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Research article

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

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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

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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.

Table 1
The composition of the various culture media in mM. The concentration of minerals was achieved by addition X cm^3 of concentrated stock solution (1 mol per 1 dm^3) of corresponding component per 1 dm^3 of medium. Numbers in the brackets indicate the pH of each nutrient solution.

1 M solution	Type of growth medium (ml/1 dm^3)							
	Full (pH 5.05)	−Ca (pH 4.89)	−S (pH 4.8)	−Mg (pH 4.88)	−K (pH 4.82)	−N (pH 4.87)	−P (pH 4.94)	−Fe (pH 5.12)
$Ca(NO_3)_2 \cdot 4H_2O$	4	–	4	4	4	–	4	4
KNO_3	6	6	6	6	–	–	6	6
$MgSO_4 \cdot 7H_2O$	2	–	–	–	2	2	2	2
$NH_4H_2PO_4$	2	2	2	2	2	–	–	2
$Mg(NO_3)_2 \cdot 6H_2O$	–	4	–	–	–	–	–	–
$MgCl_2 \cdot 6H_2O$	–	–	1	–	–	–	–	–
Na_2SO_4	–	2	–	1	–	–	–	–
$NaNO_3$	–	–	–	–	6	–	–	–
$CaCl_2$	–	–	–	–	–	4	–	–
KCl	–	–	–	–	–	2	–	–
NaH_2PO_4	–	–	–	–	–	2	–	–
NH_4NO_3	–	–	–	–	–	–	1	–
1% Iron Citrate	1	1	1	1	1	1	1	–
Microelements (solution A)[a]	1	1	–	1	1	–	1	1
Microelements (solution B)[a]	–	–	1	–	–	1	–	–

[a] See Table 2 for the components of microelements solution (A and B).

computer controlled greenhouse in 1 dm^3 dark glass pots filled with a modified Hoagland nutrient solution (see Tables 1 and 2 for the components of microelements solution A and B). Solutions were supplied by air continuously and replaced every 3 days. The medium pH was about 5.0 for all solutions types. The average temperature for day/night was 26/18 °C, respectively, relative humidity was 50–60%, the photoperiod for the day/night cycle was 16/8 h, and the maximum photosynthetically active radiation was about 1400 μmol (photons) m^{-2} s^{-1}. After 7 days of growth, the seedlings were subjected to nutrient deficiency stress. Later, 14 days after the stress application (21 days after emergence) prompt chlorophyll *a* fluorescence (PF) measurements were done on 9 fully developed leaves for each treatment. At this stage (21 days of emergence), only slight visual symptoms of nutrients deficiencies were observed.

2.2. Chlorophyll fluorescence measurement

Photo-induced transients of prompt fluorescence in leaves were measured by M-PEA (Multi-Function Plant Efficiency Analyser, Hansatech, UK). In the M-PEA instrument emitter wavelength ranges are: (1) 635 ± 10 nm, for the actinic light LED (light-emitting diode); (2) 820 ± 25 nm, for the modulated light LED, and (3) 735 ± 15 nm, for the far-red light LED; for the latter, a RG9 long pass filter was used to remove any visible light component (for more details see Goltsev et al., 2009; Strasser et al., 2010). Before measuring the experimental signals, plants were kept in dark at least for 30 min. Measurements were carried out on the abaxial surface of 3 fully developed leaves (top, middle and bottom of the plant), on the middle part of chosen leaf. Chlorophyll *a* (Chl *a*) fluorescence was recorded after illumination by red actinic light (635 nm, 5000 μmol hν m^{-2} s^{-1}). The DF dark relaxation kinetics were recorded for 3 s after turning the actinic light off.

Measured signal were analysed by M-PEA-data analyzer version 5.4 software (this software is laboratory designed in Dept. Biophysics and Radiobiology, Sofia University by Petko Chernev).

Table 2
Salts containing micronutrients (without iron) used in modified Hoagland solution.

Salts containing micronutrients	Quantity (g dm^{-3} H_2O)	
	Solution A	Solution B
H_3BO_3	2.85	2.85
$MnSO_4 \cdot 4H_2O$	1.10	–
$ZnSO_4 \cdot 7H_2O$	0.28	–
$CuSO_4 \cdot 5H_2O$	0.10	–
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.02	–
NaCl	3.12	3.12
$MnCl_2 \cdot 4H_2O$	–	0.93
$ZnCl_2$	–	0.13
$CuCl_2 \cdot 2H_2O$	–	0.07
MoO_3	–	0.002

2.3. JIP test parameters

The characteristics points of photoinduced chlorophyll fluorescence transients were used to calculate specific characteristics of the light phase of photosynthesis according to the JIP-test algorithm, described by Strasser et al. (2004, 2010). The analysed parameters are described in Table 3.

2.4. Statistical analysis

All experiments were carried out at least at 9 repetitions and data were statistically analysed. The non-parametric Kruskal–Wallis one-way analysis of variance by ranks was applied using

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
$V_J = (F_J - F_0)/(F_M - F_0)$	Relative variable fluorescence at the J-step
$\varphi_{Po} = 1 - F_0/F_M$	Maximum quantum yield of primary photochemistry (at $t = 0$)
$\varphi_{Eo} = (1 - F_0/F_M)(1 - V_J)$	Quantum yield of electron transport (at $t = 0$)
$\varphi_{Ro} = (1 - F_0/F_M)(1 - V_I)$	Quantum yield for reduction of end electron acceptors at the PSI acceptor side (RE)
$\varphi_{Do} = F_0/F_M$	Quantum yield (at $t = 0$) of energy dissipation
$\psi_{Eo} = 1 - V_J$	Probability (at $t = 0$) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-
$\delta_{Ro} = (1 - V_I)/(1 - V_J)$	Efficiency/probability with which an electron from the intersystem electron carriers moves to reduce end electron acceptors at the PSI acceptor side (RE)
$\gamma_{Rc} = Chl_{RC}/Chl_{total}$	Probability that a PSII Chl molecule functions as RC
$t(F_m)$	Time (in ms) to reach the maximal fluorescence intensity F_M
$PI_{ABS} = \gamma_{RC}/(1 - \gamma_{RC}) \cdot \varphi_{Po}/(1 - \varphi_{Po}) \cdot \psi_{Eo}/(1 - \psi_{Eo})$	Performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors
$PI_{total} = PI_{ABS} \cdot \varphi_{Ro}/(1 - \varphi_{Ro})$	Performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors
$ABS/RC = (1 - \gamma_{RC})/\gamma_{RC}$	Absorption flux (of antenna Chls) per RC
M_0	Approximated initial slope (in ms^{-1}) of the fluorescence transient $V = f(t)$
$TR_o/RC = M_0(1/V_J)$	Trapping flux (leading to Q_A reduction) per RC
$ET_o/RC = M_0(1/V_J)\psi_0$	Electron transport flux (further than Q_A^-) per RC
$RE_o/RC = M_0(1/V_J)(1 - V_I)$	Electron flux reducing end electron acceptors at the PSI acceptor side per RC
$DI_o/RC = (ABS/RC - TR_o/RC)$	Dissipated energy flux per RC (at $t = 0$)
$RC/CS_o = F_0\varphi_{Po}\, V_J/M_O$	Density of RCs (Q_A^- reducing PSII reaction centres)

Modified from Strasser et al., 2010.

Sigma Stat v.3.5 software. Principal Component Analysis was performed as per MatLab 7.5 Toolbox.

3. Results

3.1. Chlorophyll a fluorescence rise

Prompt chlorophyll fluorescence rise measured from all leaves displayed the typical OJIP transients when plotted on a logarithmic time scale (Fig. 1AB). To visualise the comparative effect of nutrient deficiency on the transient dynamics, the curves are plotted as relative variable fluorescence, $V_t = (F_t - F_o)/(F_M - F_o)$ (Strasser et al., 2004). This experimental expression is taken as a measure of the fraction of the primary quinone electron acceptor of PSII being in its reduced state $[Q_A^-]/[Q_{A.(total)}]$. The fluorescence transients showed two steps between O and P: J at about 2 ms and I at about 30 ms. The OJ phase is strongly light dependent (Neubauer and Schreiber, 1987; Schansker et al., 2006) and contains information on antenna size and connectivity between PSII reaction centres. The *J* to *P* rise is called the thermal phase (Delosme, 1967) and reflects a reduction of the rest of the electron transport chain (Schansker et al., 2005). The shape of the OJIP fluorescence transients recorded in nutrient deficient (ND) plants of both species differed from those recorded in control plants (Fig. 1AB). However, nutrient deficiency in maize plants had stress response markedly different from that of tomato plants. The major changes in prompt fluorescence of stressed plants were observed in the *J* (V_J) and *I* (V_I) levels, except for P-deficiency in maize plants, and these changes were less prominent in tomato plants.

Changes in OJIP fluorescence rise kinetics were revealed by calculating the difference in variable fluorescence curves (ΔV_t). ΔV_t curves were constructed by subtracting the normalized fluorescence values (between O and P) recorded in ND plants from those recorded in control plants (Fig. 2AB). Analysis of the fluorescence transients revealed that although the major effects of nutrient deficiency for maize and tomato plants occurred in the O-J phase, changes were also evident in multiple turnover events of PSII (i.e. the *J–P* transition) (Fig. 2AB). The appearance of three bands in fluorescence intensity in ND plants can be seen, namely a ΔK (at ~300 μs), ΔJ peak (at ~2 ms) and ΔI (at 10–30 ms), which depended on nutrient deficiency and plant species. A clearly defined ΔK-band was induced by nutrient deficiency in both maize and tomato plants (Fig. 2AB), except in P-deficient tomato plants. However, this change was more important in maize leaves than in tomato leaves. We noted here that ΔK-bands are associated with uncoupling of the oxygen evolving complex (Guisse et al., 1995), and ΔJ-bands are associated with accumulation of Q_A^-, i.e. inhibition of the Q_A^- reoxidation. Inactivation of ferredoxin-NADP$^+$ oxidoreductase (FNR) has also been suggested as a factor that could contribute towards the appearance of an *I*-peak (Schansker et al., 2003).

The appearance of ΔK-band in the fluorescence transients of stressed plants furthermore pointed at foliar nutrient limitation. The ΔJ-bands were much prominent in stressed maize plants (except in P-deficient plants) than in tomato plants. The

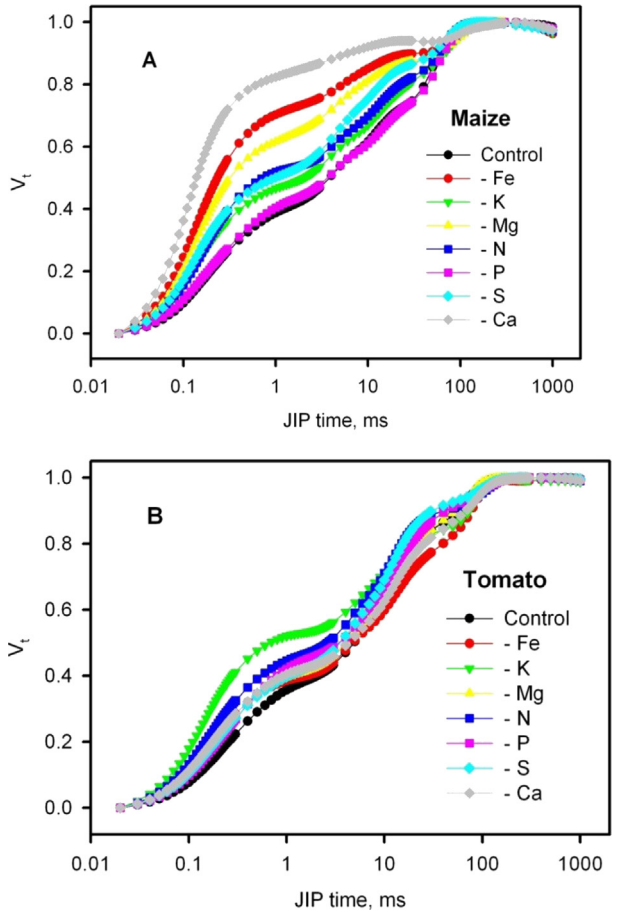

Fig. 1. Effect of nutrient deficiencies on relative chlorophyll *a* fluorescence (V_t) in leaves of maize (A) and tomato (B) plants.

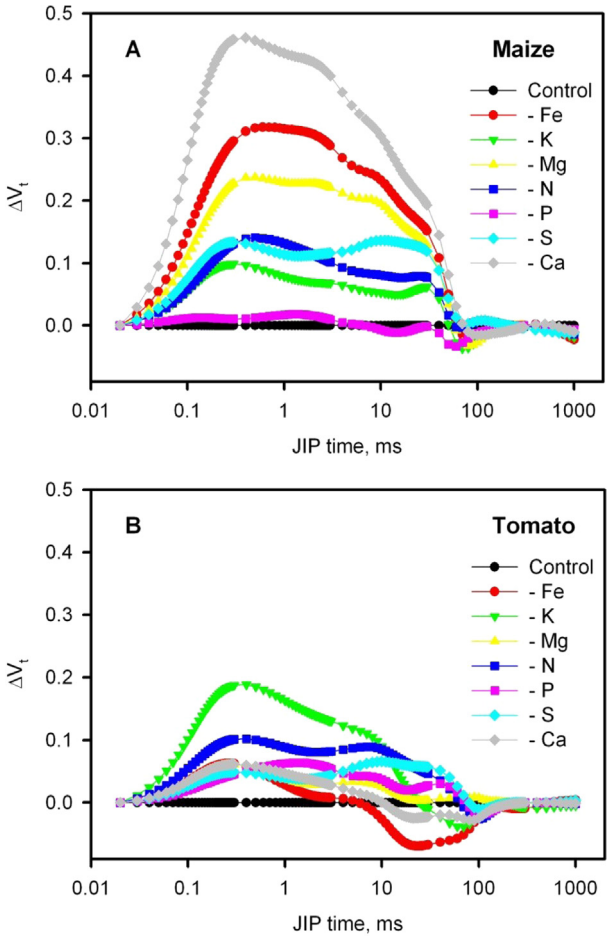

Fig. 2. Effect of nutrient deficiencies on differential plots of relative chlorophyll *a* fluorescence (ΔV_t) in leaves of maize (A) and tomato plants (B).

appearance of Δ*I*-bands in ND maize plants could be a result of an inhibition of FNR activity. However, the quantitative effect of each nutrient deficiency was different in maize and tomato plants. For example, the effect of Ca-deficiency was more prominent in maize plants than in tomato plants, as observed by the higher amplitude of Δ*K*-band, Δ*J*-band and Δ*I*-band. On the other hand, in tomato plants the *K*-deficiency had greater effect on these three parameters.

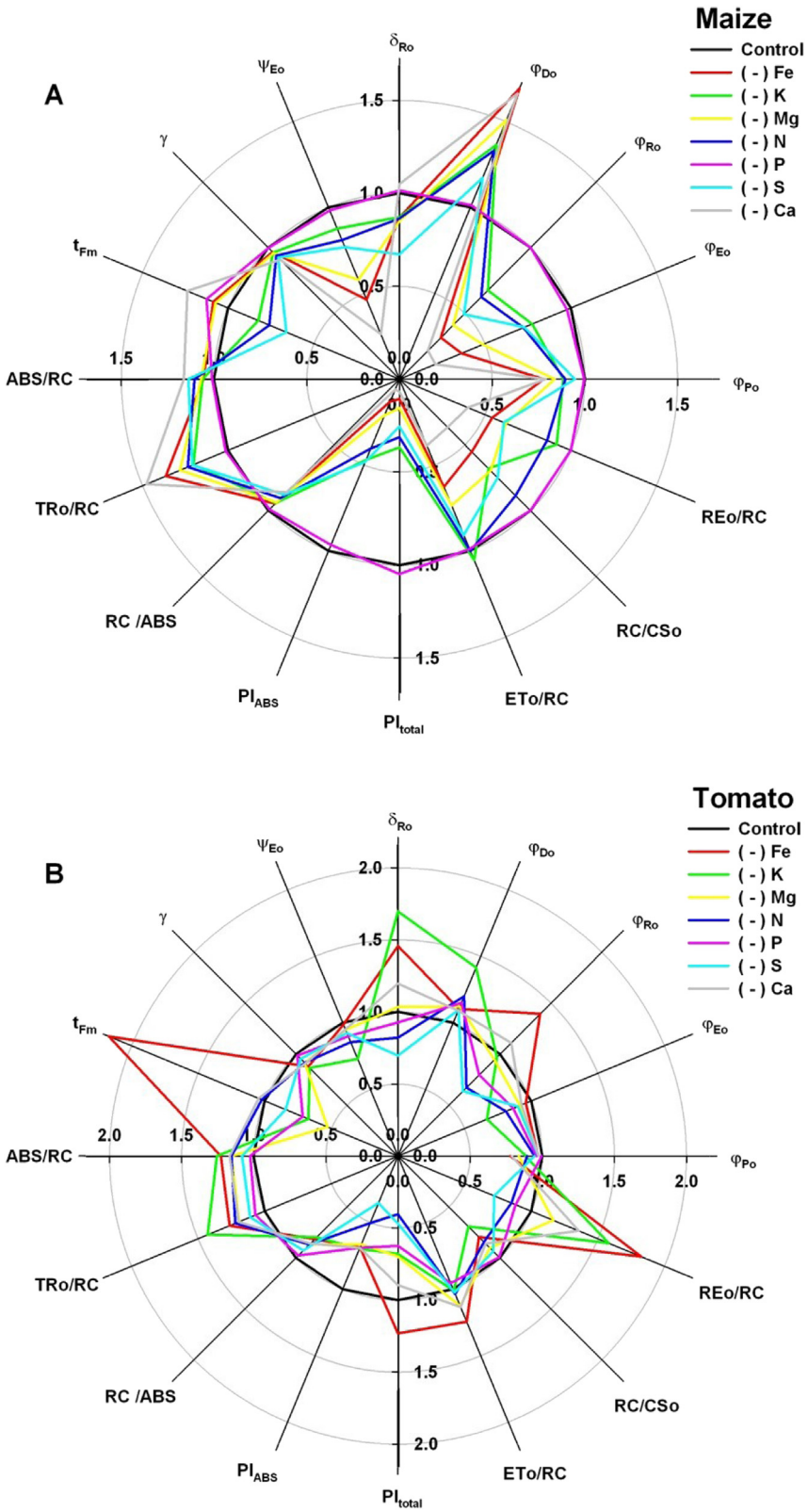

Fig. 3. Changes of shape of spider plot JIP-test parameters' images induced by fertilizers deficiency in maize (A) and tomato (B) plants.

3.2. PSII biophysical parameters derived by the JIP-test equations

The OJIP transients were also translated to biophysical parameters (Strasser et al., 2004): the quantum yields (φ_{Po}, φ_{Eo}, φ_{Ro} and δ_{Ro}); specific activities per reaction centre (RC); phenomenological fluxes per cross section (CS); and performance indexes (PI). The values of the calculated parameters were normalized to those of the control plants. The deviation of the behaviour pattern of the stressed and control plants demonstrates the impact of each nutrient deficiency on plants of each species (Fig. 3AB).

The statistical analysis of nutrient deficiency effects on different photosynthetic parameters is presented in Tables 4 and 5. We noted here that P-deficiency in maize plants did not have any significant effect on the studied photosynthetic parameters. We observed that ND had a significant effect ($p < 0.001$) on the maximum yield of primary photochemistry of PSII ($\varphi_{Po} = F_V/F_M$) for both species, except for P-deficient maize plants (Fig. 3AB, and Table 5). Possible interpretation of this decrease can be that ND had impaired PS II photochemical efficiency. The decrease of φ_{Po} was accompanied by a significant change in other JIP-test parameters. It is evident that during the experimental nutrient deficiency, effects were observed on the electron transport system too. Example for this influence are parameters, connected with electron transport – φ_{Eo} and φ_{Ro}, representing respectively the quantum yield efficiency with which a trapped exciton can move an electron to the electron transport chain (φ_{Eo}) and the quantum yield with which electrons reduce the PSI end-electron acceptors (φ_{Ro}). The significant decrease of φ_{Eo} in the leaves of stressed plants of both species suggests that the probability for electron transport beyond Q_A^- was decreased under nutrient deficiency (Fig. 3AB). φ_{Ro} decreased significantly ($p < 0.001$) in all ND leaves of maize plants, except for P-deficient ones, but it increased in Fe and Ca-deficient leaves of tomato plants. In contrast, φ_{Do} increased for both species suggests, that maximum energy was lost in the form of heat dissipation. δ_{Ro} is designed as the efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end-electron acceptors. Decrease of this parameter is associated with decrease in IP-amplitude that was shown to be a symptom either as decrease in PSI content (Ceppi et al., 2012) or decrease of PSI fraction involved in linear electron flow (Zivcak et al., 2014). The decrease of δ_{Ro} was observed in ND leaves of maize plant, except for P and Ca-deficiency; however this parameter increased in Ca, Fe and S-deficient leaves of tomato plants. An apparent increase in throughput of electron transport chain between PSII and PSI results probably from decrease in redox poise of PSII electron acceptors due to lower PSII activity, i.e. decrease in ratio between number of active PSII and PSI reaction centres. In Fig. 3, the symbol γ estimates the ratio of reaction centre chlorophylls 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 all nutrient deficiencies in both species the parameter γ slightly reduced (Fig. 3AB). It is of interest to find out if ND alters the ratio between antenna light harvesting complex (ABS) and active PSII reaction centres. Then, the increase of the absorbed energy by active RCs (ABS/RC) was observed in all ND plants for both species and an increase in this parameter means that either a fraction of RCs is inactivated or the apparent antenna size increased. These changes can easily be visualized also by reduction of the active RCs per excited cross section (RC/CS_0), which decreased in all samples except those in P deficient plants of both species. The increase of ABS/RC (or decrease of the active RCs) was accompanied by an increase of trapping per active reaction centre (TR/RC). As explained above, the appearance of K-band at 300 μs has been associated with an inactivation of the oxygen-evolving-complex (OEC). Since, the value of WK at 300 μs is calculated similarly to the TR/RC, then this parameter also could be used as an indicator of OEC impairment by stress treatment. An increase of TR/RC was observed for stressed maize and tomato plants. The ET/RC and the electron transport from Q_A^- to the PSI electron acceptors (RE/RC) of ND leaves decreased in maize plants but they increased in ND leaves of tomato plants, except for N and P deficiency. Lastly, as far as the specific reaction centre activities are concerned, the most important observed change was energy dissipation (DI/RC), which decreased for all ND leaves of both species. Thus, this parameter has proved as a general non-specific indicator of ND stress in plants.

PI_{ABS} and PI_{total}, as measures of plant performance, showed significant differences in the response to nutrient deficiency in the two species (Fig. 3AB). The performance index PI_{ABS} is used to quantify the PSII behaviour. The performance index, PI_{total}, a measure for the performance up to the reduction of PSI end-electron acceptors (RE), incorporates several electron transport steps: φ_{Po}, ET/TR, Chl_{RC}/Chl_{total} and RE/ET. In maize, PI_{total} and PI_{ABS} decreased significantly in all ND leaves, except for P-deficient plants. Over the 14 days of ND, the decreases of parameters PI_{ABS} and PI_{tot} were much more pronounced in Ca-deficient maize plants as compare to deficiencies of other minerals, the values decreased by 94% and 95%, respectively compared to the control.

Table 4
Effect of nutrient deficiencies on maize plants analysed by JIP test parameters. Kruskal–Wallis one-way analysis of variance by ranks was applied. Means ± Standard Errors values are calculated on the base $n = 9$. The significance values of difference as compare to control samples based on Dunnett's Method are presented.

Parameter	Control	−Fe	−K	−Mg	−N	−P	−S	−Ca
φ_{Po}	0.757 ± 0.002	0.589 ± 0.018**	0.669 ± 0.006**	0.634 ± 0.011**	0.677 ± 0.002**	0.754 ± 0.003^{ns}	0.716 ± 0.008**	0.597 ± 0.013**
φ_{Eo}	0.403 ± 0.007	0.146 ± 0.012**	0.312 ± 0.008**	0.196 ± 0.014**	0.291 ± 0.008**	0.394 ± 0.012^{ns}	0.294 ± 0.021**	0.084 ± 0.009**
φ_{Ro}	0.192 ± 0.003	0.061 ± 0.007**	0.129 ± 0.003**	0.079 ± 0.005**	0.120 ± 0.004**	0.192 ± 0.012^{ns}	0.094 ± 0.008**	0.042 ± 0.005**
φ_{Do}	0.243 ± 0.002	0.411 ± 0.018**	0.331 ± 0.006**	0.366 ± 0.011**	0.323 ± 0.002**	0.246 ± 0.003^{ns}	0.284 ± 0.008**	0.403 ± 0.013**
δ_{Ro}	0.477 ± 0.003	0.414 ± 0.021*	0.415 ± 0.003**	0.404 ± 0.010**	0.412 ± 0.011**	0.483 ± 0.017^{ns}	0.320 ± 0.006**	0.500 ± 0.024^{ns}
ψ_{Eo}	0.531 ± 0.008	0.246 ± 0.017**	0.466 ± 0.007**	0.307 ± 0.018**	0.430 ± 0.012**	0.522 ± 0.014^{ns}	0.408 ± 0.026**	0.139 ± 0.013**
γ_{Rc}	0.371 ± 0.003	0.359 ± 0.005^{ns}	0.357 ± 0.002**	0.355 ± 0.003**	0.348 ± 0.003**	0.370 ± 0.008^{ns}	0.343 ± 0.009*	0.337 ± 0.005**
$t(F_m)$	258 ± 5	280 ± 42^{ns}	212 ± 12**	277 ± 26^{ns}	196 ± 26*	290 ± 15^{ns}	170 ± 9**	319 ± 56^{ns}
ABS/RC	1.698 ± 0.024	1.793 ± 0.038^{ns}	1.802 ± 0.014**	1.814 ± 0.021**	1.875 ± 0.024**	1.714 ± 0.062^{ns}	1.932 ± 0.077*	1.973 ± 0.042**
TR_o/RC	2.243 ± 0.036	3.055 ± 0.074**	2.695 ± 0.038**	2.870 ± 0.067**	2.769 ± 0.034**	2.274 ± 0.090^{ns}	2.708 ± 0.129**	3.309 ± 0.024**
DI_o/RC	0.508 ± 0.021	1.046 ± 0.056**	0.549 ± 0.013^{ns}	0.939 ± 0.047**	0.686 ± 0.036**	0.538 ± 0.053^{ns}	0.853 ± 0.093**	1.514 ± 0.023**
PI_{ABS}	2.112 ± 0.110	0.277 ± 0.037**	0.994 ± 0.063**	0.445 ± 0.049**	0.854 ± 0.045**	2.038 ± 0.168^{ns}	1.019 ± 0.173**	0.127 ± 0.017**
PI_{total}	1.920 ± 0.092	0.206 ± 0.038**	0.705 ± 0.044**	0.300 ± 0.033**	0.600 ± 0.038**	2.011 ± 0.243^{ns}	0.490 ± 0.091**	0.129 ± 0.022**
ET_o/RC	1.190 ± 0.007	0.747 ± 0.048**	1.253 ± 0.012**	0.875 ± 0.043**	1.190 ± 0.032^{ns}	1.176 ± 0.012^{ns}	1.079 ± 0.020**	0.460 ± 0.043**
RC/Cs_o	4138 ± 99	2285 ± 457*	2813 ± 178*	2879 ± 253*	3665 ± 94^{ns}	4132 ± 148^{ns}	3101 ± 209^{ns}	1518 ± 139*
RE_o/RC	0.567 ± 0.004	0.307 ± 0.022**	0.520 ± 0.007**	0.352 ± 0.016**	0.489 ± 0.015^{ns}	0.567 ± 0.017^{ns}	0.346 ± 0.011**	0.277 ± 0.022**

** Significant difference, $\alpha < 0.01$.
* Significant difference, $\alpha < 0.05$.
^{ns} Non-significant differences.

Table 5
Effect of nutrient deficiencies on tomato plants analysed by JIP test parameters. Kruskal–Wallis one-way analysis of variance by ranks was applied. Means ± Standard Errors values are calculated on the base $n = 9$. The significance values of difference as compare to control samples based on Dunnett's Method are presented.

Parameter	Control	−Fe	−K	−Mg	−N	−P	−S	−Ca
φ_{Po}	0.787 ± 0.001	0.765 ± 0.002**	0.699 ± 0.026**	0.761 ± 0.003**	0.745 ± 0.004**	0.755 ± 0.013**	0.767 ± 0.003**	0.768 ± 0.003^{ns}
φ_{Eo}	0.447 ± 0.005	0.428 ± 0.005^{ns}	0.298 ± 0.038**	0.409 ± 0.006**	0.362 ± 0.011**	0.386 ± 0.020**	0.402 ± 0.007**	0.414 ± 0.006^{ns}
φ_{Ro}	0.126 ± 0.004	0.175 ± 0.008**	0.122 ± 0.010^{ns}	0.119 ± 0.006^{ns}	0.084 ± 0.006**	0.100 ± 0.009^{ns}	0.080 ± 0.013**	0.139 ± 0.005^{ns}
φ_{Do}	0.213 ± 0.001	0.235 ± 0.002**	0.301 ± 0.026**	0.239 ± 0.003**	0.255 ± 0.004**	0.245 ± 0.013**	0.233 ± 0.003**	0.232 ± 0.003^{ns}
δ_{Ro}	0.281 ± 0.008	0.409 ± 0.020**	0.476 ± 0.072^{ns}	0.290 ± 0.015^{ns}	0.231 ± 0.011^{ns}	0.260 ± 0.019^{ns}	0.196 ± 0.028^{ns}	0.336 ± 0.008^{ns}
ψ_{Eo}	0.568 ± 0.006	0.560 ± 0.006**	0.414 ± 0.042**	0.537 ± 0.006^{ns}	0.485 ± 0.012**	0.510 ± 0.022**	0.524 ± 0.008**	0.540 ± 0.006^{ns}
γ_{Rc}	0.381 ± 0.004	0.335 ± 0.005**	0.330 ± 0.004**	0.348 ± 0.003**	0.349 ± 0.005**	0.376 ± 0.005^{ns}	0.363 ± 0.002**	0.346 ± 0.004**
$t(F_m)$	318 ± 40	689 ± 26**	213 ± 48^{ns}	168 ± 43^{ns}	324 ± 30^{ns}	227 ± 36^{ns}	268 ± 57^{ns}	332 ± 39^{ns}
ABS/RC	1.625 ± 0.027	1.993 ± 0.048**	2.037 ± 0.035**	1.872 ± 0.024**	1.873 ± 0.045**	1.665 ± 0.033^{ns}	1.755 ± 0.019^{ns}	1.893 ± 0.035**
TR_o/RC	2.065 ± 0.036	2.606 ± 0.067**	2.947 ± 0.119**	2.459 ± 0.039**	2.517 ± 0.071**	2.206 ± 0.044^{ns}	2.289 ± 0.023^{ns}	2.466 ± 0.050**
DI_o/RC	0.454 ± 0.019	2.104 ± 0.093**	1.067 ± 0.228^{ns}	2.001 ± 0.099**	1.523 ± 0.135^{ns}	2.079 ± 0.236**	2.082 ± 0.092**	2.072 ± 0.102**
PI_{ABS}	3.019 ± 0.132	2.072 ± 0.102**	2.104 ± 0.093^{ns}	2.001 ± 0.099**	1.523 ± 0.135**	2.079 ± 0.236^{ns}	1.067 ± 0.228**	2.082 ± 0.092**
PI_{total}	1.184 ± 0.074	1.457 ± 0.090^{ns}	0.805 ± 0.107^{ns}	0.826 ± 0.075^{ns}	0.478 ± 0.069**	0.738 ± 0.117^{ns}	0.556 ± 0.110**	1.061 ± 0.083^{ns}
ET_o/RC	1.171 ± 0.010	1.457 ± 0.033**	1.184 ± 0.090^{ns}	1.320 ± 0.012**	1.215 ± 0.009^{ns}	1.118 ± 0.031^{ns}	1.199 ± 0.018^{ns}	1.329 ± 0.016**
RC/Cs_o	5164 ± 76	4102 ± 172**	3570 ± 217**	4654 ± 111^{ns}	4352 ± 89**	5127 ± 213^{ns}	4826 ± 120^{ns}	4438 ± 78**
RE_o/RC	0.329 ± 0.011	0.600 ± 0.040**	0.520 ± 0.047^{ns}	0.383 ± 0.021^{ns}	0.280 ± 0.013^{ns}	0.290 ± 0.022^{ns}	0.237 ± 0.037^{ns}	0.446 ± 0.010^{ns}

** Significant difference, $\alpha < 0.05$.
^{ns} Non-significant differences.

3.3. *Principal component analysis of stress effect on maize and tomato plants*

The nutrient deficiency induces modification of the plant that reflected in changes of the sixteen JIP-test parameters (φ_{Po}, ψ_{Eo}, PI_{ABS}, ETo/RC, φ_{Eo}, δ_{Ro}, PI_{total}, RE_o/RC, φ_{Ro}, γ_{RC}, ABS/RC, DI_o/RC, φ_{Do}, $t_{(Fm)}$, TR_o/RC, RC/CS_o), describing the physiological state of photosynthetic machinery. We used multiparametric analysis to evaluate the stress effects in plants in order to identify parameters that are most sensitive for plant stress response. The individual parameters are not fully independent (as is the case for the JIP-test parameters) because they are calculated on the basis of points of one experimental curve – chlorophyll fluorescence rise curve, and some of parameters are connected by mathematical expressions (e.g. φ_{Po} and φ_{Do}). An effective approach to use such a set of experimental parameters is principal component analysis (PCA). PCA evaluates variations in the values of experimental parameters and derives new complex variables that reflect maximal changes in the parameter data set. The first principal component (PC), Comp 1, is a vector in n-dimensional space that corresponds to maximal variations of parameters. The second PC, Comp 2 is a vector in the plane perpendicular to Comp 1 vector and reflects maximal change of parameters in the same plane.

The positions of points with coordinates Comp 1/Comp 2 in this parametric plane present the state of photosynthetic machinery and show the effect of stress factors (see Fig. 4AB). The projections of values of parameters on the plane of PCs, Comp 1/Comp 2 display the influence of each parameter within total stress response represented by the PCs.

For a better understanding of the stressors effect on the whole, we applied principal component analysis. This approach allows transforming the set of measured parameters into fewer variables that determine the changes in plant physiological state (Jolliffe, 2002).

The modifications in the first Principal Component (Comp 1, PC 1) determined about 70% of total changes in maize plants, the second component Comp 2 reflected 14%, and Comp 3–6%. This means that most of the stress induced variations in the plant could be connected with the three components. The analysed JIP-test parameters had different sensitivity to stressors, and different contribution in the formation of principal components. In maize plants main part for PC 1 and PC 2 have parameters shown in Table 6. The parameters presented in the left columns contribute increase the PC 1 values and those in the right columns decrease

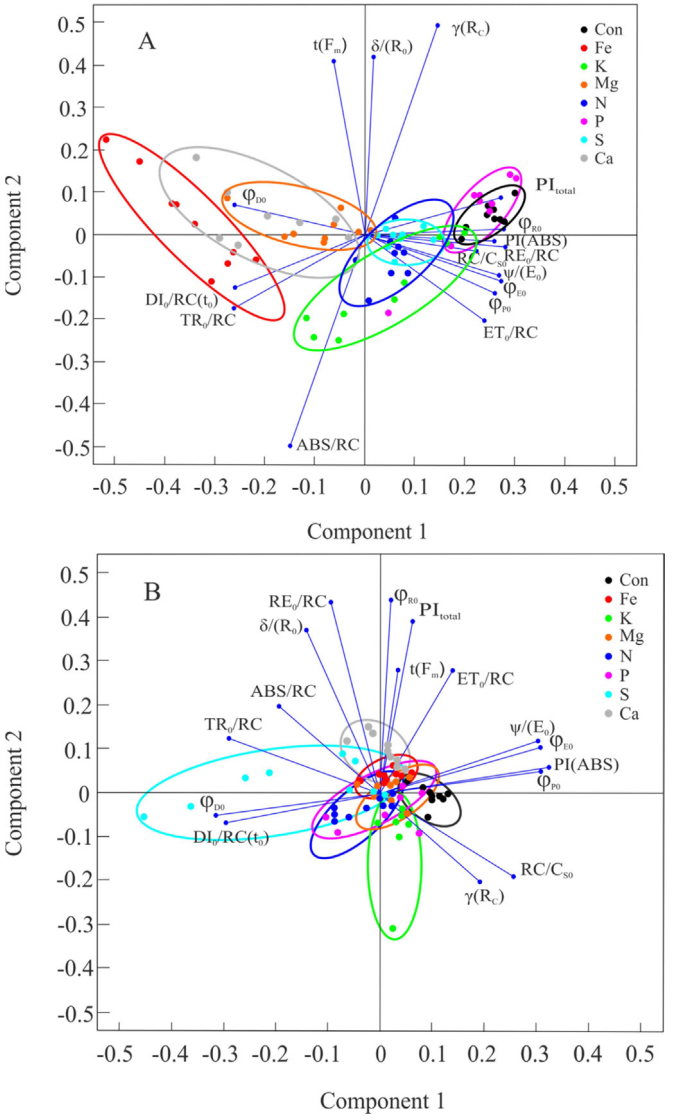

Fig. 4. Principal Component Analysis of variability of JIP-test parameters in leaves of maize (A) and tomato (B) plants grown at fertilizers' deficiency.

Table 6
Contribution of changes in different JIP test parameters into total variation of principal components in maize plants.

Parameter	PC 1	Parameter	PC 1	Parameter	PC 2	Parameter	PC 2
PI_{ABS}	0.28	φ_{Do}	−0.26	γ_{Rc}	0.49	TR_o/RC	−0.18
φ_{Ro}	0.28	DI_o/RC	−0.26	$t(F_m)$	0.49	ET_o/RC	−0.20
PI_{total}	0.28	TR_o/RC	−0.26	δ_{Ro}	0.42	ABS/RC	−0.49
φ_{Eo}	0.27						
ψ_{Eo}	0.27						
φ_{Po}	0.26						
RE_o/RC	0.26						
ET_o/RC	0.24						
RC/CS_o	0.22						

their values. Other 4 parameters do not have sufficient part in this component. The variations of the PC 2 are determined mainly due to 3 parameters in the positive direction and other 3 – in the negative direction. The sign of values in Table 6 shows what correlation exists between the parameters and principal components – positive or negative.

The stress induced variation in the investigated plants could be better visualized in 2D graph on a plane with Cartesian coordinates "Component 1" and "Component 2" (see Fig. 4). The samples are represented as points in the plane and their colour marks the experimental group the subject belongs. For maize plants the control group is positioned in a narrow region of the plane Comp 1/Comp 2 (Fig. 4A). The position of samples representing stressed plants is shifted and this shift is higher in more stressed objects. The deviations from control plants are mostly due to the reduction of the values of Comp 1 and to a minor extent – of Comp 2. Another important effect demonstrated in Fig. 4A, is that almost in all variants (except P deficiency) the stress resulted in a significant increase in the heterogeneity of the population in respect of the studied parameters.

For a better understanding of which processes and structures in photosynthetic machinery were affected by stress, we can apply a graphical approach – so-called biplot or dual graph, which describes the coordinates of points, reflecting the state of the investigated samples and simultaneously it shows vectors presenting observed variables (JIP parameters). These vectors give us information about the relative "contribution" of each variable to the formation of the principal components (Comp 1 and Comp 2). The direction and magnitude of the vector are indicators of this. Comparing these two plots, we can obtain information about the effects of nutrients.

3.3.1. Principal components analysis of maize plants

The sample distribution within Comp 1/Comp 2 plane is not homogeneous. For maize plants the samples distribution could be positioned into 5 relatively good separated clusters (Fig. 4A). The first cluster includes control group and the plants endured phosphorus deficiency. They are placed in region with positive values both of Comp 1 and Comp 2. It shows that phosphorus deficiency does not modify strongly the photosynthetic machinery as compare to control plants. The second cluster includes the samples with N, Mg and S deficiency that are distributed almost homogeneously around origin of the coordinate system. There is a slight shift of the points of N and S-deficient plants toward positive but those of Mg-deficient plant – toward negative direction. It means that despite the similarities in the fluorescence transients there are enough features that could be used as a fluorescence phenotype marker for distinguishing the samples within the group.

The third cluster is composed mainly of samples lacking K in plants and it is located in the negative region of Comp 1 and Comp 2. This means that the lack of K in maize can be easily determined by measuring the fluorescence.

The fourth and fifth clusters are formed by objects with a deficit of Fe and Ca, i.e. when maize lacks iron or calcium, plants have similar JIP-parameters and they are well separated from the others.

3.3.2. Analysis of principal components in tomato plants

As compare to maize, the tomato plants were more homogeneously distributed around the origin of the coordinate system and the disposal of individual variants are significantly overlapped (Fig. 4B). In these plants the contribution of the PC 1 into total stress induced variation is 54%, of PC 2–25% and PC 3–13%. The PC 1 and PC 2 reflect totally 79% of changes in tomato plants.

The formation of the first component is due to the changes in parameters reflecting the activity of PSII (see Table 7: PI_{ABS}; φ_{Po}; φ_{Eo}; ψ_{Eo}) and the second component is sensitive to parameters related to PSI activity (see Table 7: φ_{Ro}; δ_{Ro}; RE_o/RC; PI_{total} etc.). The parameters reflecting the concentration of reaction centres (RC/CS_o and γ_{Rc}) contributed to decrease in Comp 2.

4. Discussion

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). In this investigation, we recorded *in vivo* the chlorophyll *a* fluorescence transients to analyse the changes in light phase of photosynthesis in nutrient-deficient tomato and maize plants. Maize and tomato plants were grown hydroponically to determine possible effect of macronutrients (N, P, K, Mg, S and Ca) and micronutrient (Fe) deficiency on PSII and PSI function. The use of chlorophyll *a* fluorescence and related parameters to evaluate the effect of induced stress on photosynthetic machinery has gained considerable interest in recent years. Indeed, nutrient deficiency induced marked changes in the shape of the Chl *a* fluorescence induction curve. The photosynthetic response of maize and tomato plants to nutrient deficiency occurred at different sites of photosynthetic apparatus, and an inter-species variability was observed. The increased value of V_J and V_I parameters suggests the accumulation of reduced Q_A and plastoquinone, which cannot transfer electrons to the dark reactions. It is noteworthy that maize and tomato plants were differently affected by P-deficiency. P-deficiency in maize plants has no effect on all the studied photosynthetic parameters, on the contrary to P-deficient tomato plants. This deviation between P-deficiency effects is not based on different carbon metabolism of maize and tomato plants (C_4 and C_3, respectively) (Jacob and Lawlor, 1991) but probably partly due to species differences in operating P recycling mechanisms (Nanamori et al., 2004). The structural and functional parameters, deduced from the OJIP transients were evaluated. Differences between responses of both species were evident in the chlorophyll fluorescence parameter results. The results demonstrate the negative effect of ND on photosynthetic yield of PSII, reflected in reduction of the quantum

Table 7
Contribution of changes in different JIP test parameters into total variation of principal components in tomato plants.

Parameter	PC 1	Parameter	PC 1	Parameter	PC 2	Parameter	PC 2
PI_{ABS}	0.32	TR_o/RC	−0.28	φ_{Ro}	0.44	RC/CS_o	−0.19
φ_{Po}	0.30	DI_o/RC	−0.29	RE_o/RC	0.43	γ_{Rc}	−0.20
φ_{Eo}	0.30	φ_{Do}	−0.31	PI_{total}	0.39		
ψ_{Eo}	0.30			δ_{Ro}	0.37		
RC/CS_o	0.25			$t(F_m)$	0.28		
				ET_o/RC	0.28		

yield of PSII electron transport and the efficiency of excitation energy capture by open PSII reaction centres (φ_{Po}; φ_{Eo}; ψ_{Eo}), suggesting that ND induces some photoinhibitory damage to PSII (Baker and Rosenquist, 2004). This was suggested in previous studies where reduced PSII activity has been observed at nutrient deficiency conditions (Molassiotis et al., 2006; Redillas et al., 2011; Msilini et al., 2013). In addition, the γ_{Rc} decrease in both species caused a reduction of the amount of light harvesting complexes in PSII.

In this study, the decrease in the fraction of active RCs (estimated as an increase of ABS/RC) was observed in all nutrient deficient-plants. Then, a decrease in this parameter means that either a fraction of RCs is inactivated or the apparent antenna size increased. These changes were confirmed by a decrease of the active RCs per excited cross section (RC/CS). The inactivation of the RCs (non-Q_A reducing or heat sink centres) may be an indication of susceptibility to photoinhibition. In other word, the inactivation of RCs is considered a down-regulation mechanism, to dissipate the excess of absorbed light. Energy dissipation (φ_{Do}, DI_o/RC) is enhanced in order to protect nutrient-deficient leaves of both species from photo-oxidative damage and excess absorbed light energy was converted into heat dissipation. Nutrient deficiency resulted also in: a) damage of OEC, indicated by the appearance of a positive K-band at 300 μs; b) inactivation of some of the PSII RCs, suggested by an increase in ABS/RC; and c) decrease in both RC/ABS and RC/CSo. The re-reduction of P^{+}_{680} was markedly slower and the amplitude of the fastest delayed fluorescence component was highly suppressed as observed by DF decay in Ca-deficient maize than in the controls plants (data not shown). In the structure of the OEC, there are four manganese ions, one calcium ion, and five oxygen atoms; and calcium has also been identified as an essential cofactor in water oxidation and the calcium-binding sites in PSII (Najafpour et al., 2012). However, damage of OEC may be related to deficiency in the macronutrients. Nevertheless, response to Mg or Ca supply limitation was found to differ among maize and tomato plants. The data analysed in this paper suggest that change in the PSI end electron acceptors activity may represent parameters for monitoring nutrient deficiency effects on PSI. This can be in the form of the parameters RE/RC, RE/ABS and RE/ET changes. Decrease of PI_{abs} and $PI_{tot,}$ in conditions of ND suggested decrease in overall photosynthetic performance associated usually with decrease of leaf electron transport capacity. The differences in the response of both species to ND were also reflected by differential inhibition of PSI.

Recording of OJIP fluorescence transients in our experiments, followed by analysis with the JIP test, allowed the quantification of photosynthetic parameters that give insight into the changes occurring in PSII and PSI function. We observed clear differences in ND response between tomato and maize plants. Moreover, the application of PCA allows within a big set of JIP-test parameters to separate parameters according to their influence in the total plant stress response. It was shown that some groups of parameters seem to be sensitive to ND (see Tables 6 and 7) and could be used as a fluorescence phenotype marker. The PCA approach allows also to monitoring in specificity of the stress response in respect of the mineral deficiency type (see Fig. 4). It outlines good perspectives for the development of approaches and algorithms for rapid *in vivo* assessment of nutrient deficiency of mineral elements in the soil for crops, that could be based on constructed and trained artificial neural networks (Goltsev et al., 2012).

5. Conclusion

Deficiencies of individual nutrients affected significantly the photochemical processes of photosynthesis, as it was documented by a complex of parameters derived from chlorophyll *a* fluorescence transient recorded *in vivo*. Decrease of PSII photochemical efficiency associated with increase in non-photochemical dissipation and reduced number of active reaction centres were found almost in all samples affected by nutrient deficiency. However, there were some specific responses associated with limitation at PSII donor or acceptor side, including changes associated with activity of PSI or components at PSI acceptor side. The principal component analysis was employed to recognize nutrient deficiencies on the basis of chlorophyll fluorescence data. Surprisingly, we observed quite different responses to nutrient deficiencies in two plant species, which means that our attempt to create universal method was not successful. Nonetheless, our results suggest that the multiparametric approach for early detection of nutrient deficiency based on chlorophyll fluorescence data, including principal component analysis or similar method, might be useful, if the species-specific approach is designed.

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References

Abadía, J., 1992. Leaf responses to Fe deficiency. J. Plant Nutr. 15 (10), 1699–1713.
Baker, N.R., 2008. Chlorophyll fluorescence: a probe of photosynthesis *in vivo*. Annu. Rev. Plant Biol. 59, 89–113.
Baker, N.R., Rosenquist, E., 2004. Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. J. Exp. Bot. 55, 1607–1621.
Berry, W.L., Wallace, A., 1981. Toxicy: the concept and relationship to the dose response curve. J. Plant Nutr. 3, 13–19.
Brand, J.J., Becker, D.W., 1984. Evidence for direct roles of calcium in photosynthesis. J. Bioenerg. Biomembr. 16, 239–249.
Brestic, M., Zivcak, M., 2013. PSII fluorescence techniques for measurement of drought and high temperature stress signal in crop plants: protocols and applications. In: Rout, G.R., Das, A.B. (Eds.), Mol. Stress Physiol. Plants. Springer, pp. 87–131.
Brestic, M., Zivcak, M., Kalaji, H.M., Carpentier, R., Allakhverdiev, S.I., 2012. Photosystem II thermostability *in situ*: environmentally induced acclimation and genotype-specific reactions in *Triticum aestivum* L. Plant Physiol. Biochem. 57, 93–105.
Brestic, M., Zivcak, M., Olsovska, K., Shao, H.-B., Kalaji, H.M., Allakhverdiev, S.I., 2014. Reduced glutamine synthetase activity plays a role in control of photosynthetic responses to high light in barley leaves. Plant Physiol. Biochem. 81, 74–83. http://dx.doi.org/10.1016/j.plaphy.2014.01.002.
Ceppi, M.G., Oukarroum, A., Çiçek, N., Strasser, R.J., Schansker, G., 2012. The IP amplitude of the fluorescence rise OJIP is sensitive to changes in the photosystem I content of leaves: a study on plants exposed to magnesium and sulfate deficiencies, drought stress and salt stress. Physiol. Plant. 144, 277–288.
Ciompi, S., Gentili, E., Guidi, L., Soldatini, G.F., 1996. The effect of nitrogen deficiency on leaf gas exchange and chlorophyll fluorescence parameters in sunflower. Plant Sci. 118 (2), 177–184.
D'Hooghe, P., Escamez, S., Trouverie, J., Avice, J.-C., 2013. Sulphur limitation provokes physiological and leaf proteome changes in oilseed rape that lead to perturbation of sulphur, carbon and oxidative metabolisms. BMC Plant Biol. 13 (1), 23.
Delosme, R., 1967. Etude de l'induction de fluorescence des algues vertes et des chloroplastes au debut d'une illumination intense. Biochim. Biophys. Acta 143, 108–128.
Evans, J., Terashima, I., 1987. Effects of nitrogen nutrition on electron transport components and photosynthesis in spinach. Funct. Plant Biol. 14 (1), 59–68.
Goltsev, V., Zaharieva, I., Chernev, P., Kouzmanova, M., Kalaji, H.M., Yordanov, I., Krasteva, V., Alexandrov, V., Stefanov, D., Allakhverdiev, S.I., Strasser, R.J., 2012. Drought-induced modifications of photosynthetic electron transport in intact

leaves: analysis and use of neural networks as a tool for a rapid non-invasive estimation. Biochim. Biophys. Acta 1817 (8), 1490–1498.
Goltsev, V., Zaharieva, I., Chernev, P., Strasser, R.J., 2009. Delayed fluorescence in photosynthesis. Photosynth. Res. 101, 217–232.
Guisse, B., Srivastava, A., Strasser, R.J., 1995. The polyphasic rise of the chlorophyll *a* fluorescence (O-K-J-I-P) in heat-stressed leaves. Arch. Sci. Geneve 48, 147–160.
Hermans, C., Delhaye, J.P., Strasser, R.J., 2001. Magnesium deficiency in sugar beets probed by optical measurements *in vivo*. In: Paper presented at the Congress on Photosynthesis Brisbane, Australia, August 18–23.
Jacob, J., Lawlor, D., 1991. Stomatal and mesophyll limitations of photosynthesis in phosphate deficient sunflower, maize and wheat plants. J. Exp. Bot. 42 (8), 1003–1011.
Jolliffe, I.T., 2002. Graphical representation of data using principal components. In: Jolliffe, I.T. (Ed.), Principal Component Analysis, Springer Series in Statistics. Springer, New York, pp. 78–110.
Kalaji, H., Govindjee, Goltsev, V., Bosa, K., Allakhverdiev, S.I., Strasser, R., 2012. Experimental *in vivo* measurements of light emission in plants: a perspective dedicated to David Walker. Photosynth. Res. 114, 69–96.
Kalaji, M.H., Govindjee, Bosa, K., Koscielniak, J., Zuk-Golaszewska, K., 2011. Effects of salt stress on photosystem II efficiency and CO_2 assimilation of two Syrian barley landraces. Environ. Exper. Bot. 73, 64–72.
Laing, W., Greer, D., Sun, O., Beets, P., Lowe, A., Payn, T., 2000. Physiological impacts of Mg deficiency in *Pinus radiata*: growth and photosynthesis. New. Phytol. 146 (1), 47–57.
Lu, C., Zhang, J., Zhang, Q., Li, L., Kuang, T., 2001. Modification of photosystem II photochemistry in nitrogen deficient maize and wheat plants. J. Plant Physiol. 158 (11), 1423–1430.
Ludwig, B., Geisseler, D., Michel, K., Joergensen, R., Schulz, E., Merbach, I., Raupp, J., Rauber, R., Hu, K., Niu, L., 2011. Effects of fertilization and soil management on crop yields and carbon stabilization in soils. Agron. Sustain. Dev. 31, 361–372.
Molassiotis, A., Tanou, G., Diamantidis, G., Patakas, A., Therios, I., 2006. Effects of 4-month Fe deficiency exposure on Fe reduction mechanism, photosynthetic gas exchange, chlorophyll fluorescence and antioxidant defense in two peach rootstocks differing in Fe deficiency tolerance. J. Plant Physiol. 163 (2), 176–185.
Msilini, N., Essemine, J., Zaghdoudi, M., Harnois, J., Lachaâl, M., Ouerghi, Z., Carpentie, R., 2013. How does iron deficiency disrupt the electron flow in photosystem I of lettuce leaves? J. Plant Physiol. 170 (16), 1400–1406.
Najafpour, M.M., Moghaddam, A.N., Allakhverdiev, S.I., 2012. Biological water oxidation: lessons from Nature. Biochim. Biophys. Acta 1817 (8), 1110–1121.
Nanamori, M., Shinano, T., Wasaki, J., Yamamura, T., Rao, I.M., Osaki, M., 2004. Low phosphorus tolerance mechanisms: phosphorus recycling and photosynthate partitioning in the tropical forage grass, brachiaria hybrid cultivar mulato compared with rice. Plant Cell. Physiol. 45 (4), 460–469.
Neubauer, C., Schreiber, U., 1987. The polyphasic rise of chlorophyll fluorescence upon onset of strong continuous illumination: I. Saturation characteristics and partial control by the photosystem II acceptor side. Z. Naturforsch. 42c, 1246–1254.
Osman, K.T., 2013. Plant nutrients and soil fertility management. In: Osman, K.T. (Ed.), Soils. Springer, pp. 129–159.
Pieters, A.J., Paul, M.J., Lawlor, D.W., 2001. Low sink demand limits photosynthesis under Pi deficiency. J. Exp. Bot. 52 (358), 1083–1091.
Qu, C., Liu, C., Gong, X., Li, C., Hong, M., Wang, L., Hong, F., 2012. Impairment of maize seedling photosynthesis caused by a combination of potassium deficiency and salt stress. Environ. Exp. Bot. 75, 134–141.
Redillas, M.C.R., Jeong, J.S., Strasser, R.J., Kim, Y.S., Kim, J.-K., 2011. JIP analysis on rice (*Oryza sativa* cv Nipponbare) grown under limited nitrogen conditions. J. Korean Soc. Appl. Biol. Chem. 54 (5), 827–832.
Richardson, A.D., Duigan, S.P., Berlyn, G.P., 2002. An evaluation of noninvasive methods to estimate foliar chlorophyll content. New. Phytol. 153 (1), 185–194.
Rochaix, J.-D., 2011. Assembly of the photosynthetic apparatus. Plant Physiol. 155 (4), 1493–1500.
Schansker, G., Srivastava, A., Govindjee, Strasser, R.J., 2003. Characterization of the 820-nm transmission signal paralleling the chlorophyll *a* fluorescence rise (OJIP) in pea leaves. Funct. Plant Biol. 30 (7), 785–796.
Schansker, G., Toth, S., Strasser, R.J., 2006. Dark recovery of the Chl *a* fluorescence transient (OJIP) after light adaptation: the q_T-component of non-photochemical quenching is related to an activated photosystem I acceptor side. Biochim. Biophys. Acta 1757, 787–797.
Schansker, G., Tóth, S.Z., Strasser, R.J., 2005. Methylviologen and dibromothymoquinone treatments of pea leaves reveal the role of photosystem I in the Chl *a* fluorescence rise OJIP. Biochim. Biophys. Acta 1706 (3), 250–261.
Smethurst, C.F., Garnett, T., Shabala, S., 2005. Nutritional and chlorophyll fluorescence responses of lucerne (*Medicago sativa*) to waterlogging and subsequent recovery. Plant Soil. 270 (1), 31–45.
Stirbet, A., Govindjee, 2011. On the relation between the Kautsky effect (chlorophyll *a* fluorescence induction) and photosystem II: basics and applications of the OJIP fluorescence transient. J. Photochem. Photobiol. B: Biol. 104 (1–2), 236–257.
Strasser, B., Strasser, R., 1995. Measuring fast fluorescence transients to address environmental questions: the JIP-test. In: Garab, G. (Ed.), Photosynthesis: From Light to Biosphere, vol. 5, pp. 977–980.
Strasser, R.J., Tsimilli-Michael, M., Qiang, S., Goltsev, V., 2010. Simultaneous *in vivo* recording of prompt and delayed fluorescence and 820-nm reflection changes during drying and after rehydration of the resurrection plant *Haberlea rhodopensis*. Biochim. Biophys. Acta 1797, 1313–1326.
Strasser, R.J., Tsimilli-Michael, M., Srivastava, A., 2004. Analysis of the chlorophyll *a* fluorescence transient. In: Papageorgiou, G., Govindjee (Eds.), Advances in Photosynthesis and Respiration. Chlorophyll *a* Fluorescence: a Signature of Photosynthesis. Springer, Dordrecht, The Netherlands, pp. 321–362.
Živčák, M., Brestič, M., Olšovská, K., Slamka, P., 2008. Performance index as a sensitive indicator of water stress in *Triticum aestivum L.* Plant Soil. Environ. 54 (4), 133–139.
Zivcak, M., Kalaji, H.M., Shao, H.B., Olsovska, K., Brestic, M., 2014. Photosynthetic proton and electron transport in wheat leaves under prolonged moderate drought stress. J. Photochem. Photobiol. B: Biol. http://dx.doi.org/10.1016/j.jphotobiol.2014.01.007.