proportion of K and particularly N was allocated to leaves at the cost of reduced K and N allocation to stems, whereas in Mg-deficient plants low proportion of Mg was allocated to leaves and high proportion to stems. K and N deficiency had no effect on non-structural carbohydrate (NSC) concentration in different plant organs, whereas, NSC concentrations in sink organs (stem and roots) of Mg-deficient plants were reduced. The effect of severe Mg and N deficiency on NSC in leaves was dependent on the specific form of carbohydrates. Mg deficiency resulted in accumulation of sugars in source leaves while, N deficiency caused accumulation of starch in source leaves. N concentration was slightly reduced in stem and root of N-deficient plants, while, N concentration in leaves was not affected by N deficiency. In contrast, K and particularly Mg concentration was severely reduced in various organs of K and Mg-deficient plants.

In nutrient sufficient plants, biomass allocation to fruits increased at the expense of reduced biomass to leaves. At 90 DAT, K, Mg and N allocation to fruits. This was at the cost of reduced N allocation to leaves, whereas for K and Mg was at the expense of lower allocation to stem. Mg allocation to fruits was increased by only 10%, whereas K allocation to fruits was increased by 30% and N allocation to fruits by 25%. In K- and Mg-deficient plants, the increase of biomass allocation to fruits was similar as in the control plants. In N-deficient plants, however, biomass allocation to fruits was more increased than in control plants and biomass

allocation to leaves was more reduced than in control plants. Nutrient allocation to fruits in deficient plants was generally more increased than in control plants.

2.1 Introduction

Elucidating mechanisms, which control plant growth as a function of nutrient availability, represents a major challenge in plant biology. Progress is slow in understanding the molecular and physiological events responsible for sensing and signaling mineral resource limitation and their effects on plant development and biomass allocation (Hermans et al. 2006). Usually, mineral nutrients taken up by the roots are loaded to the xylem and further transported to the shoot. In the shoot, photoassimilates are loaded to the phloem and transported to the roots. When plants suffer from deficiencies of essential macronutrients (e.g. N, P, K and Mg) they adjust to that imbalance by preferential partitioning of carbohydrates to the roots or leaves, and modify the root-shoot dry weight (Hermans et al. 2005). In Cakmak et al. (1994a) and Ericsson and Kahr (1995) results were indicating a markedly element-specific effect of mineral nutritional status on shoot-root partitioning of photoassimilates and shoot to root dry weight ratio.

Plant cells have a very high requirement for K for photosynthesis, enzyme activation, protein synthesis, maintenance of cell turgor, and ion homeostasis (Marschner 1995). Low K level can disturb these processes and change the source-sink relationship. K deficiency treatments of tomato plants severely decreased biomass of all organs as well as depressed leaf photosynthesis and phloem transport of ^{13}C -labelled assimilates (Zhao et al. 2001). At the early production stage in tomato, K deficiency was shown to be detrimental to fruit growth (Pujos and Morard 1997). It has to be pointed out; that the impact of K deficiency on photosynthesis and phloem transport of assimilates became evident only after fruit and stem diameter expansions were reduced. These results suggested that K deficiency diminished sink activity in tomato plants prior to its effect on the source activity (Kanai et al. 2007). In different plants, other researchers have shown that mild K deficiency suppresses assimilate translocation in the phloem without affecting photosynthesis at the source (Mengel and Viro 1974). Recent publications show a decline in photosynthesis in K-deficient plants and suggest that this could be a consequence of sucrose accumulation (Cakmak et al. 1994a, b). This suggestion is consistent with the transcriptional profiles of leaves from K-deficient plants (Hampton et al. 2004). Roots of K-deficient plants have the lowest concentrations of sucrose and starch than their K-replete counterparts (Cakmak et al. 1994a, b). One reason for this is that sucrose export to the root is reduced in K-deficient plants (Cakmak et al. 1994b), which can be attributed to a requirement

for K^+ for loading sucrose into the phloem (Pilot et al. 2003, Deeken et al. 2002). Furthermore, controversial effects of K deficiency on the sink activity are reported. On one hand, it is reported that K deficiency reduces photosynthate translocation (Huber 1984) without affecting the metabolism at the sink site (Mengel 1980, Beringer and Haeder 1981). Reduction in assimilate translocation by K deficiency in sugarcane is caused neither by death of the phloem nor by diminished sink growth (Hart 1969). On the other hand, Tsuno and Fujise (1965) found that K deficiency affects photosynthate translocation through reduced growth of storage roots, which is a predominant sink in sweet potato. Geiger and Conti (1983) reported that translocation and allocation of dry matter are not changed over a wide range of K supply. These contradictions led to the suggestion that the effect of K deficiency on plant growth remains elusive, and it is necessary to re-examine the source–sink relationship of the plant (Roitsch 1999).

Mg is essential for the functioning of many enzymes and plays a fundamental role in both, the light and dark reactions of photosynthesis (Shaul 2002). Physiological studies with crop species suggest that Mg deficiency leads to inhibition of phloem loading in source organs, because this process requires Mg (Mg-ATP) is a substrate for H^+ pumps, (Cakmak et al. 1994a, b, Hermans et al. 2005, Hermans and Verbruggen 2005) and reduction of mineral and photoassimilate transport to sink organs, because of reduced sink strength. In general, contrasting results in alteration of carbohydrate concentrations in source and sink organs were found. In *Phaseolus vulgaris*, concentration of soluble sugars and starch were increased in source leaves but reduced in roots (Cakmak et al. 1994b), whereas in *Spinacia oleracea* concentrations of carbohydrates and amino acids were increased in source leaves and in roots (Fisher et al. 1998). In *Beta vulgaris*, sucrose concentrations were increased in older leaves but not in younger leaves and roots; starch concentrations were increased in all leaves under Mg deficiency (Hermans et al. 2005). When low Mg levels induces sugar accumulation this might lead to reduced chlorophyll content and/ or photosynthetic activity (Hermans et al. 2004, Hermans and Verbruggen 2005) suggesting that the reduction of photosynthesis is a response to increased sugar levels. This theory could be strengthened when researchers found down regulation of genes involved in photosynthesis, e.g. the gene for chlorophyll a/b binding protein (Cab2) (Hermans and Verbruggen 2005) under these conditions, which might account in part for the delayed decline in chlorophyll content and photochemical performance (Cakmak et al. 1994b, Hermans et al. 2005, Hermans and Verbruggen 2005). A later effect of Mg deficiency is the reduction of plant growth and a modification of the root (R) to shoot (S) biomass allocation. However, observations of the effect of Mg shortage on R:S vary according to the plant species studied and the age of the plant. Early studies report a severe decrease in the root biomass of

bean plants (Cakmak et al. 1994a,b) and spinach (Fisher and Bremer 1993) and reduced bean seed yield (Hariadi and Shabala 2004). This is in contrast to ecological theory predicting that nutrient deficiency should increase biomass and nutrient partitioning to roots and seeds to optimize mineral acquisition from soil, and seed propagation. More recent studies report the absence of an effect on the root system of sugar beet (Hermans et al. 2004, 2005), *Arabidopsis* (Hermans and Verbruggen, 2005) and rice (Ding et al. 2006) grown hydroponically. Carbon allocation to the youngest leaves is more affected than carbon allocation to roots (Hermans et al. 2005, Hermans and Verbruggen 2005). An increase in R:S ratio is observed in certain species (Hermans et al. 2004, Hermans and Verbruggen 2005, Hermans et al. 2006), which is generally attributed to Mg deficiency reducing the growth of young leaves more than the growth of roots.

N deficiency affects primarily photosynthesis, sugar metabolism and/or carbohydrate partitioning between source and sink tissues. Deficiencies of N result in accumulation of carbohydrate in leaves, higher carbon allocation to roots and an increase in R:S biomass ratio (Scheible et al. 2004, Remans et al. 2006, Hirai et al. 2004). Under N deficiency, the increase in root-shoot dry weight ratio (Marschner 1995) is not only caused by preferential phloem export of sucrose to the roots, but also by export of N, which can exceed the xylem import of N from roots to the shoot (Peuke et al. 1994). These results suggest that a higher proportion of the N taken up from the substrate was retained in the roots under N deficiency and cycling from shoot to roots might provide additional N to the roots. This cycled fraction of N may not only contribute to, but may even cause, the shift in sink strength for photosynthates of the roots at the expense of the shoot apex. The N deficiency-induced increase in plant R:S ratio, is associated with transcriptional changes in a pathway directing the accumulation of sugars and starch in shoots and increasing translocation of sucrose to the root (Scheible et al. 2004). In addition, genes associated with the metabolism and catabolism of N-containing compounds (amino acid, amine and glutamate metabolism) were significantly over-represented among the differentially regulated genes in shoots of N-deficient plants (Scheible et al. 2004). A repression of sets of genes required for photosynthesis and export of photosynthates occurs (Scheible et al. 2004, Hirai et al. 2004). The reduction of photosynthesis in N-deficient plants is probably a direct consequence of sugar accumulation, because sugars exert metabolite feedback regulation (Rook and Bevan 2003, Chiou and Bush 1998) and affect many of the genes involved in photosynthesis (Koch 2004, Blasing et al. 2005). The regulation of gene expression by sugars is also consistent with the identification of a significant number of *Arabidopsis* genes whose expression is regulated by both mineral deficiency and increased shoot sucrose concentrations.

In this study, the responses of tomato plants to different intensities of K, Mg and N deficiency with regard to allocation of biomass and nutrients to different plant organs were measured. Specifically, the addressed question was if the plant responses to nutrient deficiency are dependent on the intensity of nutrient deficiency, and if the plant responses are specific for each nutrient. For this reason, tomato plants were cultured under controlled conditions until fruit maturity at three different rates of N, K or Mg supply: nutrient supply sufficient for optimal growth, slight limitation of nutrient supply resulting in growth reduction to 80 % of optimal growth, and severe nutrient limitation resulting in growth reduction to 60 % of optimal growth. This experimental approach is different to most preceding physiological studies, which were confined to plants in the vegetative growing stage and where plants were subjected to a short period of complete nutrient withdrawal.

2.2 Material and Methods

Plants were grown in a glasshouse from mid of July to mid of November 2008. Day length varied from 16.3 h in July to 8.7 h in November. Temperatures in glasshouse varied from 25 to 20 °C during the experiment. Natural irradiance was supplemented by artificial light on cloudy days, which was used to maintain the period of irradiance 14 h at least. Through the experiment, the light intensity of artificial light at plant height was 180-220 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Idoia genotype of tomato plants was used to carry out these experiments.

2.2.1 Plant culture and nutrient deficiency treatments

Tomato plants were cultured under controlled conditions at three different levels of N, K and Mg supply: optimal supply supporting normal growth, medium supply reducing growth to 80 % and low supply reducing growth to 60 % until fruit maturity. Seeds were germinated in peat moss. After one week, seedlings were transferred to plastic pots (5 seedlings per pot), which contained 10% of the nutrient concentration of the standard nutrient solution. After one week, each plant was transferred to an individual pot at starting of treatments. The standard nutrient solution (optimal supply) had the following composition (mol m^{-3}): 1 K_2SO_4 ; 5 $\text{Ca}(\text{NO}_3)_2$; 0.1 KH_2PO_4 ; 0.6 MgSO_4 ; 0.1 KCl ; 0.1 FeEDTA ; 0.01 H_3BO_3 ; 5×10^{-4} $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$; 1×10^{-4} $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$; 3×10^{-4} $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$; 5×10^{-6} $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot \text{H}_2\text{O}$. For the treatments with medium and low N supply, Ca was supplied as CaCl_2 instead of $\text{Ca}(\text{NO}_3)_2$ to maintain the same Ca concentration. For the treatments with medium and low K supply, P was supplied as $\text{Ca}(\text{H}_2\text{PO}_4)_2$ instead of KH_2PO_4 to maintain the same P concentration. In the treatments low and medium nutrient supply, nutrients were added to nutrient solution twice per week to avoid too

long phases without nutrients in the nutrient solution. To determine the amount of nutrients which had to be added to the nutrient solution to obtain growth reduction to 80% (medium supply) and 60% (low supply) of the control plants, it was assumed that the growth rate of the control plants with optimal nutrient supply is $0.24 \text{ g DM g}^{-1} \text{ DM day}^{-1}$.

At vegetative growth phase, where exponential growth was assumed, the dry mass per plant on each day was calculated according to Eqn 1:

$$DM_t = DM_0 (r + 1)^t P \dots\dots\dots \text{Eqn.1}$$

where:

DM_t = DM after t days of treatments

DM_0 = DM at start of treatments (g plant^{-1})

r = RGR (relative growth rate) $\text{g g}^{-1} \text{ day}^{-1}$

t = the time (days)

$P = 0.8$ for plant with medium supply and 0.6 for plant with low supply

The daily increment of dry mass per plant was calculated according to Eqn 2:

$$DM_d = DM_t r \dots\dots\dots \text{Eqn.2}$$

From Eqn 1, it follows that Eqn 2 can be expressed also the following form:

$$DM_d = DM_0 (r + 1)^t Pr \dots\dots\dots \text{Eqn 3}$$

The daily nutrient requirement (NR_d) of plants on day d was calculated according to Eqn 4

$$NR_d = DM_d C \dots\dots\dots \text{Eqn.4 where:}$$

NR_d = nutrient requirement (mg d^{-1})

DM_d = dry mass increment on day d (g d^{-1}) Ladies first

C = the assumed nutrient concentration in the plant dry mass ($\text{mg g}^{-1} \text{ DM}$)

From Eqn3, It follows that Eqn4 can be expressed also the following form:

$$NR_d = DM_0 (r+1)^t rPC \dots\dots\dots \text{Eqn5}$$

The assumed nutrient concentration in the plant dry mass of plants with medium and low supply were 30 and 20 mg N g^{-1} for shoot and root dry matter respectively. 20 $\text{mg K g}^{-1} \text{ DM}$ and 2 $\text{mg Mg g}^{-1} \text{ DM}$ for both shoot and root.

For the phases of linear fruit growth and fruit maturation, the growth was assumed linear for plants with medium and low nutrient supply. We assumed that the concentration of N in shoot and root is 20 mg and 15 $\text{mg g}^{-1} \text{ DM}$ respectively. For K and Mg concentrations in plants with medium and low supply, we assumed the same values as in the vegetative growth phase.

The increment of dry matter of optimal nutrient supply was determined weekly by harvesting and drying 5 plants. The dry mass increment of plants with medium and low nutrient supply was assumed to be reduced to 80% and 60 % of the plants with optimal supply. The

amount of nutrients added to the plants with medium and low nutrient supply (NR_d) was calculated according to Eqn 6:

$$NR_d = (DM_{t_2} - DM_{t_1}) P C / t_2 - t_1 \dots \dots \dots \text{Eqn 6}$$

Where:

NR_d = daily nutrient requirements (mg d^{-1})

DM_{t_2} = dry matter of plants with optimal supply at harvest 2

DM_{t_1} = dry matter of plants with optimal supply at harvest 1

$P = 0.8$ for plant with medium supply and 0.6 for plant with low supply

t = time (day)

C = the concentration assumed of nutrient (mg g^{-1} DM in plant)

The total amount of nutrients, which was added to the nutrient solutions in the different treatments to induce nutrient deficiency, is shown in Table 2-1 for the 3 different growth periods 0-20 days after start of treatment (DAT), 20-45 DAT and 45-90 DAT.

Table 2-1: Amount of nutrients added to nutrient solution at medium and low rates of nutrient supply (g pot^{-1}) in different phases of the experiment

Experimental phase	K		Mg		N	
	Medium	Low	Medium	Low	Medium	Low
0-20 DAT	0.28	0.10	0.02	0.01	0.38	0.16
0-45 DAT	0.78	0.47	0.10	0.06	0.85	0.51
0-90 DAT	0.99	0.53	0.17	0.11	1.27	0.82

2.2.2 Plant harvests and analysis of mineral nutrient concentrations

Plants were harvested at three stages of growth: at start of flowering 20 DAT, during the phase of linear fruit growth 45 DAT, and at fruit maturity 90 DAT. At each harvest, roots, stem, leaves, trusses and fruit parts were separated and dried at 100°C for one hour and then 65°C for determination of dry mass and concentrations of N, K and Mg, and analysis of soluble sugars and starch in first harvest.

Total N concentration in different plant organs was analyzed by elemental analyzer (Elementaranalysator Elementar Vario Max, Hanau, Germany combustion after Dumas Minerals).

Total concentrations of K, Mg and other elements in plant material was determined by ICP=OES (IRS/AP) with the pretreatment of dry-ash at 550°C for 5 h. 1 ml HNO_3 solution (1 HNO_3 : 2 distilled water) was added to the sample which was put on hotplate until the solution evaporated. This was repeated three times. 2.5 ml of HCl solution (1 HCl : 2 distilled water) was added to sample, which was put on hotplate for 10 min. Samples were quantitatively transferred

to a 25 ml measuring flask and were completed by distilled water, the extraction was used to determine K, Mg and other elements by ICP.

2.2.3 Analysis of soluble sugars and starch

Dried plant material was ground to pass through a 1 mm sieve. The powdered material (0.1 g) was put into a 10 mL centrifuge tube, where 5 mL of 80% ethanol was added the mixture was incubated at 80°C in a water pass shaker for 30 min, and then centrifuged at 4000 rpm for 5 min. the pellet were extracted two more times with 80% ethanol. Supernatants were retained, combined and stored for soluble sugar determinations. The soluble sugar fraction was measured. Soluble sugars in the collected extracted were determined using the anthrone method (Seifter et al. 1950). An aliquot of the extract was hydrolyzed in 5 ml of 0.1 antrone solution (1g anthrone in 1 L 95% H₂SO₄) in a boiling water bath for 15 min. After cooling, the sugar concentration was determined spectrophotometrically at 620 nm. Glucose was used as a standard the sugar concentration was calculated basis on a fresh mass (FM).

The ethanol-insoluble pellet was used for starch extraction. Ethanol was removed by evaporation. Starch in the residue was released in 2 ml distilled water for 15 min in a boiling water bath. After cooling to room temperature 2ml of 9.2 mol L⁻¹ HClO₄ were added. Starch was hydrolysed for 15 min. Distilled water (4 ml) was added to the samples. The samples were then centrifuged at 4000 rpm for 10 min.; the pellets were extracted one more time with 2ml of 4.6 mol L⁻¹ HClO₄. Supernatants were retained, combined and filled to 20 ml. The starch concentration was measured spectrophotometrically at 620 nm using anthrone reagent, and was calculated by multiplying glucose concentration by the conversion factor of 0.9 (Osaki et al. 1991) Glucose was used as a standard. The starch concentration was expressed on a fresh basis.

2.3 Results

2.3.1 Effect of nutrient supply on total plant biomass

Did our experimental approach lead to the desired intensity of nutrient deficiency stress?

Aim of the experimental approach was to adjust and maintain similar intensities of nutrient deficiency stress to plants, whereby this stress should be due to deficiency of either K or Mg or N. As a measure for the intensity of nutrient deficiency, we compared total plant biomass of stressed plants with plants, which were optimally supplied with all nutrients. We tried to induce three intensities of stress: no stress with optimal nutrient supply leading to optimal biomass production (100%); moderate stress with “medium” supply leading to 80% of optimal biomass

production; severe stress with “low” supply leading to 60% of optimal biomass production (for details see Material and methods). We harvested the plants 20, 45, and 90 days after start of treatments (DAT).

Table 2-2: Effect of nutrient supply on dry mass of different plant organs A) 20, B) 45, C) 90 days after start of treatment (DAT); Different letters within a row indicate significant (Tukey-Kramer's test, $P < 0.05$) differences of organ mass among nutrient treatments

Plant organs		Nutrient supply						
		Optimal	K		Mg		N	
			Medium	Low	Medium	Low	Medium	Low
(A) 20 DAT								
Roots	2.1 ab	2.3 a	2.2a	1.1 bc	0.8 c	1.7 abc	1.6 abc	
Stem	6.1 a	3.4 bc	3.2bc	2.8 c	2.2 d	3.8 b	3.4 bc	
Leaves	12.1 a	10.1 ab	8.3bc	9.1 b	7.5 bc	8.2 bc	6.0 c	
Blossoms	0.4 a	0.1 b	0.1b	0.2 b	0.1 b	0.2 b	0.2 b	
Total plant	20.7 a	15.9 b	13.8bc	13.2bc	10.6c	13.9 bc	11.2c	
(B) 45 DAT								
Roots	8 a	7 ab	6 ab	5 b	4 b	6 ab	4 b	
Stem	22 a	15 b	12 b	13 b	8 c	15 b	12 b	
Leaves	33 a	30 a	27 ab	29 a	21 b	23 b	16 c	
Trusses	4 a	3 b	3 b	2 bc	1 c	3 b	2 bc	
Fruits	13 a	12 a	13 a	11 a	8 a	12 a	10 a	
Total plant	80a	67b	60b	60b	43c	58b	44c	
(C) 90 DAT								
Roots	26 a	13 b	8 b	16 b	16 b	10 b	7 b	
Stem	40 a	23 b	17 b	25 b	17 b	25 b	19 b	
Leaves	72 a	56 ab	46 bc	57 ab	39 bc	26 cd	18 d	
Trusses	8 a	5 b	3 b	6 b	3 b	4 b	4 b	
Fruits	55 a	38 ab	28 b	38 ab	34 ab	36 ab	34 ab	
Total plant	201a	135b	101bc	142b	109bc	102bc	81c	

In general, increasing duration of treatment and stronger limitation of single nutrients, biomass was reduced when compared to plants with optimal nutrient supply (Table 2-2). In case of medium K supply, biomass was reduced to 80 % of total biomass of control plants at 20 and 45 DAT and was further decreased to around 67% after 90 DAT. A low K supply resulted in biomass reduced to 70 % at 20 and 45 DAT and a reduction of biomass to around 50 % compared to control plants after 90 DAT.

Medium Mg supply decreased total biomass to around 70% of biomass under optimum supply, whereas low Mg supplied plants showed a decrease of biomass to 50 % at all harvest dates (Table 2-2). Medium N supply caused reduction in total biomass to about 70 % of total biomass of control at 20 and 45 DAT, which further decreased to 50 % at 90 DAT. Low N supply reduced total biomass of plants to 50 % at 20 and 45 DAT and to 40 % of total biomass

of control plants at 90 DAT (Table 2-2 C). With regard to our intention to reduce biomass production to 80% and 60% of control when supplying medium or low nutrient concentrations respectively, we can say that this aim was adequately reached. When comparing nutrient-specific effects on biomass and nutrient allocation in the first 20 DAT, it has to be kept in mind, that the severity of nutrient limitation as based on the percentage of total plant biomass reduction was similar for the treatments low K, medium Mg and medium N, and for the treatments low Mg and low N.

2.3.2 Effects of nutrient supply on biomass of individual organs and dry matter percentage

Were the effects of nutrient deficiency stress on individual organ biomass and dry matter percentage nutrient-specific and dependent on stress intensity?

To study plant responses to nutrient deficiency stress in more detail, the effect of nutrient treatments on biomass of individual plant organs was measured (Table 2-2). In general, severe stress (low supply) led to more pronounced reduction of individual organ biomass than moderate stress (medium supply). However, this effect of stress intensity on individual organ biomass was not significant due to large standard errors.

The nutrient deficiency-induced reduction of individual organ biomass was dependent on the specific organ, and the specific nutrient. Twenty DAT, root biomass of Mg-deficient plants was about half (0.8 to 1.1 g) of that of optimally supplied plants (2.1 g); whereas, the N deficiency-induced reduction of root biomass was less severe (1.6 to 1.7 g), and root biomass of K-deficient plants (2.2 to 2.3 g) was even slightly higher than that of optimally supplied plants (Table 2-2 A). Stem biomass of deficient plants was substantially lower than that of optimally supplied plants (6.1 g). Whereby, the deficiency induced reduction was stronger in case of Mg deficiency (2.2 to 2.8 g, i.e. 36 to 46% of optimum supply) than N deficiency (3.4 to 3.8 g, i.e. 56 to 62% of optimum supply) and K deficiency (3.2 to 3.4 g, i.e. 52 to 56% of optimum supply). Leaf biomass was most severely reduced by N deficiency (50 to 68% of optimum) with less reduction by Mg deficiency (62 to 75% of optimum) and K deficiency (69 to 83% of optimum). Biomass of blossoms, in contrast was less severely reduced by N deficiency (57 to 62% of optimum) than by Mg deficiency (30% to 49% of optimum) and K deficiency (38% of optimum).

At 45 DAT, root and stem biomass again were most severely reduced by Mg deficiency (Table 2-2B). Leaf biomass, in contrast was most severely reduced by N deficiency. The biomass of trusses was most severely reduced by Mg deficiency. In comparison to other plant

organs, fruit biomass was only little affected by nutrient deficiency (62% of optimum at low Mg supply to 98% of optimum at low K supply), whereby the deficiency-induced reduction of fruit biomass was not significant.

At fruit maturity (90 DAT) K and particularly N deficiency resulted in severe reduction of root biomass (50 to 30 % of root biomass of optimally supplied plants) (Table 2-2C). Leaf mass of N-deficient plants was about 30 % of that of optimum plants; however, leaf mass of K- and Mg-deficient plants was less severely reduced (80 % of optimum). Fruit mass was markedly reduced in all nutrient deficiency treatments to 60 to 70 % of optimum.

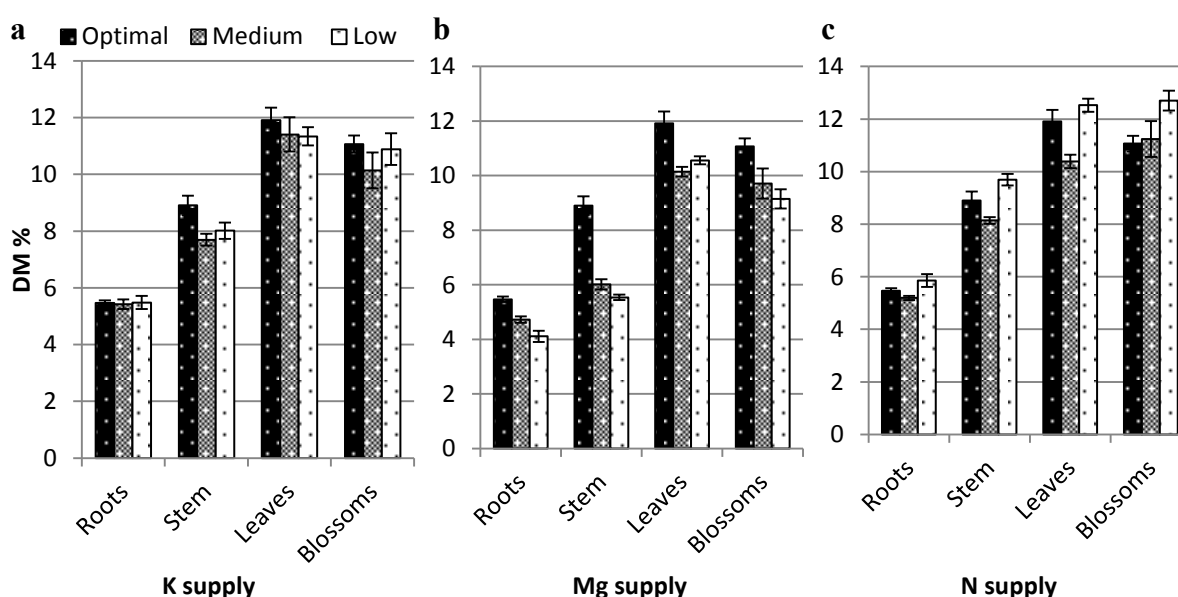


Fig. 2-1: Effect of nutrient supply on dry matter % in different plant organs at 20 DAT; vertical lines indicate standard errors of means (n=4)

K, Mg and N supply had a significant ($P \leq 0.05$) effect on dry matter percentage (DM %) of different plants organs in most cases (Fig. 2-1 a, b, c). K supply had no effect on DM % in different plant parts with exception of stem DM % which was reduced by decreasing of K supply (Fig. 2-1 a).

In comparison to optimal Mg supply, suboptimal Mg supply was associated with lower DM % in different plant organs, particularly of stems (Fig. 2-1b). DM % of roots in all treatments was 6 % with the exception of Mg-deficient plants, in which root DM % was reduced to 4 %. Stem DM % was in the range of 8 to 9 % with the exception of Mg-deficient plants where stem DM% was reduced to 6 %. Leaf DM % of Mg-deficient plants was about 10 %.

N supply had no effect on DM % in roots and blossoms, but DM % in leaves and stem was affected by low N supply. In comparison to optimal N supply, medium N supply was

associated with lower DM % in stem and leaves. In contrast, low N supply was associated with higher DM % in stem, leaves and blossoms (Fig. 2-1 c).

2.3.3 Effects of nutrient supply on carbohydrate and mineral nutritional status of individual organs

As shown in Table 2-2 the nutrient deficiency-induced reduction of biomass was dependent on the specific nutrient and plant organ. To assess if the biomass reduction was related to the carbohydrate and/or mineral nutrient status, we analyzed non-structural carbohydrate and mineral nutrient concentrations in the fresh mass of the individual organs at 20 DAT.

2.3.3.1 Non-structural carbohydrates (NSC)

Non-structural carbohydrate concentrations (soluble sugars and starch) in individual organs were not much affected by nutrient supply with the exception of Mg supply (Fig. 2-2A). In blossoms, and in particular in stem and leaves of Mg-deficient plants, concentrations of non-structural carbohydrates were markedly lower than in plants with optimal nutrient supply.

It can be expected that the immediate availability of non-structural carbohydrates for cell metabolism is higher for soluble sugars than for starch. Furthermore, in contrast to starch, soluble sugars have a function as osmotically active substances. Therefore, concentrations of soluble sugars and starch in individual organs were separately measured. The rate of K supply had no effect on concentrations of soluble sugars in individual organs (Fig. 2-2B). However, even moderate K deficiency (medium supply) was associated with a decrease of leaf starch concentrations (Fig. 2-2C).

The rate of Mg supply affected both, soluble sugar and starch concentrations. In Mg-deficient plants soluble sugar concentrations in the sink organs roots, stem and blossoms were reduced in comparison to optimal supply, whereas in leaves soluble sugar concentrations were not affected with moderate deficiency (medium supply) and significantly increased with severe deficiency (low supply) (Fig. 2-2B). Starch concentrations in blossoms were not affected by Mg supply (Fig. 2-2C). However, in roots, stem and leaves, Mg deficiency was associated with a significant reduction of starch concentration.

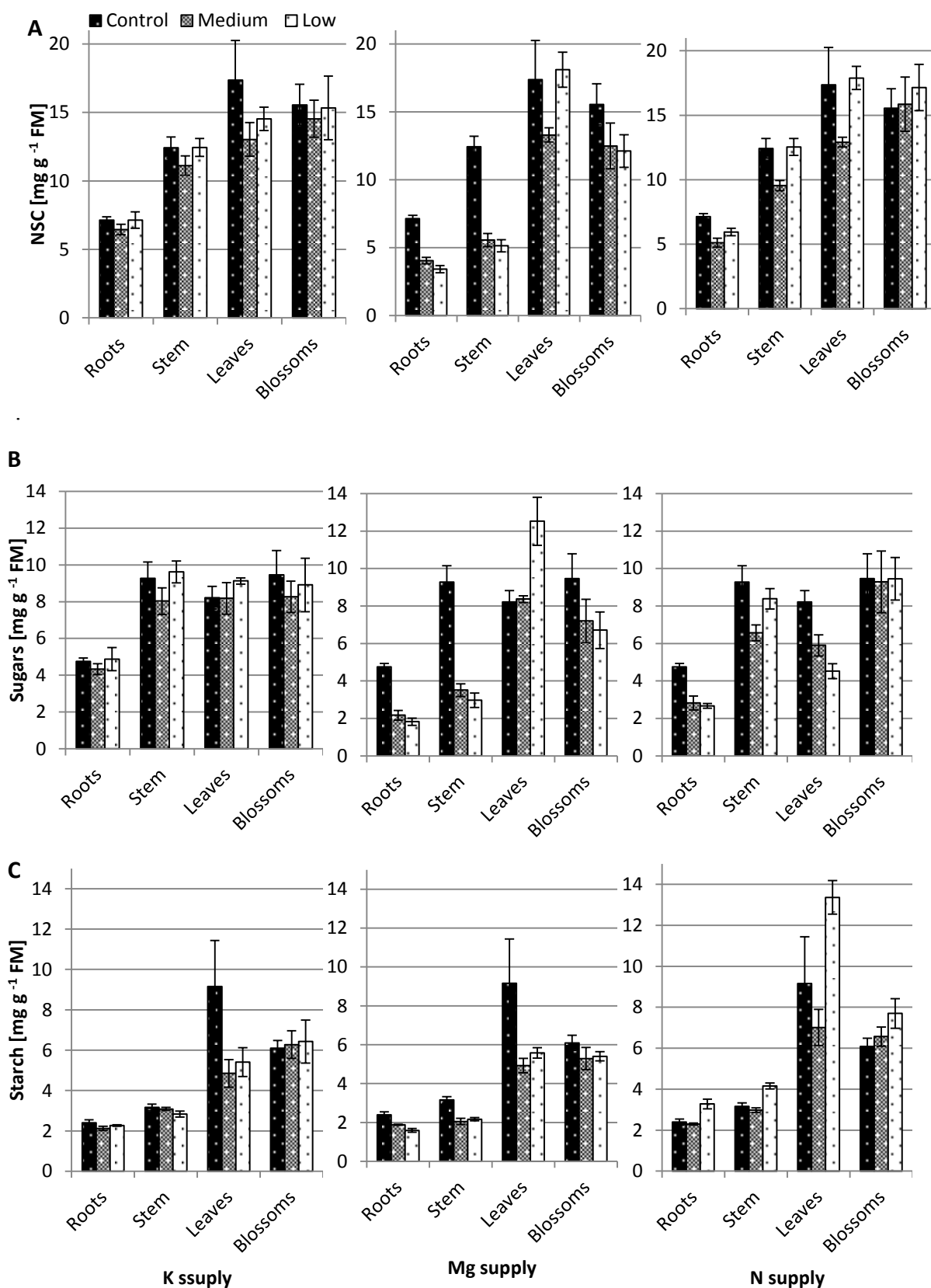


Fig. 2-2: Effect of nutrient supply on soluble sugar and starch concentrations in different plant organs at 20 DAT. Vertical lines indicate standard errors of means ($n=4$).

Similar to Mg deficiency, the effect of N deficiency on soluble sugar concentrations was also dependent on the specific organ. However, in case of N deficiency, responses could not be categorized into responses of source and sink organs. Soluble sugar concentrations in roots decreased, whereas concentrations in stem and blossoms were not affected (Fig. 2-2B). In leaves, soluble sugar concentrations decreased, particularly with severe deficiency

Starch concentrations in individual organs were little affected by moderate N deficiency (Fig. 2-2C). In severely deficient plants, however, starch concentrations in all organs were increased, whereby this increase was particularly pronounced in roots, stem and leaves.

To summarize, the effects of nutrient supply on non-structural carbohydrates were specific for each nutrient, each organ, and the form of carbohydrates (soluble sugars or starch). The K deficiency-induced reduction of organ biomass in the first 20 DAT, which was particularly pronounced for stem and blossoms (Table 2-2A), was not associated with reduction of sugar or starch concentrations in these organs (Fig. 2-2). This indicates that growth reduction was not due to availability of carbohydrates. The Mg deficiency-induced reduction of organ biomass, which was particularly pronounced for the sink organs roots, stem and blossoms (Table 2-2A) was associated with lower concentrations of soluble sugars (Fig. 2-2B). This indicates that growth reduction may be due to low sugar availability. The N deficiency-induced reduction of organ biomass was particularly pronounced for leaves (Table 2-2 A). In the leaves of N-deficient plants, soluble sugars were reduced (Fig. 2-2B), but starch concentrations were increased, at least under severe deficiency (Fig. 2-2C). This indicates, that N deficiency-induced growth reduction was not directly related to low carbohydrate availability.

2.3.3.2 Mineral nutrients

Twenty DAT, with few exceptions, mineral concentrations in all plant organs decreased in the order from optimal supply to medium supply to low supply (Fig. 2-3). The extent of concentration decrease was dependent on the specific nutrient and the specific organ.

In case of K, the decrease of concentrations was particularly drastic in roots (24 to 39% of optimal supply) and old leaves (31 to 47 % of optimal supply) (Fig. 2-3a). In stem, K concentrations decreased to 39 to 59% of optimal supply, and in young leaves (55 to 59 % of optimal supply) and blossoms (60% of optimal supply) the decrease of concentrations was comparatively small. These results indicate that K was remobilized from roots and old leaves to young leaves and blossoms.

In case of Mg, the decrease of concentrations was particularly drastic in old leaves (about 10 to 14 % of optimal supply), followed by roots (17 to 33% of optimal supply), and

stem (27 to 42 % of optimal supply) (Fig. 2-3b). The decrease of concentrations in young leaves (24 to 38% of optimal supply) and in particular, in blossoms (60 to 82% of optimal supply) was comparatively small. This indicates scarce Mg is preferentially supplied to blossoms.

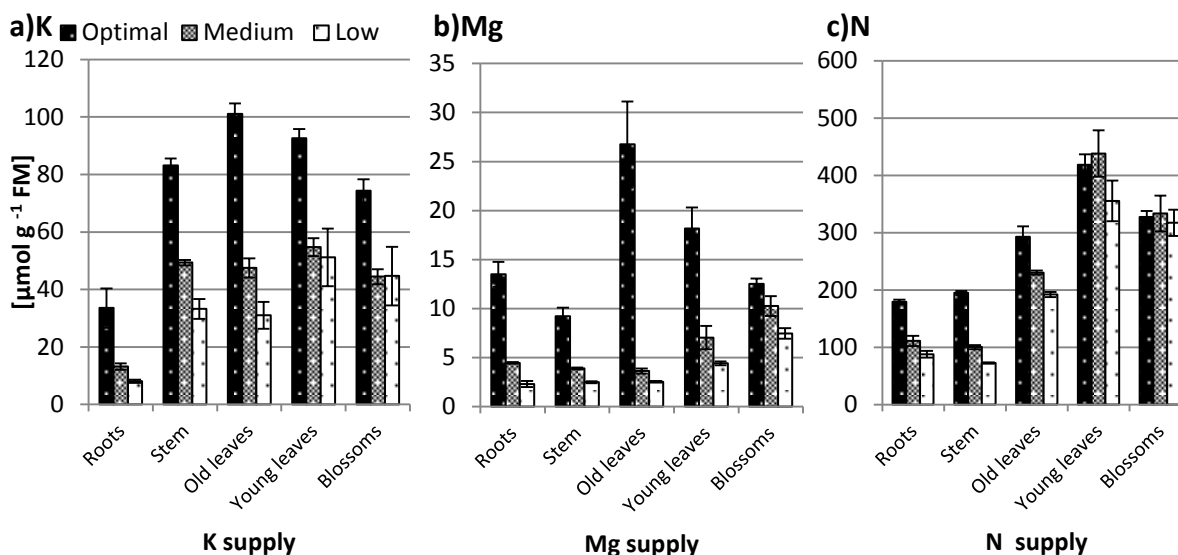


Fig. 2-3: Effect of the rate of K, Mg and N supply on K (a), Mg (b) and N (c) concentration in different plant organs respectively at 20 DAT; vertical lines represent the standard errors of means (n=4)

The N deficiency-induced decrease in nutrient concentrations (Fig. 2-3c), in general, in all organs was less pronounced than the K deficiency- (Fig. 2-3a) and Mg deficiency-induced decrease in nutrient concentrations (Fig. 2-3b). This indicates that in case of N deficiency, the reduction of N supply was more closely matched with reduction of dry mass formation. Nevertheless, N deficiency resulted in lower N concentrations in roots (49 to 62% of optimal supply), stem (37 to 52% of optimal supply) and old leaves (66 to 79 % of optimal supply) (Fig. 2-3c). N concentrations in young leaves (85 to 105% of optimal supply) and blossoms (97 to 102 % of optimal supply) were not significantly reduced under conditions of by N deficiency (Fig.2-3c).

To summarize, the nutritional status of nearly all plant organs was substantially impaired under nutrient deficiency. In general the nutritional status of young leaves, and in particular of blossoms was least affected. The nutritional status of roots was severely impaired, despite roots have the primary access to scarce nutrients. The extent of decrease of nutrient concentration in the individual organs (Fig. 2-3) was not closely associated to the decrease of organ biomass (Table 2-2). This indicates that the deficiency-induced reduction of individual organ growth was not directly regulated by the nutritional status of the individual organs.

2.3.4 Effects of nutrient supply on allocation of biomass and nutrients to individual organs

How does limited K, Mg and N supply affect biomass and nutrient allocation to different plant organs in various stages of plant development?

2.3.4.1 Biomass allocation

Previously, we described that we succeeded in reducing total biomass production by limiting supply of nutrients (Table 2-2). Now we analyze whether the reduced plant biomass is associated with a shift in biomass partitioning as described in literature for experiments with complete lack of nutrient supply. Therefore, the percentage of total biomass that was allocated to different plant organs was calculated (Fig. 2-4).

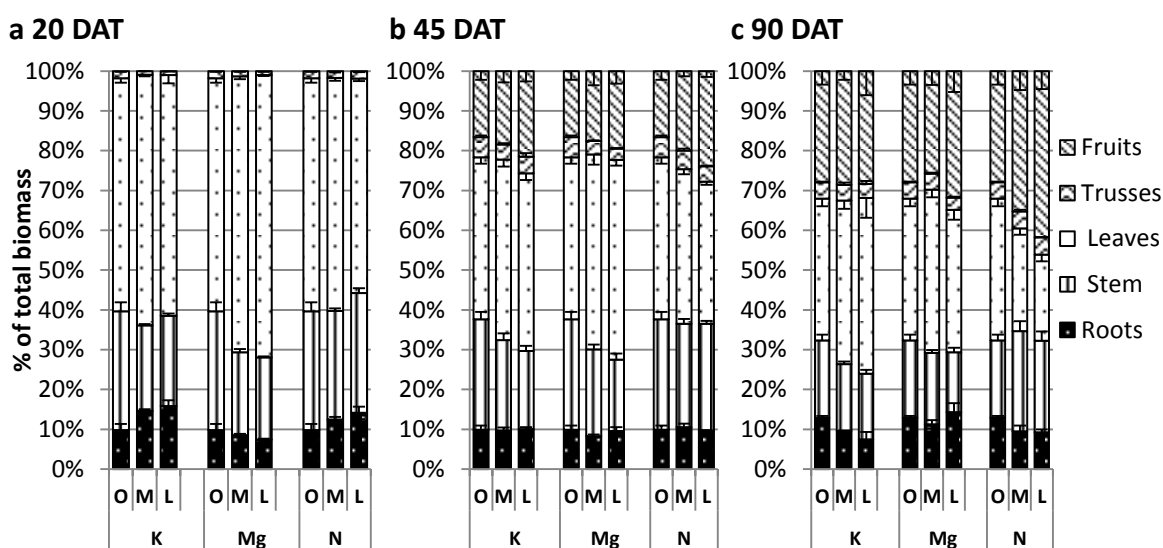


Fig. 2-4: Effect of different rates of K, Mg and N supply on biomass partitioning among plant organs during growth season. Vertical lines represent standard errors of means (n=4)

When tomato plants were optimally supplied with nutrients, total plant biomass 20 DAT was composed of about 10 % roots, 30 % stems, 58.5 % leaves and 2 % trusses (Fig. 2-4a). At low K supply, the biomass allocation to various plant organs changed in that way that roots accounted for 14 - 16 %, stems for 21-23 %, leaves for 60 – 63 % and trusses for 1 % of total plant biomass. In case of low Mg supply, biomass allocation was changed to 7.5 % roots, 20 % stems, 70 % leaves and 1 % trusses. Under N limited conditions tomato plants consisted of 12 - 14 % roots, 30 % stems, 54 % leaves and 2 % trusses. To sum up the result for biomass partitioning at 20 DAT we can say, that K-deficient plants increased biomass partitioning to leaves and roots at expense of reduced biomass partitioning to stem and trusses. In Mg-deficient

plants, a much higher proportion of biomass was partitioned to leaves at expense of lower partitioning to roots, stems and trusses. When plants suffer from low N supply, they show reduced biomass partitioning to leaves and increased biomass partitioning to roots.

The next analyzed growth period, 20 - 45 DAT, was characterized by the formation and growth of tomato fruits. 45 DAT, tomato plants under optimal nutritional conditions consisted of 10 % roots, 28 % stems, 41 % leaves, 5 % trusses and 16 % fruits (Fig. 2-4b). When plants suffered from limitations in K supply during this growth phase, biomass partitioning changed in that way that roots accounted for 10 %, stems for 20 - 22 %, leaves for 45 %, trusses for 4 % and fruits for 18 - 22 %. In Mg-deficient plants, biomass partitioning was changed to 8 - 10 % roots, 18 - 22 % stems, 50 % leaves, 3 % trusses and 18 - 19 % fruits. Plants suffering from N deficiency were composed of 10 % roots, 27 % stems, 36 - 40 % leaves, 4 - 5 % trusses and 20 - 24 % fruits. Again, it became obvious that in most cases the nutrient deficiency-induced change in biomass partitioning was more pronounced in plants suffering from strong deficiency than in plants suffering from moderate deficiency. In summary, during this phase, K and Mg-deficient plants reduced biomass partitioning to stem and trusses in favor of increased partitioning to leaves and fruits. For plants grown under N limited conditions only slight biomass partitioning changes were detected, namely decrease in leaf biomass accompanied by an increase in fruit biomass.

The third growth phase from 45 to 90 DAT was characterized by fruit maturation. 90 DAT, adequately supplied plants consisted of 13 % roots, 20 % stems, 36 % leaves, 4 % trusses and 27 % fruits (Fig. 2-4c). Under K deficiency, the composition of plants changed to 8 - 10 % roots, 16 % stems, 41 - 45 % leaves, 3 - 4 % trusses and 27 % fruits. When plants were grown with limited Mg supply, they consisted of 11 - 14 % roots, 15 - 18 % stems, 36 - 40 % leaves, 3 - 4 % trusses and 27 - 31 % fruits. In case of N limitation, plants biomass composition was characterized by 10 % roots, 23 - 25 % stems, 22 - 26 % leaves, 4 % trusses and 35 - 42 % fruits. This part of the experiment can be summarized to the result that biomass partitioning was not significantly affected by limited K supply due to the large variation. A tendency could be detected to higher allocation of biomass to leaves and lower allocation to roots and stems. Mg deficiency had no effect on biomass partitioning at 90 DAT. Meanwhile, N-deficient plants increased partitioning to fruits and stems at the expense of reduced partitioning to leaves.

An important outcome of this experiment was the nutrient-specific influence on biomass partitioning. Responses of biomass partitioning to low nutrient supply were specific for each individual nutrient. Whereas K and Mg deficiency, in general, rather increased leaves and fruits biomass proportion at the expense of roots, stems and trusses biomass, N deficiency resulted in

reduced leaves biomass percentage accompanied by increased stem biomass percentage. In all cases of starvation, proportion of fruit biomass was rather increased or similar to optimal supply condition. The shift in biomass partitioning towards roots under nutrient limitations, which is often described in literature could be observed 20 DAT only. At following growth periods, no difference in biomass partitioning compared to optimal nutrient supply was detected. In the last growth period, partitioning to roots was even decreased instead of increased for all nutrient limitations.

2.3.4.2 Nutrient allocation

Next question to be answered was, in which way and to which extent limitations of each nutrient (K, Mg and N) influence the partitioning of this limited nutrient among different plant organs. At 20 DAT, K- deficiency had no effect on K partitioning among different plant organs, meanwhile, Mg partitioning to leaves was significantly reduced and, high proportion of Mg was partitioned to stem and trusses (Fig. 2-5a). Severe Mg-deficient plants had higher proportion of Mg in leaves and lower Mg in roots as compared to moderately Mg-deficient plants. In comparison of control, N deficiency was associated with higher proportion of N in trusses and particularly leaves and lower proportion of N in stem. However, N deficiency had no effect on the N proportion, which was partitioned to roots.

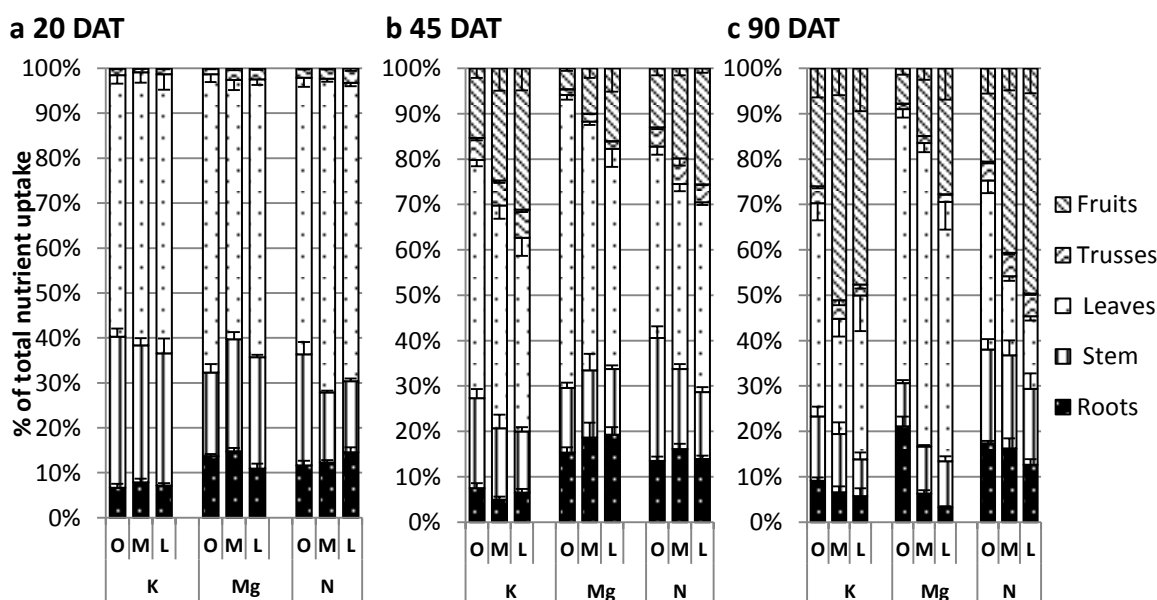


Fig. 2-5: Effect of K, Mg and N supply {optimal (O), medium (M) and low (L)} on K, Mg and N partitioning among plant organs during growth season. Vertical lines represent standard errors of means (n=4)

At 45 DAT, high proportion of K was partitioned to the fruits of K-deficient plants at expense of reduced K in leaves and stem, but K partitioning to roots and trusses was not affected by K deficiency (Fig. 2-5b). Mg deficiency resulted in an increased proportion of Mg in fruit and reduced proportion of Mg in leaves as compared to control; however, root, stem and trusses were not affected by Mg deficiency. In this period, high proportion of N was partitioned to fruits. N partitioning to stem was reduced, whereas N partitioning to roots, leaves and trusses were not affected by N deficiency.

In comparison to control, K deficiency was associated with higher proportion of K in fruits and lower proportion in leaves; however, root, stem and trusses were not affected by K deficiency at 90 DAT (Fig. 2-5c). In this period, a much higher proportion of Mg was partitioned to fruits and trusses and less Mg was partitioned to roots. Mg partitioning to stem and leaves were not affected by Mg supply. As in case of K, N deficiency was associated with higher N in fruits and lower N in leaves, but N partitioning to roots, stem and trusses were not affected by N deficiency.

Table 2-3: Effect of nutrient supply on allocation of biomass and nutrients to various plant organs (% of total plant biomass and total plant nutrient content); harvest 20, 45 and 90 DAT.

		a) 20 DAT				b) 45 DAT				c) 90 DAT			
		Nutrient supply											
Organ	Parameters	*O	-K	-Mg	-N	O	-K	-Mg	-N	O	-K	-Mg	-N
Roots	Biomass	10	15	8	13	10	10	9	10	13	9	13	9
	N	12	17	9	14	14	14	12	15	17	11	16	14
	K	7	8	9	15	8	6	7	15	9	6	12	17
	Mg	14	20	13	21	15	15	19	9	21	8	5	5
Stem	Biomass	30	22	21	29	28	21	20	26	20	17	17	24
	N	25	15	18	16	27	20	17	16	21	21	17	19
	K	34	30	31	32	20	15	16	21	14	10	13	18
	Mg	19	16	25	17	14	10	15	16	10	7	10	13
Leaves	Biomass	59	62	70	56	41	45	49	38	36	43	38	24
	N	62	68	73	68	42	47	54	41	37	43	38	17
	K	58	62	60	52	53	46	61	41	47	31	46	25
	Mg	66	63	60	61	65	71	52	63	60	77	62	63
**Generative organs	Biomass	2	1	1	2	21	24	21	26	31	31	32	43
	N	2	1	1	3	17	19	18	28	25	25	29	50
	K	2	1	1	1	20	34	15	22	30	53	28	40
	Mg	1	0.4	3	1	6	5	15	12	9	7	23	19

*O, optimal, -K, K deficiency, -Mg, Mg deficiency, -N N deficiency.

** It was blossom at 20 DAT, and was trusses and fruits at 45 and 90 DAT.

To sum up the main result of this analysis, it has to be pointed out that the partitioning of nutrient among different plant organs was significantly influenced by nutrient supply. The effect of nutrient supply on nutrient partitioning was nutrient-specific and independent on the rate of nutrient supply (low - medium) in most cases. For biomass partitioning as well as for nutrient partitioning under nutrient deficiency, we found nutrient depending effects on each process. For example, when plants suffer from Mg limitations more biomass is allocated to leaves, whereas Mg allocation in leaves was less than in plants with optimal provision of nutrients. In case of N deprivation, we found contrary effects 20 DAT, namely reduced biomass partitioning to leaves, but increased allocation of N into leaves. In case of N partitioning, first an increased allocation of N into leaves was observed, maybe due to an increased demand for chlorophyll synthesis during leaf development. In the next growth period analyzed, no differences in N starved plants compared to optimal supplied plant concerning N partitioning appeared, whereas in the last growth period N allocation into leaves was reduced. This might be due to senescence processes, where chlorophyll is degraded.

2.4 Discussion

Main aims of this study were to assess (i) if the nutrient deficiency-induced alteration of biomass and nutrient allocation among plant organs is dependent on the intensity of nutrient deficiency, and (ii) if the deficiency-induced alterations of allocation are specific for K, Mg and N deficiency, and can be classified as adaptive responses.

2.4.1 Effect of intensity of nutrient deficiency on the alteration of biomass and nutrient allocation among plant organs

It is well documented that biomass allocation within plants among organs is dependent on environmental conditions (Poorter et al. 2012). It is a widespread perception that the environment-induced modification of biomass allocation follows the principle of the balanced-growth hypothesis: biomass is preferentially allocated to those organs that acquire the resource that is limiting growth (Shipley and Meziane 2002). Unfavourable conditions for acquisition of soil resources, e.g., drought or low nutrient availability, increase biomass allocation to roots, whereas unfavourable conditions for acquisition of aboveground resources, e.g., low light intensity, increase biomass allocation to stems and leaves (Wilson 1988).

On the other hand, there is evidence that unfavourable environmental conditions like low Mg or K availability can lead to deficiency-induced disorders in plant metabolism like impairment of phloem loading in and phloem transport from source organs (Hermans et al.

2006). These disorders lead to a decrease of biomass allocation to sink organs like roots, and thus prevent biomass allocation according to the balanced-growth hypothesis. We expected, that deficiency-induced disorders mainly occur under conditions of severe deficiency, particularly, when severe deficiency is induced by complete prevention of nutrient uptake, e.g. by withdrawal of nutrients from nutrient solution. In our experimental approach, plants were continuously supplied with nutrients even under conditions of severe deficiency. This simulates the situation of low nutrient availability in soil, whereby low amounts of nutrients become continuously available for plant uptake either by slow delivery from inorganic and organic soil pools or by additional spatial exploitation of soil by root growth. This approach allowed investigating plant responses to different intensities of nutrient limitation over an extended period and in different phases of plant development from vegetative growth before flowering to fruit maturity. As intended by our experimental approach, total plant biomass was more severely suppressed by low (about 40 to 70% of optimal supply, depending on the growing phase) than by medium (about 65 to 80% of optimal supply) nutrient supply (Table 2-2). In average of all plant organs, nutrient concentrations were also more severely suppressed by low than by medium nutrient supply (Fig. 2-3). However, the effects of the intensity of nutrient deficiency on allocation of biomass (Fig. 2-4) and nutrients (Fig. 2-5) were small. This indicates that with regard to allocation of biomass and nutrients, plant responses to nutrient deficiency were not dependent on the intensity of nutrient deficiency.

With regard to non-structural carbohydrate concentrations in the vegetative growing phase, however, two observations have to be noted. First, under Mg deficiency, soluble sugar concentrations in leaves were significantly increased under severe deficiency but not under moderate deficiency (Fig. 2-2B). Sugar accumulation in leaves under Mg deficiency has been measured also in other studies (Fischer et al. 1998, Hermans et al. 2004, Hermans and Verbruggen 2005), and has been related to impairment of phloem loading (Cakmak et al. 1994b). Possibly, under conditions of severe Mg deficiency, Mg concentrations in specific leaf tissues or sub-cellular compartments fall below critical values, that are needed for phloem loading.

Second, under N deficiency, leaf starch concentrations were strongly increased under severe but not under moderate deficiency (Fig. 2-2C). N deficiency-induced accumulation of starch (but not sugars) in leaves has been measured also in other studies (Geiger et al. 1999). This effect of N deficiency has been attributed to high transcript levels and activity of ADP-glucose pyrophosphorylase, a key enzyme for the regulation of starch biosynthesis. Transcription of the gene encoding this enzyme is suppressed by nitrate (Scheible et al. 1997),

and accordingly, nitrate deficiency is associated with higher transcript levels when nitrate is missing. Possibly, under conditions of severe N deficiency, nitrate concentrations in leaf cells fall below critical values that are needed to suppress extensive starch biosynthesis in leaves.

2.4.2 Nutrient-specific alterations of biomass and nutrient allocation in response to nutrient deficiency

For the discussion, it is assumed that allocation of biomass and nutrients to various organs within the plant is regulated by the relative sink strength of each organ. To visualize effects of nutrient deficiency on allocation of biomass and nutrients, allocation to various plant organs in % of total plant content is shown in Table 2-3 a, b and c for 20 , 45 and 90 days after start of treatment (DAT) respectively. The values shown for K-, Mg- and N-deficient plants are the means of “medium” and “low” supply.

2.4.2.1 Alteration of biomass allocation during plant development

The relative sink strength of the organs for biomass changed during plant development. These changes were most prominent for leaves and generative organs. For example, in optimally supplied plants, 59% of total biomass was allocated to leaves at 20 DAT, indicating that leaves were the most important sink for biomass up to flowering (Table 2-3a). Then after, biomass allocation to leaves decreased to 41% at 45 DAT (Table 2-3b) and 36% at 90 DAT (Table 2-3c), indicating that the sink strength of leaves for biomass was decreasing during plant development. Biomass allocation to reproductive organs (blossoms at 20 DAT, fruits and trusses 45 and 90 DAT), in contrast, increased from 2% at 20 DAT (Table 2-3a) to 21% at 45 DAT (Table 2-3 b) and to 31% at 90 DAT (Table 2-3c). This indicates that the sink strength of reproductive organs strongly increased after flowering. This is in accordance with other investigations, in which the sink strength for biomass was more specifically assessed, e.g. by measuring the allocation of ¹⁴C-labelled photosynthates to various plant organs (Ho, 1984; Hocking and Steer 1994).

2.4.2.2 Alteration of nutrient allocation during plant development

Similar to the alteration of biomass allocation, nutrient allocation to various plant organs also changed during plant development (Tables 2-3 a, b, c). The ontogenetic pattern of nutrient allocation was nutrient specific. Under optimal supply, the ontogenetic changes of N allocation were most prominent for leaves (decrease of allocation from 62% of total plant N at 20 DAT to 37% at 90 DAT) and generative organs (increase from 2% at 20 DAT to 25% at 90 DAT). This indicates, that under optimal supply leaf N is the most important variable N pool which delivers N to generative organs after flowering.

For K, the ontogenetic changes of allocation were most prominent for stem (decrease of allocation from 34% of total plant K at 20 DAT to 14% at 90 DAT) and generative organs (increase from 2% of total plant K at 20 DAT to 30% at 90 DAT). This indicates that the increasing demand of generative organs after flowering was mainly buffered through the stem K pool.

For Mg, the ontogenetic changes in allocation to various organs were comparatively small. Even 90 DAT, 60% of total plant Mg remained in leaves, indicating low remobilization from leaves to other organs. The small increase of Mg allocation to generative organs during plant development (1% of total plant Mg at 20 DAT to 9% at 90 DAT) was paralleled by a small decrease of Mg allocation to the stem (19% of total plant Mg at 20 DAT to 10%).

2.4.3 Alteration of biomass and nutrient allocation in response to nutrient deficiency

2.4.3.1 Root/shoot allocation

The relative sink strength of the organs for biomass was not only influenced by ontogenetic development, but also by nutrient supply. The effect of nutrient supply was nutrient-specific. For example, during the vegetative growing phase up to flowering, K deficiency and N deficiency in comparison to optimal supply were associated with higher biomass allocation to roots, whereas Mg deficiency was associated with lower biomass allocation to roots (Table 2-3a). For N deficiency, this is in accordance with many other investigations, which show an increase of root/shoot ratio in N-deficient plants (Marschner 1995). For K deficiency, the situation is less clear. It has been found that K deficiency can be associated with a decrease (Cakmak et al. 1994a; Gerardeaux et al. 2010) or an increase of the root/shoot ratio (Peuke et al. 2002), possibly depending on plant species, plant age, and the experimental approach to induce K deficiency. Under Mg deficiency, in most studies a reduction of root/shoot ratio was found, e.g. in bean (Cakmak et al. 1994a), spinach (Fischer et al. 1998) and pepper (Riga and Anza 2003). Increased root/shoot ratio under nutrient deficiency is classified as an adaptive response, which increases the ability of plants for foraging of soil resources relative to the growth-related shoot demand for these resources (Marschner 1995). Decreases in root/shoot ratio under nutrient deficiency are assumed to be a consequence of deficiency-induced disturbances in plant metabolism rather than adaptive plant responses.

The lower biomass allocation to roots of Mg-deficient plants was associated with a decrease of non-structural carbohydrate concentrations in roots (Fig. 2-2). This indicates that the lower relative sink strength of roots was related to an impairment of carbohydrate supply from

leaves. The higher biomass allocation to roots of K- and N-deficient plants, in contrast, was not associated with significant alterations of the root carbohydrate concentrations (Fig. 2-2). This indicates that the higher relative sink strength of roots of K- and N-deficient plants was not directly controlled by carbohydrate supply. There is good evidence, that the relative sink strength of roots and shoots for biomass allocation is controlled by phytohormones, e.g. CYT and ABA, and sugar signaling (Hermans et al. 2006).

Effects of nutrient deficiency on biomass allocation to roots were often paralleled by similar effects on nutrient allocation to roots. For example, during the vegetative growing phase in N-deficient plants not only biomass allocation to roots was increased but also K allocation (from 7% under optimal supply to 15% in N-deficient plants), and Mg allocation (from 14% under optimal supply to 21% in N-deficient plants) (Table 2-3a). Similarly, K deficiency in parallel to increased biomass allocation increased also the N allocation to roots from 12 to 17%, and the Mg allocation from 14 to 20% (Table 2-3a). Mg deficiency, in parallel to reduced biomass allocation to roots, decreased also N allocation to the roots (Table 2-3a). However, nutrient and biomass allocation to roots were not always changed in parallel. For example, K deficiency did not increase K allocation, and Mg deficiency did not decrease Mg allocation to roots (Table 2-3a). Other examples: N limitation had no effect on biomass allocation to the stem, whereas N allocation was strongly decreased from 25% under optimal supply to 16% under N limitation (Table 2-3a). This is in accordance with the role of stem N pools as buffer which is filled under conditions of ample supply and depleted under conditions of deficiency (Masclaux-Daubresse et al. 2010). This indicates that sink strength for biomass and sink strength for nutrients are regulated by separate mechanisms. The mechanisms, which control sink strength of roots and shoots for minerals are not known.

2.4.3.2 Allocation to stems

Apart from root/shoot biomass ratios, little is known from literature on the effect of nutrient supply on allocation of biomass and nutrients to other organs. In our study, in which axillaries were removed, we separated the shoot into leaves and stem. Nutrient supply influenced also biomass allocation to stems and leaves, and to vegetative and generative plant organs. For example, biomass allocation to the stem was markedly decreased under K and Mg deficiency, whereas N deficiency had no effect (Tables 2-3a, b, c). Lower biomass allocation to stems under K deficiency has also been found in cotton (Gerardeaux et al. 2010). Biomass allocation was not always paralleled by nutrient allocation. For example, at 20 DAT, Mg allocation to the stem was increased from 19% under optimal supply to 25% under Mg

deficiency, despite of a decrease of biomass allocation to the stem from 30% under optimal supply to 21% under Mg deficiency (Table 2-3a). Little is known about the control of sink strength of stems for biomass and nutrients, and the ecological relevance of a change of allocation to stems. Stems are important structural elements, which support stability and erect growth habitus of plants. Furthermore, stems represent an important store for carbohydrates and nutrients which may buffer temporal imbalances between supply of resources from photosynthesis and nutrient acquisition and growth-related demand for resources (Hocking and Steer 1994; Engels et al. 2012). This would explain reduction of biomass allocation to stems under K and Mg deficiency, when photosynthesis is reduced (Table 3-1), but does not explain increased Mg allocation to stems under Mg deficiency.

2.4.3.3 Allocation to leaves

Allocation of biomass to leaves was most strongly affected by Mg deficiency at 20 and 45 DAT (Tables 2-3a, b), and by N deficiency at 90 DAT (Table 2-3c). Mg deficiency in comparison to optimal supply increased biomass allocation to leaves. Mg allocation to leaves, in contrast, was reduced. Thus, leaf Mg concentrations were drastically decreased (Fig. 2-3). The pattern of Mg deficiency induced alterations of biomass and Mg allocation to leaves was in marked contrast to the pattern of N deficiency induced alterations. In N-deficient plants, 20 DAT biomass allocations to leaves was diminished, whereas N allocation to leaves was increased (Table 2-3a). Thus, leaf N concentrations in the vegetative growing phase were only little affected by N deficiency (Fig. 2-3). Leaf N concentrations are often closely correlated with leaf photosynthetic activity (Engels et al. 2012). Thus, the pattern of N and biomass allocation under N deficiency can be interpreted as an adaptation of the plant which maintains photosynthetic activity, albeit at the cost of lower photosynthetic active leaf mass (area).

At 90 DAT, N deficiency was associated with lower allocation to leaves not only of biomass but also of N and K (Table 2-3c). It is well documented that N deficiency accelerates leaf senescence, and thus, senescence-induced remobilisation of biomass and nutrients from leaves to sink organs (Engels et al. 2012, Shi et al. 2012). Increased N remobilisation from senescent leaves, and thus, low N allocation to leaves at maturity, is an important mechanism to increase internal N use efficiency (Engels et al. 2012). The fact that Mg allocation to leaves was not reduced under N deficiency indicates that Mg, in contrast to N and K, is poorly remobilized in tomato.

In comparison to optimal nutrient supply, K deficiency at 90 DAT decreased allocation of K to leaves from 47% to 31%, but even increased allocation of N (from 37% under optimal

supply to 43% under K deficiency) and allocation of Mg (from 60% under optimal supply to 77% under K deficiency) (Table 2-3c). This indicates that the internal N use efficiency was reduced by K deficiency. K deficiency, similar as N deficiency, is also known to induce leaf senescence (Cao et al. 2006). However, there is evidence that the hormonal signals and the molecular events associated with K deficiency-induced senescence (Armengaud et al. 2004) differ from those of N deficiency-induced senescence (Criado et al. 2009). Possibly, this is the reason for the differences between N deficiency- and K deficiency-induced changes in nutrient allocation to leaves.

At 90 DAT, Mg deficiency did not change nutrient allocation to leaves in comparison to optimal nutrient supply (Table 2-3c). The leaves of Mg-deficient plants showed severe symptoms of senescence (not shown). The fact that even Mg allocation to leaves was not decreased under Mg deficiency is further evidence for the poor Mg remobilization in tomato.

2.4.3.4 Allocation to generative organs

Nutrient supply also influenced biomass and nutrient allocation to fruits. Biomass allocation to fruits was increased under N deficiency but little affected by K and Mg deficiency (Tables 2-3 b, c). The allocation of the deficient nutrient to generative plant organs was strongly increased for N, Mg and K. Under Mg deficiency, increased Mg allocation to fruits was associated with lower Mg allocation to roots. Increased K allocation to fruits of deficient plants was also found by Kanai et al. 2007). Increased allocation of biomass and nutrients to generative organs at maturity can be classified as adaptive response which improves resource availability for the succeeding generation. Interestingly, under N deficiency not only N allocation but also K and Mg allocation to fruits and trusses were markedly increased, in particular at 90 DAT (Table 2-3c). This indicates that N deficiency generally increased the relative sink strength of generative organs for biomass and all nutrients. K deficiency and Mg deficiency, in contrast, increased the relative sink strength of the generative organs only for the deficient nutrient.

3. Phenotypic plasticity of shoot and root traits of tomato in response to different rates of K, Mg and N supply

Abstract

In this chapter, the aim was to assess the physiological and morphological plasticity of shoot traits and morphological root traits in response to the intensity of nutrient deficiency and comparing plastic responses to K, Mg and N at vegetative growth phase .

Physiological and morphological shoot responses to nutrient deficiency were nutrient-specific. Net assimilation rate (NAR) of N-deficient plants was not affected, while NAR of K-deficient plants was slightly reduced, and NAR of Mg-deficient plants was severely reduced. Maintenance of high NAR in N-deficient plants was associated with severe reduction of leaf area (LA) and leaf area ratio (LAR. Leaf area per total plant biomass). Leaf area of K and Mg-deficient plants less affected and LAR was enhanced in comparison to control plants. Thus, in N-deficient plants the reduction of growth was mainly due to lower LAR, whereas, in K- and particularly Mg-deficient plants growth reduction was mainly due to lower NAR.

The morphological response of roots to nutrient deficiency also was nutrient-specific. N deficiency slightly reduced specific root length (SRL), but increased root mass ratio (RMR). Therefore, root length ratio (RLR, root length per total dry mass of plant) was not influenced by N deficiency. In contrast, Mg deficiency resulted in increased SRL, but decreased RMR. Thus, RLR was also not influenced by Mg deficiency. K deficiency was associated with higher RLR because both, RMR and SRL were increased.

3.1 Introduction:

Plants can grow in a wide range of environments by adjusting their morphological and physiological traits to cope with different environmental conditions (Lambers et al. 1989). The capacity of a given genotype to adjust biomass allocation to different plant organs, and morphological and physiological traits, and thus, to express different phenotypes in different environments is known as phenotypic plasticity (Sultan 2000).

Plastic responses may be inevitable effects of environmental limits on growth and physiology (van Kleunen and Fischer 2005). Often, however, traits involved in resource acquisition show functionally appropriate patterns of plasticity. For example, under conditions of low availability of belowground resources biomass allocation to roots is often increased, whereas under conditions of low irradiance, biomass allocation to aboveground organs is often increased (Wilson 1988). These specific adjustments of the shoot: root ratio can partly compensate functionally for the reductions in total plant growth that occurs under resource limitation, and thus, can be classified as adaptive plastic responses (Sultan 2000).

Functional shifts in response to different resource availability are not confined to alteration of shoot: root ratio but include other shoot and root traits, which are more directly related to resource acquisition. Shoot traits include physiological leaf traits such as stomatal conductance or photosynthetic rate, and morphological traits such as leaf size and specific area and whole-plant to leaf-area: biomass ratio (Nicotra et al. 2010). Physiological root traits include the root capacity for uptake of different nutrients and water or the root ability to increase nutrient availability in the rhizosphere (Marschner 1995). Morphological root traits include root length, specific root length and whole-plant biomass to root length ratio (Nicotra et al. 2010, Hodge 2004).

In agricultural and horticultural plant production, nutrient availability is an important environmental factor, which can be managed by fertilization. Phenotypic plasticity of plant traits in response to nutrient availability is an important issue as it may affect the efficiency of acquisition of belowground resources and tolerance to abiotic stresses. For example, plastic morphological root responses induced by a specific nutrient may have consequences for acquisition of other nutrients and water. Plastic morphological leaf responses may have consequences for intra- and interspecific competition for light.

N-deficient plants are typically stunted, with narrow leaves (Hawkesford et al. 2012). N deficiency results in a decrease in aboveground biomass accumulation but it did not affect belowground biomass accumulation or root morphology (Trubat et al. 2006). N deficiency was associated with reduced leaf area (LA), leaf area ratio (LAR) and specific leaf area (SLA) in

tobacco (Brueck and Senbayram 2009) and tomato (Le Bot et al. 2009). In young seedlings of *Malus huphensis*, N deficiency reduced root number, root density and the root length of the lateral roots (Wei-guo and Hong-qiang 2007).

K deficiency during vegetative development decreased plant dry matter production and LA. K deficiency reduced LA, SLA, internode length and root mass ratio (RMR), meanwhile leaf mass ratio (LMR) was increased in cotton plants (Gerardeaux et al. 2009, Gerardeaux et al. 2010). K deficiency enhanced RMR at the expense of reduced stem mass ratio in tomato plants (del Amor and Marcelis 2004). Also, low and moderate K levels affected the root morphology in pea, red clover, lucerne, barley, rye, perennial ryegrass and oilseed rape, whereby it was found that these crop species modify their root hair length in response to low K, and thereby maintain the uptake from sparingly soluble K sources (Hogh-Jensen and Pedersen 2003). Root K absorption capacity and root length proliferation are dominant mechanisms in facilitating K acquisition efficiency in tomato plants (Chen and Gabelman 2000). K-deficient wheat plants acquired more K because these plants had high root length ratio (root length/plant biomass; RLR) (Samal et al. 2010). Low K supply reduced the root growth, but moderate K deficiency increased the root length of the efficient rice genotypes (Jia et al. 2008). However, in young seedlings of *Malus huphensis* plants, with shortage of K, the number, density and the length of the lateral root were decreased (Wei-guo and Hong-qiang 2007).

Mg deficiency resulted in reduction of RGR, LA and SLA but increased LMR (Riga and Anza 2003). High Mg supply caused reduction in specific root length (SRL) in root of Norway spruce plants (Zhang and George 2008). Mg deficiency resulted in increased number, density and length of lateral roots of young seedlings of *Malus huphensis* (Wei-guo and Hong-qiang 2007). Mg deficiency results in increased root length (RL) and root diameter (RD) and root surface area (RA) and biomass allocation to the roots (Ding and Xu 2011).

The short literature review shows that the effects of nutrient deficiency on morphological shoot and root traits may considerably vary depending on not only the specific nutrient but also the specific plant species, and the specific experimental conditions. In most studies, the intensity of nutrient deficiency is not well described, and only one nutrient is considered. The aims of the investigations described in this chapter were

- i. To assess plasticity of physiological and morphological shoot traits and morphological root traits in response to the intensity of nutrient deficiency, and
- ii. To compare plastic responses to deficiency of N, K and Mg.

We expected that at moderate level of nutrient deficiency plastic responses are adaptive, whereas at severe nutrient deficiency disturbance of plant metabolism results in non-adaptive plastic responses.

3.2 Material and Methods:

Plant culture and the experimental approach to grow plants at different rates of K, Mg and N supply were described in detail in chapter 2 (see 2.2). For the assessment of plasticity of shoot and root traits, plants were harvested at start of nutrient treatment and 20 days after start of nutrient treatment (DAT). At 20 DAT, the first flowers just became visible, i.e. plants were still in the vegetative growing phase.

3.2.1 Measurement of shoot and root traits

Dry mass of individual organs, stem, internodes, leaf number and leaf area

Plants were separated into roots, leaves, stem and flowers. Dry mass of individual organs was measured after drying at 100°C for one hour and then 65°C for 24 hours. Stem length and internodes length were measured. Leaf number was counted and total leaf area was measured with a leaf area meter (Lamboa Instruments Corp. Model LI 3100).

Gas exchange measurement

Leaf gas exchange was carried out with a portable photosynthesis system (HCM-1000). Leaf gas exchange was measured on old leaf (the leaf below first truss) and young leaf (the leaf below second truss). The leaf was placed in the cuvette until stable reading was obtained.

Root analysis

Root length and mean diameter of roots were measured using WinRhizo Program 2005b (Regent Instruments, Canada). By using root length and root diameters all other root parameters can be calculated.

3.2.2 Calculation of shoot and root traits

Mean leaf area (LA) [$\text{cm}^2 \text{ leaf}^{-1}$] = total LA [cm^2] / leaf number

Specific stem length (SSL) [$\text{cm g}^{-1} \text{ stem dry mass}$] = stem length [cm] / stem dry mass [g]

Leaf mass ratio (LMR) [$\text{g leaf dry mass g}^{-1} \text{ total plant dry mass}$] = leaf dry mass [g] / total plant dry mass [g]

Specific leaf area (SLA) [$\text{cm}^2 \text{ g}^{-1} \text{ leaf dry mass}$] = total LA [cm²] / leaf dry mass [g]

Leaf area ratio (LAR) [$\text{m}^2 \text{ leaf area kg}^{-1} \text{ plant dry mass}$] = total leaf area (m^2) / total plant dry mass [kg]

Net assimilation rate (NAR) [$\text{g m}^{-2} \text{LA d}^{-1}$] = total dry mass (g) / total LA (m²) / day

Relative growth rate (RGR) [$\text{g kg}^{-1} \text{ DM d}^{-1}$] = increment in mass (g)/ total dry mass (kg)/ day

Equations

$$\text{LAR} = \text{LMR} \times \text{SLA} \times 10^{-1} \dots\dots\dots 1$$

where:

LAR = leaf area ratio ($\text{m}^2 \text{ kg}^{-1} \text{ DM}$)

LMR = leaf mass ratio ($\text{g DM leaf g}^{-1} \text{ DM plant}$)

SLA = specific leaf area ($\text{cm}^2 \text{ g}^{-1} \text{ DM leaf}$)

$$\text{RGR} = \text{NAR} \times \text{LAR} \dots\dots\dots 2$$

where

RGR = relative growth rate ($\text{g kg}^{-1} \text{ DM day}^{-1}$)

NAR = net assimilation rate ($\text{g m}^{-2} \text{ leaf area day}^{-1}$)

Using measured root parameters [root length (RL) and root diameter (RD)] other root parameters can be calculated as the following

$$\text{Root surface area (RA)} \quad \text{RA} = \text{RL} \times 2\pi r$$

$$\text{Root volume (RV)} \quad \text{RV} = \text{RL} \pi r^2$$

$$\text{where } r = \frac{1}{2} \text{RD} \quad \pi = 3.14$$

$$\text{RLR} = \text{RL} / \text{DM plant} (\text{m g}^{-1})$$

$$\text{RMR} = \text{DM root} / \text{DM plant} (\text{g. g}^{-1})$$

$$\text{SRL} = \text{RL} / \text{DM roots} (\text{m g}^{-1})$$

$$\text{F} = \text{RL} / \text{RV} (\text{m cm}^{-3})$$

$$\text{TD} = \text{DM root} / \text{RV} (\text{mg cm}^{-3})$$

where, RLR is the root length ratio, which expresses the root potential for the acquisition of below-ground resources; the RMR is the root mass ratio, which indicates the relative biomass allocated to the root; the SRL, F and TD are the specific root length, fineness and tissue density, respectively, which represent the structural root parameters. While, RL, DM and RV indicated root length, dry mass and root volume respectively (Sorgona et al. 2007). As reported by (Ryser and Lambers 1995), the following relationships obtain among the above parameters:

$$\text{RLR} = \text{SRL} \times \text{RMR}$$

$$\text{SRL} = \text{F} / \text{TD}$$

RLR = root length ratio ($\text{m g}^{-1} \text{ DM plant}$).

3.3 Results:

In chapter 2, we focused on whole plant physiological changes such as nutrient and carbohydrate distribution and their effects on biomass allocation when plants are exposed to nutrient limitation. Physiological changes precede morphological changes. The physiological changes result in biochemical changes and both influence plant morphology. In this chapter, we highlight on phenotypic plasticity of shoots, and roots in response to nutrient limitation. First, we will focus on the reduction in relative growth rate (RGR). RGR can be factorized into the physiological component net assimilation rate (NAR) and the morphological component leaf area ratio (LAR) (De Groot et al. 2001). We assessed to which extent the morphological component and physiological component are responsible for reduced RGR under nutrient limitation.

3.3.1 Effect of limited K, Mg and N supply on relative growth rate (RGR)

During the growth period from 0 to 20 DAT, nutrient limitation in comparison to optimal nutrient supply was associated with a significant reduction of the RGR (Table 3-1). In tendency, RGR was more reduced at severe nutrient limitation (low nutrient supply) than at moderate level of nutrient limitation (medium supply). For medium nutrient supply, we observed a RGR reduction to more or less 90 % of optimal supply, and for low nutrient supply RGR was further reduced to about 80% of optimal supply.

Table 3-1: Effect of K-, Mg- and N deficiency on relative growth rate (RGR) and net assimilation rate in the first 20 days after start of treatment (DAT), on leaf area ratio at 20 DAT and on rates of photosynthesis and transpiration at 17 DAT. Values followed by different letters differ significantly among treatments (Tukey-Kramer's test, $P < 0.05$)

Growth paramters		Nutrient supply						
		Optimal	K		Mg		N	
			Medium	Low	Medium	Low	Medium	Low
RGR (g g ⁻¹ d ⁻¹)		189a	176b	168bc	166bc	154c	169bc	157 c
LAR(m ² kg ⁻¹ DM plant)		16.9b	19.9a	19.5a	20.5a	20.4a	17.3b	15.8b
LMR(g g ⁻¹ DM plant)		0.58c	0.63b	0.61b	0.69a	0.71a	0.59bc	0.54d
NAR (g m ⁻² LA d ⁻¹)		11.2a	9.0bc	8.6bc	7.9 c	7.6c	9.8 ab	10.0 ab
A [*]	Young leaf	25.6a	26.9a	28.5a	29.5a	13.4b	28.6a	26.1a
	Old leaf	17.8ab	21.3ab	24.5a	12.2bc	7.0c	22.0ab	25.3a
E [*]	Young leaf	6.50a	6.16a	7.76a	7.42a	3.79b	6.27a	6.70a
	Old leaf	5.05ab	6.05a	5.40a	3.66ab	1.45b	6.29a	6.96a

*A =Photosynthetic rate ($\mu\text{mol m}^{-2} \text{LA s}^{-1}$) at 17 DAT

*E =Transpiration rate ($\text{mmol m}^{-2} \text{LA s}^{-1}$) at 17 DAT

3.3.2 Effect of limited K, Mg and N supply on NAR, rates of photosynthesis and transpiration and LAR

Nutrient limitation generally was associated with a reduction of NAR, whereby the intensity of nutrient deficiency, for all three nutrients, had no significant effect on NAR (Table 3-1). The effect of nutrient deficiency on NAR was strongly dependent on the specific nutrient (Table 3-1). NAR was more reduced by Mg deficiency (about 70 % of optimal supply) than by K deficiency (about 80% of optimal supply) and N deficiency (about 90% of optimal supply). NAR was calculated from the plant dry mass increment in the first 20 DAT and the mean leaf area during this period, and thus is a physiological leaf trait, which integrates net assimilation of all leaves over day and night, and over an extended period.

We also measured the rates of leaf gas exchange of the youngest fully developed leaf (young leaf) and the third leaf below the youngest fully developed leaf (old leaf) to assess plasticity of the rates of net photosynthesis and transpiration on day 17 after start of treatment. In general, rates of leaf gas exchange were lower in the old than in the young leaf (Table 3-1). This was to be expected, since the photosynthetic capacity of leaves decreases after leaf maturation (Engels et al. 2012). Leaf gas exchange was not significantly affected by nutrient limitation with the exception of severe Mg deficiency (low Mg supply). Low Mg supply resulted in a significant reduction of the rates of photosynthesis and transpiration in young and old leaves. Thus, our direct measurements of leaf gas exchange corroborate the data on NAR, which also showed that NAR was particularly reduced in Mg-deficient plants.

Similar to NAR, also LAR was influenced by nutrient limitation in a nutrient specific manner, whereby the intensity of nutrient limitation (medium or low supply) was of minor importance (Table 3-1). In comparison to optimal nutrient supply, LAR was significantly increased by K and Mg limitation, whereas N limitation had no significant effect.

3.3.3 Plasticity of morphological shoot traits

Data in Table 3-2 show that K deficiency (medium or low supply) resulted in reduction of stem length to about 70% of control, whereas N and Mg deficiency had no significant effect on stem length. The rate of nutrient supply did not significantly influence the rate of leaf development, and thus, leaf number 20 DAT. Accordingly, K deficiency, in parallel to reduced stem length was also associated with reduced internode length, whereas N and Mg had no significant effect on internode length.

Specific stem length (SSL) was markedly influenced by the rate of nutrient supply (Table 3-2). In general, SSL was higher under conditions of severe nutrient limitation (low

supply) than at moderate nutrient limitation (medium supply). The stem is considered as main organ for storage of photosynthates before flowering. Low availability of photosynthates due to reduction of photosynthesis is expected to reduce dry matter percentage and concentration of non-structural carbohydrates in stems (Fig. 2-1, 2-2 A), and thus, to increased SSL. The increase of SSL, was similar in K and N-deficient plants (about 150% of SSL under optimal supply). In Mg-deficient plants, SSL was nearly tripled in comparison to SSL of optimally supplied plants.

Total LA per plant and mean LA were influenced by the severity of nutrient limitation, whereby severe nutrient limitation (low supply) more strongly reduced leaf area than moderate nutrient limitation (medium supply) (Table 3-2). The effect of nutrient limitation on total LA and mean LA was dependent on the specific nutrient. Effects of K and Mg limitation were small, and with the exception of low Mg supply not significant. N deficiency, in contrast, was associated with strong reduction of total LA and mean LA to 69% (medium supply) and 46 % (low supply) of the leaf area of optimally supplied plants. In contrast to SSL, SLA was not significantly influenced by nutrient supply (Table 3-2). There was a tendency, however, that SLA was slightly increased by, K and Mg limitation, whereas SLA was not affected or slightly reduced in severely N limited plants.

Table 3-2: Effect of K, Mg and N supply on growth parameters (stem length, leaves number-internodes length, total LA mean LA, SLA and SSL). Values followed by different letters differ significantly among the treatments (Tukey-Kramer's test, $P < 0.05$)

Shoot parameters	Nutrient supply						
	Optimal	K		Mg		N	
		Medium	Low	Medium	Low	Medium	Low
Stem length (cm)	69.6a	59.6b	59.3b	68.1 a	69.9a	64.8 ab	63.6ab
Leaf No.	13.8a	16.3a	14.5a	14.8a	14.3a	14.3a	13.8a
Internode length (cm)	5.13a	3.67±b	4.10ab	4.65ab	4.93a	4.58ab	4.66ab
SSL (cm g ⁻¹ DM stem)	11.5d	16.5c	19.2c	25.1b	32.4a	17.0c	19.0c
Total LA (m ² plant ⁻¹)	0.28a	0.27a	0.22ab	0.24ab	0.18bc	0.19bc	0.13c
Mean LA (cm ² leaf ⁻¹)	208a	164ab	155abc	161abc	126bc	136bc	96bc
SLA (cm ² g ⁻¹ DM leaf)	231a	267a	269a	258a	241a	235a	217a

3.3.4 Plasticity of morphological root traits

Data in Table 3-3 show the effect of nutrient supply on root dry mass and root morphology. Root dry mass, root length (RL) and mean root diameter (RD) were directly measured. From these parameters, the other parameters were calculated (see 3.2.2). The root dry mass of K-deficient plants was very similar to that of plants with optimal nutrient supply. Root dry mass of N-deficient plants was markedly reduced to about 80% of that under optimal

supply. Root dry mass of Mg-deficient plants was even more reduced to about 50 % of optimal supply with medium and 40% of optimal supply with low Mg supply (Table 3-3).

Root length per plant is a root trait, which describes the ability of a plant for spatial exploitation of the soil. The effect of nutrient deficiency on RL was dependent on the specific nutrient (Table 3-3). K deficiency did not affect RL, whereas RL was significantly reduced by Mg and N deficiency. In general, RL was more reduced at severe nutrient deficiency (low supply) than at moderate (medium supply).

Mean root diameter is a root trait, which describes on the one hand the ability of roots to exploit small soil pores, and on the other hand, the soil volume that contributes to delivery of nutrients by diffusion (Claassen 1990). Roots with small RD are considered to be more efficient in nutrient acquisition than roots with large RD. The effect of nutrient supply on RD was nutrient specific (Table 3-3). In comparison to optimal supply, RD was reduced in K and Mg-deficient plants and increased in N-deficient plants. The intensity of nutrient deficiency had no effect on RD.

From root length and root diameter, the root surface area (RA) can be calculated. The root surface area is a measure for the size of the boundary layer between soil solution and plants under conditions of low nutrient availability, when most nutrients are absorbed by the outermost cell layer of the roots. The RA of K-deficient plants was very similar to that of plants with optimal nutrient supply (Table 3-3). The RA of Mg and N-deficient plants, in contrast, was significantly lower.

The root volume (RV) is a measure for the amount of soil nutrients, which is delivered to the root by interception. RV is closely related to the cortex volume of roots and the volume of cortical cells. Thus, RV is also a measure for the “internal” surface area of root cells, which may contribute to nutrient absorption under high nutrient supply, when not all nutrients are absorbed by the outermost cell layers of the root. Root volume was not affected by the rate of K supply, but was significantly lower in Mg and N-deficient plants (Table 3-3). In Mg and N-deficient, there was a tendency that RV was more reduced at severe deficiency (low supply) than at moderate deficiency (medium supply).

Root fineness (F), root tissue density (TD) and specific root length (SRL) are structural root traits, which together determine how much biomass (dry mass) is needed for the construction of one m root length. All these root parameters were little affected by the rate of K supply, whereas Mg and N supply had substantial effects (Table 3-3). In comparison to plants grown under optimal supply, F was increased in Mg-deficient plants and slightly reduced in N-deficient plants (Table 3-3). Tissue density was not affected by nutrient supply with the

exception of severely N-deficient plants (low supply), in which TD was increased. Specific root length (SRL) was increased in Mg-deficient plants and reduced in N-deficient plants (Table 3-3).

Root mass ratio (RMR) is a measure for the plants investment of biomass into construction of roots relative to total plant biomass (RMR). In comparison to optimal nutrient supply, there was a clear tendency that K deficiency and to a lower extent, also N deficiency increased RMR, whereas strong Mg deficiency (low supply) decreased RMR by about 30% (Table 3-3). These opposite tendencies of low Mg supply on the one hand and low N and K supply on the other hand lead to significant differences in RMR between K and Mg-deficient plants.

Root length ratio (RLR) is a measure for the root length available to supply one g total plant biomass with soil resources. In comparison to optimal nutrient supply, there was a clear tendency that K deficiency increased RLR by about 50%, whereas RLR was rather decreased by severe Mg deficiency and moderate and severe N deficiency (Table 3-3).

Table 3-3: Effect of K, Mg and N supply on root parameters {Root Length (RL), root diameters (RD), root volume (RV), root surface area (RA), root dry mass, root fineness (F), root tissue density (TD), specific root length (SRL), root mass ratio (RMR) and root length ratio (RLR)}. Values followed by different letters differ significantly among the treatments (Tukey-Kramer's test, $P < 0.05$).

Parameters	Nutrient supply						
	Optimal	K		Mg		N	
		Medium	Low	Medium	Low	Medium	Low
Measured parameters:							
RL (m)	952 ab	1,018 a	964ab	649bc	370c	476 c	380 c
RD (mm)	0.23 abc	0.21 cd	0.22 bc	0.19 d	0.20 cd	0.25 a	0.24 ab
Roots dry mass (g plant ⁻¹)	2.1 ab	2.3 a	2.2a	1.1 bc	0.8 c	1.7 abc	1.6 abc
Calculated parameters:							
RA (m ² plant ⁻¹)	0.68 a	0.67 a	0.66 a	0.37 b	0.23 b	0.37 b	0.28 b
RV (cm ³ plant ⁻¹)	38 a	35 a	36 a	17 bc	11 c	23 b	17 bc
F (m cm ⁻³)	25 bcd	29 bbcc	27 bc	37 a	32 ab	20 d	23 cd
TD (mg cm ⁻³)	76 ab	68 b	70 b	67 b	69 b	75 ab	94 a
SRL (m g ⁻¹)	332 bc	431 abc	393 abc	585 a	473 ab	274 bc	247 c
RMR (g g ⁻¹)	0.10 bc	0.15 ab	0.16 a	0.09c	0.07c	0.12abc	0.14ab
RLR (m g ⁻¹)	41 ab	63 a	64 a	51 ab	35 b	34 b	34 b

In summary, effects of nutrient deficiency on morphological root parameters were nutrient specific. K deficiency had no effect on total root biomass and morphological root parameters. As K deficiency reduced shoot biomass, RMR and RLR were increased in comparison to plants with optimal supply. Mg deficiency decreased total root biomass but roots were finer than roots of optimally supplied plants. Thus, root length of Mg-deficient plants was

less reduced than root biomass. At moderate Mg deficiency, formation of finer roots compensated the reduced root biomass with the consequence that RLR was slightly higher than RLR of optimally supplied plants. At severe Mg deficiency, formation of finer roots did not completely compensate reduced root biomass, and consequently RLR was slightly lower than RLR of optimally supplied plants. N deficiency did only slightly decrease root biomass, and in tendency increased RMR, because total plant biomass was more reduced than root biomass. However, the roots formed under N deficiency were less fine with the consequence that RLR was slightly lower than RLR of optimally supplied plants.

3.4 Discussion

In this chapter, plastic response of shoots and roots to nutrient limitation will be discussed. Results from chapter 2 showed that the effect of nutrient supply on biomass allocation to different plant organs was nutrient specific and independent on the intensity of nutrient limitation. Mg deficiency resulted in increased LMR and reduced RMR, while N deficiency resulted in reduced LMR and increased RMR. K deficiency resulted in increased both LMR and RMR. The changes in biomass allocation occurred to cope with nutrient limitation might be lead to morphological and anatomical changes in shoots and roots.

Are plastic responses specific for each nutrient and dependent on intensity of N, K and Mg deficiency?

3.4.1 Plastic response of shoots to nutrient limitation

The effect of nutrient supply on growth can be determined by factorizing RGR into the physiological component NAR and the morphological component LAR (Evans 1972). Generally, when growth is limited by irradiance, the physiological component NAR tends to be more important than the morphological component (LAR) in describing the effects on RGR. In contrast, the morphological component (LAR) is on average, more important than the physiological component (NAR) in determining a decrease in RGR due to nutrient limitation (Poorter and Nagel 2000). LAR is equal to the product of LMR and SLA, which both were increased by reducing K and Mg supply (Table 3-1, 3-2). Hence, LAR in K and Mg-deficient plants was enhanced. Therefore, the reduction in RGR was independent on morphological component (LAR) but dependent on physiological component (NAR) which was reduced in K and Mg-deficient plants (Table 3-1). In contrast, LAR was reduced in N-deficient plants but NAR was not reduced indicating that the reduction in RGR was dependent on morphological components (LAR). Thus, two different plant responses to nutrient limitation were observed.

First, N-deficient plants strongly restricted their leaf area (Table 3-2). This was associated with maintenance of adequate N concentration in leaves (Fig. 2-3c), and consequently, maintenance of high net assimilation rate (Table 3-1). The N deficiency induced decrease in leaf area growth is possibly related to a modification of the hormonal status. It is well documented that N deficiency is often associated with increased leaf abscisic acid (ABA) concentrations and reduced leaf CYT concentrations (Engels et al. 2012). These alterations of leaf phytohormone levels, in turn, may decrease leaf extension via changes in cell wall extensibility (Engels et al. 2012).

Second, Mg-deficient plants did not much reduce leaf area growth (Table 3-2). This was possible, because in Mg-deficient plants, LMR was increased, and SLA was rather increased than decreased (Table 3-1). This morphological response was associated with a strong decrease of leaf Mg concentration (Fig. 2-3b), and consequently with lower rates of net assimilation and net photosynthesis (Table 3-1). Therefore, plastic response of shoot to Mg limitation differs from the response to N limitation, because N-deficient plants were able to maintain adequate amount of N concentration in leaves by reducing leaf growth. Mg was not able to maintain adequate amount of Mg in their leaves because leaf growth was less reduced than leaf growth of N-deficient plants. This could explain the contrasting phenotype of plants responding to N and Mg deficiency (Hermans et al. 2006).

In conclusion, reduction of RGR is not necessary dependent of reduced LAR in case of nutrient deficiency, because that is true in case of N. However, in case of K and Mg, reduced RGR is dependent of severe reduction in NAR, associated with increased biomass allocation to leaves. Increasing LMR in K and Mg-deficient plants resulted in plastic response not able to restrict leaf growth and failed to maintain adequate nutrient concentrations in their leaves. Therefore, the reduction of RGR in K and Mg-deficient plants was related to severe reduction in NAR. Meanwhile, N-deficient plants were capable to stunt leaf growth to maintain adequate N concentration in leaves for photosynthesis processes. Hence, the reduction of RGR in N-deficient plants was related to severe reduction in LAR.

3.4.2 Plastic response of roots to nutrient limitation

Roots are the major organs for nutrient uptake; they play an important role in soil-plant system. Therefore, their growth is directly related with the growth and biomass yield of shoots. Generally, plants have a characteristic of enhancing their efficiency of nutrient acquisition to overcome the stress from nutrient deficiency (Lynch et al. 2007). Change of roots morphology and root distribution patterns are important adaptive mechanisms to increase acquisition of

nutrients from soil (Lynch et al. 2007, Xie et al. 2006). Important plant traits, which determine the acquisition capacity for below-ground resources, include high RMR, high F or low TD (Ryser 1998, Berendse et al. 2007). When plants are not able to increase RMR, they can increase root efficiency for acquisition of nutrients by forming roots with low RD, which can be represented by F. These roots also can have a low TD (Ryser and Lambers 1995). F and TD can be combined to SRL. Thus, RLR is determined by different morphological components: RMR and SRL (Ryser 1998).

In the present work, it was found that K-deficient plants succeeded to allocate more biomass to leaves and roots (Fig. 2-4 a). Roots of K-deficient plants were not affected, and RL was slightly higher than RL of K-sufficient plants. This explains why K deficiency had no effect on most root parameters. However, increase of RMR led to increased RLR because the latter is product of SRL and RMR (Table 3-3). Therefore, K-deficient plants increased their ability for nutrient acquisition by increase of RLR.

In contrast to K deficiency, Mg deficiency was not associated with increased allocation of biomass to roots but rather with lower biomass allocation to roots (low RMR) (Fig. 2-4 a). Consequently, RV and RA were reduced by Mg deficiency. However, Mg deficiency effects on other root parameters were dependent on the intensity of deficiency. In moderately deficient plants, plastic responses can be classified as adaptive, because RL slightly decreased leading to increase of F. As TD was not affected. Higher F resulted in increased SRL (Table 3-3). Consequently, the reduction in RMR was compensated by formation of finer roots. Therefore, RLR was enhanced by moderate Mg deficiency. These results indicate that moderately Mg-deficient plants are able to increase the efficiency of their roots. On the other hand, severe Mg deficiency was associated with severe reduction in RL. Furthermore, F was also reduced, and consequently SRL was not enhanced in comparison to optimally with Mg supplied plants. These changes of morphological root traits had the consequence that RLR was not enhanced by severe Mg deficiency. Hence, the plastic root responses under severe deficiency can not be classified as adaptive.

In contrast to K and Mg deficiency, N deficiency was associated with high RD and reduction of F, RL and RV and increase of TD. These changes resulted in severely reduced SRL (Table 3-3). Thus, although RMR increased in N-deficient plants, the severe reduction in SRL had the consequence that RLR was not enhanced by N deficiency. It is suggested that the reason for the lack of RLR increase differs from that which is responsible for the lack of RLR increase in severely Mg-deficient plants. N-deficient plants are able to allocate high proportion of

biomass to the roots (Fig. 2-4 a, Table 3-3), and their shoot biomass is restricted. Therefore, it is probable that N-deficient plants do not need to change morphological root parameters.

Plants strategies to increase nutrient acquisition were summarized previously as the following: Plants may produce longer roots either by increasing RMR as demonstrated under a low N supply (Ryser and Lambers 1995, Sorgona et al. 2007). N-deficient plants behaved this strategy where RMR was increased by N deficiency. Alternatively, plants may increase SRL when RMR is reduced or not affected by limiting resources (Ryser 1998). Mg –deficient plants behaved according to this strategy. In addition, RMR and SRL can be both increased when plants are growing in infertile soils (Yang et al. 2004). K-deficient plants followed this strategy.

We therefore need to understand the basic mechanisms for plant adaptation. It is well documented that reduction in LA and LMR and increased RMR in N-deficient plants is related to reduced CYT and increased ABA concentration (Kavanova et al. 2008, Jiang and Hartung 2008, Engels et al. 2012). Recently, CYT concentrations have been shown to decrease under K deficiency (Nam et al. 2012). The reduction of CYT in root zone results in formation of a large root system (Werner et al. 2003).

Phytohormone effects on Mg-deficient plants are still not clear. The responses of Mg-deficient plants were dependent on the deficiency intensity. Moderately Mg-deficient plants were able to adapt and increased root efficiency but severely Mg-deficient plants failed to adapt themselves. RMR and sugars in moderately Mg-deficient plants were slightly higher compared to those of severely Mg-deficient plants. It is likely that Mg-deficient roots imported sucrose. It is expected that Mg-deficient roots have low hexose to sucrose ratio because conversion of sucrose to hexose needs energy compounds (ATP), which is activated by Mg (Cakmak et al. 1994b). Sucrose is thought to promote cell differentiation and maturation, whereas hexoses favour cell division and expansion (Hermans et al. 2006). Therefore, this explains why moderate Mg-deficient plants have high F, SRL and low TD. In conclusion, it is assumed that plastic responses are adaptive; the responses may indicate successful strategies to cope with limitation of specific nutrients. However, it has to be noted that responses are not necessarily adaptive but may also be inevitable effects of limits on growth and physiology such as in case of severely Mg deficiency.

4. Effect of nutrient deficiency on fruit yield and quality

Summary:

In this chapter, focus was on the effects of nutrient deficiency on fruit yield and quality. Results showed that in K- and Mg-deficient plants, fruit growth was similarly reduced as shoot growth; however, fruit growth of N-deficient plants was less reduced than shoot growth. Therefore, the fruit index (ratio of fruits biomass to total shoot biomass) was slightly affected by K and Mg deficiency but was strongly increased particularly in severely N-deficient plants.

Nutrient deficiency had considerable impact on the nutritional status in tomato fruits. N and particularly K deficiency resulted in reduction of N and K concentration in tomato fruits, whereas Mg concentration in fruits was not affected by Mg deficiency. Lycopene concentration increased in fruits of nutrient-deficient plants irrespective the kind of nutrient deficiency. This is possibly due to lycopene concentrated in peel whereby nutrient deficiency caused reduced in fruits size. N deficiency (whether moderate or severely) resulted in increased sugars concentration in tomato fruits and had no effect on titratable acidity TA which led to increased taste values sugars/ TA . Sugars concentration increased in fruits of moderate K and Mg-deficient plants. K and Mg deficiency caused reduction in ascorbic acid (AsA) concentration in fruits whereas AsA was extremely increased in fruits of N-deficient plants, which led to increased radical scavenging activity (RSA %) in their fruits..

In conclusion, these results demonstrated that N deficiency increased tomato quality more than K and Mg deficiency. Reduction in using N fertilization can be considered the most favorable way to obtain a high quality of tomato fruits.

4.1 Introduction

Tomato (*Lycopersicon esculentum* L.) is one of the most widely grown vegetable food crops in the world. Scientific studies have shown strong inverse correlations between tomato consumption and risk of certain types of cancer, cardiovascular diseases and age-related macular degeneration (Sesso et al. 2003, Stahl and Sies 2005, Canene-Adams et al. 2005). This protective effect has been mainly attributed to provitamin A (Mayne 1996) and other carotenoids. Carotenoids provide precursors to essential vitamins and antioxidants. Because tomato is the second-most important vegetable in the world after potato (FAO 2006), this horticultural crop is the predominant source of carotenoids, major ones being lycopene, α - and β -carotene, lutein, zeaxanthin and b-cryptoxanthin. Lycopene, which constitutes about 80–90% of the total carotenoid content of red ripe tomatoes (Shi and Maguer 2000), is the most efficient antioxidant among carotenoids through its quenching activity of singlet oxygen and scavenging of peroxy radicals (Mortensen and Skibsted 1997, Sies and Stahl 1998). Other antioxidants found in tomato fruits are ascorbic acid (AsA), tocopherol and flavonoids. These mentioned bioactive compounds as well as content of minerals and flavor have to be considered when evaluating tomato fruit quality, because consumer's preference of fruits is strongly influenced by a characteristic sweet-sour tomato flavor (Krumbein et al. 2006). The desirable flavor of tomatoes is a result of a complex interaction between various aromatic volatiles and taste compounds present in the fruit (Petro-Turza, 1986, Hobson 1988). Studies on effects of taste compounds on flavor focus on titratable acidity (TA), soluble sugars, soluble solids ($^{\circ}$ brix) and the ratios of sugars to TA and $^{\circ}$ brix to TA (Wang et al. 2009). Researchers have proposed several reasons for the inferior flavor in fresh market tomatoes, and fertilization is believed to be a key factor affecting tomato flavor (Stevens 1985) and thereby quality of tomato fruits. The effect of minerals on phytonutrients and nutritional value of tomato depends on the specific mineral, the mineral form, the plant genotype, and any possible interactions with environmental conditions and agronomic practices. In this study, we are interested in the effects of different K, Mg and N supplies on fruit quality.

In literature we found that, in general, secondary plant metabolites which lack N in their structure such as lycopene, β -carotene, phenolics and flavonols are favoured under N-limiting conditions although photosynthetic activity is not simultaneously reduced (Dorais et al. 2008). Given that, it was not surprising that Dumas et al. (2003) found increased lycopene and vitamin C concentrations in fruits under N deficiency, whereas soluble solids decreased (Peet et al. 2004, Simonne et al. 2007). Fertilizing tomato plants with N leads to an increase in yield (Abou-Aziz 1968), biomass, leaf area, concentrations of nitrate, organic acids (Le Bot et al. 2009) and β -

carotene (Mozafar 1993). At the same time increased N supply lead to decreased concentrations of vitamin C (Montagu and Goh 1990), phenols and soluble sugars (Le Bot et al. 2009) and decreased acidity. With regard to carotenoids, data did not show significant and univocal differences related to different level of nitrogen supply (Benard et al. 2009).

Potassium (K) requirement of greenhouse tomatoes is high for vegetative growth (Wall 1940, Lucas 1968), fruit production (Besford and Maw 1975), and fruit quality (Winsor 1968, Trudel and Ozbun 1971). Under deficient soil K conditions, the tomato fruit will be small, drop from the plant prematurely, and lack red color (poor lycopene development), and can lead to blotchy ripening (Widders and Lorenz 1979). Low K levels in the nutrient medium limit assimilation of the element into plant parts and retard plant growth, flower development, and fruit set (Besford and Maw 1975). Furthermore, the quality of the fruit changes according to the availability of K in the growth medium (Davies and Winsor 1967, Winsor 1968, Kanai et al. 2007). High proportion of K in the nutrient solution increased quality attributes such as fruit dry matter, average fruit weight, fruit width, ten fruit weight, acidity, total soluble solid (TSS) and lycopene content of tomato fruits (Fanasca et al. 2006, Yagmur et al. 2004, Serio et al. 2007). A positive effect of K fertilization on tomato carotenoids was found in greenhouse and field experiments, with the degree of response dependent of genotype, where fruit lycopene concentration of cultivars with average lycopene concentration had not been affected by K application and high-lycopene tomato genotypes respond positively to additional soil applied K (Taber et al. 2008). Additionally, juice acidity of tomato fruit and fruit juice refractive index are positively affected by potassium fertilization; while juice pH is not affected, medium K supply seem to positively influence fruit juice vitamin C content and acidity (Si-smail et al. 2007). K supply does not have any effect on soluble solid content and pH in tomato fruit (Yurtseven et al. 2005). During the reproductive phase of growth, fruits are the strongest sink for both carbon assimilates and K. Suboptimal K levels lead to poor growth of the fruit accompanied by accumulation of sugars resulting in decreased sink strength. Feedback inhibition of photosynthesis as a result of decreased sink demand is a long known phenomenon (Roitsch 1999). Different experimental approaches have shown that sugars play a key role in this regulation by repressing the expression of photosynthetic genes (Kock 1996).

Mg is a component of chlorophyll, pectin, organic acids, and co-ferments. Mg deficiency can be observed in sandy soils, in soils high in K level where K: Mg ratio is >4.0 and in soils with poor structure or drainage (Sainju et al. 2003). Furthermore, Mg deficiency is common in tomatoes grown in the greenhouse. By applying Mg fertilizer, tomato fruit production can be significantly increased. On the other hand, in species like plum fruits, no strong effects of foliar

application with Mg and K on fruit quality are observed, but TSS tended to be lower and the acidity tended to be higher in treated plants than in controls (Vangdal et al. 2010).

The highest total antioxidant activity is observed in the treatments with a high proportion of Mg resulting in an increase of caffeic acid concentration in tomato fruits. There are no different effects between K, Mg, and Ca on vitamin C concentration in tomato fruits (Fanasca et al. 2006). KCl: MgCl₂ supply is associated with higher glucose concentration and dry matter % in tomato fruits as compared to KCl or KNO₃ supply, but TSS and Acidity were not affected by nutrient supply (Chapagain and Wiesman 2004).

High Mg/Ca or high K/Ca ratio had no effect on quality parameters such as color, TSS (°brix) sugars concentration in sweet pepper fruits (del Amor and Rubio 2009). High Mg and K had no effect on fruit color shape index total soluble solids, carbohydrate content in fruits or pericarp thickness. (del Amor and Rubio 2009). Peach fruits treated by formulation containing Ca, Mg and Ti has higher weight and pulp firmness than control fruits, while no effect was observed for either color, total soluble solid content (TSS) and titratable acidity (Serrano et al. 2004). The highest total antioxidant activity is observed in the treatment with high proportion of Mg in tomato plants (Fanasca et al. 2006).

It would be better to focus on previous theories, which illustrate the regulation of secondary metabolism under abiotic stresses. The accumulation of plant-secondary metabolites has been theorized by several hypotheses: the growth differentiation balance (GDB) theory predicts that any environmental factors that slow growth more than photosynthesis can increase the resource pool available for allocation to secondary metabolism. However, the carbon nutrient balance (CNB) theory is based upon the premise that only moderate nutrient deficiency limits growth more than photosynthesis and therefore only nutrient status can affect the accumulation of secondary metabolites (Keskitalo 2003).

According to these theories, it is expected the fruit quality of N-deficient plants superior to fruit quality of K- and Mg-deficient plants because growth was reduced in all plants, but photosynthesis process less affected in N-deficient plants as compared to K- and Mg-deficient plants, it would follow the regulation of the important secondary compounds such as (lycopene and phenols).

In former literature, it was concluded that in long-term plant cultures, meaning cultivation up to fruit maturity, nutrient deficiency increases biomass and nutrient allocation to roots and fruits to optimize mineral acquisition as ecological theory predicts. In this study, we want to increase our understanding about regulation of mineral composition and bioactive compounds such as lycopene, phenols, ascorbic acid, citric acid etc. in fruits of tomato plants.

Therefore, tomato plants were cultured up to fruit maturity in nutrient solution at optimal, suboptimal and low K, Mg and N supply.

4.2 Material and methods

Material and methods here follow the same techniques in chapter 2; the only difference will be the analysis of fresh fruit quality.

4.2.1 Titratable acidity:

The amount of alkali (KOH) utilized to neutralize the acidity in a known volume of juice is referred as titratable acidity. Ripened fruits were harvested. 10 g fresh weight sub-sampled and finely macerated with a pestle and mortar in 10-15 ml of distilled H₂O (Subramanian et al 2006). The juice was filtered through a muslin cloth to remove the turbidity and juice volume was diluted to 100 ml. A 10 ml of juice was pipetted to conical flask and 1-2 drops of phenolphthalein indicator was added and titrated against 0.01 N KOH. Based on the titer value, the titratable acidity of tomato fruits was estimated. One milliliter of 0.01 KOH consumed by titration is equivalent to 6.4 mg citric acid

4.2.2 Ascorbic acid:

Another 2.5 g of fruit sample was weighted and homogenized in 10 -15 ml of 4% oxalic acid with vortex. The juice was filtered through muslin cloth and the clear juice was filled up to 25 ml using 4% oxalic acid and 10 ml of juice were drawn and diluted to 100 ml. A 10 ml aliquot diluted juice was pipetted into a conical flask and titrated against 0.02% 2,6 Dichloroindophenol dye until the juice turned to permanent pink (Subramanian et al. 2006) . Similar titration was performed for standard ascorbic acid solution (100 mg ascorbic acid in 100 ml of 4% oxalic acid) in order to compute the ascorbic acid content of the sample juice.

4.2.3 Total phenols:

Total phenols were measured using the Folin-Ciocalteu method (Spanos and Wrolstad 1990), Modified by (Lister and Wilson 2001). In brief, 2 g of homogenized fresh tomato sample was extracted with 12.5 ml 70 % methanol, covered and shaken for 30 min, and centrifuged at 4000 rpm for 10 min. The extracts were appropriately diluted before oxidized with 2.5 ml of freshly diluted 0.2 M Folin-Ciocalteu reagent. This reaction was neutralized by adding 2.0 ml of 7.5 % w/v sodium carbonate, and the samples were vortexed for 20 s. Samples were incubated at 45 °C for 15 min and the absorbance of the resulting blue color was measured

spectrophotometrically at 765 nm. Gallic acid was used as a standard, and the results were expressed as gallic acid equivalents (GAE) per 100 FM.

4.2.4 Carotenoids

2 g of homogenized fresh tomato sample and 0.1 g CaCO_3 were put in cup 20 ml mixture of hexane–acetone–ethanol (2:1:1, v: v: v) was added to the samples which were then placed on the rotary mixer for 30 min. Agitation was continued for another 2 min after adding 10 ml of distilled water. The solution was then left to separate into distinct polar and non-polar layers and then the hexane layer was collected in a 50 ml test-tube for measurement of optical density at 663, 645, 505, and 453 nm in a spectrophotometer. Lycopene and β -carotene contents were calculated according to the Nagata and Yamashita (1992)

Equations:

$$\text{Lycopene (mg100 ml}^{-1}\text{)} = -0.0458 * A_{663} + 0.204 * A_{645} + 0.372 * A_{505} - 0.0806 * A_{453}.$$

$$\beta\text{-Carotene (mg100 ml}^{-1}\text{)} = 0.216 * A_{663} - 1.22 * A_{645} - 0.304 * A_{505} + 0.452 * A_{453}.$$

$$\text{Chlorophyll a (mg 100 ml}^{-1}\text{)} = 0.999A_{663} - 0.0989 A_{645}$$

$$\text{Chlorophyll b (mg 100 ml}^{-1}\text{)} = -0.328A_{663} + 1.77 A_{645}$$

Lycopene, β -Carotene and Chlorophyll a, b were finally expressed as mg kg^{-1} FM

4.2.5 RSA toward DPPH radical

Radical Scavenging Activity (RSA) of freshly prepared tomato juice was assayed with DPPH (2,2-diphenyl-1-picrylhydrazyl) (10^{-4} M) previously dissolved in methanol according to Ramadan et al. (2003). DPPH, in the absence of antioxidant compounds, was stable for more than 2 h of normal kinetic assay. For evaluation, 0.5 mg of juice was mixed with 5 ml methanolic DPPH radical and the mixture was vortexed for 20 sec at ambient temperature (25°C). Against a blank of pure methanol without DPPH, the decrease in absorption at 515 nm was measured in 1cm quartz cells after 30 and 60 min of mixing using spectrophotometer. RSA of DPPH radicals was estimated from the differences in absorbance of methanolic DPPH solution with or without sample (control) and the inhibition percent was calculated according to the following equation:

$$\% \text{ Inhibition} = \frac{\text{absorbance of control} - \text{absorbance of test sample}}{\text{absorbance of control}} \times 100.$$

4.3 Results

At maturity stage, as ecologist expected, plants allocated their biomass to reproductive organs (fruit). In this chapter, it would be important to see the biochemical changes in tomato fruits after a long term of nutrient stress. Plant growth responses to nutrient limitation at maturity stage have been presented in the beginning.

4.3.1 Effect of K, Mg, and N supply on growth

The effect of nutrient supply on plant growth had been discussed more details in chapter 2. In this chapter, we will discuss impact of nutrient supply on biomass and leaf area at maturity stage, and focus more details on fruit growth parameters

4.3.1.1 Dry mass in different plant organs and leaf area

At maturity stage (90 DAT), the effect of nutrient deficiency on dry mass of different plant organs was nutrient specific and dependent on the rate of nutrient supply (medium M and low L) (Fig.4-1a). In comparison to control, K, Mg and N deficiency were associated with lower biomass of all plant organs. Plant biomass (Fig. 4-1a) and leaf area (Fig. 4-1b) of N-deficient plants were more reduced than those of K and particularly of Mg-deficient plants. In N-deficient plants biomass was reduced to 51 and 40% of control in medium and low supply respectively. In K-deficient plants, biomass was reduced to 67 and 50% of control in medium and low supply respectively. In Mg-deficient plants, biomass was reduced to 70 and 54% of control in medium and low supply respectively. This indicates that the treatments to induce nutrient deficiency were associated with more intense stress for N-deficient plants in comparison to K- and Mg-deficient plants.

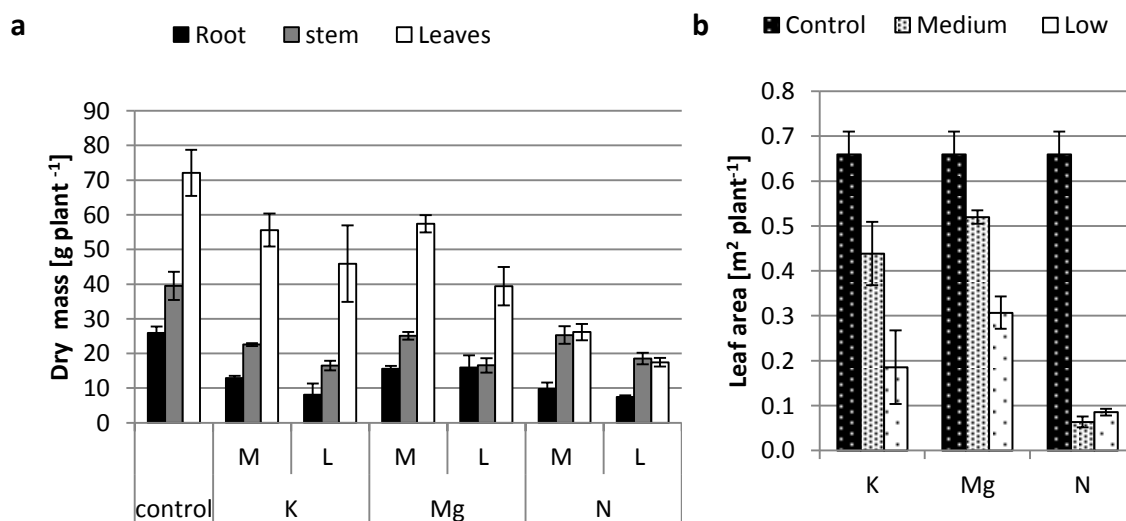


Fig. 4-1: Effect of nutrient supply on dry mass of roots, stem and leaves (a) and leaf area (b) of tomato plants at maturity. Vertical lines represent standard errors of means (4)

The extent of deficiency induced biomass reduction of the various organs was nutrient-specific indicating a nutrient-specific alteration of biomass partitioning among plant organs. For example, leaf biomass and leaf area of K and Mg-deficient plants were less affected than leaf biomass and area of N-deficient plants (Fig. 4-1 a and b). Root biomass was less affected in Mg-deficient plants than in K- and N-deficient plants (Fig. 4-1a). In contrast, stem biomass was reduced to a similar content with deficiency of K, Mg and N (Fig. 4-1a).

4.3.1.2 Fruit growth

Fruit number and fruit weight were diminished by nutrient deficiency (Table 4-1). As the standard error was quite large in this experiment, in most cases the decrease of fruit number and weight was not statistically significant. In K-deficient plants and plants grown with medium Mg supply, fruit dry weight was decreased to a similar extent as the dry weight of aboveground vegetative plant organs. Thus, the fruit index was not markedly changed in comparison with control plants. In N-deficient plants and plants grown with low Mg supply, in contrast, fruit dry weight was less affected than dry weight of aboveground vegetative plant organs. Thus, the fruit index was higher than in control plants, indicating that biomass partitioning to generative organs was increased.

In K- and Mg-deficient plants, fruit fresh weight was reduced to a similar extent as fruit dry weight. Thus, fruit dry matter percentage was similar as in control plants. In contrast, in N-deficient plants, there was a clear tendency that fruit fresh weight was more diminished than fruit dry weight, thus, leading to an increase of dry matter percentage in comparison to control plants (Table 4-1). In tendency, fruit fresh weight was more reduced by nutrient deficiency than fruit number, leading to lower means fresh weight per fruit. This was particular marked for low K and low N supply (Table 4-1).

Table 4-1: Effect of K, mg and N supply on fruit number, fresh and dry weight of fruit, mean fruit weight, fruit index and dry matter % in tomato fruits. Different letters within a row indicate significant (Tukey-Kramer's test, $P < 0.05$) differences among nutrient treatments.

Treatments	Optimal	K		Mg		N	
Fruit parameters		Medium	Low	Medium	Low	Medium	Low
Fruit No	32a	28a	26a	26a	24a	26a	26a
Fruit FW (g plant ⁻¹)	725a	514ab	350b	508ab	456ab	397ab	378b
Fruit DW (g plant ⁻¹)	55a	38ab	28b	38ab	34ab	36ab	34ab
Mean FW(g fruit ⁻¹)	23a	19a	14a	21a	19a	16a	14a
DM%	7.7a	7.5 a	7.9a	7.8a	7.4a	9.1a	9.0a
Fruit Index *	040 ab	0.47 ab	0.32 b	0.34 b	0.61 ab	0.067 ab	0.89 a

*Fruit Index Fruit DM to shoot DM

4.3.2 Effect of K, Mg and N supply on nutrient concentrations in fruits and vegetative plant organs

The different treatments markedly changed nutrient concentrations of the fruits (Fig. 4-2). N deficiency reduced fruit N concentrations by about 42% (medium supply) and 52% (low supply) in comparison to control plants (Fig. 4-2a). K deficiency reduced fruit K concentrations by about 48% (medium supply) and 62% (low supply) (Fig. 4-2b). This reduction of fruit N and K concentration was less than the reduction of N and K concentrations in leaves and stems (Table 4-2), indicating that N and K were preferably partitioned to the generative organs. Surprisingly, Mg deficiency did not reduce fruit Mg concentrations (Fig. 4-2c). In contrast to fruit concentrations, Mg concentrations in leaves, stems and particularly in roots were strongly diminished by Mg deficiency (Table 4-2).

Table 4-2: Effect of K, Mg and N deficiency on nutrient concentrations (N, K, Mg, Ca, Fe and Zn) in roots, stem and leaves of tomato plants at maturity stage. Different letters within a row indicate significant (Tukey-Kramer's test, $P < 0.05$) differences among nutrient treatments.

Treatments		Optimal	K		Mg		N	
Nutrients	organs		Medium	Low	Medium	Low	Medium	Low
N (mg g ⁻¹ DM)	Roots	35a	42a	43a	36a	40a	21b	14b
	Stem	27b	39a	40a	32b	31b	10c	7c
	Leaves	27a	30a	33a	29a	32a	8b	8b
K (mg g ⁻¹ DM)	Roots	11c	4c	3c	22b	29b	32ab	35a
	Stem	11c	4d	2d	15b	23b	17b	15b
	Leaves	21b	3c	3c	26ab	29ab	26ab	20b
Mg (mg g ⁻¹ DM)	Roots	6.3a	5.3ab	4.4b	0.9c	0.4c	1.7c	1.5c
	Stem	1.9a	2.1a	1.7a	1.0b	0.9b	1.6ab	1.5ab
	Leaves	6.5b	8.4a	8.3a	2.7c	2.2c	7.3ab	7.9ab
Ca (mg g ⁻¹ DM)	Roots	7abc	10ab	12a	12a	11ab	4bc	3c
	Stem	14b	11c	13bc	17a	16a	6d	5d
	Leaves	54ab	54ab	50b	63a	63a	37c	36
Fe (mg Kg ⁻¹ DM)	Roots	5,159a	6,535a	7,113a	4,757a	6,053a	1,600b	593b
	Stem	212ab	255a	227ab	230a	238a	160ab	88b
	Leaves	609ab	399bc	351bc	714a	651ab	277c	184c
Zn (mg Kg ⁻¹ DM)	Roots	26a	38a	30a	28a	24a	71a	49a
	Stem	20a	32a	27a	22a	37a	32a	28a
	Leaves	26a	27a	27a	29a	27a	29a	50a

Low supply of a specific nutrient also affected concentrations of other nutrients in fruits (Fig. 4-2) and vegetative plant organs (Table 4-2). Mg deficiency did not change fruit concentrations of N (Fig. 4-2a), K (Fig. 4-2b), Ca (Fig. 4-2d), Fe (Fig. 4-2e) and Zn (Fig. 4-2f). In contrast to fruits, in vegetative plant organs concentrations of Ca and in particular of K were substantially increased by low Mg supply (Table 4-2).

K deficiency also had only minor effects on fruit concentrations of other nutrients with the exception of fruit Ca concentration, which was markedly increased (Fig. 4-2d). K deficiency increased also Ca concentrations in roots but did not markedly change Ca concentrations in leaves and stems (Table 4-2).

N deficiency substantially affected fruit concentrations of other nutrients (Fig. 4-2). Low N supply reduced fruit Ca concentrations by about 30% in comparison to the control (Fig. 4-2d). Low N supply also decreased Ca concentrations in vegetative plant organs (Table 4-2). Low N supply reduced fruit Fe concentrations by about 50% (Fig. 4-2e). This decrease of fruit Fe concentrations was accompanied by a similar decrease of Fe concentrations in vegetative plant organs (Table 4-2). Low N supply reduced fruit Zn concentrations by about 55% (Fig. 4-2f). Interestingly, Zn concentrations in vegetative plant organs were not changed by low N supply (Table 4-2).

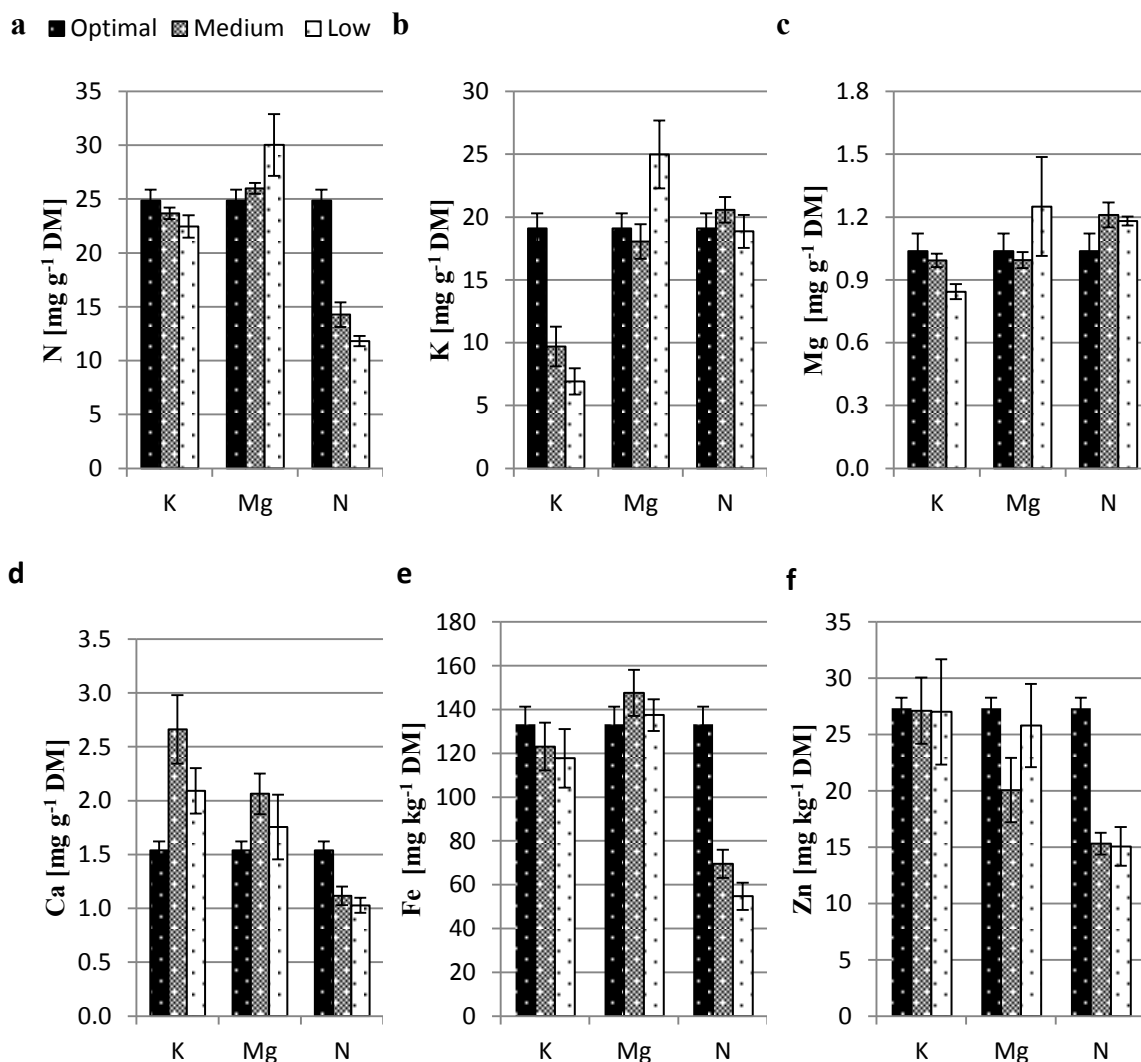


Fig. 4-2: Effect of K, Mg and N deficiency on nutrient concentration [N (a) , K(b), Mg (c), Ca(d), Fe(e) and Zn (f)] in tomato at maturity stage. Vertical lines represent standard errors of means (4)

4.3.3 Effect of K, Mg and N supply on healthy promoting substance:

Phenols and lycopene concentration in fruits were significantly increased by K deficiency (Fig 4-3 a and Table 4-3). In contrast, K deficiency resulted in reduction of ascorbic acid concentration in fruits (Fig. 4-3 b). However, K deficiency had no effect on radical scavenging activity % (RSA %) β -carotene, chlorophyll a and b concentration in fruits (Fig. 4-3 and Table 4-3).

Table 4-3: Effect of K, Mg and N supply on carotenoids (lycopene- carotene) and chlorophyll (a and b) in tomato fruits. Different letters within a row indicate significant (Tukey-Kramer's test, $P < 0.05$) differences among nutrient treatments

Treatments	Optimal	K		Mg		N	
Carotenoids		Medium	Low	Medium	Low	Medium	Low
Lycopene (mg Kg ⁻¹ FW)	26 b	34a	36 a	32ab	36a	31ab	33ab
β -Carotene (mg Kg ⁻¹ FW)	15a	17a	16a	17a	15a	14a	14a
Chl_a (mg Kg ⁻¹ FW)	2.1a	1.6a	1.6a	1.3a	1.3a	2.2a	1.7a
Chl_b (mg Kg ⁻¹ FW)	2.3a	1.4a	1.9a	1.0a	1.4a	3.2a	2.4a

In comparison to control, Mg deficiency was associated with higher concentration of lycopene in fruits (Table 4-3). In contrast, phenols and ascorbic acid (AsA) concentration were reduced by Mg deficiency (Fig. 4-3 b). Meanwhile, Mg deficiency had no significant effect on, radical scavenging activity RSA %, β -carotene, chlorophyll a and b concentration in fruits (Fig. 4-3 c).

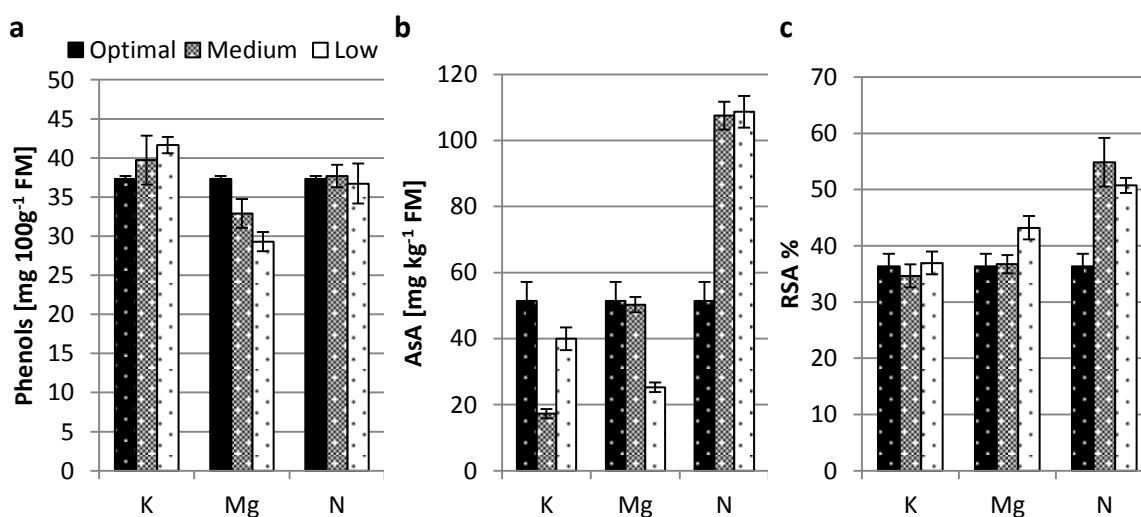


Fig. 4-3: Effect of K, Mg and N supply on phenols (a), ascorbic acid (b), radical scavenging activity (%) (c) in mature fruits. Vertical lines represent standard errors of means (n=4)

N deficiency resulted in an increase significantly in ascorbic acid, radical scavenging activity (RSA %), and lycopene concentrations in fruits (Fig.4-3 b, c and Table 4-3). N deficiency had no significant effect on, phenols and β -carotene, chlorophyll a and b concentration in fruits (Fig. 4-3 a and Table 4-3).

4.3.4 Effect of K, Mg and N supply on taste compounds :

Sugars and sugars to titratable acidity ratio (S/TA) in fruits were significantly increased by K deficiency (Fig 4-4 a, c). In contrast, K deficiency resulted in reduction of titratable acidity concentration in fruits (Fig. 4-4 b). However, K deficiency had no effect on pH and °brix to TA ratio in fruits (Fig 4 e, f). °Brix value was tendency reduced in fruits of K-deficient plants (Fig. 4-4 d). In comparison to control, Mg deficiency was associated with higher concentration of sugars in fruits (Fig. 4-4 a).

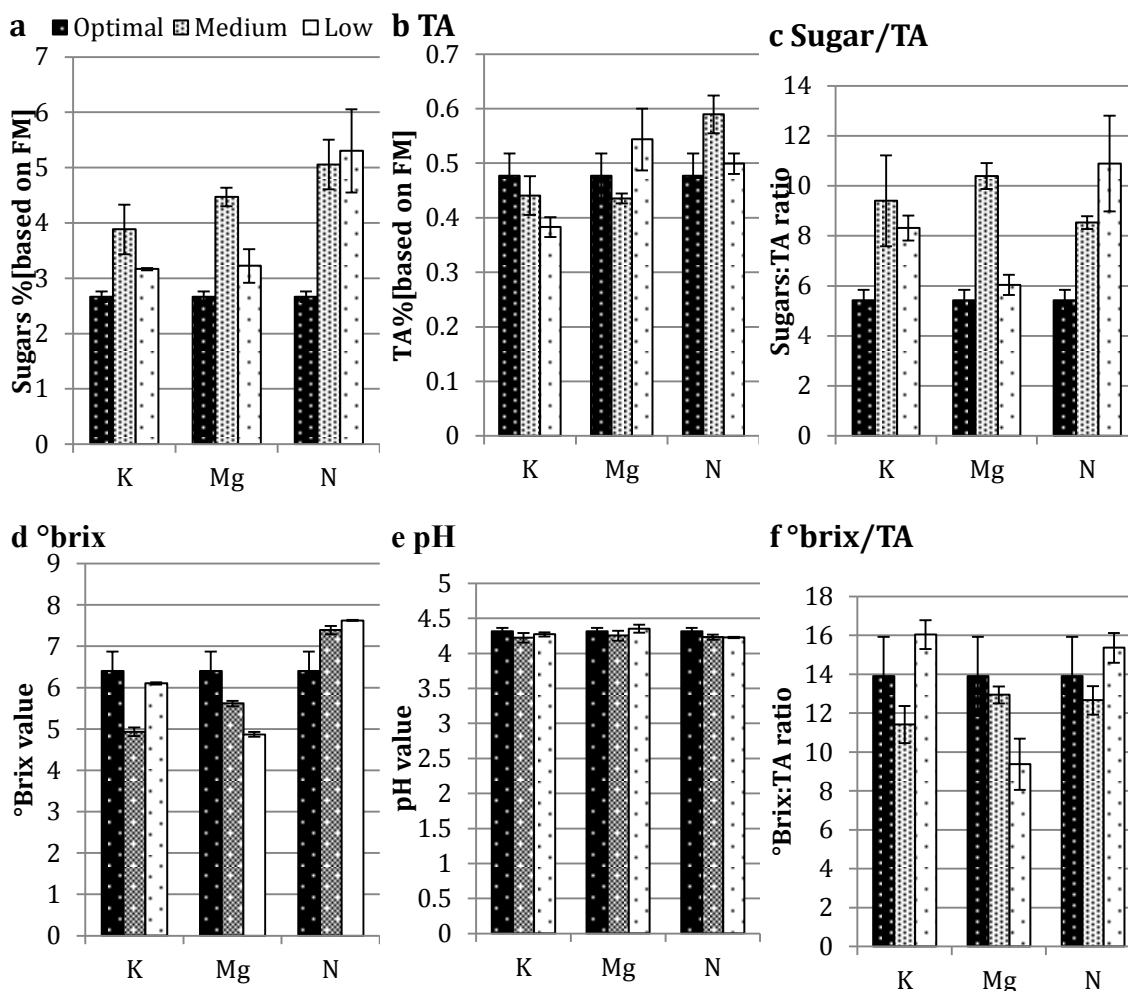


Fig. 4-4: Effect of K, Mg and N supply on soluble sugars (a) , titratable acidity (TA) (b) sugar and TA ratio (c), °brix (d) , pH (e), brix /TA ratio (f) in mature fruits. Vertical lines represent standard errors of means (n=4).

In contrast, °brix value, titratable acidity TA was low in fruits of Mg-deficient plants than those in fruit of control plants; whereas, Mg deficiency had no significant effect on pH and °brix to TA ratio in fruits (Fig. 4-4 b, d, e, f). However, Sugars to titratable acidity ratio were high in fruit of moderately Mg-deficient plants (Fig. 4-4 c).

N deficiency resulted in an increase significantly in sugars, °brix value and sugars to titratable acidity ratio in fruits (Fig.4-4 a, c, d). N deficiency had no significant effect on titratable acidity concentration in fruits (Fig. 4-4 b). N deficiency had no significant effect on pH value and °brix to TA ratio in fruits (Fig. 4-4 e, f).

4.4 Discussion

4.4.1 Effect of K, Mg, and N supply on growth

The results from this study indicated that nutrient deficiency (K, Mg and N) limited the growth more than generative production of tomato plants. Particularly, in N-deficient plants and plants were grown with low Mg supply, fruit dry weight was less affected than dry weight of aboveground vegetative plant organs (Fig.4-1 and Table 4-1). Thus, the fruit index was higher than in control plants, indicating that biomass partitioning to generative organs was increased. This is in agreement with the finding of the previous studies, which had been observed in tomato; where, lowering nitrogen supply had a low impact on fruit commercial yield but it reduced plant vegetative growth and increased fruit dry matter content (Benard et al. 2009). Whereas, K deficiency did not result in any significant decrease in dry weight of vegetative parts or in fruit production of tomato plants (Pujos and Morad 1997). Also, Increasing the K level in the nutrient solution does not change total tomato yield (Serio et al. 2007). (Zhao-Hui et al. 2008) suggested that the application of N fertilizer did not have any effect upon tomato yield, whereas application of K fertilizer increased the yield. Also, similar results had been reported in greenhouse tomato, which supplied by different levels of K and Mg nutrients, there is no difference is observed in total fruit yield, number of fruits or average fruit weight among the treatments (Chapagain and Wiesman 2004). Also, in processing tomato, N supply did not increase marketable yield (Parisi et al. 2006). These results indicated that nutrient-deficiency had slight effect on growth fruit parameters due to in nutrient- deficient plant, high proportion of biomass and nutrient is partitioned to the fruits to acquire optimally minerals from nutrient solution as ecological theory predicting.

4.4.2 Nutrient in different plant organs

The different treatments markedly changed nutrient concentrations of the fruits, K concentration in tomato fruits was severely declined (Fig.4-2). In comparison to control, K deficiency were associated with high Ca concentration in fruits (Fig 4-2), it suggested that the high concentration of Ca in nutrient solution as a result of replacement of calcium phosphate instead of potassium phosphate. These led to an increase in Ca uptake by root pressure; because there was no increment of Ca concentration in the leaves of K-deficient plants (Table 4-2). This increment of Ca in fruits of K-deficient plants caused slightly reduces in Mg concentration in this fruits as compared to the fruits of other treatments (Fig 4-2). Increasing K supply could increase Mg content in tomato fruits (Viro 1973 cited in Kirkby and Mengel 1976), this also explain why Mg concentration reduced in K-deficient plants. However, Mg accumulated in leaves of K-deficient plants at the expense of Ca, which was high in nutrient solution. The reason for that is likely to have synergistic effect between K and Mg; therefore, Mg can perform some K role in leaves of K-deficient plants such as (cation –anion balance- water relation in plant). This relation illustrated why Ca concentration increases in fruit and roots of K-deficient plants, but not in leaves. It is suggested that fruits and roots of K-deficient plants had high Ca concentration. This means, there is likely have antagonistic effect between Ca and Mg in occupation of Ca and Mg site in vacuole and cell wall. Similar results had obtained from of sweet pepper plants, high Mg reduces Ca concentration in leaves, stems, and fruits (del Amor and Rubio 2009).

Severely Mg deficiency resulted in an increase of N and K concentration in different plant organs including fruits (Table 4-2 and Fig. 4-2 a, b). The increment might be attributed to the reduction of plant growth with high N and K uptake. In addition, K remobilization from the leaves to fruits due to the high concentration of K in leaves of Mg-deficient plants resulted in an increase of K concentration in severely Mg-deficient plants than in leaves of other plants (Table 4-2). When sprayed Mg on peach trees, there was no effect of Mg supply on Ca, N, P, K, Mg, Na, Fe, Mn, Zn, Cu and Ti concentration in flesh of peach fruits (Alcaraz-Lopez et al. 2004). Although, there was no effect of Mg deficiency on Mg concentration in tomato fruits; Ca concentration increased in these fruits as compared to control (Fig. 4-3d). It might be attributed to the high concentration of Ca in all organs of Mg-deficient plant particularly in the leaves (Table 2-2). This results is agreement with that in plums plants which supplied by K and Mg, where, Mg supply did not increase the content of Mg in the fruits (Vangdal 2010). Even though Mg is mobile cation in plant, it is observed that Mg had low remobilization to the fruits as Ca (immobile cation).

The reduction of N concentration in fruits of N-deficient plants was -40% than its concentration in fruits of control plants (Fig. 4-2a). N deficiency resulted in an increase of Mg concentration in tomato fruits; this increment equivalent the reduction of Ca concentration in these fruits (Fig. 4-2 c, d) due to Ca concentration in nutrient solution was low as compared to control. As K deficiency had no effect on N concentration in fruits Also, N deficiency had no effect on K concentration in tomato fruits (Fig. 4-2 b). Similar results had been reported in muskmelon plants, K concentration is not affected by N supply (Xu et al. 2007). Fe and Zn concentration in tomato fruits were reduced by N deficiency (Fig. 4-2 e, f), which resulted in reduction of Fe concentration not only in fruits but in different plant organs (Table 4-2); meanwhile, Zn concentration was low in fruits only the rest of organs of N-deficient plants had high Zn concentration as compared to other plants (Table 4-2). In contrast, of these results, Mg concentration in fruits decrease and Ca concentration increase as N concentration increase (Xu et al. 2007). It is suggested that, if it had not been low Ca concentration in nutrient solution; it would not be any effect of N deficiency on Mg and Ca concentration within fruits. However, in kiwi plants, N and K supply increased N and K concentration in fruit respectively, in contrast, the lowest content of Ca is found on treatments with the highest N supply (Pacheco et al. 2008). Also, there are no effects of N and K supply on P, Mg, Na, Fe and Cu (Pacheco et al. 2008).

4.4.3 Healthy promoting substances:

K deficiency has direct and indirect impact on ascorbic acid (AsA). Direct effect is the effect of low concentration of K in fruits led to less demand of organic acids accumulation in tomato fruits, including AsA (Fig. 4-3 b) however, the indirect effect is shaded fruits due to the less affected leaf area by K deficiency (Fig. 4-1 b). (Yagmur et al. 2004 and Si-smail et al. 2007) obtained similar results on tomato fruit; they indicated that, there is positive relationship between K supply and AsA concentration in tomato fruits. There are no difference effects between K and Mg on ascorbic acid concentration in tomato fruits (Fanasca et al. 2006).

Lycopene concentration was higher in fruit of K-deficient plants, due to K deficiency resulted in restraining of growth more the photosynthesis process leading to increase of photosynthate which allocated to the fruits to produce secondary compounds in fruits of tomato including carotenoids such as lycopene. (Table 4-3). Due to lycopene concentrate under peel directly there is indirect effect might be the fruit size which was small according to the mean fruit weight which was low resulted in K deficiency (Table 4-1). Similar results had been reported in tomato also; that the results about lycopene concentration seem to be negatively affected by K supply (Si-smail et al. 2007), but most of researchers obtained counter results for

instance, (Serio et al. 2007) referred to lycopene content in tomato increase linearly with increasing K level in the nutrient solution. However, it was observed by (Taber et al. 2008) that lycopene concentration does not appear to be affected by K application in excess of recommended rates.

The reduction of phenols in fruits of severely Mg-deficient plants was due to the competition between phenols and nitrogen concentration in fruits (Fig 4-3 a). It is suggested that fruits of Mg-deficient plant has the highest N content therefore; the availability of N should affect concentration of phenol because it is produced in the same shikmic acid pathway as aromatic amino acids. The decline of ascorbic acid in fruits of severely Mg-deficient plants attributed to the negative relation between ascorbic acid and N and K concentrations in fruits (Fig 4-3 b). The results had agreement with which were obtained by (Liu et al. 2008) they found that ascorbic acid reduce as Mg reduce too in cabbage plants. The lycopene was higher in fruits of Mg-deficient plants as compared to fruits of control plants (Table 4-3). In fruits of severely Mg-deficient, lycopene was higher than in fruits of moderate Mg-deficient plants. Fruits of severely Mg-deficient plants had low concentration of AsA, phenols than fruits of moderate Mg-deficient plants, it superior to fruits of moderate Mg-deficient plants in lycopene and radical scavenging activity (RSA%) (Table 4-3 and Fig. 4-3 a, b, c).

The more than twofold of AsA concentration in fruits of N-deficient plants (Fig. 4-3 b), probably for indirect reason, since N supply might enhance the foliage and hence the shading of the fruit on plants unevenly illuminated by direct sunshine (Dumas et al. 2003). This explains why AsA did not increase in fruits of K and Mg deficiency because the leaf area was minor affected. The second reason is the availability of sugar in tomato fruits, which is consider the main precursor in AsA synthesis. Similar results had been reported in tomato, total AsA tended to be higher in fruit with the lowest nitrogen supply (Benard et al. 2009). Radical scavenging activity percentage (RSA %) was high in N-deficient plants resulted in increase of AsA concentration in it which consider high antioxidant compound (Fig. 4-3 c). Lycopene concentration was high in N-deficient plants as compared to control due to N deficiency limited growth more than photosynthesis as it was mentioned in former (Table 4-3). This led to increase the resource pool available for allocation to the fruits since, there is less demand to resource for fruit growth resulted lack of nitrogen in fruits that caused accumulation of secondary compound in fruit according to carbon nutrient balance hypothesis (CNB). The differences between medium and low related to the fruits volume. Lycopene is concentrated under the peel so directly and thus, when fruit volume is small, the lycopene concentration is high in the tomato fruits result in an increase in surface area. N deficiency had no effect on total phenols in tomato

fruits (Fig 4-3 a) due to the most of increment of sugars in tomato consumed to produce AsA. Similar results is obtained with tomato plants also, altering nitrogen availability had no obvious effect on the flavonol content of tomato fruits at the breaker and red stage of ripening (Stewart et al. 2001).

4.4.4 Taste compounds

K deficiency had direct effect on fruit quality. In comparison to control, K- deficiency was associated with high soluble sugar concentration in tomato fruits (Fig 4-4 a), that occurred due to less demand of resource which was allocated to the fruits because K deficiency effect the growth more than its effect on photosynthesis process according to carbon nutrient balance hypothesis (CNB) (Herms and Mattson 1992). Therefore, sugar concentration in fruit of severely K-deficient plants was lower than those was in fruit of moderate K-deficient plant. That indicated that photosynthesis process was affected by severely K deficiency. Most of researcher referred to that has positive or no effect on sugars concentration; for instance, K supply has no effect on sugars in pineapple fruits (Razzaque and hanafi 2001). Also, (Kanai et al. 2007) observed that accumulation of sugars in tomato fruits is not affected by K supply; in contrast, K fertilizer often associated with increase sugars concentration in tomato fruits (Zhao-Hui et al. 2008). The results had agreement with (Akhtar et al. 2010) who observed that potash application decrease sugars content in tomato fruits as compared to control.

There was negative relationship between acidity and K deficiency, which was associated with low acidity in tomato fruits (Fig 4-4 b), and that was attributed to less demand of organic acid to neutralize absorbed K^+ in tomato fruits (Wang et al. 2009). Most of researchers referred that the highest K dose is positively effective on acidity (Yagmur et al. 2004, Si-smail et al. 2007 and Rubio et al. 2010). Some of them indicated that K supply has no effect on acidity in tomato fruits (Akhtar et al. 2010, Razzaque and Hanafi 2001).

Mg deficiency had indirect effect on fruit quality for two reasons. 1) The fruit of Mg-deficient plants was no affected by Mg shortage. 2) These fruits were rich in N, K, and Ca (Fig 4-2a, b, d). Soluble sugars were higher in fruits of moderate Mg-deficient plants (Fig 4-4a) due to moderate deficiency of Mg in leaves might be limited growth more than photosynthesis process, which can increase resource pool available for allocation to the fruits. In contrast, low Mg supply had no effect on soluble sugars in tomato fruits (Fig. 4-4 a), because severely Mg deficiency reduced both growth and photosynthesis process. These results have agreement with carbon nutrient balance hypothesis (CNB) (Herms and Mattson 1992) and according to the conclusion of (Keskitalo 2003). Acidity was high in fruits of Mg-deficient plants, (Fig 4-4 b) K

concentration in these fruits was high (Fig. 4-2 b) consequently, it suggested that these plants tended to produce more organic acids to neutralize absorbed K^+ (Wang et al. 2009).

In comparison to other treatments, N deficiency was associated with higher soluble sugars (Fig. 4-4 a); due to N deficiency limited growth more than photosynthesis process. This led to increase the resource pool available for allocation to the fruits since, there is less demand to resource for fruit growth resulted in the lack of N in fruits that caused accumulation of sugars in fruits. It is expected that the photosynthesis process in N-deficient plants was higher than in K- and Mg-deficient plants because of N resorption was range (1- 0.7 %) in leaves that allowed to remain photosynthesis process. The resorption percentage is in this range in most plant species (Killingbeck 1996). Moderate N deficiency was associated with higher acidity in tomato fruits as compared to control (Fig. 4-4 b). It is suggested that related to K concentration in their fruits, which was high in fruits of moderate N-deficient plants. These results are in agreement with the finding of the previous studies, which had been observed in tomato (Parisi et al. 2006, Xu et al. 2007, Simonne et al. 2007).

5. Effect of the rate of Mg supply on growth and photosynthesis

Abstract

In this chapter, physiological and morphological responses to Mg-deficiency were studied in more detail at vegetative growth phase. In one experiment, in addition to the three treatments tested before (optimal supply, moderate and severe Mg deficiency) as fourth treatments Mg was completely withdrawn from the nutrient solution and responses to Mg deficiency were measured 6 and 12 DAT. In another experiment, the source/sink ratio of plants was reduced by shading of 6 basal leaves to investigate if Mg deficiency-induced increase of sugar accumulation and decrease of photosynthesis in source leaves can be prevented.

At 6 DAT, although Mg deficiency had no effect on plant growth, Mg concentration was severely reduced and reach to the same level in whole organs of Mg-deficient plants. This shows that for Mg deficiency there is no early growth response. Photosynthetic rate (A) of young source leaf was reduced simultaneously with increased sugars and reduced Mg and chlorophyll in same leaf. Despite of Mg concentration was severely reduced in old source leaf of Mg-deficient plants, A, sugar and chlorophyll concentration were not affected.

Twelve DAT, Mg deficiency resulted in reduced of plant growth, however, Leaf area was not affected when Mg deficiency was moderate, leaf area and root length RL were reduced in severely Mg-deficient plants. Specific leaf area (SLA) and specific stem length (SSL) were increased in Mg-deficient plants. Photosynthetic rate only decreased after svere reduction of leaf Mg concentration and the reduction of photosynthetic rate was associated with severe reduction of leaf chlorophyll concentration and increase of soluble sugar concentrations. Non-structural carbohydrate (NSC) was not affected, whereas, starch in source leaves of Mg-deficient plants was reduced inhered with increased fructose in these leaves.

Shading of basal source leaves reduced NSC concentration in the unshaded source leaves of Mg-sufficient plants, In Mg-deficient plants in contrast soluble sugar accumulated despite of shading of basal leaves. This proves that sugar accumulation in source leaves of Mg-deficient plants was due to Mg deficiency-induce inhibition of phloem loading.

5.1 Introduction

Mg deficiency in plants is a widespread problem, affecting yield and quality in agriculture, horticulture and forestry (Hermans et al. 2005). There are several causes for increased incidence of Mg deficiency in recent years including enhanced Mg removal with harvest products and neglecting of Mg fertilization. Mg has a high hydration radius, and thus, it adsorbs less strongly to cation exchange sites in soil colloids than other cations. Therefore, Mg^{2+} is highly prone to leaching (Mengel and Kirkby 1987). Mg^{2+} uptake is sensitive to antagonisms with other cations, e.g., K^+ and NH_4^+ , and in acidic soils Mg^{2+} uptake may also be inhibited by Mn^{2+} and Al^{3+} .

Mg is essential for the functioning of many enzymes and plays a fundamental role in both, the light and dark reactions of photosynthesis (Shaul 2002, Engels et al. 2012). Mg is the central atom of the light absorbing chlorophyll molecules in photosystems I and II. The light-induced efflux of Mg^{2+} and K^+ from the lumen to the stroma of chloroplasts may counterbalance the light-induced influx and generation of protons in the lumen, and thus maintain charge balance across the thylakoid membranes. Mg activates the ATPase in the thylakoid membranes, which can use the degradation of the proton gradient across the thylakoid membranes for ATP synthesis. The activation status of ribulose-1.5-bisphosphate carboxylase/oxygenase, the key enzyme for CO_2 assimilation in the dark reactions of photosynthesis, is regulated by Mg. Furthermore, Mg has a critical role in activation of many other enzymes such as ATPases, RNA polymerases and protein kinases (Cakmak and Kirkby 2008).

There are several reports concerning the effects of Mg deficiency on physiological processes. In Mg-deficient plants, leaf concentrations of non-structural carbohydrates are increased (Cakmak et al. 1994b, Fischer et al. 1998). Sugar accumulation in leaves often precedes any loss of chlorophyll content or photosynthetic activity (Hermans et al. 2004, Hermans and Verbruggen 2005) suggesting that the reduction of photosynthesis is a response to increased sugar levels. Various mechanisms have been suggested to explain feedback inhibition of photosynthesis by high contents of sugars and starch in the leaves (for review see Stitt 1991). These include chloroplast damage and/or negative effects on CO_2 diffusion by excessive starch accumulation, limitation of photosynthesis by phosphate deficiency within the chloroplasts, which is induced by accumulation of sugar phosphates in the cytosol and sugar-induced repression of photosynthetic genes. It is well established that high leaf carbohydrate concentrations, particularly hexose concentrations, inhibit transcription of genes coding for enzymes involved in photosynthesis (Rolland et al. 2006). In *Arabidopsis*, high glucose and

sucrose concentrations in leaves of Mg-deficient plants were associated with lower expression of *Cab2*, the gene encoding a chlorophyll protein (Hermans and Verbruggen 2005).

More detailed analysis of Mg deficiency-induced alteration of carbohydrate concentrations in source and sink organs, showed contrasting results. In *Phaseolus vulgaris*, concentrations of soluble sugars and starch were increased in source leaves but reduced in roots (Cakmak et al. 1994b) whereas in *Spinacia oleracea* concentrations of carbohydrates and amino acids were increased in source leaves and in roots (Fisher et al. 1998). In *Beta vulgaris* sucrose concentrations were increased in older leaves but not in younger leaves and roots; however starch concentrations were increased in all leaves under Mg deficiency (Hermans et al. 2005). Increased sugar concentrations in source organs were attributed to either inhibition of sucrose export in the phloem (Cakmak et al. 1994a, b, Hermans et al. 2005, Hermans and Verbruggen 2005) or lower sugar utilization of sink organs (Fisher et al. 1998).

There is evidence for Mg deficiency-induced alterations of biomass allocation between source and sink organs. Mg deficiency reduced more distinctly seed yield than plant biomass of broad bean (*Vicia faba*) indicating alteration of biomass allocation between vegetative and reproductive organs (Hariadi and Shabala 2004). The effect of Mg deficiency on the shoot/root ratio can not be generalized. In some experiments, shoot/root ratio was not changed by Mg deficiency for example in maize (Tewari et al. 2004). In other experiments, shoot/root ratio was higher in Mg-deficient than in Mg sufficient control plants, for example in spinach (Fischer et al. 1998), bean (Fischer and Bremer 1993, Cakmak et al. 1994a) and in rice (Ding et al. 2006). In other experiments, shoot/root ratio was decreased by Mg deficiency, for example in sugar beet (Hermans et al. 2005) and *Arabidopsis thaliana* (Hermans and Verbruggen 2005).

In our previous experiment we assessed the effect of moderate and strong deficiency of N, K and Mg on biomass allocation to various organs (chapter 2), and morphological shoot and root traits (chapter 3). In that experiment, plants were grown until fruit maturity. The first harvest was done 20 days after start of treatment (DAT), when the growth of deficient plants was already markedly reduced. In the first experiment described in this chapter, the effect of Mg deficiency on growth and photosynthesis was assessed already 6 DAT, when the first symptoms of Mg deficiency just became visible, but total plant growth was not yet reduced. Furthermore, we assessed plant responses not only to moderate and strong reduction of Mg supply but also to complete Mg withdrawal from nutrient solution.

In a second experiment described in this chapter, the source/sink ratio of plants grown with high or no Mg supply was modified through shading of basal leaves. Shading of basal leaves was expected to decrease the ratio of carbohydrate biosynthesis in source organs to

carbohydrate utilization in sink organs. Thus, in this experiment we assessed whether Mg deficiency effects on leaf carbohydrate accumulation and photosynthesis in non-shaded source leaves are dependent on the source to sink ratio of plants. We expected that in plants with shaded basal leaves, leaf carbohydrate accumulation in non-shaded source leaves should decrease in plants well supplied with Mg due to increased export to sink organs. In Mg-deficient plants, in contrast, leaf carbohydrate accumulation in non-shaded source leaves should remain high, if Mg deficiency-induced inhibition of phloem loading would prevent sugar export.

5.2 Material and methods:

Plants were grown in a glasshouse. Duration of the first experiments from mid of March to end of April 2011. Day length varied from 12.8 h in March to 14.8 h in April. In the second experiment from 24 of April to 24 of June 2011 day length varied from 12.4 h in April to 16.8 h in June of 2011. Temperatures in glasshouse varied from 20 to 23 °C during first experiment and from 23 to 25 °C during second experiment. Maximum natural irradiance was 1068 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and was supplemented by artificial light on cloudy days. Artificial light was also used to maintain the period of irradiance 14 h at least throughout the experiment. The light intensity of artificial light at plant height was 180-220 $\mu\text{mol m}^{-2} \text{s}^{-1}$. For both experiments, Idoia genotype of tomato plants was used.

5.2.1 Plant culture, treatments and harvests

First experiment

Seeds of Idoia genotype of tomato plants were germinated in peat moss. After two weeks, seedlings were transferred to a plastic pot with a volume of 20 L (54 seedlings), which contained 10% of the nutrient concentration of the standard nutrient solution. One week later, each plant was transferred to an individual pot with the standard nutrient solution. The standard nutrient solution had the following composition (mol m^{-3}): 1 K_2SO_4 ; 5 $\text{Ca}(\text{NO}_3)_2$; 0.5 KH_2PO_4 ; 0.6 MgSO_4 ; 0.1 KCl ; 0.1 FeEDTA ; 0.01 H_3BO_3 ; 5×10^{-4} $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$; 1×10^{-4} $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$; 3×10^{-4} $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$; 5×10^{-6} $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot \text{H}_2\text{O}$. One week later (30 days from sowing), the treatments started. For that, plants were grown under 4 different rates of Mg supply: optimal supply (standard nutrient solution), complete withdrawal of MgSO_4 from nutrient solution, daily addition of MgSO_4 at a rate to allow about 80% of optimal plant growth (“medium supply”), and daily addition of MgSO_4 at a rate to allow about 60% of optimal growth (“low supply”). In the treatments low and medium Mg supply, nutrients were added to nutrient solution daily to avoid extended phases without nutrients in the nutrient solution. To determine the amount of

nutrients, which was to be added to the nutrient solution to obtain growth reduction to 80% and 60% of the control plants, it was assumed that the growth rate of the control plants with optimal nutrient supply is $0.24 \text{ g g}^{-1} \text{ DM day}^{-1}$.

Nutrient requirement was calculated daily according to equation No. 5 in chapter 2:

$$\text{NR}_d = \text{DM}_0 (r+1)^t r \text{ PC}$$

The assumed nutrient concentration in the plant dry mass of plants with medium and low Mg supply were 1.5 and $0.9 \text{ mg g}^{-1} \text{ DM}$, respectively

NR_d = nutrient requirement (mg d^{-1})

DM_0 = DM at start of treatments (g plant^{-1})

r = RGR (relative growth rate) $\text{g g}^{-1} \text{ day}^{-1}$ of control plants

t = the time (days)

$P = (1, 0.80 \text{ and } 0.60)$ for optimal, medium and low supply respectively

C = the assumed nutrient concentration in the plant dry mass ($\text{mg g}^{-1} \text{ DM}$). It was 1.5 and $0.9 \text{ mg Mg g}^{-1} \text{ DM}$ for medium and low Mg supply, respectively. Within the 12 days of treatment, medium and low Mg supplied plants in total received 40 and 12 mg Mg , respectively.

Four plants were harvested at start of the treatments. 6 and 12 days after start of treatment (DAT), 4 plants from each treatment were harvested for analysis of fresh and dry mass, leaf area and concentrations of chlorophyll, mineral nutrients and carbohydrates. At the first harvest, plants were separated into roots, stem and leaves. At the second and third harvest, plants were separated into roots, stem, laterals and leaves. The leaves were further separated into a young and an old source leaf and the rest of the leaves. The young source leaf was leaf number 7 as counted from below (stem base). This leaf was just fully extended. In the treatment without Mg supply, this leaf showed the first visible symptoms of Mg deficiency at 6 DAT. The old source leaf was leaf number 4 from below. These two leaves were harvested separately from the rest of the leaves, because they had been used for the leaf gas exchange measurements.

Second experiment

Two weeks after germination in peat moss, seedlings were planted on one-tenth strength of nutrient solution for one week, and then each plant was transferred to an individual pot with full strength nutrient solution which contained the standard nutrient solution as in the first experiment. After two weeks in full strength nutrient solution, Mg and shading treatments were started. Half of the plants were manipulated by shading of the basal 6 source leaves, whereas the other half of the plants remained unshaded. Both sets of plants were subjected to two Mg treatments. For that, plants were fed with nutrient solutions which were either Mg-free (-Mg) or contained the Mg concentration of the full strength solution (+Mg; 0.6 mM Mg). Twenty four

DAT, plants were harvested for analysis of fresh and dry mass. Plants were separated into roots, stems, laterals, basal 6 leaves, leaf number 9 from below, and rest of the leaves. Leaf number 9 (source leaf) which had been used for measurements of leaf gas exchange before harvest, was analysed for concentrations of chlorophyll, mineral nutrients and carbohydrates.

5.2.2 Measurement of leaf gas exchange

Measurements of net photosynthetic rate (A) were carried out with a portable photosynthesis system (GFS-3000, *WALTZ*). The leaf was placed in the cuvette until stable reading was obtained. Temperature of the cuvette was 23°C and photosynthetic active radiation measured in the upper part of the cuvette (PAR top) was ($1500 \mu\text{mol m}^{-2} \text{s}^{-1}$). In the first experiment, A was measured on leaf no 4 and 7, which represented an old and a young source leaf. In the second experiment, A was measured in leaf no 9, which represented a young leaf (corresponding to leaf no 7 in the first experiment).

5.2.3 Chlorophyll analysis

0.1 g fresh leaf tissue were put into a Ultra-Turax tube with 5 ml of 80 % acetone. Three drops of KOH (0.01 N) were added to keep pH above 7. The leaf tissue was homogenized by using Ultra-Turax apparatus. The homogenate was transferred into a 10 ml centrifuge tube and centrifuged at 4000 rpm for 10 min (Lichtenthaler 1987). By using spectrometer, Chlorophyll was calculated by the absorbance of supernatant at 646, 663 and 720 nm

$$\text{Chlorophyll a (mg L}^{-1}\text{)} = 12.21 (E_{663} - E_{720}) - 2.81 (E_{646} - E_{720})$$

$$\text{Chlorophyll b (mg L}^{-1}\text{)} = 20.13 (E_{646} - E_{720}) - (E_{663} - E_{720}).$$

5.2.4 Analysis of soluble sugars and starch in fresh leaf tissue

0.1 g leaf tissue was put in Ultra-Turax tube; 5 ml of 80 % ethanol was added. It was crushed by using Ultra-Turax apparatus. Ultra-Turax tube was washed two times by 5 ml of 80% of ethanol. The extract was collected in a 20 ml centrifuge tube and centrifuged at 4000 rpm for 5 min. Supernatants were collected in 25 ml measuring flask and completed with distilled water. Soluble sugars in the ethanol extract were determined using the anthrone method (Seifter et al. 1950). An aliquot of the extract was hydrolyzed in 5 ml of 0.1 % anthrone solution (1g anthrone in 1 L 95% H_2SO_4) in a boiling water bath for 15 min. After cooling, the sugar concentration was determined spectrophotometrically at 620 nm. Glucose was used as a standard. The sugar concentration was calculated on a fresh mass basis ($\text{mg g}^{-1} \text{FM}$).

The ethanol-insoluble pellet was used for starch extraction. The pellet was dried over night at room temperature for ethanol removal by evaporation. Starch in the pellet was released

by adding 2 ml distilled water and boiling for 15 min in a water bath. After cooling to room temperature, 2ml of 9.2 molar HClO_4 was added. Starch was hydrolysed for 15 min. Distilled water (4 ml) was added to the samples. The samples were then centrifuged at 4000 rpm for 10 min.; the pellets were extracted one more time with 2ml of 4.6 molar HClO_4 . Supernatants were combined and filled to 20 ml. The starch concentration was measured spectrophotometrically at 620 nm after addition of the anthrone reagent. Starch concentration was calculated by multiplying glucose concentration by the conversion factor of 0.9 (Osaki et al. 1991). Glucose was used as a standard. The starch concentration was based on fresh mass.

5.2.5 Analysis of soluble sugars and starch in dry plant biomass

Soluble sugars and starch in dry plant biomass were measured as described in chapter 2.

5.2.6 Analysis of glucose, fructose and sucrose

The ethanol-soluble fraction of dried leaf tissue which was used for analysis of soluble sugars (see 5.2.5) was also used for glucose, fructose and sucrose analysis by using a spectrophotometric enzymatic assay according Megazyme International Ireland Limited 2005.

5.3 Results of the first experiment

5.3.1 Growth and biomass allocation to different organs

Before any noticeable growth reduction (Fig. 5-1 a), in plants grown with medium, low or without Mg supply light brightening of the young source leaf (leaf number 7) between leaf veins became visible 6 days after start of treatments (DAT) (Fig. 5-1b). Later, chlorosis symptoms and leaf rolling on the young source leaf were exacerbated and extended to leaves below and above this leaf. At 12 DAT, marked interveinal chlorosis and leaf rolling were visible in all except the basal leaves (Fig. 5-1 c, d). Leaf rolling is possibly due to increased ethylene formation under Mg deficiency (Hermans et al. 2010).

Within the first 6 DAT, total plant dry mass increment and increment of individual plant organ dry mass and leaf area were not yet influenced by the different rates of Mg supply (Table 5-1A). About 70% of total increment was due to leaf increment, 20% due to stem increment, 10% due to root increment and 1.5% due to increment of axial (Table 5-1B). In Mg-deficient plants, there was a tendency for lower contribution of stem increment to total plant increment.

From 6 to 12 DAT, total plant dry mass increment was severely reduced by Mg deficiency (Table 5-1C). The Mg deficiency-induced growth reduction became increasingly severe from medium supply (increment 58 % of optimum supply) to low supply (increment 44%

of optimum) and no supply (increment 34% of optimum) (Table 5-1C). The extent of Mg deficiency-induced decrease of dry mass increment was dependent on the plant organ. Growth reduction of leaves was less severe than that of stems, and particularly that of roots.

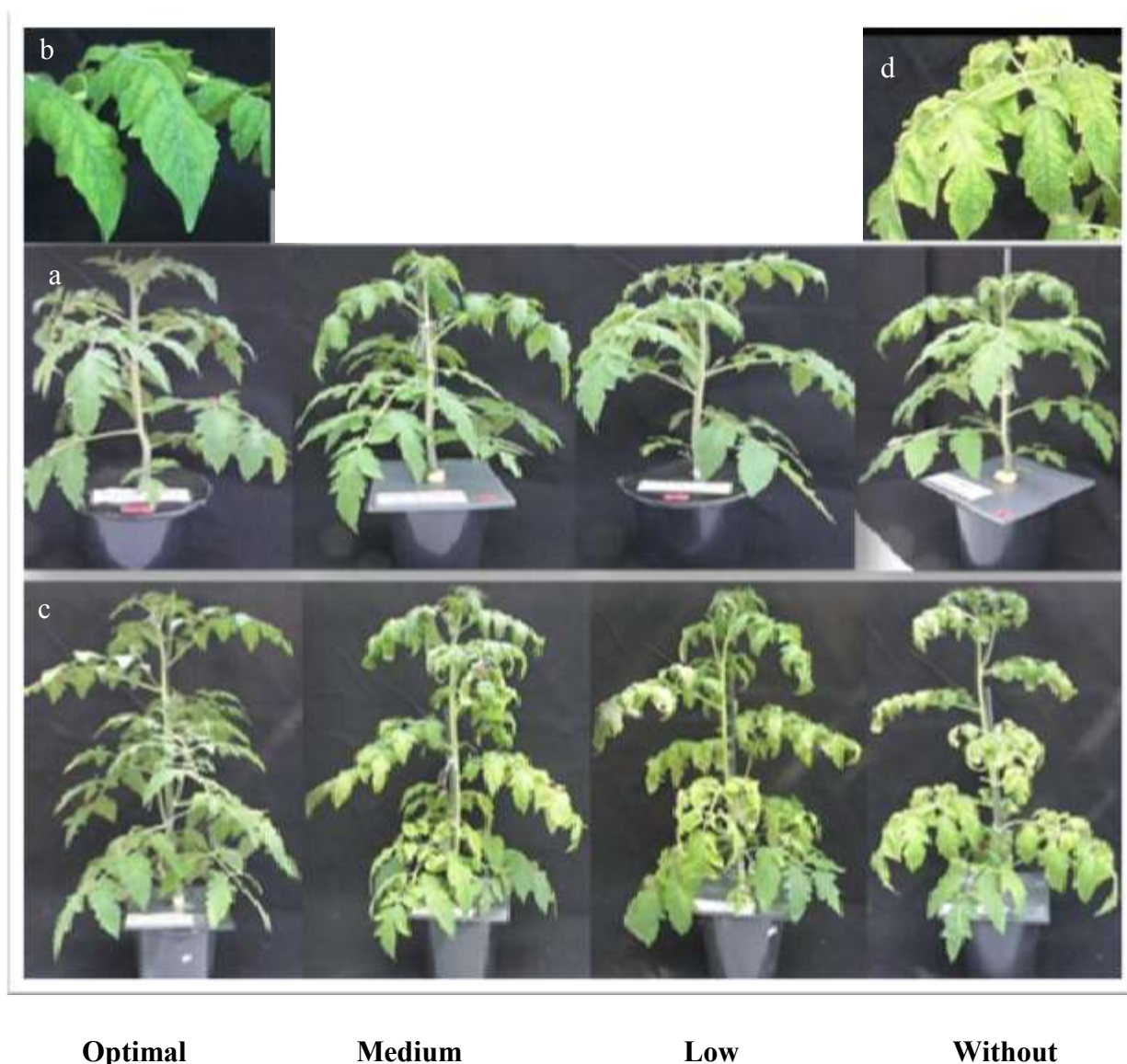


Fig. 5-1: Effect of Mg supply on plant shoots and leaf appearance. a) Total shoot appearance 6 days after start of treatment (DAT); b) Youngest source leaf of plants grown without Mg supply 6 DAT; c) Total shoot appearance 12 DAT; d) Youngest source leaf of plants grown without Mg supply 12 DAT.

Accordingly, in Mg-deficient plants the contribution of leaves to total plant increment increased to 65% at medium supply, 66% at low supply and 69% without Mg supply, whereas the contribution of roots decreased to 6% at medium, 4 at low and 2 % without Mg supply (Table 5-1D).

Leaf area increment (Table 5-2 A and B) was even less reduced than leaf dry mass increment (Table 5-1A and C). With medium Mg supply, leaf area growth was similar as with optimum supply. In the period from 6-12 DAT, with low and without Mg supply, leaf area

growth was reduced to 1913 cm² (73 % of optimum supply) and 1275 cm² (49% of optimum supply), respectively.

Table 5-1: Effect of Mg supply on absolute and relative increment of dry mass of different plant organs. Different letters within a row indicate significant (Tukey-Kramer's test, $P < 0.05$) differences among Mg treatments.

	Mg supply			
	Optimal	Medium	Low	Without
Plant organ	A) Increment of dry mass (g) 0-6 DAT.			
Roots	1.0a	1.0a	1.1a	1.1a
Stem	2.1a	1.6a	1.8a	1.7a
Leaves	7.2a	6.9a	7.6a	7.1a
Axial	0.1a	0.1a	0.2a	0.2a
Total	10.4a	9.7a	10.7a	10.1a
	B) Increment of dry mass 6-12 DAT (relative, total increment=100)			
Roots	9a	10a	11a	11a
Stem	20a	17b	17b	17b
Leaves	69b	72a	71ab	71ab
Axial	1.4a	1.5a	1.6a	1.5a
	C) Increment of dry mass (g) 6-12 DAT.			
Roots	2.9a	1.2b	0.6b	0.3b
Stem	6.8a	3.6b	2.5bc	1.7c
Leaves	18.3a	12.6b	9.7bc	7.8c
Axial	5.4a	2.0b	1.9b	1.5b
Total	33.3a	19.4b	14.7bc	11.3c
	D) Increment of dry mass 6-12 DAT (relative, total increment=100).			
Roots	9a	6ab	4bc	2c
Stem	20a	19a	18a	15a
Leaves	55b	65a	66a	69a
Axial	16a	10a	13a	13a

Table 5-2: Effect of Mg supply on absolute and relative increment of leaf area for the periods 0-6 and 6-12 DAT. Different letters within a row indicate significant (Tukey-Kramer's test, $P < 0.05$) differences among Mg treatments

	Mg supply			
Period	Optimal	Medium	Low	Without
	A) Leaf area increment (cm ² plant ⁻¹)			
0-6 DAT	1570a	1615a	1565a	1457a
6-12 DAT	2647a	2681a	1913ab	1275b
	B) Leaf area increment (relative, optimal supply =100)			
0-6 DAT	100a	104a	101a	93a
6-12 DAT	100a	102a	73ab	49b

The data in Tables 5-1 and 5-2 show that under Mg deficiency, plants allocated relatively more biomass to leaves, and that the biomass allocated to leaves was more efficiently used for

leaf area growth (see also below, specific leaf area in Table 5-4). Allocation to sink organs (roots, stem and axial), in contrast, was reduced in Mg-deficient plants, particularly in the period 6-12 DAT.

Table 5-3: Effect of Mg supply on absolute and relative Mg increment of different plant organs for the periods 0-6 and 6-12 DAT. Different letters within a row indicate significant (Tukey-Kramer's test, $P < 0.05$) differences among Mg treatments.

Plant organ	Mg supply			
	Optimal	Medium	Low	without
A) Increment of Mg content (mg) 0-6 DAT				
Roots	6.9a	0.8b	0.5b	- 0.4b
Stem	7.6a	1.9b	1.6b	0.4b
Leaves	33.9a	4.6b	3.6b	- 0.2b
Axial	0.4a	0.3a	0.2a	0.2a
Total	48.8a	7.5b	5.9b	0.0b
B) Increment of Mg content 0-6 DAT (relative, total increment =100)				
Roots	14a	10a	5a	
Stem	16b	27ab	34a	
Leaves	70a	61ab	55b	
Axial	1a	3a	7a	
C) Increment of Mg content (mg) 6-12 DAT				
Roots	25a	8b	0.7c	0.0c
Stem	18a	6b	4.1bc	- 0.2c
Leaves	83a	17b	3.6b	- 0.6b
Axial	13a	4b	2.5b	0.8b
Total	140a	36b	10.8bc	0.0c
D) Increment of Mg content 6-12 DAT (relative, total increment =100)				
Roots	18a	24a	7b	
Stem	13a	17a	32a	
Leaves	60a	48a	38a	
Axial	9b	11b	24a	

5.3.2 Mg allocation to various plant organs

We wanted to know whether the allocation of Mg to various plant organs is similar as the biomass allocation. Therefore, Mg increment in various organs was calculated from biomass increment and Mg concentrations. Already in the period from 0-6 DAT, the increment of Mg content in various plant organs was significantly influenced by the rate of Mg supply (Table 5-3 A and B). This was true not only for the absolute increment (Table 5-3A) but also for the relative increment (Table 5-3B). The contribution of roots to total Mg increment in plants decreased from 14% at optimal supply to 10% at medium supply and 5% at low supply (Table 5-3B). The contribution of leaves to total Mg increment also decreased from 70% at optimal

supply to 61% at medium supply and 55% at low supply (Table 5-3B). In plants without Mg supply, there was a net loss of Mg from roots and leaves (Table 5-3A). The contribution of stem and axial to total Mg increment increased in plants with medium and low Mg supply (Table 5-3B). In plants without Mg supply there was a slight net increase of Mg in stem and axial (Table 5-3A).

From 6-12 DAT, the rate of Mg supply in tendency had similar effects on the absolute (Table 5-3C) and relative Mg increment in various organs (Table 5-3D) as in the period from 0-6 DAT. The contribution of roots and leaves to total increment decreased, particularly at low Mg supply, whereas the contribution of stem and axial increased (Table 5-3D). In plants without Mg supply, there was a slight increase of Mg content in axial at the expense of a net loss from stem and leaves (Table 5-3C).

From the data in Tables 5-1 and 5-3 it can be concluded that Mg deficiency effects on biomass allocation were different from Mg deficiency effects on Mg allocation. Whereas Mg deficiency in the long term (6-12 DAT) decreased biomass allocation to stems and increased biomass allocation to leaves, Mg allocation to stems was increased and Mg allocation to leaves was decreased in the short term (0-6 DAT) and in the long term.

Table 5-4: Effect of Mg supply on morphological shoot traits (stem length, SSL, leaf number, SLA and LAR) at 6 and 12 DAT. Values within rows followed by different letters differ significantly among treatments (Tukey-Kramer's test, $P < 0.05$).

Parameters	Mg supply			
	Optimal	Medium	Low	Without
	6 DAT			
Stem length (cm)	43a	41a	41a	41a
Specific stem length (SSL; cm g ⁻¹ stem DM)	18a	22a	20a	21a
Leaf number	12a	11a	12a	12a
Specific leaf area (SLA; cm ² g ⁻¹ leaf DM)	247a	256a	233a	233a
Leaf area ratio (LAR; m ² kg ⁻¹ plant DM)	21a	22a	20a	20a
12 DAT				
Stem length (cm)	78a	77a	74a	76a
Specific stem length (SSL; cm g ⁻¹ stem DM)	9c	14b	16ab	20a
Leaf number	15a	15a	15a	15a
Specific leaf area (SLA; cm ² g ⁻¹ leaf DM)	177b	231a	214ab	200ab
Leaf area ratio (LAR; m ² kg ⁻¹ plant DM)	13b	17a	16ab	16ab

5.3.3 Shoot morphology

In the first 6 DAT, the rate of Mg supply had no significant effect on leaf number and morphological shoot traits (Table 5-4). Twelve DAT, leaf number and stem length were still not

affected by the rate of Mg supply. However, the specific stem length decreased with decreasing Mg supply. This indicates that Mg-deficient plants invested less dry mass into stems without losing total plant length. Similarly, the specific leaf area was also increased in Mg-deficient plants, indicating a more efficient utilization of leaf biomass for leaf area growth. The leaf area ratio of Mg-deficient plants was higher than that of plants with optimal supply.

Table 5-5: Effect of Mg supply on morphological root traits (RL, RD, SRL and RLR) at 6 and 12 DAT. Values within rows followed by different letters differ significantly among treatments (Tukey-Kramer's test, $P < 0.05$).

Parameters	Mg supply			
	Optimal	Medium	Low	Without
	6 DAT			
Root length RL (m)	214a	222a	238a	192a
Root diameter RD (mm)	0.23a	0.24a	0.25a	0.26a
Specific root length (SRL; m g ⁻¹ root DM)	181a	184a	180a	158a
Root length ratio (RLR; m g ⁻¹ plant DM)	17a	19a	19a	17a
12 DAT				
Root length RL (m)	434a	299b	286b	201b
Root diameter RD (mm)	0.28a	0.28a	0.26a	0.26a
Specific root length (SRL; m g ⁻¹ root DM)	108a	123a	147a	127a
Root length ratio (RLR; m g ⁻¹ plant DM)	10a	10a	10a	9a

5.3.4 Root morphology

In the first 6 DAT the rate of Mg supply had no significant effect on morphological root traits (Table 5-5). Twelve DAT, total root length per plant decreased with decreasing Mg supply from 434 m at optimal supply to 201 m without Mg supply. The rate of Mg supply had no significant effect on root diameter and specific root length. The root length ratio was also not influenced by the rate of Mg supply (Table 5-5). Obviously, the Mg deficiency-induced reduction of root length was similar to the Mg deficiency-induced reduction of total plant dry mass.

5.3.5 Whole plant net assimilation rate

Net assimilation rates (NAR) as calculated from whole plant dry mass increment and leaf area, were not affected by the rate of Mg supply during the first 6 DAT (Fig. 5-2). In the period from 6 to 12 DAT, NAR of Mg-deficient plants markedly decreased, whereby this decrease was most severe in plants grown without Mg, followed by low and medium Mg supply. The reduction of NAR in Mg-deficient plants shows that whole plant biomass increment was even

more strongly reduced than leaf area, indicating lower leaf photosynthetic activity and/or higher rates of whole plant respiration.

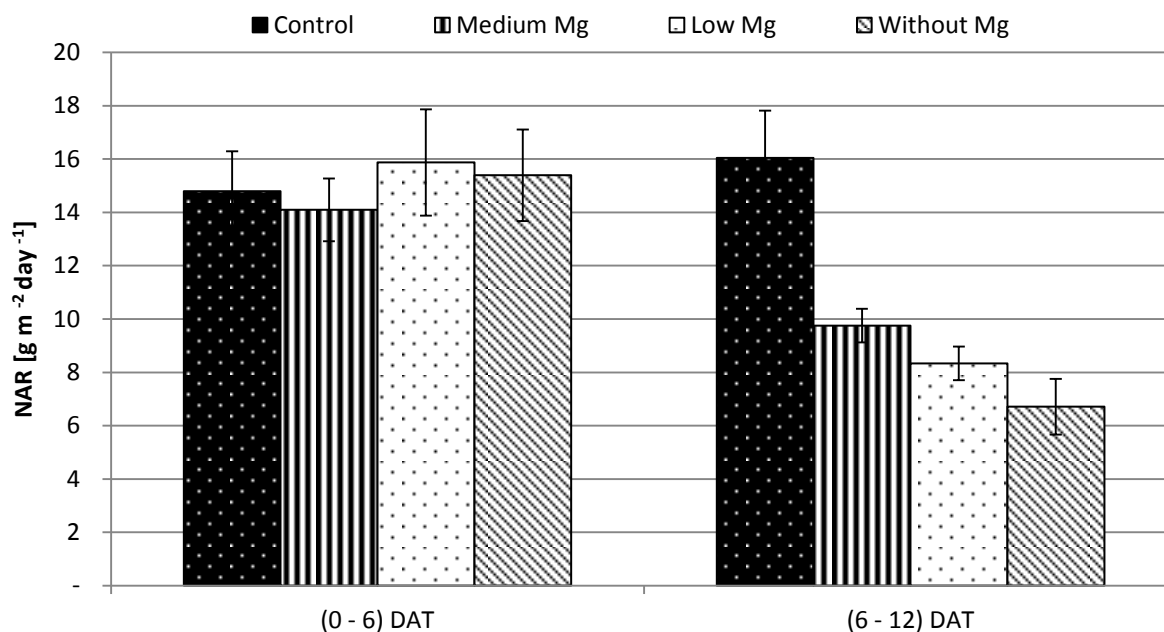


Fig. 5-2: Effect of the rate of Mg supply on whole plant net assimilation rates (NAR); vertical lines represent standard errors of the mean.

5.3.6 Mineral nutrient concentrations in young and old source leaves

In this experiment, emphasis was put on assessing early effects of Mg supply on photosynthesis and carbohydrate accumulation in source leaves. In the leaves which were used for measurement of photosynthesis and carbohydrates, mineral nutrient concentrations were also measured.

Reduction of Mg supply resulted in a drastic decrease of leaf Mg concentrations in young (Table 5-6A) and old source leaves (Table 5-6B) within the first 6 DAT, followed by a further slight decrease until 12 DAT (Table 5-6C and D). Six DAT, Mg concentrations were between 0.11 and 0.15 mg g⁻¹ leaf FM (about 1.0 mg g⁻¹ leaf DM) in young source leaves (Table 5-6A), which showed intercostal chlorosis (Fig. 5-1a), and between 0.18 and 0.20 mg g⁻¹ leaf FM (1.5 mg g⁻¹ leaf DM) in old source leaves (Table 5-6B), which did not show visible symptoms. This indicates, that the critical range of leaf Mg concentrations which leads to visible symptoms is between 0.10 and 0.20 mg g⁻¹ leaf FM (1.5 and 1.0 mg Mg g⁻¹ DM). Twelve DAT, the Mg concentration in old source leaves was 0.15 mg g⁻¹ FM (1.0 mg g⁻¹ DM) with medium Mg supply, and markedly lower (about 0.07 mg g⁻¹ FM or 0.5 mg g⁻¹ DM) with low and without

Mg supply (Table 5-6D). In young source leaves, the Mg concentrations had dropped to about 0.08 mg g⁻¹ FM (0.5 mg g⁻¹ DM), irrespective of the extent of Mg reduction (Table 5-6C).

On a physiological basis, critical leaf nutrient levels indicate the minimum amount of cell nutrient content that allows maintaining metabolic functions at non-limiting growth rate (Riga and Anza 2003). 0.6 mg g⁻¹ DM is a critical concentration of shoot for photosynthetic activity in *Pinus radiata* plants (Sun and Payn 1999). Hence, the critical level of Mg in leaves should be above 1.5 mg g⁻¹ DM in the leaves, when Mg concentration was 1.4 mg g⁻¹ DM in leaves it was 1.7 mg g⁻¹ DM in whole plant (Table 5-2), Therefore, Mg concentration in whole tomato plants should be above 1.7 mg g⁻¹ DM.

Table 5-6: Effect of Mg supply on nutrient concentration (based on fresh mass) in young and old leaf at 6 and 12 DAT. Different letters within a column indicate significant (Tukey-Kramer's test, P<0.05) differences of Mg treatments.

Treatments	Mg	Ca	K	P	Fe	Mn	Zn
	mg g ⁻¹ FM				µg g ⁻¹ FM		
	A) Young leaf at 6 DAT						
Optimal	0.55a	3.2a	4.5a	1.07a	38a	2.9a	6.0a
Medium	0.15b	3.6a	4.6a	1.14a	33a	3.1a	5.4ab
Low	0.13b	3.4a	4.5a	1.10a	27a	2.9a	4.7b
Without	0.11b	3.3a	3.9a	1.06a	19a	2.8a	4.8b
	B) Old leaf at 6 DAT						
Optimal	0.69a	6.2a	4.5a	0.57a	36a	4.9a	3.8a
Medium	0.20b	6.7a	4.9a	0.59a	31a	5.7a	3.0a
Low	0.18b	6.6a	4.6a	0.61a	27a	4.5a	3.5a
Without	0.18b	6.6a	4.5a	0.59a	22a	5.0a	3.0a
	C) Young leaf at 12 DAT						
Optimal	0.84a	5.2a	3.1a	0.62b	46a	2.4a	3.3a
Medium	0.09b	3.0b	3.5a	0.73ab	18b	2.3a	2.7a
Low	0.07b	2.7bc	3.5a	0.74ab	18b	2.3a	2.8a
Without	0.07b	2.4c	3.9a	0.91a	18b	2.8a	3.3a
	D) Old leaf at 12 DAT						
Optimal	0.87a	7.4a	3.1c	0.36b	43a	4.1a	2.6a
Medium	0.15b	7.0a	4.1b	0.44ab	28ab	4.1a	2.5a
Low	0.08b	6.6a	4.4ab	0.56a	23ab	4.1a	2.3a
Without	0.06b	5.2a	4.7a	0.57a	18b	3.9a	2.2a

The rate of Mg supply had significant effects on leaf concentrations of other macro- and micronutrients as well (Table 5-6). Twelve DAT, Ca concentrations in young source leaves were lower in Mg-deficient plants, particularly in plants grown without Mg supply (Table 5-6 C and D). This indicates lower xylem import into source leaves of deficient plants. In contrast, Mg deficiency was associated with higher K and P concentrations in young and old source leaves (Tables 5-6 C and D). In *Arabidopsis* after one week of Mg starvation (before any visible

symptom of Mg deficiency), Ca, Cu and Fe concentrations in roots and leaves were increased, whereas K and P concentrations in roots and leaves and Zn concentrations in leaves were decreased (Hermans et al. 2010). These ionic alterations are possibly related to Mg starvation induced alterations of expression of genes coding for nutrient transporters (Hermans et al. 2010). The biomass of young and old source leaves was not much affected by the Mg treatment (data not shown). Therefore, Mg deficiency induced alterations of leaf K and P concentrations may mainly result from differences in import and export. Higher K and P concentrations in leaves of Mg-deficient plants thus indicate a decrease in the ratio of export via phloem to import via xylem.

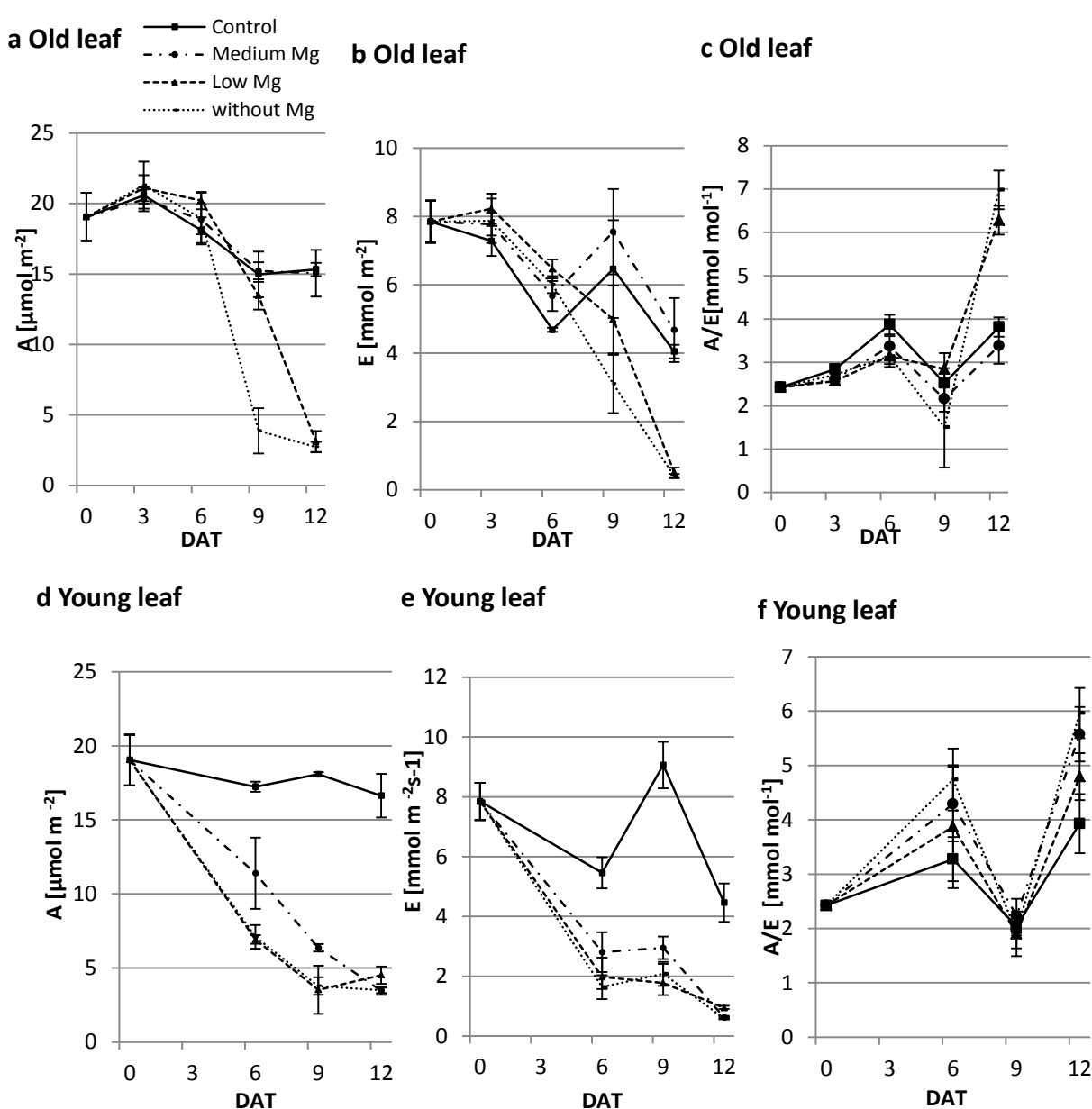


Fig. 5-3: Effect of the rate of Mg supply on CO₂ assimilation rates A (a, d), transpiration E (b, e) and water use efficiency A/E (c, f) in old and young source leaves; vertical lines represent standard errors of the means

In Mg-deficient plants, the leaf Fe concentrations were drastically reduced (Table 5-6). In leaves, Fe is mainly located in chloroplasts (Morrissey and Guerinot, 2009) suggesting that Mg deficiency-induced decrease of leaf Fe concentrations was related to lower leaf chlorophyll contents (see Fig. 5-1, and below leaf chlorophyll concentrations). In contrast to Fe, leaf Mn and Zn concentrations were not markedly changed by Mg supply (Table 5-6).

5.3.7 Rates of leaf gas exchange of young and old source leaves

The rate of Mg supply had significant effects on rates of net photosynthetic (A) and transpiration (E) in the old and young source leaf (Fig. 5-3). In the old source leaf, A was not affected by Mg supply in the first 6 DAT (Fig. 5-3a). Nine DAT in plants without and 12 DAT in plants with low Mg supply, A was drastically reduced. Interestingly, in plants with medium Mg supply, A was not lower than in plants with optimal Mg supply even 12 DAT. The effect of Mg supply on E was similar to that on A, with the exception of low Mg and without Mg 12 DAT (Fig. 5-3b). In old source leaves of these two treatments, E was even more drastically decreased than A, leading to increased photosynthetic water use efficiency (A/E; Fig. 5-3c).

In young source leaves, the Mg deficiency induced decline of A (Fig. 5-3d) and E (Fig. 5-3e) was already significant 6 DAT, which is in parallel to the stronger decline of leaf Mg concentrations in young than in old source leaves (Table 5-6 A, B). This indicates, that the critical range of leaf Mg concentrations leading to reduction of A is similar to that for the appearance of visible symptoms, namely between 0.10 and 0.20 mg g⁻¹ leaf FM (1.5 and 1.0 mg Mg g⁻¹ leaf DM). In young source leaves of Mg-deficient plants, E was even more strongly reduced than A leading to higher water use efficiency (A/E, Fig. 5-3f).

5.3.8 Chlorophyll concentrations in young and old source leaves

Leaf chlorophyll concentration in old source leaves in the first 6 DAT was not influenced by the rate of Mg supply (Fig. 5-4A). In this period, leaf Mg concentrations of plants grown with deficient Mg supply had declined to about 0.19 mg g⁻¹ FM (1.5 mg Mg g⁻¹ DM) (Table 5-6A). Twelve DAT, however, chlorophyll concentrations were lower in plants grown with medium Mg, low Mg and without Mg than in control plants (Fig. 5-4a). Until then, leaf Mg concentrations had dropped to about 0.07 mg g⁻¹ FM (low Mg, without Mg) and 0.15 mg g⁻¹ FM (medium Mg) (Table 5-6A). This indicates, that the critical leaf Mg concentrations affecting chlorophyll concentrations is between 0.15 and 0.19 mg Mg g⁻¹ FM. Interestingly, in plants grown with medium Mg supply, the drop of leaf chlorophyll concentrations to less than 1 mg chlorophyll g⁻¹ leaf FM was not associated with strong reduction of leaf photosynthesis (Fig. 5-

3a). In young source leaves, the decline of chlorophyll concentrations in Mg-deficient plants was stronger than in control plants already in the first 6 DAT (Fig. 5-4b) which was in parallel with the stronger decline in leaf Mg concentrations 0.11 to 0.15 mg g⁻¹ FM (Table 5-6A) and in rates of leaf gas exchange (Fig. 5-3 d and e).

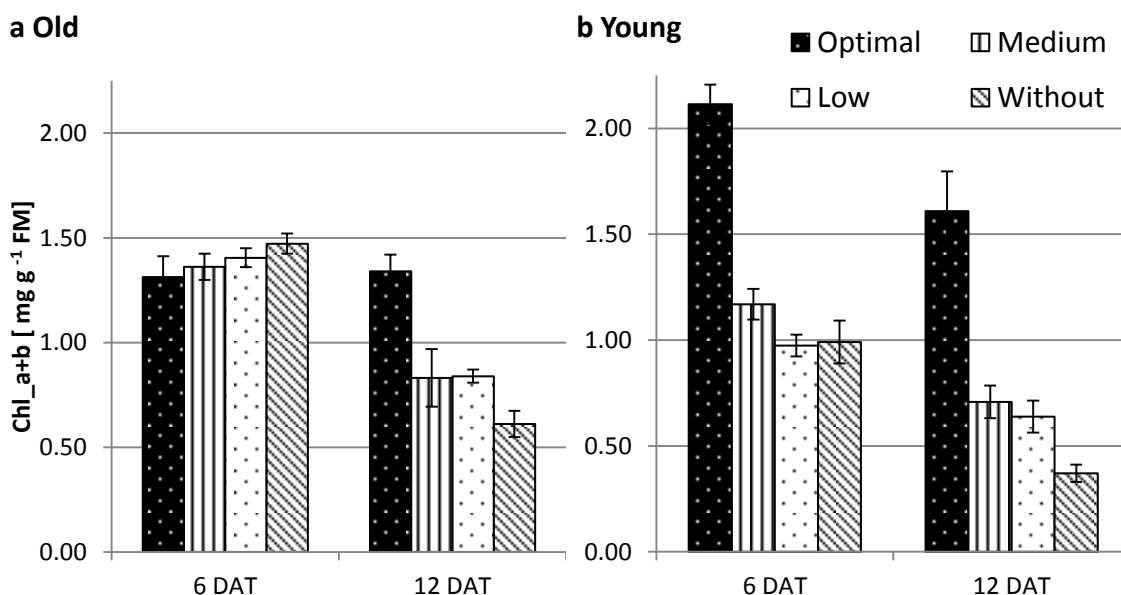


Fig. 5-4: Effect of the rate of Mg supply on chlorophyll concentrations in old (a) and young (b) source leaves; vertical lines represent standard errors of the means.

5.3.9 Concentrations of non-structural carbohydrates in young and old source leaves

During the treatment phase, leaf concentrations of soluble sugars and starch in old and young source leaves steadily increased indicating a strong effect of plant developmental stage on leaf carbohydrate status (Fig. 5-5). In leaves of plants grown with medium, low and without Mg supply, the increase of soluble sugar concentrations was stronger than in control leaves (Fig. 5-5 a and b), whereby this Mg deficiency induced increase was higher and started earlier in young (6 DAT: Fig. 5-5b) than in old source leaves (12 DAT; Fig. 5-5a). In contrast, the developmental increase of starch concentrations was weaker in leaves of Mg-deficient plants than in control leaves, whereby the Mg effects became significant only 12 DAT (Fig. 5-5 a and b). The Mg deficiency-induced reduction of leaf starch concentrations approximately compensated the Mg deficiency-induced increase of leaf soluble sugar concentrations. Thus, leaf concentrations of total non-structural carbohydrates were not significantly affected by Mg supply (Fig. 5-5 a and b), with the exception of young source leaves of Mg-deficient plants 6 DAT. In these leaves, non-structural carbohydrate concentrations were increased despite of significant reduction of photosynthetic rate (Fig. 5-3d).

Twelve DAT the concentrations of various soluble sugars were measured in the young source leaf. In this leaf, soluble sugar concentrations again were increased under Mg deficiency, whereas starch concentrations were reduced (Fig. 5-6a). In comparison to leaves from control plants, in Mg-deficient plants glucose concentrations were not affected whereas fructose concentrations were drastically increased (Fig. 5-6b). Also the concentrations of sucrose, which is the sugar form loaded into and transported within the phloem, were significantly higher in leaves of Mg-deficient plants (Fig. 5-6b).

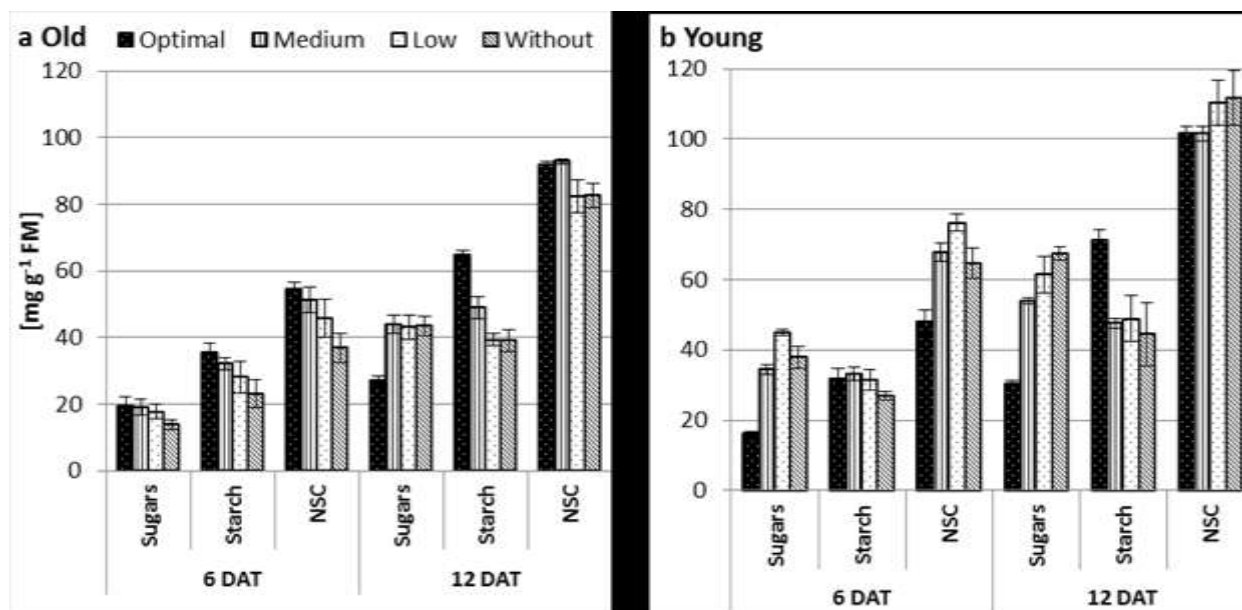


Fig. 5-5: Effect of Mg supply on concentrations of soluble sugars, starch and total non-structural carbohydrates in old (a) and young (b) source leaves; vertical lines represent standard error of the means.

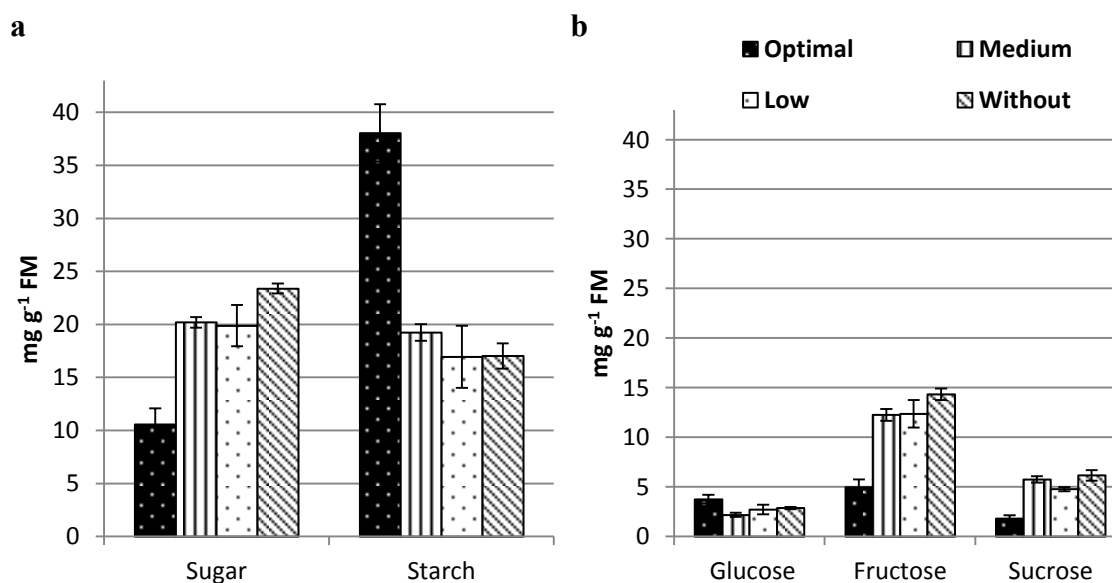


Fig. 5-6: Effect of Mg supply on concentrations of various soluble sugars and starch in the young source leaf after harvest (based on FM); vertical lines represent standard errors of the mean.

5.4 Results of the second experiment

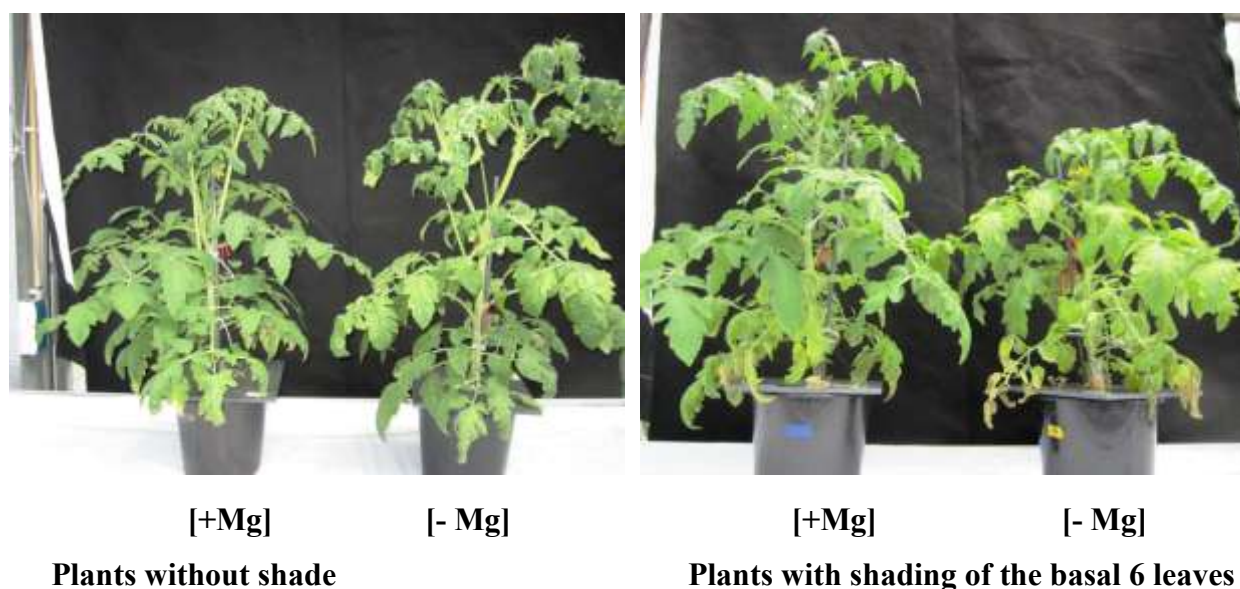


Fig. 5-7: Effect of Mg supply and shading of the basal 6 leaves on plant appearance 24 days after start of treatment.

5.4.1 Growth and biomass partitioning

In the first experiment, Mg deficiency was associated with increased soluble sugar concentrations in leaves (Figs 5-5 and 5-6), and in parallel with reduced rates of leaf photosynthesis (Fig. 5-3). In principal, this Mg deficiency effect occurred irrespectively of whether plants were subjected to medium Mg supply, low Mg supply or cultured without Mg. It has been suggested that Mg deficiency induced sugar accumulation in leaves is related to inhibition of phloem loading (Cakmak et al. 1994b), or, lower sugar demand of growing sink organs (Fischer et al. 1998). It is well documented, that sugar accumulation in leaves, in turn, may reduce photosynthesis (Engels et al. 2012). In a second experiment, the lower source leaves were either shaded or not shaded to vary the source/sink relationships. Shading should decrease the source/sink ratio in plants, and thus should decrease the plant concentrations of non-structural carbohydrates. Sugar concentrations and photosynthetic rates were measured in the upper not shaded source leaves of plants which were either optimally supplied with Mg or which did not receive any Mg after pre-culture. If phloem loading is inhibited by Mg deficiency, sugar accumulation in the upper source leaves of Mg-deficient plants should be similar in shaded and not shaded plants.

At start of Mg treatment, plants were larger in the second than in the first experiment. Consequently, also the internal Mg reserves at start of Mg treatment were larger. Therefore, first visual symptoms of Mg deficiency on young source leaves became visible only 17 DAT in the second as compared to 6 DAT in the first experiment. Interestingly, shading of the basal 6

leaves per plant accelerated the appearance of Mg deficiency symptoms in the young source leaf, and exacerbated Mg deficiency symptoms on plants 24 DAT (Fig.5-7).

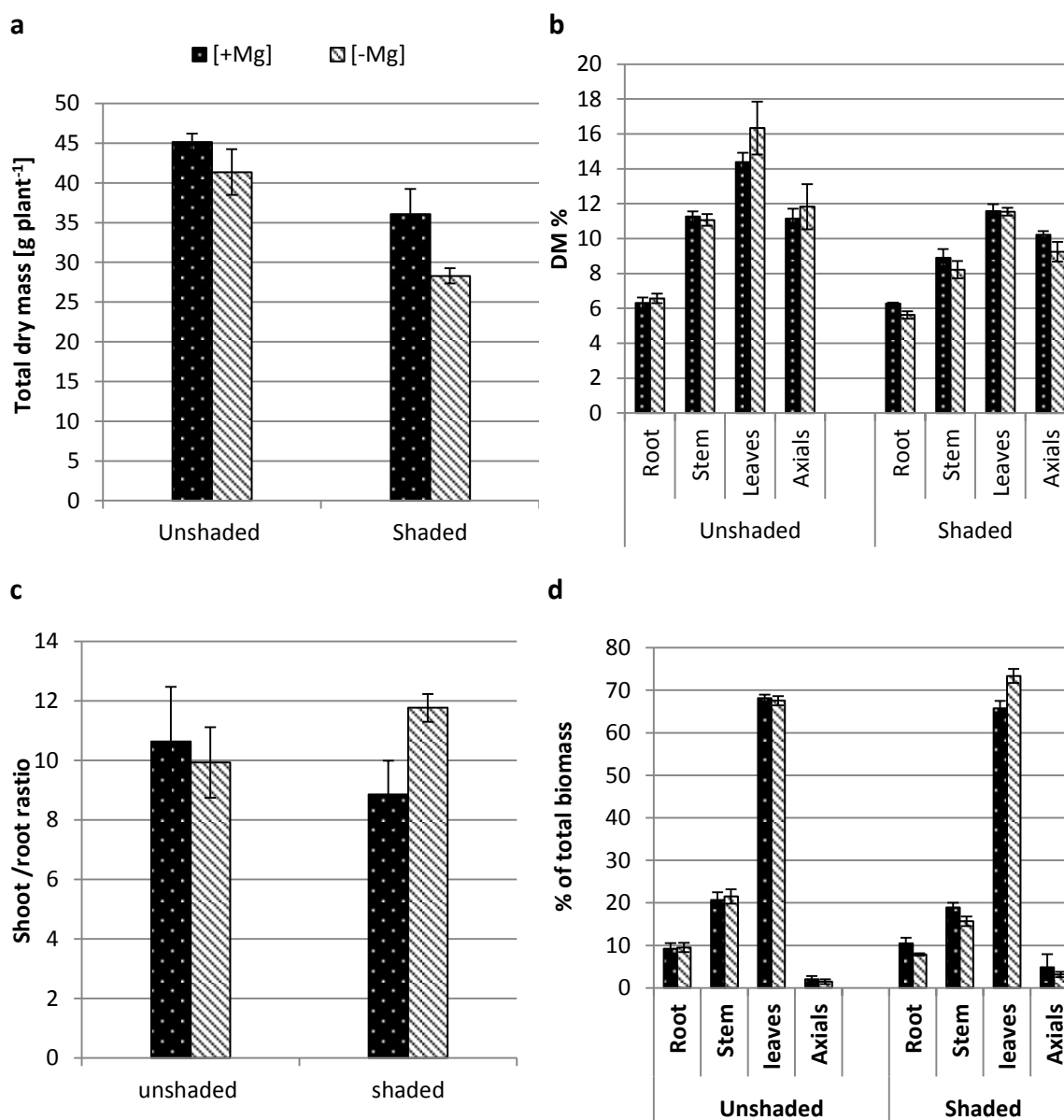


Fig. 5-8: Effect of shading and Mg deficiency on total plant dry mass (a) .dry matter content of various plant organs (b), shoot/root ratio (c) and biomass partitioning to various plant organs (d); vertical lines represent standard errors of the mean (n=4).

Mg deficiency reduced total plant biomass by about 10% in unshaded and by about 20% in shaded plants (Fig. 5-8a). The shading-induced reduction of total plant DM was about 20% with optimal Mg supply and about 30% under Mg deficiency. This indicates that Mg-deficient plants were more sensitive to the loss of photosynthetic capacity caused by shading of the basal 6 plant leaves. Was this due to inability of non-shaded leaves to increase photosynthetic rates? Was this due to inability to increase non-shaded leaf area? Shading decreased plant DM more than plant FM. Thus, dry matter percentage was significantly reduced by shading (Fig. 5-8b).

Mg deficiency influenced the shading-induced alteration of biomass partitioning among plant organs. At optimal Mg supply, shading was associated with a slight reduction of S/R-ratio (Fig. 5-8c) and percentage of leaf biomass in total plant biomass (Fig. 5-8d), whereas under Mg deficiency shading led to an increase of S/R-ratio and percentage of leaf biomass in total plant biomass

5.4.2 Nutrient concentrations in unshaded source leaves

Nutrient concentrations were measured in the source leaf in which Mg deficiency symptoms became first visible (leaf no. 9, called “young source leaf” corresponding to experiment 1) to characterize the mineral nutritional status of the leaf which was used for measurement of leaf gas exchange. Nutrient composition of the source leaf was significantly affected by shading and Mg-supply (Table 5-7). In comparison to optimal Mg supply, Mg deficiency was associated with severe reduction of Mg concentrations in the young source leaf of unshaded and shaded plants to $0.11 \text{ mg g}^{-1} \text{ FM}$ ($0.69 \text{ mg g}^{-1} \text{ DM}$) in unshaded and $0.07 \text{ mg g}^{-1} \text{ FM}$ ($0.61 \text{ mg g}^{-1} \text{ DM}$) in shaded plants.

Shading of basal leaves had little effect on concentrations of other nutrients in the young source leaf with the exception of decreased Fe concentration (Table 5-7). Mg deficiency was associated with a slight (not significant) reduction of Ca and K concentration in the young source leaf, and a reduction of Fe and Zn concentrations (Table 5-7).

Table 5-7: Effect of Mg supply and shading of the 6 basal source leaves on nutrient concentrations in the young (leaf no. 9) source leaf. Different letters within a column indicate significant (Tukey Kramer's test, $P < 0.05$) treatment effects.

		$\text{mg g}^{-1} \text{ FM}$				$\mu\text{g g}^{-1} \text{ FM}$		
		Mg	Ca	K	P	Fe	Mn	Zn
Unshaded	[+ Mg]	0.67a	3.9a	4.5a	0.78a	40a	2.2a	4.5a
	[- Mg]	0.11b	2.9a	3.5a	0.84a	32ab	2.2a	3.2ab
Shaded	[+ Mg]	0.62a	3.7a	4.4a	0.76a	28bc	1.8a	3.9ab
	[- Mg]	0.07b	2.8a	3.6a	0.67a	19c	1.9a	2.7b

5.4.3 Rates of leaf gas exchange in the young source leaf

In the first 17 DAT, the rate of net assimilation (A) of the young source leaf was not affected by shading of basal leaves and Mg supply (Fig. 5-9a). Thereafter, A was lower in Mg-deficient plants than in plants with optimal supply. Surprisingly, in Mg-deficient plants A was more strongly and earlier reduced in the young source leaf from shaded plants than from unshaded plants, whereas in plants well supplied with Mg, A of source leaves was not

consistently influenced by shading of basal leaves (Fig. 5-9a). The effects of Mg supply and shading of basal leaves on leaf transpiration rate (E) were similar to those on A (Fig. 5-9b). Thus, the ratio of A/E was not affected by leaf age, Mg supply and shading (Fig. 5-9c).

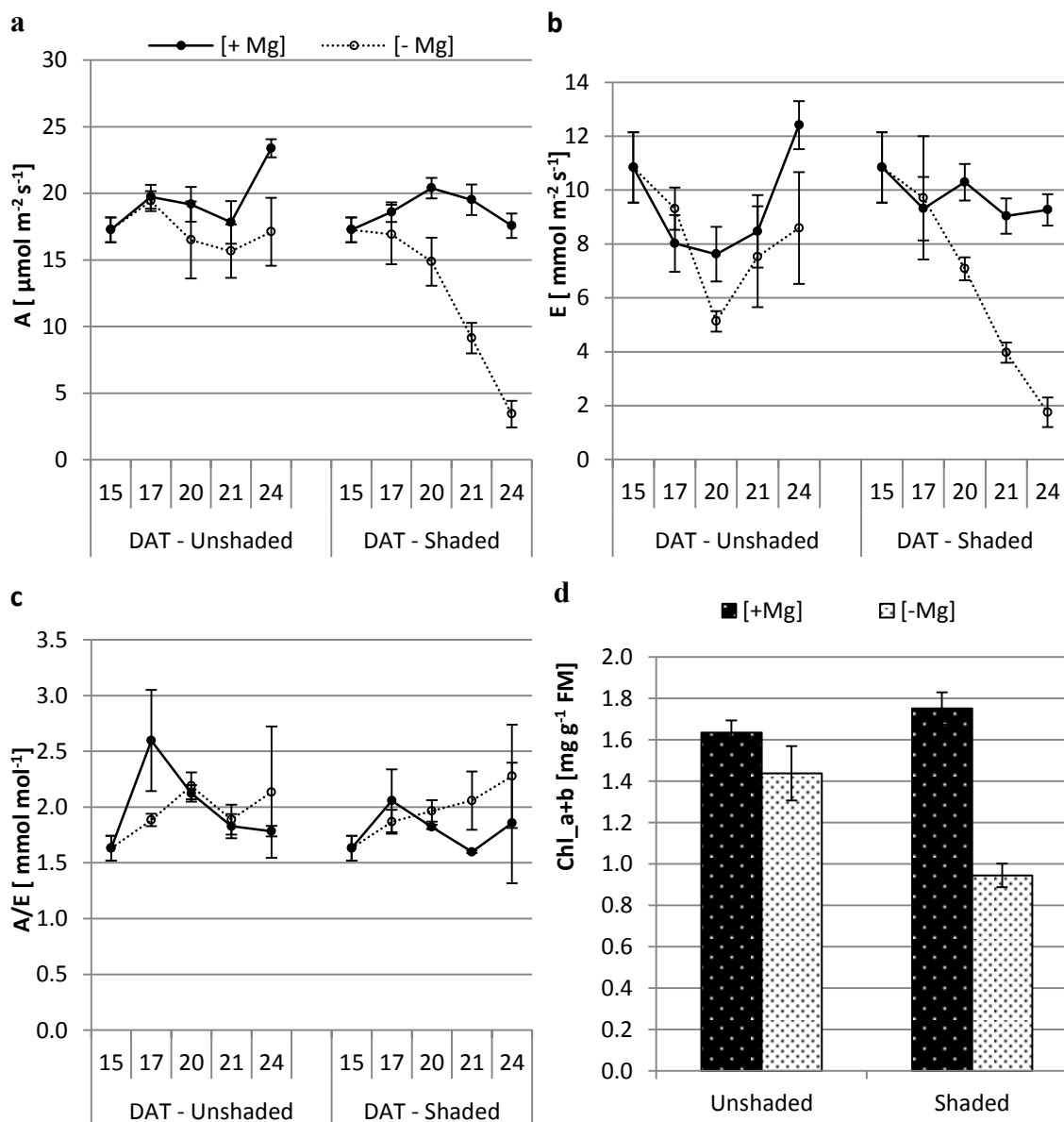


Fig. 5-9: Effect of Mg deficiency and shading on (a) the rates of net photosynthesis (A), (b) transpiration (E), (c) water use efficiency (A/E) and (d) chlorophyll a and b concentrations of the young source leaf; vertical lines represent standard errors of the mean (n=4).

5.4.4 Chlorophyll concentration in the young source leaf

In plants, which were well supplied with Mg, shading of basal leaves had no effect on chlorophyll concentration in the young source leaf at 24 DAT (Fig. 5-9 d). Mg deficiency only slightly leaf chlorophyll concentration in unshaded plants. In shaded plants, however, leaf chlorophyll concentration was significantly lower in Mg-deficient plants than in plants well supplied with Mg.

5.4.5 Concentrations of non-structural carbohydrates in the young source leaf

In plants which were well supplied with Mg, the concentrations of non-structural carbohydrates (sum of sugars and starch) in the young source leaf were significantly lower in shaded than in unshaded plants (Fig. 5-10a). This was expected, and indicates that in partially shaded plants less carbohydrate accumulated in the irradiated source leaves because the rate of sugar export to sink organs was increased. The decrease of carbohydrate concentrations in leaves of shaded plants was mainly due to lower starch concentrations. Starch concentrations in leaves of shaded plants were decreased by (64% in comparison to unshaded plants), whereas soluble sugar concentrations were only decreased by (53 % in comparison to unshaded plants).

In Mg-deficient plants, shading also reduced the concentration of starch in the young source leaf (Fig. 5-10a). However, soluble sugar concentrations were significantly higher in shaded than in unshaded plants. This increase of soluble sugar concentrations fully compensated the decrease of starch concentrations, and thus, concentrations of total non-structural carbohydrates were not significantly reduced in shaded plants (Fig. 5-10a).

A more detailed carbohydrate analysis with an enzymatic method, in principal confirmed that the Mg deficiency-induced increase of starch and soluble sugars occurred only in shaded plants but not in unshaded plants (Fig. 5-10b). Similar as in the first experiment (Fig. 5-6), Mg deficiency increased the concentrations of sucrose and in particular of fructose, whereas the glucose concentrations were only slightly increased in shaded plants and decreased in unshaded plants (Fig. 5-10b).

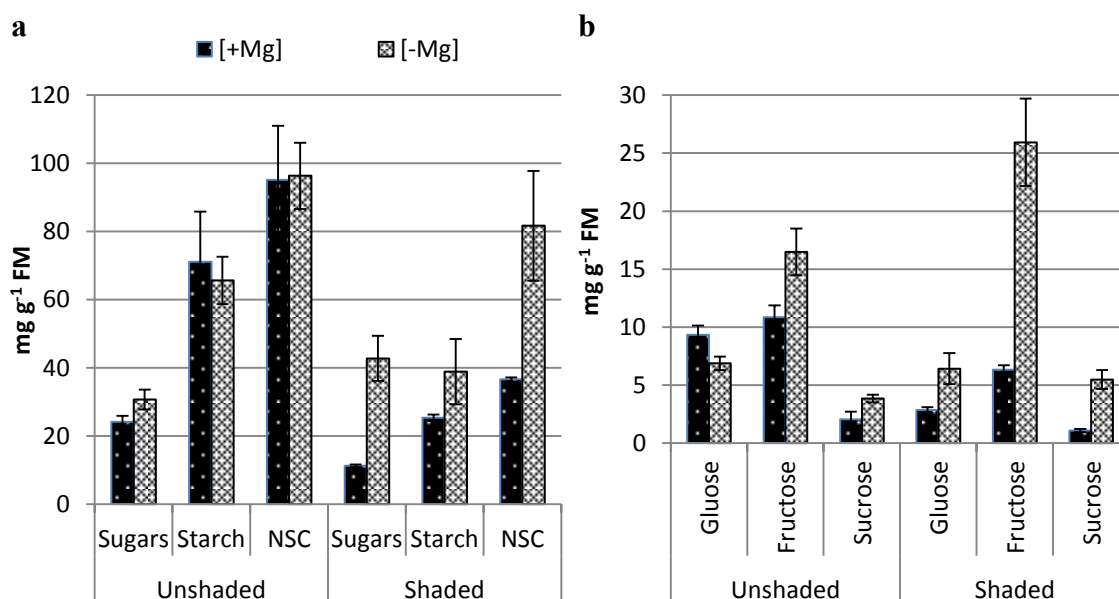


Fig. 5-10: Effect of Mg supply and shading of basal source leaves on (a) concentrations of soluble sugars, starch and total non-structural carbohydrates (NSC), and (b) concentrations of glucose, fructose and sucrose in the young source leaf; vertical lines represent standard errors of the mean (n=4).

5.4.6 Effect of Mg supply and shading on nutrient content of basal leaves

To assess if Mg supply and shading have an effect on net remobilization from basal leaves, nutrient content were determined in the basal 6 source leaves (Table 5-8). For Ca, which is not phloem mobile, and thus can not be exported from leaves, the leaf content of shaded leaves (233 mg in +Mg and 264 mg in –Mg plants) was substantially (43%) lower than the leaf content of unshaded leaves (420 mg in + Mg and 452 mg in –Mg plants). If one assumes, that Ca import via xylem was reduced to 0 after shading due to stomatal closure, this indicates that in unshaded leaves Ca import during the experimental period was about 188 mg Ca, i.e., 75% of the amount which was already present in the basal leaves at start of treatment.

Table 5-8: Effect of Mg supply and shading of the 6 basal source leaves on nutrient content of these leaves. Different letters within a column indicate significant (Tukey Kramer's test, $P < 0.05$) treatment effects.

		Nutrient content (mg [6 leaves] ⁻¹)						
Shading	Mg	Mg	Ca	K	P	Fe	Mn	Zn
unshaded	[+ Mg]	59a	420a	283a	43a	3.34a	0.25ab	0.26a
	[- Mg]	27b	452a	329a	48a	3.45a	0.28a	0.28a
Shaded	[+ Mg]	33b	233b	263a	42a	2.00a	0.18b	0.16b
	[- Mg]	28b	264b	293a	42a	2.54a	0.23ab	0.18b

In plants supplied with Mg, the basal leaves content of shaded plants (33 mg) was 56% of that of unshaded leaves (59 mg) (Table 5-8). The percentage of shade-induced reduction of Mg content was very similar to the reduction of Ca content. This indicates that Mg also was not remobilized and not exported via phloem from basal leaves. In plants, which did not receive Mg during the treatment period, the leaf content of Mg was similar in unshaded leaves from which phloem export of sugars and minerals may be expected, and shaded leaves from which phloem export should be severely reduced due to inhibition of photosynthesis. This supports the suggestion that Mg export via phloem was negligible.

In contrast to Ca and Mg, the K content (263 mg in +Mg and 293 mg in –Mg plants) and P content (42 mg) of shaded leaves was only about 10% lower than in unshaded leaves (Table 5-8). If one assumes that K and P import into unshaded leaves should increase K and P content to a similar extent as Ca content (i.e., 75% of the amount, which was already present in the basal leaves at start of treatment). This would indicate that most of the K and P, which was imported into unshaded leaves via the xylem, were exported again via the phloem.

The shade-induced decrease of leaves mineral contents leaves for Fe (34% decrease in comparison to unshaded leaves), Mn (23% decrease) and Zn (37% decrease) was between that

of phloem mobile K and P (10% decrease) and that of non-phloem mobile Ca (43% decrease) (Table 5-8). This indicates that at least part of the Fe, Mn and Zn which was imported via xylem was exported again via phloem.

There was a tendency that in Mg-deficient plants, the leaves content of all nutrients except Mg was higher in Mg-deficient than in Mg-sufficient plants (Table 5-8). If one assumes that xylem import of nutrients into basal leaves was not increased by Mg deficiency, this would indicate that mineral export via the phloem was slightly reduced by Mg deficiency.

Table 5-9: Effect of Mg supply on Mg concentration (mg g^{-1} FM) of different plant organs at 6 and 12 DAT. Different letters within a column indicate significant (Tukey-Kramer's test, $P < 0.05$) differences among Mg treatments.

Treatments	Roots		Stem		Leaves		Axial	
	6 DAT	12 DAT	6 DAT	12 DAT	6 DAT	12 DAT	6 DAT	12 DAT
	Mg concentration (mg g^{-1} FM)							
Optimal	0.32 a	0.57a	0.24 a	0.31a	0.56 a	0.71s	0.35 a	0.27a
Medium	0.08 b	0.25b	0.10 b	0.12b	0.18 b	0.18b	0.27 ab	0.23ab
Low	0.07 b	0.06c	0.10 b	0.10bc	0.17 b	0.10c	0.19 b	0.15bc
Without	0.05 b	0.04c	0.08 b	0.04c	0.16 b	0.08c	0.19 b	0.09c

5.5 Discussion

5.5.1 Early plant responses to Mg deficiency

One aim of the experiments described in this chapter was the assessment of early effects of Mg deficiency. In the first 6 DAT, total biomass growth and allocation of biomass to various plant organs were not affected by the rate of Mg supply (Optimal, Medium, Low, Without) (Table 5-1A, B), despite of severe reduction of Mg concentrations in all plant organs (Table 5-9). Moreover, appearance of Mg deficiency symptoms in young source leaves (Fig. 5-1b) of plants which were not optimally supplied with Mg. The lack of fast growth responses to Mg deficiency is in marked contrast to reports from literature on fast growth responses of tomato to N deficiency (Chapin et al. 1988). In a study with *Plantago major*, in which nutrient supply was reduced from 100% to 2%, the relative growth rate of plants was severely reduced within two days (Kuiper 1988, Kuiper et al. 1988). This fast effect of low nutrient supply on growth could be reversed by addition of CYT to the nutrient solution (Kuiper 1988, Kuiper et al. 1988) indicating that fast effects of low nutrient supply on growth were triggered by hormonal effects and not by lack of nutrients for cellular metabolism.

In the first 6 DAT the rate of Mg supply had no effect on morphological shoot traits (Table 5-4), leaf area extension (Table 5-2A), and morphological root traits (Table 5-5). In the

study of Chapin et al. (1988) with tomato, N withdrawal from nutrient solution lead to reduction of leaf area, decrease in specific leaf area (leaf area per unit leaf dry mass) and increased biomass allocation to roots already after two days. The fast response of leaf growth to N deficiency, which was also found in other plant species (Kavanová et al. 2008) has been attributed to increased leaf ABA concentrations (Chapin et al. 1988) and/or reduced CYT supply from roots to leaves (Kuiper 1988, Kuiper et al. 1988, Römheld et al. 2008). Of the nutrients N exerts the most obvious influence on the production and export of CYT to the shoots (Argueso et al. 2009), whereas Mg effects on CYT and ABA are not known from literature.

In conclusion, in contrast to deficiency of N (Chapin et al. 1988) or drought stress (Davies and Zhang, 1991), under Mg deficiency there is obviously no “fine control” of growth that enables the plant to reduce total growth or growth of individual organs before there is deficiency-induced disturbance of cellular metabolism. Growth effects were triggered only from 6 to 12 DAT (Tables 5-1, 5-2, 5-4, 5-5), when internal Mg concentrations were extremely reduced. This indicates that growth effects were related to Mg deficiency at the cellular level and lack of Mg for specific metabolic processes. Twelve DAT, in Mg-deficient plants biomass allocation to leaves was increased and biomass allocation to roots and stems was decreased (Table 5-1). This alteration of biomass allocation is consistent with results described in chapter 2 (Fig. 2-4 a, Table 2-3a) and chapter 3 (Table 3-1, 3-3). Reduced biomass allocation to roots was also found in many previous investigations with bean (Cakmak et al 1994a, b), spinach (Fisher and Bremer 1993) and *Pinus radiata* (Sun and Payn 1999). However, some recent studies report the absence of specifically negative effects of Mg deficiency on root growth of sugar beet (Hermans et al. 2004, 2005), *Arabidopsis* (Hermans and Verbruggen, 2005) and rice (Ding et al. 2006). In Mg-deficient *Arabidopsis*, carbon allocation to the youngest leaves was more reduced than carbon allocation to roots (Hermans and Verbruggen 2005, Hermans et al. 2006).

In the first 6 DAT, net assimilation rate at the whole plant level was not affected by the rate of Mg supply (Fig. 5-2). In the study of Chapin et al. (1988) N deficiency in tomato reduced rates of leaf gas exchange already after two days of N withdrawal. The decrease of leaf photosynthetic rates was associated with reduction of internal CO₂ concentration in the leaves, indicating that photosynthesis was reduced due to partial closure of stomata (Chapin et al. 1988). The authors suggested that this early N deficiency-induced decline in photosynthesis was caused by ABA-induced stomata closure. In the present study, 6 DAT, leaf gas exchange was reduced only in the young source leaf (Figs 5-3 d and e) but not in the old source leaf (Fig. 5-3a and b). In the young source leaf, Mg concentrations (Table 5-6A) were slightly lower than in the old source leaf (Table 5-6B). Possibly, the leaf Mg concentration in the young leaf just fell

below the critical value for optimal photosynthesis. Interestingly, in the young but not in the old source leaf, the leaf chlorophyll concentrations were also reduced 6 DAT (Fig. 5-4) and soluble sugar concentrations were increased (Fig. 5-5). These changes of leaf chlorophyll concentrations and soluble sugar concentrations are in agreement with the suggestion that leaf photosynthesis is reduced only after cellular Mg concentrations fell below critical values needed to directly fulfil Mg functions in cell metabolism.

An early response of plants to Mg deficiency was an alteration of Mg allocation among various plant organs (Table 5-3). Under Mg deficiency Mg allocation to roots and leaves was reduced and allocation to stems and axial increased already in the first 6 DAT (Table 5-3A, B). Obviously, effects of Mg deficiency on the relative allocation of biomass to various organs (Table 5-1) were different from effects on the relative allocation of Mg to various organs. Interestingly, the different pattern of allocation of biomass and Mg to various plant organs resulted in a remarkable equalization of Mg concentrations in all plant organs in Mg-deficient plants (Table 5-9).

5.5.2 Effects of the rate of Mg supply (medium – low – without) on plant responses

Our hypothesis was that the rate of Mg supply and particularly the mode how Mg deficiency is induced would affect plant responses to Mg deficiency. We expected that in the treatments “low supply” and “medium supply” the scarce Mg, which was continuously supplied and taken up, would be allocated among plant organs and, within cells among Mg pools serving different Mg-related functions in cellular metabolism, in a way that protects plants from severe disturbance of metabolism. We expected that such well-targeted allocation of recently acquired Mg is not possible in the treatment “without Mg supply” because in this treatment there is no recent Mg uptake.

The rate of Mg supply had a quantitative effect on the deficiency-induced growth reduction from 6 to 12 DAT (Table 5-1). The reduction of biomass growth (Table 5-1) and leaf area growth (Table 5-2) became increasingly severe from “medium” to “low” to “without” Mg supply (Table 5-1). These quantitative effects of the rate of Mg supply on growth were paralleled by similar quantitative effects of the rate of Mg supply on Mg concentrations in plant organs (Table 5-9). The rates of leaf gas exchange in old and young source leaves started earlier to decline and more strongly declined with “low” and “without” than with “medium” Mg supply (Fig. 5-3), and this was associated with more severe decrease in leaf chlorophyll concentrations with “low” and “without” than with “medium” supply (Fig. 5-4). However, beyond quantitative effects of the rate of Mg deficiency on growth and photosynthesis, we did not find qualitative

effects. In the treatments “low” and “medium” supply, the relative allocation of Mg to roots and leaves was markedly reduced, and allocation to stem and axial increased (Table 5-3). Similarly, in the treatment “without Mg supply”, during the 12 days of treatment there was net loss of Mg from leaves and net gain in axial (Table 5-3).

As an indication for disturbance of metabolism, we measured leaf carbohydrate concentrations. In previous studies in which Mg deficiency was induced by complete Mg withdrawal from nutrient solution, it was observed that Mg deficiency was associated with higher sugar and starch concentrations in source leaves (Fisher and Bremer, 1993, Cakmak et al. 1994b, Fischer et al. 1998, Hermans et al. 2005). In our experiments, leaf concentrations of total soluble sugars (Fig. 5-5) increased in Mg-deficient plants irrespective of the mode of inducing Mg deficiency. Similarly, the ratio of individual soluble sugars was affected by Mg deficiency irrespective of the mode of inducing Mg deficiency. The ratio of sucrose, and particularly, of fructose in total soluble sugars markedly increased, whereas the ratio of glucose decreased (Fig. 5-6). This indicates that disturbance of cellular metabolism which leads to sugar accumulation and alteration of sugar composition in leaf cells occurs irrespective of whether Mg is continuously supplied at suboptimal rates or whether Mg supply to roots is completely interrupted. In tomato leaves, abiotic stresses lead to higher biosynthesis of secondary compounds such as α -tomatine, which are N-containing secondary plant metabolites found in tomatoes to protect plant against biotic stress (Friedman 2002). The decrease of leaf glucose concentrations (Fig. 5-6) is possibly due to consumption of glucose for biosynthesis of α -tomatine which needs two molecules of glucose (Arneson and Durbin 1968).

In our study, leaf starch concentrations were reduced under Mg deficiency (Fig. 5-5). Low starch concentration in source leaves of Mg-deficient plants was also found in a previous study (Tewari et al. 2006). It was suggested that low starch and high sugar concentrations in leaves of Mg-deficient plants imply disturbed carbohydrate metabolism and impaired phloem transport (Tewari et al. 2006). Low starch concentrations in leaves particularly 12 DAT (Fig. 5-5) were possibly due to chloroplast degradation, because starch is synthesised and stored in chloroplasts. After Mg deficiency-induced collapse of chloroplasts there will be no starch biosynthesis. In contrast to the present observation, Cakmak et al. (1994 b) and Hermans et al. (2005) found increases of leaf starch concentrations in Mg-deficient plants. Therefore, severe reduction of Mg concentration in leaves probably caused inhibition of many enzymes that are responsible for carbohydrate metabolism.

5.5.3 Effect of shading of basal leaves on photosynthesis and carbohydrate concentrations in apical leaves of Mg sufficient and Mg-deficient plants

Other aims of the experiments described in this chapter were to assess if (i) the Mg deficiency-induced decrease of photosynthesis is due to accumulation of carbohydrates, and (ii) the carbohydrate accumulation in source leaves is due to inhibition of phloem loading.

Mg is essential for the function of many enzymes and plays a fundamental role in both, light and dark reaction of photosynthesis (Shaul 2002, Engels et al. 2012). Therefore, photosynthesis could be affected by Mg deficiency in many ways. Apart from its role as constituent of the chlorophyll molecule, Mg is required for thylakoid stacking and enzymes involved in CO₂ assimilation and assimilate partitioning between starch and sucrose (Liu et al. 2008). We were monitoring the rate of photosynthesis in specific leaves in both experiments (Figs 5-3 and 5-9). In the first experiment, 6 DAT photosynthesis of the young source leaf was reduced in all Mg-deficient plants (medium, low, and without Mg supply), whereas photosynthesis of the old source leaf was not yet affected (Fig. 5-3). The decrease of photosynthesis in the young source leaf was associated with increased concentrations of soluble sugars and total NSC, whereas in the old leaf soluble sugars and NSC were not increased (Fig. 5-5). This is in line with experiments with *Arabidopsis thaliana* which showed that Mg deficiency increased the sugar concentrations in young source leaves before any noticeable effects on photosynthetic activity (Hermans and Verbruggen, 2005). These authors suggested that the decline in photosynthetic activity might be elicited by increased sugar concentrations.

In the second experiment, we tried to avoid or reduce sugar accumulation in young source leaves of Mg-deficient plants by shading of basal leaves. We expected that shading of basal leaves should decrease source strength of the plant but not sink strength. In accordance with our expectation, in Mg sufficient plants, the concentrations of soluble sugars and starch, and thus also the concentrations of NSC in apical (unshaded) leaves were lower in shaded than in unshaded plants (Fig. 5-10a). In unshaded Mg-deficient plants, the concentrations of soluble sugars and starch in apical leaves were not yet increased (Fig. 5-10a), and accordingly, photosynthesis was not yet decreased (Fig. 5-9). Interestingly, the ratio of fructose to glucose was already higher than in Mg sufficient plants. This indicates that the alteration of leaf sugar composition is an early symptom of Mg deficiency which precedes total sugar accumulation and inhibition of photosynthesis. In Mg-deficient plants, shading of basal leaves reduced starch concentration of apical leaves, but in contrast to our expectation and intention of the experimental treatment, did not strongly change leaf concentrations of soluble sugars in comparison to unshaded Mg-deficient plants (Fig. 5-10a). Photosynthesis in apical leaves of

shaded plants, however, was strongly reduced (Fig. 5-9). This indicates that the reduction of photosynthesis in Mg-deficient plants in our experiments was not induced by high leaf sugar concentrations.

In Mg sufficient plants, the concentrations of NSC were lower in apical leaves of shaded plants than in apical leaves of unshaded plants (Fig. 5-10) despite of similar rates of photosynthesis (Fig. 5-9). This indicates that the shade induced increase of sink to source ratio resulted in increased phloem export of sugars from apical leaves to sink organs. In Mg-deficient plants, in contrast, the concentrations of NSC, and in particular of soluble sugars in apical leaves of shaded plants remained on a similarly high level as in apical leaves of unshaded plants despite of lower rates of photosynthesis at least in individual leaves. High accumulation of soluble sugars in leaves despite of low source to sink ratio is in agreement with the suggestion of other authors, that Mg deficiency leads to inhibition of phloem loading (Cakmak et al. 1994b, Hermans et al. 2005).

6. General discussion

Plants have evolved multifaceted strategies to respond to variation in nutrient availability in the soil to increase nutrient acquisition and internal nutrient utilization (Hawkesford et al. 2012). These strategies include metabolic, physiological, morphological, and developmental adaptations. These responses differ depending on the specific nutrient limitation. In the first experiment, tomato plants were cultured under controlled conditions at three different rates of nutrient supply (optimal, growth reduction to 80 % and 60 % induced either by K, Mg or N deficiency) until fruit maturity. In the second experiment, the same approach was used but only the rate of Mg supply was varied, including a treatment without Mg supply. Furthermore, in the second experiment plants were investigated only during the vegetative growth phase. The reason for this experiment was to investigate if the plant response to Mg limitation is dependent on whether plants are grown at suboptimal Mg supply or completely without Mg supply. In addition, early responses of Mg deficiency were examined. In the third experiment, the source/sink ratio of plants was reduced by shading of 6 basal leaves to investigate if Mg deficiency-induced increase of sugar accumulation and decrease of photosynthesis in source leaves can be prevented.

6.1 Effect of nutrient deficiency on photosynthesis and carbohydrates

The physiological changes are the changes of nutrient concentrations and non-structural carbohydrates (NSC; sugars and starch) in different plant organs particularly leaves and their relationship with photosynthetic rate (A) and chlorophyll concentration in source leaves. The extent to which nutrient concentrations were decreased upon reduction of nutrient supply was nutrient-specific and independent on intensity of deficiency but dependent on plant organ in most cases. K and Mg concentration in different organs of K- and particularly Mg-deficient plants were severely reduced (Fig. 2-3 a, b), and this reduction was at in the same level in all organs. This indicates that growth effects were related to K and Mg deficiency at the cellular levels and lack of K and Mg for specific metabolic processes. The results from early response to Mg deficiency (chapter 5) showed that at 6 DAT, although Mg deficiency had no effect on plant growth (Table 5-1 A), Mg concentration was severely reduced to about the same level in all plant organs (Table 5-9). In contrast, N concentration in different organs of N-deficient plants was slightly reduced (Fig. 2-3 c). The lack of fast growth responses to K and Mg deficiency is in marked contrast to growth response to N deficiency. It had been reported in literature on fast growth responses of tomato to N deficiency (Chapin et al. 1988, Magana et al. 2009). This fast

effect of low nutrient supply on growth could be reversed by addition of CYT to the nutrient solution (Kuiper 1988, Kuiper et al. 1988) indicating that fast effects of low N supply on growth were triggered by hormonal effects and not by lack of N for cellular metabolism. The absence of an effect of intensity of nutrient deficiency on nutrient concentration within plants is attributed to direct effects of nutrient-deficiency on growth, whereby, severely nutrient-deficiency reduced growth more than moderate deficiency (Table 2-2). Positive correlations are often observed between nutrient concentration of leaves and A (Engels et al. 2012). In our results therefore, A in leaves of N-deficient plants were not affected by N deficiency, while, A in leaves of K-deficient plants was relatively lower, whereas, A was severely reduced in leaves of Mg-deficient plants (Table 3-1). In conclusion, nutrient deficiency-induced reduction in photosynthesis activity is dependent directly on nutrient concentration in source leaves. Severe reduction in K concentration possibly limits A in leaf due to large mesophyll resistance or lower capacity of CO₂-fixation cycle, rather than by stomatal effects (Zhao et al. 2001, Gerardeaux et al. 2009 and Jin et al. 2011). Reduction of A might be due to severe reduction in Mg concentration in leaf with the consequence of impaired whole photosynthetic electron transport chain (Yang et al. 2012, Tang et al. 2012). Complete withdrawal of N induced decrease in A, which was caused by large decrease in amount of Rubisco and its activity (Paul and Driscoll 1997).

The A may also be indirectly affected by nutrient supply via the effects of nutrient on source-sink relationships (Engels et al. 2012). NSC accumulation in source leaves due to imbalance between source and sink at whole plant level can lead to modified expression of photosynthetic genes (Paul and Foyer 2001). It is well documented in the previous studies that the reduction of photosynthesis under deficiency of K (Cakmak et al. 1994b and Kanai et al. 2007)), Mg (Cakmak et al. 1994b, Fischer et al. 1998, Hermans et al. 2005) and N (Scheible et al. 2004 and Remans et al. 2006) is associated with accumulation of NSC in source leaves. In our study, nutrient deficiency had no effect on total NSC in source leaves but had an opposite effect on leaf sugar and starch concentrations, whereby Mg deficiency increased sugar and reduced starch in source leaves. In contrast, N deficiency reduced sugar and increased starch in these leaves. It is probable that the sugar accumulation in Mg-deficient source leaves caused reduction in A in these leaves; because A was severely decreased in source leaf of Mg-deficient plants, but did not decrease in source leaf of N-deficient plants (Table 3-1). The intensity of deficiency had an effect on sugar and starch in leaves of Mg and N-deficient plants respectively, whereby severe Mg and N deficiency resulted in increased sugar and starch in source leaves of Mg and N-deficient plants respectively (Fig. 2-2 B. C). The relationship between leaf concentrations of sugar and chlorophyll and their effect on A was assessed. Mg deficiency

resulted in increased sugar concentrations and reduced A and reduced chlorophyll concentration in source leaves. Inhibition of photosynthetic activity probably resulted from direct effect of severe reduction of Mg concentration on chlorophyll or indirect effect of Mg by increase sugars in source leaves (Hermans et al. 2004). It is suggested that the reduction of chlorophyll is a response to sugar levels, rather than a lack of Mg atoms for chelating chlorophyll (Hermans and Verbruggen 2005).

The low photosynthetic efficiency of source leaves in Mg-deficient plants is often the result of feedback regulation (Engels et al. 2012) induced by a lower demand for assimilates at the sink organs (Fischer et al. 1998) or due to inhibition of phloem loading (Cakmak et al. 1994b). Second experiment (chapter 5) proved that the reduction of source to sink ratio through shading of basal leaves did not prevent Mg deficiency-induced sugar accumulation in young source leaves (Fig. 5-10). This finding is in agreement with Mg deficiency-induced accumulation of carbohydrate in source leaves due to inhibition of phloem loading (Cakmak et al. 1994b).

6.2 Effect of nutrient supply on biomass and nutrient allocation during plant development

At 20DAT, the deficiency-induced modification of biomass and nutrient allocation were nutrient specific and independent on the intensity of nutrient deficiency. Biomass allocation to roots increased in K and N-deficient plants at expense of reduced biomass allocation to stem of K-deficient plants and reduced biomass allocation to leaves of N-deficient plants (Fig. 2-4 a). K and N deficiency induce reduction of plant CYT levels (Römheld et al. 2008, Nam et al. 2012) which leads to enhanced growth of the root system (Werner et al. 2003 and Yang et al. 2004). However, in Mg-deficient plants high proportion of biomass was allocated to leaves and lower biomass was allocated to roots (Fig. 2-4 a). Also, it was suggested that short-term K deficiency had stimulating effect on the partitioning of biomass to roots and caused rapid growth of root system (Triboulot et al. 1997). Many previous studies reported that Mg deficiency increased biomass allocation to leaves (Cakmak et al. 1994 a, Sun and Payn 1999). Low biomass allocation to roots of Mg-deficient plants is caused by low root supply with sugars indicating that phloem loading from source leaves is impaired (Cakmak et al 1994b). It is well documented that reduced leaf growth is an early response to N deficiency which is due to its regulation by reduction in CYT and increase in ABA (Fetene and Beck 1993 and Wilkinson and Davies 2002). Thus, K and N-deficient plants follow the model of ‘functional equilibrium’. According to this model, plants respond to a decrease in above-ground resources with increased allocation

to shoots (leaves), whereas they respond to a decrease in below-ground resources with increased allocation to roots (Poorter and Nagel 2000).

In comparison to optimal supply, Mg allocation to stem increased at expense of reduced Mg allocation to leaves of Mg-deficient plants (Fig. 2-5 a and Table 2-3a). In contrast, K and N allocation to stem decreased while K and N and allocation to leaves increased in K and N-deficient plants (Fig. 2-5 a and Table 2-3a). This possibly occurred because Mg mobility in phloem was lower than K and N mobility.

Therefore, it was clear that under various nutrient deficiencies, plant response with regard to biomass allocation to different plant organs was not similar to plant response with regard to nutrient allocation. Possibly, three factors controlled biomass allocation: i) changes in hormonal status within plants, ii) changes in nutrient concentration in various plant organs and iii) changes of photosynthate transport (sugar supply).

At 20DAT, There was no significant difference between the modes of how deficiency was induced that is whether plants were completely deprived from nutrient or the nutrient was supplied by low rates, because our results agreed with previous physiological studies. Moreover, in the second experiment, plant response to Mg-deprivation was similar to plant response to severe Mg deficiency. This occurred probably due to high nutrient requirement in this period. It is expected that under nutrient deprivation, plants are not able to complete their life and withdrawing nutrient from soil solution does not occur in nature.

The relative sink strength of the organs for biomass and nutrients changed during plant development. These changes were most prominent for leaves and fruits. For instance, at optimal condition, after flowering biomass allocation to fruits increased at expense of reduced biomass to leaves (Table 2-3 b, c). This is consistent with other investigations in which the allocation of ^{14}C -labeled photosynthate to different plant organs was measured (Ho 1984, Hocking and Steer 1994). At reproductive growth phase, the rate of plant growth also was relatively lower as compared to vegetative growth phase. In addition, nutrient deficient plants still received low rate of nutrient supply. Therefore, biomass and nutrient allocation to generative organs increased also in nutrient-deficient plants (Fig. 2-4, 2-5 Table 2- 3b, c).

It is suggested that low photosynthate export via phloem is reversible when the plant roots are able to uptake the nutrient. Whereby, in Mg-deficient plants resupply by Mg enhanced sucrose export via phloem (Cakmak et al. 1994b). At regenerative growth phase, Mg concentration was up to critical level 2.2 to 2.7 mg g⁻¹ DM (Table 4-2). This explains why sugars and nutrients were exported to reproductive organs (fruits) and at 90 DAT biomass allocation to different plant organs was not affected by Mg deficiency.

6.3 Effect of nutrient deficiency on processes and compounds related to fruit quality

The results indicated that nutrient deficiency (K, Mg and N) limited growth of generative organs particularly in N-deficient plants (Fig. 2-4 c Table 2-3). Despite of K, Mg and N deficiency were associated with higher K, Mg and N allocation to fruits (Fig. 2-5 c), N and particularly K concentration in fruits were reduced (Fig. 4-2 a, b). Surprisingly, Mg concentration in fruits was very low and was not affected by Mg deficiency (Fig. 4-2 c). The edible part of tomato plants for human is the fruits, therefore, their contents of nutrients, health promoting substances and taste compounds are considered the important components, which determine fruit quality. In addition, fruit quality and its potential health benefits are directly related to its chemical composition (Fernandez-Ruiz et al. 2010). Some compounds in tomato fruits are affected by nutrient concentration in fruits. For instance, decreased K concentration led to reduced organic acid concentration. Therefore, ascorbic acid (AsA) was reduced in fruits of K-deficient plants (Fig. 4-3 b). A positive relationship between K and AsA concentration in tomato fruits was also found in other studies (Fanasca et al. 2006). Also, Mg concentration in fruit of Mg-deficient plants did not differ from Mg concentration in fruits of control plants. Therefore, these fruits contain low concentration of secondary compound particularly phenols (Fig. 4-3 a), which was in direct competition with protein according to the protein completion model (PCM). This model suggests that phenolic synthesis and protein synthesis are in direct competition for their common precursor, the aromatic amino acid phenylalanine (Jones and Hartley 1999).

Moderate K and Mg deficiency and N deficiency (whether moderate or severe) resulted in increased sugars in fruits. These results were in agreement with carbon nutrient balance hypothesis (CNB) which is applicable to environmental conditions with various availability of nutrient and light. The pattern of allocation to secondary metabolism depends on the relative availability of carbon and nutrients (Keskitalo 2003). For instance when a nutrient limits plant growth the CNB hypothesis predicts that carbohydrate will accumulate in plant tissues, which lead to increased synthesis of secondary metabolites such as phenols, terpenes and other compounds (Hamilton et al. 2001). It was mentioned above that N-deficient plants maintain photosynthetic activity more than moderately K and Mg-deficient plants at vegetative growth phase. Furthermore, their leaves had high concentration of storage form of carbohydrate (starch). It is assumed that these plants can convert starch to sucrose, which can be exported to fruits. This explains why fruits of N-deficient plants contain high concentration of sugars (Fig. 4-4 a). In K- and Mg-deficient plants, sugars were higher in fruits of moderately K- and Mg-deficient plants. This might have consequences on secondary compounds in fruits and enhanced

fruit quality according to CNB hypothesis. Therefore, fruits of N-deficient plants contain high concentration of ascorbic acid and increased percentage of radical scavenging activity (RSA %) (Fig. 4-3 b, c). Ascorbic acid is increased in fruits, which are exposed to high irradiation (Dumas et al. 2003). Therefore, there was possibly an indirect effect of N deficiency on AsA because N-deficient plants had lowest leaf area (Fig. 4-1 b).

Taste is measured by the sugar to titratable acidity ratio (sugar/ TA). TA was not affected by nutrient supply. Hence, in comparison to control, nutrient deficiency was associated with higher taste values in their fruits.

In addition, nutrient-deficient plants had high concentration of lycopene in their fruits whether this nutrient was K, Mg or N (Table 4-3). This is probably attributed to fruit size because lycopene concentration in peel is about five times higher than in pulp of fruits (Sharma and Le Maguer 1996 cited in Dumas et al. 2003). Mean fruit fresh weight in fruits of nutrient deficient plants was lower than those in fruits of nutrient sufficient plants (Table 4-1). Leaf area of nutrient-deficient plants was restricted, therefore, lycopene and carotene can be increased by illuminating tomato plants during the ripening of the fruit (Dumas et al 2003). Lycopene is the carotenoid that is most severely affected by exposure to intense solar radiation. It has been suggested that radiation injury to tomato fruit might be due to the general effects of overheating on irradiated tissues (Dumas et al 2003). This explains why lycopene concentration in fruit of K- and Mg-deficient plants are slightly higher than those in fruits of N-deficient plants.

6.4 Morphological changes (shoots and roots plasticity)

In this part, the focus is on changes on morphological shoot and root traits during vegetative growth phase. Relative growth rate was high, hence, plants required high amount of nutrients. When nutrient supply rate is reduced, plant growth responds to the reduction in nutrient supply by reduced shoot and root growth resulting in modification of shoot and root characteristics. Reduction of total plant growth induced by nutrient-deficiency was similar irrespective of nutrient (K, Mg or N) and dependent of the rate of nutrient supply (Table 2-2). It well documented that N deficiency-induced changes of shoot include restriction of leaf area and increased leaf thickness (Marschner 1995). Leaf growth often is regulated by phytohormons, which are affected by N deficiency (Kuiper et al. 1989). Effects of K and Mg deficiency on phytohormon are not clear. Therefore, our hypothesis that response of shoots morphology to nutrient deficiency is nutrient-specific.

Generally, according to balanced growth hypothesis, the plants are preferentially allocating biomass to the plant organ that is harvesting the resource limiting growth. This means

that biomass allocation to leaves is favoured if light becomes more limiting, and to roots is favoured if mineral nutrients become limiting to growth (Shipley and Meziane 2002).

This hypothesis is applicable in case of N deficiency, which was associated with lower leaf area (LA), and leaf area ratio (LAR) and higher specific leaf area (SLA). In contrast, Mg- and particularly K deficiency slightly reduced LA and increased SLA and LAR. In optimal condition, the photosynthetic organs (leaves) intercept aboveground resources such as light and CO₂. Therefore, expansion of leaf area increases acquisition of aboveground resources leading to increased relative growth rate. Despite of the effect of nutrient-deficiency on LA whether absolute or relative (LAR), relative growth rate was similar in K, Mg and N-deficient plants. This indicates that the leaf efficiency for acquisition of aboveground resources was higher in N-deficient plants as compared to K- and particularly Mg-deficient plants.

Roots also intercept belowground resources such as water and nutrients. Therefore, long roots increase acquisition of belowground resources (nutrients) leading to increase nutrient uptake. Roots have strategies to cope with limiting nutrients by enhancing root system either by increased biomass allocation to roots (high carbon cost) or increased root efficiency by having fine roots. Root length ratio (RLR_c) is root length per plant dry mass. Plants may produce longer roots, either by increasing biomass allocation to roots as demonstrated under N deficiency (Ryser and Lambers 1995, Sorgona et al. 2005) or by increasing specific root length (SRL) (Ryser 1998). RLR was not affected by N deficiency due to increased biomass allocation to roots. In contrast, RLR was not affected by moderate Mg deficiency due to increased specific root length (SRL). In comparison to optimal supply, K deficiency was associated with higher RLR because both biomass allocation to roots and SRL were increased (Table 3-3). It is assumed that plastic responses are adaptive; the responses may indicate successful strategies to cope with limitation of specific nutrients. However, it has to be noted that responses are not necessarily adaptive but may also be inevitable effects of limits on growth and physiology such as in case of severely Mg deficiency.

7. References

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Declaration of originality

I, Kassem Ahmed Said Mohammed, hereby declare that this dissertation entitled “Effect of nutrient limitation on physiological and morphological plant traits related to growth and quality of tomato” is my own original work and that all sources that were used have been properly acknowledged and referenced in the text. This work has not been submitted elsewhere in any form as part of another dissertation.

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Berlin, March 2013

Kassem A. S. Mohammed