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Research article

Identification of water stress genes in *Pinus pinaster* Ait. by controlled progressive stress and suppression-subtractive hybridization

Pedro Perdiguero [a,b], Carmen Collada [a,b], María del Carmen Barbero [a,b], Gloria García Casado [c], María Teresa Cervera [a,b,d], Álvaro Soto [a,b,*]

[a] *GENFOR Grupo de investigación en Genética y Fisiología Forestal, Universidad Politécnica de Madrid, E-28040 Madrid, Spain*
[b] *Unidad Mixta de Genómica y Ecofisiología Forestal, INIA/UPM, Madrid, Spain*
[c] *Centro Nacional de Biotecnología, CNB, Madrid, Spain*
[d] *Departamento de Ecología y Genética Forestal, CIFOR-INIA, Madrid, Spain*

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ABSTRACT

Climate change is a major challenge particularly for forest tree species, which will have to face the severe alterations of environmental conditions with their current genetic pool. Thus, an understanding of their adaptive responses is of the utmost interest.

In this work we have selected *Pinus pinaster* as a model species. This pine is one of the most important conifers (for which molecular tools and knowledge are far more scarce than for angiosperms) in the Mediterranean Basin, which is characterised in all foreseen scenarios as one of the regions most drastically affected by climate change, mainly because of increasing temperature and, particularly, by increasing drought.

We have induced a controlled, increasing water stress by adding PEG to a hydroponic culture. We have generated a subtractive library, with the aim of identifying the genes induced by this stress and have searched for the most reliable expressional candidate genes, based on their overexpression during water stress, as revealed by microarray analysis and confirmed by RT-PCR.

We have selected a set of 67 candidate genes belonging to different functional groups that will be useful molecular tools for further studies on drought stress responses, adaptation, and population genomics in conifers, as well as in breeding programs.

1. Introduction

Climate change is a highly topical question nowadays. In view of the current climatic forecasts for the next hundred years, the need to study the adaptive responses of living organisms is broadly acknowledged among the scientific community. This is particularly relevant for tree species, which are sessile individuals and thus cannot flee from adverse conditions. Moreover, because of their longevity, similar to the predicted time-frame for climate change, they will have to face these perturbations with the same genetic makeup they currently possess.

Conifers, which represent approximately 34% of the world's forests (up to 61%, including mixed forest) and 60% of plantations for wood production [1], display peculiar characteristics that make the study of their genetic adaptations difficult. For instance, they usually have huge genomes, with a high percentage of repeated sequences and pseudogenes whose functions are not well known. As an illustration, whereas the genomes of *Arabidopsis thaliana* and *Populus trichocarpa* are approximately 150 and 550 Mbp long, respectively, the *Pinus pinaster* genome is about 30,900 Mbp, 70–75% of which is made up of highly repeated sequences [2]. Furthermore, angiosperms diverged from gymnosperms more than 300 million years ago. Thus, to a large extent, the knowledge and molecular tools developed for the former are not fully applicable to the latter. For these reasons, the selection of candidate genes for the study of adaptation in gymnosperms based solely on their homology with angiosperm genes, without further confirmation of their participation in the stress response, is not fully reliable.

The aim of this study was to identify and select for genes involved in the response to water stress in conifers. We used the maritime pine (*P. pinaster* Ait.), which is one of the most common conifer species in the western Mediterranean basin, as a model

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* Corresponding author. GENFOR, G.I. Genética y Fisiología Forestal, Universidad Politécnica de Madrid, Ciudad Universitaria s/n, E-28040 Madrid, Spain. Tel.: +34 91 336 63 92; fax: +34 91 336 55 56.

E-mail address: alvaro.soto.deviana@upm.es (Á. Soto).

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