PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 10(1): 140–145
https://doi.org/10.14719/pst.1916

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RESEARCH ARTICLE

Photosynthetic parameters change in *Lycopersicon esculentum* leaves under nutrient deficiencies

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OPEN ACCESS

ARTICLE HISTORY

Received: 27 May 2022
Accepted: 27 September 2022

Available online
Version 1.0 : 05 November 2022
Version 2.0 : 01 January 2023

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS etc.
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Alsharafa K Y. Photosynthetic parameters change in *Lycopersicon esculentum* leaves under nutrient deficiencies. Plant Science Today. 2023; 10(1): 140–145. https://doi.org/10.14719/pst.1916

Abstract

Lycopersicon esculentum leaves cultivated hydroponically for 24 and 48 hrs with various specific mineral deficits had their photosynthetic characteristics examined. After 24 hrs of K^+, NO_3^-, and PO_4^{2-} deficiency, a substantial induction of net photosynthetic rate was observed. The net photosynthetic rate of SO_4^{2-}, Mg^{2+}, Fe^{2+}, NO_3^-, Ca^{2+} and PO_4^{2-} deficits was significantly induced by the 48 hr exposure. After 24 hrs of deficiencies in SO_4^{2-}, Mg^{2+}, Fe^{2+}, NO_3^-, Ca^{2+} and PO_4^{2-}, stomata conductance was dramatically increased. Deficiencies in SO_4^{2-}, Fe^{2+}, NO_3^-, Ca^{2+} and PO_4^{2-} were continuously induced over 48 hrs. After 24 hrs of SO_4^{2-}, Fe^{2+}, NO_3^-, Ca^{2+} and PO_4^{2-} deficiencies, intercellular CO_2 concentration shows a considerable induction. After 48 hrs of K^+, SO_4^{2-}, Mg^{2+} and NO_3^- deficits, this behavior remained strongly induced. Water use efficiency considerably decreased in response to these changes after 24 hrs of SO_4^{2-}, Fe^{2+}, NO_3^- and PO_4^{2-} deficiencies and this effect continued after 48 hrs of Mg^{2+}, NO_3^-, Ca^{2+} and PO_4^{2-} deficiencies. Deficits in K^+, SO_4^{2-}, Mg^{2+}, Fe^{2+}, NO_3^-, Ca^{2+} and PO_4^{2-} for 24 hrs dramatically increased transpiration rate, which was modified by those deficiencies. A 48 hr exposure to NO_3^-, Ca^{2+} and PO_4^{2-} deficiency dramatically increased the transpiration rate. After 48 hrs, an SO_4^{2-} deficit drastically decreased the transpiration rate. The findings indicate that after a short term of exposure, it may be possible to diagnose a specific mineral shortage and determine which mineral influenced the parameters of photosynthesis in such a way that the selected parameters responded in a manner that was consistent with the duration of exposure.

Keywords

Abiotic stresses, acclimation, duration of exposure, mineral deficiency, photosynthetic parameters

Introduction

Plant nutrition, whether in excess or deficiency, has a significant impact on plant growth and productivity. As a result, mineral nutrient researchconcentrated on the symptoms, signals, and target molecular levels of regulation as responses to changes in soil nutrient content. The findings mainly suggested that changes in nutrient concentrations will have a significant impact on plant production and subsequently how this will affect their agricultural application, which was described as a generalized dose-response curve (1, 2).

Furthermore, the importance of minerals in biochemical and physiological functions demonstrates the severity of their limitation (3). Photosynthetic carbon assimilation is an aspect of photosynthesis that is regulated

by abiotic factors such as the availability of minerals. In response to macro- or microelement deficit, investigations are on the physiological status of the photosynthetic machinery such as decreased PSII photochemical efficiency and limitation of the activity of PSI depending on parameters derived from chlorophyll fluorescence in tomato and maize leaves (1). Early studies have been conducted to record how nutritional limitations alter photosynthetic characteristics in plant leaves (4 - 6). Through a variety of mechanisms, such as leaf gas exchange, chlorophyll fluorescence and photosynthetic machinery components such as chlorophyll contents, PSII photochemistry, PSI content and Rubisco carboxylation activity (2, 6 - 10). Light-independent photosynthetic processes are the most affected physiological process when plant circumant mineral availability varies. Further research into plant leaf gas exchange studies to measure the condition of photosynthesis in response to nutritional limitations is still needed. As part of a continuing series of investigations into the impact of mineral shortages on photosynthetic regulation in *L. esculentum* leaves, this study looks at how gas exchange changes over time in response to specific nutritional deficits.

Materials and Methods

Plant material and growth conditions

In 9 cm Petri plates with 2 layers of filter paper and 6 ml of sterile, distilled water, tomato seeds (*L. esculentum* Mill.) were put. The dishes were then incubated at 25 °C in the dark. After that, the seedlings were moved to a soil mixture of peat moss, perlite and vermiculite at a ratio of 2:1:1 and allowed to grow for two weeks under controlled monitored conditions at growth chamber (14 hrs of light with 80 µ mol quanta m^{-2} s^{-1} at 21 °C and then 10 hrs of darkness at 20 °C and ~55% relative humidity).

Plantlets that were 14 days old were transplanted into 100 ml jars with the following nutritional solution: KNO_3, 10 mM and $Ca(NO_3)_2{\cdot}4H_2O$, 0.5 mM $CuSO_4{\cdot}5H_2O$, 2 µM $ZnSO_4{\cdot}7H_2O$, 0.115 mM H_3BO_3, and 0.1 mM $H_2MoO_4{\cdot}2H_2O$. 5 mM $MgSO_4{\cdot}7H_2O$, 2.5 mM KH_2PO_4, 0.05 mM KCl, and 05.0 mM $MnSO_4$, 0.05 mM Fe–Na_2EDTA (1, 8). The implementation of a specific mineral nutrient shortage followed standard instructions (1). The deficiency of a particular mineral nutrient was created and the replacement with equivalent moles is shown in Table 1. For each deficiency of a specific mineral nutrient treatment, photosynthetic measurements were performed on 8 fully developed plant leaf samples after 24 and 48 hrs of the stress application.

Photosynthesis measurements

A CIRAS-3 portable photosynthesis device (PP Systems, Amsbury, MA, USA) was used to evaluate the net photosynthetic rate (*A*), stomatal conductance (*Gs*), intercellular CO_2 concentration (*Ci*), photosynthetic water use efficiency (*WUE*), and transpiration rate (*E*). The CIRAS-3 automatically recorded data every 5 s. On the CIRAS-3, an automatic control device was used to maintain the CO_2 concentration (380 µ mol mol^{-1}), relative humidity (60%), and leaf temperature (28 °C) under a photon flux density of 100 µ mol m^{-2} s^{-1}.

Statistical analysis

For all experiments, samples were analyzed and all the assays were carried out in 3 independent experiments (n=4). The results were expressed as mean± SD. One-way ANOVA, followed by Tukey's honest significance test, was carried out at a 95% confidence level ($p \leq 0.05$) to compare means of parameters and interactions that were statistically different. Statistical analysis of the data was carried out using GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA).

Results

The net photosynthetic rate (µmol CO^2 m^2 s^{-1})

The net photosynthetic rate (*A*) of *L. esculentum* Mill leaves was affected in different manners by the nutritional deficiencies that resulted from the treatments employed in this study (Fig. 1). The net photosynthetic rate was significantly increased in tomato leaves grown in cultures deficient in SO_4^{2-}, Fe^{2+}, NO_3^- and Ca^{2+} as the duration of specific deficiency increased (48 hr) by 1.3-, 1.3-, 1.5-, and 1.4- fold respectively. Tomato leaves grown in cultures deficient in K^+ were significantly induced 1.3- fold early (24 hr). Tomato plants grown in cultures deficient in PO_4^{2-} showed a higher significantly induced net photosynthetic rate of 1.5-fold (24 hr) and 1.4- fold (48 hr). Tomato plants grown in cultures deficient in Mg^{2+} showed the lowest significantly reduced net photosynthetic rate 0.8- fold (24 hr) compared with control.

Table 1. Treatment of specific mineral deficiencies and substitution of one salt with another, each with the final concentration specified in the materials and methods

Deficient nutrient	Replacement	Purpose of replacement
Complete medium	All minerals are	Presents
- Mg/ $MgSO_4.7H_2O$	$NaSO_4$	To keep the supply of sulfur
- Ca/$Ca(NO_3)_2.4H_2O$	$NaNO_3$	To keep the supply of nitrogen
- Fe/ Fe–Na_2EDTA	--------	--------
- P/ KH_2PO_4	KCl	To keep the supply of potassium
- K/ KH_2PO_4	$NaNO_3$	To keep the osmoticum
- N/ KNO_3 and $Ca(NO_3)_2.4H_2O$	KCl and $CaCl_2$	To keep the supply of potassium and calcium
- S/ $MgSO_4.7H_2O$	$MgCl_2$	To keep the supply of magnesium

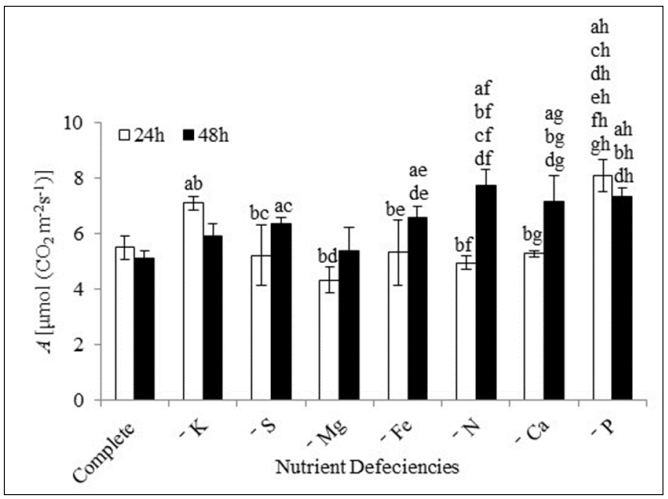

Fig. 1. Net photosynthetic rate (***A***) of leaves of *L. esculentum* Mill. leaves subjected to specific mineral deficiencies compared with that of plants grown in complete nutrient solution. Data represent mean values ± SD. Different letters denote statistically different means (Tukey's test; $P \leq 0.05$). Bars bearing different letters indicate significance difference.

Photosynthetic water use efficiency (mmol CO²/ mol H₂O)

The photosynthesis/transpiration relation of *L. esculentum* Mill plants during different nutritional disorders presented in Fig. 2. Photosynthetic Water Use Efficiency (WUE) was significantly induced in tomato leaves grown in cultures deficient in Mg^{2+} and Ca^{2+} as the duration of specific deficiency increased (48 hr) by 1.5- and 1.2- fold respectively compared with control. Tomato plants grown in cultures deficient in NO_3^- showed the lowest significantly reduced WUE to 0.3- fold (24 hr) and 0.4- fold (48 hr) compared with control.

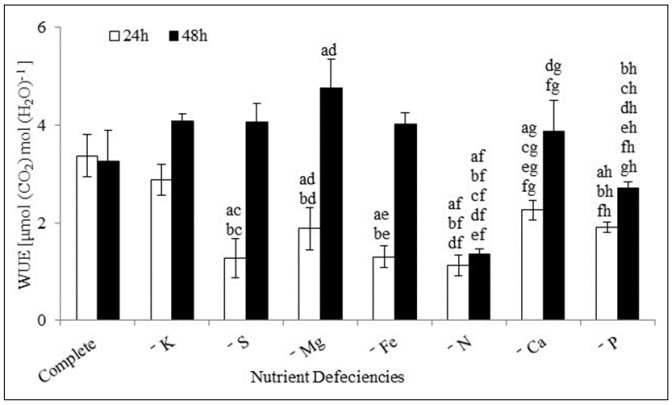

Fig. 2. Water use efficiency (WUE) of *L. esculentum* Mill. leaves subjected to specific mineral deficiencies compared with that of plants grown in complete nutrient solution. Data represent mean values ± SD. Different letters denote statistically different means (Tukey's test; $P \leq 0.05$). Bars bearing different letters indicate significance difference.

Stomatal conductance (mol CO² m² s⁻¹)

In terms of stomatal conductance (*Gs*) tomato leaves showed a specific sensitivity toward mineral deficiency similar to that of the net photosynthetic rate behavior (Fig. 3). Tomato plants grown in cultures deficient in NO_3^- had intrinsically more open stomata than other minerals-deficient plants, in respect to time of exposure, which induced 3.6- fold and 4.9- fold after 24 hr and 48 hr respectively, compared to the control. While cultures deficient in PO_4^{2-} showed a lower than NO_3^- effect that significantly induced stomatal conductance reached 2.2- fold and 1.5- fold after 24 hr and 48 hr respectively. Ca^{2+} deficient tomato plants exhibit slight increases reaching 1.3- fold and 1.2- fold after 24 hr and 48 hr respectively. Tomato leaves grown in cultures deficient in SO_4^{2-} and Fe^{2+} induced stomatal conductance as a specific duration of exposure (24 hr) that increased by 2.9- and 3- fold respectively.

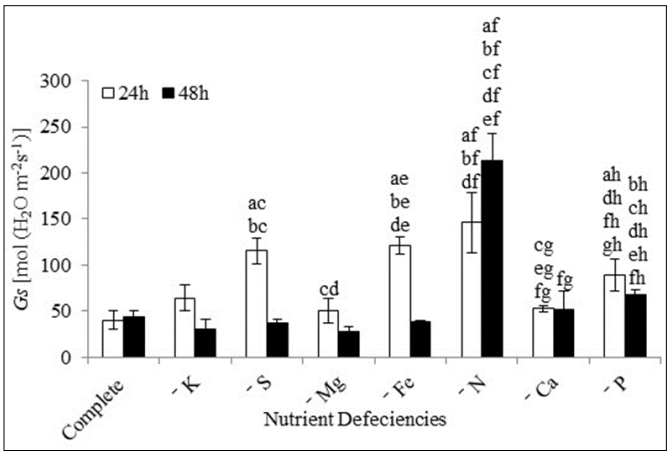

Fig. 3. Stomatal conductance (*Gs*) of *L. esculentum* Mill. leaves subjected to specific mineral deficiencies compared with that of plants grown in complete nutrient solution. Data represent mean values ± SD. Different letters denote statistically different means (Tukey's test; $P \leq 0.05$). Bars bearing different letters indicate significance difference.

Transpiration Rate (mmol H₂O m² s⁻¹)

Interestingly, the transpiration rate improved significantly in plants grown in cultures deficient in K^+, SO_4^{2-}, Mg^{2+}, Fe^{2+}, NO_3^-, Ca^{2+} and PO_4^{2-} reached 2- fold, 3.3- fold, 1.6- fold, 3.2- fold, 3.5- fold, 1.5- fold and 3.5- fold after 24 hr of treatment respectively. Then transpiration rate dramatically decreased in plants deficient in K^+, SO_4^{2-}, $Mg^{2+}$and Fe^{2+} after 48 hr of treatment. Plants were grown in cultures deficient in NO_3^-, Ca^{2+} and PO_4^{2-} they showed significant improvement reached 3.8-, 1.3- and 1.7- fold after 48 hr of treatment respectively compared to the control as shown in Fig. 4.

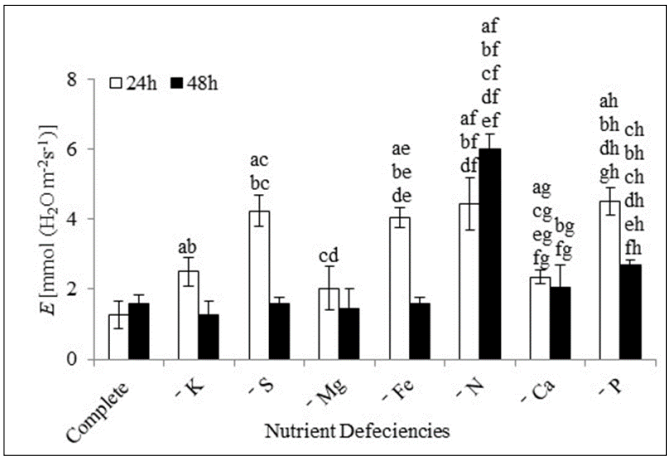

Fig. 4. Transpiration rate (*E*) of *L. esculentum* Mill. leaves subjected to specific mineral deficiencies compared with that of plants grown in complete nutrient solution. Data represent mean values ± SD. Different letters denote statistically different means (Tukey's test; $P \leq 0.05$). Bars bearing different letters indicate significance difference.

Internal CO² concentration (µmol mol⁻¹)

Intercellular CO_2 concentration (*Ci*) improved significantly

in plants grown in cultures deficient in K^+, SO_4^{2-}, Mg^{2+}, Fe^{2+}, NO_3^-, Ca^{2+} and PO_4^{2-} reached 1.3- fold, 1.5- fold, 1.3- fold, 1.6- fold, 1.6- fold, 1.2- fold and 1.3- fold after 24 hr of treatment respectively. Then transpiration rate dramatically decreased significantly in plants deficient in K^+, SO_4^{2-}, Mg^{2+} reached to 0.5-foldd for Fe^{2+} and Ca^{2+} reached 0.7- fold and 0.8- fold after 48 hr of treatment respectively compared to the control as shown in Fig. 5.

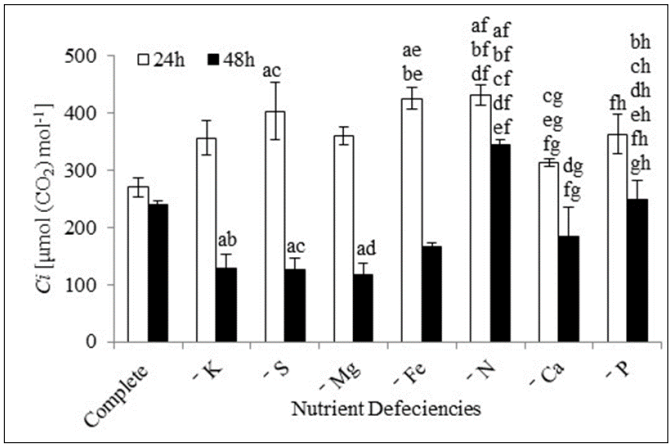

Fig. 5. Internal CO_2 Concentration (*Ci*) of *L. esculentum* Mill. leaves subjected to specific mineral deficiencies compared with that of plants grown in complete nutrient solution. Data represent mean values ± SD. Different letters denote statistically different means (Tukey's test; $P \leq 0.05$). Bars bearing different letters indicate significance difference.

Discussion

The metabolome and enzymatic regulation that indicate plant plasticity to deal with the specific variations in plant tissue mineral content are among the levels of responses that are involved in plants' acclimation to mineral shortages (8, 9). Additionally, depending on the type, duration, and severity of the abiotic stress, different mechanisms, such as rapid and long-term acclimation, express the sensitivity through photosynthetic performance in plants (2, 11-15).

In vivo gas exchange analysis is the main tool in diagnosing the photosynthesis performance of plants. Long-term N-deficiency treatment had wide effects on plant and leaves performance (16 - 18). Leaf senescence is accelerated by N-deficiency through protein degradation and photosynthetic pigments contents reduction (16, 19). Short-time exposure for N-deficiency did not exhibit leaf senescence characteristics of *L. esculentum* Mill. (8, 9). Moreover, leaf gas exchanges still exhibit significant improvement in most parameters while there was a lower reduction of WUE which may to the reduction of root surface area with diverse N--deficiency levels. For that, if the photosynthesis was affected by N- limitation is due to reduced carboxylation efficiency rather than to stomatal limitation (20 - 23).

A vital nutrient for photosynthetic metabolism is potassium. On photosynthetic characteristics, the K-deficiency stress time extension (days) had a negative impact (24 - 26). Although the physiological and biochemical functions of K^+ in plants have been established, the researchers reached the conclusion that K deficiency has a negative effect on stomatal function. However, neither the early (24 hr) net photosynthetic rate nor the transpiration rate decreased in this study. Furthermore, I draw the conclusion that stomatal conductance was not one of the elements limiting CO_2 absorption. Therefore, even with the *Ci* reduced, I propose that the K^+ internal homeostasis may control the mechanisms for carboxylation efficiency either by reducing rubisco activity or by impairing photosynthesis's photochemistry (24, 26).

Sulfur plays a key orchestral role in photosynthesis by regulating the production of antioxidants, the photosynthetic mechanism, and stomatal conductance (27). Long-term sulfur restriction causes a reduction in photosynthesis (28, 29). Photosystems were more negatively impacted by Fe deficiency (30). Most leaf gas exchange parameters significantly improved after a short-term exposure for S-deficient and Fe-deficient plants, however, there was a less dramatic decrease in WUE (24 hr). Even though *A* was unaffected by extending the exposure time, the other parameters were significantly reduced after 48 hrs, suggesting that S- and Fe-deficient plants behave similarly in terms of how they affect photosynthetic parameters, possibly because both are necessary components of iron-sulfur complexes in other proteins and the photosynthetic apparatus in the chloroplast (2, 30, 31).

Magnesium is vital in various physiological and biochemical plant processes as part of structures or enzymes activators. Mg-deficiency induced alterations of photosynthesis parameters that are not associated with stomatal conductance since the parameters changes do not flow in the same relationship as the negative relationship between *A* and C_i as shown in the results. These findings might be explained by Mg roles as part of Chl structures or photosynthesis and respiration enzymes activators as found previously (32 - 36).

Calcium plays many different roles in plants; enzyme activities, a second messenger, leaf senescence, cell membrane, tolerance to stress and photosynthesis (37, 38). Long-term Ca deficiency lowers photosynthetic performance due to lower stomatal conductance (18). In this study, tomato leaves photosynthetic performance was inducing after short-term Ca deficiency, which could be the alleviation of calcium deficiency symptoms increased with fractioning of calcium (39).

Phosphorus (P) is a key component of macromolecules, plays critical roles in the metabolism of macromolecules, signal transduction, photosynthesis and has a pivotal impact on stress resistance. Prolonged P deficiency lowers photosynthetic performance (40). Short-time exposure to P deficiency exhibits significant improvement in most parameters of photosynthesis. There are reports on the lowers photosynthetic performance of P-deficient plants to lower stomatal conductance (18). The P deficiency induction of these parameters in this study might indicate that the P deficiency exhibit calcium deficiency effects that increase with fractioning of phosphorus (41).

Conclusion

During the analysis of photosynthesis, the selected parameters showed specific responses to the selected mineral. In addition, the response was developed according to the duration of exposure. As a result, it was possible to diagnose a specific mineral deficit instantly and determine which mineral had an impact on the parameters of photosynthesis. Short-term exposure to a specific mineral deficit influenced the selected parameters of photosynthesis that responded in a manner consistent with the duration of exposure. Furthermore, the study of the photosynthesis parameters changes to specific mineral deficiency needs to be investigated with different plant species and genotypes to use it as an instant diagnosis tool.

Acknowledgements

The author is grateful to Ayah Al-maaitah for preparing the mineral hydroponics.

Authors contributions

KA designed the project, performed the research, supervised and discussed the results and wrote the article.

Compliance with ethical standards

Conflict of interest: Author does not have any conflict of interests to declare.

Ethical issues: None.

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