

Los factores de transcripción Dof en pino y la regulación de la expresión génica

MARINA RUEDA LÓPEZ

Tesis Doctoral



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Departamento de Biología Molecular y Bioquímica
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AUTOR: Marina Rueda López

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ARTÍCULO 1:

***De novo* assembly of maritime pine transcriptome: implications for forest breeding and biotechnology**

Javier Canales, Rocio Bautista, Philippe Label, Josefa Gómez-Maldonado, Isabelle Lesur, Noe Fernández-Pozo, Marina Rueda-López, Dario Guerrero-Fernández, Vanessa Castro-Rodríguez, Hicham Benzekri, Rafael A. Cañas, María-Angeles Guevara, Andreia Rodrigues, Pedro Seoane, Caroline Teyssier, Alexandre Morel, François Ehrenmann, Gregoire Le Provost, Celine Lalanne, Celine Noirot, Christophe Klopp, Isabelle Reymond, Angel García-Gutiérrez, Jean-François Trontin, Marie-Anne Lelu-Walter, Celia Miguel, María Teresa Cervera, Francisco R. Cantón, Christophe Plomion, Luc Harvengt, Concepción Ávila, M. Gonzalo Claros and Francisco M. Cánovas.

Plant Biotechnology Journal (2013)

doi: 10.1111/pbi.12136

Summary

Maritime pine (*Pinus pinaster* Ait.) is a widely distributed conifer species in Southwestern Europe and one of the most advanced models for conifer research. In the current work, comprehensive characterization of the maritime pine transcriptome was performed using a combination of two different next-generation sequencing platforms, 454 and Illumina. *De novo* assembly of the transcriptome provided a catalog of 26,020 unique transcripts in maritime pine trees and a collection of 9,641 full-length cDNAs. Quality of the transcriptome assembly was validated by RT-PCR amplification of selected transcripts for structural and regulatory genes. Transcription factors and enzyme-encoding transcripts were annotated. Furthermore, the available sequencing data permitted the identification of polymorphisms and the establishment of robust single nucleotide polymorphism (SNP) and simple-sequence repeat (SSR) databases for genotyping applications and integration of translational genomics in maritime pine breeding programmes. All our data are freely available at *SustainpineDB*, the *P. pinaster* expressional database. Results reported here on the maritime pine transcriptome represent a valuable resource for future basic and applied studies on this ecological and economically important pine species.

ARTÍCULO 2:

The family of Dof transcription factors in pine

Marina Rueda-López, Ángel García-Gutiérrez, Francisco M. Cánovas and Concepción Ávila

Trees 27: 1547-1557 (2013)

10.1007/s00468-013-0903-z

Summary

The Dof proteins are members of a major family of plant transcription factors that contain a single C2/C2 class of zinc-finger DNA-binding domain. The size of gene families and the role of transcription factors in trees have only been partially addressed. We studied the size and structure of the *Dof* gene family in loblolly and maritime pines. The presence of the conserved Dof domain was a typical feature in the ten *Dof* genes identified in both pine species. Three of the genes lack intron structures and the other seven have a single intron. An analysis of putative *cis* elements in the promoter region of the genes suggests that the members of the family play different roles throughout the life cycle of the trees in a way that is unrelated to the subfamily to which they belong. Using the information available in the maritime pine database, we have cloned the corresponding open reading frames and initiated structural and functional studies. The gene expression of maritime pine Dof factors was examined at different developmental stages: from embryo to adult tree. This study has revealed a differential pattern of gene expression based on developmental stage and tissue specificity. The interaction both *in vitro* and *in vivo* between Dof factors has been addressed and our data showed a negative effect of Dof 2 in the Dof 5-regulated expression of a target gene in maritime pine. These findings suggest that both genes interact in the regulation of gene expression.

ARTÍCULO 3

Differential regulation of two glutamine synthetase genes by a single Dof transcription factor

Marina Rueda-López, Remedios Crespillo, Francisco M. Cánovas and Concepción Ávila

Plant Journal 56: 73-85 (2008)

DOI: 10.1111/j.1365-313X.2008.03573.x

Summary

The PpDof5 transcription factor from maritime pine (*Pinus pinaster*) is a regulator of the expression of glutamine synthetase (GS) genes in photosynthetic and non-photosynthetic tissues. PpDof5 mRNA is detected almost ubiquitously during pine development with low levels of gene expression in green tissues and much higher levels in roots and lignified shoots. The PpDof5 protein expressed in bacteria binds to oligonucleotide probes containing the AAAG core sequence derived from the promoters of *GS1a* and *GS1b* genes. Transient expression experiments in agroinfiltrated tobacco leaves and in pine protoplasts demonstrated that PpDof5 is able to *trans*-regulate differentially the transcription of both *GS1a* and *GS1b*. PpDof5 activated transcription of the *GS1b* promoter and, in contrast, behaved as a transcriptional repressor of the *GS1a* promoter. These results support a regulatory mechanism for the transcriptional control of the spatial distribution of cytosolic GS isoforms in pine. Considering the precise expression patterns of GS1 genes required to fulfil the ammonium assimilation requirements during tree development, we hypothesize that PpDof5 could have a key role in the control of ammonium assimilation for glutamine biosynthesis in conifers. A regulatory model of GS1 gene expression in pine is proposed.

ARTÍCULO 4

Overexpression of PpDof5 transcription factor in *Arabidopsis* leads to increased lignin and affects carbon and nitrogen metabolism

Marina Rueda-López, Rafael A. Cañas, Javier Canales, Francisco M. Cánovas and Concepción Ávila

Manuscrito en preparación

Summary

In *Arabidopsis thaliana*, over-expression of pine Dof 5, a regulator of the expression of glutamine synthetase (GS) genes in photosynthetic and non-photosynthetic tissues of maritime pine, results in a substantial increased of lignin content. We show that, in Dof 5-overexpressing plants, a simultaneous regulation of carbon and nitrogen key genes occur. In addition carbon and nitrogen partitioning in assimilatory compounds is globally spread in various secondary metabolic pathways, suggesting pleiotropic effects of Dof 5 expression on numerous bypassing pathways branching carbon and nitrogen metabolism, like C1, flavonoids or phenylpropanoids pathways. Expressing Dof 5 plants induced the up-regulation of genes encoding enzymes for sucrose and starch biosynthesis; in addition soluble sugars content was also increased.

This allows us to hypothesize about other additional regulatory functions of Dof 5 in addition to its primary role in the control of ammonium assimilation in conifers via glutamine synthetase genes.

ANEXOS

Artículo 1

Ammonium tolerance and the regulation of two cytosolic glutamine synthetases in the roots of sorghum

Redouane El Omari, Marina Rueda-López, Concepción Ávila, Remedios Crespillo, Mohamed Nhiri B and Francisco M. Cánovas

Functional Plant Biology 37: 55-63 (2010)

doi.org/10.1071/FP09162

Artículo 2

Identification of genes regulated by ammonium availability in the roots of maritime pine trees

Javier Canales, Arantxa Flores-Monterroso, Marina Rueda-López, Concepción Ávila and Francisco M. Cánovas

Amino Acids 39: 991–1001 (2010)

DOI 10.1007/s00726-010-0483-9

Artículo 3

Molecular analysis of asparagine synthetase genes in maritime pine. Novel insights into asparagine biosynthesis in conifers

Javier Canales, Marina Rueda-López, Blanca Craven Bartle, Concepción Ávila and Francisco M. Cánovas

Frontiers in Plant Science 3 (100): 1-14 (2012)

doi: 10.3389/fpls.2012.00100