



PSII photochemistry responses to drought stress in autochthonous and modern sweet cherry cultivars

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Abstract

In this study, the JIP test was used to assess the drought tolerance of two sweet cherry cultivars (*Prunus avium* L.) (modern and autochthonous). Plants were exposed to progressive drought by withholding water and their fast (< 1 s) chlorophyll fluorescence kinetics was evaluated. JIP test analysis showed that drought stress caused a greater decrease in performance indices (PI_{ABS} and PI_{total}) in a modern cultivar, as compared to an autochthonous one. Our results suggest that limited reoxidation of primary quinone electron acceptor (Q_A), higher amount of secondary quinone electron acceptor (Q_B^-) nonreducing reaction centres, or inhibition of the electron transport between Q_A and Q_B , decreased more seriously the photosynthetic performance of the modern cultivar. Further, higher positive L- and K-bands observed for the modern cultivar also suggest lower energetic connectivity between PSII units and increased inhibition of oxygen-evolving complex over autochthonous cultivar. Our results suggest that the autochthonous cultivar Crveni hrušt had better photosynthetic performance under drought conditions, compared to the modern cultivar New Star.

Keywords: chlorophyll *a* fluorescence; chlorophyll content; malondialdehyde; photosynthesis; sweet cherry; water content.

Introduction

Fruit productions are highly dependent on water availability. In many agricultural areas, recent climate change

is a serious threat and drought is a major limitation for tree growth and productivity worldwide. Previous research demonstrated that drought stress inhibits vegetative growth (Tsuchida *et al.* 2011, Blanco *et al.* 2020) and

Highlights

- The photosynthetic performance was higher in the autochthonous sweet cherry cultivar than in the modern
- Drought caused higher damage to PSII oxygen-evolving complex in the modern cultivar
- Higher oxidative damage was found in the modern cultivar

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Abbreviations: ABS/RC – absorption flux (of PSII antenna Chl) per RC; Chl – chlorophyll; DAS – days after stress (drought application); ET_0/ABS (ϕ_{E0}) – quantum yield of electron transport from Q_A^- to PQ; DI_0/RC – dissipation flux per RC; DM – dry mass; ET_0/RC – electron transport flux per RC; ET_0/TR_0 (ψ_{E0}) – probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A ; F_0 – minimum fluorescence, when all PSII RCs are open; FM – fresh mass; F_m – maximum fluorescence, when all PSII RCs are closed; F_v – maximal variable fluorescence; F_v/F_0 – the ratio between the trapping flux and energy dissipation flux of PSII; F_v/F_m – maximum quantum yield of PSII photochemistry; M_0 – approximated initial slope of the fluorescence transient; MDA – malondialdehyde content; OEC – oxygen-evolving complex; PI_{ABS} – performance index for energy conservation from photons absorbed by PSII until the reduction of intersystem electron acceptors; PI_{total} – performance index for energy conservation from photons absorbed by PSII until the reduction of PSI end acceptors; PQ – plastoquinone; PQH_2 – plastoquinol; RC – reaction centre; RE_0/ABS (ϕ_{R0}) – quantum yield of electron transport from Q_A^- to final PSI acceptors; RE_0/ET_0 (δ_{R0}) – efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end electron acceptors; RE_0/RC – electron flux reducing end electron acceptors at the PSI acceptor side per reaction centre; ROS – reactive oxygen species; S_m – normalized total complementary area above the OJIP transient (reflecting single-turnover Q_A reduction events); TR_0/RC – trapping flux per PSII RC; V_1 – relative variable fluorescence at 30 ms (I-step); V_J – relative variable fluorescence at 2 ms (J-step); V_{OP} – relative variable fluorescence between O- and P-steps; WC – water content; W_{OJ} – relative variable fluorescence between O- and J-steps; W_{OK} – relative variable fluorescence between O- and K-steps.

Conflict of interest: The authors declare that they have no conflict of interest.

reduces fruit yield and quality (Pérez-Pérez *et al.* 2008). Under drought stress conditions, many morphological and physiological processes in plants, such as water content, membrane stability, and photosynthetic activity, are negatively affected (Abid *et al.* 2018). Plant stress responses are usually accompanied by changes in structure and function of the photosynthetic apparatus where PSII plays an important role in the response of photosynthesis to abiotic stress (Gururani *et al.* 2015). Drought stress limits the photosynthetic activity by closing the stomata, thereby decreasing CO₂ fixation (Streibet *et al.* 2018). According to Flexas and Medrano (2002), under moderate drought conditions, the decrease in photosynthesis is caused by stomatal limitation, while severe drought reduces photosynthetic rates by decreasing the ribulose-1,5-bisphosphate (RuBP) synthesis, Rubisco activity thereby inhibits CO₂ assimilation. Previous studies have shown that drought causes damage to the oxygen-evolving complex (OEC) (Gupta 2020), a decrease in electron transport rate, degradation of D1 protein (Batra *et al.* 2014), leading to the inactivation of the PSII reaction centre and inhibition in PSII activity. According to Ma *et al.* (2015), progressive drought provokes an imbalance between the energy absorbed by the source and the energy consumed by the metabolic sinks, leading to the formation of reactive oxygen species (ROS), where xanthophyll cycle-dependent thermal dissipation and the Mehler reaction are the most important pathways for dissipation excess energy from apple leaves. The photosynthetic efficiency of the plants, under stress conditions, can be estimated by the analysis of the polyphasic fast Chl fluorescence transient. The different steps of the polyphasic fluorescence transient are labelled as O, J, I, and P, and the shape of the transient reflects plant sensitivity to changes in different environmental conditions (Kalaji *et al.* 2016). Some authors reported the use of the so-called L- and K-bands, calculated from the OJIP transient, which can be used as indicators of stress (Yusuf *et al.* 2010, Redillas *et al.* 2011, Paunov *et al.* 2018). The JIP test analysis of the OJIP transient, developed by Strasser and Strasser (1995), is often used as a tool for detection and evaluation of plant tolerance to various abiotic stresses, *e.g.*, salt stress (Zushi and Matsuzoe 2017), heat stress (Jedmowski and Brüggemann 2015), cold (Franić *et al.* 2020), and drought stress (Dąbrowski *et al.* 2019). Earlier studies have demonstrated that drought stress has negative effects on the efficiency of PSII, as indicated by changes in Chl fluorescence parameters, thus this method is useful for screening genotypes for the selection of drought-tolerant cultivars (Guha *et al.* 2013, Arslan *et al.* 2020). Since the drought will become more frequent and severe in the future, due to climate changes, many efforts have been made to alleviate its impact on food production and to increase crop stability (Cattivelli *et al.* 2008). One of the strategies to reduce the impact of drought on production is a selection of drought-tolerant cultivars. Sweet cherry is an important fruit growing worldwide, with many health benefits (Kelley *et al.* 2018). Over the past decades, much work has been done for the revitalization of old

autochthonous fruit cultivars and the popularization of their products in the local area. These autochthonous varieties have been cultivated for a long time and they have been adapted to the local conditions very well. They contribute to the preservation of biological and genetic diversity as well as the stability of the agroecosystems (Radoš *et al.* 2017). Therefore, they are very important for breeding programs as a source of genetic diversity (Mondini *et al.* 2009), because of their good biological and economic characteristics, which should be used in breeding and creation of new varieties. Since they represent a very important genetic potential for future breeding programs, it is necessary to evaluate their commercial and agronomic traits. Although pomological and chemical characteristics (Radoš *et al.* 2017), as well as molecular identification of these autochthonous sweet cherry cultivars (Krmphot *et al.* 2020) were already performed, there is still a lack of studies on autochthonous varieties, particularly mechanisms of tolerance to abiotic stress. Knowledge about the adaptation to specific environmental conditions is essential for the selection of suitable varieties for future fruit production. Thus, the objective of the present study was to investigate the performance of PSII photochemistry in one-year-old trees of two contrasting sweet cherry cultivars (autochthonous and modern) under drought stress. We studied the parameters derived from the fast Chl *a* fluorescence kinetics, lipid peroxidation, as well as Chl and water content to evaluate drought stress response of sweet cherry trees and distinguish differences in drought tolerance of PSII function between the autochthonous and modern cultivars. Photosynthesis and photoprotective mechanisms are still not well understood for cherry trees (*Prunus avium* L.) during the drought period, and PSII photochemistry of the investigated autochthonous sweet cherry has not been previously studied. Thus, this work on sweet cherry leaves will provide information on the functional status of PSII in selected sweet cherry cultivars exposed to the drought.

Materials and methods

Plant material and experimental design: Two sweet cherry (*Prunus avium* L.) cultivars, one autochthonous (Crveni hrušt), and one commercial (New Star), were used for our experiment. Crveni hrušt is an autochthonous variety of unknown origin that is grown for a long time in Croatia (Požega Valley) and surrounding countries under the similar name (Crveni hrušt, Hrušt, Hrust). Despite the dominance of new, high-yielding cultivars, Crveni hrušt is still keeping its importance and is often grown in Croatia, but mostly in the backyards, as individual trees. New Star is a modern cultivar, one of the best mid-season cherry varieties, as well as one of the most represented in production in Croatia. One-year-old sweet cherry trees, grafted into rootstock Gysela 6, were grown in pots (25 L, one plant per pot). The potted substrate was composed of soil mixture: 65% white peat, 35% black peat, 150 L of clay per m³, 1,500 g of nitrogen, phosphorous, and potassium fertilizer per m³. The pots were placed in a greenhouse

located at the Agricultural Institute Osijek, Osijek, Croatia under semi-controlled conditions (mean air temperature on 3, 5, 7 d after stress (DAS): 25/16°C, 26/16°C, 25/15°C day/night; mean relative air humidity of 70/85%, 68/82%, 70/80% day/night; natural irradiance). Ten potted plants of each cultivar were normally irrigated until the experiment onset. In the experiment, five potted plants served as a control treatment and they were continually irrigated with 2 L of water every day. Another five plants were set as drought treatment and we stopped watering them to impose drought conditions. The experiment ended when wilting symptoms appeared on nonirrigated plants (drought treatment). All measurements and samplings were performed at 3, 5, and 7 d after drought application (DAS).

Chl fluorescence measurements were conducted on two leaves per plant on five plants for each cultivar and treatment. Measurements were made *in vivo*, using a *Handy PEA* (Hansatech Instruments Ltd., Norfolk, UK), on the 5th and 6th fully expanded leaves, which were not overlapping each other, on sunny days (between 08:00 to 10:00 h). After 30-min adaptation to darkness, leaves were illuminated with a 1-s pulse of saturating light intensity of 3,000 $\mu\text{mol}(\text{photon}) \text{ m}^{-2} \text{ s}^{-1}$. The fluorescence data were analyzed according to the JIP test, *see* reviews by Strasser *et al.* (2004), Stirbet and Govindjee (2011), Goltsev *et al.* (2016). Twenty JIP parameters were selected to monitor the changes that occurred in the photosynthetic apparatus. Definitions and calculations of the JIP test parameters used in the present study are given in the *Appendix*. OJIP transients in plants exposed to drought treatment and control unstressed plants were measured and analyzed. To compare the Chl fluorescence transients from two sweet cherry cultivars, we double normalized data between F_0 and F_m (O-P steps). These normalized OJIP transients represent the kinetics of the relative variable fluorescence [$V_{OP} = (F_t - F_0)/(F_P - F_0)$] and were plotted on a logarithmic time scale. For the analysis of drought-induced changes in the OJIP curve, two differential curves were calculated separately to compare changes occurring during O-P transients: the L- and K-bands. The curves for these bands were obtained by subtracting the normalized fluorescence values between O-K, and respectively, O-J, recorded in control plants from those recorded in plants under drought stress. Normalization between the O- and K-steps [$W_{OK} = (F_t - F_0)/(F_K - F_0)$] was used to calculate the L-band ($\sim 150 \mu\text{s}$): $\Delta W_{OK} = W_{OK(\text{drought})} - W_{OK(\text{control})}$. Normalization between the O- and J-steps [$W_{OJ} = (F_t - F_0)/(F_J - F_0)$] was used to calculate the K-band ($\sim 300 \mu\text{s}$): $\Delta W_{OJ} = W_{OJ(\text{drought})} - W_{OJ(\text{control})}$ (Yusuf *et al.* 2010).

Chl content: After the measurement of the Chl fluorescence of sweet cherry leaves at 3, 5, and 7 DAS, individual leaves were sampled and used for further analyses of the Chl content. Photosynthetic pigments were extracted from about 0.1 g of liquid-nitrogen-powdered leaves with absolute acetone in the presence of magnesium hydroxide carbonate. Spectrophotometrically (*Specord 200*, *Analytik*, Jena, Germany) recorded absorbances at

470, 647, and 663 nm were used for total chlorophyll [Chl (*a+b*)] calculation according to Lichtenthaler (1987).

Water content (WC): The fraction of the same leaves used for measuring Chl fluorescence were sampled from control and drought-treated plants of each cultivar and weighed to obtained fresh leaves mass (FM). The samples were then oven-dried at 75°C overnight and weighed again to determine the dry mass (DM). The water content was measured by using the following equation: WC [%] = [(FM - DM)/FM] × 100 (Kumar *et al.* 2017). Each sample was measured in biological triplicate.

MDA content: Lipid peroxidation was expressed as the MDA content and determined as 2-thiobarbituric acid (TBA)-reactive metabolites using the method of Verma and Dubey (2003). Leaves were harvested after measuring Chl *a* fluorescence and a bulk sample of the same leaves for each cultivar and treatment was used for the analysis. Fresh tissue leaf powder (0.20 g) was homogenized in 1 ml of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000 rpm for 15 min at 4°C. The reaction mixture was prepared by mixing 0.5 mL of the supernatant and 1 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA. The reaction mixture was heated at 95°C for 30 min in a heating block and then cooled in an ice bath. The samples were then centrifuged at 14,000 × g for 15 min. The absorbance of the supernatant was read at 532 and 600 nm (*Specord 200*, *Analytik*, Jena, Germany). An extinction coefficient of 155 mM⁻¹cm⁻¹ was used to calculate the amount of MDA.

Statistical analysis: Statistical differences between control and drought-treated plants and cultivars, at three time points (3, 5, and 7 DAS), were compared by analysis of variance with post-hoc Fisher's least significant difference test (LSD). Differences were considered statistically significant when $p < 0.05$. Data are means ± SE of ten biological replicates for parameters, five for chlorophyll content and MDA, and three for water content.

Results

To understand the mechanisms and to trace changes in the photosynthetic electron transport chain, we used twenty parameters from the JIP test. Significant differences under drought conditions were found between investigated sweet cherry cultivars in most of the JIP test parameters, as well as between the days of measurement and cultivars.

Fast Chl fluorescence (OJIP) curves shape: There are clear differences in the shape of OJIP transient between cultivars in drought treatment but no differences between OJIP curves in control plants (Fig. 1A). The most pronounced changes were in the modern cultivar New Star, while in the autochthonous cultivar (Crveni hrušč) OJIP shape was only slightly changed. The J- and I-steps increased under increasing drought stress, and they were more pronounced in New Star cultivar compared to Crveni hrušč (Fig. 1B). The relative variable fluorescence

at 2 ms (V_J) and the variable fluorescence at 30 ms (V_I) significantly increased under drought conditions in both cultivars from 5 DAS but significantly higher values were observed in plants of modern cultivar New Star than in plants of traditional cultivar Crveni hrušč. In this study, the kinetic difference ΔV_{OK} and ΔV_{OJ} made the L- and K-bands visible. Both investigated cultivars presented L-bands (obtained at ~ 0.15 ms) and K-band (obtained at ~ 0.3 ms) with positive amplitudes after submission to drought.

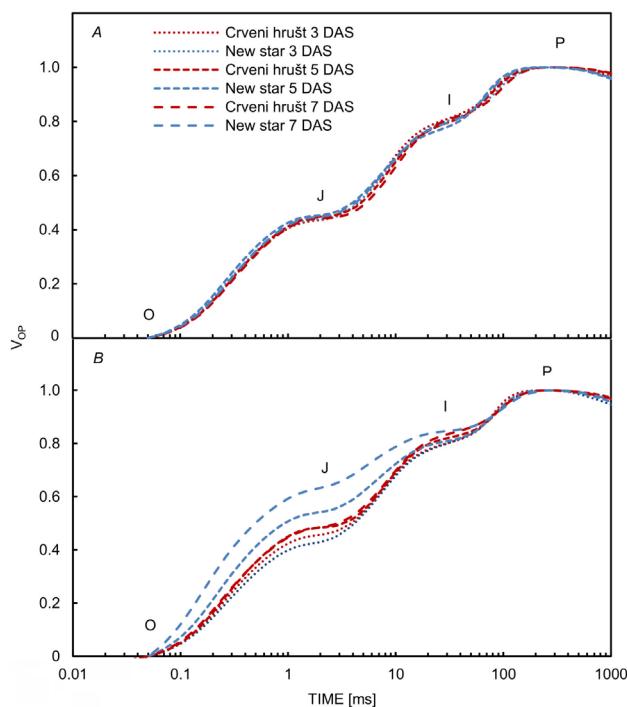


Fig. 1. Chlorophyll *a* fluorescence curves (OJIP transients) of leaves of two sweet cherry cultivars after 3, 5, and 7 DAS. O, J, I, and P indicate the specific steps in chlorophyll *a* fluorescence transient in (A) control and (B) drought-treated sweet cherry plants.

Higher K-bands were obtained for the modern cultivar New Star and similarly, a higher positive amplitude of the L-bands was observed also for the modern cultivar New Star when compared to autochthonous cultivar Crveni hrušč, at all three times of measurement (Fig. 2).

Chl fluorescence parameters: Drought stress caused a significant increase in the initial fluorescence intensity (F_0) in both investigated cultivars under drought conditions. The increase of F_0 in modern cultivar New Star was observed earlier, from 5 DAS, and it was more pronounced than in autochthonous Crveni hrušč (Fig. 3A). Drought significantly reduced the values of parameter maximal fluorescence intensity (F_m), as well as the maximal variable fluorescence (F_v), only in modern cultivar New Star from the 5th day of drought treatment (Fig. 3B,C). The normalized total complementary area above the OJIP transient (S_m) in drought treatment significantly decreased compared with those of the controls in both cultivars at 7 DAS but was significantly higher in autochthonous cultivar Crveni hrušč (Fig. 3F). A decrease in the values of the ratio between the trapping flux and energy dissipation flux of PSII (F_v/F_0) was observed in modern cultivar New Star from 5 DAS, while in autochthonous cultivar Crveni hrušč the significant decrease was observed later at 7 DAS. The decrease of this parameter was significantly higher in New Star compared to autochthonous Crveni hrušč (Fig. 3G). The initial slope of the fluorescence transient (M_0) increased in the case of both cultivars from 5 DAS with significantly higher values in New Star (Fig. 3H). A significant decrease of the parameter evaluating maximum quantum yield of primary PSII photochemistry ($F_v/F_m = TR_0/ABS$) between control and drought-stressed plants was observed in modern cultivar New Star from 5 DAS, while the autochthonous cultivar Crveni hrušč showed a significant decrease at 7 DAS (Fig. 4A). The parameter expressing quantum yield for electron transport from Q_A^- to PQ ($ET_0/ABS; \varphi_{E0}$) decreased under drought in both cultivars from 5 DAS but was significantly higher in modern cultivar New Star (Fig. 4B). The probability that a trapped exciton moves an electron into the electron

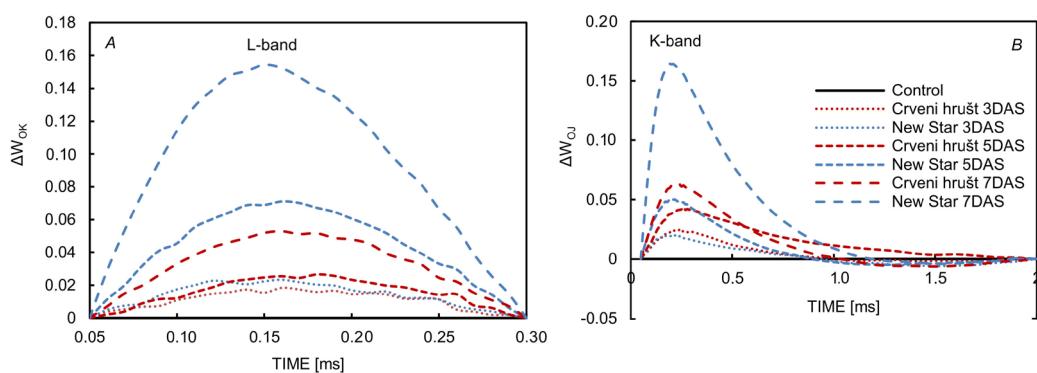


Fig. 2. (A) L-band: changes in O-K phase relative variable fluorescence intensity [$\Delta W_{OK} = V_{OK(\text{drought})} - V_{OK(\text{control})}$], (B) K-band: changes in O-J phase relative variable fluorescence intensity [$\Delta W_{OJ} = V_{OJ(\text{drought})} - V_{OJ(\text{control})}$], in two sweet cherry cultivars on 3, 5, and 7 DAS. The difference kinetics were calculated from the comparisons of the drought-stressed and control plants.

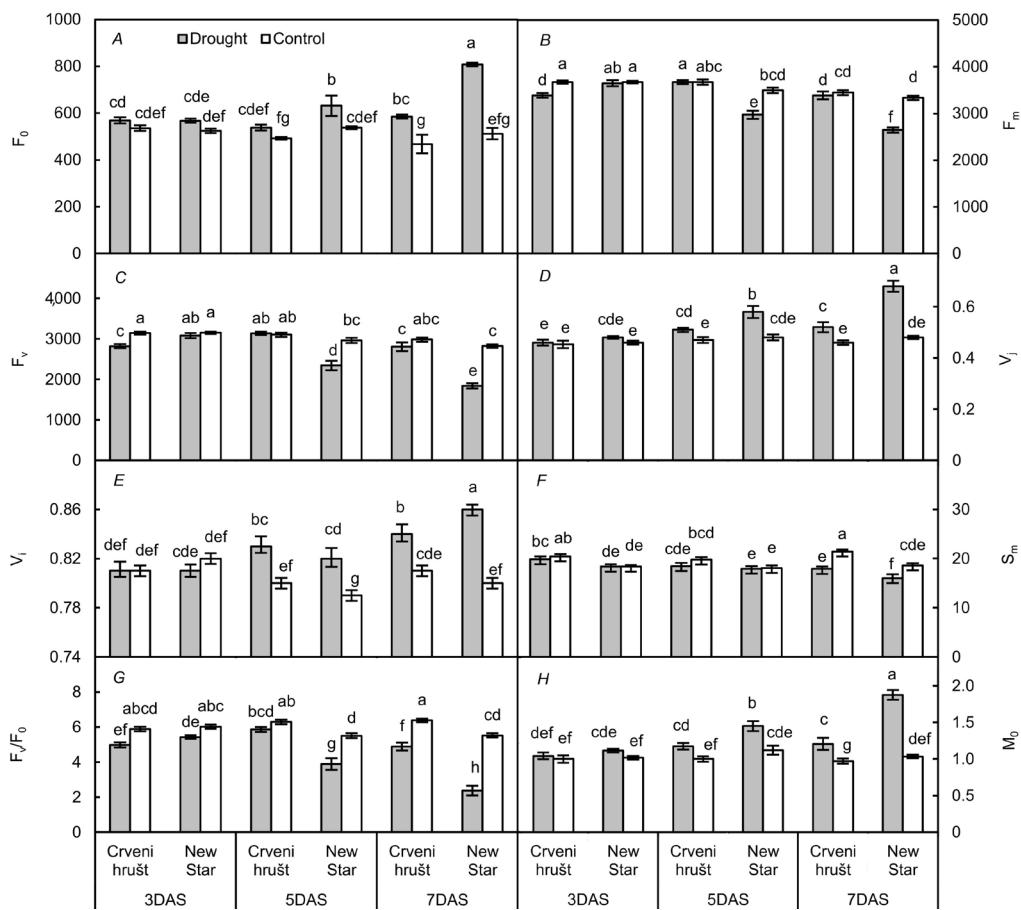


Fig. 3. OJIP-derived parameters: (A) F_0 , (B) F_m , (C) F_v , (D) V_l , (E) V_l , (F) S_m , (G) F_v/F_0 , and (H) M_0 measured at 3, 5, and 7 DAS (day after stress) in control and drought-stressed sweet cherry cultivars (Crveni hrušt and New Star). The definition for these parameters is in the Appendix. Data in the figures indicate mean of ten replicates. Different letters indicate significant difference between cultivars and treatments at $p < 0.05$.

transport chain beyond Q_A^- (ET_0/RC ; ψ_{E0}) decreased at 3 DAS in cultivar New Star, while in cultivar Crveni hrušt at 5 DAS (Fig. 4C). We also observed that the parameter reflecting the efficiency with which an electron from PQH_2 is transferred to final PSI acceptors (RE_0/ET_0 ; δ_{R0}) was constant in both cultivars with no significant differences between control and drought-treated plants (Fig. 4D). Furthermore, the parameter evaluating the quantum yield for reduction of end electron acceptors at the PSI acceptor side (RE_0/ABS ; ϕ_{R0}) of drought-treated plants significantly decreased at 7 DAS from control plants in both investigated cultivars, but without the significant difference between cultivars (Fig. 4E). The significant increase of parameters representing the absorption flux of PSII antenna per RC (ABS/RC) and the dissipation energy flux per RC (DI/RC) was observed earlier in modern cultivar New Star at 5 DAS, while in Crveni hrušt it took place at 7 DAS. Also, the highest increase of these parameters was found in modern cultivar New Star compared to autochthonous Crveni hrušt (Fig. 5A,D). The trapping per RC (TR_0/RC) increased in leaves of both cultivars at 5 DAS and prolonged at 7 DAS (Fig. 5B).

The electron transport flux per RC (ET_0/RC) of modern cultivar New Star decreased at 5 and 7 DAS, while values of that parameter in autochthonous cultivar Crveni hrušt did not significantly change (Fig. 5C). Electron flux reducing end electron acceptors at the PSI acceptor side per RC (RE_0/RC) showed significant changes only in New Star after 7 d of exposure to drought relative to controls (Fig. 5E). Under drought treatment, the performance index on absorption basis (PI_{ABS}) decreased in both cultivars at 3 DAS, while the performance index for energy conservation from exciton to the reduction of PSI end acceptors (PI_{total}) significantly decreased after 3 DAS in Crveni hrušt while in New Star at 5 DAS. Values of these parameters decreased more for modern cultivar New Star than for autochthonous Crveni hrušt (Fig. 6).

Total Chl content: The mean values of total Chl content in drought-treated leaves were significantly lower than in control leaves only in modern cultivar New Star at 7 DAS. The concentration of total Chl content in autochthonous cultivar Crveni hrušt under drought conditions increased at 3 and 5 DAS, while at 7 DAS, Chl content remained

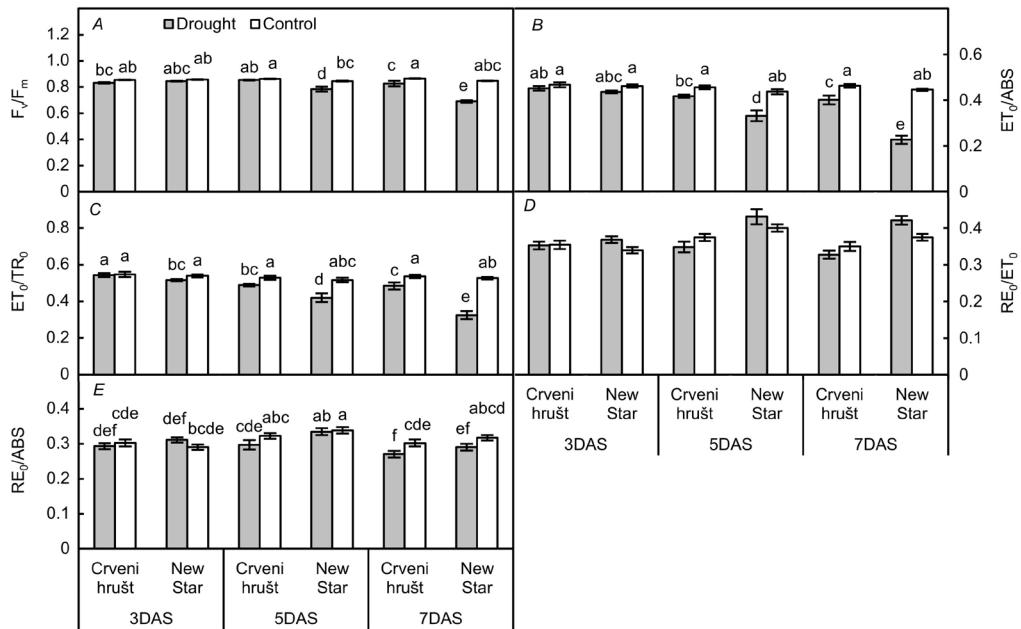


Fig. 4. OJIP-derived parameters: (A) F_v/F_m , (B) ET_0/ABS , (C) ET_0/TR_0 , (D) RE_0/ET_0 , and (E) RE_0/ABS , measured at 3, 5, and 7 DAS (day after stress) in control and drought-stressed sweet cherry cultivars (Crveni hrušt and New Star). The definition for these parameters is in the [Appendix](#). Data in the figures indicate mean of ten replicates. Different letters indicate significant difference between cultivars and treatments at $p < 0.05$.

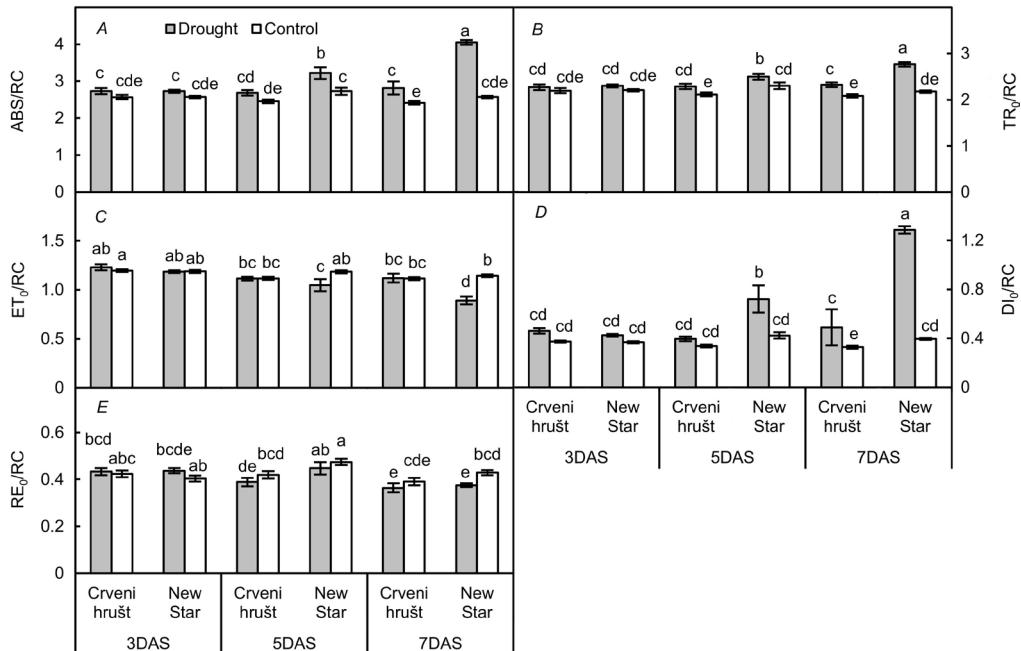


Fig. 5. OJIP-derived parameters: (A) ABS/RC , (B) TR_0/RC , (C) ET_0/RC , (D) DI_0/RC , and (E) RE_0/RC , measured at 3, 5, and 7 DAS (day after stress) in control and drought-stressed sweet cherry cultivars (Crveni hrušt and New Star). The definition for these parameters is in the [Appendix](#). Data in the figures indicate mean of ten replicates. Different letters indicate significant difference between cultivars and treatments at $p < 0.05$.

unchanged in drought-stressed leaves compared to control (Fig. 7A).

MDA content: Variations in the MDA content under drought stress were observed only in modern cultivar

New Star visualized as an increase in drought treatment compared to control at 3 DAS. Moreover, the MDA content

of modern cultivar New Star was significantly higher than that of autochthonous cultivar Crveni hrušt during the whole experiment even in control leaves (Fig. 7B).

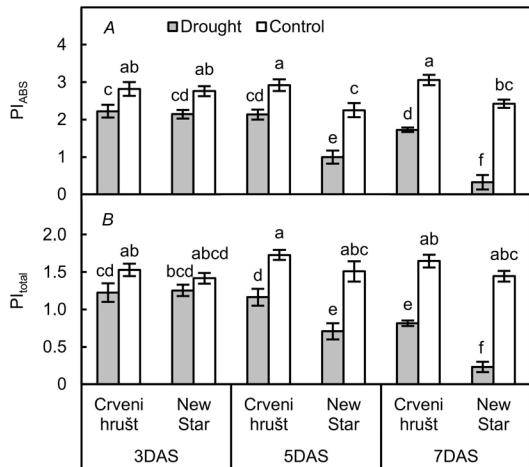


Fig. 6. OJIP-derived parameters: (A) PI_{ABS} and (B) PI_{total} measured at 3, 5, and 7 DAS (day after stress) in control and drought-stressed sweet cherry cultivars (Crveni hrušt and New Star). The definition for these parameters is in the Appendix. Data in the figures indicate mean of ten replicates. Different letters indicate significant difference between cultivars and treatments at $p < 0.05$.

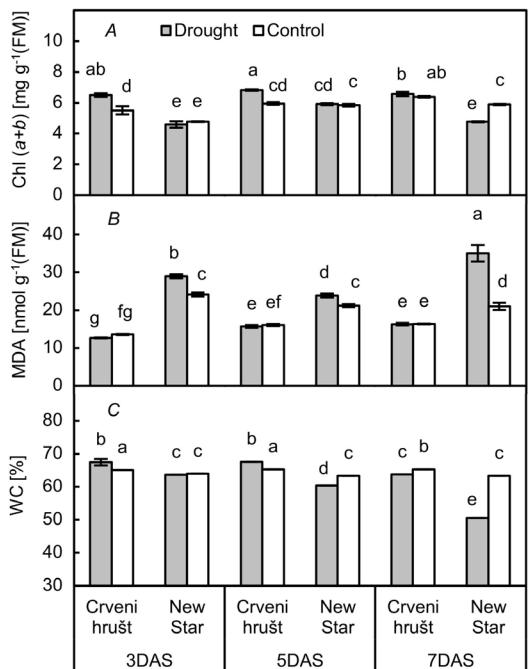


Fig. 7. (A) Total chlorophyll content [Chl (a+b)], (B) malondialdehyde content (MDA), and (C) water content (WC) measured at 3, 5, and 7 DAS (day after stress) in control and drought-stressed sweet cherry cultivars (Crveni hrušt and New Star). Data in the figures indicate mean of five replicates for chlorophyll content and malondialdehyde and three replicates for water content. Different letters indicate significant difference between cultivars and treatments at $p < 0.05$.

Water content (WC): The water content in the leaves of the modern cultivar New Star decreased significantly from 5 DAS. In the leaves of autochthonous cultivar Crveni hrušt, a significant increase in water content was recorded at 3 and 5 DAS. The significant decrease of water content in this cultivar was observed at 7 DAS (Fig. 7C).

Discussion

In our study, we used progressive drought stress in sweet cherry plants to induce changes in the light phase of photosynthesis. Obtained results showed that drought stress-induced changes, demonstrated by the JIP test, were different between two investigated sweet cherry cultivars. The effect of drought stress was reflected in the change of the OJIP curves of investigated cultivars and showed the differences between them. The J- (parameter V_J) and I-step (parameter V_I) gradually increased along with the increasing duration of drought. Considerable changes in fluorescence occurred at J- and I-steps in modern cultivar New Star, whereas only minor changes in fluorescence at J- and I-steps occurred in autochthonous cultivar Crveni hrušt. The higher increase in the J-step of modern cultivar New Star indicates the stronger decrease of electron transport on the acceptor side of PSII (Strasser 1997) and a large accumulation of reduced Q_A⁻ in PSII RCs (Lopes *et al.* 2019). A higher increase at I-step of fluorescence transient curve observed in New Star at 7 DAS may be due to a higher accumulation of the amount of the Q_B⁻ nonreducing centres (Lazár 2006), which are unable to transfer electrons efficiently from electron acceptor Q_A to Q_B (Lavergne 1982). These results are similar to previous findings which revealed a rise of V_I, which occurred in drought-stressed barley plants (Ghotbi-Ravandi *et al.* 2016). Under drought, Chl *a* fluorescence analyses have shown positive values for L- and K-bands in both cultivars of sweet cherry. According to previous research (Strasser *et al.* 2004, Jedmowski *et al.* 2014) appearance of the K-band indicates that drought stress caused partial inactivation of the oxygen-evolving complex (OEC), while the appearance of the L-band indicates lower PSII energetic connectivity of antennae of PSII RC units (Oukarroum *et al.* 2007, Stirbet 2013). Higher positive L-band observed in the leaves of modern cultivar New Star suggests less connectivity within the PSII units compared to autochthonous cultivar Crveni hrušt, while higher positive K-band of that cultivar suggests the reduced efficiency of OEC due to slower electron flow between OEC and acceptor side of the RC (Yusuf *et al.* 2010). This result indicates that autochthonous cultivar Crveni hrušt maintained better utilization of excitation energy and greater stability of the system when exposed to progressive drought. Similar positive L- and K-bands occurred in the leaves of *Passiflora edulis* L. (Gomes *et al.* 2012) and in the leaves of rubber trees (Falqueto *et al.* 2017) under drought conditions. S_m represents the normalized area of the Chl fluorescence induction curve, it is proportional to

the pool size of electron carriers. This parameter was significantly lower in drought-stressed leaves of both cultivars at 7 DAS, indicating the reduced transport from Q_A^- to Q_B , as it was observed in the drought-stressed apple leaves (Yuan *et al.* 2013). Since more light energy was used to reduce Q_A , this resulted in an increase of M_0 . The significant increase in the net rate of RC closure observed in New Star suggested that the reduction of Q_A to Q_A^- was higher under drought treatment in the leaves of New Star cultivar than in autochthonous Crveni hrušt. This finding is supported by previous results that the net rate of RC closure increased in drought-sensitive mung bean plants after exposure to drought (Bano *et al.* 2021). Significantly higher values of F_0 due to drought stress in New Star could occur because of a partial reduction of the PQ pool in darkness. Increased value of F_0 was reported in drought-stressed mulberry plants (Guha *et al.* 2013). According to Kalaji *et al.* (2011), low values of F_m indicate the accumulation of inactive RC at PSII, which was confirmed in our study, only in the leaves of modern cultivar New Star. In previous research, a large increase was observed in the parameters ABS/RC, DI_0/RC , and TR_0/RC and a large decrease in ET_0/RC and PI_{ABS} in drought-sensitive plants of potato subjected to drought (Plich *et al.* 2020). Similarly, we also noticed the increase in parameters ABS/RC, DI_0/RC , and TR_0/RC by drought in both cultivars, but these were more obvious in modern cultivar New Star. The higher values of ABS/RC in New Star implied that a fraction of active reaction centres was more inactivated because of higher inhibition of electron transport from Q_A^- to Q_B , and transformation of RCs to 'silent' RCs (Yusuf *et al.* 2010). This corroborated with reduced values of ET_0/RC in New Star under drought as it was observed in drought-stressed plants of *Abies koreana* (Je *et al.* 2018). Since the inactive centres could not trap photons, the increase in the rate of energy dissipation of untrapped excitations causes an increase of DI_0/RC (Sousaraei *et al.* 2021). According to previous research, an increase in ABS/RC and TR_0/RC indicates that some RCs were inactivated due to inactivation of OEC as well as the transformation of active RCs to silent (Liang *et al.* 2019); an increase in TR_0/RC can indicate impairment of the oxygen-evolving complex (Braga *et al.* 2020). In our study, we also observed higher increases of TR_0/RC and higher K amplitude in cultivar New Star compared to Crveni hrušt. Significantly higher values of DI_0/RC and decreased values of ET_0/RC in modern cultivar New Star showed that excess energy was dissipated, which is indicated as an adaptive strategy to reduce photodamage to photosynthetic apparatus (Akhter *et al.* 2021). Furthermore, a similar but more efficient strategy can be seen in cultivar Crveni hrušt, which resulted in untouched ET_0/RC in drought-stressed leaves referring that this can be the base of its tolerance, implying that it could protect its photosynthetic apparatus through efficient utilizing absorbed light energy rather than through thermal dissipation. The parameter F_v/F_m describing the efficiency of energy trapping in PSII reaction centres is the most commonly analyzed Chl fluorescence parameter. The more pronounced decline in F_v/F_m ratio was observed in modern

cultivar New Star and similar reducing trends were also observed in the parameter F_v/F_0 . The parameter F_v/F_0 is very sensitive due to water deficit (Pereira *et al.* 2000). A higher decrease of this parameter in the modern cultivar New Star indicates inactivity of the water-splitting complex at the PSII donor side in this cultivar as was observed in wheat under salinity stress (Maswada *et al.* 2018). A decrease in F_v/F_m and F_v/F_0 parameters has been reported earlier in cultivated barley compared to wild type under drought stress (Ghotbi-Ravandi *et al.* 2016). Drought stress inhibited the electron transport rates at the PSII acceptor side as evidenced by decreasing ET_0/ABS and ET_0/TR_0 . A higher decrease of these two parameters in modern cultivar New Star is in agreement with significantly higher values of V_J and V_I of this cultivar. Liu *et al.* (2020) also reported that drought stress caused a decrease of these parameters in drought-sensitive maize plants. Values of parameter RE_0/ET_0 depend on the electrons transferred to PSI from PQH_2 and the electron influx from the upper electron carrier (Yan *et al.* 2013). Unchanged values of parameter RE_0/ET_0 in both cultivars and a small decline of RE_0/ABS at 7 DAS indicated that electron flow was still proceeding normally from PQH_2 to the PSI end electron acceptors (Luo *et al.* 2016). Like in previous reports (Boguszewska-Mańkowska *et al.* 2018, Botyanszka *et al.* 2020), our data also showed that PI_{ABS} and PI_{total} were found to be more sensitive to drought than F_v/F_m . Although the parameter fluorescence quantum yield (F_v/F_m) is the most widely used parameter for the efficiency of PSII, decreasing of this parameter was less pronounced compared to PI_{ABS} and PI_{total} in both cultivars. Particularly in autochthonous cultivar Crveni hrušt, where the decrease of this parameter was significantly lower only at 7 DAS. PI_{ABS} has been used by many researchers to evaluate plants' health status and identify cultivars' tolerance to various abiotic stresses including drought stress (Živčák *et al.* 2008, Boureima *et al.* 2012). In this study, drought caused a significant decrease of photosynthetic activity in both investigated cultivars where decreases in PI_{total} , as water deficit increased, were similar to those observed for PI_{ABS} . A stronger decline of these parameters during the drought period in modern cultivar New Star confirmed its higher drought sensitivity compared with autochthonous cultivar. In our previous research, we also observed that autochthonous apple cultivar had better photosynthetic efficiency compared to modern apple cultivar under drought conditions (Mihaljević *et al.* 2021).

In general, drought stress resulted in changes in Chl fluorescence parameters in both modern and autochthonous cultivars, but these alterations were more pronounced in modern cultivar New Star. A greater increase in the energy dissipation per active reaction centre (DI_0/RC) revealed that RCs were inactivated under drought stress because the RCs dissipated most of the energy that was absorbed as heat and not used in the photochemistry of photosynthesis. Autochthonous cultivar Crveni hrušt is capable of better sustaining the photosynthetic electron transport machinery under drought conditions in comparison with the modern cultivar New Star, therefore it has less declining trends of PI_{ABS} and PI_{total} .

Chl molecules are essential pigments for photosynthesis. A reduction in the Chl content is a common effect of different abiotic stress conditions including drought (Viljevac *et al.* 2013), therefore it is a reliable indicator of water stress, particularly in stress-sensitive varieties (Guo *et al.* 2016, Umar and Siddiqui 2018). The total amount of leaf Chl content [Chl (a+b)] directly influences the photosynthetic capacity of plants and high concentrations of Chl increase photosynthesis. According to our results, we can conclude that in the autochthonous cultivar Crveni hrušt which was the more stress-tolerant cultivar, Chl contents increased and it caused a better stress tolerance of this cultivars. A previous study also reported that Chl content in leaves of tolerant cultivar increased under drought conditions (Mensah *et al.* 2006) and that tolerant cultivars have a higher Chl content (Zaefyzadeh *et al.* 2009).

It is known that drought stress in plants disturbs the photosynthetic electron transport, leading to a decrease in photosynthesis and causing excessive production of ROS (Takahashi and Murata 2008). Oxidative injuries caused by drought stress resulted in lipid peroxidation and MDA content is used to examine the oxidative damage in the cell membranes (Bandurska and Józwiak 2010). Increases in the MDA content of the modern cultivar New Star suggest that drought stress induced oxidative damage of the membrane system in this cultivar. Unchanged MDA content in the leaves of autochthonous cultivar during drought treatment did not lead to lipid peroxidation, indicating better protection from oxidative damage. According to Xu *et al.* (2008), MDA is one of the best physiological components of drought tolerance in plants and low MDA content was associated with drought tolerance in previous research (Bhardwaj and Yadav 2012). It was reported that an increase in lipid peroxidation of the plants, under stress, may cause Chl degradation (Karimpour 2019). In our study, a significant reduction in Chl content observed in modern cultivar New Star (cultivar with lower photosynthetic performance) was accompanied by increased lipid peroxidation.

Under drought conditions, the modern cultivar New Star showed a significant reduction in WC at an earlier period of drought applied, indicating that the water status in this cultivar was more sensitive to drought stress compared to autochthonous. The increase in WC at 3 and 5 DAS in autochthonous cultivar Crveni hrušt indicates the better acclimation of this cultivar to water stress conditions, probably because of better ability to mention water loss from the leaves. We presumed that higher water content in this cultivar was due to activation of some defense mechanisms and accumulation of some compounds, such as proline, causing a decrease of osmotic potential and increase leaf turgor (Bandurska and Józwiak 2010). Our results are consistent with other reports which presented that drought-tolerant plants have higher water content and photosynthetic efficiency (Kumar *et al.* 2014, Braga *et al.* 2020).

Conclusion: The water deficit had a significant effect on the photochemical activity of investigated sweet cherry

cultivars, reducing photosynthetic apparatus performance. The inhibition of electron transport rate, accompanied by increasing of MDA and decreasing of photosynthetic pigments content, is an indicator of limited photochemistry of PSII in modern cultivar New Star. The autochthonous cultivar Crveni hrušt maintained higher WC, Chl content, photosynthetic efficiency, and lesser oxidative damage during drought stress. Therefore, the autochthonous cultivar showed an advantage over modern in terms of the efficiency of PSII under progressive drought conditions.

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Appendix. Definitions and calculations of the JIP test parameters used in the present study.

Technical fluorescence parameters

F_0	Initial fluorescence intensity at 50 μ s, when all PSII RCs are open
F_m	Maximal fluorescence intensity, when all PSII RCs are closed
$F_v = F_m - F_0$	Maximal variable fluorescence
$V_J = (F_J - F_0)/(F_m - F_0)$	Relative variable fluorescence at the J-step (2 ms)
$V_I = (F_I - F_0)/(F_m - F_0)$	Relative variable fluorescence at the I-step (30 ms)
$S_m = \text{Area}/(F_m - F_0)$	Normalized total complementary area above the OJIP transient (reflecting single-turnover Q_A^- reduction events)
$F_v/F_0 = k_p/k_N = \text{TR}_0/\text{DI}_0$	The ratio between the trapping flux and energy dissipation flux of PSII; where k_p and k_N are the photochemical and nonphotochemical de-excitation rate constants of PSII antenna in dark-adapted samples
$M_0 = 4(F_{300\mu s} - F_0)/(F_m - F_0)$	Initial slope of the fluorescence transient
Quantum efficiencies or flux ratios	
$\text{TR}_0/\text{ABS} = F_v/F_m = [1 - (F_0/F_m)]$	Maximum quantum yield of primary PSII photochemistry
$\text{ET}_0/\text{ABS} = \varphi_{E0} = (1 - F_0/F_m)(1 - V_J)$	Quantum yield for electron transport from Q_A^- to PQ
$\text{RE}_0/\text{ET}_0 = \delta_0 = (1 - V_I)(1 - V_J)$	The efficiency with which an electron from PQH_2 is transferred to final PSI acceptors
$\text{ET}_0/\text{TR}_0 = \psi_{E0} = 1 - V_J$	Probability that trapped exciton moves an electron into the electron transport chain beyond Q_A^-

$RE_0/ABS = \varphi_{R0} = \varphi_{P0} \times \psi_{E0} \times \delta_{R0}$	Quantum yield for reduction of end electron acceptors at the PSI acceptor side
Specific fluxes (per active PSII)	
$ABS/RC = M_0(1/V_J)(1/\varphi_{P0})$	Absorption flux (of antenna Chls) per RC
$TR_0/RC = M_0(1/V_J)$	Trapped energy flux per RC
$ET_0/RC = M_0(1/V_J) \times \psi_0$	Electron transport flux per RC
$DI_0/RC = ABS/RC - TR_0/RC$	Dissipated energy flux per RC
$RE_0/RC = M_0(1/V_J) \times \psi_{E0} \times \delta_{R0}$	Electron flux reducing end electron acceptors at the PSI acceptor side, per RC
Performance indexes	
$PI_{ABS} = (RC/ABS)(TR_0/DI_0)[ET_0/(TR_0 - ET_0)]$	Performance index on absorption basis
$PI_{total} = PI_{ABS}[(RE_0/ET_0)/(1 - RE_0/ET_0)]$	Performance index for energy conservation from exciton to the reduction of PSI end acceptors

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