Agència de Gestió d'Ajuts Universitaris i de Recerca

AGAUR

Memòria justificativa de recerca de les convocatòries BCC, BE, BP, CTP-AIRE, INEFC i PIV

La memòria justificativa consta de les dues parts que venen a continuació:
1.- Dades bàsiques i resums
2.- Memòria del treball (informe científic)

Tots els camps són obligatoris

1.- Dades bàsiques i resums

Nom de la convocatòria

BP-2009

Llegenda per a les convocatòries:

BCC	Convocatòria de beques per a joves membres de comunitats catalanes a l'exterior
BE	Beques per a estades per a la recerca fora de Catalunya
BP	Convocatòria d'ajuts postdoctorals dins del programa Beatriu de Pinós
CTP-AIRE	Ajuts per accions de cooperació en el marc de la comunitat de treball dels Pirineus. Ajuts de mobilitat de personal investigador.
INEFC	Beques predoctorals i de col·laboració, dins de l'àmbit de l'educació física i l'esport i les ciències aplicades a l'esport
PIV	Beques de recerca per a professors i investigadors visitants a Catalunya

Títol del projecte: ha de sintetitzar la temàtica científica del vostre document.

Estudi de la regulació del procés de formació de la fusta per a l'obtenció de biomassa i biocombustibles: el paper clau de dos factors de transcripció MYB amb funcions antagòniques.

Dades de l'investigador o beneficiari

Nom: Marçal

Cognoms: Soler Del Monte

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Dades del centre d'origen

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Número d'expedient

2009 BP-A 00185

Paraules clau: **cal que esmenteu cinc conceptes que defineixin el contingut de la vostra memòria.**

Regulació transcripcional, factor de transcripció, paret cel·lular secundària, fusta, eucaliptus.

Data de presentació de la justificació

24/09/2012

Nom i cognoms i signatura del/de la investigador/a

Vist i plau del/de la responsable de la sol·licitud

Marçal Soler del Monte

Resum del projecte: cal adjuntar dos resums del document, l'un en anglès i l'altre en la llengua del document, on s'esmenti la durada de l'acció

Resum en la llengua del projecte (màxim 300 paraules)

Durant l'estada de recerca de dos anys a l'LRSV (Laboratoire de Recherche en Sciences Végétales – CNRS/UPS) he pogut complir els principals objectius del projecte de recerca sobre la regulació de la formació de les parets secundàries en un arbre d'importància econòmica, l'eucaliptus. He caracteritzat dos factors de transcripció, EgMYB1 i 2, comparant el seu patró d'expressió espaciotemporal a nivell cel•lular i estudiant l'efecte fenotípic de la pèrdua de funció d'aquests gens. A més, he construït una llibreria de xilema d'eucaliptus adaptada a la tècnica dels dos híbrids i he descobert alguns del partners proteics d'EgMYB1 i 2, els quals estic actualment validant per mètodes alternatius.

Augmentant els objectius inicials de la sol•licitud, he aprofitat la recent publicació del genoma de *l'Eucalyptus grandis* per identificar i caracteritzar el patró d'expressió de vàries famílies gèniques. Gràcies a això, 4 articles científics, un dels quals com a primer autor i dos com a segon, estan a punt de ser enviats per publicació. A més, he publicat un capítol de llibre (segon autor) i he presentat els resultats en congressos internacionals en forma d'una presentació oral i un pòster.

He millorat les meves competències en les tècniques més novadores emprades en genòmica funcional, biologia molecular, genètica, microscòpia i bioinformàtica. També he supervisat dos estudiants de màster i ha enquadrat un tècnic, augmentant així les meves capacitats en la transferència de coneixement. A part d'això, he millorat les meves competències en idiomes, essent capaç de parlar i escriure fluentment en francès.

Gràcies a les qualitats científiques adquirides, m'han ofert un contracte de recerca de 18 mesos al laboratori d'acollida, per acabar de treure profit dels resultats obtinguts i per augmentar encara més les meves competències en ciència. Aquesta expertesa adquirida en un laboratori exterior hauria de permetre'm reintegrar-me al sistema de ciència i tecnologia català.

Resum en anglès (màxim 300 paraules)

During my two-year research training period in the LRSV (Laboratoire de Recherche en Sciences Végétales – CNRS/UPS) I have accomplished the main objectives of my research project dedicated to the regulation of the secondary cell wall formation in a tree of economic importance, eucalyptus. I characterized two transcription factors, EgMYB1 & 2, comparing their spatiotemporal expression pattern at tissular and cellular level and studying the phenotypic effect of their loss-of-function in transgenic lines. I also constructed a eucalyptus xylem yeast-two-hybrid library and discovered some candidate protein partners for each of the two genes, which I am currently validating by alternative methods.

Enlarging the objectives of the initial grant proposal, I took profit of the recently released *Eucalyptus grandis* genome sequence to identify and characterize the expression of several whole gene families. Four scientific papers, one as first author and two as a second author, are ready to be submitted. Besides, I also published a book chapter (second author) and I presented one oral communication and one poster in two international scientific congresses.

I mastered state-of-the-art techniques in functional genomics of secondary cell wall formation, and I considerably enlarged my competences and skills in molecular biology, genetics, microscopy and bioinformatics. In addition, I supervised two master students for their training period as well as a lab technician, improving my competences in transferring knowledge. I also acquired language skills being able to speak and write fluently French.

Thanks to my scientific qualities and competences, I was offered an 18 month-research contract from the hosting lab, in the same research field, to finish exploitation of the obtained results and to expand my competences. With all the expertise and reinforced skills acquired during this long term stay in a foreign lab, my final career's objective is to integrate the Catalan system of science and technology.

2.- Memòria del treball (informe científic sense limitació de paraules). Pot incloure altres fitxers de qualsevol mena, no més grans de 10 MB cadascun d'ells.

Thanks to the Beatriu de Pinós grant, I spent postdoctoral training period of two years (1/09/2010 to 31/08/2012) at the LRSV (Laboratoire de Recherche en Sciences Végétales – CNRS/UPS) in the "Eucalyptus Functional Genomics" team to study the regulation of the secondary cell wall formation in eucalyptus, a tree of high economical importance. The main objective of my work was to elucidate the mechanisms underlying the regulatory network ruled by two master regulators *EgMYB1 & 2* during wood formation in *Eucalyptus* and to investigate if secondary wall biosynthesis is controlled by the combinatorial action of positive and negative regulators. The completions of each of the specific objectives of this project are detailed below:

Objective 1: to get an accurate picture of the spatiotemporal expression pattern of *EgMYB1 & 2* in order to precisely localize their respective sites of expression at the cellular level.

Around 2.5 Kb of the promoters of EgMYB1 & 2 have been cloned using specific primers, designed thanks to the publication of the Eucalyptus genome sequence. These promoter regions were subsequently fused before the reporter gene GUS, and the corresponding constructs were introduced in the genome of *Arabidopsis* plants. Histochemical studies showed that both genes were expressed in vascular tissues, but meanwhile EgMYB1 is widely expressed in a large set of tissues in the plant, EgMYB2 expression pattern is much more restricted, and it is found only is some vessels and fibers in the stem, similarly as it was found by Zhong et al. (2007) when studying AtMYB46, its functional ortholog in Arabidopsis. EgMYB1 has no direct functional ortholog in Arabidopsis, but AtMYB4, the most similar gene in this plant, is expressed in many different tissues like stems, leaves, siliques and seeds, and it is thought to be involved in regulating the biosynthesis of phenylpropanoid and lignin genes (Jin et al. 2000). Therefore, and even if more detailed studies are currently running to verify this results, it seems that EgMYB1 is a general regulator of the phenylpropanoid and lignin genes including secondary cell wall formation, whereas EgMBY2 is regulating more specifically only secondary cell wall formation in vascular tissues.

Objective 2: to evaluate and compare the effect of up- and down-regulation of these two genes.

Several Arabidopsis transgenic lines carrying the full-length coding region of EgMYB1 & 2 fused with the 35S CaMV promoter and the EAR element (ethylene-responsive element binding factor-associated amphiphilic repression) have been obtained. These constructions, strongly expressed in the transgenic lines, produce modified versions of the proteins of interest that bind and block the specific cis-regulatory elements, inhibiting not only the function of the desired proteins, but also the function of the potential homolog proteins. This is especially helpful when working with multigenic families because impairs the function complementation by homolog proteins.

As a general result, dominant repression of EgMYB1 strongly impairs the growth of the plants and the secondary cell wall formation, much stronger than overexpressing the same gene. Moreover, these transgenic lines show problems in seed formation and maturation, probably by a partial male sterility of pollen grains similarly as observed in mutant lines of the close gene AtMYB32 (Preston et al. 2004). Dominant repression of EgMYB2 showed an opposed phenotype of the overexpression of this gene (Goicoechea et al. 2005), resembling somewhat the overexpression of EgMYB1 (Legay et al. 2010). It is worth noting that the complementation of the double mutant AtMYB46/AtMYB83 with EgMYB2 has been performed by another group during the course of this work strengthening the fact that EgMYB2 is indeed a master regulator controlling the biosynthesis of lignin, cellulose and hemicellulose in secondary cell walls (Zhong et al. 2010). To complement and validate these results, similar analysis will be performed in the following months using transgenic poplar lines (instead of *Eucalyptus* for the reasons cited below), which has been recently transformed and currently being regenerated.

Objective 3: to detect the direct target genes of *EgMYB1 & 2*.

To detect the direct target genes of *EgMYB1 & 2*, we used a steroid receptor-based inducible system. It consists of fusing the coding sequence of the transcription factor with a steroid binding domain and transfect cells with the construct. The transcription factor will remain sequestered in the cytoplasm by a protein complex until the application of a steroid. The steroid disrupts the complex and allows the migration of the transcription factor into the cell nucleus to modulate expression of downstream genes. By using cycloheximide, which inhibits the *de novo* protein synthesis, only transcription levels of direct target genes will be altered. As a control, the *GFP* (Green fluorescent protein), which does not affect the expression of any plant gene, will be fused to the steroid binding domain. All the constructs have been made as describe here. However, this objective has been delayed due to the difficulties arisen during the transformation of *Eucalyptus*, which impaired the obtaining of the transgenic eucalyptus suspension cells, necessary for the completion of this objective. Lot of efforts has been put in setting up an efficient protocol for carrying out this, and eventually it

was possible to get transformed suspension cells carrying an inducible system to express EgMYB1 & 2. Now, the suspension cells are growing and ready to start the induction and the first tests to ensure efficiency. It is foreseen to perform the induction and detect the expression of the direct target genes in the following year.

Objective 4: to identify and confirm the interactions of *EgMYB1 & 2* (protein partners).

The yeast-two-hybrid technology, based on testing the interaction in yeast between a given protein fused with the DNA binding domain of the transcription factor GAL4 against a library of proteins fused with the activator domain of GAL4, was used for the identification of the protein partners. First of all, I constructed a two yeast hybrid library of eucalyptus xylem proteins fused with the activator domain. Then, I fused EgMYB1 & 2 cDNAs to the binding domain of GAL4. However, the intrinsic repressive or activator domain of EgMYB1 & 2 impaired the obtaining of proper results using this technique, so truncated versions of these proteins without these domains were used. Several putative interacting proteins were identified for EgMYB1 & 2, and for the best candidates, repeatability and specificity tests were performed to avoid false positives. Co-expression of best candidates with EgMYB1 & 2 was also analyzed by qPCR and by co-localization in a transient expression experiment in *Nicotiana benthamiana* leaves. Only when both proteins co-localize in the same compartment of the cell, I selected them to validate the interaction *in vivo* using FRET-FLIM (Fluorescence Resonance Energy Transfer – Fluorescence Lifetime Imaging Microscopy). This technique, which is a powerful way to validate protein-protein interactions, measures the fluorescence emission lifetime of a fluorescent protein fused with EgMYB1 or 2, and when EgMYB1 or 2 interact with another protein fused with a compatible fluorescent protein, a transfer of energy is produced and the fluorescence emission lifetime decreases. Now, FRET-FLIM validation is being performed for a partner protein of EgMYB1, and in the following months this will be done for 3 or 4 other proteins. Since many promising results are being obtained thanks to this approach, a PhD student will start her thesis next October helping in validating the candidate partners of EgMYB1 & 2 identified, and characterizing them by a reverse genetic approach. Moreover, the incorporation of a new biochemical specialist researcher in the team (from January 2012) has been related in set up tools to study protein-protein interactions. All this together will add an increased value to the results I found.

Objective 5: to map the partners and targets genes onto *Eucalyptus* genetic maps and seek for potential QTL co-localisation.

This part of the project which has been delayed specially for the direct targets (objective 3) will be pursued next year in tight collaboration with the CIRAD FORÊT, which had already mapped EgMYB2 and shown a co-localisation with a QTL for lignin composition and content.

Side project:

Taking profit of the sequencing of the *Eucalyptus grandis* genome, I had the opportunity to enlarge the objectives initially included in the grant proposal by identifying and characterizing the expression of several whole multigenic families in *Eucalyptus*. In fact, I was charged to study the R2R3-MYB family of transcription factors, and this was the initial work that guided the other studies carried out in the lab studying other gene families. Concerning the R2R3-MYB transcription factors family, we found some subgroups apparently only present in woody species and we showed some interesting specificities from *Eucalyptus*, like that there is a big influence of tandem duplications in some subgroups related to woody species. This kind of work was especially interesting because it helped me enlarging my competences in bioinformatic tools and analysis. Moreover, I also collaborated in studying the AUX/IAA, the ARF and the lignin biosynthetic enzymes families. Besides, I collaborated in studying the best eucalyptus housekeeping genes to be used in qPCR experiments and in analyzing the effect of different nitrogen treatments in the secondary cell wall formation. All this work is nearly to be published in 4 scientific papers, one as a first author and two as a second author (this without considering the other papers referred to the grant proposal objectives, which will be prepared in the following months).

In addition, a book chapter reviewing the recent insights in the regulation of lignin biosynthesis has been recently published (second author). I also presented one oral communication and one poster in two international scientific congresses, I gave some informal seminars in the lab and in the frame of an european KBBE project and I also collaborated in other communications from the team. Besides, I also supervised two master students, teaching them not only the basics of the research we were doing and how to work in the lab, but also helping them to write scientific reports and to orally present the results. All this complementary work helped to enlarge my scientific competences, together with improving my scientific curriculum vitae, which will be much more enriched after the complete exploitation of the results obtained thanks to the Beatriu de Pinós grant. In fact, I was offered an 18 month-research contract from the hosting lab, to finish the exploitation of the results presented hereby. With all the expertise and reinforced skills acquired during this long term stay in a foreign lab, my final career's objective is to integrate the Catalan system of science and technology.

Book chapters:

Jacqueline Grima-Pettenati, Marçal Soler, Eduardo Camargo and Hua Wang (2012). Transcriptional Regulation of the Lignin Biosynthetic Pathway Revisited: New Players and Insights. In Lise Jouanin and Catherine Lapierre, editors: Advances in Botanical Research, Vol. 61, Burlington: Academic Press, 2012, pp. 173-218.

Articles submitted or in preparation for peer-reviewed journals:

Hua Wang, Marçal Soler, Hong Yu, Eduardo Camargo, Victor Carocha, Nathalie Ladouce, Jorge Pinto Paiva, Jean-Charles Leplé and Jacqueline Grima-Pettenati (submitted). Selection of reference genes for high-throughput quantitative RT-PCR analysis of gene expression in organs and tissues of *Eucalyptus* grown in various environmental conditions. BMC Plant Genomics.

Marçal Soler, Eduardo Camargo , Hua Wang, Hélène San Clemente, Nathalie Ladouce, Charles Hefer, Alexander Myburg, Jorge Pinto Paiva and Jacqueline Grima-Pettenati (in preparation). Comparative genome-wide analysis of the R2R3-MYB transcription factor family reveals extensive duplication of *Eucalyptus grandis* genes in clades specific or expanded in woody species.

Victor Carocha, Marçal Soler, Charles Hefer, Hua Wang, Alexander Myburg, Pedro Fevereiro, Jorge Pinto Paiva and Jacqueline Grima-Pettenati (in preparation). Genome-wide analyses of the ten multigene families involved in the *Eucalyptus grandis* phenylpropanoid metabolism and lignin branch pathway.

Eduardo Camargo, Leandro Costa, Marçal Soler, Marcela Salazar, Jorge Lepikson, Marcelo Carazzolle, Yves Martinez, José-Carlos Rodrigues, Ana-Carolina Deckmann, Jacqueline Grima-Pettenati and Gonçalo Pereira (in preparation). Effects of limiting and luxuriant nitrogen fertilization on xylem global gene expression and secondary cell walls in *Eucalyptus urophylla* x *E. grandis* hybrids.

Congress proceedings:

Hua Wang, Marcal Soler, Hong Yu, Eduardo Leal Oliveira Camargo, Hélène San Clemente, Bruno Savelli, Nathalie Ladouce1, Jorge Pinto Paiva and Jacqueline Grima-Pettenati. Master regulators of wood formation in *Eucalyptus*. BMC Proceedings 2011, 5(Suppl 7):P110.

Eduardo Camargo, Leandro Costa, Marçal Soler, Marcela Salazar, Jorge Lepikson, Danieli Gonçalves, Wesley Marques, Marcelo Carazzolle, Yves Martinez, Jacqueline Grima-Pettenati and Gonçalo Pereira. Effects of nitrogen fertilization on global xylem transcript profiling of *Eucalyptus urophylla* x *grandis* evaluated by RNA-seq technology. BMC Proceedings 2011; 5(Suppl 7): P106.

Oral communication in conferences

Marçal Soler, Eduardo Camargo, Hélène San Clemente H, Bruno Savelli, Nathalie Ladouce, Matthieu Bensussan, Hong Yu, Charles Hefer , Alexander Myburg, Jorge Pinto Paiva, Hua Wang and Grima-Pettenati. R2R3-MYB transcription factors regulating wood formation in *Eucalyptus*. 9th SFBV meeting, Clermont-Ferrand, France, 12-14 December 2011. Oral communication.

Posters

Marçal Soler, Eduardo Camargo, Hélène San Clemente, Bruno Savelli, Nathalie Ladouce, Matthieu Bensussan, Hong Yu, Jorge Pinto Paiva, Hua Wang and Grima-Pettenati J. *Eucalyptus* MYB transcription factors regulating wood formation. Bioenergy trees 26th New phytologist Symposium, INRA Nancy, France, 17-19 May 2011. Poster

Hua Wang, Marcal Soler, Hong Yu, Eduardo Leal Oliveira Camargo, Hélène San Clemente, Bruno Savelli, Nathalie Ladouce1, Jorge Pinto Paiva and Jacqueline Grima-Pettenati. Master regulators of wood formation in *Eucalyptus*. IUFRO Tree Biotechnology Conference 2011: From Genomes to Integration and Delivery. Arraial d'Ajuda, Brazil, 26 June - 2 July 2011. Poster.

Eduardo Camargo, Leandro Costa, Marçal Soler, Marcela Salazar, Jorge Lepikson, Danieli Gonçalves, Wesley Marques, Marcelo Carazzolle, Yves Martinez, Jacqueline Grima-Pettenati and Gonçalo Pereira (in preparation). Effects of nitrogen fertilization on global xylem transcript profiling of *Eucalyptus urophylla* x *grandis* evaluated by RNA-seq technology. IUFRO Tree Biotechnology Conference 2011: From Genomes to Integration and Delivery. Arraial d'Ajuda, Brazil, 26 June - 2 July 2011. Poster.

Other seminars given:

Finding protein-protein interactions using the Yeast-Two-Hybrid approach. Tree for Joules kick-of meeting, April 2011, Toulouse, France.

Eucalyptus R2R3 MYB transcription factors regulating wood formation. Tree for Joules meeting in Lisboa, October 2011, Lisboa, Portugal..

Looking for protein-protein interactions in wood-related transcription factors. Tree for Joules meeting in Málaga, April 2012, Málaga, Spain.

R2R3-MYB transcription factors regulating wood formation in *Eucalyptus*. Tree for Joules meeting in Málaga, April 2012, Málaga, Spain.

R2R3-MYB transcription factors regulating wood formation in *Eucalyptus*. LRSV lab day, July 2012, Toulouse, France.

References:

Goicoechea, M., Lacombe, E., Legay, S., Mihaljevic, S., Rech, P., Jauneau, A., Lapierre, C., Pollet, B., Verhaegen, D., Chaubet-Gigot, N. and Grima- Pettenati, J. (2005). EgMYB2, a new transcriptional activator from *Eucalyptus* xylem, regulates secondary cell wall formation and lignin biosynthesis. The Plant Journal 43, 553–567.

Jin, H., Cominelli, E., Bailey, P., Parr, A., Mehrtens, F., Jones, J., Tonelli, C., Weisshaar, B. and Martin, C. (2000). Transcriptional repression by AtMYB4 controls production of UV-protecting sunscreens in *Arabidopsis*. The EMBO Journal 19, 6150–6161.

Legay, S., Sivadon, P., Blervacq, A. S., Pavy, N., Baghdady, A., Tremblay, L., Levasseur, C., Ladouce, N., Lapierre, C., Se´guin, A., Hawkins, S., Mackay, J. et al. (2010). EgMYB1, an R2R3 MYB transcription factor from eucalyptus negatively regulates secondary cell wall formation in Arabidopsis and poplar. The New Phytologist 188, 774–786.

Zhong, R., Richardson, E. A. and Ye, Z. H. (2007). The MYB46 transcription factor is a direct target of SND1 and regulates secondary wall biosynthesis in *Arabidopsis*. The Plant Cell 19, 2776–2792.

Zhong, R., Lee, C. and Ye, Z. H. (2010a). Evolutionary conservation of the transcriptional network regulating secondary cell wall biosynthesis. Trends in Plant Science 15, 625–632.