Roles of cytochromes P450 in plant reproductive development

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Abbreviations:

ABA: Abscisic acid
BR: Brassinosteroid
CK: Cytokinin
CL: Carlactone
CR: Campesterol
CYP: Cytochrome P450 monooxygenase
GA: Gibberellic acid
IAOx: Indole-3-acetaldoxime
JA: Jasmonic acid
SAM: Shoot Apical Meristem
SLs: Strigolactones
Trp: Tryptophan

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Abstract

The cytochrome P450 superfamily is a large enzymatic protein family that is widely distributed along diverse kingdoms. In plants, CYPs participate in a vast array of pathways leading to the synthesis and modification of multiple metabolites with variable and important functions during different stages of plant development. This includes the biosynthesis and degradation of a great assortment of compounds implicated in a variety of physiological responses such as signaling and defense, organ patterning and the biosynthesis of structural polymers among others. In this review we summarize the characteristics of the different families of plant CYPs, focusing on the most recent advances in elucidating the roles of CYPs in plant growth and development and more specifically, during plant gametogenesis, fertilization and embryogenesis.

Cytochromes P450 in plants: Diversity and classification

Cytochrome P450 monooxygenases (CYPs) are widely distributed in all domains of organisms such as plants, animals, fungi, protists, bacteria, archaea, and even viruses (Lamb et al., 2009). Although they share low sequence identity, they show a common three dimensional structure. They all present a proline-rich membrane hinge, an I-helix oxygen binding domain, a K-helix and a “PERF” consensus, which is involved in locking the heme pocket (Graham and Peterson, 1999). CYPs catalyze extremely diverse reactions and they are involved in numerous biosynthetic and xenobiotic pathways with distinct and complex functions.

Cytochrome P450 encoding genes are present in the nine plant taxa, as are found in algae, liverworts, hornworts, mosses, lycophytes, ferns, gymnosperms, non-eudicot angiosperms and eudicots (Nelson 2018). The CYP family is one of the largest gene families in plants. It includes 39 genes in Chlamydomonas reinhardtii (Nelson 2006), 71 genes in Physcomitrella patens (Nelson 2006), 244 genes and 28 pseudogenes in Arabidopsis (Figure 1,(Bak et al., 2011)), and 332 full-length genes and 378 pseudogenes in soybean (Guttikonda et al., 2010). The large number of CYPs is associated with the biosynthesis of a great assortment of metabolites implicated in a variety of physiological responses such as signaling and defense, with the biosynthesis of important structural polymers and with the emergence of complex anatomical structures. It is currently accepted that P450s diversification had a significant biochemical impact on the emergence of new metabolic pathways during the evolution of land plants. In accordance, the numbers of CYPs genes in plant genomes has increased through evolution.
CYP genes from all organisms are named based on protein sequence identity and phylogeny. As mentioned before, sequence identity among CYPs can be very low (less than 20% in Arabidopsis (Bak et al., 2011). P450s from the same family typically share at least 40% identity, and at least 55% identity within a subfamily. When gene duplication is detected, which is common in plants, family assignment is based on phylogeny and gene organization (Nelson and Werck-Reichhart, 2011). Based on phylogenetic classification, a type of CYP is distinguished for its family number and subfamily letter. Orthologs in different species shared numbers and unique numbers are used for paralogs in the same species.

Based on reported sequences, members of the CYPs gene superfamily in plants were grouped into ten clans (Nelson and Werck-Reichhart, 2011). Four of these clans include multifamily members and are named taking into account the lowest-numbered family member. They are CYP71, CYP72, CYP85 and CYP86. The remaining clans are named according to their family number: CYP51, CYP74, CYP97, CYP710, CYP711 and CYP727. CYP727 is only present in monocots (Nelson and Werck-Reichhart, 2011).

Physiological and metabolic functions of plants cytochromes P450

Plant CYPs participate in a variety of biochemical pathways leading to the production of multiple metabolites such as terpenoids, alkaloids, glucosides and a variety of hormones that allow plants to cope with variable conditions at different stages of development (Bak et al., 2011). Although some CYP families are involved in specific pathways (Table 1, (Bak et al., 2011, Banerjee and Hamberger, 2018, Bell, 2019, Bishop and Koncz, 2002, Ehling et al., 2008, Franke et al., 2012, Ghosh, 2017, Höfer et al., 2008, Kim et al., 2009, Kim et al., 2005b, Koch et al., 2013, Ma and Tredway, 2013, Mathur et al., 1998, Morikawa et al., 2009, Nakamura et al., 2005, Pollmann et al., 2019, Quinlan et al., 2012, Sauveplane et al., 2009, Schaller and Stintzi, 2009, Stumpe and Feussner, 2006)), many CYPs from different families are known to participate catalyzing multiple steps in a common metabolic pathway. Some examples of this include the synthesis of camalexin in Arabidopsis, involving CYP79B2, CYP79B3, CYP71A12, CYP71A13 and CYP71B15 (Mucha et al., 2019) and the GA biosynthetic pathway from ent-kaurenoic acid involving CYP88 members and CYP701 (Helliwell et al., 2001). Similarly, the metabolism of important compounds such as JA, BR, glucosinolate, lutein and terpenoid, is also regulated by CYPs from different families (Guo et al., 2013).

CYPs play important roles in plant defense against pathogens and herbivores through their involvement in the synthesis of antimicrobial compounds and toxins, such as phytoalexins, which are compounds synthetized in plants in response to pathogen attack. Arabidopsis plants synthetize camalexin in a
pathway that involves five P450 enzymes (Glawischnig, 2006). Other examples of phytoalexins include diterpenoids from rice (Bathe and Tissier, 2019), glyceollins from soybean (Kinzler et al., 2016) and serotonin, produced in wheat (Du Fall and Solomon, 2013). CYPs are also involved in the biosynthesis of jasmonic acid (JA), a phytohormone that plays important roles in the plant response after wounding and biotic attacks (Glausser et al., 2008, Koo et al., 2014). CYP79D6 and CYP79D7 from poplar participate in the synthesis of volatile compounds such as aldoximes, which not only repel herbivores but also attract herbivore predators (Irmisch et al., 2013). In conifers, CYPs participate in the synthesis of resin acids, which are also involved in insect defense (Hamberger et al. 2011). The synthesis of alkaloids and derivatives with antimicrobial action is also dependent on CYPs activities in diverse species (Ikezawa et al., 2003).

So CYPs are involved in the synthesis of a plethora of defensive signaling molecules, most of them involved in innate immunity. However, they are also crucial to protect plants from abiotic stresses. CYPs are important players in detoxification, responding to heavy metal salts and herbicides (Rai et al., 2015). In addition, they are required for dehydration tolerance and in response to osmotic stress. Several CYPs are involved in Abscisic acid (ABA) metabolism. ABA is a sesquiterpene phytohormone that controls numerous adaptive responses to environmental stresses. CYP707A, which encodes an ABA 8’-hydroxylase, modulates ABA contents in Arabidopsis and barley and responds specifically to drought stress (Kushiro et al., 2004). Members of the CYP75 and CYP93 families are flavonoid biosynthetic enzymes. Flavonoids are phytochemical compounds with ultraviolet-absorbance properties and antioxidant activities, conferring stress tolerance to a broad number of plant species (Tohge et al., 2018, Yonekura-Sakakibara et al., 2019). The high number and variability of CYP proteins responding to plant stress are subjected to a fine-tuned regulation, establishing a complex signaling web that allow plants to cope with changing environmental conditions along their life cycle. We will focus now, specifically, on the different aspects of plant growth and regulation affected directly by CYPs’ activities.

Cytochromes P450 regulate phytohormone homeostasis

CYPs also regulate many important cell processes that affect plant growth and development. Among them, is crucial their role modulating plant hormone metabolism (Fig. 2). Phytohormone homeostasis is essential for proper growth and development of plants. Specifically, they regulate shoot and root patterning, flower development, stems and leaves growth and development, gametophytic development, fertilization and the development and ripening of fruits.
Two Arabidopsis cytochromes P450, CYP79B2 and CYP79B3, participate in one of the L-Trp-dependent pathways proposed for auxin biosynthesis, by converting tryptophan (Trp) into indole-3-acetaldoxime (IAOx) in vitro (Ljung, 2013, Zhao et al., 2002). SUR2, encoding CYP83B1, modulates auxin homeostasis (Barlier et al., 2000). Furthermore, CYP77A4 is involved in the auxin response pattern in embryos by regulating the distribution of the auxin efflux carrier, PIN1 (Kawade et al., 2018).

Strigolactones (SLs) have been classified as a new group of plant hormones essential for shoot branching inhibition. SLs are synthesized from carotenoid via the precursor carlactone (CL). MAX1 encodes a CYP711A1, which catalyzes the conversion of carlactone into carlactonoic acid in the SLs biosynthesis pathway (Abe et al., 2014, Challis et al., 2013, Lazar and Goodman, 2006). Concordantly, mutants in MAX1 exhibit abnormally abundant branches and aberrant patterns of auxin influx and efflux carriers’ expression in the stems.

Also, several CYPs are involved in Gibberellic acid (GA) homeostasis. Gibberellins are essential plant growth regulators that are active in many stages of plant development. Two cytochromes P450, ELA1 (CYP714A1) and ELA2 (CYP714A2) catalyze the deactivation of bioactive GAs in Arabidopsis (Zhang et al., 2011). Similarly, the rice gene EUI1 encodes a CYP that epoxidizes gibberellins (Zhu et al., 2006). Furthermore, CYP735A1 and CYP735A2 function as cytokinin (CK) hydroxylases, catalyzing the biosynthesis of trans-zeatinis, which are isoprenoid CKs in Arabidopsis (Takei et al. 2004). In addition, CYP707A encodes an ABA 8’-hydroxylase, an enzyme that regulates ABA content in Arabidopsis and barley and thus controlling dormancy and seed germination (Kushiro et al., 2004, Millar et al., 2006).

The synthesis of brassinosteroids (BRs), which are a type of polyhydroxysteroids that are essential for plant growth and development (Planas-Riverola et al., 2019) basically depends on CYPs’ activities (Ohnishi, 2018). BRs act regulating the division, elongation and differentiation of numerous cell types throughout different stages of the plant life cycle (Fig. 2). BRs are synthetized from campesterol (CR), a phytosterol that possesses a methyl group at the C-24 position in its side chain. CR is first converted to campestanol and then to brassinolide (BL, the most potent brassinosteroid known so far) via two parallel pathways that consist in a series of hydroxylation steps catalyzed by CYPs: the early C-6 oxidation pathway (where the C-6 position is oxidized early) or the late C-6 oxidation pathway (where the C-6 position is oxidized in the final step) (Oh et al., 2015, Zhao and Li, 2012). DWF4/CYP90B1 catalyzes the C-22 hydroxylation of CR, CPD/CYP90A1 (Ohnishi et al., 2012) is involved in the C-3 dehydrogenation of steroid skeletons and CYP90C1/ROT3 and CYP90D1 have redundant functions as C-23 hydroxylases (Kim et al., 2005a). CYP85A1 and CYP85A2 were found to catalyze the C-6 oxidation reaction (Ohnishi,
As cytochromes P450 are involved in the metabolism of most phytohormones, including auxins, GAs, cytokinins, BRs, ABA, JA, as well as in the synthesis of a wide plethora of metabolites, they play crucial roles in different stages of plant development, all along their life cycle (Fig. 2). We will focus now on the functions described for CYPs in reproductive development.

**Roles of cytochromes P450 in floral development**

CYPs regulate floral development by catalyzing the synthesis or deactivation of hormones involved in the process, but also by catalyzing the synthesis of specific metabolites that were found essential for proper floral patterning. CYP78A9, for instance, participates in a pathway that controls floral organ size and ovule integument development (Sotelo-Silveira et al., 2013). Plants defective in CYP78A9 and in its closer paralog, CYP78A8, present a reduction in floral organ size compared with the WT. Interestingly, this is the opposite effect seen from when CYP78A9 is overexpressed (Sotelo-Silveira et al., 2013). Although metabolic profiling using overexpressing and mutant plants revealed that CYP78A9 is able to alter the flavonoid pathway, the observed phenotypes are not caused by alterations in flavonoid content. This suggests that CYP78A9 might be involved in the synthesis of a novel signal other than the known hormones controlling these aspects of floral development (Sotelo-Silveira et al., 2013).

Mutants in SPS, a gene encoding CYP79F1, show multiple shoot developmental defects, including curly and serrated leaves, high levels of chlorophyll, abnormal vasculature patterns and aberrant floral development. Anthers are usually indehiscent and stigmas remain underdeveloped. In addition, some flowers show reduction or absence of petals and stamens (Tantikanjana et al., 2001). CYP79F1 is required for aldoxime formation in the biosynthesis of glucosinolates in Arabidopsis (Hansen et al., 2001). Although glucosinolates have been specially connected with defense function, as they are precursors of defensive metabolites, their role in plant development is now widely recognized (Jeschke et al., 2019).

A special case is the cytochrome P450 KLUH/CYP78A5. KLUH promotes organ growth via a non-cell-autonomous signal that is distinct from the known classical phytohormones. The KLUH-dependent signal moves beyond individual organs in a flower, coordinating their growth and determining final organ size (Eriksson et al., 2010). In addition, overexpression of KLUH/CYP78A5, as well as of ENHANCER OF DA1-1 (EOD3)/CYP78A6, and of CYP78A9 genes, all produced similar phenotypes that include large siliques and short stamens, a delay in bud opening and reduced fertility, which is more severe in basipetal
flowers. As the overexpression of these genes produce a similar phenotype, it was suggested that they might be part of the same metabolic network (Fang et al., 2012, Marsch-Martinez et al., 2002). Although the catalytic function of the CYP78A enzymes remains unknown, their expression pattern and mutagenesis analysis suggest that they might be involved in the biosynthesis of a new type of plant growth regulator (Sotelo-Silveira et al., 2013).

Among the CYPs involved in floral development by regulating hormone homeostasis is CYP715A1. CYP715s constitute a family of duplication-resistant cytochrome P450 genes in seed plants, present as singletons in most plant genomes (Liu et al., 2015). In Arabidopsis, CYP715A1 regulates petal development, floral GA and JA homeostasis and volatile terpenoid emission. It was proposed as a key regulator of flower maturation, synchronizing petal expansion and the emission of sesquiterpene (Liu et al., 2015). Upon flower opening, JA content declines rapidly. Detailed genetic studies identified CYP94C1 as the major player in the oxidative JA turnover pathway involved in this process (Widemann et al., 2016). CYP94C1 is dominantly expressed in mature anthers, which is consistent with the established role of JA signaling in male fertility.

CYPs also play important roles in the biosynthesis of flavonoids and anthocyanins, both of which are major floral pigments. The number of hydroxyl groups on the B-ring of anthocyanidins, which determines the blue color of these pigments, depends on the activity of two CYPs, flavonoid 3'-hydroxylase (F3'H) and flavonoid 3',5'-hydroxylase (F3'5'H). F3'H and F3'5'H belong to the CYP75B and CYP75A families respectively. An exception is the F3'5'Hs in Compositae, where was originated from gene duplication of CYP75B (Tanaka and Brugliera, 2013). The enzyme FLAVONE SYNTHASE II (FNSII), which catalyzes flavone biosynthesis from flavanones is also a cytochrome P450 (CYP93B) and also contributes to flower color, as flavones act as co-pigments to anthocyanins (Tanaka and Brugliera, 2013).

**Roles of cytochromes P450 during gametogenesis, fertilization and embryogenesis**

The specification of the germ-line is essential for sexual reproduction. In the ovules of most flowering plants, a single hypodermal cell enlarges and differentiates into a megaspore mother cell (MMC), which undergoes meiosis to render a functional megaspore (FM) and three spores that die soon after. The mitotic division of the FM, followed by coordinated events of cellularization and cell specification, promotes the formation of the haploid female gametophyte or embryo sac, which is enclosed in the sporophytic maternal tissues of the ovule.
The CYP gene KLU is required in developing ovules for female meiosis and maternal control of seed size (Eriksson et al., 2010, Zhao et al., 2018). In the ovule, KLU is specifically expressed in the inner integument, at the proximal end of ovule primordia. It was found that KLU restricts MMC specification to a single cell by promoting the expression of the transcription factor gene WRKY28, which in turn prevents somatic cells from differentiating into MMCs (Zhao et al., 2018).

Members of the CYP85A family, like CYP85A2, were shown to catalyze the oxidation of castasterone (Cs) to BL (Kim et al., 2005b). Mutations in the gene encoding CYP85A2 result in severe dwarfism. However, that is not the case for CYP85A1. Insertional lines defective in CYP85A1 do not show any obvious sporophytic defect. However, they are semi-sterile and display female gametophytes arrested before the first nuclear mitotic division. Translational pCYP85A1-GUS fusions showed GUS expression restricted to the female gametophyte, suggesting that CYP85A1 function might be required specifically inside the embryo sac. Although CYP85A1 catalytic activity remains unknown, as the cyp85a2 mutant phenotype is exacerbated in cyp85a1 cyp85a2 double mutants, it was suggested that CYP85A1 and CYP85A2 might have overlapping functions in brassinosteroid synthesis (Perez-Espana et al., 2011).

The already mentioned CYP78A9 participates in a pathway that not only contributes to floral organ size but also to ovule integument development (Sotelo-Silveira et al., 2013). Furthermore, the expression pattern of CYP78A9 suggests that the signal produced by CYP78A9 coordinates integumental growth with gametophytic development. Plants carrying a mutation in CYP78A9 and in its closer paralog, CYP78A8, presented ovules with short integuments that do not accompany the growth of the developing embryo sac, which results in physical restriction of the gametophyte leading to female sterility (Sotelo-Silveira et al., 2013). As expression studies using GUS transcriptional fusions under the control of the CYP78A9 promoter show that the promoter responds to the fertilization event, it was suggested that CYP78A9 might coordinate the developmental growth of the ovule during and after the fertilization process (Sotelo-Silveira et al., 2013).

CYPs also participate in male reproductive development. Anthers and pollen grains are protected from desiccation by a cuticle and by exine layers respectively. The synthesis of cutin monomers and wax components depends essentially on the activity of CYP703A3. CYP703A3 functions as an in-chain hydroxylase of lauric acid, preferably generating 7-hydroxylated lauric acid. Arabidopsis, maize and rice plants defective in CYP703A3 display defective pollen exine and anther epicuticular layer (Morant et al, 2007; Somaratne et al., 2017, Yang et al., 2014). The expression of OsCYP703A3 is directly regulated by Tapetum Degeneration Retardation, a known regulator of tapetum programmed cell death and pollen exine formation (Yang et al., 2014). In maize, mutants impaired in Abnormal Pollen Vacuolation1
(APVI), a tapetum-specific gene, are also defective in anther cuticle and pollen exine formation and completely male sterile. The microspores of apvl mutants are swollen and less vacuolated. APVI encodes a member of the P450 subfamily, ZmCYP703A2, which is widely expressed in the tapetum at the vacuolation stage. AVP1 is involved in the synthesis of sporopollenin precursors and cutin monomers that are essential for the formation of pollen exine and for the anther cuticle in maize (Somaratne et al., 2017). From another CYP family, CYP704B is an omega fatty acid hydroxylase that is also required to make precursors of the tough pollen wall polymer sporopollenin in Arabidopsis, rice, maize and bread wheat (Singh et al., 2017).

During pollen development, the tapetum provides the precursors required for the formation of the pollen wall. Microsporogenesis starts with the differentiation of a microspore mother cell (MMC), which becomes enclosed by a thick callose wall and undergo meiosis, resulting in a tetrad of four haploid microspores. Microgametogenesis starts with the expansion of the microspore which is associated with the formation of a large vacuole. At this stage, the microspore nucleus is displaced to position against the microspore wall, where it undergoes the first mitotic division (pollen mitosis I). This mitosis is asymmetrical, resulting in the formation of a large vegetative cell and a small generative cell. The generative cell is subsequently engulfed by the vegetative cell and divides once more by mitosis (pollen mitosis II) to form the two sperm cells. Depending on the species, this last mitotic division can take place in the anther or within the pollen tube. Right after the first mitosis, pollen grains are enclosed by an outer-wall (exine) and an inner-wall (intine). The intine is the innermost layer of the pollen wall and is secreted by the microspore. It is composed of cellulose, pectin, and various proteins. In Arabidopsis, CYP715A1 was shown to be required for normal intine deposition. Mutants in CYP715A1 show intine layers that are severely undulated, probably as a result of perturbations of the microspore vesicular trafficking (Liu et al., 2015). CYP715A1 showed a restricted tissue-specific expression. In developing flowers it is exclusively expressed in the tapetum. Comparative expression analysis revealed that the expression of genes involved in pollen development and cell wall biogenesis are downregulated in CYP715A1 mutants, which suggest a role for this cytochrome regulating different aspects of pollen development (Liu et al., 2015).

Upon fertilization, the embryogenesis program is activated, which is coordinated with the growth of protective structures that cover the developing seeds. This coordinated and synchronous development of the embryo and the surrounding integuments, largely relies in the communication between maternal tissues and the embryo (Robert et al., 2018). Orientation of cell division planes and expansion, cell-cell communication events and cell fate specification are tightly regulated through the embryogenesis process. Auxin gradients play a central role in embryo patterning. The direction of auxin transport is determined by the asymmetric membrane localization of the efflux carriers, the PIN proteins.
An abnormal distribution of the auxin efflux carrier PIN1 is found in mutants defective in CYP77A4. This cytochrome has fatty-acid epoxidation activity in the microsomal fraction and it is located in the endoplasmic reticulum, where presumably acts as an epoxidase of unsaturated fatty acids. Plants carrying a mutation in the CYP77A4 gene exhibit developmental defects in embryonic patterning from stage 8-cell on. By using auxin-related reporters, it was shown that CYP77A4 is required to establish the normal auxin response pattern in embryos. Since CYP77A4 has fatty-acid epoxidation activity, it is probable that its activity on the membrane-included fatty acids determines the transient localization of PIN1, which in turn might affect the establishment of polarity in the developing plant embryos (Kawade et al., 2018). This should not be surprising, as previous studies have found that the homeostasis of several membrane lipids regulates trafficking of PIN1 and PIN3 (Wang et al., 2017).

The GIANT EMBRYO (GE) gene encodes a CYP78A, a subfamily of P450 monooxygenases that was shown to coordinate rice embryo and endosperm development. GE mutants display enlarged embryos, as a result of an excessive expansion of scutellum cells while post-embryonic growth was severely inhibited due to defective shoot apical meristem (SAM) maintenance (Yang et al., 2013). GE is localized to the endoplasmic reticulum and is expressed predominantly in the interface region between the embryo and the endosperm. Overexpression of GE promoted rice plant growth and grain yield, but reduced embryo size. As overexpression of the GE homolog CYP78A10 in Arabidopsis also yield bigger seeds, a conserved role for this class of P450 proteins in facilitating seed growth was proposed (Yang et al., 2013). In addition, another report showed that GE functions in the embryo to control cell size, and in the endosperm to regulate cell death via ROS signaling (Nagasawa et al., 2013). As GE also regulates SAM but GE mRNA is not detected in that tissue, it was suggested that GE might generate a mobile signal to regulate SAM development in a non-cell autonomous manner, as suggested for KLUH/CYP78A5 (Eriksson et al., 2010). In addition, GE expression in either the embryo or in the endosperm can control embryo and endosperm size (Nagasawa et al., 2013). The catalytic functions of CYP78As are still unknown, although their characterization may reveal a novel mechanism underlying plant growth and seed development.

Concluding remarks

The CYP superfamily plays crucial roles regulating many important cell processes that affect plant growth and development. CYP proteins are involved in the biosynthesis, modification, activation and deactivation/degradation of multiple compounds in various metabolic pathways. These include flavonoids, steroids, terpenoids, phenylpropanoids, glucosinolate and glycosides that are known to play major roles along the plant life cycle (Fig. 2). CYPs are also strictly involved in the regulation of plant
hormone metabolism and thus are vital for processes that include seed germination, shoot patterning, growth, flower development and reproduction. From the analysis of specific knockout mutants, some CYPs are known to be essential for crucial developmental events, although their catalytic activities are still under study. The characterization of the catalytic functions of these CYPs and the signal molecule(s) they produce may reveal new mechanisms underlying different aspects of plant development. As many of these still uncharacterized CYPs are essential for growth and reproduction, their study might also provide new biotechnological approaches for improving yield in species of agronomical interests.

References

ABE, S., SADO, A., TANAKA, K., KISUGI, T., ASAMI, K., OTA, S., KIM, H.I., YONEYAMA, K., XIE, X., OHNISHI, T. et al. (2014). Carlactone is converted to carlactonoic acid by MAX1 in Arabidopsis and its methyl ester can directly interact with AtD14 in vitro. Proc Natl Acad Sci U S A 111: 18084-9.

BAK, S., BEISSON, F., BISHOP, G., HAMBERGER, B., HÖFER, R., PAQUETTE, S. and WERCK-REICHHART, D. (2011). Cytochromes P450. The Arabidopsis Book / American Society of Plant Biologists 9: e0144.

BANERJEE, A. and HAMBERGER, B. (2018). P450s controlling metabolic bifurcations in plant terpene specialized metabolism. Phytochemistry Reviews 17: 81-111.

BARLIER, I., KOWALCZYK, M., MARCHANT, A., LJUNG, K., BHALERAO, R., BENNETT, M., SANDBERG, G. and BELLINI, C. (2000). The SUR2 gene of Arabidopsis thaliana encodes the cytochrome P450 CYP83B1, a modulator of auxin homeostasis. Proceedings of the National Academy of Sciences of the United States of America 97: 14819-14824.

BATEH, U. and TISSIER, A. (2019). Cytochrome P450 enzymes: A driving force of plant diterpene diversity. Phytochemistry 161: 149-162.

BELL, L. (2019). The Biosynthesis of Glucosinolates: Insights, Inconsistencies, and Unknowns. In Annual Plant Reviews online, pp.969-1000.

BISHOP, G.J. and KONCZ, C. (2002). Brassinosteroids and Plant steroid hormone signaling. Plant Cell 14: 97-110.

CHALLIS, R., HEPWORTH, J., MOUCHEL, C.L., WAITES, R. and LEYSER, O. (2013). A role for MAX1 in evolutionary diversity in strigolactone signalling upstream of MAX2. Plant Physiology 161: 1885-1902.

DU FALL, L.A. and SOLOMON, P.S. (2013). The necrotrophic effector SnToxA induces the synthesis of a novel phytoalexin in wheat. New Phytologist 200: 185-200.

EHLTING, J., SAUVEPLANE, V., OLRY, A., GINGLINGER, J.-F., PROVART, N.J. and WERCK-REICHHART, D. (2008). An extensive (co-)expression analysis tool for the cytochrome P450 superfamily in Arabidopsis thaliana. BMC Plant Biology 8: 47.

ERIKSSON, S., STRANSFELD, L., ADAMSKI, N.M., BREUNINGER, H. and LENHARD, M. (2010). KLUH/CYP78A5-dependent growth signaling coordinates floral organ growth in Arabidopsis. Curr Biol 20: 527-532.

FANG, W., WANG, Z., CUI, R., LI, J. and LI, Y. (2012). Maternal control of seed size by EOD3/CYP78A6 in Arabidopsis thaliana. The Plant journal : for cell and molecular biology 70: 929-939.

FRANKE, R.B., DOMBRINK, I. and SCHREIBER, L. (2012). Suberin goes genomics: use of a short living plant to investigate a long lasting polymer. Frontiers in Plant Science 3: 4-4.

GHOSH, S. (2017). Triterpene Structural Diversification by Plant Cytochrome P450 Enzymes. Frontiers in Plant Science 8: 1886-1886.
GLAUSER, G., GRATA, E., DUBUGNON, L., RUDAZ, S., FARMER, E.E. and WOLFENDER, J.-L. (2008). Spatial and Temporal Dynamics of Jasmonate Synthesis and Accumulation in Arabidopsis in Response to Wounding. Journal of Biological Chemistry 283: 16400-16407.

GLAWISCHNIG, E. (2006). The role of cytochrome P450 enzymes in the biosynthesis of camalexin. Biochemical Society transactions 34: 1206-1208.

GRAHAM, S.E. and PETERSON, J.A. (1999). How Similar Are P450s and What Can Their Differences Teach Us? Archives of Biochemistry and Biophysics 369: 24-29.

GUO, R., QIAN, H., SHEN, W., LIU, L., ZHANG, M., CAI, C., ZHAO, Y., QIAO, J. and WANG, Q. (2013). BZR1 and BES1 participate in regulation of glucosinolate biosynthesis by brassinosteroids in Arabidopsis. Journal of Experimental Botany 64: 2401-2412.

GUTTIKONDA, S.K., TRUPTI, J., BISHT, N.C., CHEN, H., AN, Y.-Q.C., PANDEY, S., XU, D. and YU, O. (2010). Whole genome co-expression analysis of soybean cytochrome P450 genes identifies nodulation-specific P450 monooxygenases. BMC Plant Biology 10: 243.

HANSEN, C.H., WITTSTOCK, U., OLSEN, C.E., HICK, A.J., PICKETT, J.A. and HALKIER, B.A. (2001). Cytochrome P450 CYP79F1 from Arabidopsis Catalyzes the Conversion of Dihomomethionine and Trihomomethionine to the Corresponding Aldoximes in the Biosynthesis of Aliphatic Glucosinolates. Journal of Biological Chemistry 276: 11078-11085.

HELLIWELL, C.A., CHANDLER, P.M., POOLE, A., DENNIS, E.S. and PEACOCK, W.J. (2001). The CYP88A cytochrome P450, ent-kaurenoic acid oxidase, catalyzes three steps of the gibberellin biosynthesis pathway. Proceedings of the National Academy of Sciences of the United States of America 98: 2065-2070.

HÖFER, R., BRIESEN, I., BECK, M., PINOT, F., SCHREIBER, L. and FRANKE, R. (2008). The Arabidopsis cytochrome P450 CYP86A1 encodes a fatty acid omega-hydroxylase involved in suberin monomer biosynthesis. Journal of Experimental Botany 59: 2347-2360.

IKEZAWA, N., TANAKA, M., NAGAYOSHI, M., SHINKYO, R., SAKAKI, T., INOUE, K. and SATO, F. (2003). Molecular cloning and characterization of CYP719, a methylenedioxy bridge-forming enzyme that belongs to a novel P450 family, from cultured Coptis japonica cells. J Biol Chem 278: 38557-38565.

IRMISCH, S., MCCORMICK, A.C., BOECKLER, G.A., SCHMIDT, A., REICHELT, M., SCHNEIDER, B., BLOCK, K., SCHNITZLER, J.-P., GERSHENZON, J., UNSICKER, S.B. et al. (2013). Two herbivore-induced cytochrome P450 enzymes CYP79D6 and CYP79D7 catalyze the formation of volatile aldoximes involved in poplar defense. The Plant Cell 25: 4737-4754.

JESCHKE, V., WEBER, K., MOORE, S.S. and BUROW, M. (2019). Coordination of Glucosinolate Biosynthesis and Turnover Under Different Nutrient Conditions. Frontiers in Plant Science 10: 1560-1560.

KAWADE, K., LI, Y., KOGA, H., SAWADA, Y., OKAMOTO, M., KUWAHARA, A., TSUKAYA, H. and HIRAI, M.Y. (2018). The cytochrome P450 CYP77A4 is involved in auxin-mediated patterning of the <em>Arabidopsis thaliana</em> embryo. Development 145: dev168369.

KIM, G.-T., FUJIOKA, S., KOZUKA, T., TAX, F.E., TAKATSUTO, S., YOSHIDA, S. and TSUKAYA, H. (2005a). CYP90C1 and CYP90D1 are involved in different steps in the brassinosteroid biosynthesis pathway in Arabidopsis thaliana. The Plant journal : for cell and molecular biology 41: 710-721.

KIM, J., SMITH, J.J., TIAN, L. and DELLAPENNA, D. (2009). The evolution and function of carotenoid hydroxylases in Arabidopsis. Plant Cell Physiol 50: 463-479.

KIM, T.-W., HWANG, J.-Y., KIM, Y.-S., JOO, S.-H., CHANG, S.C., LEE, J.S., TAKATSUTO, S. and KIM, S.-K. (2005b). Arabidopsis CYP85A2, a Cytochrome P450, Mediates the Baeyer-Villiger Oxidation of Castasterone to Brassinolide in Brassinosteroid Biosynthesis. The Plant Cell 17: 2397-2412.

KINZLER, A.J., PROKOPIAK, Z.A., VAUGHAN, M.M., ERHARDT, P.W., SARVER, J.G., TRENDEL, J.A., ZHANG, Z. and DAFOE, N.J. (2016). Cytochrome P450, CYP93A1, as defense marker in soybean. Biologia Plantarum 60: 724-730.

KOCH, A., KUMAR, N., WEBER, L., KELLER, H., IMANI, J. and KOGEL, K.-H. (2013). Host-induced gene silencing of cytochrome P450 lanosterol C14α-demethylase-encoding genes confers strong
resistance to Fusarium species. *Proceedings of the National Academy of Sciences of the United States of America* 110: 19324-19329.

KOO, A.J., THIREAULT, C., ZEMELIS, S., POUDEL, A.N., ZHANG, T., KITAOKA, N., BRANDIZZI, F., MATSUURA, H. and HOWE, G.A. (2014). Endoplasmic Reticulum-associated Inactivation of the Hormone Jasmonoyl-L-Isoleucine by Multiple Members of the Cytochrome P450 94 Family in Arabidopsis. *Journal of Biological Chemistry* 289: 29728-29738.

KUSHIRO, T., OKAMOTO, M., NAKABAYASHI, K., YAMAGISHI, K., KITAMURA, S., ASAMI, T., HIRAI, N., KOSHIBA, T., KAMIYA, Y. and NAMBARA, E. (2004). The Arabidopsis cytochrome P450 CYP707A encodes ABA 8'-hydroxylases: key enzymes in ABA catabolism. *The EMBO journal* 23: 1647-1656.

LAMB, D.C., LEI, L., WARRILOW, A.G., LEPESHEVA, G.I., MULLINS, J.G., WATERMAN, M.R. and KELLY, S.L. (2009). The first virally encoded cytochrome p450. *J Virol* 83: 8266-9.

LAZAR, G. and GOODMAN, H.M. (2006). MAX1, a regulator of the flavonoid pathway, controls vegetative axillary bud outgrowth in Arabidopsis. *Proc Natl Acad Sci USA* 103: 472-476.

LIU, Z., BOACHON, B., LUGAN, R., TAVARES, R., ERHARDT, M., MUTTERER, J., Demais, V., PATEYRON, S., BRUNAUD, V., OHNISHI, T. *et al.* (2014). A Conserved Cytochrome P450 Evolved in Seed Plants Regulates Flower Maturation. *Molecular Plant* 8: 1751-1765.

LIJUNG, K. (2013). Auxin metabolism and homeostasis during plant development. *Development* 140: 943-50.

MA, B. and TREDWAY, L.P. (2013). Induced overexpression of cytochrome P450 sterol 14α-demethylase gene (CYP51) correlates with sensitivity to demethylation inhibitors (DMIs) in Sclerotinia homoeocarpa. *Pest management science* 69: 1369-1378.

MARSCH-MARTINEZ, N., GRECO, R., VAN ARKEL, G., HERRERA-ESTRELLA, L. and PEREIRA, A. (2002). Activation Tagging Using the <em>En-I</em> Maize Transposon System in Arabidopsis. *Plant Physiology* 129: 1544-1556.

MATHUR, J., MOLNAR, G., FUJIOKA, S., TAKATSUTO, S., SAKURAI, A., YOKOTA, T., ADAM, G., VOIGT, B., NAGY, F., MAAS, C. *et al.* (1998). Transcription of the Arabidopsis CPD gene, encoding a steroidogenic cytochrome P450, is negatively controlled by brassinosteroids. *Plant J* 14: 593-602.

MICHEAL, S., HEINZLMEIR, S., KRIECHBAUMER, V., STRICKLAND, B., KIRCHHELLE, C., CHAUDHARY, M., KOWALSKI, N., EICHMANN, R., HÜCKELHOVEN, R., GRILL, E. *et al.* (2019). The Formation of a Camalexin Biosynthetic Metabolon. *The Plant Cell* 31: 2697.

NAGASAWA, N., HIBARA, K.-I., HEPPARD, E.P., VANDER VELDEN, K.A., LUCK, S., BEATTY, M., NAGATO, Y. and SAKAI, H. (2013). GIANT EMBRYO encodes CYP78A13, required for proper size balance between embryo and endosperm in rice. *The Plant Journal* 75: 592-605.

OHNISHI, T. (2018). Recent advances in brassinosteroid biosynthetic pathway: insight into novel brassinosteroid shortcut pathway. *Journal of pesticide science* 43: 159-167.
OHNISHI, T., GODZA, B., WATANABE, B., FUJIOKA, S., HATEGAN, L., IDE, K., SHIBATA, K., YOKOTA, T., SZEKERES, M. and MIZUTANI, M. (2012). CYP90A1/CPD, a brassinosteroid biosynthetic cytochrome P450 of Arabidopsis, catalyzes C-3 oxidation. *J Biol Chem* 287: 31551-31560.

PEREZ-ESPAÑA, V., SANCHEZ-LEON, N. and VIELLE-CALZADA, J. (2011). CYP85A1 is required for the initiation of female gametogenesis in Arabidopsis thaliana. *Plant Signal Behav* 6: 321-326.

PLANAS-RIVEROLA, A., GUPTA, A., BETEGÓN-PUTEZ, I., BOSCH, N., IBAÑES, M. and CAÑO-DELGADO, A.I. (2019). Brassinosteroid signaling in plant development and adaptation to stress. *Development* 146: dev151894.

POLLMANN, S., SPRINGER, A., RUSTGI, S., VON WETTSTEIN, D., KANG, C., REINBOTHE, C. and REINBOTHE, S. (2019). Substrate channeling in oxylipin biosynthesis through a protein complex in the plastid envelope of Arabidopsis thaliana. *Journal of Experimental Botany* 70: 1483-1495.

QUINLAN, R.F., SHUMSKAYA, M., BRADBURY, L.M.T., BELTRÁN, J., MA, C., KENNELLY, E.J. and WURTZEL, E.T. (2012). Synergistic Interactions between Carotene Ring Hydroxylases Drive Lutein Formation in Plant Carotenoid Biosynthesis. *Plant Physiology* 160: 204-214.

RAI, A., SINGH, R., SHIRKE, P.A., TRIPATHI, R.D., TRIVEDI, P.K. and CHAKRABARTY, D. (2015). Expression of Rice CYP450-Like Gene (Os08g01480) in Arabidopsis Modulates Regulatory Network Leading to Heavy Metal and Other Abiotic Stress Tolerance. *PLoS ONE* 10: e0138574-e0138574.

ROBERT, H.S., PARK, C., GUTIÈRREZ, C.L., WÓJCIKOWSKA, B., PĚNČÍK, A., NOVÁK, O., CHEN, J., GRUNEWALD, W., DRESSELHAUS, T., FRIML, J. et al. (2018). Maternal auxin supply contributes to early embryo patterning in Arabidopsis. *Nature plants* 4: 548-553.

SAUVEPLANE, V., KANDEL, S., KASTNER, P.-E., EHLTING, J., COMPAGNON, V., WERCK-REICHHART, D. and PINOT, F. (2009). Arabidopsis thaliana CYP77A4 is the first cytochrome P450 able to catalyze the epoxidation of free fatty acids in plants. *The FEBS journal* 276: 719-735.

SCHALLER, A. and STINTZI, A. (2009). Enzymes in jasmonate biosynthesis – Structure, function, regulation. *Phytochemistry* 70: 1532-1538.

SINGH, M., KUMAR, M., THILGÉS, K., CHO, M.-J. and CIGAN, A.M. (2017). MS26/CYP704B is required for anther and pollen wall development in bread wheat (Triticum aestivum L.) and combining mutations in all three homeologs causes male sterility. *PLoS ONE* 12: e0177632.

SOMARATNE, Y., TIAN, Y., ZHANG, H., WANG, M., HUO, Y., CAO, F., ZHAO, L. and CHEN, H. (2017). ABNORMAL POLLEN VACUOLATION1 (APV1) is required for male fertility by contributing to anther cuticle and pollen exine formation in maize. *Plant J* 90: 96-110.

SOTELO-SILVEIRA, M., CUCINOTTA, M., COLOMBO, L., MARSCH-MARTÍNEZ, N. and DE FOLTER, S. (2013). Toward understanding the role of CYP78A9 during Arabidopsis reproduction. *Plant Signaling & Behavior* 8: e25160.

STUMPE, M. and FEUSSNER, I. (2006). Formation of oxylipins by CYP74 enzymes. *Phytochemistry Reviews* 5: 347-357.

TANAKA, Y. and BRUGLIERA, F. (2013). Flower colour and cytochromes P450. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 368: 20120432-20120432.

TANTIKANJANA, T., YONG, J.W.H., LETHAM, D.S., GRIFFITH, M., HUSSAIN, M., LJUNG, K., SANDBERG, G. and SUNDARESAN, V. (2001). Control of auxillary bud initiation and shoot architecture in Arabidopsis through the SUPERSHOOT gene. *Genes & Development* 15: 1577-1588.

TOHGE, T., PEREZ DE SOUZA, L. and FERNIE, A.R. (2018). On the natural diversity of phenylacylated-flavonoid and their in planta function under conditions of stress. *Phytochemistry Reviews* 17: 279-290.

WANG, Y., YANG, L., TANG, Y., TANG, R., JING, Y., ZHANG, C., ZHANG, B., LI, X., CUI, Y., ZHANG, C. et al. (2017). Arabidopsis choline transporter-like 1 (CTL1) regulates secretory trafficking of auxin transporters to control seedling growth. *PLoS biology* 15: e2004310-e2004310.

WIDEMANN, E., SMIRNOVA, E., AUBERT, Y., MIESCH, L. and HEITZ, T. (2016). Dynamics of Jasmonate Metabolism upon Flowering and across Leaf Stress Responses in Arabidopsis thaliana. *Plants* 5: 4.
YANG, W., GAO, M., YIN, X., LIU, J., XU, Y., ZENG, L., LI, Q., ZHANG, S., WANG, J., ZHANG, X. et al. (2013). Control of Rice Embryo Development, Shoot Apical Meristem Maintenance, and Grain Yield by a Novel Cytochrome P450. *Molecular Plant* 6: 1945-1960.

YANG, X., WU, D., SHI, J., HE, Y., PINOT, F., GRAUSEM, B., YIN, C., ZHU, L., CHEN, M., LUO, Z. et al. (2014). Rice CYP703A3, a cytochrome P450 hydroxylase, is essential for development of anther cuticle and pollen exine. *J Integr Plant Biol* 56: 979-94.

YONEKURA-SAKAKIBARA, K., HIGASHI, Y. and NAKABAYASHI, R. (2019). The Origin and Evolution of Plant Flavonoid Metabolism. *Frontiers in Plant Science* 10.

ZHANG, Y., ZHANG, B., YAN, D., DONG, W., YANG, W., LI, Q., ZENG, L., WANG, J., WANG, L., HICKS, L.M. et al. (2011). Two Arabidopsis cytochrome P450 monooxygenases, CYP714A1 and CYP714A2, function redundantly in plant development through gibberellin deactivation. *The Plant Journal* 67: 342-353.

ZHANG, Y., ZHANG, B., YAN, D., DONG, W., YANG, W., LI, Q., ZENG, L., WANG, J., WANG, L., HICKS, L.M. et al. (2011). Two Arabidopsis cytochrome P450 monooxygenases, CYP714A1 and CYP714A2, function redundantly in plant development through gibberellin deactivation. *The Plant Journal* 67: 342-353.

ZHAO, B. and LI, J. (2012). Regulation of Brassinosteroid Biosynthesis and Inactivation. *J Integr Plant Biol* 54: 746-759.

ZHAO, L., CAI, H., SU, Z., WANG, L., HUANG, X., ZHANG, M., CHEN, P., DAI, X., ZHAO, H., PALANIVELU, R. et al. (2018). *KLU* suppresses megasporocyte cell fate through SWR1-mediated activation of *WRKY28* expression in *Arabidopsis*. *Proceedings of the National Academy of Sciences* 115: E526-E535.

ZHAO, Y., HULL, A.K., GUPTA, N.R., GOSS, K.A., ALONSO, J., ECKER, J.R., NORMANY, J., CHORY, J. and CELENZA, J.L. (2002). Trp-dependent auxin biosynthesis in Arabidopsis: involvement of cytochrome P450s CYP79B2 and CYP79B3. *Genes & Development* 16: 3100-3112.

ZHU, Y., NOMURA, T., XU, Y., ZHANG, Y., PENG, Y., MAO, B., HANADA, A., ZHOU, H., WANG, R., LI, P. et al. (2006). ELOGATED UPPERMOST INTERNODE encodes a cytochrome P450 monooxygenase that epoxidizes gibberellins in a novel deactivation reaction in rice. *The Plant Cell* 18: 442-456.
| Clan   | Metabolic pathways/activities                        | References                                                                 |
|--------|------------------------------------------------------|----------------------------------------------------------------------------|
| CYP51  | Synthesis of sterols and triterpenes                | Kim et al. 2005; Koch et al. 2013; Ma and Tredway 2013; Gosh, 2017         |
| CYP71  | Terpene bifurcation                                  | Banerjee and Hamberger, 2018                                              |
| CYP72  | Brassinosteroids inactivation                        | Nakamura et al., 2005; Bak et al., 2011;                                  |
| CYP73  | Phenylpropanoid biosynthesis pathway                 | Ehlting et al., 2008                                                      |
| CYP74  | Synthesis of oxylipin derivatives                    | Schaller and Stintzi, 2009; Stumpe and Feussner, 2006; Pollmann et al., 2019 |
| CYP77  | Fatty acid oxidases, cutin biosynthesis              | Sauveplane et al., 2009                                                   |
| CYP79  | Biosynthesis of glucosinolates                       | Bell, 2019                                                                |
| CYP85  | Brassinosteroid biosynthesis                         | Kim et al. 2005; Bishop and Koncz, 2002; Bak et al., 2011                 |
| CYP86  | Hydroxylation of fatty acids, suberin biosynthesis   | Höfer et al., 2008; Franke et al., 2012                                   |
| CYP90  | Brassinosteroid biosynthesis                         | Kim et al. 2005; Bishop and Koncz, 2002; Bak et al., 2011; Mathur et al., 1998 |
| CYP97  | Hydroxylation of carotenoids                         | Kim et al. 2009; Quinlan et al., 2012                                     |
| CYP710 | Sterol C-22 desaturases                              | Morikawa et al., 2009                                                     |
Fig.1. Circular cladogram of cytochrome P450s from A. thaliana.

Alignment was performed in MEGA7 package using Muscle (Parameters were: gap opening penalty, 10; a gap extension penalty, 0.1; Gonnet matrix). The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 72.64941847 is shown. The evolutionary distances were computed using the Poisson correction method. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are not shown. The analysis involved 242 amino acid sequences. Evolutionary analyses were conducted in MEGA7.
Fig. 2. Involvement of Cytochromes P450 in different aspects of plant growth and development along the life cycle. The different hormones that regulate the developmental events shown are also indicated.