Advances in Biology & Earth Sciences
Vol.4, No.3, 2019, pp.206-213

THE DIFFERENT FACTORS INDUCED OXIDATIVE DAMAGES OF PS II AND PROTECTION BY Na-ASCORBATE

J.R. Jafarova*

Cell Biophysics laboratory, Institute of Botany, Baku, Azerbaijan

Abstract. To create the increased content of reactive species of oxygen (ROS) in the photosynthesizing plants the seedlings of wheat (*Triticum aestivum* L.) and pumpkin (*Cucurbita pepo* L.) were treated by methylviologen (MV), high intensity light and heavy metals (Zn^{2+}, Ni^{2+}, Cu^{2+}). The ability of Na-asc to prevent the activity changes of photosynthetic electron transport chains of PS II was investigated. On the base changes characteristics of millisecond delayed fluorescence of chlorophyll (ms DF Chl *a*) was shown that effect of Na-asc as an ROS inactivator is displayed on donor and acceptor sides of PS II electron transfer chain of PS II depending on generating stress.

Keywords: *Na-asc – ascorbic acid sodium salt, delayed chlorophyll fluorescence, oxidative stress, high light stress, heavy metals, methylviologen.*

Corresponding Author: *Jeyhuna Jafarova, Institute of Botany, Badamdar 40, Baku-1004, Azerbaijan, Tel.: +994553966414, e-mail: j.jafarova@yahoo.com*

Received: *25 October 2019;* ***Accepted:*** *5 December 2019;* ***Published:*** *12 December 2019.*

1. Introduction

The excitations of light reactions of photosynthesis lead to enhanced production of ROS (Barber, 2003). As highly reactive substances ROS is known to cause significant damage to biological structures of cell and biological-biochemical processes occurring in it. The excess generation of ROS and damage by them cell content is designated in literature as oxidative stress. The reason of oxidative stress as it is considered is a spatial-time in balance between generation and removal of ROS that seriously disturbed the cell normal metabolism (Meloni *et al.*, 2003; Blokhina *et al.*, 2003; Ahmad *et al.*, 2008). The main target of plants under stress is known to be photosynthetic membrane of chloroplast. The most vulnerable are the photochemical reactions occurring at complex molecular reaction and structure of PS II and chlorophyll-protein complex of PS II especially (Andersson & Barber 1996; Tyystjärvi *et al.*, 2005; Renger & Renger, 2008). The optimal rate of photosynthesis is fulfilled at narrow range of light intensity. The light absorbed by photosynthetic apparatus is not fully used by photochemical reactions. That leads to disbalance between quantum of absorbed light and its utilization during photosynthesis. As a result of processes the generation of OH and $O_2^{-\bullet}$, H_2O_2 are rapidly increased (Alscher *et al.*, 1997; Ort, 2001; Scandalios, 2002). This condition is appeared also under other stress factors in plant organism.

The toxic concentrations of heavy metals create a problem that became fundamental for elucidation of survival and adaptation of plants to damaging factor caused by them. Heavy metals ions actively catalyzed reactions of free radical formation. The oxidative burst in plants is caused by herbicides used in agriculture in

particular methylviologen (MV). MV accepts the electrons from electron transport chain of PS I and forming bipiridin radicals, that easily react with O_2, formed superoxide and in a number of reactions produced H_2O_2 and hydroxyl radicals (Takizawa *et al.*, 2007). The important role of ROS in detoxification have an enzymes and nonenzymatic compounds possessing by antioxidant properties. Inspite of important role of enzymes in detoxification of ROS at present time it is thought that low molecular organic antioxidants at a number of cases were found more effectively protect of biological structures from ROS (Wang *et al.*, 2012; Zeng *et al.*, 2018).

Na-asc is an active antioxidant capable to suppress of one-electron restoration of O_2 at metabolism. Na-asc being donor of electrons to ascorbate peroxidase activating this antioxidant enzyme (Smirnoff, 2000; Smirnoff & Wheeler, 2000; Mano *et al.*, 2004).

The aim of this work is to demonstrate an ability of Na-asc to resist to accumulation of ROS having different levels and sites in dependence from created stresses so protecting the functional activity of PS II.

2. Materials and methods

Object: 7-8 days of wheat seedlings (*Triticum aestivum* L.) and pumpkin (*Cucurbita pepo* L.) were grown in water medium at the white light illumination with intensity 250 mcW/cm^2 and temperature +26°C.

Oxidative damage conditions: The oxidative stress in chloroplasts was created: 1) by means of spray of leaves during 5 min by water solution of methylviologen (MV) in concentration 100 μM; 2) by action of light with high intensity (4000 μmol photons $m^{-2}s^{-1}$) within 2 h; 3) by incubation of seedlings in heavy metals solutions during 24 h ($NiCl_2$, $ZnCl_2$ in concentration 10^{-3}, $CuSO_4$ concentration 13 and 26 mg/l). Low molecular antioxidant sodium salt of ascorbic acid (Na-asc at concentration $4\cdot10^{-4}$ m) serve as protector from oxidative stress. Na-asc was used at variants of simultaneous and successive action with oxidants.

Delay fluorescence measurements. The functional activity of photosystem II was evaluated on the base of analyses induction transition of kinetic curves of delayed fluorescence in millisecond range (ms DF Chl *a*), reflecting partial reactions of chloroplasts electron transport chain of PS II (Goltsev *et al.*, 2005).

The kinetics of millisecond DF was measured using a phosphoroscope. A sample in quartz cuvette was irradiated with continuous white light (250 mcW/cm^2) passing through a 2 cm of $CuSO_4$ solution. The light was passing through holes on the rotating wheel of the phosphoroscope (three holes, 120° apart) in such way that 0.3 ms of registration was following by 1.25 ms of dark and 0.3 ms of registration/dark/registration per one full turnover of phosphoroscope wheel. The delayed light emission was measured with a photomultiplier shielded by red cutoff filter with λ>680 nm (KC9, LOMO Russia). Signal was amplified and recorded by chart recorder (Gasanov *et al.*, 2007).

3. Results

The interaction of dark and light processes of photosynthesis are strikingly reveals itself in induction process, when the transition of adapted to dark plant to stationary photosynthesis under action of excited light.

The investigation of fluorescent characteristics demonstrated that induction curves of ms DF Chl *a* changes depend on acting factors.

Oxidative stress induced by methylviologen. The oxidative stress, caused by action of MV, catalyzing of formation superoxide anion radicals leads to sharp fall of ratio fast and slow phases to stationary level on induction curve DF (f./s. and sl./s.). Such state leads to changes of oxidation-reduction equilibrium in the PS II chain. Na-asc was used by simultaneous (Na-asc +MV) and successive action of MV and after 30 min Na-asc.

The dynamics of restoration changes of first acceptor of PS II and stability of electrons flow on Q_A and Q_B reveals that protecting effect was observed under successive influence MV→Na-asc. Simultaneously treatment of leaves by Na-asc+MV depressed the MV aggression. The restoration of value f./s. on 75% relatively to MV action was observed at this case. The value sl./s. is increased relatively to action of MV and 6 times exceed a control variant (Fig. 1).

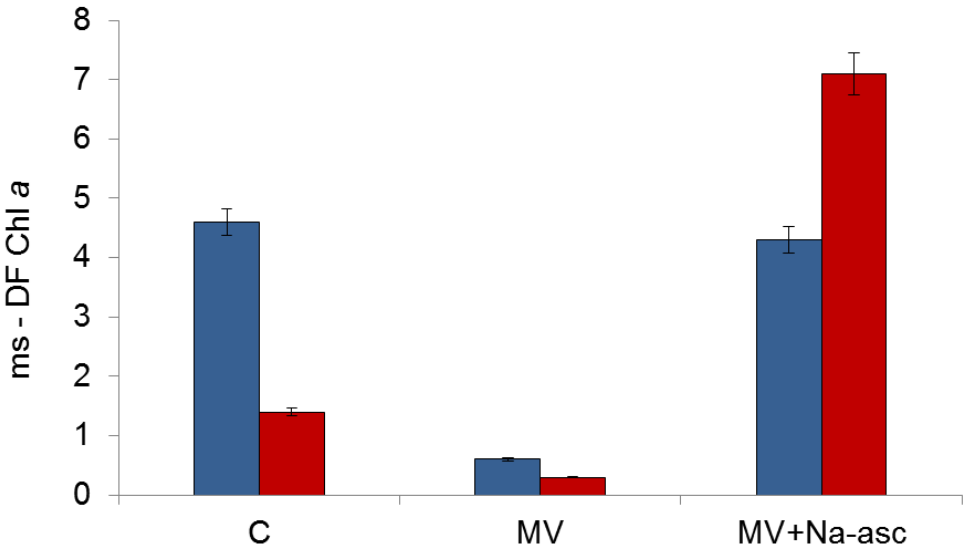

Fig.1. The columns demonstrates the changes of ratio for fast and slow phases to stationary phase (■-f./s., ■-sl./s.) of chlorophyll DF. C- control; MV- after 2 h after treatment of pumpkin leaves (*Cucurbita pepo* L.) by methylviologen (MV) during 5 min; MV+Na-asc - after 2 h after treatment by Na-asc of pumpkin leaves (*Cucurbita pepo* L.), treated by MV during 5 min.

Oxidative stress induced by high light intensity illumination. The state of electron transport chain of PS II under action by high light intensity on seedlings (4000 μmol $m^{-2}s^{-1}$ photon) is identical to action of MV. The sharp drop of f./s. and sl./s. values on 76,7% and 98% relatively to control was observed. The exposition of photoinhibited seedlings under normal illumination during 5 h restored given values: the value f./s. on 32%, the value sl./s. on 56% relatively to photoinhibition. The Na-asc action effectively restored these parameters after 5 h exposition on control exposition on 59% and 81% relatively to restoration at absence of antioxidant (Fig. 2).

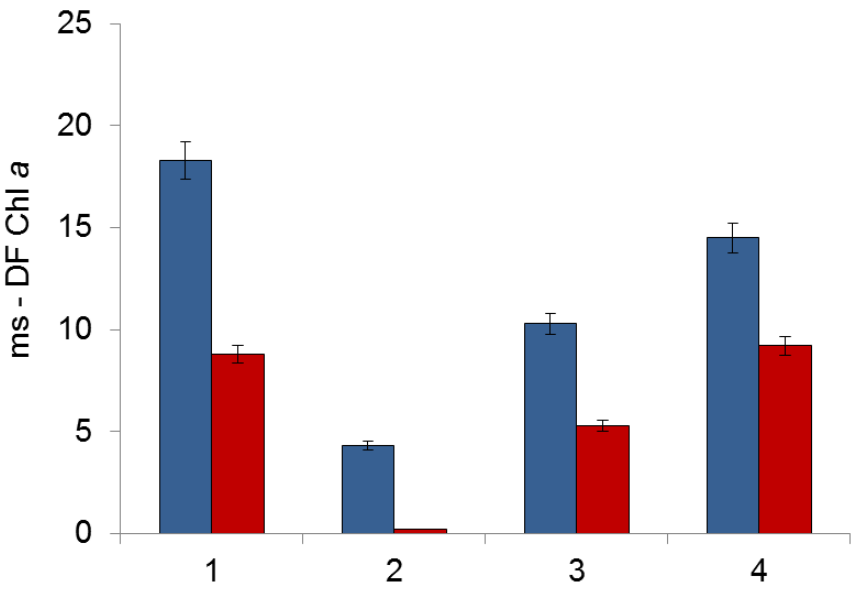

Fig. 2. The columns demonstrates the changes of ratio of fast and slow phases to stationary phase (■-f./s., ■-sl./s.) of DF Chl *a*. 1) control leaves; 2) leaves of pumpkin seedlings (*Cucurbita pepo* L.), subjected to photoinhibition (4000 μmol photons $m^{-2}s^{-1}$); 3) after photoinhibition during 5 h exposed to light of normal intensity (250 mcW/cm^2); 4) at exposed to normal illumination photoinhibited seedlings of pumpkin after treatment by Na-asc after 5 h.

Oxidative stress induced by heavy metals action. The observed changes in PS II electron transfer chain under action of metals were depended from their toxicity. Incubation of seedlings in $CuSO_4$ solution at concentration 13 mg/l and 26 mg/l during 24 h induced decrease of f./s. and sl./s. on 60% and 55% and 55% and 30% accordingly to concentration. The Zn^{2+} ions action during 24 h suppressed f./s. up to 30% and more aggressively manifest itself in relation to value sl./s. (on 50%). The Ni ions suppressed at more extent the value f./s. (on 30%) and only to 10% the value sl./s. (Fig. 3*A,B*, 4*A,B*).

The reaction of Na-asc in protection of PS II activity was depended from acting metal and experiment variants (Fig. 3*A, B*). Under action of the Cu^{2+} effect of Na-asc manifest itself at case of successive action ($CuSO_4$→Na-asc). The restoration of value f./s. above control level and on 85% is promoted relatively to action of Cu^{2+}. The value sl./s. was restored only to 28% relatively to action of Cu^{2+} and put up below of control. Effect of Na-asc in relation to Zn^{2+} action reveal itself also under successive incubation of seedlings in solutions Na-asc→Zn^{2+}. In given variant the value f./s. was restored and was above of control, on 30% inrelation to action Zn^{2+} was increased up to on 58% relatively to suppression value of Zn^{2+} (Fig. 4*A*). The value sl./s. is increased unsignificantly.

The effect of Na-asc under Ni^{2+} action manifest itself on simultaneously incubation of seedlings in solution Na-asc+Ni^{2+} only on acceptor side of PS II chain. The value sl./s. was increased in 2 times relatively to suppression by Ni and on 18% was higher on control (Fig. 4 *B*).

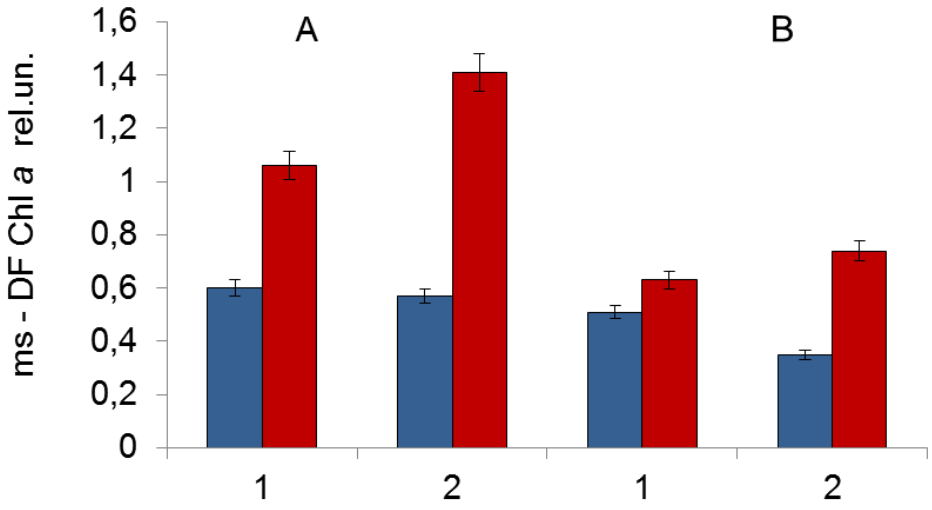

Fig. 3. The columns reflect the values of relation fast phase to stationary phase (f./s.) (A) and slow phase to stationary phase (sl./s.) (B) on induction curve of ms DF Chl *a* under action of Cu^{2+} at concentration 1) 13 mg/l; 2) 26 mg/l during 24 h. ■-$CuSO_4$; ■-addition of Na-asc. The value of relation in control is taking to one.

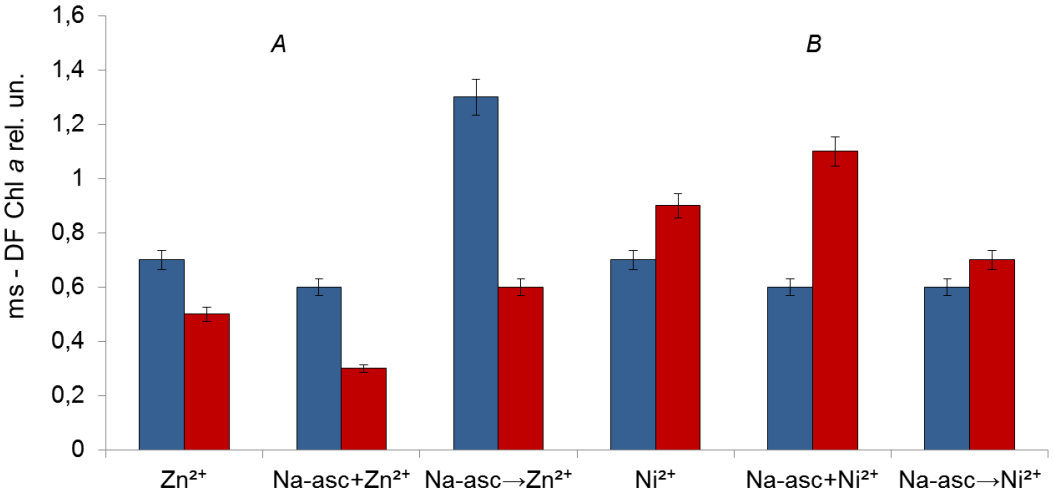

Fig. 4. The column reflects the changes value of ratios f./s. (■) and sl./s. (■) of ms DF Chl *a* under action within 24 h. The value of ratio in control is taking to one.
A) Zn^{2+}; B) Ni^{2+} to wheat seedlings (*Triticum aestivum* L.) and after action of Na-asc in different variants.

So, on the base of obtained results it is may concluded that Na-asc can protect an activity of electron transport chain of PS II under stress (Table 1).

Table 1. The restoration f./s. and sl./s. phases ratios of DF of Chl *a* by Na-asc action under different stresses in %. Control is – 100%

Conditions / Parameters	MV	MV+ Na-asc	HLI	HLI+ Na-asc	Cu^{2+}	Cu^{2+}→ Na-asc	Zn^{2+}	Na-asc→ Zn^{2+}	Ni^{2+}	Na-asc+ Ni^{2+}
f./s.	18	96	24	83	60	110	70	130	70	60
sl./s.	25	350	2.3	83.3	52	62	50	60	90	100

4. Discussion

Significant amount of investigations at present revealed the negative role of ROS and oxidative stress. This processes leads to disturbance of photochemical reactions in electron transport chain of PS II. The inactivation of donor side (f.ph) took place as a result generation of long lived $P680^{+*}$ and Tyr Z^{+*} radicals that cannot be reduced at the absence of sufficient electron flow from Mn_4O_5Ca-cluster. These radicals have a highly oxidize energy and damaged their protein surrounding with sequential leading to lowering of electron transfer between Tyr Z and P680. The donor side (sl.ph) inactivation in PS II chain leads to disturbance of acceptors function Q_A and Q_B and formation of singlet oxygen (Gasanov *et al.*, 2007; Gasanov *et al.*, 2012). Studied stress factors such as MV, photoinhibition and heavy metals leads to inactivation of electron transport chain as on donor side as well as on acceptor sides followed to direct or indirect molecular damage through the formation of ROS. The primary sites of ROS action is shown to be electron transport chain of PS II. The inhibitors are linked to D_1 protein of RC of PS II and so blocking the electron transfer to plastoquinone. The photoinhibition primarily damage the PS II that leads to decreasing of electron transfer rate in photosynthetic electron transport chain and damage the structure of D_1 protein (Anderson & Aro, 2001; Vass & Aro, 2008; Tyystjärvi, 2008). It is known that MV weaken the activity of antioxidant enzymes superoxidedismutase and ascorbate peroxidase (Choi *et al.*, 2001). MV has a capacity to self-oxidation on light generating ROS. As it is seen on Fig. 1 the protective effect of Na-asc is expressed less active than under photoinhibition (Fig. 2). Under photoinhibition effect of Na-asc to become stronger during time exposition. It is known that under photoinhibition ROS and $^1O_2$, generated during excitation energy transfer in reaction centre of PS II are regarded as a trigger of D_1 protein degradation (Anderson & Aro, 2001). The heavy metals toxicity depends from ability to inactivate of enzymes and other macromolecules connected with SH-groups and to block the prosthetic groups by substitution of functionally important metal ions (Mohanty *et al.*, 1989; Ganiyeva *et al.*, 2018). The sites of toxic action of Zn^{2+}, Ni^{2+}, Cu^{2+} in electron transfer way of PS II are presented by contradictory data concerning of their toxic action place. The Zn ions can affect on oxidative side of PS II and inhibit Mn_4O_5Ca cluster. It is suggested second site of damage in electron transport chain on the PQ level. The Ni ions as it is demonstrated in a number of works reduced the Chl content caused by inhibition of its synthesis and disturb the transport of electrons from pheophytin through the quinine Q_A and Fe to quinone Q_B. It is a result of carries change or reaction centre proteins (Vass & Aro, 2008). At high concentrations of Cu^{2+}, denaturation of PS II leads to a significant disruption of the electron transfer and inhibition of O_2 release (Baron *et al.*, 1995; Boucher & Carpentier, 1999; Burkhead *et al.*, 2009). Heavy metals ions involving in the photosynthetic electron transport pathway, binding to several sites within the PSII electron transfer chain with subsequent

suppressing of its activity (Fig. 3*A, B*). Action of Cu^{2+} and Zn^{2+} caused inactivation both on donor and acceptor sides whereas the toxicity of Ni^{2+} is manifested on donor side of PS II. In the case of given stress Na-asc also manifest antioxidative properties as a key oxidant, reacting with $^{\bullet}OH$ radicals, O_2 and $^1O_2$, generating under toxic action of metals (Chen & Gallie, 2004).

It is known, that Na-asc neutralized formed under stress superoxide anion radical and detoxified H_2O_2 as an electron donor to ascorbate peroxidase or O_2^- and $^*OH$, preventing by its inactivation of enzymes and keep up the oxidation-restoration equilibrium between photosystems. Besides, Na-asc takes part in protection of enzymes activity, containing of prostatic metal ions, maintaining them to reduced forms (Wang *et al.*, 2012). The mechanism of Na-asc action under all stresses is known to be its capacity to suppress a reaction of one electron oxygen reduction, breaking off the chain of reacting-leading to plant cell death.

So, Na-asc being an universal antioxidant plays a decisive role in keeping of oxidation-restoration equilibrium between photosystems, that are evidenced by submitted investigations.

References

Ahmad, P., Sarwat, M., & Sharma, S. (2008). Reactive oxygen species, antioxidants and signaling in plants. *Journal of Plant Biology*, *51*(3), 167-173.

Alscher, R.G., Donahue, J.L., & Cramer, C.L. (1997). Reactive oxygen species and antioxidants: relationships in green cells. *Physiologia Plantarum*, *100*(2), 224-233.

Andersson, B., & Aro, E. M. (2001). Photodamage and D1 protein turnover in photosystem II. In *Regulation of photosynthesis* (pp. 377-393). Springer, Dordrecht.

Andersson, B., Barber, J. (2003). Mechanisms of Photodamage and Protein Degradation During Photoinhibition of Photosystem II. – N.R. Baker (ed): *Photosynthesis and the Environmen*t. (pp.101–121) Springer, Dordrecht.

Barber, J. (2003). Photosystem II: the engine of life. *Quarterly reviews of biophysics*, *36*(1), 71-89.

Barón, M., Arellano, J. B., & Gorgé, J. L. (1995). Copper and photosystem II: a controversial relationship. *Physiologia Plantarum*, *94*(1), 174-180.

Blokhina, O., Virolainen, E., & Fagerstedt, K. V. (2003). Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Annals of Botany*, *91*(2), 179-194.

Boucher, N., & Carpentier, R. (1999). Hg2+, Cu2+, and Pb2+-induced changes in photosystem II photochemical yield and energy storage in isolated thylakoid membranes: a study using simultaneous fluorescence and photoacoustic measurements. *Photosynthesis Research*, *59*(2-3), 167-174.

Burkhead, J. L., Gogolin Reynolds, K. A., Abdel-Ghany, S. E., Cohu, C. M., & Pilon, M. (2009). Copper homeostasis. *New Phytologist*, *182*(4), 799-816.

Chen, Z., & Gallie, D. R. (2004). The ascorbic acid redox state controls guard cell signaling and stomatal movement. *The Plant Cell*, *16*(5), 1143-1162.

Ganiyeva, R., Atakishiyeva, S., Zeynalova, N., Abdullayev, Kh., Bayramova, S. (2018). Na-ascorbate in protection of PS II activity, suppressed by copper ions. *Advances in Biology & Earth Sciences*, 3, 39-46.

Gasanov, R.A., Alieva, S., Arao, S., Ismailova, A., Katsuta, N., Kitade, H., Yamada, Sh., Kawamori, A., Mamedov, F. (2007). Comporative study of the water oxidizing reactions and the millisecond delayed chlorophyll fluorescence in photosystem II at different pH. *J. of Photochem.Photobiol*., 86, 160-164.

Gasanov, R.A., Aliyeva, S.A., Mamedov, F. (2012). Delayed Fluorescence in a Millisecond Range – a Probe for the Donor Side-Induced Photoinhibition of Photosystem II. − In:

Photosynthesis: Overview on Recent Progress and Future Perspectives, (Eds: Guruprasad K.N., Hot S., Mohanty P.) K. Publisher. Chapter 7, 101-107.

Goltsev, V., Chernev, P., Zaharieva, I., Lambrev, P., & Strasser, R. J. (2005). Kinetics of delayed chlorophyll a fluorescence registered in milliseconds time range. *Photosynthesis Research*, *84*(1-3), 209-215.

Mano, J. I., Hideg, É., & Asada, K. (2004). Ascorbate in thylakoid lumen functions as an alternative electron donor to photosystem II and photosystem I. *Archives of Biochemistry and Biophysics*, *429*(1), 71-80.

Meloni, D. A., Oliva, M. A., Martinez, C. A., & Cambraia, J. (2003). Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environmental and Experimental Botany*, *49*(1), 69-76.

Mohanty, N., Vass, I., & Demeter, S. (1989). Impairment of photosystem 2 activity at the level of secondary quinone electron acceptor in chloroplasts treated with cobalt, nickel and zinc ions. *Physiologia Plantarum*, *76*(3), 386-390.

Ort, D. R. (2001). When there is too much light. *Plant Physiology*, *125*(1), 29-32.

Park, S. M., Suh, K. H., Kim, J. S., & Park, Y. I. (2001). Inactivation of Photosystem I in Cucumber Leaves Exposed to Paraquat-Induced Oxidative Stress. *Journal of Photoscience*, *8*(1), 13-17.

Renger, G., & Renger, T. (2008). Photosystem II: the machinery of photosynthetic water splitting. *Photosynthesis Research*, *98*(1-3), 53-80.

Scandalios, J. G. (2002). The rise of ROS. *Trends in Biochemical Sciences*, *27*(9), 483-486.

Smirnoff, N. (2000). Ascorbic acid: metabolism and functions of a multi-facetted molecule. *Current Opinion in Plant Biology*, *3*(3), 229-235.

Smirnoff, N., & Wheeler, G. L. (2000). Ascorbic acid in plants: biosynthesis and function. *Critical Reviews in Plant Sciences*, *19*(4), 267-290.

Takizawa, M., Komori, K., Tampo, Y., & Yonaha, M. (2007). Paraquat-induced oxidative stress and dysfunction of cellular redox systems including antioxidative defense enzymes glutathione peroxidase and thioredoxin reductase. *Toxicology in Vitro*, *21*(3), 355-363.

Tyystjärvi, E. (2008). Photoinhibition of photosystem II and photodamage of the oxygen evolving manganese cluster. *Coordination Chemistry Reviews*, *252*(3-4), 361-376.

Tyystjärvi, E., Hakala, M., & Sarvikas, P. (2005). Mathematical modelling of the light response curve of photoinhibition of photosystem II. *Photosynthesis Research*, *84*(1-3), 21-27.

Vass, I., Aro, E.-M. (2008). Photoinhibition of photosynthetic electron transport. In: *Primary Processes of Photosynthesis: Basic Principles and Apparatus*. Ed. Renger G. Publ. Roy. Soc. Chem., Cambridge, UK. 393-411.

Wang, L. Y., Zhang, Q. Y., Wang, F., Meng, X., & Meng, Q. W. (2012). Ascorbate plays a key role in alleviating low temperature-induced oxidative stress in Arabidopsis. *Photosynthetica*, *50*(4), 602-612.

Zeng, L. D., Li, M., Chow, W. S., & Peng, C. L. (2018). Susceptibility of an ascorbate-deficient mutant of Arabidopsis to high-light stress. *Photosynthetica*, *56*(1), 427-432.