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

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Abstract: 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. 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).
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.
Keywords: Phenotypic plasticity, shoot and root traits, tomato.

Introduction:

Plants can grow in a wide range of environments by adjusting their morphological and physiological traits to cope with different environmental conditions[1]. 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[2].

Plastic responses may be inevitable effects of environmental limits on growth and physiology[3]. 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. 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[2].

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[4]. 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[5]. Morphological root traits include root length, specific root length and whole-plant biomass to root length ratio[4, 6].

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[7]. N deficiency results in a decrease in aboveground biomass accumulation but it did not affect belowground biomass accumulation or root morphology[8]. N deficiency was associated with reduced leaf area (LA), leaf area ratio (LAR) and specific leaf area (SLA) in tobacco[9] and tomato[10]. In young seedlings of *Malushuphensis*, N deficiency reduced root number, root density and the root length of the lateral roots[11].

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[12,13]. K deficiency enhanced RMR at the expense of reduced stem mass ratio in tomato plants[14]. 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[15]. Root K absorption capacity and root length proliferation are dominant mechanisms in facilitating K acquisition efficiency in tomato plants[16]. K-deficient wheat plants acquired more K because these plants had high root length ratio (root length/plant biomass; RLR)[17]. Low K supply reduced the root growth, but moderate K deficiency increased the root length of the efficient rice genotypes[18]. However, in young seedlings of *Malushuphensis* plants, with shortage of K, the number, density and the length of the lateral root were decreased [11].

Mg deficiency resulted in reduction of RGR, LA and SLA but increased LMR[19]. High Mg supply caused reduction in specific root length (SRL) in root of Norway spruce plants[20]. Mg deficiency resulted in increased number, density and length of lateral roots of young seedlings of Malushuphensis[11]. Mg deficiency results in increased root length (RL) and root diameter (RD) and root surface area (RA) and biomass allocation to the roots[21].

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 study 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.

Material and Methods:

Plant culture and the experimental approach to grow plants at different rates of K, Mg and N supply. 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 Ca $(NO_3)_2$; 0.1 KH_2PO_4; 0.6 $MgSO_4$; 0.1 KCl; 0.1 FeEDTA; 0.01 H_3BO_3; $5x10^{-4}$ MnSO4* $4H_2O$; 1 x 10^{-4} $CuSO_4*5H_2O$; $3x10^{-4}$ $ZnSO_4*7H_2O$; $5x10^{-6}$ (NH_4) $6Mo_7O_{24}*H_2O$. For the treatments with medium and low N supply, Ca was supplied as $CaCl_2$ instead of Ca $(NO_3)_2$ to maintain the same Ca concentration. For the treatments with medium and low K supply, P was supplied as Ca $(H_2PO_4)_2$ 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. The amount of nutrients which were added to nutrient solution at medium and low rates of nutrient supplywere described in detail in (Table 1). For the assessment of plasticity of shoot and root traits, plants were harvested at 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.

Table 1: Total amount of nutrients were added to nutrient solution at medium and low rates of nutrient supply

Total amount of nutrients were added to nutrient solution during vegetative growth phase					
K (mg pot^{-1})		Mg (mg pot^{-1})		N (mg pot^{-1})	
Medium	**Low**	**Medium**	**Low**	**Medium**	**Low**
280	100	20	10	380	160

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 65^0C 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).

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.

Calculation of shoot and root traits

Mean leaf area (LA) [cm^2 $leaf^{-1}$]) = total LA [cm^2] / leaf number
Specific stem length (SSL) [cm g^{-1} stem dry mass] = stem length [cm] / stem dry mass [g]
Leaf mass ratio (LMR) [g leaf dry mass g^{-1} total plant dry mass] = leaf dry mass [g] / total plant dry mass [g]
Specific leaf area (SLA) [cm^2 g^{-1} leaf dry mass] = total LA [cm]/ leaf dry mass [g]
Leaf area ratio (LAR) [m^2 leaf area kg^{-1} plant dry mass] = total leaf area (m^2) / total plant dry mass [kg]
Net assimilation rate (NAR) [g m^{-2}LA d^{-1}] = total dry mass (g) / total LA (m) /day
Relative growth rate (RGR) [g kg^{-1} DM d^{-1}] = increment in mass (g)/ total dry mass (kg)/ day

Equations

$LAR = LMR \times SLA \times 10^{-1}$
Where:
LAR =leaf area ratio (m^2 kg^{-1} DM)
LMR= leaf mass ratio (g DM leaf g^{-1} DM plant)
SLA= specific leaf area (cm^2 g^{-1} DM leaf)
RGR= NAR x LAR. Where:
RGR =relative growth rate (g kg^{-1} DM day^{-1})
NAR = net assimilation rate (g m^{-2} leaf area day^{-1}).

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

Root surface area (RA) $RA = RL * 2\pi r$

Root volume (RV) RV= $RL\ \pi r^2$

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

RLR = RL /DM plant (m g^{-1})
RMR = DM root/ DM plant (g. g^{-1})
SRL = RL/ DM roots (m g^{-1})
F= RL / RV (m cm^{-3})
TD = DM root / RV (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 (31). As reported by (29), the following relationships obtain among the above parameters:

RLR = SRL x RMR SRL= F/ TD RLR = root length ratio (m g^{-1} DM plant).

Results:

Physiological changes precede morphological changes. The physiological changes result in biochemical changes and both influence plant morphology. In this study, 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). We assessed to in which extent, the morphological component and physiological component are responsible.

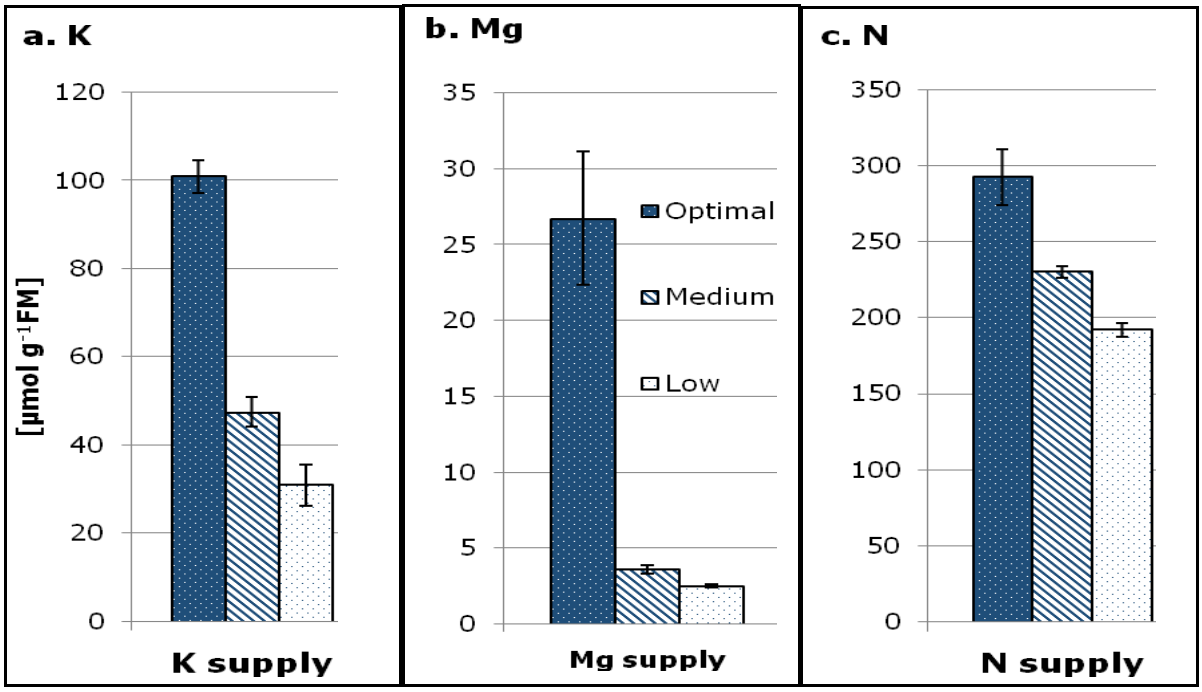

Fig.1. Effect of K, Mg and N supply on K (a), Mg (b) and N (c) concentration in source leaves.Vertical lines indicate standard errors of means (n=4)

K, Mg and N concentration in source leaves of K, Mg and N deficient plants.

Before describing the plasticity of shoot and roots, it should be recognize nutrient concentration in photosynthetic organ (leaves), which has direct effect on photosynthesis process and consequently net assimilation rate (NAR). Effect of nutrient limitation on nutrient concentration in photosynthetic organ (leaves) was nutrient specific. Where, medium and low K supply caused reduction in K concentration in source leaves (about 50% and 25% of optimal supply, respectively) (Fig.1), whereas, Mg deficiency resulted in sever reduction of Mg concentration in source leaves (about 10% of optimal concentration). In contrast K and Mg efficiency, N deficiency slightly reduced N concentration in source leaves.

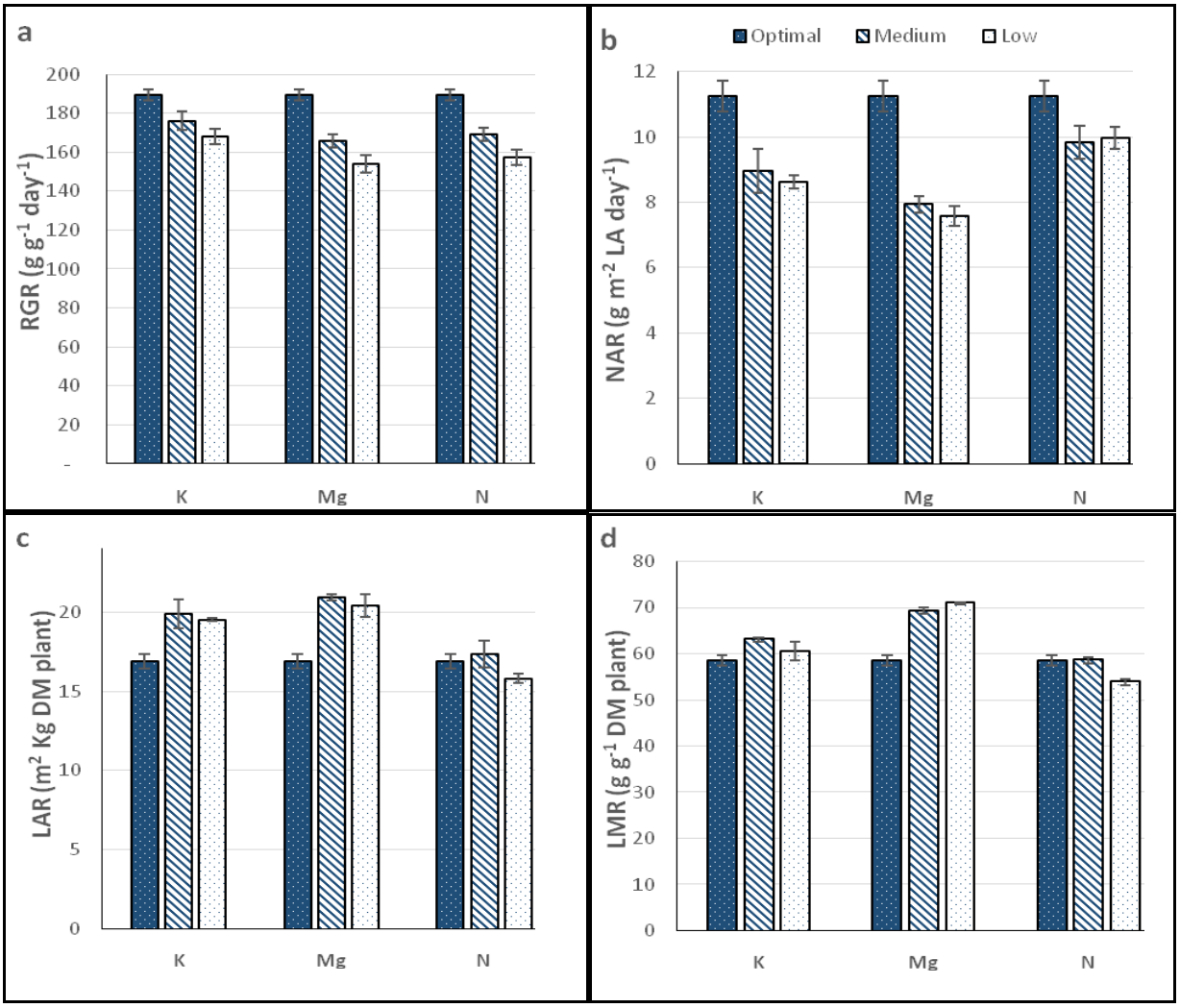

Fig. 2. Effect of K-, Mg- and N- deficiency on relative growth rate (RGR), leaf area ratio (LAR), leaf mass ratio (LMR) and net assimilation rate (NAR). Vertical lines indicate standard errors of means (n=4)

Effect of nutrient Limitation on RGR, LAR, LMR and NAR

Nutrient limitation in comparison to optimal nutrient supply was associated with a significant reduction of the RGR (Fig. 2a).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.

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 (Fig.2b). The effect of nutrient deficiency on NAR was strongly dependent on the specific nutrient. 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 days after start of treatment 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.

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 (Fig. 2c). In comparison to optimal nutrient supply, LAR was significantly increased by K and Mg limitation, whereas N limitation had no significant effect (Fig. 2c). LMR increased by Mg limitation to compensate severe reduction of NAR, whereas, K and Mg deficiency had no effect on LMR (Fig. 2d).

Plasticity of morphological shoot traits

Data in (Table 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). 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 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. In contrast to SSL, SLA was not significantly influenced by nutrient supply (Table 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.

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 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.

Table 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 be 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^2g^{-1} DM leaf)	231a	267a	269a	258a	241a	235a	217a

Plasticity of morphological root traits

Data in Table 3 show the effect of nutrient supply on root dry mass and root morphology. Root dry mass, root length (RL) and mean root diameters (RD) were directly measured. From these parameters, the other parameters were calculated. 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).

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). 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). 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). 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). 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). In comparison to plants grown under optimal supply, F was increased in Mg-deficient plants and slightly reduced in N-deficient plants (Table 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).

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). 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).

Table 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).

Paramters	Nutreint 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
Caluclated 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.

Discussion

In this study, plastic response of shoots and roots to nutrient limitation will be discussed. The effect of nutrient supply on leavesmassratio (LMR) androotmassratio was nutrient specific and independent on the intensity of nutrient limitation. Mgdeficiency 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?

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 [22]. 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 [23]. LAR is equal to the product of LMR and SLA, which both were increased by reducing K and Mg supply (Fig.2c, d and Table 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 2). 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). This was associated with maintenance of adequate N concentration in leaves (Fig.1), and consequently, maintenance of high net assimilation rate (Fig.2 b). 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. These alterations of leaf phytohormone levels, in turn, may decrease leaf extension via changes in cell wall extensibility [24].

Second, Mg-deficient plants did not much reduce leaf area growth (Table 2). This was possible, because in Mg-deficient plants, LMR was increased, and SLA was rather increased than decreased (Table 2). This morphological response was associated with a strong decrease of leaf Mg concentration (Fig.1b), and consequently with lower rates of net assimilation (Fig. 2b). 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[25].

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.

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 [26]. Change of roots morphology and root distribution patterns are important adaptive mechanisms to increase acquisition of nutrients from soil [26,27]. Important plant traits, which determine the acquisition capacity for below- ground resources, include high RMR, high F or low TD[28]. When plants are not able to increased 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[29]. F and TD can be combined to SRL. Thus, RLR is determined by different morphological components: RMR and SRL[28].

In the present work, it was found that K-deficient plants succeeded to allocate more biomass to leaves and roots (Fig2d andTable 3). 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). 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. 2d and Table 3). 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). 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 cannot 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). 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 allocated high proportion of biomass to the roots (Table 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 [29,31]. 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[28]. Mg –deficient plants behaved according to this strategy. In addition, RMR and SRL can be both increased when plants are growing in infertile soils [32]. 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[33,34,24]. Recently, CYT concentrations have been shown to decrease under K deficiency [35]. The reduction of CYT in root zone results in formation of a large root system[36].

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[37]. Sucrose is thought to promote cell differentiation and maturation, whereas hexoses favour cell division and expansion [25]. 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.

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