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DEFICIENCY OF SOME NUTRIENT ELEMENTS IN BEAN AND MAIZE PLANTS ANALYZED BY LUMINESCENT METHOD

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Abstract

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A deficiency of any essential macro (N, P, S, Ca, Mg, K) and micro (Zn, Cu, B, Mo, Cl, Mn and Fe) elements has a major influence on the development of plants. Deficits of some elements result in external features in plants. These physical marks often overlap with each other or are similar to those obtained as a result of an infection, and it is why they cannot serve as a sign for accurate diagnosis. The determination of lacking elements requires analysis of the soil or plant tissue content, or a combined analysis. Plants react to shortage of the nutrient components and, therefore, their functional analysis is preferable to soil analysis for monitoring of the nutrient deficiency. In this study the mineral deficiency in nutrient solution was evaluated by the stress response of the plants estimated by leaves photosynthetic activity. Bean *(Phaseolus vulgaris*) and maize (*Zea mays*) plants were grown hydroponically in the Hoagland nutrient medium – full or lacking K, Ca or Fe. All plants were grown in a full Hoagland nutrient solution for 10 days, and then the experimental plants were transferred to modified solution. The bean plants were decapitated after 7 days of growth in the unmodified medium. The photosynthetic activity was estimated by analysis of the chlorophyll fluorescence using JIP-test approach that reflects functional activity of Photosystems I and II and of electron transfer chain between them, as well as the physiological state of the photosynthetic apparatus as whole. The comparison of *Phaseolus vulgaris* and *Zea mays* showed different impact of each deficiency on the photosynthetic machinery of the two species. The high sensitivity of plants and specificity of primary stress reactions of the photosynthesis to mineral deficiencies outline good perspectives for fluorescent analysis application in agricultural industry. This approach is fast and cheap, and can be implemented *in vivo* and *in situ* measuring conditions.

Key words: nutrient deficiency, JIP test, plant stress, chlorophyll *a* fluorescence
Abbreviations: PSI – Photosystems I; PSII – Photosystems II; PF – prompt chlorophyll a fluorescence; MR_{820} – modulated reflection at 820 nm; ETR – electron transport rate; Q_A – quinon A; Q_B – quinon B; PQ – plastoquinone; PC – plastocyanin; Chl – chlorophyll; RC – reaction center; OEC – oxygen evolving complex; RE – electron acceptors; P700 – the first electron donor in PS I reaction center; All other abbreviations are given in Table 3.

Introduction

In addition to carbon, oxygen and hydrogen, another 13 elements are essential for all vascular plants: N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Cl, B and Mo. The iron and the elements Mn, Zn, Cu, Cl, B and Mo are required in very small amounts (<100 mg/kg dry matter) and are called trace elements or micronutrients. Other elements (N, P, K, Ca, Mg, S) are required in larger quantities (>1000 mg/kg dry matter) and are called macronutrients. Plants cannot complete their

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life cycles and accomplish normal physiological functions in the absence of the elements that are considered to be essential (Osman, 2013).

A deficiency of some of these elements causes metabolic defects leading to stunting or deformity of roots, stems, or leaves, chlorosis or necrosis of various organs and even death of the plant. Soil pH also affects the availability of mineral nutrients. All of these elements are available for plants in the pH range of 5.5–6.5 (Lucas and Davis, 1961).

In higher plants the deficiencies of some nutrient minerals depress photosynthesis and disrupts efficient operation of photosynthetic apparatus, cause a reduction of photochemical efficiency of PSII and changes in values of chlorophyll fluorescence (Bottrill et al., 1970).

In this work, we evaluated the influence of deficiency of K, Ca and Fe on photosynthetic machinery of higher plants.

The first observable symptom of *potassium* deficiency is marginal chlorosis, which then develops into necrosis primarily at the leaf tips, then at the margins, and between veins. The younger leaves are most sensitive and these symptoms are initially appeared there. The stems in these plants may be weak (Taiz and Zeiger, 2010). The K ions play a very important role in cells osmoregulation and in pH gradient formation in the thylakoid membrane. In deficiency of this element conditions stomatal resistance increases and carbon dioxide diffusion becomes difficult. Potassium activates more than 50 enzymes and it is important for RuBisCO functioning in Calvin-Benson cycle. The potassium ions are not involved directly in photosynthetic metabolism, but they are required in relatively high concentrations for other biophysical and biochemical processes, which affect photosynthesis. The deficiency of K^+ induces a reduction of the PS II electron transport efficiencies at dark and light adapted states (Tuffers et al., 2001). It affects chlorophyll antennae complexes, decrease the PSII cooperativity and results in inhibition of PSII reaction centers (Qu et al., 2012).

There are several defects that can be associated with low levels of *calcium*: poor root development, leaf necrosis and curling. Young leaves can be deformed. Calcium is very important to plants metabolism. It regulates plant structure and functions (Hepler, 2005). Ca ions (Ca^{2+}) play an important role in membrane stabilization, in the regulation of enzyme synthesis (protein kinases or phosphatases), in the synthesis of new cell walls. They are an activator of several enzymes: phospholipase, arginine kinase, adenosine triphosphatase, adenyl kinase, and amylase **(**Osman, 2013)**.** Calcium is involved directly in several aspects of photosynthetic process. Calcium realizes very important function in the water-splitting complex, because Ca is involves in Mn_4CaO_5-cluster. In the last years have been found, that Ca ion plays a functional role in binding substrate water molecules, rather than a structural role. (Koua et al., 2013)

Iron (Fe) could be placed between the macro- and micronutrients. This element has major physiological functions. The deficiency of Fe causes chlorosis, usually first in younger leaves, due to a reduced amount of chlorophyll. It is also observed, that young leaves in plants with deficiency of iron are smaller than normal (Osman, 2013). The leaf photosynthesis is highly sensitive to lack of the iron. It is very important for the process of chlorophyll synthesis. The iron participates in Fe–S proteins and heme proteins ferredoxin and cytochromes in photosynthetic electron transport chain (Yruela, 2013). Ferredoxin is involved in the formation of NADPH (Osman, 2013). The iron is represented in PSII in the cytochrome b559 and like non-heme Fe in the PS II acceptor side and in the stromal part of core proteins between quinones Q_A and Q_B (Yruela, 2013).

The loss of PSII activity in leaves of plants with iron limitation could be due to the loss of D1 protein, 33 kDa, 28/25 kDa, and 23 kDa polypeptides (Bertamini et al., 2002).

In recent years, the chlorophyll fluorescence techniques appear to be more sensitive than other techniques for the study of plant physiology under stress conditions (Kaladji et al., 2012). Few papers have shown that chlorophyll fluorescence is a good indicator for nutrient deficiency (Bürling et al., 2011; Bélanger et al., 2006; Adams et al., 1993).

In this study, we investigated influence of K, Ca and Fe deficiency on the photosynthetic machinery in leaves of bean and maize plants at *in vivo* conditions.

Materials and Methods

In our experiments we used maize (*Zea mays* L. cv "Knezha") and bean (*Phaseolus vulgaris* L. cv. "Cheren Starozagorski"). The plants were grown on dark glass pots at controlled temperature (22–23ºC), light (200 μmol photons m^{-2} s^{-1}), and humidity (40–50%) at photoperiod 12/12 h. The plants were grown initially in a full Hoagland nutrient solution (pH around 5), and after 7 days of growth, the plants were transferred to modified solution (Tables 1 and 2). Solutions were supplied by air. Later, 10 days after stress application, prompt chlorophyll *a* fluorescence and modulated reflection at 820 nm (MR_{820}) measurements were done. The bean plants were decapitated periodically by removing the stem, buds, apex and the newly appearing leaves. Primary leaves of decapitated plants were used in the experiments (Yordanov et al., 2008).

The kinetics of prompt chlorophyll *a* fluorescence (PF) and modulated reflection at 820 nm (MR_{820}) were simultaneously recorded by the Multifunctional Plant Efficiency Ana-

Table 1

Composition of a modified Hoagland nutrient solution for growing plants (macronutrients)

1 M solution	Type of growth medium, ml/dm^3			
	Full	– Ca	–K	– Fe
$Ca(NO_3)_2 \cdot 4H_2O$	4	–	4	4
KNO_3	6	6	–	6
$MgSO_4 \cdot 7H_2O$	2	–	2	2
$NH_4H_2PO_4$	2	2	2	2
$Mg(NO_3)_2 \cdot 6H_2O$	–	4	–	–
$MgCl_2 \cdot 6H_2O$	–	–	–	–
Na_2SO_4	–	2	–	–
$NaNO_3$	–	–	6	–
1% Iron Citrate	1	1	1	–
Microelements	1	1	1	1

Table 2

Composition of a modified Hoagland nutrient solution for growing plants (micronutrients)

Salts containing micronutrients	Quantity, g dm^{-3} H_2O
	Solution
H_3BO_3	2.85
$MnSO_4 \cdot 4H_2O$	1.10
$ZnSO_4 \cdot 7H_2O$	0.28
$CuSO_4 \cdot 5H_2O$	0.10
MoO_3	0.02
NaCl	3.12

lyzer, M-PEA (Hansatech Instrument Ltd, UK). Before measuring, the plants were dark adapted for 30 minutes. Then chlorophyll *a* fluorescence was recorded after illumination for 1s by red actinic light with intensity of 4000 μmol hν m^{-2} s^{-1}.

For a detailed analysis of the photosynthetic machinery, we used JIP-test algorithm described by (Strasser et al., 2004; Strasser et al., 2010). The analysed parameters are described in Table 3.

Results and Discussion

Three different rise components were distinguished when Chl *a* fluorescence induction curves had been measured *in vivo* (Strasser and Govindjee, 1991, 1992; Strasser et al., 1995). The fluorescence transients showed three steps between O and P (between initial (zero) and maximal levels of fluorescence). The O to J phase, lasting about 2 ms, reflects the reduction of Q_A to Q_A^- (Schreiber and Neubauer, 1987). The J to I (about 30 ms) and I to P phases are due to PQ-pool reduction (Strasser et al., 1995) and reduction of PS I end acceptors (Strasser et al 2004, 2010). The induction curves of the OJIP fluorescence transients recorded in plants with deficits of Fe, K and Ca of both species differ from those recorded in the control plants (Figure 1). The induction curves in deficient maize plants were different from that of bean plants grown in the solution with the same deficiency. The major changes in prompt fluorescence of stressed plants were observed during the J and I phases in maize plants but these changes were less pronounced in bean plants.

The OJIP curve were also translated to biophysical parameters, as shown in Table 3 (Strasser et al., 2004): specific activities per reaction center (RC); the quantum yields (φ_{Po}, φ_{Eo}, φ_{Ro}, φ_{Do} and ψ_{Eo}); phenomenological fluxes per cross section (CS); and performance indexes. The values of the calculated parameters were normalized to those of the

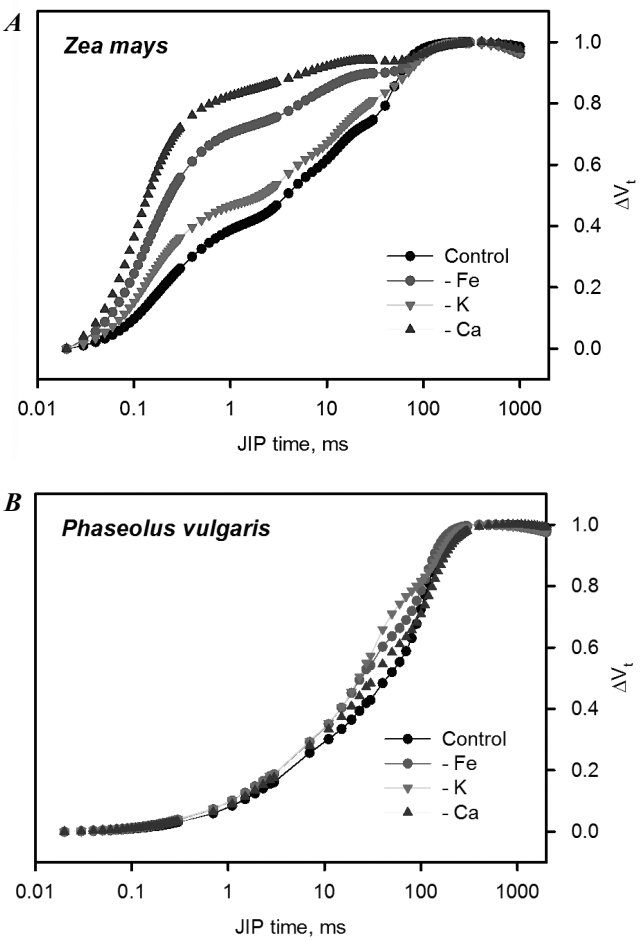

Fig. 1. Effect of Fe, K and Ca deficiencies on relative chlorophyll a fluorescence (Vt) in leaves of *Phaseolus vulgaris* (A) and *Zea mays* (B)

Table 3

Definition of terms and formulae for calculation of the JIP-test parameters from the Chl a fluorescence transient OJIP emitted by dark-adapted leaves

Fluorescence parameters	Description
F_0	minimal fluorescence, when all PS II RCs are open (at $t = 0$)
F_M	maximal fluorescence, when all PS II RCs are closed
$V_J = \frac{F_J - F_0}{F_M - F_0}$	relative variable fluorescence at the J-step
$\varphi_{Po} = 1 - \frac{F_0}{F_M}$	maximum quantum yield of primary photochemistry (at $t = 0$)
$\varphi_{Eo} = \left(1 - \frac{F_0}{F_M}\right)(1 - V_J)$	quantum yield of electron transport (at $t = 0$)
$\varphi_{Ro} = \left(1 - \frac{F_0}{F_M}\right)(1 - V_I)$	quantum yield for reduction of end electron acceptors at the PSI acceptor side (RE)
$\psi_{Eo} = 1 - V_J$	probability (at $t = 0$) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-
$\delta_{Ro} = \frac{1 - V_I}{1 - V_J}$	efficiency/probability With which an electron from the intersystem electron carriers moves to reduce end electron acceptors at the PSI acceptor side (RE)
$\gamma_{Rc} = \frac{Chl_{RC}}{Chl_{total}}$	probability that a PSII Chl molecule ·functions as RC
k_n is proportional to $\frac{1}{F_M}$	Non-photochemical de-excitation constant
$PI_{ABS} = \frac{\gamma_{RC}}{1 - \gamma_{RC}} \cdot \frac{\varphi_{Ro}}{1 - \varphi_{Ro}} \cdot \frac{\psi_{Eo}}{1 - \psi_{Eo}}$	performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors
$PI_{total} = PI_{ABS} \frac{\delta_{Ro}}{1 - \delta_{Ro}}$	performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors
$ABS/RC = \frac{1 - \gamma_{RC}}{\gamma_{RC}}$	absorption flux (of antenna Chls) per RC
M_0	approximated initial slope (in ms^{-1}) of the fluorescence transient $V = f(t)$
$TR_o/RC = M_0 \left(\frac{1}{V_J}\right)$	trapping flux (leading to Q_A reduction) per RC
$ET_o/RC = M_0 \left(\frac{1}{V_J}\right)\psi_0$	electron transport flux (further than Q_A^-) per RC
$RE_o/RC = M_0 \left(\frac{1}{V_J}\right)(1 - V_I)$	electron flux reducing end electron acceptors at the PSI acceptor side, per RC
$RC/CS_o = F_0 \varphi_{Po} \frac{V_J}{M_0}$	density of RCs (Q_A^- reducing PSII reaction centres)

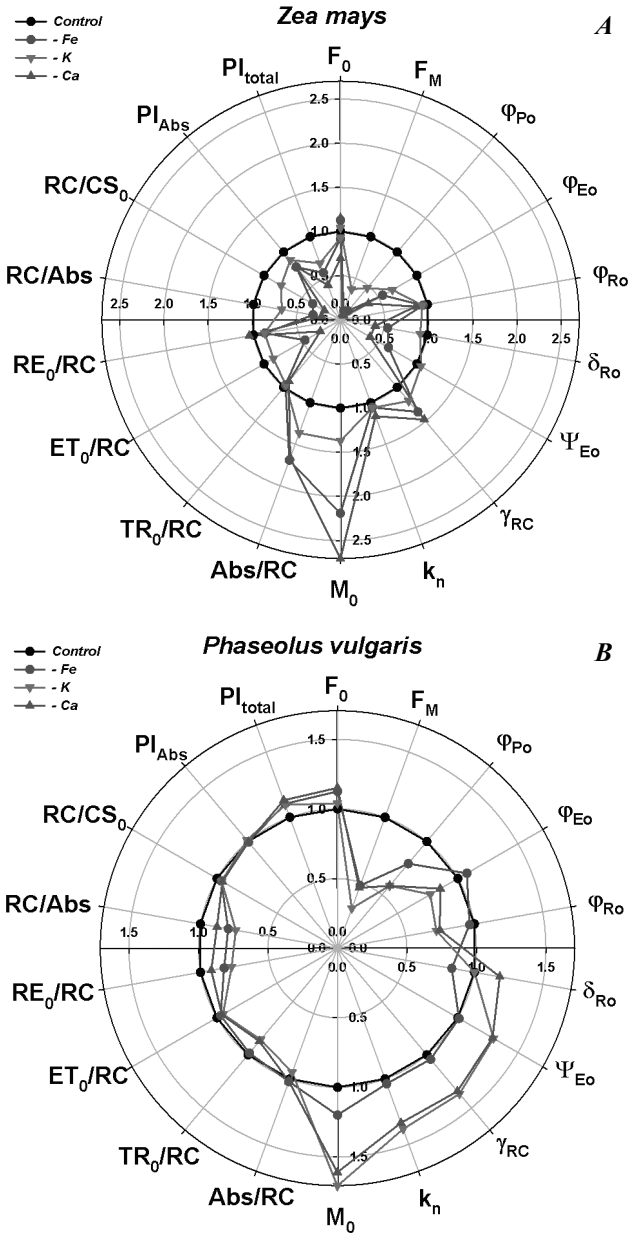

Fig. 2. Changes in the shape of the spider plot of the JIP-test parameters' images induced by deficiency of Fe, K and Ca in leaves of *Phaseolus vulgaris* (A) and *Zea mays* (B)

control plants. The alterations in φ_{Eo} indicate that under the nutrient deficiency electron transport beyond Q_A^- decreased in stressed maize but not in bean plants (Figure 2). φ_{Ro} decreased in all stressed maize plants, while it increased in stressed bean plants. The parameter φ_{Ro} describes the ability of an electron to move from the reduced intersystem electron acceptors to the end electron acceptors at the PSI acceptor side. The decrease of φ_{Ro} was observed in nutrient deficient maize plants except for Ca-deficient plant; however this parameter increased in nutrient deficient bean plants. The parameter γ represents the ratio of reaction center chlorophylls (Chl_{RC}) and the total chlorophyll of PSII (Chl_{RC}/Chl_{total}, where Chl_{total} is the sum of Chl_{RC} and Chl of the antenna). For every nutrient deficiency in both species, γ increased (Figure 2). The ψ_{Eo} decreased in stressed bean and maize plants. This means that the efficiency of a trapped exciton in the movement of an electron further than Q_A^- in the electron transport chain is decreased (Strasser et al., 2000).

An increase of the fraction of active RCs (ABS/RC) was observed in all nutrient deficient maize plants. The increase of ABS/RC means that either a fraction of RCs is inactivated or the apparent antenna size increased. These changes can be confirmed also by the decrease of the active RCs per excited cross section (RC/CS_0). The spider plots also showed increase of trapping per active reaction center (TR/RC). The differences in kinetics at the O to J phase also revealed the so called K-band which, when is positive, indicates either damage in the oxygen evolving complex (OEC) or increase in functional antenna size (Redillas et al., 2011). The appearance of K-band can be explained by an imbalance between the electron flow leaving the reaction centers on the acceptor side and the electron flow coming to the reaction centers from the donor side. K-band corresponds to the calculated parameter TR/RC. The value of TR/RC is slightly affected in stressed maize and bean plants. The ET_0/RC, which reflects the electron transport from Q_A^- to the PSI, is decreased in nutrient deficient maize plants but not in nutrient deficient bean plants. PI_{ABS} and PI_{total} showed differences in the response to nutrient deficiency in the both species (Figure 2). The performance index PI_{ABS} is used to quantify the PSII behaviour. The performance index PI_{total}, which measures the performance up to the reduction of PSI end electron acceptors (RE), incorporates several electron transport steps: φ_{Po}, Chl_{RC}/Chl_{total} and RE/ET. PI_{total} and PI_{ABS} decreased significantly in all nutrient deficient maize plants.

Oxidation of the reaction center chlorophylls of PSI, P700, is known to cause an increase in absorbance in the 800–850 nm range. The simultaneous measurement of the chlorophyll *a* fluorescence and the transmission at 820 nm makes possible to evaluate the electron transfer processes during induction within the two functional complexes: PS II and PS I (Schansker et al., 2003). We investigated the changes in PS I activity of plants exposed to deficiency of Fe, K and Ca (Figure 3). MR_{820} changes were used here to monitor electron flow through PS I. Figure 3 showed alterations both in the amplitude and the rate of photoinduced changes of

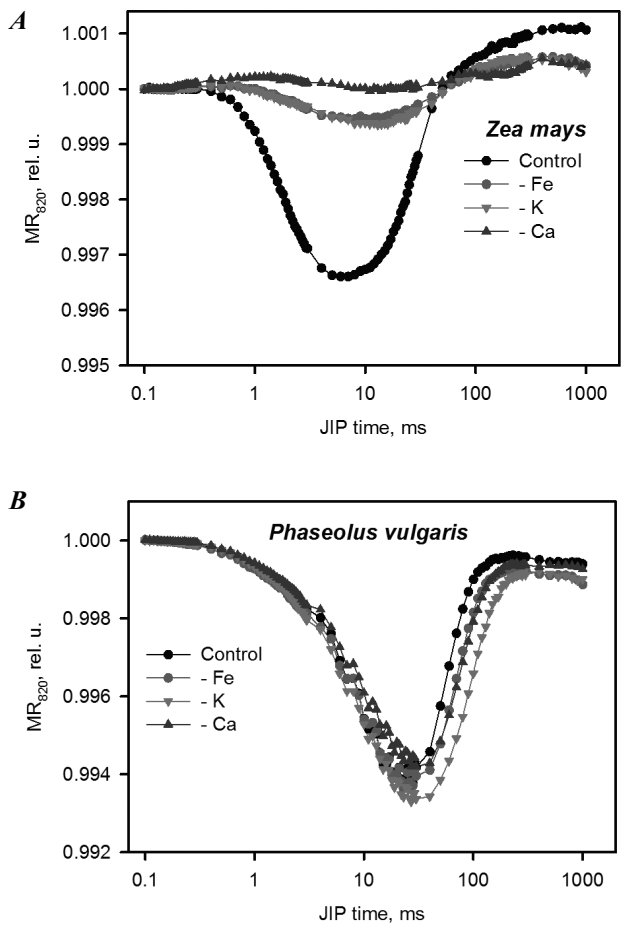

Fig. 3. Effect of Fe, K and Ca deficiency on relative changes of modulated reflection at 820 nm (MR820) in leaves of *Phaseolus vulgaris* (A) and *Zea mays* (B)

MR_{820} in nutrient-deficient bean and maize plants compared to control plants. In maize plants, grown in control condition, re-reduction kinetics of $P700^+$ and PC^+ started after 8 ms, however, in the leaves of the nutrient-deficient plants re-reduction kinetics of $P700^+$ and PC^+ were slower and occurred after 20 to 30 ms, indicating PS I inhibition. Changes in the kinetics of MR at 820 nm in nutrient deficient bean plants showed that re-reduction of $P700^+$ and PC^+ occurred after 10 ms. However the changes of MR at 820 nm in bean plants are not so pronounced as in maize plants. MR_{820} decline and the oxidation of PC and P700 were faster in Fe and K deficient plants, than in control ones

The availability of micro and macro-elements during plant growth and development is essential for the normal physiological state of the plant as a whole, including for the maintenance of photosynthetic processes (Smethurst et al., 2005; Osman, 2013). Differences between responses of both species were evident in the chlorophyll fluorescence parameters. Our results demonstrate the negative effect of nutrient deficiency on photosynthetic yield of PS II which reflected in reduction of the quantum yield of PSII electron transport; in the efficiency of excitation energy capture by open PSII reaction centers (φ_{Po}; φ_{Eo}; ψ_{Eo}), and suggest that nutrient deficiency induces some photoinhibitory damages to PSII (Baker and Rosenqvist, 2004). We suppose that the changes in the PSI end electron acceptors activity may be used for monitoring of nutrient deficiency effects on PS I. Changes in the parameters RE/RC and MR_{820} can be sign for specific nutrient deficiency. We observed clear differences in nutrient deficiency response between bean and maize plants.

Conclusion

The deficiency of all analysed elements changed the physiological state of bean and maize plants that was displayed in modifications of the chlorophyll fluorescence transients and the dynamics of MR at 820 nm. The effects of the lack of these elements included the impairments in electron transport chain in both donor and acceptor sides of PSII and of PSI. Each of these sites responded in a different way. The sensitivity of maize and bean plants to the lack of K, Ca and Fe was different too. Furthermore, the analysis of fluorescence from *in vivo* plants provides enough information for detecting the nutrient deficiency stress and for recognition of the type of limitation nutrient element.

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