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

José Hélio Costa ^a, Dirce Fernandes de Melo ^a, Zélia Gouveia ^b, Hélia Guerra Cardoso ^b, Augusto Peixe ^c and Birgit Arnholdt-Schmitt ^{b,*}

^b EU Marie Curie Chair, ICAM, University of Évora, Apartado 94, 7002-554Évora, Portugal

Correspondence to * e-mail: eu_chair@uevora.pt

ABSTRACT

'Genomic design' refers to the structural organization of gene sequences. Recently, the role of intron sequences for gene regulation is being better understood. Further, introns possess high rates of polymorphism that are considered as the major source for speciation. In molecular breeding, the length of gene-specific introns is recognized as a tool to discriminate genotypes with diverse traits of agronomic interest. 'Economy selection' and 'time-economy selection' have been proposed as models for explaining why highly expressed genes typically contain small introns. However, in contrast to these theories, plant-specific selection reveals that highly expressed genes contain introns that are large. In the presented research, 'wet' Aox gene identification from grapevine is advanced by a bioinformatics approach to study the species-specific organization of Aox gene structures in relation to available expressed sequence tag (EST) data. Two Aox1 and one Aox2 gene sequences have been identified in Vitis vinifera using grapevine cultivars from Portugal and Germany. Searching the complete genome sequence data of two grapevine cultivars confirmed that V. vinifera alternative oxidase (Aox) is encoded by a small multigene family composed of Aox1a, Aox1b and Aox2. An analysis of EST distribution revealed high expression of the VvAox2 gene. A relationship between the atypical long primary transcript of VvAox2 (in comparison to other plant Aox genes) and its expression level is suggested. V. vinifera Aox genes contain four exons interrupted by three introns except for Aox1a which contains an additional intron in the 3'-UTR. The lengths of primary Aox transcripts were estimated for each gene in two V. vinifera varieties: PN40024 and Pinot Noir. In both varieties, Aox1a and Aox1b contained small introns that corresponded to primary transcript lengths ranging from 1501 to 1810 bp. The Aox2 of PN40024 (12 329 bp) was longer than that from Pinot Noir (7279 bp) because of selection against a transposable-element insertion that is 5028 bp in size. An EST database basic local alignment search tool (BLAST) search of GenBank revealed the following ESTs percentages for each gene: Aox1a (26.2%), Aox1b (11.9%) and Aox2 (61.9%). Aox1a was expressed in fruits and roots, Aox1b expression was confined to flowers and Aox2 was ubiquitously expressed. These data for V. vinifera show that atypically long Aox intron lengths are related to high levels of gene expression. Furthermore, it is shown for the first time that two grapevine cultivars can be distinguished by Aox intron length polymorphism.

Received 7 April 2009; revised 16 June 2009

DOI: 10.1111/j.1399-3054.2009.01267.x

Full article available: http://www3.interscience.wiley.com/journal/122498653/abstract

^a Department of Biochemistry and Molecular Biology, Federal University of Ceará, PO Box 6029, 60455-900, Fortaleza, Ceará, Brazil

^c Laboratory of Plant Breeding and Biotechnology, University of Évora–ICAM, Apartado 94, 7002-554Évora, Portugal