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Photosynthetic responses of heat-stressed apple leaves to foliar application of salicylic and ascorbic acid

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Abstract

High temperatures have significant impacts on fruit tree production. Foliar spraying application of promoting agents can be a sustainable approach to managing high-temperature stress in orchards. The mechanism of certain improving agents on photosynthesis is not yet well understood, particularly in fruit trees. Photosynthesis, as a vital and very sensitive process in plants, is a pivotal component in fruit production. Therefore, in this study, we explored the potential of two different promoting agents, salicylic acid (SA) and ascorbic acid (AsA), to alleviate oxidative stress caused by high temperature in controlled conditions (37°C for 1 h) at the photosynthetic level. For studying photosynthetic responses, we used chlorophyll fluorescence measurements. According to our findings, foliar application of promoting agents effectively increased the high-temperature tolerance of apple leaves, when compared to sole heat stress treatment. Both promoting agents significantly increased photosynthetic efficiency under stress, while the maximum was observed with AsA. In general, AsA and SA applications had a positive effect on the photosynthesis of apple leaves at high temperatures.

Keywords: chlorophyll *a* fluorescence; chlorophyll content; electron transport chain; high temperature; photosystem II.

Introduction

Apple (*Malus domestica* Borkh.) is one of the most significant and widespread fruits cultivated in regions with temperate climates (Duan *et al.* 2017). Due to its many health benefits and versatility, it is one of the favourites and very often consumed fruits worldwide. Apples, like other fruit trees in orchards, are subjected to many different abiotic stressors, among which high temperatures have one of the greatest effects on apple production. High-temperature stress can cause excessive production of reactive oxygen species (ROS), resulting in oxidative stress in plants, which may adversely impact many metabolic processes and lower productivity (Mittler 2002). As a result of global warming, daily temperatures have increased significantly, and apple trees frequently experience temperature stress, which could lead to reduced yields and lower fruit quality (Felicetti and Schrader 2008). During the summer months, high irradiance and elevated temperatures caused physiological disorders, such as apple sunburn (Schrader *et al.* 2008), bitter pit (Jemrić *et al.* 2016), a decrease in acid concentration, and fruit firmness (Sugiura *et al.* 2013). Under these stressful conditions, a decrease in plant growth is frequently accompanied by a reduction in photosynthetic capacity and damage to the leaf photosynthetic system (Mathur *et al.* 2011, Yan *et al.* 2011). High temperatures could directly damage the PSII oxygen-evolving complex (OEC) (Allakhverdiev

Highlights

- High temperature caused photosystem II inhibition in apple leaves
- Salicylic and ascorbic acid mitigate high-temperature impact on photosystem II
- Salicylic and ascorbic acid increase pigment contents and photosynthetic efficiency

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Abbreviations: AsA – ascorbic acid; I step – fluorescence intensity measured at around 30 ms (F_I); J step – fluorescence intensity measured at 2 ms (F_J); OEC – oxygen-evolving complex; O step – fluorescence intensity measured at 50 µs, when all PSII RCs are open (F_0); P step – maximal fluorescence intensity when all PSII RCs are closed at 1 s (F_m); PQ – plastoquinone; PQH_2 – plastohydroquinone; RC – reaction centre; SA – salicylic acid; V_{OJ} – relative variable fluorescence between O and J steps; V_{OK} – relative variable fluorescence between O and K steps; V_{OP} – relative variable fluorescence between O and P steps.
Conflict of interest: The authors declare that they have no conflict of interest.

et al. 2008, Shanker *et al*. 2022) and reduce electron transport throughout the PSII reaction centres (RCs) (Wahid *et al*. 2007). According to Haldimann and Feller (2005), the reduction of photosynthesis by high temperatures is caused by decreases in stomatal conductance and Rubisco carboxylation activity. Since the increase in temperature can seriously affect fruit tree productivity, the behaviour of fruit trees under these extreme environmental conditions needs to be extensively studied. Beside the priority of developing heat-tolerant varieties that can survive and adapt to extreme temperatures, it is also important to take specific actions in fruit production such as soil and crop management practices, and use different substances to alleviate the harmful effects of high temperatures. Therefore, taking actions to help plants survive and adapt to climate change is crucial for ensuring global food security. This is especially important because sustainable fruit production demands environmentally friendly substances.

Using some promoting substances and practices can help reduce heat stress and improve fruit production. Many previous studies reported that various exogenous applications, such as plant hormones, antioxidants, and osmoprotectants, are very effective in mitigating adverse environmental stress factors in many plant species. SA is a phenolic compound that acts as a plant hormone and signalling molecule with beneficial effects on the resistance to many abiotic stressors, including salt (Nazar *et al*. 2011), heat (Wang *et al*. 2010), and drought (Iqbal *et al*. 2022). During high-temperature stress, exogenous SA applications improve photosynthesis (Wang *et al*. 2010), pigment concentration, and antioxidant systems by regulating enzymes, such as peroxidases, superoxide dismutase, and catalases, which lower the formation of reactive oxygen species and reduce oxidative stress (Khavari *et al*. 2021). A foliar spray of AsA has been demonstrated to enhance plant growth and development. It functions as an antioxidant, defending plants against oxidative stress and enhancing their general health and productivity (Akram *et al*. 2017). According to some previous studies, AsA is useful in reducing different abiotic stressors in many plant species. Exogenous AsA treatments enhance growth under water scarcity (Farooq *et al*. 2020), and high-temperature conditions (Ergin *et al*. 2014). Stomatal closure is an adaptive response in plants facing both drought and heat stress, helping conserve water by minimizing transpiration. While essential for maintaining water balance and preventing dehydration, this defence mechanism limits the intake of carbon dioxide necessary for photosynthesis. The reduction in stomatal conductance during stress can result in decreased carbon assimilation, leading to a decline in overall photosynthetic activity and an increased risk of photooxidative stress (Wujeska-Klause *et al*. 2015). Treatment with AsA and SA followed by heat stress enhanced gas-exchange parameters and effectively alleviated the adverse effects on net photosynthesis, intercellular CO_2 concentration, transpiration rate, stomatal conductance, and stomatal limitation (Jahan *et al*. 2019, Zong *et al*. 2023). Recent studies suggest that AsA has positive effects on photosynthesis under high-temperature conditions. Zong *et al*. (2023) demonstrated that the application of exogenous AsA enhances the photosynthetic capacity of alfalfa plants under both drought and heat stress. They found that in alfalfa plants, the application of exogenous AsA maintains a higher net photosynthetic rate by improving stomatal openness. The research findings of Chen *et al*. (2017) indicate that AsA improves PSII behaviour under heat stress by reducing oxidative damage, thereby enhancing photosynthetic efficiency and overall plant growth. Photosynthesis, a vital process in plants, is also a very sensitive and complex process that can be easily disrupted by various stress factors. Measuring chlorophyll fluorescence is a commonly used technique for evaluating stress tolerance since it provides valuable information on how plants react to different stress factors (Zare Bavani *et al*. 2015, Shin *et al*. 2021, Viljevac Vuletić *et al*. 2022). SA and AsA applications are effective in improving apple yield and quality (Kazemi *et al*. 2011, Allahveran *et al*. 2018), but there are not many findings regarding how these promoting agents affect the physiology of apples under various stresses, particularly their impact on PSII. Considering the facts presented so far, we hypothesised that exogenously applied SA and AsA will compensate the impact of high-temperature stress on photosynthesis in apple leaves. Therefore, our study aimed to evaluate the effect of SA and AsA applied exogenously as the protection of the photosynthetic process in apple leaves subjected to high temperatures. Photosynthetic parameters and chlorophyll content were monitored to explain and compare the influence of ascorbic and salicylic acids on apple leaf photosynthesis.

Materials and methods

Design of the experiment and growth conditions: To investigate the effects of AsA and SA on the photosynthetic efficiency of apple leaves at high temperature, experiment was conducted in July 2022 at Agricultural Institute Osijek, Croatia. The research was done on two-year-old apple trees (variety Red Topaz) at a planting distance of 4.0 × 1.5 m in an experimental orchard. During vegetation, standard horticultural measures were applied, including irrigation with a drip irrigation system and integrated disease and pest control. Climatic data for the 7 d before the experiment are presented in the text table.

Day	Maximum air temperature [°C]	Maximum air moisture [%]	Maximum solar radiation [W m^{-2}]
5 July	30.4	53.3	1,420
4 July	34.3	37.9	1,060
3 July	32.7	46.8	1,160
2 July	29.4	55.8	1,710
1 July	36.0	42.9	1,010
30 June	34.5	43.4	1,100
29 June	35.6	36.4	1,070

The experiment was performed to test the effect of two different promoting agents (AsA and SA) on the alleviation of negative high-temperature impact on photosynthesis in apple leaves. Five control trees were sprayed with distilled water (1 L per tree) (CK), five trees were sprayed with AsA (0.5 mM), and five trees were sprayed with SA (0.5 mM). Effective doses of SA and AsA were selected based on previous studies, considering the positive effects of the chosen concentrations. Trees were sprayed using a hand-pump sprayer. On the following day, three leaves per plant were cut, including both treated and untreated trees, along with their stalks and immersed in water for 24 h for acclimatization in an air chamber (*Clima Plus 9000* at 25°C and 60% humidity) for further investigation. Heat-stress treatment (HS, SA+HS, AsA+HS) was performed as follows: leaves were enclosed in 0.5-L glasses for 1 h and placed in the water bath at 37°C, while control leaves (CK, SA, AsA) were placed in the water bath at 25°C. The whole experiment consisted of six treatments as shown in the text table. After treatments, chlorophyll (Chl) fluorescence and Chl content were measured.

CK	Control-foliar spray with water
HS	Heat stress treatment
SA	Application with 0.5 mM SA without heat stress
SA+HS	Application with 0.5 mM SA + heat stress
AsA	Application with 0.5 mM AsA without heat stress
AsA+HS	Application with 0.5 mM AsA + heat stress

Chl fluorescence: The effect of promoting agents (SA and AsA) on the primary photosynthetic processes of heat-stressed apple leaves was evaluated using OJIP curves and Chl *a* fluorescence (ChlF) parameters. A *Handy PEA* (*Hansatech Instruments Ltd*., Norfolk, UK) was used to record the Chl *a* fluorescence in apple leaves. Before the measurements, the leaves were attached with leaf clips and left in the dark for 30 min. This process ensured the complete oxidation of plastoquinone (Q_A), the primary electron acceptor, and the opening of reaction centres, which is necessary for measuring the minimum fluorescence intensity (F_0). In our previous study on apple leaves, where we used the parameter PI_{ABS} as an indicator of stress, we found that a dark adaptation time of 30 min was sufficient for measuring the Chl *a* fluorescence transient in apple leaves (Mihaljević *et al*. 2020). For each treatment, fifteen measurements were recorded from five different trees. Red light with an intensity of 3,200 µmol(photon) m^{-2} s^{-1} was used for the induction of fluorescence, and during the first seconds of the measurement, the OJIP transient recorded changes from which various fluorescence parameters were calculated to analyse the photochemistry of PSII (Strasser *et al*. 2010, Chen *et al*. 2021). Among the various parameters that were calculated according to the JIP-test, we used 18 parameters that were significantly affected by treatments and are explained in Appendix 1.

To represent the changes in fluorescence intensity between the OJ and OK phases, ChlF transient data were double-normalized between F_0 and F_J (2 ms) and between F_0 and F_K (0.3 ms) to visualize the K and L bands, calculated as $V_{OJ} = [(F_t - F_0)/(F_J - F_0)]$ and $V_{OK} = [(F_t - F_0)/(F_K - F_0)]$. To indicate the difference between the control groups and the treatments, we calculated the differences in relative variable fluorescence values between treatments and reference (control treatment): $\Delta V_{OK} = V_{OK(treatment)} - V_{OK(control)}$ and $\Delta V_{OJ} = V_{OJ(treatment)} - V_{OJ(control)}$ (Ghaffar *et al*. 2023).

Chl content: For the estimation of Chl content in apple leaves, 0.2 g of fresh leaf sample was mashed and mixed in a mill, with the addition of magnesium carbonate. Absolute acetone was used as a solvent for pigment extraction. After extraction, the supernatant was gathered, and a spectrophotometer (*Specord 200*, *Analytik*, Jena, Germany) was used to read the absorbance at 663 nm for the determination of Chl *a* and 647 nm for the determination of Chl *b*. Total Chl content [Chl (*a*+*b*)] was estimated according to a previously described method using formulae according to Lichtenthaler (1987).

Statistical analysis: The results obtained from this study were analysed using the analysis of variance (*ANOVA*). Significant differences between treatments were identified by the LSD test, and differences were considered significant when $p \leq 0.05$. The results of photosynthetic parameters are shown as the mean of 15 biological replications, while the results of Chl content are shown as the mean of five replicates.

Results

OJIP curve is a fluorescence transient curve that represents changes in Chl fluorescence intensity. In this study, we examined the photochemical processes of primary photosynthesis in the apple leaves under heat stress using characteristic fluorescence parameters obtained from the OJIP curves. Furthermore, we analysed how salicylic and ascorbic acid treatments influenced changes in these parameters and whether they had a positive effect on photosynthetic reactions under high temperatures.

OJIP transients: Chl fluorescence of apple leaves was measured after treatments, and the OJIP induction curve was plotted on a logarithmic time scale, where in control plants, a characteristic O–J–I–P shape might be seen (Fig. 1*A*). A greater decrease in Chl fluorescence in heat-stressed apple leaves was found at J and I steps, while treatments with SA and AsA on heat-stressed leaves significantly increased J and I steps when compared to heat-stressed leaves. In plants treated just with SA and AsA, there was no significant change in typical OJIP kinetics compared with control (Fig. 1*A*). We showed the differential curves separately for the L (Fig. 1*B*) and K (Fig. 1*C*) bands to analyse alterations in OJIP fluorescence rise kinetics in more details. The highest positive L and K bands were observed in heat-stressed plants, while leaves sprayed with SA and AsA under heat stress exhibited lower positive L and K bands compared to heat-stress treatment. However, exogenous application of SA and AsA on apple leaves reduced stress effects and increased fluorescence transients when compared to leaves exposed only to heat treatment.

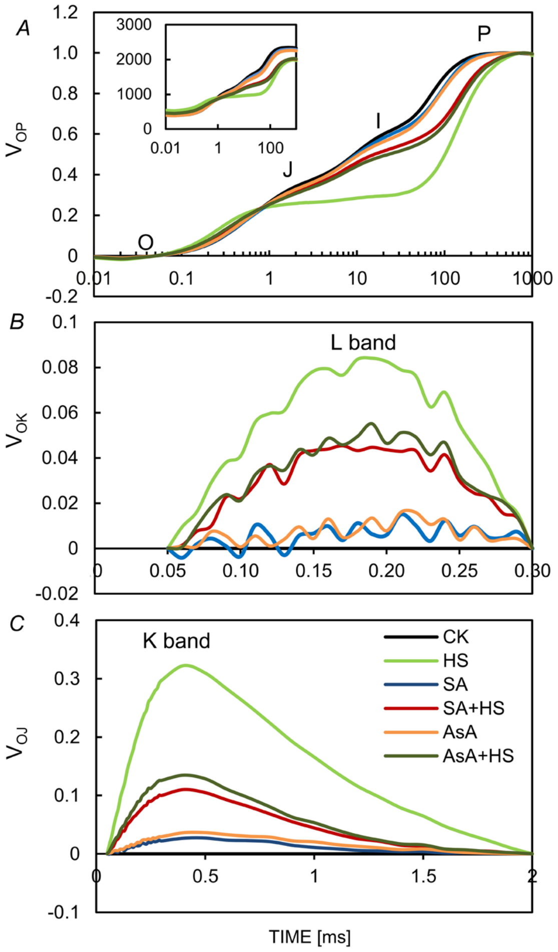

Fig. 1. Double-normalised OJIP curves of chlorophyll *a* fluorescence kinetics between O and P steps expressed as $V_{O-P} = [(F_t - F_0)/(F_P - F_0)]$, inserted graph represents a native curves (*A*); differences in data curves at O and K steps (L band) obtained after normalization between the steps O–K [$\Delta V_{OK} = V_{OK(treatment)} - V_{OK(control)}$] (*B*); differences in data curves at O and J steps (K band) obtained after normalization between the steps O–J [$\Delta V_{OJ} = V_{OJ(treatment)} - V_{OJ(control)}$] (*C*) in apple leaves with or without exogenous salicylic (SA) and ascorbic acid (AsA) spraying, *n* = 15. CK (control) – foliar spray with water; HS – heat-stress treatment; SA – application of 0.5 mM SA without heat stress; SA+HS – application of 0.5 mM SA + heat stress; AsA – application of 0.5 mM AsA without heat stress; AsA+HS – application of 0.5 mM AsA + heat stress.

JIP-test parameters: Heat stress significantly ($p \leq 0.05$) reduced relative variable fluorescence intensity at J step (V_J) and relative variable fluorescence intensity at I step (V_I) in apple leaves, while application of SA and AsA significantly increased these parameters in heat-stressed conditions (Table 1). Relative variable fluorescence at the L step (V_L) and relative variable fluorescence at the K step (V_K) under high temperature significantly increased and treatments with SA and AsA under heat stress significantly decreased those values in comparison with heat-stressed apple leaves (Table 1). When apple leaves were subjected to high temperatures the approximate initial slope of the fluorescence transient (M_0) significantly increased. In comparison to heat stress treatment, exogenous SA and AsA reduced the values of that parameter under stress (Table 1).

As shown in Fig. 2*A–E*, parameters that represent activities per reaction centres: absorption flux per RC (ABS/RC), trapped energy flux per RC (TR_0/RC), electron transport flux per RC (ET_0/RC), dissipated energy flux per RC (DI_0/RC), and electron flux reducing end electron acceptors at the PSI acceptor side, per RC (RE_0/RC), in apple leaves at high-temperature stress, showed significant increments in comparison with control plants. High values of these parameters in apple leaves under heat stress were significantly reduced by applying exogenous SA and AsA. The application of SA reduced these parameters' values similar to those found in the control plants. The density of active reaction centres per cross-section (RC/CS_0) was also affected by high temperature, which can be seen in the significant decrease of this parameter compared to control plants. However, exogenous application of SA and AsA enhanced values of RC/CS_0 in heat-stressed leaves (Fig. 2*F*).

Stress treatment significantly increased quantum yield parameters: efficiency with which an electron from PQH_2 is transferred to final PSI acceptors (RE_0/ET_0) (Fig. 3*A*), quantum yield for reduction of end electron acceptors at the PSI acceptor side (RE_0/ABS) (Fig. 3*B*), the probability that trapped exciton moves an electron into the electron transport chain beyond Q_A^- (ET_0/TR_0) (Fig. 3*C*) but did not influence the parameter quantum yield for electron transport (ET_0/ABS) (Fig. 3*D*), showing that these parameters are not sensitive to high-temperature stress. The stress treatment induced a significant increase in the quantum yield of energy dissipation (F_0/F_m) compared to control leaves. Although this parameter showed similar values in leaves treated with SA and AsA and in the control treatment, the application of these two promoting agents in heat-stressed leaves caused a significant decrease compared to heat-stressed leaves (Fig. 3*E*).

The most sensitive photosynthetic parameters are the performance index on absorption basis (PI_{ABS}) and maximum quantum yield of primary PSII photochemistry (F_v/F_m), which are frequently used for detecting plant tolerance to various abiotic stresses. PI_{ABS} and F_v/F_m showed significant decreases in heat-stressed leaves compared to the control, while their values in leaves treated with SA and AsA, without heat stress, were similar to CK leaves. SA and AsA application mitigated the decline in the values of these two parameters under stress, more precisely; exogenous SA and AsA increased the PI_{ABS} and F_v/F_m values when exposed to high temperature, as seen in Fig. 4*A*,*B*.

Chl content: Total chlorophyll content Chl (*a*+*b*) in apple leaves was significantly reduced as a result of high temperature (Fig. 5). Application of SA and AsA mitigated the degradation of Chl due to high temperature. Fig. 5 shows that, in both treatments with and without heat stress, the application of AsA significantly enhanced

Table 1. JIP parameters deduced from chlorophyll *a* fluorescence OJIP transient curves in apple leaves with or without exogenous ascorbic (AsA) and salicylic acid (SA) spraying. Mean values (± SE) were calculated from 15 independent measurements. Significant differences between means are indicated by *different letters*, according to the *Fischer*'s Least Significant Differences test at a p=0.05 level. CK (control) – foliar spray with water; HS – heat-stress treatment; SA – application of 0.5 mM SA without heat stress; SA+HS – application of 0.5 mM SA + heat stress; AsA – application of 0.5 mM AsA without heat stress; AsA+HS – application of 0.5 mM AsA + heat stress. V_J – relative variable fluorescence intensity at the J step; V_I – relative variable fluorescence intensity at the I step; V_L – relative variable fluorescence at 0.15 ms; V_K – relative variable fluorescence at 0.3 ms; M_0 – approximated initial slope of the fluorescence transient.

Treatments	V_J	V_I	V_L	V_K	M_0
CK	0.35 ± 0.00^a	0.64 ± 0.01^a	0.31 ± 0.00^c	0.31 ± 0.00^c	0.45 ± 0.01^c
HS	0.28 ± 0.01^c	0.32 ± 0.03^c	0.64 ± 0.00^a	0.64 ± 0.02^a	0.71 ± 0.02^a
SA	0.33 ± 0.01^b	0.62 ± 0.01^a	0.34 ± 0.00^c	0.34 ± 0.00^c	0.46 ± 0.01^c
SA+HS	0.33 ± 0.01^b	0.55 ± 0.02^b	0.43 ± 0.00^b	0.43 ± 0.01^b	0.56 ± 0.01^b
AsA	0.34 ± 0.00^{ab}	0.60 ± 0.01^a	0.35 ± 0.00^c	0.35 ± 0.01^c	0.47 ± 0.01^c
AsA+HS	0.32 ± 0.01^b	0.52 ± 0.02^b	0.45 ± 0.00^b	0.45 ± 0.01^b	0.59 ± 0.02^b

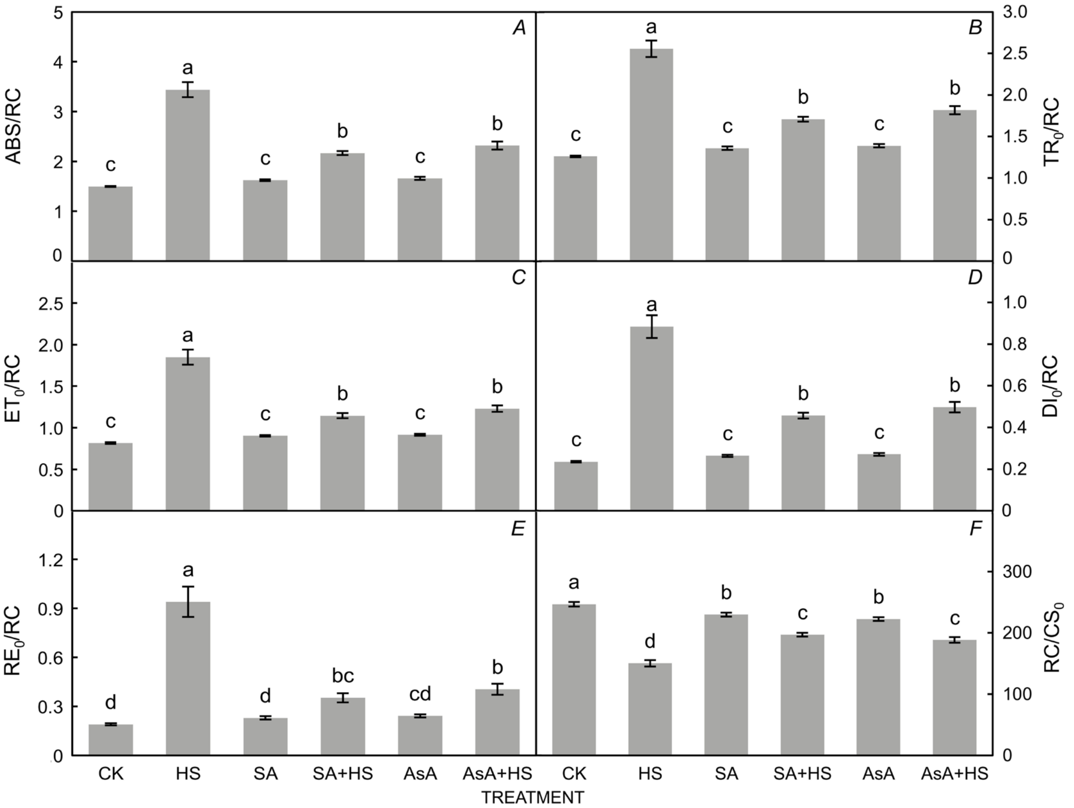

Fig. 2. Influence of SA and AsA on absorption flux per RC, ABS/RC (*A*), trapped energy flux per RC, TR_0/RC (*B*), electron transport flux per RC, ET_0/RC (*C*), dissipated energy flux per RC, DI_0/RC (*D*), electron flux reducing end electron acceptors at the PSI acceptor side, per RC, RE_0/RC (*E*), density of RCs, RC/CS_0 (*F*) in apple leaves. Data presented as mean values (± SE) were calculated from 15 measurements. Significant differences between means are indicated by *different letters*, according to the *Fischer*'s Least Significant Differences test at a p=0.05 level. CK (control) – foliar spray with water; HS – heat-stress treatment; SA – application of 0.5 mM SA without heat stress; SA+HS – application of 0.5 mM SA + heat stress; AsA – application of 0.5 mM AsA without heat stress; AsA + HS – application of 0.5 mM AsA + heat stress.

Chl (*a*+*b*) content in comparison to control and heat-stressed plants, whereas treatment with SA significantly increased Chl (*a*+*b*) content only when combined with heat stress. The greatest Chl (*a*+*b*) content was obtained with AsA treatment [4.5 mg g^{-1}(FM)].

Discussion

Many studies have shown that photosynthesis is seriously affected by high temperatures, as evidenced by Chl fluorescence measurements (Xu *et al.* 2014, Jedmowski

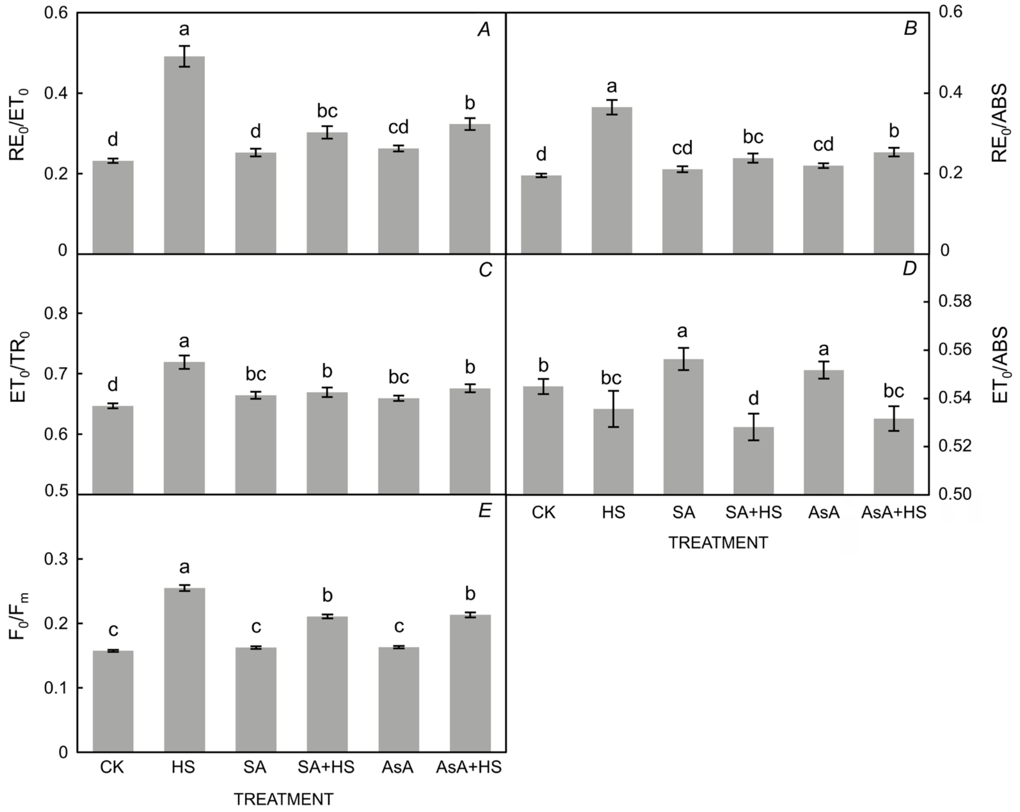

Fig. 3. Influence of SA and AsA on efficiency with which an electron from PQH_2 is transferred to final PSI acceptors, RE_0/ET_0 (*A*), quantum yield for reduction of end electron acceptors at the PSI acceptor side, RE_0/ABS (*B*), probability that trapped exciton moves an electron into the electron transport chain beyond Q_A^-, ET_0/TR_0 (*C*), quantum yield for electron transport, ET_0/ABS (*D*), quantum yield of energy dissipation, F_0/F_m (*E*) in apple leaves. Data presented as mean values (± SE) were calculated from 15 measurements. Significant differences between means are indicated by *different letters*, according to the *Fischer*'s Least Significant Differences test at a p=0.05 level. CK (control) – foliar spray with water; HS – heat-stress treatment; SA – application of 0.5 mM SA without heat stress; SA+HS – application of 0.5 mM SA + heat stress; AsA – application of 0.5 mM AsA without heat stress; AsA + HS – application of 0.5 mM AsA + heat stress.

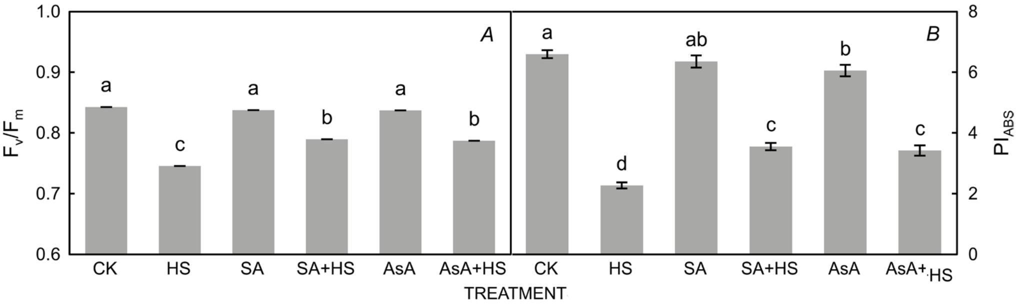

Fig. 4. Influence of SA and AsA on maximum quantum yield of primary photochemistry, F_v/F_m (*A*), performance index on absorption basis, PI_{ABS} (*B*) in apple leaves. Data presented as mean values (± SE) were calculated from 15 measurements. Significant differences between means are indicated by *different letters*, according to the *Fischer*'s Least Significant Differences test at a p=0.05 level. CK (control) – foliar spray with water; HS – heat-stress treatment; SA – application of 0.5 mM SA without heat stress; SA+HS – application of 0.5 mM SA + heat stress; AsA – application of 0.5 mM AsA without heat stress; AsA + HS – application of 0.5 mM AsA + heat stress.

and Brüggemann 2015). In this research, two different promoting agents (SA and AsA) were applied to apple leaves with or without heat stress treatment to determine their influence on photosynthetic activity.

Effect of high temperature on photosynthetic activity in apple leaves: As we observed in our previous study on apple trees, high temperatures harm PSII performance, resulting in lower photosynthetic efficiency (Mihaljević

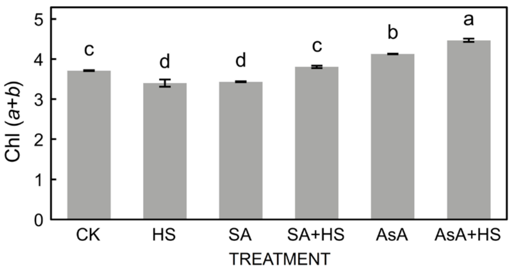

Fig. 5. Total chlorophyll content [mg g^{-1}(FM)] in apple leaves with or without exogenous ascorbic (AsA) and salicylic acid (SA) spraying. Data presented as mean values ± SE ($n = 5$). Significant differences between means are indicated by *different letters*, according to the *Fischer*'s Least Significant Differences test at a p=0.05 level. CK (control) – foliar spray with water; HS – heat-stress treatment; SA – application of 0.5 mM SA without heat stress; SA+HS – application of 0.5 mM SA + heat stress; AsA – application of 0.5 mM AsA without heat stress; AsA + HS – application of 0.5 mM AsA + heat stress.

et al. 2020). The most widely used parameters, PI_{ABS} and F_v/F_m, are very popular and sensitive indicators used for quantifying abiotic stress reactions, including heat stress in many plant species (Xu *et al.* 2014, van der Westhuizen *et al.* 2020). These parameters provide valuable information regarding the physiological and functional status of photosynthesis. A decrease in these two parameters induced by high temperatures has been observed in our study. We noticed that the values of parameter F_v/F_m were 0.74, indicating that PSII was significantly affected by high temperature. It is considered that F_v/F_m ratios between 0.75 and 0.83 indicate normal functionality of PSII in most plant species, while values lower than 0.75 signify a decline in the quantum yield of PSII or disruption of PSII function (Krause and Weis 1991). The high temperature changed the shape of the ChlF induction curves of apple leaves. The major changes were observed in the J (V_J) and I (V_I) steps, where a significant decrease in the J and I steps was detected. Many previous studies have documented an elevation in V_J and V_I parameters in plants subjected to heat stress. Ji *et al.* (2022a) suggested that the rise in V_J values signifies constrained Q_A reoxidation, leading to an accumulation of Q_A reduction and a decline in electron transport. Jiang *et al.* (2006) utilized parameter V_I to evaluate the inhibition of electron transport at the acceptor side of PSII due to heat. They observed that V_I in heat-stressed leaves was significantly higher, leading to the conclusion that heat markedly reduces electron transport at the acceptor side of PSII. Yan *et al.* (2013) observed an increase in both J and I steps at high temperatures in sweet sorghum plants, suggesting an inhibition of electron transport beyond Q_A and PQH_2, implying that the fragment from Q_A to PQH_2 is the most heat-sensitive. In contrast to their findings, our research revealed that, following heat stress, these parameters were lower than those observed in control plants, and a decline in the fluorescence intensity of the OJIP transient was observed. This aligns with the findings of Chen and Cheng (2009), who observed that, based on the lower V_I and V_J in heat-treated leaves, the donor side (OEC) of PSII was more severely damaged than the acceptor side under heat stress. They concluded that the reduction of PSII activity by heat stress primarily results from the inactivation of OEC. Similarly, in an experiment with tall fescue, the J and I step dropped, after heat stress, compared to the control treatment (Chen *et al.* 2014). Some *Amaranthus* plants showed a decline in the fluorescence intensity of the OJIP transient at higher temperatures (Netshimbupfe *et al.* 2022). The presence of the K band (at 300 μs) is widely acknowledged as an indicator of heat stress, usually associated with inhibition of the OEC and impairment of electron donation to the reaction centres of PSII (Jedmowski and Brüggemann 2015, Strasser *et al.* 2000). The positive K band indicates a reduction in the efficiency of the OEC to split water and supply electrons to a P_{680} PSII RCs. This change may be associated with an increased antennae size of PSII and disruption between the donor and acceptor sides, potentially causing an imbalance in electron flow. The findings suggest that higher temperatures compromise the stability of the OEC (Yusuf *et al.* 2010, Oukarroum *et al.* 2016). Therefore, in our study, a characteristic positive K band and increased V_K value at high temperature, suggesting also that the OEC is one of the most sensitive components of the photosynthetic electron transport chain. The K band has been also found in many previous studies as a typical characteristic that represents the destruction of the PSII structure induced by heat (Martinazzo *et al.* 2012, Giorio and Sellami 2021). The positive L band was noticed in heat-stressed leaves, suggesting a reduction in the energetic connectivity of LHCII and PSII reaction centres in leaves subjected to high temperature. It signifies the separation of the pigment complex from the antenna, resulting in an increased distance between PSII antennas and, consequently, less efficient energy exchange (Ji *et al.* 2022a). The decline in cooperativity also signals a reduction in the proportion of RCs (Dimitrova *et al.* 2020). This disruption suggests a negative impact of high temperature on the normal functioning of the photosynthetic process in apple leaves, affecting the efficiency of energy transfer and utilization. The increment of the parameter (M_0) points to a larger decrease in Q_A to Q_A^- in heat-stressed leaves and a reduction in photosynthesis, as was confirmed in the study of Shanker *et al.* (2022) on heat-stressed pearl millet plants. A significant increase in ABS/RC, TR_0/RC, ET_0/RC, and RE_0/RC noticed in heat-stressed apple leaves may be linked to a lack of active RCs in PSII because certain RCs have become inactive, as shown in the heat-stressed leaves of *Prunus persica* by Martinazzo *et al.* (2012). ET_0/RC reflects the activity of only the active RCs in the PSII of plants during photosynthesis. Unlike most studies where high temperature reduced parameter ET_0/RC, we observed an increase in this parameter under higher temperatures. An increase in ET_0/RC was observed in the research of Ji *et al.* (2022b), who noted that under high-temperature stress, ET_0/RC was higher than that of the control *Paeonia* plants. According to previous studies by Martinazzo *et al.* (2012) and Mathur *et al.* (2013), an increase in the parameter ET_0/RC under high temperatures suggests that the proportion of active centres capable of efficiently participating in electron transport is decreasing. Their findings suggest that a higher proportion of inactive reaction centres in PSII results in less efficient transfer of electrons from the primary electron acceptor (Q_A^-) to the secondary electron acceptor (Q_B). This reduction in electron transfer efficiency can harm the overall efficiency of photosynthesis which was confirmed also in our study. Further, a higher rise in DI_0/RC demonstrated that RCs were inactivated at high temperatures probably because they dissipated most of the energy as heat (Zushi *et al.* 2012). An increase of ABS/RC in heat-stressed apple leaves correlated with a reduced RC/CS_0, which also implied that the number of active RCs in PSII was reduced significantly (De Ronde *et al.* 2004). The inactivation of certain RCs in PSII also increased parameter F_0/F_m, which is considered an important adaptation technique that helps plants cope with high temperatures while maintaining efficient photosynthesis and avoiding oxidative damage (Kalaji *et al.* 2011). According to the study of Maxwell and Johnson (2000), photoinhibition has been demonstrated by a decrease in the F_v/F_m ratio and an increase in the F_0/F_m ratio, as was observed in our study. Parameters, marked as RE_0/ABS and RE_0/ET_0, describe electron transport to the end electron acceptors of PSI. Antunović Dunić *et al.* (2023) reported that lower RE_0/ET_0 and RE_0/ABS values imply on reduced electron flow rate between reduced intersystem electron acceptors and PSI. Our results showed that these values were higher in heat-stressed leaves, indicating that electron transport in these leaves was efficient and electron flow from PQH_2 to the PSI was normal. Similarly, Markulj Kulundžić *et al.* (2022) and Chen *et al.* (2016) observed that the parameters RE_0/ET_0 and RE_0/ABS increased in sunflower and crofton weed plants at higher temperatures, respectively. Unchanged values of parameter ET_0/ABS showed that high temperature did not reduce the efficiency of electron transport beyond Q_A^- (Xu *et al.* 2014). A significant increase of parameters RE_0/ET_0 and RE_0/ABS could be interpreted as high temperature in this study increased PSI activity. Our results are consistent with the previous studies where PSI activity increased under moderate heat stress (Huther *et al.* 2013, Yang *et al.* 2021). Along with the changes in all of the above parameters, high temperature caused a reduction in total PSII activity, which was confirmed by a significant decrease in PI_{ABS}.

The Chl content in plants is an important factor that influences photosynthetic capacity, and plants with higher Chl content can absorb more light energy, improving their photosynthetic efficiency (Wang *et al.* 2022). High temperatures reduce the amount of photosynthetic pigments (Feng *et al.* 2014). These findings corresponded with our findings on apple leaves, where reduced Chl (*a*+*b*) in heat-stressed leaves were probably due to enhancement in Chl degradation and weakening of Chl synthesis.

Effect of SA and AsA on photosynthetic activity in heat-stressed apple leaves: The positive effect of SA in plants and its significant role in preventing heat stress has already been reported (Sangwan *et al.* 2022). Although some previous studies showed that SA inhibits photosynthetic activity, others found that photosynthesis increases when SA is present in small concentrations (Gao *et al.* 2018, Janda *et al.* 2014). The benefit of AsA in protecting of photosynthetic function stability was also observed in rice plants (Zhang *et al.* 2018). According to the results obtained in our study, SA and AsA applications enhanced photosynthetic capacity and Chl concentration in heat-stressed apple leaves. It increased PI_{ABS} and F_v/F_m. We assumed that the increase of PI_{ABS} and F_v/F_m parameters could be linked to the effects of SA and AsA on the increasing number of RCs per PSII antenna chlorophyll and F_v/F_m, as was observed in water-stressed *Brasica napus* plants treated with fluvic acid (Lotfi *et al.* 2015). According to Wang *et al.* (2010), the increased thermostability of PSII caused by SA treatment is correlated with an increase in the thermostability of the PSII centre. Apple leaves sprayed with SA and AsA showed enhancement of electron transport under heat stress. Similarly, Moustakas *et al.* (2022) also noticed that SA improved electron transport with an increase of F_v/F_m. As previously stated, the heat stress treatment had a significant disruptive impact on parameters ABS/RC and DI_0/RC. The positive effects on photosynthesis resulting from SA and AsA treatments in our study were observed through a reduction in ABS/RC and DI_0/RC values, almost reaching levels comparable to those observed in control plants. We believe that AsA and SA played an important role in regulating the utilization and expenditure of energy absorbed by PSII RCs in heat-stressed apple leaves, allowing for the efficient transfer of energy from heat dissipation to photochemical reactions (Chen *et al.* 2021). In this way, SA and AsA contributed to improving the dissipation process, thereby preventing damage to the photosynthetic system caused by heat stress. Our findings align with those of Bukhat *et al.* (2020), who observed similar outcomes. In their study on salt-stressed radish plants, the exogenous application of SA enhanced the quantum yield of PSII. This improvement was attributed to an increase in the density of active reaction centres per antenna chlorophylls, leading to a reduction in ABS/RC and DI_0/RC. The application of SA on heat-stressed pepper leaves was observed to significantly alleviate these parameters, as was connected by the mitigation of heat stress injuries of PSII (Zhang *et al.* 2020). Both promoting agents mitigated the increase of V_K (K band), which is a specific marker of OEC damage on the PSII electron donor side. Tomato leaves treated with SA were shown to have higher OEC efficiency than untreated leaves (Moustakas *et al.* 2022). Leaves treated with AsA and SA also exhibited a lower L band when subjected to heat stress, which indicates that both promoting agents influenced better energetic connectivity and stability. Similar results, with lower L and K bands, were shown for tomato plants treated with AsA under salt stress conditions (Chen *et al.* 2021).

In both heat-stressed and non-stressed treatments, the application of AsA and SA on apple leaves increased photosynthetic pigments in comparison to control plants. Our findings are consistent with those of Wang *et al*. (2014) who observed that foliar application of SA decreased Chl degradation, suggesting that SA may have a protective effect against heat stress. Furthermore, spraying with AsA has been shown to improve the Chl content of cotton leaves by detoxifying hydrogen peroxide (Kamal *et al*. 2017).

These mechanisms together contribute to the maintenance of optimal photosynthetic rates and could help fruit trees cope with the harmful effects of high temperatures on photosynthesis. Overall, the combination of OJIP curves and the analysis of basic fluorescence parameters provide valuable insights into the photochemistry and responses of apple leaves to high-temperature stress, as well as the potential positive effects of SA and AsA treatments on the photosynthetic reactions under such conditions.

Conclusion: Exposure of apple leaves to high temperatures resulted in the disruption of various components within PSII, including damage to the OEC, deactivation of RCs in PCII, and a reduction in the connections between PSII units. In general, the application of SA and AsA to apple leaves when subjected to high temperatures had a positive impact on their photosynthetic activity. Our investigation revealed that these promoting agents effectively mitigate the detrimental effects of high temperatures on PSII by improving both its structure and function, leading to an overall enhancement in photosynthetic activity. These findings suggest that both agents may be employed to raise pigment concentration and enhance photosynthetic efficiency, ultimately enhancing the response of apple leaves to heat stress.

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Appendix 1. Photosynthetic parameters derived from the JIP-test analysis.

Fluorescence parameters and formulae	Explanations
$V_J = (F_J - F_0)/(F_m - F_0)$	Relative variable fluorescence intensity at the J step
$V_I = (F_I - F_0)/(F_m - F_0)$	Relative variable fluorescence intensity at the I step
$V_L = (F_L - F_0)/(F_m - F_0)$	Relative variable fluorescence at 0.15 ms
$V_K = (F_K - F_0)/(F_m - F_0)$	Relative variable fluorescence at 0.3 ms
$M_0 = 4(F_{300\mu s} - F_0)/(F_m - F_0)$	Approximated initial slope of the fluorescence transient
Flux ratios of PSII	
$TR_0/ABS = \varphi_{P0}\ F_V/F_m = [1 - (F_0/F_m)]$	Maximum quantum yield of primary photochemistry
$ET_0/ABS = \varphi_{E0} = [1 - (F_0/F_m)] \times (1 - V_J)$	Quantum yield for electron transport
$F_0/F_m = \varphi_{D0} = 1 - F_v/F_m$	Quantum yield (at t = 0) of energy dissipation
$ET_0/TR_0 = \psi_{E0} = 1 - V_J$	Probability that trapped exciton moves an electron into the electron transport chain beyond Q_A^-
Flux ratios of PS I	
$RE_0/ET_0 = \delta_0 = (1 - V_I)(1 - V_J)$	Efficiency with which an electron from PQH_2 is transferred to final PSI acceptors
$RE_0/ABS = \varphi_{R0} = \varphi_{P0} \times \psi_{E0} \times \delta_{R0}$	Quantum yield for reduction of end electron acceptors at the PSI acceptor side
Activities per reaction center (RC)	
$ABS/RC = M_0\ (1/V_J) \times (1/\varphi_{P0})$	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
$RE_0/RC = M_0\ (1/V_J)\ \psi_{E0}\ \delta_{R0}$	Electron flux reducing end electron acceptors at the PSI acceptor side, per RC
Density of reaction centers	
$RC/CS_0 = \varphi_{P0}\ (V_J/M_0)\ F_0$	Density of RCs (Q_A reducing PSII reaction centres)
Vitality index	
$PI_{ABS} = (RC/ABS)(TR_0/DI_0)[ET_0/(TR_0 - ET_0)]$	Performance index on absorption basis