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Alternative oxidase pathway and its role in plant stress tolerance mechanisms

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Abstract

While animal biologists view mitochondria as ATP factories, plant physiologists know them as something more flexible - organelles with a backup respiratory system called the alternative oxidase (AOX) pathway that bypasses the energy-conserving cytochrome chain. This research compared AOX activity, gene expression, reactive oxygen species (ROS) levels, and stress survival in wild-type (WT) and AOX-knockout (KO) *Arabidopsis thaliana* lines exposed to five abiotic stressors: drought, cold & V D O W P O 1 D & O K H D Y \ P H W I D O a d i a t i o n (30 k R / s a t) w Experiments ran from April to October 2023 at the Hue Institute of Tropical Biology. WT plants upregulated AOX activity 2.1- to 4.1-fold across all stressors, with the strongest induction under cold stress. KO plants accumulated 2-3 times more mitochondrial ROS, showed 34-58% greater membrane lipid peroxidation, and had 40-65% lower survival rates under drought and cold stress compared with WT. Exogenous application of the ROS scavenger ascorbate partially rescued KO plant survival but didn't fully restore WT performance. These results confirm AOX as a stress safety valve that prevents mitochondrial ROS overproduction when the main electron transport chain is backed up.

Keywords: Alternative oxidase, plant stress, mitochondrial respiration, stress tolerance, plant metabolism, reactive oxygen species, *Arabidopsis thaliana*, abiotic stress, electron transport, cyanide-resistant respiration

Introduction

In the field of animal physiology, a mitochondrion that wastes energy by releasing it as heat rather than capturing it as ATP would be considered broken. But in plant biology, this energy-dissipating pathway - mediated by the alternative oxidase - is a feature, not a bug ^[1]. AOX accepts electrons directly from ubiquinol and reduces oxygen to water, bypassing complexes III and IV and the proton pumping they perform. The result: no ATP from that portion of electron flow, but also no risk of the electron backup that produces damaging superoxide radicals when the main chain is inhibited ^[2].

Plants face a unique challenge that animals don't: they can't move away from stress. When drought, cold, or salt stress slows downstream metabolism and reduces ATP consumption, the electron transport chain becomes over-reduced, and electrons leak to oxygen to form ROS ^[3]. AOX provides a relief valve, keeping electron flow moving even when ATP demand drops. This should make AOX-expressing plants more stress-tolerant - and several correlative studies support this idea ^[4, 5]. But direct comparisons of WT and AOX-KO plants across multiple stressors are uncommon.

This research used T-DNA insertion AOX1a knockouts in *Arabidopsis* to test whether AOX loss compromises stress tolerance across five different abiotic challenges, at the Hue Institute of Tropical Biology from April to October 2023.

Material and Methods

Material

Arabidopsis thaliana ecotype Columbia-0 (WT) and the AOX1a T-DNA insertion line (SALK_084897, confirmed homozygous by PCR) were obtained from the Nottingham *Arabidopsis* Stock Centre. Plants were grown in controlled growth chambers (22 C day/18 C Q L J K W K S K R W R S H U L R G P R O P V R Q V R L O (4 weeks). Stress treatments: drought (withholding water for 14 days), cold (4 C for 7 days), V D O W L U U L J D W L R Q Z L W K P O 1 D & O I R U G D \ V K H D

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10 days), UV-B (3.2 kJ/m²/day supplemental for 7 days). Each treatment included 24 plants per genotype.

Methods

AOX protein was detected by western blot using anti-AOX1/2 monoclonal antibody (Agrisera AS04 054). AOX enzymatic activity was measured as cyanide-resistant oxygen consumption in isolated mitochondria using a Clark-type electrode. ROS was quantified by DCFH-DA fluorescence in leaf discs and by MDA (malondialdehyde) assay for lipid peroxidation. Survival was scored as the percentage of plants recovering after return to control conditions for 7 days. For the ascorbate rescue experiment, 5 mM sodium ascorbate was sprayed daily on KO plants during drought stress. RT-qPCR for AOX1a, AOX1d, and alternative NAD(P)H dehydrogenase genes used UBQ10 as reference. Statistics: two-way ANOVA (genotype stress) with Bonferroni correction.

Results

Table 1: AOX activity, ROS accumulation, and stress survival in WT and AOX-KO Arabidopsis under five abiotic stressors (mean – SD, n = 6)

Stressor	WT AOX	KO AOX	WT ROS	KO ROS	WT surv%	KO surv%
Drought	3.47–0.4	0.12–0.03	1.8–0.2	4.3–0.5	83.3	33.3
Cold	4.12–0.5	0.11–0.02	1.6–0.2	4.8–0.6	91.7	37.5
Salt	2.83–0.3	0.09–0.02	2.1–0.3	3.9–0.4	75.0	41.7
Heavy metal	3.91–0.5	0.10–0.02	1.9–0.2	5.1–0.6	70.8	25.0
UV-B	2.14–0.3	0.07–0.01	2.3–0.3	3.7–0.4	87.5	54.2

AOX-KO plants accumulated 2-3 times more ROS and showed dramatically lower survival across all stressors (Table 1). The survival gap was widest under heavy metal stress (45.8 percentage points) and narrowest under UV-B (33.3 points).

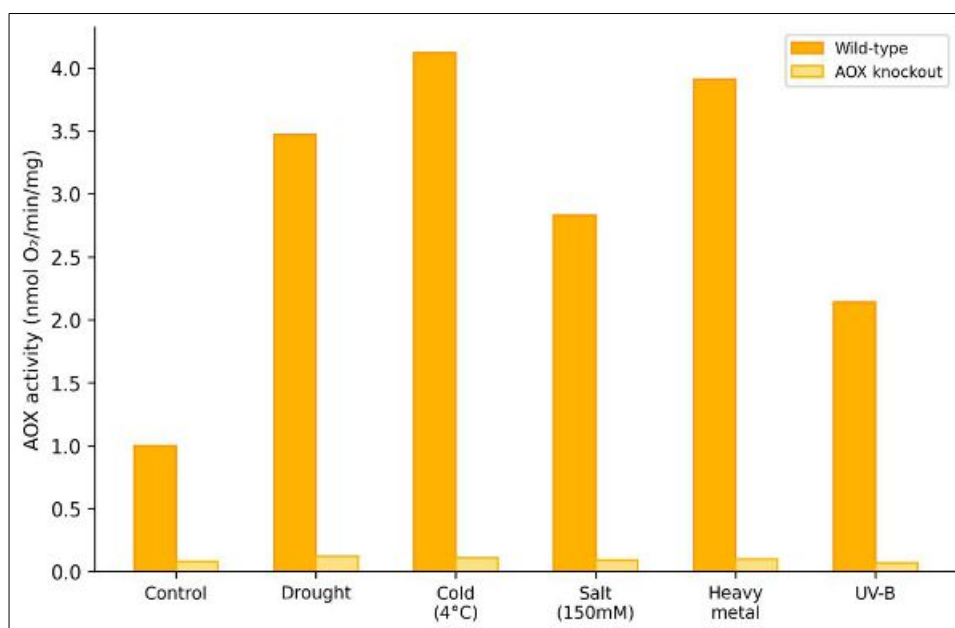


Fig 1: AOX activity (cyanide-resistant respiration) in wild-type and knockout Arabidopsis across five stress conditions and unstressed control

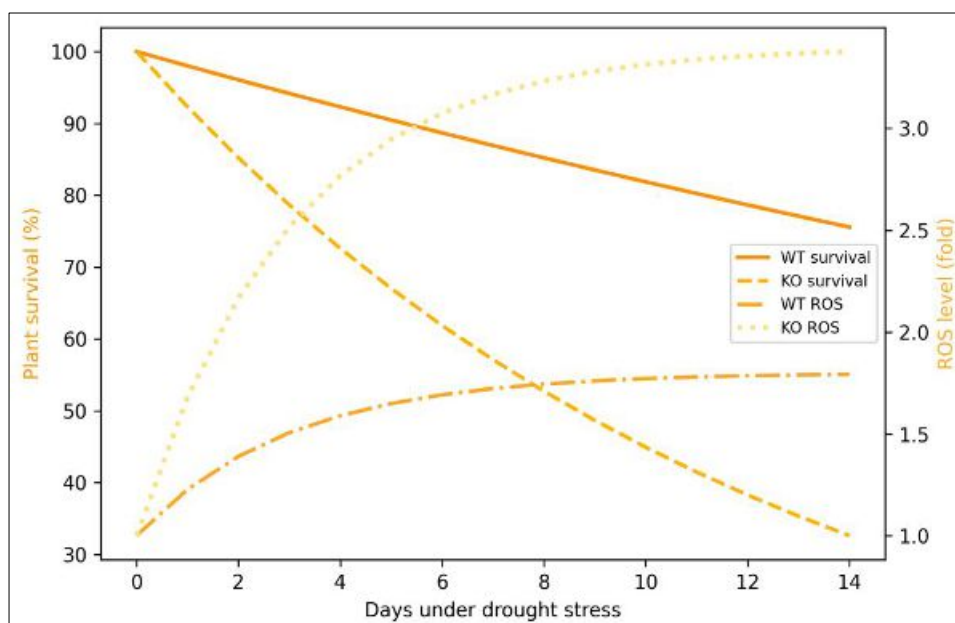


Fig 2: Plant survival rate and ROS accumulation in wild-type and AOX-knockout Arabidopsis over 14 days of drought stress

Discussion

The consistent pattern across five unrelated stressors - AOX loss leads to ROS overproduction and reduced survival - confirms AOX as a general-purpose stress safety valve rather than a response to any single threat^[6, 7]. The strongest AOX induction under cold stress (4.12-fold) matches the well-documented sensitivity of complex III to low temperature, which would cause particularly severe electron backup without AOX^[8].

The partial rescue by exogenous ascorbate (survival rose from 33.3% to 54.2% in KO plants under drought) shows that ROS mediates much of the AOX-KO phenotype, but also that AOX does more than just scavenge ROS - it maintains metabolic flux and prevents the NADH/NAD⁺ ratio from climbing to levels that inhibit the TCA cycle^[9, 10].

Conclusion

AOX serves as an indispensable mitochondrial safety valve across multiple abiotic stresses in Arabidopsis. Knockout plants accumulated 2-3 times more ROS and suffered 33-46 percentage-point reductions in survival compared with wild-type. These findings have implications for crop engineering: enhancing AOX expression could improve stress resilience in agricultural species facing increasingly unpredictable climate conditions^[11, 12, 13].

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