Abscisic acid induced protection against photoinhibition of PSII correlates with enhanced activity of the xanthophyll cycle

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Abstract The exogenous application of abscisic acid (ABA) to barley seedlings resulted in partial protection of the PSII photochemistry against photoinhibition at low temperature, the effect being most pronounced at 10^{-5} M ABA. This was accompanied by higher photochemical quenching (qP) in ABA-treated leaves. A considerable increase (122%) in the amount of total carotenoids and xanthophylls (antheraxanthin, violaxanthin and zeaxanthin) was also found in the seedlings subjected to ABA. The activity of the xanthophyll cycle measured by the epoxidation state of xanthophylls under high-light treatment was higher in ABA-treated plants compared with the control. This corresponds to a higher value (0.411) of non-photochemical quenching (qNP) observed in ABA-treated than in control (0.306) leaves.

Key words: Abscisic acid; Carotenoid; Chlorophyll fluorescence; Low temperature; Photoinhibition; Xanthophyll cycle

1. Introduction

It is generally believed that plant growth substances, especially abscisic acid (ABA) and related compounds, play a key role in plant responses to low and high temperatures, freezing, water deficit, etc., and in the process of acclimation of the photosynthetic apparatus to various environmental stresses [1,2]. Acclimation of a number of plant species to low temperatures was found to be associated with elevation in the endogenous ABA levels [3–6], and exogenous application of ABA-induced increased freezing and chilling tolerance [7–10].

The phenomenon of photoinhibition of photosynthesis has been reported in many plant species when exposed to light conditions that exceed the photon requirements for photosynthesis [11]. It is well established that low temperatures in combination with even moderate or low photon fluence rates increase the susceptibility of photosynthesis to photoinhibition [12–14].

In the recent years, the role of the xanthophyll cycle (light-inducible, dark reversible de-epoxidation of violoxanthin to zeaxanthin) in the protection of photosynthetic apparatus from photoinhibition has been also well documented [15,16]. Zeaxanthin-dependent non-photochemical chlorophyll fluorescence quenching (qN) has been suggested as a major protective mechanism against photoinhibition [16–18].

Since the strong evidence for the indirect pathway of ABA biosynthesis from epoxy-carotenoids has been reported [19–22], it was of interest to investigate the influence of exogenously applied ABA on the light-dependent zeaxanthin formation, the related capacity for non-photochemical chlorophyll fluorescence quenching and the possible involvement of ABA in the protection of PSII photochemistry from excessive radiation at low temperatures.

2. Materials and methods

Seeds of barley (*Hordeum vulgare* L. var. *cadette*) were germinated for 3 days and grown in aqueous solutions of ABA (10^{-5} M and 10^{-6} M) as in [23]. ABA treatments lasted 7 days. The solutions were changed daily. During the experimental period, seedlings were grown in a growth chamber under white fluorescent light (250 μmol m^{-2} s^{-1}) with 16-h light/dark period. Day/night temperatures were 20/26°C. RH was 50%.

Susceptibility of control (non-treated) and ABA-treated barley seedlings to photoinhibition at low temperature was monitored as follows: leaves were placed on a moist filter-paper in an ice-cooled aluminum tray and were exposed to an irradiance of 1800 μmol m^{-2} s^{-1} using high-pressure sodium lamps (Lucalox L4-400, CGE, Toronto, Ont., Canada) in a cold room set for on air temperature of 5°C. A heat filter of circulating cold water was placed between the light source and the samples and two circulating fans provided continuous air circulation over the samples. Under these conditions, the temperature of the leaves was maintained at 5–7°C during exposure to the photoinhibitory treatment.

Pigments were extracted from leaf samples by homogenization with 4×2.5 ml 100% acetone and 25 mg $CaCO_3$ at 4°C and dim light [24]. The supernatant was filtered through a 0.22-μm syringe filter and samples were stored on ice in the dark until analysed. Carotenoids were analysed by high-performance liquid chromatography (HPLC). The system contained of a Beckman System gold programmable solvent module 126, diode array detector module 168 (Beckman Instruments, San Ramon, CA), CSC-Spherisorb ODS-1 reverse-phase column (5 μm particle size, 25×0.46 cm i.d.) with an Upchurch Perisorb A guard column (both columns from Chromatographic Specialties, Concord, Ont., Canada). Samples were injected using a Beckman 210A sample injection valve with a 20-μl sample loop. Separation was performed following the procedure in [24] with some modifications. Pigments were eluted isocraticly for 6 min with a solvent system acetonitrile/methanol/0.1 M Tris-HCl (pH 8.0) (74:11:3.5 v/v/v), followed by a 2-min linear gradient to 100% methanol/ethylacetate (68:32 v/v) which continued isocraticly for 4 min. Total run time was 12 min. Flow rate was 2 cm^3 min^{-1}. Absorbance was detected at 440 nm and peak areas were integrated by Beckman System Gold software. Retention times and response factors of Chl *a*, Chl *b*, lutein and β-carotene were determined by injection of known amounts of pure standards purchased from Sigma (St. Louis, MO). The retention times of zeaxanthin, antheraxanthin, violoxanthin and neoxanthin were determined by using pigments purified by thin-layer chromatography as described in [25]. The epoxidation state (EPS) of the leaf samples was estimated as in [26] using the equation: $EPS = (V + 0.5A)/(V + A + Z)$, where V, A and Z correspond to the concentration of violaxanthin, antheraxanthin and zaexanthin, respectively.

Chl *a* fluorescence of dark-adapted (30 min) barley leaves was measured using a PAM 101 chlorophyll fluorescence measuring system (Walz, Effeltrich, Germany) as described in [27]. Instantaneous (dark) chlorophyll fluorescence at open PSII centres (F_o) was excited by non-actinic modulated measuring beam (650 nm, 0.12 μmol m^{-2} s^{-1}) at

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1.6 kHz in the dark and 100 kHz in the light. Maximum fluorescence at closed PSII centres (F_m) was induced by saturating white light pulses (800 ms, 2800 μmol m^{-2} s^{-1}) provided by a Schott lamp (KL 1500, Schott Glaswerke, Mainz, Germany) and controlled from a Walz PAM 103 Trigger Control Unit. The actinic light beam had a PPFD of 230 μmol m^{-2} s^{-1}. The photochemical (qP) and non-photochemical (qNP) fluorescence quenching parameters were calculated using the procedure described in [28], taking into account the quenched F_o during the dark relaxation. The fluorescence characteristics were evaluated when the steady-state F_s level was reached.

3. Results and discussion

The effects of high-light (1800 μmol m^{-2} s^{-1} PFD), low-temperature (5°C) treatments on the photochemical efficiency of PSII (F_v/F_m) in control and ABA-grown barley leaves are presented in Fig. 1. The mean F_v/F_m values of dark-adapted leaves of control and 10^{-6} M and 10^{-5} M ABA-treated leaves do not differ significantly and were in the range of 0.78–0.81 depending on the experiment. Moreover, ABA treatment alone did not exhibit effects on the yield of PSII electron transport ($qP \times F_v'/F_m'$) as calculated from modulated fluorescence measurements (line 3 in Table 2). In line with a number of previous studies, the F_v/F_m gradually decreased to 58.1% of the initial value in control plants over the 4-h exposure to high light, the F_v/F_m being declined significantly even over the first hour (73.5%). Treatment with ABA markedly affected the high light-induced F_v/F_m decline, showing a concentration-dependent decreased susceptibility to photoinhibition of photosynthesis. Although F_v/F_m changed in a similar fashion, ABA-treated leaves exhibited significantly lower decreases in F_v/F_m than in control plants over the same photoinhibition period. The most pronounced effect was observed in 10^{-5} M ABA-treated plants (71.3%). Furthermore, exposure of ABA-treated leaves to high-light irradiance had only minor effect on the yield of PSII electron transport compared with the significantly decreased level of $qP \times F_v'/F_m'$ in control plants under the same conditions. It has been suggested that the sustained increase in F_o observed as a result of high-light treatment could be associated with 'photoinhibitory damage' [16,29], resulting from a decrease in the rate constant for photochemistry or a decrease in the rate constant for energy transfer to the PSII reaction center [30]. Actually, a considerable increase of F_o was found in the control leaves exposed to high-light, whereas 10^{-5} M ABA treatment almost completely prevented the 'photoinhibitory damage' (line 4 in Table 2).

The data presented above clearly indicates certain protective role of ABA on the photochemical apparatus against low temperature photoinhibition. As proposed earlier, the increased resistance to photoinhibition could be ascribed to an increased capacity of the photosynthetic machinery to maintain an increased portion of the PSII reaction centers in an open state (i.e. the ratio of oxidized to reduced Q_A, determined as qP from the modulated fluorescence measurements) under given light and temperature conditions [30,31]. In fact, ABA-treated plants exhibited higher values of qP upon both non-photoinhibitory and photoinhibitory conditions (line 1 in Table 2).

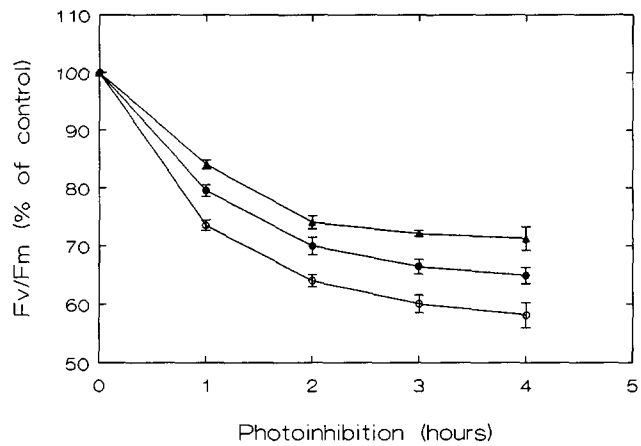

Fig. 1. The effects of high-light (1800 μmol m^{-2} s^{-1}) treatments at 5°C on the F_v/F_m ratios in control (○), 10^{-6} M ABA (●) and 10^{-5} M ABA (▲)-treated barley leaves. The data are presented as percentage of F_v/F_m values in dark-adapted (30 min) samples of non-treated and ABA-treated leaves. Mean ± S.E. values were calculated from 10–14 measurements in 5–7 independent experiments.

On the other hand, the radiationless dissipation of excess excitation energy in the chlorophyll pigment bed associated with the formation of the xanthophyll pigment zeaxanthin (Z) has been proposed as one of the major protective mechanism against photoinhibitory damage of PSII [16,18]. As can be seen from the data representing a comparative HPLC analysis (Table 1) of the quantitative changes in the carotenoid composition and content of control and ABA-treated barley seedlings, the amounts of neoxanthin (N), violaxanthin (V), lutein and β-carotene were significantly higher in 10^{-5} M ABA-treated leaves. The sum of total carotenoids and especially the total amount of the three components of the xanthophyll cycle (V + A + Z) were also increased to 122.1 and 121.9%, respectively. Assuming that oxygenated carotenoids have been suggested as precursors in the ABA biosynthetic pathway [19–22], it could be speculated that exogenously applied ABA would

Table 1
Carotenoid composition of dark-adapted (30 min) control and ABA (10^{-6} M and 10^{-5} M)-treated barley leaves

Pigments	Control (μg·g FW^{-1})	10^{-6} M ABA (μg·g FW^{-1} %)		10^{-5} M ABA (μg·g FW^{-1} %)	
Neoxanthin	10.6 ± 0.4	11.5 ± 0.6	108.6	14.8 ± 0.7*	139.6
Violaxanthin	30.5 ± 0.8	31.2 ± 0.7	102.1	36.3 ± 1.6*	119.0
Antheraxanthin	0.3 ± 0.2	0.4 ± 0.2	116.6	T.A.	
Leutein	65.6 ± 1.8	67.1 ± 2.1	102.2	77.4 ± 2.5*	118.0
Zeaxanthin	2.1 ± 0.7	1.8 ± 0.6	86.0	3.7 ± 0.9	176.0
β-Carotene	46.1 ± 0.9	44.9 ± 2.3	97.3	57.3 ± 1.9*	124.3
Carotenoids	155.2 ± 0.7	156.9 ± 0.9	101.0	189.5 ± 1.3*	122.1
V+A+Z	32.9 ± 1.2	33.4 ± 1.1	101.5	40.1 ± 1.3*	121.9

The results are presented as μg pigment·g fresh weight $(FW)^{-1}$. Mean ± S.E. values were calculated from 7–13 measurements in 5 independent experiments. Significance levels were calculated from t test on the differences between control and ABA-treated mean values (*$P < 0.001$).

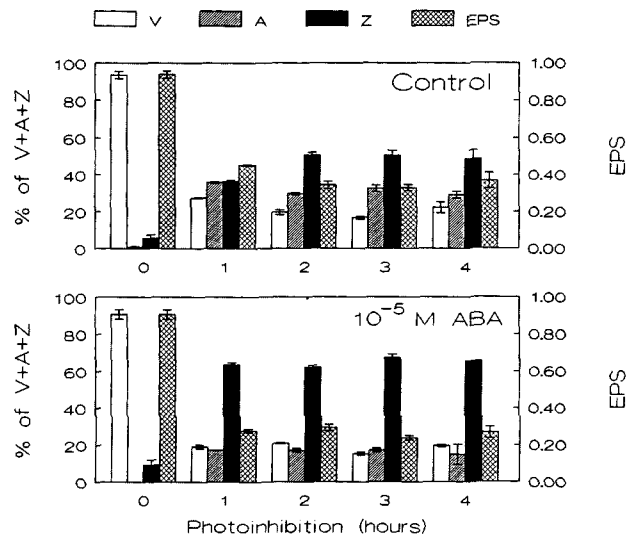

Fig. 2. High light-induced changes of xanthophyll cycle activity in control and ABA (10^{-5} M)-treated barley seedlings. For high-light treatments, leaves from control and ABA-treated plants were exposed to illumination of 1800 μmol m^{-2} s^{-1} at 5°C. Non-photoinhibited plants were dark-adapted for 30 min. Violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z) contents are presented as percentage of the total xanthophylls (V + A + Z) found in each sample. EPS = $(V + 0.5A)/(V + A + Z)$. Mean ± S.E. values were calculated from 7–13 measurements in 3–5 independent experiments.

reduce the requirement of carotenoid precursors for its biosynthesis resulting in an increase of the xanthophyll pool size in plant subjected to ABA. It is of interest to note that, although the sum of V + A + Z was higher in ABA-treated plants under control non-photoinhibitory (dark-adapted for 30 min) conditions, this increase is due exclusively to elevated amount of V. The relative amounts of both antheraxanthin (A) and Z were very small in all samples measured, i.e. non-treated and ABA-grown plants. It should be also mentioned that a small but not statistically significant increase, especially in the level of neoxanthin, was found even in 10^{-6} M ABA-treated plants. These results are in agreement with the xanthophyll imbalances associated with *aba* mutant alleles of *Arabidopsis thaliana* reported in [33]. In addition, the data are in close relation with the finding that most (up to 80%) of the carotenoids violaxanthin, neoxanthin and lutein were found in the LHCII-associated polypeptides [34], and with the recently proposed ABA-induced increase of the LHCII level in barley chloroplast membranes [23]. This is also consistent with the hypothesis that Z and quite possibly A [35] are both involved in non-radiative dissipation of the excess excitation energy in the antenna of PSII [18].

The dynamics in the relative quantitative changes in the three components of the xanthophyll cycle and the EPS corresponding to the conversion of V to Z (which causes EPS to approach 0) upon high-light treatment are presented in Fig. 2. It is seen that, besides the initially higher level of the xanthophyll pool size (V + A + Z) and especially of V in 10^{-5} M ABA-treated barley leaves (see Table 1), a considerable larger fraction of the violaxanthin pool had been converted into zeaxanthin (65.6%) after 4 h of exposure to high-light as compared with the control (48.6%). Interestingly, the relative amounts of V in both control and ABA-treated plants after 4 h of photoinhibition were found almost equal, i.e. 22.3 and 19.5%, respectively. However, the level of A was twice higher in control (29.2%) than in plants subjected to ABA (15%) under the same conditions. Bearing in mind that in the xanthophyll cycle the final product Z is formed through de-epoxidation of the di-epoxide V via a monoepoxide A [16], it appears evident that the lower levels of Z might be due to less effective de-epoxidation of A to Z in the control plants as compared with these subjected to ABA. The corresponding EPS mean values for control and 10^{-5} M ABA-treated leaves under the same photoinhibitory conditions (4 h) are 0.368 and 0.268, respectively, thus indicating an enhanced activity of the xanthophyll cycle in the leaves subjected to ABA. It should be also mentioned that the higher stable levels of zeaxanthin formation and enhanced activity of the xanthophyll cycle in 10^{-5} M ABA-treated plants were registered upon 1 h of high-light treatment and the levels remain relatively constant during the whole period tested, as distinct from the control plants where the stable levels of Z and EPS were reached after 2 h of exposure to excessive light (Fig. 2).

Zeaxanthin-dependent non-photochemical quenching (qNP) reflecting non-radiative energy dissipation of excess absorbed light is generally thought to play an essential role in protecting the PSII against the damaging effects of excess light [16,18]. The exact molecular mechanism(s) is still a matter of debate, but it has been reported very recently that the light-inducible enzymatic de-epoxidation of V (possessing 9 conjugated bonds) into Z causes extension of the π-conjugated bonds to 11, thus lowering the energy of the lowest excited singlet state of Z below that of Chl *a*. The calculated excited state energies suggests that it is thermodynamically feasible for Z to act as a trap for the excess excitation energy on Chl *a* molecules [36]. The observed higher qNP values in ABA-treated leaves (0.411) than in control (0.306) upon high-light exposure (line 2 in Table 2) directly correlated with the lower EPS level, reflecting the conversion of a substantially higher fraction of the xanthophyll pool to Z (Fig. 2).

To summarize, the experimental data presented here clearly indicate that the exogenously application of ABA induces enhanced resistance of barley seedlings to photoinhibition at low temperatures. Although the exact mechanism of this ABA-protective action has to be clarified more precisely, it is evident that it could be related to the increased re-oxidation rate of Q_A and to the increased activity of the xanthophyll cycle.

Table 2
Effects of photoinhibition (4 h) at 5°C on the steady-state chlorophyll fluorescence characteristics (qP and qNP) and steady-state yield of electron transport over PSII (Φe) of control (non-treated) and 10^{-5} M ABA-treated barley leaves

Parameter	Control		10^{-6} M ABA	
	D	HL	D	HL
qP	0.768 ± 0.025	0.718 ± 0.012	0.823 ± 0.015	0.767 ± 0.008
qNP	0.725 ± 0.014	0.306 ± 0.053	0.764 ± 0.015	0.411 ± 0.038
Φe	0.308 ± 0.027	0.202 ± 0.006	0.272 ± 0.022	0.267 ± 0.006
F_o^{HL}/F_o^D	1.14 ± 0.02		1.01 ± 0.01	

The photon fluence rate of the photoinhibition light was 1800 μmol $m^{-2} \cdot s^{-1}$. $\Phi e = qP \times F_v'/F_m'$, where F_v' and F_m' are the variable fluorescence and maximum fluorescence, respectively, during steady-state illumination of 230 μmol $m^{-2} \cdot s^{-1}$. qP, qNP and F_o were measured under the same conditions as described in section 2. Mean ± S.E. values are calculated from 3–5 measurements.
D, non-photoinhibited sample; HL, photoinhibited sample.

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