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Roles of cytochromes P450 in plant reproductive development

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ABSTRACT The cytochrome P450 superfamily is a large enzymatic protein family that is widely distributed along diverse kingdoms. In plants, cytochrome P450 monooxygenases (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.

KEY WORDS: *cytochromes P450, plant development, plant reproduction*

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 *Physcomitrium patens* (Nelson 2006), 244 genes and 28 pseudogenes in *Arabidopsis* (Fig. 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

Abbreviations used in this paper: 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; SL, strigolactones; Trp, tryptophan.

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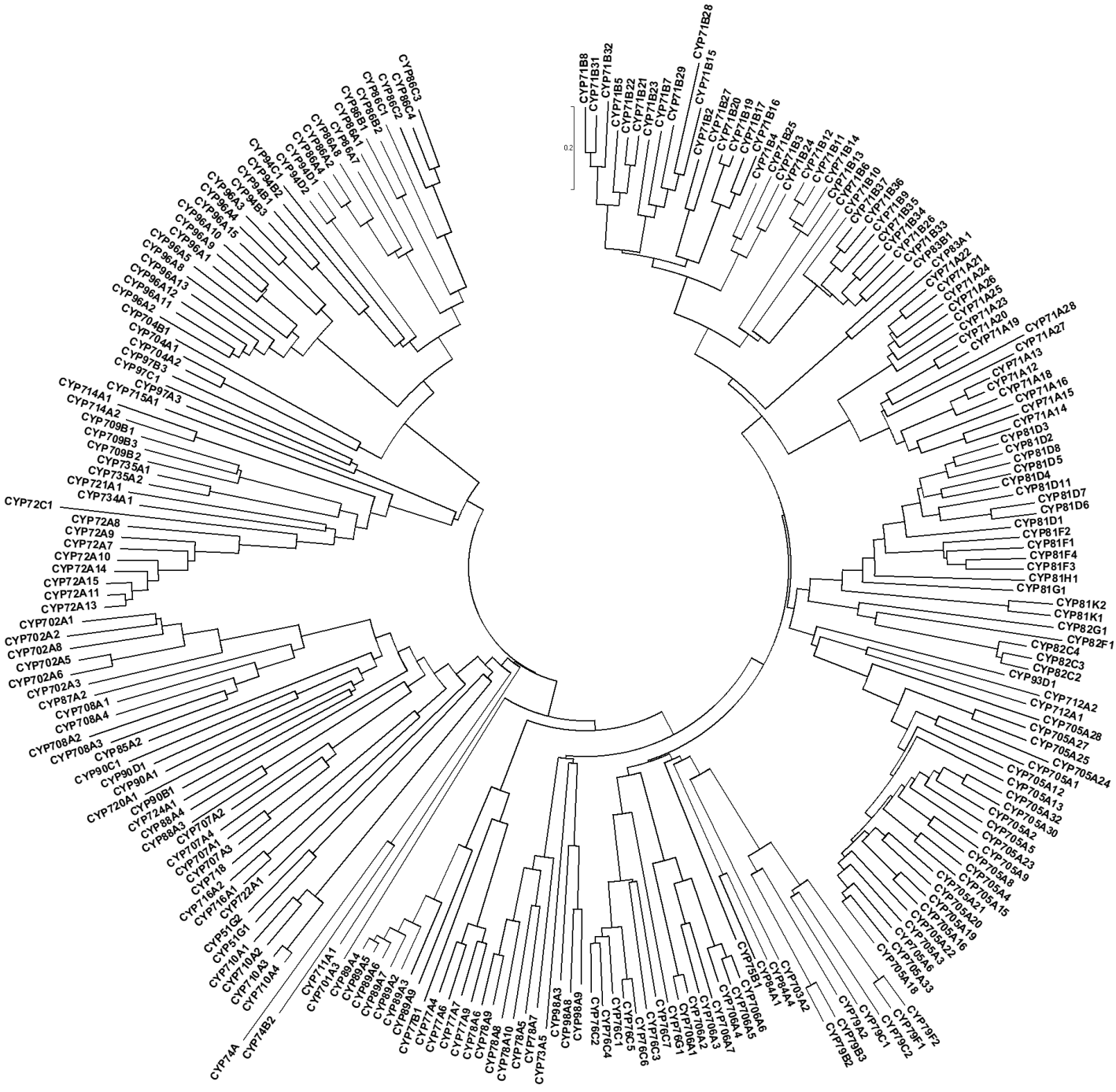

Fig. 1. Circular cladogram of cytochrome P450s from *A. thaliana*. *Alignment was performed in MEGA7 package using Muscle (Parameters were: gap opening penalty, 10; a gap extension penalty, 0.1; Gonnet matrix). The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree 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.*

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 plant 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

TABLE1

FUNCTION OF P450 FAMILIES IN SPECIFIC PLANT METABOLIC PATHWAYS

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

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, Ehlting *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 (Glauser *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-zeatins, which are isoprenoid CKs in

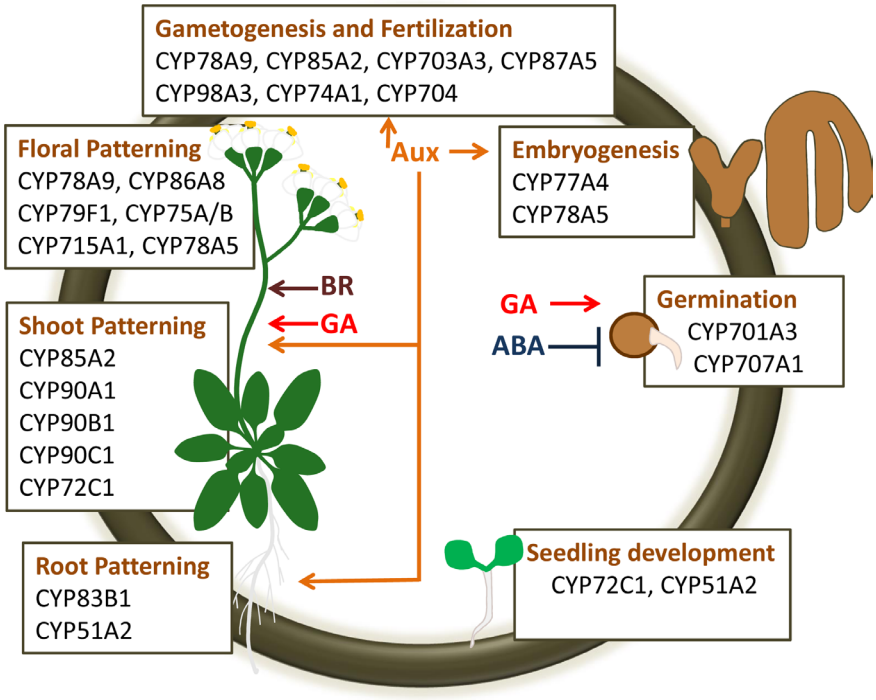

Fig. 2. Involvement of Cytochromes P450 in different aspects of plant growth and development along the life cycle. *The different hormones that regulate the developmental events shown are also indicated.*

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, 2018). The structural diversity of BRs is also generated by the action of several groups of CYPs (Ohnishi, 2018).

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, they 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* (*APV1*), a tapetum-specific gene, are also defective in anther cuticle and pollen exine formation and completely male sterile. The microspores of *apv1* mutants are swollen and less vacuolated. *APV1* 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.

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