XX Congresso Brasileiro de Fruticultura
54th Annual Meeting of the Interamerican Society for Tropical Horticulture
12 a 17 de Outubro de 2008 - Centro de Convenções – Vitória/ES

BIOINFORMATIC ANALYSIS OF THE GENES CONTROLLING DEVELOPMENTAL TRANSITIONS IN GRAPEVINE

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INTRODUCTION

The physiology and metabolism of the plants are drastically affected by daily and seasonal environmental changes, which control the major developmental transitions by integrating information from endogenous time-keeping mechanism and environmental cues. In grapevine breeding programs, developmental processes like flowering time, inflorescence development, ripening initiation and length of maturation period are target traits. The timing of developmental transitions allows staggering the harvest along growing season, expanding production periods and ensuring plant adaptation to climatic and geographic conditions. These traits result from complex developmental processes controlled by the integration of genetic, physiological and environmental factors. In order to provide a molecular framework to study the genetic control of developmental traits in *Vitis*, we have identified candidate genes to temporal programming and its integrative pathways to physiology and metabolism by using combined *in silico* EST profiling and domain structural data analysis.

MATERIAL AND METHODS

Database Searches and Phylogenetic Analysis Vitis ortologs of functionally characterized regulators of the developmental transitions from model species were identified in BLAST searches (ALTSCHUL et al., 1997) against open-access databases. The resulting alignments were filtered by a threshold e value of 1^{e-15} and the hits were further analyzed according to functional domain description. Validated sequences were translated and protein alignments were performed using ClustalX (THOMPSON et al., 1997). Phylogenetic analyses were performed using the software PAUP* 4.0b10 (http://paup.csit.fsu.edu/), with default parameters. Re-sampling bootstrap trees containing 1000 random samples were constructed using PSIGNFIT software (http://www.bootstrap-software.org/).
Motif analysis and in silico characterization

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The identified orthologs were investigated for the presence and sequence conservation of recognizable functional domains described in protein analysis and gene function databases (EMBL-EBI www.ebi.ac.uk/interpro/; ExPaSy from SIB http://www.expasy.org/prosite/; GO, http://www.godatabase.org/cgi-bin/amigo/go.cgi; Pfam http://www.sanger.ac.uk/Software/Pfam/).

In silico gene expression analysis

Qualitative gene expression profiling was performed by virtual northern blot. The gene of interest was used in queries against reference databases, generating an alignment of the input gene to its paralogs. It was used to find probe-specific sequences in the mRNA input. A correlation matrix between EST contigs and libraries was generated and gene expression patterns among ESTs and libraries were obtained by hierarchical clustering based on Spearman Rank correlation matrix using the software Cluster v.2.11 (EISEN et al., 1998), by substituting the clusters by their average expression pattern. Graphic outputs were generated using Tree View v.1.6 (http://rana.lbl.gov/EisenSoftware.htm) and are presented in grayscale.

RESULTS AND DISCUSSION

In the present study, we have identified 74 *Vitis* orthologs of genes from model species that are involved in the control of the genetic programs responsible for phenological traits by *in silico* analysis (Table 1). *Vitis* genome contained orthologs of all the searched genes, although in higher number, indicating the presence of sequence conservation and differential gene expansion during evolution as observed in similar studies (MATUS et al., 2008) and in the grape genome sequencing project (JAILLON et al., 2007).

TABLE 1 - Number of *Vitis* sequences identified by BLAST searches of public databases showing sequence similarity to regulators of developmental transitions in model species.

Biological process	Developmental transition	Gene/Family	*Vitis* genes
1. Photoperception and light signaling	Germination, shade avoidance, flowering time, dormancy/release	*CRY, PHOT, PHY, ZTL*	18
2. Temperature sensing and signal transduction	Germination, flowering, fruiting dormancy control, ripening	*FLC, GI, PIF3, PFT1, CO, FT*	26
3. Endogenous time-keeping mechanism	Flowering time, dormancy/release, berry development	*CCA1/LHY, LUX/PCL1, CDF, ELF3, SOC1, FD, TOC1/PRR*	30
Total of identified genes			**74**

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The candidate sequences identified in *Vitis* were functionally assigned to gene ontology classes according to their cellular localization and molecular function in comparison to *Arabidopsis thaliana*, woody model-species *Populus* and perennial fruit tree *Citrus* expressed sequence data from public databases (Figure 1). In general, *Vitis* sequence distribution in ontology classes is more similar to *Populus* and *Citrus* than to *Arabidopsis* in agreement with previous reports (DODDAPANENI et al., 2008). In *Vitis* genome, the functional motifs of proteins involved in environmental sensing, signaling and in the endogenous time-keeping mechanism presented extensive sequence conservation in comparison to their functionally characterized counterparts in *Arabidopsis*, however differential gene family expansion events were often observed (Figure 2), suggesting an important adaptive role of these genes in grapevine throughout evolution. Similarly, *in silico* expression profiling demonstrated shared and distinct patterns of gene expression between grapevine and other species.
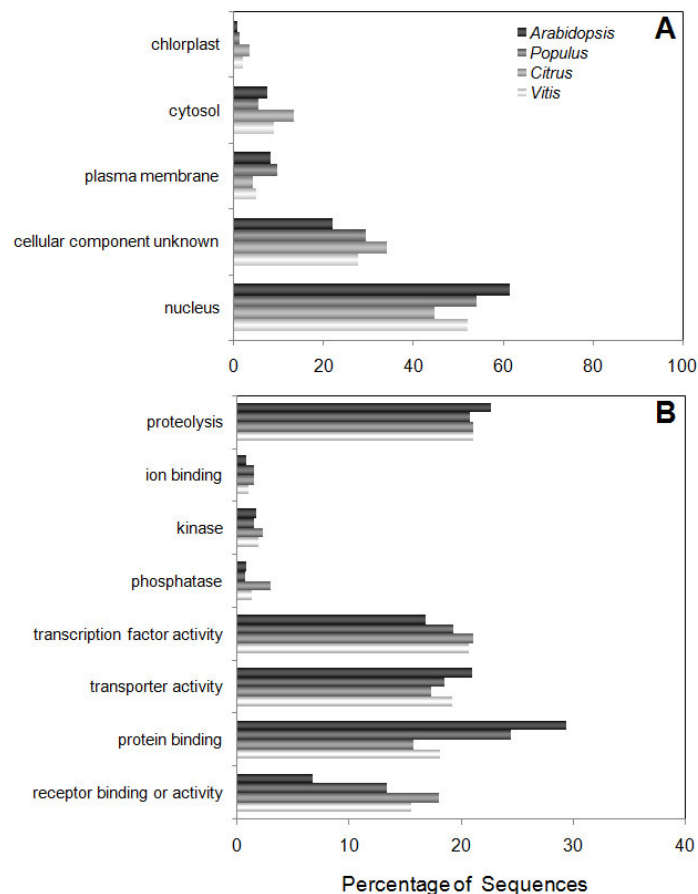

FIGURE 1 - Functional categories of *Vitis* candidate genes to control developmental transitions using gene ontology analysis: **A**, sub-cellular localization and **B**, molecular function. Assignments are based on the data available at the TIGR Gene Index version 13.0.
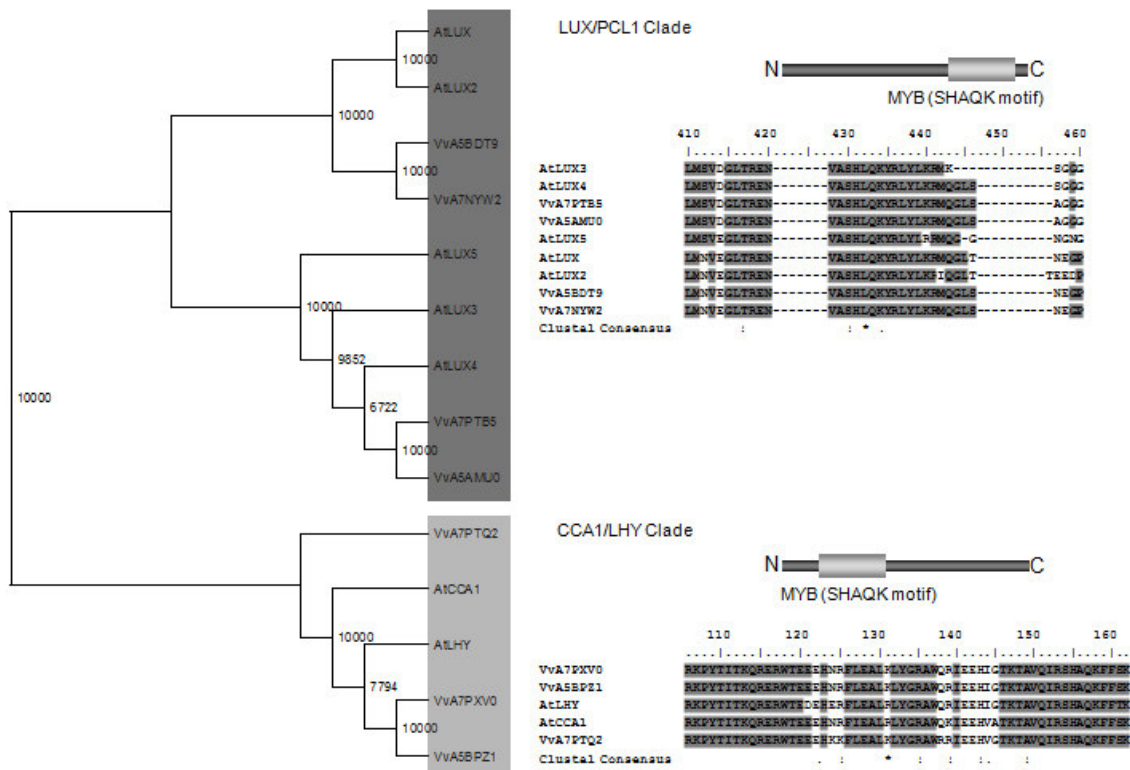
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FIGURE 2 - MYB transcription factors involved in daylenght perception in *Vitis* and *A. thaliana*. Phylogenic analysis of *Vitis* and *Arabidopsis* LUX/PCL1 and CCA1/LHY families. Clades are represented by highlighted gray boxes. Neighbor-joining tree for full length sequences were aligned with ClustalX. Bootstrap values are indicated above each branch. Schematic representation of protein domains and MYB domain alignment in *Vitis* and *A. thaliana*. At, *Arabidopsis thaliana*; Vv, *Vitis vinifera*.

CONCLUSIONS

The present work was designed to investigate the temporal programming and its integrative pathways to physiology and metabolism in grapevine, employing combined *in silico* EST profiling and domain structural data analysis. The results presented here demonstrate that the vast majority of the gene products involved in developmental control in *Arabidopsis* is present in *Vitis*. Moreover, genetic distance and domain structure analyses have uncovered extensive amino acid sequence conservation, providing indications of functional equivalence. Taken together, the results have demonstrated the feasibility of incorporating *in silico* analyses for gene discovery in non-model species.

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ACKNOWLEDGMENTS

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20080731_144716