

# Polymorphisms in alternative oxidase (AOX) genes – a useful tool to investigate genetic diversity across plant populations and species



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## INTRODUCTION

Targeting polymorphisms in candidate genes that are crucial for genotype–environment interactions will advance studies on biodiversity, population dynamics and ecological relationships. This knowledge is also fundamental to support the development of new cultivars by a non-transgenic approach using functional markers and to protect existing natural resources and regional products. Here, we focus on genomic variations in alternative oxidase (AOX) genes in carrot (*Daucus carota* L.), St John's Wort (*Hypericum perforatum* L.), olive (*Olea europaea* L.), grapevine (*Vitis vinifera* L.) and pine trees (*Pinus pinaster* L.). AOX is an inner-mitochondrial membrane protein, encoded by the nuclear genome that takes part of the respiratory chain, acting as terminal oxidase in the alternative pathway of respiration. Thus, AOX is in a central and up-stream position in energy metabolism involved in perceiving and transducing abiotic and biotic environmental stress signals (Arnholdt-Schmitt et al., 2006. Trends Plant Sci 11: 281-287).

## RESULTS

### EU Marie Curie Chair (Lab since January 2006) University of Évora



#### Our group identified in candidate AOX genes:

- Natural variability in exons, introns and 3'-UTRs, with some genes presenting an overall higher degree of polymorphisms than others
- Differences in intron sizes (intron length polymorphisms, ILPs) in individual plant genomes and between plants of the same species by EPIC-PCR (Exon-Primed-Intron-Crossing PCR)
- Insertions/deletions (InDels), single-nucleotide-polymorphisms (SNPs) and retrotransposons by sequencing and bioinformatic approaches
- Links between the occurrence of InDels and SNPs
- Differential expression related to physiological plant responses as part of the functional analysis

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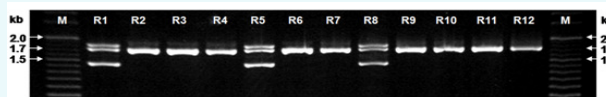
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#### Carrot alternative oxidase gene AOX2a demonstrates allelic and genotypic polymorphisms in intron 3

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*D. carota* L. shows a unique AOX genes pattern not found in other species (Costa et al., 2009. Plant Physiol Biochem 47: 753-759).

Individual plants can be discriminated by intron 3 of *DcAOX2a* length and sequence polymorphism that allows grouping of genotypes. Both events were linked.



An InDel of 286bp in intron 3 of *DcAOX2a* interferes with the predictability of a pre-miRNA.

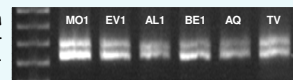
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#### Intron polymorphism pattern in AOX1b of wild St John's wort (*Hypericum perforatum*) allows discrimination between individual plants

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EPIC-PCR revealed natural variability in *HpAOX1b*. ILPs were identified at individual plant level from different locations with a common two band pattern.



Intron 1 and intron 2 of *HpAOX1b* demonstrated high sequence diversity. This was a result of InDels and SNPs. The overall polymorphic pattern distinguished individual plants.

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#### Physiologic responses and gene diversity indicate olive alternative oxidase as a potential source for markers involved in efficient adventitious root induction

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3'-UTR size (bp)	Number of clones
76	1
113	1
151	1
259	1
297	1
298	2
301	2

Highest variability was identified in the 3'-UTR region of *OeAOX2*, followed by the ORF and the intron due to SNPs and InDels.

Sequence analyses revealed differences at the 3'-UTR length in *OeAOX2*.

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#### The gymnosperm *Pinus pinaster* contains both AOX gene subfamilies, AOX1 and AOX2

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The discovery of AOX in gymnosperms supports the hypothesis that AOX1 and AOX2 arose prior to the separation of gymnosperms and angiosperms. Thus, AOX2 absence in monocots suggests a gene loss event.

PpAOX1b	DNA	PpAOX1b2		G	A	A	T	T	T	T	A	T	T	G	G	A	C	T	C	A
		PpAOX1b15		G	A	A	T	T	T	T	A	T	T	G	G	A	C	T	C	A
				194	195	204	216	244	259	268	270	278	279	311						
	A. nesi	PpAOX1b2		A	I	K	L	S	I	I	G	Y	S	A						
		PpAOX1b15		G	M	K	L	F	I	V	G	H	S	S						

Variability in exons is revealed by the existence of some SNPs. Non-synonymous and synonymous translation sites of partial *PpAOX1b* sequences were observed.

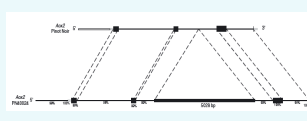
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#### The alternative oxidase family of *Vitis vinifera* reveals an attractive model to study the importance of genomic design

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Intron polymorphism can be a major source for speciation. Atypically long AOX intron lengths are related in *V. vinifera* to high levels of gene expression.



Atypical *VvAOX2* intron length variation between genotypes was caused by a retrotransposon in intron 2.

## OUR MAIN GOAL

- The establishment of phylogenetic studies and functional analysis of polymorphic regions in AOX genes that will allow exploring the significance of the identified polymorphisms for plant adaptation during evolution as a crucial base for population dynamics.
- The screening of functional polymorphic AOX markers for their potential to support marker-assisted plant breeding.

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