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JIP-test as a tool for early detection of the macronutrients deficiency in *Miscanthus* plants

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Abstract

The optimization of cultivation process and improving the yield of perspective energy crops in Europe could be one of the ways to overcome the problem of limited amount of conventional fuel. The aim of this work was to check if the use of JIP-test, as a noninvasive method for early detection of the changes in photosynthetic apparatus, can be applied to detect nutrients deficiency in miscanthus (*Miscanthus* × *gigantheus* Anderss.) plants. The experiment was performed in fully randomized design with the following experimental variants: CaNPK (full fertilization, control), NPK (without calcium), CaKN (phosphorus poor), CaPK (nitrogen poor), CaPN (potassium poor), and Ca (without NPK). Our results revealed that the reaction of photosynthetic apparatus of miscanthus plants grown under certain macronutrient deficiency was associated with exclusive significant modifications in the measured chlorophyll fluorescence signals, analysed further by JIP-test. Analysis of chlorophyll fluorescence induction curves discovered substantial deficiency of phosphorus and potassium ahead of the standard chemical method.

Additional key words: abiotic stress; chlorophyll *a* fluorescence; energy crops; photosynthetic apparatus.

Introduction

Miscanthus (*Miscanthus* × *gigantheus* Anderss.) is one of the key energy crop species (Brosse *et al.* 2012), which is characterized by low maintenance and high yield/energy content, since it could play an important role in the sustainable production of different kinds of renewable fuels in very near future (de Vrije *et al.* 2002, Brosse *et al.* 2012, Cadoux *et al*. 2012). The possibility of obtaining high-yield miscanthus is reduced substantially by the stress factors (*e.g.*, nutrient deficiency), influencing it very negatively. One of the key elements of avoiding stresses of plants cultivated at high scale, especially for energy purpose, is their optimal fertilization. It is important that it should be suitable for current growth phase and conditions on which the plant is cultivated. Traditional diagnostic methods based on visual observations connected with laboratory analysis are of very low precision. Moreover, observed visual changes of some plant organs always generate a decrease in the quantity and quality of the final yield. Blackmer and Shepers (1995) suggest using chlorophyll (Chl) meters to control N status of corn plants for monitoring efficacy of fertigation process under field conditions. Kalaji *et al.* (2014, 2018) and Goltsev *et al*. (2016) suggest that more precise and sensitive for diagnostic purpose are the methods based on Chl fluorescence measurements. They show that some of the basic parameters of that phenomenon are sensitive and can be used for the diagnostic of plant mineral status. For example, the maximum quantum efficiency of PSII (F_v/F_m)

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Abbreviations: F_O – initial fluorescence yield; F_m – maximal fluorescence yield; F_v/F_m – maximum quantum efficiency of photosystem II; PCA – principal component analysis; PF – prompt fluorescence.

observed in barley plants cultivated without N decreased significantly as compared to the control. However, the analysis of these parameters may be not sufficient. To assess quickly the photosynthetic function in a high number of field-grown plants, the analysis of polyphasic fast Chl fluorescence transient was developed by Professor Reto Strasser (1995). That method is also known as prompt fluorescence (PF) and is based on high-frequency record of Chl 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 provides valuable information on the functional and structural attributes of components involved in photosynthetic electron transport, mainly PSII (Kalaji *et al*. 2011, Stirbet and Govindjee 2011). The fluorescence rise during the first second of illumination shows a sequence of phases (labeled 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 is known as JIP-test (Strasser and Strasser 1995). It enables the calculation of specific biophysical parameters, quantum yields, and probabilities characterizing the structure and function of PSII. Many studies have demonstrated the ability of the JIP-test to reveal changes in PSII photochemistry initiated by environmental factors, *e.g*., effects of stresses, especially in grasses (Dąbrowski *et al*. 2015, 2016, 2019).

Some studies prove that the deficiency of some macro- and micronutrients impacts the phases of OJIP curves of corn and tomato (Kalaji *et al*. 2014), as well as actinidia (Swoczyna *et al.* 2019). The evidence of sensitivity of photosynthetic apparatus to different form of stress is provided also by Strasser *et al.* (1995, 2000, 2004) and Bussotti *et al.* (2010). However, there is a lack of the detailed knowledge how the deficiency of chosen macronutrient/macronutrients affects the behaviour of the OJIP curves measured in the leaves of different age, which are the mirror view what is performing within machinery of photosynthetic apparatus. The aim of this paper was to estimate the changes of functional characteristic of photosynthetic apparatus of miscanthus plant caused by selected macronutrient deficiency assessed by JIP-test parameters.

The hypothesis of this work is that the specific physiological effects of deficiencies of chosen nutrients correlate with different effects on photochemical processes, so the JIP-test parameters would respond in a way that depends on the given deficiency (one out of four selected macronutrients: Ca – calcium, N –nitrogen, P – phosphorus, and K – potassium).

Materials and methods

Experimental conditions: The experiment was set on the experimental field of Prof. Marian Górski, Skierniewice Experimental Station, WULS-SGGW Faculty of Agriculture and Biology, Poland (51°57'N, 20°9'E). The plants were grown on a luvisol, class IVa in 2003 in the frames of the rain-fed, long-term static fertilizer experiment. The soil at Skierniewice is a stagnic luvisol (Podlaski *et al.* 2017) with the following fractions in the 0–25 cm soil layer: > 0.05 mm: 87%, 0.002–0.05 mm: 5%, and < 0.002 mm: 7% (Sosulski *et al.* 2015). The following treatments were studied: CaNPK (full fertilization, control), NPK (acid soil, without calcium), CaKN (phosphorus poor), CaPK (nitrogen poor), CaPN (potassium poor), and Ca (without NPK). Control conditions were: 1,000 kg(Ca) ha^{-1}, 90 kg(N) ha^{-1}, 60 kg(P) ha^{-1}, 110 kg(K) ha^{-1}. The experiment was performed for one season from May to August in 2016 in fully randomized design with three replicates (6 × 3 m plots).

Weather conditions: During the years 1955–2005, the average annual rainfall in Skierniewice was equal to 480–532 mm with a mean of 516 mm. In 2016, the annual rainfall was about 724 mm, so it eliminated one of the stress factors which could have the potential impact on plants and in consequence the results of measurements.

Chl fluorescence was measured with *Pocket-PEA* fluorimeter (*Hansatech*, King's Lynn, Norfolk, UK), and the indices obtained were presented as OJIP curves and spider plot data. The measurements were carried at three the most important stages of development of photosynthetic apparatus: (*1*) the beginning of the development of layer structure of plant canopy; (*2*) maximal development of photosynthetic apparatus and canopy structure; (3) first visual symptoms of destruction of photosynthetic apparatus and canopy structure, at three canopy levels. The leaves from the top, middle, and bottom of canopy were chosen randomly, while the measurements were performed on the middle part of the chosen leaves. In the *PocketPEA*, instrument emitter wavelength ranges were as follow: (*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). Before measuring the experimental signals, the leaves were adapted in dark for 15 min at least. Chl *a* fluorescence was recorded after the illumination by red actinic light [635 nm; 3,500 µmol(photon) m^2 s^{-1}]. Measured signals were analysed by *PEA Plus* software. The characteristic points of JIP-test were used to calculate the specific characteristics of the light phase of photosynthesis according to algorithm, described by Strasser *et al*. (2004). The analysed parameters are described in Appendix.

Statistical analysis: The *Fisher*'s Least Significant Differences test was used as a post hoc at a 0.05 confidence level. *Statistica 10.0* program (*Statsoft Inc.*, Tulsa, USA) was used to perform the differences between the means of measured JIP-test parameters.

Evaluation of relationships between selected variables, which describes crop fluorescence in different growth stages, were conducted using Principal Component Analysis (PCA). Moreover, using PCA multivariate characteristics of the fertilization objects was presented using the first two principal components (PC1 and PC2). Results were

presented by the mean of the biplots which allow evaluating multivariate relationships as well as multivariate variability.

Results

OJIP curves and differential curves of ΔV_t: During the 1st measurement, done on the top layer of the canopy, JIP-test analysis and differential curves of of ΔV_t showed only slight influence of P deficiency on the last part of I–P phase. Those curves after the differentiation process (Fig. 1*B*) showed that deficiency in NPK and Ca caused moderate growth of those curve ratios in the last stages of O–J and in the beginning of the stages of J–I. The OJIP curves from previously mentioned measurement term and from middle canopy level (Fig. 1*C*) did not show any visible differences. The differential curves of ΔV_t (Fig. 1*D*) showed the substantial influence of some nutrient deficiency. The deficiency of P caused strong, and in case of N, slight growth of curves at the last stages of O–J and in the

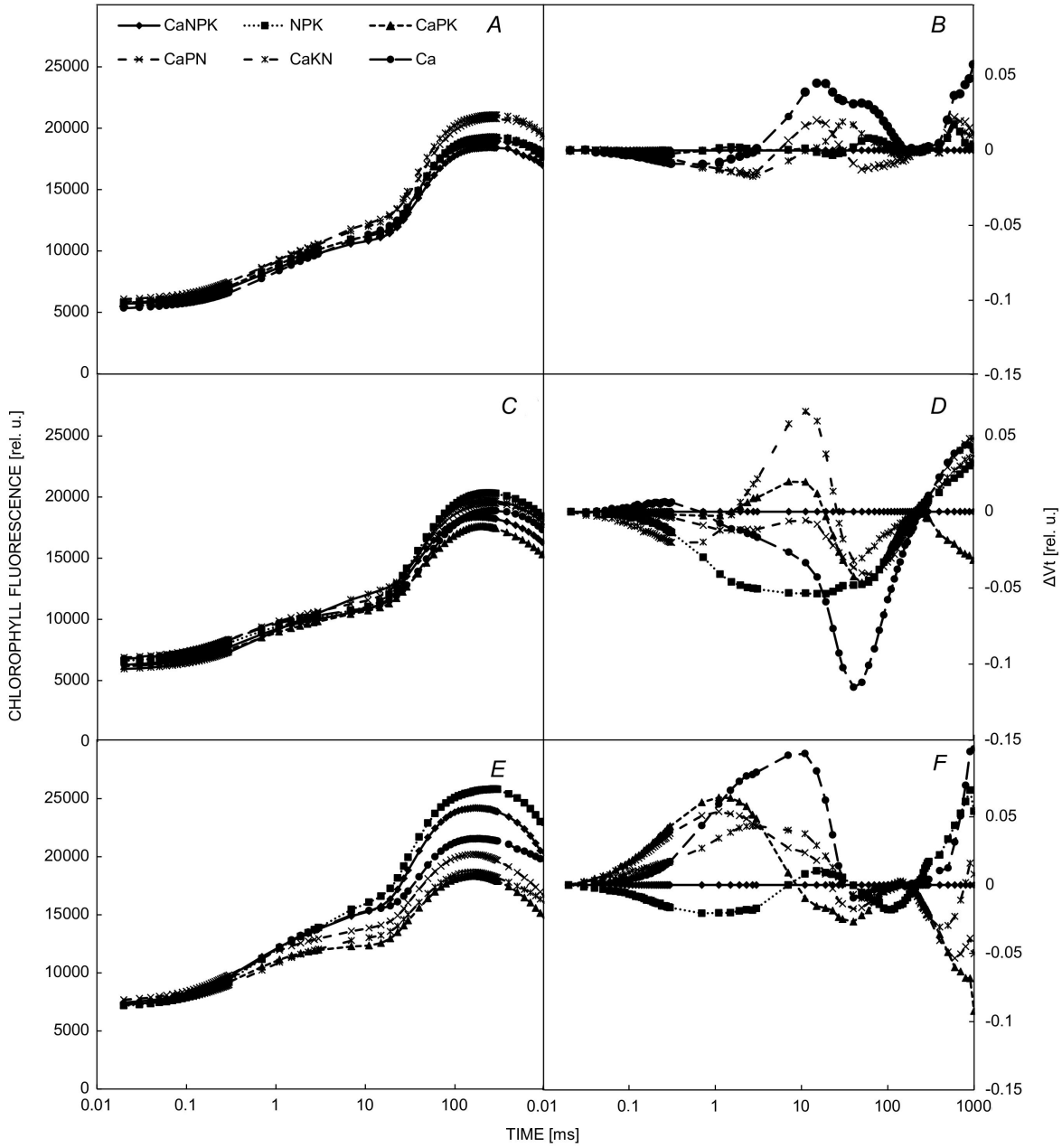

Fig. 1. The induction and differential curves of chlorophyll *a* fluorescence 1st-term measurement of *Miscanthus* × *giganteus* cultivated under six fertilization treatments. (*A*) Induction curves for leaves of canopy top layer; (*B*) differential curves after double normalization for leaves of canopy top layer; (*C*) induction curves for leaves of canopy middle layer; (*D*) differential curves after double normalization for leaves of canopy middle layer; (*E*) induction curves for leaves of canopy bottom layer; (*F*) differential curves after double normalization for leaves of canopy bottom layer.

beginning stages of J–I phase. The OJIP curves from the bottom layer of canopy from the 1st measurement (Fig. 1*E*) were influenced by stress at the last stages of I–P phase. Ca deficiency caused the slight growth on the background of control. Stress generated by other treatments involved the reduction of curve course in relation to control. On those curves after differentiation process (Fig. 1*F*), the strong influence of NPK deficiency was observed, it caused growth of the curve ratio at last stages of O–J and at the beginning stages of J–I and I–P phase. The moderate influence of N, K, and P was also indicated by the growth of curves course at the last stages of O–J, and at the beginning stages of J–I phase. The influence of Ca, which caused slight reduction of curve course, was also noticed.

Curves from the 2nd term and top layer of canopy (Fig. 2*A*) were different from the whole long lines, but the strongest influence of each treatment was observed by the end of I–P phase. The deficiency of P and Ca caused slight increase in the curve course. In that phase, quite opposite effect as compared to other treatments might be observed. On previously mentioned differential curves of ΔV_t (Fig. 2*B*), the influences of each treatment were observed. NPK deficiency involved strong and N deficiency slight influence on OJIP curves by the end of O–J and at the beginning of J–I. During those phases, the reductive influence on the curve course was also observed – strong for P, moderate for K, and mild for Ca deficiency. On the OJIP curves from the 2nd term and middle layer of canopy

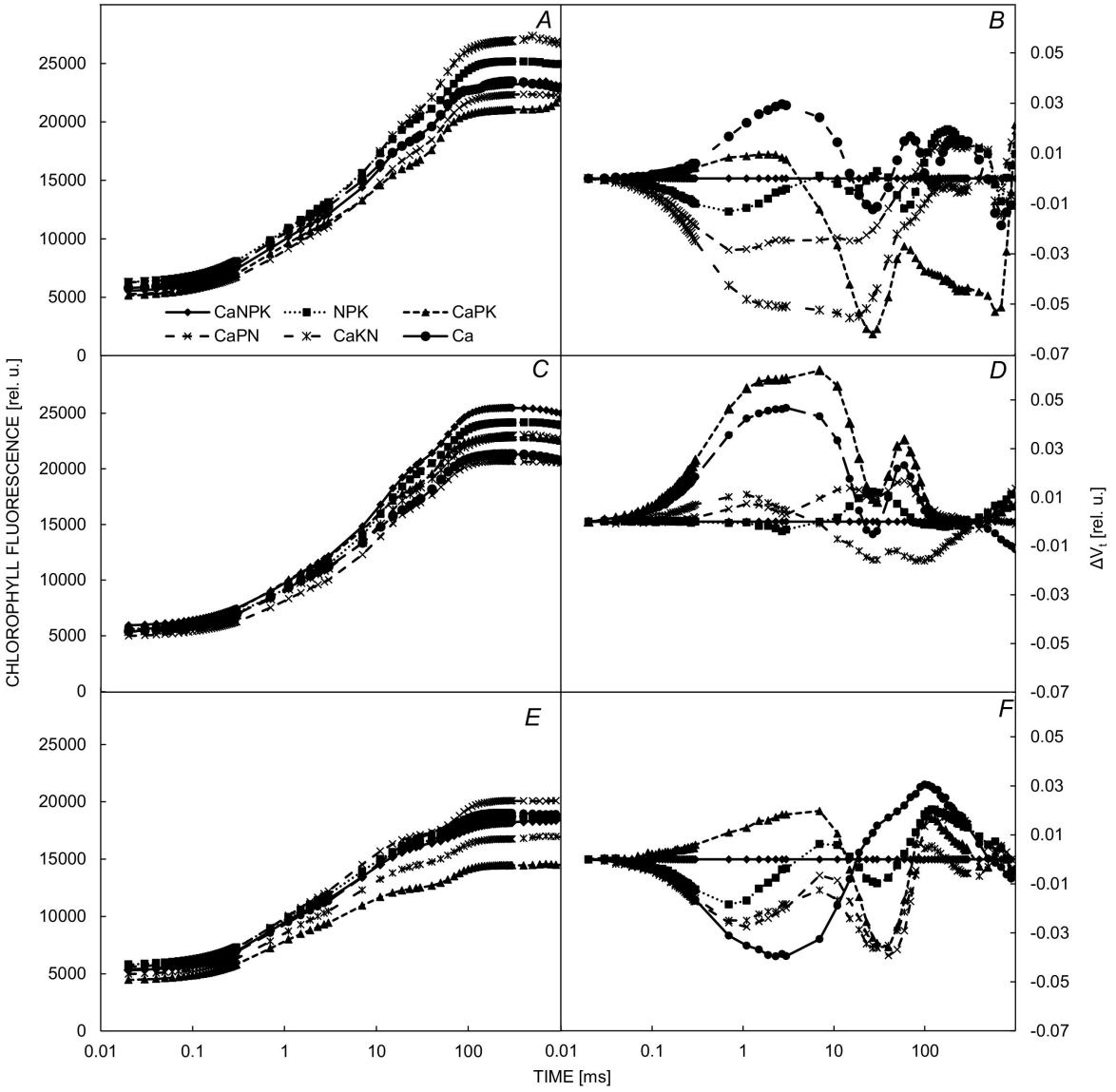

Fig. 2. The induction and differential curves of chlorophyll *a* fluorescence 2nd-term measurement of *Miscanthus* × *gigantheus* cultivated under six fertilization treatments. (*A*) Induction curves for leaves of canopy top layer; (*B*) differential curves after double normalization for leaves of canopy top layer; (*C*) induction curves for leaves of canopy middle layer; (*D*) differential curves after double normalization for leaves of canopy middle layer; (*E*) induction curves for leaves of canopy bottom layer; (*F*) differential curves after double normalization for leaves of canopy bottom layer.

(Fig. 2*C*), we could not observe any visual differences. On the previous curves after the differentiation process (Fig. 2*D*), the strong influence of N and NPK deficiency could be seen. The both deficiencies involved the curve course increase in relation to control at the last stages of O–J and at the beginning stages of J–I phase. On the OJIP curves from the 2nd term and bottom layer of the canopy (Fig. 2*E*), some differences were observed. The most pronounced visual differences between the curves were noticed by the end of I–P phase. The course of the curves for plants cultivated without N and P were moderately and slightly lower than that of the control. It is possible to notice more details on all those differential curves of ΔV_t (Fig. 2*F*). The moderate influence of N deficiency for increasing the curve course in the last stages of O–J phase was observed.

There were no visual differences between OJIP curves from the 3rd term of measurement with leaves in top layer of the canopy (Fig. 3*A*). On those curves after the differentiation process (Fig. 3*B*), some differences were observed between those from experimental treatments and control. The course of curves for the plants cultivated without

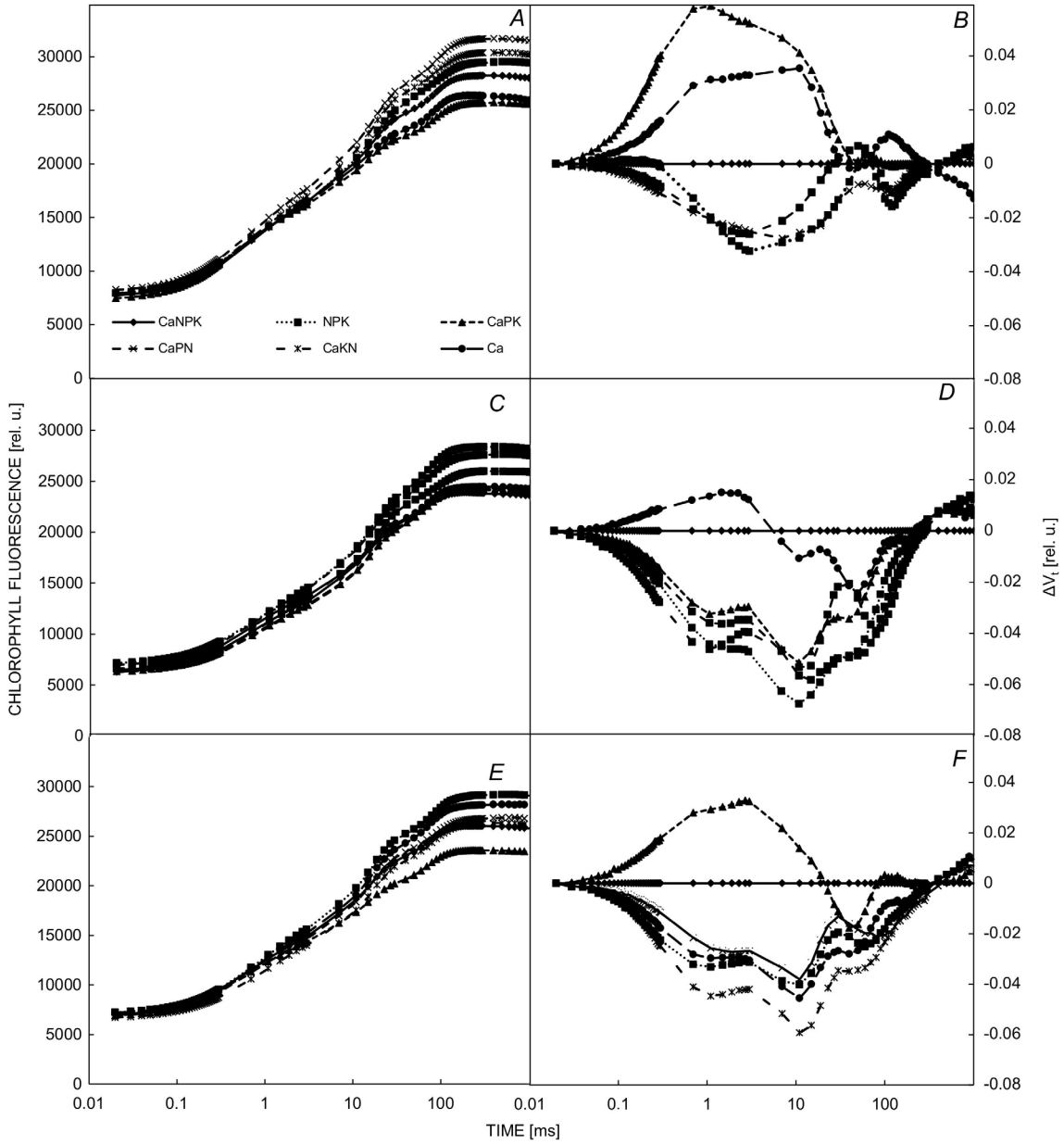

Fig. 3. The induction and differential curves of chlorophyll *a* fluorescence 3rd-term measurement of *Miscanthus* × *giganteus* cultivated under six fertilization treatments. (*A*) Induction curves for leaves of canopy top layer; (*B*) differential curves after double normalization for leaves of canopy top layer; (*C*) induction curves for leaves of canopy middle layer; (*D*) differential curves after double normalization for leaves of canopy middle layer; (*E*) induction curves for leaves of canopy bottom layer; (*F*) differential curves after double normalization for leaves of canopy bottom layer.

N was strongly higher, and those for the ones cultivated without NPK were moderately higher than that in control. The differences were seen by the end of O–J and at the beginning of the J–I phase. No influence of any treatment was observed on the curves measured on the leaves from middle layer of the canopy (Fig. 3*C*), but after the following differentiation process (Fig. 3*D*), some differences arose between those from experimental treatments and control. The course of curve for the plant cultivated without NPK was slightly higher than that in control by the end of O–J phase. Other curves were strongly lower than that in control and that scheme was observed between the end O–J and the beginning J–I phase. OJIP curves for the bottom layer of the canopy differed only at the final stages of I–P phase. At OJIP curves from the 3rd measurement term and middle layer of the canopy (Fig 3*E*), in plants cultivated on soil without N, the course of Chl fluorescence curve at I–P phase was slightly lower than in others. In those differential curves of ΔV_t, the reciprocal differences were more detailed. The courses of the curves for the plants cultivated without N were strongly higher by the end of O–J and at the beginning of J–I phase. There was no influence of others treatments on the shape of the curves.

Spider plots of JIP-test parameters: The first analysed spider plot showed that DI_0/RC and PI_{total} parameters were significantly affected in the plants cultivated without NPK in the 1st term of measurement in the case of the leaves from top layer of canopy (Fig. 4*A*). In that date and layer, PI_{total} was significantly influenced in plants of CaPK treatment. The second one indicates that, in leaves from middle layer of canopy, the highest differences were caused by K deficiency. DI_0/RC and N parameters were the most influenced ones (Fig. 4*B*). The PI_{abs} and PI_{total} for these plants and those from NPK and Ca treatments showed the same values. In the leaves from bottom layer, the most pronounced changes were denoted under CaPK, CaPN, and Ca treatments (Fig. 4*C*). DI_0/RC was the most changed/affected parameter.

The highest differences in Chl *a* fluorescence parameters on the 2nd term of measurement for leaves in top layer of canopy (Fig. 5*A*) were observed in Area, S_m, and N parameters for the plants treated with CaPK. High differences between control and CaKN treatment were observed in S_m. The greatest differences in parameters for leaves in middle layer of canopy (Fig. 5*B*) were observed in PI_{abs} and PI_{total} of these plants and those from CaKN and CaPN treatments. The highest differences in parameters for leaves in bottom layer of canopy (Fig. 5*C*) were observed in PI_{total} of these plants and those from CaKN and CaPN treatments.

The parameters measured for leaves in the top layer of canopy during the 3rd term were not affected by any treatment (Fig. 6*A*). In Fig. 6*B*, t_{Fm}, Area, and S_m/t_{Fm} in plants without Ca and Area in plants without P were much higher than that in control. In the last spider plot (Fig. 6*C*), the higher DI_0/RC of plants without N and NPK was observed. Some differences in DI_0/CS_0 parameter between control and the plants from NPK, CaPK, CaKN, and Ca treatments were also observed.

During the early growth stage in the 1st term of measurements, very strong positive correlations were found between the following variables: $\varphi_{(P0)}$, $\psi_{(E0)}$, $\varphi_{(E0)}$, F_m, F_v, F_v/F_m, TR_0/CS_0, ET_0/CS_0, TR_0/CS_m, ET_0/CS_m, RE_0/CS_m, and PI_{abs}. These all variables were strongly negatively

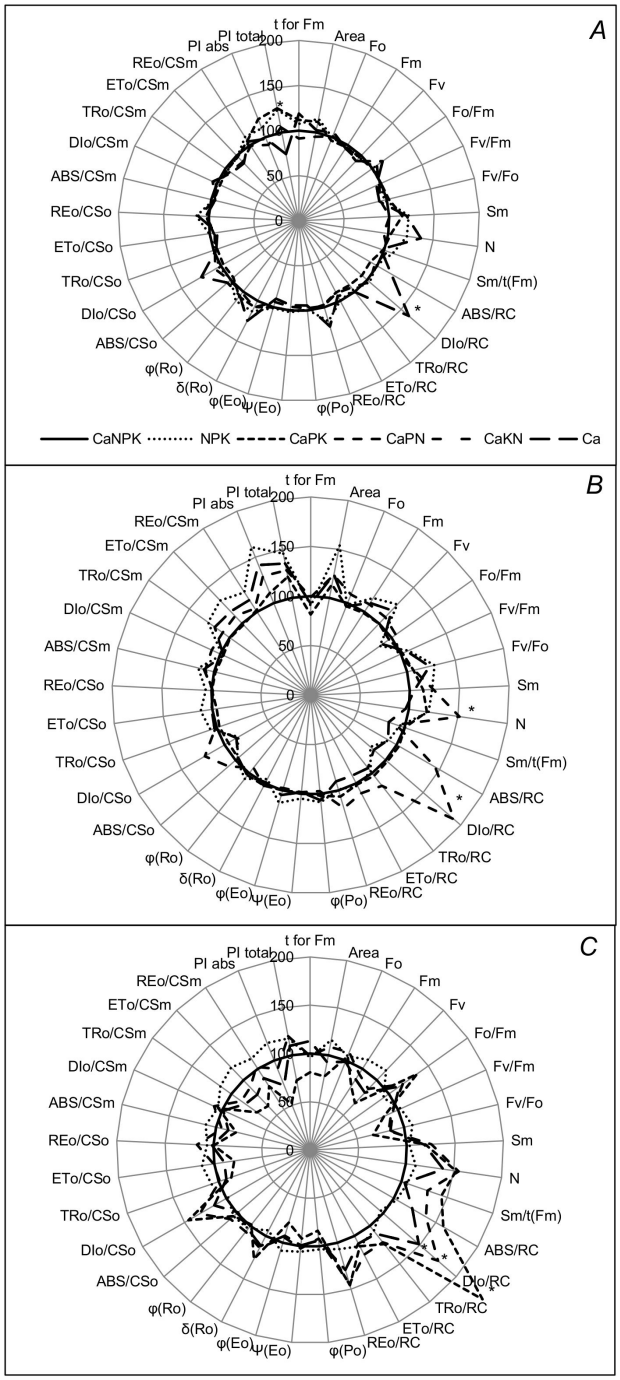

Fig. 4. The spider plot JIP-test parameters for the 1st term of measurements of *Miscanthus* × *gigantheus* cultivated under six fertilization treatments. (*A*) Top, (*B*) middle, and (*C*) bottom layer of the canopy. The means of one parameter marked by an *asterisk* differ significantly from the control one (p<0.05). For the meaning of the parameters *see* Appendix.

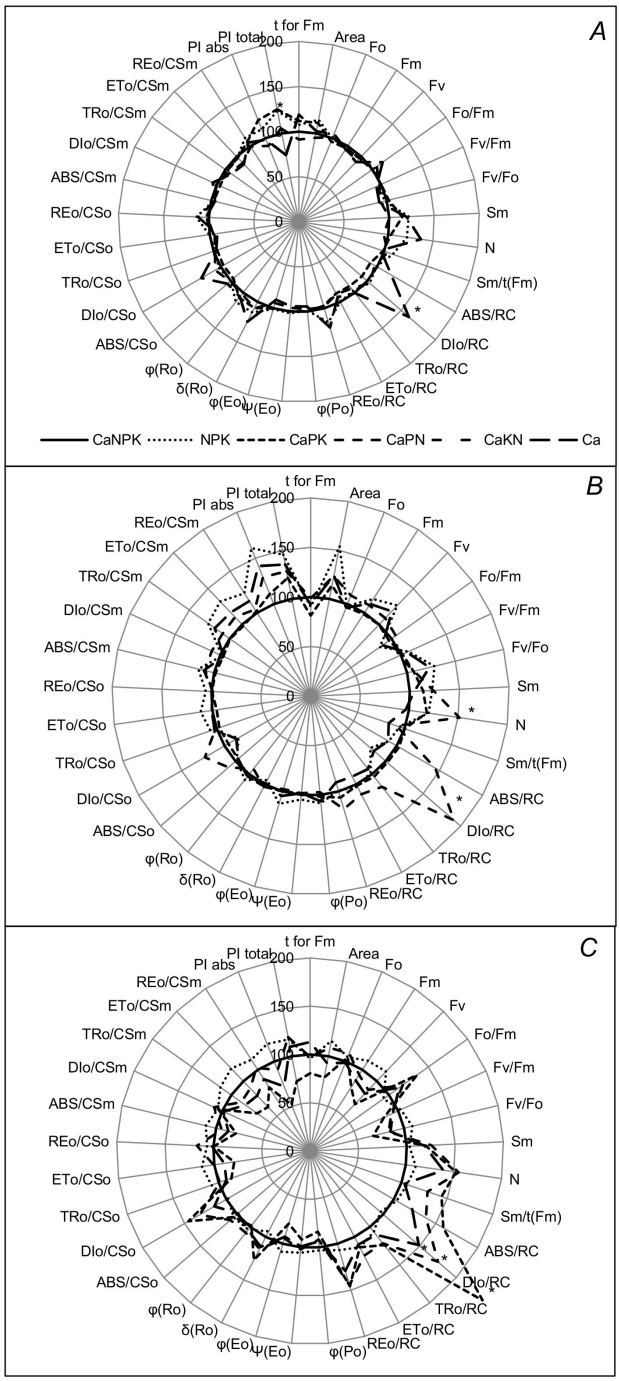

Fig. 5. The spider plot JIP-test parameters on the 2nd term of measurements of *Miscanthus* × *giganteus* cultivated under six treatments. (*A*) Top, (*B*) middle, and (*C*) bottom layer of the canopy. The means of one parameter marked by an *asterisk* differ significantly from the control one (p<0.05). For the meaning of the parameters *see* Appendix.

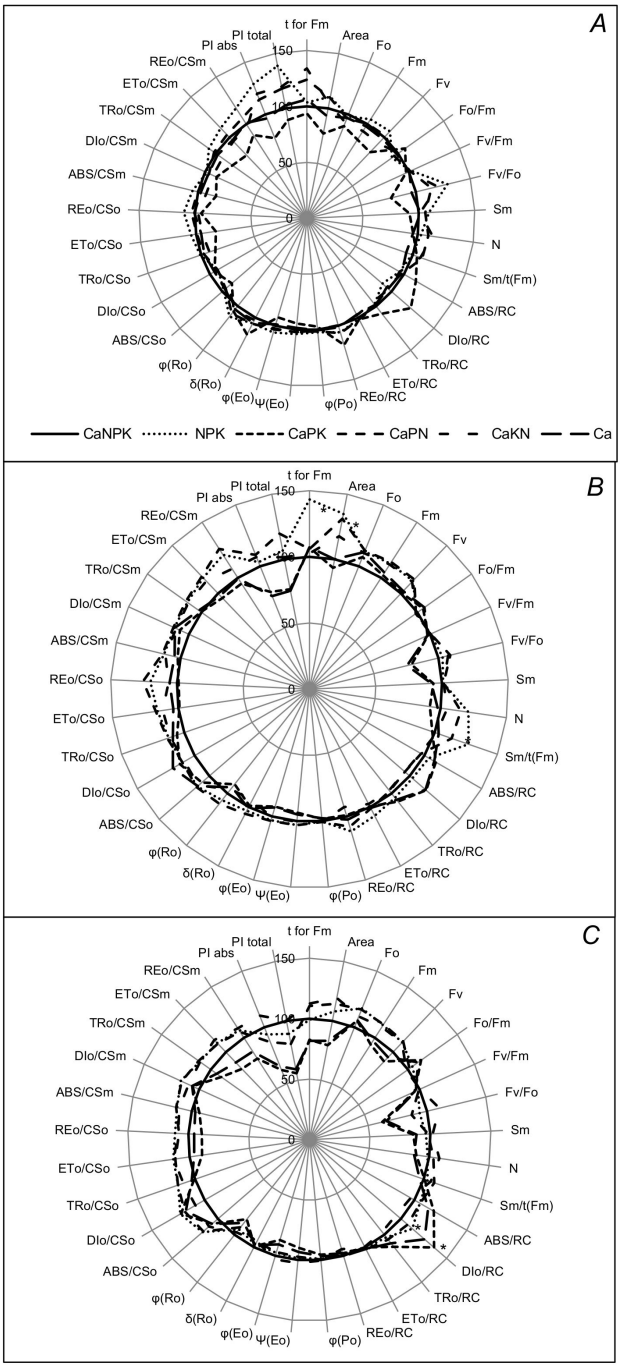

Fig. 6. The spider plot JIP-test parameters on the 3rd term of measurements of *Miscanthus* × *giganteus* cultivated under six treatments. (*A*) Top, (*B*) middle, and (*C*) bottom layer of the canopy. The means of one parameter marked by an *asterisk* differ significantly from the control one (p<0.05). For the meaning of the parameters *see* Appendix.

correlated with $\delta_{(R0)}$ and quite strongly negatively correlated with ABS/RC, DI_0/RC, TR_0/RC, and RE_0/RC. Because most of the total multivariate variability (about 61%) is explained by PC1, the multivariate differences are more important during horizontal axis. The largest multivariate differences were observed between NPK fertilization *vs*. CaPK fertilization. These fertilization objects were differentiated according the variables strongly correlated with PC1. Very similar fertilization effects were caused by Ca and CaKN, which characterized themselves

by low values of most variables. CaPN and CaNPK were mainly different according variables which were strongly correlated with PC2, *e.g.*, t for F_m, ABS/CS_0, and F_O (Fig. 7*A*).

During the later growth stage in the 2nd term (Fig. 7*B*), very strong positive correlations were found between the following variables: F_O, F_m, F_v, ABS/CS_0, DI_0/CS_0, TR_0/CS_0, ET_0/CS_0, RE_0/CS_0, ABS/CS_m, DI_0/CS_m, TR_0/CS_m, ET_0/CS_m. These all variables were strongly correlated with PC1, what explained about 41% of total multivariate variability. PC2 explained a large part of total variability, *i.e.*, about 34%. It means that the differences according horizontal and vertical axes were almost equally important. The largest multivariate differences were observed between CaNPK *vs*. CaPN fertilization treatment. In the case of CaNPK, most of variables were of high values, especially the ones strongly negatively correlated with PC1. Similar effects of the fertilization treatments were proved for Ca and CaPK which characterized themselves by low values of most variables. CaKN and NPK were mainly different according variables which were strongly correlated with PC2, *e.g.*, PI total, ABS/RC, and t for F_m.

During the latest growth stage in the 3rd (Fig. 7*C*) term, very strong positive correlations were found between the following variables: t for F_m, Area, F_m, F_v, F_v/F_m, $\varphi_{(P0)}$, $\psi_{(E0)}$, $\varphi_{(E0)}$, TR_0/CS_0, ET_0/CS_0, RE_0/CS_0, ABS/CS_m, TR_0/CS_m, ET_0/CS_m, RE_0/CS_m, PI_{abs}, and PI_{total}. These all variables were strongly positively correlated with PC1, what explained about 75% of total multivariate variability. It means that these variables were the most important in multivariate characteristics of the objects. PC2 explained a small part of total variability, *i.e.*, about 19%. It means that differences according horizontal axis were more important than the ones along the vertical axis. The largest multivariate differences were observed between CaPN and NPK *vs*. CaPK fertilization treatments. In the cases of CaPN and NPK, variables strongly negatively correlated with PC1 had the highest values. Plants fertilized with CaNPK or CaKN had much higher values of PC2 which means that these two fertilization treatments had lower values of most variables included in the analysis.

Discussion

The availability of suitable nutrients for the current growth phase and conditions of cultivation during plant vegetation are crucial for the normal physiological state of the plant as a whole, especially for the photosynthesis (Kalaji *et al.* 2014, Mastalerczuk *et al*. 2017). In this work, we investigated *in vivo* on three occasions the Chl *a* fluorescence transients at three leaf ages to analyze the changes in light-dependent phase of photosynthesis in nutrient-deficient *Miscathus* × *gigantheus* Anderss. plants.

Based on our results, we may say that the macronutrient deficiency highly influences the functioning of photosynthetic apparatus, both in young and old leaves. However, this influence was more clear in the case of young leaves, since they were not adapted to such a stress, yet. The changes in photosynthetic apparatus functioning were clearly documented by the changes on the standard OJIP curves shape, differential ones, and changes in spider plot parameters and the PCA graphs. The sensitivity of photosynthetic apparatus to nutrient deficiency was proved by many researchers. During our studies, we observed that the young leaves were much more sensitive to a synergic effect of deficiency in three macronutrients than to each one of them individually. The similar phenomena were observed by Kalaji *et al*. (2018) in rapeseed plants which were suffering from a strong deficiency of iron. On fully developed leaves from old plants of miscanthus, the strong positive influence of Ca deficiency was observed on the OJIP curve course after the differentiation process. Quite opposite effect was observed by Kalaji *et al.* (2014) for the leaves of maize. Strong negative influence was noticed concerning the deficiency on functioning of photosynthetic apparatus in the leaves from fully developed plants in middle part of the canopy. Nearly the same result was found by Redillas *et al.* (2011) for the leaves of rice cultivated without N fertilization. There was no N deficiency influence on the value of F_v/F_m measured in young and fully developed leaves of miscanthus. The same effect of N deficiency on the young and fully developed leaves was reported by Živčák *et al*. (2014). The young and fully developed leaves from young miscathus plants cultivated without N did not differ to that measured in control and quite similar phenomena were observed by Ciompi *et al.* (1996) for the leaves of sunflower.

The structural and functional parameters deduced from the JIP-test were also analyzed in this work. The differences between the leaves from three layers were significant (Figs. 4–6). The results demonstrate the negative effect of stress on parameters, such as DI_0/RC, N, DI_0/CS_m, PI_{total}, PI_{abs}, or Area. However, Kalaji *et al*. (2014) or Baker and Rosenquist (2004) demonstrated that nutrient deficiency influenced photosynthetic yield of PSII, but it was the result of a reduction of the quantum yield of PSII electron transport and the efficiency of excitation energy capture by open PSII reaction centers. Those authors suggested that nutrient deficiency induces some photoinhibitory damage of PSII. The reduction of PSII activity has been observed at nutrient deficiency conditions also by Redillas *et al*. (2011) and Msilini *et al*. (2013). The decrease in PI_{abs} and PI_{total} in the plants under nutrient deficiency stress suggested the decrease in overall photosynthetic performance associated usually with decrease in leaf electron transport capacity. Our data, especially those obtained from the double normalization of Chl fluorescence curves, showed that the most visible changes were noted in the case of lack of a single nutrient, except potassium and phosphorus deficiency.

For the whole set of PCA graphs, it was found that the large variability was caused by the first component PC1. The correlations of the majority of the studied fluorescence parameters with PC1 mainly, permit to name the component as 'primary reactions of PSII'. The increased distance of PI_{total} parameter from *x*-axis indicates to the identification of the second component PC2, as photosynthetic apparatus efficiency. It seems to be that, during vegetation period, both photosystems (PSI and PSII) played similar role in keeping the performance of

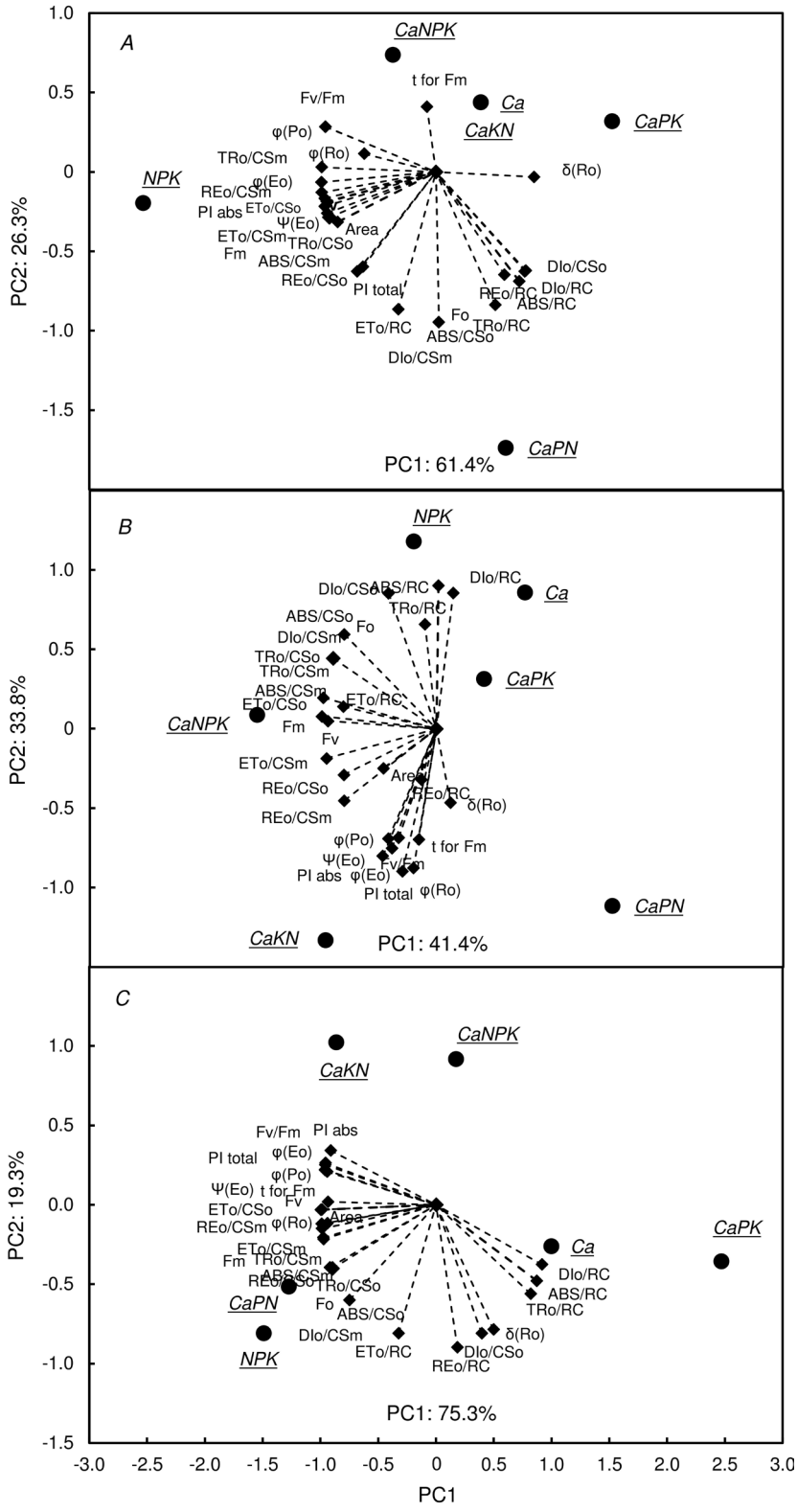

Fig. 7. The biplot presenting multivariate relationships and multivariate variability of the fertilization treatments based on the principal component analysis (PC1 and PC2) in the 1st (*A*), 2nd (*B*), and 3rd (*C*) terms of measurements. For the meaning of the parameters *see* Appendix.

photosynthetic machinery. However, at the beginning and the end of vegetation period, PSI played more important role as compared to PSII.

In conclusion, the response of photosynthetic apparatus of miscanthus plants grown under prearranged macronutrient deficiency in soil was correlated with significant modifications of chlorophyll fluorescence signals and changes in OJIP induction curves. Applying double normalization of OJIP curves allowed revealing the substantial deficiency of phosphorus and potassium much

earlier as compared to visual identification methods and the application of standard destructive chemical analysis. The studied chlorophyll fluorescence parameters could be classified into two principal components: primary reactions of PSII and activity of photosynthetic apparatus (reactions of PSII and PSI). They were responsible for 75–95% (depending on the growing phase) of explainable variability. Due to the fact that plant photosynthetic efficiency response significantly differed at various canopy layers, measurements of chlorophyll fluorescence for selection purpose must be performed at the whole canopy level, *i.e.*, at three canopy layers: top, middle, and bottom.

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

Fluorescence parameter	Description
t for F_m	Time (in ms) to reach the maximal fluorescence intensity F_m
$\varphi_{(P0)} = 1 - Fo/F_m$	Maximum quantum yield of primary photochemistry (at t = 0)
$\varphi_{(E0)} = (1 - Fo/F_m)(1 - V_J)$	Quantum yield of electron transport (at t = 0)
$\varphi_{(R0)} = (1 - Fo/F_m)(1 - V_I)$	Quantum yield for reduction of end electron acceptors at the PSI acceptor side (RE)
$PI_{abs} = \frac{\gamma_{RC}}{1-\gamma_{RC}} \times \frac{\varphi_{(Po)}}{1-\varphi_{(Po)}} \times \frac{\psi_{(Eo)}}{1-\psi_{(Eo)}}$	Performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors
$PI_{total} = PI_{abs}\ \varphi_{(R0)}/(1 - \varphi_{(R0)})$	Performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors
$TR_0/RC = M_0(1/V_J)$	Trapping flux (leading to Q_A reduction)/RC
$ET_0/RC = M_0(1/V_J)\psi_0$	Electron transport flux (further than Q_A)/RC
$RE_0/RC = M_0(1/V_J)(1 - V_I)$	Electron flux reducing end electron acceptors at the PSI acceptor side/RC
$DI_0/RC = (ABS/RC - TR_0/RC)$	Dissipated energy flux/RC (at t = 0)
$S_m = (Area)/(F_m - Fo)$	Normalized total area above the OJIP curve
$N = (S_m/S_s) = S_mM_0(1/V_J)$	Turnover number, that is the number of Q_A reductions from time 0 to tF_m
$ABS/CS_m \approx F_m$	Absorption of energy per excited cross-section (CS) approximated by F_m
ET/CS_m	Electron flux transported by PSII of photosynthesizing sample cross-section at t = max
$TR_0/CS_m = \varphi_{(Po)}(ABS/CS_m)$	Excitation energy flux trapped by PSII of a photosynthesizing sample cross-section at t = max
RE_0/CS_m	Electron reduction of PSII of a photosynthesizing sample cross-section (CS) at t = max
$DI_0/CS_m = (ABS/CS_m) - (TR_0/CS_m)$	Heat dissipation of excitation energy by PSII of a photosynthesizing sample cross-section (CS) at t = max
$ET/CS_0 = \varphi_{(Eo)}(ABS/CS_0)$	Electron flux transported by PSII of a photosynthesizing sample cross-section (CS) at t = 0
RE_0/CS_0	Electron reduction of PSII of a photosynthesizing sample cross-section (CS) at t = 0
$TR_0/CS_0 = \varphi_{(Po)}(ABS/CS_0)$	Excitation energy flux trapped by PSII of photosynthesizing sample cross-section (CS) at t = 0
$DI_0/CS_0 = (ABS/CS_0) - (TR_0/CS_0)$	Heat dissipation of excitation energy by PSII of photosynthesizing sample cross-section (CS) at t = 0