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Research article

Identification of nutrient deficiency in maize and tomato plants by *in vivo* chlorophyll *a* fluorescence measurements

Hazem M. Kalaji [a], Abdallah Oukarroum [b], Vladimir Alexandrov [c], Margarita Kouzmanova [c], Marian Brestic [d], Marek Zivcak [d], Izabela A. Samborska [a], Magdalena D. Cetner [a], Suleyman I. Allakhverdiev [e,f,*], Vasilij Goltsev [c,**]

[a] Department of Plant Physiology, Warsaw University of Life Sciences SGGW, Nowoursynowska 159, 02-776 Warsaw, Poland
[b] Department of Chemistry and Biochemistry, University of Québec in Montréal, Montréal, Quebec, C.P. 8888, Succ. Centre-Ville, H3C 3P8 Canada
[c] Department of Biophysics and Radiobiology, University of Sofia, 8 Dragan Tzankov Blvd., 1164 Sofia, Bulgaria
[d] Department of Plant Physiology, Slovak Agricultural University, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic
[e] Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya Street 35, Moscow 127276, Russia
[f] Institute of Basic Biological Problems, Russian Academy of Sciences, Pushchino, Moscow Region 142290, Russia

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ABSTRACT

The impact of some macro (Ca, S, Mg, K, N, P) and micro (Fe) nutrients deficiency on the functioning of the photosynthetic machinery in tomato (*Solanum lycopersicum* L.) and maize (*Zea mays* L.) plants grown in hydroponic cultures were investigated. Plants grown on a complete nutrient solution (control) were compared with those grown in a medium, which lacked one of macro- or microelements. The physiological state of the photosynthetic machinery *in vivo* was analysed after 14-days of deficient condition by the parameters of JIP-test based on fast chlorophyll *a* fluorescence records. In most of the nutrient-deficient samples, the decrease of photochemical efficiency, increase in non-photochemical dissipation and decrease of the number of active photosystem II (PSII) reaction centres were observed. However, lack of individual nutrients also had nutrient-specific effects on the photochemical processes. In Mg and Ca-deficient plants, the most severe decrease in electron donation by oxygen evolving complex (OEC) was indicated. Sulphur deficiency caused limitation of electron transport beyond PSI, probably due to decrease in the PSI content or activity of PSI electron acceptors; in contrary, Ca deficiency had an opposite effect, where the PSII activity was affected much more than PSI. Despite the fact that clear differences in nutrient deficiency responses between tomato and maize plants were observed, our results indicate that some of presented fluorescence parameters could be used as fluorescence phenotype markers. The principal component analysis of selected JIP-test parameters was presented as a possible species-specific approach to identify/predict the nutrient deficiency using the fast chlorophyll fluorescence records.

1. Introduction

Over the last century, agricultural production has steadily increased, mainly due to improved nutrient availability (Ludwig et al., 2011). Macro- and microelements such as Ca, S, Mg, K, N, P and Fe have so far been recognized as essential for plants. Plants cannot complete their life cycles and accomplish their physiological functions in the absence of these nutrients. Their deficiencies reduce growth and yield of crops (Osman, 2013). Plant growth in relation to the concentration of an essential nutrient element is described by the "generalized dose–response curve" (Berry and Wallace, 1981). There is a nutrient-concentration window where plant growth is optimal. Concentrations below this optimal range are considered sub-optimal, consequently plant growth is reduced.

Photosynthetic carbon assimilation is the key process of plant metabolism, strongly influenced by environmental conditions. Photosynthesis consists of two main parts: the photochemical processes running at the level of thylakoid membranes producing NADPH and ATP, as well as CO_2 reduction pathways (mainly Calvin cycle) using ATP and NADPH for CO_2 assimilation. The photochemical processes are driven by protein complexes embedded in the thylakoid membranes of chloroplasts (PSII, the cytochrome b_6/f

* Corresponding author. Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya Street 35, Moscow 127276, Russia.
** Corresponding author.
E-mail addresses: suleyman.allakhverdiev@gmail.com, sallakhverdiev@yandex.ru (S.I. Allakhverdiev), goltsev@biofac.uni-sofia.bg (V. Goltsev).

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Abbreviations[1]

Chl	chlorophyll
CS	cross section
ET	electron transport
FNR	ferredoxin-$NADP^+$ oxidoreductase
LED	light-emitting diode
M-PEA	Multi-Function Plant Efficiency Analyser, Hansatech, UK
ND	nutrient deficiency/deficient
OEC	oxygen evolving complex
PC	principal component
PCA	principal component analysis
PI	performance index
PF	prompt chlorophyll fluorescence
PSI, PSII	Photosystem I, II
RC	reaction centre
RE	reduction of PSI end electron acceptors

complex, and PSI) linked in series through the photosynthetic electron transport chain. Incident light energy is captured by the light-harvesting complex of photosystems. The energy is transferred to the central chlorophyll molecule of the reaction centre (RC), ensuring a charge separation across the membrane and splitting water into molecular oxygen, protons, and electrons on the donor side of PSII. The electrons are moved from PSII to the plastoquinone pool (Q_A, Q_B), *Cyt* b_6/f, plastocyanin, and PSI where a second charge separation occurs, followed by reducing PSI electron acceptor ferredoxin that subsequently reduces $NADP^+$ to NADPH. The reactions of electron transport are coupled to proton pumping through the thylakoid membrane producing pH gradient that drives synthesis of ATP by ATP synthase (Rochaix, 2011).

Lack of main nutrients specifically affect photosynthetic functions at different levels, including PSII photochemistry. Nutrient deficiency directly influences the photosynthetic apparatus, mainly through biosynthesis and functioning of key photosynthetic components. Direct effects on synthesis of protein complexes involved in photosynthetic reactions were documented mostly for nitrogen, sulphur and iron deficiencies (Abadía, 1992; Ciompi et al., 1996; D'Hooghe et al., 2013). The chlorophyll synthesis was directly affected under deficit of nitrogen, magnesium and iron (Abadía, 1992; Ciompi et al., 1996; Laing et al., 2000). Calcium is necessary for the membrane stability and together with potassium, they play a central role in the maintenance of osmotic homaeostasis and cell signalling, associated with stress tolerance and proper photosynthetic functions (Brand and Becker, 1984; Qu et al., 2012).

In addition to direct effects on photosynthetic structures, a feedback effect caused by a low sink demand in conditions of nutrient deficiency can play a very important role. Generally, mineral deficiency leads to decrease in growth and accumulation of biomass, which is associated with down-regulation of photosynthesis due to lower demand for assimilates. Thus, a lower CO_2 assimilation under conditions of nutrient deficiency may lead to greater excess of excitation energy that may lead to over-reduction of photosynthetic electron transport chain (Evans and Terashima, 1987). To maintain high efficiency of photosynthetic energy conversion, the photochemical structures in the chloroplast are adjusted so that the photosynthetic electron and proton transport related to the production of ATP and NADPH can be in equilibrium with decreased requirement of energy for carbon assimilation (Lu et al., 2001). The low sink strength was shown to be the primary limitation on photosynthesis in phosphate deficiency (Pieters et al., 2001), but it may importantly contribute to photosynthetic limitation caused by deficit of any other nutrient.

Recently, in addition to costly biochemical analyses and slowly gas exchange records, the parameters based on optical measurements of chlorophyll content have been used as a measure of status of the photosynthetic apparatus (Richardson et al., 2002). However, they do not express fully the photosynthetic structure and contain almost no direct information on the photosynthetic activity. On the other hand, the chlorophyll fluorescence techniques were shown to be reliable, non-invasive, powerful and simple tools for assessment of photosynthetic electron transport and related photosynthetic processes (Kalaji et al., 2012). The most common are the fast measurements of F_v/F_m parameter (i.e. the maximum quantum yield of photochemistry), but this parameter was shown to be non-specific (Baker, 2008) and often insensitive (Živčák et al., 2008). Much more useful and also broadly accepted are parameters obtained by the saturation pulse method in light adapted leaves; the measurements are, however, time consuming, more suitable for purposes of basic research than for practical applications in field conditions (Brestic and Zivcak, 2013). To assess quickly the photosynthetic function in a high number of field grown plants, the non-destructive analysis of polyphasic fast chlorophyll transient was developed (Strasser and Strasser, 1995; Strasser et al., 2004). This method is based on high-frequency record of chlorophyll fluorescence emitted by dark adapted leaf during short (usually one second lasting) pulse of strong actinic light by fluorimeter. The fluorescence kinetics reflects the photochemical efficiency of the photosynthetic apparatus and it provides valuable information on the functional and structural attributes of components involved in photosynthetic electron transport, mainly photosystem II (Stirbet and Govindjee, 2011). The fluorescence rise during the first second of illumination shows a sequence of phases (labelled as O, K, J, I, P) from the initial (F_o) to the maximal (F_m) fluorescence value. The mathematical model of the polyphasic transient was developed and named as JIP-test (Strasser and Strasser, 1995). It enables calculation of specific biophysical parameters, quantum yields and probabilities characterizing structure and function of PSII.

Numerous studies have demonstrated the ability of the JIP method to uncover changes in PSII photochemistry caused by environmental or genetic factors, e.g. effects of stresses, mutations, etc. (Kalaji et al., 2011; Brestic et al., 2012, 2014). There are some examples of application of fast chlorophyll fluorescence kinetics in nutrition-deficiency studies (Lu et al., 2001; Hermans et al., 2001), however, a complex study comparing deficiency of the main nutrients is still lacking.

Therefore, the main aim of this study was a detailed *in vivo* analysis and comparison of nutrient deficiency-induced changes in PSII photochemistry in two different plant species by means of parameters derived from the fast chlorophyll fluorescence records. We could assume that the specific physiological effects of deficiencies of individual nutrients would be accompanied by different effects on photochemical processes. Moreover, we also tested whether the chlorophyll fluorescence data could be used to distinguish type of nutrient deficiency by using principal component analysis.

2. Materials and methods

2.1. Plants, growth conditions, and experimental design

Maize (*Zea mays* L.), cultivar "Marignan" and tomato (*Solanum lycopersicum* L.), cultivar "Maeva F1" plants were grown in a

[1]See Table 3 for other symbols representing chlorophyll fluorescence parameters.