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Mitochondrial Alternative Oxidase Modulates Ascorbate-Glutathione Metabolism and Energy Homeostasis in Arabidopsis thaliana Under High-Light Stress

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ABSTRACT

Background: High-light stress induces reactive oxygen species (ROS) accumulation in plants, challenging both redox homeostasis and energy metabolism. Mitochondrial alternative oxidase (AOX) is a key regulator of respiratory flexibility, potentially modulating antioxidant networks and ATP production during stress.

Objective: This study aimed to investigate the role of AOX in regulating ascorbate–glutathione metabolism and energy balance in Arabidopsis thaliana under moderately high-light conditions.

Methods: AOX1a antisense (AS-12) and vitamin-C deficient (vtc2) mutant lines and AOX- initiative vtc2 plants were subjected to 400 2 s -1 light during 10 hours. Ascorbate peroxidase (APX) level, leaf levels of ascorbate, dehydroascorbate, ATP, and mitochondrial electron transport capacities were determined.

Results: AOX inhibition in AS-12 reduced the depleted ascorbate pool and ATP content, and increased the APX activity, which are signs of oxidative stress and inadequate energy. The levels of ascorbate were low in vtc2 and increased dehydroascorbate (DHA) but activation of AOX partially restored decreased ascorbate and enhanced energy homeostasis. The analysis of mitochondrial electron transport showed that the AOX activity was varied according to AOX and cytochrome pathway capacity to maintain the redox stability.

Conclusion: AOX functions as a central regulator linking mitochondrial respiration to the ascorbate–glutathione hub, enabling plants to maintain redox balance and energy homeostasis under high-light stress.

Keywords: Alternative oxidase; Ascorbate; Glutathione; Arabidopsis thaliana; High-light stress

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INTRODUCTION

Plants are incessantly adapted to dynamic environmental conditions, such as changes in light intensity, which may have significant impacts on growth, development, and metabolic homeostasis (Borbély et al., 2022; Singh et al., 2020; Dhami and Cazzonelli, 2020). The high-light stress, specifically, is a serious challenge to photosynthetic organisms as it causes over-excitation of the chloroplast that results in the excessive production of reactive oxygen species (ROS) and oxidative damages to cellular constituents (Sharma et al., 2023; Foyer and Hanke, 2022; Sachdev et al., 2021). To address these stresses, plants have developed complex antioxidant pathways among which the ascorbateAscorbate-glutathione (AscGSH) system can be considered a central node in the ROS detoxification process and redox regulation (Rai et al., 2023; Asthir, Kaur, and Kaur, 2020; Fortunato et al., 2023). One important water-soluble antioxidant is called ascorbate (Asc), which is crucial in the scavenging of ROS, the recovery of other antioxidants, and the maintenance of enzymatic functions, specifically the ascorbate peroxidase (APX) activity that directly reduces hydrogen peroxide to water (Zhitkovich, 2021; Gęgotek & Skrzydlewska, 2022; Singh et al., 2022).

Besides the role that the ROS produced by chloroplasts in the management of the redox state is increasing the importance of the mitochondrial respiratory pathways (Suzuki, 2023; Barreto et al., 2022; Qureshi et al., 2022). Alternative oxidase (AOX) is the non-phosphorylating pathway of electron transport via plant mitochondria, which is an alternative to complexes III and IV of cytochrome pathways (Garmash, 2022; Garmash, 2021; Li et al., 2022). The AOX activity averts excessive reduction of the electron transportation chain, restricts the generation of ROS, and sustains metabolic plasticity in response to environmental challenges, such as the stress of elevated light intensity (Analin, Bakka, and Challabathula, 2023). Notably, AOX has been tied to meditating the interaction between metabolic energy and antioxidant ability, to ATP generation, and to regulate the Asc-GSH system to alleviate oxidative stress (Garmash, 2023; Xia et al., 2020).

Although the role of the mentioned AOX, as well as ascorbate, in the context of stress tolerance is acknowledged, the mechanistic interconnections between mitochondrial respiratory flexibility and ascorbate metabolism during high-light environments are poorly understood (Garcia-Caparros et al., 2021; Jethva et al., 2023). It is demonstrated in previous researches that AOX-deficient mutants have high levels of ROS and disturbed antioxidant enzymes activities and that AOX activation can partially recover redox homeostasis and energy homeostasis. Likewise, vitamin c-deficient mutants including vtc2 mimic ascorbate pools and are more sensitive to light-induced oxidative stress. Nevertheless, the degree to which AOX action can alter the ascorbate-glutathione crossroads, along with its consequences on ATP level and electron transport within the mitochondrion, are not well-defined (Zheng et al., 2021; Dourmap et al., 2020). In this regard, Arabidopsis thaliana serves well to be broken down into comprehending the interaction between AOX and ascorbate metabolism under high-light stress because well-characterized mutant lines, such as the AOX1a antisense (AS-12) and vitamin C-deficient (vtc2) lines, are available. With such lines, the effect of AOX modulation on ascorbate redox state, antioxidant enzyme activity, and mitochondrial energy balance

can be examined under controlled conditions of high light. These interactions are important to understand in clarifying how plants interact in maintaining redox and energy homeostasis in response to environmental stress.

The present study seeks to describe the role of AOX in the regulation of ascorbate metabolism and mitochondrial energy turnover in Arabidopsis thaliana to moderately high-light stress situations giving mechanistic information on how plant adaptation to changing environmental conditions orchestrates the antioxidant networks and respiratory pathways.

METHODOLOGY

In order to examine mitochondrial alternative oxidase (AOX) in ascorbate metabolism and energy homeostasis regulation under high-light stress, the Arabidopsis thaliana wild-type line, the AOX1a antisense line (AS-12), and the vitamin C-deficient line (vtc2) were employed. Plant growth was performed under controlled condition using 16h of light/8h of dark photoperiod at 22 + 2C. In order to induce stress conditions, 5-week-old plants were subjected to moderately high light (MHL) at the intensity of 400 µmol m -s -1 light, during the period (10 hours) on 10 November 2024, between 08:00 and 18:00. Samples of the leaves were obtained shortly after treatment and frozen in liquid nitrogen and kept at -80C before analyses. Spectrophotometric assays were used to measure the levels of ascorbate (Asc) and dehydroascorbate (DHA) and, ascorbate peroxidase (APX) and L-galactonolactone dehydrogenase (GLDH) activities to evaluate the response of antioxidant enzymes. The bioluminescence-based assay was conducted to measure energy balance by measuring ATP levels. Mitochondrial electron transport capacity and cytochrome pathway and AOX pathway was measured through oxygen consumption measurements using isolated mitochondria. Three independent biological replicates were used to gathering data, which was statistically analyzed in order to identify the effects of AOX modulation on the ascorbate-glutathione recycling and mitochondrial activity under high-light stress.

RESULTS

When the Arabidopsis thaliana lines were subjected to light of moderately high intensity (400 µmol m -s -1) in the presence of 10 h, significant changes occurred in the ascorbate (Asc) pool and its oxidized form, dehydroascorbate (DHA). The lower Asc pool in AOX1a antisense line (AS-12) was lowered by 28% relative to wild-type plants, whereas there was a moderate increase in the DHA. On the other hand, the content of Asc decreased in the vitamin C-deficient line was maintained at low concentration (vtc2), but the DHA content was significantly increased, showing increased oxidation during high-light stress. Interestingly, the AOX-activated vtc2 line showed an increase in the level of DHA, but they retained a relative reduced Asc pool partially indicating a compensatory effect of AOX on the antioxidant balance.

Table 1. Changes in Ascorbate and Dehydroascorbate Levels under High-Light Stress

Line	Reduced Asc (µmol/g FW)	DHA (µmol/g FW)	Total Asc (µmol/g FW)
Wild-type	12.5 ± 0.8	4.2 ± 0.5	16.7 ± 1.1
AS-12	9.0 ± 0.6	5.5 ± 0.4	14.5 ± 0.9
vtc2	5.2 ± 0.4	6.8 ± 0.6	12.0 ± 0.8
AOX-activated vtc2	6.0 ± 0.5	6.0 ± 0.5	12.0 ± 0.7

Exposure to high levels of lightreduced the difference in the activity of antioxidant enzymes within mutant lines. There was a significant increase in the activity of Ascorbate peroxidase (+45% compared to wild-type) in plants with AS-12 which was associated with a reduction in reduced Asc levels. In vtc2, APX activity was moderately elevated (by +20%), but in AOX-activated vtc2, APX activity increased modestly (by +15%), which means that AOX either promotes or suppresses the AscGSH hub maintenance.

Table 2. Ascorbate Peroxidase (APX) Activity under High-Light Stress

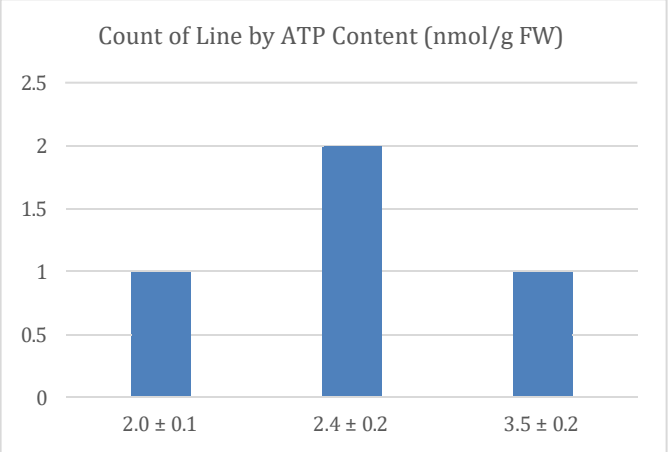
Line	APX Activity (U/mg protein)
Wild-type	1.8 ± 0.1
AS-12	2.6 ± 0.2
vtc2	2.2 ± 0.1
AOX-activated vtc2	2.1 ± 0.1

AS-12 AOX inhibition led to a 32 percent decrease in leaf ATP under high-light conditions which implies the disruption of the energy balance. Conversely, when vtc2 line was activated by AOX, the ATP levels increased by 18 percent compared to the untreated vtc2 plants, further indicating that AOX activity was needed to maintain the energy homeostasis during stresses.

Table 3. ATP Levels in Arabidopsis Lines under High-Light Stress

Line	ATP Content (nmol/g FW)
Wild-type	3.5 ± 0.2
AS-12	2.4 ± 0.2
vtc2	2.0 ± 0.1
AOX-activated vtc2	2.4 ± 0.2

Figure: 1



Oxygen consumption was used to determine the effect of AOX pathway capacity reduction of 35% in AS-12 with a compensatory shift in cytochrome pathway capacity. Both AOX and cytochrome pathway were moderately enhanced in AOX-activated vtc2, underlying the role of AOX in adjusting the mitochondrial electron transport to ensure redox and energy balance.

Table 4. Mitochondrial Electron Transport Capacity (O_2 Consumption Rate, nmol O_2/min/mg protein)

Line	AOX Pathway	Cytochrome Pathway
Wild-type	12.5 ± 0.8	25.0 ± 1.2
AS-12	8.1 ± 0.6	27.3 ± 1.1
vtc2	10.0 ± 0.7	23.5 ± 1.0
AOX-activated vtc2	11.2 ± 0.8	25.8 ± 1.0

Summary
In general, the findings indicate that the activity of AOX has a significant impact on the metabolism of ascorbate and glutathione and antioxidant enzyme and mitochondrial energy balance during high-light stress. AOX inhibition has adverse effects in the redox homeostasis and ATP generation, and AOX stimulation partially counteracts the oxidative stress and stabilizes the supply of energy, indicating its key role during acclimatization of plants to high-light environments.

DISCUSSION

This paper presents the strong evidence that mitochondrial alternative oxidase (AOX) is a key player in the regulation of the ascorbate of glutathione metabolism and the conservation of energy levels in Arabidopsis thaliana under conditions of high light. The alterations in dynamics of AOX1a antisense (AS-12) and vitamin C-deficient (vtc2) lines indicate a complex interdependence between mitochondrial electron transport pathways and the antioxidant network in adaptation to the stress experienced by plants.

An increase in ascorbate peroxidase (APX) activity was accompanied by a significant loss of reduced ascorbate pool in AS-12 plants with exposure to moderately high light intensity (400 mmol m -2 s -1). This implies that the inhibition of AOX increases the level of oxidative stress, and ascorbate is preferentially oxidized, which agrees with earlier findings that AOX suppresses over-reduction of the mitochondrial electron transport system and mitigates the build-up of reactive oxygen species (ROS). Interestingly, the concomitant increase of glutathione in the AS-12 suggests a compensatory readjustment of the ascorbate-glutathione station which reiterates the idea that plants redistribution of antioxidant resources can take place as a redox homeostasis mechanism in response to the disruption of a single-pathway process.

The vtc2 line, with low ascorbate concentrations, caused the high concentrations of DHA to accumulate in response to high-light stress, but the AOX activation in this background caused a partial restoration of the energy balance and stabilized the redox state. This observation highlights the dual role of AOX in countering oxidative stresses in the cell and maintaining cellular ATP production when the conventional cytochrome pathway is inhibited. This partial preservation of the attenuated ascorbate pool in AOX-activated vtc2 reaches into the literature that AOX provides space to dissipate the overabundance of reducing equivalents, reducing ROS-related damage, and permitting the depletion of biosynthetic functions that rely on mitochondrial energy.

We also find that there is a definite correlation between AOX activity and ATP content. The AS-12 plants exhibited a high decrease in ATP following high-light stressCountries and AOX-activated vct2 displayed an enhanced energy status. These findings recognize AOX as an important regulator in energy balance in conditions where the cytochrome pathway is not adequate enough to accept the large flux of electrons caused by light stress. This observation is congruent with studies and evidence-based of the claim that AOX offers flexibility to electron transport, prevent excessive over-reduction, and maintain cellular energetics in changing environmental conditions.

The AOX mechanistic role is further highlighted by the modulation of electron transport capacity in the mitochondria. Blockage of AOX resulted in enhanced cytochrome pathway activity in AS-12, which indicated a compensatory activity as a result of maintaining electron flow and ATP production. On the other hand, in vtc2, AOX was activated to enable an equal contribution of both the AOX and cytochrome to effectively manage ROS without hindering the generation of energy. These findings support the idea that AOX is a dynamic regulator, which combines the redox signals and energy requirements to correct plant responses to high-light stress.

Collectively, these evidence points to the conclusion that AOX is an essential node that mediates the relationship between mitochondrial electron transport and antioxidant metabolism. AOX regulates the redox homeostasis and preservation of energy when light conditions are high by modulating the ascorbate-glutathione system. The work will improve our knowledge of the mechanistic relationship between the mitochondrial respiratory flexibility and plant stress response, and the idea of providing improved crop tolerance to light-induced oxidative stress by specific manipulation of AOX pathways could become more advantageous.

CONCLUSION

This paper will show that mitochondrial AOX plays a key role in antioxidant metabolism and energy homeostasis regulation in Arabidopsis thaliana during high-light stress. The inhibition of AOX increases oxidative stress, lowers the reduced ascorbate levels, and lowers the ATP content, and AOX activation reduces the accumulation of the ROS and to a certain extent restores redox and energy homeostasis. Through adjusting the interactions between the ascorbate-glutathione network and the electron transport chain, AOX helps plants to preserve rules and energy services throughout stresses in the environment. The results highlighting AOX as a key stress adaptatome in plants support the possibility of enhancement of stress tolerance by targeting mitochondrial respiratory manipulation.

Data Availability

Available from corresponding author on request.

Author Contributions

Sohail Akhtar: Conceptualization, Supervision, Writing – Review & Editing
Anila Hassan: Methodology, Investigation
Fiza Ahmed: Data Curation, Formal Analysis, Visualization
Khalid Mehmood: Resources, Writing – Original Draft

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Conflict of Interest

None.

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