

THE NATURE OF PSII REACTIONS STABILITY UNDER OXIDATIVE STRESS

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Abstract

Comparative investigations of millisecond delayed fluorescence of chlorophyll *a* (msec-DF Chl *a*) characteristics under oxidative stress induced separately by high intensity light and methyl viologen (MV) were investigated in pumpkin leaves (*Cucurbita pepo* L.). The inactivation of electron transport chain of PSII was observed in both experiments. The restoration of given characteristics observed at normal illumination with photoinhibited seedlings was found to enhance under action Na-ascorbate (Na-asc). The restoration of fluorescence characteristics on treated MV seedlings was recorded only at presence of Na-asc. Effect of Na-asc to restore inactivated processes is considered to be due to its ability to actively neutralize reactive forms of oxygen generated under oxidative stress. It is suggested that mechanism leading to changes of induction pattern of msec-DF Chl *a* of PSII has common nature in both cases.

Introduction

The abiotic stresses of environment have generated oxidative stress in plant cells. As a result of excitation of light reaction of photosynthesis the intensive production of reactive oxygen species (ROS) ($^{\bullet}\text{O}_2$, $\text{O}_2^{-\bullet}$, OH^{\bullet} , H_2O_2) occur. Accumulation of ROS are unavoidable as a result of photosynthesis even under favourable conditions because the chloroplasts are potential source of oxygen toxic derivatives (Asada 1996). The damage of chloroplast components and decrease of photosynthesis activity start with inactivation of PSII, the most vulnerable link of photosynthetic apparatus (Bukhov 2004, Tyystjärvi *et al.* 2005, Anderson *et al.* 1996). Damage of oxygen evolving complex leads to denaturation and proteolysis of proteins, peroxidation of reaction centre lipids of PSII and suppression of electron transport in PSII (Biswal 2005, Biswal *et al.* 2011). Action of excess light on photosynthetic apparatus has been researched in detail, but it is not common opinion about photoinhibition mechanism (Tyystjärvi 2008, Vass 2012). Photoinhibition lead to inactivation of PSII due to damage of D₁ protein involved in processes of formation of ROS (Andersson *et al.* 2001, Anja *et al.* 2011, Jafarova *et al.* 2012). Increasing of light intensity induce enhance formation oxidative components on donor side and reduce component on acceptor side of reaction transport chain of PSII that results in disruption of oxidation-reduction balance. Inactivation of PSII induced by donor side under photoinhibition took place because of non-coordinated transport of electrons from P680 and donation of electron from Mn₄O₅Ca-cluster on RC chlorophyll. The accumulation of long lived oxidizing radical P680⁺⁺ and Tyrz⁺⁺ on donor side of PSII leads to degradation of D₁ protein (Gasnov *et al.* 2007, Antal *et al.* 2009).

Inactivation of PSII by acceptor mechanism takes place as a result of stabilization of Q_A⁻ by protonation (Tyystjärvi 2008, Hakala *et al.* 2006). At investigation oxidative stress as a chemical model is used MV as an oxidation mediator on acceptor side of PSI. Primary target of oxidative stress induced by MV are antioxidative enzymes sensitive to action of ROS (Sun *et al.* 2001).

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Also, superoxide is formed on acceptor side PSI, PSII particularly of OEC is more receptive to treatment by MV (Song *et al.* 2006). MV inhibited PSII by cyclization of electron flow through PSI, suppressing electron transport (Pavel 2009). Plant cells are protected against oxidative damage by broad spectrum of radical scavenging systems including antioxidant enzymes as well as non-enzymatic antioxidants, like glutathione and ascorbate. Ascorbic acid is considered as the most popular and powerful ROS-detoxifying compound because of its ability to donate electrons in a number of reactions (Nijs *et al.* 1991).

Ascorbate detoxifies H_2O_2 as electron donor to ascorbate reductase or O_2^- and $\cdot OH$ by non-enzymatic way and thus preventing enzymes inactivation.

At present most attention is devoted to kinetic investigations of photosynthetic activity. The interaction of light and dark processes of photosynthesis is outstanding in induction processes when the transition of adapted to dark plants to stationary photosynthesis under action of excited light. For investigation of induction processes the delayed fluorescence of Chl *a* related to PSII is used on photosynthetic objects (Gasarov *et al.* 2012). The delayed fluorescence method makes it possible to rapid test of plant stability to stress conditions and gives possibility to reveal most vulnerable part of electron transport chain. The method of msec-DF of Chl *a* permits to track of restoration effect of antioxidants at stress situation that was aimed at the present work.

Materials and Methods

The study was conducted in Biophysics laboratory of the Botany Institute of Azerbaijan National Sciences Academy in 2016.

The leaves of 10-day seedlings of pumpkin (*Cucurbita pepo* L.) were grown as water culture in factorostate conditions at 22°C, 80% humidity and illumination 250 $\mu V/cm^2$.

For creation of oxidative stress due accumulation of ROS in chloroplasts the leaves were sprinkled by MV solution (1,1'-dimethyl-4,4'-bipyridinium dichloride) at concentration 100 μM ; by means of high intensity light (4000 μM photon/ m^2/s) the photodamage of PSII was caused that characterised the stability of photosynthetic apparatus to oxidative stress, induced by photoinhibition. To protect from oxidative stress the low molecular compound Na-asc at concentration of $4 \cdot 10^{-4} M$ was used.

The functional state of photosynthetic apparatus was evaluated by means of delayed fluorescence of Chl *a* at millisecond time rang *in vivo* to be conditional on recombination processes in reaction centre of PSII (Gasarov *et al.* 2007, Goltsev *et al.* 2012). The kinetics of msec-DF of Chl *a* was measured by phosphoroscope with time interval between excitation and luminescence equal 1,25 ms.

Results and Discussion

In leaves incubated in MV solution the drop of intensity of fast (f.ph) and slow phase (sl.ph) of msec-DF of Chl *a* was observed (Fig. 1). After 1 hr treatment pumpkin leaves with MV solution the intensity of f.ph decreased by 3.6 times, sl.ph by 1.8 times relatively to control (Fig. 1A), after 2 hrs the intensity of f.ph were decreased by 4.4 times, sl.ph by 2.2 times (Fig. 1B). The rise of induction curves stationary phase (s.ph) by 1.6 times in relation to control in both variants were observed that gives reason to suppose violation in oxidation-reduction reactions at PSII chain (Fig. 1A, B).

To elucidate suppression mechanism of photosynthetic reaction in PSII the low molecular antioxidant Na-asc was used, its protected mechanism as it is known to be implemented by suppression of ROS formation (Sankhalkar *et al.* 2002). Action of Na-asc after 1 hr leads to rise f.ph intensity by 2.5 times and sl.ph by 4.6 times relatively to MV action (Fig.1C). The intensity of both phases was decreased after 2 hrs (Fig.1D).

The formation of highly oxidize radical pair $P680^+$ and $TyrZ^+$ under stress is known to induce inactivation of donor side of PSII chain. The inactivation of acceptor side is known to take place due to accumulation of reduce first acceptor Q_A . Probably, action of MV inducing oxidative stress and accumulation of ROS have led to inactivation of electron transport chain of PSII. It is known that MV depressed the activity of antioxidant enzymes of superoxide dismutase and ascorbate peroxidase which are sensitive to action of ROS (Daisuke *et al.* 2016). The absence of phase reduction intensity during time exposition after action of MV are likely to be the result of ability of MV to self-oxidation on light, continuing to generate ROS. That is why protective effect of Na-asc appears after 1 hr MV action was weakened to 2 hrs.

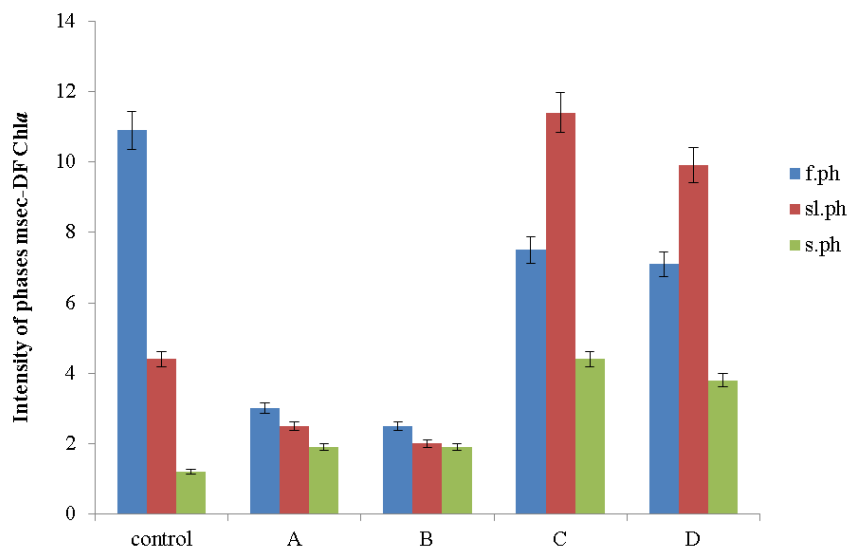


Fig.1. Results of induction of msec-DF Chl *a* changes of pumpkin leaves seedlings treated by MV solution at concentration $100 \mu\text{M}$. Intensity of phase of msec-DF Chl *a*: fast (f.ph), slow (sl.ph), stationary phase (s.ph) were conducted 30 min after incubation of leaves in MV after 1 (A), 2 hrs (B) and after action of Na-asc solution in concentration $4 \cdot 10^4 \text{ M}$ on treated by MV leaves also after 1 (C) and 2 hrs (D).

By means of objective analysis of equilibrium between acceptor and donor side of electron transfer chain of PS II the changes of relation value f.ph. and sl.ph to s.ph (f.ph/s.ph: sl.ph/s.ph) were observed (Table 1). After 1 and 2 hrs treatment of leaves by MV the drop of these values relative to control was observed. The value of f.ph decreased by 5.8 times after 1 hr and the value of sl.ph by 3 times, after 2 hrs by 7 and 3 times. Action of Na-asc was shown to restored given values and f.ph/s.ph increased by 1.5 times and value sl.ph/s.ph by 2.5 times relatively to action of MV.

The observed rise of stationary level on induction curve is considered to be associated in condition with MV Na-asc did not can fully support oxidation-reduction balance between photosystems and fully neutralized superoxide radical (life time $\sim 2\text{ms}$) which has time to migrate to surrounding of PSII. Displacement of redox state enhanced of electron flow PSI resulting in increase of s.ph level and according decreasing value of ratio to stationary phases.

After treatment of seedlings of pumpkin during 2 hrs by light of high intensity the changes of phases msec-DF Chl *a* was measured (Fig. 2). The drop of f.ph intensity relatively to control up to 4.2 times was registered. The slow phase almost did not register and was by 10 times lower

relatively to control. Photoinhibited seedlings were transferred on definite time to normal illumination and restoration phase of msec-DF Chl *a* were observed (Fig. 2). After 1.5 of light exposition the partial restoration of f.ph intensity it was observed that it increased by 1.6 times, intensity of sl.ph increased by 1.5 times higher relatively photoinhibition (Fig. 2). After exposition of seedlings on normal illumination during 5 hrs more greater restoration of intensities as f.ph (by 3.4 times), so sl.ph (by 9 times) that becomes nearly equal to control (Fig. 2). The stationary phase (s.ph) level on induction curve in both variants was equal to control and only in variant of seedling exposition during 5 hrs its insignificant rise (by 1.4 times) was observed (Fig. 2).

Table 1. Changes of value of relation f.ph and sl.ph to s.ph (f.ph/s.ph and sl.ph/s.ph) of msec-DF Chl *a* after incubation of pumpkin seedlings leaves in MV solution for 1 and 2 hrs without and with treatment by Na-asc after 1 and 2 hrs of restoration.

Msec-DF Chl <i>a</i>	Control	MV (100 μ M)		Na-asc ($4 \cdot 10^{-4}$ M)	
		1h	2h	1h	2h
f.ph/s.ph	9.2	1.6	1.3	1.7	1.9
sl.ph/s.ph	3.6	1.3	1.05	2.6	2.6

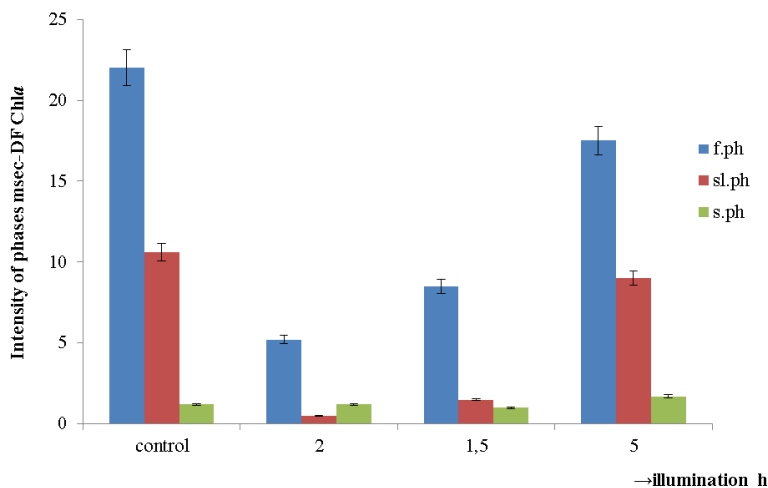


Fig. 2. The results of changes of induction character of msec-DF Chl *a* of pumpkin seedlings leaves subjected to action of high intensity light ($4000 \mu\text{M photons/m}^2/\text{s}$) during 2 hrs (photoinhibition of PSII) and transferred to normal illumination (250 mkV/sm^2). The changes of phase intensity were determined: fast (f.ph), slow (sl.ph), stationary (s.ph) after 1.5 and 5 hrs of restoration.

The uncoordinated transport of electron transport chain of PSII observed during photoinhibition partly was restored dependently from exposition time under transfer of plants to normal illumination. Under treatment of seedlings by Na-asc the restoration of millisecond intensity of phases took place significantly (Fig.3). The intensity f.ph and sl.ph were restored after 1.5 hr and was equal to 2.3 and 9 times, after 5 hrs by 2.4 and 14 higher relatively to photoinhibition. s.ph remained on control level in all variants (Fig. 3).

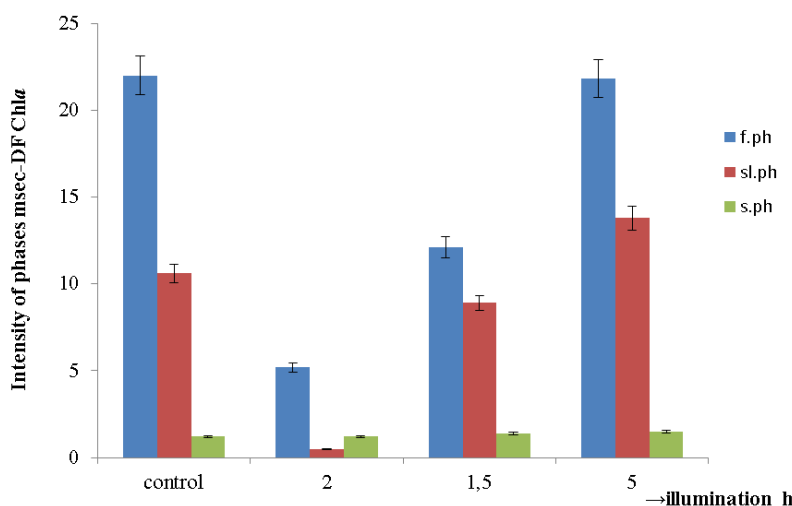


Fig. 3. The results of induction character changes of msec-DF Chl *a* of pumpkin seedlings leaves subjected to action of high intensity light ($4000 \mu\text{M photons/m}^2/\text{s}$) during 2 hrs (photoinhibition of PSII) and transferred after a treatment by Na-asc photoinhibited seedlings to normal illumination (250 mkV/sm^2). The changes of phase intensity were determined: fast (f.ph), slow (sl.ph), stationary (s.ph) after 1.5 and 5 hrs of restoration.

Action of Na-asc under photoinhibition enhanced reduction of fluorescence activity characteristic during time exposition. ROS, involved to photoinhibition drive to D_1 protein degradation slowly than the PSII inactivation. ROS has inhibited synthesis the protein D_1 *de novo* (Nishiyama *et al.* 2015).

The restoration of values f.ph/sl.ph and s.ph/sl.ph observed after photoinhibition (after 1.5 hr by 2 and 7.5 times; after 5 hrs by 2.4 and 2.6 times, correspondingly) was found to increase in the presence of Na-asc (Table 2). After 1.5 hrs action of Na-asc values f.ph/s.ph restored by 2 times, after 5 hrs by 3.4 times. Restoration of sl.ph/s.ph value was more intensive and to 5 hrs exceed of control variant.

Table 2. The changes of relation values of f.ph. and sl.ph to s.ph of msec-DF Chl *a* in leaves seedling of pumpkin photoinhibited during 2 hrs ($4000 \mu\text{M photons/m}^2/\text{s}$) and then transferred to normal illumination (250 mkV/sm^2) on 1.5 and 5 hrs after treatment by Na-asc.

Msec-DF Chl <i>a</i>	Control	2 hrs photoinhibition ($4000 \mu\text{M photons/m}^2/\text{s}$)	Normal illumination (250 mkV/sm^2)		Normal illumination (250 mkV/sm^2) Na-asc	
			1.5 h	5 h	1.5 h	5 h
f.ph/s.ph	18,3	4,3	8.5	10.3	8.6	14.5
sl.ph/s.ph	8,8	0,2	1.5	5,3	6.4	9.2

Restoration by Na-asc processes suppressed by photoinhibition and resulted by action of MV took place in induction period of msec-DF Chl *a* and appeared to be associated with result effective neutralization formed ROS.

So, it may be suggested that mechanism leading to change of induction picture of DF Chl *a* as a results of action of both factors has common nature. One of the key ways to enhance resistance

of photosynthetic apparatus to stress under action of protective system of plant is generally accepted to increase antioxidant enzymes activity and/or the low molecular antioxidant action effectivity. The photosynthetic apparatus switched over to adaptive program and thus is providing its resistance to stress.

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