

Plant Gene Register

Sequencing of a Soybean Alternative Oxidase cDNA Clone¹

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The alternative oxidase is a respiratory chain component of higher plants and fungi that catalyzes cyanide-insensitive oxygen consumption (Moore and Siedow, 1991). It is a quinol oxidase that does not pump protons and therefore does not contribute to, nor is it controlled by, cellular energy charge. In the floral appendages of certain plants, the oxidase contributes to thermogenesis and its expression is regulated by salicylic acid (Rhoads and McIntosh, 1992). Its function in nonthermogenic tissues is unknown, but it may serve as an overflow pathway that allows substrates to be oxidized when cell energy charge is high. This may be useful in the recycling of cofactors and the production of carbon intermediates for synthesis of cellular compounds, especially secondary metabolites. It may also serve as a protection mechanism to prevent overreduction of the ubiquinone pool and consequent production of superoxide in the mitochondrial electron transport chain. Soybean (*Glycine max* L.) shows tissue-specific expression of the alternative oxidase protein (Kearns et al., 1992) and therefore is an ideal system with which to investigate the regulation of the oxidase.

We have isolated a full-length clone from a soybean shoot cDNA library, with the characteristics outlined in Table I. The predicted amino acid sequence shows 69% homology to the *aox* clone of *Sauromatum guttatum* (Rhoads and McIntosh, 1991) and 77% to that of *Arabidopsis thaliana* (Kumar and Soll, 1992), including two conserved Cys residues. The predicted plant sequences show only 30 to 40% homology with an *aox* cDNA from *Hansenula anomala* (Sakajo et al., 1992). The description of the *aox* gene from several organisms paves the way for functional characterization of motifs essential for its function.

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The EMBL data base accession number for the sequence reported in this article is X68702.

LITERATURE CITED

Kearns A, Whelan J, Young S, Elton TE, Day DA (1992) Tissue-specific expression of the alternative oxidase in soybean and sirato. *Plant Physiol* **99**: 712-717

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Table I. Characteristics of the alternative oxidase (*aox*) cDNA clone from soybean

Organism:	<i>Glycine max</i> L. cv Williams.
Function:	Cyanide-insensitive terminal oxidase of the respiratory electron transport chain.
Genome Localization:	Nuclear encoded.
Cloning Techniques:	A 1.2-kb cDNA clone was isolated from a soybean cDNA library (Clontech Laboratories, Inc.) using an <i>aox</i> cDNA from <i>S. guttatum</i> as a probe (Rhoads and McIntosh, 1991). The insert was subcloned into pGem-3Z and overlapping restriction fragments were sequenced using the dideoxy chain termination method.
Method of Identification:	Sequence identity to predicted amino acid sequences from <i>S. guttatum</i> and <i>Arabidopsis</i> .
Features of Predicted Amino Acid Sequence:	Protein begins with five in-frame Met's and contains 321 amino acids with a predicted presequence of 42 amino acids. The mature protein has a molecular mass of 32.2 kD.
Subcellular Localization:	Mitochondrial inner membrane.
Antibodies:	Monoclonal antibodies against the <i>S. guttatum</i> protein.

Kumar AM, Soll D (1992) *Arabidopsis* alternative oxidase sustains *Escherichia coli* respiration. *Proc Natl Acad Sci USA* **89**: 10842-10846

Moore AL, Siedow JN (1991) The regulation and nature of the cyanide-resistant oxidase of plant mitochondria. *Biochim Biophys Acta* **1059**: 121-140

Rhoads DM, McIntosh L (1991) Isolation and characterisation of a cDNA clone encoding the alternative oxidase protein of *Sauromatum guttatum* (Schott). *Proc Natl Acad Sci USA* **88**: 2122-2126

Rhoads DM, McIntosh L (1992) Salicylic acid regulation of respiration in higher plants: alternative oxidase expression. *Plant Cell* **4**: 1131-1139

Sakajo S, Minagawa N, Komiyama T, Yoshimoto A (1991) Molecular cloning of cDNA for antimycin A inducible mRNA and its role in cyanide resistant respiration in *Hansenula anomala*. *Biochim Biophys Acta* **1090**: 102-108