Possible function of ABA in protection against photodamage by stimulating xanthophyll cycle in sorghum seedlings

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The effect of high light at 30°C on chlorophyll fluorescence, xanthophyll cycle and abscisic acid (ABA) was determined to study the relationship of xanthophyll cycle and ABA in protection against photodamage. Sorghum seedlings of 8 days were grown on Hoagland medium supplemented with ABA (10−3 M) and photoinhibited at 2200 and 3600 μmol m−2 s−1 PAR light intensities, and measurement of chlorophyll fluorescence (Fv/Fm, ratio, qN, ΦPS II and Φ exe), xanthophyll cycle content and ABA content were made. The results obtained show that seedlings grown on ABA-supplemented medium had better energy dissipation (greater qN) and much greater level of de-epoxidation (high level of Z) than control seedlings. The level of endogenous ABA in seedlings grown on ABA-supplemented medium or otherwise remained the same. The results indicate a possible role of ABA in energy dissipation, probably by stimulating the xanthophyll cycle.

EXPOSURE of leaves to light levels where the excitation energy exceeds the capacity for orderly dissipation by the photosynthetic system can lead to photoinhibition. The effect of plants to different light environment has profound influence on the structure and function of photosynthetic apparatus. Plants grown under full sunlight have much greater photosynthetic capacity, which saturates at higher photon flux density (PFD), than plants grown in shade conditions. Shade leaves are considered to be more prone to photoinhibition than sun leaves. Photoinhibition is characterized by a sustained decrease in the efficiency of photon utilization by photosystem II (PS II) photochemistry, due to absorbing more excitation energy than could be dissipated by photochemical processes.

Under normal conditions, the light energy absorbed by the pigments in chlorophyll protein complexes of PS II and photosystem I (PS I) is utilized in a controlled manner leading to generation of NADPH and ATP. However, under photoinhibitory conditions, this process is not able to dissipate all the available energy resulting from the decrease in efficiency of the photosynthetic system in utilizing the energy. There are several built-in mechanisms of energy dissipation such as carotenoids, photorespiration, Mehler reaction, reducing substances, SOD, etc. which play a role in providing some protection against oxidative damage to the PS II reaction centre against photoinhibition. Xanthophylls (violaxanthin, antheraxanthin and zeaxanthin) present in the light-harvesting complex II are supposed to play an important role in the photoprotection of plants. A well-known function of zeaxanthin is to promote rapid thermal energy dissipation, as revealed by energy-dependent fluorescence quenching (qE, which reflects the mechanism associated with both the PS II reaction center and the light-harvesting antennae of the PS II) which allows rapid down-regulation of PS II energy capture under excess irradiance.

Studies using time-resolved chlorophyll fluorescence also suggest that non-photochemical energy dissipation is related to the binding of de-epoxidized xanthophylls (A + Z) to the inner PS II antenna.

Recent reports suggest that synthesis of ABA from the oxidative cleavage of zeaxanthin is of great interest as ABA is a plant hormone involved in many physiological and developmental processes such as transpiration, germination, dormancy and also with the adaptation of plants to environmental stresses (e.g. drought, chilling and pathogen attack).

The proposed biosynthetic pathway of ABA in higher plants shows close relationship between xanthophyll cycle and ABA. Here we have studied the effect of ABA treatment on the xanthophyll cycle and thus, on the process of energy dissipation and photoprotection in sorghum seedlings.

Seeds of *Sorghum bicolor* L. cv MSH-51 were obtained from Mahyco Seeds Corporation, Jalna and were stored in a desiccator at room temperature. The seeds were soaked in tap water for one hour and were surface sterilized with HgCl2 (0.1%). They were germinated in glass petri plates (10 cm diameter) containing moist cotton pad and blotting paper, soaked in half strength Hoagland solution. The control plants were irrigated with the solution daily. ABA treatment (10−3 M) in 0.1 mM KOH was given to 4-day-old seedlings of normal growth. Seedlings were allowed to grow up to 8 days of age in a growth chamber illuminated with incandescent and fluorescence light (200 μmol m−2 s−1) measured using radiometer model LI-189, Licor, USA. The day/night temperature was maintained at 30 ± 2°C and relative humidity at 50%.

Experiments were carried out with intact leaves of 8-day-old seedlings. Leaves were exposed to high irradiance of 3600 and 2200 μmol m−2 s−1 photosynthetic active radiation (PAR) using KL-1500 electronic cold light source. Temperature during the treatment was maintained at 30°C. Chlorophyll fluorescence was measured using a pulse amplitude modulation fluorometer (PAM 101 & 102 from Walz, Germany) according to Schreiber et al. Prior to the measurements, leaves were dark-adapted for

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10 min. The 1 cm$^2$ portion of the leaves was exposed to modulated light (4 μmol m$^{-2}$ s$^{-1}$) to measure initial fluorescence ($F_0$) followed by the exposure of the leaves to a saturating pulse of white light (4000 μmol m$^{-2}$ s$^{-1}$) to measure maximum fluorescence ($F_m$). After the measurement of $F_m$ the leaf was exposed to an actinic light of 800 μmol m$^{-2}$ s$^{-1}$ to allow it to reach a steady state of fluorescence ($F_t$). Another burst of saturating light was used to measure secondary maximum chlorophyll fluorescence ($F_m'$. $F_o'$ was measured by exposing the leaves to infra-red radiation. The photochemical and non photochemical quenching coefficients were calculated according to Schreiber et al.$^{11}$ and φPS II and φexc were calculated according to Habash et al.$^{12}$.

The exposed portion of the treated leaves was cut and weighed accurately. The leaf tissue was thoroughly extracted in 0.5 ml acetone (containing 0.1% butylhydroxytoluene, BHT) in 1.5 ml eppendorf tube. The extract was centrifuged at 5000 g for 5 min at 4°C. The supernatant was filtered through 30 μm syringe filter and 10 μl of filtrate was used for HPLC analysis for carotenoids as well as for ABA.

Identification and separation of carotenoids was carried out according to Sharma and Hall$^{13}$ using HPLC (Spectra Physics, UK), with C-18 reverse-phase column (ET-250/4 nucleosil 100-5 C-18 ODS) Spectra Physics SP-termy HPLC pump with SP-4270 integrator and Spectra 100 variable wave length detector. Ten μl of the filtered tissue extract was injected onto the HPLC column at 25°C. The separation was carried out using linear gradient of ethyl acetate and acetonitrile:water (9:1) over 25 min with 1.2 ml min$^{-1}$ flow rate, at 445 nm. Identification of carotenoid was carried out on the basis of retention time and spectral characteristics of the standards.

Part of the leaf extract (see above) was used for the ABA analysis. The separation and quantitative estimation was carried out according to Rodrigues et al.$^{14}$ using HPLC (for details of the machine and specification, see above). The separation was carried out using a linear gradient of 0–100% methanol, containing 1% acetic acid throughout and quantified with peak integration after calibration with external standard ABA.

The effect of photoinhibition treatment (2200 and 3600 μmol m$^{-2}$ s$^{-1}$ PAR) at 30°C on the $F_0$/$F_m$ ratio in sorghum seedlings grown with and without exogenously supplemented ABA is presented in Figure 1. Six hours of high light treatment (2200 μmol m$^{-2}$ s$^{-1}$ PAR) of sorghum seedlings grown without ABA resulted in a decrease of 69% in $F_0$/$F_m$ ratio compared to only 27% decrease observed in ABA-grown seedlings treated at the same light level and the duration (Figure 1a). Similarly, photoinhibition treatment at 3600 μmol m$^{-2}$ s$^{-1}$ PAR for six hours resulted in a decrease of 75% in $F_0$/$F_m$ ratio for (−) ABA-grown seedlings compared to only 49% decrease in the seedlings grown with ABA and treated to the same high light level and the duration of the treatment (Figure 1a).

Recovery of the photosynthesis ($F_o$/$F_m$ ratio) following the photoinhibition treatment at 3600 μmol m$^{-2}$ s$^{-1}$ PAR for 6 h was also greater (76% recovery) in ABA-grown seedlings than observed in seedlings grown without ABA (63% recovery; Figure 1b).

Relative quantum yield of PS II electron transport (ϕ PSII; Figure 2a and b) and the efficiency of excitation capture by open PSII centre, (ϕexc; Figure 2c and d) calculated according to Habash et al.$^{12}$ from fluorescence measurements, was also decreased as a result of photoinhibition. The ϕPSII was decreased to 71% due to the photoinhibition of (−) ABA-grown seedlings when photoinhibited at 3600 μmol m$^{-2}$ s$^{-1}$ PAR for 6 h compared to a decrease of only 20% as a result of the same photoinhibition treatment of (+) ABA-grown seedlings (Figure 2a and b). Similar results were obtained for ϕexc. The ϕexc gradually decreased to 80% in (−) ABA-grown seedlings over the six hour exposure to high light compared to control. Though ϕexc changed

![Figure 1](image-url)
in a similar fashion in ABA-grown seedlings, the extent of decrease in $\Phi_{\text{exc}}$ was only 63% (Figure 2c and d).

Table 1 shows a comparative HPLC analysis of the quantitative changes in the carotenoid composition and content in (-) ABA and (+) ABA grown sorghum seedlings. Sorghum seedlings grown with ABA showed a greater level of de-epoxidation (conversion of violaxanthin to zeaxanthin). A six-hour photoinhibition treatment of (+) ABA-grown seedlings at 2200 and 3600 $\mu$mol m$^{-2}$ s$^{-1}$ PAR resulted in 71% and 76% de-epoxidation level, respectively compared to 30% and 37% de-epoxidation in seedlings grown without ABA and photoinhibited at 2200 and 3600 $\mu$mol m$^{-2}$ s$^{-1}$ PAR.

The $V + A + Z$ pool was higher in ABA-grown control plants and it further increased as a result of high light treatment. However, the $V + A + Z$ pool in control of (-) ABA-grown seedlings was 15% less than that seen in control of ABA-grown seedlings. The photoinhibition of (-) ABA-grown seedlings did not result in an increase in the $V + A + Z$ pool, as observed in the (+)
ABA-grown seedlings. Chlorophyll to carotenoids ratio was slightly greater in (-) ABA-grown seedlings compared to the seedlings grown with ABA and did not change significantly as a result of high light treatment in either case, (-) ABA and (+) ABA-grown seedlings.

Table 2 shows the effect of photoinhibition on non-photochemical quenching (qN) in (-) ABA and (+) ABA grown plants and its relationship with the xanthophyll cycle. The results show that photoinhibition of plants grown with exogenously supplemented ABA exhibited a greater level of qN than observed in plants grown without ABA. Also, the greater increase in qN was matched with the greater level of de-epoxidation of violaxanthin to zeaxanthin, showing a relationship between the two.

Table 3 shows the level of endogenous ABA contents in (-) ABA and (+) ABA grown plants. The endogenous ABA contents of seedlings grown without exogenously supplemented ABA showed a slightly lower level of endogenous ABA in comparison to that of seedlings grown with exogenously supplemented ABA medium. However, photoinhibition treatment of both types of seedlings (grown with exogenously supplemented ABA or without exogenously supplemented ABA) showed more or less the same level of endogenous ABA.

Chlorophyll fluorescence gives indication about the health of the photosynthetic system in leaves. In this study, we observed that photoinhibition of sorghum seedlings at 2200 and 3600 μmol m⁻² s⁻¹ PAR at 30°C resulted in a decrease in Fv/Fm ratio, which suggests that the efficiency of PS II electron transfer declined as a result of photoinhibition. This decline in the efficiency of the PS II was much greater in seedlings grown without ABA than in seedlings grown with exogenously supplied ABA, suggesting relatively less photoinhibitory damage to PS II in ABA-fed plants. The Fv/Fm level recovered, following the photoinhibition treatment, to a much greater level in plants grown with ABA than in those which were grown without ABA (Figure 1b), indicating that ABA provided some protection to PS II damage under high light condition. The results shown in Table 2 further suggest that the protection to PS II provided by the application of exogenous ABA is due to better energy dissipation, as qN (which indicates dissipation of excessive energy in the pigment bed, thereby preventing over-energization of reaction centre under high light conditions) was much higher in plants grown with ABA than in plants grown without ABA. The increase in qN was also matched by greater level of de-epoxidation of violaxanthin to zeaxanthin, showing the relationship between qN and the xanthophyll cycle. These results were further substantiated with measurements of ΦPS II (relative quantum yield of PS II indicating PS II activity) as the photoinhibition of sorghum seedlings grown without ABA resulted in greater decrease in the ΦPS II than seen in seedlings grown with exogenously supplied ABA (Figure 2a and b). Similarly φexc (efficiency of excitation capture by open PSII) was also decreased to a greater extent in seedlings grown without exogenously supplied ABA than in seedlings grown with ABA, indicating higher efficiency of energy transduction in open PS II reaction centres. These data clearly indicate certain protective roles of ABA on the PS II (as room temperature fluorescence is largely generated from PS II only) under high light conditions. Thus, the increased resistance to photoinhibition in ABA-grown seedlings could be ascribed to an increased capacity of the photosynthetic machinery to maintain an increased portion of the PS II reaction centre in an open state by efficient dissipation of absorbed excitation (seen as higher qN and ΦPS II).

Ivanov et al. have also shown ABA-induced protection against photoinhibition of PS II in barley seedlings. They observed that exogenous application of ABA resulted in partial protection of PS II under high light condition at low temperature. They also observed 122% increase in the amount of xanthophyll in seedlings subjected to ABA. The results presented here are in confirmation with the work published by Ivanov et al. The radiation-less dissipation of excess excitation energy in the chlorophyll pigment bed has been proposed to be one of the major protective mechanisms against photoinhibitory damage of PS II which is supposed to be regulated by the xanthophyll cycle. The ABA-grown seedlings upon photoinhibition showed much greater level of de-epoxidation; thus better energy dissipation in ABA-grown plants. The increase in the V + A + Z pool was largely on account of greater amount of V present in ABA-grown seedlings than in seedlings grown without ABA (Table 1).

Schwartz et al. and others have reported that violaxanthin also acts as precursor to the ABA biosynthetic pathway. Thus higher V + A + Z pool in ABA-grown plants could be explained, as exogenously supplied ABA would reduce the requirement of violaxanthin for the biosynthesis of ABA resulting in an increase of the xanthophyll pool size in plants grown with ABA-supplemented medium, largely on account of less conversion of violaxanthin to ABA in ABA-grown seedlings compared to that seen in seedlings grown without ABA. Another interesting fact is that ABA is also proposed to induce increase in LIIC II level, a site where most (80%) of the V, A and Z is localized; this may also result in an increase in the level of carotenoids (V + A + Z) in ABA-grown seedlings as observed in this study.

Our results also show that the endogenous level of ABA in both type of seedlings after photoinhibition remains more or less constant (Table 3). As discussed earlier, a common pool of violaxanthin acts as precursor to ABA as well as zeaxanthin formation; thus violaxanthin would have to convert either to ABA or to zeaxanthin or both depending on environmental factors. If the violaxanthin pool is also used for ABA synthesis, then it
Table 2. Non-photochemical quenching and xanthophyll cycle (violaxanthin and zeaxanthin) content in sorghum seedlings grown without and with ABA and photoinhibited at 2200 and 3600 μmol m⁻² s⁻¹ PAR at 30°C for 6 h. Xanthophyll contents are given as μg pigments FW⁻¹ using β-carotene as external standard.

<table>
<thead>
<tr>
<th></th>
<th>Without ABA</th>
<th>With ABA</th>
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<tbody>
<tr>
<td></td>
<td>Control (0 h)</td>
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<tr>
<td>Non-photochemical quenching (qN)</td>
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<td>0.443</td>
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<tr>
<td>Xanthophylls</td>
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<td></td>
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<tr>
<td>Violaxanthin</td>
<td>19.33</td>
<td>17.56</td>
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<tr>
<td>Zeaxanthin</td>
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Table 3. Effect of photoinhibition on ABA content measured using HPLC in 8-day-old sorghum seedlings grown with or without endogenous ABA (10⁻⁵ M) supplemented Hoagland's solution. Values of ABA are presented as μg mg FW⁻¹ calculated using ABA as external standard. Each reading represents average of two repetitions.

<table>
<thead>
<tr>
<th>Duration of treatment (h)</th>
<th>Without ABA</th>
<th>With ABA</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>2200 μmol m⁻² s⁻¹</td>
<td>3600 μmol m⁻² s⁻¹</td>
</tr>
<tr>
<td>0</td>
<td>0.104 ± 0.0</td>
<td>0.104 ± 0.02</td>
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<tr>
<td>1</td>
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<tr>
<td>3</td>
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<td>0.283 ± 0.05</td>
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<td>6</td>
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Other articles cited in the text:

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