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Common bean (*Phaseolus vulgaris* L.) gas exchange capacity under nutrient deficiency

Kapacitet izmjene plinova graha (*Phaseolus vulgaris* L.) u uvjetima nedostatka hranjiva

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ABSTRACT

The lack of plant nutrients is a major problem for agriculture. Because of their essential role in the most important metabolic processes of plants, their deficiency depresses photosynthesis and disrupts the efficient operation of the photosynthetic apparatus. In this study, we investigated the gas exchange of common beans bean under the N, P, K, Mg, and Fe deficiency. The experiment was set up as a hydroponic, fully aerated, floating system in 6 hydroponic tubs. Each hydroponic tub contained 10 plants and was filled with a modified Hoagland nutrient solution. One group of plants was grown in a complete nutrient solution (control), while the other treatments lacked one of the following nutrients: N, P, K, Mg and Fe. During the experiment, gas exchange parameters: net photosynthetic rate (A), transpiration rate (E), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were measured. At the end of the experiment, the nutrient content of the plant tissue was determined. Nutrient deficiency significantly affected all measured photosynthetic parameters and visual symptoms indicated that a lack of different nutrients affected the photosynthetic machinery at different points. Potassium deficiency lower stomatal conductance and increased mesophyll resistance to CO_2 diffusion and/or RubisCO activity. Nitrogen and Mg deficiency affected chlorophyll synthesis and accelerated leaf senescence. Phosphorus deficiency caused less damage to gas exchange parameters probably due to protective mechanisms of reduced leaf area. Seed Fe content was surplus to sustain photosynthetic machinery during the early developmental phase.

Keywords: transpiration, net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration

SAŽETAK

Nedostatak biljnih hranjiva je veliki problem za poljoprivredu. Zbog njihove bitne uloge u ključnim metaboličkim procesima biljaka, nedostatak hranjiva smanjuje fotosintezu i remeti učinkoviti rad fotosintetskog sustava. U ovome radu smo mjerili izmjenu plinova kod graha u uvjetima nedostatka N, P, K, Mg i Fe. Pokus je bio postavljen kao hidropon, plutajući sistem u 6 hidroponskih kada. Svaka hidroponska kada sadržavala je 10 biljaka, te je bila ispunjena modificiranom Hoaglandovom hranjivom otopinom. Jedna grupa biljaka je uzgajana u kompletnoj hranjivoj otopini (kontrola), dok je kod drugih tretmana nedostajalo neko određeno hranjivo: N, P, K, Mg ili Fe. Tijekom pokusa mjereni su parametri izmjene

plinova: stopa fotosinteze (A), stopa transpiracije (E), provodljivost puči za H_2O (g_s), te intercelularna koncentracija CO_2 (C_i). Pri završetku pokusa određen je sadržaj hranjiva u biljnome materijalu. Nedostatak hranjiva statistički je značajno utjecao na sve izmjerene fotosintetske parametre, a vizualni simptomi ukazuju da nedostatak različitih hranjiva različito utječe na fotosinetski sustav. Nedostatak K smanjuje provodljivost puči i povećava otpor mezofila za difuziju CO_2 i/ili RubisCO aktivnost. Nedostatak N i Mg je utjecao na sintezu klorofila i ubrzavao starenje listova. Nedostatak P manje je naštetio parametrima izmjene plinova vjerojatno zbog zaštitnog mehanizma smanjene površine lista. Sadržaj Fe u sjemenu bio je dovoljan za održavanje fotosintetskog sustava tijekom rane faze razvoja.

Ključne riječi: transpiracija, stopa fotosinteze, provodljivost puči, intercelularna koncentracija CO_2

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a protein-rich grain legume important for human consumption (Broughton et al., 2003), and is cultivated worldwide, with even greater importance in developing countries. Increasing agricultural demand combined with a growing population will require a doubling of crop yields by 2050 (Valin et al., 2014). Understanding photosynthetic responses can lead to increased crop productivity to meet growing agricultural demands (Niinemets et al., 2017; Kubis and Bar-Even, 2019). Photosynthesis is a highly complex and crucial process for all life in the biosphere and is under the strong influence of light, temperature, water, and nutrients (Thornley, 2002). Previous studies on common beans have shown that increased photosynthetic rate and stomatal conductance can lead to higher yields (Ribeiro et al., 2004; Wentworth et al., 2006).

Aside from carbon, oxygen, and hydrogen, all vascular plants require an additional 13 elements to complete their life cycle: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), chlorine (Cl), boron (B), and molybdenum (Mo). Macronutrients content (N, P, K, Ca, Mg and S) in the plant is higher than 0.05% in DM and micronutrients (Fe, Mn, Zn, B, Cu, and Cl) content in the plant is lower than 0.05% in DM (Marschner, 1986). Deficiency of plant nutrients causes metabolic defects leading to stunting or deformation of roots, stems, or leaves, chlorosis, or necrosis of various organs and even death of the plant. Although all essential nutrients play an irreplaceable role in plant metabolism lack of some nutrients has a more profound effect on the photosynthetic machinery. Of the most important macro elements, photosynthesis is most affected by the lack of N, P, K and Mg, while among the microelements, the lack of Fe is most severe (Marschner, 1986; Kalaji et al., 2018)).

Nitrogen (N) serves as a constituent of many plant cell components, including amino acids and nucleic acids. It is also important in the biochemistry of coenzymes, photosynthetic pigments and polyamines. Nitrates play an important role in stomatal movement. Stomatal conductance (g_s) increases with increasing N supply by regulating turgor and hormone synthesis (Broadley et al., 2001). Nitrogen deficiency promotes the biosynthesis of abscisic acid (ABA) which is thought to be responsible for stomatal closure (Mu and Chen, 2021). Besides stomatal factors, nitrogen deficiency also affects the non-stomatal factors of photosynthesis. The non-stomatal factors mainly include the content of photosystem I (PSI), photosystem II (PSII) and light harvesting complexes (LHCs), cytochrome b6f complex (Cyt b6f), adenosine triphosphate (ATP) synthase and photosynthetic enzymes (Dwyer et al., 2012), which reduces light absorption and conversion and increases non-photochemical quenching. Von Caemmerer and Farquhar (1981) concluded that nitrogen deficiency in *Phaseolus* sp. reduces both RubisCO activity and the electron transport chain to the same relative extent *in vitro*.

Potassium (K) is the most abundant cation in plants (up to 6% of plant dry weight) and is involved in several physiological functions related to plant growth and development (Pettigrew, 2008). The osmotic function of K in guard cell regulation is well known, so it is not surprising that stomatal conductance often decreases under K-deficient conditions (Zhao et al., 2001; Jákli et

al., 2017). Regardless of the importance of K^+ in regulating osmotic potential in guard cells, changes in stomatal conductance do not primarily limit photosynthesis, even when total K concentration in the leaf is below critical levels (Zhao et al., 2001; Jin et al., 2011). Potassium deficiency reduces the carboxylation activity of RubisCO in chloroplasts. This has been proven *in vitro* (Hu et al., 2015; Zahoor et al., 2017) and by estimates of the maximum RubisCO carboxylation velocity (Vc_{max}), from combined measurements of leaf gas exchange and chlorophyll fluorescence *in vivo* (Jin et al., 2011; Erel et al., 2015; Jákli et al., 2017). Occasionally, K deficiency is claimed to inhibit RubisCO activation (Oosterhuis et al., 2013), resulting in decreased rates of ribulose-1,5-biphosphate (RuBP) carboxylation. In addition to the amount of RubisCO, K supply may also indirectly affect RubisCO activity. Due to reduced CO_2 diffusion in K-deficient mesophyll tissue at low chloroplast CO_2 concentrations down-regulation of RubisCO activity may occur (Jákli et al., 2017)

Phosphorus (P) is an essential element in compounds such as ATP, nicotinamide adenine dinucleotide phosphate (NADPH), nucleic acids, sugar phosphates and phospholipids, which are all important for photosynthesis (Hammond and White, 2007). Studies of the long-term limitations of Pi on photosynthesis and carbon partitioning have shown that inhibition of photosynthesis is largely due to limitations of the photosynthetic carbon reduction (PCR) cycle on RuBP regeneration (Rychter and Rao, 2005). Pieters et al. (2001) have shown that in Pi deficiency, the low sucrose synthesis rates are caused by the low requirements for sinks that limit Pi recycling to chloroplasts and restrict photosynthesis. Long-term insufficient Pi supply reduces photosynthetic rates by limiting the ability to regenerate RuBP, although reduced activation of RubisCO may also play an important role (Jacob and Lawlor, 1992).

Magnesium (Mg) is a key component of the chlorophyll molecule, and its deficiency is associated with leaf yellowing in the form of interveinal chlorosis in older leaves (Cakmak and Yazici, 2010). The occurrence of these symptoms is explained by the involvement of magnesium in the synthesis of chlorophyll. Magnesium is crucial for CO_2 fixation as it is directly involved in the activation and activity of RubisCO. In addition, Mg is critical for protein synthesis, which affects RubisCO protein levels. Magnesium is very important for the export of carbohydrates from source to sink (Cakmak et al., 1994). Sugar accumulation in leaves is an early symptom of Mg deficiency (Cakmak et al., 1994). Sugar accumulation in leaves can suppress the expression of genes encoding photosynthetic enzymes, resulting in a decrease in chlorophyll content and photosynthetic capacity (Sheen, 1994).

Almost all of the iron in a plant is located in the chloroplasts. The remainder of a plant's iron is distributed in the cytoplasm and other organelles that contain additional heme and/or iron-sulphur proteins (Miller et al., 1995). Iron deficiency leads to the decline of many photosynthetic components, such as iron-containing proteins involved in essential oxidoreductive pathways of chloroplasts (Tognetti et al., 2007). A specific symptom of iron deficiency is chlorosis, which is attributed to the inhibition of chlorophyll synthesis (Reinbothe et al., 2006). Therefore, chloroplasts are targets of iron deficiency, which leads to a decrease in photosynthetic activity as well as pigments and proteins (Winder and Nishio, 1995). Both the utilisation of RuBP by RubisCO and its regeneration by the Calvin cycle appear to be impaired in iron-deficient plants (Winder and Nishio, 1995).

Due to the essential role of nutrients in regulating photosynthesis and because of the importance of photosynthesis in yield formation, this study aims to compare the effects of N, P, K, Mg and Fe deficiency on gas exchange parameters of common beans.

MATERIALS AND METHODS

Experimental design

The experiment was conducted at the University of Zagreb Faculty of Agriculture, Croatia. Seeds of *Phaseolus vulgaris* 'Ferguson' belonging to 'Trešnjevac' type, were obtained from the Department of Seed Science and Technology. In order to obtain sufficient plant material

for the experiment, seeds were planted in germination trays (containers). Germination and the initial growth phase were carried out in a growth chamber at 25/22 °C, a photoperiod of 16/8 h per day/night, 60% relative humidity, and a photosynthetic photon flux density (PPFD) of 250 µmol/m^2 per s provided by Valoya L35, NS12 spectrum LED (Valoya Oy, Helsinki Finland).

After 10 days, 60 uniformly developed seedlings were selected for the experiment. The experiment was set up as a hydroponic, fully aerated, floating system in 6 hydroponic tubs with a volume of 45 L. Each hydroponic tub contained 10 plants and was filled with a ½ strength modified Hoagland nutrient solution (Hoagland and Arnon, 1950). One group of plants was grown in a complete nutrient solution (control), while others lacked one of the following nutrients: N, P, K, Mg or Fe. The final concentration of the nutrients in nutrient solutions is shown in Table 1. The solutions were replaced every 3 days.

Measurement of physiological traits

Leaf gas exchange parameters, net photosynthetic rate (A), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were measured using a LI-6800 Portable Photosynthesis System (LI-COR Biosciences, Lincoln, NE, United States). Plants were grown in a nutrient solution for 10 days prior to measurement, allowing characteristic deficiency symptoms to develop in all treatment solutions. Measurements were made on fully developed leaves with a 2 x 3 cm clamp-on leaf cuvette at the 1100 µmol/m^2 per s light intensity, 400 µmol/mol per CO_2 concentration, a temperature of 25 °C, and a 60% relative humidity and the flow rate through the chamber was regulated to 600 µmol/s. The measurements were taken after the stability criteria were met. Stability criteria were set as the standard deviation limit of ΔH_2O mmol/mol per min for 20 seconds less than 0.1 and the standard deviation limit of ΔCO_2 µmol/mol per min for 20 seconds less than 0.1.

Table 1. Composition of a modified Hoagland nutrient solution for treatments

Nutrient concentration (mg/l)	Treatment solution					
	Control	-N	-P	-K	-Mg	-Fe
N	98	/	98	105	98	95
P	15.5	15.5	/	15.5	15.5	15.5
K	97.75	97.75	97.75	/	97.75	97.75
Ca	100.25	100.25	100.25	100.25	100.25	100.25
Mg	24.3	24.3	24.3	24.3	/	24.3
S	64.24	64.24	72.24	32.24	32.24	64.24
Fe	13.96	13.96	13.96	13.96	13.96	
Cl	/	177.25	/	/	/	/
B	0.5	0.5	0.5	0.5	0.5	0.5
Zn	0.05	0.05	0.05	0.05	0.05	0.05
Cu	0.02	0.02	0.02	0.02	0.02	0.02
Mn	0.36	0.36	0.36	0.36	0.36	0.36
Mo	0.01	0.01	0.01	0.01	0.01	0.01

Analysis of nutrient content in plant material

At the end of the experiment, shoots of the experimental plants were harvested and dried at 70 °C to a constant weight (VENTI-Line 180 Prime) and grounded. To obtain sufficient plant material for mineral content analysis, three composite subsamples were created from each treatment. To determine seed nutrient content, seed samples were dried and grounded using a laboratory mill. Nitrogen content was determined by the Kjeldahl method (AOAC, 2015). After digestion with concentrated HNO_3 and $HClO_4$ (MILESTONE 1200 Mega Microwave Digester), phosphorus content was determined using a spectrophotometer (EVOLUTION 60S UV-VISIBLE), potassium with a flame photometer (JANWEY PFP 7), while calcium, magnesium, iron, zinc, manganese and copper were analysed with an atomic absorption spectrophotometer (AAS SOLAR THERMO SCIENTIFIC) (AOAC, 2015).

Statistical analysis

Data were analysed using the general linear model in the statistical package JMP® PRO 16 (SAS Institute Inc., Cary, NC). One-way analysis of variance (ANOVA) was used to compare the gas exchange parameters (A, E, g_s, and C_i) among the different nutrient deficiency treatments. The nutrient deficiency treatments were used as fixed effects and the plants were treated as pseudo replicates and were set as random effects. In the case of a significant F test (P<0.05) mean values were compared by Tukey's HSD (Honestly Significant Difference) test.

RESULTS AND DISCUSSION

Effect of nutrient deficiency on the common bean mineral nutrition

According to (Bergmann, 1992) appropriate ranges for minerals in dry matter of common bean are N (3.0 – 6.0%), P (0.25 – 0.50%), K (2.0 – 3.0%), Ca (0.50 – 2.0%), Mg (0.25 - 0.70%), Cu (7 - 15 mg/kg), Mn (40 – 100 mg/kg), and Zn (30 - 70 mg/kg). Our results were within these optimal nutrient ranges (except for nutrients that were absent in certain treatments) (Table 2). The results of the chemical analysis of plant shoots were in accordance with the applied treatments, which was proven by lower dry matter concentrations of N, P, K and Mg. The sufficiency range for Fe in leaf tissue for most plants is from 50 to 100 mg/kg (ppm) of dry matter; the so-called critical concentration is 50 mg/kg (Marschner, 1986; Jones Jr., 2016). Compared to these levels, Fe concentration in the dry matter of the plants was higher, except for the -Fe treatment, which was close to the threshold levels. This indicates that the Fe content in the seed is surplus to satisfy common bean needs during early developmental phase. Due to the large genetic diversity of 'Trešnjevac', the mineral content of the seeds varies. It is common for Andean landraces, such as ´Trešnjevac´, to have seeds with higher nitrogen, iron, and potassium content (Palčić et al., 2018). Our results are consistent with this and are similar to the results of other authors who had analyzed 'Trešnjevac' (Moraghan and Grafton, 2001; Mario et al., 2009)

Effect of nutrient deficiency on gas exchange parameters

The lack of K, N and Mg caused the greatest decrease in stomatal conductance (g_s) compared to the control. In the case of K deficiency, this decrease was 96%, in the case of N 91% and in the case of Mg 86% (Figure 1a). C_i values were lowest in the Mg, K, and N-deficient treatments compared to the control. There were no significant differences between these treatments. The average reduction of intercellular CO_2 concentration (C_i) in the Mg deficiency treatment was 38%, in the K deficiency treatment 36% and in the N deficiency treatment 32% (Figure 1b). The treatments with K, N and Mg deficiency had a significant effect on the reduction of transpiration rate (E). Compared to the control, K deficiency caused a 96% decrease in E, N deficiency 90% and Mg deficiency 85% (Figure 1c). The lowest photosynthetic rate (A) was found in the -K and -N treatments. Potassium deficiency caused a 91% decrease in A, and N deficiency caused an 89% decrease compared to the control (Figure 1d). For all parameters measured, there were no significant differences between

Table 2. The concentration of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) in seed samples and shoot dry matter of common bean plants grown in complete nutrient solution (control) and in nutrient solutions in which nitrogen (-N), phosphorus (-P), potassium (-K), magnesium (-Mg) and iron (-Fe) were omitted

Treatments and seed	Mineral nutrient content in dry weight of common bean shoots								
	N	P	K	Ca	Mg	Fe	Zn	Mn	Cu
	%					mg/kg			
Control	5.27	1.04	3.78	1.50	0.23	214.26	43.49	166.18	7.61
-N	2.46	1.63	4.91	3.15	0.19	141.73	37.31	101.98	9.26
-P	4.80	0.09	2.19	0.89	0.20	141.16	19.24	56.84	10.04
-K	5.01	1.55	0.62	2.08	0.42	161.80	26.45	114.56	11.20
-Mg	4.16	0.86	3.14	1.33	0.04	321.18	25.07	177.98	6.54
-Fe	5.05	0.89	4.39	1.27	0.29	54.71	51.57	339.55	12.17
	*Seed samples								
	3.98	0.30	1.47	0.04	0.11	88.42	28.32	11.55	7.67

* The average seed samples used in the experiment, seed samples were collected before the experiment to determine its nutrient content

the control and the -Fe treatment. The average values of stomatal conductance (g_s) were much higher (P<0.0001) for plants in the -Fe and control treatments than for those in other treatments. In addition, plants from -P had higher g_s compared to -Mg, -N, -K treatments (Figure 1a). The values of intercellular CO_2 concentration (C_i) were lowest (P<0.0001) in -N, -K and -Mg treatments and were not significantly different among these treatments (Figure 1b). The average transpiration rate (E) was significantly higher (P<0.0001) in control and -Fe plants than in plants from treatments -N, -P, -Mg and -K. Plants from -P treatment had higher E compared to -N, -K, and -Mg (Figure 1c). The average values of net photosynthetic rate (A) were highest (P<0.0001) in control plants; however, it was not significantly higher compared to -Fe plants. On the other hand, the lowest A was found in the -N and -K treatments (Figure 1d).

Although, the individual effects of nutrient deficiencies on the process of photosynthesis are well studied, direct comparisons of the effects of several key nutrients on the gas exchange are less common. Together with water, light and soil, plant nutrition is one of the most important factors in supporting plant performance and increasing yields. Adequate nutrition can lead to an increase in photosynthesis, which can ultimately translate into higher biomass and yield (Ribeiro et al., 2004; Wentworth et al., 2006). Plants grown in treatment solutions showed impaired gas exchange parameters. Namely the average values of A, E, g_s and C_i were considerably lower in plants from treatments -N, -P, -K, -Mg compared to plants from treatment -Fe and the control (Figure 1a-1d). The lowest values of all measured parameters were found in the -N, -Mg and -K treatments. In the vacuole, K is mainly involved regulating osmotic potential and turgor, which regulates stomatal opening (Talbott and Zeiger, 1996; Kang et al., 2007; Ni, 2012).

Our results show that K deficient plants had low E and A which was caused by low g_s. However, reduced stomatal conductance cannot solely explain reduced A in K deficit plants. Because results show that C_i was less affected in these plants, other factors such as mesophyll resistance to CO_2 diffusion (Zhao et al., 2001) and RubisCO activity may also play a role in the decrease in A. Although there is no evidence that K is involved in RubisCO activation, it is possible that this element can alter RubisCO activity by affecting the cell's pH optimum for its activation and the amount of substrate for its reaction (CO_2 and possibly RuBP) (Tränkner et al., 2018). Magnesium and N

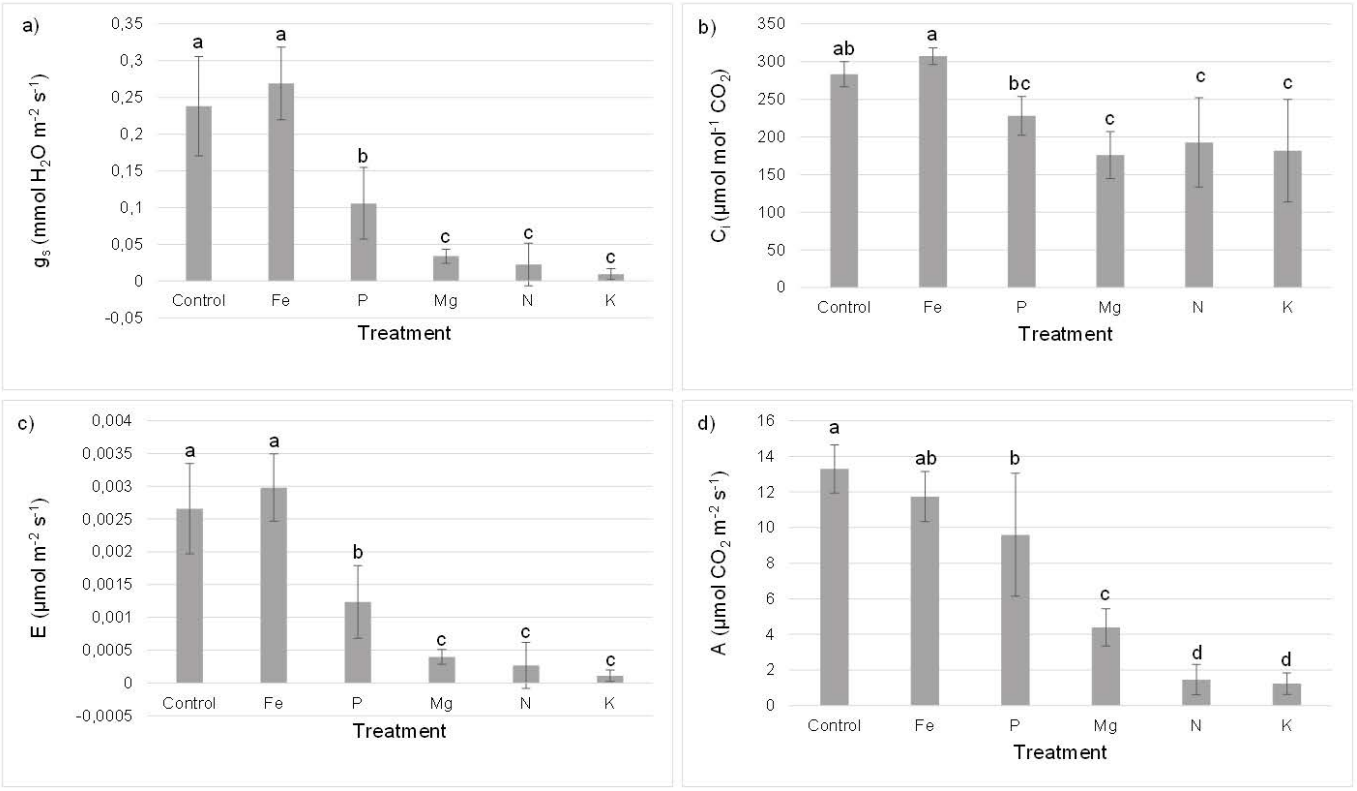

Figure 1. Average values and standard deviation of stomatal conductance (g_s) (a), intercellular CO_2 concentration (C_i) (b), transpiration rate (E) (c), and net photosynthetic rate (A) (d), in common bean grown for 10 days in ½ complete Hoagland's nutrient solution (control) and in nutrient solutions in which nitrogen (-N), phosphorus (-P), potassium (-K), magnesium (-Mg) and iron (-Fe) were omitted. Different letters indicate significant differences (P<0.05) between treatments based on Tukey's HSD test

deficiency had similar negative effects on all gas exchange parameters, probably due to similar negative effects on chloroplasts. Crafts-Brandner et al. (1998) shoved that N deficiency leads to a decrease in proteins located in chloroplasts, peroxisomes, and cytosol which increases leaf senescence and decreases leaf photosynthetic capacity. During the leaf senescence RubisCO is the first enzyme degraded in the Calvin cycle (Deng et al., 2001). Therefore, disturbance of the carbohydrate balance between source and sink organs may accelerate leaf senescence in Mg deficit plants and similarly affect RubisCO (Walker and Weinstein, 1991).

Phosphorus deficiency also affected photosynthetic parameters, but not as drastically as Mg, N, and K deficiency. Although leaf area was not presented in this paper, we have noticed that P-deficient plants had the smallest leaf area compared with all other plants in the experiment. The earliest and most often described symptom of P deficiency is the reduction of shoot growth and leaf area (Rychter and Rao, 2005). This seems to be a plant strategy to cope with P starvation and represent plants trade off to protect its significant metabolic processes. In addition, P-deficient plants have a lower ability to convert light into chemical energy, slower electron transport, and lower ATP and NADPH synthesis rate, which all together result in a lower RuBP carboxylation rate (Jacob and Lawlor, 1993).

In addition, P is known to affect the activation of numerous enzymes involved in the Calvin cycle that participate in CO_2 fixation. The -Fe treatment had no significant effect on the measured gas exchange parameters compared to the control, which is probably because the Fe concentration was near the critical threshold values. Moreover, all measurements were conducted at the visual detection phase of nutrient deficiency on older, fully developed leaves, whereas deficiency symptoms (chlorosis) were visible on younger leaves. In addition, nutrient content was analysed on

the whole shoot basis, so it is possible that older leaves on which measurements were performed contained sufficient iron to support photosynthesis.

CONCLUSIONS

The nutrient solutions correspond to the nutrient deficiencies of each treatment, except for the -Fe treatment, where the plants were near threshold values. This indicates that the common bean iron supply is adequate to support plant growth and gas exchange during the early developmental phase. Although the nutrient deficiency treatments caused similar reductions in gas exchange parameters, measured parameters and visual symptoms indicate that deficiencies of different nutrients affect the photosynthetic machinery at different points. The greatest reduction in all measured traits was found in plants deficient in K, N and Mg. The reduction in photosynthetic rates in K-deficient plants was not only related to the reduction in stomatal conductance but probably also to higher resistance of the mesophyll to CO_2 diffusion and a possible reduction in RubisCO activity. The reduction in gas exchange parameters in Mg- and N- deficient plants was probably related to reduced pigment synthesis and increased senescence-induced RubisCO degradation. Although gas exchange parameters were significantly reduced in the P-deficient plants compared to the control, the P-deficient plants had higher gas exchange parameters compared to the K-, N-, and Mg-deficient plants, but had smaller leaves.

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