Alternative oxidase alleviates mitochondrial oxidative stress during limited nitrate reduction in *Arabidopsis thaliana*[1]

Daisuke Otomaru[a], Natsumi Ooi[a,b], Kota Monden[a], Takamasa Suzuki[c], Ko Noguchi[d], Tsuyoshi Nakagawa[a] and Takushi Hachiya[a†]

[a]Department of Molecular and Functional Genomics, Interdisciplinary Center for Science Research, Shimane University, 1060 Nishikawatsu-cho, Matsue, Shimane 690-8504, Japan

[b]Division of Biological Science, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan

[c]College of Bioscience and Biotechnology, Chubu University, 1200 Matsumoto-cho, Kasugai, Aichi 487-8501, Japan

[d]Department of Life Sciences, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi Hachioji, Tokyo 192-0392, Japan

[†]To whom correspondence should be addressed.

Takushi Hachiya

E-mail: takushi.hachiya@life.shimane-u.ac.jp

Tel.: +81-852-32-6288

Fax: +81-852-32-6109

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[1]AOX, Alternative oxidase; FW, Fresh weight; GO, Gene Ontology; GST, Glutathione S-transferase; mETC, Mitochondrial electron transport chain; MS, Murashige and Skoog; N, Nitrogen; ND_{ex}, Type II NAD(P)H dehydrogenases located in the cytosol; ND_{in}, Type II NAD(P)H dehydrogenases located in the matrix; NR, Nitrate reductase; PPFD, Photosynthetic photon flux density; qPCR, Quantitative PCR; ROS, Reactive oxygen species; RT, Reverse transcription

Abstract

The conversion of nitrate to ammonium, i.e., nitrate reduction, is a major consumer of reductants in plants. Previous studies have reported that the mitochondrial alternative oxidase (AOX) is upregulated under limited nitrate reduction conditions, including no/low nitrate or when ammonium is the sole nitrogen (N) source. Electron transfer from ubiquinone to AOX bypasses the proton-pumping complexes III and IV, thereby consuming reductants efficiently. Thus, upregulated AOX under limited nitrate reduction may dissipate excessive reductants and thereby attenuate oxidative stress. Nevertheless, so far there is no firm evidence for this hypothesis due to the lack of experimental systems to analyze the direct relationship between nitrate reduction and AOX. We therefore developed a novel culturing system for *A. thaliana* that manipulates activities of nitrate reduction and AOX separately without causing N starvation, ammonium toxicity, and/or lack of nitrate signal. Using this system we examined genome-wide gene expression and plant growth to better understand the relationship between AOX and nitrate reduction. Here we show that AOX alleviates mitochondrial oxidative stress and sustain plant growth under limited nitrate reduction.

Keywords: alternative oxidase; hypoxia; nitrate reduction; oxidative stress

1. Introduction

Plants use nitrate and ammonium as main sources of nitrogen (N). The conversion of nitrate to ammonium, i.e., nitrate reduction, requires eight moles of electrons per mole of nitrate, which accounts for about half of the energy required for protein synthesis from nitrate [1]. Thus, ammonium is an energetically superior N source, but most crops prefer nitrate [2]. In herbaceous plants, nitrate reduction generally occurs in the shoots [3–5], and acts as a sink of reductants generated by the chloroplasts. Hence, when the nitrate supply is limited, excessive reductants may accumulate within illuminated photosynthetic cells. Recent studies have shown that the NAD(P)H/NAD(P)$^+$ ratio and the levels of hydrogen peroxide are increased in *A. thaliana* leaves of ammonium-grown plants following nitrate restriction [6–8]. Moreover, N starvation treatments have been found to enlarge the ascorbate pool in several plant species (summarized in [9]). Taken together, these findings suggest that a decrease in nitrate reduction can lead to oxidative stress in plants.

Nitrate reduction significantly alters the action of the plant mitochondrial electron transport chain (mETC) [6]. In photosynthetic tissues, the expression and activities of several enzymes that bypass energy conservation steps in mETC are induced under limited nitrate reduction conditions; i.e., if the plant is experiencing N starvation or if ammonium is the sole N source [10–13]. One enzyme is the alternative oxidase (AOX), which allows direct electron transfer from ubiquinone to molecular oxygen, bypassing the proton-pumping complexes III and IV [14]. Therefore, upregulation of AOX under limited nitrate reduction may dissipate excessive reductants without being limited to steep proton gradients across the mitochondrial inner membrane, thereby attenuating reactive oxygen species (ROS) production and oxidative stress. Actually, in *Arabidopsis* plants under low nitrate conditions, the shoot expression of antioxidant enzyme genes was induced by disrupting the major isoform *AOX1a* [12]. Antisense suppression of *Arabidopsis AOX1a* was found to greatly decrease the reducing state of ascorbate under ammonium but not under nitrate [8]. Moreover, in barley grown under low nitrate, chemical

inhibition of AOX activity elevated the levels of hydrogen peroxide [15].

The above studies suggest that AOX is linked to nitrate reduction. However, these studies manipulated nitrate reduction activity by transferring plants to the growth conditions that included no/low nitrate or the use of ammonium as the sole N source. Reduced nitrate reduction may therefore be accompanied by N starvation or ammonium toxicity [16], causing plant growth suppression and/or the initiation of stress responses. Moreover, since nitrate acts as a signal to alter genome-wide gene expression [17], a decrease in the nitrate supply also depletes the nitrate signal. For these reasons, it is impossible to distinguish whether the above-mentioned effects of AOX deficiency are caused by reduced nitrate reduction, N starvation, ammonium toxicity, or the lack of nitrate signal. To solve this problem, we developed a novel culturing system to manipulate the degree of nitrate reduction and AOX activity while excluding the effects of N starvation, ammonium toxicity, and the lack of nitrate signal. Using this system we examined genome-wide gene expression and plant growth to better understand the relationship between AOX and nitrate reduction.

2. Materials and Methods

2.1. Plant materials and growth conditions

In this study we used *A. thaliana*, accession Columbia (Col-0) as a control line as well as the *aox1a-1* (SALK_084897 [18]), *aox1a-2* (SAIL_030_D08 [18]), *nr* (*nia1-1/chl3-5* [17]), *aox1a-1 nr*, and *aox1a-2 nr* mutants. The homozygous multiple mutants of *aox1a-1 nr* and *aox1a-2 nr* were produced via crossing. The surface-sterilized seeds were placed in the dark at 4°C for three days to break seed dormancy. For *in vitro* culturing, we first grew plants on a sterile cellophane sheet [19] placed on a solid medium containing 30 mL half-strength Murashige and Skoog (1/2-MS) salts without nitrogen supplemented with 2.5 mM $(NH_4)_2SO_4$, 0.1% (w/v) MES-KOH (pH 6.7), 0.5% (w/v) sucrose, and 0.25% (w/v) gellan gum (Fujifilm Wako, Osaka, Japan). Plants were grown horizontally at 22°C for 18 days under a fixed photosynthetic photon flux density

(PPFD) of 30 μmol m^{-2} s^{-1} (i.e., constant light). Plants-on-cellophane were then transferred to a medium containing 30 mL of 1/2-MS salts without nitrogen supplemented with 0.05% (w/v) MES-KOH (pH 5.7), 0.5% (w/v) sucrose, and 0.25% (w/v) gellan gum (Fujifilm). The resulting mixture was incubated at 22°C for 24 h, also under a PPFD of 30 μmol m^{-2} s^{-1} (i.e., constant light). Finally, the plants-on-cellophane were transferred to a medium containing 30 mL of 1/2-MS salts supplemented with 0.05% (w/v) MES-KOH (pH 5.7), 0.5% (w/v) sucrose, and 0.25% (w/v) gellan gum (Fujifilm) and then incubated at 22°C under a PPFD of 150–200 μmol m^{-2} s^{-1} (constant light). For soil cultivation, plants were grown for 14 or 24 days under a PPFD of 100–130 μmol m^{-2} s^{-1} (i.e., on a 16h/8h light/dark cycle) at 22–23°C using an Arasystem 360 kit (Betatech BVBA, Gent, Belgium) in a 1 : 1 mixture of nutrient-rich soil (Supermix A; Sakata Seed, Kyoto, Japan) and vermiculite.

2.2. Determination of in vitro nitrate reductase activities

First, we assessed *in vitro* nitrate reductase (NR) activity using a previously published method [20] with slight modification. Frozen samples were first ground in a Multi-Bead Shocker (Yasui Kikai, Osaka, Japan) using zirconia beads. The resulting powder was then mixed with 10 vol. of extraction buffer (50 mM HEPES-KOH, pH 7.6, 1 mM EDTA, 7 mM cysteine) and the extracts were centrifuged at 20,400×g at 4°C for 10 min. Next, a 30 μl aliquot of the supernatant was added to 90 μl of assay buffer (50 mM HEPES-KOH, pH 7.6, 133 μM NADH, 2 mM EDTA, 6.67 mM KNO_3). After incubation at 30°C for 2 and 17 min, 24 μl of 1% (w/v) sulfanilamide solution in 1 N HCl and 24 μl of 0.02% (w/v) *N*-(1-naphthyl)ethylenediamine dihydrochloride solution were added to 48 μl aliquots of reaction mixture. Finally, nitrite content was determined based on absorbance readings at 540 nm. *In vitro* NR activity was calculated by determining the nitrite amount produced over a 15 min period.

2.3. Determination of in vivo nitrate reductase activities

First, we submerged fresh shoots in 50 vols. of reaction buffer (i.e., 100 mM sodium phosphate buffer, pH 7.4, 10 mM KNO_3, 4% (v/v) n-propanol), followed by vacuum-infiltration. The reaction mixture was then incubated at 30˚C for 1 h in the dark. The amount of nitrate produced was then visualized by mixing the supernatant with 1% (w/v) sulfanilamide solution in 1 N HCl, and 0.02% (w/v) *N*-(1-naphthyl)ethylenediamine dihydrochloride solution in a 2 : 1 : 1 ratio.

2.4. Determination of nitrate concentration

Nitrate content was quantified as per a previously published protocol [20] with slight modifications. Nitrate was first extracted with 10 vol. of deionized water at 100°C for 20 min. Next, 10 μL of the supernatant was mixed with 40 μL of a reaction reagent (i.e., 5% (w/v) salicylic acid in concentrated sulfuric acid), and the resulting mixture was then incubated at room temperature for 20 min. A mock treatment of 40 μL of concentrated sulfuric acid was also produced. Finally, 1 mL of 8% (w/v) NaOH solution was added to the mixture, and nitrate was determined based on absorbance at 410 nm.

2.5. Determination of total protein

Total protein was determined according to a previously described method [20]. Frozen samples were first homogenized with a Multi-Beads Shocker (Yasui Kikai) using zirconia beads. Total proteins were then extracted with 10 vol. of sample buffer [2% (w/v) SDS, 62.5 mM Tris-HCl (pH 6.8), 10% (v/v) glycerol, and 0.0125% (w/v) bromophenol blue] and Halt™ protease inhibitor cocktail (ThermoFisher Scientific, Tokyo, Japan), followed by incubation at 95°C for 5 min. Extracts were then centrifuged at 20,400×g at 8°C for 10 min and 10 μL aliquots were suspended in 500 μL deionized water. Next, 100 μL 0.15% (w/v) sodium deoxycholate was added, and the mixture was incubated at room temperature for 10 min. 100 μL of 72% (v/v) trichloroacetic acid was then added, followed by incubation at room temperature for 15 min and centrifugation at 20,400×g for 10 min. Precipitates were then air-dried and suspended in 25 μL water. This

suspension was used to determine total protein concentrations using Takara BCA Protein Assay Kits (TaKaRa, Kusatsu, Japan).

2.6. Immunodetection of AOX and ACTIN proteins

12.35 µL of total protein extract was mixed with 0.65 µl of 1 M DTT, followed by incubation at 95˚C for 3 min. 5 µL of mixture (~0.43 mg fresh weight) was then subjected to SDS-PAGE using a 12% Mini-PROTEAN TGX Gel (Bio-Rad, Hercules, CA, USA) before transferring to a Trans-Blot Turbo Mini PVDF membrane using the Trans-Blot Turbo Transfer System (Bio-Rad). Membranes were incubated for 1h in blocking buffer (50 mM Tris-HCl, and 150 mM NaCl, pH 7.6, 0.1% (v/v) Tween-20) containing 5% (w/v) ECL Prime Blocking Agent (Cytiva, Tokyo, Japan). Membranes were then incubated for 1h with a 1/200 dilution of monoclonal antibodies against AOX (AS10 699, Agrisera, Vännäs, Sweden) or a 1/2,500 dilution of polyclonal antibodies against ACTIN (AS13 2640, Agrisera). Finally, the antigen–antibody complex was detected using a 1/10,000 dilution of horseradish peroxidase conjugated with anti-mouse IgG sheep antibodies (Cytiva) for AOX or anti-rabbit IgG donkey antibodies (Cytiva) for ACTIN by ECL Prime chemiluminescent detection (Cytiva). Proteins blotted on membranes were visualized using TaKaRa CBB Protein Safe Stain (TaKaRa) and chemiluminescence and CBB staining were visualized using ImageQuant LAS 500 mini (Cytiva).

2.7. RNA extraction

Samples were first homogenized as described above. Next, total RNA was extracted using an RNeasy Plant Mini Kit (Qiagen, Tokyo, Japan). For RNA-seq library preparation, RNA was purified using on-column DNase digestion (Qiagen).

2.8. RNA-seq

RNA quality was first evaluated using a Qubit RNA IQ Assay Kit (ThermoFisher Scientific).

RNA samples with RNA IQs 8.7–10.0 were used for library preparation. cDNA libraries were constructed using a NEBNext Ultra II RNA Library Prep Kit with Sample Purification Beads (New England Biolabs, Tokyo, Japan), a NEBNext Poly(A) mRNA Magnetic Isolation Module (New England Biolabs), and NEBNext Multiplex Oligos for Illumina (New England Biolabs). cDNA libraries were then sequenced using a NextSeq 500 (Illumina, Tokyo, Japan), and resulting bcl files were converted to fastq files using bcl2fastq (Illumina). Finally, obtained read counts were analyzed using iDEP version 096 [21], Metascape [22], and GeneCloud [23] using the default settings.

2.9. RT-qPCR

Reverse transcription (RT) was performed using a ReverTraAce qPCR RT Master Mix with gDNA Remover (Toyobo, Osaka Japan). Synthesized cDNA was then diluted tenfold with water and used for quantitative PCR (qPCR). RT-qPCR was performed on a QuantStudio 1 (ThermoFisher Scientific) with KOD SYBR qPCR Mix (Toyobo). Relative transcript levels were calculated using the comparative cycle threshold method with *ACTIN3* as an internal standard. Primer sequences are shown in Supplementary Table 1.

3. Results and Discussion

3.1. Manipulation of nitrate reduction and AOX activities

We manipulated the activities of nitrate reduction and AOX via the transfer experiment (Fig. 1A) using Col-0 and the mutants deficient in either or both of NR and AOX activities. First, plants were grown for 18 days with ammonium as the sole N source to ensure uniform growth independently of NR activity. Higher medium pH and lower light intensity were used to reduce ammonium toxicity and AOX expression [10,11,14,16], allowing uniform growth regardless of AOX activity. Second, plants were subjected to N starvation at pH 5.7 for 24 h to induce AOX expression [10,11]. Finally, plants were transferred to a medium containing adequate nitrate and

incubated at an elevated light intensity, which induces NR expression and fills photosynthetically-derived reductants to the cell [14,17]. The NR activity of Col-0 increased rapidly from 0 to 7 h after nitrate supply and slightly more from 7 to 24 h (Supplementary Fig. 1A). Both *in vitro* and *in vivo* NR activities were increased 7 h after nitrate supply in the shoots of Col-0 and *aox1a-1*, but not in *nr* and *aox1a-1 nr* (Fig. 1B, C). Thus, we focused on the 7 h after nitrate supply to identify the early effects of nitrate reduction for subsequent experiments. We found that nitrate accumulated in the shoots of all lines after nitrate supply, with concentrations ranging from 14.7 to 21.6 μmol g^{-1} (Fig. 1D), indicating an adequate supply of nitrate for signaling [17]. In addition, higher nitrate concentrations in shoots of *nr* and *aox1a-1 nr* than Col-0 and *aox1a-1* would reflect a deficiency in nitrate reduction. Shoot protein concentrations were comparable among all lines before and after nitrate supply, ranging from 27.0 to 29.7 mg g^{-1} (Fig. 1E), suggesting that no N starvation occurred. Western blot analysis confirmed that AOX protein signals were negligible in *aox1a-1* and *aox1a-1 nr* (Supplementary Fig. 1B), suggesting that knockout of *AOX1a* is sufficient to diminish AOX activity, as reported earlier [11,18]. Taken together, these culturing conditions permit comparisons of plants differing nitrate reduction and AOX activity while excluding the effects of ammonium toxicity, N starvation, and lack of nitrate signaling.

3.2. AOX alleviates mitochondrial oxidative stress under limited nitrate reduction

Next, to dissect the role of AOX in limited nitrate reduction, we performed three independent RNA-seq analyses using shoots from Col-0, *aox1a-1*, *aox1a-2*, *nr*, *aox1a-1 nr*, and *aox1a-2 nr* 7 h after nitrate supply (Supplementary Table 2). A k-means clustering analysis classified transcripts into four groups according to their expression patterns (Fig. 2A and Supplementary Table 3). In cluster D, *AOX1a* deficiency consistently induced gene expression in the *nr* background, but not in the Col-0 background (Fig. 2A, B). Moreover, 42 of 141 genes in cluster D were statistically significantly upregulated in *aox1a-1 nr* and *aox1a-2 nr* relative to *nr* (Fig. 2C and Supplementary Table 4). Further enrichment analyses of the 42 genes identified significant overrepresentation of the terms “toxin catabolic process,” “glutathione metabolism” (Fig. 2D),

"interpro-ipr004046 (glutathione S-transferase, C-terminal)," and "gst" (Fig. 2E). These terms were derived from the glutathione S-transferase (GST) genes *AT2G29460* (*GSTU4*), *AT1G17170* (*GSTU24*), *AT1G17180* (*GSTU25*), and *AT1G02920* (*GSTF7*) (Supplementary Table 4). *GSTU4*, a plant-specific tau class GST, may contribute to hydrogen peroxide degradation by using glutathione as an electron donor [24]. The terms "response to hypoxia" and "response to oxidative stress" were also enriched in these 42 genes (Fig. 2D). Furthermore, genes induced by hypoxia [25] and H_2O_2 treatments [26] were significantly upregulated in *nr*, and their induction was enhanced by *AOX1a* deficiency (Fig. 2F, G and Supplementary Table S5, 6). The NAC transcription factor ANAC017 mediates ROS-related mitochondrial retrograde signaling, thereby activating mitochondrial dysfunction stimulation genes including *AOX1a*, *UPOX*, and *ANAC013* [27,28]. The expression of ANAC017-inducible genes [27] and mitochondrial dysfunction stimulation genes [28] reached a maximum level in *aox1a nr and aox1a-2 nr* (Fig. 2H, I and Supplementary Table S7, 8). Further RT-qPCR analyses revealed that the expression levels of hypoxia-inducible genes (Fig. 2J–M) and oxidative stress marker genes (Fig. 2N–Q) were induced by nitrate supply in *nr*, and this induction was enhanced by *AOX1a* deficiency. Meanwhile, before nitrate supply, little difference was observed among all lines (Fig. 2J–Q). Since hypoxia elevates intracellular $NADH/NAD^+$ ratio via mETC inhibition [29], the upregulation of hypoxia-inducible genes may reflect reductant accumulation. Consequently, our transcriptome analysis suggests that AOX dissipates excessive reductants and mitigate oxidative stress under limited nitrate reduction.

The plant mETC possesses type II NAD(P)H dehydrogenases located on the cytosol side (ND_{ex}) or matrix side (ND_{in}) of the inner mitochondrial membrane [6]. Since these dehydrogenases transfer electrons to ubiquinone and bypass the proton-pumping complex I, they can dissipate excessive reductants like AOX. Of these, the expression of *NDB2*, the primary ND_{ex} contributor to NADH oxidation [6], was significantly induced in *nr* after nitrate supply, and this induction was intensified by *AOX1a* deficiency (Supplementary Fig. 2A, B). Since cytosolic NR

has a much lower K_m for NADH than ND_{ex} [10], NDB2 may operate only under limited nitrate reduction. Furthermore, the compensated induction of *NDB2* by *AOX1a* deficiency (Fig. 2A, B) suggests a significant contribution of AOX1a-NDB2 module to balance cellular redox.

3.3. AOX sustains plant growth under limited nitrate reduction

Finally, we analyzed shoot growth parameters following nitrate supply. Over 6 days, we found that *AOX1a* deficiency significantly reduced (−15%) shoot fresh weight in *nr*, but not in Col-0 (Fig. 3A). Meanwhile, rosette diameter was little affected by *AOX1a* deficiency (Fig. 3B). When plants were grown in nutrient-rich soil, shoot appearance and shoot fresh weight were decreased in *aox1a-1 nr* and *aox1a-2 nr* relative to the others (Fig. 3C–E). However, in NR-deficient plants, leaves were pale green (Fig. 3C). Thus, it is plausible that long-term limitation of nitrate reduction may cause N starvation, which complicates the interpretation of the relationship between nitrate reduction, AOX, and shoot growth.

3.4. Conclusion

We have successfully developed a cultural system to manipulate activities of nitrate reduction and AOX while excluding the effects of ammonium toxicity, N starvation, and lack of nitrate signaling. Analyses using this system suggest that AOX alleviates mitochondrial oxidative stress and sustain plant growth under limited nitrate reduction.

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CRediT authorship contribution statement

Daisuke Otomaru: Writing – review & editing, Investigation. Natsumi Ooi: Writing – review & editing, Investigation. Kota Monden: Writing – review & editing, Investigation. Takamasa Suzuki: Writing – review & editing, Investigation. Ko Noguchi: Writing – review & editing, Resources. Tsuyoshi Nakagawa: Writing – review & editing, Supervision. Takushi Hachiya: Writing – original draft, Conceptualization, Validation, Formal analysis, Visualization, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that this study was conducted in the absence of any commercial relationships that could lead to any potential conflicts of interest.

Data availability statement

The RNA-seq raw data are available in ArrayExpress under accession number E-MTAB-14027 (https://www.ebi.ac.uk/biostudies/arrayexpress).

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Figure legends

Fig. 1. Manipulation of nitrate reduction without depleting nitrate signal and organic N.

(A) Schematic diagram of experiment. (B-E) *in vitro* NR activity (B), *in vivo* NR activity (C), nitrate concentration (D), and total protein concentration (E) in plant shoots before and 7 h after nitrate supply. Two plants of each line (eight in total) per plate were grown, and two shoots were pooled as one biological replicate. Data: mean ± SD (n = 3 (B), n = 5–9 (D), n = 4 (E)). For (C), color intensity is proportional to NR activity. Different lowercase letters indicate significant differences determined via Tukey–Kramer tests at $P < 0.05$. ND and NS denote "not detected" and "not significant." FW denotes "fresh weight."

Fig. 2. Transcriptomic alteration by *AOX1a* deficiency under limited nitrate reduction conditions.

(A–I) One plant from each line (six in total) per plate was grown. Six shoots from six plates were pooled as one biological replicate. Shoots from plants 7 h after nitrate supply were subjected to RNA-seq. Numbers indicate the order of RNA-seq experiments. (A) Heat map from a k-means clustering of normalized transcript levels. Magenta and green represent higher and lower expression levels, respectively. (B) Plots of normalized transcript levels in cluster D. Normalized transcript levels of splice variants were averaged for each gene. (C) Venn diagram showing gene number from cluster D and genes significantly upregulated in *aox1a-1 nr* or *aox1a-2 nr* relative to *nr*. (D, E) Significantly enriched terms identified by Metascape (D) and GeneCloud (E) from the 42 genes in (C). (E) shows the number of genes containing the term (left) and the fold enrichment (right). (F–I) Shoot expression of genes upregulated under hypoxia (F), by H_2O_2 (G), by ANAC017 (H), and included in mitochondrial dysfunction stimulon (I) 7h after nitrate supply. Normalized transcript levels of splice variants were averaged for each gene using means from three independent experiments. (J–Q) RT-qPCR analysis of marker genes upregulated under

hypoxia (J–M) and by oxidative stress (N–Q) in shoots before and 7 h after nitrate supply. Two plants of each line (eight in total) per plate were grown, and two shoots were pooled as one biological replicate. Data: mean ± SD (n = 5).

Fig. 3. Effects of *AOX1a* deficiency on shoot growth under limited nitrate reduction conditions.

(A, B) Shoot fresh weight (A) and rosette diameter (B) of plants 6 days after nitrate supply. One plant of each line (four in total) per plate were grown and one shoot was pooled as one biological replicate. (C–E) Shoot appearance of 24-day-old plants (C) and shoot fresh weights of 14-day-old (D) and 24-day-old (E) plants grown in pots containing nutrient-rich soil. Data: mean ± SD (n = 17 (A, B), n = 18 (D), n = 7 (E)).

Figure 1

A

Procedure for transfer experiment

	Condition 1 (preculture) →	Condition 2 (preculture) →	Condition 3 (NO_3^- supply)
Duration	18 days	24 hours	0, 2, 4, 7, 24 hours or 6 days
N source	5 mM NH_4^+	No nitrogen	19.7 mM NO_3^- & 10.3 mM NH_4^+
pH	6.7	5.7	5.7
PPFD	30 µmol m^{-2} s^{-1}	30 µmol m^{-2} s^{-1}	150-200 µmol m^{-2} s^{-1}

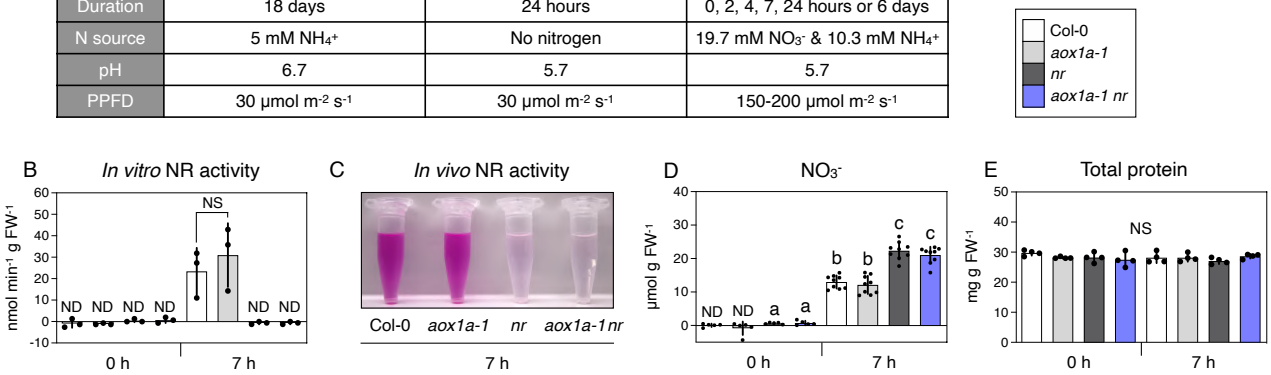


Figure 2

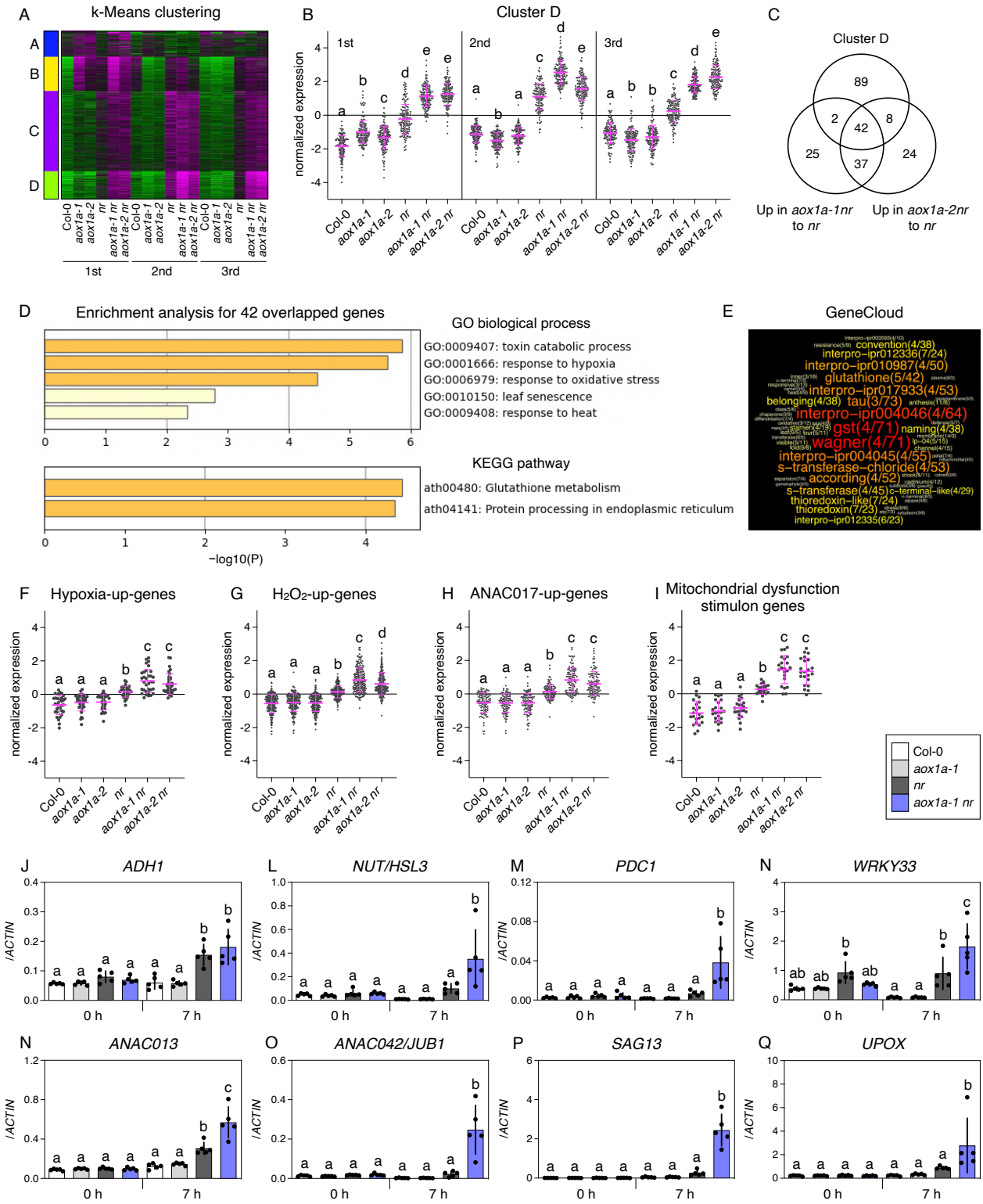
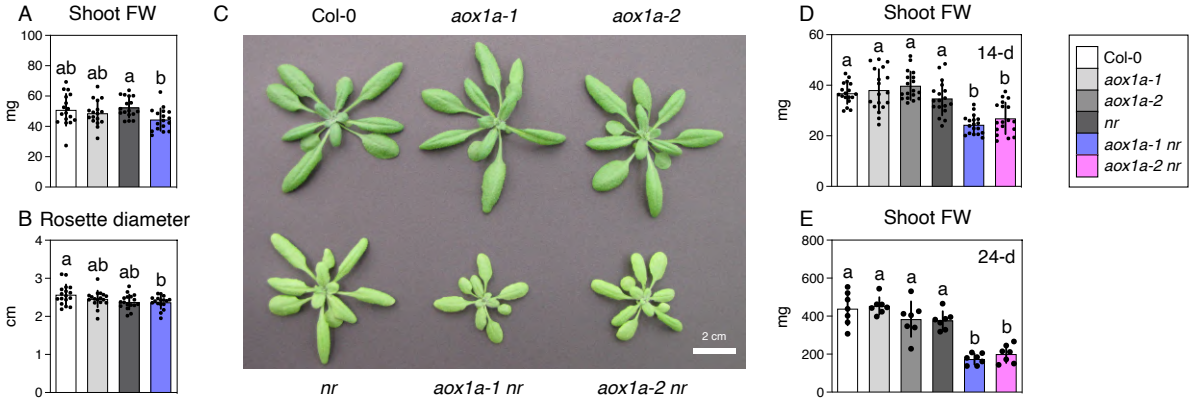

A
k-Means clustering
B
Cluster D
1st
2nd
3rd
normalized expression
C
Cluster D
89
2
8
42
25
24
37
Up in aox1a-1nr to nr
Up in aox1a-2nr to nr
D
Enrichment analysis for 42 overlapped genes
GO biological process
GO:0009407: toxin catabolic process
GO:0001666: response to hypoxia
GO:0006979: response to oxidative stress
GO:0010150: leaf senescence
GO:0009408: response to heat
KEGG pathway
ath00480: Glutathione metabolism
ath04141: Protein processing in endoplasmic reticulum
−log10(P)
E
GeneCloud
F
Hypoxia-up-genes
G
H2O2-up-genes
H
ANAC017-up-genes
I
Mitochondrial dysfunction stimulon genes
Col-0
aox1a-1
aox1a-2
nr
aox1a-1 nr
aox1a-2 nr
J
ADH1
L
NUT/HSL3
M
PDC1
N
WRKY33
N
ANAC013
O
ANAC042/JUB1
P
SAG13
Q
UPOX
/ACTIN
0 h
7 h

Figure 3


A
Shoot FW
mg
ab
ab
a
b
B
Rosette diameter
cm
a
ab
ab
b
C
Col-0
aox1a-1
aox1a-2
nr
aox1a-1 nr
aox1a-2 nr
2 cm
D
Shoot FW
14-d
mg
E
Shoot FW
24-d
mg
Col-0
aox1a-1
aox1a-2
nr
aox1a-1 nr
aox1a-2 nr