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Structural and functional response of photosynthetic apparatus of radish plants to iron deficiency

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Abstract

In this work, we tried to identify some specific chlorophyll *a* fluorescence (ChlF) parameters, that could enable detection of iron deficiency (Fe_{def}) in radish plants (*Raphanus sativus* L.), before any visual symptoms appear. Changes in ChlF kinetics, JIP-test parameters, and chlorophyll content revealed that iron deficiency negatively affected PSII activity mainly *via* disruption of light absorption in light-harvesting complexes and by decreasing the activity of the primary quinone acceptor of PSII (Q_A). Iron deficiency was clearly reflected in the changes of some JIP-test parameters, such as time to reach maximal fluorescence (F_M), Area, normalized total area under the OJIP curve, and number of Q_A redox turnovers until F_M is reached. The visible symptoms of Fe_{def} appeared after 7 d of stress application, while ChlF measurements allowed us to detect iron deficiency during 1–3 d. Our results suggest that analysis of ChlF signals has a high potential for early detection of iron deficiency in radish plants.

Additional key words: nutrient deficiency; OJIP curves; photosynthetic efficiency; plant physiological status; principal component analysis.

Introduction

Under natural environments, plants are exposed to various environmental stresses simultaneously, *e.g.*, changes in temperature, light spectrum and intensity, and various combinations of macro- and micronutrients deficiency (Goltsev *et al.* 2012, 2016; Mathur *et al.* 2016, 2018; Kalaji *et al.* 2018). Nutrients availability in growth medium is among the most important factors that affect plant growth and development. Their deficiency estimation/identification is

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Abbreviations: A_M – total complementary area between the fluorescence induction curve; Chl – chlorophyll; CS – cross section; DF_{abs} – driving forces of PSII; DF_{total} – driving force of photosynthesis calculated on cross section basis; DI_0/CS_0 – dissipated energy flux per cross section at t = 0; DI_0/RC – dissipated energy flux per reaction center at t = 0; ET_0/CS_M – electron transport flux per cross section; ET – electron transport; ETC – electron transport chain; F_0 – fluorescence at time 0; F_0/F_M – quantum yield (at t = 0) of energy dissipation; Fe_{def} – iron deficiency; F_M – maximal fluorescence recorded under saturating illumination at the peak P of OJIP, when all PSII RCs are closed; F_t – fluorescence at time t; F_V – maximum variable fluorescence; F_V/F_0 – ratio of photochemical to nonphotochemical quantum efficiencies; F_V/F_M – maximum quantum yield of primary PSII photochemistry; N – number of Q_A redox turnovers until F_M is reached; P_{2G} – grouping probability, takes into account all possible ways of energetic communication between neighbouring PSII core antenna; PI_{abs} – performance index on absorbance basis; PI_{total} – performance index, the performance of electron flux to the final PSI electron acceptors; Q_A – primary quinone acceptor of PSII; RC – reaction center; $S_M/t_{(FM)}$ – measure of the average excitation energy of open RCs from time 0 to $t_{(FM)}$, that is the time needed to obtain total RC closure; TR_0/RC – trapped energy flux per reaction center; $t_{(FM)}$ – time to reach maximal fluorescence (F_M); V_K – relative variable fluorescence at K-step (300 μs, K-band); V_L – relative variable fluorescence at L-step (150 μs, L-band); V_t – relative variable fluorescence at time t; $W_{(E)}$ – model-derived value of relative variable fluorescence in 100 ms calculated for unconnected PSII units; $\varphi_{(Eo)}$ – quantum yield of electron transport; $\varphi_{(Ro)}$ – quantum yield of electron transport flux until PSI electron acceptors; Ψ_0 – probability of an electron to reach the electron transport chain outside Q_A^-.
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usually based on the presence of visual alterations, which appear too late and indicate a substantial damage of the plants.

Iron plays an essential role in plant biochemistry, mainly in energy transformation needed for syntheses and other physiological processes, such as photosynthesis, respiration, nitrogen fixation, and uptake mechanisms (Kabata-Pendias 2011). Moreover, it takes part in DNA synthesis through the action of the ribonucleotide reductase (Reichard 1993). It belongs to an active cofactor of many enzymes that are necessary for plant hormone synthesis, such as ethylene, lipoxygenase, 1-aminocyclopropane acid-1-carboxylic oxidase (Siedow 1991), or abscisic acid (compounds that are active in many plant development pathways and their adaptive responses to fluctuating environment conditions). Iron (Fe) absorbed by plants is one of the major sources for human and animal nutrition (Abadía 1992, Briat *et al.* 2015). Its deficiency is one of the most common shortages of micronutrients globally, constituting a problem for agriculture by limiting crop yields and posing a serious threat to public health (Finkelstein *et al*. 2017). Most of Fe-deficient soils occurred in arid climate on calcareous soils (Kabata-Pendias 2011). The main reason of Fe deficiency is its low accessibility to plants, especially on neutral and alkaline soils (Zuo and Zhang 2011). Iron deficiency (Fe_{def}) results in characteristic visual symptoms – interveinal chlorosis mainly in young leaves (Chen *et al.* 2015). Plants could absorb Fe^{2+} and Fe^{3+} species as well as Fe chelate, while the main process of iron absorption is a reduction of Fe^{3+} to Fe^{2+} in roots. Many antagonistic relationships of Fe with other microelements, such as Mn, Ni, Co, Zn, Si, and Se, were observed in plants. Moreover, Ca can suppress the Fe absorption (Kabata-Pendias 2011).

Photosynthesis is a very sensitive process, which can be disturbed by any stressor, including nutrients deficiency (Kalaji *et al.* 2017a, 2018). Although, there are many studies related to the effect of plant mineral status on photosynthetic apparatus (PSA) functioning and performance (Kalaji *et al.* 2016, 2018), detailed mechanisms of this effect are still not well-known. Usually, the first visible stress symptoms in plants appear when they already have significant changes in the structure and functioning of photosynthetic apparatus (Kalaji *et al.* 2014b, 2017a). These changes are often irreversible, especially those related to electron transport chain. Therefore, there is a strong need to elaborate a low-cost and noninvasive method for early nutrient deficiency detection.

Chlorophyll (Chl) *a* fluorescence is a quick, reliable tool for *in vivo* assessment of bioenergetic status of plants (Baker 2008, Cetner *et al.* 2017, Kalaji *et al*. 2017a, Samborska *et al.* 2018, 2019; Bąba *et al.* 2019). The changes in fluorescence signals are related, directly or indirectly, to various stages of photosynthetic light reactions: photolysis of water, reduction of photosynthetic machinery components, electron transport, generation of the pH gradient across thylakoid membranes, and ATP synthesis (Goltsev *et al.* 2016, Kalaji *et al.* 2017a,b). The technique based on detailed analysis of Chl *a* fluorescence signals is known as JIP-test (Strasser *et al.* 2004), which is frequently used to study the changes in structure and function of photosynthetic apparatus (Kautsky and Hirsch 1931, Stirbet and Govindjee 2011, Kalaji *et al*. 2014a,b; Chen *et al.* 2015, Bąba *et al.* 2016, Goltsev *et al.* 2016, Kalaji *et al.* 2017a). Illumination of dark-adapted leaf tissue leads to an increase of a prompt Chl fluorescence (PF) signal, which curves are named as chlorophyll fluorescence induction curves. They are based on the theory of energy flux in thylakoid membranes (Strasser *et al.* 2000). The name of the JIP-test originates from the specific points on the curves of Chl *a* fluorescence signal (Stirbet and Govindjee 2012, Tsimilli-Michael and Strasser 2013). JIP-test parameters have been categorized into four groups: data extracted from the recorded fluorescence transient, quantum yields and probabilities, vitality indices, and energy fluxes, which are divided in phenomenological and specific ones (Strasser *et al.* 2000, 2004; Goltsev *et al.* 2016).

In this work, we executed an experimental procedure to identify some specific JIP-test parameters obtained by measuring Chl *a* fluorescence, which enable us to detect early Fe_{def} in radish plants at early growth stages, before appearance of any visual symptoms. For better understanding, we conducted additional chlorophyll content measurements on plants under Fe_{def}.

Materials and methods

Plant material: Radish is a common root vegetable plant from Brassicaceae family, closely related to the model plant *Arabidopsis thaliana* (L.) Heynh., characterized by short life cycle (6 weeks) and low cultivation demands and its genome is already sequenced (Kitashiba *et al.* 2014). Two hybrid cultivars of radish plants (*Raphanus sativus* L.) were used: 'Fluo HF1' and 'Suntella F1'. However, since responses of both cultivars to the applied stressor (Fe_{def}) were similar (no significant statistical differences were found between the two cultivars), in the subsequent analyses, we used the calculated average values obtained from both of them.

Growing conditions: Plants were grown under controlled conditions in a growth chamber in hydroponic system filled with modified Hoagland solution (Hoagland and Arnon 1950), which allowed precise controlling of the mineral composition and concentrations. Radish plants were grown either on full solution (control, Cs) or on iron-deficient solution (Fe_{def}). Both solutions were developed and modified according to procedure described by Hoagland and Arnon (1950). Fe_{def} was generated by deprivation of iron ions, while leaving the other components as in control solution concentration. Their detailed composition is presented in the text table.

Polyethylene pellets (PE), made from a synthetic resin, were used as root growth medium. Comparing to other growth substrates, it is characterized by a very low ion absorption capacity (innovative method by Cetner *et al.* 2017, Samborska *et al.* 2018, 2019). Radish seeds were germinated for 7 d and after that sprouts were moved into plastic multi-pot trays (48 seedlings per tray: 24 of 'Fluo HF1' and 24 of 'Suntella F1' cultivars) filled with PE. The

Component		Molar mass [g mol^{-1}]	Molar concentration of a stock solution [M]	Amount of stock solution to prepare 1 L of nutrient solution [ml]
Control solution				
1	1 M KNO_3	101.10	1.0	3.5
2	1 M $Ca(NO_3)_2$•$4H_2O$	236.15	1.0	4.0
3	1 M NaH_2PO_4	119.98	1.0	2.0
4	1 M $MgSO_4$•$7H_2O$	246.47	1.0	2.0
5	1 M KCl	74.55	1.0	1.0
6	1 M K_2SO_4	174.26	0.5	2.0
7	iron chelate	-	-	2.0
8	micronutrient	-	-	1.0
Iron-deficient solution				
1	1 M KNO_3	101.10	1.0	3.5
2	1 M $Ca(NO_3)_2$•$4H_2O$	236.15	1.0	4.0
3	1 M NaH_2PO_4	119.98	0.5	1.5
4	1 M $MgSO_4$•$7H_2O$	246.47	1.0	2.0
5	1 M KCl	74.55	1.0	1.0
6	1 M K_2SO_4	174.26	0.5	1.5

trays were set on the top of six plastic containers filled with 20 L of aerated modified Hoagland solution (control solution).

The experiment consisted of three phases. During the first phase, which lasted 16 d (measuring Chl fluorescence at term: t_0), all plants grown on control solution. During the next phase that lasted 13 d, Fe_{def} was applied to plants of three containers (half of the experimental set), while the other three containers were kept on a full (control) solution (measuring Chl fluorescence at terms: t_1–t_5). During the last 11 d of the experiment (recovery phase), the control solution was applied again to all the plants (measuring Chl fluorescence at terms: t_6–t_7) (Fig. 1).

Altogether, plants were grown for 43 d. During this time, in particular treatments, all solutions were changed every week to avoid nutrient depletion (Fig. 1). The pH of the nutrient solutions in both treatment was maintained between 5–6. The photoperiod was 14-h and day/night air temperature was 18/13°C, respectively. PAR was about 250 µmol(photon) m^{-2} s^{-1}. Average (24 h) relative air humidity was about 50%.

Visual symptoms: All the plants were visually checked three times a day and presence of any visible symptoms of the Fe deficiency, on leaves, stalks or roots were spotted.

Chl content was measured *in vivo* by a portable meter *Dualex* (*Force-A Inc*., France). Measurements were conducted three times during germination (at 0, 4, 11 d), two times during stress phase (4 and 11 d), and two times during the recovery phase (at 17 and 24 d). Measurements were performed with eight technical replicates, on the same leaves chosen for Chl *a* fluorescence measurements for both Cs and Fe-deficient plants.

Chlorophyll fluorescence (ChlF) measurements were done *in vivo* at the middle part of leaf blade, every time on the same leaves. Twenty plants were chosen randomly from one container before introducing the stress phase. Leaves were dark-adapted for 20–30 min, using leaf clips attached in the middle part of the leaf. The measurements were repeated by keeping 2–5-d intervals. The first measurement was done before stress phase (t_0), then repeated five times during stress phase (t_1, t_2, t_3, t_4, t_5), and two times during recovery phase (t_6, t_7). The experimental timetable is described in Fig. 1. Prompt fluorescence measurements were recorded after sample illumination with red actinic light intensity of *ca*. 2,500 µmol(photon) m^{-2} s^{-1} using *Handy PEA* fluorometer (*Hansatech Instruments*, *Ltd*., UK). From the measured primary parameters (F_0, F_m, V_K, V_L, t_{FM}), the secondary ones, *i.e.* specific energy fluxes per one PSII reaction center (RC), quantum yields and efficiencies, phenomenological energy flux per excited cross section (CS), and performance indexes (PI_{total} and

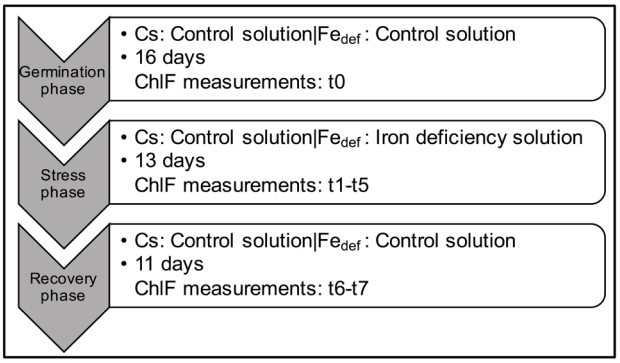

Fig. 1. The experiment schedule with germination, stress, and recovery phases showing the duration and treatments applied (Cs – control, Fe_{def} – iron deficiency) during each phase. During germination phase and recovery phase, radish plants were grown in full solutions. The stress phase started by introducing solutions without iron on 17 d of vegetation (t_1).

PI_{abs}), were calculated by JIP-test described in Table 2S, *supplement* (Tsimilli-Michael and Strasser 2007, Strasser *et al.* 2004).

Statistical analysis: To estimate the significance of differences between average values of particular ChlF parameters in the control (t_0) *vs.* Fe_{def} plants (t_1–t_5) and recovery (t_6–t_7), *Student*'s *t*-test was applied. The significance of differences in average values of Chl content was tested with one-way *ANOVA*. The null hypothesis on lack of differences between means was rejected at $p<0.01$. In order to gain insight into the changes in ChlF parameters in Cs and Fe_{def} radish plant, PCA analysis was performed by *IBM SPSS Statistics ver. 23* for *Windows*.

Results

Visual symptoms: The first visual symptoms were observed on younger leaves, because iron is weakly reutilized micronutrient. The first visual symptoms were observed after 23 d of plant vegetation and 7 d after stress treatment introduction (t_3). Plants grown under Fe_{def} conditions were smaller than control plants and young leaves were less green (more yellow). Leaf veins became brighter and later almost white (Fig. 2).

Chl content: The data for relative Chl content are shown in Fig. 3. During whole experimental cycle (from the 3rd d of stress application), significantly lower values of Chl content were observed in Fe-deficient plants in comparison with control plants. However, during the recovery period, these values were similar in both control and Fe_{def} plants (Fig. 3).

Prompt fluorescence induction curves: The differential ChlF induction curves were calculated to evaluate the influence of Fe_{def} on PSA. Each differential curve value was calculated as a difference between the values of the relative variable fluorescence [$V_t = (F_t - F_0)/(F_M - F_0)$] recorded in the Fe-deficient plants minus the respective values for the control plants at a given time period t_0–t_7, [*i.e.*, for t_0 period: $\Delta V_{t(t0)} = V_{t(t0)}(-Fe) - Vt_{(t0)}(Cs)$]

Fig. 2. The radish plant growing under control conditions (*left*) and iron deficiency (*right*) at the end of the stress phase (t_5). The visual symptoms were observed on younger leaves.

(Samborska *et al.* 2019).

The Fe deficit-induced alterations were clearly visualized by differential curves. The differential curves showed peaks at: L (0.2 ms), K (0.3 ms), J (around 2 ms), I (around 5 ms) points, and H (10–20 ms), and more pronounced peak G (around 100 ms). This confirmed the strong and complex impact of Fe_{def} on almost all light energy transfer and conversion during the light-dependent phase of photosynthesis (Fig. 4).

Principal component analysis: The selected JIP-test parameters are shown in two PCA figures (Figs. 5, 6), where

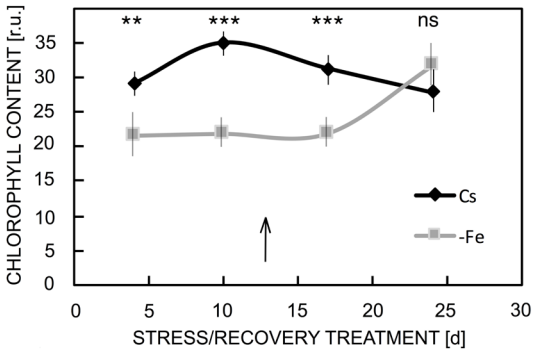

Fig. 3. Dynamics of chlorophyll content in leaves in *Raphanus sativus* L. for both cultivars (averaged values for 'Fluo HF1' and 'Suntella F1') under control condition (Cs) and iron-deficiency (Fe_{def}) stress. The stress and recovery phase (*arrow*) is shown. The asterisks are related to statistical significance level of differences between Cs and Fe_{def} plants according to analysis of variance (*ANOVA*): ns – not significant ($p \geq 0.05$), ** – $0.001 < p < 0.01$; *** – $p < 0.001$. The values are means ± SE.

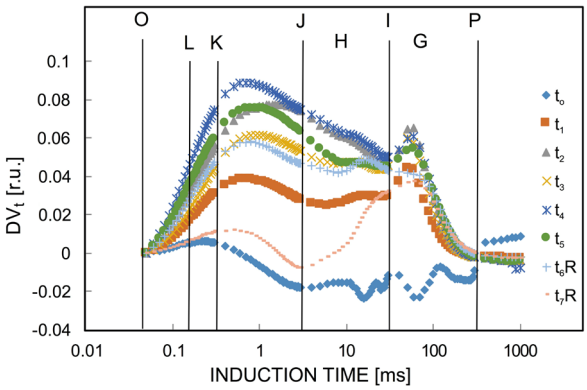

Fig. 4. Effect of iron deficiency on the shape of ChlF transients normalized between the induction phases 'O' (20 µs) and 'P' (300 ms) measured on radish plants during the stress phase (t_1–t_5) and after transferring the plants back to a full nutrient medium (recovery phase, t_6– t_7). Each point is an averaged value recorded from 60–118 samples. The differential Chl fluorescence induction curves were calculated to evaluate the influence of Fe_{def} on photosynthetic apparatus. Each differential curve value was calculated as a difference between the values of the relative variable fluorescence [$V_t = (F_t - F_0)/(F_M - F_0)$] recorded in the Fe-deficient plants minus the respective values for the control plants at a given time period t_0–t_7.

PC1 is on the bottom, on the *x* axis. The position of points, related to a particular ChlF parameter on the PCA biplot in Fig. 5*A* and *B* is determined by the PC1–PC3 values, calculated on the basis of all values of that parameter. On the other hand, the point, related to a given measurement is outcome of all values of ChlF parameter, recorded at that time (Fig. 5).

The PC1, which explains nearly a half of all variability in PCA, reflected the temporal changes in ChlF parameters during the experiment, while the PC2, which explained 21.8% of variability is related to the differences between control and Fe_{def} plants. Fe_{def} conditions imposed on plants during the t_1–t_5 period resulted in a strong increase the value of some ChlF parameters, such as antenna organization and electron transport rate, number of active RCs, and energy dissipation DI_0/RC and DI_0/CS_0. However, the maximal differences between control and Fe-deficient plants were observed at the end of stress phase (t_4–t_5) (Fig. 5).

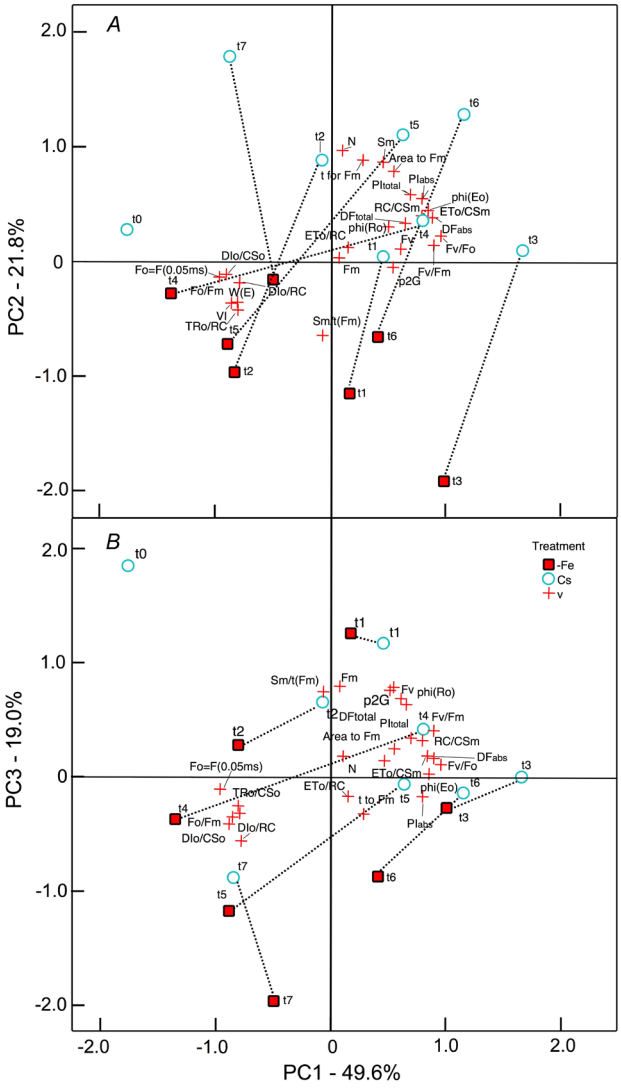

Fig. 5. Dynamics in biplots for parameters (PC1 and PC2) of chlorophyll fluorescence for control (*circles*) and iron-deficient (*squares*) radish plants during stress phase and recovery phase (*A*). *Red cross symbol* (+) describes chlorophyll fluorescence parameters, which are correlated with principal components (PC1 and PC3). Significant differences between control and iron deficiency appeared after 5 d of iron-deficiency stress (t_2) (*B*).

During the whole study period, the control plants were characterized by higher density of RC per sample area, and higher values of Area, S_M, PSII efficiency (F_V/F_M), PI_{abs}, PI_{total}, and other ChlF parameters, indicating the higher efficiency of PSII in comparison to Fe-deficient plants.

During the experimental period, values of ChlF parameters were not constant and depended on plant age as indicated by fluctuations of ChlF parameters even in the control plants. Their reactions were differentiated during the period of growth and development, especially, when senescence started.

In order to find the parameters most sensitive to Fe-deficiency stress, we compared the number of statistically significant ($p<0.01$) ChlF parameters between control and Fe-deficient plants during the stress t_1–t_5 and recovery t_6–t_7 periods. Shortly after stress application (t_1), the Cs and Fe_{def} plants differed in 17 ChlF parameters, and this value increased up to 22 in t_2 and to 23 in t_3–t_5 period. During the recovery phase, the strong decrease of 20 ChlF parameters at the t_6 and only nine at the t_7 period was observed. Among ChlF parameters studied we found that F_0, F_V, F_0/F_M, F_V/F_M, F_V/F_0, DI_0/CS_0, DI_0/RC, DI_0/CS_0, ET_0/CS_M, PI_{abs}, PI_{total}, DF_{abs}, DF_{total} were sensitive to Fe-deficiency stress and changes of their values followed the control–stress–recovery treatments.

The data from JIP-test obtained only for Fe_{def} plants were also compared with control plants (t_0). Results are presented on radar plot (Fig. 6). Studied parameters showed various changes during the whole vegetative period. The most significant changes in JIP-test parameters were observed at the end of stress phase (t_5) and at the end of experimental cycle, during recovery phase (t_6, t_7). DF_{total} and PI_{total} parameters decreased significantly under Fe_{def}. The highest increases were observed in the case of DI_0/RC, V_L, and $W_{(E)}$ parameters, reflecting antenna organizational changes, and DF_{total} parameter, which were strongly correlated with PC2 (Figs. 5, 6).

Discussion

In this work, we used a combination of the Chl content measurements, elaborated prompt fluorescence differential curves, and application of multivariate analyses (PCA) in order to find parameters that can be used for early detection of changes related to iron-deficiency stress in photosynthetic apparatus of radish plants before any visual stress symptoms appear. Our results showed that the 14-d period of Fe-deficiency stress had a strong and significant impact on growth and development of radish plants. Although iron is not a component of Chl molecule itself, it is essential for Chl synthesis and stabilization of chloroplast structure and function and approximately 80% of Fe is deposited in photosynthetic cells (Kruk and Szymańska 2012, Connorton *et al.* 2017). The iron and Chl contents in plant leaves are thus often correlated (Rout and Sahoo 2015). We confirmed a significantly lower total Chl content in leaves of Fe_{def} plants during the

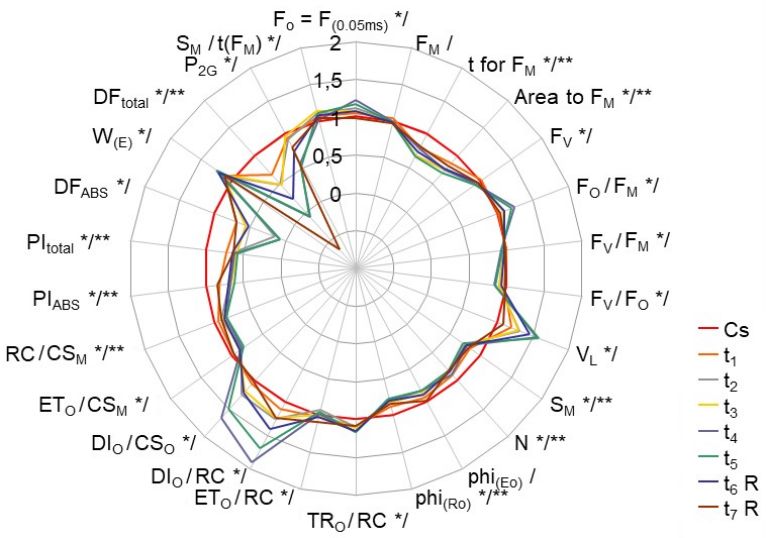

Fig. 6. The dynamics of JIP-test parameters (selected by coefficient of variation) between iron-deficient and control (Cs) plants in time (t_1–t_7) presented in radar plot. Significant differences, based on *Student*'s *t*-tests, between t_0 and the end of stress phase (t_4–t_5) are marked by * ($p<0.01$) and significant differences between t_0 and the recovery phase (t_6–t_7) are marked by ** ($p<0.01$).

Table 1. Temporal changes in selected ChlF parameters during the control conditions (full Hoagland solution), Fe-deficiency stress (full Hoagland solution without Fe), and recovery period (full Hoagland solution) in radish plants. ●, ↑, ↓ – no difference, significantly higher, and significantly lower average value ($p<0.01$) of ChF parameters in Fe-deficient radish plant as compared with control, respectively.

ChlF parameters	Control	Fe-deficiency stress					Recovery	
	t_0	t_1	t_2	t_3	t_4	t_5	t_6	t_7
F_0	●	↑	↑	↑	↑	↑	●	●
$t_{(FM)}$	●	↓	↓	↓	↓	↓	↓	↓
A_M	●	↓	↓	↓	↓	↓	↓	↓
F_V	●	●	●	↓	↓	↓	↓	●
F_0/F_M	●	●	↑	↑	↑	↑	↑	●
F_V/F_M	●	●	↓	↓	↓	↓	↓	●
F_V/F_0	●	●	●	↓	↓	↓	↓	●
V_L	●	↑	↑	↑	↑	↑	↑	●
S_M	●	↓	↓	↓	↓	↓	↓	↓
N	●	↓	↓	↓	↓	↓	↓	↓
$\varphi_{(Eo)}$	●	↓	↓	↓	↓	↓	↓	●
$\varphi_{(Ro)}$	●	↓	↓	↓	↓	↓	↓	↓
TR_0/RC	●	↑	↑	↑	↑	↑	↑	↑
ET_0/RC	●	●	↓	●	●	●	●	↑
DI_0/RC	●	↑	↑	↑	↑	↑	↑	●
DI_0/CS_0	●	●	↑	↑	↑	↑	●	●
ET_0/CS_M	●	●	↓	↓	↓	↓	↓	●
RC/CS_M	●	↓	↓	↓	↓	↓	↓	↓
PI_{abs}	●	↓	↓	↓	↓	↓	↓	●
PI_{total}	●	↓	↓	↓	↓	↓	↓	●
DF_{abs}	●	↓	↓	↓	↓	↓	●	●
DF_{total}	●	↓	↓	↓	↓	↓	↓	●
$W_{(E)}$	●	↑	↑	↑	↑	↑	↑	↑
$S_M/t_{(FM)}$	●	↑	↑	↑	●	↓	●	●
P_{2G}	●	●	●	●	↓	●	↓	●

whole stress phase and its increase during the recovery phase. The first visual symptoms were observed 7 d after stress introduction. However, the significant decrease of Chl content in Fe-deficient plants was detected already 3 d after stress application, confirming that Chl content could be a sensitive indicator of Fe_{def} in radish plants.

Iron is a part of Fe-S proteins, distributed across thylakoid membrane: cytochrome b_6f complex (Rieske protein), ferredoxins, and PSI, which are involved in electron transport chain in light-dependent phase of photosynthesis

(Briat *et al.* 2015). The cytochrome b_6f complex catalyses the transfer of electrons between plastoquinol and plastocyanin, while the ferredoxin takes part in electron transport beyond the PSI and transfers electrons to $NADP^+$ reductase (Blankenship 2014). Moreover, iron takes part in oxygen transport or regulation of protein stability (Connorton *et al.* 2017). Therefore, the shape of ChlF curves as well as some JIP-test ChlF parameters were affected by iron deficiency reflecting its strong and complex negative impact on the structure of photosynthetic apparatus. This could be explained by increasing Chl *a*/*b* ratio and decrease in antenna size, changes in their organization, and light-harvesting efficiency (M'sehli *et al.* 2014).

The effect of decreasing Fe content in plant tissue was detectable when we compared the shape of prompt fluorescence differential curves of control and Fe-deficient radish plants. The positive appearance of L-band showed the effects of iron deficiency on PSII connectivity. This can indicate the possible physiological role of this nutrient in connectivity changes in photoprotection as shown in shade-acclimated plants (Živčák *et al.* 2014). Moreover, the appearance of K-band indicated ungrouping reaction centers and inactivation of the oxygen-evolving complex (OEC), particularly, Mn-complex in PSII donor side (Yusuf *et al.* 2010, Stirbet *et al.* 2014). On other hand, the appearance of the positive band around J-step after Fe removal reflected the withdrawal of electrons from Q_A, more closed reaction centers at that time, *i.e.*, reduced electron flux rate from Q_B to plastoquinone. The drops in J-step during recovery phase indicated that it was probably no longer blocked by stress and accelerations of electron transport reactions in the recovering plants. The J–I phase reflects the dynamics of the reduction of the plastoquinone pool between the PSII and PSI, weaker and slower reduction between Q_A to Q_B. It consists of two phases: the fast (around 5 ms – I-band) and slow (around 10–20 ms, H-band), reflecting the existence of two molecules with different rate of reduction – fast and slow reducing by PSII. The comparison between the two groups of plants confirmed that variations in the differential curves appear as a result of changes in relative volumes of the PQ pool (the number of electrons required to fully reduce the PQ pool to I-step). At a decreased PQ pool capacity, the rate of reduction was higher and this resulted in positive values of the transient band (as we observed in Fe-deficient plants). Conversely, during the late recovery phase (t_6–t_7), the relative size of a PQ pool increased in the fast phase (J-step), which resulted in slightly negative values for that band. In the last I–P phase, Fe_{def} was detected by much more pronounced positive peaks (G), which reflected the reduced size pool and faster reduction PSI end acceptors (*i.e.*, $NADPH^+$). During the recovery phase, while J and I peak disappeared, the peaks around K, H, and G were still visible on the differential curve after the addition of Fe, which indicated, among others, the slower recovery of PSI-end acceptors pool size. The detailed analyses of the shape of Chl *a* fluorescence combined with PCA analysis are very sensitive tools for early detection of environmental stress (Allakhverdiev 2011, Kalaji *et al.* 2016, 2018; Cetner *et al.* 2017, Samborska *et al.* 2018, 2019). The PCA analysis and inspection of the Table 1 of temporal changes in ChlF parameters during control–stress–recovery sequence provided additional insight into the reaction of photosynthetic apparatus to iron deficiency in radish. Fe deficiency strongly decreased both PSII and PSI activity and electron transport rate (Terry and Abadía 1986, Molassiotis *et al.* 2006), which is indicated by a decrease of some JIP-test parameters, such as maximum efficiency of PSII (F_V/F_M) and ratio of photochemical to nonphotochemical quantum efficiencies (F_V/F_0).

During the initial period (t_0), we did not found any significant differences in measured ChF parameters between radish plants, which grew with the control solution (Table 1). However, just at t_1, a strong decrease of values related to efficiency of photosynthetic apparatus parameters, such as $t_{(FM)}$, Area, S_M, N, $\varphi_{(Eo)}$, $\varphi_{(Ro)}$, RC/CS_M, PI_{abs}, PI_{total}, DF_{abs}, DF_{total}, and an increase of some parameters, such as F_0, W_E, V_L, TR_0/RC, DI_0/RC, S_M/t_{FM}, were observed. In the t_2, additionally, an increase of F_0/F_M and a decrease of maximum quantum yield of primary photochemistry reactions in PSII RC were denoted. Among the parameters, which we found to be the most correlated to changes along control–stress–recovery sequence, were: F_0, F_V, F_0/F_M, F_V/F_M, F_V/F_0, DI_0/CS_0, DI_0/RC, DI_0/CS_0, ET_0/CS_M, $\varphi_{(Eo)}$, PI_{abs}, PI_{total}, DF_{abs}, DF_{total}, S_M/t_{FM}. By taking into account their high sensibility, in combination, they could be used in as reliable indicator of Fe deficiency in radish plant.

We found a few JIP-test parameters, such as A_M, F_M, RC/CS_M, which were at the end of recovery phase still significantly lower in Fe-deficient plants compared to control and probably more time was needed for their full recovery (Table 1).

Conclusion: Iron deficiency affected photosynthetic machinery functioning and performance in radish plants. However, this effect could be reversed after elimination of the studied stress factor. We found some JIP-test parameters connected with the appearance of visible symptoms, which can be used as an early sensitive indicator for iron-deficiency stress detection (Kalaji *et al.* 2017b).

The earliest observed changes were denoted in JIP-test parameters (26 parameters related to photochemical reaction). PSII activity was interrupted at the level of photon absorption in light-harvesting complexes and Q_A reduction at PSII acceptor side. Parameters, such as t_{FM}, Area, S_M, N, and V_L, were the most indicative ones to iron deficiency in radish plants. However, we confirmed that it should not be diagnosed only on the basis of these chosen parameters. Taking into account the complex role of iron in plant biological and biochemical processes, iron deficiency imposed a strong stress, but did not provoke any irreversible changes to photosynthetic apparatus in radish plants.

Our results showed that the visible symptoms of iron deficiency in radish plants appeared after 7 d of stress application. On the other side, chlorophyll *a* fluorescence allowed us to detect the stress earlier (1–3 d). Both observation (visual and that based on ChlF) were noticeable only in direct comparison with the control plants grown in

optimal growth medium.

The early detection of the macro- and micronutrients deficiency can help to prevent its further shortage and prevent irreversible changes in plants. The earliest measurable effects of Fe shortage in growth medium were noted by changes of chlorophyll *a* fluorescence parameters. We confirmed that the measurement of chlorophyll *a* fluorescence can be used for early detection of iron deficiency in radish plants.

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