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Plastid alternative oxidase functions as an alternative electron acceptor in *Arabidopsis* and may protect young plastids from photodamage.

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Introduction

The transfer of electrons from the plastoquinone pool to oxygen, chlororespiration, has been demonstrated in green algae and plants. No physiological function for this pathway has been clearly demonstrated, although it has been suggested that it could function as a protective pathway under environmental stress conditions. Characterization of the *immutans* mutant of *Arabidopsis* identified a plastidic terminal oxidase (PTOX) whose first identified function was in carotenoid desaturation (Carol et al., 1999; Josse et al., 2000). PTOX is related to the mitochondrial alternative oxidase and is sensitive to inhibition by n-propyl-gallate (Cournac et al., 2000). PTOX is a good candidate for a component of the chlororespiratory pathway that might operate under conditions of a highly reduced plastoquinone pool and regulate photosynthetic electron flow in addition to it established role in carotenoid biosynthesis.

One approach to help determine the role of alternate electron sinks in photosynthesis would be to identify mutants that showed an over-reduction of the quinone pool. We have identified and characterized a mutant of *Arabidopsis* that develops pale green leaves under high light and that has a highly reduced plastoquinone pool. In this mutant, the plastoquinone pool eventually becomes reoxidized under high actinic light, presumably by electron transfer to an alternative acceptor. This reoxidation event is sensitive to n-propyl-gallate, suggesting that PTOX is involved.

Materials and methods

Arabidopsis wild type (*Ler*) and mutant plants were grown in soil under 100, 300 or 600 μmol photons m^{-2} s^{-1}. Seeds of the mutant called *arc2* were obtained from the Arabidopsis Biological Resource Center. Chlorophyll fluorescence was measured using a Waltz PAM fluorimeter.

Results

Identification of a mutant with an over-reduced quinone pool. *Arabidopsis* plants were screened to identify mutants that have an over-reduced plastoquinone pool. The screen involved an analysis of chlorophyll fluorescence quenching over a range of actinic light intensities that allowed a determination of the photosynthetic efficiency of PSII (ϕPSII), the relative level of reduction of the quinone pool (1-qP), and the extent of nonphotochemical quenching (NPQ). In leaves of 14-day old wild-type plants, PSII decreases as the actinic light intensity increases and an increasing proportion of the PSII reaction centers are in a reduced state, as identified by 1-qP (Figure 1). In addition, with increasing light intensity, the efficiency of light use for photosynthesis decreases concomitant with an increase in the non-photochemical fluorescence quenching parameter, NPQ. During screening of *Arabidopsis* mutant plants, we identified a mutant that showed an unusually over-reduced quinone pool (Figure 1). This phenotype is observed only in young leaves, suggesting the mutant gene is expressed at early stages of chloroplast development.

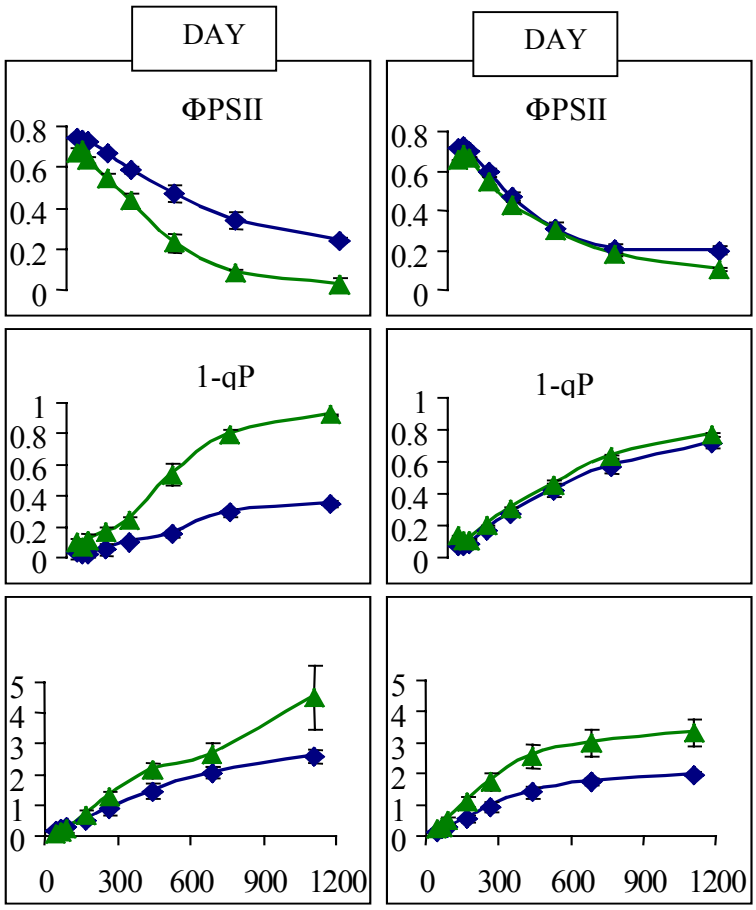

Figure 1. Fluorescence parameters measured from leaves of *Ler* (◊) and *arc2* (Δ).

Thylakoid ultrastructure of arc2. Figure 2 shows that the thylakoids in *arc2* chloroplasts of day 14 leaves are significantly more stacked than in wild-type (*Ler*). A similar increase in stacking is observed when grown at 300 µmol photons m^{-2} s^{-1}. This increased stacking could be one reason for the over-reduction of the quinone pool, as more light may be funneled to the PSII reaction center than is required for optimal photosynthesis.

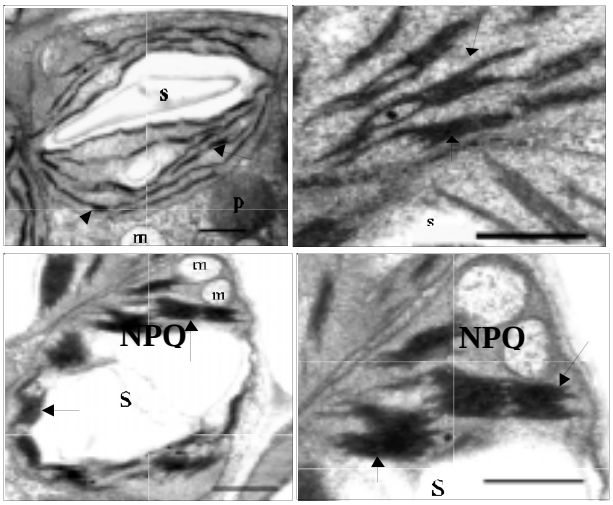

Figure 2. Thylakoid ultrastructure in wild-type (upper left and right) and arc2 (lower left and right).

Arc 2 shows unusual changes in plastoquinone redox state under

high light. When *arc2* plants are grown at higher light intensities, the profile of chlorophyll fluorescence quenching parameters at different actinic light intensities is highly unusual (Figure 3). Instead of a progressive increase in the reduction state of the plastoquinone pool (1-qP) with increasing actinic light intensity, there is a rapid re-oxidation of the quinone pool before it again becomes reduced with further increasing light intensity (Figure 3). This reoxidation event is unaffected by KCN but is inhibited by n-propyl-gallate (Figure 3). It is further shown in Figure 3 that addition of n-propyl-gallate to young wild-type leaves causes an increase in the reduction state of the quinone pool, suggesting a role of PTOX as an alternate electron sink in developing chloroplasts.

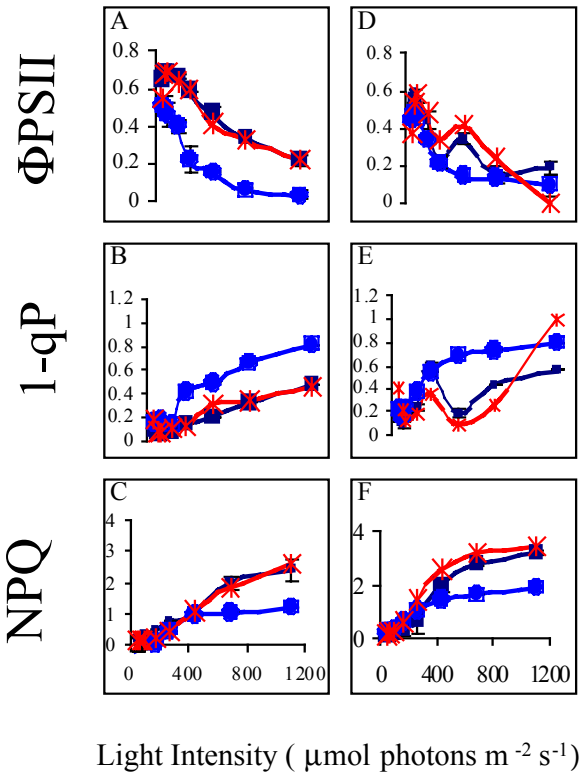

Figure 3. Fluorescence parameters from *Ler* (left) and *arc2* (right) leaves incubated with n-propyl-gallate (¤), KCN(□) or H_20 (X).

Mapping the ARC2 loci. The initial mapping of the ARC2 loci began with using a small mapping population to identify which of the five chromosomes the ARC2 loci was located. By using available markers from the top and bottom arms of each chromosome, it was found that the ARC2 locus was on the top arm of Chromosome 1. The mapping population was then increased and the mapping refined using SSLP and CAPS analysis on a population of F2 mutant plants. ARC2 has now been fine mapped to the top arm of chromosome 1 between markers nga59 and ATHACS (Bell and Ecker, 1994) which span 3 BACs (T25K16, F6F3, F22L4).

Discussion

The *arc2* mutant of *Arabidopsis* has an over-reduced quinone pool at early stages of leaf and plastid development. The precise reason for over-reduction of the quinone pool is unknown at this time. It likely reflects an increased excitation energy pressure on PSII due to the increased stacking of the thylakoid membranes. The ARC2 protein is presumably required for normal assembly of thylakoid membranes at early stages of plastid development. Further fine mapping and analysis will identify the defective gene.

The unusual pattern of the fluorescence parameter 1-qP in *arc2* leaves suggests that there is an alternative electron sink that operates when the quinone pool becomes over-reduced in young plastids. One candidate for an alternative electron acceptor is PTOX. Although the only defined role for PTOX is in carotenoid biosynthesis, it is feasible that it could also act as an alternate electron transfer intermediate in the transfer of electron to oxygen when the quinone pool is over-reduced. PTOX is similar to the alternative oxidase found in mitochondria and can be inhibited by n-propyl-gallate. Even in wild-type leaves, we have shown that n-propyl-gallate inhibition of PTOX can cause an increase in the reduction state of

the quinone pool. In the *arc2* mutant, we also show that the reoxidation event that occurs when the quinone pool is over-reduced is inhibited by n-propoyl-gallate. This suggests that PTOX may serve as an electron carrier to prevent over-reduction of the plastoquinone pool in developing plastids.

Young leaves emerging into the light contain chloroplasts that may be particularly susceptible to damage by high light. One reason for this is that the photosynthetic apparatus is still poorly developed and rates of CO_2 fixation are low. An alternative electron acceptor such as PTOX can protect the photosynthetic apparatus from over-reduction by direct transfer of electrons to oxygen. We also observed that n-propyl-gallate treatment resulted in a decrease in NPQ. This would suggest that in young chloroplasts under high light stress, PTOX is needed to alleviate "excitation pressure" on PSII so that a proton gradient can be formed and NPQ protective mechanisms can be activated.

Acknowledgments

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