Application of chlorophyll fluorescence performance indices to assess the wheat photosynthetic functions influenced by nitrogen deficiency

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ABSTRACT

Nitrogen deficiency influences importantly the plant photosynthetic capacity and crop productivity. Here, we employed the rapid, non-invasive measurements of chlorophyll *a* fluorescence kinetics for calculation of the integrative fluorescence parameters related to the leaf photosynthetic performance. In pot experiments with winter wheat (*Triticum aestivum* L.) we cultivated plants during the whole growing period in the soil substrate supplied with four different doses of nitrogen. The leaf nitrogen and chlorophyll content as well as the plant dry mass were analyzed after chlorophyll fluorescence records in three growth stages. Our results indicate that the commonly used parameter F_v/F_m (the maximum quantum yield of photochemistry) was almost insensitive to nitrogen treatment. In contrary, the performance index (PI_{abs}) and total performance index (PI_{tot}) were much more responsive and significant differences among plants of different nitrogen treatments as well as between the youngest and third leaf from the top were observed. Parameter PI_{tot} was shown to express only small diurnal changes, thus being more reliable and more useful for comparison of different samples in field conditions than more frequently used parameter PI_{abs}.

Keywords: performance index; photosynthesis; plant ecophysiology; non-invasive techniques; JIP-test

It was well documented that the nitrogen nutrition influences the plant photosynthetic capacity (Terashima and Evans 1988). Thus, the membrane processes must be balanced to maintain high efficiency in the conversion of energy and to avoid the over-reduction of photosynthetic electron chain in conditions with different nitrogen supply (Lu et al. 2001).

To assess quickly the photosynthetic function in a high number of field grown plants, the non-destructive analysis of polyphasic fast chlorophyll *a* fluorescence (ChlF) transient was developed (Strasser et al. 2000, reviewed in Stirbet and Govindjee 2011). The method is based on high-frequency record of ChlF emitted by dark adapted leaf during short (usually one second lasting) pulse of strong actinic light by fluorimeter. The ChlF 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

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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 the photosynthetic electron transport system as well as some integrative parameters related to plant photosynthetic performance. Strasser et al. (2000) introduced the performance index (PI_{abs}) as the product of three independent parameters combining structural and functional criteria: density of reaction centers, the quantum efficiency of primary photochemistry and conversion of excitation energy in electron transport. This parameter was found to be much more sensitive to environmental effects than commonly used parameter F_v/F_m (Brestič and Živčák 2013) and it was shown to be well correlated with photosynthetic capacity measured as CO_2 assimilation (van Heerden et al. 2003, Ripley et al. 2004). Later, the total performance index (PI_{tot}) was introduced (Smit et al. 2009). This parameter is based on PI_{abs} but it is extended by the quantum efficiency of reduction of photosystem I (PS I) end acceptors (Redillas et al. 2011).

The analysis of fast ChlF transient was applied in numerous studies in crop plants, e.g. to assess the environmental effects in wheat, such as drought (Živčák et al. 2008a), high temperature (Brestič et al. 2012), light stress (Kalaji et al. 2012, Živčák et al. 2014). The JIP-test analyses were applied several times also in studies dealing with nitrogen deficiency in plants and the effect of poor nitrogen supply on PS II is recently well described (Lu et al. 2001, Redillas et al. 2011, Li et al. 2012, etc.). Although in many of published works the rapid ChlF is denoted as a useful tool for assessing the physiological effects of nitrogen deficiency on plants, there is still a lack of data on the usefulness of the method in assessment of plant photosynthetic performance in crop trials with different nitrogen supply. In our study we present the results obtained in three growth stages of winter wheat exposed either to full or partial nitrogen deficiency compared to plants grown in normal nitrogen supply as well as in the excess of nitrogen. We focused on practical aspects and we tried to suggest the useful way to utilize the rapid chlorophyll *a* fluorescence method for detecting nitrogen deficiency or monitoring effects of nitrogen nutrition on wheat photosynthetic performance.

MATERIAL AND METHODS

Cultivation of plants. Pot experiment with winter wheat (*Triticum aestivum* L., cv. Akteur) was established on the 22nd October 2012 in a vegetation cage of the Slovak University of Agriculture, Nitra. Each pot contained 20 kg of medium heavy Cambisol. Owing to the fact that there was good content of N_{min} (15 mg/kg), P (90 mg/kg) and K (224 mg/kg) in the soil autumn fertilization with mineral fertilizers was not performed.

Sowing of 32 grains per pot was realized by hand. After wintering regenerative fertilization of winter wheat with nitrogen was done in spring (18th April 2013) as follows:

- treatment 1 0.0 g N per pot (control);
- treatment 2 0.5 g N per pot;
- treatment 3 1.0 g N per pot;
- treatment 4 2.0 g N per pot.

Nitrogen was applied in the form of liquid fertilizer DAM-390 (UAN-390) diluted with water in a ratio of 1:5 (1 part of fertilizer in 5 parts of water). Each treatment was repeated 6 times, i.e. each treatment consisted of 6 pots. During the growing period soil moisture in pots was maintained at the level of 50–60% of full soil water capacity. Aboveground phytomass of plants from one pot in each treatment was sampled three times (3rd May, 21st May, and 7th June) during the vegetation period.

Analysis of total nitrogen. Total nitrogen per dry mass (DM) unit in wheat leaves was analyzed in dry samples (after drying at 60°C). The content of total nitrogen (mg/g of DM) was analyzed using the standard Kjeldahl method. The mean value of two parallel samples is presented.

Determination of chlorophyll content. The leaf chlorophyll content was measured spectrophotometrically from acetone extract, and calculated using the equation of Lichtenthaler (1987), as described elsewhere (Živčák et al. 2014). Six leaves of each treatment/leaf position were analyzed.

Chlorophyll fluorescence measurements. ChlF measurements were performed using the Handy-PEA continuous excitation plant efficiency analyser (Hansatech Instruments Ltd, Kings Lynn, UK). The leaf clips were placed on the leaves 20 min to prior the measurements to provide dark adaptation. The light pulse intensity used was 3500 μmol/m^2/s for 1 s. Leaf segment measurements were performed in the middle part of a leaf blade, away from the main leaf vein. ChlF transient data were used to calculate parameters. The maximum quantum yield (efficiency) of PS II photochemistry (F_v/F_m), performance index and total performance index were calculated according to the equations reviewed by Stirbet and Govindjee (2011).

ChlF measurements were done in 3 cycles (in the growth stages of tillering, stem elongation and before anthesis). Measurements were done always on the newest, fully developed leaf (leaf 1) and on the third leaf from the top (leaf 3). Moreover, after anthesis, the test of parameter stability during hot and sunny day was performed.

Statistical analysis. The reported data represent the mean ± standard error (SE). Statistical analysis was done using the analysis of variance (ANOVA) followed by the Tukey's *HSD* test (α = 0.05) by the Statistica version 9.0 (Statsoft Inc., Tulsa, USA).

RESULTS

Different doses of nitrogen fertilizers lead to differences in the nitrogen content in plants as well as to significant differences in production of biomass and some related parameters, as shown in Table 1.

The highest difference was between treatment 0N and other three treatments in nitrogen content as well as in all growth parameters, the differences among ½N, 1N and 2N were lower but in many cases significant, confirming the positive relationship between nitrogen supply, the leaf chlorophyll content and biomass production.

We have examined an effect of different nitrogen nutrition on values of three ChlF parameters in two leaf positions (Figure 1). Our results indicate, that in the youngest, fully developed leaf there is no decrease of maximum quantum yield of PS II photochemistry (F_v/F_m) due to nitrogen deficiency; moreover, the average values of F_v/F_m were the highest in nitrogen depleted plants in early growth stages. On the other hand, the older leaves (leaf 3) had slightly lower F_v/F_m in treatments with no or insufficient nitrogen supply. The trend of F_v/F_m in leaf 1 neither in leaf 3 did not follow the trend of plant nitrogen content or nitrogen supply.

Values of PI_{abs} parameter derived from ChlF records (Figures 1d–f) confirmed a much higher responsiveness of PI_{abs} compared to F_v/F_m. The values in 0N treatment were significantly lower in both leaf 1 and leaf 3 in all three growth stages. It corresponds with the lowest leaf nitrogen and chlorophyll content as well as low production of biomass (Table 1). Moreover, the PI_{abs} values indicated lower photosynthetic performance of leaf 3 compared to leaf 1, especially in 0N and ½N treatments; the difference was getting higher in later growth stages. On the other hand, there were only insignificant differences among PI_{abs} values in leaf 1 of treatments ½N, 1N and 2N in stages of tillering and stem elongation (Figures 1d,e).

The results of PI_{tot} parameter (Figures 1g–i) were similar to PI_{abs} (Figures 1d–f); however, we could observe some differences. Firstly, the differences between leaf 1 and leaf 3 were much higher in PI_{tot}. Moreover, in later growth stages we could observe also significant differences between 1N and 2N treatments in top leaf, which was not found in PI_{abs}.

We examined the reliability of the measured parameters in field conditions expressed by the stability of the values during hot and sunny day (Figure 2). We could see that the PI_{abs} values progressively decreased due to heat stress and photoinhibition (similarly as shown by decrease of F_v/F_m values). On the other hand, the values of PI_{tot} (Figure 2c) were much more stable and there were only insignificant differences between measurement in the morning and any other measurement during the day.

Table 1. Nitrogen content and plant growth parameters in evaluated growth stages

Growth stage treatment	Tillering (BBCH 25-29)				Stem elongation (BBCH 35-39)				Pre-anthesis (BBCH 55-60)			
	0N	½N	1N	2N	0N	½N	1N	2N	0N	½N	1N	2N
Nitrogen – leaves (mg/g)	33.6	41.9	40.6	45.5	24.9	36.0	37.0	43.1	13.5	23.8	30.4	34.7
Dry mass – total (g/plant)	0.36	0.57	0.75	0.86	0.58	1.09	1.19	1.55	0.65	1.78	2.14	2.77
Chlorophyll, leaf 1 (mg/m^2)	172.8^a	381.9^{bc}	457.7^c	433.8^c	233.5^a	497.7^{bc}	596.8^c	576.4^c	182.0^a	361.8^b	517.3^c	619.6^d
Chlorophyll, leaf 3 (mg/m^2)	126.9^a	316.9^b	368.1^{bc}	398.1^{bc}	131.1^a	419.7^b	488.7^{bc}	538.4^c	87.5^a	363.8^b	382.8^b	448.5^c

Small letters indicate statistically homogenous groups (Tukey *HSD* test, α = 0.05)

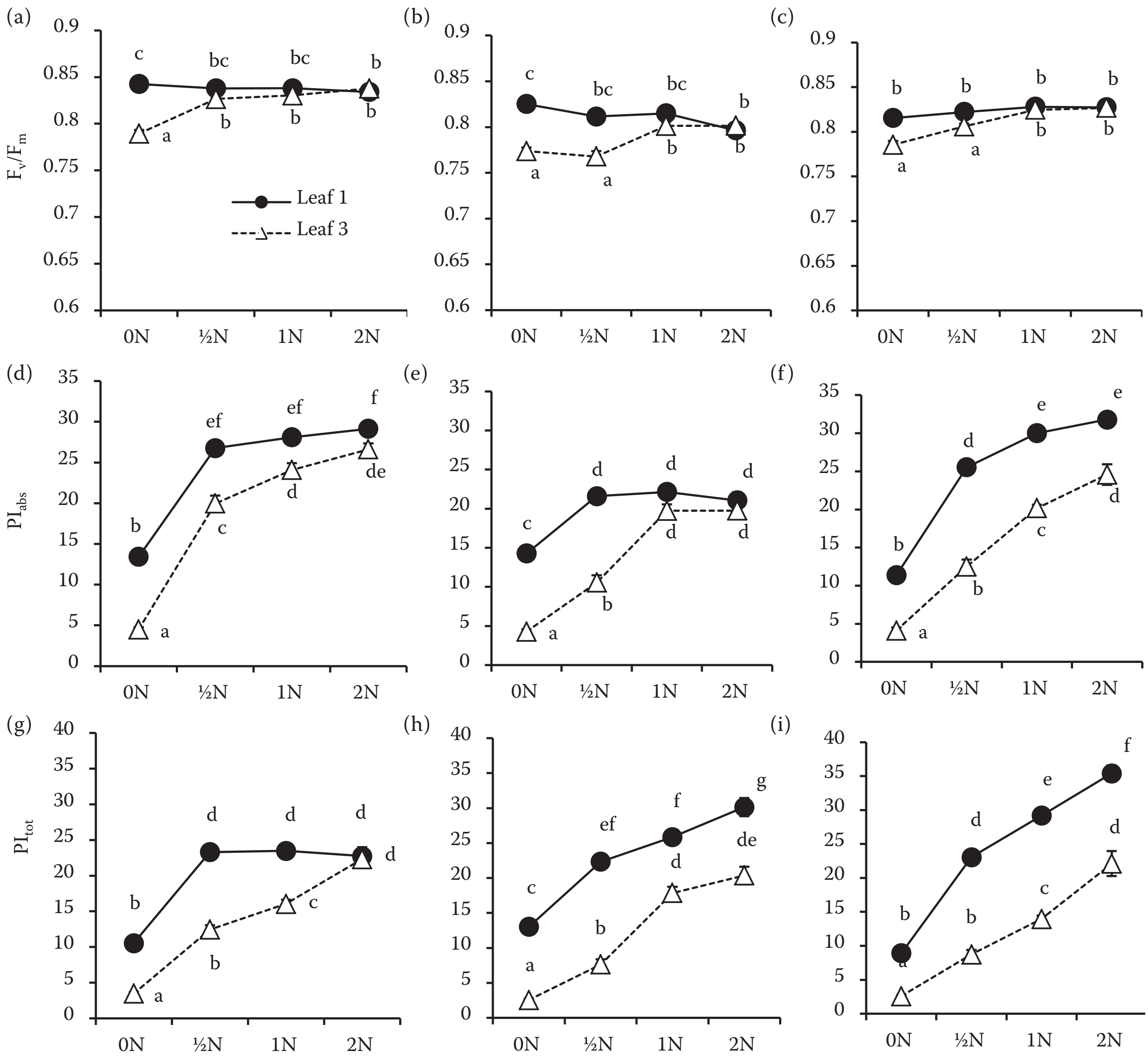

Figure 1. The calculated parameters derived from fast fluorescence transients recorded in the first, youngest fully developed leaf (leaf 1) or the third leaf (leaf 3) on the wheat main stem, counted from the top. The ChlF measurements were done in three different growth stages: in the stage of tillering (column left), during stem elongation (middle column) and before anthesis (column right). Figures (a), (b) and (c) represent the values of the maximum quantum yield of PS II photochemistry (F_v/F_m), figures (d), (e) and (f) the performance index (PI_{abs}) and figures (g), (h) and (i) the total performance index (PI_{tot}). Means ± SE are presented; the small letters indicates statistically homogenous groups (Tukey *HSD* test, $\alpha = 0.05$, $n = 50$)

DISCUSSION

ChlF techniques are frequently employed in physiological analyses of nitrogen deficiency in plants; however, the most of papers focus on F_v/F_m parameter as it is simple to measure and interpret. However, F_v/F_m is insensitive to many factors that negatively affect plant photosynthesis, such as drought (Živčák et al. 2008b). Unlike to results of some authors (Guidi et al. 1997, Janušauskaite and Feiziene 2012), our results indicate that F_v/F_m is insensitive even to nitrogen deficiency, except the decrease in old leaves indicating initiation of leaf senescence.

On the other hand, performance indices much better reflected expected changes in leaf photosynthetic performance, as it was shown even previously (van Heerden et al. 2003, 2004). This was mainly

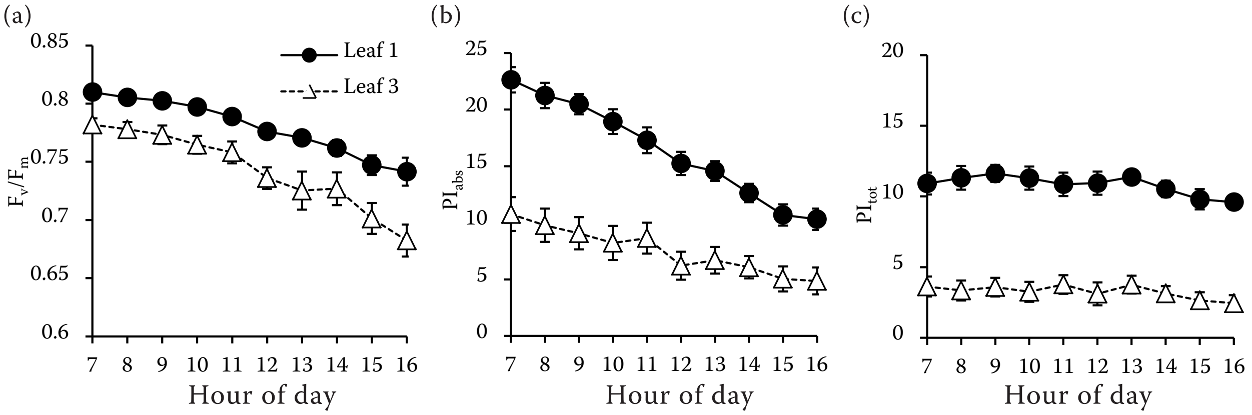

Figure 2. The daily trend of mean values of the maximum quantum yield of photochemistry (F_v/F_m) (a); performance index (PI_{abs}) (b), and total performance index (PI_{tot}) (c). The records were done during the sunny and hot day (air temperature ranged from 18°C at 7:00 to 32°C afternoon). Measurements for assessment of diurnal changes were done in plants with optimum nitrogen nutrition (1N) after anthesis (BBCH 70–75). Means ± SE from 15 measurements are presented

due to the significant effect of nitrogen deficiency on the density of reaction centers (Lu et al. 2001, Li et al. 2012, etc.). The differences between the PI trends found in the youngest leaf (leaf 1) and old leaf (leaf 3) represent a nice example of the effects of nitrogen redistribution on photosynthetic performance, which was dominant in plants poorly supplied with nitrogen. After emergence of the spike, the nitrogen can be re-distributed also from leaves, as disassembly of photosynthetic apparatus under stress conditions can be a valuable source of nitrogen for the grain (Vassileva et al. 2012).

Recently introduced parameter total performance index (Smit et al. 2009) extended the recorded effects also to the capacity of electron transport between photosystems (Schansker et al. 2005), which is not considered in PI_{abs}. In terms of the mechanism, the nitrogen deficiency was proven to increase the cyclic electron transport and there is less PS I available for accepting electrons from PS II (Antal et al. 2010). Similar to our results, Nikiforou and Manetas (2011) attributed the positive correlation between leaf nitrogen content and PI_{tot} values to decrease of PS I activity in linear electron transport caused by nitrogen deficiency. In parallel, Redillas et al. (2011) observed the decrease of PI_{tot} during N-depletion as a result of the decrease in the reduction of end electron acceptors on PS I.

Another important issue is the reliability of the measured parameters. As the performance indices are very sensitive, they can also respond to fluctuating environmental factors, such as actual temperature or incident light intensity. High light, especially when associated with high temperature, can lead to photoinhibition or slow relaxation of PS II photochemistry, which causes a decrease of all three components included in PI_{abs}, mostly of the reaction center number (Kalaji et al. 2012). It makes this parameter particularly suitable to observe diurnal changes in the status of PS II photochemistry (e.g. in ecophysiological studies), but less reliable to compare the intrinsic properties of photosynthetic apparatus in different samples. The values of PI_{tot} were much more stable during the day, thanks to the fact that the fewer electrons released by decreased number of reaction centers were balanced by higher probability that these electrons will be accepted by photosystem I (as indicated by individual components of PI_{tot}, not shown here). Therefore, we can consider PI_{tot} to be more useful for comparison of plant photosynthetic properties in field conditions, including photosynthetic performance of plants grown under different nitrogen supply.

In conclusion, our results indicate that parameters derived from rapid and non-invasive measurements of the ChlF kinetics can serve for assessment of leaf photosynthetic performance influenced by different nitrogen nutrition, useful for crop research and practical applications. We suggest using the integrative parameter total performance index, which was shown to be sufficiently sensitive and reliable.

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