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# Characterization of photosynthetic performance during natural leaf senescence in winter wheat: Multivariate analysis as a tool for phenotypic characterization

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## Abstract

In the current research, we used chlorophyll fluorescence measurements and photosynthetic pigment content to evaluate onset and rate of the flag leaf senescence every 7 d beginning at the flowering stage (0 d after flowering, DAF) until late senescence stage (35 DAF) on three winter wheat field-grown varieties (Alka, Žitarka, and Olimpija) with the similar maturation time. OJIP curves implied that flag leaves of variety Alka detained the longest photosynthetic efficiency, while the earliest symptoms of senescence onset were indicated by positive L-band at 7 DAF and K-band at 14 DAF in variety Olimpija. A shift in I–P phase in the wheat flag leaves suggested the reduction of first acceptors in PSI, implying degradation of photosynthetic apparatus. Performance index ($PI_{total}$) proved to be the sensitive indicator of senescence; it decreased very early at 7 DAF in the flag leaves of variety Olimpija compared to Alka and Žitarka. Principal component analysis elucidated quantum yield of energy dissipation and dissipated energy flux per reaction centre as the earliest indicators of senescence in wheat flag leaves and thus showed that multivariate approach may be useful in detection of senescence onset. Sustainability indexes (SIs) corroborated the statistical significance results of evaluated parameters implying that SIs can be used as user friendly data analysis for screening purposes. Selection for functional stay-green trait could contribute to increasing crop yields.

*Additional key words*: fluorescence transient; grain filling; JIP-test; leaf maturation; photoinhibition; photosystem II.

## Introduction

Senescence is natural process of aging of plant cells, tissues, and organs characterized as the last developmental stage in plants (Distelfeld *et al.* 2014). In wheat plants, the flag leaf senescence onset very often coincides with the grain-filling period, so it is the important factor in wheat production (Zhang *et al.* 2006) because approximately 30–50% of photoassimilates in wheat grain is coming from the flag leaf photosynthesis (Sylvester-Bradley *et al.* 1990). Leaf senescence is characterized by degradation of photosynthetic pigments and disassembling of photosynthetic apparatus as well as chloroplast protein degradation (Lu *et al.* 2001, Gregersen *et al.* 2008). These events negatively influence photosynthetic process, consequently resulting in disturbances in all aspects of the plant metabolism and physiology (Kalaji *et al.* 2018a). In previous research of Lu *et al.* (2001, 2002, 2003), during natural

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*Abbreviations*: ABS/RC – absorption flux (of antenna chlorophylls) per reaction centre; Car – carotenoids; ChlF – chlorophyll *a* fluorescence; DAF – days after flowering; $DI_0$/RC – dissipated energy flux per reaction centre; $ET_0$/RC – electron transport flux (further than $Q_A^-$) per reaction centre; $F_0$ – minimal fluorescence intensity (50 μs); FM – fresh mass; $F_V$ – maximal variable fluorescence; OEC – oxygen-evolving complex; PCA – principal component analysis; $PI_{ABS}$ – performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors; $PI_{total}$ – performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors; RC – reaction centre; RC/$CS_0$ – density of reaction centres per cross section; $RE_0$/RC – electron flux reducing end electron acceptors at the PSI acceptor side per reaction centre; SI – sustainability index; $TR_0$/RC – trapping flux (leading to $Q_A$ reduction) per reaction centre; $W_{OJ}$ – relative variable fluorescence between O- and J-steps; $W_{OK}$ – relative variable fluorescence between O- and K-steps; $W_{OP}$ – relative variable fluorescence between O- and P-steps; $\delta_{R0}$ – probability that an electron from the electron transport chain is transferred to reduce end electron acceptors at the PSI acceptor side; $\varphi_{D0}$ – quantum yield of energy dissipation at t = 0; $\varphi_{P0}$ – maximum quantum yield for primary photochemistry at t = 0; $\varphi_{R0}$ – quantum yield for reduction of end electron acceptors at the PSI acceptor side; $\varphi_{E0}$ – quantum yield of electron transport at t = 0; $\psi_{E0}$ – probability that a trapped exciton moves an electron into the electron transport chain beyond $Q_A^-$.
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flag leaf senescence, photosynthetic performance of the field-grown wheat plants was investigated. By the use of modulated chlorophyll (Chl) fluorescence technique, they found that PSII apparatus during senescence progressing remained functional, but downregulated under the steady state of photosynthesis. They associated such a downregulation with the closure of PSII centres and an enhanced xanthophyll cycle-related thermal dissipation in the PSII antennae. PSII downregulation and activation of xanthophyll cycle occurred after degradation of Chl, followed by neoxantin and β-carotene pigments, accompanied by increased Chl *a*/*b* ratio at one side and lutein and xanthophyll pigments maintenance at the other side. Zhang *et al.* (2006) found that later onset and slower rate of senescence in wheat variety NM9 grown in the field may be responsible for its higher grain yield than that in variety NM8 ageing earlier. Similar results were obtained by Yang *et al.* (2007) who showed that the higher photosynthetic capacity can accumulate more dry matter in the flag leaf of wheat hybrid compared to its parents, and might be the physiological basis for the higher grain yield.

Photosynthesis deals with energy flow through the plant, starting with light absorption, its conversion to chemical energy, and dissipation of unused energy trough thermal dissipation or/and Chl fluorescence (ChlF). Therefore, ChlF, as concomitant process to energy absorption, conversion, and thermal dissipation, can be used for estimation of photosynthetic efficiency (Maxwell and Johnson 2000). Recently, modern fluorimeters, which record emitted fluorescence in high frequency, made ChlF technic a user friendly scientific tool because it performs fast and noninvasive analysis and gives powerful data about photosynthetic process. Such fluorimeters induce ChlF by amplification of short pulse (1-s lasting) of strong actinic light on the dark-adapted leaf. Recorded fluorescence data (118 data in 1 s) present fast polyphasic fluorescence transient and this kinetics (OJIP kinetics) is basis for the photosynthetic efficiency interpretation (Kalaji *et al.* 2014). Analysis of OJIP kinetics, called the JIP-test (Strasser *et al.* 2004), gives us numerous structural and functional parameters that quantify photosynthetic performance through PSII complexes (Yusuf *et al.* 2010).

Important advantage of OJIP kinetics is that it can capture delicate alterations in photosynthetic performance which other technics based on ChlF measurement cannot. JIP-test calculations describe specific energy fluxes, phenomenological fluxes, and performance indexes (Strasser *et al.* 2004) altogether with quantum yields, photochemical and nonphotochemical quenching obtained by saturation pulse method (Maxwell and Johnson 2000). Furthermore, the shape of characteristic OJIP curve can be changed under nonoptimal conditions (biotic or abiotic stress) for photosynthesis, in the way that unusual shift of fluorescence can be observed at 300 μs (K-band), 150 μs (L-band) as well as shift in I-step (about 30 ms) (Yusuf *et al.* 2010, Kalaji *et al.* 2018a, Pavlović *et al.* 2019). Above mentioned facts enable to detect alterations in photosynthetic process, while the most frequently used parameters, such as maximal quantum yield of PSII ($F_v/F_m$) and performance index (PI), stay still unchanged. Since ChlF is fast, noninvasive, informative, and cheap technic, it is widely eligible tool for screening of breeding material for different purposes (Kovačević *et al*. 2017, Kalaji *et al.* 2018b, Galić *et al.* 2019, Rapacz *et al.* 2019).

ChlF transient completed by JIP-test is very informative and might give a detailed explanation of downregulation of photosynthesis during the senescence process. According to our best knowledge, such investigations on the wheat flag leaves are still missing. Our main goal was to identify one or more JIP parameters sensitive enough for the early detection of senescence onset because it could be an important tool for wheat breeders who are targeting selection for 'stay-green' genotypes. Furthermore, the main objective of this study focused on the onset of the senescence in three winter wheat varieties with similar maturation time through chlorophyll fluorescence transient followed by JIP-test and concentrations of photosynthetic pigments. Above mentioned analyses provided numerous parameters, so we hypothesised that the use of multivariate method of data analysis should elucidate the most informative parameter(s) for use in wheat breeding selection. Furthermore, wheat varieties could be differentiated with explanation of relations among the investigated parameters during the flag leaf maturation and senescence.

## Materials and methods

**Plant material and experimental design**: The experiment was conducted at Agricultural Institute Osijek (AIO), Osijek, Croatia, on three winter wheat (*Triticum aestivum* L.) field-grown varieties (Alka, Žitarka, and Olimpija) with similar maturation time in vegetation season 2016–2017. The varieties were sown during October in 2016 in 7.56 $m^2$ randomized plots using a *Seedmatic* seeding machine (*Hege*, Germany) at the experimental field (45°32'N, 18°44'E). Seed density was 330 seeds $m^{-2}$ for all varieties, where each variety was replicated in two plots. To meet the winter wheat plant nutrient requirements, fertilization was done during the study (N:P:K, 120:80:120 kg $ha^{-1}$). Pesticides and herbicides were used as necessary to minimise the effects of pests and weeds. The mean annual temperature during the vegetation season was 10.0°C with the sum of annual precipitation during this period of 481.5 mm (Fig. 1). The date of flowering (growth stage 65, Zadoks *et al.* 1974) was determined when plants shed at least 50% of anthers of the spike throughout each plot. Measurements and samplings were performed in 7-d intervals from flowering to 35 d after flowering (DAF).

**Measurement of the fast Chl *a* fluorescence transient**: Measurements of ChlF were made on the one fully expanded flag leaf of five different plants in each plot using a plant efficiency analyser (*Handy PEA*, *Hansatech Instruments Ltd.*, Norfolk, UK). Flag leaves were dark-adapted for 30 min using special leaf clips. ChlF transient was induced by red actinic light [wavelength at peak 650 nm; 3,200 μmol(photon) $m^{-2}$ $s^{-1}$] and 1 s of transient fluorescence was recorded. Fluorescence signals have been collected from 10 μs up to 1 s with data acquisition every 10 μs for the first 300 μs, then continued every 100 μs

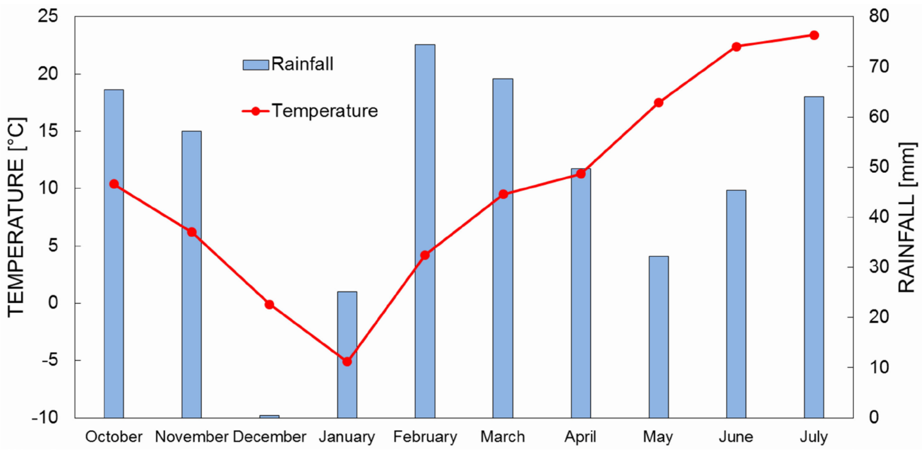


Fig. 1. Climatic conditions during vegetative season 2016/2017 for each month separately.

up to 3 ms, and later every 1 ms, 118 points within 1 s in total. ChlF transient data were demonstrated as OJIP curves and used for calculation of JIP-test parameters (Table 1S, *supplement*).

**Analysis of Chl fluorescence data**: The OJIP transients were double-normalized between O- (50 µs) and P-steps and presented as the relative variable fluorescence [$W_{OP} = (F_t - F_0)/(F_P - F_0)$] on a logarithmic scale. Native fluorescence induction curves were also presented. Furthermore, normalization between O- and K- (300 µs) steps detected L-band (150 µs) which was demonstrated as variable fluorescence [$W_{OK} = (F_t - F_0)/(F_K - F_0)$] plotted with difference kinetics $\Delta W_{OK} = W_{OK} - (W_{OK})_{ref}$, where $(W_{OK})_{ref}$ represented reference values obtained from measurements at 0 DAF. Similarly, normalization between O- and J- (2 ms) steps detected K-band (300 µs) which was demonstrated as variable fluorescence [$W_{OJ} = (F_t - F_0)/(F_J - F_0)$] on the linear time scale plotted with difference kinetics $\Delta W_{OJ} = W_{OJ} - (W_{OJ})_{ref}$, where $(W_{OJ})_{ref}$ represented referent values obtained from measurements at 0 DAF (Strasser *et al.* 2004, Yusuf *et al.* 2010).

The particular data points of ChlF transients were used to calculate JIP-test-derived parameters according to Strasser *et al.* (2000). The analysed parameters and their equations are described in Table 1S.

**Determination of photosynthetic pigments content**: After measurement of the ChlF, the same flag leaves were taken, pooled together, and stored at –80°C until further analysis. Leaf tissue was homogenized into a fine powder by liquid nitrogen with addition of magnesium hydroxide carbonate and five replicates were separated (0.1 g) from each composed sample. Photosynthetic pigments were extracted with the cold absolute acetone and then reextracted several times until plant tissue was completely uncoloured. The concentrations of Chl *a*, Chl *b*, and carotenoids (Car) were determined spectrophotometrically (*Specord 200*, *Analytik Jena*, Germany) at 470, 661.6, and 644.8 nm according to Lichtenthaler (1987) and expressed as mg $g^{-1}$ of fresh mass. The Chl *a*/*b* was calculated.

**Statistical analysis**: Statistical differences between all parameters in three winter wheat varieties at six time points (0, 7, 14, 21, 28, and 35 DAF) were analysed using analysis of variance followed by *post hoc Fisher*'s Least Significant Difference (LSD) test. Data presented in the text, figures, and tables are means ± SE of ten biological replicates. Differences were considered significant at $P<0.05$. Correlations among JIP parameters, photosynthetic pigments and winter wheat varieties were explored by principal component analysis (PCA) in order to distinguish parameters, which could be indicators of the senescence onset. The analyses were performed on ten cases for each sampling point (DAF) in *Statistica* 7.0 software (*Statsoft Inc.*, Tulsa, OK, USA).

## Results

The first visible symptoms of senescence were seen as yellowing of apical parts of flag leaves at 7 DAF in Olimpija (Fig. 2). In flag leaves of varieties Alka and Žitarka, the first symptoms of senescence occurred at 14 and 21 DAF, respectively, exhibited as sporadic yellow blur on the whole leaf surface.

**Chl *a* fluorescence**: Variable fluorescence curves were constructed to find changes in the photosynthetic performance of winter wheat varieties during natural leaf

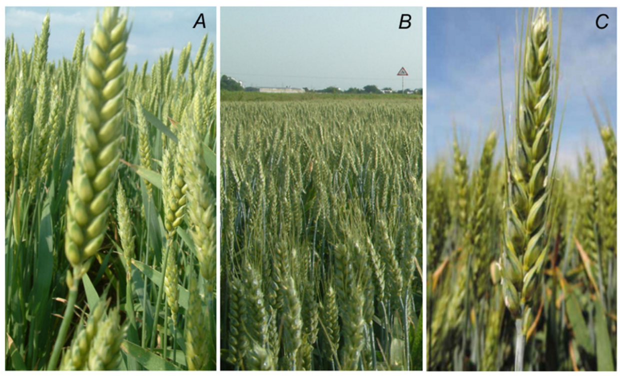


Fig. 2. Status of senescence in winter wheat varieties Alka (*A*), Žitarka (*B*), and Olimpija (*C*) 7 d after flowering.

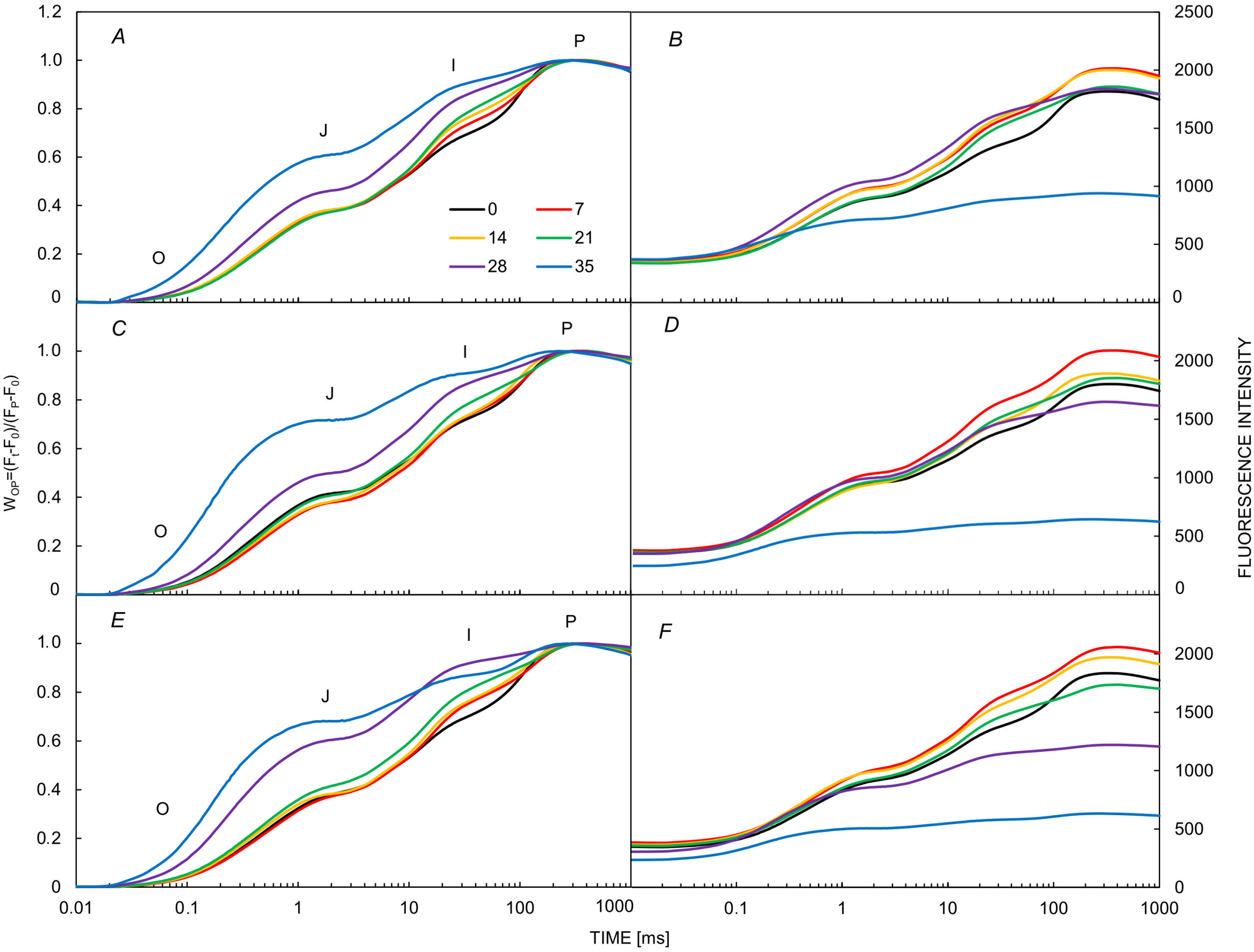


Fig. 3. Double O–P normalized OJIP transients (*A*,*C*,*E*) and native fluorescence induction curves (*B*,*D*,*F*) in the flag leaves of winter wheat varieties Alka (*A*,*B*), Žitarka (*C*,*D*) and Olimpija (*E*,*F*) at 0, 7, 14, 21, 28, and 35 d after flowering (DAF). Each curve presents average kinetics of ten replicates.

senescence (Fig. 3). The first visible changes between variable fluorescence curves occurred at I-step starting already at 7 DAF in the flag leaves of Alka and the same trend continued until the end of the experiment accompanied by alterations in the whole OJIP curve which occurred at 28 and 35 DAF (Fig. 3*A*). In the flag leaves of variety Žitarka (Fig. 3*C*), the first visible changes on OJIP curve were observed in I-step at 21 DAF followed by alterations in the whole OJIP curve starting at 28 DAF. Flag leaves of variety Olimpija during experimental period revealed alterations in the whole OJIP curve starting already at 7 DAF (Fig. 3*E*). Fluorescence induction curves (Fig. 3 *B*,*D*,*F*) revealed distinct differences between varieties. Shape of OJIP induction curve at 28 DAF showed that in Alka, photochemistry remained functional, while in Žitarka and Olimpija, flattening of fluorescence induction curve occured, more markedly in Olimpija. Fluorescence induction curves were flattened in all three varieties at 35 DAF. A clear differences in L-bands (Fig. 4*A*,*C*,*E*) and K-bands (Fig. 4*B*,*D*,*F*), generated from the normalized O–K and O–J curves, were found within varieties. The first negative effects of natural senescence on photosynthesis were seen as the positive L-bands in the flag leaves of variety Olimpija at 7 DAF (Fig. 4*E*) accompanied with positive K-bands at 14 DAF (Fig. 4*F*). Other two varieties reacted similarly but the L-bands prolonged time to positive level at 28 DAF; while K-bands in Žitarka were negative until 28 DAF, in Alka, this was until 14 DAF.

The significant differences were found between varieties in most of the JIP-test parameters during natural flag leaf senescence of winter wheat varieties (Fig. 5; Table 2S, *supplement*) as well as between the time of measurements. JIP-test parameters shown in radar plots were normalized to enable the comparison of the variables measured at a different scale. Values at the radar plot presented percentage of values obtained for measurements at 0 DAF. At the 7 DAF, no significant differences were between fluorescence indices ($F_0$ and $F_V$), quantum yields and efficiencies ($\varphi_{D0}$, $\varphi_{P0}$, $\varphi_{R0}$, $\varphi_{E0}$, $\psi_{E0}$, and $\delta_{R0}$), specific energy fluxes, and phenomenological fluxes ($ABS/RC$, $TR_0/RC$, $ET_0/RC$, $DI_0/RC$, $RE_0/RC$, and $RC/CS_0$), and performance indexes ($PI_{ABS}$ and $PI_{total}$) in the flag leaves of Alka (Fig. 5*A*). The first significant changes

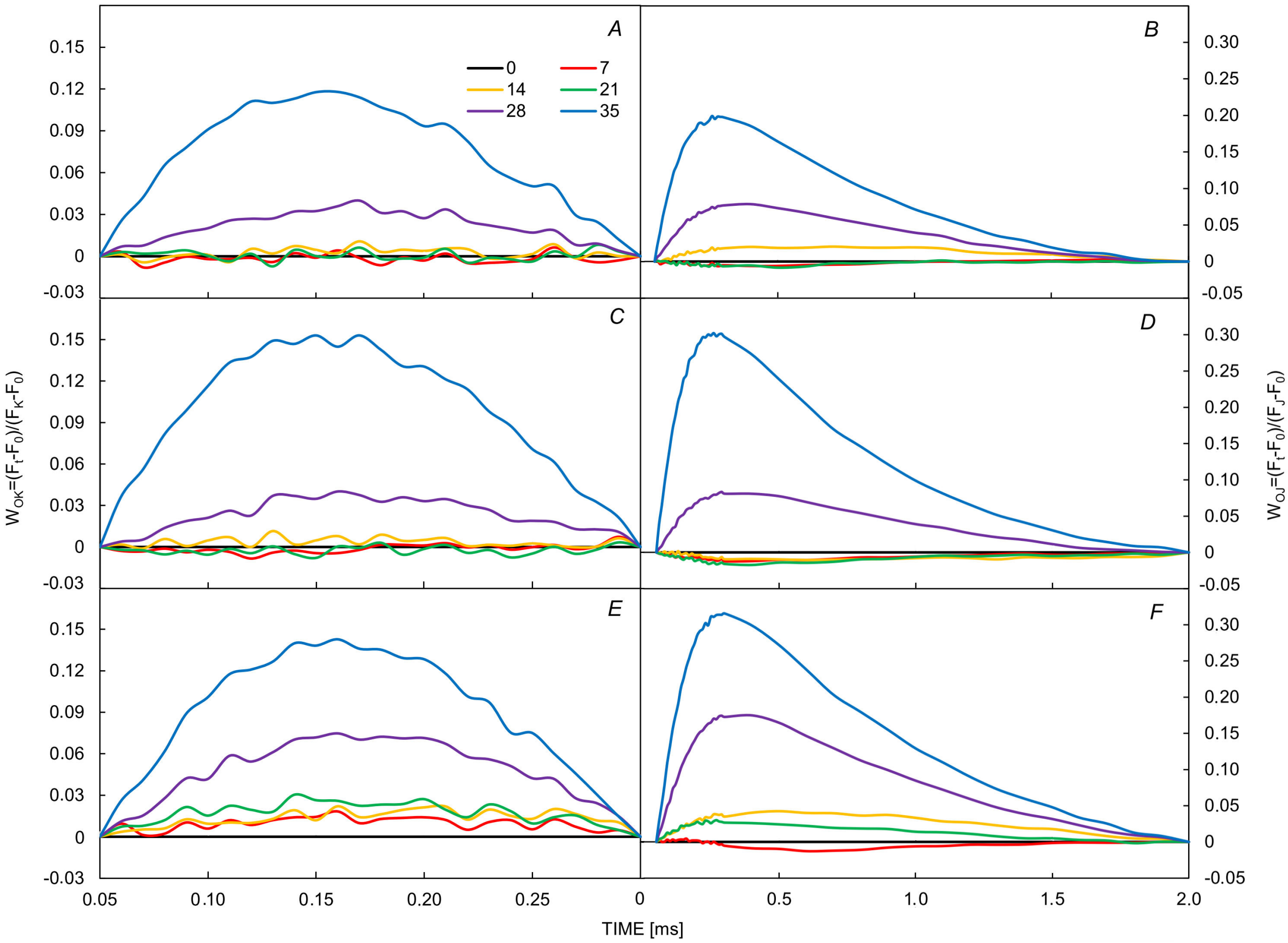


Fig. 4. Chlorophyll *a* fluorescence transient curves in the flag leaves of winter wheat varieties Alka (*A*,*B*), Žitarka (*C*,*D*) and Olimpija (*E*,*F*) at 0, 7, 14, 21, 28, and 35 d after flowering (DAF). Fluorescence data are normalized between O- and K-steps (L-band) (*A*,*C*,*E*) and plotted as difference kinetics [$\Delta W_{OK} = W_{OK} - (W_{OK})_{ref}$] in the 0.05–0.3 ms time range. Fluorescence data are normalized between O- and J-steps (K-band) (*B*,*D*,*F*) and plotted as difference kinetics [$\Delta W_{OJ} = W_{OJ} - (W_{OJ})_{ref}$] in the 0.05–2 ms time range. Referent values were obtained from measurements at the time of flowering (0 DAF). Each curve presents average kinetics of ten replicates.

appeared at 14 DAF as the decrease of $\varphi_{R0}$, $\delta_{R0}$, $RE_0/RC$, and $PI_{total}$ compared to 0 DAF. All ChlF parameters remained significantly at the same level until 28 DAF, when significant decreases were found in $\varphi_{R0}$, $\varphi_{E0}$, $\psi_{E0}$, $RE_0/RC$, $PI_{ABS}$, and $PI_{total}$, while the increase occurred in $TR_0/RC$. At 35 DAF, all investigated parameters were significantly altered with exception of $F_0$, which remained at the same level.

ChlF-derived parameters in the flag leaves of winter wheat variety Žitarka changed already at 7 DAF, compared to 0 DAF (Fig. 5*B*), namely $F_V$, $\varphi_{R0}$, $\delta_{R0}$, $RE_0/RC$, $RC/CS_0$, and $PI_{total}$. At 14 DAF, only $PI_{ABS}$ decreased, while at 21 DAF, the larger significant alterations started to be seen as decrements of $F_V$, $\varphi_{R0}$, $\delta_{R0}$, $RE_0/RC$, $PI_{ABS}$, and $PI_{total}$. All traced ChlF parameters significantly changed at 28 DAF in the way that most parameters decreased, except increase of $\varphi_{D0}$, $ABS/RC$, $TR_0/RC$, and $DI_0/RC$. Similar significant alteration also occurred at 35 DAF.

Several of the ChlF parameters in the flag leaves of winter wheat variety Olimpija decreased already at 7 DAF, namely $F_V$, $\varphi_{R0}$, and $PI_{ABS}$ (Fig. 5*C*). Following significant changes appeared at 14 DAF as diminution in $\varphi_{R0}$, $RE_0/RC$, and $PI_{total}$ and decrement continued at 21 DAF in $\varphi_{E0}$, $\psi_{E0}$, $\delta_{R0}$, $RC/CS_0$, and $PI_{ABS}$. At 35 DAF, all traced ChlF parameters significantly altered with exception of $\varphi_{R0}$ and $PI_{total}$ which remained at the same level. Parameters $\varphi_{D0}$, $ABS/RC$, $TR_0/RC$, and $DI_0/RC$ increased, while others decreased, similarly as it was in the flag leaves of varieties Alka and Žitarka.

**Photosynthetic pigments**: Chl *a* concentration increased at 7 DAF in the flag leaves of Alka and Žitarka, but started decreasing continuously from 14 DAF till the end of experiment (35 DAF) (Fig. 6*A*, Table 2S). In the flag leaves of Olimpija, a significant diminution of Chl *a* started at 14 DAF and it continued during the experiment. In variety Žitarka, a similar trend was observed also for Chl *b* (Fig. 6*B*), while in the flag leaves of varieties Alka and Olimpija, the concentration of Chl *b* remained unchanged until 28 DAF and 21 DAF, respectively, then started decreasing and diminution continued until the end of the experiment. Carotenoids (Car) in the flag leaves of variety Alka were

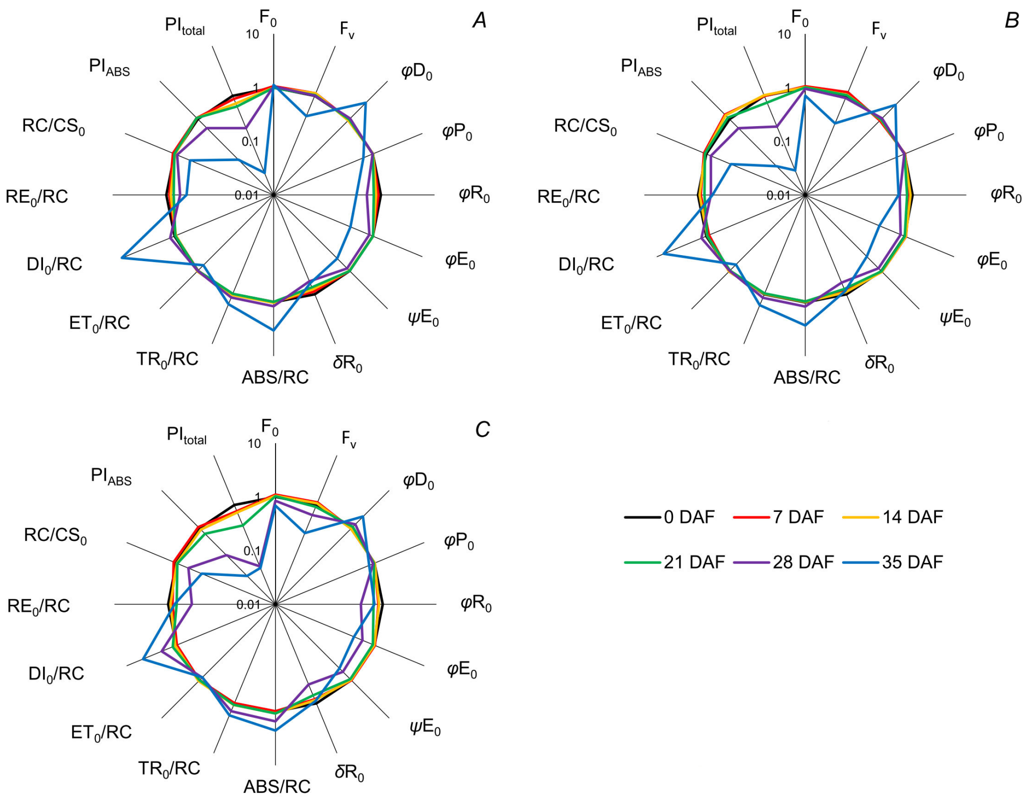


Fig. 5. Radar plots of structural and functional JIP-test parameters in the flag leaves of winter wheat varieties Alka (*A*), Žitarka (*B*) and Olimpija (*C*) at 0, 7, 14, 21, 28, and 35 d after flowering (DAF). Each data presents average value of ten replicates normalized and shown as percentage of values obtained at 0 DAF, enabling the comparison of variables measured on different scales. Raw data shown in radar plots and statistical differences obtained by *ANOVA* followed by *Fisher*'s LSD test ($P$<0.5) are presented in Table 2S. $F_0$ – minimal fluorescence intensity (50 μs); $F_V$ – maximal variable fluorescence; $\varphi D_0$ – quantum yield of energy dissipation at t = 0; $\varphi P_0$ – maximum quantum yield for primary photochemistry at t = 0; $\varphi R_0$ – quantum yield for reduction of end electron acceptors at the PSI acceptor side; $\varphi E_0$ – quantum yield of electron transport at t = 0; $\psi E_0$ – probability that a trapped exciton moves an electron into the electron transport chain beyond $Q_A^-$; $\delta R_0$ – probability that an electron from the electron transport chain is transferred to reduce end electron acceptors at the PSI acceptor side; ABS/RC – absorption flux (of antenna chlorophylls) per RC; $TR_0$/RC – trapping flux (leading to $Q_A$ reduction) per RC; $ET_0$/RC – electron transport flux (further than $Q_A^-$) per RC; $DI_0$/RC – dissipated energy flux per RC; $RE_0$/RC – electron flux reducing end electron acceptors at the photosystem I (PSI) acceptor side per RC; RC/$CS_0$ – density of reaction centres per cross section; $PI_{ABS}$ – performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors; $PI_{total}$ – performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors.

at the same level until 28 DAF when they significantly decreased and the same trend continued till 35 DAF (Fig. 6*C*). In the flag leaves of Žitarka and Olimpija, the first significant change in the Car content was found at 14 DAF, seen as diminution which continued until 35 DAF. Values of Chl *a*/*b* (Fig. 6*D*) in the leaves of Alka increased signifi-cantly at 35 DAF, while in varieties Žitarka and Olimpija at 21 DAF and 28 DAF, respectively; after that it continued increasing until the end of experiment.

**Sustainability index (SI)**: The SI of photosynthetic pigment contents and photosynthetic parameters of the winter wheat varieties (Alka, Žitarka, and Olimpija) at 7, 14, 21, 28, and 35 DAF, compared to 0 DAF, are shown in Table 1. The SI of Chl *a* and Car remained at the similar level (1) at 7, 14, and 21 DAF in Alka, while in Žitarka and Olimpija, those parameters decreased already at 14 DAF. In contrary, SI of Chl *b* in Alka decreased at 14 DAF, while in Žitarka and Olimpija remained at the same level until 21 DAF. Furthermore, SI of Chl *a*/*b* was generally increasing during the experiment, but more pronounced increment was noticed in Žitarka and Olimpija, compared to Alka at 35 DAF. In variety Alka, SI of $F_0$ remained at the similar level (1) through the whole experiment, while in Žitarka and Olimpija, it started decreasing at 28 DAF. Pronounced increments of SI for ABS/RC and $DI_0$/RC at 35 DAF was observed in variety Alka, compared to Žitarka and Olimpija. The SI of performance indexes ($PI_{ABS}$ and $PI_{total}$) behaved differently in those varieties: SI of $PI_{ABS}$ remained at the similar level at 7 and 14 DAF in all varieties and decreased afterward in Olimpija, while in Alka and Žitarka, decrements were observed at 28 DAF. The SI of $PI_{total}$ was more sensitive, with decrement already at 7 DAF in Alka and Olimpija and at 21 DAF in Žitarka.

**Principal component analysis (PCA)**: The PCA was performed on a correlation matrix of photosynthetic pigment contents and photosynthetic parameters for each

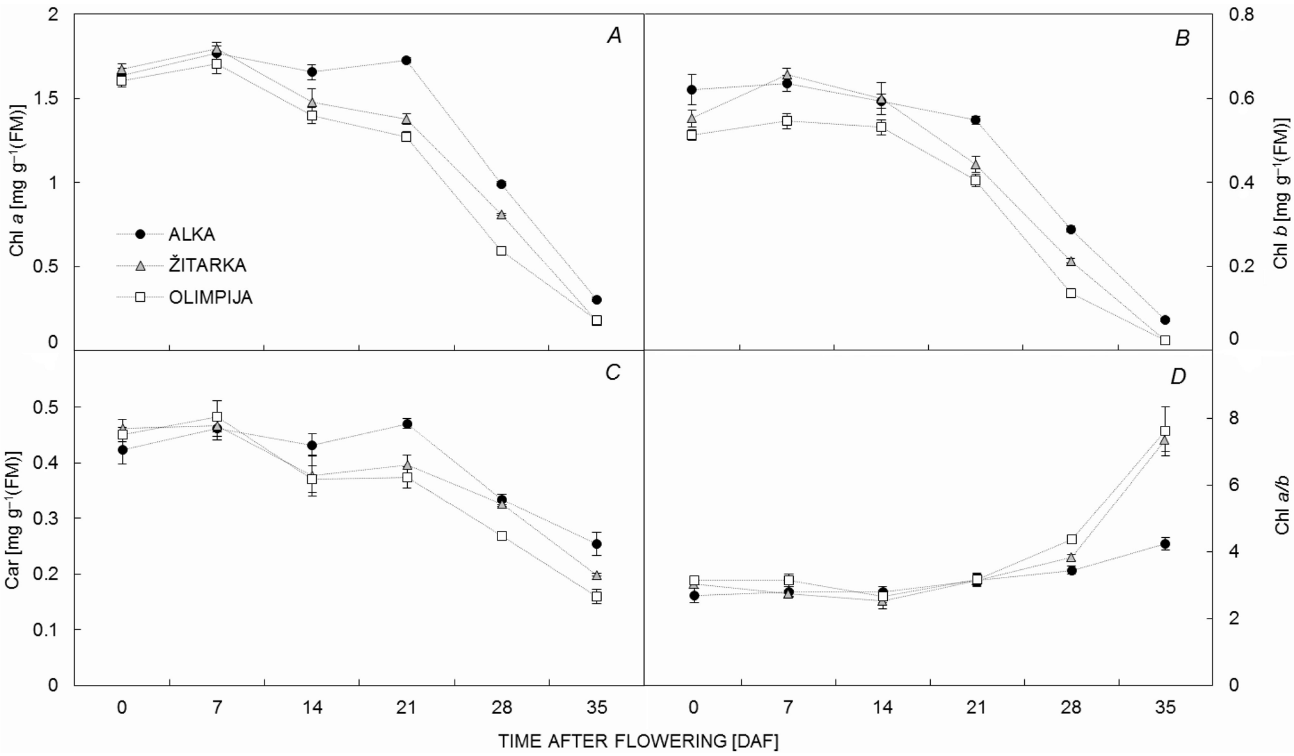


Fig. 6. Changes of photosynthetic pigments content in the flag leaves of winter wheat varieties Alka, Žitarka and Olimpija at 0, 7, 14, 21, 28, and 35 d after flowering (DAF). Content of chlorophyll *a* (*A*), chlorophyll *b* (*B*), carotenoids (*C*), and chlorophyll *a*/*b* ratio (*D*) are presented as means ± SE of ten biological replicates. Raw data and statistical differences obtained by *ANOVA* followed by *Fisher*'s LSD test ($P$<0.5) are presented in Table 2S.

experimental point separately (0, 7, 14, 21, 28, and 35 DAF) with the aim (*1*) to find parameters, which indicate the onset of senescence, (*2*) to separate wheat varieties according senescence onset, and (*3*) to explain relations among the parameters at flag leaf maturation and senescence (Fig. 7).

At 0 DAF (Fig. 7*A*), parameters were grouped as follows: specific energy fluxes (ABS, $TR_0$, $ET_0$, $DI_0$, $RE_0$ per RC) at the right side, and efficiencies and performance indexes at the left side of biplot. Variety Žitarka highly correlated with Chl *a*, Car, and Chl *a*/*b* content and parameters $F_0$ and $DI_0$/RC. Varieties Alka and Olimpija were located at the opposite side of biplot, close to parameters $PI_{ABS}$, $\varphi_{R0}$, and $\varphi_{E0}$. Varieties were distinctly separated at 7 DAF (Fig. 7*B*); whereas Žitarka correlated with specific energy fluxes and Chls, Alka with parameters describing dissipation and electron acceptors at the PSI acceptor side, and Olimpija with $PI_{ABS}$, Car, Chl *a*/*b*, $RC/CS_0$, and parameters describing electron transport. At 14 and 21 DAF (Fig. 7*C*,*D*), Alka was located at the opposite side of biplot, compared to Olimpija and Žitarka, and highly correlated with $\varphi_{P0}$ at 14 DAF and with $RC/CS_0$, Chl *a* and *b*, Car, and $PI_{ABS}$ at 21 DAF. At the other side, Žitarka and Olimpija correlated with parameters describing dissipation and electron acceptors at the PSI acceptor side at 14 DAF and dissipation at 21 DAF. PCA biplot for 28 DAF (Fig. 7*E*) is crucial for understanding of senescence onset because experimental parameters and varieties were divided by their high correlation in the way that variety Olimpija was isolated at the left side of biplot close to parameters Chl *a*/*b*, $\varphi_{D0}$, $DI_0$/RC, and ABS/RC. Žitarka and Alka were positioned at the right side of biplot, whereas Žitarka was close to performance indexes, parameters describing electron transport and electron acceptors at the PSI acceptor side, while Alka correlated with $\varphi_{P0}$, $F_0$, $F_V$, Chl *a* and *b*. At 35 DAF (Fig. 7*F*), variety Alka was in the correlation with Chl *a* and *b*, Car and $F_0$, while Žitarka and Olimpija correlated with Chl *a*/*b* and parameters describing electron acceptors at the PSI acceptor side.

## Discussion

In the wheat, recent studies of senescence focused on the flag leaf because it has been proven that flag leaf is the main source of assimilates during the grain-filling period which coincides with the onset of senescence (Wardlaw 1990, Zhang *et al.* 2006). Flag leaf senescence can be visualised as a leaf yellowing due to Chl loss but numerous additional molecular and biochemical processes contribute to the senescence syndrome as well (Distelfeld *et al.* 2014). The most significant syndrome of senescence is the decrease of photosynthetic capacity caused by disintegration of photosynthetic apparatus resulting from degradation of pigments and proteins (Gregersen *et al.* 2008). In the present study, changes in photosynthetic efficiency and photosynthetic pigment contents in the flag leaves of three winter wheat varieties grown in the field were investigated during period from the flowering stage

Table 1. Sustainability index (SI) of photosynthetic pigment contents and photosynthetic parameters in flag leaves of varieties Alka, Žitarka, and Olimpija at 7, 14, 21, 28 and 35 d after flowering (DAF), compared to data taken at the time of flowering (0 DAF); SI = (the values at 7, 14, 21, 28 or 35 DAF)/(the values at 0 DAF). Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; Car – carotenoids; $F_0$ – minimal fluorescence intensity (50 µs); $F_V$ – maximal variable fluorescence; $\varphi D_0$ – quantum yield of energy dissipation at t = 0; $\varphi P_0$ – maximum quantum yield for primary photochemistry at t = 0; $\varphi_{R0}$ – quantum yield for reduction of end electron acceptors at the PSI acceptor side; $\varphi_{E0}$ – quantum yield of electron transport at t = 0; $\psi_{E0}$ – probability that a trapped exciton moves an electron into the electron transport chain beyond $Q_A^-$; $\delta_{R0}$ – probability that an electron from the electron transport chain is transferred to reduce end electron acceptors at the PSI acceptor side; ABS/RC – absorption flux (of antenna chlorophylls) per RC; $TR_0$/RC – trapping flux (leading to $Q_A$ reduction) per RC; $ET_0$/RC – electron transport flux (further than $Q_A^-$) per RC; $DI_0$/RC – dissipated energy flux per RC; $RE_0$/RC – electron flux reducing end electron acceptors at the photosystem I (PSI) acceptor side per RC; RC/$CS_0$ – density of reaction centres per cross section; $PI_{ABS}$ – performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors; $PI_{total}$ – performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors.

| Parameters | Alka | | | | | Žitarka | | | | | Olimpija | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 7 DAF | 14 DAF | 21 DAF | 28 DAF | 35 DAF | 7 DAF | 14 DAF | 21 DAF | 28 DAF | 35 DAF | 7 DAF | 14 DAF | 21 DAF | 28 DAF | 35 DAF |
| Chl *a* | 1.081 | 1.012 | 1.054 | 0.606 | 0.186 | 1.072 | 0.882 | 0.822 | 0.485 | 0.104 | 1.064 | 0.872 | 0.794 | 0.370 | 0.113 |
| Chl *b* | 1.024 | 0.956 | 0.882 | 0.465 | 0.116 | 1.188 | 1.084 | 0.802 | 0.385 | 0.043 | 1.063 | 1.034 | 0.789 | 0.265 | 0.047 |
| Car | 1.089 | 1.020 | 1.111 | 0.788 | 0.600 | 1.008 | 0.816 | 0.857 | 0.706 | 0.429 | 1.073 | 0.822 | 0.830 | 0.594 | 0.354 |
| Chl *a*/*b* | 1.043 | 1.045 | 1.174 | 1.283 | 1.576 | 0.900 | 0.826 | 1.030 | 1.255 | 2.414 | 1.006 | 0.850 | 1.014 | 1.394 | 2.434 |
| $F_0$ | 1.062 | 1.005 | 0.991 | 1.048 | 1.134 | 1.061 | 1.020 | 1.018 | 0.953 | 0.711 | 1.104 | 1.045 | 1.032 | 0.847 | 0.686 |
| $F_V$ | 1.120 | 1.002 | 0.918 | 0.977 | 0.386 | 1.182 | 1.057 | 1.031 | 0.909 | 0.280 | 1.125 | 1.080 | 0.919 | 0.627 | 0.273 |
| $\varphi_{D0}$ | 0.966 | 0.949 | 1.068 | 1.058 | 2.586 | 0.916 | 0.971 | 0.991 | 1.048 | 2.372 | 0.986 | 0.977 | 1.097 | 1.283 | 2.039 |
| $\varphi_{P0}$ | 1.007 | 1.018 | 1.004 | 0.992 | 0.645 | 1.019 | 1.006 | 1.002 | 0.989 | 0.695 | 1.003 | 1.005 | 0.979 | 0.940 | 0.778 |
| $\varphi R_0$ | 0.898 | 0.813 | 0.733 | 0.546 | 0.350 | 0.880 | 0.913 | 0.786 | 0.574 | 0.547 | 0.828 | 0.860 | 0.672 | 0.393 | 0.703 |
| $\varphi_{E0}$ | 1.003 | 1.010 | 1.014 | 0.853 | 0.355 | 1.072 | 1.060 | 1.014 | 0.836 | 0.311 | 1.019 | 0.991 | 0.926 | 0.571 | 0.386 |
| $\psi_{E0}$ | 0.996 | 0.994 | 1.009 | 0.860 | 0.471 | 1.052 | 1.052 | 1.012 | 0.846 | 0.413 | 1.016 | 0.986 | 0.945 | 0.606 | 0.489 |
| $\delta_{R0}$ | 0.892 | 0.799 | 0.730 | 0.550 | 0.600 | 0.863 | 0.907 | 0.784 | 0.580 | 0.861 | 0.826 | 0.857 | 0.687 | 0.417 | 0.897 |
| ABS/RC | 0.984 | 1.023 | 0.982 | 1.177 | 3.330 | 0.959 | 0.979 | 0.963 | 1.193 | 2.669 | 0.983 | 1.071 | 1.083 | 1.522 | 2.261 |
| $TR_0$/RC | 0.990 | 1.041 | 0.986 | 1.168 | 1.590 | 0.977 | 0.984 | 0.965 | 1.180 | 1.660 | 0.986 | 1.077 | 1.062 | 1.428 | 1.739 |
| $ET_0$/RC | 0.986 | 1.033 | 0.994 | 1.001 | 0.710 | 1.026 | 1.030 | 0.971 | 0.987 | 0.674 | 1.000 | 1.059 | 1.001 | 0.852 | 0.842 |
| $DI_0$/RC | 0.953 | 0.939 | 0.967 | 1.220 | 11.591 | 0.878 | 0.957 | 0.955 | 1.252 | 7.218 | 0.969 | 1.042 | 1.182 | 1.959 | 4.696 |
| $RE_0$/RC | 0.885 | 0.831 | 0.733 | 0.557 | 0.421 | 0.891 | 0.941 | 0.764 | 0.577 | 0.539 | 0.829 | 0.907 | 0.690 | 0.362 | 0.754 |
| RC/$CS_0$ | 1.077 | 0.981 | 1.005 | 0.890 | 0.486 | 1.109 | 1.050 | 1.065 | 0.810 | 0.319 | 1.124 | 0.976 | 0.956 | 0.568 | 0.307 |
| $PI_{ABS}$ | 1.068 | 1.061 | 1.074 | 0.581 | 0.085 | 1.325 | 1.261 | 1.107 | 0.582 | 0.057 | 1.076 | 0.924 | 0.716 | 0.196 | 0.055 |
| $PI_{total}$ | 0.864 | 0.702 | 0.610 | 0.223 | 0.028 | 1.001 | 1.028 | 0.730 | 0.239 | 0.031 | 0.755 | 0.711 | 0.388 | 0.058 | 0.054 |

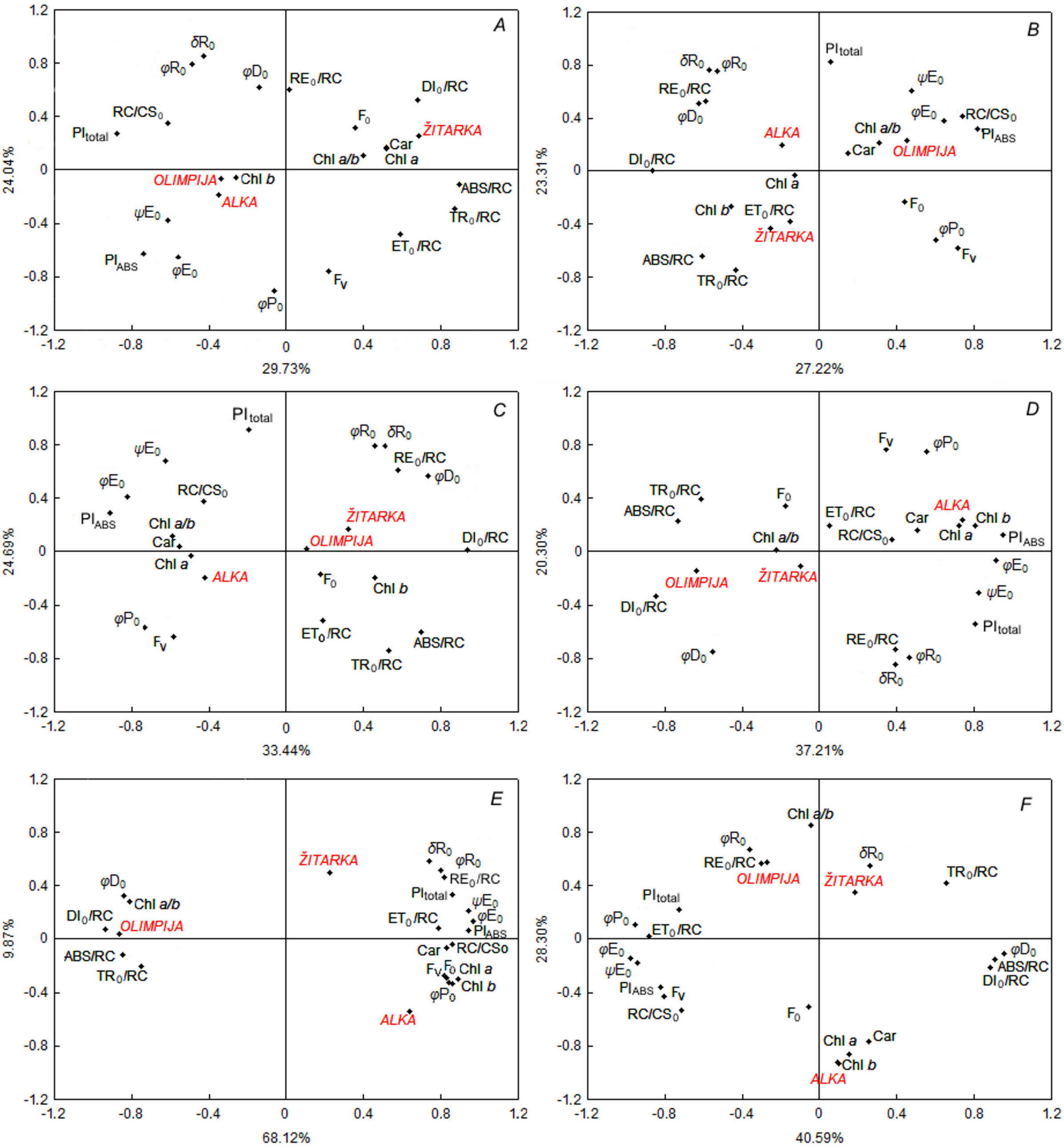


Fig. 7. Results of two-dimensional principal component analysis (2D-PCA) of structural and functional JIP-test parameters and photosynthetic pigments in the flag leaves of winter wheat varieties Alka, Žitarka and Olimpija obtained at 0 (*A*), 7 (*B*), 14 (*C*), 21 (*D*), 28 (*E*), and 35 (*F*) days after flowering (DAF). Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; Car – carotenoids; $F_0$ – minimal fluorescence intensity (50 µs); $F_V$ – maximal variable fluorescence; $\varphi D_0$ – quantum yield of energy dissipation at t = 0; $\varphi P_0$ – maximum quantum yield for primary photochemistry at t = 0; $\varphi R_0$ – quantum yield for reduction of end electron acceptors at the PSI acceptor side; $\varphi E_0$ – quantum yield of electron transport at t = 0; $\psi E_0$ – probability that a trapped exciton moves an electron into the electron transport chain beyond $Q_A^-$; $\delta R_0$ – probability that an electron from the electron transport chain is transferred to reduce end electron acceptors at the PSI acceptor side; ABS/RC – absorption flux (of antenna chlorophylls) per RC; $TR_0$/RC – trapping flux (leading to $Q_A$ reduction) per RC; $ET_0$/RC – electron transport flux (further than $Q_A^-$) per RC; $DI_0$/RC – dissipated energy flux per RC; $RE_0$/RC – electron flux reducing end electron acceptors at the photosystem I (PSI) acceptor side per RC; $RC/CS_0$ – density of reaction centres per cross section; $PI_{ABS}$ – performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors; $PI_{total}$ – performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors.

until senescence (35 DAF). Although three investigated wheat varieties were similar according to their maturation time, the present study revealed delicate distinctions in their flag leaf photochemistry by analysis of OJIP kinetics.

Numerous studies proved that the shape of OJIP curve is strongly modified under different stresses (Mehta *et al.* 2010, Yusuf *et al.* 2010, Fan *et al.* 2014, Kalaji *et al.* 2018a, Paunov *et al*. 2018), in phenotyping mutants in contrast to wild types (Lepeduš *et al.* 2009, Yusuf *et al.* 2010), during diurnal changes in the photochemistry (Mlinarić *et al.* 2017) or during the growing season (Sitko *et al.* 2019). In the current study, senescence onset induced shifts in all parts of OJIP curve. The increase in O–J phase started at 28 DAF in Alka, much earlier in Olimpija and Žitarka, and consequently reflecting reduction of the acceptor side of PSII (Kalaji *et al.* 2016). Better understanding of changes in O–J phase and significant differences in photochemistry of experimental wheat varieties have been obtained by visualisation of two additional steps (K- and L-band) (Yusuf *et al.* 2010). The difference kinetics $\Delta W_{OK}$ revealed positive L-bands in all measurements on the leaves of Olimpija indicating low energetic connectivity and stability of PSII units. This means that onset of senescence in Olimpija started with a partial disruption of thylakoid membranes as evidenced in decrease of energy transfer between PSII units, similarly as Paunov *et al.* (2018) found for Cd and Zn toxic impact on durum wheat leaf photochemistry. Positive L-bands in the leaves of Alka and Žitarka were found at the later growth stages. In addition, K-band revealed by $\Delta W_{OJ}$ difference kinetics in Olimpija was shifted in positive way already at 14 DAF, earlier than that of two other varieties indicating that Alka and Žitarka detained functional PSII antenna longer than Olimpija, where imbalance between electron donor and electron acceptor side of PSII occurred and caused impaired oxygen-evolving complex (OEC) function. In the recent literature, the negative K-band is widely used as a sign of tolerance to various stresses (Mathur *et al.* 2011, Fan *et al.* 2014, Krüger *et al*. 2014, Begović *et al.* 2016). Similar positive K- and L-band occurred in the leaves of quick-leaf senescence compared to stay-green *Zea mays* L. inbred lines when senescence was artificially induced by ethephon (Zhang *et al.* 2012) suggesting that appearance of positive K- and/or L-band can be characterised as a senescence onset marker. J–I phase is characterised by reduction of the intersystem electron carriers, *e.g*., secondary electron acceptor $Q_B$, plastoquinone, cytochrome, and plastocyanin (Yusuf *et al.* 2010, Kalaji *et al.* 2016). Increase in J–I transient during the experiment was visible in the flag leaves of Olimpija earlier than that in other varieties which supported previous evidence of senescence onset in the flag leaves of Olimpija. In the flag leaves of Alka and Olimpija at 7 DAF, the increase of fluorescence in I-step and positive peak of I–P transient was found which could be connected with a smaller pool of plastoquinone and/or PSI end electron acceptors per active PSII RC (Kalaji *et al.* 2016, Paunov *et al.* 2018), resulting in delay in further electron transfer among electron transport chain. Failure of PSI to oxidise reduced plastoquinone leads to an accumulation of the reduced states which is typical reaction to heat stress in wheat (Mathur *et al.* 2011). Delicate differences in Chl fluorescence changes, seen on native fluorescence curve during experimental period in the flag leaves of wheat varieties, suggested that first symptoms of alterations in photochemistry occurred in Olimpija, followed by Žitarka and Alka. Flattened curves at 35 DAF were almost linear, suggesting a complete disassembly of photosynthetic apparatus and offset of photosynthesis (Sitko *et al*. 2019).

In order to quantify the changes in light absorption, its utilisation, and conversion to chemical energy and to analyse fluorescence transient, JIP-test was conducted and parameters shown on radar plots gave the cleaner picture of senescence onset in different wheat varieties. The most prominent changes of JIP parameters during natural senescence were visible in the flag leaves of Olimpija which was in accordance with shifts in its OJIP curves and early appearance of positive K- and L-bands although statistically significant difference in JIP parameters was found only for $PI_{total}$ at 7 DAF. Subsequent significant alteration was found in parameters explaining the state of end electron acceptors at the PSI acceptor side ($\varphi_{R0}$ and $RE_0/RC$) suggesting that although other JIP parameters did not significantly disturbed, appearance of positive K- and L-bands was early indicator of impaired photochemistry in the senescence process. Furthermore, significant decline in $PI_{total}$, performance index for overall energy flow from exciton to the reduction of PSI end acceptors, coincided with the decrease of parameters $\varphi_{R0}$ and $RE_0/RC$. Pavlović *et al*. (2019) also found that under the salinity stress in *Brassica* species, the most significant impact on $PI_{total}$ had the parameter which reflects the efficiency of processes involving PSI and its ability to reduce its end acceptors $[RE_0/(ET_0 – RE_0)]$.

However, the most used parameter in ChlF, $\varphi_{P0}$, was not affected by senescence process until senescence symptoms become pronounced. $\varphi_{P0}$ decreased at 28 DAF in Olimpija, compared to other varieties where decrease of $\varphi_{P0}$ occurred at 35 DAF. Performance indexes ($PI_{ABS}$ and $PI_{total}$) decreased earlier than $\varphi_{P0}$. $PI_{total}$ proved to be much more sensitive than $PI_{ABS}$ under senescence conditions. Stirbet *et al.* (2018) emphasised that sensitivity of $PI_{ABS}$ and $PI_{total}$ is different depending on factors, which caused photochemical alterations in photosystem. Panda and Sarkar (2013) found that $\varphi_{P0}$ remained unchanged with the progression of senescence in rice flag leaves which supported previous findings by Falqueto *et al.* (2009) and Lu *et al.* (2003). In addition, $PI_{ABS}$ appeared to be sensitive indicator of senescence onset and progression in rice leaves (Zhang *et al.* 2010, Panda and Sarkar 2013). In the current research, the highest increase was observed in parameters describing light absorption (ABS/RC) and dissipation of unutilized energy ($\varphi_{D0}$ and $DI_0/RC$) which is characteristic in senescence process as well as under stress conditions. Lu *et al.* (2001, 2002) showed that in the field conditions 25 d after anthesis, photochemical quenching of wheat flag leaves decreased significantly, while at the same time, nonphotochemical quenching increased to dissipate surplus captured energy suggesting downregulation of PSII and activation of photoinhibition process. Photoinhibition is following process when downregulation of PSII occurs due to disassembly of photosynthetic apparatus. The major loss of Chls, seen as the decline in their concentrations during the experimental period, and increase in Chl *a*/*b* ratio implied alterations in the photosynthetic pigments stoichiometry during leaf senescence (Lu *et al.* 2001).

Despite earlier Chl loss (at 14 DAF), Chl *a*/*b* remained unchanged until 28 DAF as well as majority of JIP parameters, suggesting that ChlF is remarkably insensitive to changes of the leaf Chl content until Chl *a*/*b* stayed unaffected (Dinç *et al*. 2012). According to our results, Chl *a* was more affected than Chl *b* and taken together with earlier decline of Car in the flag leaves of Olimpija (21 DAF), it suggests that photoinhibition occurred in this variety earlier than that in other two varieties. The decline in Car content implied that the xanthophyll cycle was not activated properly to protect PSII from photoinhibition (Jahns and Holzwarth 2012).

Among all results, it is hard to distinguish importance of any certain parameter. Hence, we calculated sustainability indexes (SIs) per sampling points to alleviate result interpretation. SIs corroborated with the results of statistical significances (Table 1. *vs*. Table 2S) implying that SIs can be used as user friendly data analysis for screening purposes of wheat material as it was concluded in previous research of Panda and Sarkar (2013).

Analyses of OJIP curve and JIP-test assigned numerous findings and conclusions, with the requirement of a significant knowledge and understanding of photosynthetic process in details. However, numerous parameters obtained by ChlF are confusing with the need of multivariate approach in statistical analysis. JIP-test parameters are calculated on the basis of fluorescence transient curve points and some of them were in high correlation because of their mathematical expressions (*e.g.*, $\varphi_{D0}$ and $\varphi_{P0}$). Thereby, multivariate statistical method such as PCA is method of choice because it is based on calculation of new complex variables that reflect maximal changes in the experimental data set. Usually, first two principal components explain the majority of variations which in our case were induced by senescence. In addition, visualization in 2D graph on a plane with Cartesian coordinates PC1 and PC2 gave distinct picture of relations within parameters and/or among wheat varieties (Kalaji *et al*. 2014, 2018a,c; Španić *et al*. 2017, Pavlović *et al.* 2019, Rusinowski *et al.* 2019).

PCA analysis elucidated a moment of senescence onset at 21 DAF in Olimpija which was confirmed by high correlation of variety Olimpija and parameters describing dissipation process ($\varphi_{D0}$ and $DI_0$/RC). Furthermore, at 28 DAF, the same parameters ($\varphi_{D0}$ and $DI_0$/RC) grouped with Chl *a*/*b*, ABS/RC, and $TR_0$/RC indicating progressive photoinhibition of PSII in the flag leaves of Olimpija which is in accordance with flattened fluorescence intensity OJIP curve. Similar results were obtained by Kalaji *et al.* (2018a) in drought-stressed *Tillia cordata* plants. On contrary, variety Alka correlated with Chl contents, performance indexes, and parameters describing electron transport.

As photosynthesis is of a great importance during wheat grain-filling period and directly influences grain yield, delayed flag leaf senescence is desirable trait in wheat breeding programs. Zhang *et al.* (2006) found correlation between delayed flag leaf senescence and higher grain yield of wheat. Summarizing the results presented in current study, alterations in flag leaf photochemistry strongly suggested that variety Alka managed to delay flag leaf senescence onset whereas modifications of photosynthetic apparatus and thus photosynthetic efficiency decline started earlier in Žitarka and particularly Olimpija. This coincided with the grain yield potential of those three varieties, whereas according to catalogues yearly published by Agricultural Institute Osijek (www.poljinos.hr), Alka was the most yielding variety with genetic potential of 11 t ha$^{-1}$, followed by Žitarka and Olimpija with 9 and 8 t ha$^{-1}$, respectively. We confirmed hypothesis that 'stay-green' trait enabled Alka to retain green leaves longer after anthesis, thus improving grain yield potential, compared to Žitarka and notably Olimpija with lower grain yield potential. Alka showed that duration of photosynthetic processes in wheat flag leaves influence the grain yield significantly, which was previously found by many authors (Zhang *et al.* 2006, Yang *et al.* 2007, Gregersen *et al.* 2013, Thomas and Ougham 2014, Viljevac Vuletić *et al.* 2019).

Based on presented data, although investigated wheat varieties have similar maturation time, senescence onset was triggered earlier in the flag leaves of Olimpija, compared to Žitarka and Alka. OJIP curves, with L- and K-bands presented, implied conclusions that flag leaves of variety Alka detained the longest photosynthetic efficiency, while the first symptoms of senescence onset were visible through positive L- and K-bands in the flag leaves of variety Olimpija. Furthermore, a shift in I–P phase in the wheat flag leaves suggested a reduction of the first acceptors in PSI, implying a degradation of photosynthetic apparatus beside appearance of photoinhibition. $PI_{total}$ proved to be a much more sensitive indicator of senescence than $PI_{ABS}$. Parameters $\varphi_{D0}$ and $DI_0$/RC highly correlated with variety Olimpija suggesting photoinhibition onset followed by disassembly of photosynthetic apparatus. The flag leaves of Olimpija could not maintain their photosynthetic activity for a longer period of time, experiencing very early leaf senescence. A consequence of that is the lower genetic potential for grain yield, compared to Žitarka and particularly Alka. Along with $PI_{total}$, principal component analysis elucidated $\varphi_{D0}$ and $DI_0$/RC as early indicators of senescence in the wheat flag leaves and thus showed that multivariate approach may be useful in the senescence onset detection. Multivariate approach for analysing the data obtained by ChlF can be useful tool for wheat breeders allowing them to enhance breeding selection from physiological point of view to obtain lines with 'stay-green' trait thus improving productivity.

## References

Begović L., Mlinarić S., Antunović Dunić J. *et al*.: Response of *Lemna minor* L. to short-term cobalt exposure: The effect on photosynthetic electron transport chain and induction of oxidative damage. – Aquat. Toxicol. **175**: 117-126, 2016.

Dinç E., Ceppi M.G., Tóth S.Z. *et al*.: The chl *a* fluorescence intensity is remarkably insensitive to changes in the chlorophyll content of the leaf as long as the chl *a*/*b* ratio remains unaffected. – BBA-Bioenergetics **1817**: 770-779, 2012.

Distelfeld A., Avni R., Fischer A.M.: Senescence, nutrient remobilization, and yield in wheat and barley. – J. Exp. Bot. **65**: 3783-3798, 2014.

Falqueto A.R., Cassol D., de Magalhães Júnior A.M. *et al.*:

Physiological analysis of leaf senescence of two rice cultivars with different yield potential. – Pesqui. Agropecu. Bras. **44**: 695-700, 2009.

Fan X., Zhang Z., Gao H. *et al.*: Photoinhibition-like damage to the photosynthetic apparatus in plant leaves by submergence treatment in the dark. – PLoS ONE **9**: e89067, 2014.

Galić V., Franić M., Jambrović A. *et al.*: Genetic correlations between photosynthetic and yield performance in maize are different under two heat scenarios during flowering. – Front. Plant Sci. **10**: 566, 2019.

Gregersen P.L., Culetić A., Boschian L., Krupinska K.: Plant senescence and crop productivity. – Plant Mol. Biol. **8**: 603-622, 2013.

Gregersen P.L., Holm P.B., Krupinska K.: Leaf senescence and nutrient remobilisation in barley and wheat. – Plant Biol. **10**: 37-49, 2008.

Jahns P., Holzwarth A.R.: The role of xanthophyll cycle and of lutein in photoprotection of photosystem II. – BBA-Bioenergetics **1817**: 182-193, 2012.

Kalaji H.M., Bąba W., Gediga K. *et al.*: Chlorophyll fluorescence as a tool for nutrient status identification in rapeseed plants. – Photosynth. Res. **136**: 329-343, 2018c.

Kalaji H.M., Jajoo A., Oukarroum A. *et al.*: Chlorophyll fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. – Acta Physiol. Plant. **38**: 102, 2016.

Kalaji H.M., Oukarroum A., Alexandrov V. *et al.*: Identification of nutrient deficiency in maize and tomato plants by *in vivo* chlorophyll *a* fluorescence measurements. – Plant Physiol. Bioch. **81**: 16-25, 2014.

Kalaji H.M., Račková L., Paganová V. *et al.*: Can chlorophyll-*a* fluorescence parameters be used as bio-indicators to distinguish between drought and salinity stress in *Tilia cordata* Mill? – Environ. Exp. Bot. **152**: 149-157, 2018a.

Kalaji H.M., Rastogi A., Živčák M. *et al.:* Prompt chlorophyll fluorescence as a tool for crop phenotyping: an example of barley landraces exposed to various abiotic stress factors. – Photosynthetica **56**: 953-961, 2018b.

Kovačević J., Mazur M., Drezner G. *et al.*: Photosynthetic efficiency parameters as indicators of agronomic traits of winter wheat cultivars in different soil water conditions. – Genetika **49**: 891-910, 2017.

Krüger G.H.J., De Villiers M.F., Strauss A.J. *et al.*: Inhibition of photosystem II activities in soybean (*Glycine max*) genotypes differing in chilling sensitivity. – S. Afr. J. Bot. **95**: 85-96, 2014.

Lepeduš H., Tomašić A., Jurić S. *et al.*: Photochemistry of PSII in CYP38 *Arabidopsis thaliana* deletion mutant. – Food Technol. Biotech. **47**: 275-280, 2009.

Lichtenthaler H.K.: Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. – Method. Enzymol. **148**: 350-382, 1987.

Lu C., Lu Q., Zhang J., Kuang T.: Characterization of photosynthetic pigment composition, photosystem II photochemistry and thermal energy dissipation during leaf senescence of wheat plants grown in the field. – J. Exp. Bot. **52**: 1805-1810, 2001.

Lu Q., Lu C., Zhang J., Kuang T.: Photosynthesis and chlorophyll *a* fluorescence during flag leaf senescence of field-grown wheat plants. – J. Plant Physiol. **159**: 1173-1178, 2002.

Lu Q., Wen X., Lu C. *et al.*: Photoinhibition and photoprotection in senescent leaves of field-grown wheat plants. – Plant Physiol. Bioch. **41**: 749-754, 2003.

Mathur S., Jajoo A., Mehta P., Bharti S.: Analysis of elevated temperature-induced inhibition of photosystem II using chlorophyll *a* fluorescence induction kinetics in wheat leaves (*Triticum aestivum*). – Plant Biol. **13**: 1-6, 2011.

Maxwell K., Johnson G.N.: Chlorophyll fluorescence – a practical guide. – J. Exp. Bot. **51**: 659-662, 2000.

Mehta P., Jajoo A., Mathur S., Bharti S.: Chlorophyll *a* fluorescence study revealing effects of high salt stress on Photosystem II in wheat leaves. – Plant Physiol. Bioch. **48**: 16-20, 2010.

Mlinarić S., Antunović Dunić J., Skenderović Babojelić M. *et al.*: Differential accumulation of photosynthetic proteins regulates diurnal photochemical adjustments of PSII in common fig (*Ficus carica* L.) leaves. – J. Plant Physiol. **209**: 1-10, 2017.

Panda D., Sarkar R.K.: Natural leaf senescence: probed by chlorophyll fluorescence, $CO_2$ photosynthetic rate and antioxidant enzyme activities during grain filling in different rice cultivars. – Physiol. Mol. Biol. Pla. **19**: 43-51, 2013.

Paunov M., Koleva L., Vassilev A. *et al.*: Effects of different metals on photosynthesis: Cadmium and zinc affect chlorophyll fluorescence in durum wheat. – Int. J. Mol. Sci. **19**: 787, 2018.

Pavlović I., Mlinarić S., Tarkowská D. *et al.*: Early *Brassica* crops responses to salinity stress: A comparative analysis between Chinese cabbage, white cabbage, and kale. – Front. Plant Sci. **10**: 450, 2019.

Rapacz M., Wójcik-Jagła M., Fiust A. *et al.*: Genome-wide associations of chlorophyll fluorescence OJIP transient parameters connected with soil drought response in barley. – Front. Plant Sci. **10**: 78, 2019.

Rusinowski S., Szada-Borzyszkowska A., Zieleźnik-Rusinowska P. *et al.*: How autochthonous microorganisms influence physiological status of *Zea mays* L. cultivated on heavy metal contaminated soils? – Environ. Sci. Pollut. R. **26**: 4746-4763, 2019.

Sitko K., Rusinowski S., Pogrzeba M. *et al*: Development and aging of photosynthetic apparatus of *Vitis vinifera* L. during growing season. – Photosynthetica **58** (SI): 1-8, 2020.

Španić V., Viljevac Vuletić M., Abičić I., Marček T.: Early response of wheat antioxidant system with special reference to *Fusarium* head blight stress. – Plant Physiol. Bioch. **115**: 34-43, 2017.

Stirbet A., Lazár D., Kromdijk J., Govindjee: Chlorophyll *a* fluorescence induction: Can just a one-second measurement be used to quantify abiotic stress responses? – Photosynthetica **56**: 86-104, 2018.

Strasser R.J., Srivastava A., Tsimilli-Michael M.: The fluorescent transient as a tool to characterise and screen photosynthetic samples. – In: Yunus M., Pathre U., Mohanty P. (ed.): Probing photosynthesis: Mechanisms, Regulation and Adaptation. Pp. 445-483. Taylor & Francis, London 2000.

Strasser R.J., Tsimilli-Michael M., Srivastava A.: Analysis of the chlorophyll *a* fluorescence transient. – In: Papageorgiou G.C., Govindjee (ed.): Chlorophyll *a* Fluorescence: A Signature of Photosynthesis. Advances in Photosynthesis and Respiration. Pp. 321-362. Springer, Dordrecht 2004.

Sylvester-Bradley R., Scott R.K., Wright C.E.: Physiology in the Production and Improvement of Cereals. Home-Grown Cereals Authority Research Review, Vol. 18. Pp. 156. HGCA, London 1990.

Thomas H., Ougham H.: The stay-green trait. – J. Exp. Bot. **65**: 3889-3900, 2014.

Viljevac Vuletić M., Marček T., Španić V.: Photosynthetic and antioxidative strategies of flag leaf maturation and its impact to grain yield of two field-grown wheat varieties. – Theor. Exp. Plant Phys. **31**: 387-399, 2019.

Wardlaw I.F.: The control of carbon partitioning in plants. – New Phytol. **116**: 341-381, 1990.

Yang X., Chen X., Ge Q. *et al.*: Characterization of photosynthesis of flag leaves in a wheat hybrid and its parents grown under field conditions. – J. Plant Physiol. **164**: 318-326, 2007.

Yusuf M.A., Kumar D., Rajwanshi R. *et al.*: Overexpression of γ-tocopherol methyl transferase gene in transgenic *Brassica juncea* plants alleviates abiotic stress: physiological and chlorophyll *a* fluorescence measurements. – BBA-Bioenergetics **1797**: 1428-1438, 2010.

Zadoks J.C., Chang T.T., Konzac F.C.: A decimal code for the growth stages of cereals. – Weed Res. **14**: 415-421, 1974.

Zhang C.J., Chen G.X., Gao X.X., Chu C.J.: Photosynthetic decline in flag leaves of two field-grown spring wheat cultivars with different senescence properties. – S. Afr. J. Bot. **72**: 15-23, 2006.

Zhang M.-P., Zhang C.-J., Yu G.-H. *et al*.: Changes in chloroplast ultrastructure, fatty acid components of thylakoid membrane and chlorophyll *a* fluorescence transient in flag leaves of a super-high-yield hybrid rice and its parents during the reproductive stage. – J. Plant Physiol. **167**: 277-285, 2010.

Zhang Z., Li G., Gao H. *et al*.: Characterization of photosynthetic performance during senescence in stay-green and quick-leaf-senescence *Zea mays* L. inbred lines. – PLoS ONE **7**: e42936, 2012.