EFFECTS OF RESPIRATION AND PHOTOSYNTHESIS INHIBITORS ON THE STRUCTURE AND FUNCTION OF BARLEY CHLOROPLAST AND MITOCHONDRIA UNDER HEAT SHOCK

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Abstract. *The structural and functional characteristics of thylakoid and mitochondria membranes under impact of respiration (NaF, NaN_3) and photosynthesis (DCMU) inhibitors and heating (40°C, 3 h) were studied. Suppression of respiration on 90% by NaF induced 40-50 % inhibition of photosynthetic activity, rise of low-temperature chlorophyll fluorescence maximums ratio (I_{740}/I_{685}), and partial suppression of PS II activity in barley seedlings. The effects of NaN_3 on the photosynthetic membrane structure and PS II activity were similar. DCMU inhibited of O_2 evolving activity of chloroplasts more than 80%, and reduced insignificantly respiration. In these conditions the change in fluorescence of rhodamine 123 incorporated in mitochondrial membranes was detected. Heat shock (HS) reduced slightly the photosynthesis and respiration activity and changed structure of photosynthetic membranes. At the same time, HS improved a state of electron-transport chain both mitochondria and chloroplast after inhibition of respiration. The received data give the methodological tool for further investigation of mitochondria and chloroplast interaction under heat stress.*

MATERIAL AND METHOD

Barley (*Hordeum vulgare* L.) plants were grown on tap water at 100 μmol photons $m^{-2} s^{-1}$, 16-h light/ 8-h dark period, 70 % humidity, and a thermal regime of 24/ 22°C day/ night. Primary leaves of 7-day-old seedlings were cut and were put in glass with water or solution with inhibitors during 2 h.. Heat stress was obtained by the heating of leaves during 3 h under 40 ℃ and continuous illumination at 100 μmol $m^{-2} s^{-1}$.

Glycolysis was suppressed by sodium fluoride (NaF, $5 \cdot 10^{-2}$M). Sodium azide (NaN_3, $5 \cdot 10^{-3}$M) was used for inactivation of final stages of respiration in mitochondria [1, 2]. DCMU (10^{-5} - 10^{-6} M) suppressed electron transport in chloroplast between PSII and PSI.

Protoplasts were isolated from mesophyll of 7-days-old barley leaves according [3] with some modification. Inhibitors were infiltrated in leaves and protoplasts were isolated after 2 h incubation. The protoplasts were heated during 15 min at 37°C with rhodamine 123 (R 123, $2{,}6 \times 10^{-6}$M). Then chloroplast was separated by centrifugation at 3000 g (15 min), and mitochondria were sedimented from supernatant at 18000 g (20 min). The degree of mitochondria pollution by chloroplast fragments was controlled by chlorophyll fluorescence and did not exceed 5 %. Fluorescence spectra of R 123 were registered in various intracellular fractions with fluorometer “Solar LSF 222” (Belarus) at 20°C.

Oxygen evolution activity of leaves homogenate was measured in a closed thermostatic cell using a Clark-type electrode (Hansatech, UK) under continuous illumination with white light (600 μmol photons m^{-2} s^{-1}) at 20°C and in presence of 1 mM 1-4-benzoquinone and 0.5 mM $K_3Fe(CN)_6$. Respiration activity was determined in presence of 20 μM $NADH_2$. The low-temperature (-196°C) fluorescence spectra of intact barley leaves were registered according to [4]. Chlorophyll fluorescence of intact barley leaves was measured at room temperature with a PAM 201 chlorophyll fluorometer (Walz, Germany). The basic chlorophyll fluorescence terms were calculated according to [5, 6]. The kinetics of photochemical (qP) and non-photochemical (qN) quenching of chlorophyll fluorescence were measured with actinic light PFD of 120 μmol m^{-2} s^{-1}.

RESULTS AND DISCUSSIONS

The inhibition of glycolysis by sodium fluoride resulted in essential suppression of photosynthesis (figure 1, a). At the same time suppression of photosynthetic activity by DCMU was poorly reflected on intensity of respiration (figure 1, b). It is possible to propose, that inhibition of respiration affected photosynthetic activity by means of reduction common pool of ATP in cell. The photosynthesis suppression was not influenced on respiration activity probably due to short time of DCMU incubation. In these conditions presumable decrease in assimilate content was so much small, so that was not affect respiration.

Heat treatment of barley leaves at constant illumination caused suppression both photosynthetic evolving of oxygen and respiration oxygen uptake (figure 1). It is necessary to note, that respiration in green leaves appeared more thermostability than photosynthesis. Heating increased a degree of suppression of photosynthetic function in leaves with inhibited respiration. High temperature inhibited respiration in untreated and in DCMU-treated leaves in equal degree. Received data shown, the intensity of a respiratory metabolism supervises activity of photosynthetic reactions. At the same time inhibition of photosynthesis influences poorly on intensity of leaves respiration.

Structural state of thylakoid membranes under respiration inhibitors impact was estimated by means of low temperature chlorophyll fluorescence. High temperature did not affected the relative ratio of chlorophyll fluorescence maximum I_{740}/I_{685} (figure 2). Sodium azide and sodium fluoride treatment increased ratio I_{740}/I_{685}. Heating of leaves treated by NaF and NaN_3 caused the additional rise of ratio I_{740}/I_{685}. Changes in ratio I_{740}/I_{685} occurred due to increase in fluorescence maximum at 740 nm. These data indicate the rise of PSI emission. It is possible in case of phosphorylation of light harvesting complex (LHC) and migration of phospho-LHC to PSI. This process is considered as protective mechanism preventing overreduction of electron transport rate in chloroplasts and PSII photoinhibition. In case of inhibited respiration the flow of assimilates from chloroplast is decreased and photosynthesis is suppressed feedback.

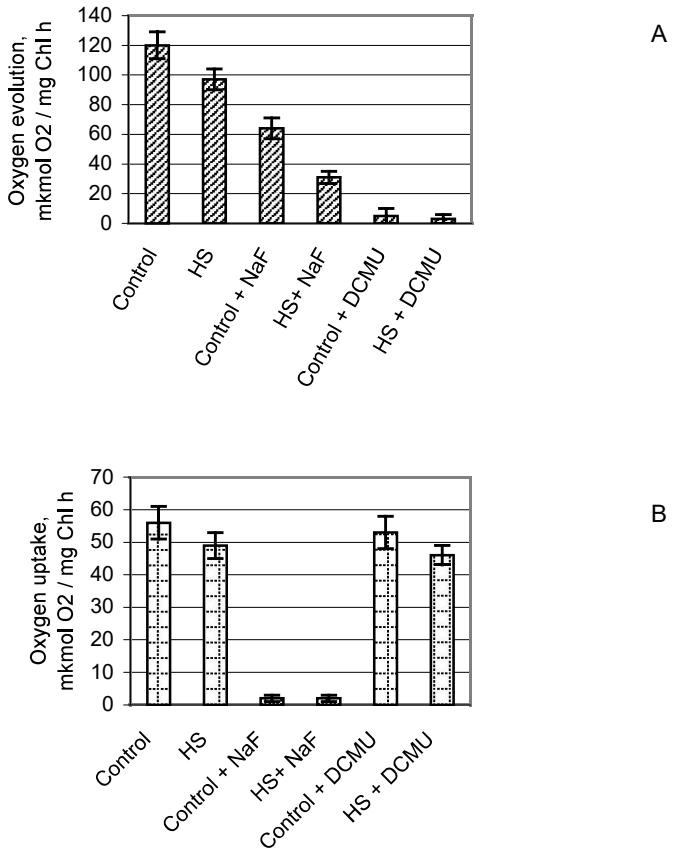

Figure 1 - Photosynthetic activity (A) and respiration intensity (B) in homogenates of 7-day-old barley leaves treated by photosynthesis (10^{-5} - 10^{-6} M DCMU) and respiration ($5 \cdot 10^{-2}$M NaF, $5 \cdot 10^{-3}$M NaN_3) inhibitors and after heating (3 h, 40˚C).

The changes of PS II activity in green barley leaves measured by PAM fluorometry is shown in table 1. The inhibition of glycolysis by NaF resulted in diminution of the effective quantum yield of PSII photochemistry and essential rise of nonphotochemical quenching of chlorophyll fluorescence (qN). These data indicate limited effectiveness of as PS II as electron transport chain. The increasing of phosphoglycerine acids concentration as a result of pyruvate deficit (end-product of glycolysis) due to sodium fluoride effect, probably, is a signal for decrease in photochemical activity of PS II. It is also known, that sodium fluoride is effective inhibitor of phosphoserine- and phosphothreonine phosphotases in chloroplasts and mitochondria in plants [7]. Therefore this inhibitor could influence directly on redox dependent phosphorylation of D1 and D2 proteins and cause the release Q_A from pigment-protein complex. HS weakened the inhibition

effect of NaF on PS II activity in green barley leaves. Effect of NaN_3 treatment was analogical but feebly marked (table 1).

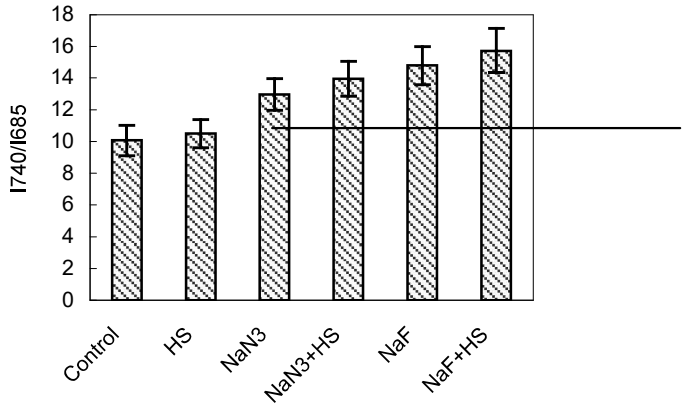

Figure 2 - Effect of HS and inhibitors of respiration on I_{740}/I_{685} ratio in low-temperature fluorescence spectra of barley leaves.

Table 1

Effect of respiration inhibitors on parameters of chlorophyll a fluorescence in barley leaves

	F_v/F_m	Φ_{PSII}	qP	qN
Control	0,799	0,663	0,913	0,333
HS	0,752	0,680	0,941	0,267
NaF	0,799	0,462	0,917	0,745
NaF+HS	0,745	0,633	0,897	0,279
NaN_3	0,789	0,584	0,887	0,519
NaN_3+HS	0,751	0,711	0,927	0,312

Interdependence between mitochondria and chloroplasts was studied in protoplasts with lipophylic fluorescent probe R 123. It is known R 123 is accumulated specifically by mitochondria in proportion to $\Delta\psi$ [8]. In our experiments the incorporation of R 123 in chloroplast was shown too. Heat shock was not affected essentially fluorescence of R 123 accumulated both in chloroplasts and mitochondria (table 2).

Table 2

Effect of inhibitors and HS on the rhodamine 123 fluorescence intensity incorporated in isolated mitochondria and chloroplasts

	Mitochondria	Chloroplasts
Control	2,70	3,02
NaN_3, 10^{-2}M	3,10	3,48
DCMU, 10^{-6}M	3,72	3,41
HS	2,68	2,82
NaN_3 +HS	2,94	4,71
DCMU + HS	3,75	4,15

Note: The data was given as ratio of rhodamine 123 fluorescence intensity (λ_{ex}=495 nm, λ_{em}=530-532nm) normalized on the intensity of total protein fluorescence (λ_{ex} =280nm, λ_{em}=330nm) in sample.

DCMU increased linkage parameters of R 123 with mitochondria in more degree than with chloroplasts. NaN_3 treatment changed fluorescence intensity of R 123 incorporated in chloroplasts and mitochondria in same degree. Heating of inhibitor treated protoplasts was not affect on R 123 incorporation in mitochondria and increased linkage of this probe in chloroplast. Obtained data indicate that inhibitors impact changed the structural state of both chloroplast and mitochondria membranes.

CONCLUSIONS

1. It is established, the glycolysis intensity influenced on photosynthetic activity. The inhibition of the glycolysis by NaF caused the reduction of oxygen uptake (on 90 %), 40 – 50 % decrease in photosynthetic oxygen evolution, increase of the chlorophyll fluorescence ratio I_{740}/I_{685} and some suppression of the PS II photochemical activity.

2. The inhibition of mitochondria electron transport chain by NaN_3 acted on chlorophyll fluorescence ratio I_{740}/I_{685} and activity of PS II to a lesser extent than NaF.

3. The inhibition of photosynthesis by DCMU resulted in strong reduction of photosynthetic oxygen evolution (more than 80 %) was poorly reflected on intensity of respiration (5 %). At the same time parameters of linkage of fluorescent probe R 123 with mitochondria membranes varied.

4.The received data show new proofs of interdependence between photosynthesis and respiration on cell level. This investigation has great importance for development of the strategy of crop plants tolerance increasing by methods of gene engineering and selection.

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