

Alternative oxidase, a determinant of plant gametophyte fitness and fecundity

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The alternative oxidase (AOX) is the terminal oxidase that comprises the cyanide-resistant respiratory pathway in plant mitochondria. While the role of AOX in plant thermogenesis is well established, its role in the reproductive development of non-thermogenic species is not well understood. *AOX* genes can be separated into two groups based on sequence homology, *AOX1* and *AOX2*. Reverse genetic experiments carried out primarily in *Arabidopsis* and tobacco have largely focussed on examining the role of *AOX1*-type genes in stress responses. We recently reported a systematic characterisation of the reproductive phenotypes of three *AOX2* antisense lines of soybean. This addendum summarizes the key evidence in our recent paper that points to a role for AOX in the development and function of both male and female gametophytes. Furthermore, we discuss the relative importance of AOX in the reproductive biology of plant species examined to date and highlight practical implications of our findings to crop improvement research.

The alternative oxidase (AOX) is a cyanide-resistant terminal oxidase that participates in the electron transport chain found in the inner mitochondrial membrane of numerous organisms, including plants. The free energy released by electron flow through AOX is released as heat. The liberation of heat through AOX activity underlies the prominent role of AOX in thermogenesis in the floral tissues of thermogenic plant species.¹

In non-thermogenic plant tissues, AOX has been proposed to function as an

“energy overflow” pathway, wherein AOX permits continued turnover of the tricarboxylic acid cycle when activity of the cytochrome-mediated electron transport pathway is constrained.² A second potential role of AOX is to prevent the generation of reactive oxygen species (ROS) in the mitochondria, thus protecting against oxidative stress.^{3,4} AOX may also provide a flexible control on respiratory efficiency, hence maintaining cellular energy charge under changing environmental conditions.^{5,6}

AOX is encoded by a small multigene family in plants. These genes can be separated into two groups, *AOX1* and *AOX2*, based on sequence homology. All higher plants examined carry at least one *AOX1*-type gene, but only eudicots carry the *AOX2*-type genes.^{1,7} Current understanding of the role of AOX in plants has benefited greatly from the analyses of mutants with altered AOX expression. Prior to our study, nearly all reverse genetic experiments on AOX had focused on the role of the enzyme in various stress responses, providing little information on the role of AOX in normal plant development. Moreover, studies had been restricted to the manipulation of stress responsive *AOX1*-type genes in only *Arabidopsis*,⁸ tobacco⁹ and rice,¹⁰ species that produce little AOX protein under benign growth conditions. It is uncertain whether the conclusions drawn from these studies are transferable to other plant species.

We recently characterised three soybean (*Glycine max*) lines which had been transformed with an antisense (AS) *GmAox2b* cDNA driven by the cauliflower mosaic virus 35S promoter.¹¹ Soybean AOX

Key words: alternative oxidase, soybean, plant fertility, reproductive development, gametophyte

Abbreviations: AOX, alternative oxidase; AS, antisense; *GmAox*, soybean alternative oxidase; ROS, reactive oxygen species

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is encoded by three genes, *GmAox1*, *GmAox2a* and *GmAox2b*.^{7,12} The production of AOX1 protein is induced by stress treatments that increase ROS formation, while the expression of AOX2a and AOX2b proteins is regulated in a development- and tissue-dependent manner.¹³⁻¹⁵ Hence, our research not only added to the range of species used in AOX mutant analyses, but also extended AOX mutant analyses to a developmentally-regulated *AOX2* gene type and to a species that produces much more AOX in some tissues, such as cotyledons and leaves, under benign conditions than rice, Arabidopsis or tobacco.

The paper summarized here presents four lines of evidence that argue for a role of AOX in plant fecundity and gametophyte development.¹¹

(a) Repression of AOX protein expression and AOX-dependent respiration, measured in leaves and presumably also repressed in other organs, was associated with reduced seed set. Prior to our work, evidence supporting a role for AOX in the fecundity of non-thermogenic plants was inconclusive and the work that was done was not substantiated by information about seed set, the outcome of plant sexual reproduction.

(b) Gametophyte development and function were compromised in AOX AS soybean. Increased rates of pollen abortion in AOX AS soybean indicated that AOX plays a role in pollen development, supporting previous findings on AOX-repressed tobacco.¹⁶ Furthermore, the results of in vitro pollen germination and in vivo fertilisation assays suggested that pollen of AOX AS plants is functionally compromised. A higher proportion of immature-sized, non-fertile ovules in AS flowers indicated that AOX is important to female gametophyte development, lending weight to previous speculations that AOX has a role in this reproductive process.^{17,18}

(c) The rates of ovule abortion were increased in AOX AS soybean.

(d) The non-Mendelian transgene segregation patterns among the progeny of two of the three AS lines were also suggestive of gametophytic defects.¹⁹

Our research highlights the likelihood that soybean is more dependent on AOX protein expression for reproductive development than other non-thermogenic

species, specifically, Arabidopsis, tobacco and rice. Two lines of evidence support this notion: Firstly, AOX protein was readily detected in crude membrane preparations from wild-type soybean leaves but was barely or not detectable in the wild-type leaves of Arabidopsis,⁸ rice¹⁰ or tobacco⁹ under benign growth conditions. Secondly, repression of AOX protein expression and AOX respiration compromised the reproductive phenotype of AOX AS soybean, but not that of Arabidopsis,⁸ rice¹⁰ or tobacco.⁹

Our work raises several questions for further research. Is the apparent dependence of soybean reproductive development on AOX protein expression a species-dependent response? Can the conclusions drawn from soybean be extended to other legume species, or perhaps even more widely? Generation of AOX-repressed mutants in other legume species should provide useful tools to address these questions. A good candidate for these experiments is cowpea (*Vigna unguiculata*), a legume that has two *AOX2* genes whose expression patterns resemble those of the *AOX2* genes of soybean.²⁰

Beyond the scope of AOX biology, our research is pertinent to crop improvement programs that are searching for genes that control important agronomic traits, such as seed yield. Motivated by the increasing global demand for food, numerous efforts have been made to identify candidate genes that potentially increase crop yield.²¹ Based on our findings that antisense repression of the *GmAox2b* gene impairs gametophyte development and reduces seed set in soybean, the possibility that AOX overexpression, perhaps in specific tissues, particularly in the flower, may increase seed yield is an area for future research.

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