Molecular Plant
Research Article

CellPress
PARTNER JOURNAL

A Conserved Cytochrome P450 Evolved in Seed Plants Regulates Flower Maturation

Zhenhua Liu[1,11], Benoît Boachon[1], Raphaël Lugan[1,12], Raquel Tavares[2], Mathieu Erhardt[1], Jérôme Mutterer[1], Valérie Demais[3], Stéphanie Pateyron[4], Véronique Brunaud[5], Toshiyuki Ohnishi[6], Ales Pencik[7], Patrick Achard[1], Fan Gong[8,13], Peter Hedden[8], Danièle Werck-Reichhart[1,9,10,*] and Hugues Renault[1,9,10]

[1]Institute of Plant Molecular Biology, Centre National de la Recherche Scientifique (CNRS), University of Strasbourg, 67084 Strasbourg, France

[2]Laboratoire de Biométrie et Biologie Évolutive, Université Lyon 1, CNRS, 69622 Villeurbanne, France

[3]Plateforme d'Imagerie In Vitro, IFR 37 de Neurosciences, 67084 Strasbourg, France

[4]Transcriptomic Platform, Unité de Recherche en Génomique Végétale (URGV), INRA, Université d'Evry Val d'Essonne, CNRS, 91057 Evry, France

[5]Bioinformatics for Predictive Genomics, URGV, INRA, Université d'Evry Val d'Essonne, CNRS, 91057 Evry, France

[6]Graduate School of Agriculture, Shizuoka University, Shizuoka, 422-8529 Japan

[7]Laboratory of Growth Regulators & Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University & Institute of Experimental Botany AS CR, 771 47 Olomouc, Czech Republic

[8]Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

[9]University of Strasbourg Institute for Advanced Study (USIAS), 67084 Strasbourg, France

[10]Freiburg Institute for Advanced Studies (FRIAS), University of Freiburg, 79104 Freiburg, Germany

[11]Present address: 158 Emerson Hall, Section of Plant Biology, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853, USA

[12]Present address: Laboratoire Physiologie des Fruits et Légumes – EA 4279, Campus Agroparc, Avignon, France

[13]Present address: Home Office Science – Centre for Applied Science and Technology, Woodcock Hill, Sandridge, St Albans, Herts AL4 9HQ, UK

***Correspondence: Danièle Werck-Reichhart (daniele.werck@ibmp-cnrs.unistra.fr)**

http://dx.doi.org/10.1016/j.molp.2015.09.002

ABSTRACT

Global inspection of plant genomes identifies genes maintained in low copies across taxa and under strong purifying selection, which are likely to have essential functions. Based on this rationale, we investigated the function of the low-duplicated *CYP715* cytochrome P450 gene family that appeared early in seed plants and evolved under strong negative selection. *Arabidopsis CYP715A1* showed a restricted tissue-specific expression in the tapetum of flower buds and in the anther filaments upon anthesis. *cyp715a1* insertion lines showed a strong defect in petal development, and transient alteration of pollen intine deposition. Comparative expression analysis revealed the downregulated expression of genes involved in pollen development, cell wall biogenesis, hormone homeostasis, and floral sesquiterpene biosynthesis, especially *TPS21* and several key genes regulating floral development such as *MYB21*, *MYB24*, and *MYC2*. Accordingly, floral sesquiterpene emission was suppressed in the *cyp715a1* mutants. Flower hormone profiling, in addition, indicated a modification of gibberellin homeostasis and a strong disturbance of the turnover of jasmonic acid derivatives. Petal growth was partially restored by the active gibberellin GA_3 or the functional analog of jasmonoyl-isoleucine, coronatine. *CYP715* appears to function as a key regulator of flower maturation, synchronizing petal expansion and volatile emission. It is thus expected to be an important determinant of flower–insect interaction.

Keywords: flower development, phylogenomics, negative selection, jasmonate, gibberellins, volatile compounds

Liu Z., Boachon B., Lugan R., Tavares R., Erhardt M., Mutterer J., Demais V., Pateyron S., Brunaud V., Ohnishi T., Pencik A., Achard P., Gong F., Hedden P., Werck-Reichhart D., and Renault H. (2015). A Conserved Cytochrome P450 Evolved in Seed Plants Regulates Flower Maturation. Mol. Plant. **8**, 1751–1765.

INTRODUCTION

The availability of the first plant genomes revealed extensive duplication in some gene families, and predicted an unsuspected complexity of plant metabolism and regulation networks. Sequencing of a larger number of plant genomes brings a new outlook on the global picture and, for example, highlights some

Published by the Molecular Plant Shanghai Editorial Office in association with Cell Press, an imprint of Elsevier Inc., on behalf of CSPB and IPPE, SIBS, CAS.

genes that are under strong purifying selection with low duplication number in most plant genomes, and well conserved across plant taxa and sometimes in other organisms (De Smet et al., 2013). Such genes are most often involved in essential housekeeping functions such as DNA- or RNA-related processes, photosynthesis and plastid organization, cofactor metabolic processes, or embryonic development (De Smet et al., 2013). Although less frequent, some single-copy genes can also be found in large superfamilies encoding transcription factors or enzymes (Nelson and Werck-Reichhart, 2011; Airoldi and Davies, 2012; De Smet et al., 2013). In the latter case, a comparative genomics approach might thus support identification of genes with important developmental functions. A survey of the largest family of genes coding for metabolic enzymes, cytochromes P450 (P450s), on eight land plant genomes showed that most P450 families with essential housekeeping functions, involved for example in the biosynthesis of lignin precursors or hormone homeostasis, are present in low-copy, sometimes single-copy number, and broadly distributed across plant taxa (Nelson and Werck-Reichhart, 2011). It also indicated a few orphan P450 genes with similar characteristics.

CYP715A1 (At5g52400) is the sole member of its P450 family in *Arabidopsis thaliana*. A single CYP715 family member is also found in larger dicot or monocot genomes such as those of grapevine or rice (Nelson and Werck-Reichhart, 2011). The CYP715 family, in addition, belongs to the CYP72 clan of P450 enzymes that encompasses several families (CYP734, CYP735, CYP714) contributing to hormone homeostasis (Bak et al., 2011). CYP734s are brassinolide 26-hydroxylases, involved in the catabolism of the brassinosteroid hormones (Neff et al., 1999; Turk et al., 2003), while CYP735s catalyze hydroxylation of the isoprenoid chain of cytokinin precursors for the biosynthesis of *trans*-zeatin (Takei et al., 2004). Members of the CYP714 family were recently shown to function in gibberellin (GA) deactivation and homeostasis in rice via 16α,17-epoxidation or 13-hydroxylation (Zhu et al., 2006; Magome et al., 2013). Genetic evidence suggests that CYP714s play a similar role in *Arabidopsis* (Zhang et al., 2011; Nomura et al., 2013). The function of the CYP715 proteins, however, has not been reported. The strong selection pressure maintaining the single-copy status of *CYP715* genes and their membership of the CYP72 clan led us to postulate that they play a role in plant hormone metabolism and development. Here, we provide evidence that *CYP715A1* in *Arabidopsis* regulates petal development, floral hormone homeostasis, and volatile terpenoid emission.

RESULTS

CYP715 Is a Single or Low-Copy Gene that Evolved with Early Seed Plants

A systematic mining of genomic data available in Phytozome (http://www.phytozome.org) and in the OnekP sequencing project database (http://www.onekp.com; Matasci et al., 2014) was first carried out. It indicated a broad distribution of the CYP715 family across seed plants (Figure 1). The CYP715 family is detected in all spermatophytes (i.e. seed plants) including gymnosperms and angiosperms (Nelson and Werck-Reichhart, 2011). The CYP714 family, which is found exclusively in angiosperms, seems to have a more recent origin. In most cases (21 out of 32), a single CYP715 member could be retrieved for each taxon. In some of them, however, usually plants that have undergone recent whole-genome duplications, a few gene duplicates can be found, for example in Fabaceae (i.e. legumes), with up to six copies in the paleoploid soybean genome (Schmutz et al., 2010) (Figure 1). Single-copy genes also usually exhibit high sequence conservation (De Smet et al., 2013). To investigate the selection regimes acting on the remarkably few CYP715 genes, we calculated the ratios of non-synonymous to synonymous substitutions ($\omega = d_N/d_S$) in the whole family using the one-ratio model from PAML software (Yang, 2007). This model assumes the same ω value for all the lineages. The calculated ω of 0.11781 indicates a strong purifying selection for the CYP715 family in angiosperms (Supplemental Table 1). Furthermore, site models in PAML allowing the ω ratio to vary among sites were tested using the nearly neutral model (M1a) and the selection model (M2a). Within both models, 85% of the sites have an ω value of 0.09073 and nearly 15% of the sites have an ω value of 1. No sites under positive selection were significantly identified on the CYP715 sequences (Supplemental Table 1). The CYP715 family thus seems to have evolved under high purifying selection. The reasons for such high conservation and negative selection pressure were investigated by studying the function of *CYP715A1* in *A. thaliana*.

CYP715A1 Expression Is Restricted to Anther Filaments and Tapetal Cells

CYP715 mutants have not so far emerged from genetic screens with a major impact on plant development or viability. It was therefore necessary to focus on particular developmental stages and tissues for a functional analysis of *CYP715* using mutants. A survey of publicly available transcriptome data (http://www-ibmp.u-strasbg.fr/~CYPedia/CYP715A1/CoExpr_CYP715A1_Organs.html) and a qRT–PCR analysis (Figure 2A–2C) indicated flowers as the main site of *CYP715A1* expression. *CYP715A1* was highly expressed at anthesis (Figure 2B) and in stamens of mature flowers (Figure 2C). Plants transformed with a $CYP715A1_{pro}$:*GUS* fusion construct further revealed restricted and tissue-specific expression in the tapetal cells during pollen development (flower stages 5–9; Figure 2D–2F) and in the anther filament upon flower maturation (flower stages 12–15; Figure 2D and 2G). Staining did not reveal any gene expression in other organs of the plant grown under standard conditions.

CYP715A1 Regulates Petal Development and Intine Deposition

The two *cyp715a1* T-DNA insertion mutants (*cyp715a1-1* and *cyp715a1-2*) and a *CYP715A1* overexpressor line (OE-2) (see Supplemental Figure 2 for molecular validation) did not display any significant alteration of whole plant development and architecture. Focus on flower development, however, revealed a striking inhibition of petal growth in both *null* mutants, with reduced petal surface area (Figure 3A–3D, 3I) associated with reduced cell size (Figure 3E–3G, and 3J). Absence of curvature of the shortened petals and defective flower opening (Figure 3B–3D) was typical of mutations affecting petal growth. The petal growth phenotype could be rescued when plants