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Review

Expression and signal regulation of the alternative oxidase genes under abiotic stresses

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Plants in their natural environment frequently face various abiotic stresses, such as drought, high salinity, and chilling. Plant mitochondria contain an alternative oxidase (AOX), which is encoded by a small family of nuclear genes. *AOX* genes have been shown to be highly responsive to abiotic stresses. Using transgenic plants with varying levels of *AOX* expression, it has been confirmed that *AOX* genes are important for abiotic stress tolerance. Although the roles of AOX under abiotic stresses have been extensively studied and there are several excellent reviews on this topic, the differential expression patterns of the *AOX* gene family members and the signal regulation of *AOX* gene(s) under abiotic stresses have not been extensively summarized. Here, we review and discuss the current progress of these two important issues.

Keywords alternative oxidase; abiotic stresses; respiration; mitochondria

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Introduction

Abiotic stresses, such as drought, high salinity, and chilling, limit the growth, development, and production of plants [1]. Considering the importance of mitochondria in the energy and substance metabolism of plant cells, mitochondrial respiratory metabolism is expected to play important roles in abiotic stress tolerance.

A key to understand the role of mitochondria under abiotic stresses is to learn the differences in the features of respiratory metabolism between plant and animal mitochondria. In the plant mitochondrial electron transport chain, there is a unique component, alternative oxidase (AOX), which is located in the mitochondrial inner membrane and catalyses the alternative respiratory pathway. In higher plants, electrons produced by the respiratory oxidation of nicotinamide adenine dinucleotide can flow from ubiquinone directly to AOX and thus bypass two of the three sites of energy conservation supporting oxidative phosphorylation (complexes III and IV). This causes plants to dissipate the redox energy into heat instead of ATP production [2].

Many studies showed that most, if not all, abiotic stresses, including drought, high salinity, chilling, high light, high temperature, metal toxicity, and nutrient limitation can increase the amounts of AOX protein or mRNA [3–11]. In recent years, transgenic plants with varying levels of *AOX* gene expression have provided molecular evidence that AOX actually contributes to abiotic stress tolerance [10,12–18]. Furthermore, the following mechanisms by which AOX is involved in the tolerance of plants to abiotic stress have also been studied: (i) AOX can limit the excessive generation of reactive free radicals, such as reactive oxygen species (ROS) and reactive nitrogen species, and maintain the redox balance in plant cells [12,14,19,20]; (ii) AOX is important for optimizing photosynthesis, especially when photosynthesis is impaired by abiotic stresses [3,21,22]; (iii) AOX plays important roles in plant metabolic adaptation to abiotic stresses by modulating carbon-use efficiency and the balance of carbon and nitrogen, the NAD(P)H/ATP ratio, and the ATP/ADP ratio [7,9,23–25]. Readers who are interested in the roles and mechanisms of AOX in resisting abiotic stresses are suggested to refer to recently published papers [24,26–28]. Up to now, however, two important issues regarding the role of AOX under abiotic stresses have not been extensively summarized and reviewed. First, AOX is encoded by a small gene family in higher plants [29]. Some studies have shown that the *AOX* genes display differential expression patterns under abiotic stresses (see below). To learn which *AOX* gene(s) is/are the potential candidate(s) for selecting abiotic stress-tolerant cultivars for agronomic traits, the expression patterns of the *AOX* genes under abiotic stresses is very important. Secondly, many signal molecules related to abiotic stresses have been reported to induce *AOX* gene expression (see below), indicating that there might be a complex regulatory signaling mechanism for the induction of *AOX* gene(s) under abiotic stresses. Thus, this review will primarily focus on the expression and regulation of *AOX* gene(s) under abiotic stresses.

Expression of *AOX* Genes under Abiotic Stresses

The family of nuclear genes encoding AOX

It is known that there is a small family of nuclear genes encoding AOX in higher plants. Molecular identification studies of *AOX* genes from different plant species have revealed that there are at least two discrete *AOX* gene subfamilies, *AOX1*-type and *AOX2*-type genes. *AOX1* is present in both monocot and eudicot plant species, but *AOX2* is absent in all monocot species [29]. Many members of the *AOX* gene family have been isolated and characterized, especially in model plants and some important crops (**Table 1**). There are three *AOX* genes identified in the soybean [*AOX1*, *AOX2a* (previously named *AOX2*), and *AOX2b* (previously named *AOX3*)] [30], rice (*AOX1a*, *AOX1b*, and *AOX1c*) [31], and maize (*AOX1a*, *AOX1b*, and *AOX1c*) [32]; two *AOX* genes in tobacco (*AOX1* and *AOX2*) [33] and wheat (*AOX1a* and *AOX1c*) [34]; and five *AOX* genes in *Arabidopsis thaliana* (*AOX1a*, *AOX1b*, *AOX1c*, *AOX1d*, and *AOX2*) [35] (**Table 1**).

Differential expression of *AOX* genes under abiotic stresses

In some studies, specific probes were used to precisely show differential expression of *AOX* gene family member(s) under abiotic stresses. For example, in leaves of *A. thaliana*, the *AOX1a* gene was induced by chilling stress, while the *AOX1b*, *AOX1c*, and *AOX2* genes were not responsive. *AOX1d* even exhibited decreased expression under this stress [35]. In the roots and leaves of rice seedlings, the *AOX1a* and *AOX1b* genes were induced by chilling, drought, and high salt, whereas the *AOX1c* gene was not responsive to these stresses [36,37]. Studies in rice seedlings further showed that the *AOX1b* gene exhibited a similar expression profile to *AOX1a* under chilling, drought, and high salt, but the transcript abundance of *AOX1b* was relatively lower [36,38]. These observations indicated that there are qualitatively and quantitatively different expression patterns among different *AOX* genes under abiotic stresses. Thus, a study that only investigates the changes of total AOX protein or mRNA expression cannot reflect the actual changes of transcripts of different *AOX* genes under abiotic stresses. More importantly, considering that different isoforms of AOX proteins encoded by different *AOX* genes have different catalytic properties and activities [39], information about the changes of different *AOX* expression levels under abiotic stresses is valuable in evaluating their physiological functions.

The induction of *AOX* genes is associated with plant tolerance of abiotic stresses

Table 1 summarizes the available information about the expression of various *AOX* genes induced by abiotic stresses in plant species that are often used in plant physiology research. Among these *AOX* genes, *AOX1a* is the most intensively studied and is the most responsive to stress. In *Arabidopsis*, this gene is induced by most abiotic stresses including drought, chilling, high salt, high light, and limitation of nitrogen [4,6,23,40,41]. Furthermore, using transgenic *A. thaliana* with varying expression levels of *AOX1a*, it demonstrated that the induction of *AOX1a* under abiotic stresses is actually associated with the tolerance of plants. It was observed that *Arabidopsis* plants expressing antisense *AOX1a* showed greater susceptibility to drought [12], low temperature [13], and high light [6] compared with wild-type plants. Furthermore, *Arabidopsis* plants constitutively overexpressing *AOX1a* gene had greater tolerance to high salt and low temperature stress than wild-type plants [14,15].

In addition to *AOX1a*, other *AOX* genes are induced by abiotic stresses. For example, the transcript of *Arabidopsis AOX1b* displayed similar increase to *AOX1a* under high light stress [6]. Furthermore, compared with wild-type *Arabidopsis*, the leaves of both *AOX1a*-deficient and *AOX1b*-deficient mutants were more severely photodamaged by high light [6]. This indicates that *AOX1b*, possibly in combination with *AOX1a*, may have important benefits for the adaptation of plants to high light stress. The level of *AOX1a* mRNA in *Arabidopsis* leaves under high light was higher than that of *AOX1b* mRNA, and the photodamage in *AOX1a*-deficient mutants was also more severe than that in *AOX1b*-deficient mutants under high light [6]. These observations indicated that different *AOX* genes with quantitatively different induction levels under abiotic stresses could have different contributions to plant tolerance to abiotic stresses. In conclusion, qualitative and quantitative analysis of the expression patterns of *AOX* gene family members in various plant species and under various types of abiotic stresses are helpful in understanding the importance or contribution of different *AOX* genes to plant abiotic stress tolerance, especially when we need to learn which *AOX* gene(s) could be used as potential candidates for breeding abiotic stress-tolerant cultivars.

Possible Signal Transduction Inducing *AOX* Gene Expression under Abiotic Stresses

Reactive oxygen species

One early response of plant cells to abiotic stresses is the significant increase of the production of ROS, including singlet oxygen ($^1O_2$), the superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (HO•) [57]. It has been found that treatment with exogenous H_2O_2 or O_2^- generators can induce *AOX* expression [58,59], while the addition of H_2O_2 or O_2^- scavengers partially suppresses the induction of the AOX transcript under drought and salt stresses [60,61]. These observations

Table 1 Summary of the main observations of the expression of *AOX* genes in some representative plant species under abiotic stresses

AOX gene	Chilling	Drought	High salt	Light stress	Nutrient limitation	Ozone	Metal toxicity	Heat stress	Reference
Monocot									
Wheat									[34,42]
AOX1a	+ in leaves	nd	nd	nd	nd	nd	nd	nd	
AOX1c	+ in leaves	nd	nd	nd	nd	nd	nd	nd	
Rice									[31,36–38,43–45]
AOX1a	+ in leaves, roots, and shoot	+ in leaves, roots, and shoot	+ in leaves, roots, and shoot	− in leaves	nd	nd	nd	+ in shoot	
AOX1b	+ in leaves, roots, and shoot	+ in leaves, roots, and shoot	+ in leaves, roots, and shoot	− in leaves	nd	nd	nd	+ in shoot	
AOX1c	− in leaves, roots, and shoot	− in leaves, roots, and shoot	− in leaves, roots, and shoot	+ in leaves	nd	nd	nd	+ in shoot	
Maize									
AOX1a	nd	nd	nd	nd	nd	nd	nd	nd	
AOX1b	nd	nd	nd	nd	nd	nd	nd	nd	
AOX1c	nd	nd	nd	nd	nd	nd	nd	nd	
Dicots									
Arabidopsis thaliana									[4,6,14,35,40,41,46–50]
AOX1a	+ in leaves, root, callus, and suspension cells	+ in leaves and roots	+ in leaves, roots, and callus	+ in etiolated seedlings	+ in shoot and root by low N	nd	+ in mesophyll protoplasts by Al stress	nd	
AOX1b	− in leaves, callus, and suspension cells	− in leaves	nd	+ in etiolated seedlings	+ in shoot by low N; − in roots by low N	nd	nd	nd	
AOX1c	− in leaves; + in callus and suspension cells	nd	nd	+ in etiolated seedlings	+ in shoot by low N; − in roots by low N	nd	nd	nd	
AOX1d	↓ in leaves; − in callus	nd	+ in seedlings	+ in etiolated seedlings	+ in shoot and root by low N	nd	nd	nd	
AOX2	− in leaves, callus, and suspension cells	nd	nd	− in etiolated seedlings	+ in shoot by low N; − in roots by low N	nd	nd	nd	

Continued

Table 1 *Continued*

AOX gene	Chilling	Drought	High salt	Light stress	Nutrient limitation	Ozone	Metal toxicity	Heat stress	Reference
Soybean									[30]
AOX1	+ in suspension cells	nd	nd	nd	nd	nd	nd	nd	
AOX2a	nd	nd	nd	nd	nd	nd	nd	nd	
AOX2b	nd	nd	nd	nd	nd	nd	nd	nd	
Tobacco									[10,16,17, 51–54]
AOX1	+ in leaves	+ in leaves particularly combined with increased irradiance	+ in leaves	nd	nd	+ in leaves	+ in suspension cell by Al stress; ↓ in leaves by $CoCl_2$	nd	
AOX2	nd	nd	nd	nd	nd	+ in leaves	nd	nd	
Cowpea									[55,56]
AOX1	+ in leaves	+ in leaves; − in roots	− in roots	nd	nd	nd	nd	nd	
AOX2a	− in leaves	− in leaves	− in roots	nd	nd	nd	nd	nd	
AOX2b	+ in leaves	+ in leaves; + in roots of the drought-/salt-tolerant cultivar; − in the roots of sensitive cultivar	+ in roots of the drought-/salt-sensitive cultivar; − in the roots of tolerant cultivar	nd	nd	nd	nd	nd	

'+' indicates an increase in the transcript abundance of this *AOX* gene under the given abiotic stress; '↓' indicates a decrease in the transcript abundance of this *AOX* gene under the given abiotic stress; '−' indicates that this *AOX* gene is not responsive (or undetectable) under the given abiotic stress. 'nd' indicates that the expression of this *AOX* gene under the given abiotic stress has not been determined.

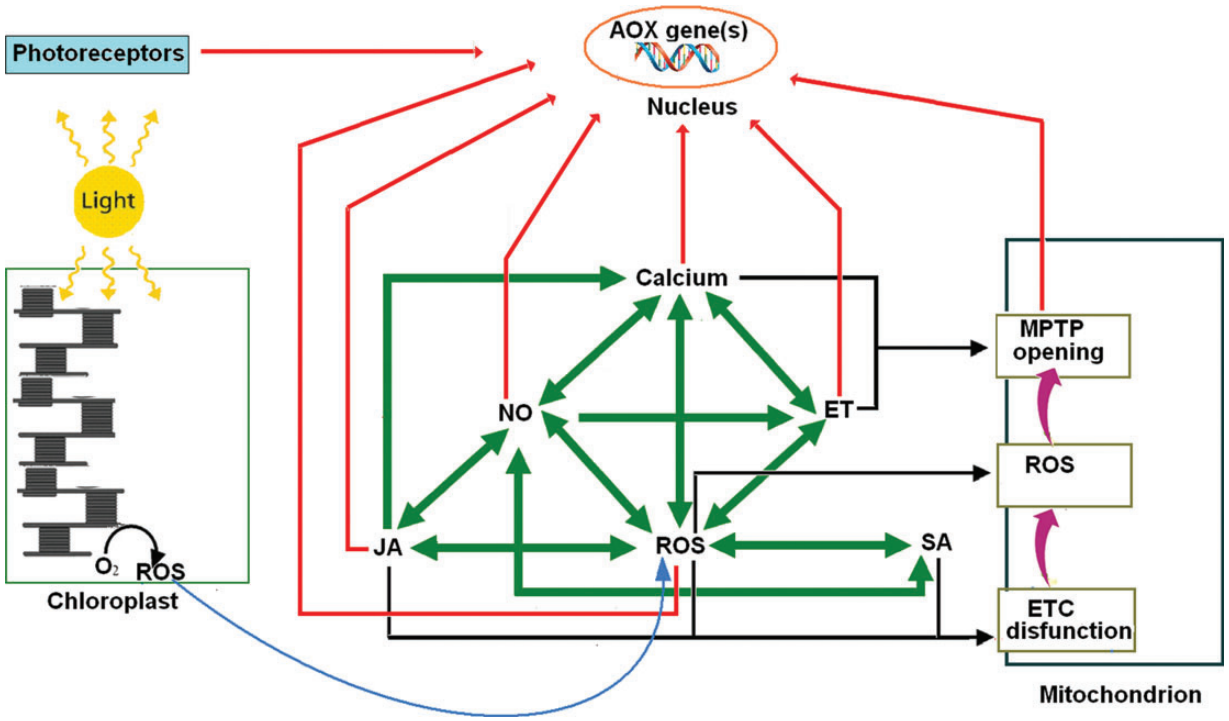

Figure 1 A working model of the potential signaling pathways for the induction of *AOX* gene(s) under abiotic stresses The model suggests that abiotic stress-induced signaling molecules, which include ROS (active oxygen species), SA (salicylic acid), NO (nitric oxide), jasmonic acid (JA), calcium, and ET (ethylene), could be involved in the induction of *AOX* gene(s) by opening the MPTP (directly or in directly) or by other mechanisms that do not involve mitochondria, as indicated by red arrows. Potentiating interactions among these signaling molecules, which are indicated by the green arrows, would report sufficient intensity of the physiological events to activate the *AOX* gene(s). Light may induce the *AOX* gene(s) by increasing ROS production (shown by blue arrow) or by photoreceptors.

suggested that ROS can initiate signaling for *AOX* gene expression under abiotic stresses.

In plant cells, O_2^- dismutates very rapidly into H_2O_2 either spontaneously or through the action of superoxide dismutase (SOD) [62]. Among these ROS, H_2O_2 has the longest half-life and the ability to permeate through cell membranes [57]. One hypothesis originally suggested that H_2O_2 is responsible for the ROS-induced expression of *AOX* genes by directly oxidizing transcription factors or modulating the phosphorylation processes of transcription factors [58,63,64]. However, exogenous H_2O_2 treatment caused a faster increase in cytoplasmic H_2O_2 than treatment with the artificial mitochondrial ROS (mtROS) generator antimycin A (a chemical inhibitor of mitochondrial electron transport). However, antimycin A caused faster induction of *AOX1* expression than exogenous H_2O_2 treatment [65]. In a recent study, it was found that mitochondrial SOD, rather than cytoplasmic and chloroplast SOD, attenuated the expression of rice *AOX1a* and *AOX1b* under conditions of drought, high salinity, and chilling [37]. These findings indicated that the mtROS production is more efficient in inducing *AOX* gene expression than cytoplasmic or chloroplastic ROS, and thus the induction of *AOX* genes can hardly be explained by the above hypothesis alone. Currently, an alternative hypothesis to explain the ROS-induced expression of *AOX* genes is that ROS can cause the opening of the mitochondrial permeability transition pore (MPTP), which is a crucial step in *AOX* gene expression [65,66]. It is supported by observations that the opening of the MPTP may be promoted by ROS [65,67], and pre-treatment with bongkrekic acid, a known inhibitor of MPTP, completely blocked the expression of *AOX* genes induced by antimycin A and H_2O_2 [65]. Such a mechanism can explain the faster induction of *AOX* genes by mtROS-inducing factors, because ROS produced directly from mitochondria is more efficient in opening the MPTP than cytoplasmic or chloroplastic ROS [65,66].

Other signal molecules

In addition to ROS, salicylic acid (SA), nitric oxide (NO), jasmonate (JA), calcium ion (Ca^{2+}), and ethylene (ET) are reported to be accumulated under abiotic stresses [68]. All these signaling molecules have the ability to induce *AOX* genes [51,69–71]. These signaling molecules may also be responsible for the induction of *AOX* genes under abiotic stresses. SA, NO, and JA have been reported to cause an increase in mtROS by inhibiting cytochrome oxidase or disrupting mitochondrial electron transport [72–74]. ET and Ca^{2+} are known to induce ROS production or directly increase mitochondrial permeability [75,76]. Thus, these signaling molecules induce *AOX* genes either by increasing ROS production or by directly affecting the MPTP (**Fig. 1**).

Table 2 Summary of the main studies of the potentiating interactions among the signaling molecules

Signal molecule	Experimental material	Event	Reference
SA and NO	*Arabidopsis* roots and cultured cells	SA treatment resulted in a strong increase of NO production	[84]
	Arabidopsis leaves	Treatment with NO resulted in accumulation of SA	[85]
SA and ROS	Tobacco leaves	After infiltration with H_2O_2, the levels of SA were increased	[86]
	Rice leaves	SA treatment resulted in an increase in the contents of endogenous H_2O_2	[87]
Calcium and NO	Maize leaves	Treatments with $CaCl_2$ induced increases in the generation of NO and the activity of nitric oxide synthase. NO donor sodium nitroprusside also led to increases in the concentration of cytosolic Ca^{2+}	[88]
Calcium and ROS	*Arabidopsis* leaves and roots	H_2O_2 triggered a $[Ca^{2+}]_{cyt}$ elevation	[81]
	Arabidopsis roots	Ca^{2+} activated the production of ROS	[82]
Calcium and ET	Tobacco suspension cells	Ethylene-releasing compound (ethephon) induced elevation of $[Ca^{2+}]_{cyt}$	[89]
	Apple fruit	Ca^{2+} stimulated ethylene production	[90]
	Apple fruit, mung bean hypocotyls	Ca^{2+} stimulated ethylene precursor, ACC, (1-aminocyclopropane-1-carboxylic acid) dependent ethylene production	
Calcium and JAs	*Arabidopsis* leaves	Exogenous application of jasmonic acid increased concentration of $[Ca^{2+}]_{cyt}$	[91]
NO and ROS	Maize leaves	NO and H_2O_2 reciprocally enhanced the production of each other	[92]
NO and JAs	*Sophora flavescens* suspension cells	Treatment with NO enhanced jasmonic acid levels. External application of jasmonic acid stimulates NO generation	[83]
NO and ET	Tobacco leaves	NO donor boosted ET accumulation, whereas ET did not induce NO emission	[51]
ROS and JAs	*Panax ginseng* suspension cells	H_2O_2 stimulated JA accumulation	[93]
	Date palm leaves	Treatment with jasmonic acid increased levels of H_2O_2	[94]
ROS and ET	*Vicia faba* leaves	Exogenous ethylene induced H_2O_2 production	[95]
	Cotton fiber	Exogenous H_2O_2 induced ethylene production	[96]

However, because NO, Ca^{2+}, JA, ET, and SA have also been reported to alter the expression of nuclear genes by other mechanisms that are not involved in ROS or mitochondria [64,77–80], it is possible that these signaling molecules can induce *AOX* genes via multiple signaling pathways that may be ROS (or mitochondria)-dependent and -independent (**Fig. 1**).

Interaction among the signaling molecules that induce *AOX* gene expression

Under abiotic stresses, ROS, SA, NO, JA, Ca^{2+}, and ET are simultaneously existed and accumulated in plant cells. Many studies have revealed that these signal molecules can interact with each other *in vivo*. For example, H_2O_2 treatment enhanced the level of cytoplasmic Ca^{2+} in *Arabidopsis* roots, while Ca^{2+} also activated the production of H_2O_2 [81,82]. Treatment with NO enhanced the level of JA level in *Sophora flavescens* suspension cells, and the external application of JA also stimulated NO generation [83]. The potentiating interactions among the signaling molecules are summarized in **Table 2** and **Fig. 1**. We suggested that the signal transduction that induces the expression of *AOX* genes under abiotic stresses could be a self-amplifying cycle, in which these signaling molecules could potentiate each other. The culmination of this self-amplifying cycle or potentiating interaction will render sufficient intensity of the physiological or biochemical events to trigger the MPTP or other mechanisms responsible for the induction of *AOX* gene expression.

Light signals

Svensson and Rasmusson [97] found AOX protein in mitochondria isolated from light-grown potato leaves, whereas its amount was dropped to an undetectable level with dark treatment. In tobacco leaves, *AOX* gene transcripts showed a marked diurnal rhythm [98]. These observations indicated that light up-regulates the expression of AOX by changes of the protein and mRNA abundance. Furthermore, in many studies, high or constant light further increased the expression levels of *AOX* genes [6,44]. It is well known that light, particularly excess light stress, can increase the leakage of electrons from the photosynthetic electron transport chain, which can reduce molecular oxygen to ROS [57]. Considering that ROS can induce the expression of *AOX* genes, the induction of *AOX* genes under light or excess light could be a result of the increase of intercellular ROS. Zhang *et al*. [6] revealed that photoreceptors, including phytochromes, phototropins, and cryptochromes, also play important roles in the light signal transduction pathway for *AOX* gene expression. Thus, it is reasonable to assume that these two mechanisms could coordinate to amplify the intensity of light signals that lead to the expression of *AOX* genes (**Fig. 1**).

Summary and Prospective

AOX is an important component of plant tolerance towards abiotic stresses. In this review, we focused on the current understanding of the expression and signal regulation of *AOX* genes under abiotic stresses. *AOX* genes under abiotic stresses show differential expression patterns and are induced by multiple signaling pathways. These characteristics may allow AOX to flexibly deal with the challenge of different types of abiotic stresses. Considering that plants in their natural environment have to face multiple, simultaneous, and inconsistent abiotic stresses, AOX could be a powerful tool to attain multiple or optimal tolerance to abiotic stresses. Ultimately, a full understanding of the significance of AOX should ideally come from research combining biological, molecular, and agricultural perspectives.

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